OCHRATOXICOSIS IN QUAILS
(*COTURNIX COTURNIX JAPONICA*)
WITH SPECIAL REFERENCE TO IMMUNOPATHOLOGICAL RESPONSE

By

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THESIS

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To

Those who think lovingly, speak lovingly

and act lovingly towards all mankind

DECLARATION

I hereby declare that this thesis entitled **"Ochratoxicosis in Quails (Coturnix coturnix japonica) with Special Reference to Immunopathological Response"** 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 of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Introduction

INTRODUCTION

The association of toxic fungal metabolites, which are popularly known as mycotoxins with human and animal health has been known since biblical times when ergotism was identified to be of mycotoxic origin. The recent awareness on the problem came when Sakaki, a Japanese physician pointed out that rice grains infected with fungi were responsible for the development of cardiac beri beri. He demonstrated that spoiled rice grains contained a neurotoxin and later the neurotoxin was identified to be a mycotoxin, Citreoviridin produced by Penicillium species. Since then, mycotoxins have been considered to be hazardous agents for human and animal health. But it was the discovery of aflatoxins in the early 1960s that really focussed attention on the problem of mycotoxins and led to the discovery of many toxigenic fungi as well as many toxic fungal metabolites.

During the past three decades a wealth of information has accumulated on toxigenic fungi, their toxins and resulting toxicosis. The impact of the discovery of aflatoxicosis as a disease entity with a wide spectrum of deleterious effects in man and animals has resulted in the intensification of research on other mycotoxicoses and their cause effect relationship.

The identification of the carcinogenic and teratogenic effects of some of the mycotoxins have added a new dimension to the problem of mycotoxicosis. There are many well known mycotoxins and new mycotoxins are being identified. Their aetiological role in diseases of man and animals is being established. There is now an organised effort devoted to understand and hopefully control mycotoxicoses.

In India, recognizing the magnitude of the problem in recent years, considerable attention has been paid by scientists working in various institutions to investigate on various aspects of mycotoxicosis. In Kerala, mycotoxicosis was encountered for the first time in 1964 when aflatoxicosis was diagnosed in ducks and pigs. Since then, a variety of mycotoxic conditions have been identified in the state affecting different classes of livestock. The warm and humid climatic conditions existing in Kerala are ideally suited for fungal multiplication and toxin production in feed stuff. One important aspect that has to be considered is the presence of mycotoxins in feed in sublethal or negligible doses which over a period of time cripple the production performance of livestock. Synergistic action of various mycotoxins present in feed commodities, even when they are present in low doses cause an adverse effect on livestock health. The presence of residues of mycotoxins or their metabolites in livestock products like meat and milk highlights the public health

significance of the problem. The immunosuppressive effect of aflatoxin has been identified to be responsible for the outbreak of many diseases and often breakdown of immunity following vaccination particularly in poultry has been pinpointed to the consumption of aflatoxin contaminated diet. The mycotoxins, therefore, have emerged as important factors which cripple economic livestock production programmes in the country.

The interest in ochratoxins dates from the discovery of toxigenic Aspergillus ochraceus and the subsequent identification of ochratoxin A (OA) by Van der Merwe et al. (1965). The ochratoxins comprise of a series of nephrotoxic and hepatotoxic 3, 4-dihydro-3-methyl isocoumarin derivatives produced by several species of fungi belonging to the genera Aspergillus and Penicillium. OA producing fungi are widespread in nature and have been characterised as storage fungi. The OA contamination of many agricultural commodities has alerted scientists about the potential hazard of these toxins to human health. The discovery of the presence of OA in meat and milk of animals consuming OA has focussed attention on the public health significance of the problem.

When OA was discovered during the course of studies for identification of new toxic metabolites from fungi there was no association with animal or human disease. The toxin

was encountered as a natural contaminant in maize in 1968 in USA but there also it was not associated with any disease problem. The importance of OA and its association with disease was known when it was revealed that OA was the determinant causative factor in mycotoxic porcine nephropathy encountered in the Scandinavian countries. Since then, OA has been identified as a contaminant of grains in many countries. Human exposure has been reported in many European countries by the detection of OA in the blood of people suffering from endemic Balkan nephropathy.

The pathogenic effects of OA have been demonstrated in various other species of livestock also. The investigations undertaken on OA have surprisingly revealed the similarity in the biological effects of aflatoxin and OA. If the aflatoxin bites the liver and licks the kidney the OA licks the liver and bites the kidney. Like aflatoxin, OA has been demonstrated to have carcinogenic and teratogenic effects in certain species. The synergistic toxic effects of OA and other important mycotoxins have also been the subject of investigation. The investigations undertaken in this laboratory have shown that feed ingredients in Kerala are contaminated with OA and it could be an important problem which can adversely affect livestock health.

Disease outbreaks, in spite of vaccination against contagious diseases, is a threat to farm economy. This breakdown of immunity particularly in chicken is a curse on the farmers and they loose faith in vaccination programmes. Although, there are ever so many reasons for such breakdown of immunity, mycotoxins have been recognized to be very important factors.

There have not been much published works on the immunotoxicity of OA particularly in quails. This study has been designed to investigate into the pathological effects of OA in quail embryos, and quails with particular reference to the immune system. The objective also includes an effort to modulate the immunodeficiency induced by OA.

Review of Literature

REVIEW OF LITERATURE

2.1 Ochratoxins

Ochratoxin is a toxic metabolite produced by certain species of Aspergillus and Penicillium. Three strains of Aspergillus ochraceus were isolated from grains by Scott (1965) and the grain inoculated with these strains was found to be toxic to ducklings, mice and rats. Van der Merwe et al. (1965) identified the toxic principle in the grain and named it as ochratoxin A (OA). Subsequently ochratoxin B and C were also extracted from strains of Aspergillus ochraceus Wilh. Two strains of A. ochraceus from mouldy barley and hay were isolated by Staron et al. (1965). Lai et al. (1968) reported that six other species of A. ochraceus, group also produced ochratoxins. OA was later isolated from several species of Penicillium also (Van Walbeek et al. 1969 and Ciegler et al. 1972). Shotwell et al. (1969) isolated OA as a natural contaminant of corn for the first time. Scott et al. (1970) identified the fungal species responsible for ochratoxin production in wheat in Penicillium viridicatum.

Ciegler (1972) and Hesseltine et al. (1972), reported that A. sclerotiorum, A. alliaceus, A. ostianus and A. petrakii were capable of producing OA, and ochratoxin B.

Other species of Penicillium, like P. palitans, P. commune, P. variabile, P. purpurescens and P. cyclopium also were found to elaborate ochratoxin as their metabolite (Ciegler et al. 1972). Josefsson et al. (1975) isolated Aspergillus and Penicillium species in varying number in samples of rice and wheat flour. Three strains of fungi were isolated by Torrey and Marth (1977) which were found to be capable of producing OA.

Vesela et al. (1978), isolated OA from fodder barley contaminated with P. verrucosum, var. verrucosum in Czechoslovakia. Lillehoj and Goransson (1980) found that four strains of P. purpurscens, one of P. verrucosum and one of A. ochraceus species produced ochratoxin in barley.

Krogh (1987) described the chemical properties of ochratoxins, which were classified according to biosynthetic origin as pentaketides within the group of polyketides. The acids including 4-hydroxy-ochratoxin A, the methyl and ethyl esters, and the isocoumarin part of ochratoxin B, have all been isolated from fungal cultures. On acid hydrolysis OA yielded phenylalanine and the isocoumarin part. Ochratoxin , a cleavage product was also found in the intestine, faeces, urine and liver of rodents experimentally fed OA. OA was a stable compound, it could be stored in ethanol in the refrigerator for more than a year without loss, however, such

solution on exposure to light for several days decomposed. OA was a colourless crystalline compound, obtained by crystallization from benzene, with a melting point about 90°C, and contained approximately one mole of benzene, after drying for one hour at 60°C it had a melting point in the range of 168-173°C. OA was soluble in polar organic solvents, slightly soluble in water and soluble in dilute aqueous bicarbonate.

2.2 Effect of OA on embryo

2.2.1 Japanese quail

When Japanese quail hens were fed a diet containing 16 ppm OA, the hatchability of the eggs was reduced (Prior et al. 1978). In a study, Prior et al. (1979) injected fertile Japanese quail eggs with graded doses of OA, and incubated for 7 days and examined macroscopically for viability. The percentage of early embryonic deaths in eggs injected with either 1600 or 5000 µg OA per egg was similar to the incidence of early embryonic death observed in the in vivo groups in which the females received 16 ppm of OA in their feed. They opined that embryotoxicity effect of OA was exerted through the presence of OA in the egg rather than an alteration of gametes.

2.2.2 Other avian species

Gilani et al. (1978) reported teratogenic effect of OA

on chick embryos which were administered 0.0005 to 0.0007 µg/egg at 0-4 days. The percentage of survival ranged from 100 to 7. Short and twisted limbs, short and twisted neck, eye defects, exencephaly, reduced body size and everted viscera were the malformations observed.

Lalithakunjamma (1987) observed 75 per cent mortality in chick embryos which were inoculated with 0.5 µg OA/egg. The embryos which survived were stunted in size and had reduced body weight. The embryos which were dead during the early period showed imperfect organogenesis.

According to Niemiec et al. (1990) OA in the feed of hens (0, 1, 2 and 4 ppm) influenced embryo mortality and embryonic anomalies were seen with predominant changes in the liver and kidney.

2.2.3 Laboratory animals

OA was demonstrated to cause foetal resorption in rats (Still et al. 1971). Intraperitoneal injection of OA in mice (5 mg/kg OA) on one of the gestation days 7-12 resulted in increased prenatal mortality, decreased foetal weight, and various foetal malformations (Hayes et al. 1973). Exencephaly and anomalies of the eyes, face, digits, and tail were the most common defects observed by them.

When pregnant golden hamsters were injected intraperitonealy with 2.5-20 mg/kg on gestation days 7-10, Hood et al. (1976) observed malformations such as micrognathia, hydrocephalus, short tail, digodactyly, syndactyly, cleft lip, micromelia and heart defects.

Brown et al. (1976) stated that OA is teratogenic to rats at lower dose and embryocidal at higher doses.

Hood et al. (1978) observed an increase in fetocidal effects in mice treated prenatally with high dose combination of OA and T2 toxin.

Szezech and Hood (1979) discovered that OA in CD-1 mice caused necrosis of cells in fetal cerebrum. There were many necrotic cells in the cerebrum in fetuses from dams given 3 or 4 mg/kg OA.

Arora (1981) indicated that OA was most effective in producing congenital anomalies when given to mice on day 9 of pregnancy. Nearly all the foetuses whose dams were exposed to a single oral dose of 4 mg/kg were malformed.

The visible defects were principally localized in the head and face region and the brain invariably was exencephalic. It was presumable that the OA primarily interfered with the process of neural tube closure.

According to Szezech and Hood (1981) single dose of OA given to mouse on day 8, 9 or 10 of gestation were teratogenic and single dose given on 15th, 16th or 17th day of gestation was insufficient to induce cerebral necrosis in offsprings.

OA caused increased prenatal mortality, reduced foetal growth and wide variety of malformations when given to CBA mice by stomach tube in single dose (8 mg/kg) on pregnancy day 8 or 9 (Arora et al. 1981).

Arora and Frolen (1981) observed craniofacial anomalies only by exposure on day 8 or 9. The incidence, multiplicity and severity increased with increasing dosage; the peak effect was on day 9 in mouse.

Mayura et al. (1982) reported that internal hydrocephaly and shift in position of oesophagus were the main internal soft tissue defects. Major skeletal defects involved sternebrae, vertebrae and ribs in rats which were given OA (1.75 mg/kg) on one of the gestation days 4-10.

When 4 mg/kg of OA was given on day 8 or 9 of pregnancy to mice there was higher incidence of malformation and foetotoxic effects were noticed (Appelgren and Arora, 1983 a).

Appelgren and Arora, (1983 b) by autoradiographic

studies traced the distribution of 14c ochratoxin in pregnant mice. The concentration of radioactivity in the tissues was found in decreasing order in the liver, kidney, blood, salivary glands, large vessels, brown fat myocardium, uterus and lymphatic tissues. The toxin was shown to cross the placental barrier on day 9 of pregnancy at which time it was most effective in producing foetal malformation.

On the other hand Arora et al. (1983) stated that the teratogenic effects of OA were either not seen or occurred in relatively small percentage when the toxin was combined with zearalenone or diethylstilboestrol in mouse.

Mayura et al. (1984) reported that a single, subcutaneous teratogenic dose of OA (1.75 mg/kg) on gestation 7, resulted in significantly increased foetal resorption, decreased foetal body weights and increased foetal malformation in impaired renal function and sham operated female rats.

The incidence of malformed offspring was about 11 per cent after exposure on day 8 of gestation but was zero after exposure on day 9, in mice exposed to OA (Arora, 1985).

Hoshino et al. (1989) observed developmental disturbance in the cerebral cortex of mouse offspring from dams treated with OA, during pregnancy. Significant changes were

found in the brain weight, antero-posterior length of cerebrum, mean distance from perikaryon to the tips of dendrites and number of intersections of dendrites with the zonal boundaries.

Growth retardation, hypoplasia of the telencephalon, poor flexion, stunted limb bud development, underdeveloped sensory primordia and decreased mandibular and maxillary size, were found in the rat embryos in culture treated with OA. Histological examination demonstrated extensive OA induced necrosis of embryonal mesodermal structures and neuroectoderm (Mayura et al. 1989). They recommended the rat embryo in culture as a sensitive indicator of OA toxicity which could be useful for predicting developmental hazards associated with OA.

2.3 Effect of OA on body and organ weight

2.3.1 Japanese quail

Doster et al. (1973) reported reduction in weight gain in quails fed both on pure OA as well as a chloroform extract of A. ochraceus culture at 4, 8 and 16 ppm levels.

Prior et al. (1976) observed no reduction on weight gain at dose levels below 10.9 mg/kg though the reduction was proportional to the dose at higher levels of OA.

Prior et al. (1978) reported in five experiments testing the response of Japanese quail to graded doses of OA, a significant depression in body weight at 16 ppm. A rapid increase in body weight was observed in hens receiving 16 ppm when OA was removed from their diet.

2.3.2 Other avian species

Huff et al. (1973) reported that graded doses of OA (0, 0.5, 1.0, 2.0, 4.0 and 8.0 µg/g) when incorporated in feed of broiler chickens from hatching until three weeks of age resulted in a decrease in growth rate, enlarged kidney, crop, proventriculus, gizzard and liver, while, the bursa of Fabricius was regressed.

Tucker and Hamilton (1971) reported depressed growth rate in broilers even at 0.5 ppm of ochratoxin. High mortality was observed at 4 and 8 ppm. They recorded increase in the relative weights of the crop, pancreas, liver and gizzard.

Huff and Ruff (1982) observed that OA caused alteration in the relative weight of the kidney in broiler chicks.

Golinski et al. (1983) observed that when OA was fed to broiler chicken, there was depression of the body weight which was found to be proportional to the level of OA.

There was a trend towards an elevated relative kidney weight in growing chicks, dosed with OA, (Kubena et al. 1983).

Campbell et al. (1983) reported a synergistic toxicity of aflatoxin in broiler chicken and ochratoxin and there was a significant decrease in body weight and relative weight of bursa of Fabriciüs and number of follicles for a given area of the folds of the bursa of Fabricius.

Dwivèdi and Burns (1984 a) observed that OA caused a significant enlargement of the kidney, liver and proventriculus, whereas the thymus and bursa of Fabricious were reduced in size in broiler chicks.

In broiler chicks given OA in the diet the relative kidney and liver weights were significantly increased, whereas body weights were significantly depressed (Manning and Wyatt, 1984).

Huff et al. (1984) studied the synergistic toxicity of aflatoxin and OA and observed significant decrease in the body weight in broiler chickens.

Kubena et al. (1985) observed that OA and vanadium toxicity in growing chicks caused a significant increase in the relative weights of the liver, kidney, gizzard and proventriculus and a significant decrease in the relative

weights of the bursa of Fabricious. The decrease in the bursal weight was due to atrophy of lymphoid follicles.

Kubena et al. (1986) reported decreased body weight gains in chicks receiving diet containing OA.

Aleksandrov and Dzhurov (1987) observed that when a standard feed mixture containing 4 mg/kg OA in pure substance, was offered to the birds in the course of 21 days both the growth and the development of the birds were arrested along with an increase in the relative weight of the kidneys.

Kubena et al. (1988) studied the effect of OA and deoxynivalenol on growing broiler chicks. They opined that with regard to the relative weight of the liver, gizzard and spleen, OA and deoxynivalenol appeared to interact additively. However, in body weight and body weight gain there was significant interactions.

Huff et al. (1988) reported that the toxicity of OA to broiler was evident as early as six days of age, when significant growth depression occurred at 4.0 ug dietary OA/g of feed. It also increased the relative weights of the liver, kidney, spleen, pancreas and gizzard.

Kubena et al. (1989) reported depressed body weight in OA fed broiler chickens. However, the relative weight of the liver and kideny was increased.

Increased relative weight of the liver and kidney was also observed in OA toxicity in growing chicks by Sreemannarayana et al. (1989).

Gibson et al. (1989) noted that the body weight decreased with rising levels of OA in three-week-old broilers. There was an increase in the relative weight of the liver, kidney, spleen, but the relative weight of the bursa of Fabricious decreased significantly.

Gibson et al. (1990) reported that, broilers receiving OA weighed less and had poor feed conversion. The relative weight of the liver, proventriculus, gizzard and heart increased, while the relative weight of the bursa decreased.

Ruff et al. (1990) observed significantly decreased body weight, in chukar partridges at the highest level of OA by two weeks of age when given diet containing 1, 2 or 4 ppm OA.

Singh et al. (1990) reported decreased weight of thymus, bursa of Fabricious and spleen in ochratoxicosis in broiler chicks.

2.4 Haematological changes in ochratoxicosis

2.4.1 Avian species

Huff and Ruff (1982) reported reduction in the

haemoglobin concentration and packed cell volume in ochratoxicosis in broiler chicken.

Anaemia, characterised by a significant decrease in packed cell volume and haemoglobin levels was observed in ochratoxicosis in broilers, when OA was given at the rate of 1, 2 and 4 ug of feed (Huff et al. 1988).

Kubena et al. (1989) reported that when OA was given to broiler chicks at the rate of 2.0 mg/kg from day one to three weeks there was microcytic hypochromic anaemia.

Bailey et al. (1989) reported that adding OA at 2 and 4 mg/kg body weight to the diets of broilers, decreased the haemoglobin concentration and corpuscular volume.

2.4.2 Other animal species

Ribelin et al. (1978) reported decline in lymphocytes and increase in neutrophils in ochratoxicosis in goats.

Gupta et al. (1979) studied the effect of OA (5 mg/kg, intraperitoneal) on haematological parameters in mice. They reported that OA caused a significant decrease in the haemoglobin level and in the total count of red blood cells. Among white blood cells there was a significant increase in lymphocytes and a significant fall in the count of neutrophils,

basophils and monocytes. The clotting time was also prolonged by more than 6 fold.

2.5 Effect of OA on the immune system

2.5.1 Avian species

Chang (1982) reported that in chicken OA at 4 ppm and above caused significant decrease in locomotive activity of monocytes. The percentage and mean phagocytic activity were also decreased.

Total serum protein level was significantly depressed by OA in growing chicks (Kubena et al. 1983).

When OA was administered to broiler chicken at the rate of 2.0 µg/g from one day to three weeks of age, and immunized with sheep red blood cells and Brucella abortus, antibody titre and phagocytic activity of heterophils, were not significantly altered. However, the number of follicles for a given area of the folds of the bursa of Fabricious were significantly decreased (Campbell et al. 1983).

Dwivedi and Burns (1984 b) observed that OA caused regression of and a drastic reduction in the lymphoid cell population in the immunological organs. Thymus and bursa of Fabricious were reduced in size in broiler chickens, when given 2 and 4 ppm of toxin. In broiler chicks when they fed

graded dietary levels of OA up to 4 ppm for 20 days from hatch OA caused a significant depression in immunoglobulin containing cells in all the lymphoid organs. Deposition of immunoglobulins, especially IgG in the glomerular basement membrane was seen with greater frequency in kidneys. Immunoglobulin containing lymphoid cells occurred more frequently in the kidney parenchyma.

Manning and Wyatt (1984) analysed the serum from broiler chicks which were given OA. There was significant decrease in total protein, albumin and globulin.

Kubena et al. (1985) reported that OA caused atrophy of the lymphoid follicles of bursa of Fabricious in growing chicks.

Harvey et al. (1987) observed that OA treated chick embryos (2-5 µg) had slight but significant changes in the number of immunoglobulin-bearing cells in the bursa.

In Khaki Campbell ducklings Burns and Maxwell (1987) observed that OA caused thickening of the glomerular basement membrane and infiltration of lymphoid cells in the kidney. There was increased deposition of IgG in the glomeruli, regression of thymus, and subepithelial migration of positive cells in the bursa of Fabricius.

Huff et al. (1988) reported significant reduction in the serum level of total protein, albumin and globulin during ochratoxicosis in broiler chickens as observed by Sreemannarayana et al. (1989) in growing chicks fed OA.

Adding OA to the diet of broilers decreased the concentrations of serum total protein as well as the albumin (Bailey et al. 1989).

Singh et al. (1990) reported that OA in broiler chicks caused significant reduction in cell-mediated immunity which was indicated by diminished skin sensitivity, graft versus host reaction and T lymphocyte counts. On the other hand the overall HA titres differed significantly. Total lymphocyte count, total serum proteins, serum albumin and serum globulin were significantly depressed on the 21st day of intoxication.

2.5.2 Other species

Luster et al. (1987) studied the effect of OA on the immune mechanism associated with tumour resistance in mice using dose level similar to those that caused neoplasia. OA was shown to specifically inhibit natural killer cell activity. It was suggested that OA suppressed the natural killer cell activity by inhibiting production of basal interferon. This was suggested as a possible role for altered natural killer cell function in the development of mycotoxin-induced carcinogenesis.

The effect of OA on pig lymphocytes stimulated by Concanavalin A, was studied by Holmberg et al. (1988). They reported that OA inhibited the mitogenic response to concanavalin A, in a dose dependent way by measuring the rates of 3H - thymidine incorporation in DNA. The immunosuppressive effect of OA was only altered by different contents of bovine serum albumin (0.1 or 3.0 per cent) in cell culture medium.

Lea et al. (1989) studied the mechanism of immunosuppression of OA. They reported that both IL_2 and production and IL_2 receptor expression of activated T lymphocytes were severely unpaired. The inhibitory effect of OA on antibody production was not only due to blocking of T helper cell function. It was suggested that the OA caused immunosuppression through interference with essential processes in cell metabolism irrespective of lymphocyte population or subpopulation.

Gremmels and Leistner (1989) stated that the specific mode of action of OA was inhibition of the phenylalanine-TRNA synthetase, resulting in an inhibition of the elongation step in protein synthesis and therefore, they suggested reduced immunoglobulin synthesis. The hypothesis was supported by histopathological findings demonstrating necrotic lesions in various lymphoid tissues as well as by several in vitro tests.

2.6 Pathological features of ochratoxicosis

2.6.1 Japanese quail

In an experimental study Doster et al. (1973) observed high mortality in groups of Japanese quail fed 4, 8 and 16 ppm of pure OA. Autopsy revealed pale tan to bright orange coloured liver, with focal haemorrhages. Histologically swollen nucleus, bile duct proliferation and diffuse vacuolation of hepatocytes were reported.

Prior et al. (1976) calculated the seven day LD 50 value for Japanese quail to be 16 $\pm$ 0.56 mg/kg weight. They reported progression of symptoms from listlessness, huddling, occasionally diarrhoea, ataxia, prostration and death in acute ochratoxicosis.

After intravenous injection of OA at the rate of 70 µg/kg body weight Fuchs et al. (1988) found high concentration of OA in the gastric and intestinal contents.

Toxico-kinetics of OA was studied by Hagelberg et al. (1989). After the administration of 50 µg/g body weight, they found that the distribution volume to be 1500 ml/kg in quail. Plasma clearance was found to be most rapid in quails when compared to fish and mouse.

Piskorska and Juszkiewicz (1990) gave oral doses of 0, 1, 5 and 20 mg of OA/kg of the body weight, and evaluated the

residues of the toxin in various organ. The highest concentration of OA was found in the kidney and lowest in the muscle. Four days after the administration of OA it could still be detected in the kidney, liver, muscle, yolk and egg. Even after six days traces of OA were found in the muscle of birds given 20 mg/kg.

2.6.2 Other avian species

Choudhury et al. (1971) reported delayed sexual maturity and lower rate of egg production in pullets fed OA at 1, 2 and 4 ppm.

Huff et al. (1973) fed broiler chickens graded doses of OA (0, 0.5, 1.0, 2.0, 4.0 and 8.0 µg/g). They observed reduced hatchability, petechiae of papillae of the proventriculus, slight gizzard erosions and paleness of the liver, pancreas, and especially the kidney.

Elling et al. (1975) observed that out of 14 birds with macroscopic renal changes collected from the poultry slaughter house, five birds had OA residues ranging from 4.3 to 29.2 ug/kg and in four of those birds of toxic nephropathy characterised by atrophy and degeneration of proximal and distal tubules and interstitial fibrosis were evident.

When one day old chicken were fed OA contaminated diets at two levels, 0.3 and 1.0 mg OA per kg of feed for

341 days according to Krogh et al. (1976) the only observable lesion to develop was a kidney damage and this was associated with impairement of glomerular and tubular functions.

Dwivedi and Burns (1984 a) stated that OA, principally affected the proximal convoluted tubules of the kidney and caused severe distension, enlargement and hypertrophy. There was also thickening of the glomerular basement membrane. In the liver vacuolation and glycogen accumulation in the hepatocytes were observed.

Duff et al. (1987) observed histological changes in the diaphyseal cortices which accounted for reduced breaking strength of the bone in chicks and turkey poults when fed a diet contaminated with 4 ppm OA for 10 weeks. They suggested that osteoporosis was a result of direct toxic effect of OA on osteoblasts.

Chicken exhibited hypocarotenoidemia in ochratoxicosis as a result of lipid malabsorption (Osborne et al. 1982).

Hamilton et al. (1982) recorded the incidence of ochratoxicosis in chicken when contaminated feed was found to have 2 to 16 ppm of OA. Histologically there was oedema and necrosis of the proximal convoluted tubules.

Golinski et al. (1983) identified residues of OA in

the liver and white muscles of birds fed 1.0, 1.5 and 2.0 mg OA per kg feed and in red muscles of birds fed 1.5 and 2.0 mg OA per kg feed.

When an intravenous dose of OA was administered to broiler chicken after they were fed a diet containing 2.0 and 4.0 µg/OA, significant decrease in the heart rate and diastolic, systolic and mean blood pressure occurred. The severity and duration of OA administration were significantly enhanced as the level of dietary OA increased (Richardi and Huff, 1983).

Micco et al. (1987) indicated that when broiler and laying hens were fed a diet contaminated with 5.0 ppm, from 14 days of age onwards after 64 and 169 days respectively, residues in the liver were higher in broilers up to 11.0 ppb than hens 1.5 ppb whereas reverse occurred in the kidney.

2.6.3 Other animal species

Kitchen et al. (1977a) described the pathology of OA in dogs. When OA was given at 0.1 and 0.2 mg/kg dose levels, they observed necrosis of lymphoid tissues in the spleen, tonsil, thymus and peripheral lymphnodes and lymph nodules of the ileum, colon and rectum. Kidneys were degenerated and necrosed with desquamation of tubular epithelial cells, primarily in the straight segment of the proximal tubules.

Zimmermann et al. (1978) documented the gross lesion found in rats fed isolates of A. ochraceus. Focal necrosis in the liver, greenish discolouration of the kidney, gastric ulceration, ulceration of the scrotal epidermis, and corneal opacity were seen. Histological changes observed included necrosis of the epithelium of biliary ducts, periductal oedema, pericholangitis, periductal fibrosis and disseminated focal hepatocellular necrosis. Necrosis of epithelium occurred also in the extrahepatic ducts and was accompanied by an interstitial pancreatitis in certain rats. Foci of leukocytes and macrophages were found in the dermis of scrotum. Renal lesions consisted of tubular necrosis and hyaline biliary casts within convoluted tubules, when fed on a diet containing 1, 2 and 3 per cent fungal mat.

Maryamma (1983) described the histological changes in goats in ochratoxicosis. There was degeneration and necrosis of the epithelial cells of the proximal convoluted tubules of the kidney.

Changes in the liver were mainly necrotic in nature. Parenchymatous degeneration and fatty changes were observed in paracentral hepatocytes. Lymphoid cells in the lymphnodes and spleen, revealed varying degree of necrotic changes.

2.7 Ultrastructural pathology

2.7.1 Japanese quail

Maxwell et al. (1987) reported ultrastructural changes in the liver and kidney of 11-week old Japanese quail, fed OA from day-old, at 4 and 8 ppm levels. Abnormal mitochondria and excessive numbers of lipid droplets were seen in the proximal convoluted tubules. Glomeruli showed thickening of the basement membrane. Liver showed swollen mitochondria and variable glycogen deposition. They suggested, OA was more hepatotoxic in quail than in broilers besides being nephrotoxic.

2.7.2 Other avian species

Dwivedi et al. (1984) reported ultrastructural changes in the kidney and liver of 20-day old broiler chicks fed OA at 2 and 4 ppm levels. Kidney showed abnormally shaped mitochondria in the proximal convoluted tubules, increase in size and number of mitochondrial dense granules and cytoplasmic peroxisomes, intranuclear and cytoplasmic lipid droplets and electron dense round bodies in the dilated endoplasmic reticulum. Regional thickening and degeneration of glomerular basement membrane were also observed. Liver showed an increased accumulation of cytoplasmic glycogen in the hepatocytes. Abnormal mitochondrial ring forms in the kidney and accumulation of glycogen in the liver were

considered to be of diagnostic significance in ochratoxicosis in young broiler chicks.

Brown et al. (1986) reported that feeding of OA at the level of 3.0 µg from 0 to 21 days of age in layer chicks was associated with proximal tubular intranuclear membrane-bound inclusions, elongated mitochondrial matrix granules with hyaline centers and increase in number and size of peroxisomes and secondary lysosomes.

Burns and Maxwell (1987) studied the electron microscopic changes seen in Khaki Campbell ducklings fed with OA at 2 ppm level. They observed, accumulation of glycogen and presence of mis-shapen mitochondria in the hepatocytes in the liver, and thickening of glomerular basement membrane in the kidney.

Lalithakunjamma (1987) described the ultrastructural changes in chick embryo which received 0.5 µg OA/egg. There was damage to the membrane system mainly of the liver and kidney. Mitochondria showed changes varying from swelling to severe destruction of the organellar structures. There was fragmentation and destruction of the rough endoplasmic reticulum. Nucleus was severely damaged and moderate destruction of the proximal convoluted tubules was noticed. In the glomeruli there was an increase in the fenestrated

nature of the endothelial surface of the basement membrane and damage to the foot processes of the podocytes.

2.7.3 Other animal species

Szczech et al. (1974) described the ultrastructural changes in ochratoxicosis of Beagle dogs. Cytomorphological alterations were seen primarily in the endomembrane system of renal epithelial cells of the proximal convoluted tubules. Increased amounts of smooth-surfaced membranes were present as linear and concentric arrays and as large and small residues. Fewer basilar infoldings of the plasma membrane were present in the proximal tubular epithelial cells. Many tubular epithelial cells contained lipid droplets and cytoplasmic accumulations of phospholipids. The prominent alterations of interstitium included separation between tubular basement membrane. Few alterations were found in the epithelial cells of the distal convoluted tubules, some contained more lipid droplets, than normal when OA given daily at an oral dose rate of 0.3 mg/kg body weight for a period of 11-15 days.

Munro et al. (1974) observed thickened basement membrane in the proximal tubules of a male rat fed 5.0 ppm OA in the diet for 90 days. The basement membrane appeared layered and measured up to 4 micrometer in thickness. Myelin figures were observed by Kitchen et al. (1977b) in the proximal epithelial cells of dogs given OA.

Maryamma and Nair (1990) reported that OA caused organellar damage, particularly in the epithelial cells of the kidney of goats. The intensity of changes was more in the proximal convoluted tubules.

Alterations in the mitochondrial morphology and structure along with changes in the nucleus and endoplasmic reticulum were noticed. There was increase in cytolysosomes and peroxisomes. The foot processes of podocytes showed configurations indicating their fusion. Basement membrane showed thickening and fragmentation.

2.8 Effect of OA on DNA

Cooray (1984) studied the effect of OA on the DNA synthesis in phytohaemagglutinin stimulated human peripheral blood lymphocytes. Total inhibition was obtained with 20 micrograms of OA and 50 per cent inhibition with 14 micrograms of OA.

On the other hand Bendele *et al*. (1985) observed no evidence of unscheduled DNA synthesis in primary cultures of rat hepatocyte exposed to concentration of OA, ranging from 0.00025 to 500 microgram/ml.

Creppy *et al*. (1985 a) reported that OA, induced DNA damages. Damages were dose dependent, reversible, and varied

upon the time and according to the tissue. They opined that OA, could possibly induce leukemia.

Creppy et al. (1985 b) demonstrated evidence of DNA single-strand breaks induced by OA, in mice spleen cells.

Stetina and Votava (1986) reported that OA at the concentration over 4 µg/ml elicited mild inhibition of DNA synthesis upto 20 per cent. OA produced DNA breaks in CHO cells at very high concentration of 200 µg/ml.

Kane et al. (1986) studied the distribution of single low dose of (3H) OA in different tissues of male Wistar rats, and they observed that at 5 h the highest specific label was found in the stomach contents and in increasing order in intestinal contents, lung, liver, kidney, heart, fat, intestine, testes and the lowest in muscle, spleen and brain. After a 12 week feeding experiment with doses of 4 ppm in feed each 48 h, DNA in the liver and kidney was estimated by alkaline elution method, combined with micro-spectro fluorimetric method. There was evidence for DNA single-strand breaks.

2.9 Effect of OA on mitochondrial function

Aled et al. (1991) studied the effect of OA on isolated rate renal proximal tubules in suspension. OA

decreased tubule viability in a concentration (0-1 n^M) and time (0-4 h) dependent manner. The initial decrease occurred 1 h after exposure. Tubule basal and nystatin stimulated oxygen consumption decreased before cell death after OA (0.5 and 1 n^M) exposure. Direct probing of mitochondrial function within the proximal tubules confirmed the toxicity of OA, to mitochondria.

2.10 Immunomodulation

2.10.1 Levamisole

Levamisole (L-2,3,5,6-tetrahydro-6-phenylimidazo [2, 1-b] thiazole), an anthelmintic drug was demonstrated to be useful in correcting defective leukocyte reactivity and to bring about clinical improvement in chronic infections, inflammatory disease, and malignancy (Burner and Muscoplat, 1980).

Koller (1982) reported that levamisole acted as a nonspecific stimulant of the immune system. It stimulated anitbody production, enhanced graft-vs-host reactivity, increased phagocytosis by macrophages, and inhibited tumour growth.

Vanselow (1987) stated that levamisole behaved physiologically as a thymomimetic agent and restored compromised immune response by restoring the effector functions of T lymphocytes and macrophages.

2.10.1.1 Immunomodulatory effect on avian species

Hassan et al. (1989) observed that when levamisole was injected before or after vaccination with Lasota strain, increased the macrophage migration inhibition for two weeks in chicken. They also reported increased mitogenic response of peripheral blood lymphocytes to phytohaemagglutinin-P, Concanavalin-A, and purified protein derivative. Administration of levamisole before vaccination increased the antibody titre to Newcastle disease virus vaccine.

When levamisole was administered simultaneously with Ranikhet disease vaccine in chicks, there was significant immunostimulatory effect (Vyas et al. 1987). They also concluded that levamisole could be of practical importance in enhancing the capability of individuals to resist infection or disease outbreak.

AbdulRahman et al. (1989) reported that immunity against Eimeria tenella infection developed in levamisole treated groups prior to infection within two weeks and reached peak within 3 to 4 weeks. Enhanced cell-mediated immunity was observed as measured by leukocyte migration inhibition test.

Palanski et al. (1990) reported that levamisole when administered in drinking water at the rate of 2.5 mg/kg body weight, given to broiler chicks at 1-3 days of age for

24 hours before immunization with Lasota Newcastle disease vaccine, increased the T· and B· lymphocytes count.

Gavkare et al. (1991) observed that when chickens treated with levamisole were vaccinated against Ranikhet disease showed greater immunocongultinin response as compared to those which were not treated with levamisole.

2.10.1.2 Immunomodulatory effect on other animal species

Irwin et al. (1976) reported that in calves vaccinated against Infectious Bovine Rhinotracheitis and simultaneously given levamisole, there was mild suppression of the group geometric mean serum neutralization titre, which occurred between 12 and 59 days.

Forsyth and Jones (1980) indicated that levamisole could be combined with polyvalent clostridial vaccine in sheep. Antibody response was increased, and the anthelmintic activity of levamisole was unimpaired.

Hogarth et al. (1980) observed a significant heightened antibody response to five antigenic components of clostridial vaccine, when it was given with levamisole in sheep.

Levamisole appeared to have a beneficial effect on

antibody response in cattle after vaccination to bovine herpes virus-1 (Babiuk and Misra, 1982).

Jayappa and Loken (1982) investigated the effect of levamisole on random migration chemotaxis, phagocytosis, and intracellular killing by bovine polymorphonuclear leukocytes. Levamisole enhanced the chemotactic response of polymorphonuclear leukocytes and increased random migration. Chemotaxis and phagocytosis were also observed.

Confer et al. (1985) reported that when cattle given 6 mg of levamisole per kg subcutaneously, either at the time of vaccination with Brucella abortus or 7 days later, increased the antibody titre.

Levamisole enhanced the immune status during infection with Escherichia coli, Pseudomonas aeruginosa, coronavirus and rotavirus, which caused neonatal diarrhoea in calves (Krasnikov et al. 1986).

Nalini Kumari and Choudhari (1986) reported that when buffalo calves vaccinated with Rinderpest tissue culture vaccine, were given levamisole on the day of vaccination, showed a nonsignificant increase in total leukocyte count and a significant increase in lymphocyte percentage from first to third week.

Anilkumar and Rajan (1986) studied the *in vivo* stimulatory effect of levamisole in kids, affected with pneumonia as well as the healthy kids. They concluded that levamisole at the dose rate of 3.75 mg/kg body weight caused immunostimulation in healthy kids, no immunomodulation was observed when there was sufficient immunostimulation due to infections. They also recommended levamisole for stimulating the immune system of healthy goats when outbreak of infections are prevalent or expected in a herd.

Hennessy *et al.* (1987) reported that levamisole enhanced the skin test responses and mitogen induced lymphocyte proliferation in pigs reared artificially.

2.11 Immunological investigations

2.11.1 Acid alphanaphthyl acetate esterase(ANAE) activity

ANAE activity was demonstrated in human lymphocytes (Li *et al.* 1973) and subsequently it was used as a T cell marker by Mueller *et al.* (1975) and Osbaldiston *et al.* (1978). They successfully used this to evaluate the cell-mediated immunity in the cat, dog, goat, guinea pig, hamster, rabbit, rat, sheep and pigs. The activity was seen as reddish brown granules in the cytoplasm.

Reddi *et al.* (1980) demonstrated ANAE activity in the peripheral blood lymphocytes of cattle. They recommended this

test for routine evaluation of T cell population. Dhingra et al. (1982) observed the reaction as spherical or oval reddish brown granules product adjacent to cell membrane in cattle. ANAE was used as a T cell marker in the peripheral blood of pigs in evaluating the immunopotentiation response of pigs sensitized with 2,4-dinitrochlorobenzene (Rajan et al. 1982). Sulochana et al. (1982) compared the ANAE activity and E-rosette forming cells in the peripheral blood of goats. They recorded the same number of ANAE positive lymphocytes and E-rosette forming cells.

Vishalakshan et al. (1984) and Nair (1986) assessed the cell-mediated immune response of pigs in experimental aflatoxicosis using ANAE activity in the lymphocytes of the peripheral blood.

Jayaprakasan (1986) used ANAE activity to assess the cellular immunity during Corynebacterium pseudotuberculosis infection in the peripheral blood of goats.

Prajapati and Heranjal (1987) employed ANAE as a T cell marker for evaluating the cell-mediated immunity in cattle affected with horn cancer.

2.11.2 Serum protein fractions

2.11.2.1 Japanese quail

Matsumoto et al. (1978) reported significant difference

with regard to albumin, alpha 1 and beta 1 globulin and albumin globulin ratio in quails infected with Aspergillus fumigatus.

Chaudhary (1987) carried out paper electrophoresis on sera of quails infected with Aspergillus fumigatus and reported increase in the relative percentage of different globulin fractions in the sera of infected birds. This was mainly due to increase in beta globulin from day one to seven post infection.

2.11.2.2 Other avian species

Brandt et al. (1951) carried out gel electrophoresis of chicken sera at different age groups. They stated that the low gamma globulin exhibited by the young birds is in keeping with the fact that these birds may not have developed the antibodies which would be produced by later exposure to various organisms. However, the possibility could not be excluded that a large portion of the gamma globulin found in older birds might be a normal development as the birds matured.

Glick (1968) studied the serum protein electrophoresis patterns in acrylamide gel electrophoresis of the chicken sera. All the gel studied contained a heavy stained band of transferrin, approximately midway between the origin and albumin, the origin was the separation point between the

stacking and separating gel. The first two bands beyond the origin, and a stained area 4-8 mm below the origin represented immunoglobulins. They concluded that the absence of the stained area in the electropherograms of chemically bursectomised birds indicated reduced immunoglobulin levels.

Morgan and Glick (1972) employed polyacrylamide gel electrophoresis to sera of chickens. They reported that at 2-3 weeks of age birds attained ability to produce IgG in appreciable quantities. IgM which was generally absent or very low at hatching increased rapidly during the first week of postembryonic life. both surgical and hormonal bursectomy resulted in delay in IgG production whereas IgM and transferrin were higher. They also observed that surgical bursectomy plus 700 r of the whole body gamma-irradiation resulted in decrease in both IgG and IgM levels.

2.11.3 Leukocyte migration inhibition test (LMIT)

Tompkins et al. (1970) demonstrated the application of the Macrophage migration inhibition test in assessing the cellular immunity induced by Fibroma virus.

Kantoch et al. (1979) studied the cell mediated immune response to measles and mumps viruses in monkey and guines pigs using LMIT.

Falecka (1979) assessed the cell-mediated immunity using LMIT in guinea pigs vaccinated with measles virus.

Hussain and Mohanty (1979) assessed the cell-mediated immunity to bovine rhinovirus type-1 in calves employing LMIT.

Karpe (1982) employed the LMIT for the assessment of cell mediated immunity in lambs, vaccinated and infected with sheep-pox. LMIT was used to evaluate the cellular immunity of sheep to attenuated sheep-pox virus (Kalra and Sharma, 1984).

McCorkle and Simmons (1984) studied the ability of peripheral blood leukocytes from young poults to migrate *in vitro* using LMIT.

Yadav *et al.* (1986) used LMIT for the assessment of cell mediated immunity following vaccination with goat-pox vaccine.

Chaturvedi and Sharma (1986) compared the LMIT and delayed hypersensitivity tests to assess the cell mediated immunity in *Salmonella dublin* infection.

Patel *et al.* (1987) employed the LMIT in the diagnosis in Brucellosis in cattle.

Bansal *et al.* (1989) used LMIT in assessing the cellular immunity in rabbits to lapinised Rinderpest virus.

Chandrasekar et al. (1989) demonstrated the cell mediated immune response by employing LMIT to Ranikhet disease vaccine in chicks.

Pani et al. (1989) assessed the cell mediated immunity in chick hatched from embryos which were inoculated via chorioallantoic membrane with Bryan standard strain of Rous sarcoma virus. He reported that chicks that developed tumors, had a positive cell mediated immune response and those that were negative for liver tumor were negative based on LMIT.

LMIT was used in the assessment of immune response of guinea pigs during experimental leptospirosis (Ramkrishna et al. 1990).

2.11.4 Response to 2,4-dinitrochlorobenzene (DNCB)

Eliber and Morton (1970) stated that DNCB test could be considered as one of the reliable tests to assess the cellular immune response in man.

Brummerstedt and Basse (1973) employed the DNCB test for the first time to assess the cell mediated immunity in calves. Later Jennings (1979) evaluated the DNCB response in calves.

Reddi et al. (1981) standardised the DNCB skin test in cattle. Subsequently a study was undertaken to assess the

efficacy of DNCB sensitization test in evaluating the cell mediated response in goats (Rajan et al. 1981).

Valsala et al. (1981) first described the DNCB test for assessing the cell mediated immune response of ducks.

Rajan et al. (1982) used the DNCB test for evaluating the cell mediated immunity in pigs. They also reported that DNCB induced a generalised stimulation of cellular immunity which was indicated by an increase in the number of ANAE positive T lymphocytes in the peripheral blood.

Nair (1986) evaluated the cell mediated immunity in experimental aflatoxicosis in pigs using DNCB test.

Yadav et al. (1986) used DNCB test to measure the cell mediated response following vaccination with goat-pox vaccine.

Chaudhary (1987) employed the DNCB test to measure the cell- mediated response in Japanese quail in experimental Aspergillosis.

Cell- mediated immunity was evaluated using DNCB in experimentally infected lambs with Mycoplasma arginini cell antigen (Sreeramalu and Krishnaswamy, 1989).

Ashturkar et al. (1989) used the DNCB test to assess

the cellular immunity in experimental diaphragmatic herniaplasty in buffalo calves.

2.11.5 Response to phytohaemagglutinin-M (PHA-M)

Nowell (1960) observed the effect of an extract of the red kidney bean Phaseolus vulgaris (Phytohaemagglutinin) for inducing lymphocyte transformation.

Jannossy and Greaves (1971) reported that PHA is a T cell specific mitogen.

The response of Porcine T lymphocytes to PHA was observed (Binns et al. 1972). PHA was used as a skin test for the evaluation of cell mediated immunity in cancer patients (Zuckerman and Lo Bugilo, 1977).

Marchalonis (1978) observed that PHA acted directly without initial sensitization and recommended it for evaluating the cell-mediated immunity in man.

Intradermal PHA response was used in iodine toxicity in cattle (Haggard et al. 1980).

Cell-mediated immunity was assessed in horses using PHA and the reaction was stated to be an indication of delayed hypersensitivity (Thein et al. 1981).

Rajan et al. (1982) used PHA for the assessment of cell mediated immunity in goats.

The effect of heat and cold on the immune system was evaluated by Kelley et al. (1982) using PHA.

Reddi and Rajan (1984) used intradermal PHA test to assess the cell mediated immunity status of cattle bearing carcinoma of the ethmoid mucosa.

Graft versus host reaction (GVHR)

Chauhan et al. (1984) studied the cell mediated immunity in chicken with Marek's disease, using DNCB skin reaction, T lymphocyte numbers in peripheral blood and GVHR. The GVHR had a smaller coefficient of variation than the other two tests and it was concluded that GVHR could be the most reliable indicator of Marek's disease infection.

Singh et al. (1990) evaluated the cell mediated immunity in broiler chicks during chronic ochratoxicosis by using GVHR, and found the test to be of significance in assessing the cell mediated immunity.

Ghosh et al. (1991) employed GVHR as a parameter to assess the cell mediated immune status of broiler chicks given aflatoxin B_1. They observed suppression of cell mediated immunity.

Materials and Methods

MATERIALS AND METHODS

3.1 Experimental design

The study comprised of three parts.

1. Pathological response induced by OA in Japanese quail embryo
2. Pathological response induced by OA in Japanese quail
3. Immunomodulatory effect of levamisole in OA induced immunodeficiency in Japanese quail

3.1.1 Toxin

Pure OA supplied by Sigma (St. Louis, USA) was used for the experimental studies, for each one mg of crystalline benzene free OA, one ml of propylene glycol (Sarabhai, India) was used as diluent (Lalithakunjamma, 1987).

3.1.2 Pathological response induced by OA in Japanese quail embryo

Two hundred, ten day old Japanese quail embryos were obtained from the University Poultry Farm, Mannuthy, in different batches of twenty each.

OA was administered to 150 embryos through the air sac route at the dose rate of 0.02 µg/egg. The eggs were first

candled and cleaned with spirit, then they were drilled and inoculated, sealed by paraffin wax and incubated at 37°C.

Fifty embryos were administered 0.02 µg of diluent by the same route.

Embryos were sacrificed on the 13th and 16th day of incubation. The weight of the embryos were recorded, the bursa of Fabricius, spleen and thymus were dissected out, the weights were recorded and then the organs were fixed in 5 per cent buffered formalin for histopathological examination. For electron microscopical examination, small bits of tissues from the organs were fixed in 2.5 per cent glutaraldehyde.

3.1.3 Pathological response induced by OA in Japanese quail

3.1.3.1 Experimental birds

One hundred and twenty, three weeks old Japanese quail chicks were obtained from the University Poultry Farm, Mannuthy.

3.1.3.2 Management

The birds were reared upto one month of age under ideal brooding conditions. The experimental room and cages were thoroughly cleaned with 2.5 per cent phenol and subsequently fumigated with formaldehyde gas (35 ml of

commercial formalin plus 17.5 g potassium permanganate per 100 cubic feet area).

The birds were fed commercial balanced quail mash which was supplied by Meat Products of India, Koothattukulam, Ernakulam district, throughout the experiment. The feed and water were given ad libitum. The feed samples were screened for aflatoxin and OA and found free of these toxins, were fed to the quails.

3.1.3.3 Experimental groups

When birds attained one month of age, they were divided at random into three groups.

1. Group I - OA fed (54 quails)
2. Group II - control (48 quails)
3. Group III - OA fed group subjected to immunostimulation (18 quails)

3.1.3.4 OA administration

OA was administered through oesophageal intubation to Group I and Group III, at the dose rate of 50 µg/bird/day. The Group II was given the diluent (Propylene glycol) at the dose rate of 50 µg/bird/day, by the same route.

3.1.3.5 Group I and II

At each fortnight (1st, 2nd, 3rd and 4th), six birds each were sacrificed from group I and II. Before bleeding, the body weights were recorded and blood smears from the peripheral blood were made. Blood was collected for haematology and serum was separated for estimation of protein, albumin, globulin and serum protein fractions.

The weight of liver, bursa of Fabricius, spleen and thymus were recorded.

At the end of the 60th day (4th fortnight), twelve birds from group I and six birds from group II were vaccinated with Fowl pox vaccine (BAIF, India). After seven days the blood was collected and LMIT was performed.

Response to DNCB was evaluated in six birds each from group I and II. Similarly six birds each from group I and II were subjected to PHA-M response.

The GVHR was evaluated in six birds of group I and II.

3.1.3.6 Group III

Eighteen numbers of one month old Japanese quail were fed OA for 60 days. Then levamisole (Helmonil, Alved, India) was administered subcutaneously at the rate of 0.1 mg per

quail, two doses at four day intervals. After a fortnight, haematological parameters were evaluated, six birds were vaccinated with Fowl pox vaccine (BAIF, India) and LMIT was done. On the remaining birds DNCB and PHA-M responses were measured. Prior to sacrifice the body weights were recorded, blood smears were made from the peripheral blood. The blood was collected for haematology and serum was separated for estimation of total protein, albumin, globulin and protein fractions.

On autopsy the weights of the liver, bursa of Fabricius, spleen and thymus were recorded.

3.1.3.7 Haematological parameters

The study included the determination of haemoglobin (Hb), concentration, packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC).

Hb concentration was estimated by Sahli's method (Schalm et al. 1975). For determination of PCV, Microhaematocrit method of Cohen (1967) was used. The TEC and TLC were determined as per the method described by Sastri (1976). The DLC was done with the Copper peroxidase method of Sato and Sekiya (1965).

3.1.3.8 Determination of T lymphocytes in the peripheral blood smear (ANAE staining technique)

Peripheral blood smears were immediately fixed in the fixative which contained six parts of acetone and four parts of 0.038 in sodium citrate (pH-5.4). The smears were kept in the fixative for 30 seconds, rinsed in distilled water and dried. The labelled smears were stored at room temperature (Giorno and Beverly, 1980).

For staining the smears, a reaction mixture was prepared as follows:

In 40 ml of 0.067 m phosphate buffer (pH 5.0), 2.4 ml of hexazotised pararosaline and 10 mg of alphanaphthylacetate (Loba, India) dissolved in 0.4 ml acetone was added, and the final pH of the reaction mixture was adjusted to 5.8 with 2 N sodium hydroxide.

The hexazotised pararosaline was prepared in the following manner:

Equal volumes of two solutions

1. Freshly prepared 4 per cent sodium nitrite in distilled water.
2. One gram of pararosaline hydrochloride (Sigma) dissolved in 20 ml of distilled water and 5 ml of 12 N hydrochloric acid

These two solutions were combined. The hexazotised pararosaline which formed was shaken and then allowed to stand for one minute before adding it to the reaction mixture (Knowles et al. 1978).

The slides were incubated in the reaction mixture for 18-21 hours at room temperature then rinsed thoroughly with distilled water and then counterstained with 1 per cent toludine blue for 45-60 minutes. The slides were then rinsed thoroughly with distilled water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in DPX and examined under oil immersion objective of a microscope. Those lymphocytes with localised orange and nodular reaction product in the cytoplasm were considered as positive cells (T lymphocytes). The number of positive cells in every hundred cells was counted and recorded.

3.1.3.9 Serum, protein, albumin and globulin

The estimation of total serum protein (TSP), serum albumin (SAl) was done by using Autopack commercial kits (Miles India) and Chemetrics analyser (Miles India, USA). By deducting SAl from TSP, serum globulin (SGl) value was determined.

3.1.3.10 Serum protein fractions

3.1.3.10.1 Polyacrylamide gel electrophoresis (PAGE)

The pooled serum samples were subjected to PAGE to

study the serum protein fractions. The PAGE was performed as per the method described by Davis (1964).

3.1.3.10.2 Reagents

1. Preparation of electrode buffer (Solution A)

Tris, 36.6 g and 0.23 ml of NNN'N' tetramethyl ethylene diamine (TEMED) with 48 ml of 1.0 N hydrochloric acid were taken in a 100 ml volumetric flask. The contents were dissolved and the volume was made up to the mark with distilled water. The pH of the solution was adjusted to 8.9.

2. Gel solution (Solution B)

Acrylamide (28 g) and bisacrylamide (0.735 g) were dissolved in 100 ml of distilled water.

3. Ammonium persulphate (APS) solution (Solution C)

APS (0.14 g) was dissolved in 100 ml of distilled water to make 0.14 per cent solution.

4. Electrolyte buffer (Solution D)

Six grams of glycine and 28.3 g of tris, were dissolved in one litre of distilled water, the pH of the solution was adjusted to 8.3.

5. Staining solution (Solution E)

One gram amidoblack (10 B) was dissolved in a minimum amount of methanol, and the volume of 100 ml was made up with 7 per cent acetic acid solution.

6. Destaining solution (Solution F)

Seven per cent acetic acid solution.

7. Sucrose solution (Solution G)

Twenty per cent sucrose solution.

3.1.3.10.3 Preparation of gel solution

One part of solution A, two parts of solution B, one part of water, and four parts of solution C were mixed well and immediately used for polymerization.

