EMBRYO MORTALITY IN CHICKEN

By

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THESIS

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To save unborn-ennobled task--
Needs morbid causes to unmask.

DECLARATION

I hereby declare that this thesis entitled 'EMBRYO MORTALITY IN CHICKEN' 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.

Lalitha

Place: Mannuthy, C.R. LALITHAKUNJAMMA

Date : 25-10-1987.

CERTIFICATE

Certified that this thesis entitled 'EMBRYO MORTALITY IN CHICKEN' is a record of research work done independently by Smt. C.R. Lalithakunjamma under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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Introduction

1. INTRODUCTION

Poultry production is increasingly becoming a specialized and an organized industry of great economic importance. Concomitant with intensification of poultry rearing, hatcheries have been established for supply and distribution of chicks. An efficient hatchery management is sine qua non for a successful poultry industry. Very often hatchability becomes poor resulting in heavy economic loss. Various factors, exogenous and endogenous, are associated with embryonic mortality and lower hatchability. The identification of causes becomes very essential to formulate suitable preventive measures.

Numerous reports are available on embryo mortality and abnormalities in chicken caused by individual agents. The work of Romanoff and Romanoff (1972) could be considered as a pioneering attempt to consolidate the available knowledge on the pathogenesis of embryo abnormalities in poultry.

The avian embryo has an extra uterine existence and since all its requirements are met with from within, the incubation period or prenatal period is very sensitive and highly important for the existence of the developing chick. During incubation the embryo is continuously subjected to the various environmental factors which may facilitate or interfere with the normal development or survival of the chick before or after hatching. Disturbed incubating conditions,

unhygienic handling of eggs and infections passed on from the hen can result in embryo malformations and mortality. Very often eggs become infected before they are laid, such as in avian encephalomyelitis, lymphoid leukosis, mycoplasmosis, fowl typhoid and salmonellosis while other agents like Escherichia coli, Staphylococcus aureus and Clostridia cause shell contamination resulting in embryonic mortality (Gordon and Jordan, 1982).

The avian embryo is considered as an ideal experimental system since it is free from maternal dependence and influences Any drug or chemical introduced into the egg system remains there throughout the developmental period and is not lost externally unlike in other animals. The teratological effects, if any, can be continuously evaluated and the effects of the etiological agent can be directly observed. The avian embryo is thus very suitable for investigation into the role played by extrinsic environmental factors in the production of abnormalities. It has been reported that mycotoxins and other chemical residues get accumulated in the foetus when the dam is exposed to these agents (Arora, 1982) and these cause morbidity and mortality when the embryos develop. Eventhough malformations and mortality of the chicken embryos have been documented (Rolle and Bevelander, 1966; Smith et al., 1975) reports on the detailed investigation on the pathogenesis of the various teratological defects are not available. Similarly, knowledge on the subcellular changes due to the

direct effect of mycotoxins in the developing embryo is lacking. Information on the specific changes and the mechanism of production of lesions would give some indication to identify etiological factors. This would help to formulate measures to correct hatchery conditions and possibly to eliminate factors which cause disturbance of the growth and mortality of the embryo.

The objectives of this investigation in chicken embryo are to study

i) the occurrence of mortality in hatcheries,

ii) the pathoanatomical features of embryopathies, and

iii) the cellular and subcellular changes after experimental inoculation of agents suspected to produce pathological changes.

Review of Literature

2. REVIEW OF LITERATURE

The avian embryo, has long been a most popular experimental material. In the nineteenth century scientists were mainly interested with the experimental teratogenesis of the avian embryo. The eggs were subjected to such varied treatments as temperature, atmospheric pressure, poisonous gases and vapours, mechanical shocks, electricity and magnetism. On the whole these different treatments seemed to produce similar types of abnormalities. Towards the beginning of the twentieth century attention of the workers was turned to the mode of occurrence of structural anomalies and their specificity with individual teratogenic agents. Sufficient proof began to accumulate to show the chick embryo as the most suitable experimental material among vertebrates for the preliminary quantitative evaluation of the teratogenic and other detrimental effects of various harmful agents in the prenatal development (Romanoff and Romanoff, 1972).

Malformations

Spontaneous malformations are those abnormalities which occur without any experimental intervention. They have been observed by several investigators in a large number of eggs with dead embryos (Byerly, 1930 ; Blackwood, 1960; Clement et al., 1969; Romanoff and Romanoff, 1972). Markaryan (1978) studied the type and frequency of malformations in chick embryos. The malformations of feet and legs were the most

common, then head abnormalities like acrania, single defects, double defects and triple or more defects were also encountered. Yoshida et al. (1981) classified about 28 types of teratological deformity including deformities of beak, brain and eye and umbilical hernia.

Dwarfism

Spontaneous occurrence of dwarf embryos might be due to general alteration of physiological functions or to thyroid abnormalities or nutritional deficiencies or hereditary or non-hereditary or lethal mutation (Asmundson, 1945).

Embryonic malpositions

Under ordinary conditions various forms of malpositions have been reported with consistent frequency. It was shown that the most frequent malposition was the embryo positioned upside down with the head at the small end of the egg. The least frequent was the head of the embryo at the blunt end of the egg turned to the right and with the beak over the right wing (Byerly, 1930). The frequency of malposition was related to the total mortality. The failure of embryos to attain normal position for hatching might account for the peak of mortality at the last period (Romanoff and Romanoff, 1972). Jain and Gangwar (1973) reported abnormal position of wings and legs in dead in shell eggs.

Genetic mutations

It was reported that a large number of congenital malformations in birds were caused by genetic factors. The

embryonic mortality was found to increase significantly by inbreeding. The incidence of abnormalities observed by Goff and Tumlin (1950) in 18 days incubated embryos was found to be higher in inbred hens. Many reports have appeared describing the deleterious consequences of inbreeding (Sittmann et al., 1966). Susceptibility to all the natural teratogens like alteration in the atmospheric temperature, nutritional deficiencies, susceptibility to chemicals, pathogenic microorganisms etc. was found to be increased due to inbreeding (Davis et al. 1938; Purchase et al. 1966). Spontaneous mutations were also reported to be due to chromosomal aberration (Donner et al. 1969). Embryos with anophthalmia, brachygnathia superia and hernia umbilicus and retarded growth were found to be carriers of triploidy with ZZZ sex chromosome.

Lethals

Creeper, a genetic abnormality, which is a homozygous mutant was extensively studied by Landauer (1939). It was reported that the embryo weighed only one quarter of the normal at the 18th day of incubation and that the length of the long bones was about one quarter of the normal embryos of the corresponding age. It has also been reported that many hereditary abnormalities manifested themselves by degenerative changes which appeared somewhat characteristic for each lethal gene involved.

Temperature during incubation is an important factor in the avian embryonic development. Deviation from the optimum

temperature towards higher or lower levels, greatly affected the rate of growth, livability of the embryo and the manifestation of some malformation of the whole body of the embryo or its individual organ. At extreme levels retardation of growth occurred. Similar effects were seen with the eggs of pheasants, quail, turkeys and ducks. Embryo mortality and malformations were also found to be caused by temperature alterations. Beyond certain limits of high and low temperature, none of the embryos survived (Romanoff and Romanoff, 1972). Similarly an increased atmospheric pressure, high and low humidity, exposure to gases like carbon dioxide, also produced malformations and embryonic mortality. Dobrowolskie et al. (1978) reported that low environmental temperature and humidity together with an increased concentration of ammonia led to a higher mortality rate in the avian embryo.

Ionizing radiation

It was observed that X-rays acted directly, destroying the cells and the embryo itself and that the mortality due to X-radiation was concentrated at certain fixed timings. Doses ranging from 22000 to 35000 R produced death in about one hour of exposure while 800 to 950 R produced maximum mortality on the 6th day. The survivors showed remarkable recovery but none of them hatched out and all of them were found to be dead on the twenty-third day. The most sensitive period of the embryos to X-rays was occurred on the eighth

to nineth day and the next most sensitive period was on the sixteenth day. Various malformations were also reported due to the direct and indirect effects of X-irradiation (Romanoff and Romanoff, 1972).

Nutritional deficiency

Vitamins.

Vitamins and minerals were found to be specifically related to the embryonic development. Embryonic mortality and malformations occurred when Vitamin D deficient diets were fed to hens (Bethke et al. 1936). Reports have appeared indicating that riboflavin (Romanoff and Bauernfeind, 1942), biotin (Couch et al. 1948), folic acid (Karnofsky et al. 1949) and pantothenic acid (Beer et al. 1963) produced malformations and mortality when deficient in the diet of the hen. The administration of vitamin A in excess was found to be equally harmful to the growing embryos (Fell and Mellanby, 1953). Vitamin E deficient diet in turkeys produced smaller embryos and produced malformations like cloudy-lens, oedematous areas on the neck and feet and haemorrhagic vitreous humor (Ferguson et al. 1954). Embryos from hens fed with vitamin B_{12} deficient diet were smaller than the normal. Malformations like decrease in size of the embryo, oedema and haemorrhages, local areas of necrosis in the liver, brain and spinal cord and a marked increase in the parenchymatous tissue were also noticed in embryos from hens fed a vitamin B_{12} deficient diet. The

thyroid gland in the vitamin B_{12} deficient embryos appeared more vascular than those of the controls and appeared to be hypofunctional (Ferguson et al. 1955). Vitamin A deficient diet produced embryo mortality and malformations (Fell, 1960). The highest percentage of embryonic mortality during the first week of incubation was recorded in groups deficient in riboflavin, biotin, pantothenic acid and vitamin B_{12} and the lowest with folic acid deficiency (Ferguson et al. 1961). According to Romanoff and Romanoff (1972) deficiencies of organic and inorganic compounds in the egg seriously disturbed embryonic development and led to various structural malformations and premature death of embryos. Sunde et al. (1978) reported the essentiality of vitamin D metabolites for embryonic development of chick.

Minerals.

Minerals like Manganese (Lyons and Insko, 1937; Caskey et al. 1939), iodine (Rogler et al. 1961) and zinc (Blamberg et al. 1960; Kienholz et al. 1961) produced embryonic mortality and malformation like microphthalmia, coelosomia, abnormalities of beak and external head structures and extreme oedema when they were deficient in the diet of hen.

Chemicals

Reports have appeared describing various malformations and embryonic mortality by inorganic compounds like sodium molybdate, lithium and thallium salts and boric acid

(Landauer, 1954). Williams et al. (1963) recorded various abnormalities in chick embryos following administration of thalidomide and insoluble compounds like ground glass, colloidal alumina, clay and sand and carbon suspension and colloidal silica. Hirano and Kochen (1973) experimentally administered lead salts to the chick embryo and reported neurotoxic effects.

Sukra et al. (1976) studied the effect of selenium and mercury on gross morphology and histopathology of chick embryos and found that they produced lesions in the liver and kidney. Fitzsimmons and Phalaraksh (1978) injected fresh fertile eggs with various levels of selenium and reported that the wet weight of the embryos was decreased and there was embryonic mortality. Shamshad et al. (1980) evaluated the teratogenic potential of nickel chloride to developing chick embryos. The malformations reported were exencephaly, everted viscera, short and twisted neck and limbs, microphthalmia, haemorrhage and reduced body size. The toxicity was found to be highest in groups inoculated on the 2nd day of incubation.

Sulpha drugs and antibiotics

Sulpha drugs used as an antibacterial agent was found to produce mortality and malformations when administered into the embryo (Zwilling and De Bell, 1950).

Reports on the toxic effects of various antibiotics to embryonated chicken eggs have given different results (Gentry, 1958). The incidence of embryonic malformations and mortality decreased with increase in the age of the embryo at the time of injection. The effect of tetracyclines on growth was manifested by an overall decrease in size, particularly marked in the limbs characterized by inhibition of mineralisation and erosion of the long-bone cartilage (Rolle and Bevelander, 1966).

Insecticides

Dunachie and Fletcher (1969) studied the toxic effects of twenty-five insecticides at various concentrations for their toxicity to chicken embryos and recorded malformations and mortality. The malformations encountered were cartilagenous and osseous skeletal dwarfism, micromelia, ectrosyndactyly, and irregular beak growth. Paul and Vadlamudi (1976) studied the teratogenic effect of Fenitrothion on chick embryos. Teratogenic effects of methyl parathion were tested in pheasant embryos. The pathological alterations encountered were hypoplasia or atrophy of the cervical musculature accompanied by lordosis and scoliosis of the cervical spine (Varnagy et al. 1984).

Solvents and diluents

Various diluents and solvents were commonly used in experiments for diluting the chemical teratogens. Among the

inorganic solvents, the most commonly used are distilled water and sodium chloride and other physiological preparations, such as Ringer's solution. Organic solvents which have been used as solvents and tested for toxicity and teratogenic effects were mineral oils, sesame oils and corn oils (Romanoff and Romanoff, 1972).

Naber and Smathers (1975) described the pattern of toxicity and teratogenicity in the chick embryo resulting from administration of certain nutrients and food additives.

Mycotoxins

Mycotoxins have long been known as the major cause of feed toxicosis in poultry and livestock and are now being recognized to constitute a serious environmental hazard to human health as well (Wilson and Hayes, 1973). Many of these toxins apart from their deleterious effects in the adults were found to interfere with the growth and development during embryonic life. The deviation of embryogenesis was manifested by one or more characteristics such as late foetal death, complete resorption of the implant, growth inhibition and structural abnormalities or functional defects of the offspring (Arora, 1982).

Injection of about 0.05 mg of aflatoxin into the air cell of the fresh egg resulted in reduction in size of the embryo at hatching to almost half of the control (Shibko et al. 1968). The retardation in growth began on the twelfth

day of development. There was a decrease in weight of the liver which was parallel to the decrease in the weight of the body. Smith et al. (1975) conducted a comparative histopathological study of tissues exposed to Aflatoxin B_1 and palmotoxin B_0 and G_0 and found that Aflatoxin B_1 and palmotoxin B_0 were of similar toxicity to the developing chick embryo and revealed lesions in the heart, liver, skeletal muscles, brain and cartilage at varying severity and G_0 was relatively nontoxic. Ogbadu and Bassir (1979) compared the toxicity of gamma-irradiated and non-irradiated fractions of Aflatoxins on chick embryos and found that toxicity decreased with increased dose of irradiation.

There were many reports about the teratogenic and foeticidal as well as growth retarding effect of Ochratoxin A in the embryo of mouse (Hayes et al. 1974a; Hood et al. 1978; Arora and Frolen, 1981; Arora et al. 1981), rat (Still et al. 1971; More and Galtier, 1974; Brown et al. 1976) and hamster (Hood et al. 1976). Ochratoxin A was found to be an inhibitor of mitochondrial transport systems (Meisner and Chan, 1974). Gilani et al. (1978) reported the teratogenicity of ochratoxin A in chick embryos. The malformations reported were short and twisted limbs and necks, microphthalmia, exencephaly, everted viscera and reduced body size. The cardiac anomalies observed were ventricular septal defects, aortic stenosis, thin ventricular walls, atrial septal defects and malformations of valves. Burns and Dwivedi (1984) studied

the effect of ochratoxin A in Japanese quail and reported teratogenic effects. Dwivedi et al. (1984) reported the ultrastructural changes in liver and kidney in ochratoxicosis A in young broiler chicks as an increase in liver glycogen and abnormally shaped mitochondria. Mayura et al. (1984) studied the effect of simultaneous prenatal exposure to ochratoxin A and citrinin in the rat foetus and reported that the combined effect resulted in an increase in the gross malformations, visceral abnormalities and skeletal defects. Brown et al. (1986) reported the individual and combined effect of citrinin and ochratoxin A on renal ultrastructure in layer chicks. Maxwell et al. (1987) reported the ultrastructural changes of liver and kidneys of quail in ochratoxicosis. The changes in the kidneys were limitedto the proximal convoluted tubules and glomeruli. In the proximal convoluted tubules abnormal mitochondria and excessive numbers of lipid droplets were the principal findings.

Vesely et al. (1982, 1985) tested the teratogenic effect of nineteen mycotoxins on chicken embryos and that of cyclopiazonic acid. Ivanosky (1985) observed various teratological abnormalities in chick embryos when sterigmatocystine was administered. Kurmanov and Kostyunina (1985) found that the LD 50 of Zearalenone for chick embryos was 109/µg/egg. Karavaev (1986) inoculated T-2 mycotoxin in chick embryos and found that the LD 50 was 74 micrograms.

Microorganisms

Many infectious agents like viruses, bacteria and fungi were found to produce developmental anomalies and embryonic mortality. The more specific effects of these pathogens took place during a definite stage of embryonic development. Romanoff and Romanoff (1972) reported that the chicken embryo was most susceptible at the age of 10 to 15 days.

The effect of infectious bronchitis virus on the avian embryo was studied and found to produce dwarfing, stunting, curling and death of the embryos (Beaudette and Hudson, 1937; Delaplane and Stuart, 1939; 1941; Fabricant, 1949; Loomis et al. 1950). Development of heart and lung was decreased and the spleen was enlarged than the normal in these embryos. Simpson (1958) found the incubation temperature to markedly alter the virus growth, virulence and population stability in embryonated eggs. The microscopic lesions in the embryos were perivascular cuffing in the liver, extensive necrosis and congestion of the kidneys and oedema of amnion and chorioallantoic membrane. Following inoculation of laryngo-tracheitis virus to embryonating chicken eggs through the chorioallantoic membrane, small areas of grey thickening were visible on the membrane by the third day. The lesions became larger upto the fifth or sixth day when the embryo usually died (Burnet, 1934; Brandly, 1935). The embryos that lived to the sixth day were stunted. Microscopically the membrane had areas of cellular proliferation and oedema. Intranuclear

inclusion bodies were observed in groups of ectodermal epithelial cells between the first and third days. As the lesion progressed, the epithelium underwent necrosis. Newcastle Disease virus infection of the embryonated eggs by chorio-allantoic membrane route resulted in minute fine gray foci in the exposed portion of the membrane, distinct petechiae on the skin, cranial haemorrhage and congestion of yolk sac (Jungherr et al. 1946; Blattner and Williamson, 1951). Avian encephalomyelitis virus was inoculated in the embryonated chicken eggs by the allantoic and yolk sac routes and the lesions observed were decreased movement and retardation of growth (Jungherr et al. 1956; Sumner et al. 1957a; 1957b; Calneck and Jehnich, 1959a; Moore and Flowers, 1959; Taylor and Schelling, 1960). The histopathological lesions in the embryo were encephalomalacia and muscular dystrophy. The neural lesions were local oedema, gliosis, vascular proliferation and pyknosis. The muscular changes consisted of eosinophilic swelling and necrosis, fragmentation and loss of striations of the affected fibers with rare sarcolemmal proliferation and heterophil infiltration (Jungherr et al. 1956). When infectious sinusitis virus was inoculated by yolk sac route, the embryos died 4-10 days post-inoculation showing oedema and haemorrhage. The liver, kidney, spleen were enlarged and there were necrotic foci (Lecce et al. 1955). When duck virus hepatitis virus was inoculated into 9 day old chicken embryo it was found that the embryos were stunted and oedematous

and there was hepatic necrosis (Hwang, 1965; Levine, 1967). Viruses like Distemper, Herpes simplex, Vaccinia, Influenza A (Heath et al. 1956) and Mumps virus (Williamson et al. 1956, 1957) were found to produce various malformations and mortality of chick embryos. The malformations were dwarfing, incomplete closure of the abdomen, microcephaly, defects of the neural tube and lens cataracts and abnormalities of organs. Williamso et al. (1956) reported that a strain of Influenza A virus caused specific organ defects in the lens and auditory vesicles in addition to axis twist and flattening of the encephalon. Price et al. (1976) reported a virus induced arthrogryposis in chick embryos. Yolk sac route of inoculation of reovirus into embryonated chicken eggs resulted in death of the embryo along with extensive subcutaneous haemorrhage (Glass et al. 1973; Olson, 1975; Levisohn and Weisman, 1980). Inoculation of chorioallantoic membrane of 10 day old chicken embryo produced death of embryos 3-5 days after inoculation with development of large necrotic plaques on the chorioallantoic membrane. The surviving embryos were stunted and had greenish discolouration of liver, splenomegaly and heart lesions (Mustaffa Babjee et al. 1973; Bains et al. 1974; Spradbrow and Bains, 1974). The avian reovirus was vertically transmitted from infected hens to chicks and produced embryomortality and reduced hatchability (Glass et al. 1973; Mendes et al. 1975a; Hussain et al. 1981; Jones et al. 1981). Yamada et al. (1977) isolated avian reovirus from dead in shell chick embryos.

Chute and Cole (1954) reported the embryonic malformations and mortality produced by mycoplasma. Echymotic haemorrhages were noticed frequently in the skin of the head, neck and ventral thoracic region. Meningococcal endotoxin produced mortality in chick embryos (Smith and Thomas, 1956). Harry (1957) reported embryo mortality due to bacterial infection of egg yolk. He showed that yolk infection is usually initiated by bacteria, relatively non-pathogenic in sites other than the yolk which possess enzymes capable of degrading the yolk protein complexes, and occasionally by toxigenic bacteria capable of stimulating the production of an inflammatory exudate from the yolk sac lining. The chick embryo was found to survive doses of Clostridium botulinum toxin which would be lethal to adult birds. This was presumably due to the special mechanism of respiratory gas exchange, which in the avian embryo was accomplished by passive diffusion across the chorioallantoic membrane (Romanoff, 1960). The embryos injected with toxin showed severe ankylosis, short upper beak and atrophy and degeneration of the skeletomuscular system (Drachman, 1964). Chicken embryos were found to be highly susceptible to some strains of staphylococci and relatively resistant to others. The LD 50 was found to be less than ten bacteria. The lesions produced were frequent haemorrhage in the kidney, haemorrhage with distortion of structure in liver, haemorrhagic encephalosis and necrosis of nervous tissue (Macabe (1962). Russell and Cottew (1972)

isolated Mycoplasma gallisepticum from embryonated chicken eggs. Renes and Szalay (1974) conducted a bacteriological examination of dead in shell eggs. Ochsenhirt (1974) localised Salmonella typhimurium in the chick embryo after experimental infection of the eggs. Karim and Ali (1976) conducted a survey of bacterial flora from chicken embryo and their effect on low hatchability. Flade (1977) isolated E. coli serotypes from the yolk sac of dead in shell embryos. Nashed (1981) conducted a bacteriological study on unhatched chicken eggs and reported that there was an average contamination of 60.8%. The contaminated flora comprised of Enterobacteriacae, Micrococci, Streptococci, Pseudomonas and Anthracoid species. McClenaghan et al. (1981) inoculated fertile eggs with mycoplasma broth and reported embryonic death between 19 and 21 days of incubation showing lesions of congestion, stunting and oedema of head. Orajaka and Mohan (1985) conducted a bacteriological survey from dead in shell chicken embryos from Nigeria and isolated E. coli and Staphylococcus aureus, Micrococcus, Klebsiella, Pseudomonas and Proteus. They suggested that the high incidence of pathogenic bacteria might have contributed to the embryonic mortality and reduced hatchability.

Hormones

Injection of hormones like estradiol, cortisone, insulin (Parhon et al. 1956) caused chick embryo mortality and malformations like shortening and bending of femur and humerus,

exteriorised viscera, shortened beak, bent upper beak, delayed ossification of bones of the roof of the skull. The avian embryo was found to be very responsive to a lack of thyroid hormone as well as an excess of thyroxine. It was shown that thyroxine accelerated the growth of the embryo and the time of hatching was delayed by one to one and a half days (Beyer, 1952).

Antithyroid drugs like thiourea prevented the synthesis of thyroid hormone and so produced a state of hypothyroidism and resulted in retarded growth, delayed hatching, increased mortality, thyroid hypertrophy and other anomalies (Adams and Bull, 1949; Adams and Buss, 1952). Histological changes like hyperaemia, hyperplasia, marked increase in height of follicular epithelium and vacuolation of colloid and colloid release were also produced in the avian embryo by antithyroid drugs like thiourea and thiouracil. Christensen (1985) injected fertile turkey eggs with thyroid hormones and found that the injection at setting depressed hatchability and injection at later days of incubation improved hatchability.

Materials and Methods

3. MATERIALS AND METHODS

Incidence and nature of embryopathy

In order to study the incidence and pattern of embryo-mortality dead embryos from the following hatcheries were studied.

1. Hatchery attached with the University Poultry Farm, Mannuthy.
2. Hatchery attached with the All India Coordinated Research Project on Poultry for Eggs, Mannuthy.
3. The Central Hatchery, Chengannur belonging to the Animal Husbandry Department, Kerala State.

Five thousand four hundred and forty embryos which failed to hatch were examined.

Pathoanatomical studies

The dead in shell embryos were opened and the embryos were examined for gross pathological abnormalities. The malformed embryos were classified with detailed description of their pathoanatomy. Out of these, tissues from 500 representative embryos were subjected to detailed histopathological studies. The tissues were fixed in 10% neutral formalin and processed by paraffin embedding. Sections cut at 5 micron thickness were routinely stained by Haematoxylin and Eosin. Wherever necessary serial sections were cut to study organogenesis along with histopathological alterations. In suspected

cases of fatty changes, staining for fat was done with Sudan III in sections taken in a cryostat microtome.

Bacteriological studies

One hundred and fifty dead embryos which showed lesions of suspected infections, were subjected to cultural examination to isolate bacteria, if any. All the embryos were collected under sterile conditions and the embryonic fluid was cultured in Muller Hinton Agar. The plates were observed for 48 hours and the organisms were identified upto the Genus level as described by Cowan (1974).

Experimental studies

The experimental schedule is given in table 1.

Oxytetracycline.

Pure oxytetracycline (obtained from Hindustan Antibiotics Limited, Pimpri, Pune) was diluted in sterile distilled water. Eight-days embryonated eggs were used. Two mg of tetracycline diluted in 0.2 ml of distilled water was administered into the air cell to each of the 20 embryonated eggs. An equal number of eggs was inoculated with 0.2 ml of sterile distilled water. Another 20 numbers of uninoculated eggs were also incubated along with the inoculated eggs. All the eggs were candled daily. The dead ones were opened. The embryos were weighed and then subjected to detailed pathological investigation. After the gross examination, tissues were collected for histopathology. The experiment of oxytetracycline administration was repeated in another set of 20 eggs.

Table 1. Schedule of experimental inoculation

Sl. No.	Materials used	Diluent used	Dose/egg	Route of administration	Age of embryo at the time of inoculation	Number of eggs used
1	Oxytetracycline	Distilled water	2 mg	Air cell	8 days	40
2	Ochratoxin A	Propylene glycol	0.5 µg	Air cell	4 days	40
3	Citrinin	Propylene glycol	5 µg	Air cell	4 days	40
4	Ochratoxin A + Citrinin	Propylene glycol	0.25 µg+ 2.5 µg	Air cell	4 days	40

Ochratoxin A.

Pure ochratoxin A was obtained from Makor Chemicals, Israel. Ochratoxin A was diluted in propylene glycol at the rate of 5 µg per ml and 0.1 ml was inoculated into the air cell of each embryos. Twenty numbers of four-day incubated eggs were used. Another 20 embryonated eggs were inoculated with 0.1 ml of propylene glycol into the air cell. Twenty number of uninoculated embryos were also incubated. The experiment of ochratoxin A administration was repeated. All the embryos were candled daily. The dead ones were removed and weighed. After gross examination, tissues were collected for histopathological examination.

Citrinin.

Citrinin in pure form obtained from Makor Chemicals, Israel was diluted in propylene glycol at the rate of 50 µg per ml and 0.1 ml of this solution was inoculated into the air cell of 20 numbers of 4-day incubated eggs. Another 20 numbers of 4-day incubated eggs were inoculated with 0.1 ml of propylene glycol. Twenty numbers of uninoculated eggs were also incubated. The experiment of citrinin administration was repeated in another set of 20 embryonated eggs as before. All the eggs were candled daily. The dead embryos were removed and weighed. All the dead embryos were examined grossly and tissues were collected for histopathological examination.

Ochratoxin A and Citrinin.

Ochratoxin A and citrinin were diluted in propylene glycol so as to contain 2.5 µg of ochratoxin A and 25 µg of citrinin per ml. This mixture was given at the rate of 0.1 ml per egg into the air cell of 20 numbers of 4-day incubated eggs. Another 20 eggs were inoculated with 0.1 ml of propylene glycol alone. Twenty numbers of uninoculated eggs were also incubated along with the inoculated ones. All the eggs were candled daily and dead embryos were removed. Recorded the weight and studied the lesions grossly and microscopically.

Virus inoculation.

Embryos infected with Influenza virus - A/duck/India/ 1/85:H_9N_2 - received from the Department of Microbiology were used for studying the tissue reaction. Twenty numbers of 9 day incubated eggs were inoculated with 0.2 ml of virus suspension into the chorioallantoic cavity. These embryos were collected at 18 h, 36 h, 48 h and 72 h after inoculation.

Ultrastructural studies

Liver and kidney from embryos inoculated with Ochratoxin citrinin and ochratoxin A and citrinin were fixed in 3 per cen glutaraldehyde buffered with cacodylate. The glutaraldehyde fixed tissues were post fixed in Osmium tetroxide buffered with S-collidin. The material was embedded in Epon. Section were cut with glass knives on a LKB ultratome. Thin sections

were picked up on uncoated copper grids, stained with uranyl acetate and examined in a Philips EM 420 at 60 KV. The electron micrographs were taken in the Department of Pathology, Faculty of Veterinary Medicine, Uppsala, Sweden.

Results

4. RESULTS

Occurrence and nature of embryopathy

The present study was conducted on 5440 embryos which failed to hatch on the 21st day out of 32,700 eggs set. These embryos were subjected to detailed pathoanatomical investigation. The percentage of occurrence of various abnormalities is given in table 2.

Sixty per cent of the embryos had double or multiple defects. Values for each defect were recorded separately and the percentage was calculated on the total number. The significant pathoanatomical features of some of these conditions are described here.

Twentyseven per cent of cases had oedema in the region of head and neck (Figs. 1 and 2). The oedema was gelatinised and was seen involving the dorsal aspect of the cranium and the orbital region and was found to be extending dorsaly and laterly in the neck region upto the thoracic inlet. The gelatinous coagulum was seen involving both the subcutis and musculature. Petechiae and streaks of haemorrhage were noticed in the oedematous region in many cases.