3.1.3.10.4 Procedure

One end of the gel tube was closed and was filled 3/4th with the prepared gel mixture. A drop of distilled water was added above the gel, to prevent contact with oxygen of the atmosphere. The tubes were then kept in upright position for about 30 minutes for polymerization. After polymerization the tubes were fixed in upper compartment of the electrophoretic apparatus, the water layer on the top of

the gel was removed with a strip of filter paper, the lower compartment was filled with electrode buffer. Ten microlitre of a 1 per cent serum solution in 20 per cent sucrose was applied to the tube. As the marker to one of the tubes a little bromophenol blue was added to indicate the movements of the run. The tubes were than filled up with the electrode buffer and also the upper tank, the upper tank was closed with lead and then electrical connections were given. Initially a current of 2 MA per tube was given until sample entered the separation gel in a concentrated band, and then it was raised to 4 MA per tube till the end of the run, it was run for one hour, after which tubes were removed. The gel was removed from the tube by immersing the tube in distilled water and inserting a fine guage needle between the gel and the inner wall of the tube. By fine rotation of the needle along the inner wall from both sides, the gel was removed and placed in staining solution (solution F) for one hour, then it was destained in 7 per cent acetic acid repeatedly till the interspace between the fractions were cleared. The gel was subjected to Ultroscan XL Laser Densitometer (Pharmacia LKB) and different readings of fractions were recorded.

3.1.3.11 LMIT

The test was carried out according to the procedure described by McCoy *et al*. (1976).

Three ml of blood was collected from each Japanese quail through the cardiac route in sterile syringe containing 20 IU of sodium heparin per ml of blood, and then transferred to clean sterile tubes.

Four ml of ficoll paque (Pharmacia, Sweden) was taken in each tube, over which three ml of blood was slowly layered from the wall of the tubes. The tubes were centrifuged in refrigerated centrifuge at 600 xg for 20 minutes. The supernatant was removed gently by a pasture pipette and resuspended in RPMI 1640 (Rosewell Park Memorial Institute 1640) tissue culture medium (Sigma). Leukocytes so separated were washed in RPMI 1640, thrice at 300 xg for 10 minutes each. The cell concentration was adjusted to 2×10^7, viable leukocytes per ml. The capillaries (1 mm diameter) were charged with the cell suspension, and one end of the capillary tubes were sealed with plasticin. Cappilaries were centrifuged at 300 xg for five minutes. Each capillary was cut with a file at 1/3 of the way below the cell-pellet-liquid interface. The capillaries were immediately placed with the help of non-toxic grease in the migration chambers (Laxbro Co., India). The chambers were charged with RPMI 1640 medium with antigen (1.5 mg of Fowl pox antigen) and other chambers without antigen for the replicate samples. The chambers were covered with coverslips, with the help of non-toxic grease, and incubated on level shelf at 37°C for 18 h in a humid chamber.

The areas of migration were examined under low magnification (40x). The migration index (MI) values were calculated as per the formula gtiven below.

$$MI = \frac{\text{Area of spread of leukocytes in the presence of antigen}}{\text{Area of spread of leukocytes in the absence of antigen}} \times 100$$

3.1.3.12 Response to DNCB

The response to DNCB was determined by the method described by Rajan et al. (1982).

Approximately 4-5 cm^2, relatively featherless elliptical areas were chosen on the left and right side of the abdomen. The areas were first cleaned with alcohol and then 0.25 ml of the sensitizing dose of 1 per cent DNCB (Loba, India) in acetone was applied to each site. This was dropped slowly drop by drop and allowed to evaporate quickly by blowing, the area was demarcated by holding a metal ring on to the skin at the site. The metal ring was kept at the site until the solution evaporated. The birds were challenged on the 15th day.

The reaction was assessed by measuring the skin thickness at the site of the test with the help of a vernier caliper. The measurement of the skin at the site of application was done at 24, 48 and 72 hours post challenge,

each measurement was repeated thrice on a constant area. The challenged area of the skin was examined for erythema, induration and vesication, if any. Skin biopsies were taken at each 24, 48, and 72 hours post challenge and preserved in neutral buffered formalin for histopathology.

3.1.3.13 Response to PHA-M

The method described by Rajan et al. (1982) was employed. Five micrograms of PHA-M (Difco Laboratories, USA) were dissolved in 0.1 ml distilled water. The feathers over the left and right side of the abdomen were clipped. Two sites were marked for injecting PHA-M. The thickness of the skin was measured using a vernier caliper. At each site 0.05 ml of distilled water containing 2.5 micrograms of PHA-M was administered intradermally. The skin thickness at these sites were measured at 24, 48 and 72 hours. Skin biopsies were taken at the same intervals and fixed in neutral buffered formalin for histopathology.

3.1.3.14 GVHR

This test was performed as described by Singh et al. (1990) with modifications.

Thirty ml of blood was taken from three, two month old chicken through the cardiac route in sterile syringe

containing 20 IU of sodium heparin per ml of blood, and then transferred to clean sterile tubes. Four ml of ficoll paque was taken in each tube over which three ml of blood was slowly layered from the wall of the tube. The tubes were centrifuged in refrigerated centrifuge at 600 xg for 20 minutes. The lymphocytes separated were then washed three times with chilled RPMI 1640 tissue culture medium at 300 xg for 10 minutes each, and finally prepared as 15 per cent (v/v) suspension. An aliquot (0.2 ml) of cell suspension was injected intravenously into six birds from group I and six birds of group II. The inoculated quails were weighed and sacrificed 96 h later and their spleens were weighed individually. The spleen index was calculated by the following formula

$$\text{Spleen Index} = \frac{\text{Spleen weight in g}}{\text{Body weight in g}} \times 100$$

3.1.3.15 Gross and histopathology

Birds which sacrificed at different intervals or those died during the course of the experiment were subjected to detailed autopsy examination. Gross lesions in different organs were recorded. Representative pieces of the liver, kidney, bursa of Fabricius, spleen, thymus, brain and heart were collected for histopathological studies.

Tissues were fixed in 10 per cent neutral buffered formalin and were processed by the routine method. Paraffin

sections cut at 4-5 microns thickness were stained with Harri's haematoxylin and eosin, described by Sheehan Hrapchak, (1980).

3.1.3.16 Ultrastructural studies

Bursa of Fabricius, spleen and thymus from the embryos inoculated with OA as well as the controls and liver, kidney, bursa of Fabricius, spleen, thymus and brain from the adult groups, OA fed as well as their controls were studied at different intervals.

For electron microscopy fresh tissues were immediately fixed in 2.5 per cent glutaralaldehyde. The glutaralaldehyde fixed tissues were washed three times in phosphate buffer pH 7.4, and post fixed in 1 per cent osmium tetraoxide in 4°C for 24 h, then they were dehydrated in acetone and embedded in spur.

Sections were cut with glass knives on a LKB ultratome. Thin sections were cut and picked up on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined in a Hitachi 600 A electron microscope at 75 KV and the electron micrographs were taken.

3.1.3.17 Statistical analysis

Statistical analysis was done using students 't' test according to the method described by Snedecor and Cochran

Results

RESULTS

4.1 Ochratoxicosis in Japanese quail embryo

4.1.1 Embryo mortality

The mortality in OA inoculated embryos was 68.66 per cent and in control embryos 12 per cent.

4.1.2 Body weight and organ weight

4.1.2.1 Thirteen day old embryo

The average body and organ weight of control and experimental groups are given in Table 1. There was a highly significant reduction in the body weight (Fig.1) and organ weights of bursa of Fabricius (Fig.2), spleen (Fig.3) and thymus (Fig.4) in the experimental group ($P < 0.01$).

4.1.2.2 Sixteen day old embryo

The average body and organ weight of the control and experimental groups are furnished in Table 1. Highly significant reduction ($P < 0.01$), was observed in the body weight (Fig.1) and weights of bursa of Fabricius (Fig.2), spleen (Fig.3) and thymus (Fig.4) of the experimental group.

Table 1. Average (Mean ± SE) body and organ weight of Japanese quail embryo (Control and Experimental)

Days of incubation	Body weight (g)		Bursa of Fabricius weight (g)		Spleen weight (g)		Thymus weight (g)	
	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental
13th	5.416588 ± 0.098452	4.410291** ± 0.081827	0.001425 ± 0.000100	0.000875** ± 0.000067	0.001250 ± 0.000096	0.000833** ± 0.000048	0.033250 ± 0.003310	0.019041** ± 0.001082
16th	5.934770 ± 0.067882	5.013217** ± 0.078284	0.001681 ± 0.000093	0.000956** ± 0.000096	0.001636 ± 0.000102	0.000869** ± 0.000070	0.031272 ± 0.003133	0.014347** ± 0.000917

** ($P < 0.01$)

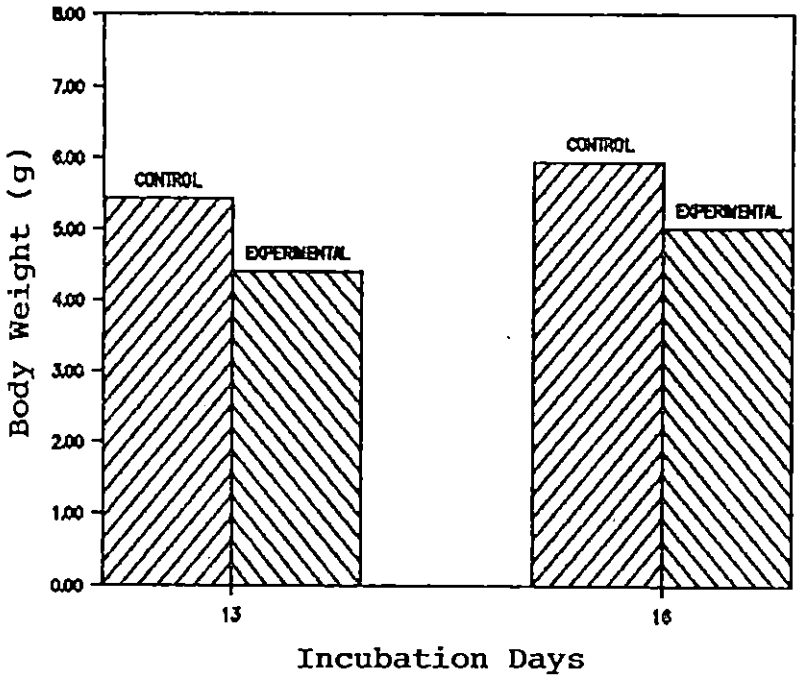


Fig.1 Average weight of embryos

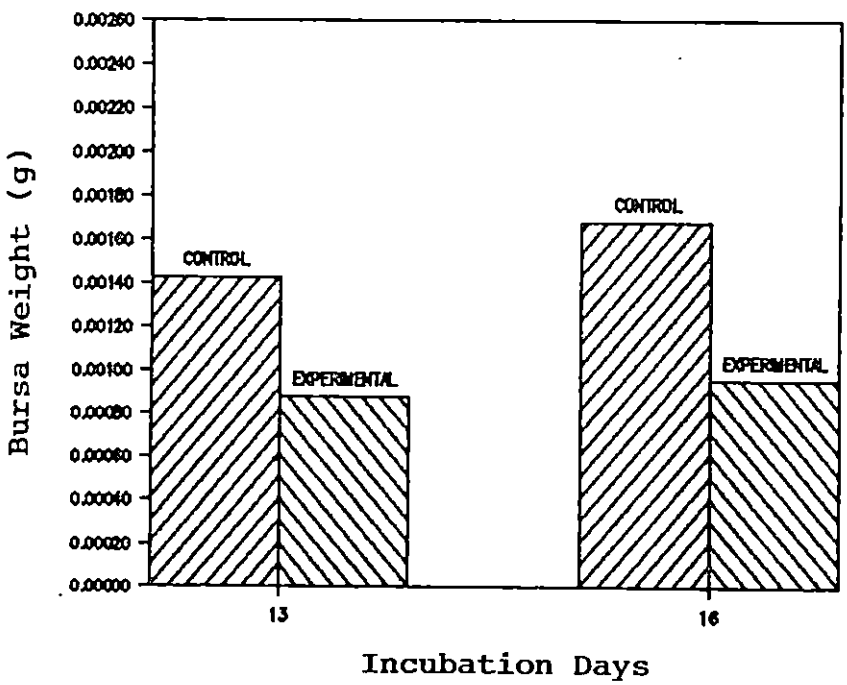


Fig.2 Average weight of bursa of embryos

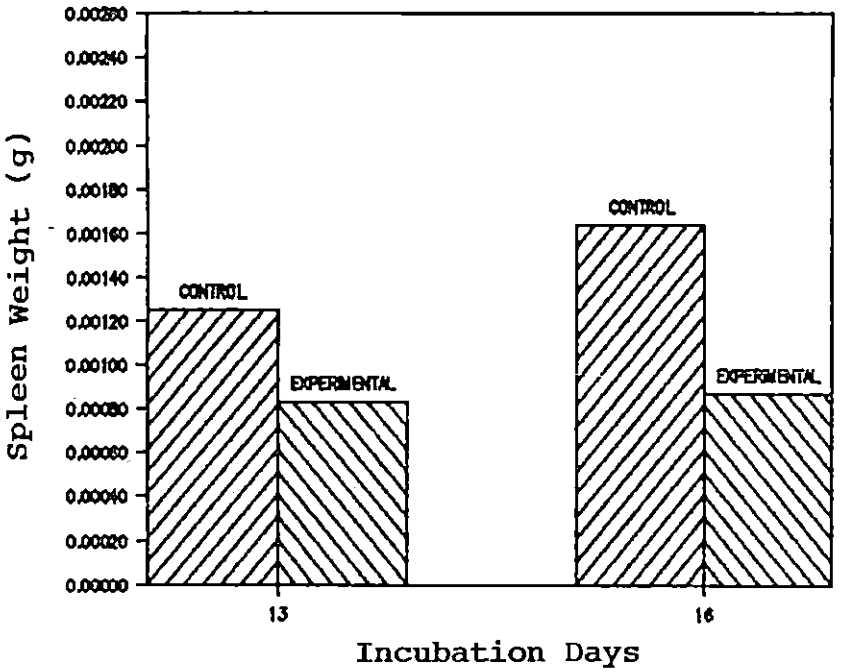


Fig.3 Average weight of spleen of embryos

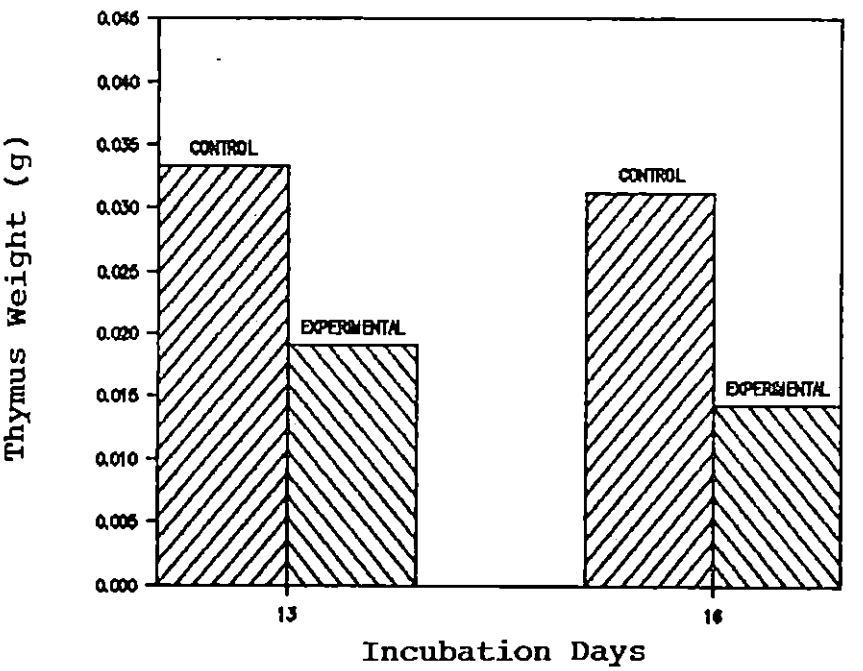


Fig.4 Average weight of thymus of embryos

4.2 Ochratoxicosis in Japanese quail

4.2.1 Clinical signs

All the OA fed birds were emaciated and they were smaller in size when compared to control birds. The OA fed quails were hyperexcited in the later part of the experiment.

4.2.2 Body weight and organ weight

4.2.2.1 Group I and II

The average body and organ weight of quails in group I and II during the course of the experiment are furnished in Table 2.

4.2.2.1.1 Body weight

There was highly significant reduction in the body weight of quails in group I (Fig.5) during the course of the experimental observation period ($P < 0.01$).

4.2.2.1.2 Liver weight

In the first fortnight no variation was observed in the liver weight. In the 2nd, 3rd and 4th fortnights the liver weight showed an increase in the quails belonging to the group I, but it was not significant (Fig.6).

Table 2. Average (Mean ± SE) body and organ weight (Group I, II and III)

Fortnight	Group	Body weight (g)	Weight of liver (g)	Weight of bursa of Fabricius (g)	Weight of spleen (g)	Weight of thymus (g)
First	Group I	91.6666 ± 4.9534**	3.0298 ± 0.1279	0.0491 ± 0.0104*	0.0618 ± 0.0057	0.1701 ± 0.0065
	Group II	134.1666 ± 4.1527	3.0453 ± 0.1912	0.1040 ± 0.0131	0.0900 ± 0.0044	0.1950 ± 0.0016
Second	Group I	90.8333 ± 5.5798**	2.8818 ± 0.1401	0.0351 ± 0.0072**	0.0368 ± 0.0045**	0.1216 ± 0.0111**
	Group II	145.8333 ± 2.7428	2.7183 ± 0.1522	0.0906 ± 0.0029	0.0828 ± 0.0052	0.1898 ± 0.0023
Third	Group I	115.0000 ± 4.5643**	3.8908 ± 0.2300	0.0426 ± 0.0061**	0.0768 ± 0.0103*	0.1701 ± 0.0190*
	Group II	150.0000 ± 3.1180	3.0584 ± 0.2036	0.0968 ± 0.0046	0.1115 ± 0.0089	0.2655 ± 0.0229
Fourth	Group I	129.1666 ± 2.9853**	3.4966 ± 0.2544	0.0270 ± 0.0034**	0.0885 ± 0.0070	0.1888 ± 0.0147**
	Group II	149.1666 ± 2.1783	2.7900 ± 0.1069	0.0853 ± 0.0032	0.1113 ± 0.0081	0.3010 ± 0.0238
Fifth	Group III	148.6111 ± 1.1657**	2.9288 ± 0.0506**	0.0279 ± 0.0017	0.1281 ± 0.0048**	0.1912 ± 0.0021

* ($P < 0.05$)

** ($P < 0.01$)

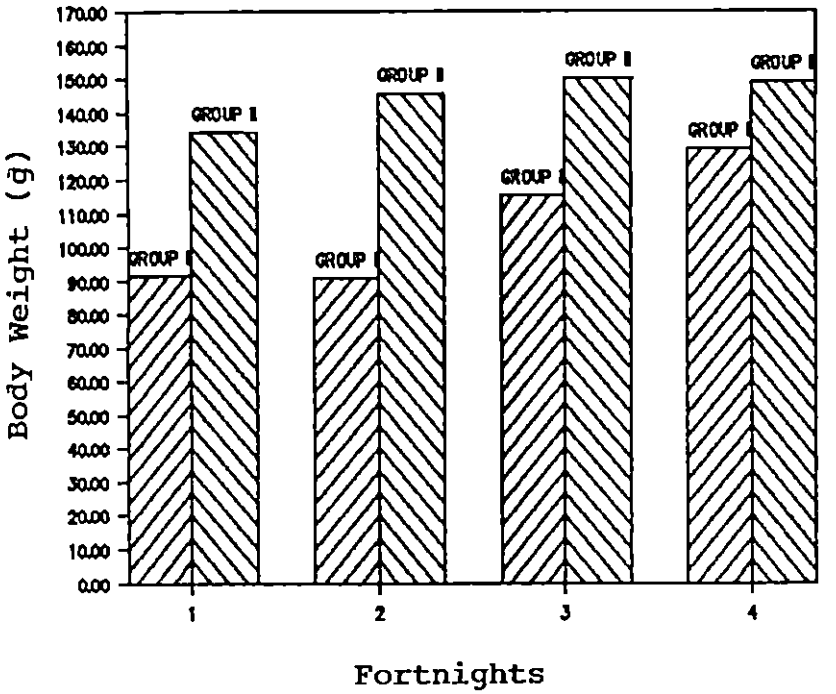


Fig.5 Average body weight (Group I and II)

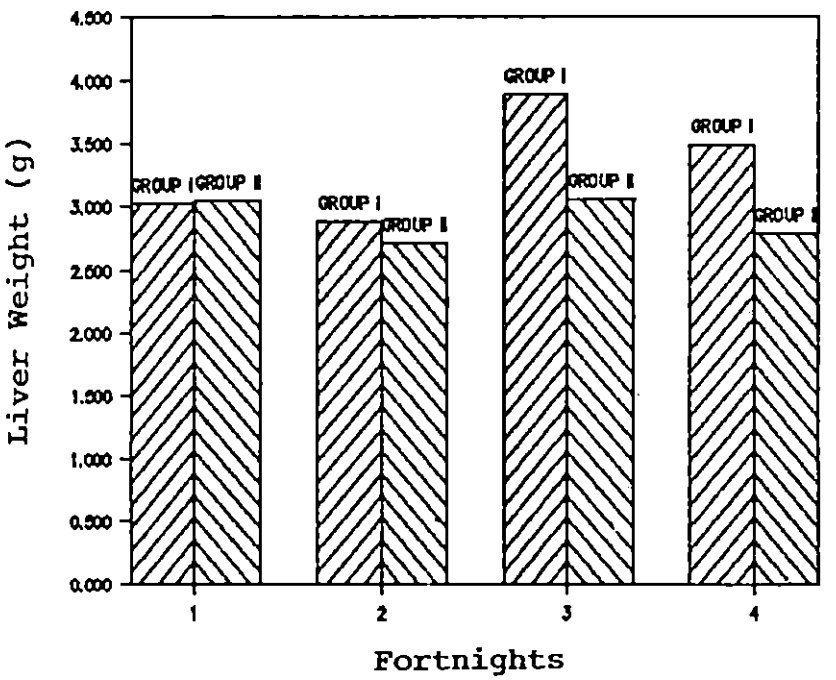


Fig.6 Average liver weight (Group I and II)

4.2.2.1.3 Weight of bursa of Fabricius

During the first fortnight there was reduction in the weight of bursa of Fabricius in the group I. This was significant ($P < 0.05$). The reduction was more appreciated in the 2nd, 3rd and 4th fortnights (Fig.7), which was found to be highly significant ($P < 0.01$).

4.2.2.1.4 Weight of spleen

Quails in goup I showed significant reduction in the weight of spleen during the first fortnight ($P < 0.05$). In the 2nd fortnight the reduction was highly significant in group I ($P < 0.01$). This reduction became significant ($P < 0.05$) at the 3rd fortnight. Though the reduction persisted during the 4th fortnight it was not statistically significant (Fig.8).

4.2.2.1.5 Weight of thymus

The reduction in the weight of thymus in group I quails was not significant in the first fortnight, but it was highly significant ($P < 0.01$) in the 2nd fortnight, significant ($P < 0.05$) in the 3rd fortnight and once again highly significant ($P < 0.01$), in the 4th fortnight (Fig.9).

4.2.2.2 Group III

The mean body and organ weight of quails in group III are given in Table 2. They were compared with group I

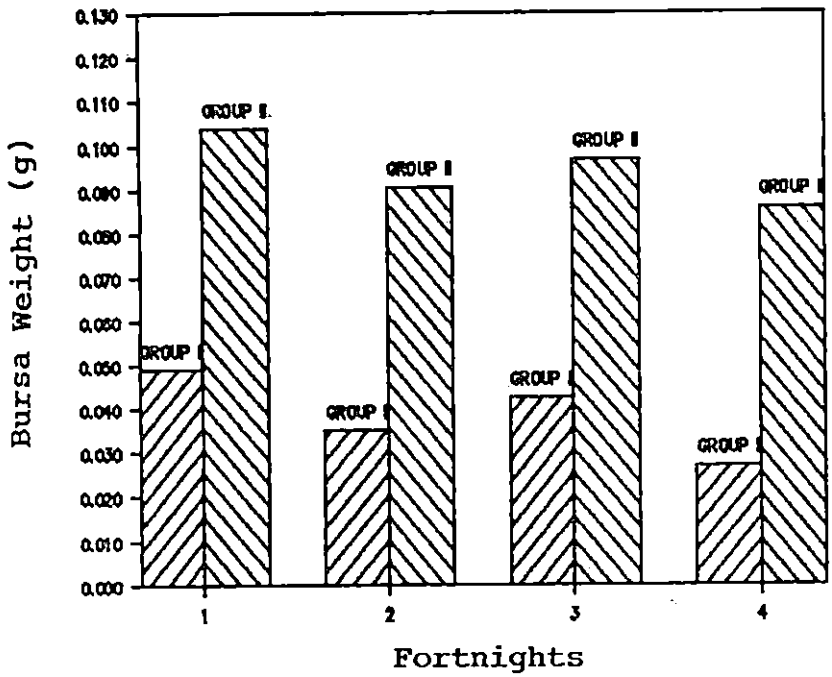


Fig.7 Average bursa weight (Group I and II)

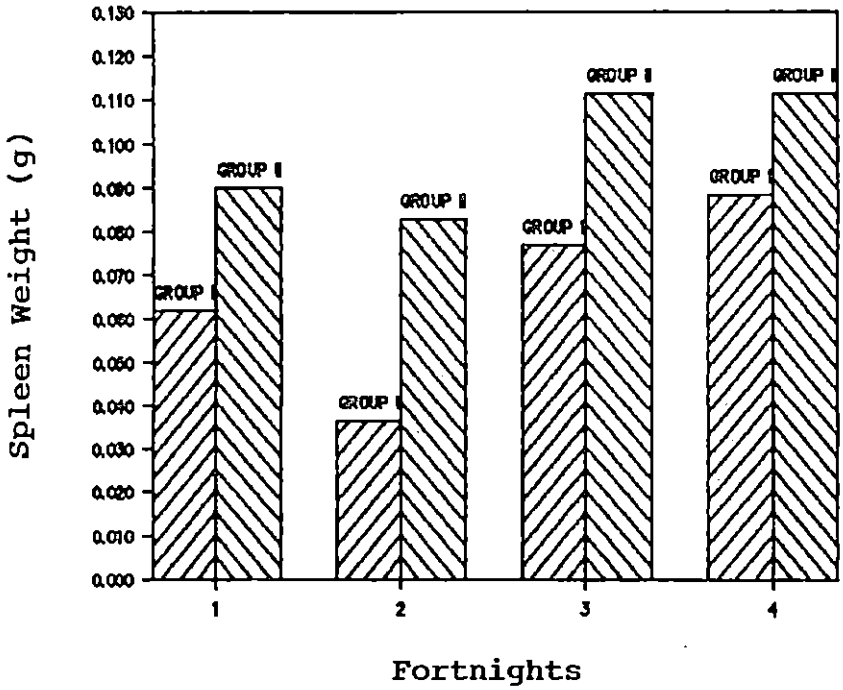


Fig.8 Average spleen weight (Group I and II)

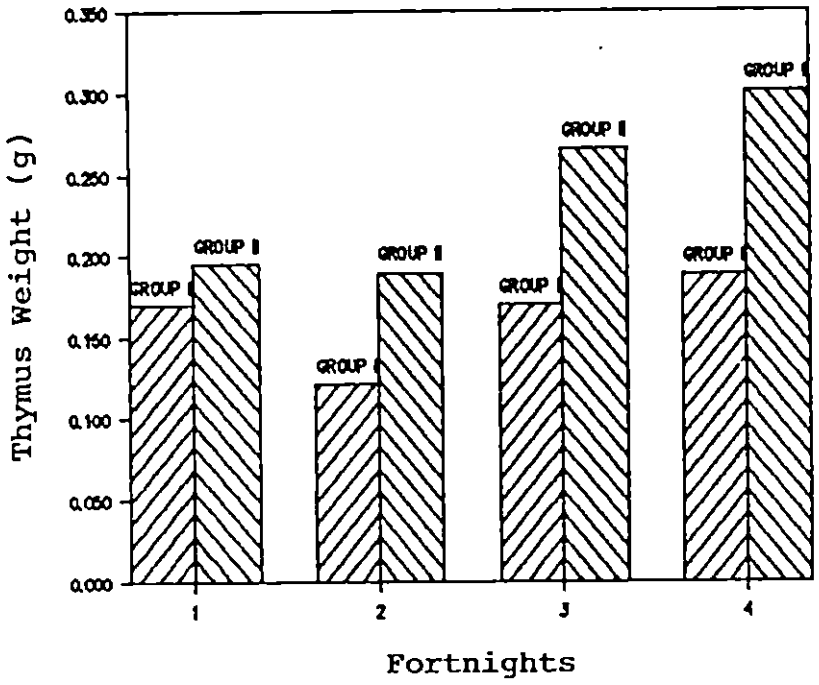


Fig.9 Average thymus weight (Group I and II)

individuals at the 4th fortnight. The increase in the body weight (Fig.10) and spleen weight (Fig.13) and reduction of the liver weight in group III quails (Fig.11) were found to be highly significant ($P < 0.01$). However, there was no significant changes in the weight of bursa of Fabricius (Fig.12) and thymus (Fig.14).

4.2.3 Haematology

4.2.3.1 Group I and II

The average values for TEC, Hb, PCV, MCV, MCH and MCHC are given in Table 3.

4.2.3.1.1 TEC

During the first fortnight there was reduction in TEC in group I which was not significant. But the reduction in TEC values of group I was found to be highly significant ($P < 0.01$) during the 2nd, 3rd and 4th fortnights (Fig.15).

4.2.3.1.2 Hb

There was reduction in the Hb concentration during the course of the experiment (Fig.16). In group I, this was found to be significant ($P < 0.05$) in the first fortnight and highly significant ($P < 0.01$) during the 2nd, 3rd and 4th fortnights.

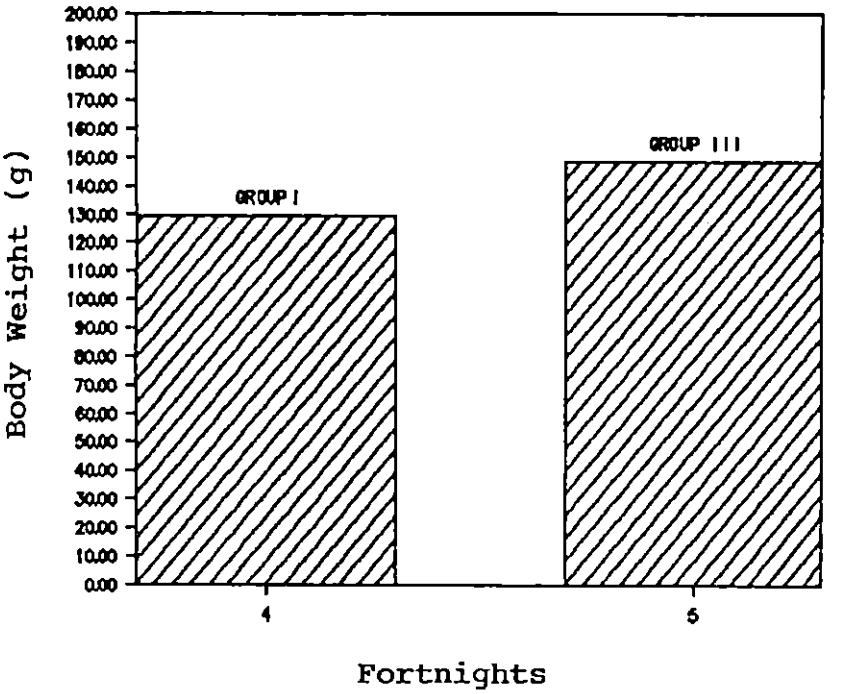


Fig.10 Average body weight (Group I and III)

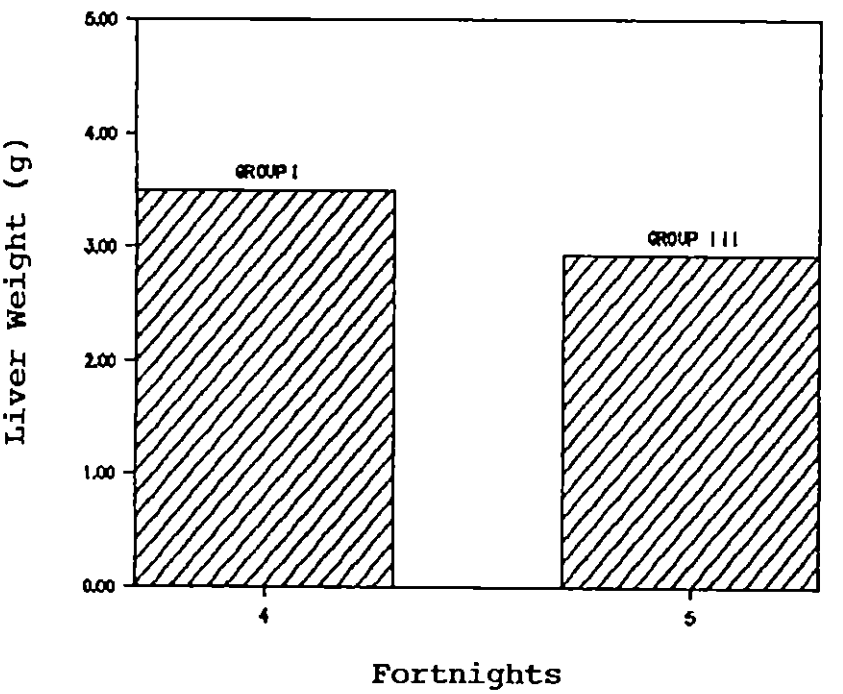


Fig.11 Average liver weight (Group I and III)

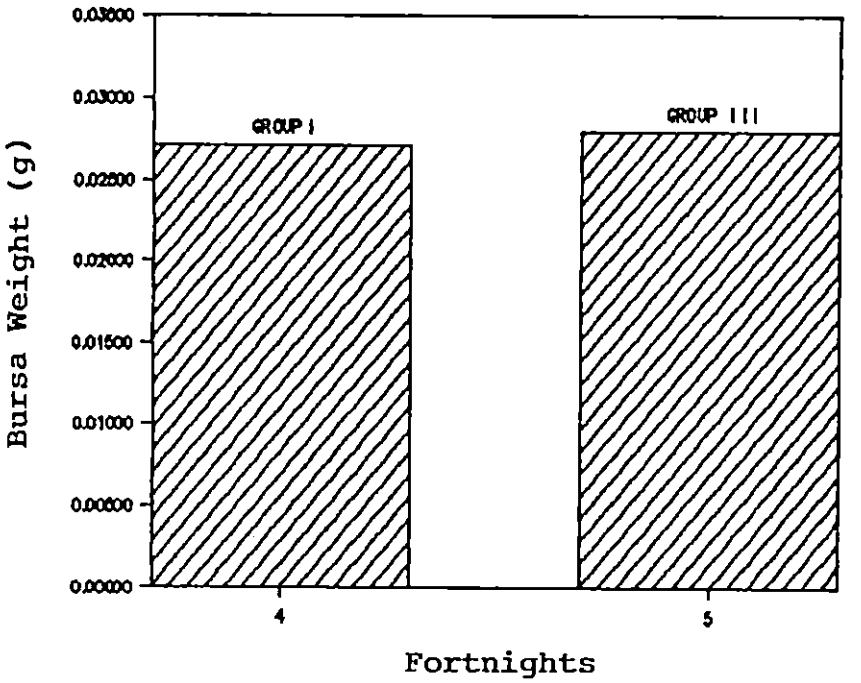


Fig.12 Average bursa weight (Group I and III)

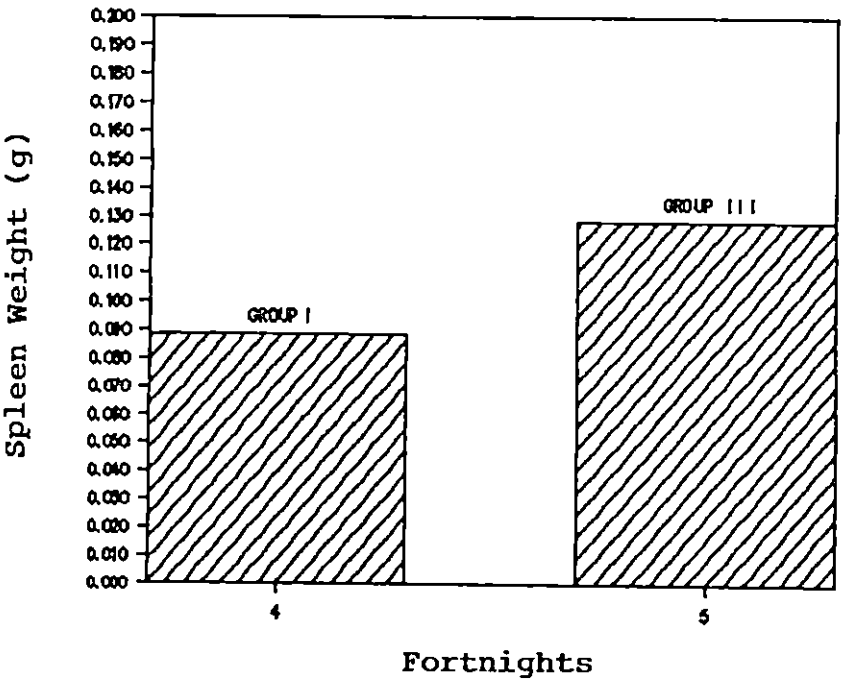


Fig.13 Average spleen weight (Group I and III)

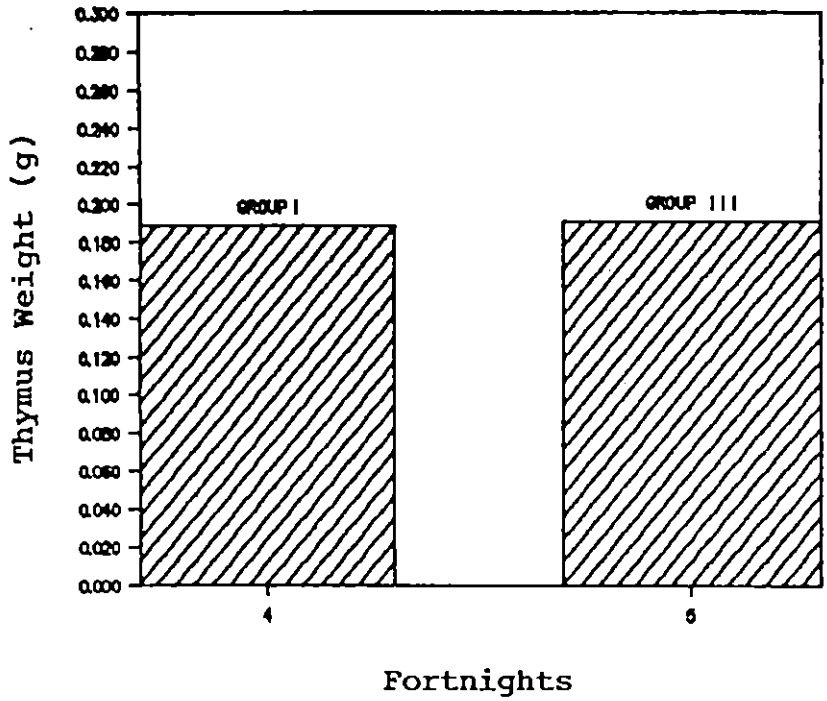


Fig.14 Average thymus weight (Group I and III)

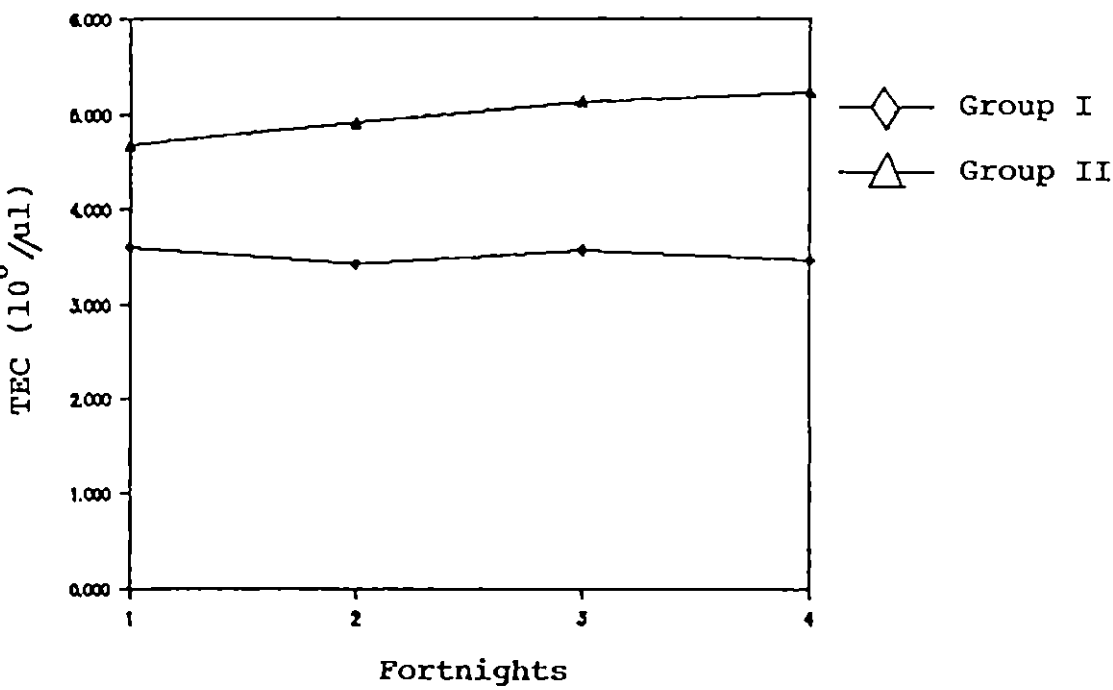


Fig.15 Average TEC (Group I and II)

Table 3. Average (Mean ± SE) of TEC, Hb, PCV, MCV, MCH and MCHC (Group I, II and III)

Fortnight	Group	TEC ($10^6/\mu l$)	Hb (g/dl)	PCV (%)	MCV (μ^3)	MCH ($\mu\mu g$)	MCHC (%)
First	Group I	0.4638 ± 3.6083	* 10.5000 ± 0.6141	** 29.6660 ± 0.5610	86.8000 ± 7.9124	31.6933 ± 3.7248	35.4266 ± 2.0515
	Group II	4.6816 ± 0.2850	13.3000 ± 0.6476	36.6666 ± 1.5396	79.1750 ± 3.5074	28.9066 ± 2.1186	36.8316 ± 2.7366
Second	Group I	** 3.4266 ± 0.1879	** 8.7833 ± 0.5376	** 30.1666 ± 2.5212	87.4950 ± 3.8708	25.8033 ± 1.2431	29.6750 ± 1.6061
	Group II	4.9116 ± 0.1368	13.3333 ± 0.3220	45.1666 ± 0.9254	92.2866 ± 2.7584	27.3316 ± 0.9851	29.5916 ± 0.2777
Third	Group I	** 3.5750 ± 0.2079	** 9.7666 ± 0.4700	* 33.5000 ± 3.2123	76.5466 ± 11.3292	27.4466 ± 0.5481	29.6966 ± 1.6105
	Group II	5.1333 ± 0.1576	14.4333 ± 0.2883	46.5000 ± 0.8416	90.9650 ± 1.8886	28.2516 ± 0.9228	31.0766 ± 0.7047
Fourth	Group I	** 3.4766 ± 0.1994	** 10.1000 ± 0.5390	35.3333 ± 3.2116	100.7483 ± 4.9248	29.7316 ± 2.4289	31.0933 ± 2.3590
	Group II	5.2316 ± 0.1209	14.4333 ± 0.2805	46.0000 ± 0.8983	88.2866 ± 2.2629	27.6766 ± 0.8622	31.4416 ± 0.8704
Fifth	Group III	** 4.4066 ± 0.1584	11.8000 ± 0.4888	** 42.0000 ± 1.2765	144.6166 ± 46.8133	27.0255 ± 0.7130	30.7188 ± 2.5131

* ($P < 0.05$)

** ($P < 0.01$)

4.2.3.1.3 PCV

The PCV percentage was reduced in group I (Fig.17), during the experimental period, this reduction was found to be highly significant ($P < 0.01$), in the first and 2nd fortnights and significant ($P < 0.05$) in the 3rd fortnight, but not significant in the 4th fortnight.

4.2.3.2 Group III

The average values for TEC, Hb, PCV, MCV, MCH and MCHC are given in Table 3. There was highly significant ($P < 0.01$) increase in the TEC (Fig.18). The Hb concentration (Fig.19) although increased was not significant and there was increase in PCV (Fig.20) which was significant ($P < 0.05$). The group III values were compared to group I values at the 4th fortnight.

4.2.4 Leukogram

4.2.4.1 Group I and II

The average values of TLC, DLC and T lymphocytes during the course of experiment are given in Table 4.

4.2.4.1.1 TLC

There was highly significant ($P < 0.01$) decrease in the TLC in group I (Fig.21) during the first, 2nd, 3rd and 4th fortnights.

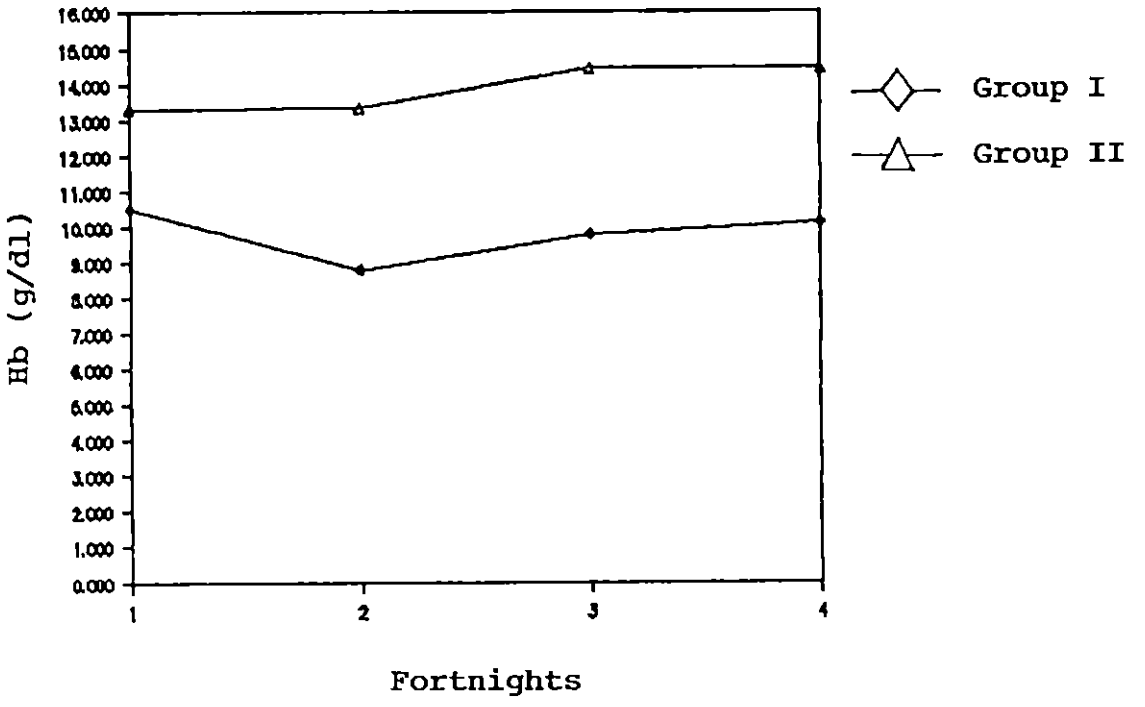


Fig.16 Average Hb (Group I and II)

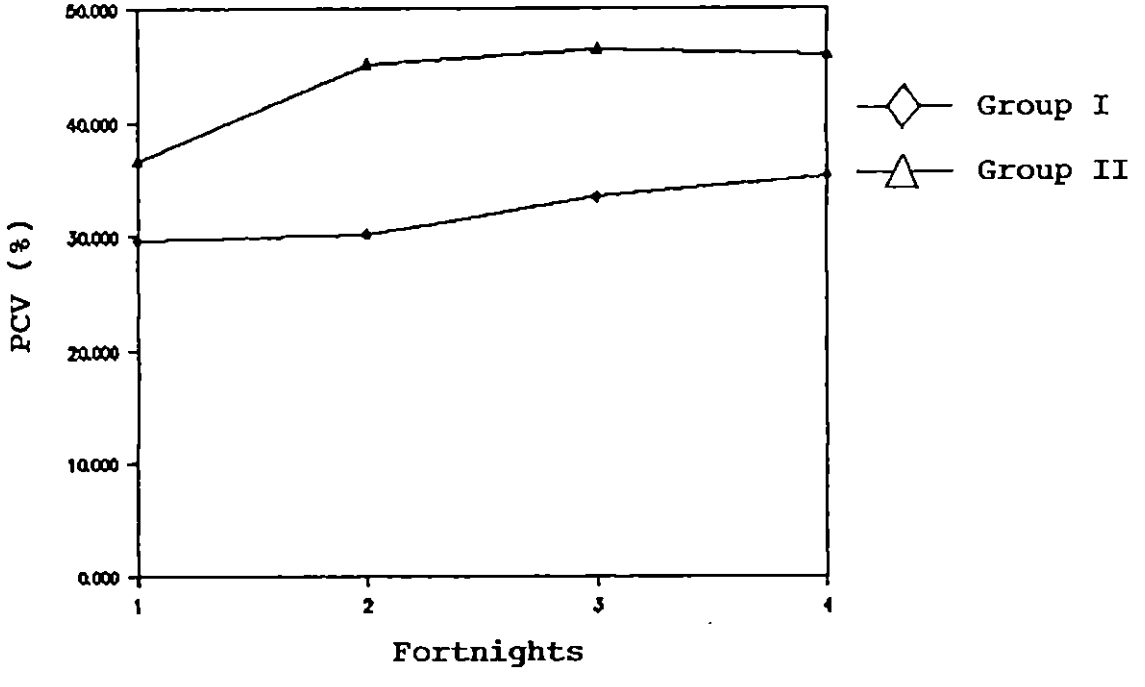


Fig.17 Average PCV (Group I and II)

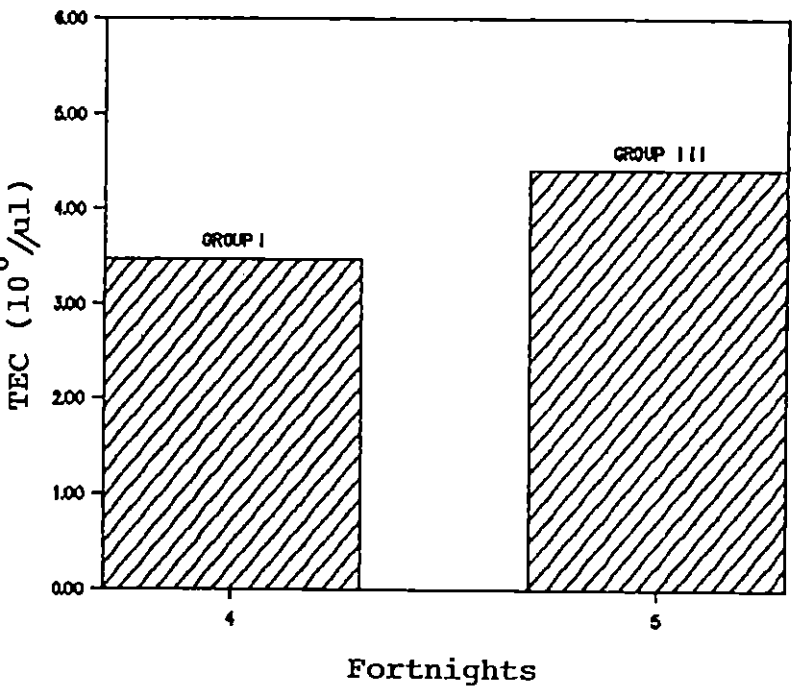


Fig.18 Average TEC (Group I and III)

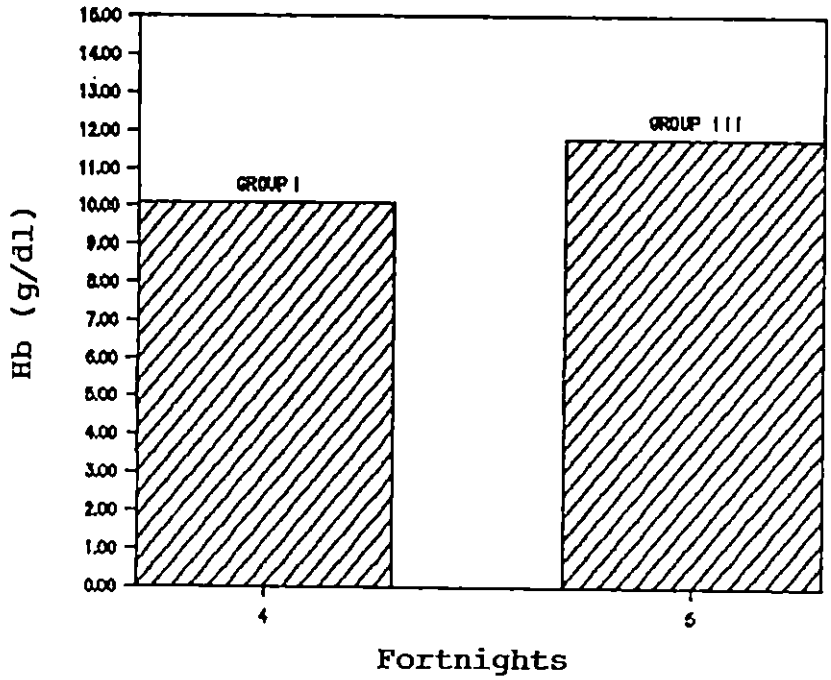


Fig.19 Average Hb (Group I and III)

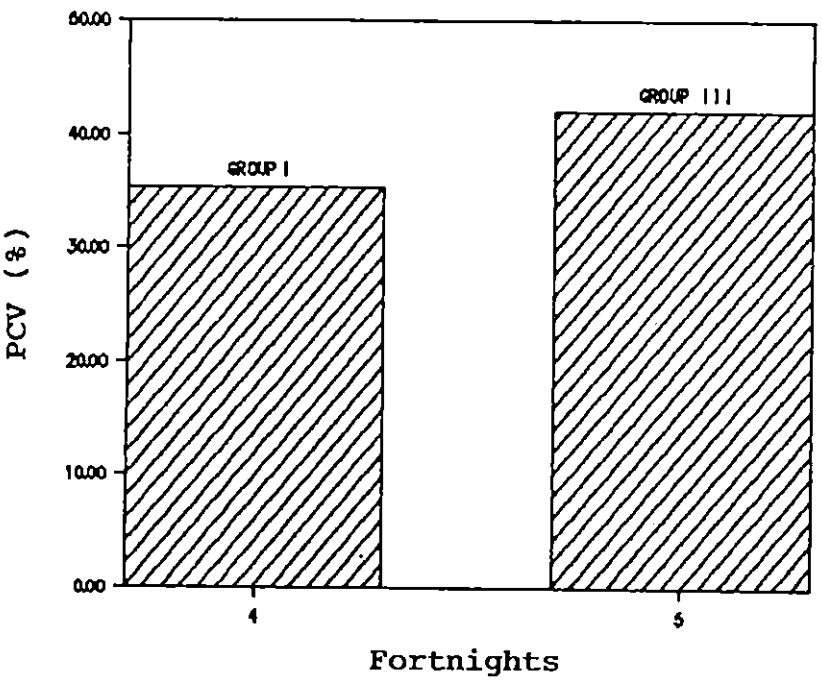


Fig.20 Average PCV (Group I and III)

Table 4. Average (Mean ± SE) of TLC, DLC and T lymphocytes (Group I, II and III)

Fortnight	Group	TLC ($10^3/\mu l$)	Hetero-phils (%)	Eosino-phils (%)	Baso-phils (%)	Lympho-cytes (%)	Mono-cytes (%)	T lympho-cytes (%)
First	Group I	28166.6600 ± 894.9446**	33.0000 ± 0.6236**	3.1666 ± 0.2805	0	61.0000 ± 0.5773**	2.8333 ± 0.2805	11.5000 ± 0.8079
	Group II	33666.6666 ± 932.9364	24.3333 ± 1.0715	2.6666 ± 0.3042	0	69.6666 ± 1.2171	3.3330 ± 0.5610	13.6666 ± 1.0971
Second	Group I	27666.6600 ± 732.8281**	35.6666 ± 0.8050**	2.6666 ± 0.1924	0	59.5000 ± 1.1242**	2.6666 ± 0.3042	9.1666 ± 1.0115
	Group II	32475.0000 ± 688.3706	26.0000 ± 1.5456	2.6666 ± 0.3042	0	68.5000 ± 1.2638	2.8333 ± 0.5970	14.5000 ± 0.9646
Third	Group I	27516.6600 ± 695.3882**	34.3333 ± 0.9329**	2.8333 ± 0.2805	0	59.6660 ± 0.9329**	3.1666 ± 0.2805	8.1666 ± 0.5970**
	Group II	34416.6666 ± 557.9808	25.5000 ± 1.9112	2.3333 ± 0.1924	0	69.5000 ± 2.1048	2.6666 ± 0.1924	14.8333 ± 1.3625
Fourth	Group I	24000.0000 ± 666.6666**	37.1666 ± 0.5485**	3.0000 ± 0.2357	0	56.8333 ± 0.7964**	3.0000 ± 0.2357	7.8333 ± 0.6838**
	Group II	35000.0000 ± 1000.0000	23.6666 ± 0.9329	2.1666 ± 0.1521	0	71.1666 ± 0.7964	2.6666 ± 0.1924	15.6666 ± 0.8713
Fifth	Group III	30666.6600 ± 509.1750**	26.7777 ± 1.0139**	2.7777 ± 0.1851	0	67.6111 ± 1.1114**	2.8333 ± 0.1800	14.2222 ± 0.5064**

* (P <0.05)

** (P <0.01)

4.2.4.1.2 DLC

4.2.4.1.2.1 Heterophils

The percentage of heterophils showed an increase in group I (Fig.22), in all the fortnights till end of the experiment. This increase was found to be highly significant ($P < 0.01$).