Microscopically the oedematous area consisted of homogenous granular eosinophilic contents with few strands of fibrin. When muscular tissue was involved there was separation, fragmentation and myolysis of fibres. Focal areas of

Table 2. Occurrence of various abnormalities in the chick embryos (Total 5440 embryos examined)

Name of condition	Total number	Percentage
Oedema of head and neck	1473	27.1
Gastroschisis and herniation of yolk sac	2380	43.7
Early embryonic death	2065	38.0
Curled embryos	577	10.6
Dwarf embryos	615	11.3
Omphalitis and Septicaemia	272	5.0
Coelesoma	428	7.9
Dead in shell	816	15.0
Live sticky	1087	20.0
Beak abnormalities:		
Parrot beak	125	2.3
Crossed beak	201	3.7
Short upper beak	138	2.5
Brachygnathia	15	0.3
Agnathia	1	0.02
Brachycephaly	89	1.7
Cranioschisis	600	11.03
Limb abnormalities:		
Micromelia	80	1.5
Thoracomelia	1	0.02
Polymelia	1	0.02
Phocomelia	1	0.02
Ectromelia	1	0.02
Curled toe	244	4.5
Eye abnormalities:		
Microphthalmia	142	2.6
Anophthalmia	20	0.4
Cyclopia	18	0.3
Streptosomia	82	1.5
Diprosopus	1	0.02
Duplicatus (Twinning)	2	0.04

haemorrhage as small petechiae or as large streaks between muscle bundles were noticed. Few heterophils, some of them fully or partially degranulated, were found in the oedematous area. The brain did not reveal malacic foci. Some of these embryos concomitantly had other types of developmental abnormalities also.(Fig. 3).

Herniation of yolk associated with gastroschisis was seen in 2380 (43.7%) embryos. Many of such embryos had other lesions also.

Thirtyeight per cent of cases because of their poor development indicated early embryonic death. The size of the embryos indicated death had occurred before 10 days of incubation. Because of the advanced autolytic changes it was not possible to identify the specific pathological features.

Ten per cent of the embryes were small in size and had a curled appearance. The size of the embryos indicated that the death had occurred between 10 and 18 days. Histopathological examination did not reveal any lesion suggestive of viral infections.

Six hundred and fifteen (11.3%) embryos were reduced in size and weight and were considered as dwarfs (Figs. 4 and 5). The reduction in overall size was also reflected in the reduced size of the internal organs. Fiftyfive cases showed extreme shortness of long bones of the extremities and

seventy cases showed haemorrhages in the liver, kidney and heart. The liver, kidney and heart when examined histopathologically revealed haemorrhages and degenerative and necrotic changes of varying degrees. Among the dwarf embryos 22 had generalized oedema.

Five per cent of the cases revealed omphalitis and lesions of general septicaemia (Figs. 6, 7 and 8). This was observed where there was herniation of yolk sac as well as in embryos in which yolk was retained within the body. The yolk in these cases was creamy in consistency, very often with a greenish colouration. The visceral organs especially the liver, kidneys, lungs and spleen were congested and very often petechiae were also seen (Fig. 9). Histologically there was marked parenchymatous degeneration and necrosis of hepatic cells as well of renal tubular cells. There was severe congestion with petechiae in many organs. The yolk sac was severely hyperaemic with focal areas of necrosis. Leukocytic reaction was minimal. In two cases the lungs were very severely pneumonic with the presence of fibrinous inflammatory exudate. In the exudate the number of heterophils was very few, the predominant cell being mononuclear cells, probably of macrophage type. In three cases the kidneys revealed basophilic bodies with the morphology of corpora amylacea.

Coelosoma, characterised by the eventration of viscera, was seen in 7.8% of cases. The eventration was in varying degrees and severity. In some cases only loops of intestines

were seen outside the body (Fig. 10) while in extreme cases the entire viscera of the abdomen was found eventrated.

Fifteen per cent of the embryos were classified as 'dead in shell' and did not reveal any gross malformation either by external appearance or gross examination of the internal organs. On histopathological examination of representative samples, no specific lesions were seen except moderate degree of congestion and degenerative changes in the liver and kidney.

Twenty per cent of the embryos were classified as 'live sticky'. In general they were reduced in size and appeared weak. The feathers were moist and sticky and were found to be partially adherent to the egg shell membrane. Except for general congestion and slight degenerative changes no specific lesions were seen in the organs.

Abnormalities and deformities of the bone and appendages included beak abnormalities, polymelia (Figs. 11 and 12), thorocomelia, phocomelia, ectromelia (absent or rudimentary limbs), brachycephalia, cranioschisis and curled toe (Fig. 13). The important beak abnormalities were parrot beak (2.3%), crossed beak (3.7%)(Fig. 14), short upper beak (2.5%) (Fig.13), brachy gnathia (0.3%) and agnathia (0.02%) (Fig. 15).

Cranioschisis was seen in 11.03% of cases. In this condition there was complete or partial failure of fusion of the cranium and the skull bones were imperfectly formed (Fig.16

This had resulted in partial herniation of the brain. Many of these embryos had other concomitant deformities. Histopathological examination of the herniated brain revealed neuronal degeneration and extensive malacia.

Microphthalmia 2.6% and anophthalmia 0.4% were seen along with other abnormalities like crossed beak or herniation of yolk.

Other developmental abnormalities seen were cyclopia (both eyes fused medially), Streptosomia (twisting of the body), diprosopus (embryo with two heads) and thoracomelus (an embryo with supernumerary wing attached to the thorax). One of the embryos which was cyclopia had two upper beaks and one lower beak with cranioschisis and herniation of yolk also (Fig. 17). Twinning or duplicatus was seen in two cases.

The normal position of the body of the embryo parallel to the long axis of the egg with the head toward the blunt end was found disturbed in 2430 cases in which malpositions were recorded.

Out of the 2430 malpositions seen the following types were recorded.

i) Embryo upside down (head at the sharp end of the egg).

ii) The head of the embryo at the blunt end, turned to the right and positioned over the right wing.

iii) Head of the embryo at the blunt end but turned to the left.

iv) Embryo rotated away from the air cell

v) Head between the thighs

The malpositioned embryos were with or without other pathological alterations. One consistent observation was the oedema in the region of the head and neck.

Bacteriological examination

One hundred and fifty dead embryos which showed lesions of suspected infections were subjected to bacteriological examination by cultural methods; 59.3% of them (89 embryos) were positive for bacteria. In 26 samples, more than one type of organism was found to be involved. A total of 115 bacterial isolates was obtained during the study. Out of these 115 isolates 66.95% (77 isolates) were gram positive and 33.05% (38 isolates) were gram negative. Among the gram positive bacterial isolates 43 (55.84%) were Staphylococcus spp. 20 (25.97%) Bacillus spp., 7 (9.09%) Corynebacterium, 4 (5.19%) Micrococcus spp. and 3 (3.9%) Aerococcus spp. The gram negative bacterial isolates obtained were identified as Coliforms (17), Proteus spp. (7), Pseudomonas spp. (6), Alkaligenus spp. (6) and Aeromonas spp. (2) and the percentages were 44.74, 18.42, 15.79, 15.79 and 5.26 respectively. The results are presented in table.

Bacterial isolates from embryos

Total number of embryos examined	: 150
Number of positive samples	: 89 (59.3%)
Number of total bacterial isolates	: 115

Total number of gram positive bacteria isolated	: 77
Staphylococcus spp.	: 43 (55.84%)
Bacillus spp.	: 20 (25.97%)
Corynebacterium spp.	: 7 (9.09%)
Micrococcus spp.	: 4 (5.19%)
Aerococcus spp.	: 3 (3.90%)
Total number of gram negative bacteria isolated	: 38
Coliform	: 17 (44.74%)
Proteus spp.	: 7 (18.42%)
Pseudomonas spp.	: 6 (15.79%)
Alkaligenus spp.	: 6 (15.79%)
Aeromonas spp.	: 2 (5.26%)

Experimental studies

Oxytetracycline.

The mortality pattern and gross lesions of the inoculated embryos are given in table 3. There was a mortality of 52.5% in the experimental group. There was no mortality in the control group. In general the embryos that survived upto 21 days (47.5%) were reduced in size, especially the limbs appeared shorter. The mean average weight of the embryos that survived on the 21st day of incubation was 15 grams and that of normal or control group was 25 grams. This reduction in weight was found to be statistically significant. Irrespective of the day of examination, most of the embryos had an

Table 3. Mortality pattern and gross lesions of embryos inoculated with Oxytetracycline (2 mg/egg) (Inoculated on 0th day)

Age of embryo	Dead/Live	Gross lesions
10th day	4 dead	Generalised congestion (3 Nos.) Generalised congestion and Microophthalmia (1 No.)
11th day	2 dead	Generalised congestion
13th day	4 dead	Generalised oedema and congestion (3 Nos.) Oedema, congestion and crossed beak (1 No.)
14th day	1 dead	Oedema - Embryo smaller than normal
15th day	3 dead	Oedema and congestion (2 Nos.) Oedema, congestion and Microophthalmia (1 No.) All the embryos smaller than the normal
16th day	2 dead	Embryos smaller than normal
18th day	1 dead	Oedema, smaller in size, short upper beak
21st day	4 dead	Oedema of head and neck (4 Nos.)
	19 live	Sticky (6 Nos.), Herniation of yolk sac (7 Nos.), Oedema of head and herniation of yolk (6 Nos.) All the embryos were smaller than normal.

Controls: (Inoculated with distilled water and uninoculated ones) did not show any significant pathological alteration.

oedematous appearance and were reduced in size (Figs. 18, 19, 20 and 21).

Histopathological examination revealed moderate to severe degenerative changes in the liver (Fig. 22) and kidneys (Fig. 23). Numerous fatty vacuoles were seen in hepatic cells. Congestion and occasional petechiae were noticed in most of the internal organs. The digestive tract epithelium showed degeneration and desquamation. There was submucosal oedema. The brain was oedematous with prominent perivascular spaces and separation of neuronal cells. This was particularly seen in the cerebral hemispheres. Other organs did not reveal any pathological lesions.

It was found that there was interference in the development of bones. The normal embryonic bone rudiments mainly consisted of hyaline cartilage matrix which has a basophilic matrix. In the embryos inoculated with oxytetracycline, the condensation of the unoriented mesenchymal cells appeared normal but the nuclei in the condensed area were arranged in a haphazard manner. The deposition of matrix around the cells was less in quantity compared to the normal (Fig. 24). It was found that chondrogenesis was not uniform in all areas even though the formation of perichondrium appeared normal. The orderly orientation and flattening of the chondroblast and the appearance of intercellular matrix were seen disturbed (Fig. 25). The arrangement of cells between the epiphysis and diaphysis was irregular. Stellate cells were seen in

Table 4. Mortality pattern and gross lesions of embryos inoculated with ochratoxin A (0.5 ug/egg) (Inoculated on 4th day)

Age of embryo	Dead/Live	Gross lesions
5th day	4 dead	Mild congestion
6th day	2 dead	Mild congestion
7th day	2 dead	Congestion and oedema
9th day	4 dead	Severe congestion (2)
10th day	2 dead	Severe congestion (2)
11th day	2 dead	Severe congestion (2)
13th day	4 dead	Severe congestion
15th day	2 dead	Severe congestion Crossed beak (1)
21st day	8 dead	Herniation of yolk (7) Herniation of yolk and cranioschisis (1)
	10 live	Herniation of yolk (6) Curled toe (1) Oedema of head and neck (2) Crossed beak (1) All the embryos were reduced in size

Controls: Two embryos inoculated with propylene glycol died on the 7th and 10th day while one uninoculated embryo died on the 18th day due to unrelated causes.

with the circumference of the cartilageneous rudiment was maintained, but in some areas this arrangement was lost and was mixed up with tongues of advancing blood vessels (Fig.27). During the formation of the bony cylinder, the conversion of the osteoblasts to osteocytes was slightly inhibited and numerous chondroblasts were seen and mixed with osteoblasts and osteocytes. Calcification was partial.

Endochondral ossification was also seen slightly interfered. The osteoblasts failed to arrange themselves in a regular manner. Large number of capillaries was seen in the epiphysis and there was partial failure of calcification of epiphysis. Endochondral ossification of the vertebrae and cartilage bones of the skull were also found interfered with. Only part of the cartilage matrix was calcified before it was resorbed.

Ochratoxin A.

The details of mortality of the embryos inoculated with ochratoxin A and the gross lesions encountered are given in table 4. The embryos which survived were stunted in size and mean weight was 14.6 grams and was found to have significantly lower weight compared to the controls (Figs. 28, 29 and 30).

The embryos which were dead during the early period showed imperfect organogenesis. The differentiation of cells was not found complete compared with the embryos of

the epiphyseal region in the matrix, but they were not clearly demarcated from the surrounding tissue.

Signs of ossification were seen beneath the perichondrium around the hypertrophic zone only by the 15th day (Fig. 26). The matrix was not of uniform density in the epiphysis and the transformation of stellate cells to round cells was not uniform in all locations. Eventhough there was cellular hypertrophy, degenerated cells were seen in large numbers. Between the enlarged cells of the metaphysis there was less matrix. The proliferating zones seen between the epiphysis and diaphysis were irregular. Eventhough in the normal embryo, the first signs of calcification were seen on the 14th day, in the experimental group they were not seen when examined on the 16th day. Even in the 21st day embryo the extent of calcification was much less than in the normal and the distribution was also irregular and patchy. The initial foci of calcification were noticed immediately beneath the periosteal bone near the extremities of the diaphysis. Perichondrial ossification was also of not uniform pattern. As the chondroblast started to enlarge, the perichondrium covering the zone of hypertrophy was seen differentiating into an outer layer of fibroblast and an inner layer of osteoblast. It was noticed that in the long bones the formation of these layers was quantitatively inhibited to a slight degree and of not uniform pattern. In many locations the normal arrangement of osteoblast with a long axis parallel

corresponding age. There was marked degeneration and necrosis of the cells in the various developing structures.

Abnormalities were particularly marked in the developing bones. The long bones as well as the various craniofacial bones were found affected. The epiphyseal region of the long bones showed uncoordinated proliferation of cartilage cells in comparison with the tissues from the controls of the same age. There was quantitative reduction in the level of ossification in the bones. The orderly transition into bone differentiation and ossification was singularly lacking (Fig. 31). Necrosis of mesenchymal cells was noticed in very early stages, thus affecting orderly chondrogenesis. There were focal areas of lysis of chondrocytes in the long bones. In three embryos, it was seen that the irregularity of the proliferating cartilage of the vertebrae had partially obliterated the neural canal resulting in partial compression of the spinal cord (Fig. 32). The spinal cord posterior to this region showed neuronal degeneration.

Histopathological lesions were consistently seen in the eye (Fig. 33). The portion of the membrane extending from the pupillary border to the lens had disappeared during the early stages and the portion over the lens had fused with the underlying capsule of lenticular origin. The retina was either found detached or there was folding of the ganglionic cell layer of neural retina (Fig. 34). The choroid and sclerotic layers were continuous with the ciliary body which

was showing degenerative changes but were found slightly detached (Fig. 35). In many locations the transition between the ganglionic and the inner reticular layers was not very evident. Similarly differentiation of the inner nuclear layer as the amacrine cells (correlation neurons) and the outer cells of the inner nuclear layer to become the basal cells was also not evident. The pigment glial cells of the pecten showed moderate degree of degenerative changes and there was accumulation of oedematous fluid between the folds.

The brain, in all cases, was slightly oedematous resulting in loosely arranged cells. In one case there was accumulation of fluid in the lateral ventricles with consequent pressure atrophy of the brain cells. In cases of craniochisis, the brain showed numerous foci of liquifaction necrosis with only a few intact neuronal cells. Presence of glial cells was prominent (Fig. 36). Some of the neurons of the nerve ganglia had undergone degeneration but without exhibiting neuronophagia (Fig. 37). Consistent degenerative and necrotic changes were seen in the kidney and liver. The kidneys showed interstitial oedema and degeneration and necrosis of tubular epithelial cells(Figs. 38 and 39). Many of the tubules revealed epithelial debris. The glomeruli appeared hypercellular and in a few cases there was desquamation of both visceral and parietal epithelial cells. The extent and degree of damage to renal epithelium was more in embryos that survived than in those that died during

development. The liver was severely engorged with focal haemorrhages. The hepatic cells showed islands of degeneration and necrosis (Fig. 40). In other areas individual hepatic cells were found laden with fat.

The cardiac muscles showed oedema and occasional bundles showed fragmentation (Fig. 41). The endocardial surface had irregular button like protrusions of cardiac muscles in four embryos which survived (Fig. 42). There was necrosis and degeneration of both epithelial and lymphoid components of the bursa of Fabricius (Fig. 43). Moderate degree of degeneration of the lymphoid elements was seen in the thymus.

Citrinin.

The mortality pattern of embryos which were inoculated with citrinin and the lesions observed are given in table 5. The embryos which survived were smaller in size and had a mean weight of 14.2 gram and was significantly low compared with the controls.

Histopathological examination of the embryos which died before 21 days, showed general congestion, degeneration and necrosis. The developing kidney and liver revealed severe degeneration and necrosis (Fig. 44). The developing bones were apparently normal with the usual chondrogenesis, osteoblastic proliferation, osteocytic replacement and calcification. Except for slight oedema of the diencephalon (Fig. 45), there was no significant alteration in the developing brain.

Table 5. Mortality pattern and gross lesions of embryos inoculated with citrinin (inoculated on 4th day) (5 µg/egg)

Age of embryo	Dead/live	Gross lesions
5th day	4 dead	No gross lesion
6th day	3 dead	Mild congestion and oedema
7th day	3 dead	Petechial haemorrhage and oedema
8th day	3 dead	Petechial haemorrhage and oedema
9th day	4 dead	Congestion and oedema
13th day	1 dead	No gross lesion, small in size
15th day	2 dead	General oedema and small in size
21st day	7 dead	Oedema at the neck region
	13 live	Small in size
		Herniation of yolk (4)

Controls: One embryo inoculated with propylene glycol and one uninoculated embryo died on the 12th and 15th days respectively due to unrelated causes.

On histopathological examination, changes were noticed in the kidneys of embryos which survived for 21 days. The glomeruli were swollen and had a hypercellular appearance. A few of the tubules contained eosinophilic, granular proteinaceous contents mixed with cellular debri. Liver showed moderate degenerative changes and many hepatic cells were laden with lipid droplets. Occasional cells appeared necrotic. The epithelial components of the alimentary tract also showed moderate degree of damage in the form of degeneration and necrosis. The extent of leukocytic reaction was minimal. Submucosal oedema was seen in a few cases.

Two of the embryos had calcified foci in the kidneys and liver (Figs. 46 and 47). They were present as granular deposits near necrotic cells. The calcium deposits were also seen as granular precipitates in individual cells or as basophilic deposits on the basement membrane of renal tubules.

In seven cases there was severe subcutaneous and muscular oedema in the region of the head and neck. The oedema caused disruption and degeneration of muscles especially in the neck region and also haemorrhage (Fig. 48). No inflammatory cells were seen in these locations.

The epithelial cells of the ciliary body in the eye (in 3 cases) showed slight degeneration between the conjunctive-vascular layer of the ciliary bodies and the sclerotic coat. There was oedema and fibrinous deposits in the region.

The pulmonary tissues showed slight oedema. The brain was slightly to moderately oedematous. A few of the neurons showed moderate degree of degeneration. Other organs including thymus and bursa of Fabricius did not reveal any significant change.

Ochratoxin A and Citrinin.

The mortality and gross lesions of the embryos inoculated simultaneously with ochratoxin A and citrinin are given in table 6. The embryos were smaller in size and had a mean weight of 14.5 grams and was found to be significantly less than the controls.

Histological changes were very severe in the kidney and liver. Degeneration and necrosis of the tubular epithelium of the kidneys were consistently observed (Fig. 49) when examined at different time intervals. The necrotic changes were more pronounced in the embryos that survived upto 21 days than in those that succumbed earlier. The glomeruli were swollen (Fig. 50) and endothelial cells appeared very prominent. The increase in the number of mesangial cells gave an appearance of hypercellularity of the glomeruli. There was extensive hepatic cell degeneration and focal necrosis. Many of the hepatic cells were laden with lipid droplets. The hepatic reticulum appeared broken in many locations. Haemorrhagic foci were noticed and occasionally they became confluent to form large areas of interconnecting sinusoids (Fig.51). Two cases showed small focal areas of calcification both in the

Table 6. Mortality pattern and gross lesions of embryos inoculated with Ochratoxin A and citrinin (0.25 + 2.5 µg/egg)

Age of embryo	Dead/Live	Gross lesions
5th day	5 dead	No gross lesions
6th day	4 dead	Mild congestion
7th day	5 dead	Mild congestion
8th day	4 dead	Mild congestion
9th day	3 dead	Mild congestion
11th day	2 dead	Slight oedema and very small in size
12th day	3 dead	Eventration of viscera (1) All are small in size
15th day	1 dead	Crossed beak - small in size
16th day	4 dead	Congestion and slight oedema, Very small sized embryos
21st day	4 dead	Cranioschisis (2) All are small in size
	5 live	Herniation of yolk (3) Curled toe (2) All are very small in size

Controls: One embryo inoculated with propylene glycol died on the 6th day while 3 uninoculated embryos died on the 18th day due to unrelated causes.

kidney and liver. They appeared in the form of granular deposits. There was extensive epithelial damage in various developing organs, especially in the alimentary organs. Degeneration and necrosis were seen in the thymus as well as in the lymphoid areas of the bursa of Fabricius (Fig. 52). Interference in chondrogenesis, osteogenesis, ossification and calcification was noticed in the bones (Fig. 53). In two cases in the skull the membraneous neurocranium was ill developed with distortion of the ethmoidal complex. The chondrocranium was also not well formed. The ill developed cranial bones resulted in imperfect fusion resulting in herniation of brain tissue. The long bones depicted histological changes associated with imperfect bone formation in the epiphysis and diaphysis. Imperfect ossification was seen in the vertebrae (Fig. 54). In the developing brain it was found that the diencephalon, mesencephalon and pons had undergone necrotic changes (Fig. 55). In the fully developed brain there was atypical and excessive proliferation of cells protruding into the ventricle as aggregates admixed with necrotic debri. The brain was oedematous and the neurons in the brain as well as in the ganglia showed moderate degenerative changes. In the eye there was marked degeneration of the ganglionic cell layers of the retina with fibrinous deposits. There was moderate degree of degenerative changes of the epithelial cells of the ciliary body. Fibrinous oedema was seen between the folds of the pecten and also

adjacent to the ciliary body. Degenerative changes were also seen on the corneal and scleral layers. Fibrin deposits were seen in the anterior chamber.

Avian Influenza virus.

At 18 hours post inoculation there was generalised oedema, and acute congestion of the various developing tissues. Eventhough the blood vessels were engorged emigration of granulocytic cells was not characteristic. The sinusoids in the liver were markedly dialated and engorged with erythrocytes. The hepatic cells showed moderate degree of degenerative changes. Small number of mononuclear cells were noticed (Fig. 56). The kidney showed congestion, focal haemorrhages and moderate amount of mononuclear reaction. The renal epithelium showed degenerative changes. The gut epithelium had a sloughed up appearance, and the subepithelial mesenchymal cell layer had a loosened appearance because of oedema (Fig.57). The pericardial sac was patent and small quantity of fluid admixed with fibrinous material was noticed along with some stellate and fusiform cells. Individual myocardial fibers and the syncytial groups revealed fragmentation. These had a very loose arrangement and moderate numbers of mononuclear cells were found in the tissue. Undifferentiated cells were seen along with well differentiated myocardial fibers. In the lungs there was slight oedema and presence of numerous mesenchymal types of cells. The respiratory bronchioles contained cellular debri.

Thirtysix hours post inoculation the intensity of the reaction was more than at 18 hours. Petechiae were noticed in the kidney, liver, pancreas and in the pulmonary tissue. Myocardial fibers showed extensive degeneration and fragmentation. There was haemorrhage and mononuclear reaction (Fig. 58) could be well discerned. There was degeneration of the parenchymatous organs and diffuse infiltration of mononuclear cells.

The nature of changes were qualitatively same at 48 hours post-inoculation but the intensity was more severe and involvement more wide. The degenerative changes were more pronounced in the liver and kidney. Many foci of necrosis were seen in the liver. The renal epithelial cells also showed severe necrosis and there was interstitial mononuclear reaction (Fig. 59). The myocardial fibers were widely separated with oedema and presence of round and stellate cells. There was partial lysis of the fibers. Numerous spindle shaped cells were seen in between the muscle bundles, but it was not quite evident whether they were undifferentiated mesenchymal cells or developing fibroblasts.

Different parts of the gut showed degeneration of the epithelium and cellular infiltration in the interstitial tissue (Figs. 60 and 61). There was proteinaceous oedema and occasional areas of haemorrhage. The orbital fossa contained protein rich fluid containing large numbers of round cells. The changes in the brain were mainly characterised

by oedema and liquifactive necrosis. There was a loosening of the cytoarchitecture and the chambers contained degenerated cells. The epithelium lining the respiratory pathway showed degenerative changes with mononuclear infiltration. In the lungs there was oedema and the parenchyma contained large number of mononuclear cells.

At 72 hours post-inoculation the changes were more intense than seen at 48 hours. In the eye the layers of the retina were found separated and there was accumulation of small quantity of protein rich fluid (Fig. 62). In the anterior chamber and near the ciliary body numerous round cells were seen. In the brain tissue there was degeneration of neuronal cells, liquifactive necrosis and haemorrhage (Fig. 63). Infiltration of glial elements around the degenerating neurons was not seen. The changes in the respiratory organs were more intense than seen at 48 h. The pulmonary tissue especially the peribronchial area had few mononuclear cellular reaction.

Lesions in the heart were more severe with degeneration and fragmentation of muscle fibers and massive infiltration of mononuclear cells (Fig. 64). The pericardial surface had fibrin rich fluid along with erythrocytes and mononuclear cells. The epithelial cells in other locations, especially the gut showed varying degree of degeneration. There was marked degeneration and necrosis of hepatic cells and renal epithelial cells. The glomeruli appeared hypercellular (Fig. 65).

Occasional fragmentation of the glomerular tuft was seen. Inside the Bowman's capsule eosinophilic granular material along with desquamated and degenerated cells were present.

The bursa of Fabricius had focal necrosis, and it was more prominent in the lymphoid areas. Except for the infiltration of large number of mononuclear cells no significant change was seen in the thymus.

In a few locations in the developing bone there was chondrolysis and infiltration with few number of mononuclear cells.

ULTRASTRUCTURAL CHANGES

The ultrastructural changes were evaluated after comparing them with those in the control tissues.

Ochratoxin A

Liver.

The changes manifested by the hepatic cells varied from necrosis of some cells to apparently mild ones as manifested by swelling of the mitochondria. There was varying amount of membrane damage. The frank necrosis with complete destruction of cytoplasmic structures and nuclear configuration was noticed only in few cells (Fig. 66). In general the significant alteration noticed was the increased number of lipid droplets of varying sizes. They were of different electron density. Some of them were uniformly electron dense while

others were electron lucent with an outer rim of electron dense material (Fig. 67 and 68). The mitochondria were numerous. They were oval, round or elongated and occasionally giant mitochondria were also seen. The outer mitochondrial membranes were intact but the cristae appeared swollen and the matrix was of increased electron density (Fig. 69). Foci of homogenous electron lucent areas with depletion of organelles were also noticed. The amount of glycogen varied from cell to cell. Peroxisomes were few and when present did not reveal any nucleoid structures. The nuclei did not show any uniform alteration. Flocculent aggregation of chromatin or partial lysis was seen. Nucleoli were single or multiple and of different sizes and configurations. Euchromatin was predominant, heterochromatin was sparse and the number of nuclear pores varied (Fig. 70). There was fragmentation and degranulation of rough endoplasmic reticulum. Smooth endoplasmic reticulum was not evident. Golgi complex was poorly developed. Structures similar to lysosomal structures were seen, but only sparsely. The plasma membrane at the endothelial surface was intact but on the bile canalicular part they had a ruffled appearance and appeared damaged. The bile canaliculi contained bits of damaged cellular structures (Fig. 71).

Kidney.

The changes were found to be more intense in the convoluted tubules, more specifically in the proximal convoluted tubules. In many areas the tubular structures were not

well formed, only groups of cells with epithelial characteristics and forming tight junctions were seen. In general, ultrastructural alterations were more severe in cells which had been differentiated to form definite structures than in undifferentiated cells. In many of these cells which had not been organized the organellar components were generally sparse. Mitochondria were few in number. The nuclei were generally very large with the chromatin mainly of euchromatin type.

In the proximal convoluted tubular epithelium, there was moderate destruction of the brush border with full or partial lysis of the brush border. In many locations the plasma membrane showed irregularity and disruption and in some places there was concentration of granular electron dense deposits close to its attachment near the basement membrane. Few strands of endoplasmic reticulum were noticed. Ribosomes were few in these endoplasmic fragments. The structural changes in the mitochondria showed great variation. Some of them had extreme organellar disfiguration with loss of cristae while others presented only slight swelling with increased electron density of matrix. Few dense bodies and myelinated structures resembling secondary lysosomes were noticed. Numerous vacuoles were present (Fig. 72).

The nuclei were large with irregular nuclear membrane. Aggregation of chromatin was seen in some cells while others had partially lytic chromatin. Nucleoli were prominent, but

very often there was segregation of nucleolonema and the granular components. In a few cells nucleoli appeared as condensed mass. The cells in other parts of the nephron revealed varying degrees of degeneration. Cytoplasmic damage was comparatively more severe in the distal convoluted tubules. Few lysosomes were present (Fig. 73).

The changes in the glomeruli were mainly confined to the basement membrane and the podocytes. There appeared to be an increase in the fenestrated nature of endothelial surface of the basement membrane and damage to the foot processes of the podocytes. The foot processes were either found absent or had fused to form large sucker like protrusions (Fig. 74). Except for slight swelling of the mitochondria, and increased electron density of the cytoplasmic matrix the organellar changes in the cytoplasm were not very pronounced. The nuclei, in general, appeared normal. The cytoplasm of the mesangial cells except for increased amount of lysosomal structures apparently did not manifest severe damage. Occasionally the cisternae of the endoplasmic reticulum were dilated and formed vesicular structures. The nuclear chromatin did not show much evidence of damage.

Citrinin

Liver.

The cytoplasm had increased electron density and granular contents. The mitochondria were fairly numerous with round or oval morphology. Some appeared swollen with slightly

disorganized cristae. A few of the mitochondria had completely lost their identity and only remenants of cristae could be seen inside them (Fig. 75). In general endoplasmic reticulum was intact and degranulation was not prominent. The endoplasmic reticulum was thin and there was no contents in most of them. Few lipid droplets were present (Fig. 76). The nuclei appeared normal or had only slight changes in the form of aggregations of chromatin and loss of interchromatin granules. In a few cells ring-shaped nucleoli with desegregation of nucleolenemma and granular components was noticed. Lysosomes were few and peroxisomes were scanty. The quantity of glycogen appeared normal and uniformly distributed mainly as beta-particles. The bile canaliculi contained cellular debri. The canalicular part of the cytoplasm was more electron dense in appearance. Plasma membrane adjoining adjacent hepatic cells was interdigitated and showed partial lysis in places. Golgi complex was illdeveloped.

Kidney.

The tubular epithelial cells showed the severest form of damage. Some of the cells had organellar changes indicative of advanced necrobiotic changes while in others the changes were only minimal. Even in cells which were not showing advanced necrobiotic changes numerous vacuoles of varying sizes were present in the cytoplasm.

The necrobiotic cells showed severe condensation of chromatin or chromatolysis. Chromatolysis was complete or

partial. Complete disintegration and lysis of the nuclear membrane was occasionally seen. There was slight damage to the brush border. The tubular epithelial cells showed different grades of destruction, from slight changes to severe destruction of organelles and nucleus (Figs. 77, 78, 79, 80). The changes in the glomeruli were not very prominent except that there was mild to severe swelling of the mitochondria of podocytes, mesangial cells, endothelial cells and epithelium lining the Bowman's capsule. Numerous vacuoles were present in the endothelium and their attachment to the underlying basement membrane appear loose. The nuclei in these cells did not manifest any severe change. The podocyte foot processes showed slight disorientation in their arrangement and occasionally there was fusion of foot processes (Fig. 81).

In many areas the tubular architecture was not clearly defined and groups of epithelial cells were seen only as collections. But organogenesis and structural identification could be made out. In these cells the changes were mainly manifested as bulged out plasma membrane with a clear area under the plasma membrane or with the presence of numerous vacuolar structures and electron lucency of the cytoplasm (Fig. 82).

Citrinin and Ochratoxin A

Liver.

The hepatic cells had varying degrees of organellar changes including presence of lipid droplets (Fig. 83).

Some of the cells were frankly necrotic while others had only minor alterations. In cells not necrotic, profiles of rough endoplasmic reticulum were seen mostly near mitochondria. These appeared as fragmented units. Ribosomes with polysomal configurations were found lying free in the cytoplasm. Plasma membrane appeared disrupted in many places. Numerous vacuoles most of them in the cytoplasm beneath the plasma membrane, were present. There was marked oedema within the cytoplasm. The mitochondria were numerous in number and most of them were elongated and enlarged. A few oval or ring shaped mitochondria were also noticed. The chromatin appeared partially condensed or flacculent (Fig. 84). The cristae were oriented in different directions and the normal spatial arrangement was seen lost. The matrix was very dense. The intramitochondrial granules were few. The inner and outer mitochondrial membranes were clearly discerned and in some mitochondria the outer membranes were irregularly thrown into protrusions. Giant mitochondria with irregular outline were also seen. The bile canaliculi contained granular contents (Fig. 85). Lipid droplets were more in number than seen in the normal liver. They showed varying size and electron density of the contents (Fig. 86). Some of these droplets had an outer rim of increased electron density while others had uniform contents. In a few cells the lipid contents were segmented and compartmentalised. A few were enclosed in membraneous structures indicating their liposomal nature.

The cytoplasm contained fairly large quantities of glycogen particles, both alpha and beta particles. Golgi complex was not prominent - the lamellar, vacuolar and vesicular components were not well developed. Lysosomes were seldom present and if seen they occurred as small discrete granules. Numerous membrane bound structures containing electron dense crystalline contents were seen. The outer membranes of these structures were very irregular. In addition to the crystalline structures, some amount of electron lucent granular contents was noticed within the membranes (Figs. 87 and 88).

The nuclei were usually large, oval with irregular nuclear membranes. Euchromatin was the predominent type and the little aggregates of heterochromatin were located at the inner nuclear membrane. The karyoplasm had a limpid appearance. Lipid inclusions were seen in some cells within the nucleus. In all the cells more than one nucleolus was found. The granular and fibrillar components were clearly discerned. Occasional cells with ring shaped nucleoli were also seen.

The villous protrusions of the plasma membrane were not uniform and they had a disrupted appearance. The canaliculi in addition to containing slightly amorphous electron lucent material had also disrupted cytoplasmic components from the villous surfaces. The structural changes seen in the kupffer cells were mainly an increase of lysosomes, both autophagolysosomes and heterolysosomes and of the rough surfaced endoplasmic reticulum.

Kidney.

The tubular epithelial cells especially those of the convoluted tubules showed extensive cytoplasmic changes. Numerous vacuoles were present (Fig. 89). The mitochondria were swollen and in many the cristae were completely disorganized. There was degranulation of ribosomes from the few strands of endoplasmic reticulum. The cytoplasm was electron dense with a granular appearance. There was partial destruction of basement membrane (Fig. 90).

The nucleus was prominent with irregular nuclear membranes. Heterochromatin was arranged as clumps on the inner nuclear membranes as well as lying as small blocks elsewhere. The cell junctions were not prominent at the tenth day embryo but they were clearly discerned in the fifteenth day embryo. Lysosomes were scanty and golgi complex were not generally seen. The nucleoli were usually large and prominent and the nucleolonema was seen surrounded by islands of granular components. In some, there was condensation of the nucleoli (Fig.91) The histological pattern of tubules of the mature kidney was not seen in some locations and sheets of epithelial cells with attempted tubule formations were noticed. The cytoplasm appeared more electron dense (Fig. 92).

In the proximal convoluted tubules the brush border appeared disrupted. Along the border of the cells the appearance of degenerated cytoplasmic components was indicated by myelin figures. Some of these cells showed advanced

degenerative phenomenon with numerous dark dense bodies suggestive of secondary lysosomes. The basement membrane of the collecting tubules and the cell junctions appeared more or less intact. The changes in the cells of the proximal and distal convoluted tubules appeared more severe than in other parts of the nephron and collecting tubules (Figs. 93 and 94).

In the glomeruli, the basement membrane was found to have lost its homogenous nature and in some places there was fragmentation while in others there was increased electron density. There was fusion of the foot-processes of the podocytes. The nuclei of podocytes appeared normal but the cytoplasm revealed increased amount of lysosomal granules. A few lipid droplets were also noticed in the cytoplasm. The endothelial cells were swollen with increased number of vacuoles. The cytoplasm of the endothelial cells were thrown out into numerous folds. The mesangial cell cytoplasm was granular and electron dense and contained numerous vacuoles. The endoplasmic reticulum was slightly fragmented (Fig. 95). The protruded portion contained mitochondria which was larger than those seen in other locations. The nuclear membrane was very prominent and was seen as cisternae in some places.

Figs. 1 & 2. Chick embryo - Oedema in the region of head and neck. Subcutaneous tissue and muscles affected.

Fig. 3. Chick embryo - Subcutaneous and muscular oedema. Herniation of yolk sac also seen

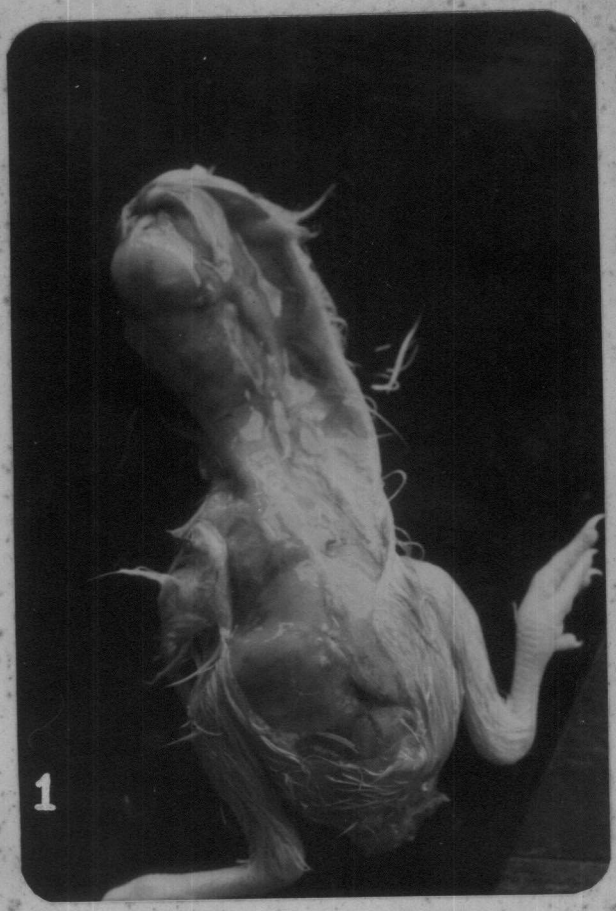
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Fig. 4. Dwarf embryo with curled toe and oedema in the neck region

Fig. 5. Dwarf embryo with illdeveloped appendages


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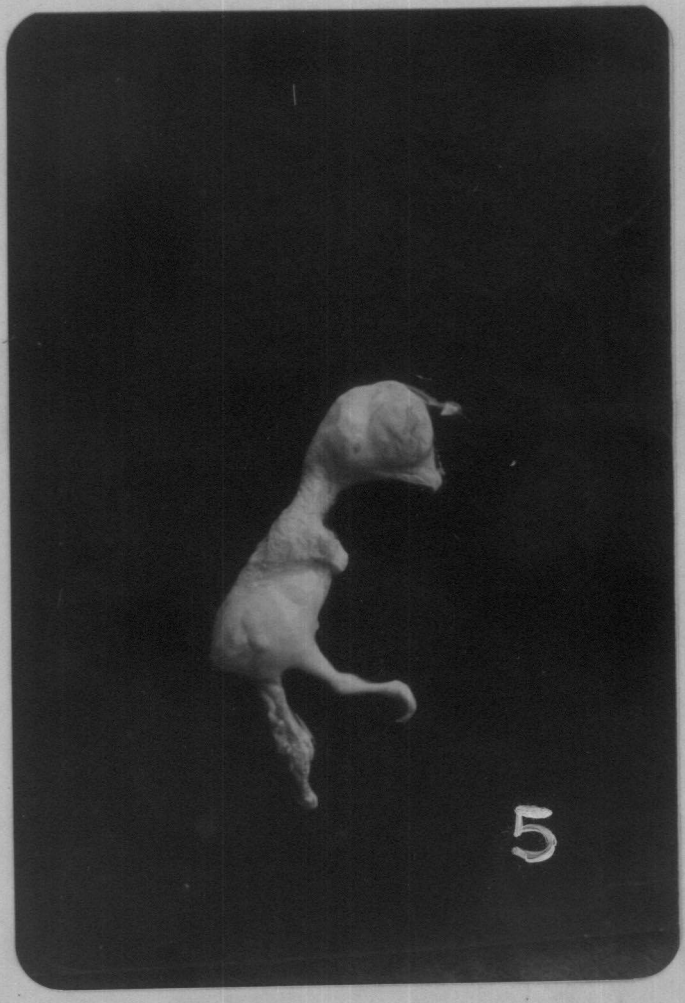
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Figs. 6, 7 & 8. Embryos with omphalitis

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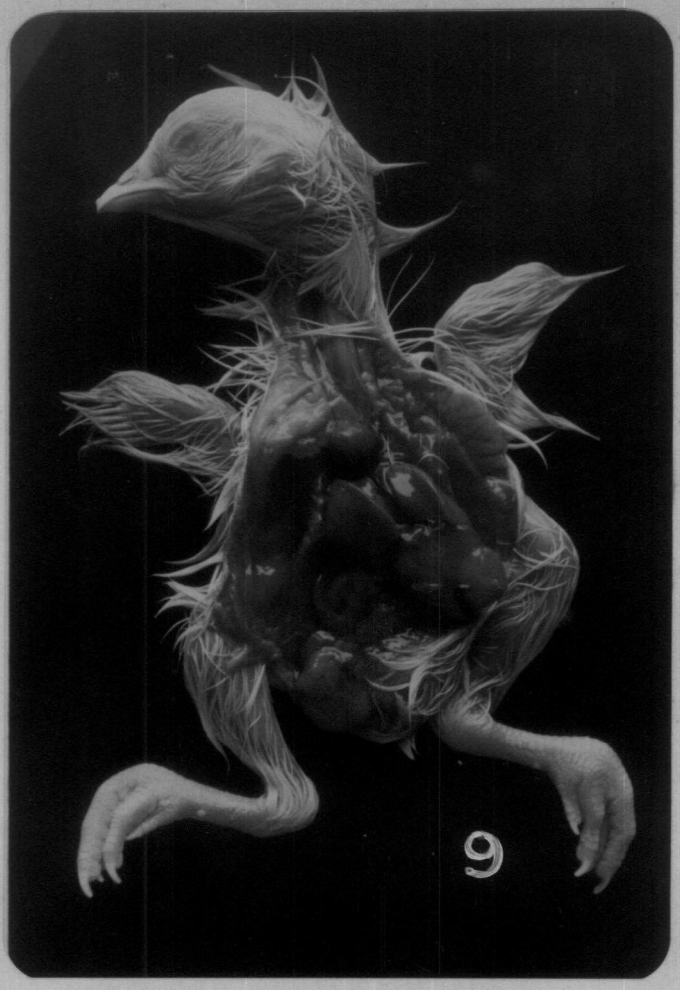
Fig. 9. Omphalitis with associated congestion and haemorrhage of visceral organs

Fig. 10. Coelosoma with eventration of intestine and yolk sac. The embryo on the left shows beak abnormality and partial herniation of brain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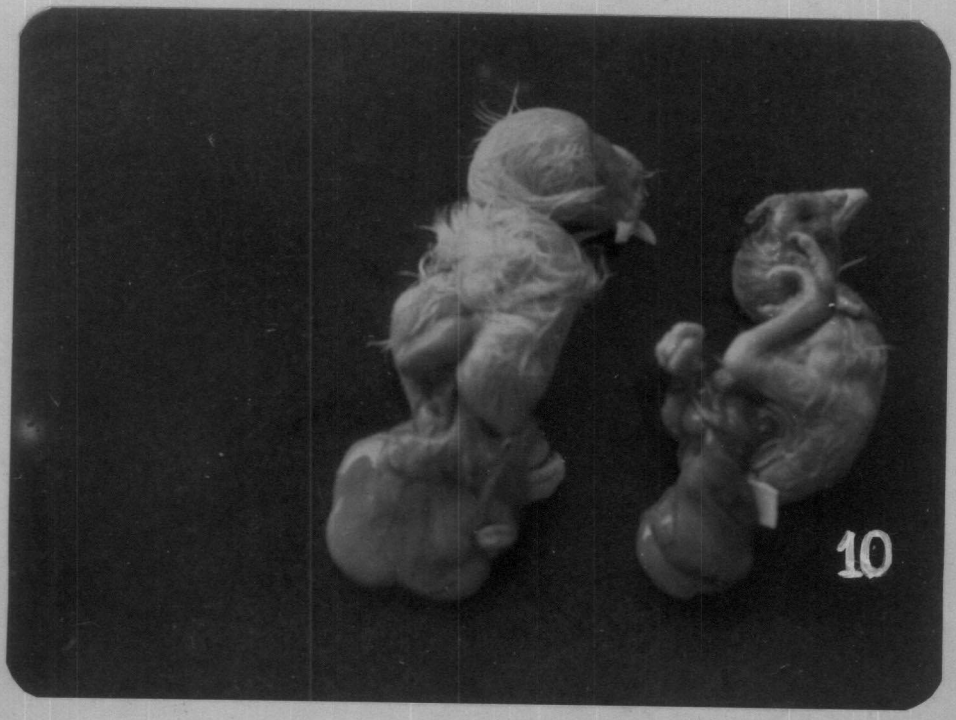
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Fig. 11. Embryo with polymelia and coelosoma

Fig. 12. Dorsal view of the same embryo showing partial cranioschisis


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Fig. 13. Embryo with curled toe, short upper beak and herniation of brain

Fig. 14. Embryo with crossed beak


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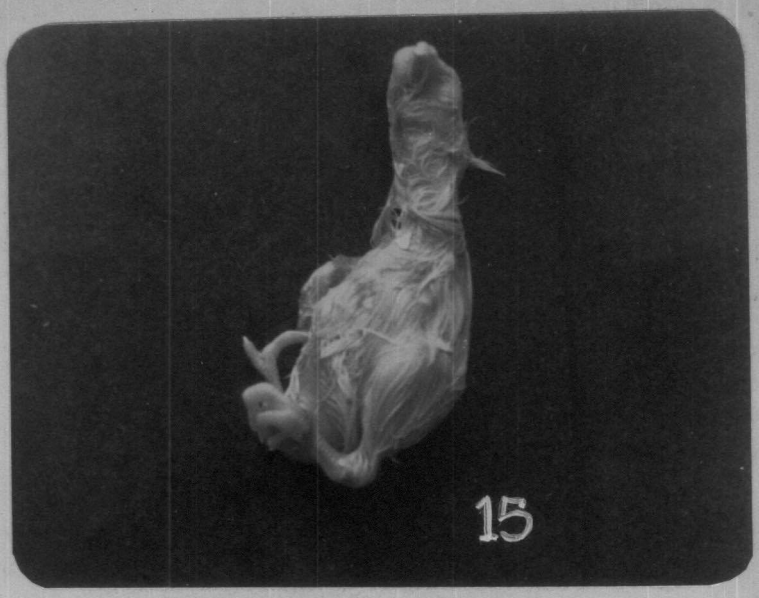
Fig. 15. Embryo showing agnathia

Fig. 16. Embryo with cranioschisis and imperfect development of skull bones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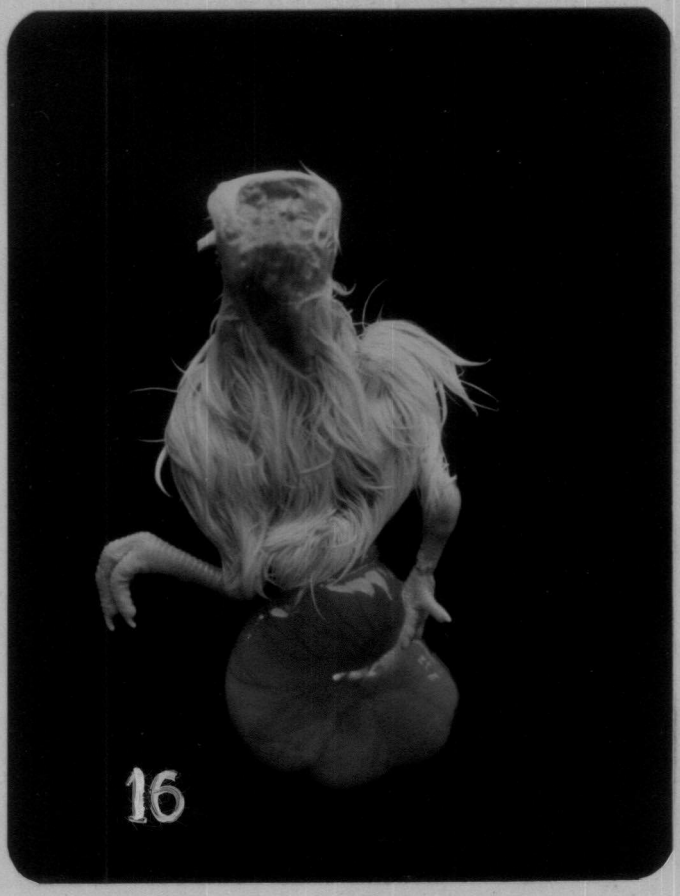
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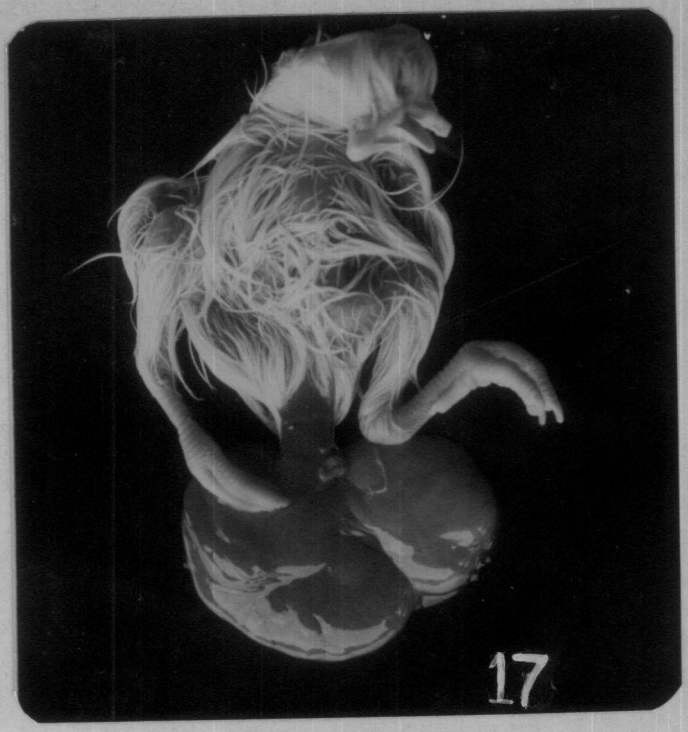
Fig. 17. Cyclopic embryo with two upper beaks, cranioschisis and herniation of yolk

Figs.18 & 19. Embryo showing reduction in the size and generalised oedema (Oxytetracycline administration)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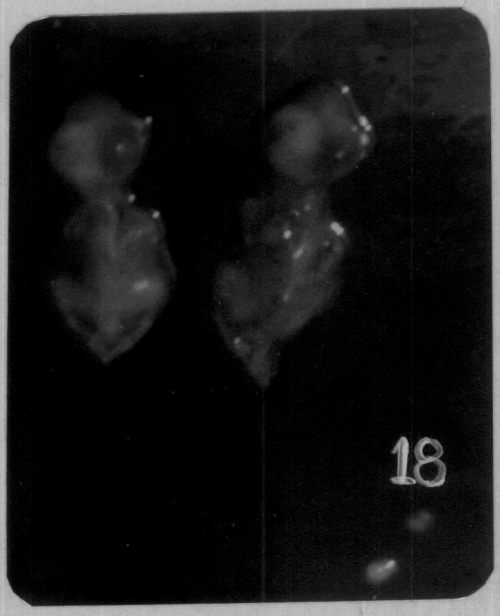
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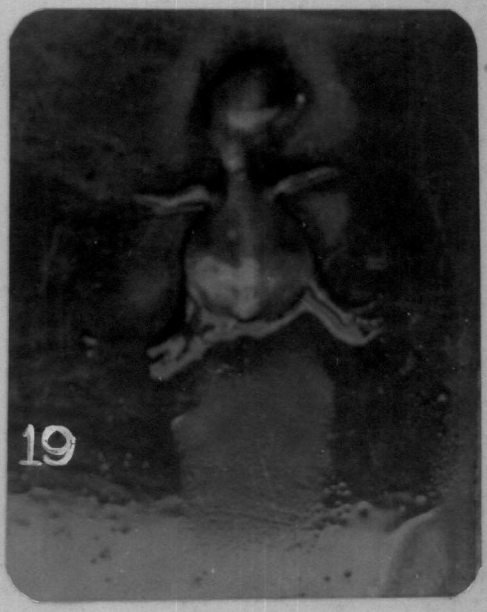
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Fig. 20. Two embryos on the left (Oxytetracycline administration) which are reduced in size compared to the normal on the right

Fig. 21. Reduction in size of the embryo with herniation of the yolk (Oxytetracycline administration)


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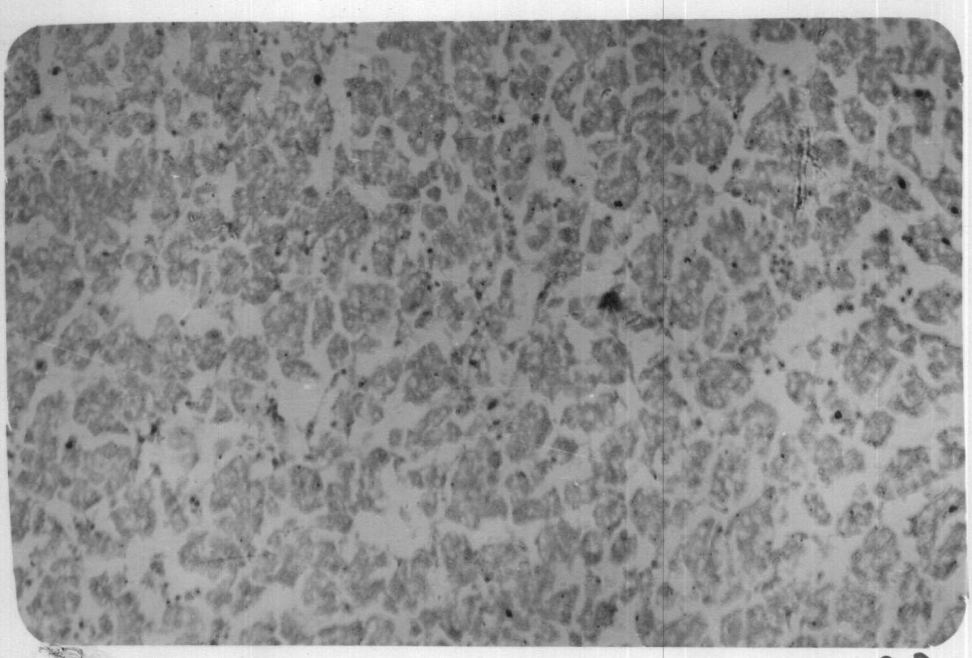
21

Fig. 22. Liver - 18 day old embryo (Oxytetracycline) - There is diffuse parenchymatous degeneration and fatty change. H & E x 400

Fig. 23. Kidney - 18 day old embryo (Oxytetracycline) - Tubular epithelium shows severe degenerative changes. H & E x 600



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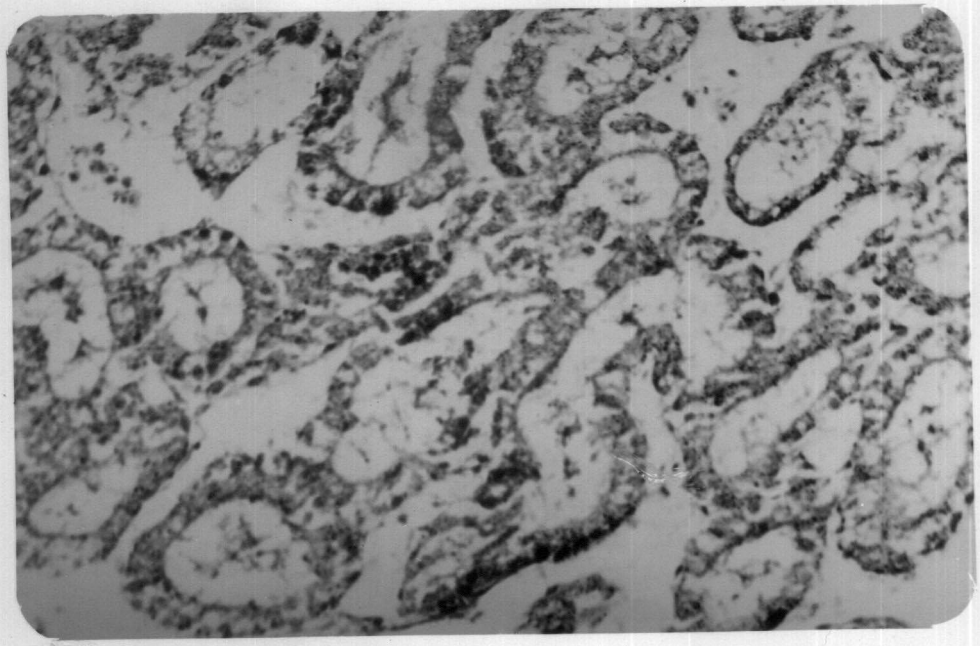

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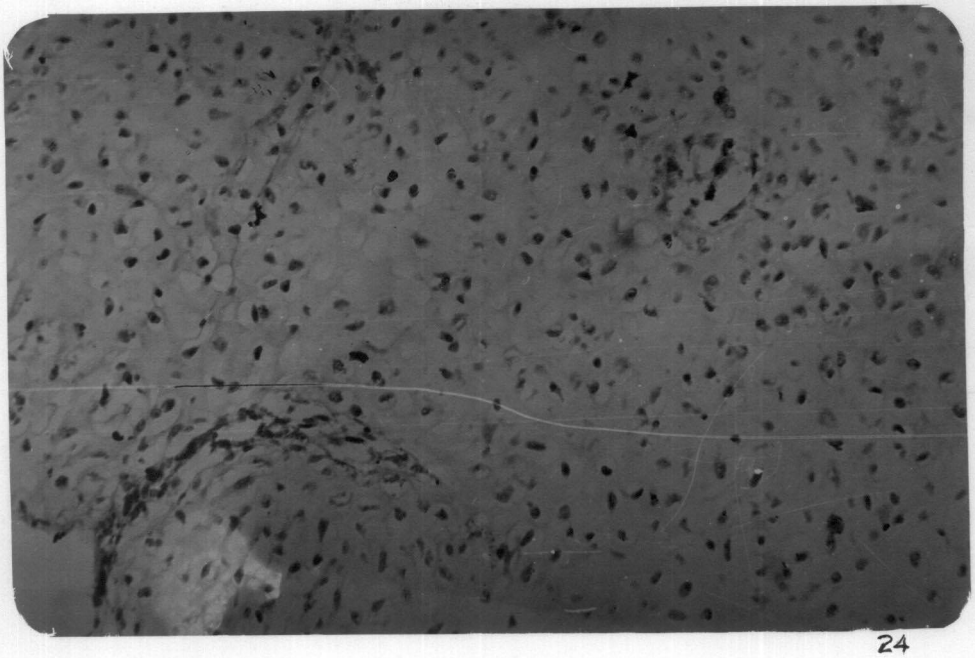
Fig. 24. Basal cranium - 18 day old embryo (Oxytetracycline) Irregular arrangement of cartilage cells with imperfect matrix formation. H & E x 400

Fig. 25. Bone - 18 day old embryo (Oxytetracycline). Note imperfect chondrogenesis. Many of the chondroblasts appear flattened. H & E x 400