4.2.4.1.2.2 Eosinophils

The percentage of eosinophils during the first fortnight was increased in group I, but it was not significant. In the 2nd fortnight there was no variation between the eosinophils percentage of group I and II. The percentage was again increased in group I during the 3rd and 4th fortnights, but statistically remained insignificant.

4.2.4.1.2.3 Basophils

No basophil could be detected in the blood smears of both group I and II during the course of the experiment.

4.2.4.1.2.4 Lymphocytes

The percentage of lymphocytes declined in group I (Fig.23), during all the fortnights. This decrease was highly significant ($P < 0.01$).

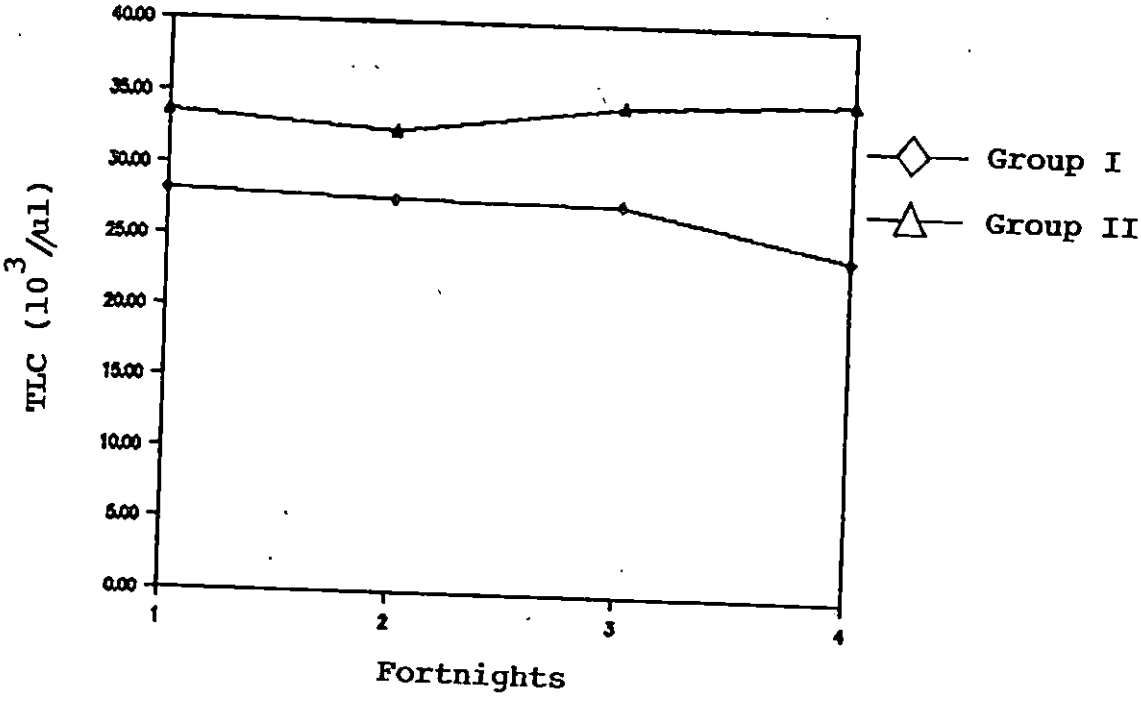


Fig.21 Average TLC (Group I and II)

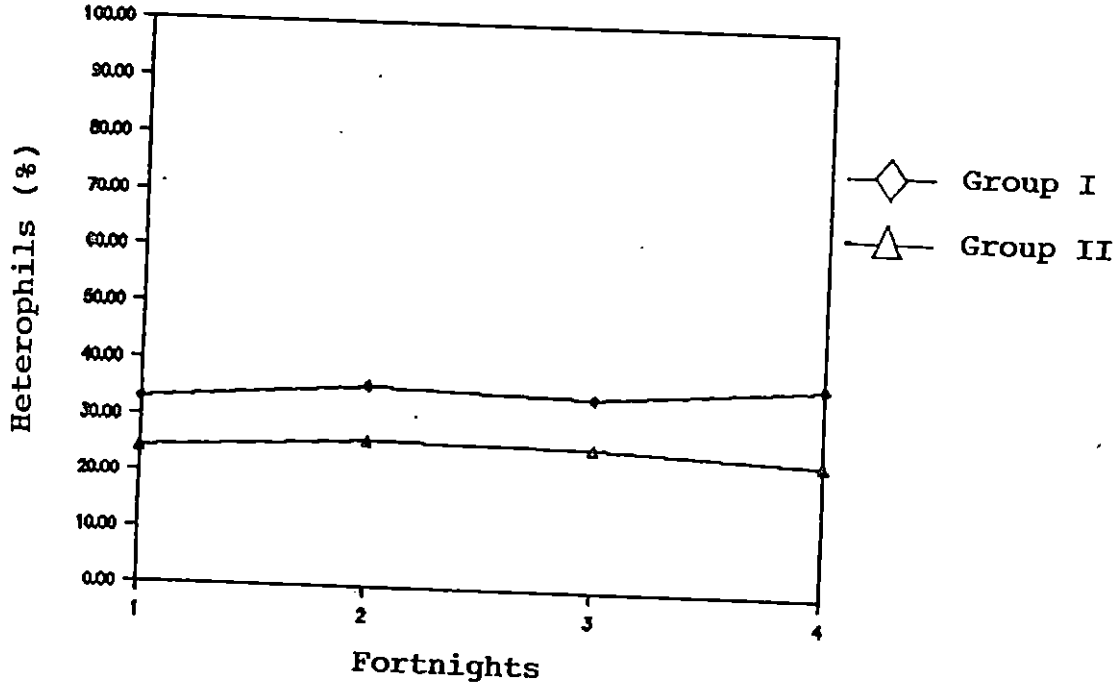


Fig.22 Average heterophils (Group I and II)

4.2.4.1.2.5 Monocytes

The variation between the percentage of monocytes in group I and II remained insignificant during the course of the experiment. In the first and 2nd fortnights the number was more in group II and in 3rd and 4th fortnights group I had higher values.

4.2.4.1.2.6 T lymphocytes

The ANAE positive T cells (Fig.48) showed decline in the percentage (Fig.24) in group I during the first fortnight, but it was not significant. This reduction became significant ($P < 0.05$) in the 2nd fortnight, and highly significant ($P < 0.01$) in the 3rd and 4th fortnights.

4.2.4.2 Group III

The average values of leukogram parameters of group III were compared with group I at the 4th fortnight. There was highly significant ($P < 0.01$) increase in TLC (Fig.25) of group III. The reduction in heterophils percentage (Fig.26) in group III was also highly significant ($P < 0.01$). Reduction of eosinophils in group III was not significant. No basophils could be detected in blood smears of quails belonging to group III. There was highly significant ($P < 0.01$) increase in the lymphocytes percentage

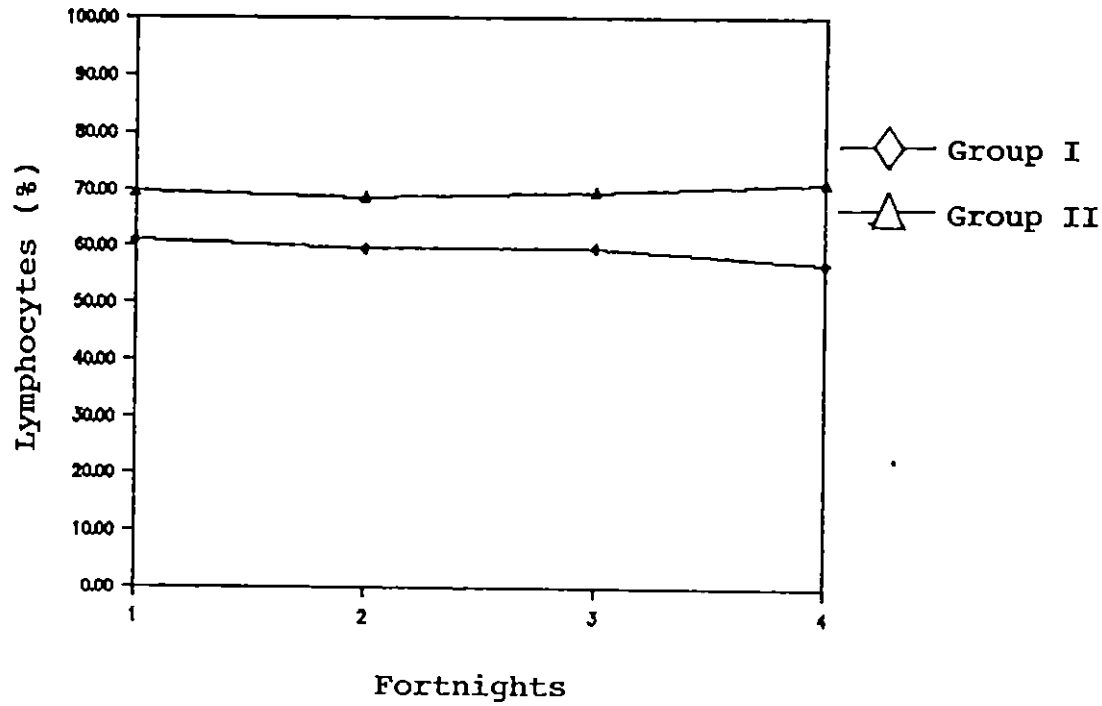


Fig.23 Average lymphocytes (Group I and II)

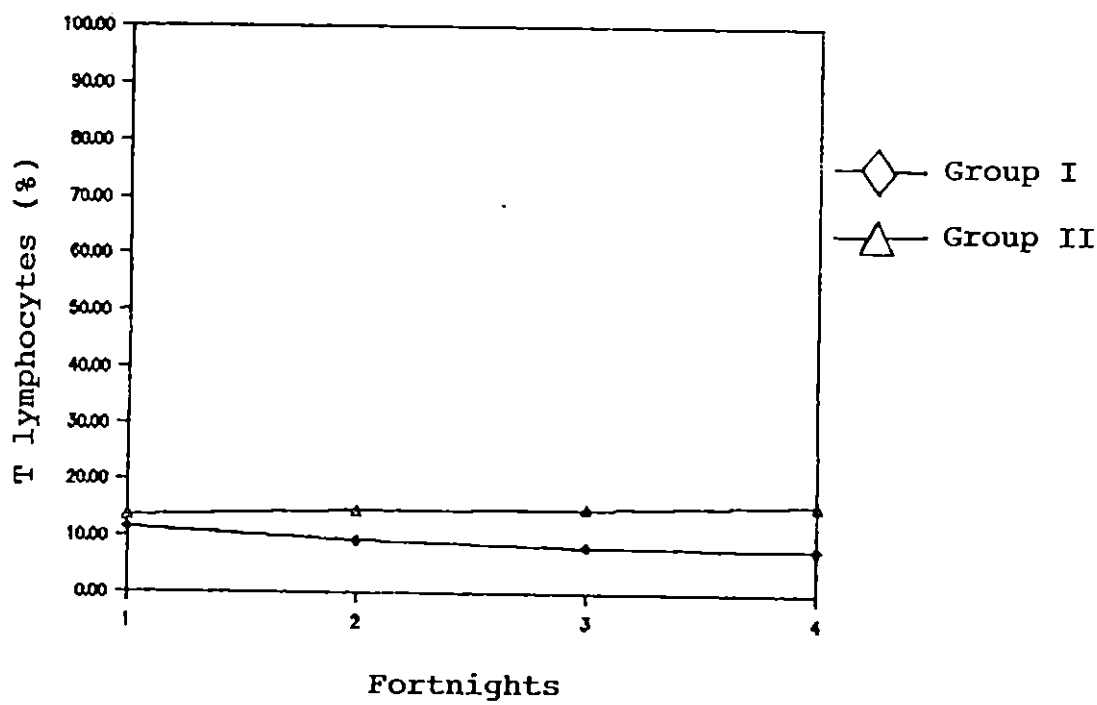


Fig.24 Average T lymphocytes (Group I and II)

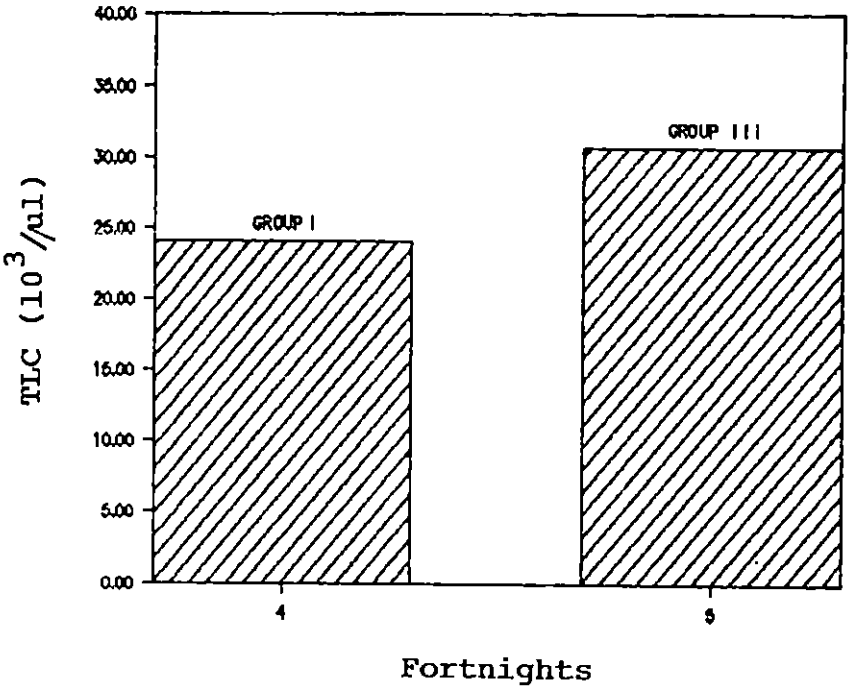


Fig.25 Average TLC (Group I and III)

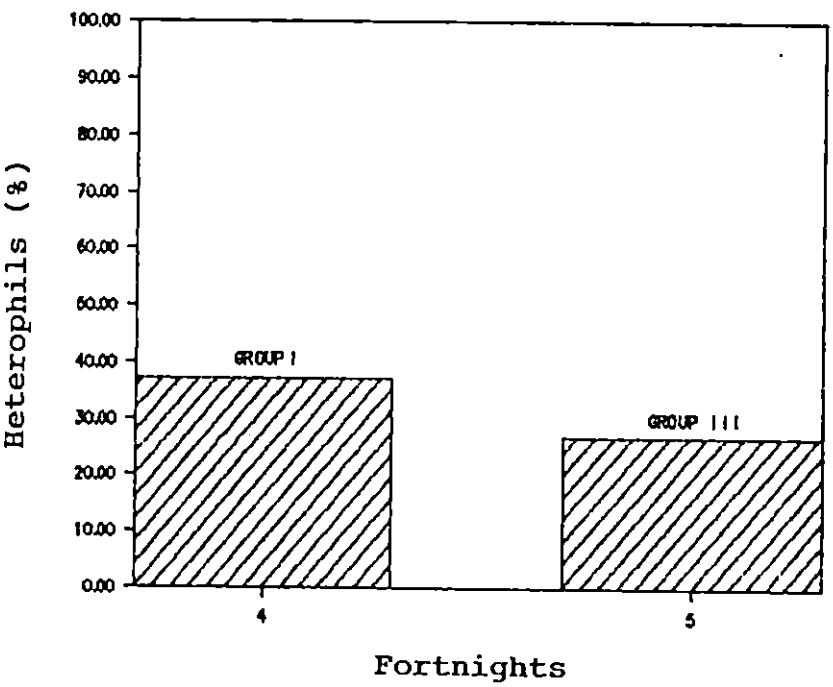


Fig.26 Average heterophils (Group I and III)

in group III (Fig.27). The reduction in monocytes percentage was not significant. Highly significant (P <0.01) increased in T lymphocytes percentage was seen in group III (Fig.28).

4.2.5 Serum protein

4.2.5.1 TSP, SA1 and SG1

4.2.5.1.1 Group I and II

The mean values of TSP, SA1 and SG1 are given in Table 5.

4.2.5.1.1.1 TSP

There was highly significant (P <0.01) reduction of TSP in group I individuals (Fig.29), at the 1st, 2nd, 3rd and 4th fortnights.

4.2.5.1.1.2 SA1

The increase in SA1 was seen in all the fortnights in group I individuals. This increase was highly significant (P <0.01) at 1st and 2nd fortnights. At the 3rd and 4th fortnights the SA1 increase in group I was not significant (Fig.30).

4.2.5.1.1.3 SG1

In the group I there was decrease in SG1 levels (Fig.31), which was found to be highly significant (P <0.01)

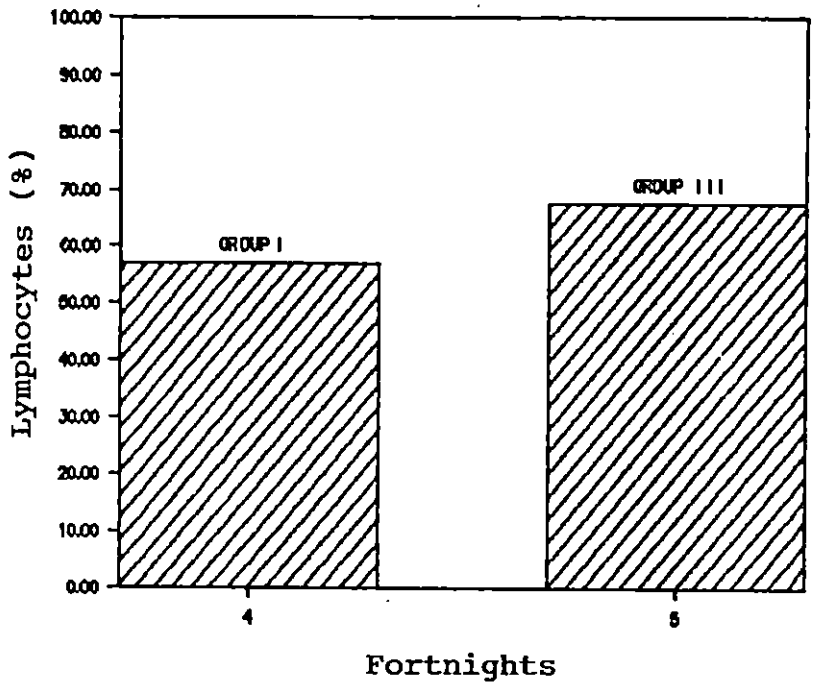


Fig.27 Average lymphocytes (Group I and III)

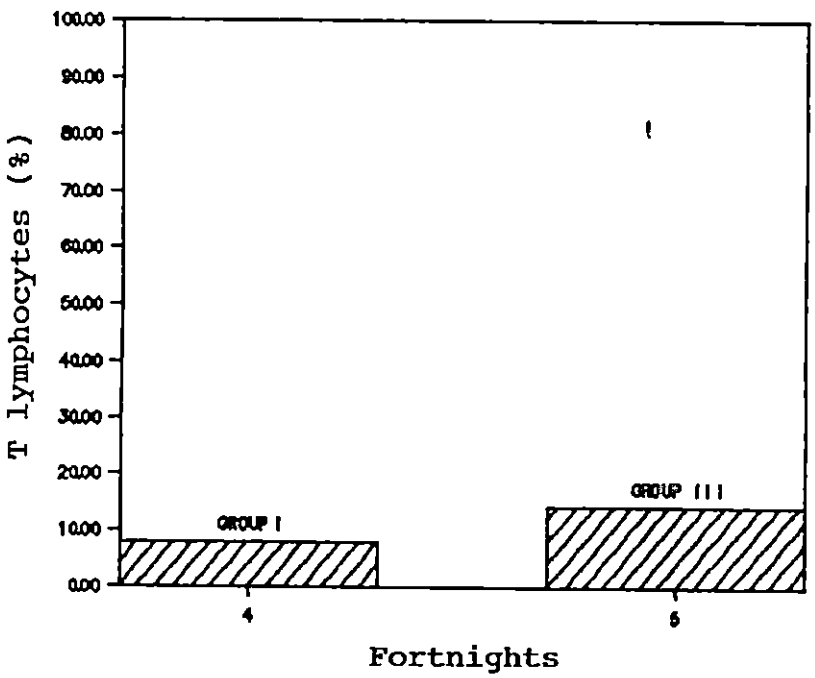


Fig.28 Average T lymphocytes (Group I and III)

Table 5. Average (Mean ± SE) of TSP, SAl, SGl and albumin globulin ratio (Group I, II and III)

Fortnight	Group	TSP (g/dl)	SAl (g/dl)	SGl (g/dl)	Albumin Globulin ratio
First	Group I	2.3016 ± 0.1163**	1.0633 ± 0.0353**	1.2383 ± 0.1188**	0.9106 ± 0.1035
	Group II	3.6766 ± 0.1644	0.8316 ± 0.0217	2.8633 ± 0.1527	0.2931 ± 0.0087
Second	Group I	2.7433 ± 0.8646**	1.1633 ± 0.0598**	1.5783 ± 0.1104**	0.7719 ± 0.0931
	Group II	4.0550 ± 0.6826	0.8283 ± 0.0106	3.2266 ± 0.0720	0.2577 ± 0.0082
Third	Group I	2.7533 ± 0.1409**	1.1066 ± 0.0691	1.6466 ± 0.1262**	0.7061 ± 0.0821
	Group II	4.3783 ± 0.1126	0.9583 ± 0.0277	3.4200 ± 0.0934	0.2806 ± 0.0067
Fourth	Group I	3.1833 ± 0.1321**	1.7383 ± 0.1887	1.5283 ± 0.2228**	1.4873 ± 0.4060
	Group II	5.2166 ± 0.2639	1.3366 ± 0.1295	3.8800 ± 0.2156	0.3494 ± 0.0348
Fifth	Group III	4.9166 ± 0.2579**	1.5822 ± 0.0828	3.3344 ± 0.2270**	0.5057 ± 0.0354

** (P <0.01)

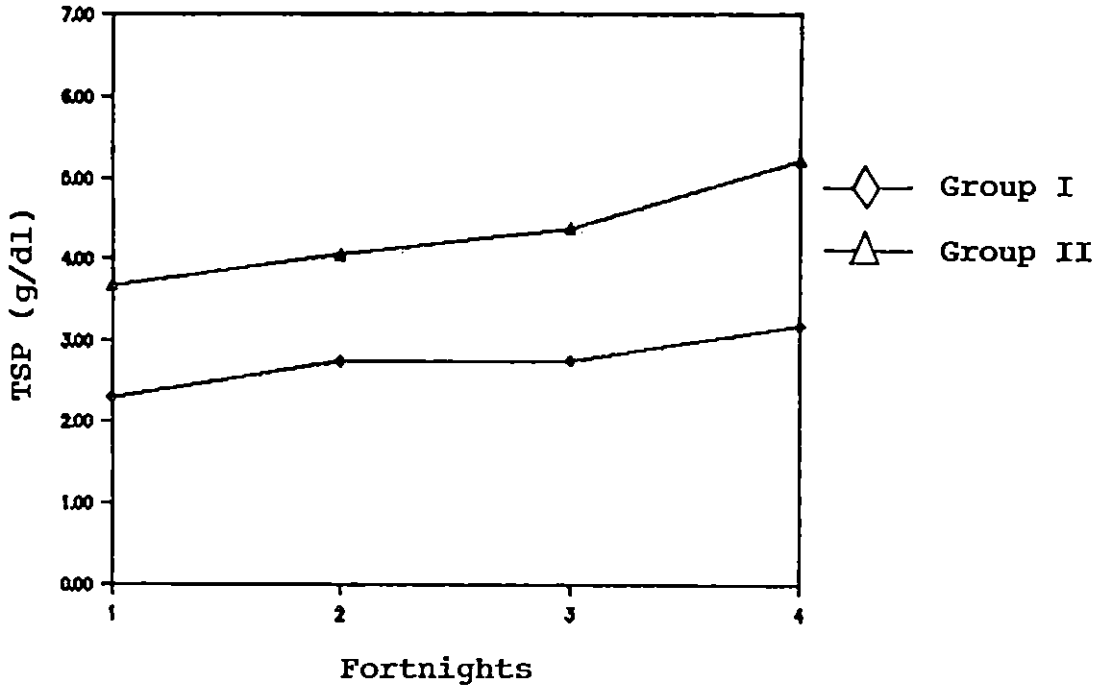


Fig.29 Average TSP (Group I and II)

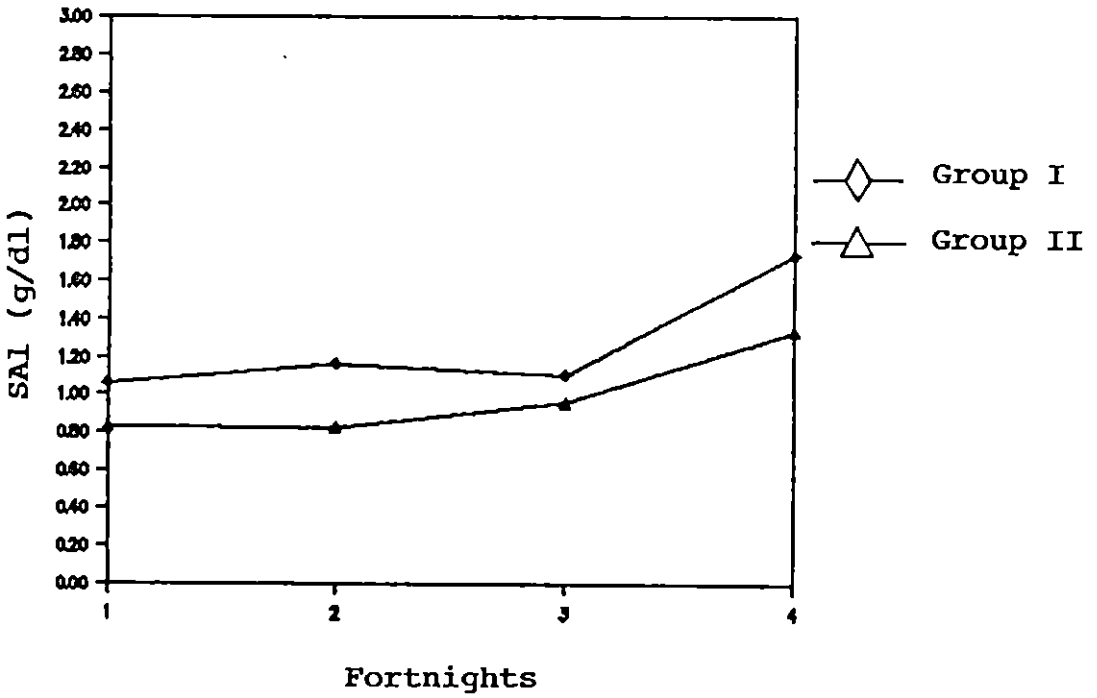


Fig.30 Average SAl (Group I and II)

4.2.5.1.2 Group III

The mean values for TSP, SA1 and SG1 are given in Table 5. The group III values were compared with group I at the 4th fortnight and it was found that the TSP and SG1 levels increased (Fig.32 and 34) which was highly significant ($P < 0.01$), whereas the decrease in SA1 values was not significant (Fig.33).

4.2.5.2 Serum protein fractions

4.2.5.2.1 Group I and II

The result of the PAGE for group I and II with the illustrations are furnished (Fig.35, 36, 37, 38, 39, 40, 41 and 42). During the 1st, 2nd, 3rd and 4th fortnights, there was reduction in the relative percentage of immunoglobulins, IgM and IgG in group I birds, compared to that of group II quails.

4.2.5.2.2 Group III

The PAGE illustration for the group III is furnished (Fig.43), which was compared with the group I in the 4th fortnight. There was increase in both IgM and IgG fractions of the immunoglobulins after immunostimulation.

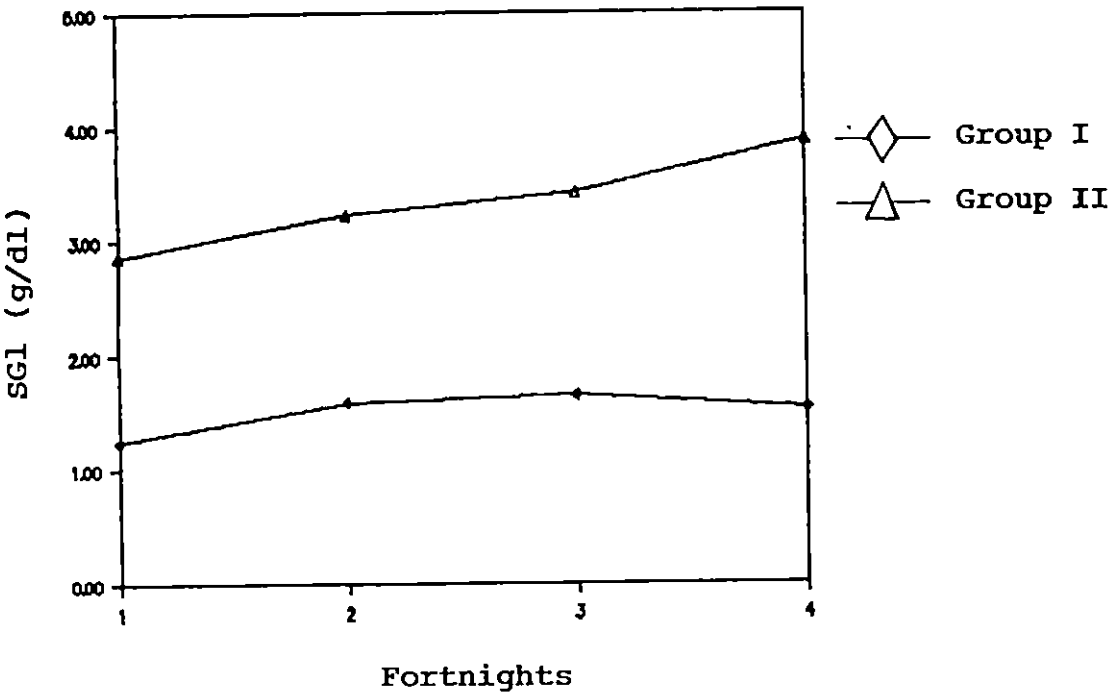


Fig.3l Average SGl (Group I and II)

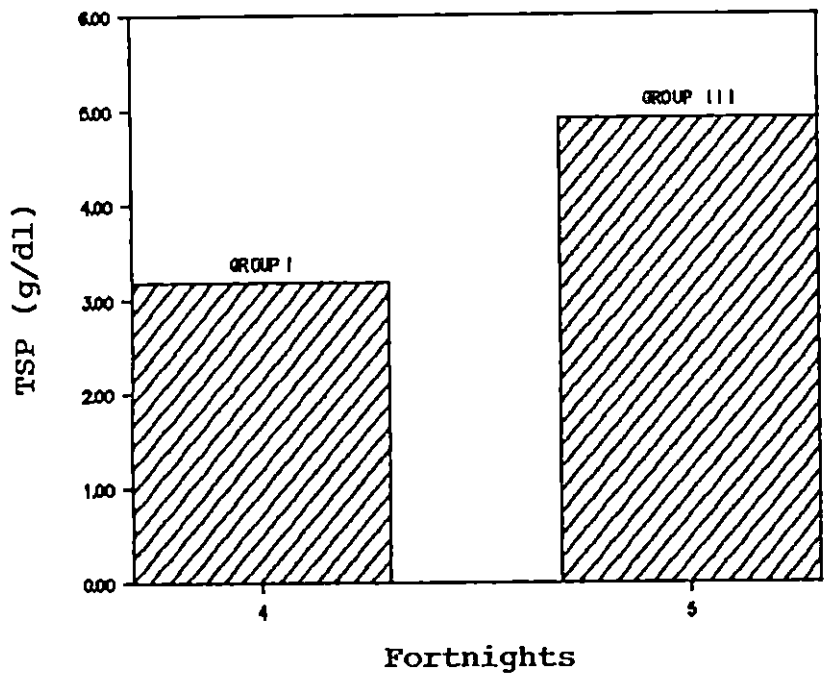


Fig.32 Average TSP (Group I and III)

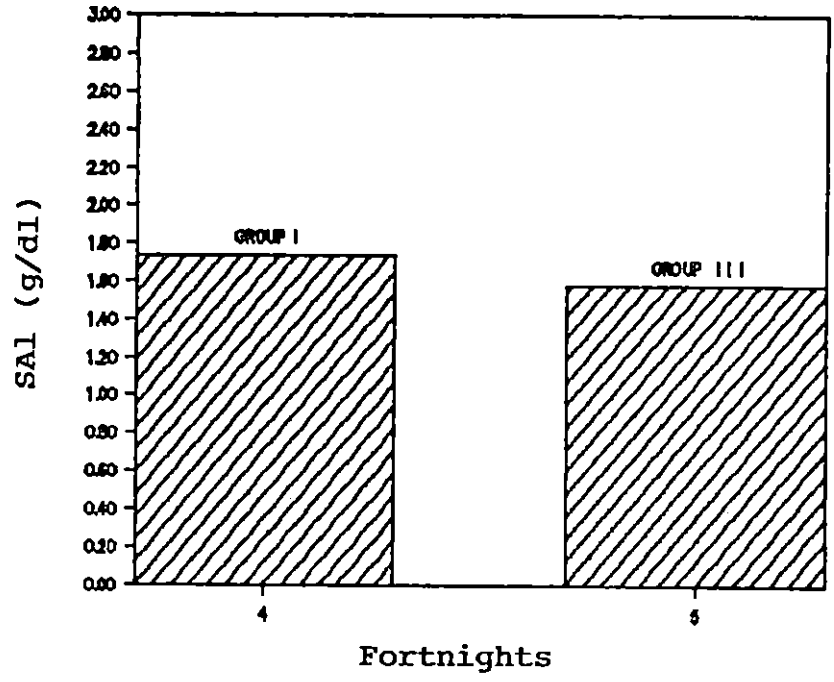


Fig.33 Average SA1 (Group I and III)

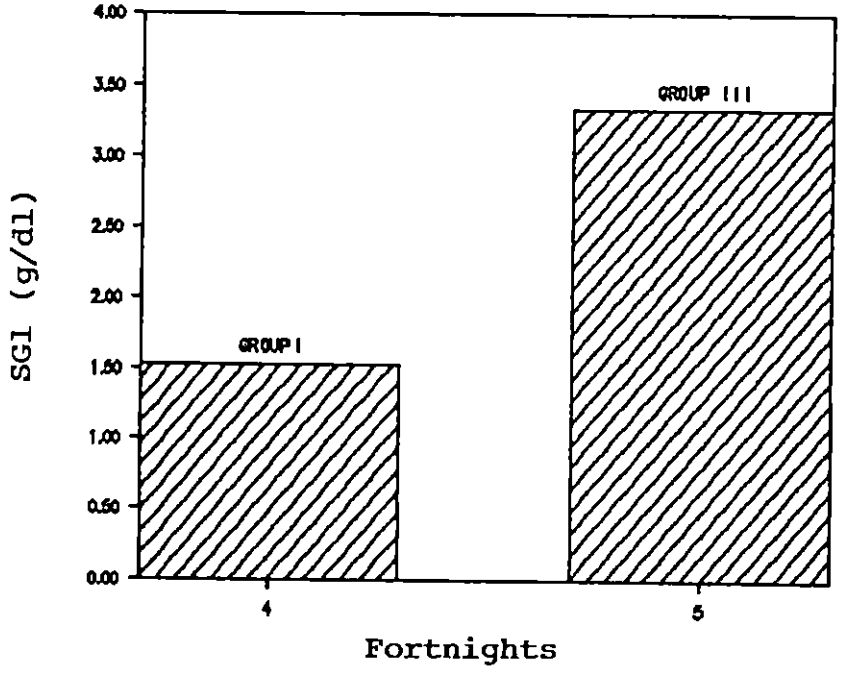


Fig.34 Average SG1 (Group I and III)

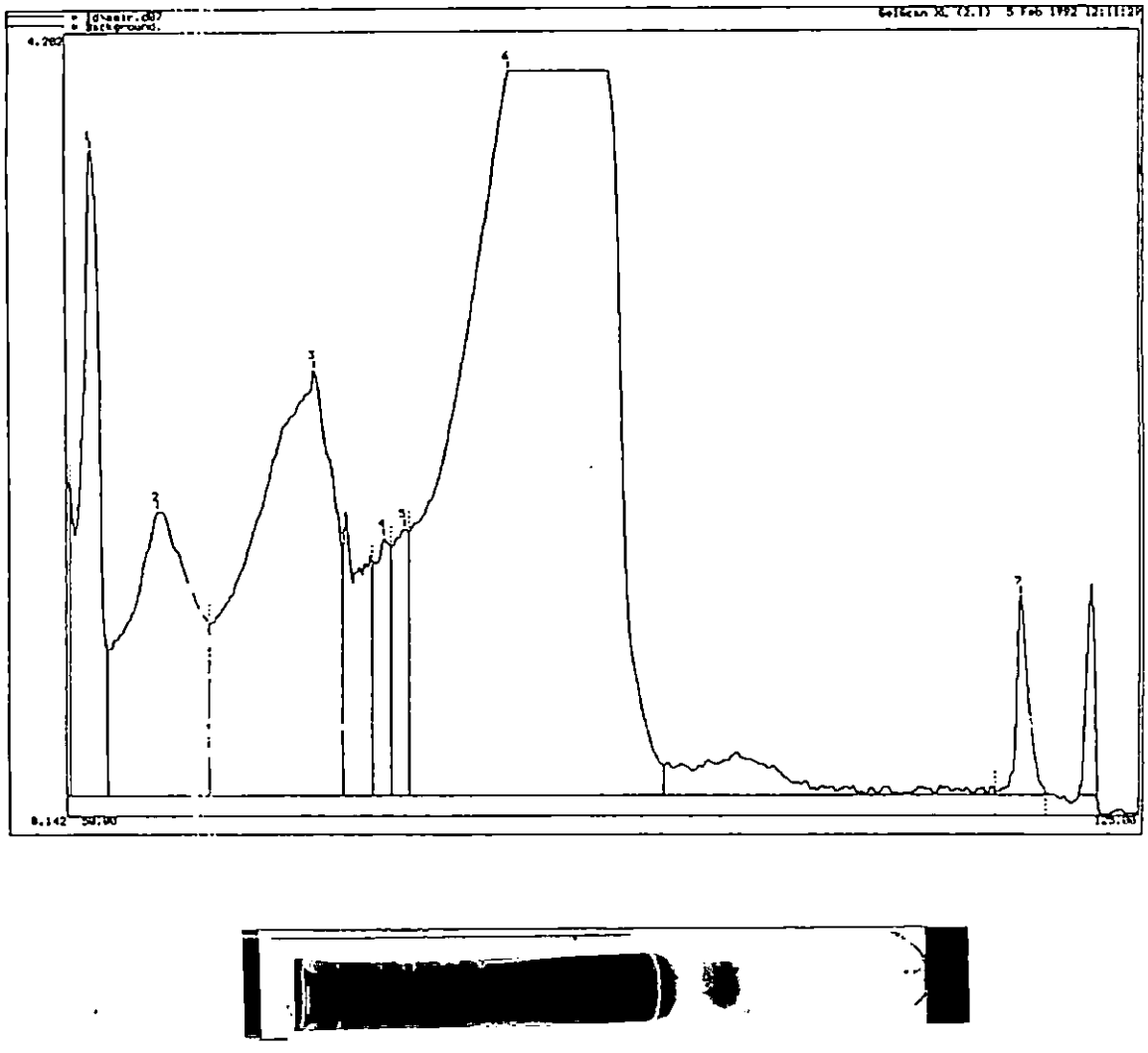


Fig.35 Serum electrophoretic pattern (Group II - lst fortnight)

GelScan XL (2.1) - Fig.36

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	54.780

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	52.48	1.507	2.27960	4.2
2	56.96	0.787	1.98940	3.6
3	59.08	0.884	3.78540	6.9
4	69.44	1.353	11.00948	20.1
5	74.12	0.733	0.86320	1.6
6	77.20	0.840	1.75004	3.2
7	85.20	2.606	13.05180	23.8
8	88.88	3.775	17.35860	31.7
9	117.88	1.361	1.06448	1.9
10	119.92	1.885	1.62772	3.0

GelScan XL (2.1) - Fig.35

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	79.373

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	51.72	3.363	5.39152	6.8
2	56.36	1.482	7.89584	9.9
3	67.32	2.205	14.74860	18.6
4	72.20	1.338	1.73188	2.2
5	73.60	1.385	1.67516	2.1
6	81.04	3.760	46.94944	59.2
7	116.68	1.035	0.98096	1.2

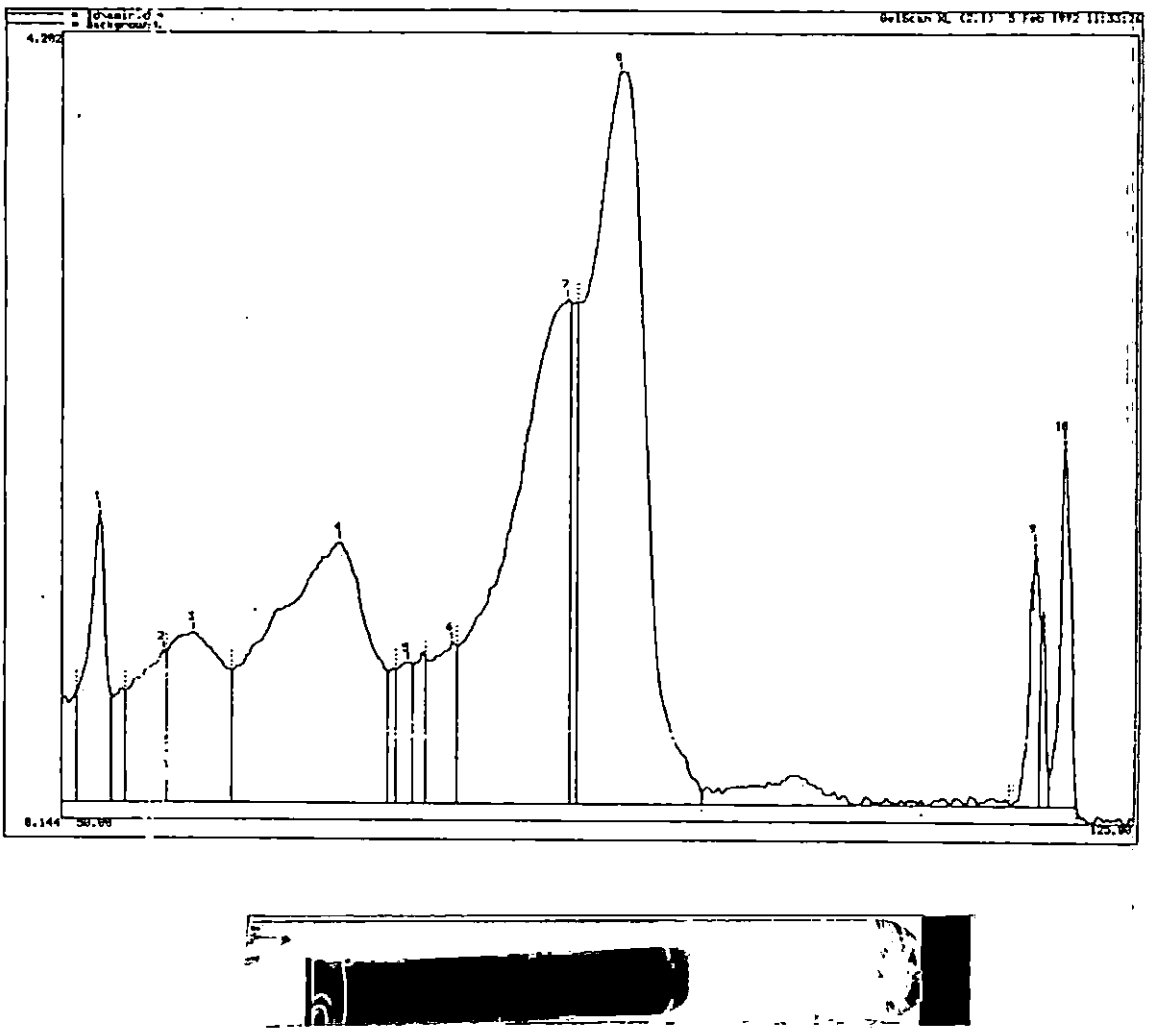


Fig.36 Serum electrophoretic pattern (Group I - 1st fortnight)

GelScan XL (2.1) - Fig.37

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	83.419

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	50.48	2.496	2.10000	2.5
2	51.48	1.102	1.44556	1.7
3	53.76	1.884	2.81704	3.4
4	60.16	2.130	14.12256	16.9
5	66.72	1.981	6.10740	7.3
6	68.60	2.084	4.55076	5.5
7	70.32	2.064	2.29268	2.7
8	71.04	2.118	6.51352	7.8
9	76.00	1.149	1.09144	1.3
10	77.24	1.170	1.48208	1.8
11	78.16	1.077	1.17376	1.4
12	86.16	3.761	37.32212	44.7
13	119.28	1.527	1.47604	1.8
14	120.36	1.277	0.92384	1.1

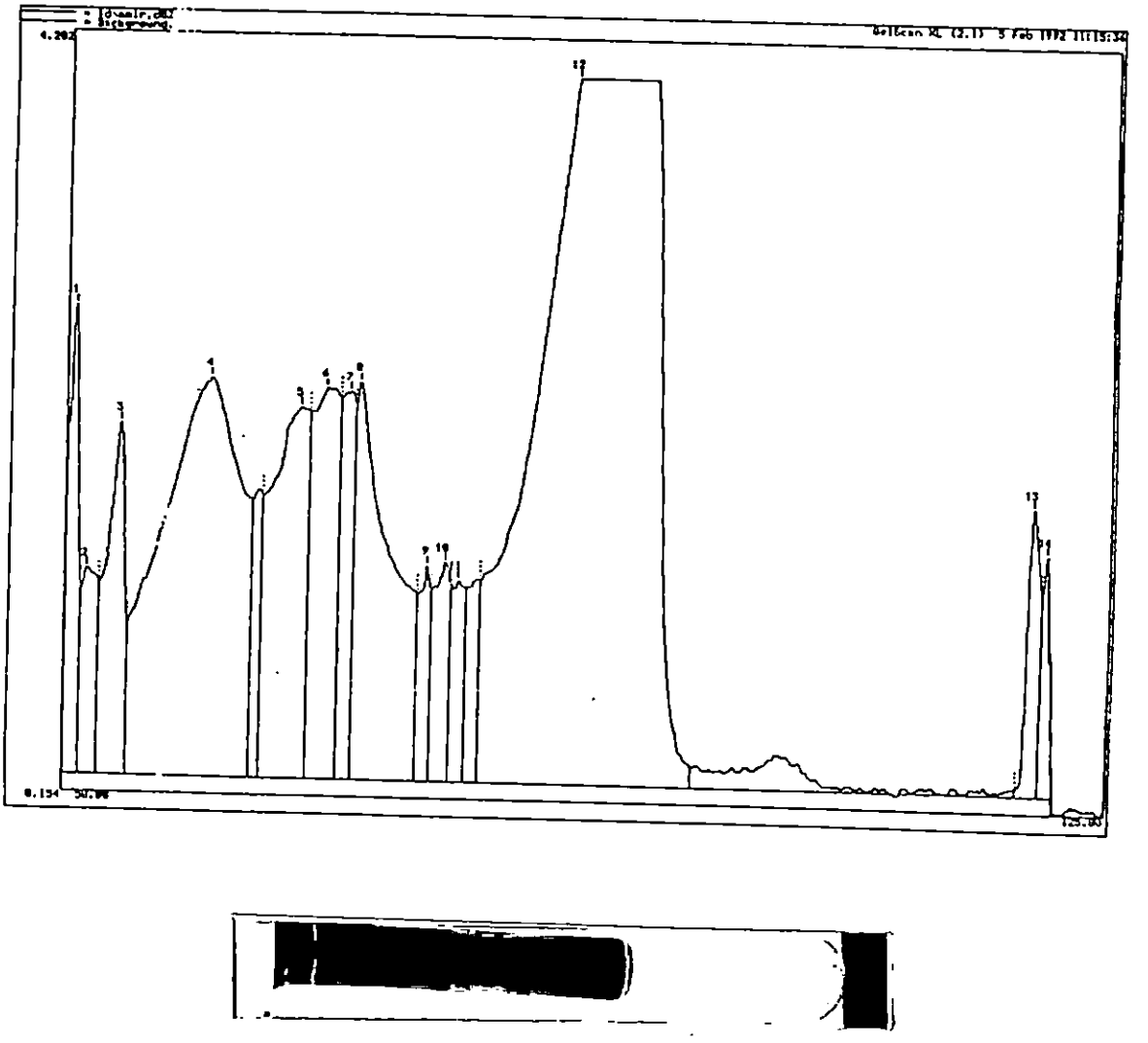


Fig.37 Serum electrophoretic pattern (Group II - 2nd fortnight)

GelScan XL (2.1) - Fig.38

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	79.987

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	50.48	2.489	2.87920	3.6
2	52.76	3.009	5.96496	7.5
3	54.52	0.946	1.24940	1.6
4	55.92	0.924	1.63512	2.0
5	58.40	0.926	1.03272	1.3
6	62.16	1.341	4.30584	5.4
7	62.72	1.365	1.84188	2.3
8	65.56	1.456	3.39304	4.2
9	70.08	1.502	7.24884	9.1
10	83.52	3.651	49.46616	61.8
11	122.92	1.242	0.96964	1.2

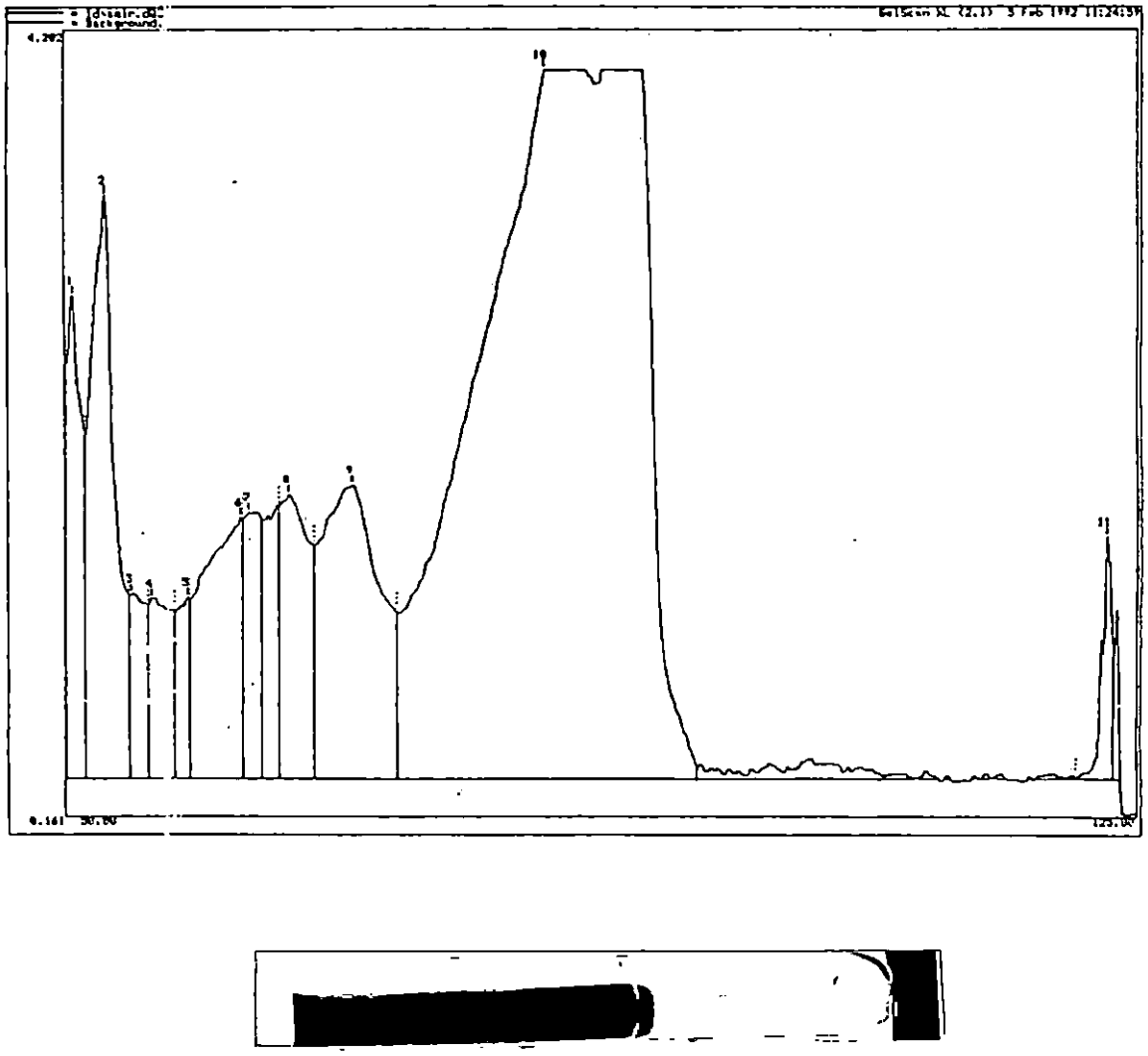


Fig.38 Serum electrophoretic pattern (Group I - 2nd fortnight)

GelScan XL (2.1) - Fig.39

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	92.887

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	52.56	2.636	3.31472	3.6
2	57.68	1.586	5.34180	5.8
3	58.36	1.584	4.19676	4.5
4	67.72	2.570	12.08608	13.0
5	69.20	2.697	10.22988	11.0
6	80.32	3.759	55.06569	59.3
7	98.84	0.412	1.30500	1.4
8	120.00	1.999	1.34672	1.4