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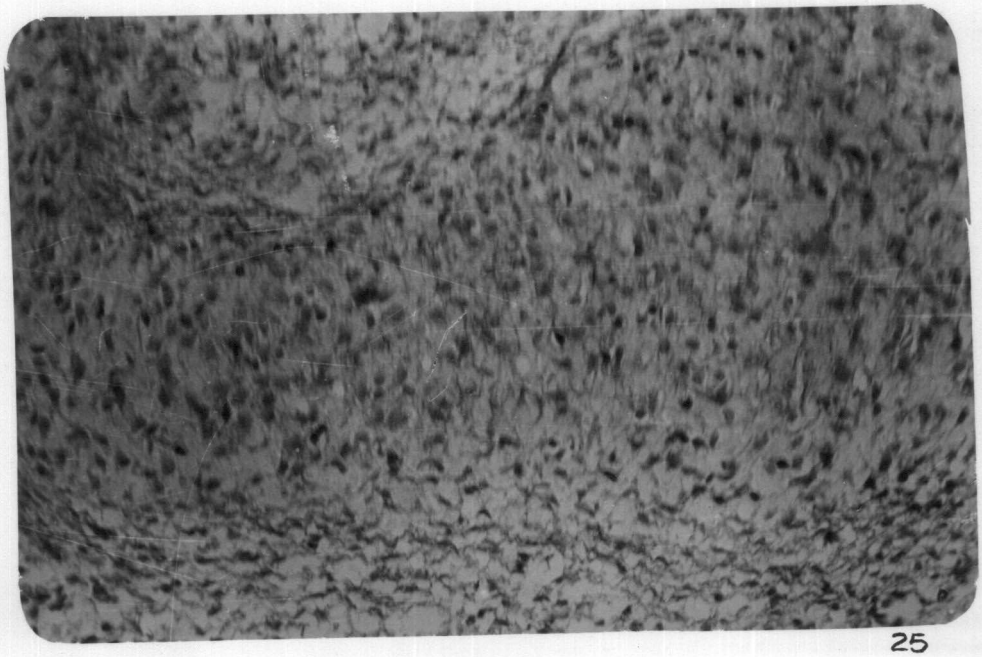

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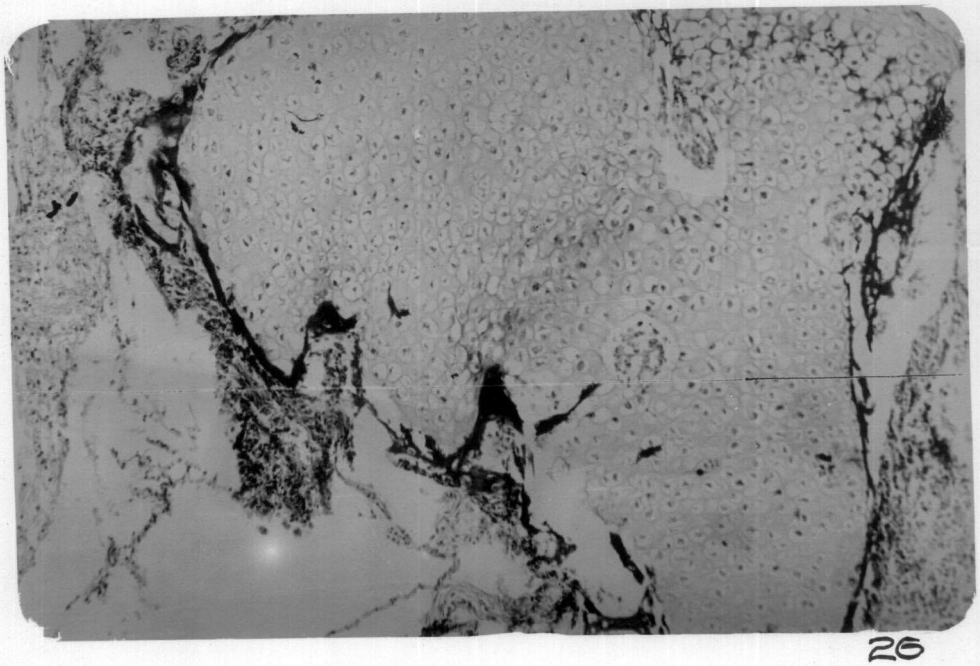
Fig. 26. Bone - 21 day old embryo (Oxytetracycline) - There is irregular calcification beneath the perichondrium. H & E x 200

Fig. 27. Bone - 18 day old embryo (Oxytetracycline. Numerous mesenchymal cells which have not differentiated noticed. There is irregular arrangement of osteoblasts. Few blood vessels are also seen. H & E x 400



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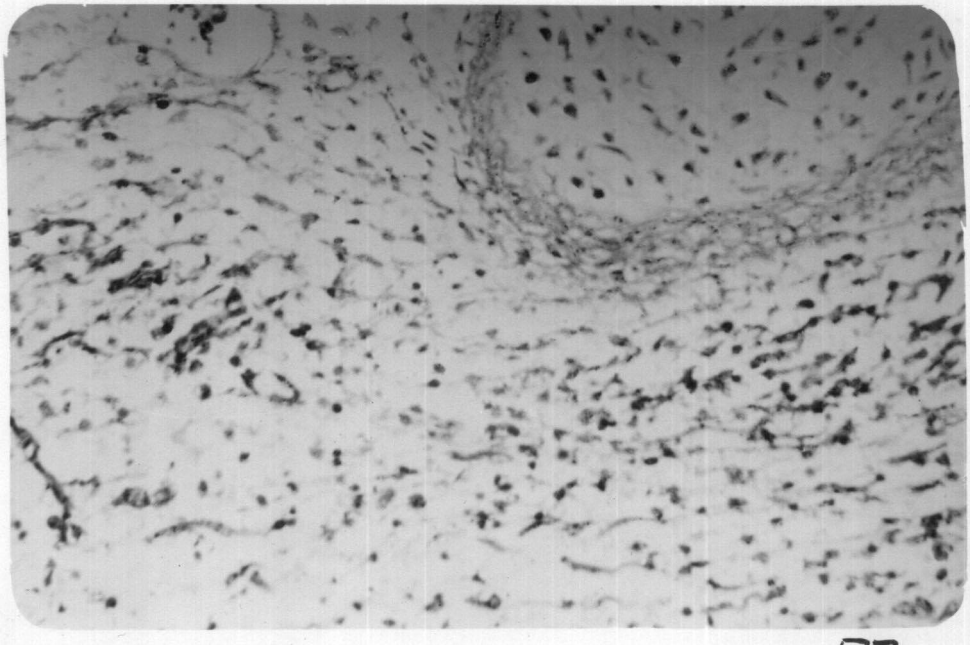

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Fig. 28. Reduction in the size of the embryo on the right (Ochratoxin administration) in comparison with the normal embryo (control)

Fig. 29. Reduction in size and cranioschisis (Ochratoxin A administration)

Fig. 30. Embryo showing reduction in size and beak abnormalities (Ochratoxin A administration)


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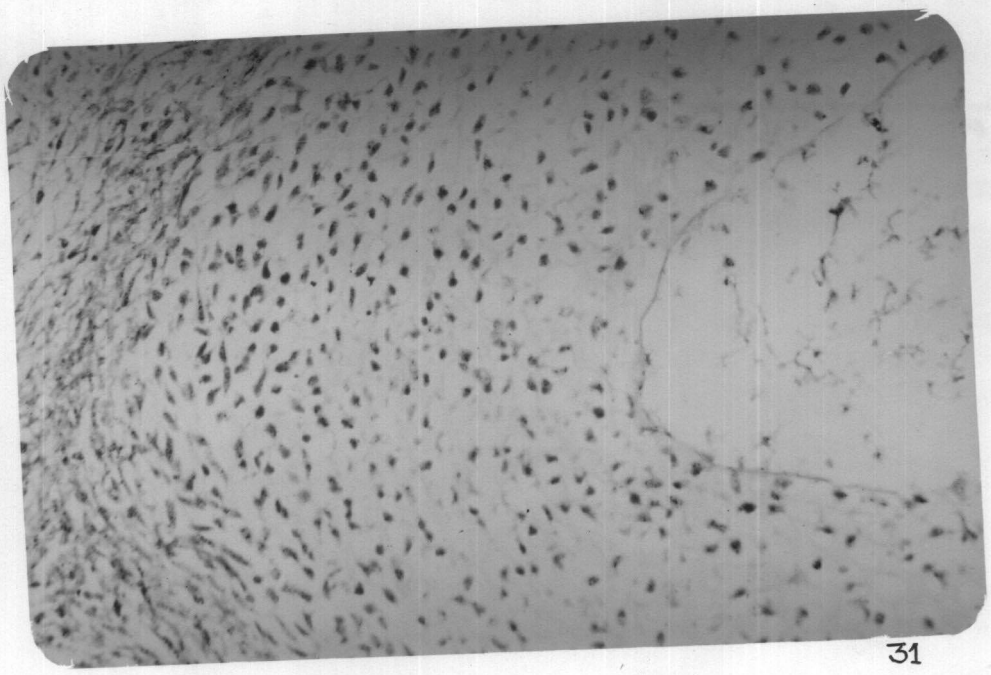
Fig. 31. Bone - 21 day old embryo (Ochratoxin A). Note numerous mesenchymal cells which have not differentiated into chondroblasts and osteoblasts. H & E x 400

Fig. 32. 21 day old embryo (Ochratoxin A). Irregular cartilagenous thickening of the vertebrae with narrowing of the neural canal. H & E x 100



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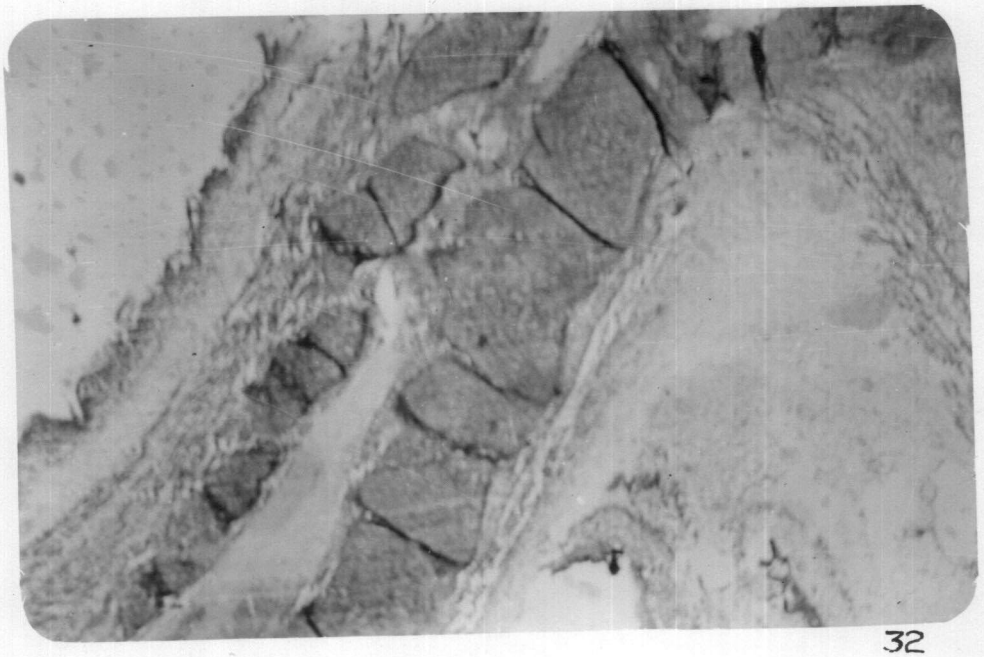

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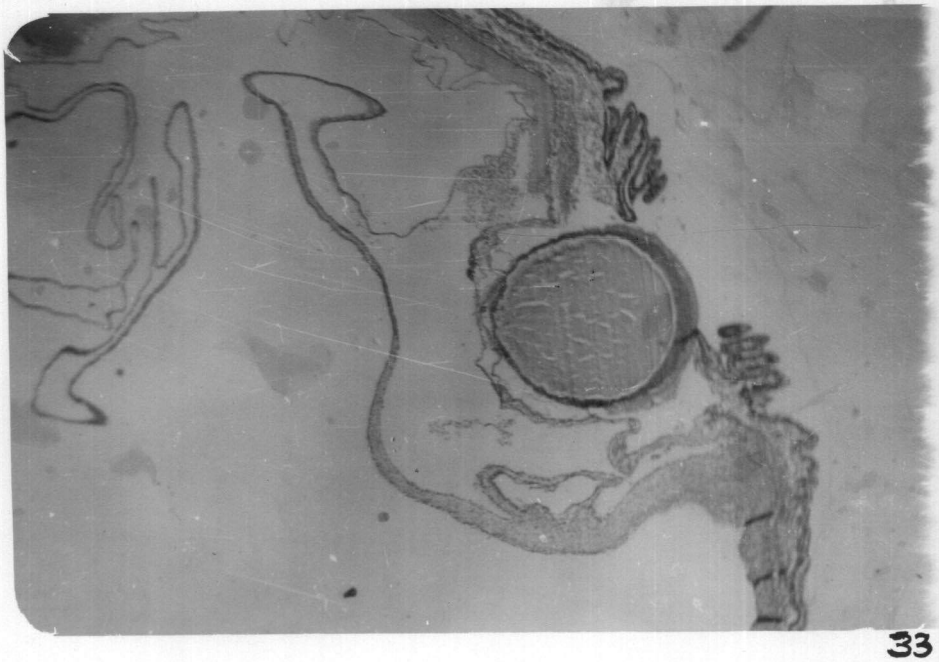
Fig. 33. Eye - 15 day old embryo (Ochratoxin A). Note detachment of retina. H & E x 100

Fig. 34. Eye - 15 day old embryo (Ochratoxin A). Degeneration of the ganglionic layer of Retina. H & E x 400



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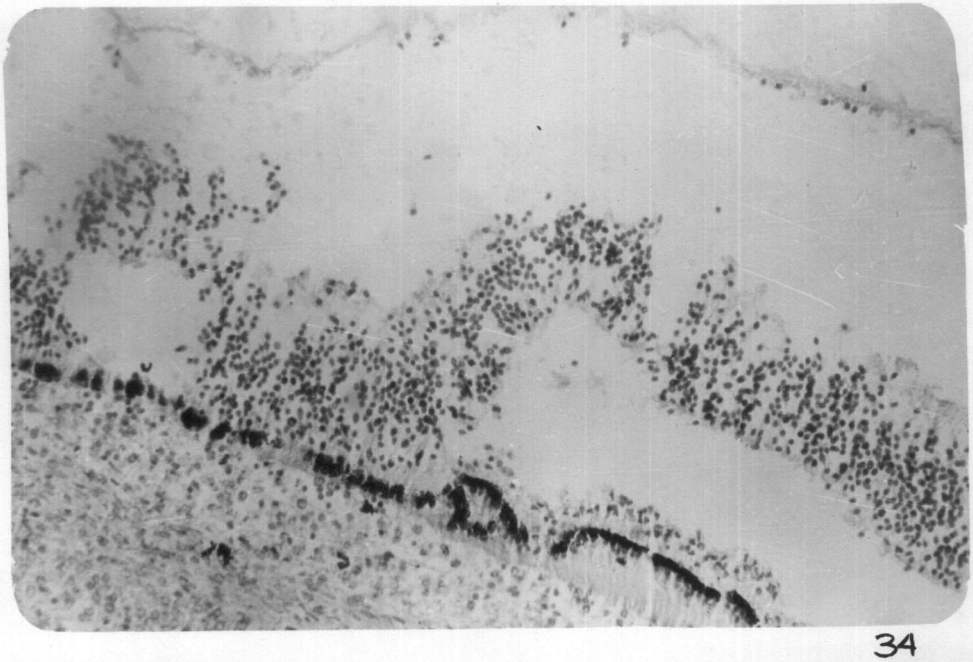

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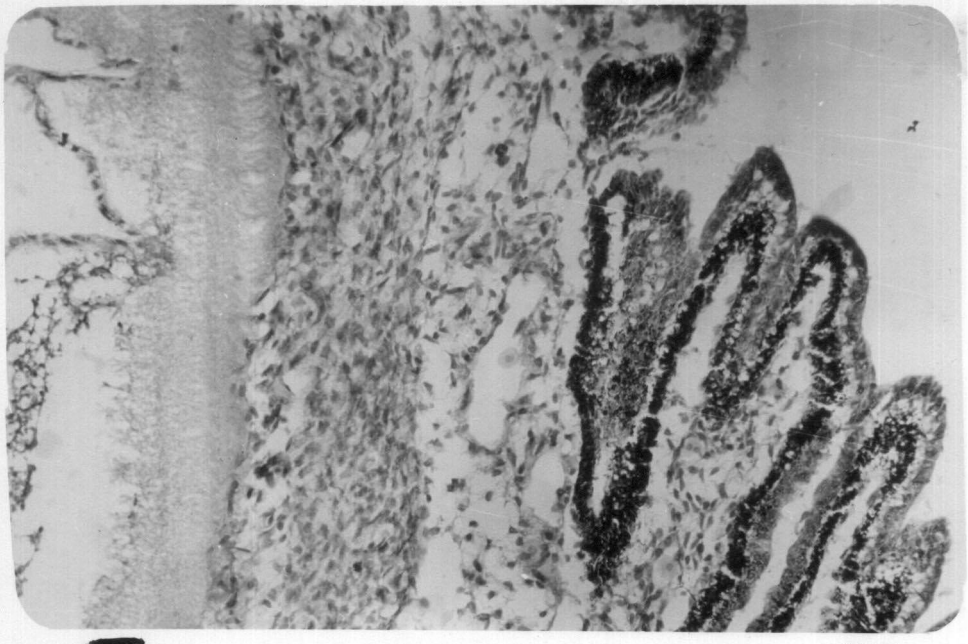
Fig. 35. Eye - 15 day old embryo (Ochratoxin A).
Degenerative changes in the ciliary body.
H & E x 600

Fig. 36. Brain - 21 day old embryo with cranioschisis
(Ochratoxin A). There is liquifactive necrosis.
Note the presence of numerous glial cells.
H & E x 400



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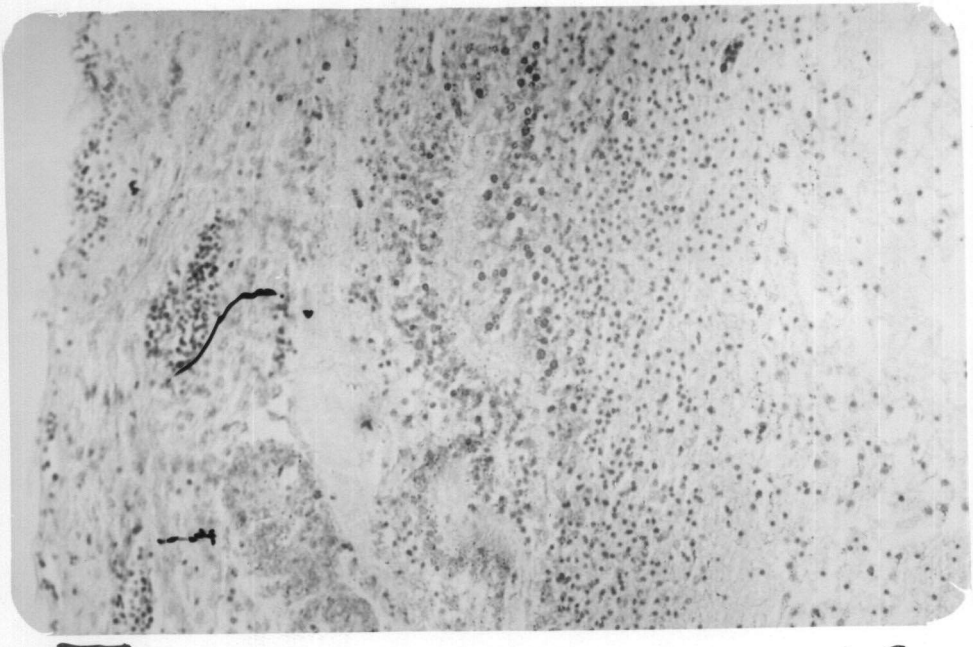

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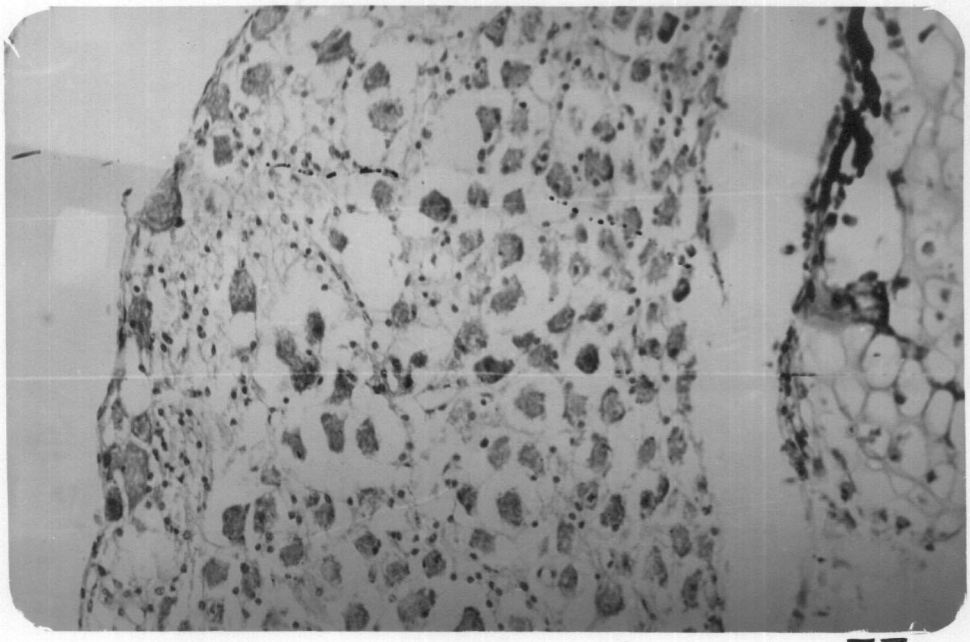
Fig. 37. Nerve ganglion - 21 day old embryo (Ochratoxin A). Neurons showing degenerative changes. H & E x 400.

Fig. 38. Kidney - 15 day old embryo (Ochratoxin A). There is degeneration and necrosis of the tubular epithelial cells. There is interstitial oedema. H & E x 200



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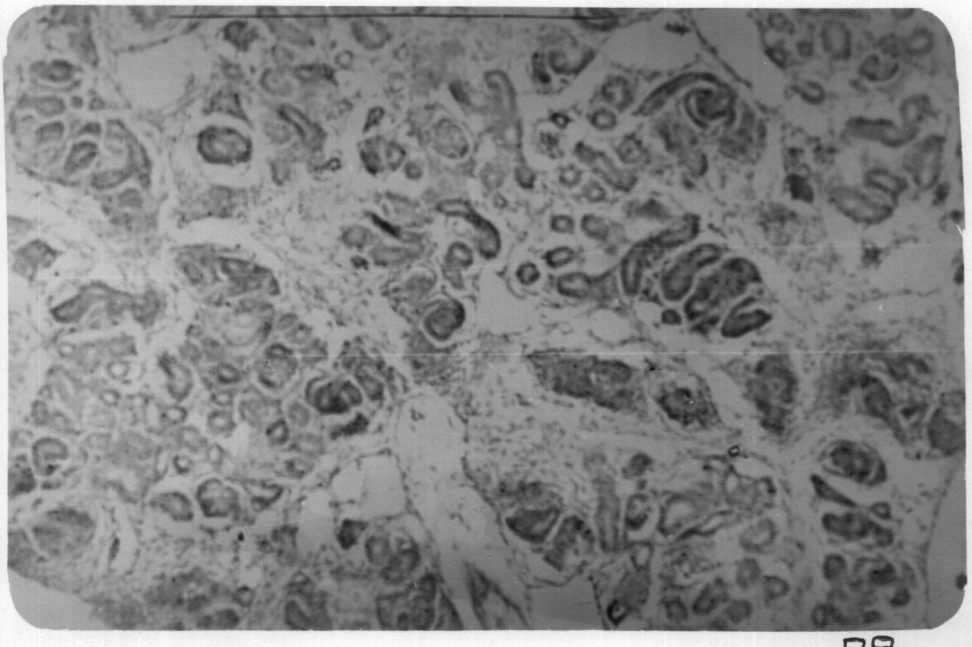

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Fig. 39. Kidney - 21 day old embryo (Ochratoxin A). Extensive degeneration and necrosis of tubular epithelium. H & E x 400.

Fig. 40. Liver - 21 day old embryo (Ochratoxin A). Hepatic cells showing degeneration and necrosis. H & E x 200

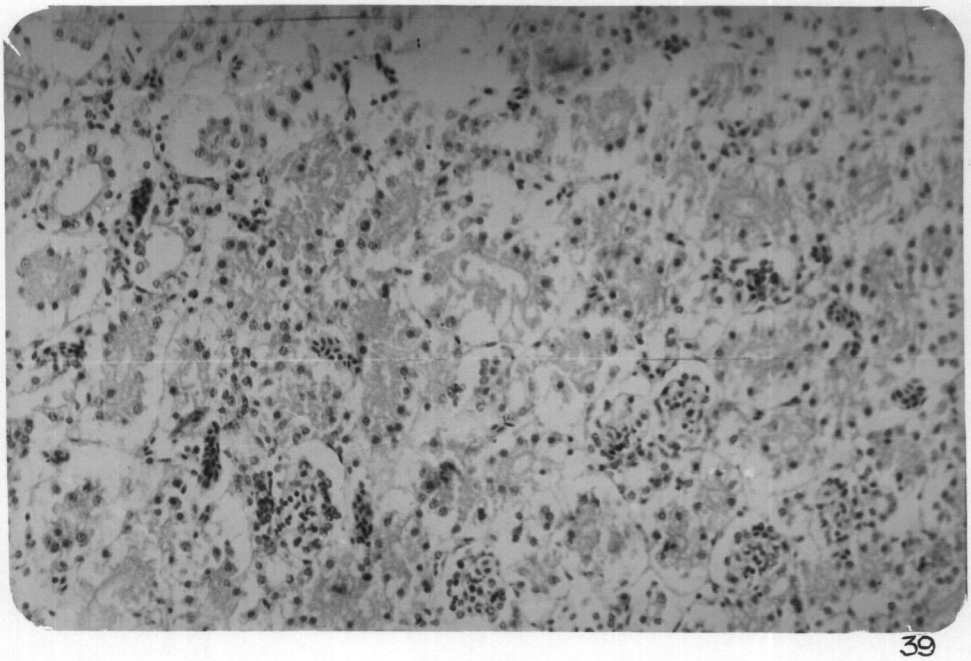

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Fig. 41. Heart - 21 day old embryo (Ochratoxin A). Cardiac fibres showing myolysis and fragmentation. H & E x 400

Fig. 42. Heart - 21 day old embryo (Ochratoxin A). Endocardium showing button like protrusions of cardiac muscles. H & E x 400

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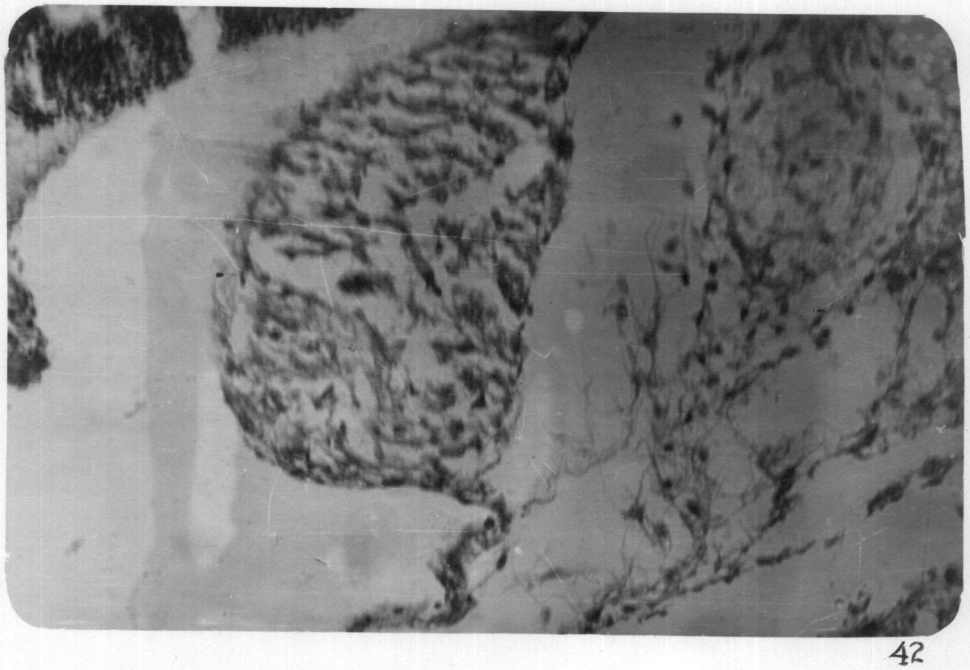

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Fig. 43. Bursa of Fabricius - 21 day old embryo (Ochratoxin A). There is necrosis of epithelial and lymphoid cells. H & E x 200

Fig. 44. 15 day old embryo (Citrinin). Liver and kidney showing degenerative changes. H & E x 100

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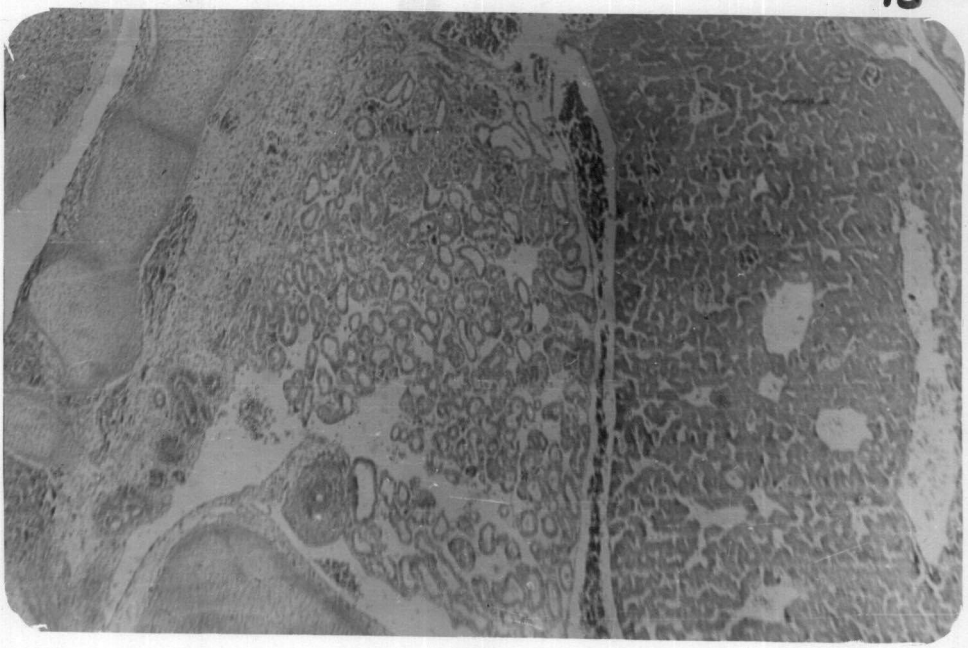

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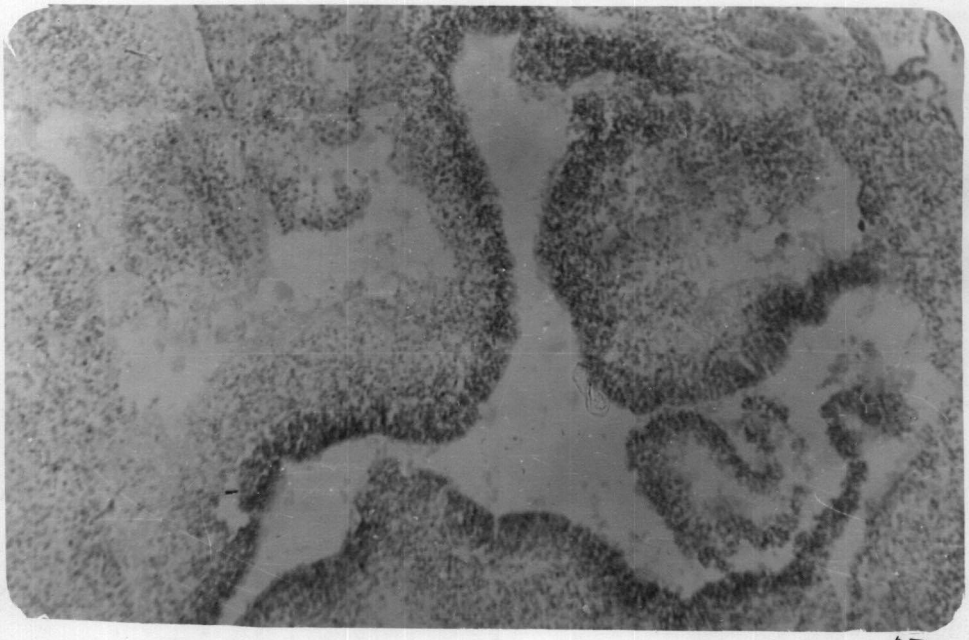
Fig. 45. Brain - 13 day old embryo (Citrinin). Note oedema of diencephalon. H & E x 100

Fig. 46. Kidney - 21 day old embryo (Citrinin). Note areas of calcification. H & E x 400



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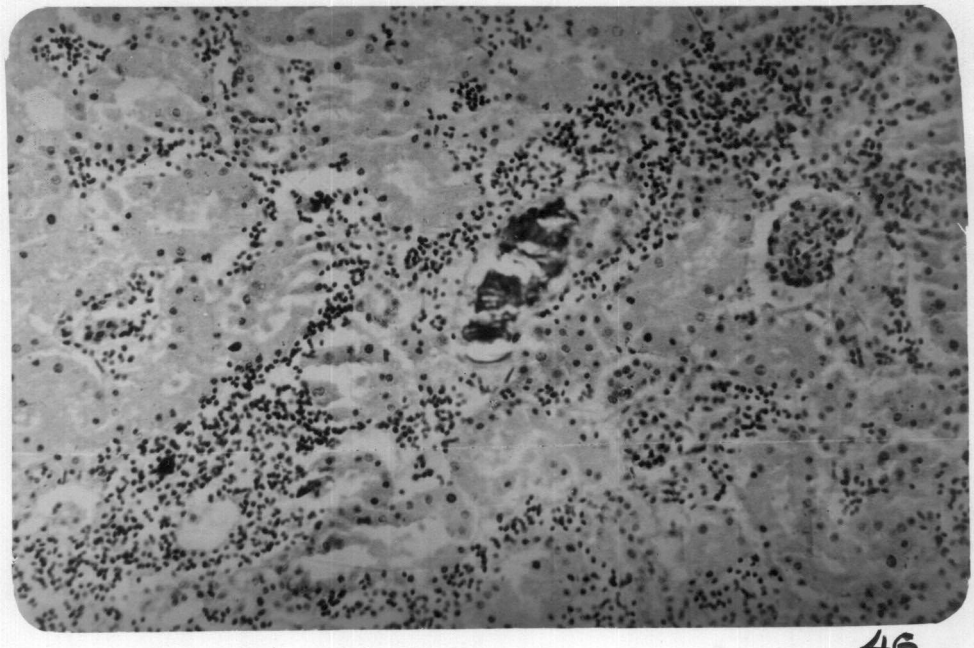

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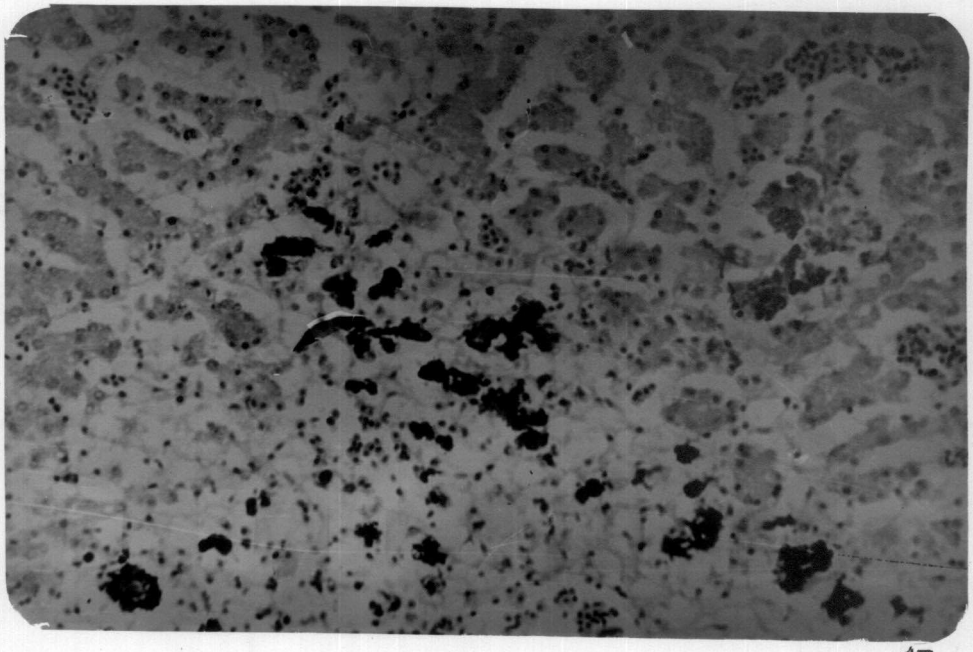
Fig. 47. Liver - 21 day old embryo (Citrinin). Numerous foci of calcification seen. H & E x 400

Fig. 48. 21 day old embryo (Citrinin) showing oedema and haemorrhage in the subcutis and muscles of the neck region. H & E x 400



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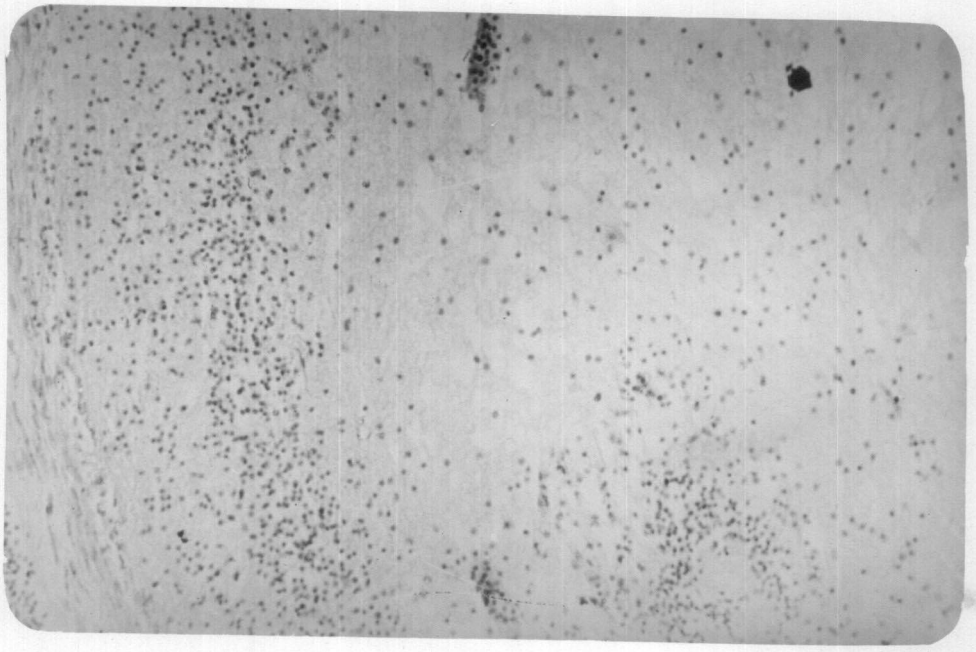

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Fig. 49. Kidney - 21 day old embryo (Ochratoxin A and Citrinin). Diffuse necrosis of the tubular epithelium of the kidney. H & E x 400

Fig. 50. Kidney - 21 day old embryo (Ochratoxin A and Citrinin). Extensive degeneration and necrosis of tubular epithelium. Note the swollen glomeruli. H & E x 600

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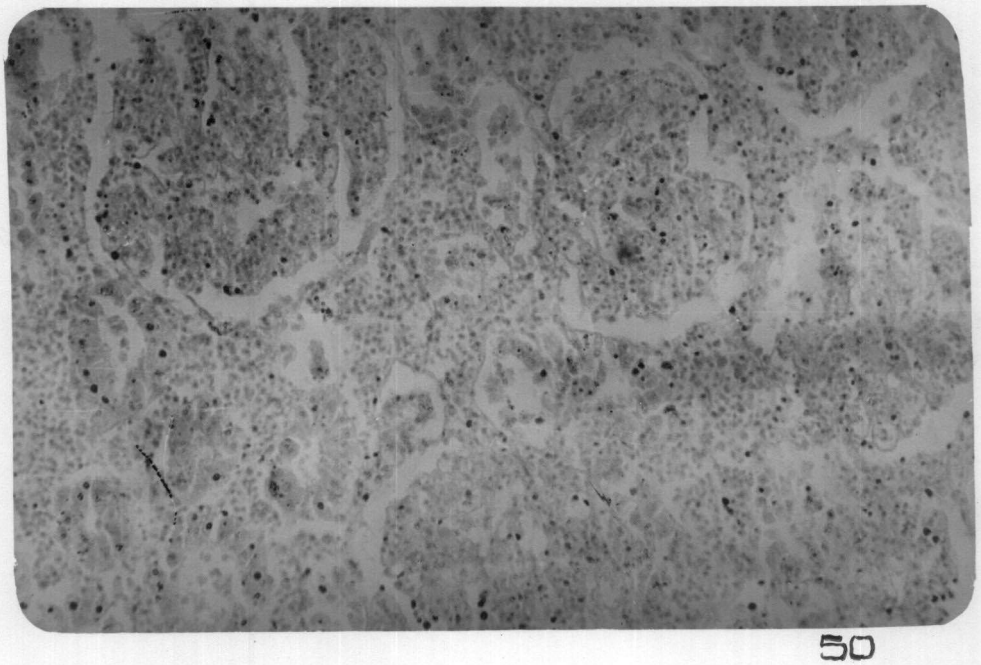

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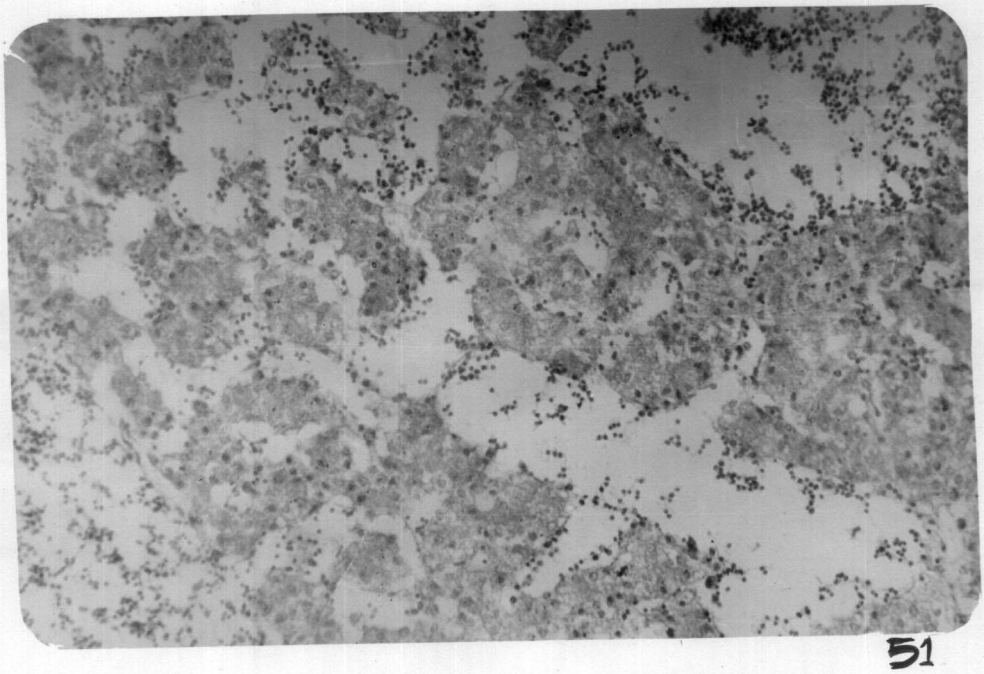
Fig. 51. Liver - 16 day old embryo (Ochratoxin A and Citrinin). Diffuse necrosis with confluent areas of haemorrhage. H & E x 400

Fig. 52. Bursa of Fabricius - 21 day old embryo (Ochratoxin A and Citrinin). Note diffuse degeneration of lymphoid elements. H & E x 400



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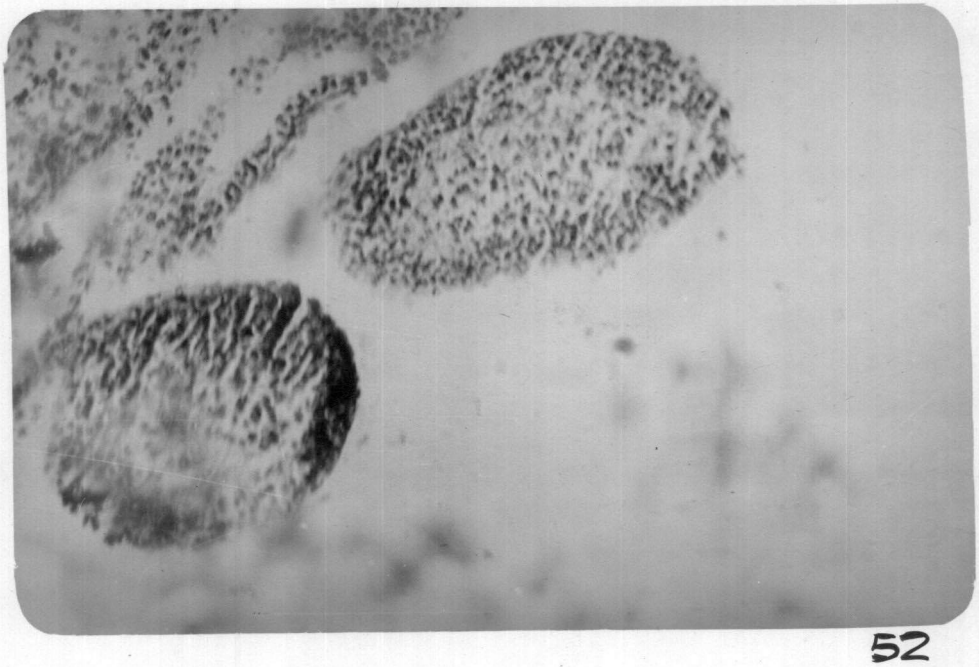
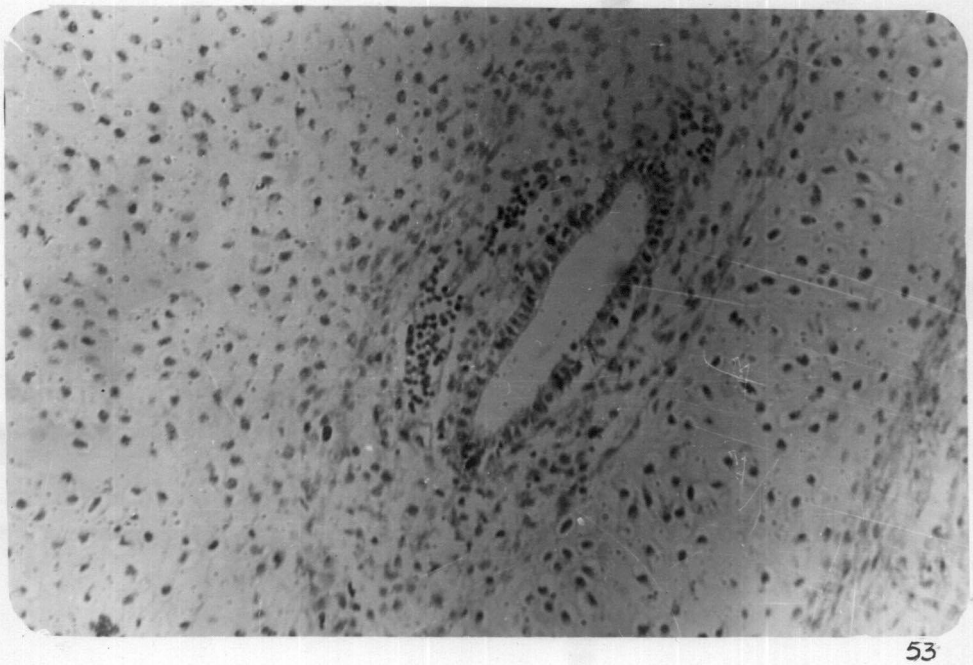


52

Fig. 53. Bone - 21 day old embryo (Ochratoxin A and Citrinin). There is disturbed chondrogenesis, osteogenesis and calcification. H & E x 400

Fig. 54. 16 day old embryo (Ochratoxin A and Citrinin). Vertebra showing imperfect osteogenesis. H & E x 200



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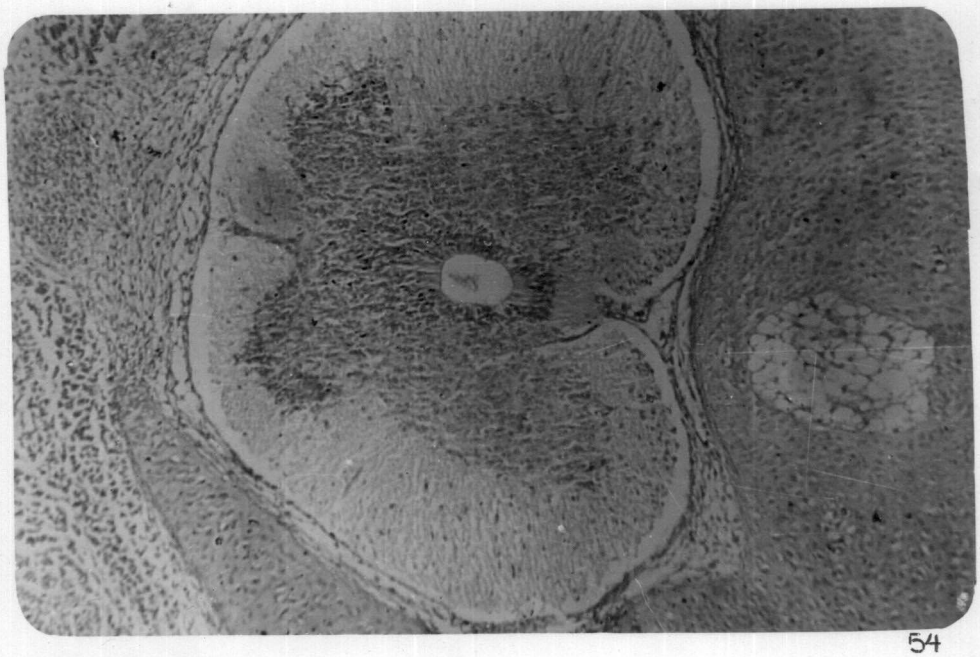
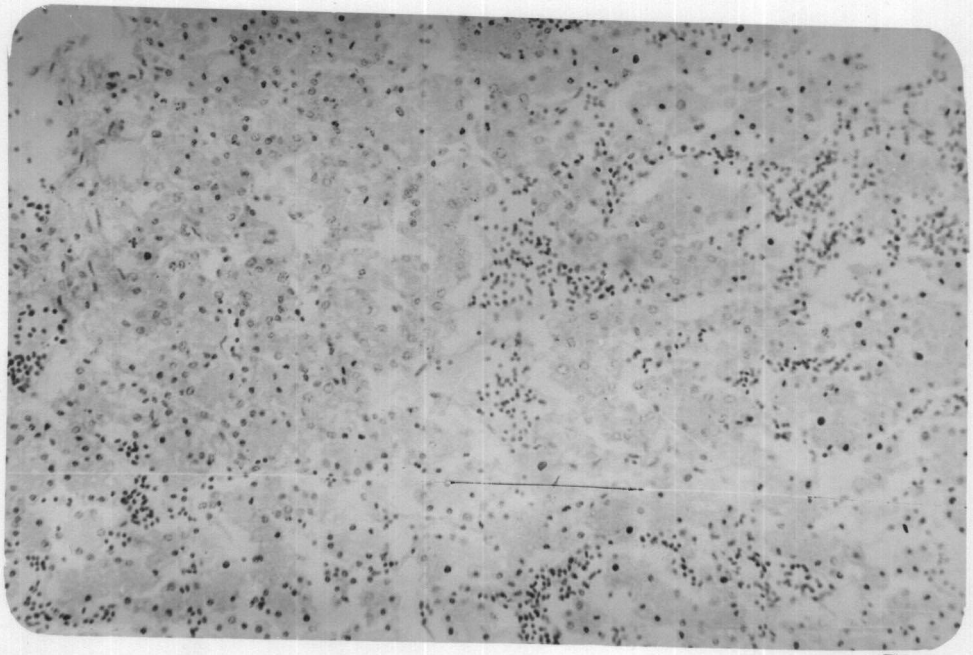


54

Fig. 55. Brain - 11 day old embryo (Ochratoxin A and Citrinin). Note degeneration, necrosis and oedema. H & E x 100

Fig. 56. Liver - 10 day old embryo (Avian influenza virus). 18 hours post-inoculation. Hepatic cells showed degenerative changes. There is congestion and haemorrhage. H & E x

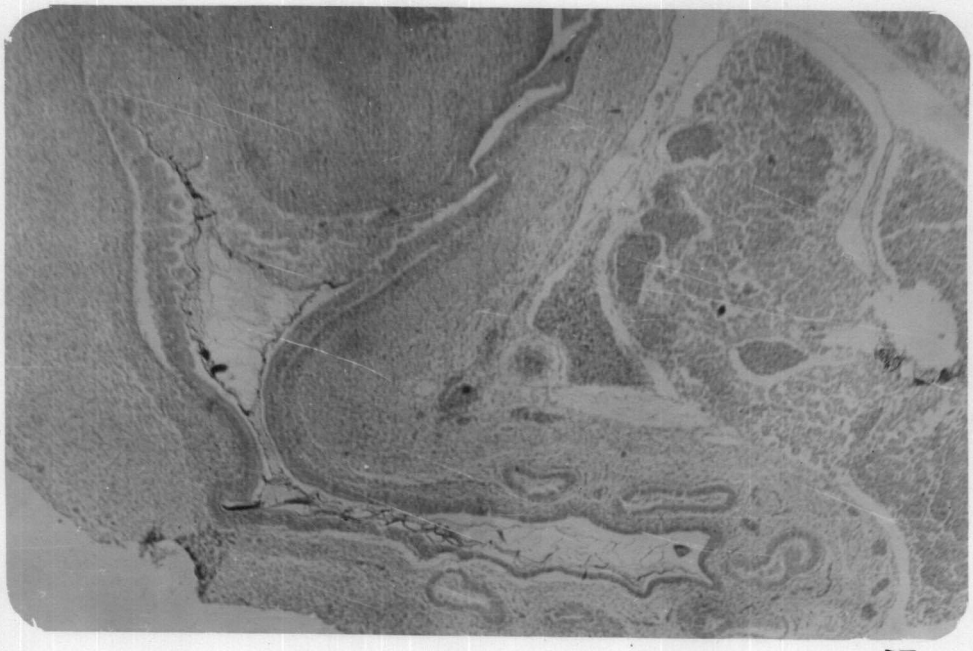
55



56

Fig. 57. 10 day old embryo (Avian influenza virus) - 18 hours post inoculation. Degeneration and sloughing of gut epithelium. H & E x 100

Fig. 58. Heart - 11 day old embryo (Avian influenza virus) - 36 hours post-inoculation - Myocardium showing degeneration and fragmentation of muscle fibers. Mononuclear infiltration seen. H & E x 200



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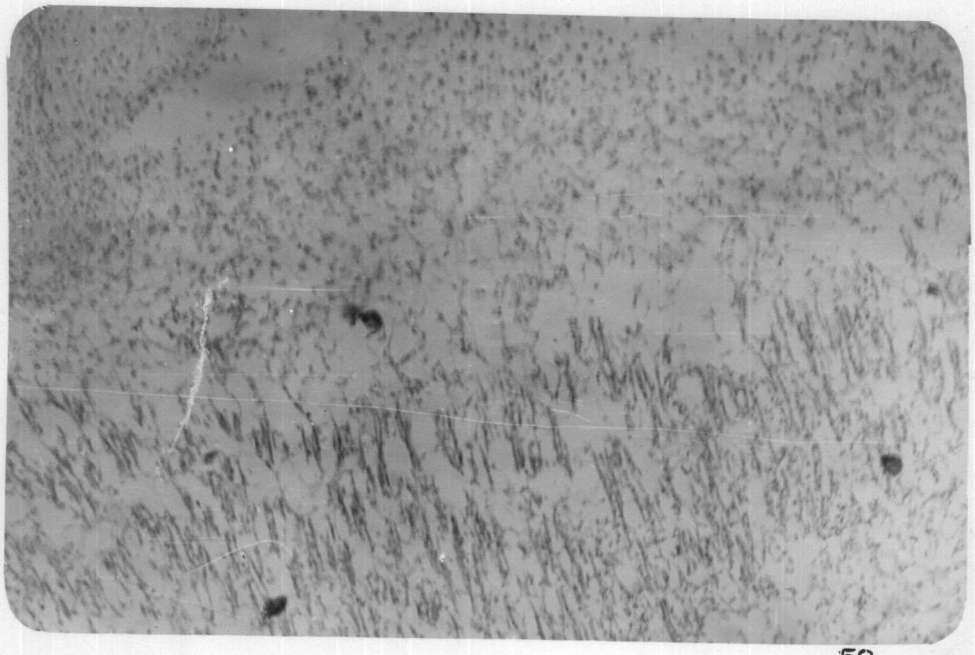
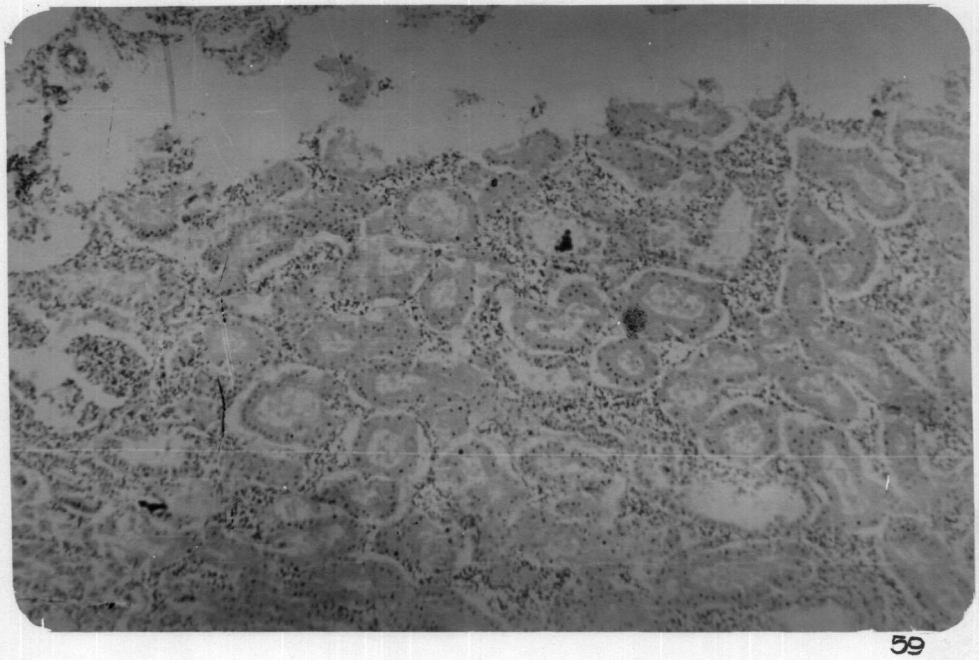


58

Fig. 59. Kidney - 11 day old embryo (Avian influenza virus) - 48 hours post-inoculation. Necrosis of tubular epithelial cells and interstitial mononuclear reaction. H & E x 400

Fig. 60. 11 day old embryo (Avian influenza virus) - 48 hours post-inoculation. Portion of the gut showing degeneration of lining epithelium. H & E x 400