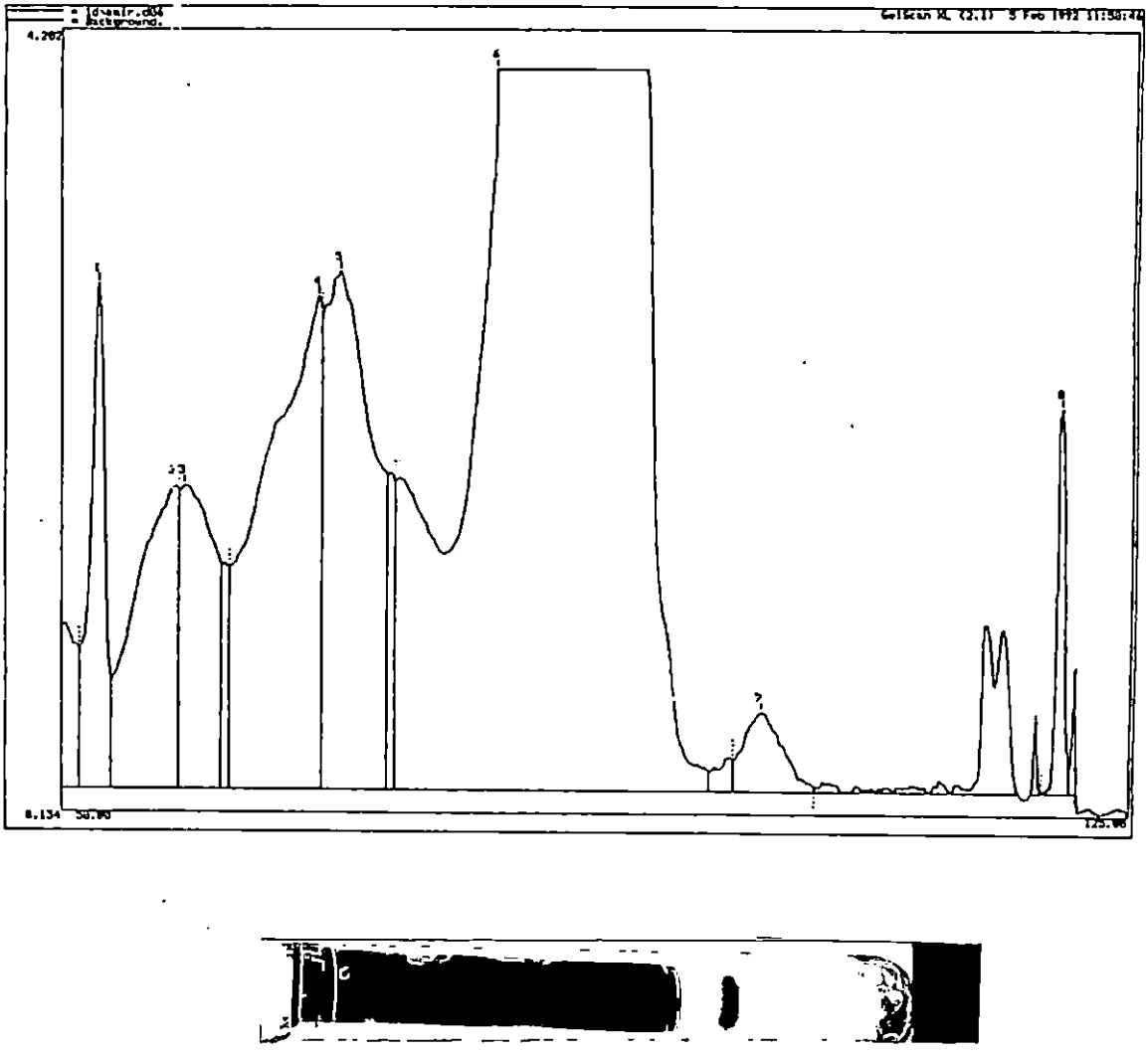


Fig.39 Serum electrophoretic pattern (Group II - 3rd fortnight)

GelScan XL (2.1) - Fig.40

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	98.737

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	53.04	2.348	4.71316	4.8
2	59.32	1.470	7.52408	7.6
3	60.48	1.435	2.90408	2.9
4	67.40	1.862	7.08464	7.2
5	68.60	1.994	2.21252	2.2
6	69.96	2.312	3.37616	3.4
7	70.72	2.322	3.99864	4.0
8	74.64	2.158	3.16892	3.2
9	79.84	3.840	62.23689	63.0
10	115.92	1.493	1.51760	1.5

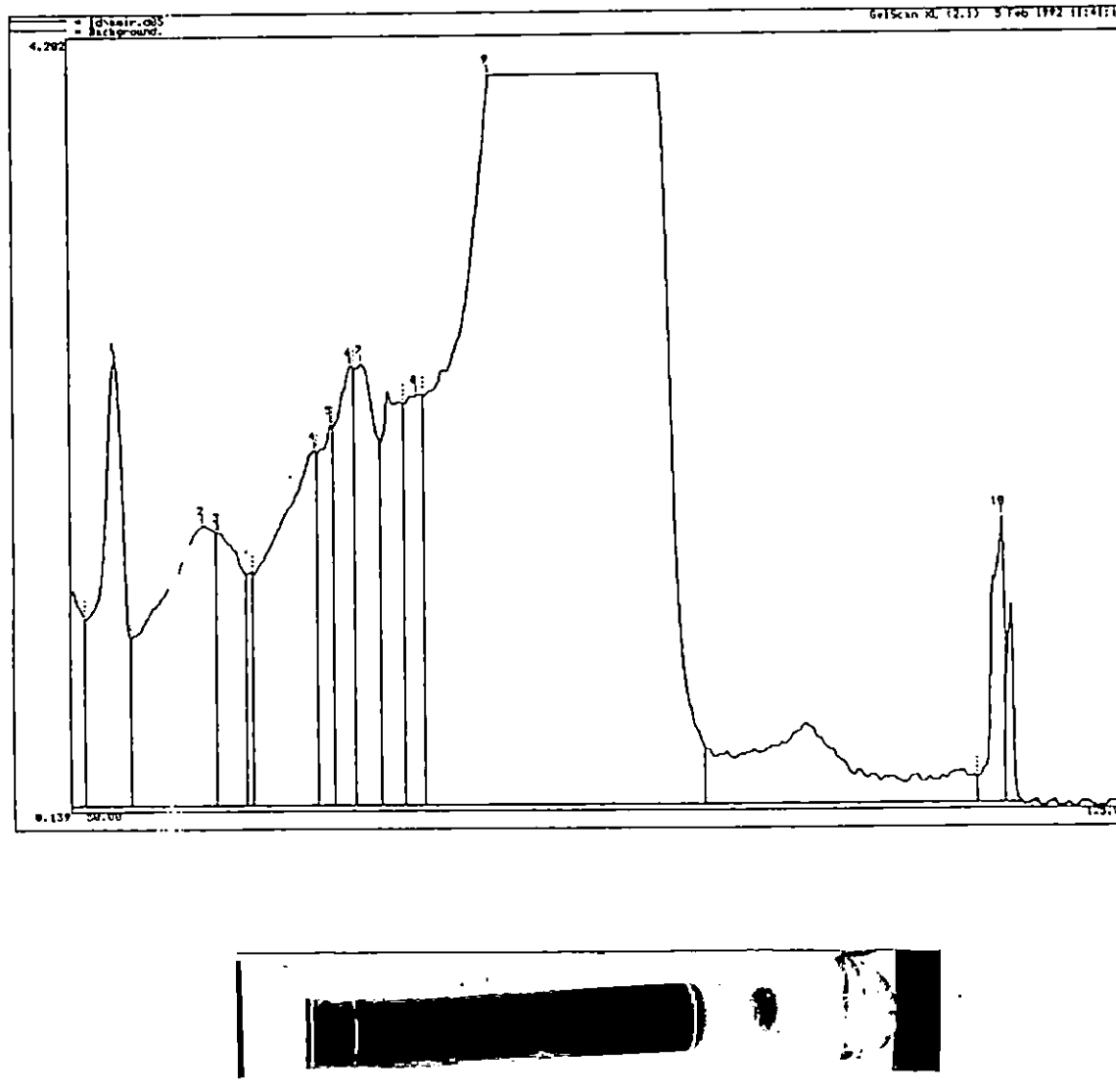
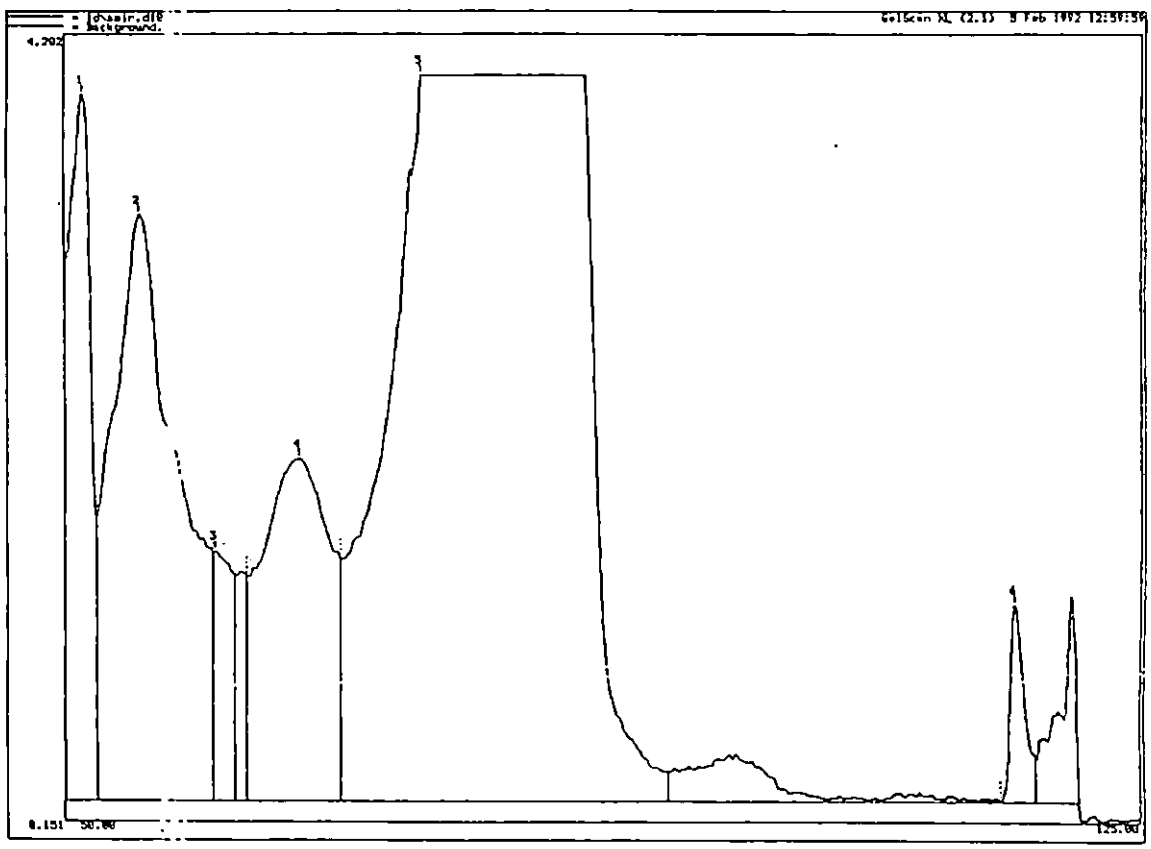


Fig.40 Serum electrophoretic pattern (Group I - 3rd fortnight)

GelScan XL (2.1) - Fig.4l

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	95.113

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	51.12	3.656	6.42036	6.8
2	55.12	3.032	16.38876	17.2
3	60.40	1.299	1.99768	2.1
4	66.20	1.778	9.82224	10.3
5	74.72	3.755	59.32444	62.4
6	116.20	1.030	1.15944	1.2



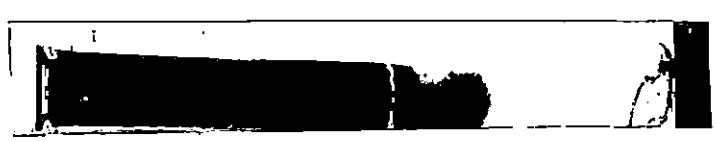


Fig.41 Serum electrophoretic pattern (Group II - 4th fortnight)

GelScan XL (2.1) - Fig.42

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	100.475

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	51.00	0.925	1.263388	1.3
2	53.92	3.809	5.54796	5.5
3	58.92	1.927	8.61484	8.6
4	64.08	2.077	6.55520	6.5
5	68.36	3.008	14.85852	14.8
6	78.72	3.811	62.47448	62.2
7	117.24	1.196	1.16036	1.2

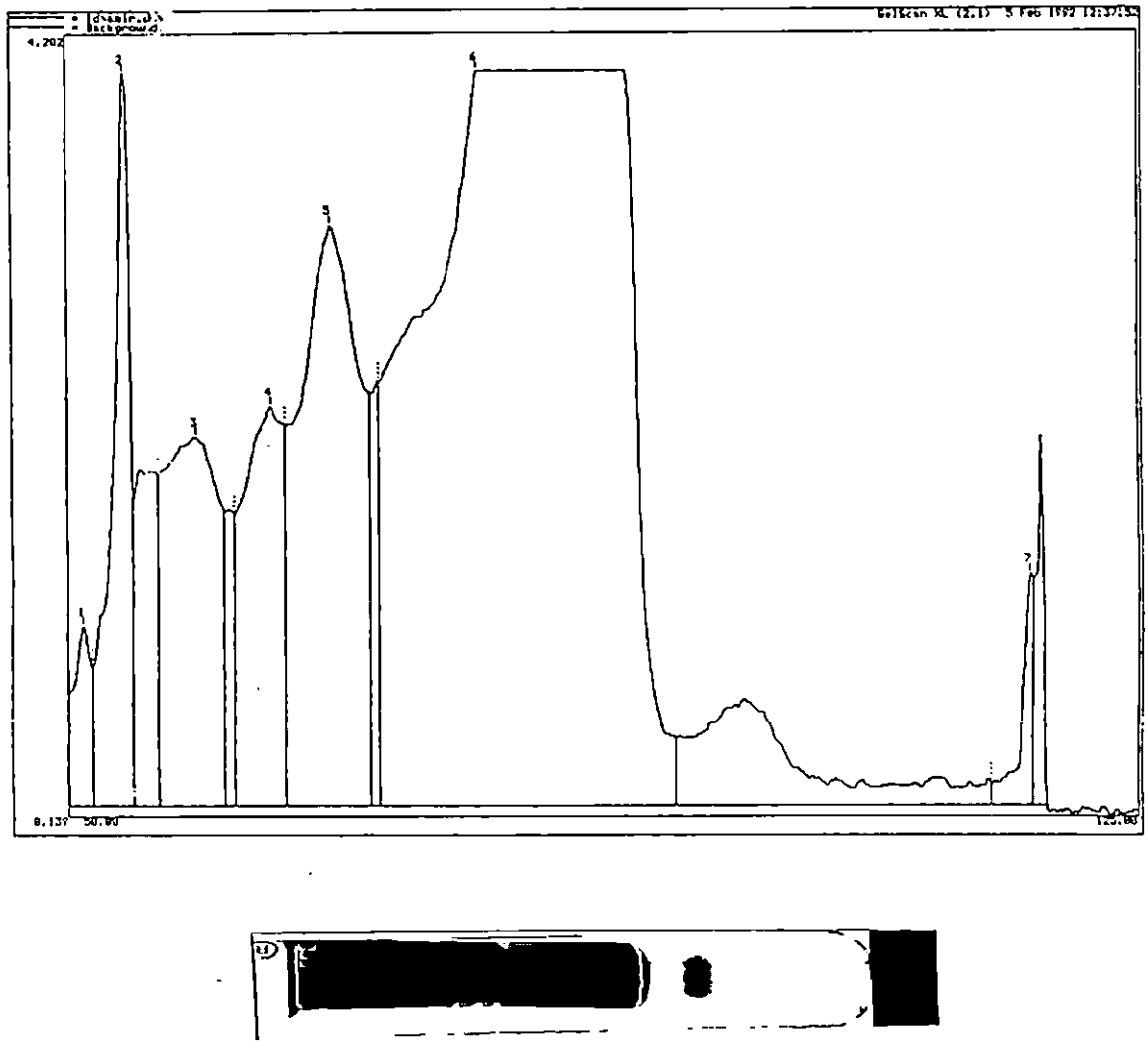


Fig.42 Serum electrophoretic pattern (Group I - 4th fortnight)

GelScan XL (2.1) - Fig.43

Integration method	:	Signal
Background option	:	Horizontal
Peak width for peak search (mm)	:	1.000
Filtering (no. of points)	:	None
Area reject (as per cent of total)	:	1.0
Peak height limit (AU)	:	0.00
Maximum number of peaks to handle	:	400
Start peak search from (mm)	:	50.00
End peak search at (mm)	:	125.00
Area sum (AU*mm)	:	80.667

Number	Location (mm)	Height (AU)	Area (AU*mm)	Relative area (%)
1	51.96	3.741	6.52488	8.1
2	56.48	2.047	5.25816	6.5
3	60.00	1.248	1.26224	1.6
4	63.60	1.734	3.97252	4.9
5	64.80	2.152	2.24560	2.8
6	66.20	2.289	3.04484	3.8
7	66.72	2.284	7.32592	9.1
8	73.16	1.348	3.32432	4.1
9	73.92	1.336	1.93940	2.4
10	80.64	3.741	44.04032	54.6
11	120.04	1.475	1.72912	2.1

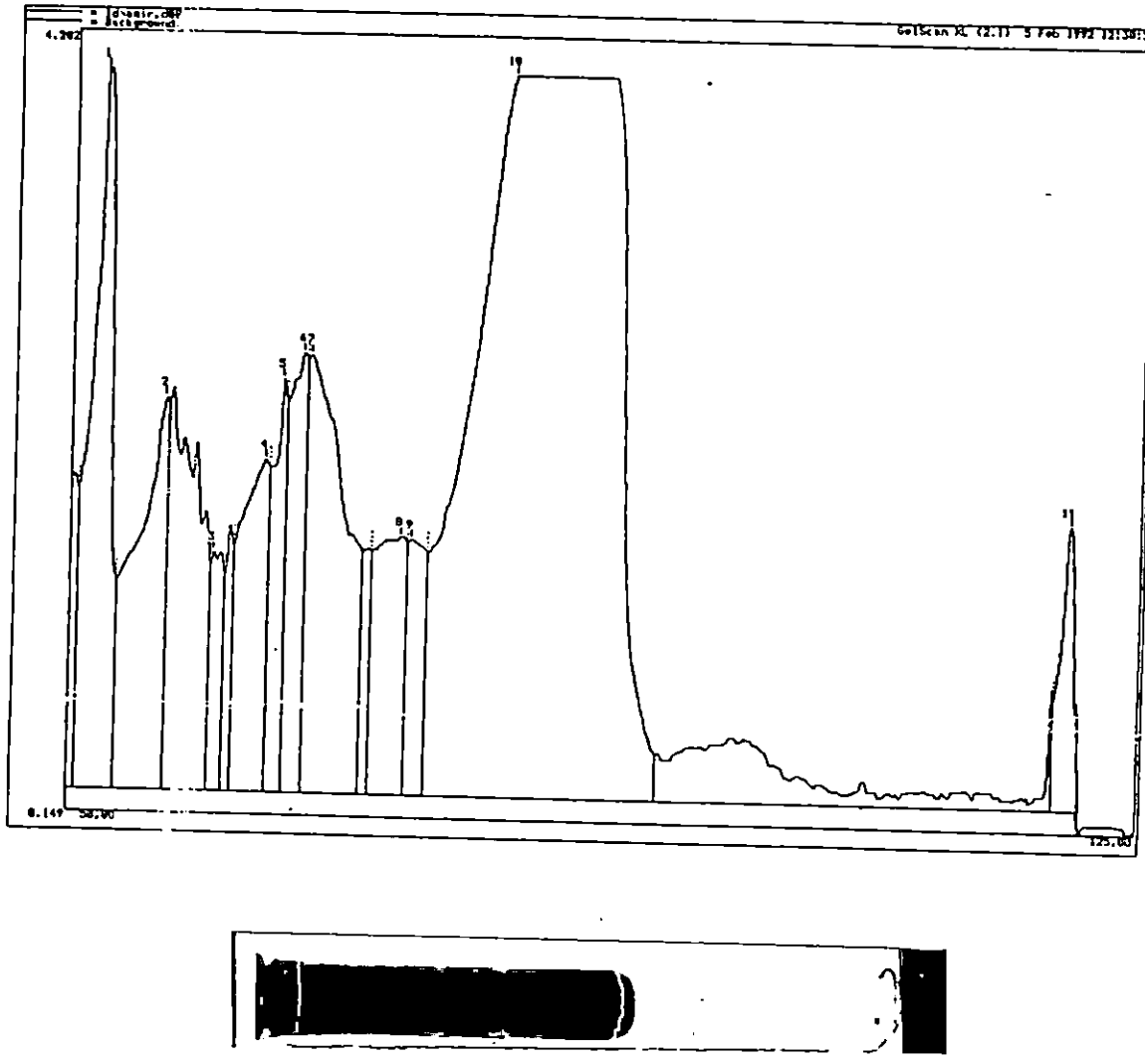


Fig.43 Serum electrophoretic pattern (Group III - 5th fortnight)

4.2.6 LMIT

4.2.6.1 Group I, II and III

The migration patterns are shown (Fig.49, 50 and 51). The average migration indices are furnished in Table 6. There was increase in the migration indices in group I which was highly significant ($P < 0.01$) compared to group II. The increase in migration indices of group III was highly significant ($P < 0.01$) when compared to group II. There was significant ($P < 0.01$) decrease in migration indices of group III when compared to group I (Fig.44).

4.2.7 Response to DNCB

4.2.7.1 Group I, II and III

The average values are given in Table 7. There was no variation of skin thickness before challenge between the three groups (Fig.45).

4.2.7.1.1 Twenty four hours

Twenty four hours after challenge there was highly significant ($P < 0.01$) reduction in the skin thickness in group I when compared to the group II. Increase in the skin thickness in group II was significant ($P < 0.05$) when compared to the group III. There was increase in skin thickness in group III compared to group I which was not significant.

Table 6. Average (Mean ± SE) of migration Index (Group I, II and III)

	Group I	Group II	Group III
	**	**	*
Migration Index	34.5390 ± 4.8999	6.0210 ± 1.1526	17.1576 ± 2.6351

* (P <0.05)

** (P <0.01)

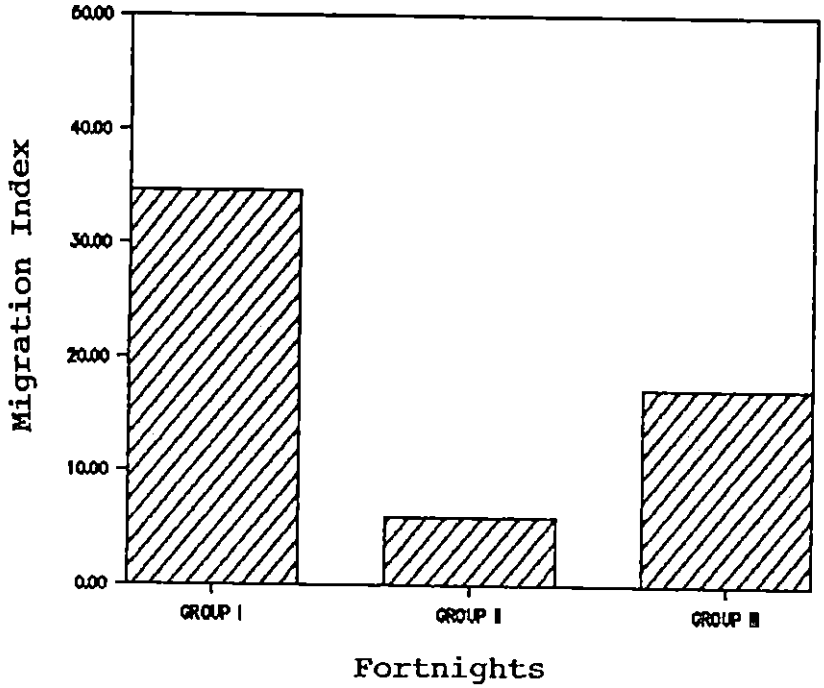


Fig.44 Average migration index of leukocytes (Group I, II and III)

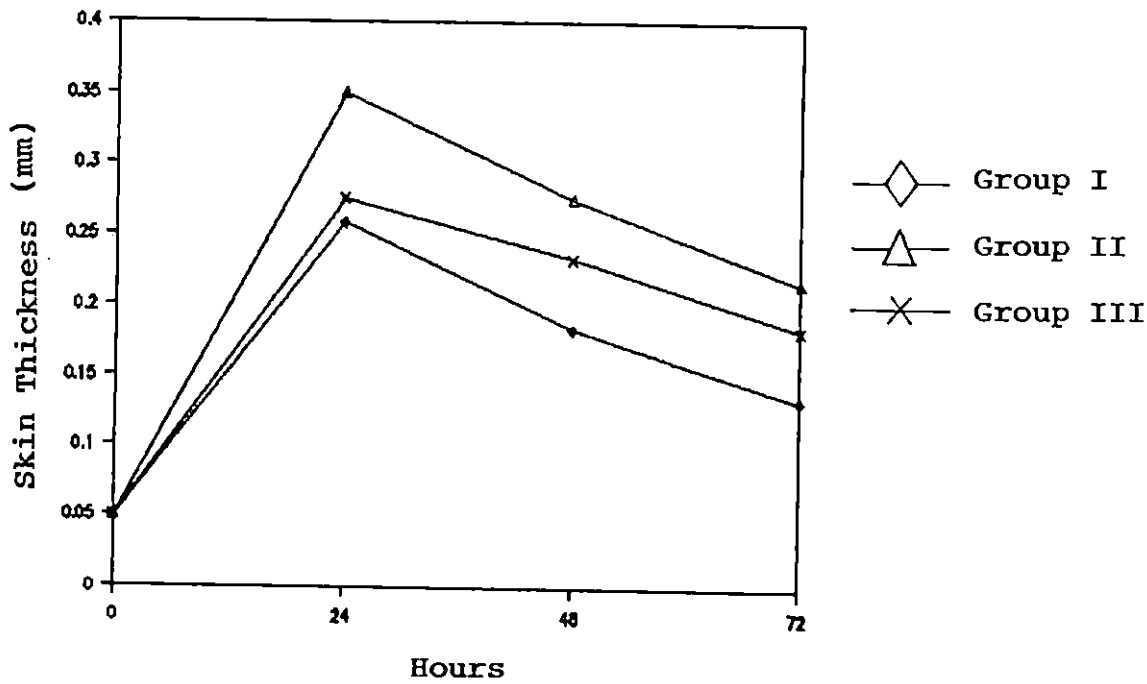


Fig.45 Average skin thickness in response to

Table 7. Average (Mean ± SE) skin thickness in response to DNCB (Group I, II and III)

Groups	Skin thickness (mm) before challenge	Skin thickness (mm) after challenge		
		24 h	48 h	72 h
I	0.05	** 0.2583 ± 0.0076	** 0.1833 ± 0.0096	** 0.1333 ± 0.0152
II	0.05	0.3500 ± 0.0166	0.7250 ± 0.0155	0.2166 ± 0.0096
III	0.05	* 0.2750 ± 0.0155	0.2333 ± 0.0192	0.1833 ± 0.0280

* ($P < 0.05$)

** ($P < 0.01$)

4.2.7.1.2 Forty eight hours

Highly significant (P <0.01) increase in the skin thickness was seen in group II when compared to group I. The average skin thickness was less in group III compared to group II, which was not significant. The group III showed more thickness than group I but it was not significant.

4.2.7.1.3 Seventy two hours

At 72 h the persistent reduction in skin thickness in group I when compared to group II was found to be highly significant (P <0.01). Group II showed more thickness than group III but was not significant. The group I skin thickness was less than group III but it was not statistically significant.

4.2.8 Response to PHA-M

4.2.8.1 Group I, II and III

The average skin thickness values for the three groups are given in Table 8. No variation was observed betwen the three groups before the PHA-M administration (Fig.46).

4.2.8.1.1 Twenty four hours

There was highly significant (P <0.01) increase in the skin thickness in group II when compared to group I.

Table 8. Average (Mean ± SE) skin thickness in response to PHA-M (Group I, II and III)

Groups	Skin thickness (mm) before administration	Skin thickness (mm) after administration		
		24 h	48 h	72 h
I	0.05	0.10 ± 0.00**	0.05 ± 0.00	0.05 ± 0.00
II	0.05	0.15 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
III	0.05	0.1166 ± 0.0096**	0.0833 ± 0.0096**	0.05 ± 0.00

** ($P < 0.01$)

Group III showed lesser skin thickness than group II which was highly significant (P <0.01). On the other hand although there was increase in the skin thickness in group III, compared to group I, it was not significant.

4.2.8.1.2 Forty eight hours

No variation was seen between group I and II. Group III had a skin thickness which was more than group II and group I. In both the cases the increase was highly significant (P <0.01).

4.2.8.1.3 Seventy two hours

At 72 h no variation was recorded between the three groups.

4.2.9 GVHR

4.2.9.1 Group I and II

The average values of body weight, spleen weight and spleen indices are given in Table 9. The body weight was more in group II individuals but it was not significant. There was increase in the weight of the spleen and spleen indices (Fig.47), which were found to be statistically highly significant (P <0.01).

Table 9. Average (Mean ± SE) body weight, spleen weight and spleen Index (Group I and II)

Groups	Body weight (g)	Spleen weight (g)	Spleen Index
I	134.1666 ± 6.3919	** 0.0396 ± 0.0037	** 0.2948 ± 0.0216
II	147.5000 ± 2.5685	0.0588 ± 0.0015	0.3988 ± 0.0110

** ($P < 0.01$)

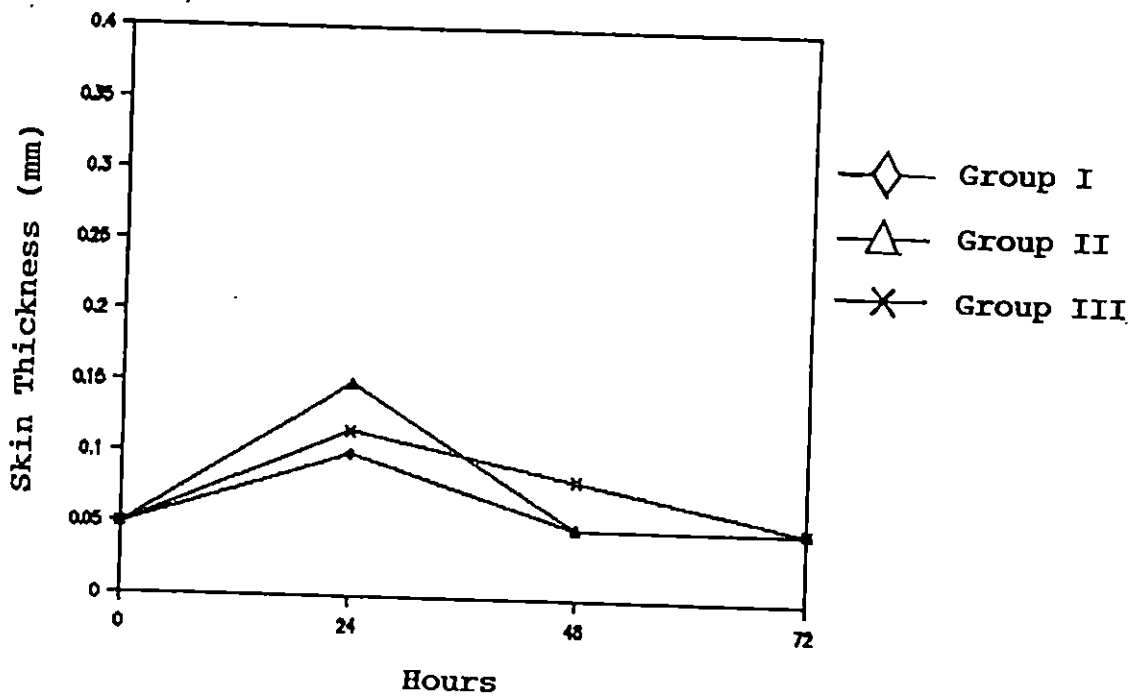


Fig.46 Average skin thickness in response to PHA-M (Group I, II and III)

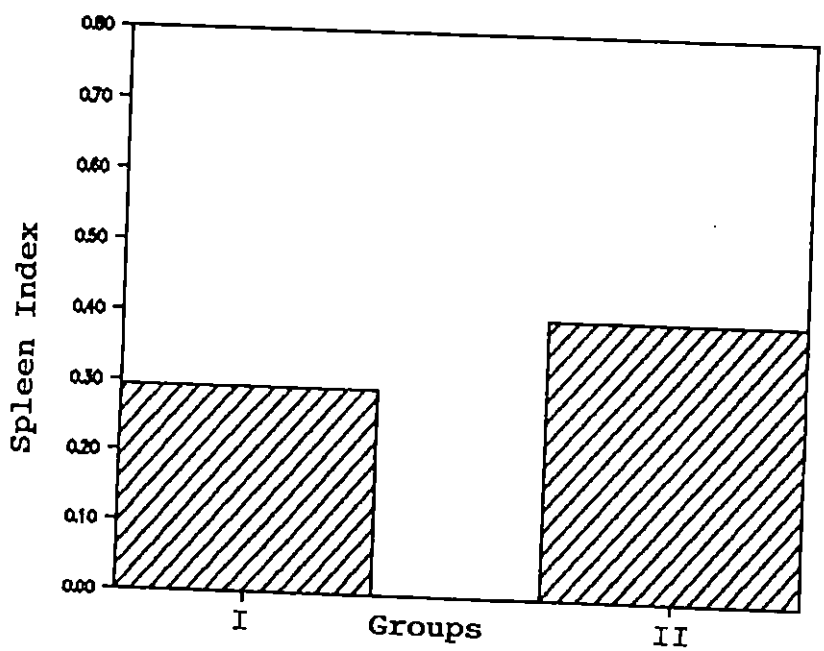


Fig.47 Average spleen index (Group I and II)

4.2.10 Pathology

4.2.10.1 Japanese quail embryo

4.2.10.1.1 Gross and histopathology

4.2.10.1.1.1 Bursa of Fabricius

The bursa of Fabricius on the 13th and 16th day of incubation was smaller in size in experimental group when compared to the control group. No other gross changes seen.

Histologically the lymphoid follicles were few and smaller in size. There was severe depletion of lymphoid elements from the follicles (Fig.52 and 53). The bursa of most of the experimental embryos revealed atrophy, and necrotic changes. There was slight interstitial oedema. The eptithelial cells showed mild vacuolation and granularity of the cytoplasm. There was no variation in the intensity of the lesions on the 13th and 16th day of incubation.

4.1.10.1.1.2 Spleen

Grossly the size of the organ in the experimental groups was similar, both on the 13th and 16th day of incubation.

Hitologically there was less cellularity in the experimental group. There was severe depletion of lymphoid cells from the white pulp, and necrosis of the lymphoid cells was seen. The trabeculae were less distinct in the

experimental group. The severity of the lesion was similar on the 13th and 16th day of incubation.

4.2.10.1.1.3 Thymus

The thymic chain in the experimental embryos was comparatively smaller than that of the control. Some were showing petechial haemorrhages.

Histologically, congestion and focal areas of haemorrhages were seen. There was depletion of the lymphoid elements, stromal oedema, isolated small foci of necrosis and degenerative changes were seen both on the 13th and 16th day of incubation. The epithelial cells also revealed granularity and vacuolation of the cytoplasm.

4.2.10.1.2 Ultrastructural pathology

4.2.10.1.2.1 Bursa of Fabricius

The ultrastructual changes noticed in the lymphoid cells and epithelial cells of the bursa, were qualitatively more intense on the 16th day than on the 13th day embryos. The number of cells showing organellar damage was also more.

In the follicular area the lymphoid cells showed spectra of changes from almost a normal appearance with least organellar damage to severe cytoplasmic and nuclear damage (Fig.78). On the 13th day, many of the cells had swollen

mitochondria with partial or complete disorganization and lysis of cristae. Matrix showed focal electron density or lysis. Many of the cells also had mitochondria with normal morphology. Cells with blastoid features had only free ribosomes. Mature cells had few strands of rough endoplasmic reticulum and occasionally there was degranulation. Golgi zone was not prominent. Lysosomes were few. The morphology of the nucleus showed great variation depending on the maturity of the cell. In general, nucleus was large with irregular nuclear membrane which in some of the cells showed fragmentation. In most of the cells there was condensation of nuclear chromatin in focal areas. Euchromatin was the predominant type. Perichromatin granules were seen increased in cells showing advanced organellar changes (Fig.79). The nucleoli were predominant but were characterised by a condensation of the granular and fibrillar components. In such cases it was very difficult to delineate. The nucleolonema, the nucleolar associated chromatin could not also be clearly discerned in such cells. Amidst normal looking lymphoid cells and with moderate cellular damage, few cells with changes like condensation and lysis of chromatin and other severe organellar destruction in the cytoplasm were also seen. Many autophagosomes were seen in such cells (Fig.79). On the 16th day, the nature of changes in the lymphoid cells were the same except there was more number of lymphoid cells showing severe organellar changes especially of

mitochondria (Fig.80). Frank evidence of nuclear damage was seen in more number of cells (Fig.81). Cells undergoing mitosis were also noticed (Fig.82).

The epithelial cells of the bursa also showed alterations in the mitochondria, endoplasmic reticulum and other cytoplasmic organelles. Numerous vacuoles of varying size were found in the cytoplasm. Nuclear damage was not as intense and extensive as in the lymphoid cells. The plasma membrane of many of these cells showed irregularity and bleb formation. Electron dense structures, probably secondary lysosomes and residual bodies were also seen. Marked oedema was seen in the subepithelial tissue.

4.2.10.1.2.2 Spleen

The organellar changes were more or less same, both in the lymphoid cells and macrophages. There was no qualitative change in the lymphoid cells in the follicular area and around the blood vessles. Cells with minimum cytoplasmic and nuclear damage along with those showing severe alterations indicative of cell death were seen (Fig.83 and 84). The intensity and extent of changes were more in the 16th day embryos than in the 13th day embryos. As in the lymphoid cells of the bursa of Fabricius the characteristic features seen in the lymphoid cells were large condensation of the nucleolar region resulting in massive electron dense area. The nuclear

envelope was more or less intact in these cells. In the macrophages heterochromatin was seen as blocks along the inner nuclear membranes. Vacuoles and lipid granules were seen in some cells (Fig.85). Lysosomes were very few or absent (Fig.86). The golgi zone was not prominent, with only remnants of dictyosomes, even though a few vacuoles and vesicles were seen. Glycogen particles were very scanty. Some of the macrophages had few short strands of endoplasmic reticulum with attached ribosomes. Evidence of degranulation was also present as indicated by free ribosomes in the cytoplasm near the endoplasmic reticulum. In cells showing advanced retrogressive changes, the nucleus was seen partially lysed. Endocytic vesicles were few. The heterophil leukocytes also showed varying grades of damage. Some of them had damaged plasma membrane and completely pyknotic nucleus. The heterophilic granules were either intact or showed partial lysis and fusion. In a few cells except for the presence of partially damaged granules the cytoplasm had a washed out appearance. In general heterophil leukocytes had very few mitochondria.

4.2.10.1.2.3 Thymus

The ultrastructural changes in the thymus showed varying degree from very little to severe organellar damage. The mitochondria were swollen and some showed partial or complete loss of cristae, in some cells mitochondria with

normal morphology were seen. The rough endoplasmic reticulum was scanty and free ribosomes were seen. The golgi zone was absent and when present was not very prominent. There was variation in the shape of the nucleus with condensation of the granular and fibrillar components of the nucleoli. The pattern of changes were more or less the same in the 13th and 16th days.

4.2.10.1.2.4 Control group

No ultrastructural abnormalities were observed in this group.

4.2.10.2 Japanese quail

4.2.10.2.1 Gross pathology

4.2.10.2.1.1 Group I

The early change in the liver was variation in the colour. The liver of the OA fed birds was pale, friable and enlarged. In some cases petechial haemorrhages were also noticed.

Kidney was congested, enlarged and bulged out from the lumbar fossa. Some kidney showed petechial haemorrhages and in the later part of the experiment some of them were smaller in size and showed focal areas of haemorrhages.

No gross changes were seen in the heart. Some of the OA fed birds showed mild enteritis. Brain showed cerebral congestion in all the OA fed birds. There was sporadic muscular haemorrhages in the breast and thigh muscles in few of the birds.

The bursa of Fabricius was atrophied and it was smaller than that of the control birds. At the end of the experiment some birds showed almost total atrophy of the bursa of Fabricius.

Spleen of the OA fed birds was smaller in size and no other gross changes were seen.

Thymus was smaller in size, and petechial haemorrhages were seen in OA fed birds, specially at the 4th fortnight.

4.2.10.2.1.2 Group II

The control birds showed no gross abnormalities at autopsy.

4.2.10.2.1.3 Group III

The gross changes seen in the internal organs of group III were similar to that of group I birds with the lower intensity.

4.2.10.2.2 Histopathology

4.2.10.2.2.1 Group I

4.2.10.2.2.1.1 Bursa of Fabricius

The changes in the bursa of Fabricius were dose and time dependent. The initial changes observed were reduction in the number of follicles. They were also small in size. In the follicles the distribution of lymphoid cells particularly in the germinal centre (Fig.54) was sparse and loosely arranged. There were thin bands of fibro-collagenous tissue encircling the follicles. Mild to moderate degree of interstitial oedema was present (Fig.55). The interstitial tissues contained sparsely distributed heterophils, monocytes, plasma cells and the small population of lymphocytes. The germinal centre of the follicles was inactive and cells in mitosis were only few. Amidst the loosely arranged lymphoid cells, degenerative and necrotic cells were seen along with the macrophages (Fig.56). The epithelial lining did not show any significant change. By the second and third fortnight the changes were almost the same but more severe. Besides this, the epithelial lining cells showed in certain sections, degeneration and desquamation. Follicular pattern of lymphoid cells was not very much evident. In certain cases there were only diffuse collections of lymphoid cells without differentiation into lymphoid follicles (Fig.57). Focal areas of necrosis were evident. There was moderate degree of

reticulocyte response in the germinal centre of the follicles. By the 4th fortnight changes were more extensive. There was isolated foci of cystic dilatation of the epithelial cells, and the interstitial oedema was more pronounced. The dilated cystic spaces contained degenerated and desquamated epithelioid cells (Fig.58). Follicular pattern was less evident and degenerated and necrotic foci were extensive.

4.2.10.2.2.1.2 Spleen

The lymphoid follicles were numerically less in the experimental group. The differentiation into lymphoid follicles was less distinct in the 3rd and 4th fortnights. In the lymphoid follicles the germinal centre was less active. Lymphoid collection in the periarterial sheath became progressively reduced, as the duration of treatment prolonged. This was more evident in the 4th fortnight. Sheathing of the lymphoid cells was a significant finding (Fig.59). The wall of the vessels were relatively thick (Fig.60). There was slight swelling of the vascular endothelial cells and mononuclear cells were seen in the lumen of the vessels. The lymphoid cells were loosely scattered in the parenchyma (Fig.60). Congestion of the spleen was occasionally seen. The sinusoids were engorged. The vascular endothelium showed mild degree of degenerative changes particularly in the 3rd and 4th fortnight.

4.2.10.2.2.1.3 Thymus

Thymus was moderately congested. Scattered focal areas of haemorrhage were seen particularly in the 3rd and 4th fortnights. There was depletion of lymphoid cells in the parenchyma and it was more evident in the 3rd and 4th fortnights. Some of the lymphoid cells were degenerated. The epithelial cells showed degeneration and slight vacuolation. Heterophilic infiltration was evident in certain areas. Slight but perceptible oedema was seen in the 4th fortnight (Fig.61 and 64). Degeneration and necrotic foci were seen in the parenchyma in the 4th fortnight (Fig.63).

4.2.10.2.2.1.4 Kidney

The changes in the kidney revealed significant qualitative and quantitative differences at specific time intervals. These time dependent changes were qualitatively the same but the degree of change was more severe as the time passed. The most predominant change was seen in the proximal convoluted tubules. The tubules were distended in size and shape. Some were very much dilated. They were lined with hypertrophic columnar cells with acidophilic cytoplasm. Necrotic individual cells were clearly evident. There were scattered areas of lymphoid and mononuclear infiltration (Fig.64 and 65). In the 3rd and 4th fortnights there was frank necrosis of the epithelial cells. In certain areas the

cells were degenerated and desquamated. The lumen of the tubules were filled with desquamated, degenerated cells, hyaline casts and necrotic debris. In certain other foci the tubules were smaller in size with narrow lumen and lined with the basophilic, cuboidal to flattened epithelial cells. There was degeneration, necrosis and desquamation of epithelial cells in these tubules also (Fig.66). In some of the tubules the cells had undergone vacuolar degeneration.

The glomeruli contained granular degenerating materials in the Bowman's space. The glomerular tufts were atrophic and the Bowman's space appeared enlarged. There was moderate to severe thickening of the glomerular basement membrane (Fig.67).

Congestion and focal areas of haemorrhages were seen.

4.2.10.2.2.1.5 Liver

There was moderate to severe degree of granular degeneration of hepatocytes and focal areas of fatty change (Fig.68). The sinusoids were engorged by the 4th fortnight and focal areas of necrosis were seen. The fatty change was mild in the 1st fortnight and it was more pronounced by the 4th fortnight (Fig.69). Occasionally there were scattered foci of lymphoid infiltration.

4.2.10.2.2.1.6 Brain

The meningeal vessels were engorged. In the cerebrum which was subjected to detailed histopathological examination, there was focal oedema (Fig.70). This was evident by the 3rd and 4th fortnights. The neurons in certain areas showed pyknosis and karyorrhexis, and satellitosis of microglial cells was seen (Fig.71 and 72). The vascular endothelium was swollen (Fig.73) and vacuolated.

4.2.10.2.2.1.7 Heart

No histological changes could be seen.

4.2.10.2.2.2 Group II

The organs of the control group did not show any histological changes.

4.2.10.2.2.3 Group III

The bursa of quails in which levamisole was administered, revealed lymphoid hyperplasia of diffuse nature. The lymphocytes were active and blastoid transformation was evident. The interstitial tissues revealed macrophages and plasma cells. The lymphoid follicles with active germinal centres were seen (Fig.74). In the spleen there was focal

proliferating large hypertrophic lymphocytes (Fig.75). The thymus revealed diffuse lymphoid hyperplasia and scattered heterophilic and mononuclear reactions. In the interstitial tissues, there were plasma cells and mononuclear infiltration along with a few heterophils.

4.2.10.2.3 Response to DNCB

4.2.10.2.3.1 Gross and histopathology of skin

After 24 h, the area on the skin was warm, hyperaemic and diffusely oedematous. This change was very severe in group II and it was mild in OA fed group. In the group III there was enhancement of this reaction when compared to the OA fed group I birds.

Histologically in the control birds at 24 h there was slight focal necrosis of the epidermis and pronounced diffuse oedema. Heterophils in large numbers were seen along with some lymphocytes and macrophages (Fig.76). Blood vessels were engorged. Scattered foci of haemorrhages also were seen. At 48 h, congestion and oedema was less evident, there was lymphocytic infiltration, focal and diffuse, associated with macrophages. By 72 h, the oedema was less, it had almost subsided, scattered macrophages and lymphocytes were seen in the dermis.

In the OA fed birds the same sequence of reactions were

observed, with less severe cellular response (Fig.77). This was much evident in the measurement of the skin thickness.

After immunostimulation in group III there was enhanced cellular reaction compared to OA fed birds, but not to the extent of the control birds.

4.2.10.2.4 Response to PHA-M

4.2.10.2.4.1 Gross and histopathology of skin

Twenty four hours after the administration of the PHA-M the control birds showed hyperaemia and oedema of the skin.

Histologically in the control birds, by 24 h there was congestion and oedema of the capillaries. Also, there was infiltration of lymphocytes, macrophages which was more pronounced in the perivascular locations. By the 48 h and 72 h the reaction subsided. There was oedema but it was less evident. The cellular infiltration of lymphocytes persisted.

In OA fed birds the same reaction was observed but there was only less intense reaction by 24 h. After immunostimulation, in group III birds there was enhancement of cellular response to PHA-M and measurement of skin thickness also indicated the same.

4.2.10.2.5 Ultrastructural pathology

4.2.10.2.5.1 Group I

4.2.10.2.5.1.1 Bursa of Fabricius

The nature of structural alterations seen in the bursal cells, both lymphoid and epithelial, were more or less the same as described in the embryos (Fig.87). Bursal follicles showed large number of lymphoid cells with cytological alterations indicative of cell death (Fig.88 and 89). Some of the cells showed only a lytic cytoplasm with a bit of condensed nucleus. The packing of lymphocytes was very loose with large spaces between them indicating that there had been cell loss. Definite indications of transformation into plasmocytoid series were lacking.

4.2.10.2.5.1.2 Spleen

The spleen when examined on the 60th day, showed large number of cells with advanced cytological alterations. In many locations the lymphoid cells and macrophages showed large vacuolated areas with rupture of plasma membrane. Many lymphoid cells showed a homogenous organellar free cytoplasm (Fig.90). The nucleus of some of the lymphoid cells were represented only by the envelope in which the outer and inner membrane could not be distinguished and only some flocculent aggregates of chromatin were seen. In other cells the

karyoplasm had a homogenous consistency with only an occasional heterochromatin clumping. The vacuoles within a cell and also those of adjacent cells had become confluent to present large electron lucent or electron light areas (Fig.91).

4.2.10.2.5.1.3 Thymus

Ultrastructural alterations were not uniform in the lymphoid cells of the thymus. Cells with blastoid features as well as those with mature characteristics were seen. Most of these cells showed condensation of the nucleoli as a compact mass along with chromatin particles (Fig.92, 93 and 94). In a few, there was segregation of the granular and fibrillar components forming delineated structures. Both in the immature and mature cells, clumping of chromatin along the inner nuclear membrane was a constant feature (Fig.95). Fragmentation of nucleus was seen in some cells. Occasionally the perinuclear cisternae were found very prominent. Vacuoles of different sizes were found in some cells. Even though, a few free ribosomes were noticed in the cytoplasm, presence of rough surfaced endoplasmic reticulum was not a feature of these cells. Lysosomes were very scanty. In cells showing advanced organellar damage, a few dense bodies were seen (Fig.96). The thymic tissue had increased amount of stromal tissue which was oedematous in many locations (Fig.97).

4.2.10.2.5.1.4 Kidney

The epithelium of the proximal convoluted tubules showed advanced cytoplasmic and nuclear changes. The cell surface villi showed full or partial destruction. There was fragmentation and lysis of the plasma membrane at the luminal surface. Numerous vacuoles were seen in the cytoplasm and they had become confluent to form large areas giving a honey combed pattern (Fig.98). In some cells the cytoplasm had become extremely electron dense with partial or complete loss of organellar structures. Numerous lysosomes were seen (Fig.99). Mitochondria showed varying stages of damage and destruction. Some cells had few lipid droplets. Similar changes also were seen in the epithelial cells of the other parts of the tubules. But the extent and intensity of damage were not as severe as in the proximal convoluted tubules. Cells with intense alterations showed swollen nucleus with condensed nucleoli.

In the glomerulus, there was swelling of the endothelial cells with swollen mitochondria. Pinocytic vesicles were few. The foot processes of podocytes appeared fused and club shaped in many locations (Fig.100 and 101). In some areas there appeared to be thickening and fragmentation of basement membrane. The nucleolus and cytoplasm of pedocytes and the parietal epithelium of the Bowman's capsule did not show severe alterations. Occasionally some cells in

the glomeruli showed necrotic changes involving both the endothelial cells and podocytes. The changes in the mesangial cells were comparatively of lesser intensity.

4.2.10.2.5.1.5 Liver

The cytologic changes in the hepatocytes varied from mild swelling of mitochondria to severe cytolysis and nuclear fragmentation (Fig.102 and 103). The cytoplasmic matrix was granular and electron dense. Most of the mitochondria, were swollen with partial disorganization or lysis of cristae (Fig.104). Giant mitochondria and ring shaped or cup shaped mitochondria were also noticed (Fig.105). The outer mitochondrial membrane was disrupted in some, while in others there was complete damage of both the inner and outer membranes. Few mitochondria showed swollen cristae with electron dense matrix. Occasionally intramitochondrial granules were seen. In cells showing advanced cytologic changes, profiles of autophagosomes with remnants of mitochondrial structure were seen. There was vesiculation and rupture of rough surface endoplasmic reticulum with varying grades of degranulation. Glycogen particles were not conspicuous. As occasional microbody with partial damage to the outer membrane was seen. Large vesiculated cavities containing electron lucent granulated material and myelinated structures were visible. In some locations these vesicles had become confluent to present large irregular cytoplasmic

cavities with granular contents. The plasma membrane had become vesiculated or discontinuous and cell functions were not very evident. Lipid droplets of varying sizes were seen in some cells. At the sinusoidal surface there was discontinuity of the plasma membrane and the space of Disse was dilated and contained granulated electron lucent material. The bile canaliculi were seen blocked in some areas with cellular debris. Lysosomes were few.

In most of the hepatic cells the nuclear envelope was intact with prominent perinuclear cisternae in some. The nuclear pores appeared normal in size and number. The changes in the chromatin showed varying patterns depending on other cytological changes. In general, there was more euchromatin with few aggregations of heterochromatin at the inner nuclear membrane. There was prominent nucleolus where the granular and fibrillar components could be discerned.

4.2.10.2.5.1.6 Brain

The most characteristic picture was the separation of the neuronal elements to form loose cytoarchitecture with fragmentation and vacuolation of perineuronal elements indicating oedema (Fig.106). The myelinated and nonmyelinated axons showed extensive alterations (Fig.107). The cytoplasm of some of the Schwann cells showed washed out appearance

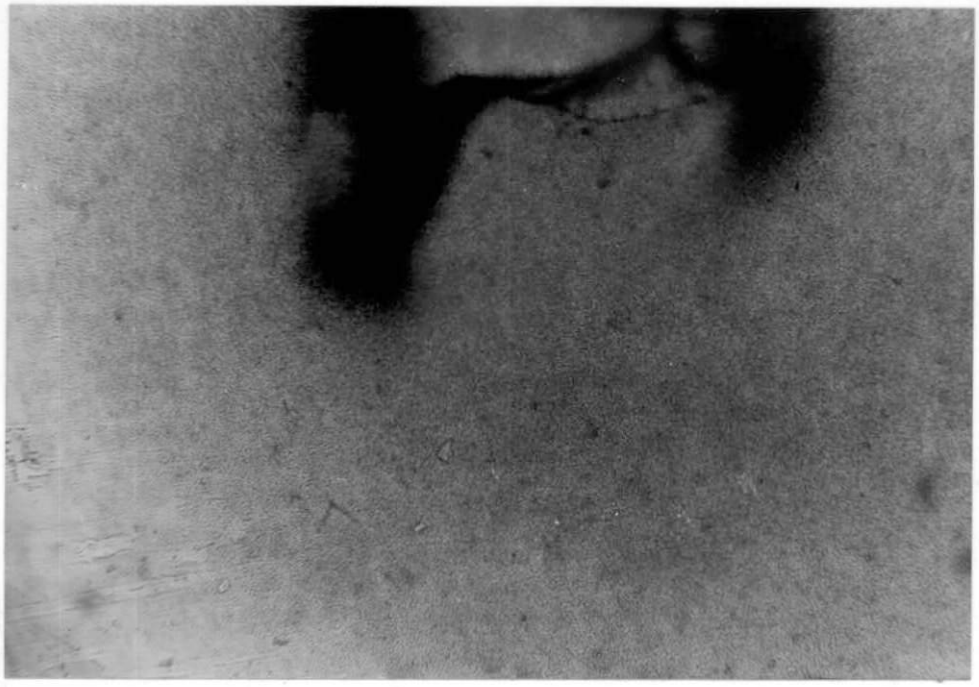
while some others showed only mild organellar damage. Swollen mitochondria, chromatin clumping, and focal distribution of nuclear membrane were seen. The multilayer appearance of myelin sheath was not evident in many locations. In these areas the myelin sheath appeared as homogenous electron dense areas. In some of these the myelin sheath had assumed a wrinkled nature. In general, fragmentation was not very much evident. The infolding of plasmalemma to form the outer mesaxon could not be clearly demonstrated in many locations. Compared to the neurons and axons the organellar integrity of glial cells was found not much affected. Cytoplasm of neurons, showed mild organellar changes. The endothelial cells of the blood vessels showed nuclear swelling with partial loss of chromatin (Fig.108).