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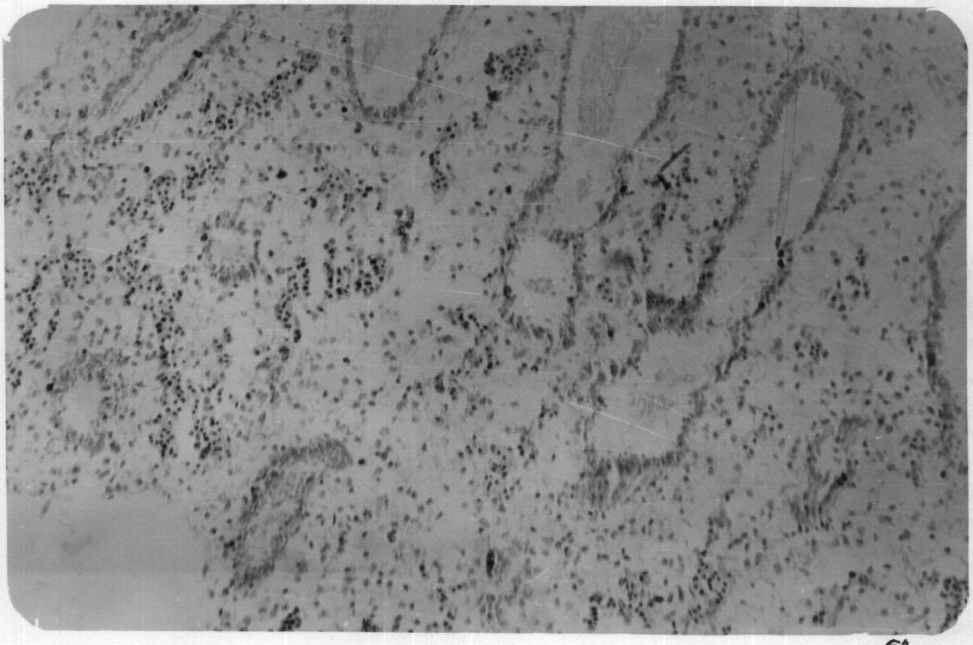
60

Fig. 61. 11 day old embryo (Avian influenza virus) - 48 hours post-inoculation portion of the gut showing presence of mononuclear cells in the interstitial tissue. H & E x 300

Fig. 62. Eye - 12 day old embryo (Avian influenza virus) - 72 hours post-inoculation. There is separation of layers of retina and accumulation of protein rich fluid. H & E x 100



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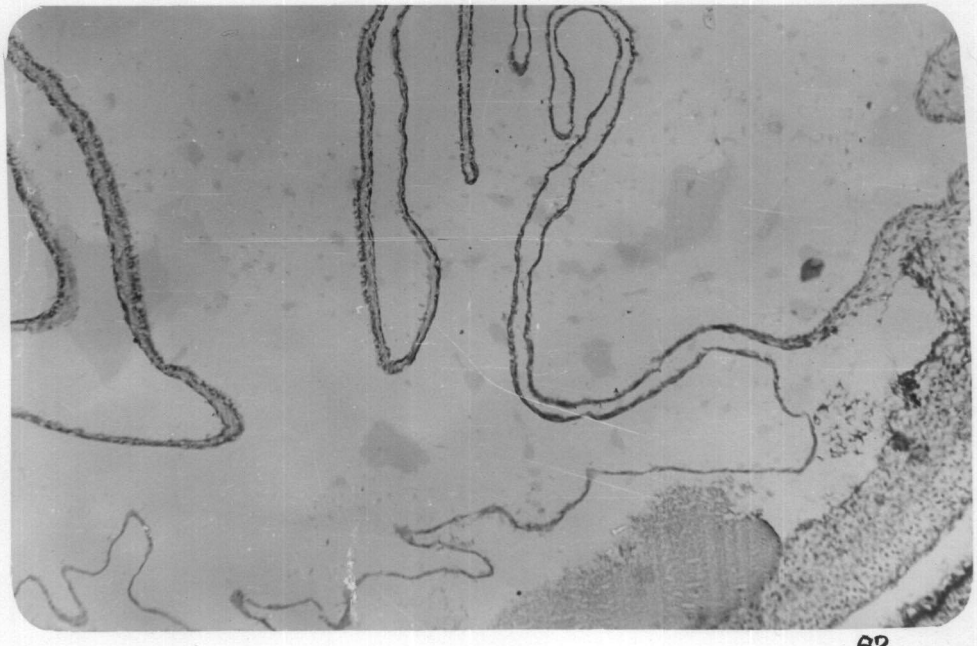
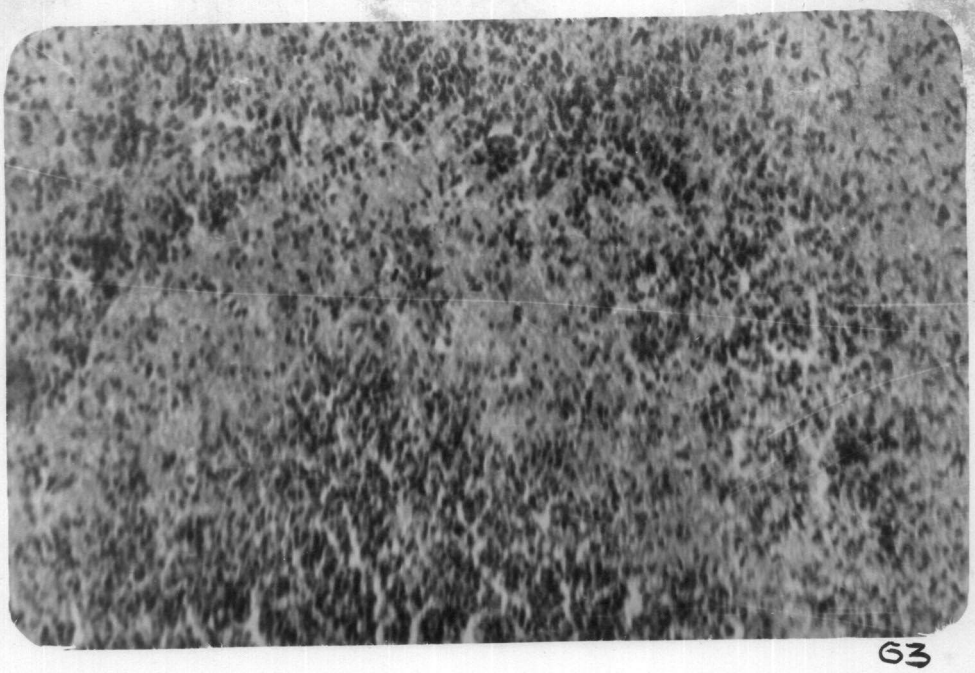


62

Fig. 63. Brain - 12 day old embryo (Avian influenza virus) - 72 hours post-inoculation - liquifactive necrosis and haemorrhage. H & E x 400

Fig. 64. Heart - 12 day old embryo (Avian influenza virus) - 72 hours post-inoculation. Fragmentation and necrosis of cardiac fibres - Interstitial infiltration of mononuclear cells. H & E x 400

Fig. 65. Kidney - 12 day old embryo (Avian influenza virus) - 72 hours post-inoculation. Necrosis and tubular epithelial cells with hypercellularity of glomeruli. H & E x 400



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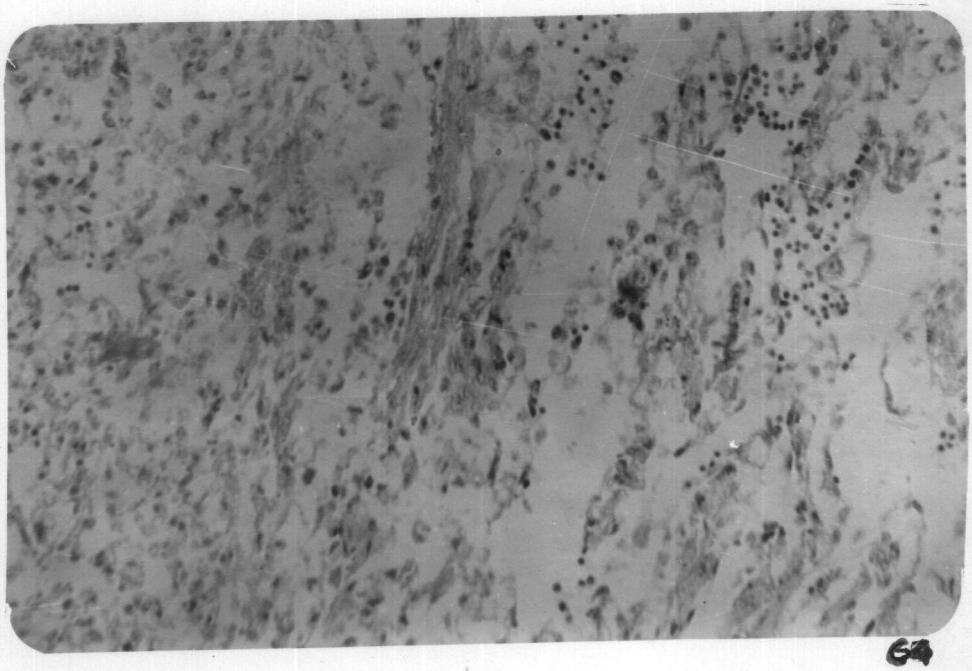

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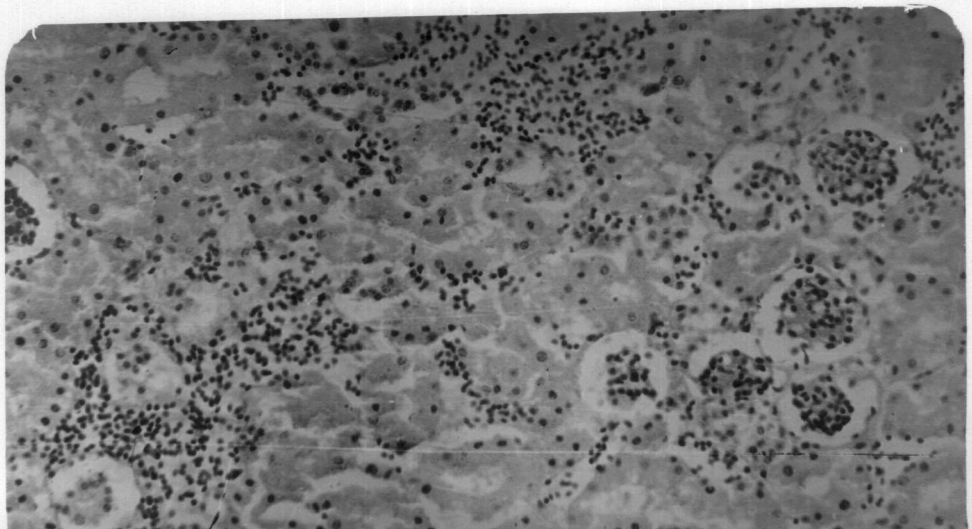


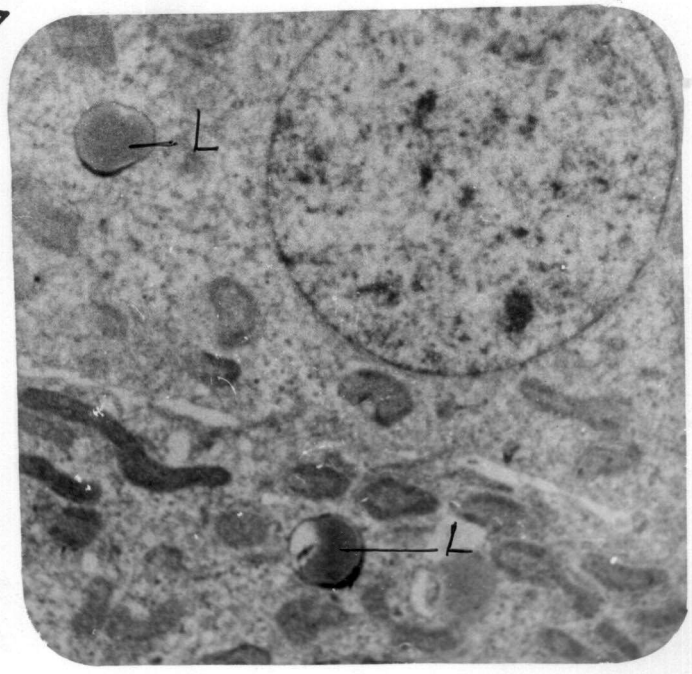
Fig. 66. Electron micrograph. Liver (Ochratoxin A). Hepatic cell showing extensive destruction of cytoplasmic organelles partial loss of nuclear membranes and condensation of chromatin. x 18,000.

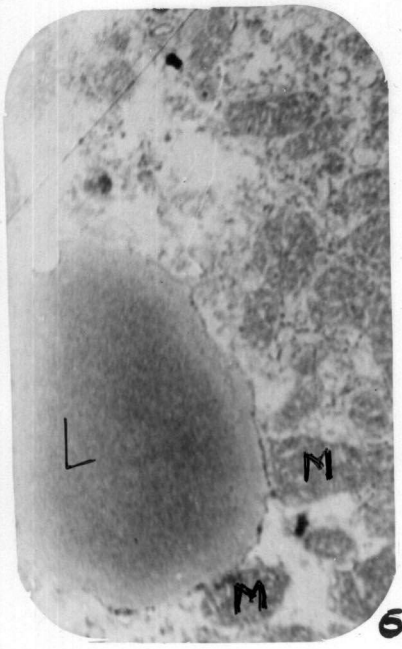
Fig. 67. Electron micrograph. Liver (Ochratoxin A). Presence of lipid droplets (L) in the cytoplasm of hepatocytes. x 12,000

Fig. 68. Electron micrograph. Liver (Ochratoxin A). Irregular lipid droplets (L) adjacent to mitochondria (M) in a hepatic cell. x 18,000.

66

67





68

Fig. 69. Electron micrograph. Liver (Ochratoxin A). Mitochondria (M) with deformed internal structures. Giant mitochondria (G) also noticed. x 18,000

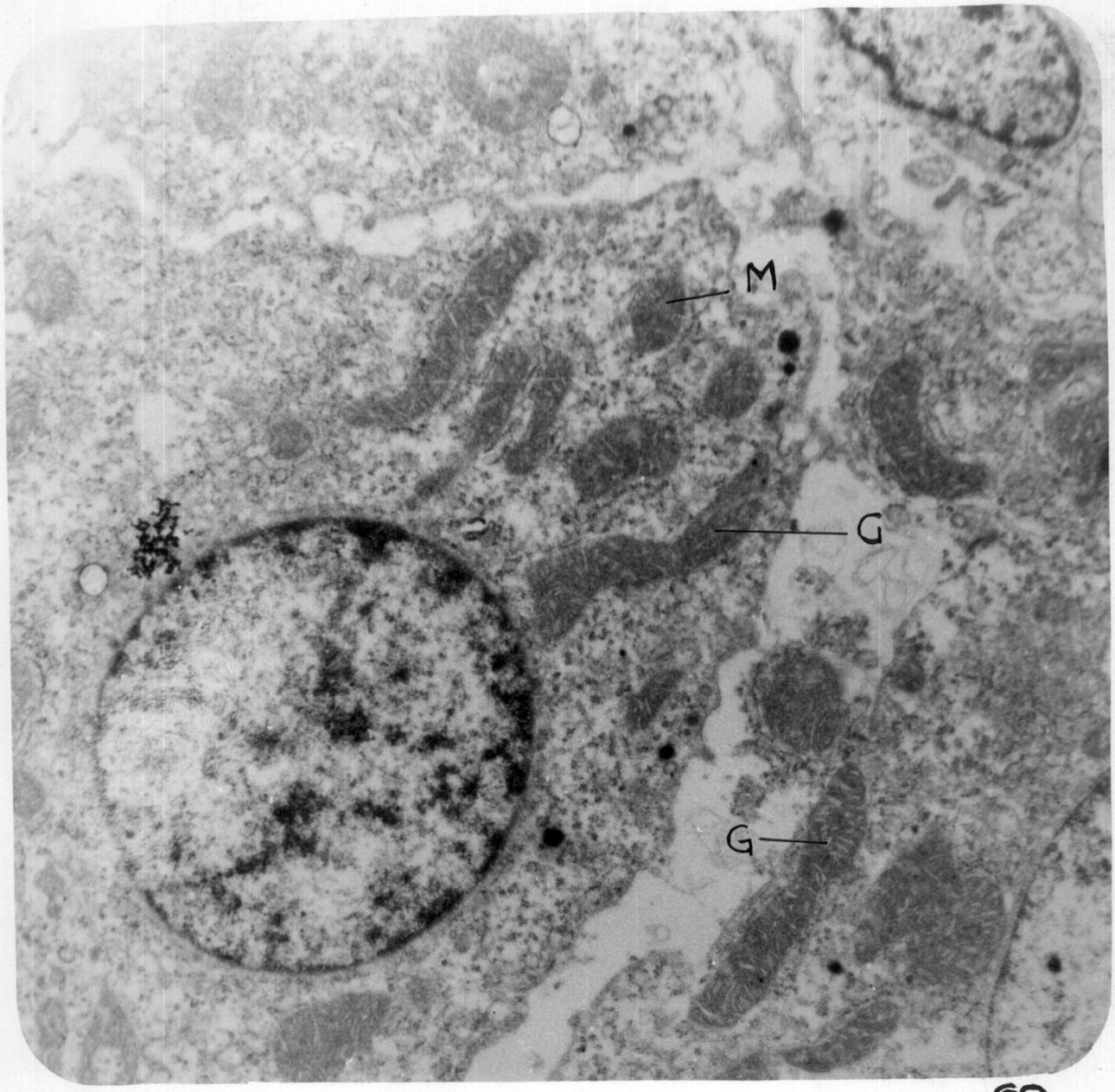

M
G
G

Fig. 70. Electron micrograph (Ochratoxin A). Hepatic cell showing prominent nucleoli (NL) and flocculent euchromatin. x 12,000

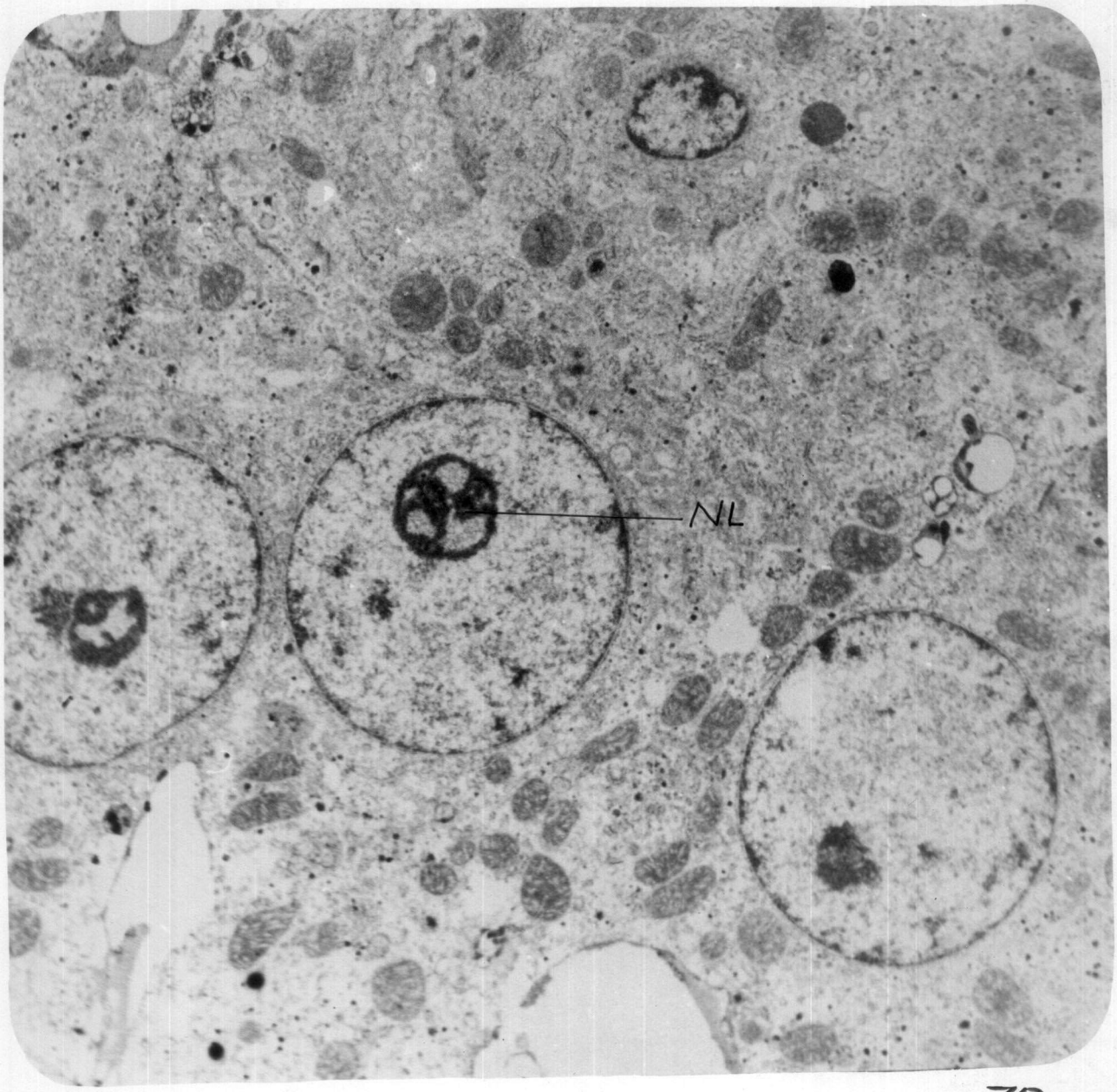
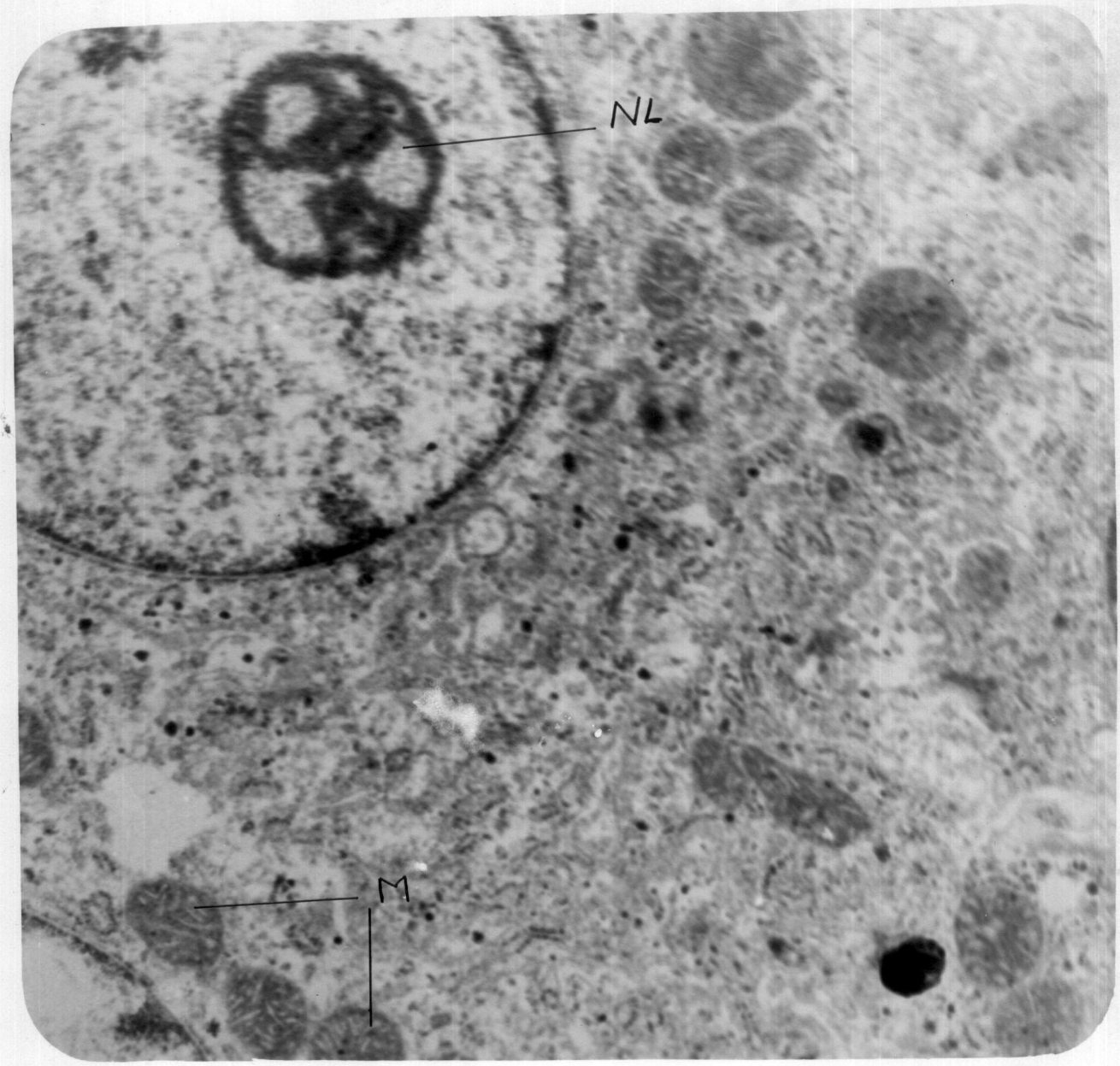

NL

Fig.71. Electron micrograph. Liver (Ochratoxin A). Hepatocyte adjacent to the bile canaliculi (BC). Prominent nucleoli (NL) and partially deformed mitochondria (M) are also seen. x 25,000



71

Fig. 72. Electron micrograph. Kidney (Ochratoxin A). Proximal convoluted tubular epithelium. Partial destruction of the brush border with the plasma membrane showing disruption at places. Deformed mitochondria (M) and lysosomes(Ly) are also seen. x 25,000

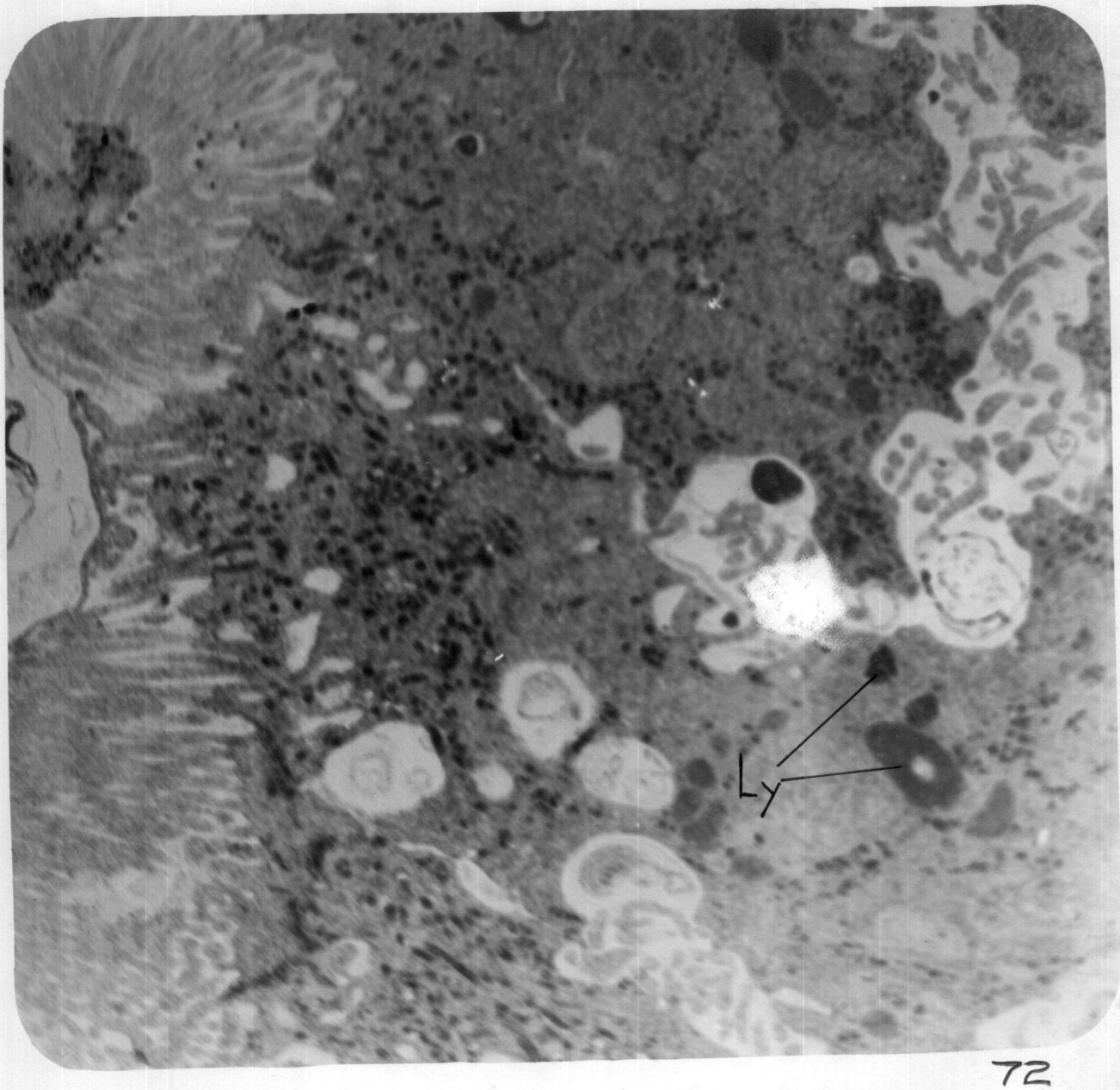
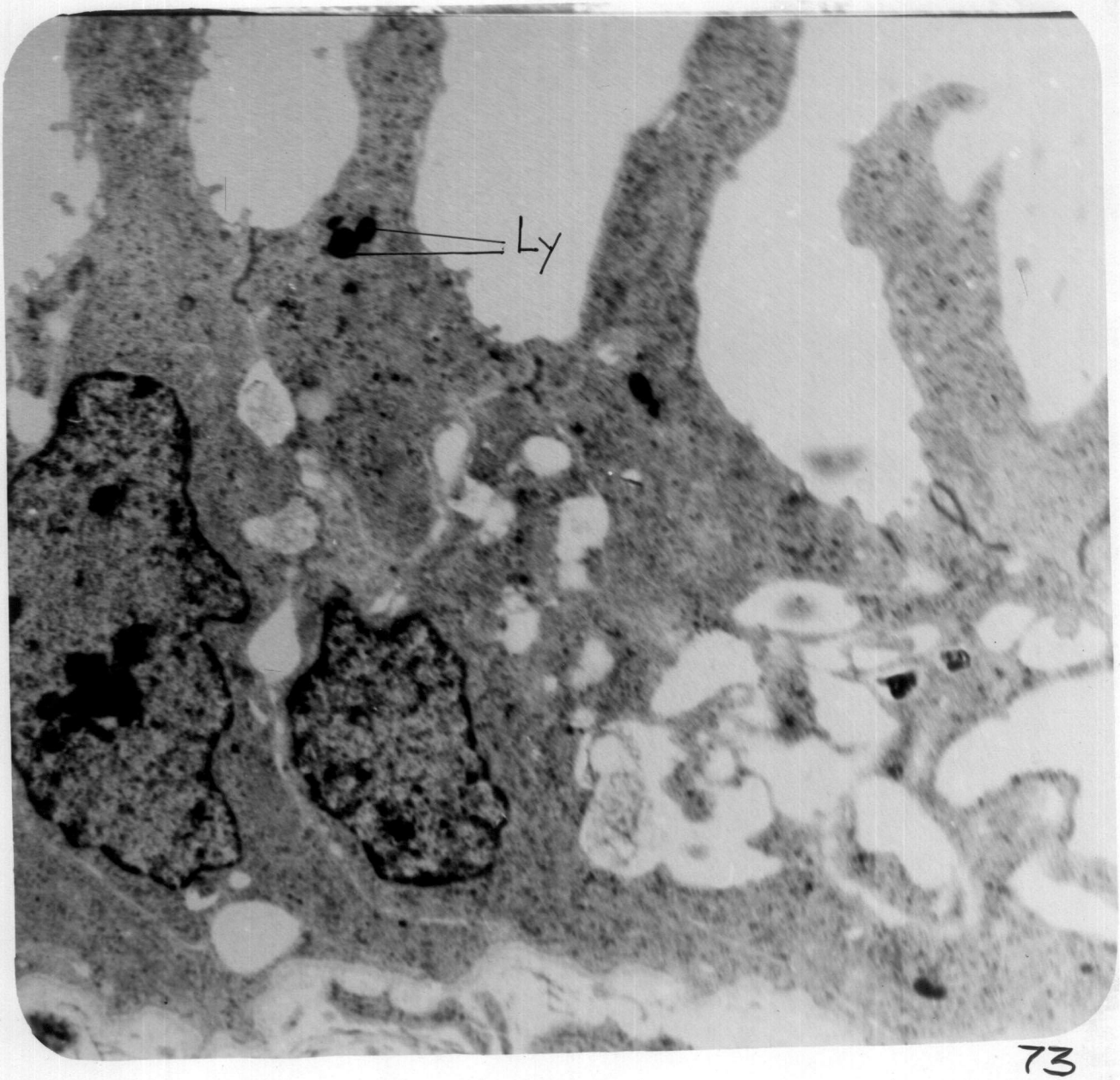

Ly

Fig. 73. Electron micrograph. Kidney (Ochratoxin A). Distal convoluted tubular epithelium showing extensive cytoplasmic destruction. Few lysosomes (Ly) seen. x 14,000



73

Fig. 74. Electron micrograph. Kidney (Ochratoxin A). Fenestrated appearance of the endothelial surface of the basal membrane of glomeruli. A leukocyte (LE) and an erythrocyte (E) are also seen within the capillary. x 12,000.