4.2.10.2.5.2 Group II

No ultrastructural alterations were noticed in this group.

Fig.48 Blood smear - ANAE positive T lymphocytes - Orange red nodular reaction products in the cytoplasm x 1000

Fig.49 Normal leukocyte migration in the absence of antigen - Group II x 100



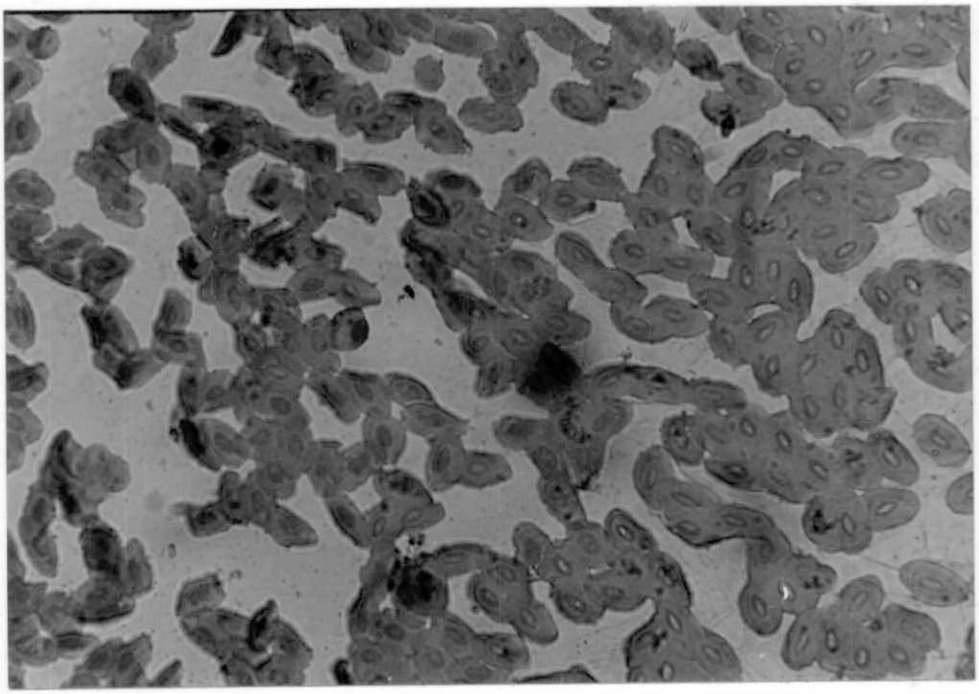
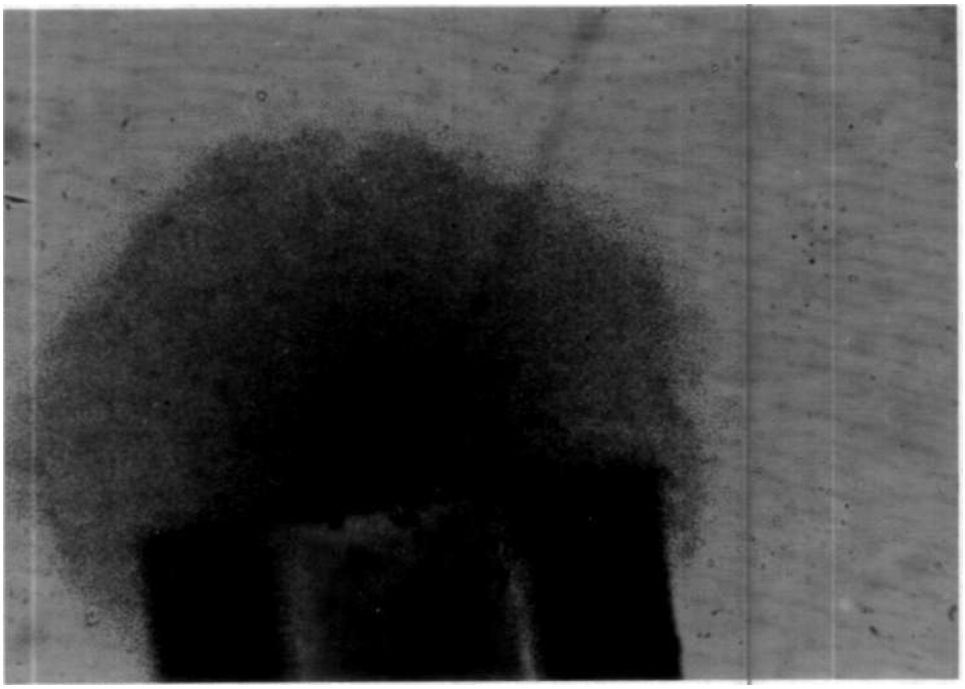


Fig.50 Leukocyte migration in the absence of antigen - Group I x 100

Fig.51 Leukocyte migration inhibition in the presence of antigen - Group I x 100



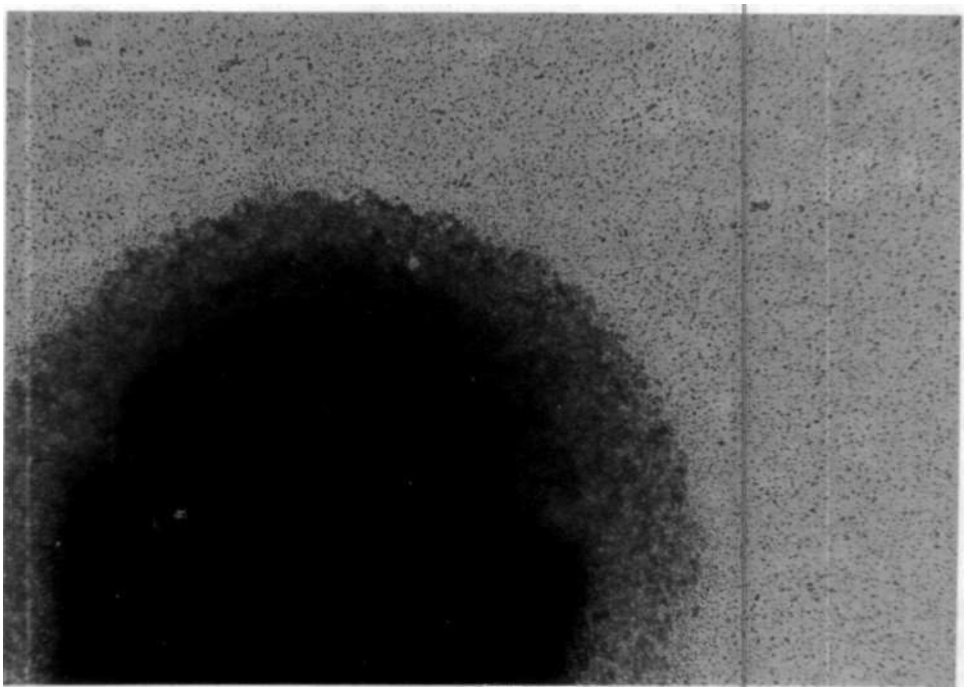
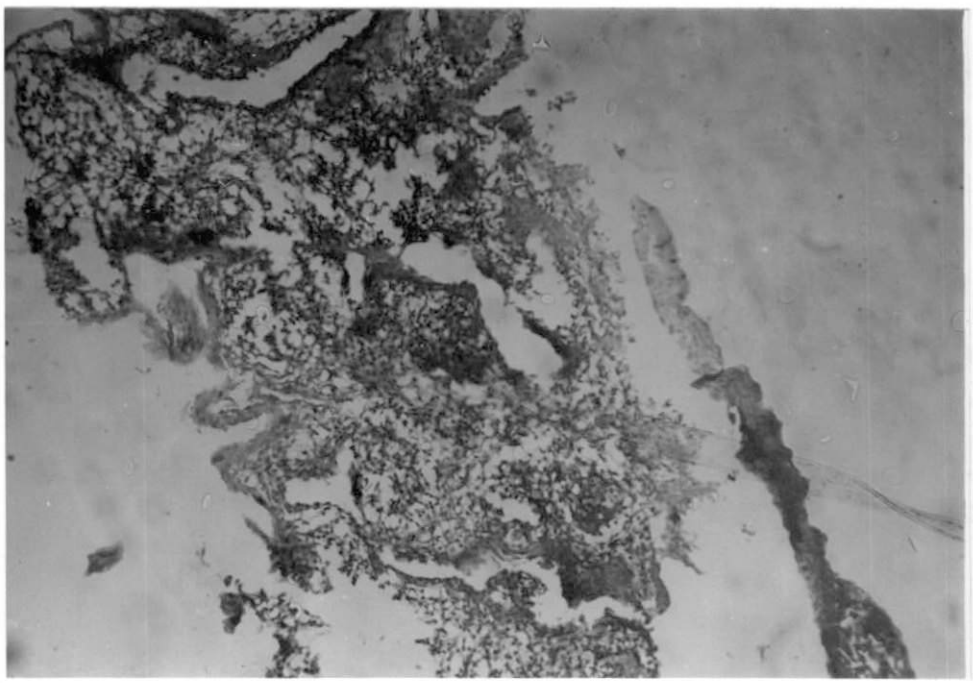


Fig.52 Bursa of Fabricius - Experimental - 13 day old embryo - Atrophy - Very few lymphoid cells - H&E x 250

Fig.53 Bursa of Fabricius - Experimental - 16 day old embryo - Depletion of lymphoid elements - Vacuolated appearance - H&E x 400



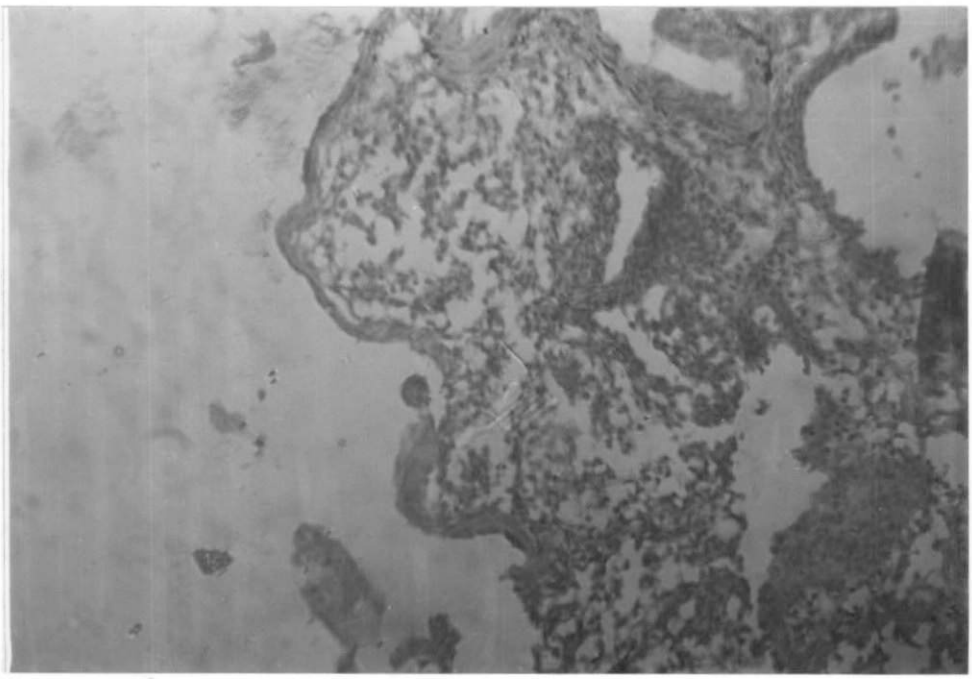
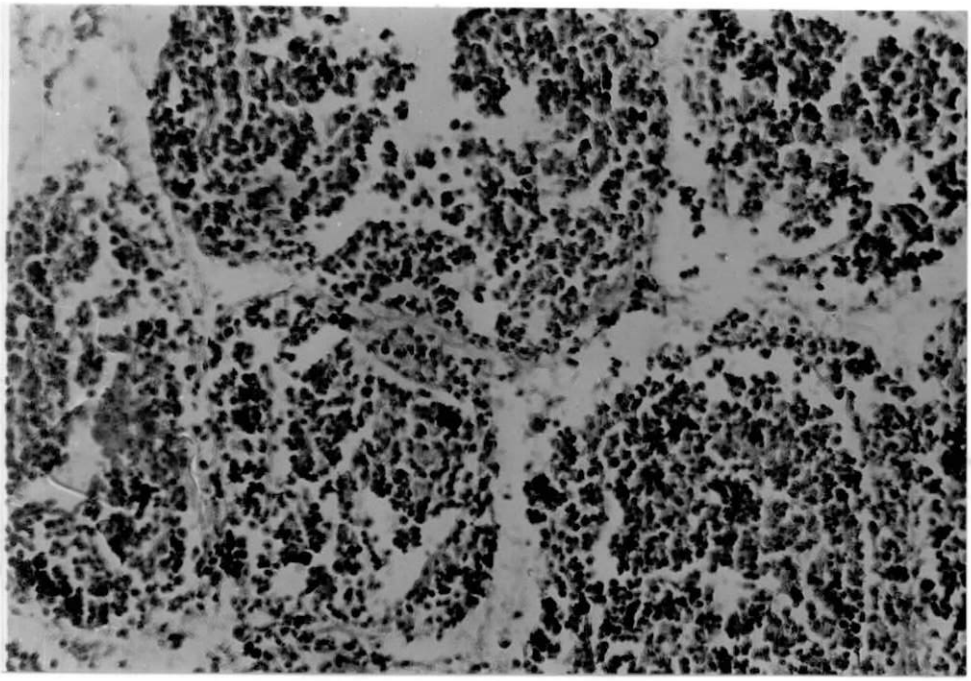


Fig.54 Bursa of Fabricius - Group I - Depletion of lymphoid cells - H&E x 400

Fig.55 Bursa of Fabricius - Group I - Interstitial oedema and sparse distribution of lymphoid cells - H&E x 400



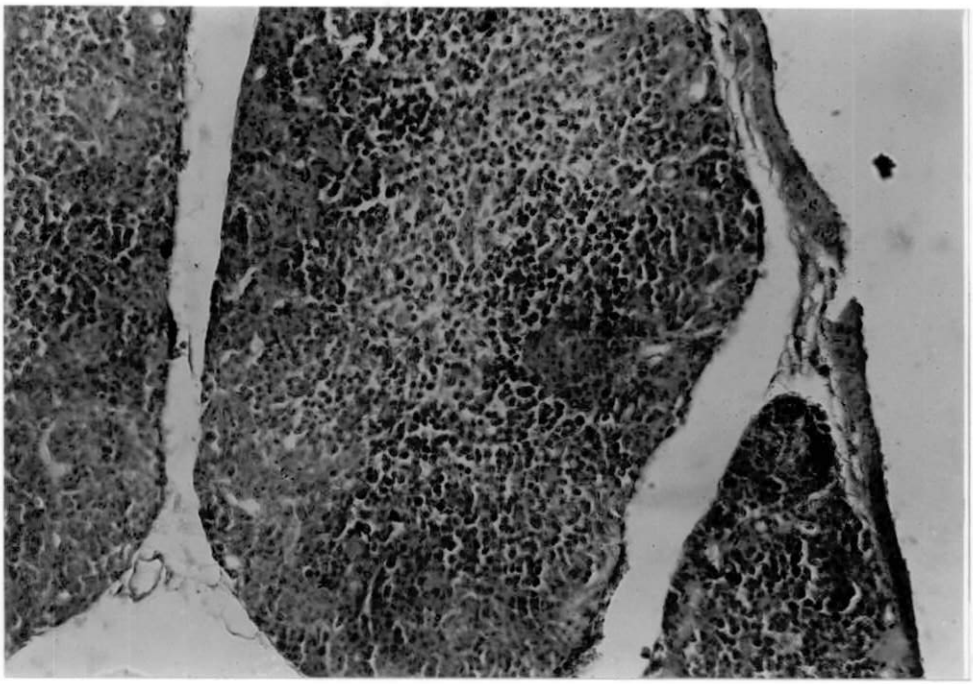
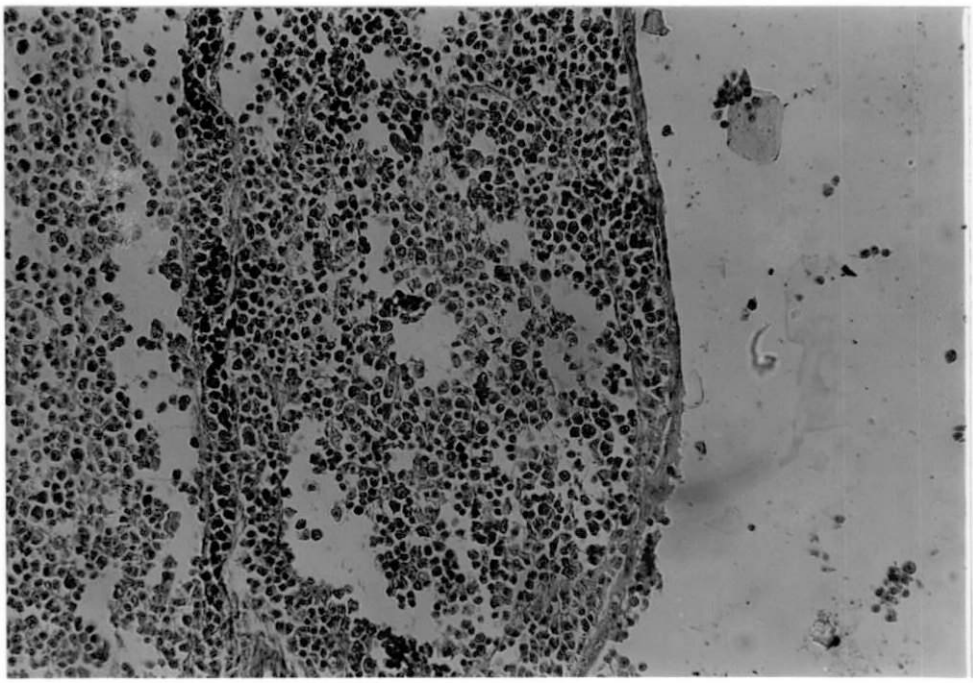


Fig.56 Bursa of Fabricius - Group I - Degenerative and necrotic cells along with macrophages - No follicular pattern - Loosely arranged sparse lymphocytes - H&E x 400

Fig.57 Bursa of Fabricius - Group I - Diffuse collection of undifferentiated lymphoid cells - No distinct follicular pattern - H&E x 400



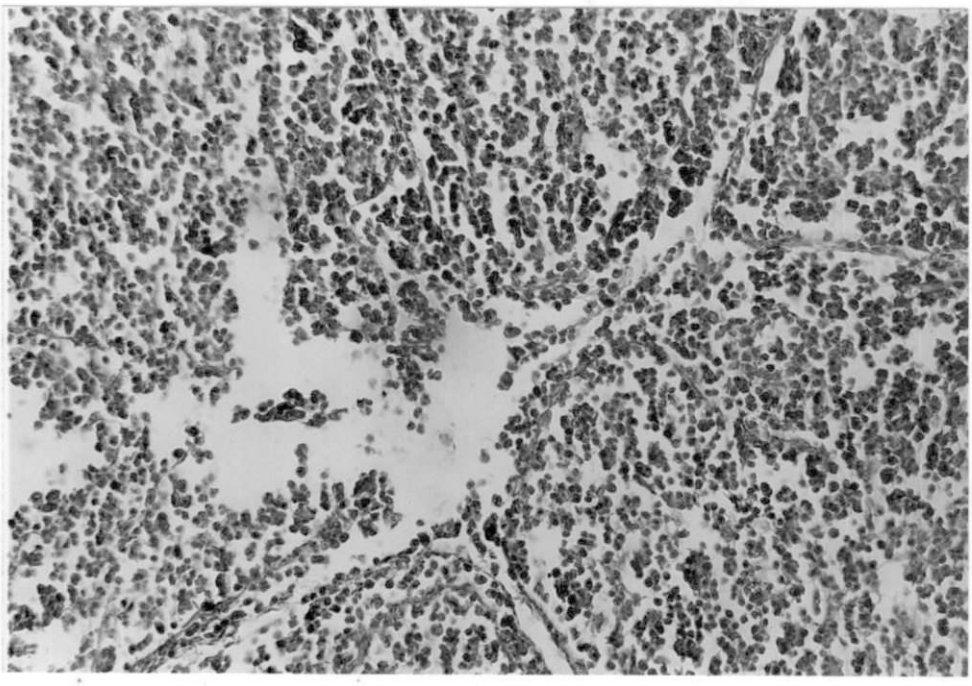
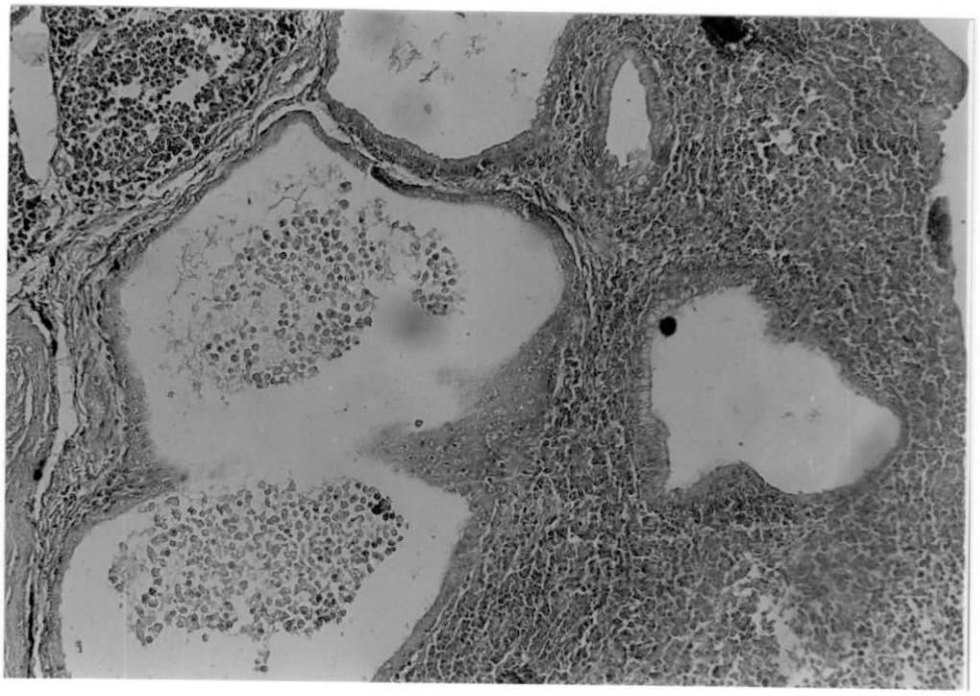


Fig.58 Bursa of Fabricius - Group I - Dilated cystic spaces containing degenerated and desquamated epithelial cells - H&E x 250

Fig.59 Spleen - Group I - Loss of lymphoid cells from periarterial sheath and sheeting of the lymphoid cells - H&E x 250



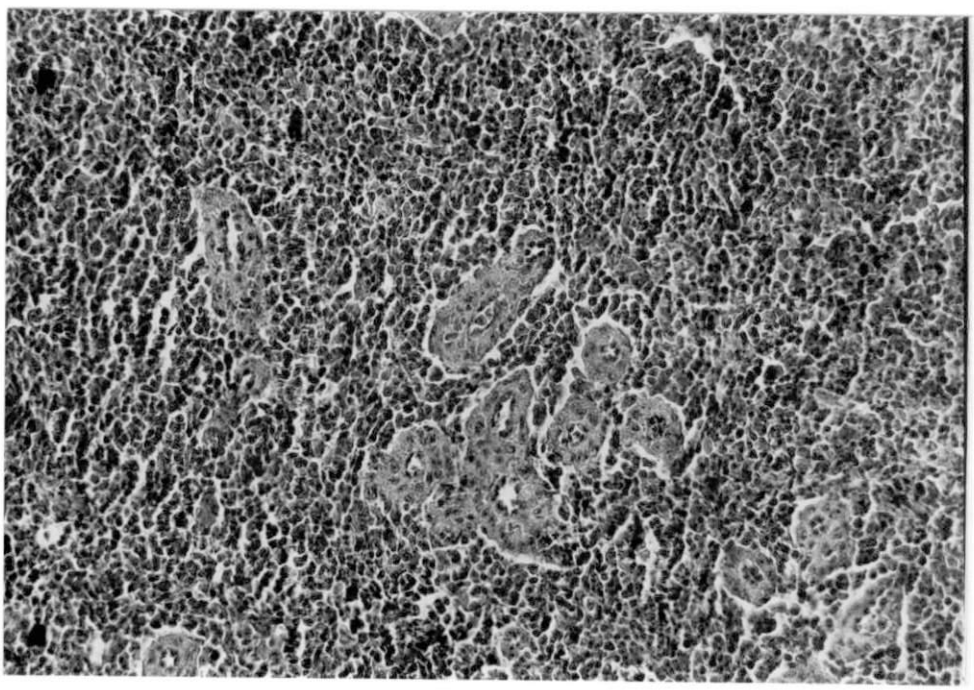
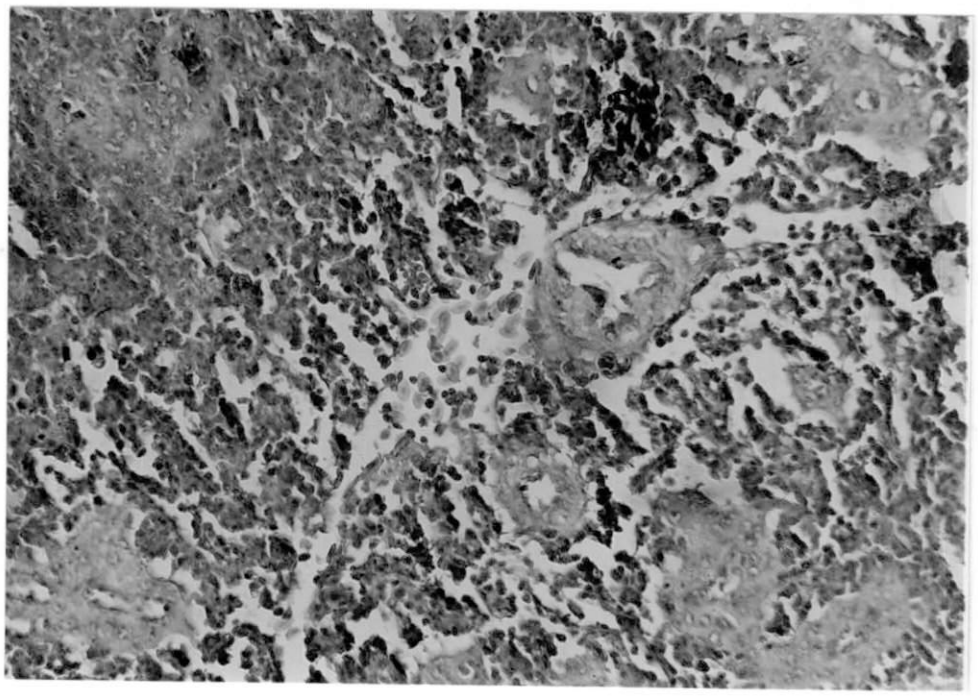


Fig.60 Spleen - Group I - Loosely scattered lymphoid cells with thickened vessel walls - H&E x 250

Fig.61 Thymus - Group I - Loosely packed lymphoid cells and oedema - H&E x 250



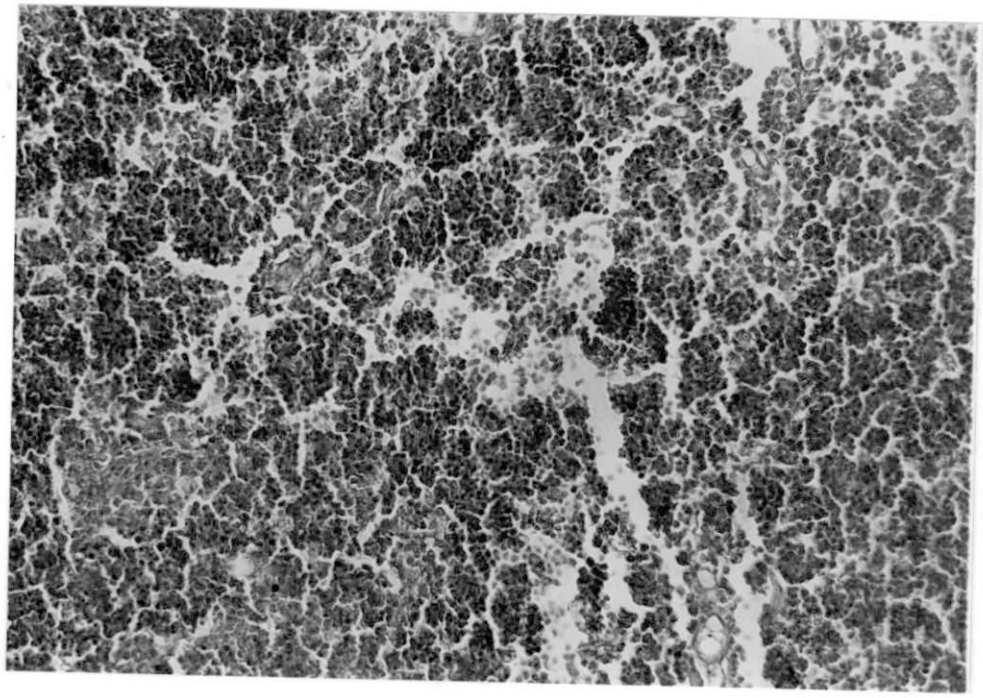
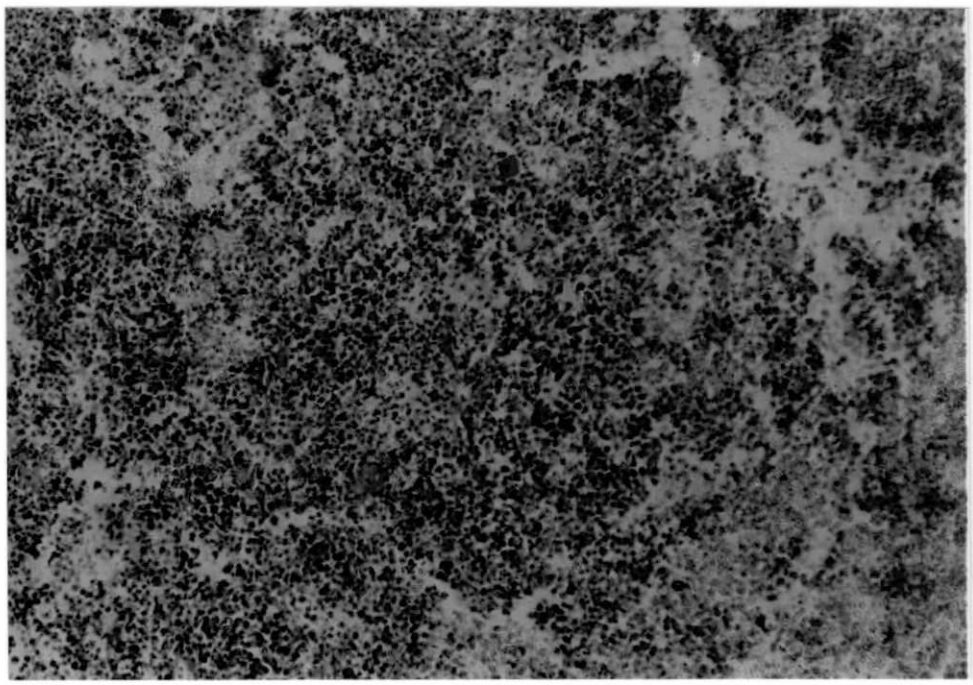


Fig.62 Thymus - Group I - Diffusely scattered lymphoid cells and focal areas of haemorrhages - H&E x 250

Fig.63 Thymus - Group I - Degenerative and necrotic foci in the parenchyma - H&E x 250



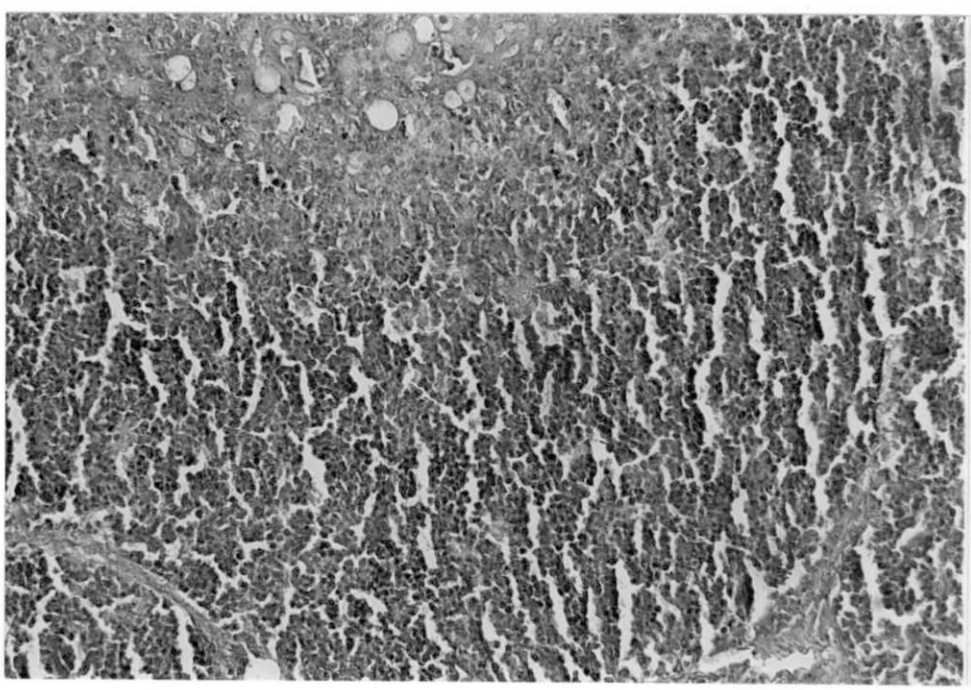
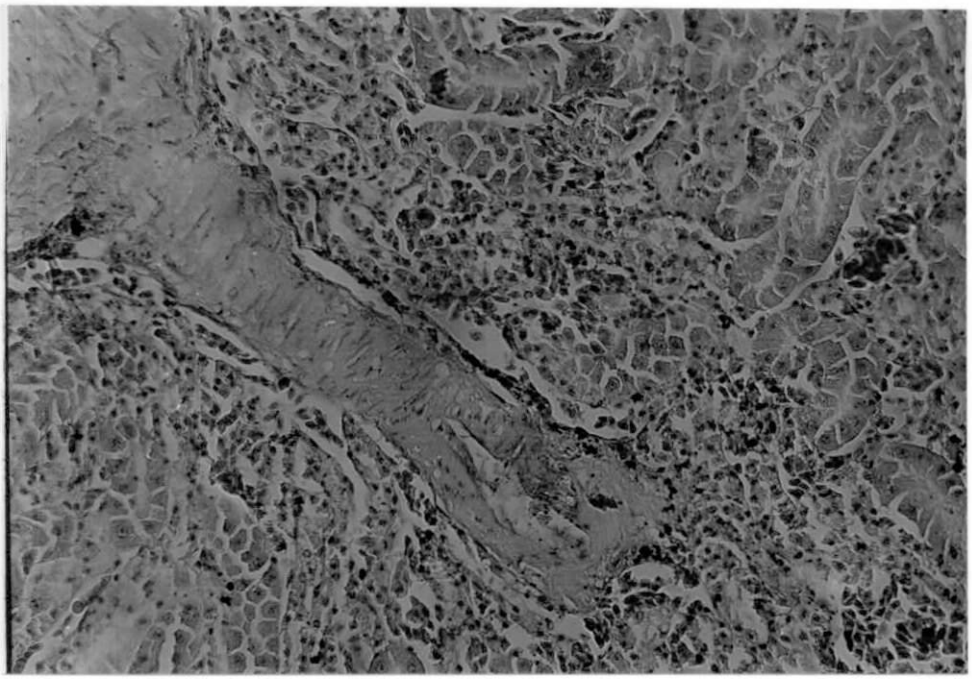


Fig.64 Kidney - Group I - Scattered areas of lymphoid infiltration - Degenerated epithelial cells lining the tubules - H&E x 400

Fig.65 Kidney - Group I - Necrosis of epithelial cells - H&E x 400



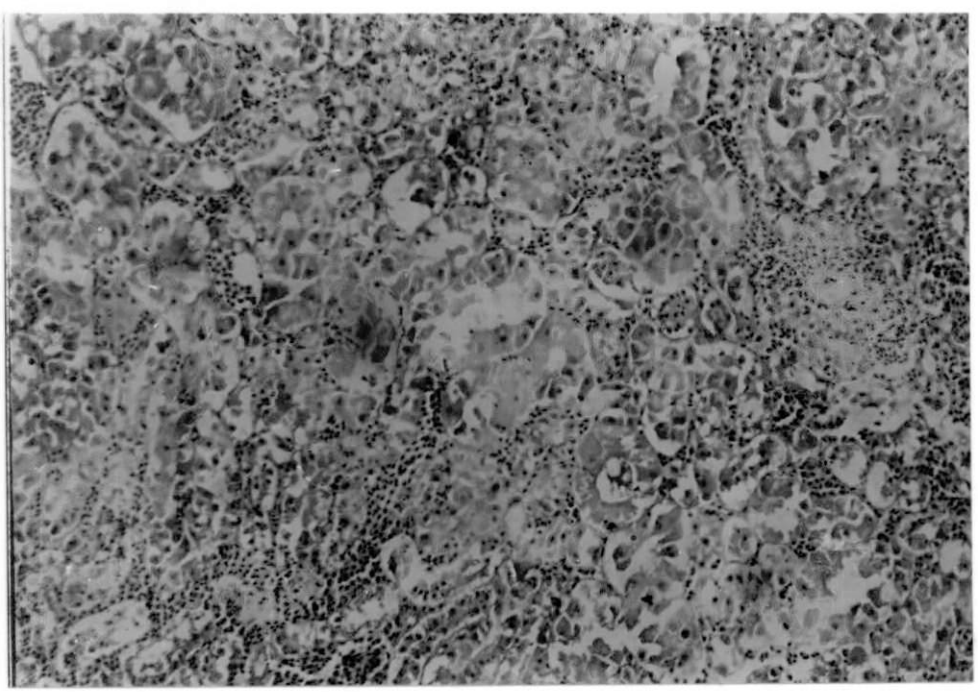
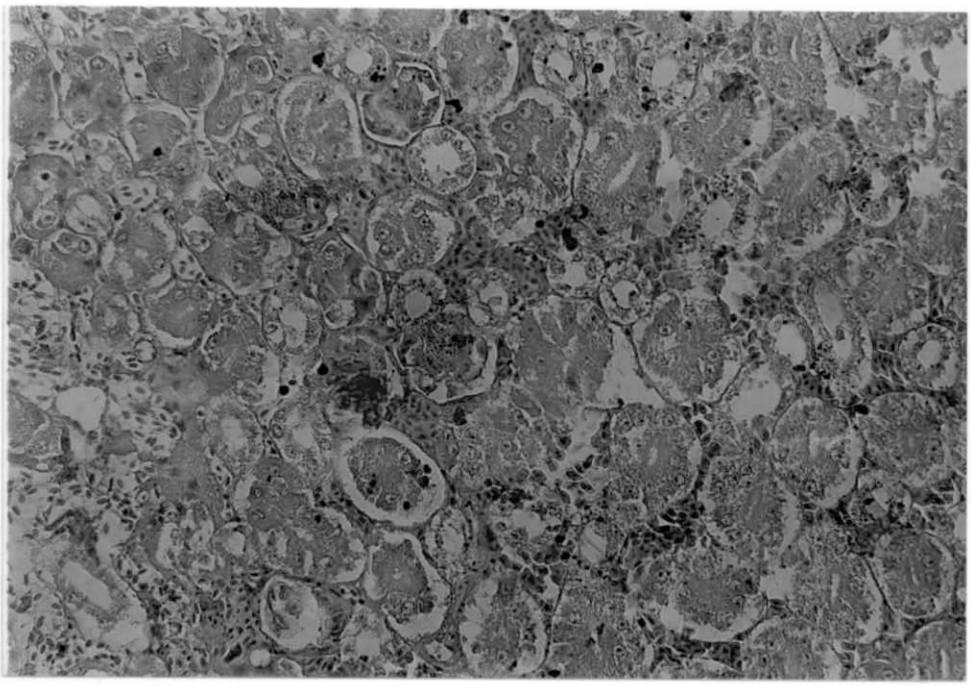


Fig.66 Kidney - Group I - Degeneration, necrosis and desquamation of epithelial cells in the proximal convoluted tubules - H&E x 600

Fig.67 Kidney - Group I - Thickening of the glomerular basement membrane - Tubules showing degenerative and necrotic changes - H&E x 400



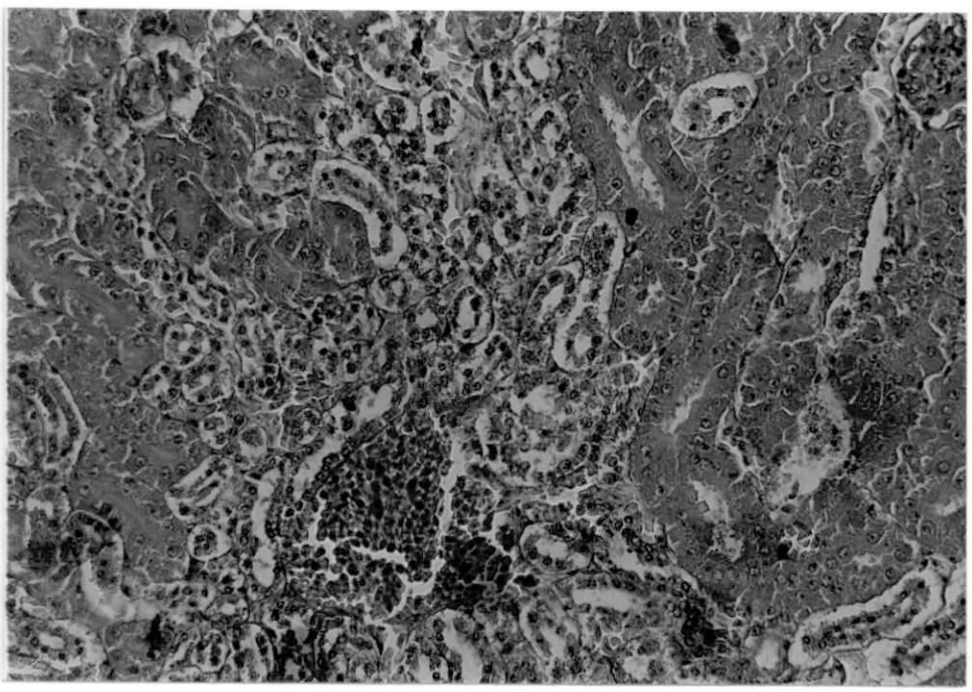
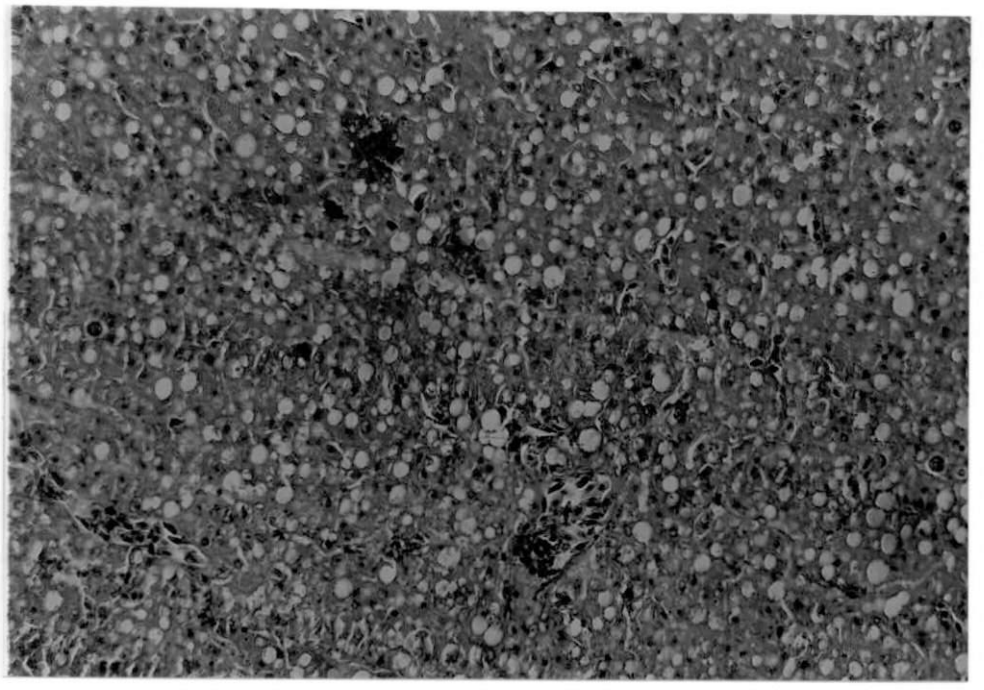


Fig.68 Liver - Group I - Focal areas of fatty change - H&E x 400

Fig.69 Liver - Group I - Diffuse fatty change - H&E x 250



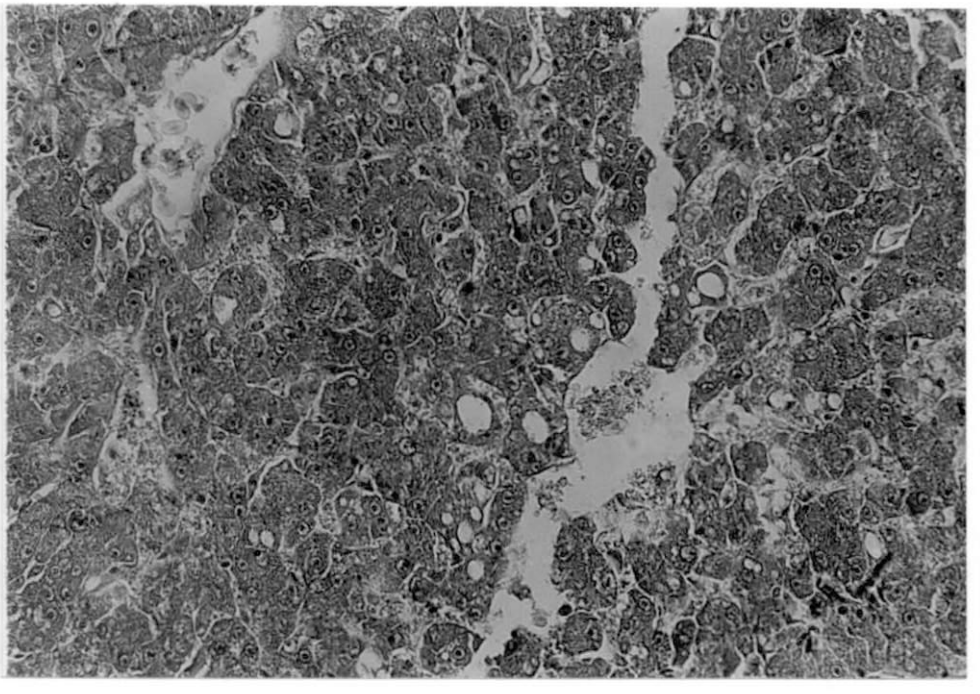
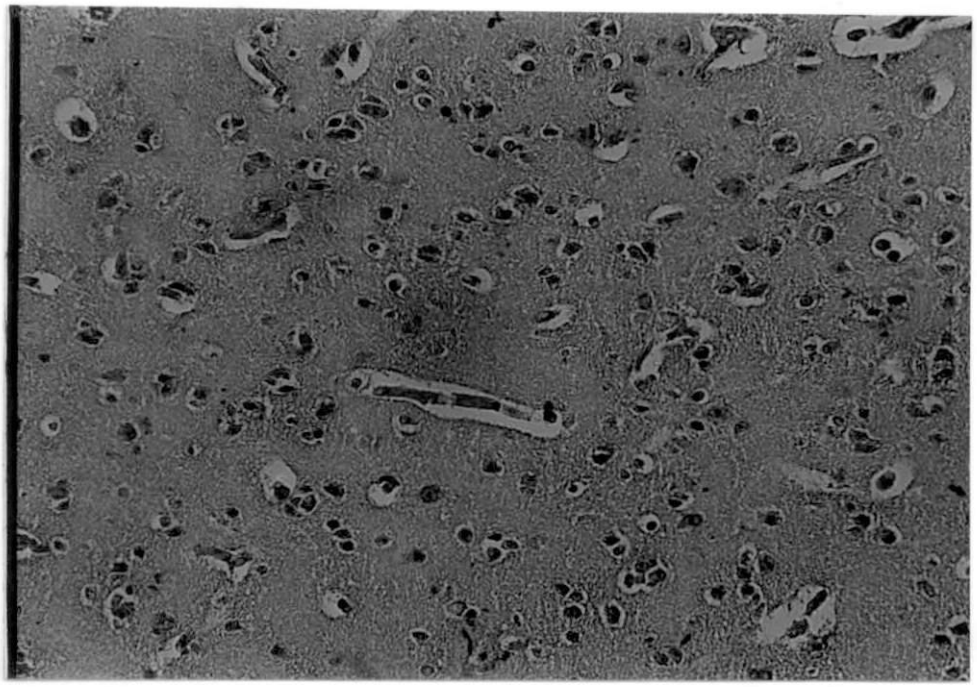


Fig.70 Brain - Group I - Focal areas of oedema - H&E x 250

Fig.71 Brain - Group I - Satellitosis - H&E x 250



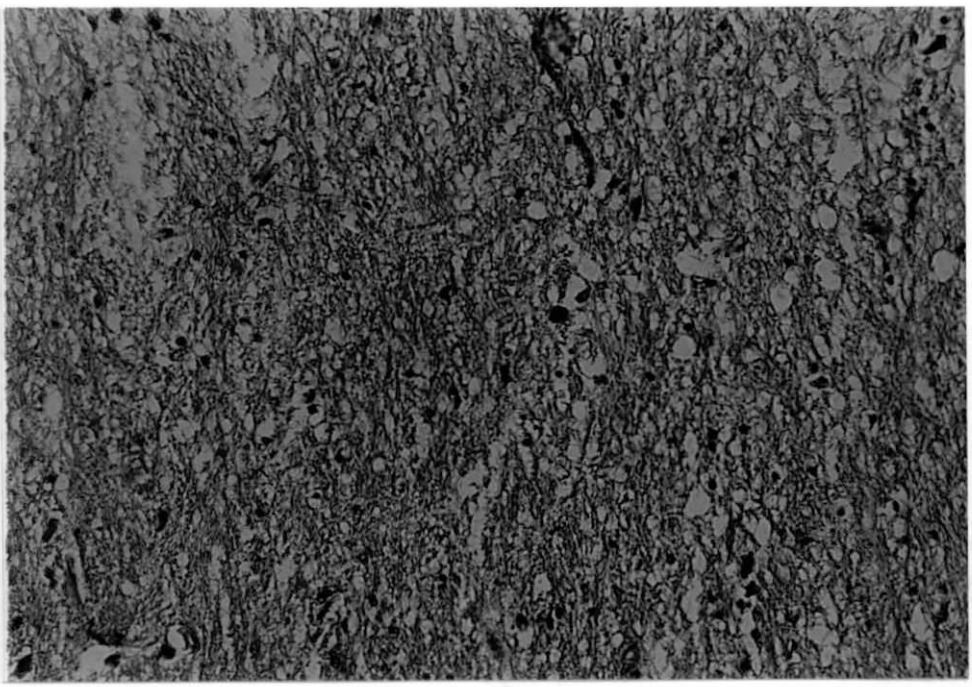
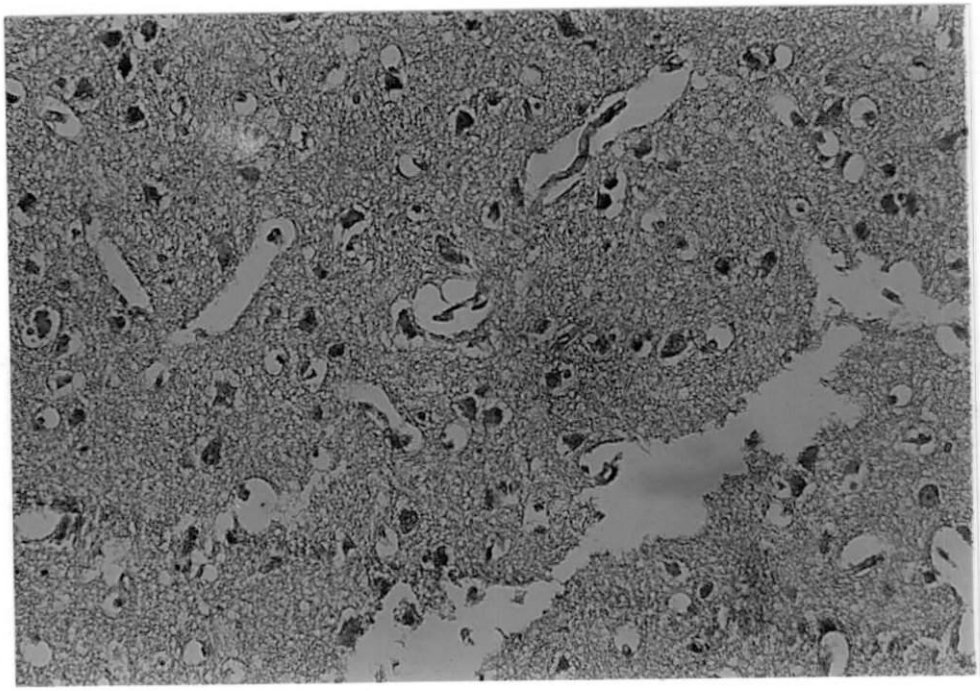


Fig.72 Brain - Group I - Pyknosis of neurons and perineuronal oedema - H&E x 250

Fig.73 Brain - Group I - Congestion of vessels and swollen vascular entothelium - H&E x 250



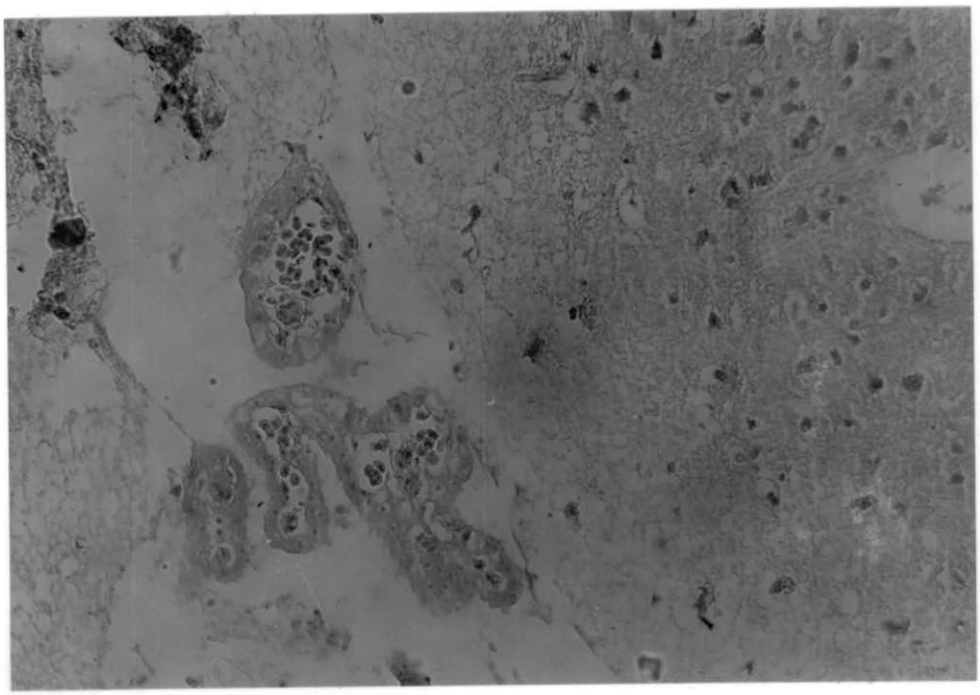
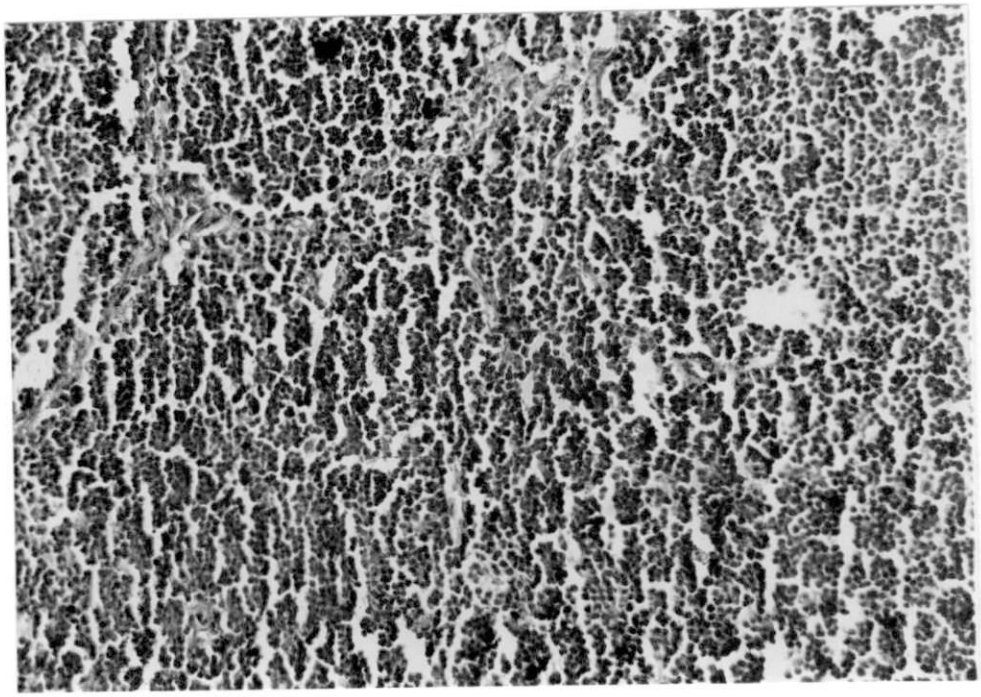


Fig.74 Bursa of Fabricius - Group III - Lymphoid hyperplasia of diffuse nature - H&E x 250

Fig.75 Spleen - Group III - Focal proliferating large hypertropic lymphocytes - H&E x 400



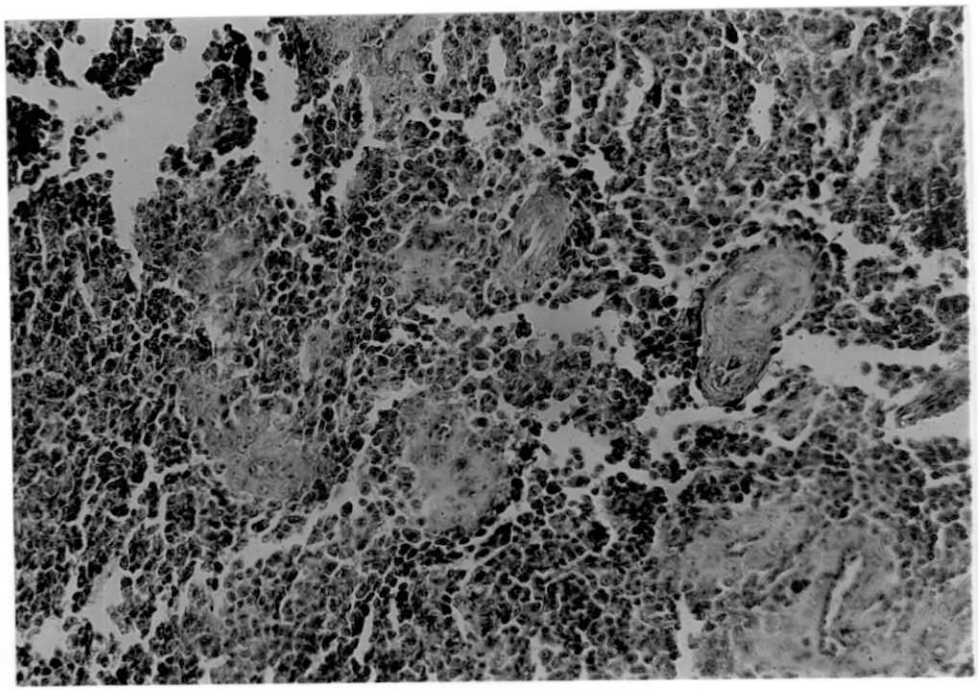
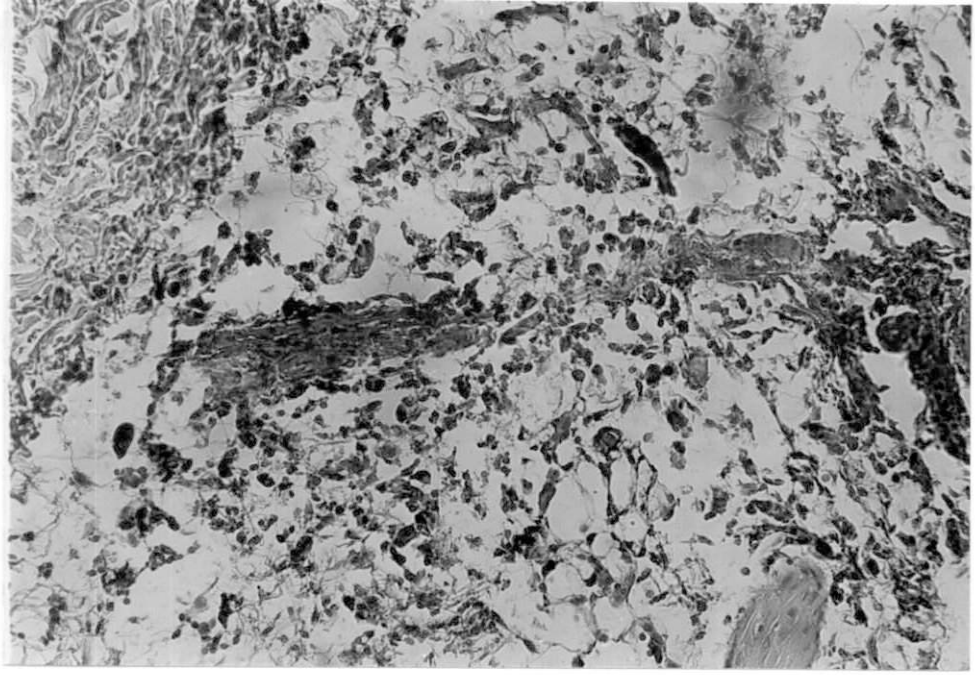


Fig.76 Skin - Group II - DNCB response - 24 h - Diffuse infiltration of mononuclear cells, lymphocytes and few heterophils - H&E x 250

Fig.77 Skin - Group I - DNCB response - 24 h - Mild infiltration with mononuclear cells and heterophils - H&E x 250



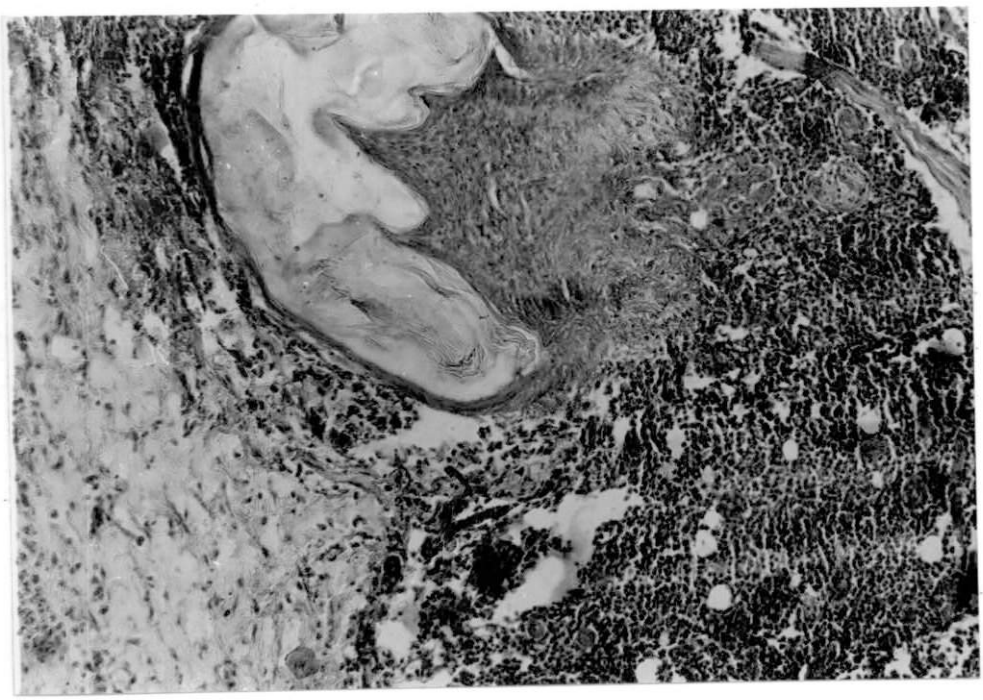


Fig.78 Electron micrograph - Quail embryo - 13th day - OA inoculated - Bursa of Fabricius - Lymphoid area with cells showing varying grades of organellar changes x 13,000

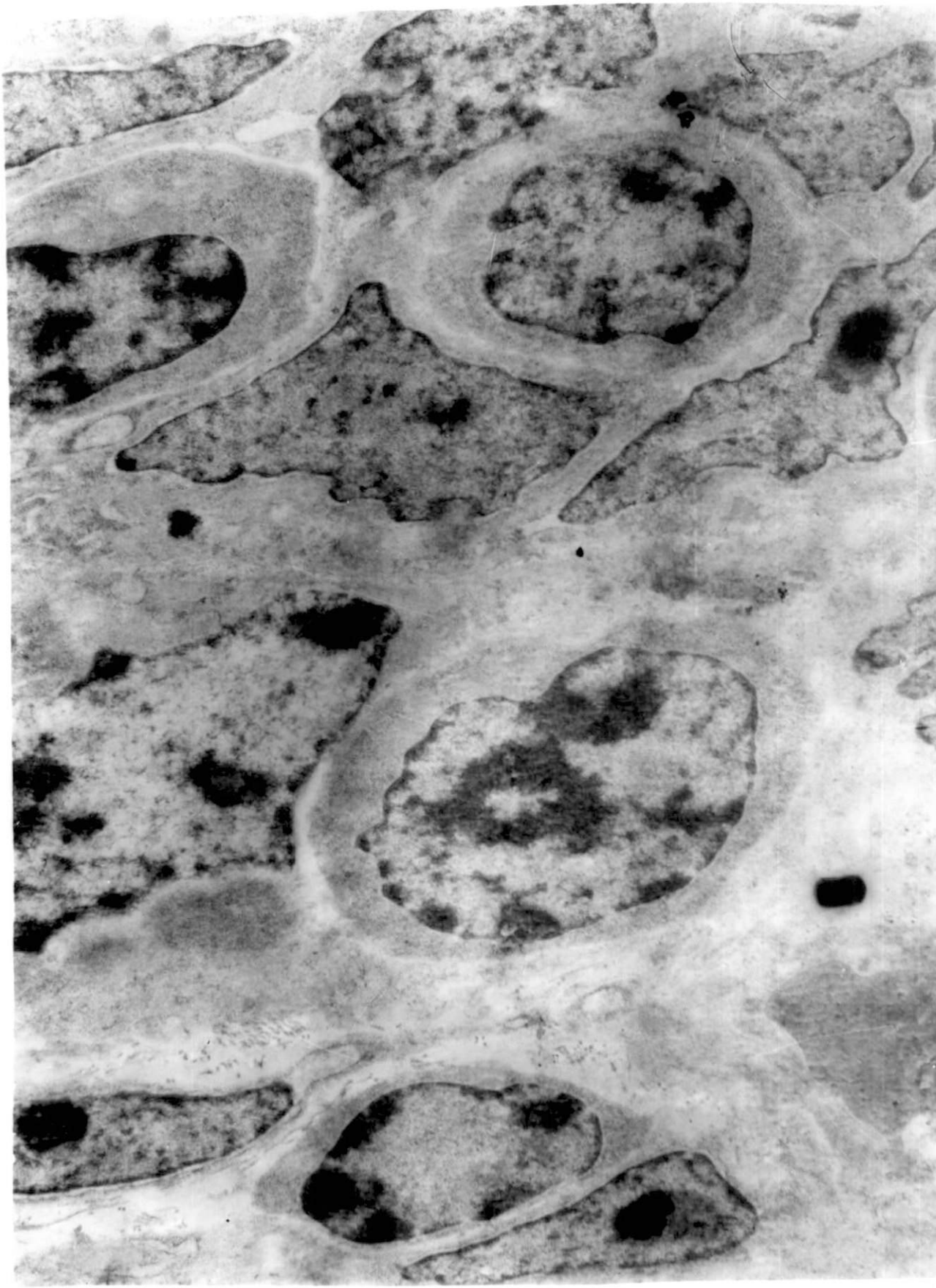


Fig.79 Electron micrograph - Quail embryo - 13th day - OA inoculated - Bursa of Fabricius - Lymphoid cell with severe organellar destruction in the cytoplasm- Dense bodies (DB) suggestive of phagolysosome - Nucleolus (NL) showing condensation of granular and fibrillar contents x 21,000

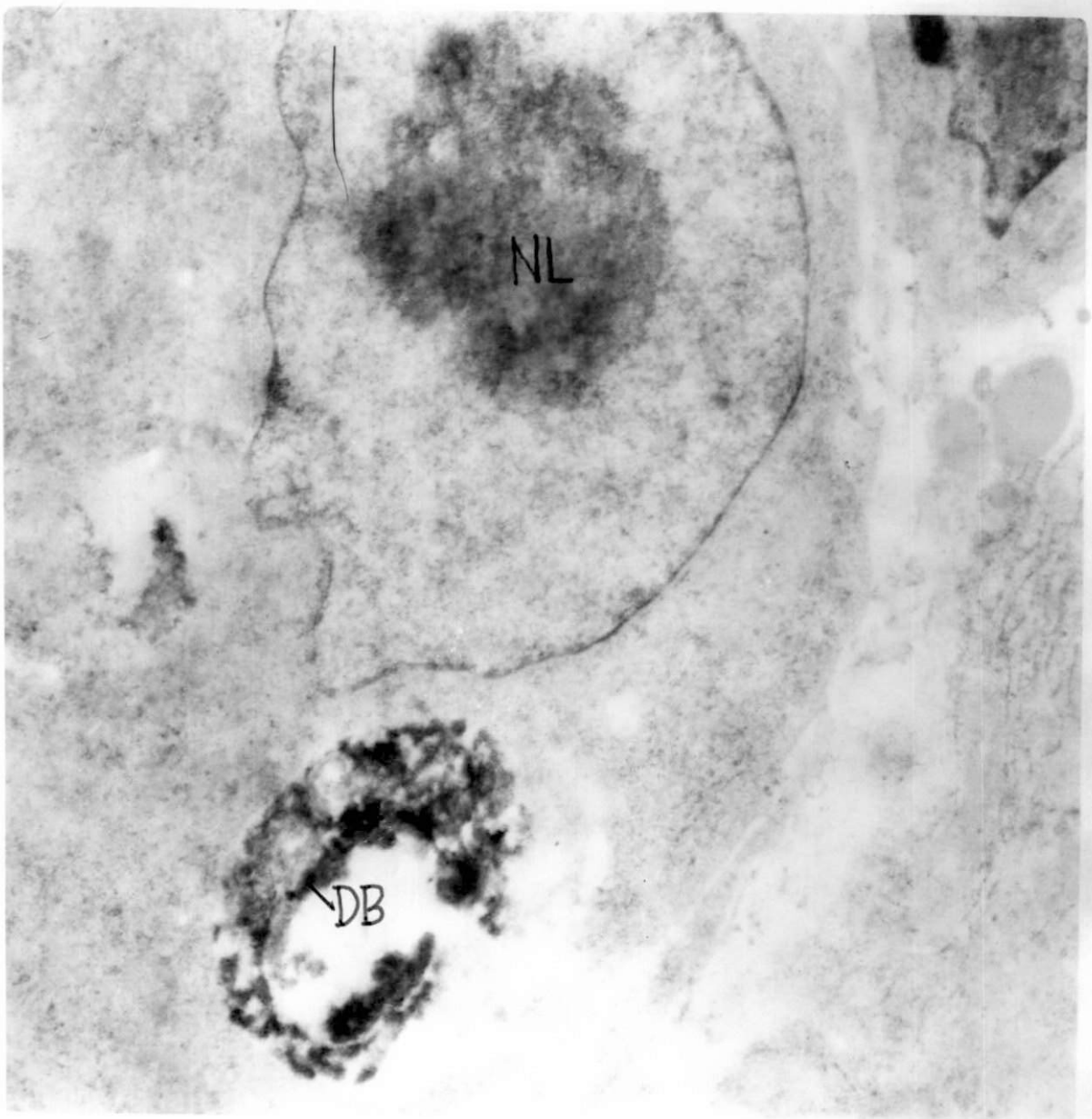

NL
DB

Fig.80 Electron micrograph - Quail embryo - 16th day - OA inoculated - Bursa of Fabricius - Lymphocytes showing damaged mitochondria (M) and nucleolar condensation (NL) x 40,000

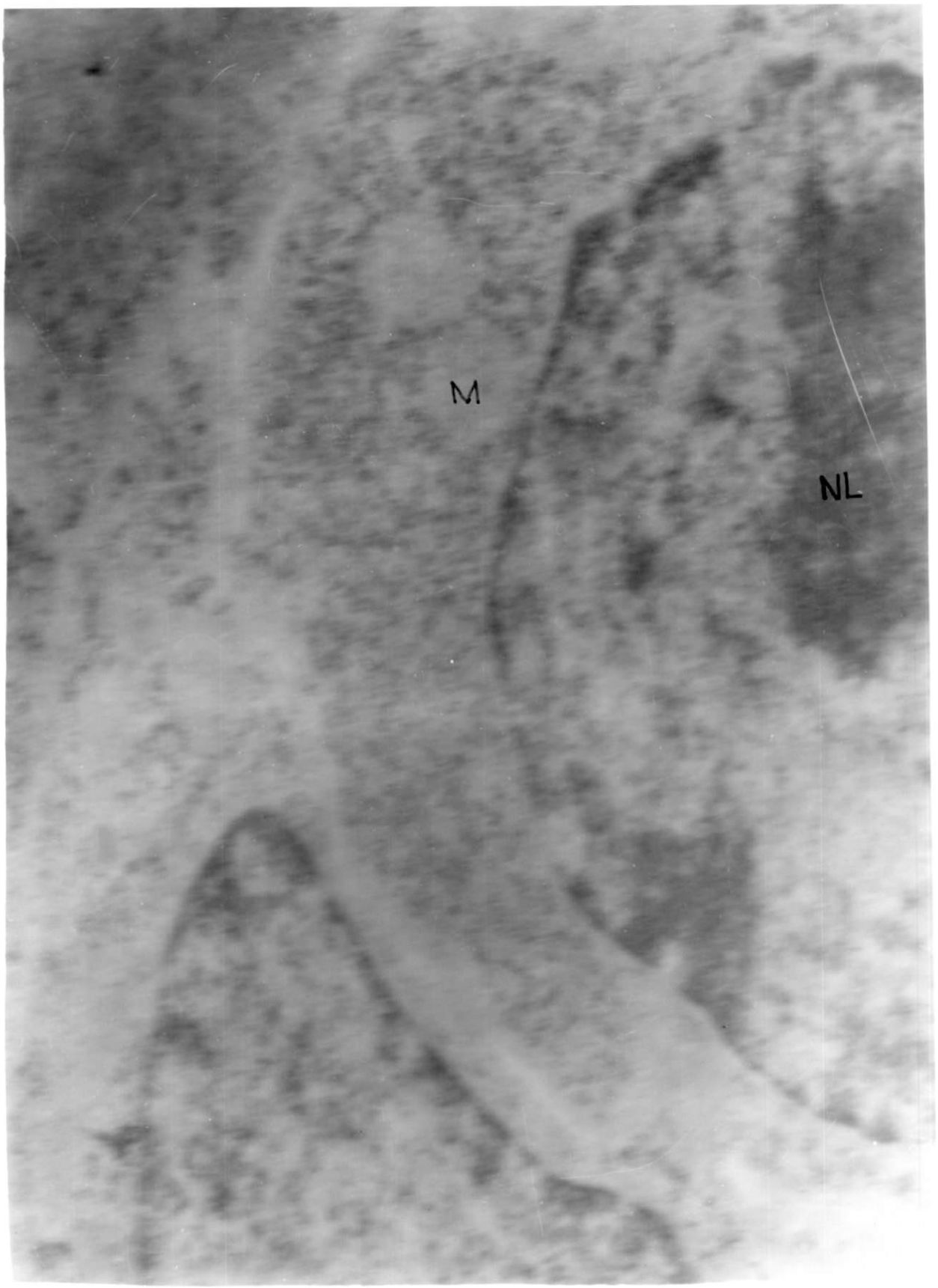

M
NL

Fig.81 Electron micrograph - Quail embryo - 16th day - OA inoculated - Bursa of Fabricius - Lymphocyte showing clumping of chromatin and nucleolar condensation - Loss of cytoplasmic organelles noticed x 40,000

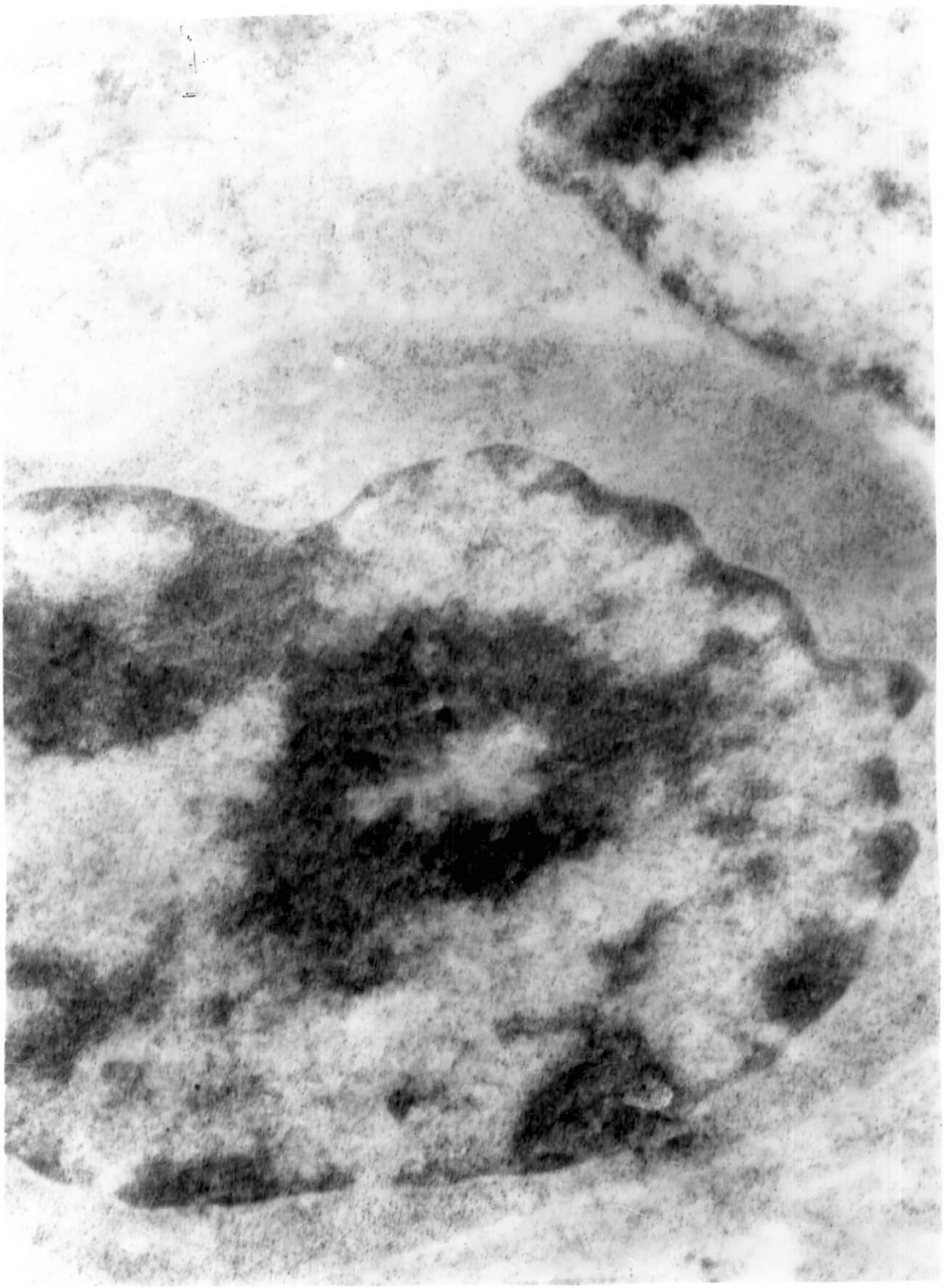


Fig.82 Electron micrograph - Quail embryo - 16th day - OA inoculated - Bursa of Fabricius - Lymphoid area showing a cell undergoing mitosis (C) - Partial degranulation of ribosomes seen x 16,000

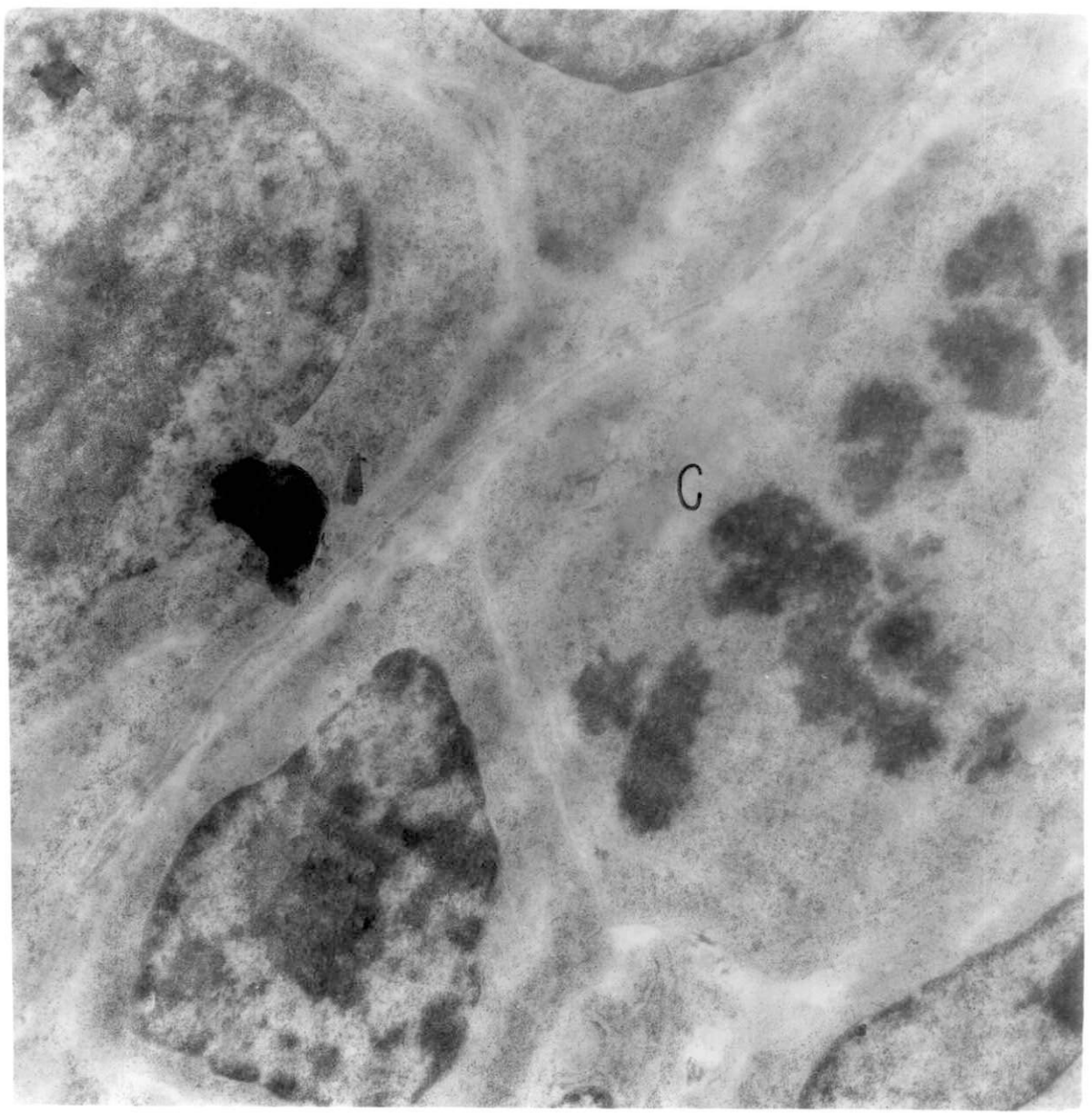

C

Fig.83 Electron micrograph - Quail embryo - 13th day - OA inoculated - Spleen - Lymphocyte showing clumping of chromatin along with inner nuclear membrane - Occasional fragmentation of endoplasmic reticulum and degranulation of ribosomes x 40,000

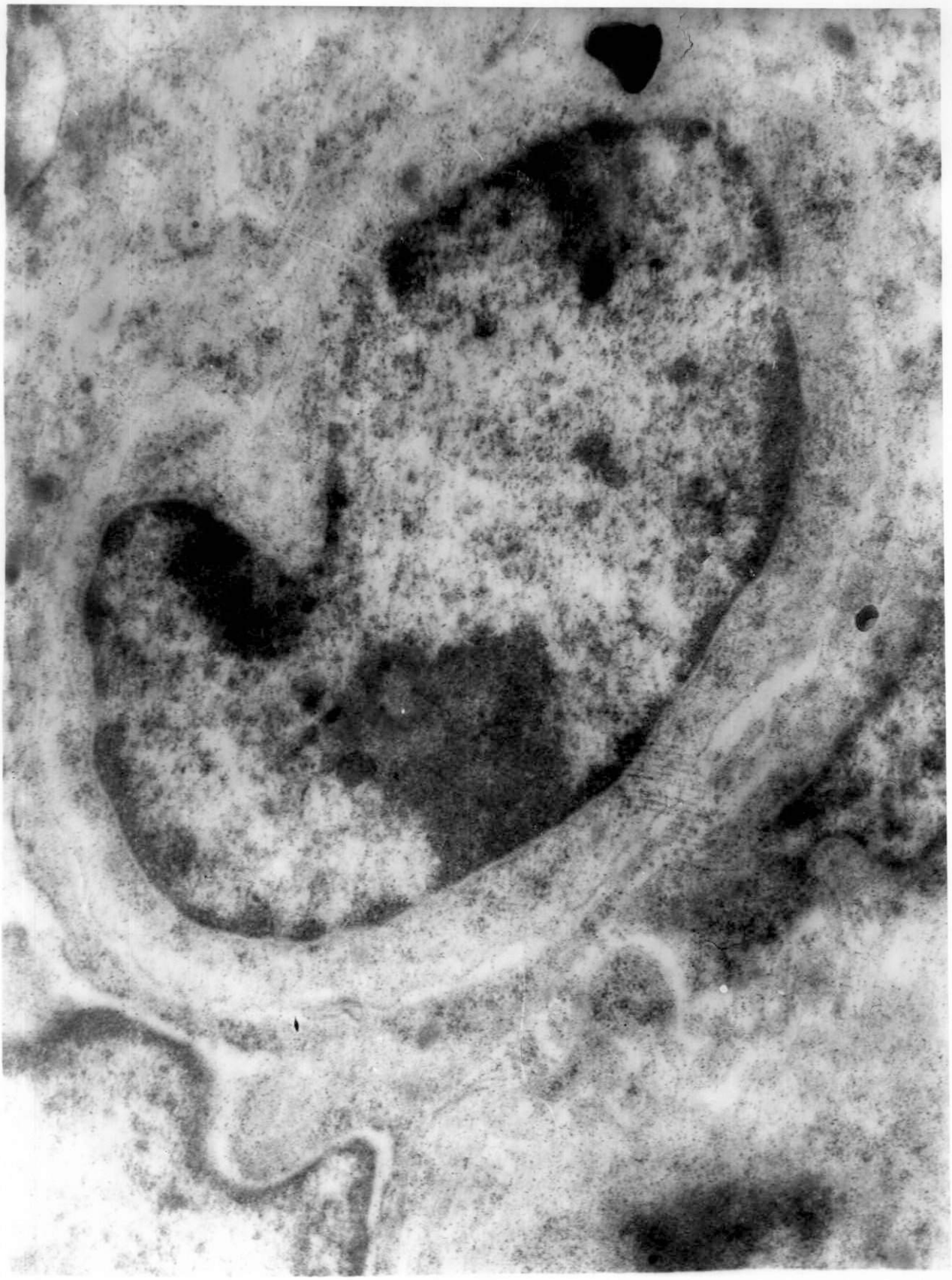


Fig.84 Electron micrograph - Quail embryo - 16th day - OA inoculated - Spleen - Lymphoid cell showing necrobiotic changes - Cytoplasm with a homogenous appearance - Numerous vacuoles (V) seen x 46,000

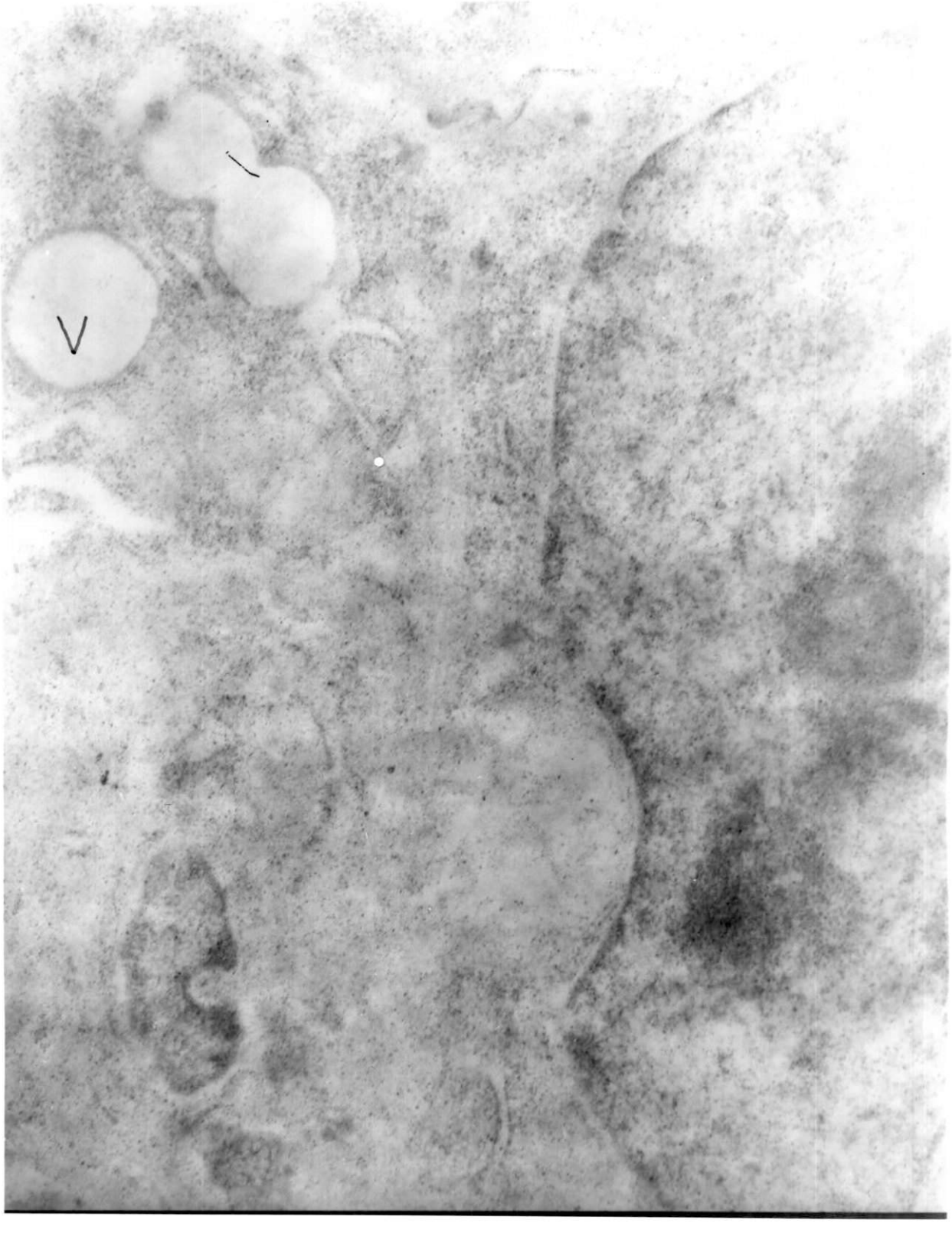

V

Fig.85 Electron micrograph - Quail embryo - 16th day - OA inoculated - Spleen - Macrophages with few strands of endoplasmic reticulum (ER), some of which are dilated - Lipid droplets (L) and Vacuoles (V) noticed - Nucleoli very markedly condensed x 40,000

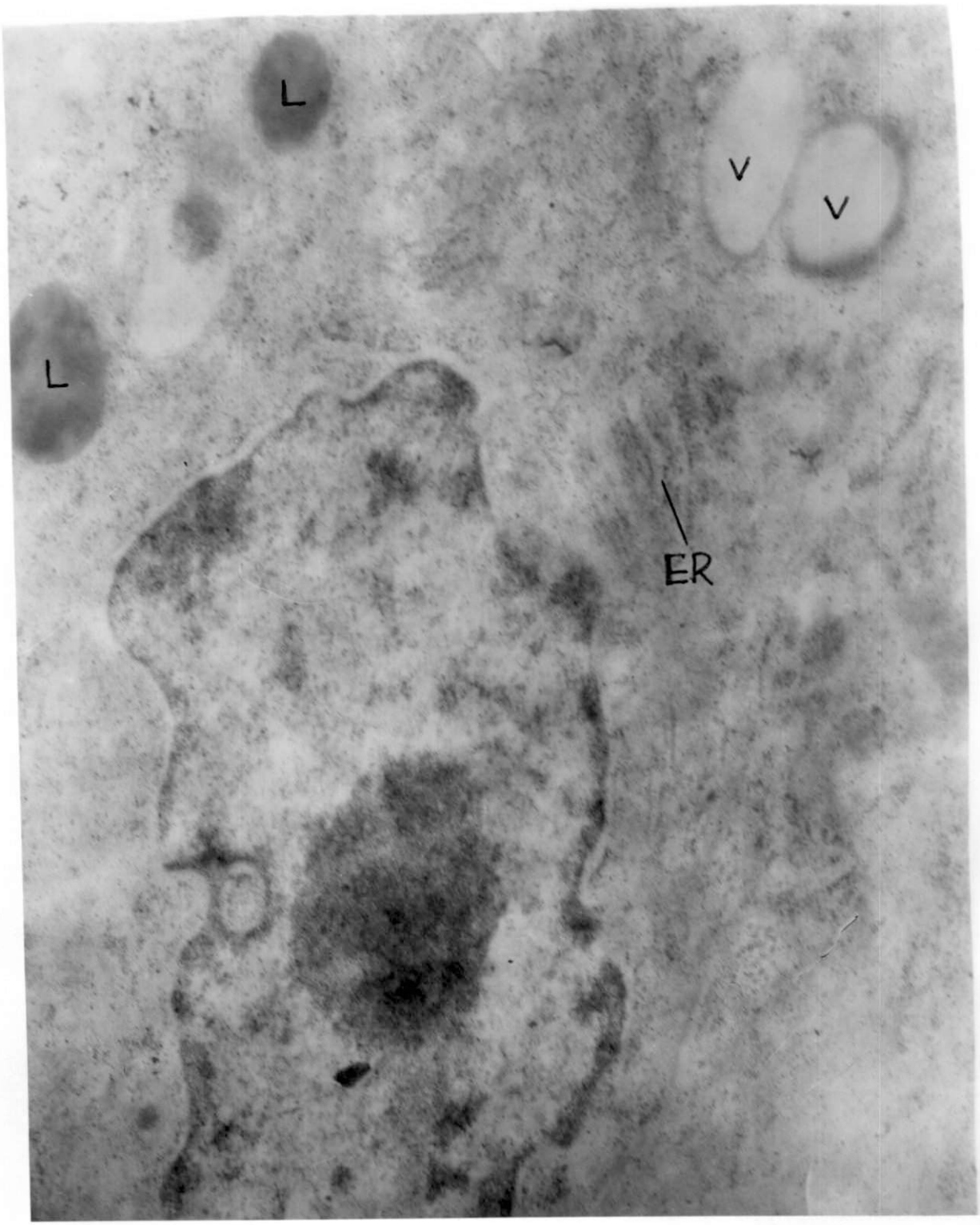

L
V
V
L
ER

Fig.86 Electron micrograph - Quail embryo - 16th day - OA inoculated - Spleen - Macrophage with a nucleus showing occasional clumping of chromatin - Loss of cytoplasmic organelles x 40,000

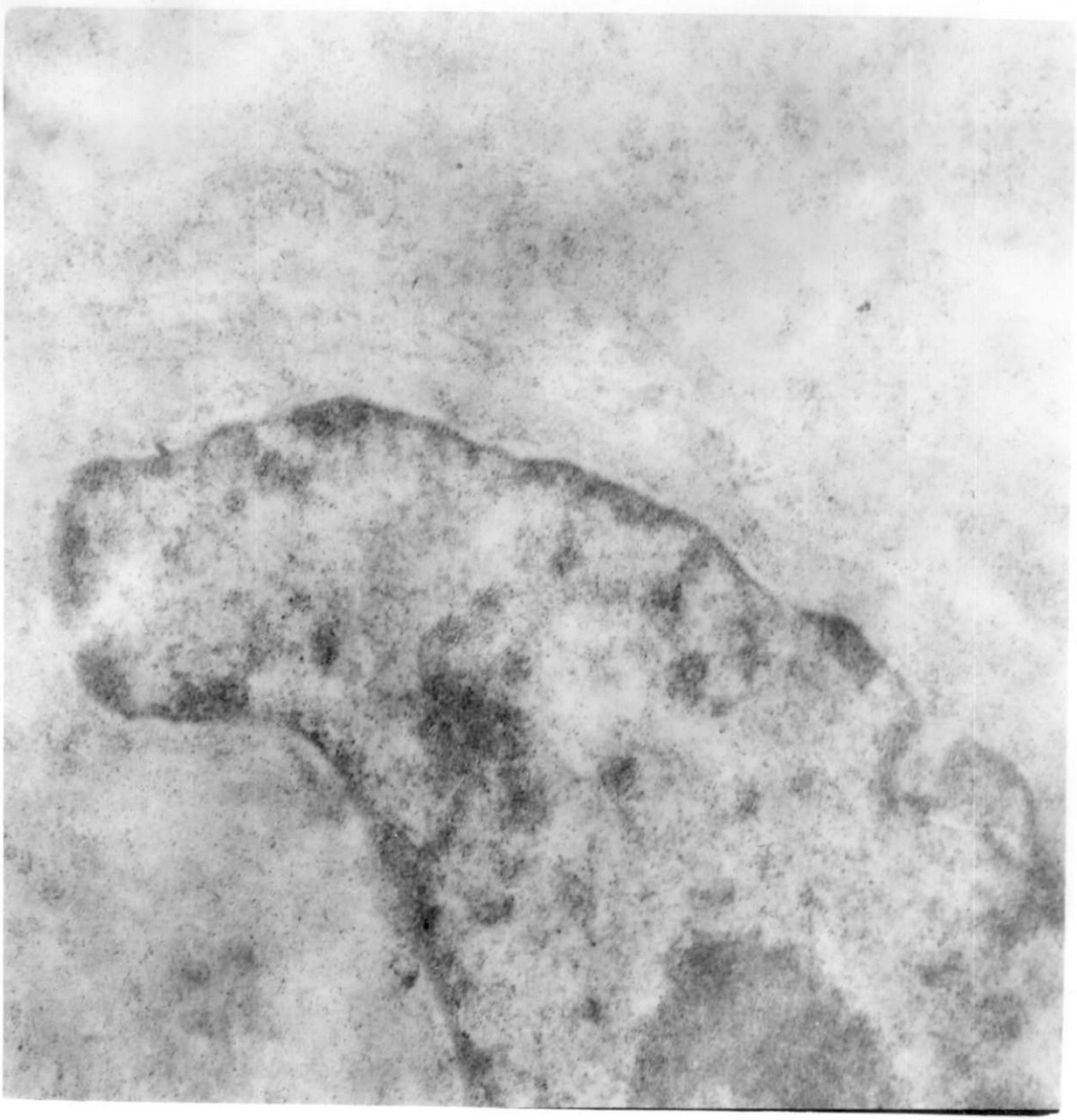


Fig.87 Electron micrograph - Quail - OA fed - Bursa of Fabricius - Lymphoid (L) and epithelial (E) components - The cells show different degrees of alteration - Cytoplasm of some cells (C) show lytic changes and condensed nucleus x 9,000

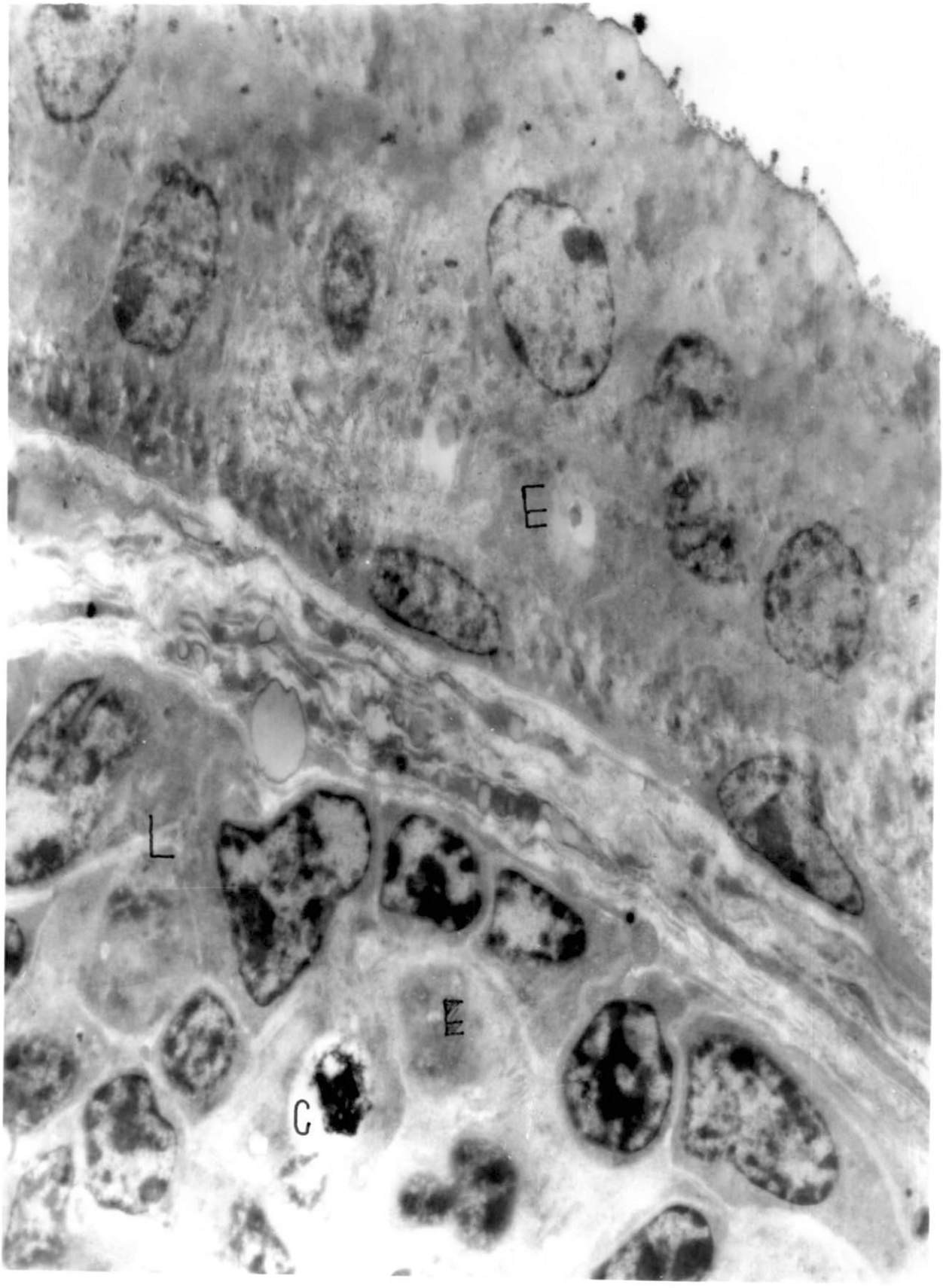

E
L
E
C

Fig.88 Electron micrograph - Quail - OA fed - Bursa of Fabricius - Lymphoid area showing heterogenous type of cells showing varying degrees of nuclear and cytoplasmic alterations x 12,000

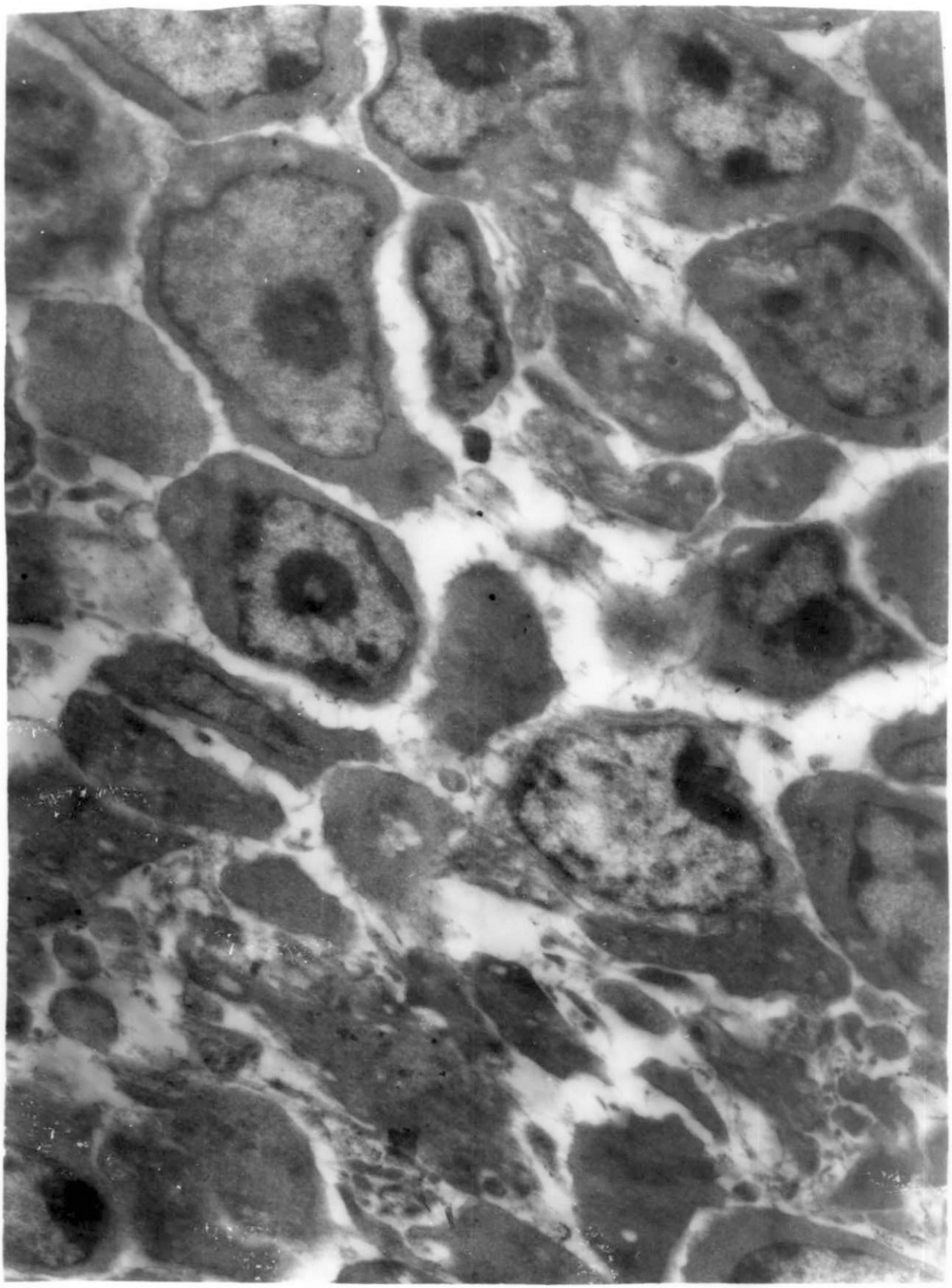


Fig.89 Electron micrograph - Quail - OA fed - Bursa of Fabricius - Higher magnification of Fig.88 - Degranulated endoplasmic reticulum (ER) and condensed nucleoli (NL) x 32,000