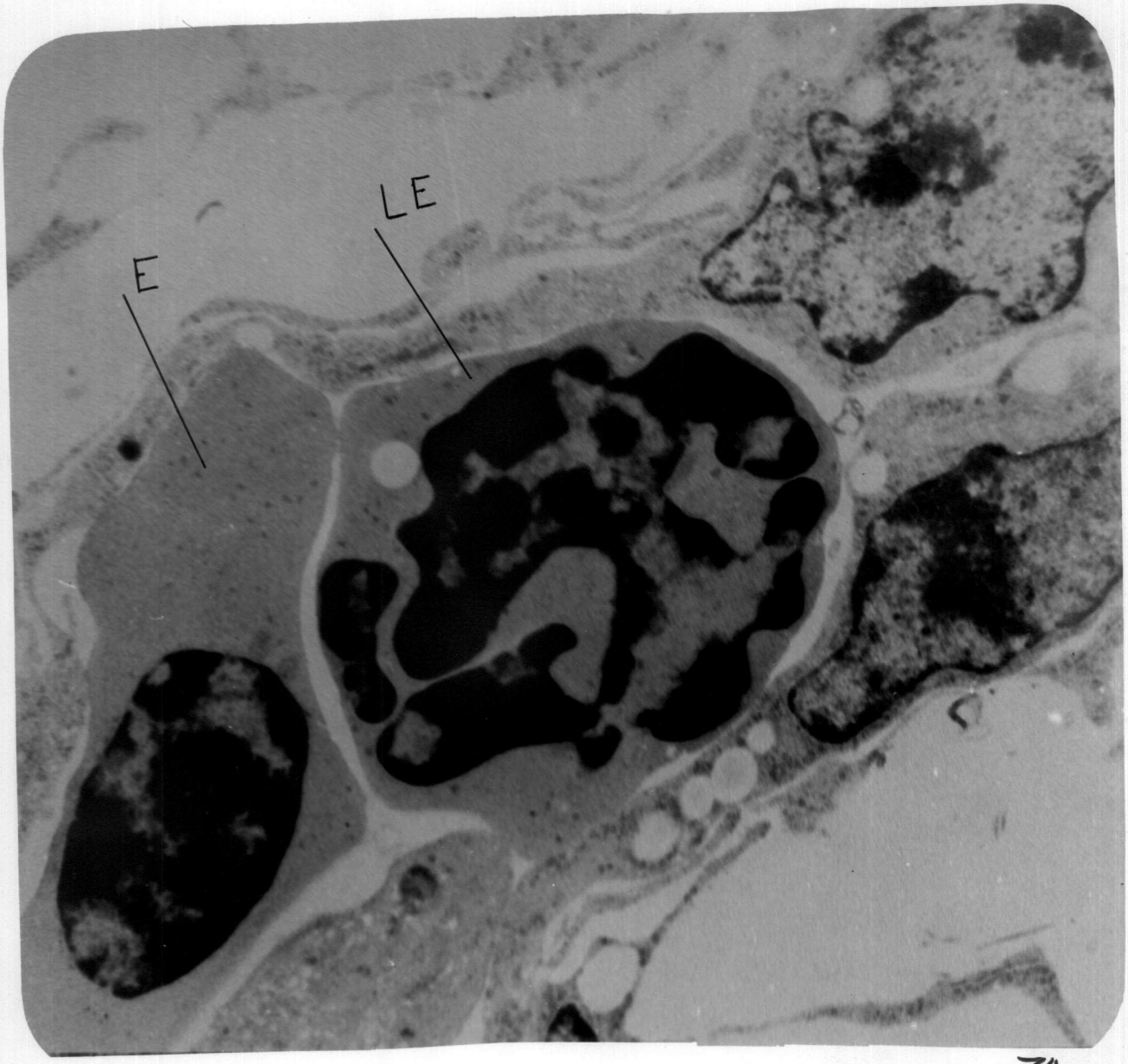

E
LE

Fig. 75. Electron micrograph. Hepatic cell (Citrinin). Numerous swollen mitochondria (M) with deformed cristae seen. x 25,000.

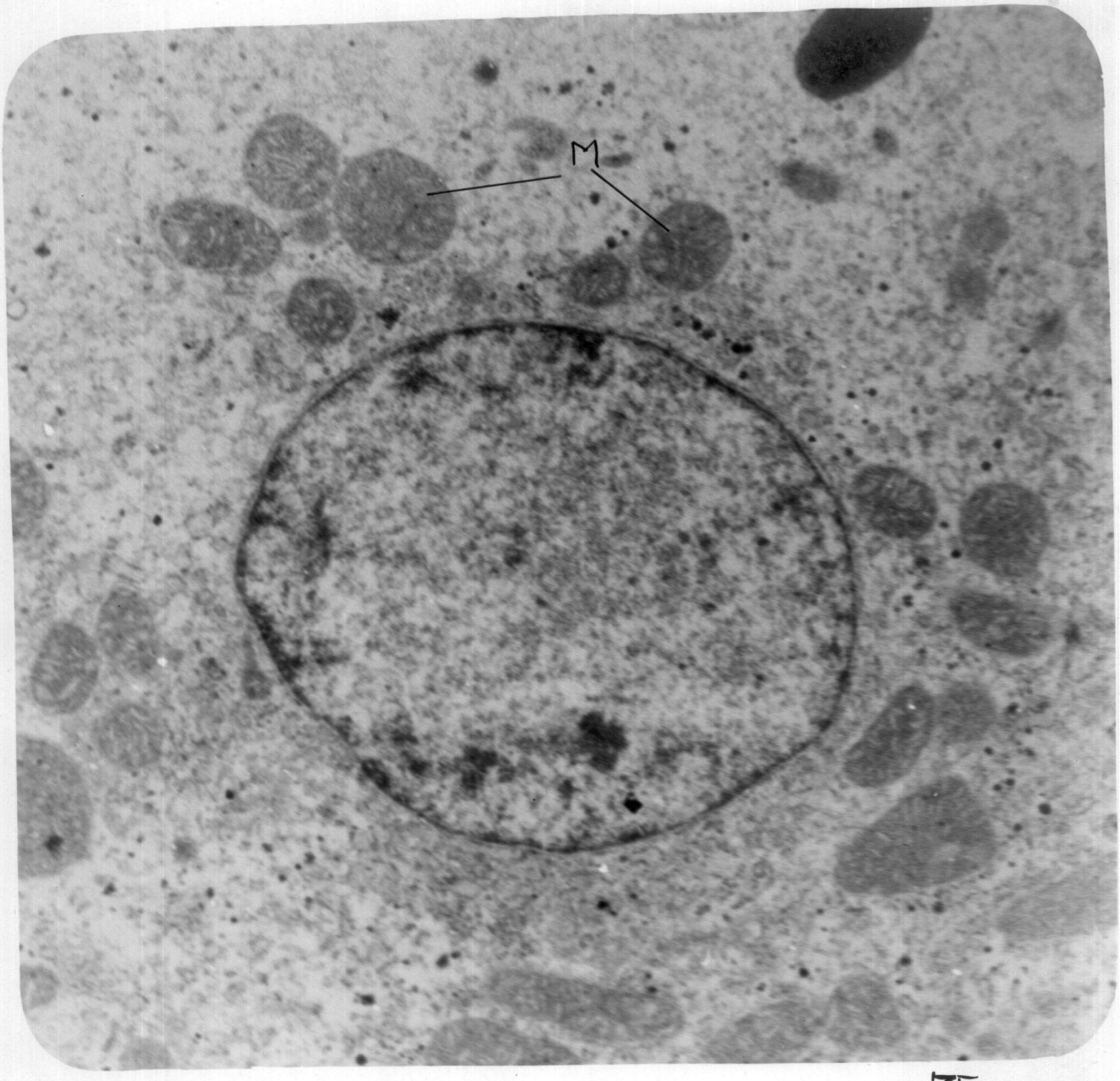

M

Fig. 76. Electron micrograph. Liver (Citrinin). Hepatic cell - Thin endoplasmic reticulum and lipid droplet (L) noticed. x 16,000

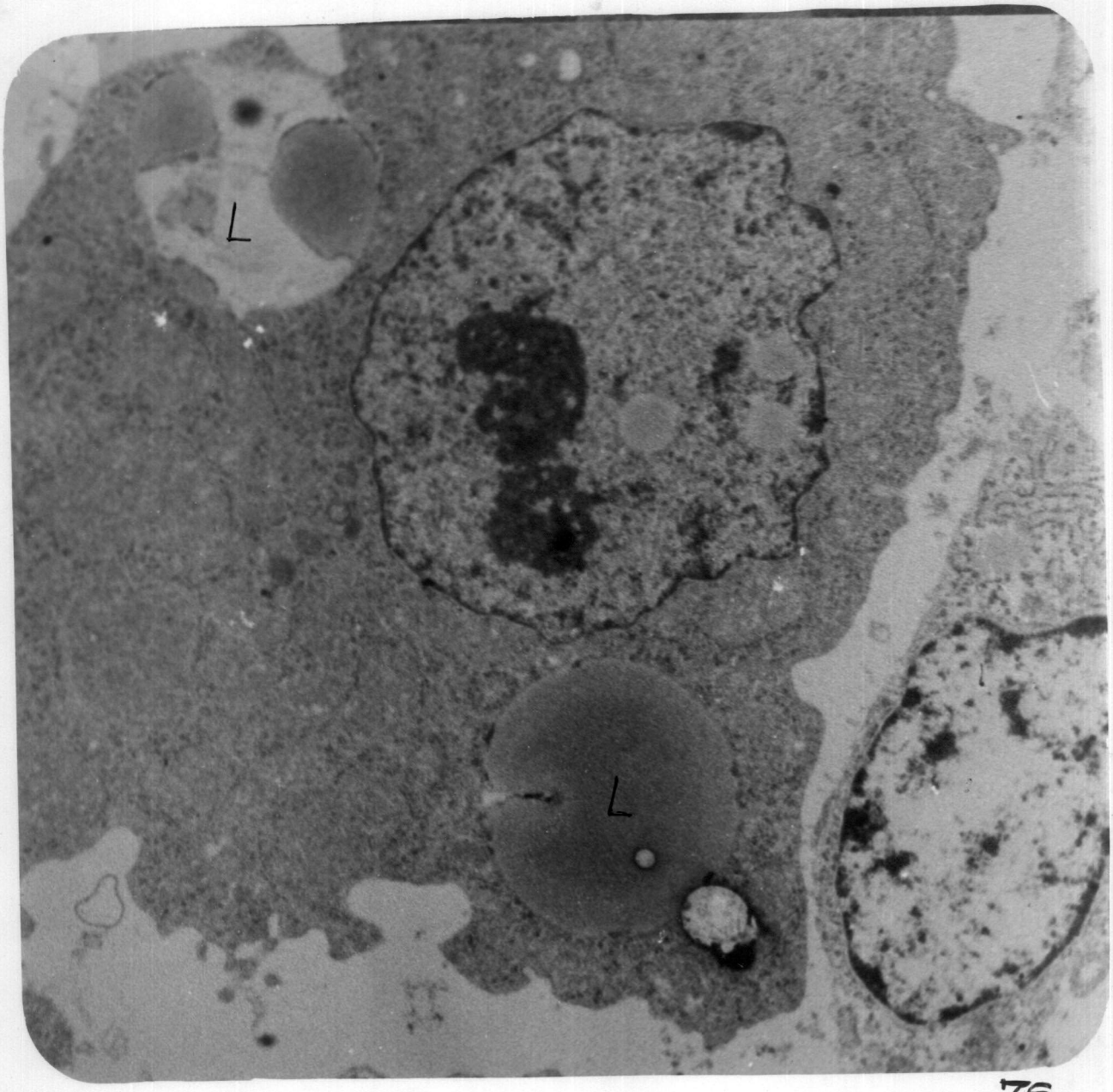
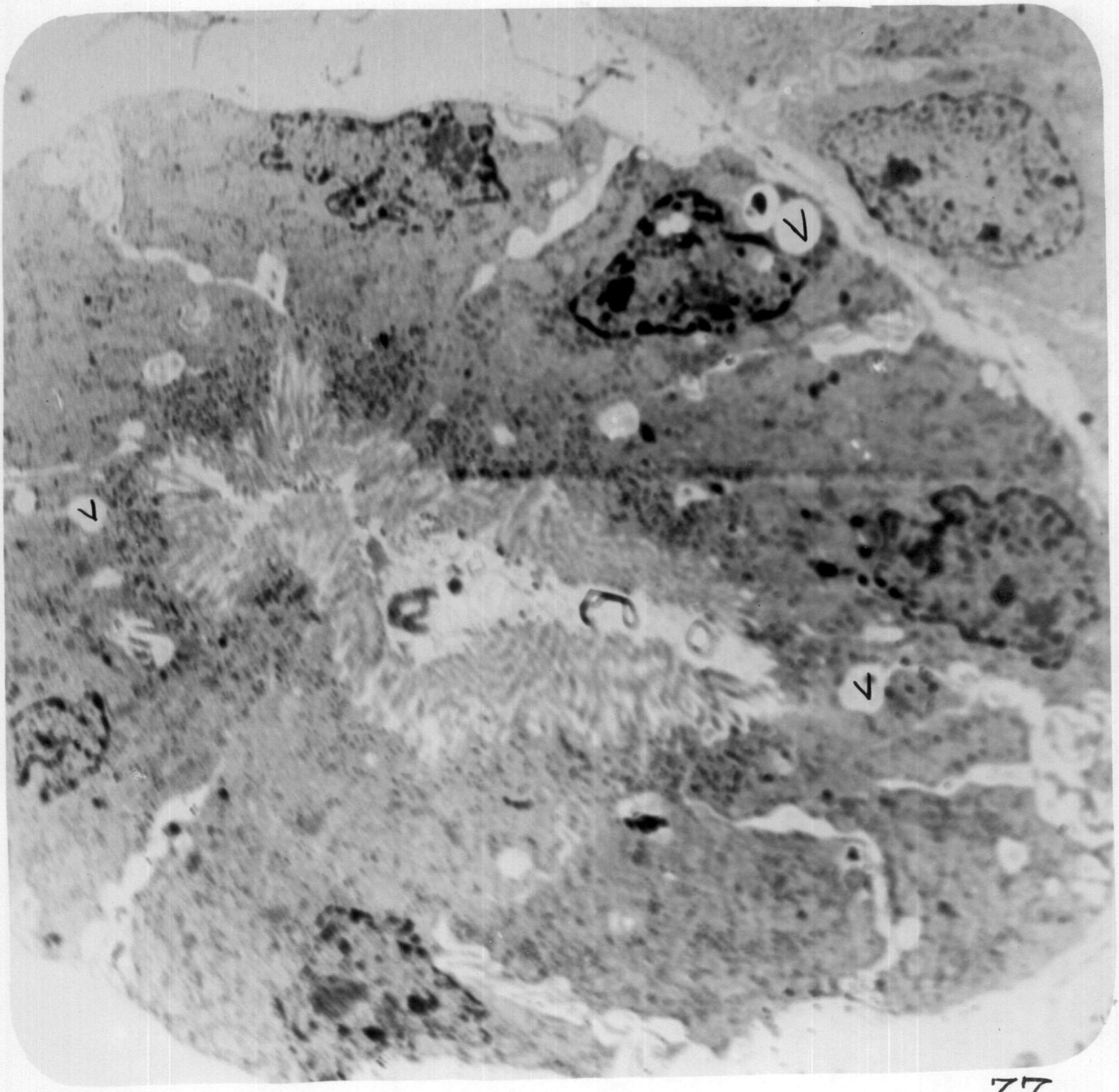

L
L

Fig. 77. Electron micrograph. Kidney (Citrinin). Proximal convoluted tubules showing epithelial cells with varying grades of organellar changes in the cytoplasm. Numerous vacuoles (V) seen. Nuclear chromatin showing varying grades of segregation. x 10,000



77

Fig. 78. Electron micrograph. Kidney (Citrinin). Tubular epithelial cells showing organellar destruction. Slight condensation of chromatin. x 12,000

Fig. 79. Electron micrograph (Citrinin). Epithelium of distal convoluted tubule showing partial lysis of nuclear membrane and condensation of nucleolus. x 20,000

78

79

Fig. 80. Electron micrograph. Kidney (Citrinin). Undifferentiated tubular epithelial cells showing aggregations and lysis of chromatin. Cytoplasmic organelles show severe destructions. x 14,000

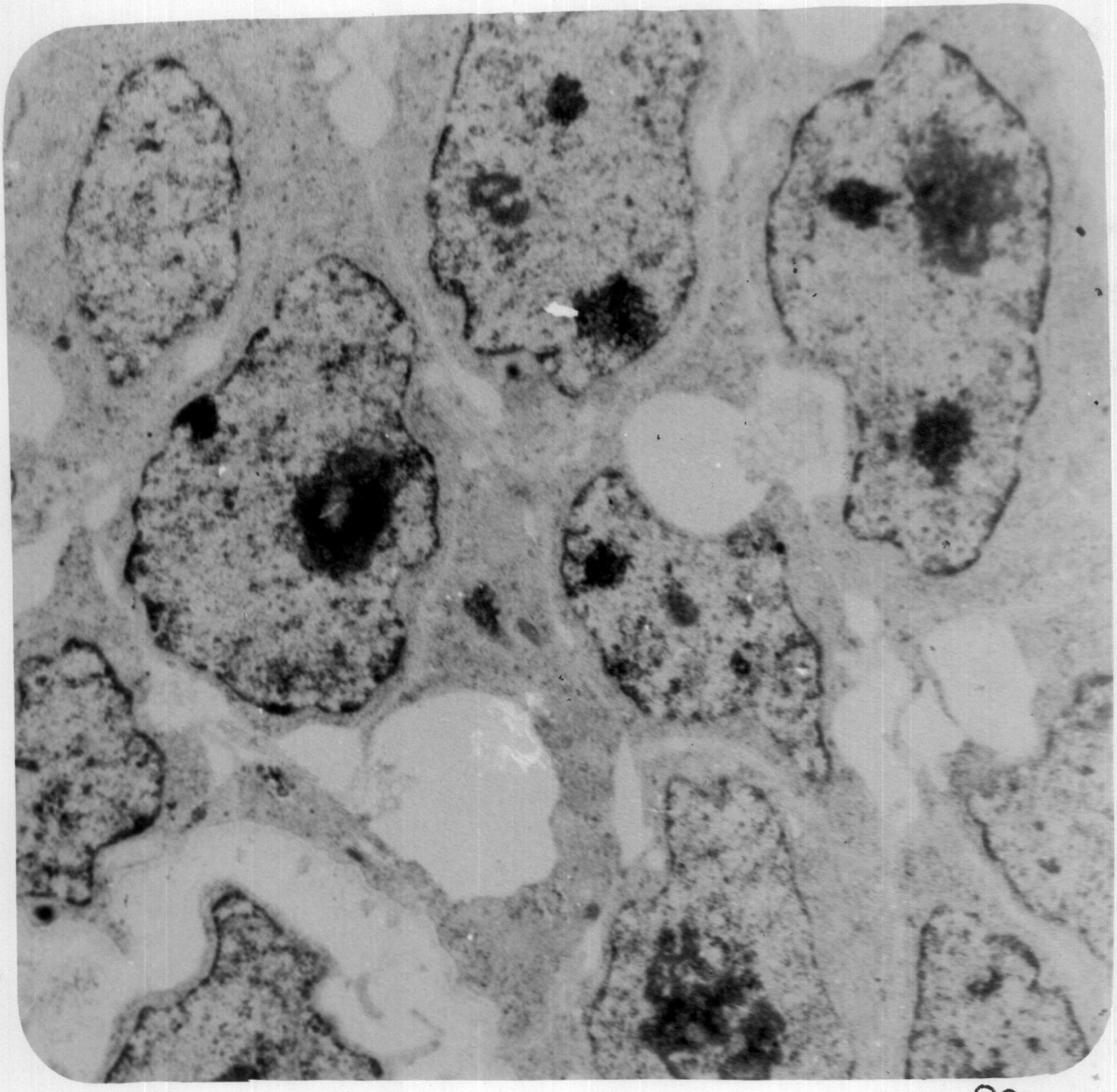


Fig. 81. Electron micrograph. Kidney (Citrinin). Glomerulus with podocytes (P) showing fusion of foot-processes (FP) x 14,000

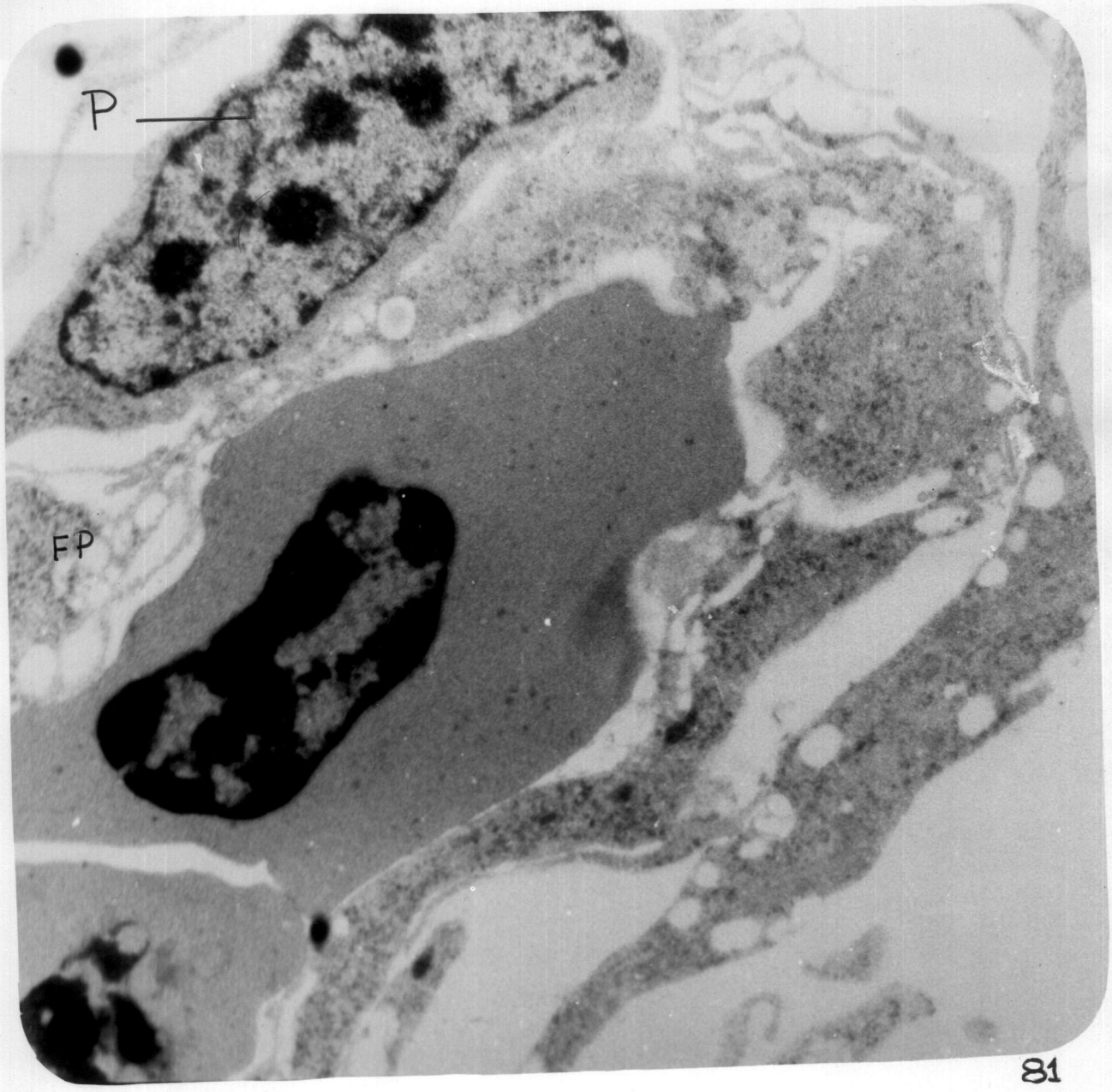
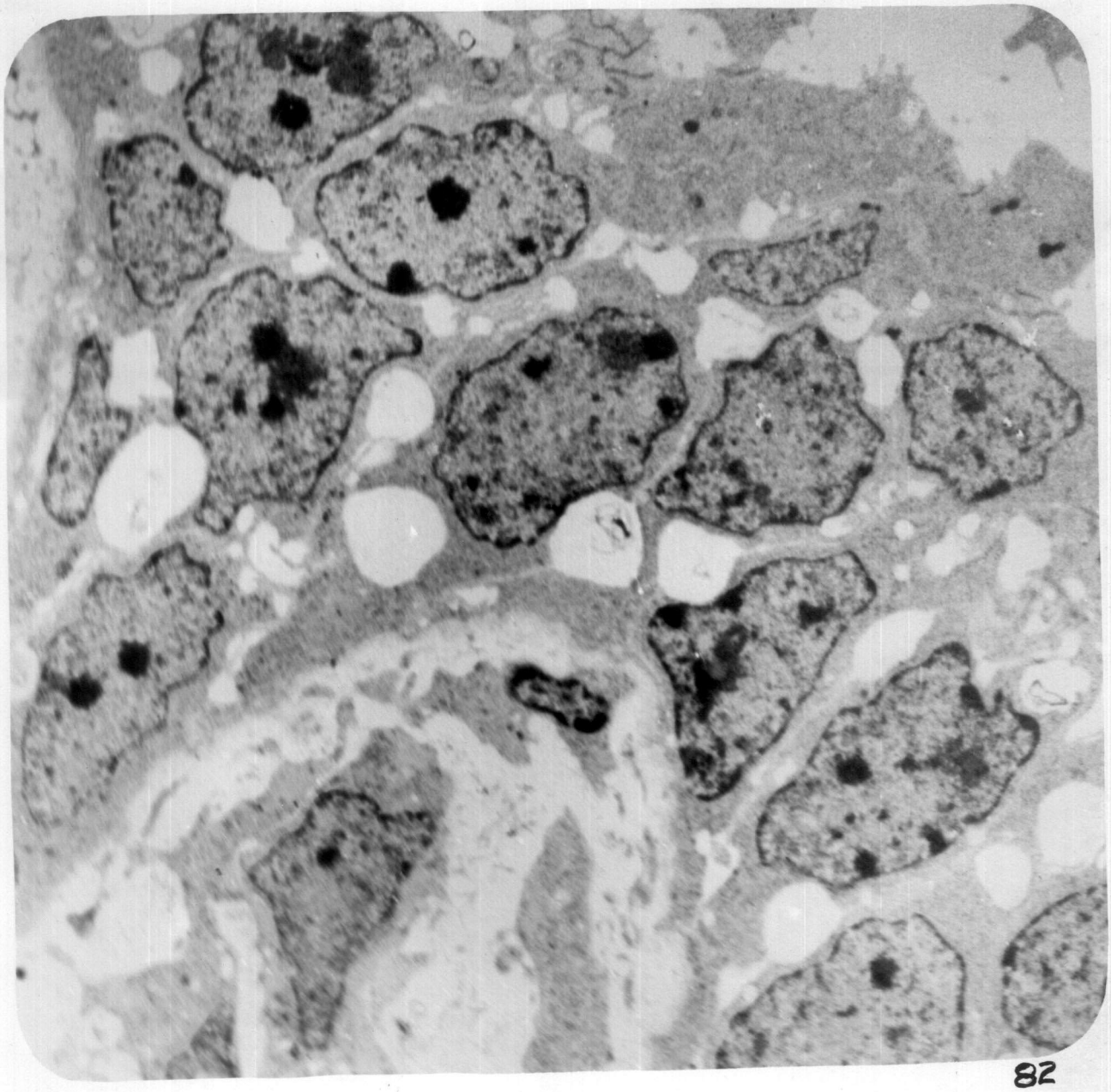