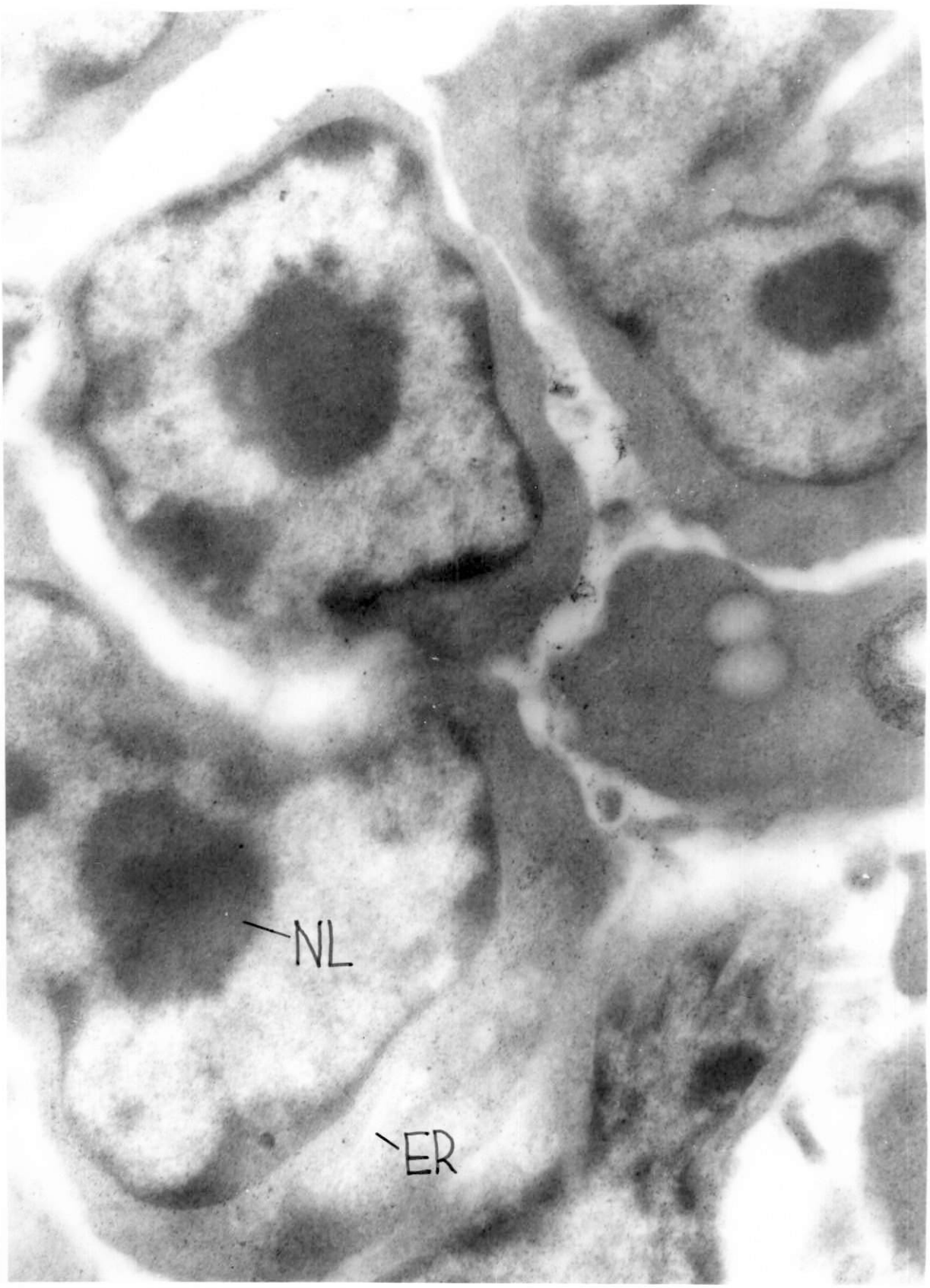

NL
ER

Fig.90 Electron micrograph - Quail - OA fed - Spleen - Heterophils (H) and Lymphocytes (L) - There is partial degranulation of heterophilic granules and lysis of plasma membrane x 24,000

H
H

Fig.91 Electron micrograph - Quail - OA fed - Spleen - Extensive necrobiotic changes in the lymphoid cells - Loss of cytoarchitecture - Organellar destruction noticed - Vacuole formation also seen x 9,000

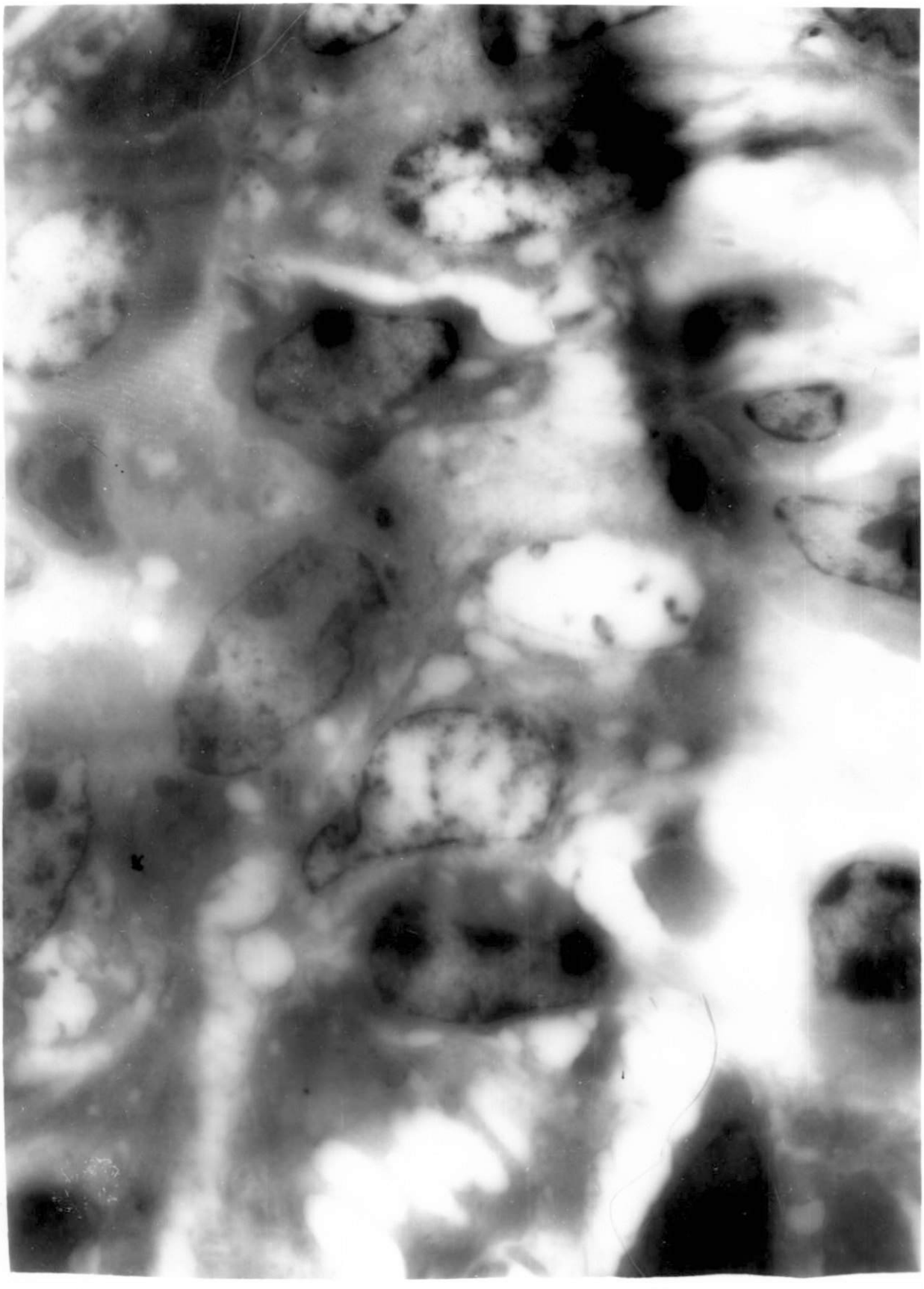


Fig.92 Electron micrograph - Quail - OA fed - Thymus - Lymphocytes - Fragmented nucleus (N) seen in a cell - Washed out appearance of cytoplasm in some other cells x 26,000

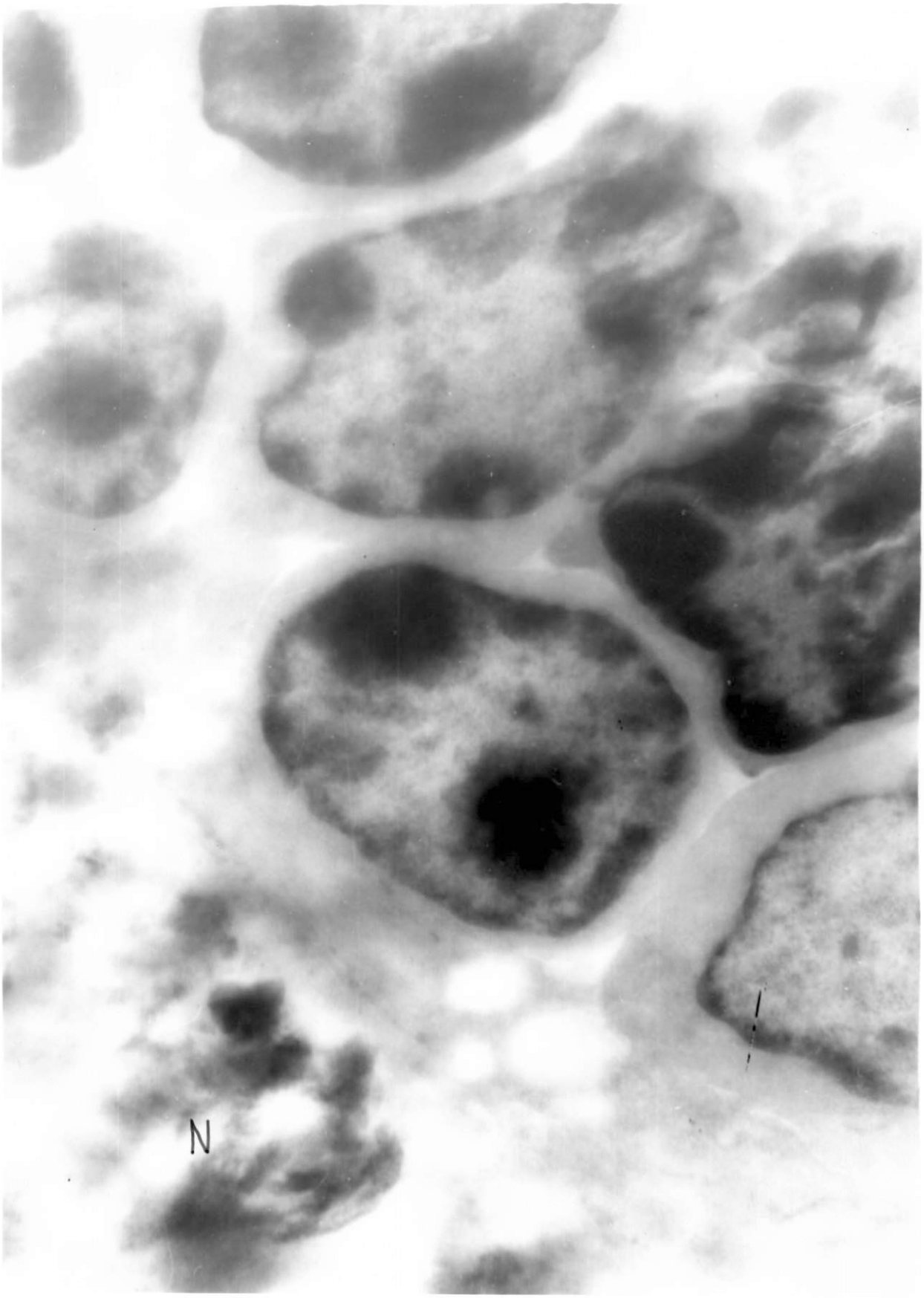

N

Fig.93 Electron micrograph - Quail - OA fed - Thymus - Lymphocytes showing heterochromatin (HC) blocks in nuclear membrane and condensation with relative absence of organelles x 27,000

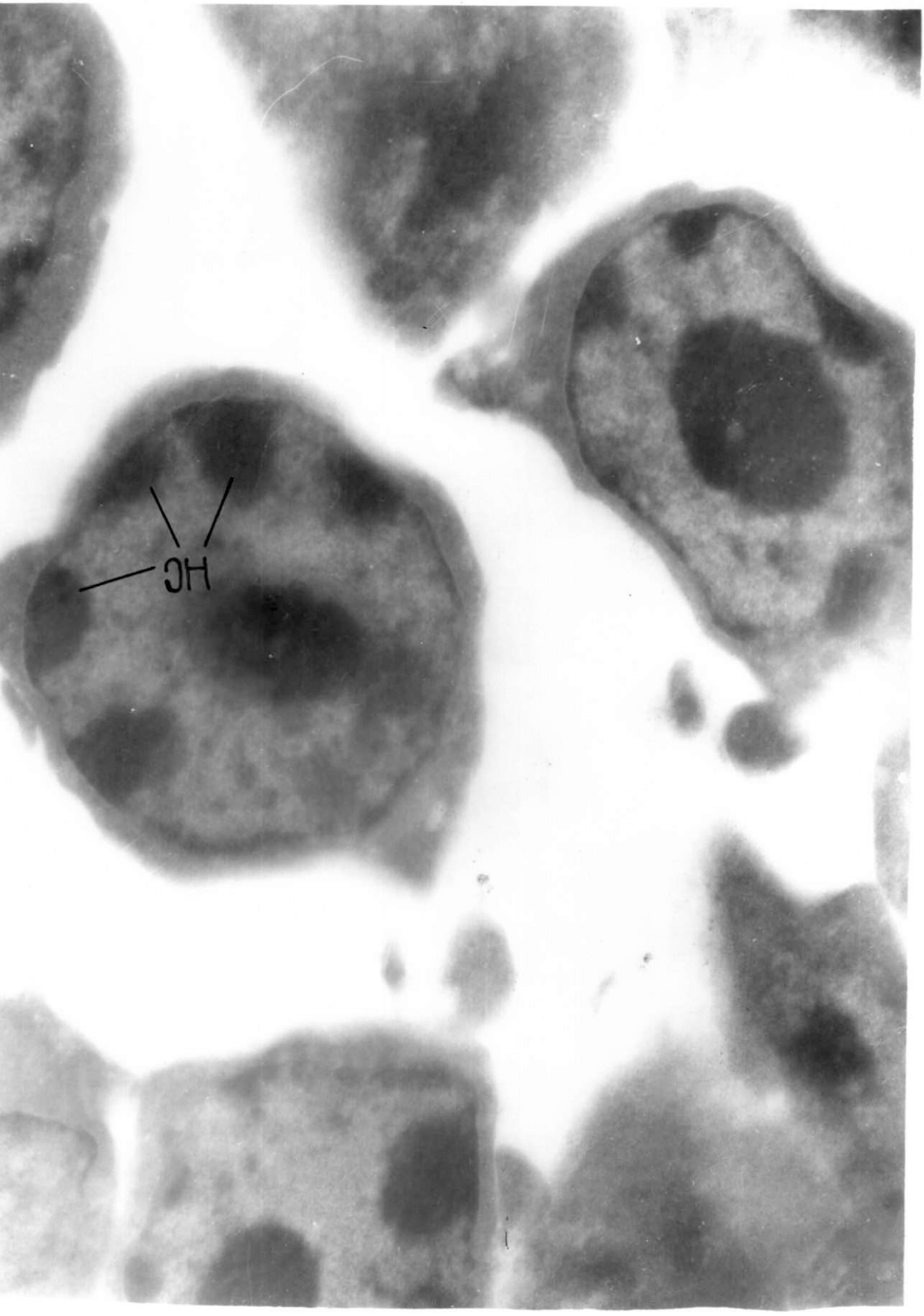

HC

Fig.94 Electron micrograph - Quail - OA fed - Thymus - Lymphocytes showing electron dense homogenous cytoplasm and marked condensation of nucleoli x 16,000

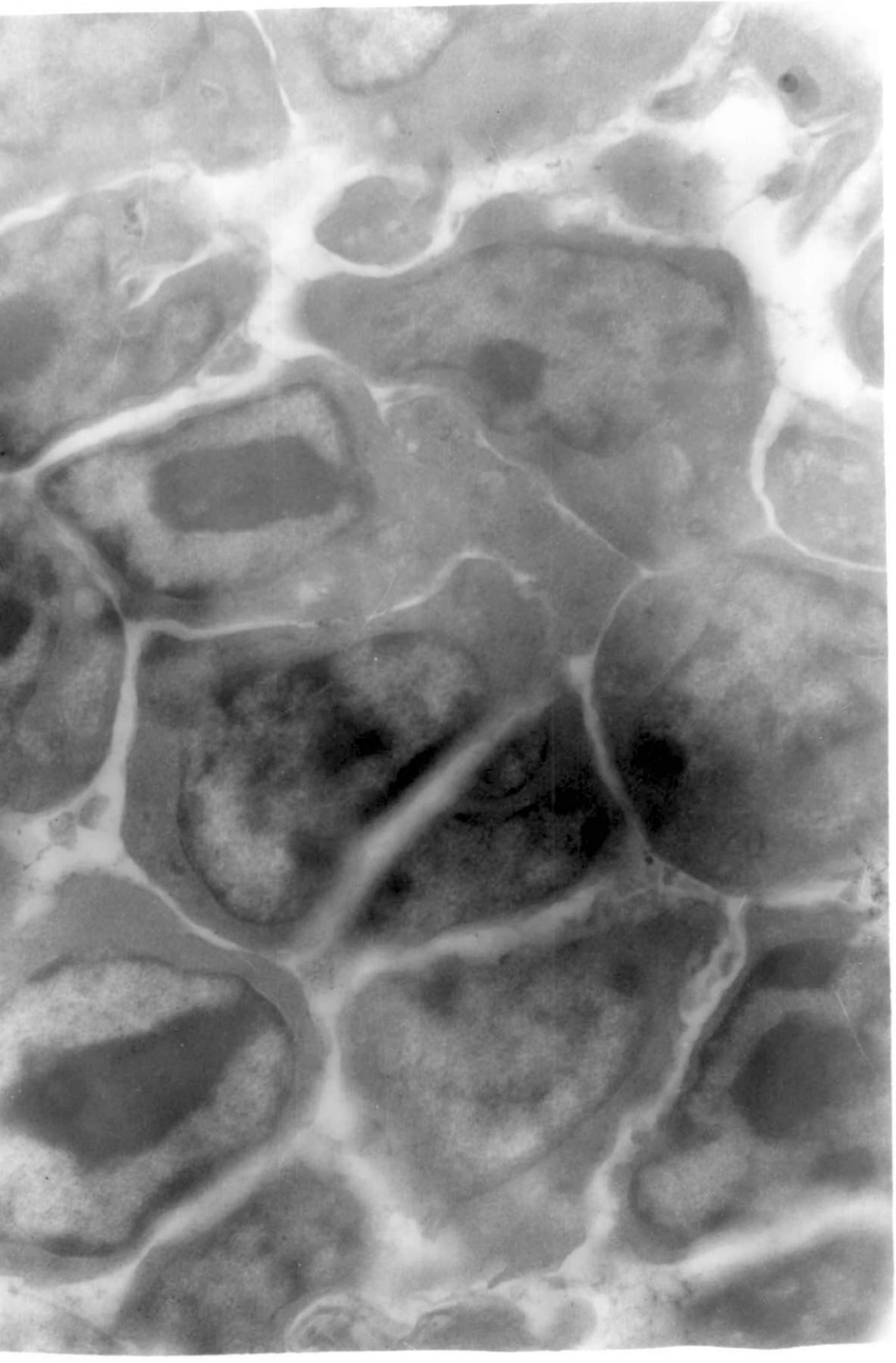


Fig.95 Electron micrograph - Quail - OA fed - Thymus - Lymphocytes with pleomorphic morphology - A macrophage (MP) is seen with dilated endoplasmic reticulum (ER) and lysosomes (L) x 27,000

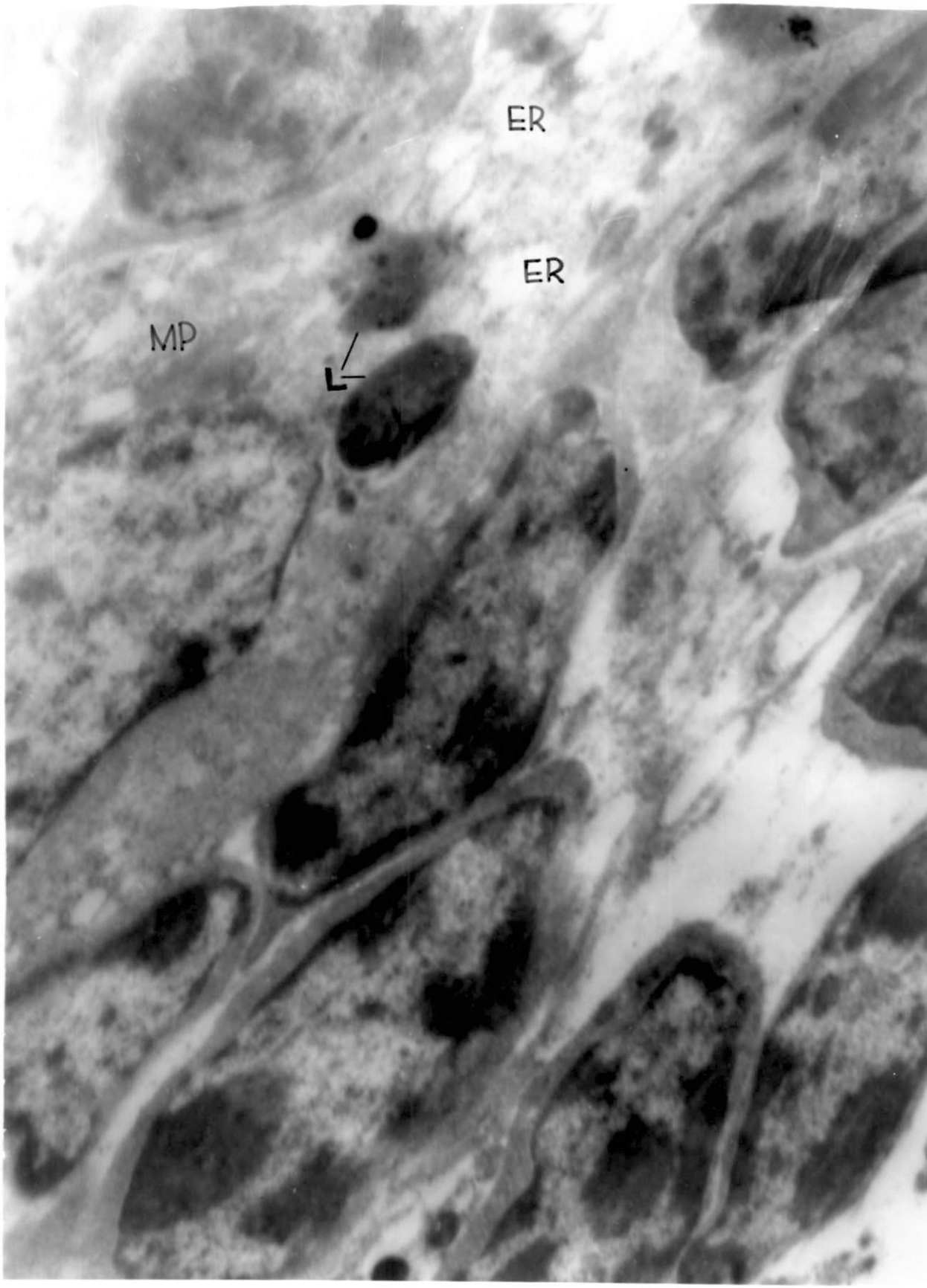

ER
ER
MP

Fig.96 Electron micrograph - Quail - OA fed - Thymus - Thymic lymphocytes - Extensive cytoplasmic changes in some cells - Lysosomal structures (L) seen x 21,000

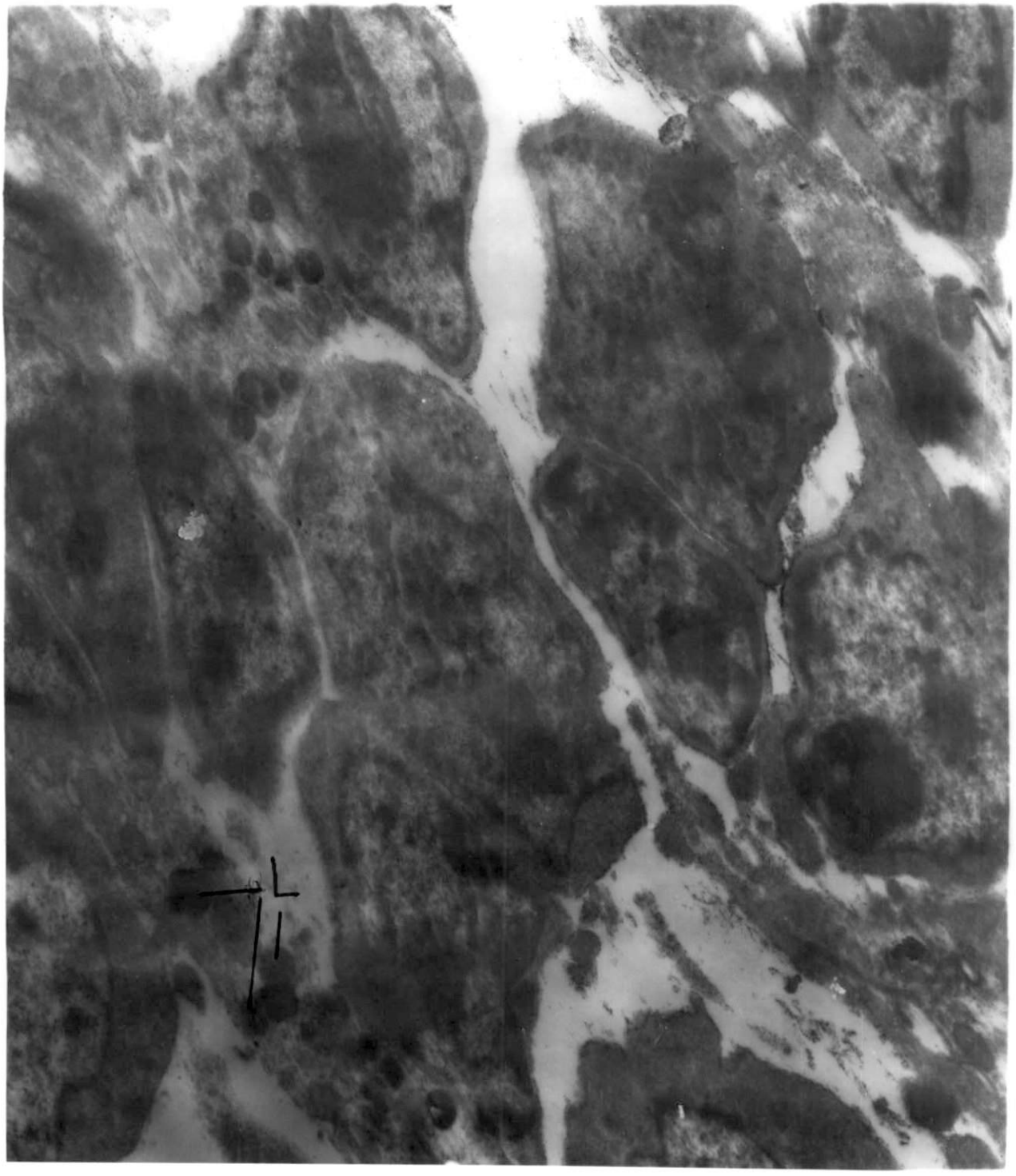


Fig.97 Electron micrograph - Quail - OA fed - Thymus - Thymic lymphocytes separated by oedematous fluid and few strands of connective tissue - Nucleolar condensation very prominantly seen x 13,000

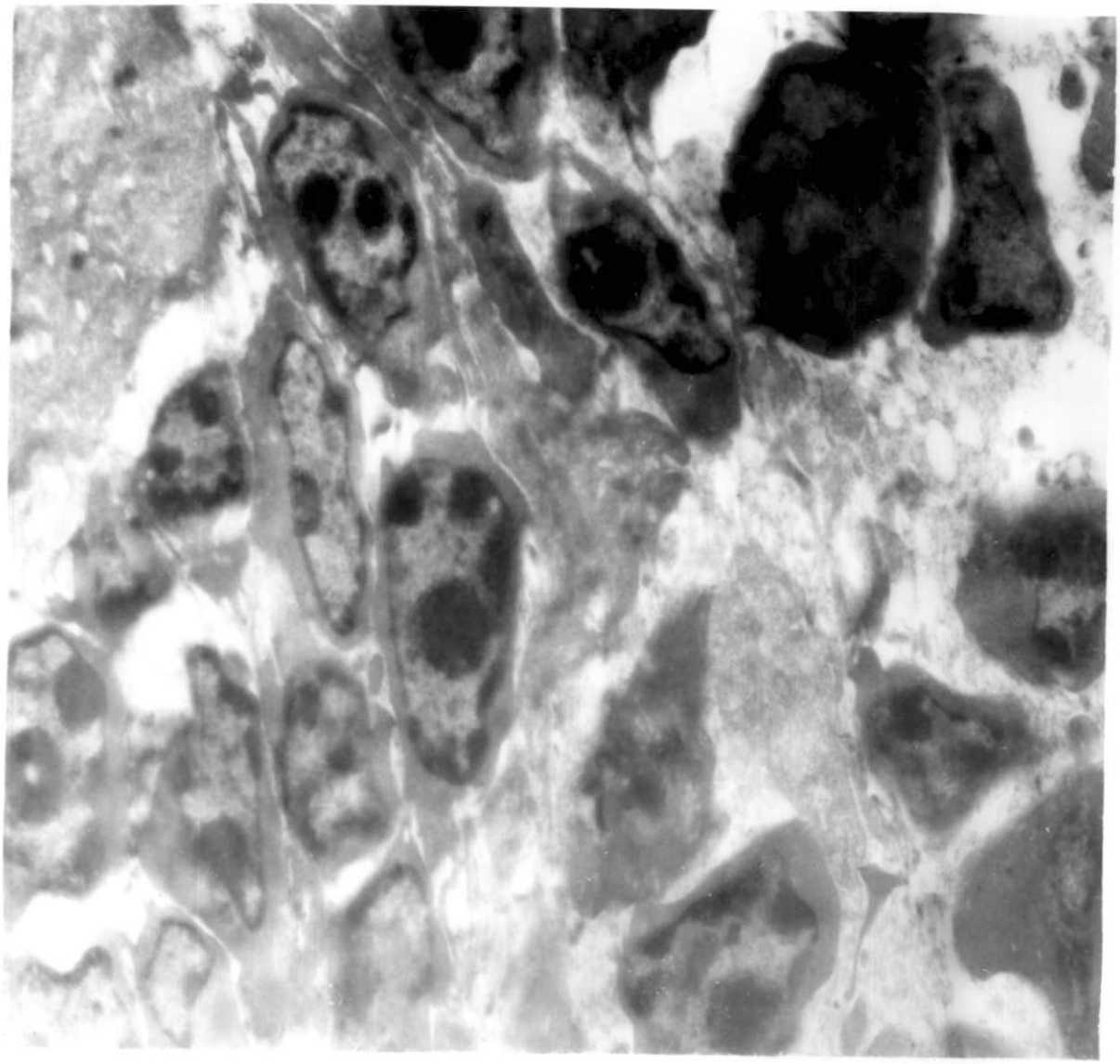


Fig.98 Electron micrograph - Quail - OA fed - Kidney - Epithelial cells of proximal convoluted tubule with partial lysis of plasma membrane and villi (V) - Numberous confluent vacuoles (CV) in the cytoplasm- Lumen of tubule (LU) x 16,000

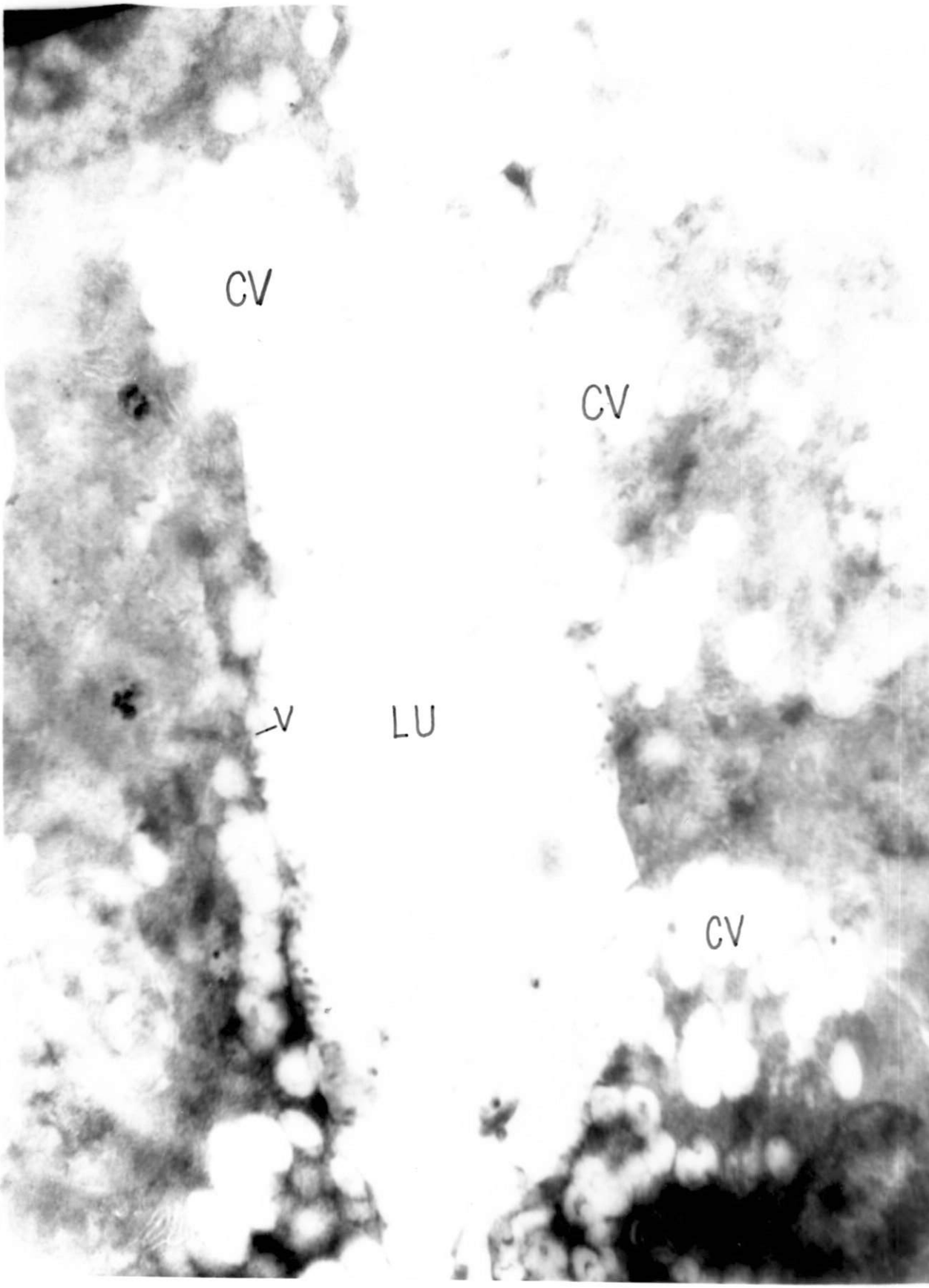

CV
CV
V
LU
CV

Fig.99 Electron micrograph - Quail - OA fed - Kidney - Renal epithelial cell with swollen and homogenous mitochondria (M) with loss of cristae - Vacuoles (V) and Lysosomes (L) seen x 27,000

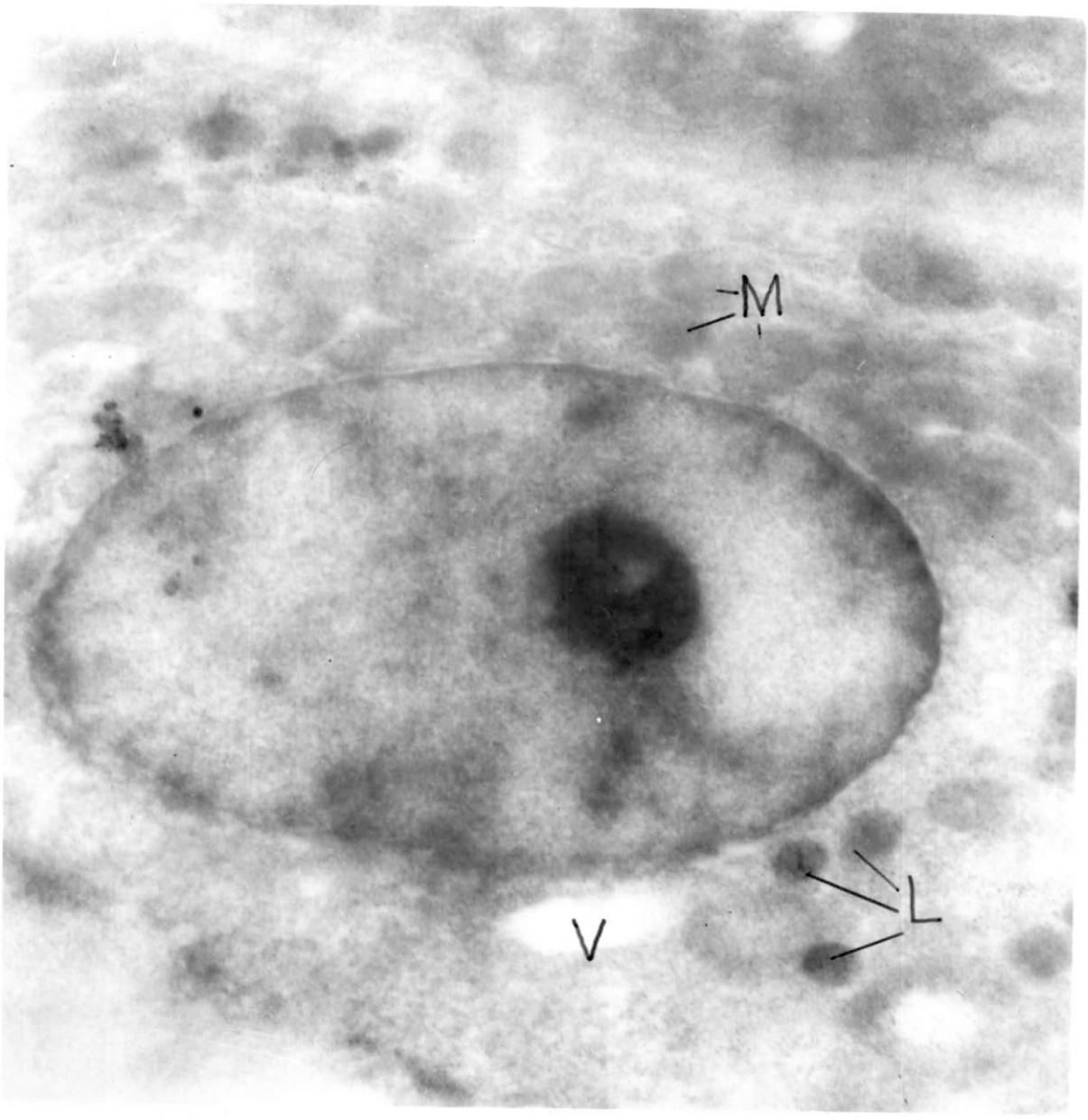

M
V
L

Fig.100 Electron micrograph - Quail - OA fed - Kidney - Glomeruli showing swollen endothelium and fusion of some foot processes (F) of podocytes seen - Erythrocyte (E) x 27,000

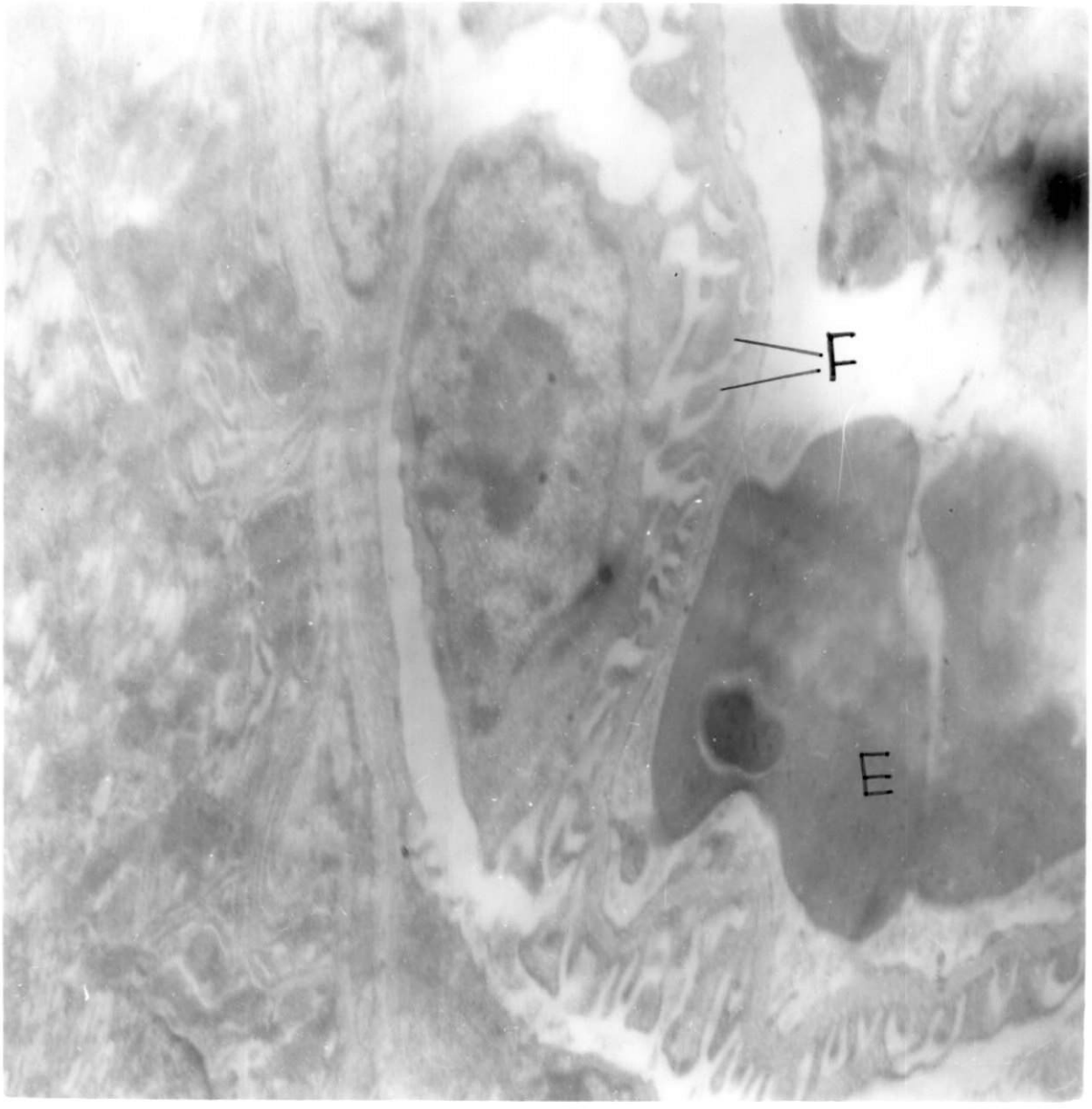

F
E

Fig.101 Electron micrograph - Quail - OA fed - Kidney - Podocyte foot processes (F) some of which appear fused x 40,000

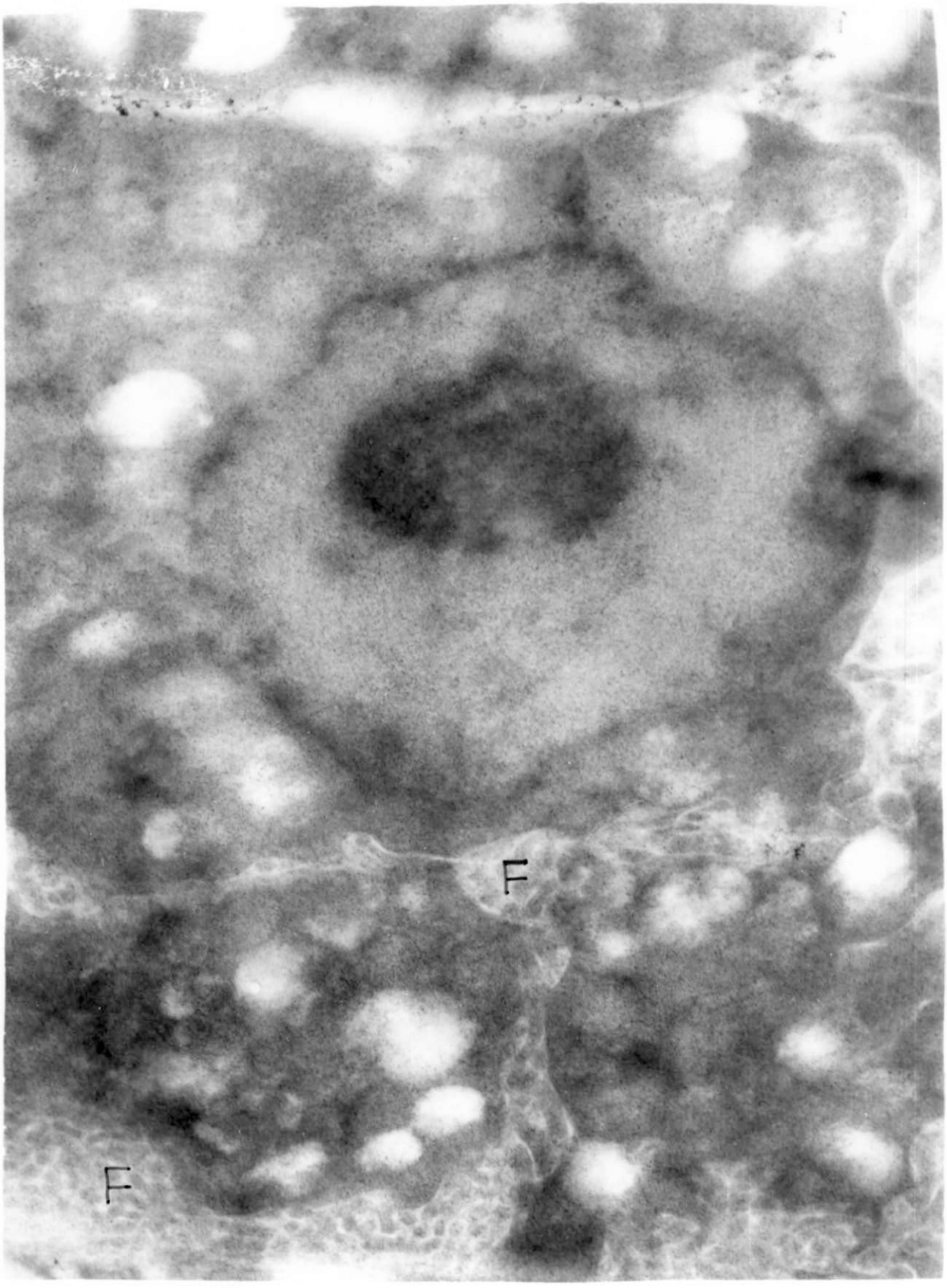

F
F

Fig.102 Electron micrograph - Quail - OA fed - Liver - Hepatic cells showing cytocavitation (C) - Loss of mitochondrial integrity - Myelinated structures (MY) noted - Erythrocyte (E) x 13,000

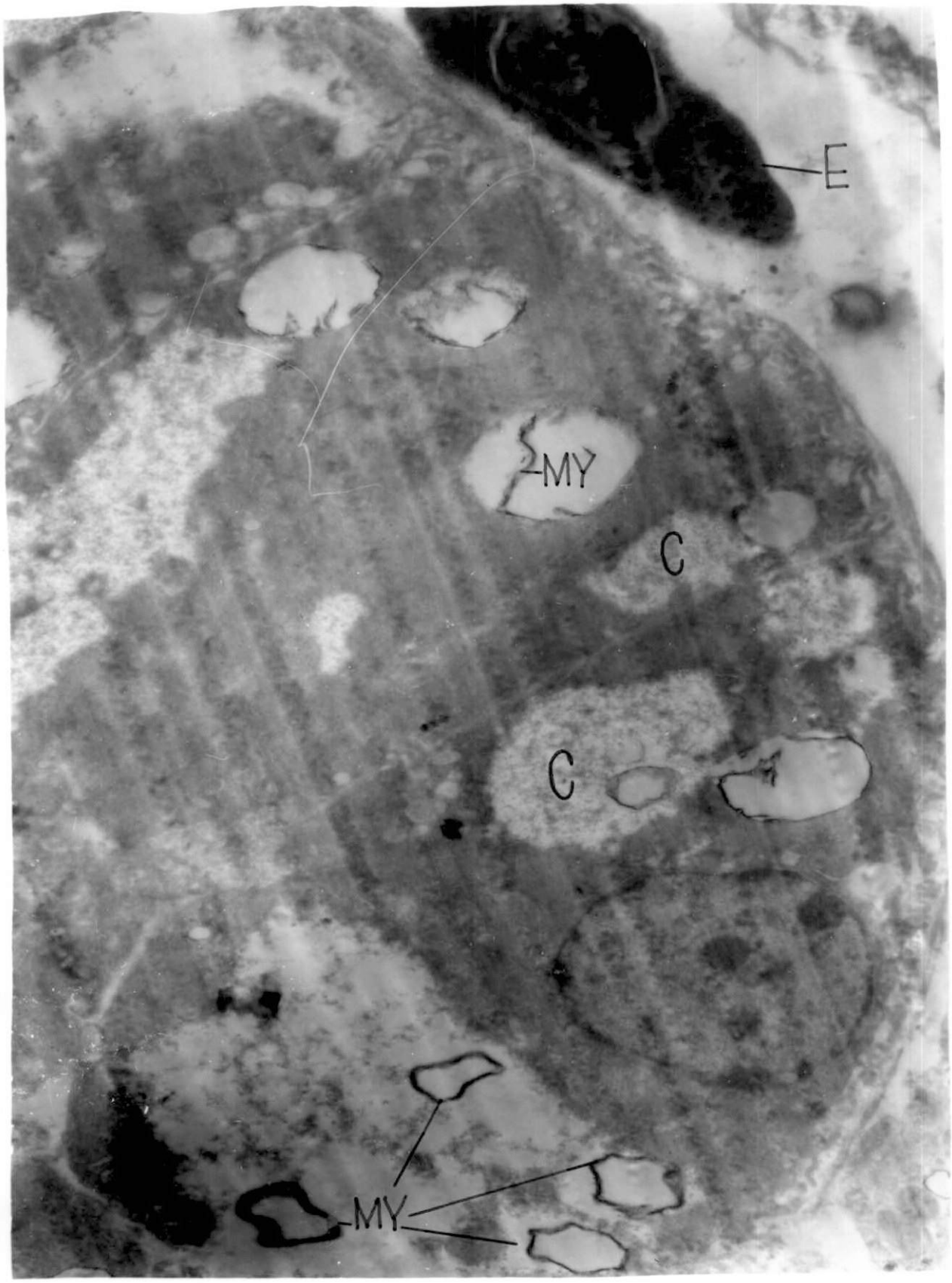

E
MY
C
C
MY

Fig.103 Electron micrograph - Quail - OA fed - Liver - Hepatic cell showing numerous damaged mitochondria (M) and other cytoplasmic organelles - Numerous lipid droplets (L) seen - Bile canaliculi (BC) x 21,000

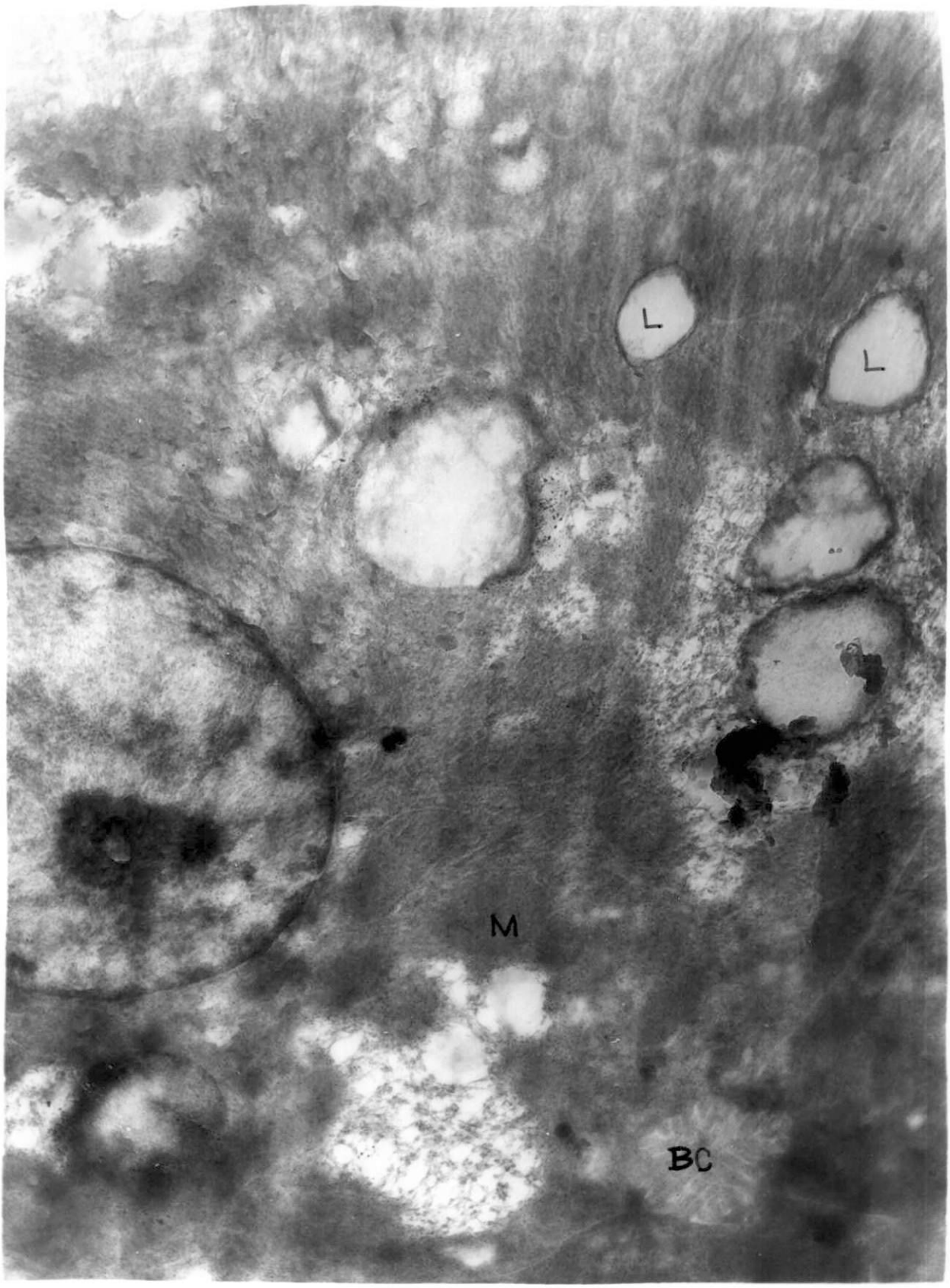

L
L
M
BC

Fig.104 Electron micrograph - Quail - OA fed - Liver - Hepatocyte with swollen mitochondria (M), some of which have lost their structural integrity x 27,000

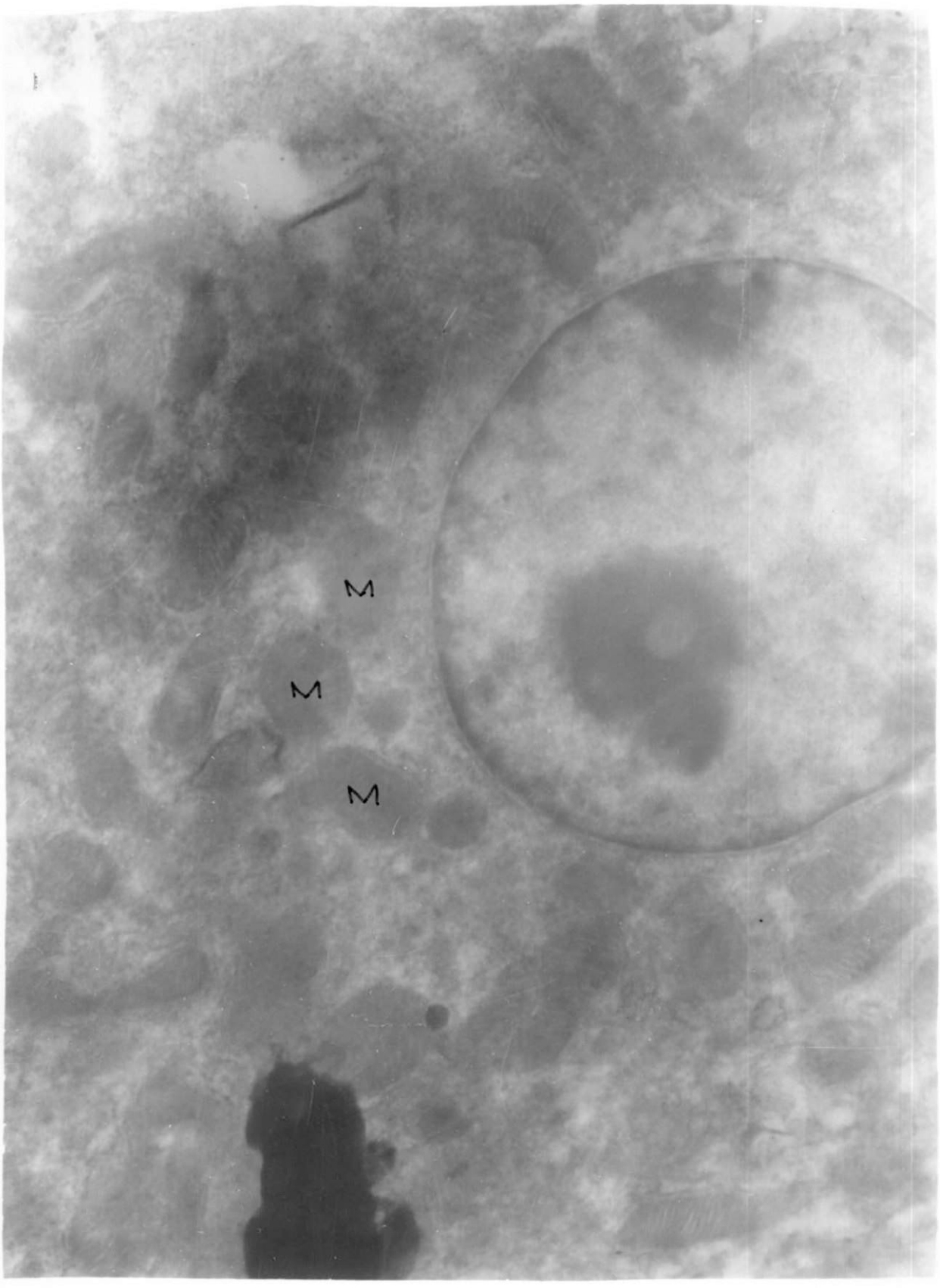

M
M
M

Fig.105 Electron micrograph - Quail - OA fed - Liver - Mitochondria (M) showing swelling of the inner mitochondrial structure and loss of cristae in some - Giant mitochondria (GM) seen x 68,000

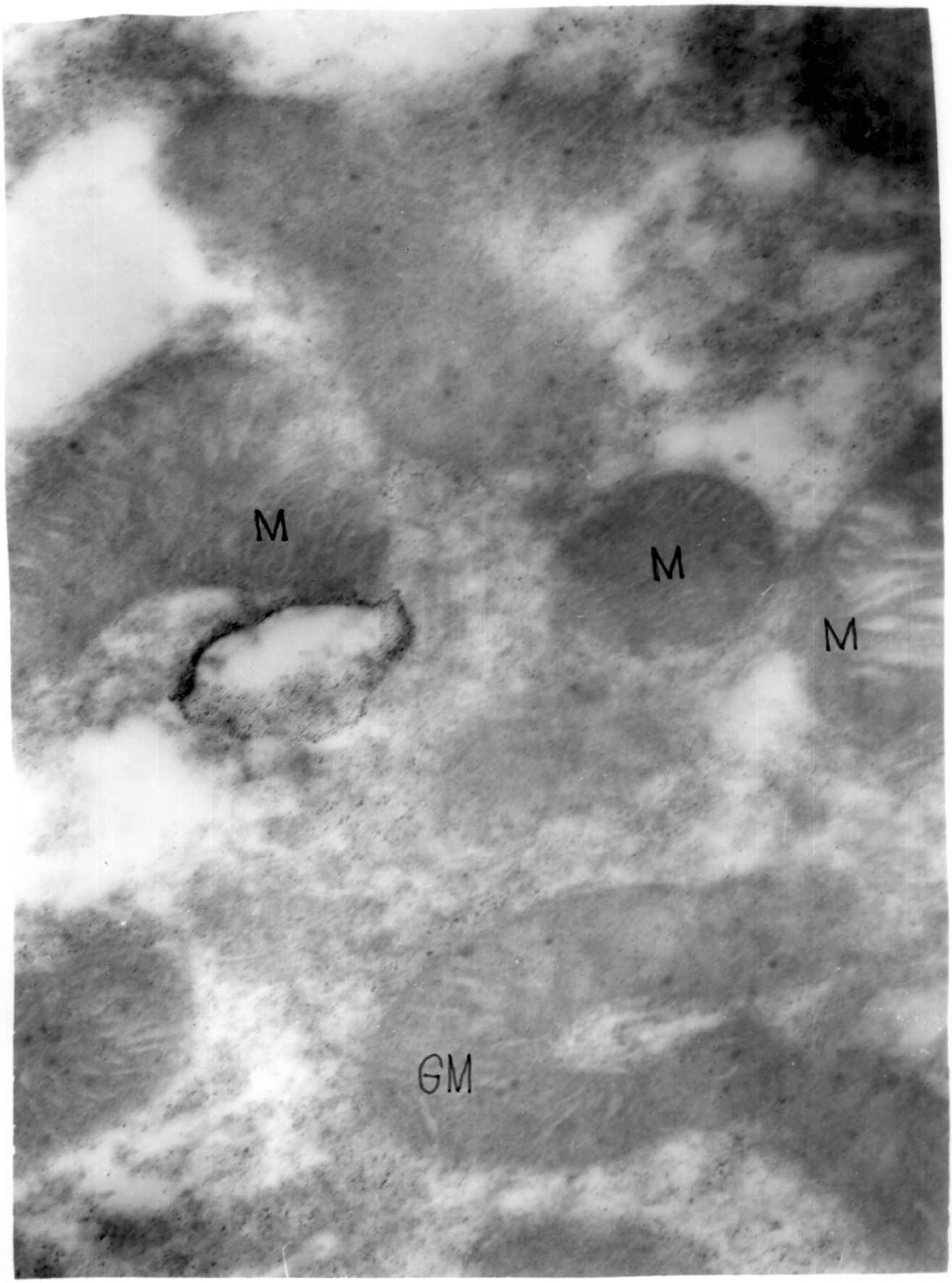

M
M
M
GM

Fig.106 Electron micrograph - Quail - OA fed - Brain - Cerebrum showing loss of cytoarchitecture and vacuolation (V) of perineuronal elements x 21,000

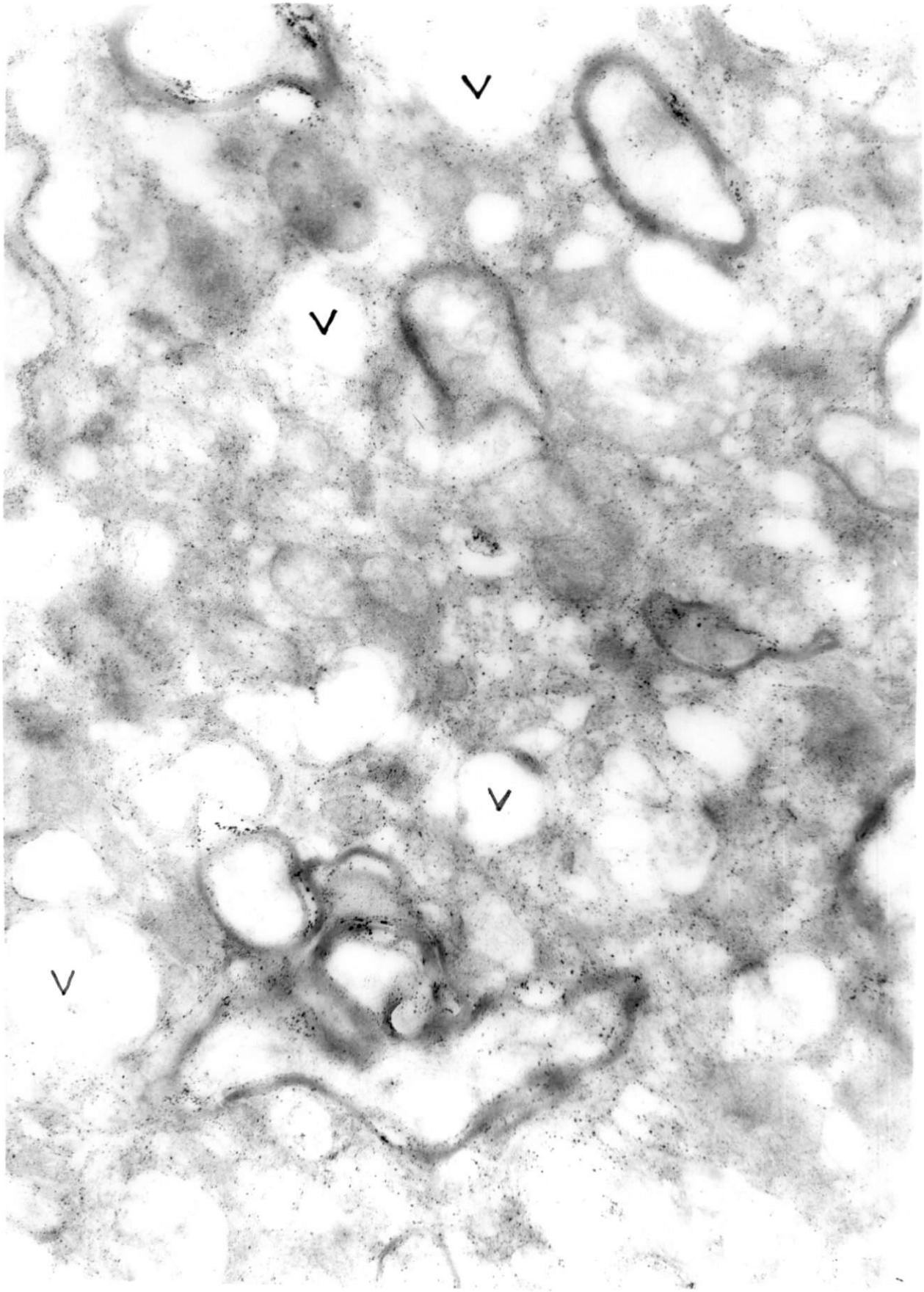

V
V
V
V

Fig.107 Electron micrograph - Quail - OA fed - Brain - Myelinated and non-myelinated axons show degenerative changes - Homogenisation and fragmentation of myelin seen x 40,000

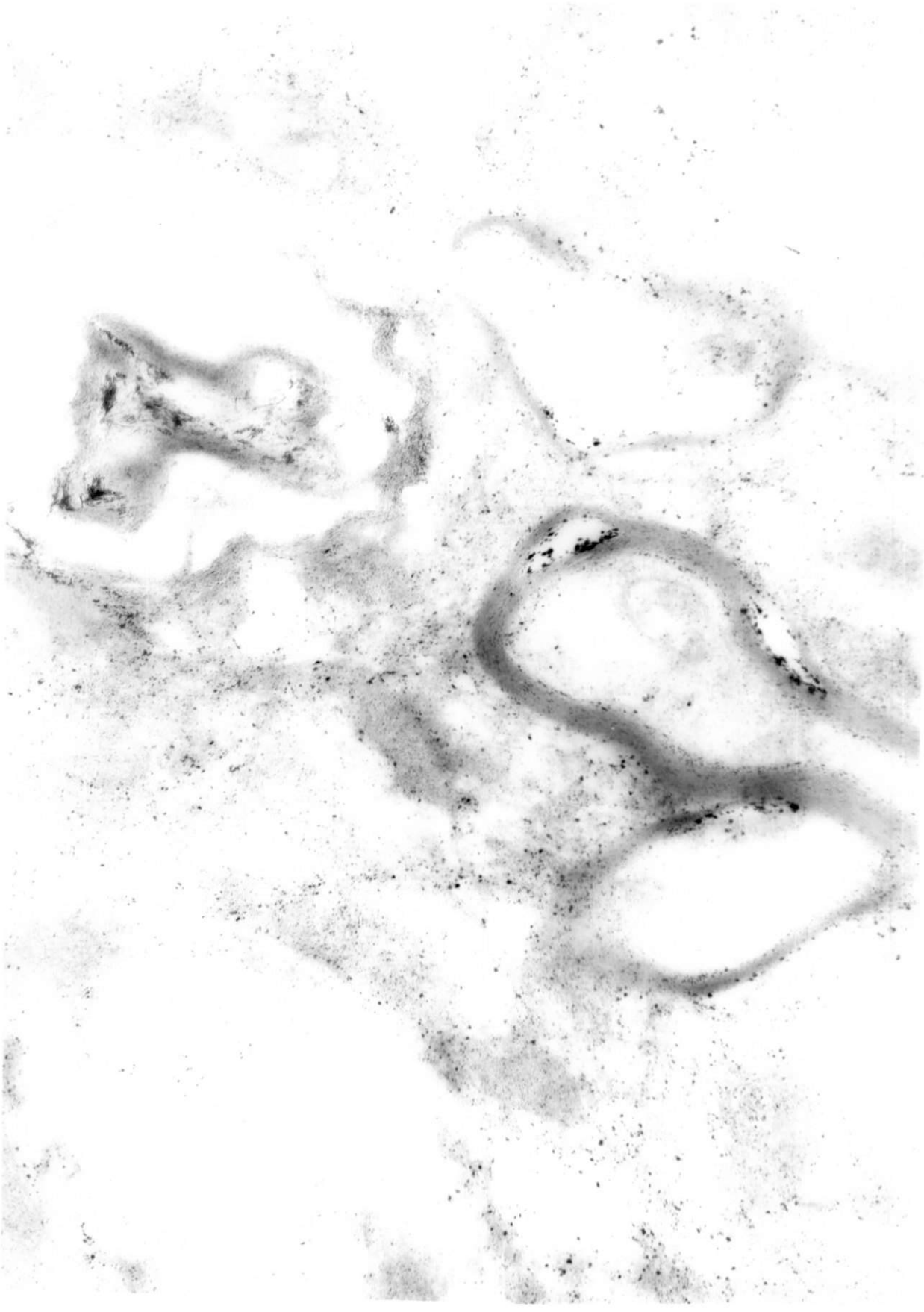
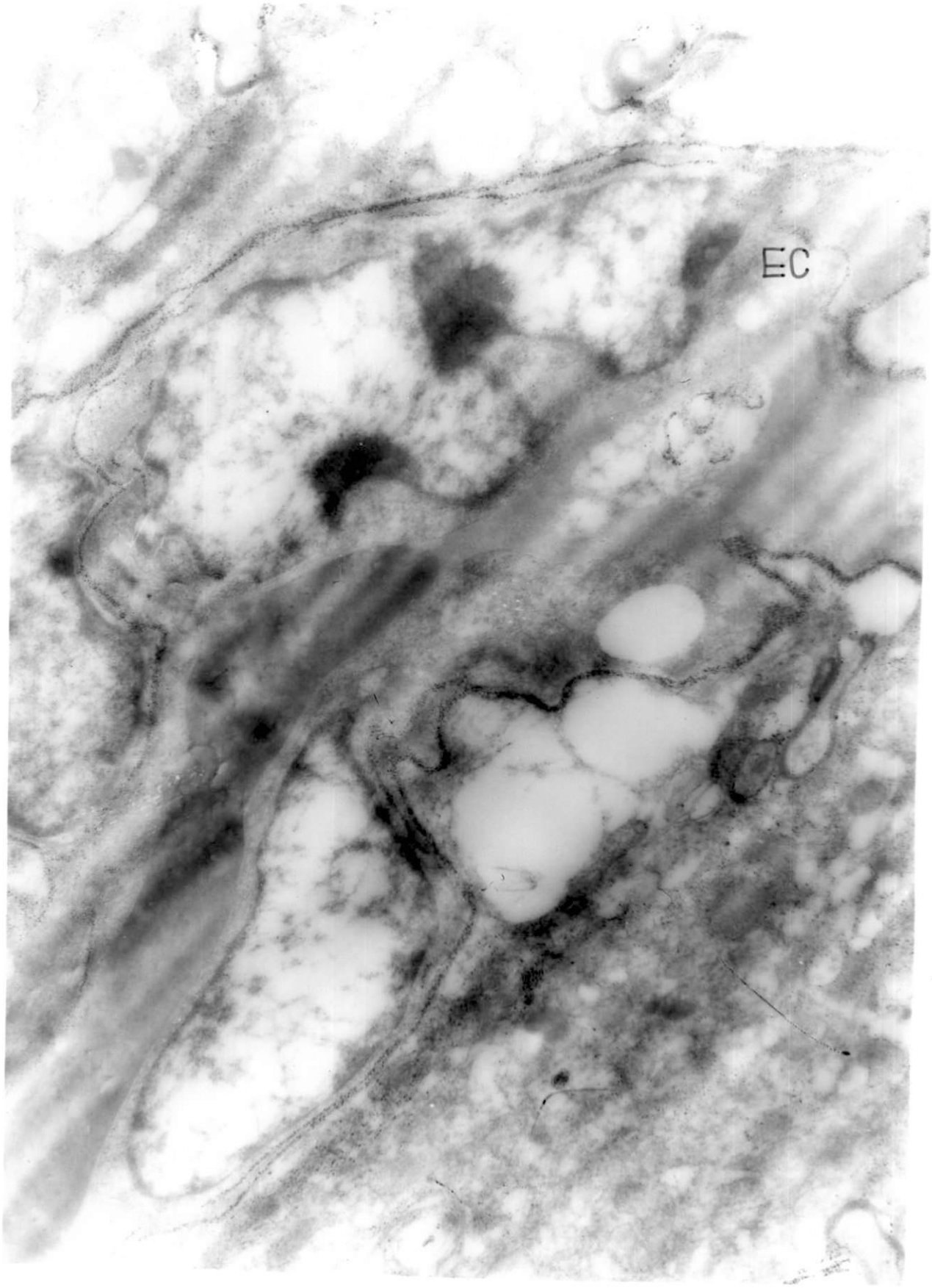


Fig.108 Electron micrograph - Quail - OA fed - Brain - Endothelial cells (EC) of blood vessel showing swelling and partial loss of nuclear chromatin x 16,000


EC

Discussion

DISCUSSION

The administration of OA caused 68.66 per cent mortality in Japanese quail embryos. This would imply that the dose level employed was toxic and the quail embryo is equally susceptible to the toxic effects of OA as in the case of chicken embryo. Prior et al. (1978) reported reduced hatchability, when Japanese quail hens were fed a diet containing 16 ppm of OA. The early eUnbryonic death is perforce a direct action of the OA on the embryo. In this context it may be pointed out that Lalithakunjamma (1987) observed 75 per cent mortality in chick embryos when they were administered 0.5 ug of OA/egg. Still et aj.. (1971) reported that OA caused foetal resorption in rats. Mayura et al. (1989) demonstrated that OA induced necrosis of embryonal mesodermal structure and neuroectoderm in rat embryos in culture.

There was highly significant decrease in the weight of the embryos and weight of bursa of Fabricius, spleen and thymus of embryos sacrificed on 13th and 16th day of incubation compared to the control embryos. The reduction in the body and organ weight was associated with severe atrophy and depletion of lymphoid elements and defective differen-tiation of lymphoid cells in the lymphoid organs. This direct

immunotoxic effect of the OA on the lymphoid cells would bring about immunosuppression , if these embryos were allowed to hatch. The changes in the lymphoid organs convincingly clarified the immunotoxic effect of OA. The young quails which may hatch out from eggs obtained from quails exposed to OA for a reasonable period of time , even at lower level, is likely to pick up infection in the immediate neonatal period due to immunodeficiency. This will lead to significant young stock mortality. Hence it is possible that depending upon the level of OA exposure either embryo mortality or immunosuppressive effect will be manifested. This has far reaching efj-ec^ en the economy of quail farming. The neonatal mortality often is erroneously diagnosed as diseases caused by pathogens, overlooking the immunosuppressive circumstances which have led to the precipitation of the disease.

The histological changes were predominantly of lympholysis in the central and peripheral lymphoid organs and akin to the observations of Lalithakunjamma (1987) in chicken embryos.

Ultrastructurally, it was clearly demonstrated that there was significant progressive organellar damage in the lymphoid cell population and arrest of maturation and differentiation of lymphoid cells. These observations clarified that OA has got direct cytolytic effect on the developing lymphoid cells of the immune system which was in agreement

with the observations of Holmberg (1992) who reported that OA caused inhibition of mitogen-induced blastogenesis of porcine blood lymphocytes in an in vitro study.

This observation has great significance since now-a-days quails are reared on an intensive system with compounded diet for commercial production of eggs on a large scale. When quails are fed a commercial ration it is likely to contain groundnut cake and other cereals which are important sources of OA. The dietary OA is likely to gain en\jry into the quail eggs and when they are set for hatching it will cause embryo mortality. This is likely to be one of the reasons for poor hatchability obtained in quail embryos. This can be explained due to the carry over of OA into the eggs. the result of this investigation, therefore, stresses on the need for checking the feed of quails for the OA level. It has to be ensured that the dietary level of OA is not exceeding the permissible level. Many times, the significance of OA as an embryotoxic mycotoxin is overlooked as aflatoxin is only attributed as a mycotoxin which causes embryopathies. This attitude has to be changed as this investigation provides ample evidence to prove the embryotoxicity of OA. Beside this, the danger of the cumulative residual effect of OA in quail eggs, on the human population consuming quail eggs containing OA has also to be borne in mind.

Besides the studies on quail embryos, systematic investigations were also undertaken to evaluate the pathological effects of OA in one month old Japanese quail.

During the experimental period quails which were fed OA, showed highly significant reduction in their body weight compared to the control group. The reduction in the growth rate appeared to be a consistent finding. While studying the effect of OA in chicken, Goliniski et al. (1983), Kubena et al. (1983), Manning and Wyatt (1984) and Huff et al. (1988) also made similar observations. Gibson et al. (1989) observed reduction in body weight in chukar partridge during ochra-toxicosis. The reduction in growth rate and consequent reduced egg production are features which affect the economy of quail farming.

Observations made in this investigation indicated an apparent increase in the weight of the liver which was mainly due to fatty change. However, this was not statistically significant. Increased liver weight during ochratoxicosis was observed by Tuker and Hamilton (1971), Huff et al. (1973), Dwivedi and Burns (1984 a), Manning and Wyatt (1984), Kubena et al. (1985), Huff et al. (1988), Kubena et al. (1989) and Gibson et al (1989, 1990) in chicken.

In OA fed quails, there was significant reduction in the weight of bursa of Fabricius in the 1st fortnight and

subsequently this reduction in the weight of bursa became highly significant, in the 2nd, 3rd and 4th fortnights. On histological examinations the reduction was demonstrated to be due to progressive loss of the lymphoid elements, during the course of the experiment. The response of the bursa of Fabricius was therefore, both time and dose dependent. It is also of significance to point out that the decrease in the bursa weight was mainly due to the atrophy of the lymphoid follicles. This observation is similar to that observed in chicken by Campbell et a_l. (1983). They reported reduced number of follicles for a given area of folds of bursa of Fabricius in experimental ochratoxicosis. The findings in quails are also in agreement with the observations of Dwivedi and Burns (1984 a) and Gibson et al. (1990) who observed reduction in the weight of bursa of Fabricius in chicken in ochratoxicosis. The reduction in the size of the bursa of Fabricius which was mainly due to loss of lymphoid elements histologically, was clarified to be associated with lack of maturation and differentiation of B lymphocytes by electron microscopic studies. The ultrastructural observations, would therefore suggest that OA has a specific affinity for the humoral system as in both embryos and adult quails, bursa was found to be affected. Immunotoxicity of OA appears to be targeted against this important lymphoid organ irrespective of the stage of the growth of the quail and it was clarified to be a specific lympholytic effect.

The weight of the spleen showed significant reduction during the 1st fortnight, this reduction turned out to be highly significant in the 2nd and 3rd fortnights. The reduction persisted in the 4th fortnight. The histological observations of less cellularity and loss of lymphoid cells in the spleen clarified the cause of reduction in weight. Similar findings were also reported by Singh et al. (1990) in broiler chicken in ochratoxicosis. In contrast to these findings Campbell et al. (1983) observed increase in the weight of spleen in chicken when given simultaneously OA and aflatoxin B, . Similarly Huff et al. (1988) and Gibson et al. (1989) also observed increase in the spleen weight during ochratoxicosis in chicken.

The weight of the thymus was not significantly reduced during the first fortnight in OA fed quails, but this reduction was highly significant in the 2nd fortnight, significant in the 3rd and in the 4th fortnight was found to be highly significant. This reduction of weight towards the end of the experiment was due to progressive loss of lymphoid cells leading to thymic atrophy. This fact is well supported by the histological evidence. Similar findings were also observed by Dwivedi and Burns (1984 a) and Singh et al. (1990) during ochratoxicosis in broiler chicken. Besides this, there was qualitative deficiency of T cells as evidenced by ultra-structural changes. The changes observed in the thymus were indicative of the immunosuppressive effects of OA and this

observation led to the conclusion that OA also induces cell-mediated immunodeficiency. It would, therefore, lead to the surmise that OA affects both humoral and cell-mediated immunity.

The data on TEC, Hb concentration and PCV pointed out an anaemic status in quails on dietary OA. This significant and progressive reduction of the TEC, Hb concentration and PCV, clearly demonstrated the action of OA on the haemopoietic system which resulted in anaemia in OA fed group throughout the experimental period. This observation is similar to the observations reported by Huff and Ruff (1982) and Bailey et al. (1989). They reported reduction in Hb concentration and PCV in ochratoxicosis in broiler chicken. Huff et al. (1988) also reported anaemia in chicken given OA. Similarly Kubena et al. (1989) reported microcytic hypochromic anaemia in broiler chicken, during ochratoxicosis. Gupta et al. (1979) reported that OA brought about significant decrease in Hb level and total red blood cells, during ochratoxicosis in mice. They clarified that anaemia could be due to the direct action of OA on the bone marrow or defective absorption of nutrients.

There was highly significant reduction in the TLC in OA fed birds, this is in contrast to the findings of Gupta et al. (1979) in mice. They observed leukocytosis when OA was given weekly at the dose rate of 5 mg/kg for 6 weeks,

intraperitoneally. The leukopenia was associated with relative heterophilia. The leukopenia must be a reflection of the immunotoxic action of OA on the lymphoid organs and the bone marrow.

The humoral immune status of OA fed quails was assessed by TSP, SAl, SGI and immunoglobulin fractions. During the course of the experiment, there was highly significant reduction in the TSP and SGI in OA fed birds. There was compensatory increase of SAl during the 1st and 2nd fortnights The significant reduction in TSP and SGI in the experimental group is a reflection of the morphological changes in the lymphoid organs and the hepatic lesions. This provides sufficient evidence for the reduction in the humoral immune response. These findings are in agreement with the observations of Kubena et al. (1984), in growing chicks and Manning and Wyatt (1984), Huff et al. (1988), Sreemannarayana et al- (1989) and Bailey et al. (1989), in chicken during ochratoxicosis. The PAGE analysis clearly demonstrated reduction in the serum protein especially the globulin fractions. This is a direct evidence which would clarify the immunosuppressive effect of OA. In this context it is relevant to point out that Singh et al. (1990) observed lowered HA titre against sheep red blood cells during ochratoxicosis in broiler chicks.

Further the PAGE analysis indicated reduction in the

immunoglobulins, IgM and IgG fractions in OA fed quails. The histologic and ultrastructural changes showed cytolysis and progressive loss of lymphoid elements in the bursa of Fabricius, particularly of the B lymphocytes, which are responsible for the immunoglobulin synthesis. This would naturally lead to defective immunoglobulin synthesis. The electron microscopic changes which revealed condensation of chromatin in the lymphocytes and lack of differentiation into plasma cells again supported this conclusion. It can, therefore, be concluded that OA has specific destructive effect on the target B cells and this adversely affects the immunoglobulin synthesising capability of B lymphocytes. The immunoglobulin synthesis in chicken is dependent on the bursa of Fabricius and other associated lymphoid organs. Dwivedi and Burns (1984 b) opined that the decreased production of immunoglobulin containing cells could be considered to be a sequel to the regression of the lymphoid organs found in chicken in ochratoxicosis. This investigation suggests that the effect of the OA on the immunoglobulin producing cells and related lymphoid organs in quails is similar to that seen in the case of chicken. The result of this observation clearly indicates the adverse biological effect of OA on the humoral immune system.

The enumeration of T lymphocytes showed reduction in T lymphocytes percentage, this reduction was not significant initially but as the experiment proceeded the reduction became

pronounced by the 4th fortnight, which was highly significant. These findings are very much similar to the observation of Singh et al. (1990) in broiler chicken during ochratoxicosis. Ghosh et al. (1991) also reported reduction of T lymphocytes during toxicosis caused by aflatoxin B, . The decrease in the T lymphocytes must be due to the severe depletion of these cells from the thymus in OA fed quails. This was very much evident in the histological picture of the thymus which revealed severe depletion of the lymphoid elements. The ultrastructural changes observed also gave evidence for the reduced and defective functioning of the T lymphocyte population. The reduction of T lymphocyte population due to administration of OA is one of the important factors in bringing down the cell-mediated immunity in quails.

The immunological response to Fowl pox antigen is predominantly cell-mediated type. Therefore the response of OA fed quails to Fowl pox antigen was assessed by LMIT. A highly significant increase in the migration indices in the OA fed birds in response to the Fowl pox antigen was observed. The LMIT is a very sensitive index of the cell-mediated immunity, Kantoch et al. (1979), Falecka (1979), Karpe (1982), Yadav et al. (1986), Chaturvedi and Sharma (1986), Chandrasekar et al. (1989) and Pani et al. (1989). Therefore, this increase in migration indices in OA fed birds, no doubt is a fool - proof indication of severe reduction in the cellular immunity.

The cutaneous response to DNCB was more intense in quails of the control group. While, there was highly significant reduction in skin thickness of OA fed birds compared to the controls. This difference in skin thickness was highly significant in 24 h, 48 h and 72 h post challenge. The DNCB skin test is one, of the reliable tests for assessing the cellular immunity according to Eliber and Morton (1970), Brummerstedt and Basse (1973), Reddi et al. (1981) and Rajan et al. (1981). This observation gives proof to the surmise that there was reduction of cell-mediated immune response in quails dosed with OA. Nair (1986) evaluated the cell-mediated immunity in aflatoxicosis in pigs employing DNCB and reported lowered immune response in toxin fed pigs. Chaudhary (1987) employed this test in assessing the cellular immune response during Aspergillosis in Japanese quail and recorded significant lowering of cell-mediated immunity.

There was decreased response to PHA-M in OA fed birds compared to that of controls. The response was less in OA fed birds which was appreciated during the first 24 h. This evidently shows that the OA fed birds were less competent in their cellular immune reactions. Marchalonis (1978) in man, Thein et al. (1981) in horse, Rajan et al. (1982) in goat and Reddi and Rajan (1984) in cattle, observed that response to PHA-M was a reliable test to monitor and evaluate the cellular immunity. Similarly in aflatoxicosis of pigs, Nair

(1986) assessed the cell-mediated immune response, employing PHA-M.

The result of GVHR which was represented by the changes in the spleen indices in OA fed quails and control birds, revealed highly significant reduction in OA fed birds. This finding is in agreement with the finding of Singh et al. (1990). They observed significant reduction in the spleen indices of broiler chicken fed OA and Ghosh £t a_l. (1991) in chicken during aflatoxicosis.

The various reliable immune markers employed to assess the humoral and cell-mediated immunity have very precisely indicated that there has been significant reduction in both responses in the quails fed OA and the hypothesis that OA is an immunosuppressive mycotoxin has been proved. The response of the immune system of quails appear to be in no way different from chicken as the findings were similar to that reported in chicken. This would mean that OA is also equally immunosuppressive in quails as in the case of chicken. The observations made in this investigation has brought to light the importance of OA as an immunosuppressive agent. This is an important observation as this will explain the outbreak of diseases in flocks of quails and breakdown of immunity following vaccination. Quails when they are in their natural habitat are considered to be relatively resistant to diseases, and this is a plus point in quail farming. However, the

experience in the intensive system of rearing of quails has shown that they also do get the disease like Marek's disease, coccidiosis, Ranikhet disease etc. which are not seen in the free habitat. However, the domestication have made them susceptible to these infections- These infections might be due to the adverse effect of dietary mycotoxins like OA on the immune system. This investigation clearly indicated that OA is as potent immunosuppressive as aflatoxin. The immuno-toxicity of OA has to be looked into and its effect in precipitating diseases should not be overlookd nor under-estimated. The farmers have to be cautioned, and the need for prescribing a diet containing safe level of the mycotoxins in maintaining an economically viable quail farming has to be stressed.

The histological changes in the bursa of Fabricius, thymus and spleen of embryos inoculated with OA, were characterised by degenerative and necrotic changes. This was clarified by ultrastructural studies. In adults which were fed OA, there was severe cytolytic damage to lymphocytes and even lympholysis in the bursa of Fabricius, spleen and thymus. These changes could be attributed to the toxic action of OA on the lymphoid cells and consequent effect on .protein synthesis. It has been established that the primary effect of OA is inhibition of protein synthesis, this effect is specific and involves a competetive inhibition of phenylalanine-tRNA^{Phe} synthetase which is necessary for fundamental amino-acylation

steps in the protein synthesis. Secondary to this, both RNA- and DNA synthesis may be inhibited (Holmberg, 1992).

The gross, light microscopic and ultrastructural studies undertaken in this investigation have shown lack of differentiation of lymphoid cells in the quail embryos inoculated with OA. OA has been shown to interfere with the normal histogenesis of lymphoid organs in chicken embryos (Lalithakunjamma, 1987). In the adult chicken similar observations were made by Dwivedi and Burns (1984 a). The changes observed in quail embryos appear to be identical to that reported in chicken embryos.

The degenerative changes which were observed in the bursa of Fabricius, spleen and thymus will have adverse effect on the immunocompetency of quails. Ultra$tructural observation of moderate to severe organellar damage in the lymphoid cells of the bursa of Fabricius, spleen and thymus, both in the embryos inoculated with OA and in the adults which were fed dietary OA, gives adequate proof of this surmise. The quails which hatch out from such embryos will be immunologically deficient and are likely to get perinatal infections leading to mortality.

According to Meister and Chan (1974) OA is an inhibitor of the mitochondrial transport system and this could very well be the reason for the structural change observed in the

mitochondria. In addition, the direct action of toxin on the membrane system could itself result in series of structural defects (Lalithakunjamma, 1987). The necrosis of the cells is related to the inhibition of the phosphorylation and arrest of mitochondrial respiration. Khan et al. (1989) suggested another mechanism for the toxic effect of OA. They stated that the toxin perturb microsomal calcium homeostasis by an impairment of the endoplasmic reticulum membrane probably via enhanced lipid peroxidation. This could alter calcium-stimulated enzymes and hormones which can produce structural and functional changes of the cell, leading to cellular necrosis. Maryamma (1983) suggested that OA damages the membrane of mitochondria, impairing enzyme activities with consequent disturbances of cellular functions. The nucleolar fragmentation could be the result of metabolic disturbances due to ATP deficiency or inhibition of RNA synthesis, (Shinozuka et al. 1970). Similarly Simard and Bernhad (1966) postulated that nucleolar segregation probably reflected DNA binding and inhibition of DNA dependent RNA synthesis due to the loss of template activity of DNA. There was ample fine structural damages in the lymphocyte population in the chief lymphoid organs and these would substantiate the conclusion that there is immunosuppression in OA toxicity at this dose level.

The histological and ultrastructural changes in the

kidney indicated as affinity of OA for the proximal convoluted tubules. This must be due to the selective deposition of the toxin in this region. OA was demonstrated at this site by immunofluorescence technique (Elling, 1977). Chu (1971) observed that there is interaction between OA and albumin and suggested that the albumin may serve as a transport molecule for OA. The albumin in the glomerular filtrate is absorbed by the proximal tubules and this was suggested to be the mechanism by which OA got localised in the proximal convoluted tubules. The observation made in this study showed increase in SA1 levels in OA fed quails, hence there could be correlation between the rise in SAl and proximal tubular damage. Similar findings were reported by Krogh etal.t 1976), Dwivedi and Burns (1984 a) in chicken, Maryamma (1983) in goat, Kitchen et al. (1977a) in dog and Munro et al. (1974) in rat. There was proteinaceous material in the proximal convoluted tubules as a result of necrosis and desquamation of epithelial cells in the tubules, as observed by Huff et al. (1975) in chicken. The effect of tubular changes together with thickened glomerular basement membrane reflect impaired renal function in the OA fed quails.

The ultrastructural alterations which included advanced cytologic and nuclear changes mainly in the proximal convoluted tubular epithelium, supported the histological findings. The organellar destruction and loss of cell surface villi, with numerous cytoplasmic vacuoles and mitochondrial

damage were all suggestive of severe adverse action of OA on the cellular components of the kidney. Similar observations were also made by Dwivedi et al. (1984) in chicken who reported changes in the mitochondrial configurations. According to Meisner and Chan (1974), the mitochondrial damage with loss of cristae is mainly due to the action of the OA on the mitochondrial transport system. The nuclear changes reflect the metabolic disturbances either due to the lack of ATP or inhibition of RNA synthesis of OA (Shinozuka et al. 1970). The glomeruli showed swollen endothelial cells with swollen mitochondria and fused foot processes. Maryamma and Nair (1990) stated that this alteration of podocytes foot processes might be the major factor responsible for aiding passage of macromolecular substances through the glomerular membrane. The result of this investigation clearly demonstrated that OA is a potent nephrotoxin capable of inducing damages to the epithelial cells, specially the proximal convoluted tubules in Japanese quail. The changes in general resembled those earlier reported in chicken.

The histological observations of the liver in the OA fed quails, revealed moderate to severe degenerative changes, focal area of necrosis and fatty change were more pronounced on the 4th fortnight. These observations are in agreement with the findings of Dwivedi and Burns (1984 a) in chicken and Zimmermann et al. (1978) in rat. In support of the histological observations, the ultrastructural investigations of the

liver, revealed various organellar changes mainly mitochondrial damages. The changes varied from mild swelling of the mitochondria to severe cytolysis and nuclear fragmentation with granular and electron dense cytoplasmic matrix. The changes although varied from mild to severe, were similar to the observations of Dwivedi et a_l. (1984 a) in OA toxicity of chicken. They reported misshapened mitochondria in the kidney. However, in the present study the glycogen particles in the liver were not as conspicuous as observed by them, whereas presence of lipid droplets were very well correlated to their observations in chicken. Vesiculation and rupture of the rough surface endoplasmic reticulum and the presence of changes in chromatin, which was more of euchromatin with few aggregation of heterochromatin suggested an impaired protein synthesis. The mallory bodies as described by Maryamma (1983) in the liver of goats during ochratoxicosis, were not observed in this study. The investigations in the kidney and liver suggested that OA is more nephrotoxic than hepatotoxic in quails as was reported in chicken (Dwivedi et al. 1984 a) .

The histological observations in the brain revealed engorged blood vessels, focal oedema, pyknosis, karyorrhexis and satellitosis. The vascular endothelium was swollen. These observations were futher clarified by the electron microscopic findings which included, vacuolations indicating

oedema and ' extensive alterations in axons. The multilayer appearance of myelin sheath was not evident. These findings indicated the toxic action of OA on the brain tissues. OA was reported to cause oedema and degeneration of components of the developing brain in chicken embryos (Lalithakunjamma, 1987). The results of this investigation indicated that dietary OA at this dose level can inflict damages to the brain tissues. Similarly cerebral necrosis was found in mouse foetuses from dams treated with OA (Krogh, 1987). There was swelling of the endothelial cells of the blood vessels in the brain. This indicated that the OA had entered the brain by haematogenous route and by binding to the histones and other macromolecules of these cells caused nuclear changes.

There was immunosuppression following the administration of OA and an experimental study was undertaken to assess the possibilities of averting the immunosuppression induced by OA using levamisole as an immunostimulator.

There was highly significant increase in the body weight and weight of spleen following the withdrawal of OA. The increase in the body weight could be mainly due to the withdrawal effect of OA leading to better absorption of nutrients and increased protein metabolism. Besides this, the kidney and liver were free of the toxic effects of OA. The increase in the weight of the spleen could be due to the

arrest of the degenerative processes since the OA was withdrawn and simultaneous lymphocytic stimulation by levamisole. The weights of the bursa of Fabricius and thymus did not show any increase after stimulation. This can be attributed to the physiological involution that had set in these organs after three months of age, in addition to the pathological effect brought about by OA, though the stimulation in these organs was triggered by levamisole.

There was increase in the TEC and PCV following the levamisole administration. However, the increase in Hb concentration was not of significance. This could be directly associated with the regenerative processes in the spleen and liver following the withdrawal of OA and stimulatory effect of levamisole.

There was significant leukocytosis and this was associated lwith lymphocytosis and heteropenia. Similar observations were made in calves by Nalinikumari and Choudhari (1986) following levamisole administration.

Vanselow (1987) stated that levamisole is physiologically a thymomimetic agent and restored compromised immune response by restoring the effector functions of T lymphocytes and macrophages. The levamisole induced T lymphocytosis observed in this experiment, could be attributed to the direct

action of levamisole on this subpopulation of lymphocytes. Similarly Palanski et al. (1990) also reported increased T lymphocytes in chicken when given levamisole. Antibody formation to most bacterial antigens is T cell dependent and an increase in T lymphocytes could enhance antibody production (Confer et al. 1985).

There was highly significant increase in TSP and SGI after immunostimulation by levamisole. It was clarified further that this increase was associated with an increase in the IgM and IgG levels. This is an observation supported by findings of Hassan et al. (1989). They observed increased antibody titre in chicken following Newcastle disease virus vaccine after levamisole administration. In chicken, Palanski et al. (1990) reported increased B lymphocyte count after levamisole administration. These findings indicate that levamisole enhances the humoral immune response. Therefore, the increase in IgM and IgG levels in quails must be an after effect of stimulation of B lymphocytes by levamisole.

The immunostimulatory response induced by levamisole was further convincingly proved by the significant reduction in the migration indices of leukocytes against Fowl pox antigen after the levamisole administration. This in turn clarifies that levamisole could be used to stimulate the cell-mediated immunity in immunosuppressed quails. Abdul Rahman et al. (1989) reported enhanced cell-mediated immunity in

chicken, following levamisole administration which was assessed by LMIT.

The increase in the skin reaction to DNCB and PHA-M after the stimulation by levamisole further clarified the immunostimulatory effect of levamisole. Hassan et al. (1989) reported increased delayed hypersensitivity in chicken given levamisole. Similarly levamisole enhanced the skin test response and mitogen induced lymphocyte proliferation in pigs (Hennessy et al_. 1987).

The results of this investigation employing various immune markers have, therefore, clearly indicated that levamisole has stimulatory action on both cell-mediated and humoral immune response in immunologically deficient quails. An analysis of data suggested that levamisole stimulated the immune system of OA fed quail but not to or above the normal immune status of the individuals. According to Bruner and Muscoplat (1980) the effect of levamisole depends on the dose level, time of administration and condition of the animal. The dose employed in this study appears to be the correct dose needed to induce an immunostimulatory effect. This observation has importance since levamisole can be advocated in field situations to restore the immunosuppression induced by OA. To a certain extent, depending upon the degree of immunosuppression, levamisole could be used to prevent the

breakdown of immunity induced by OA in the field conditions. Symoens et al. (1979) stated that levamisole can be used to restore cell-mediated immunity in mice or in elderly humans as it protects against variety of immunosuppressing diseases. Levamisole, which was orginally used as a therapeutic drug has been shown to have immunostimulatory effect in different animal systems (Vanselow, 1987) and it has been used more as an immunorestorative agent.

The light microscopic and ultrastructural studies have clearly demonstrated lymphocidal effect and poor differentiation of lymphoid cells into plasma cells in the lymphoid organs, and the PAGE analysis has indicated defective immunoglobulin synthesis and consequent decreased humoral immune response. The assessment of the cell-mediated immune response employing various elegant markers has convincingly demonstrated a defective cell-mediated response. Therefore, the immune markers employed to assess the function of both the cell-mediated and humoral immune response, have specifically brought to light the adverse effect of OA on the dichotomous immune system. The objective of the study was to assess the effect of OA on the immune system of quails. This investigation, therefore, has brought to light that OA besides having a direct nephrotoxic effect has also an immunotoxic effect not only on the lymphoid system of adult quails but also on the developing lymphoid organs of quail embryos.

The histological changes induced by OA in various organ systems of quails like the kidney, liver have been well documented earlier, in addition to these organ systems the adverse biological effect of OA on the brain tissues of quail was demonstrated in this investigation. However, there has not been any systematic investigation so far, to assess the immunotoxic effect of OA in quails. This pioneering study has focussed attention on the adverse effect of OA on the humoral and cell-mediated immune response of quails. The changes observed in quails appear to be almost similar to that observed in chicken.

The observations made during the course of this investigation and conclusion drawn have a great bearing on the economy of quail farming. The personnel in charge of the health care programme has to bear in mind the immunotoxic effect of OA and also the synergistic effect of OA and aflatoxin. The immunotoxic effects of OA and aflatoxin are almost similar and the simultaneous occurrence of these toxins in the feed could be very much dangerous and this could lead to immunological crisis and outbreak of various diseases in quail farms leading to severe economic loss.

The objective of this investigation also included an assessment of the effect of levamisole as an immunomodulator.

Employing various immunological markers, it was demonstrated that levamisole has an important immunoregulatory function and it could be used to boost up the immune system in th OA induced immunodeficiency. This observation has practical relevance as it could be advocated in field situations, to stimulate the immune system and overcome the immunological deficiency, so that infections can be reduced and mortality can be prevented.

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SUMMARY

An experimental study was undertaken for the first time to investigate into the immunopathological aspects of ochratoxicosis in Japanese quail embryo and Japanese quail. This investigation also included the study of immunoregulatory effect of levamisole in immunosuppression induced by OA in quail.

Quail embryos were administered OA by the intra-airsac route at the dose rate of 0.02 /Ug per embryo. There was 68.66 per cent mortality which clearly indicated the direct toxic action of the OA.

The embryos which were sacrificed on the 13th and 16th day of incubation showed significant reduction in the body weight and weight of bursa of Fabricius, spleen and thymus. This clarified that OA has sufficient adverse biological effect on the lymphoid organs.

Histological changes in these lymphoid organs consisted of atrophy, necrosis and depletion of lymphoid elements.

Ultrastructurally the changes were time dependent. The changes were characterised by variation in the shape of

the nucleus and condensation of the granular and fibrillar components of the nucleoli. It was, therefore, established that OA can cause severe damage to the developing lymphoid organs of the embryos and bring about immunodeficiency. It was pointed out that if these embryos were to hatch out, the immunodeficiency would lead to various disease outbreaks. The significance of this observation in economic quail farming was brought to light.

OA at the dose rate of 50 /ug/bird/day was administered through oesophageal intubation to quails and pathological changes were sequentially documented with special reference to the immune system.

In the OA fed quails, the clinical signs included emaciation and hyperexcitation at the later part of the experiment. There was highly significant reduction in the body weight, weight of the bursa of Fabricius, spleen and thymus. The reduction in the weight of the lymphoid organs gave evidence to the adverse effects of OA on the immune system of quails and immunosuppression.

It was demonstrated by employing various haematolo-gical markers like total erythrocyte count (TEC), haemo-globin (Hb) concentration and packed cell volume (PCV), that the OA has significant damaging effect on the haemopoietic system.

Employing various elegant monitoring tools to evaluate the humoral immune sytem like the estimation of total serum protein (TSP), serum globulin (SGI) and various serum immuno-glubulin fractions, it was clarified that OA at this dose level had significant biological effect on the humoral immune system.

The highly significant reduction in total leukocyte count (TLC) and T cell dependent lymphopenia, proved the immunosuppressive effect of OA. The reduction in T cells, increase migration indices in response to Fowl pox antigen in leukocyte migration inhibition test (LMIT) and reduction in the cutaneous response to 2,4-dinitrochlorobetnzene (DNCB) and phytohaemagglutinin-M (PHA-M), the decrease gpleen indices in graft versus host reaction (GVHR), clarified the immuno-toxicity of OA on the cell-mediated immune sytem.

By clarifing the effect of OA on the humoral and cell-mediated immune response, the immunosuppressive effect of OA was brought to light and the role in the precipitation of disease outbreaks was highlighted.

The gross and histological features of OA induced toxicity consisted of, pale and friable livers, congested and haemorrhagic kidney, mild enteritis, atrophied bursa of Fabricius, spleen and thymus. Thymus showed petechial haemorrhages and brain revealed cerebral congestion. These observations highlighted the effect of OA on these organs.

It was clarified by histological and ultrastructural studies that OA has direct effect on the lymphoid organs and kidney. Histologically the lymphoid organs showed degenerative changes and loss of lymphoid elements. The histological changes observed, were confirmed by the ultrastructural studies. Ultrastructurally the bursa of Fabricius showed alteration in the lymphoid and epithelial components. Lytic cytoplasm and nuclear condensation clearly demonstrated that OA had inhibitory effect on the protein synthesis. The definite indication of transformation of the lymphoid cells into plasmacytoid series of cells was lacking. Similar cellular changes were observed in the spleen and thymus. The demonstration in the lymphoid organs of defective blast transformation and poor differentiation into plasma cells gave evidence for the clinical manifestation of defective humoral and cell-mediated immune response in OA fed quails.

Kidney tubules were dilated and necrosis of the proximal convoluted tubules were seen along with congestion and focal haemorrhages. Thickening of the glomerular basement membrane was evident. Ultrastructurally advanced cytoplasmic and nuclear changes, fragmentation and lysis of plasma membrane at the luminal surface were seen. Numerous vacuoles in the cytoplasm gave the appearance of a honey comb to the cytoplasm. Fusion of the podocyte foot processes was evident. This investigation specifically brought to light the

effect of OA on the kidney of quail which was similar to that reported in chicken.

Liver showed varying degree of fatty change. Ultra-structurally mitochondrial swelling and abnormal mitochondria to severe cytolytic changes were observed.

The changes in the brain included focal oedema, pyknosis, karyorrhexis, satellitosis of microglial cells and swelling of vascular endothelium. Ultrastructurally the brain of OA fed quails showed separation of neuronal elements which formed a loose cytoarchitecture with fragmentation and vacuolation of perinueronal elements, indicating oedema.

The novel study undertaken to assess the biological effects of OA in quail in organ system other than the kidney demonstrated taht OA had significant biological effect on the nervous, haemopoietic and hepatic systems. The biological effects of OA on the nervous system of quails have not been recorded earlier.

No gross and histological changes were seen in the heart.

Immunostimulation with levamisole at the dose rate of 0.1 mg/quail two doses at 4 day interval, caused lymphocytic leukocytosis. There was significant increase in the TSP and

SGI levels with increase in IgM and IgG. These observations indicated enhanced humoral immunity. The cellular immune response was also elevated after the administration of levamisole. This was characterised by T cell associated lymphocytosis, increased cutaneous response to DNCB and PHA-M and decreased migration indices in response to Fowl pox antigen in LMIT.

There was also elevation of TEC, Hb concentration and PCV following levamisole administration. Light microscopic studies revealed hyperplasia and blastoid transformation in the bursa of Fabricius, spleen and thymus. This gave support to the observation of increased immunological response. It was, therefore, clarified that levamisole had specific immuno-stimulatory effect in immunodeficiency induced by OA and it could be recommended for immunostimulation in the field situations v/here immunodeficiency is encountered following OA toxicity.

By this investigation, it was demonstrated, that OA besides having proven nephrotoxic effect has also significant adverse biological effect on the dichotomous immune system. It was also demonstrated to have adverse effect on the hepatic, haemopoietic and nervous systems.

The immunodeficiency induced by OA was shown to be beneficially modulated by the administration of levamisole and levamisole was recommended as a immunorestorative.

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OCHRATOXICOSIS IN QUAILS
(COTURNIX COTURNIX *JAPONICA)*
WITH SPECIAL REFERENCE TO IMMUNOPATHOLOGICAL RESPONSE

By

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ABSTRACT OF A THESIS

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ABSTRACT

In this investigation the pathological effects of OA in quail embryo and adult quail were studied with special reference to the immune system. Ochratoxin A (OA) was inoculated into the quail embryos, at the dose rate of 0.02 ^g per embryo. There was 68.66 per cent mortality which clearly indicated the direct action of OA. The embryos which were sacrificed on the 13th and 16th day of incubation showed significant reduction in the body weight, weight of bursa of Fabricius, spleen and thymus. Histological changes in these lymphoid organs were, atrophy, necrosis and depletion of lymphoid elements.

Ultrastructurally the changes were time dependent. There were changes in the shape of the nucleus with condensation of granular and fibrillar components of the nucleoli. The adverse immunobiological effect of OA on the developing lymphoid organs was established by histological and ultrastructural studies.

OA at the dose rate of 50 /ag/bird/day for a period of 60 days was administered through oesophageal intubation. Pathological changes were sequentially documented with special reference to the immune system.

In the OA fed quails, the clinical signs included emaciation and hyperexcitation at the later part of the experiment. There was highly significant reduction in the body weight, weight of bursa of Fabricius, spleen and thymus. The reduction in the weight of lymphoid organs gave evidence to the adverse effects of OA on the immune system.

There was reduction in the total erythrocyte count (TEC), haemoglobin (Hb) concentration and packed cell volume in OA fed birds indicating the significant damaging effect on the haemopoietic sytem. Estimation of total serum protein (TSP), serum globulin (SGI) and serum immunoglobulin fractions clarified the biological adverse effect of OA on the humoral immune system. Highly significant reduction in total leukocyte count (TLC) and T cell dependent lymphopenia proved the immunosuppressive effect of OA.

There was reduction in T cells, increase migration indices in response to Fowl pox antigen in leukocyte migration inhibition test (LMIT), the reduction in the cutaneous response to 2,4-dinitrochlorobenzene (DNCB) and phytohaema-gglutinin-M (PHA-M) and spleen indices in the graft versus host reaction (GVHR), clarified the immunotoxicity of OA on the cell-mediated immune system.

Grossly the OA fed birds had pale, friable liver, congested and haemorrhagic kidney, mild enteritis, atrophied

bursa of Fabricius, spleen and thymus. Thymus showed petechial haemorrhages and brain revealed cerebral congestion. The lymphoid organs showed degenerative changes and loss of lymphoid elements. Ultrastructurally the bursa of Fabricius showed alteration in lymphoid and epithelial components, lytic cytoplasm and nuclear condensation. The transformation of lymphoid cells into plasmacytoid series of cells was lacking. Similar cellular changes were also seen in the spleen and thymus. These changes gave evidence for the clinical manifestation of defective humoral and cell-mediated response. Kidney tubules were dilated and necrosis of the proximal convoluted tubules was seen along with congestion and focal haemorrhages. Thickening of the glomerular basement membrane was evident. Ultrastructurally fragmentation and lysis of plasma membrane at the luminal surface, numerous cytoplasmic vacuoles and fusion of podocyte foot processes were seen. Liver showed varying degree of fatty change. Ultrastructurally mitochondrial swelling and abnormal mitochondria to severe cytolytic changes we're observed. Brain showed, focal oedema, pyknosis, karyorrhexis, satellitosis of microglial cells and swollen vascular endothelium. This was supported by electron microscopic observations of separation of neuronal elements, fragmentation and vacuolation of perineuronal elements. No gross or histological changes were seen in the heart.

Immunostimulation with levamisole at the dose rate of 0.1 mg/quail two doses at 4 day interval, resulted in

lymphocytic leukocytosis. TSP and SGI levels were increased significantly associated with increase in IgM and IgG. T cell associated lymphocytosis, decreased migration indices in response to Fowl pox antigen in LMIT, increased cutaneous response to DNCB and PHA-M, were also recorded. There was also elevation of TEC, Hb concentration and PCV.

The histological examination of lymphoid organs of immunostimulated quails revealed hyperplasia and blastoid transformation in the bursa of Fabricius, spleen and thymus.

This study brought to light the immunotoxicity of OA on the humoral and cell-mediated immune system. ' In addition to this,adverse effects on hepatic, haemopoietic and nervous systems were also documented. Levamisole was demonstrated to regulate the immunodeficiency induced by OA in quails.