P
FP

Fig. 82. Electron micrograph. Kidney (Citrinin). Undifferentiated tubular epithelium with bulged out plasma membranes and vacuoles. x 10,000



82

Fig. 83. Electron micrograph. Hepatocyte (Ochratoxin A and Citrinin). Mitochondria (M) showing varying degrees of damage. Lipid (L) droplets present. x 18,000

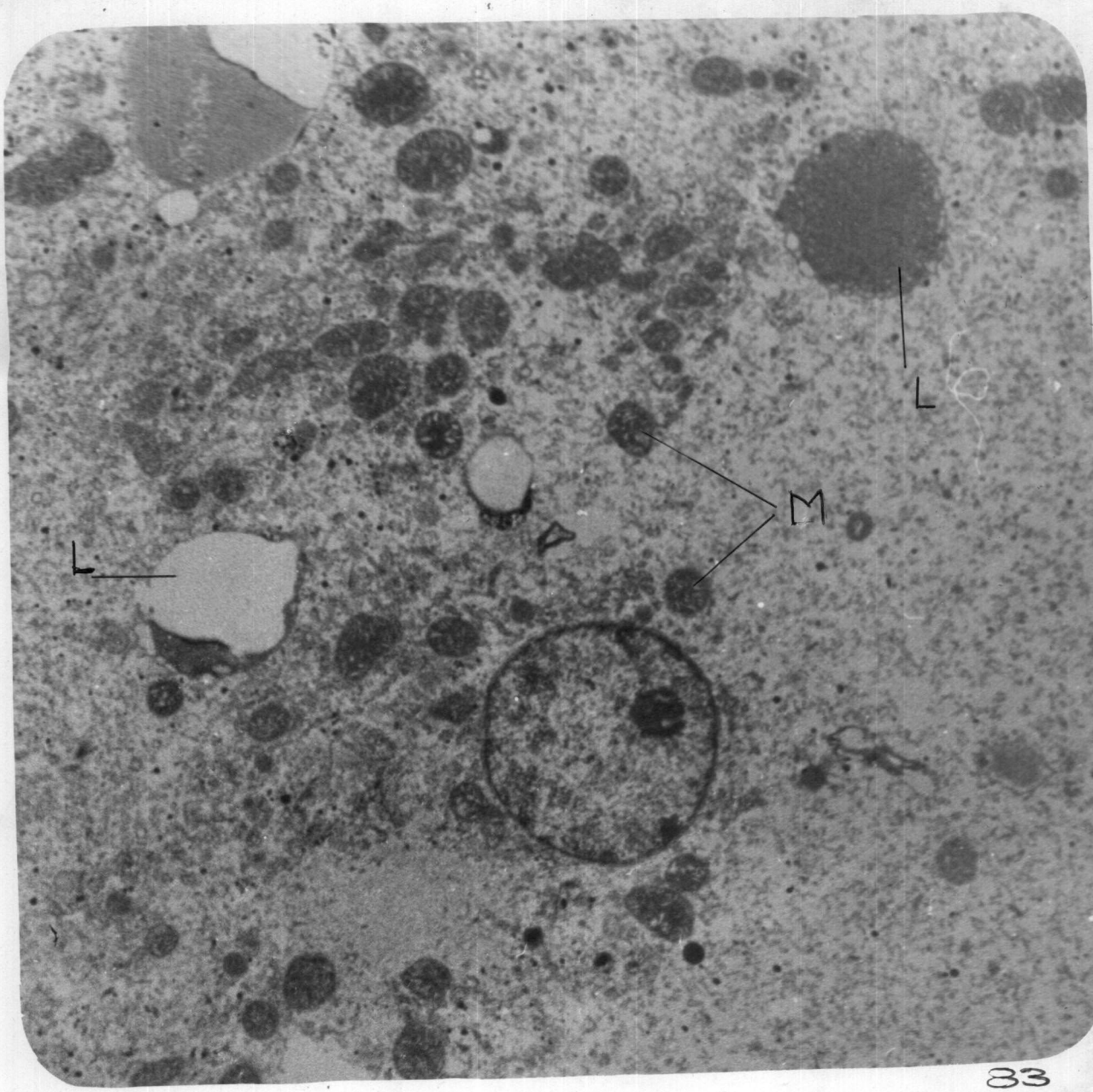

L
M
L

Fig. 84. Electron micrograph. Liver (Ochratoxin A and Citrinin). Ring shaped mitochondria (RM) and lipid (L) seen in a hepatocyte-Endoplasmic reticulum (ER) show partial degranulation. x 24,000

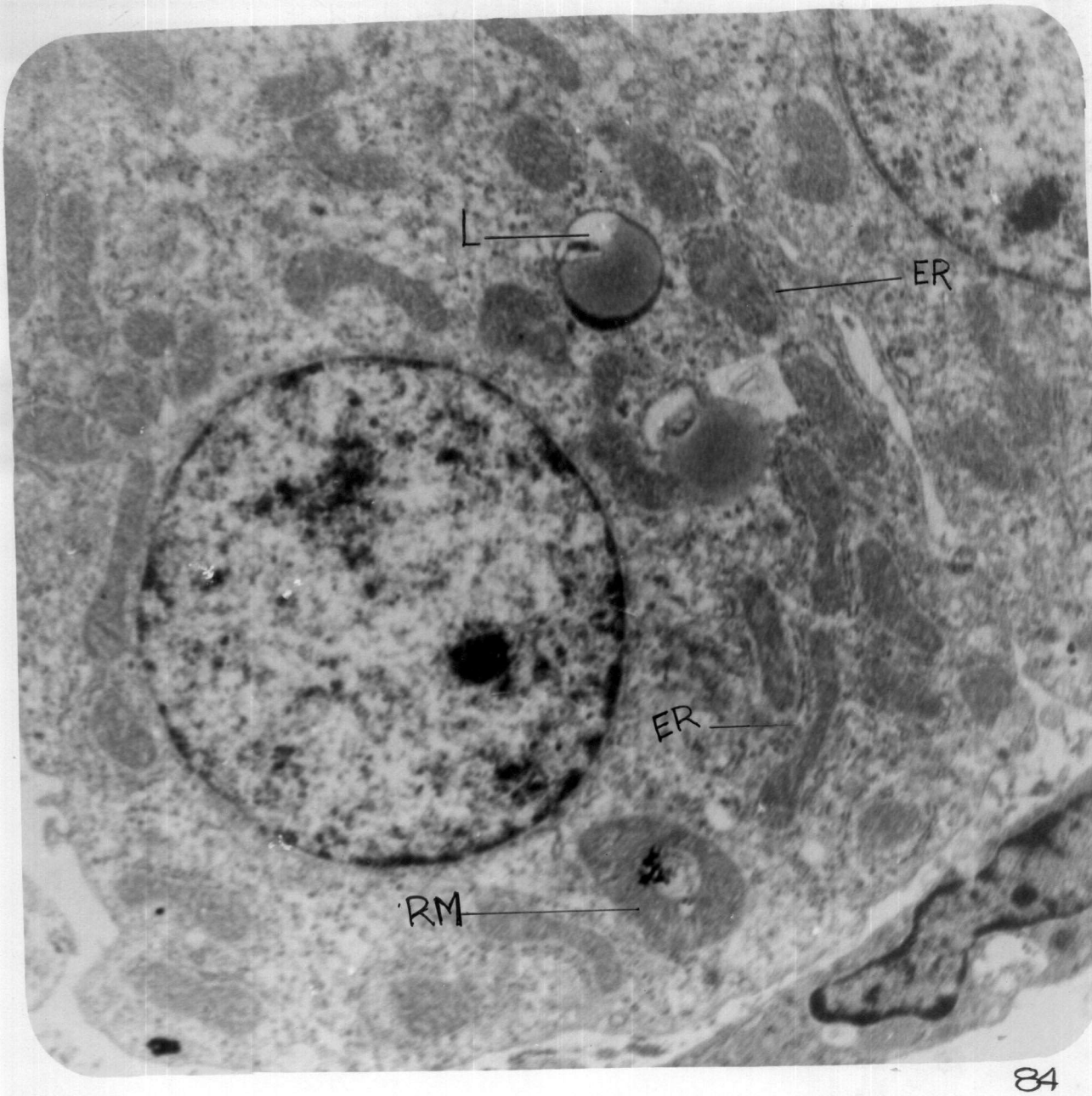
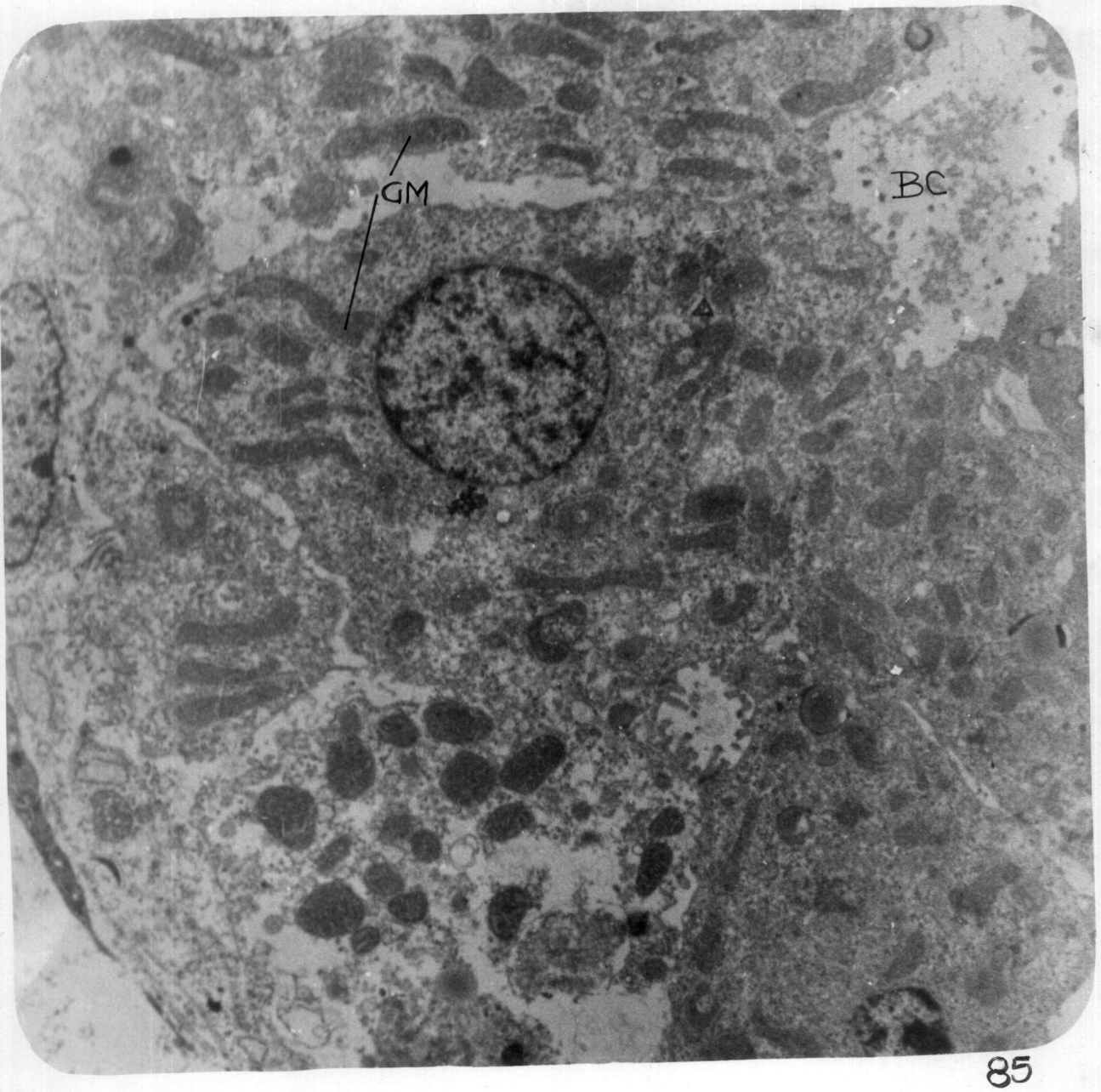

L
ER
ER
RM

Fig. 85. Electron micrograph. Liver (Ochratoxin A and Citrinin). Numerous giant mitochondria (GM) seen in the cytoplasm of hepatocytes. Plasma membrane disrupted at places. Bile canaliculus (BC) contains granular contents. x 14,000



85

Fig. 86. Electron micrograph. Liver (Ochratoxin A and Citrinin). Hepatic cell showing large lipid (L) droplet. x 28,000.

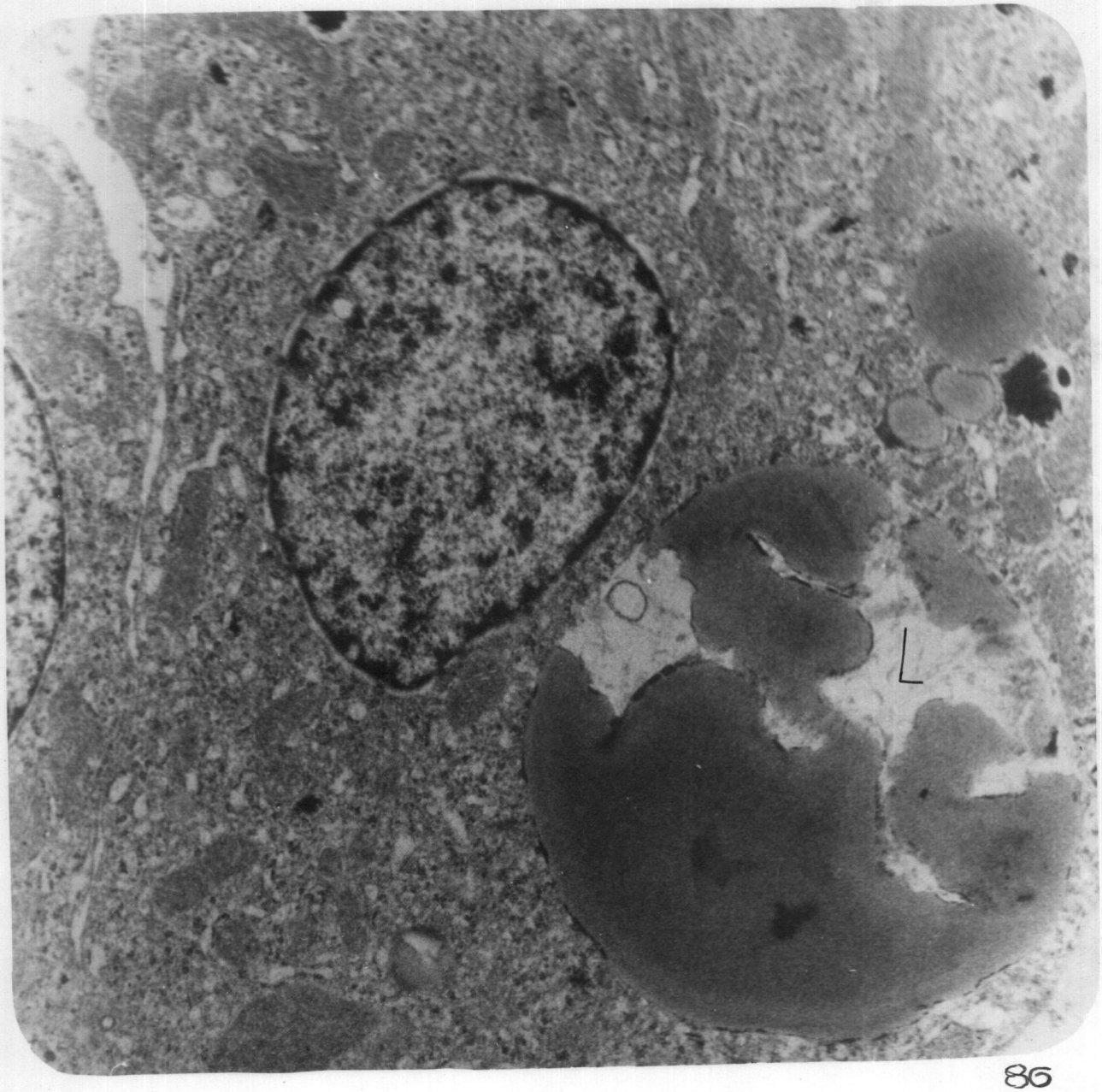
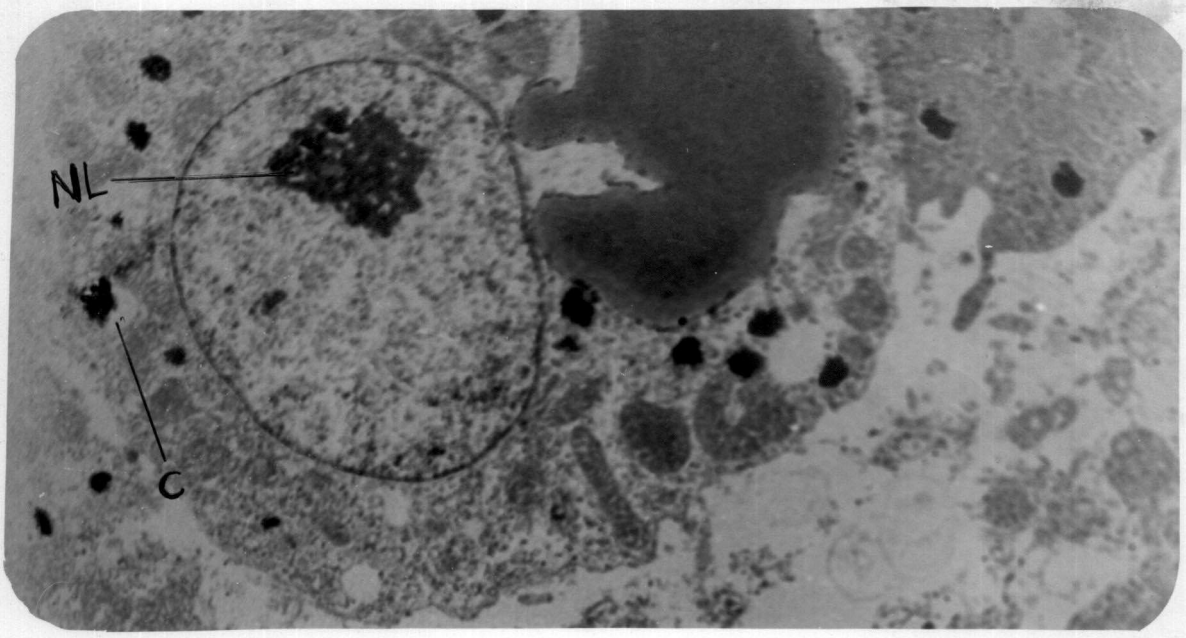

L

Fig. 87. Electron micrograph. Liver (Ochratoxin A and Citrinin). Hepatic cell showing lipid droplet. Note deformed mitochondria, prominent nucleolus (NL) and numerous electron dense crystalline structures (C) bounded by membraneous structures. x 14,000.

Fig. 88. Electron micrograph. Liver (Ochratoxin A and Citrinin). Mitochondria (M) with swollen cristae and crystalline electron dense structures (C) bounded by membranes seen. x 40,000.



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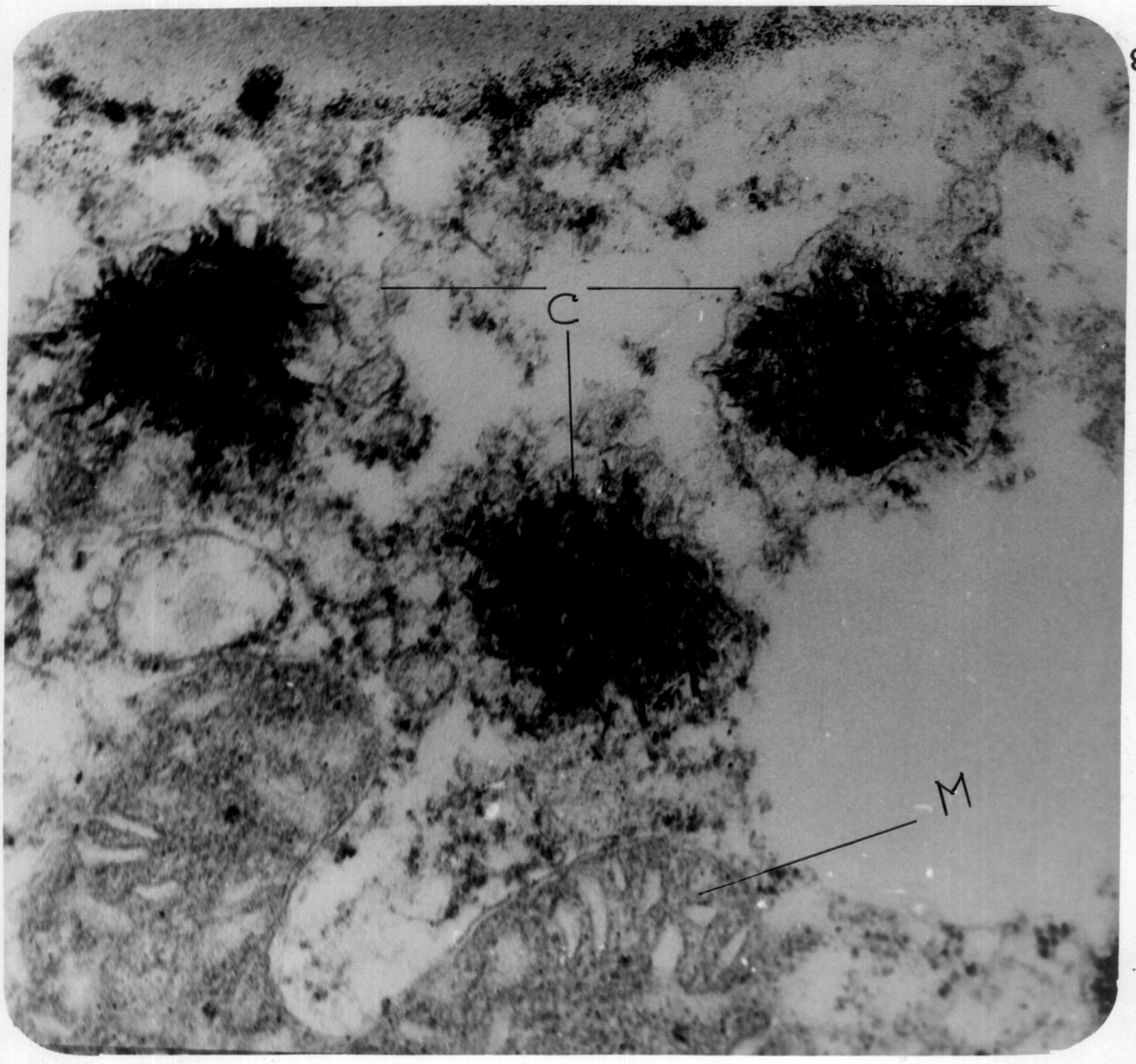


88

Fig. 89. Electron micrograph. Kidney (Ochratoxin A and Citrinin). Tubular epithelium showing numerous vacuolation (V) of the cytoplasm. x 14,000

V

Fig. 90. Electron micrograph. Kidney (Ochratoxin A and Citrinin). Tubular epithelium showing vacuoles (V). There is partial degranulation of endoplasmic reticulum and increased electron density of the cytoplasm with partial destruction of basement membrane (BM). x 20,000

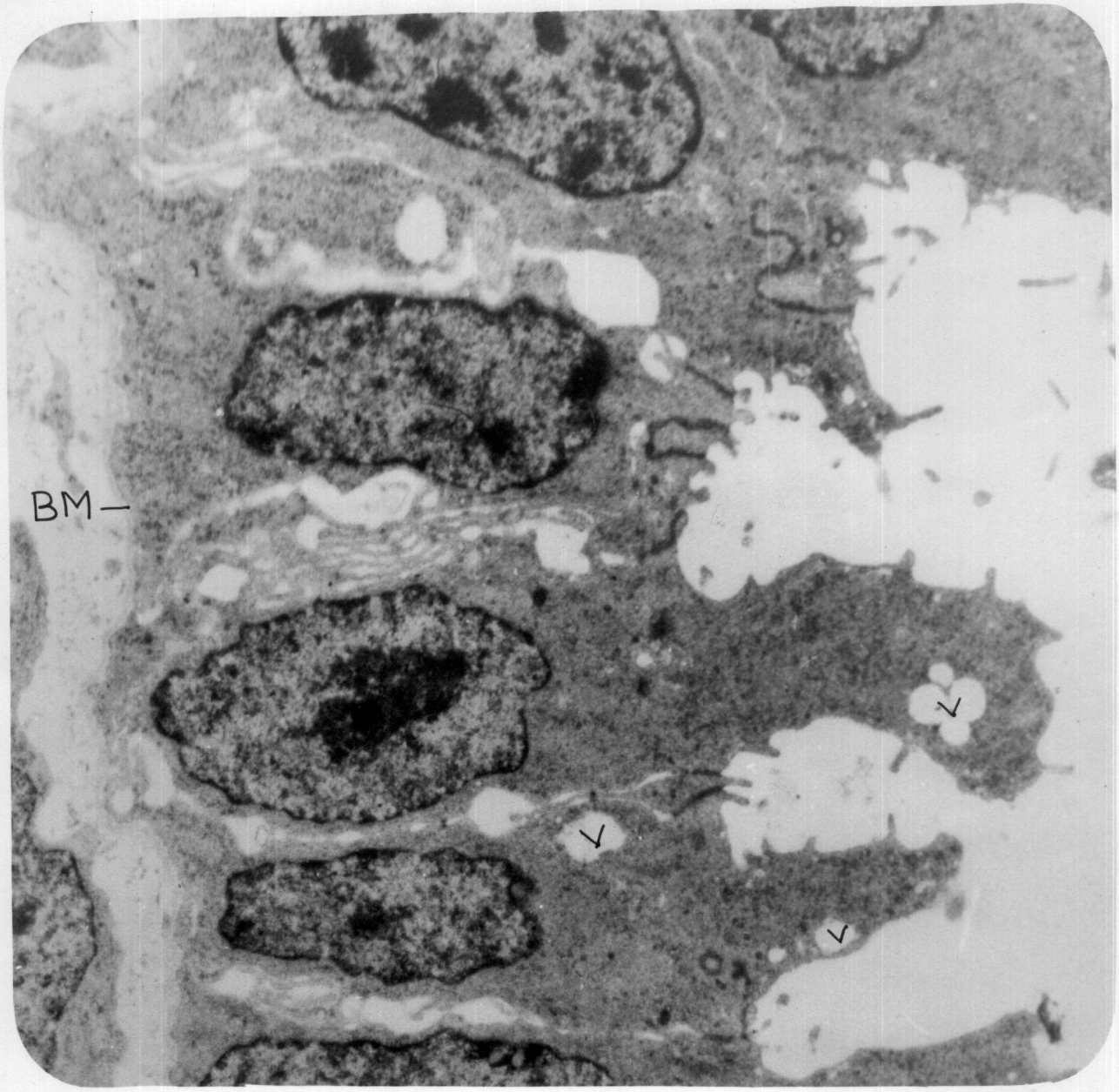
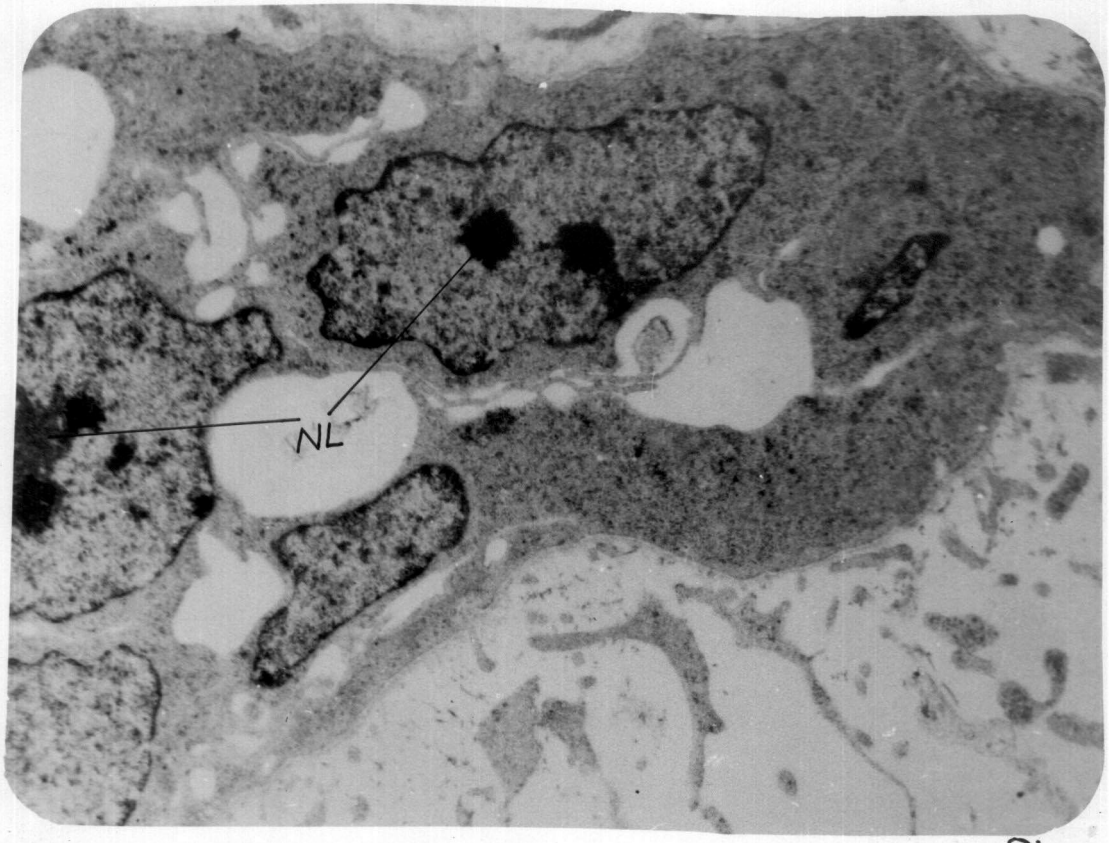

BM
V
V
V

Fig. 91. Electron micrograph. Kidney (Ochratoxin A and Citrinin). Tubular epithelial cells showing increased density of the cytoplasm. Note condensation of nucleolus (NL). x 12,000

Fig. 92. Kidney (Ochratoxin A and Citrinin). Undifferentiated renal epithelial cells. Homogenous appearance of the cytoplasm with few lysosomes (Ly). x 14,000



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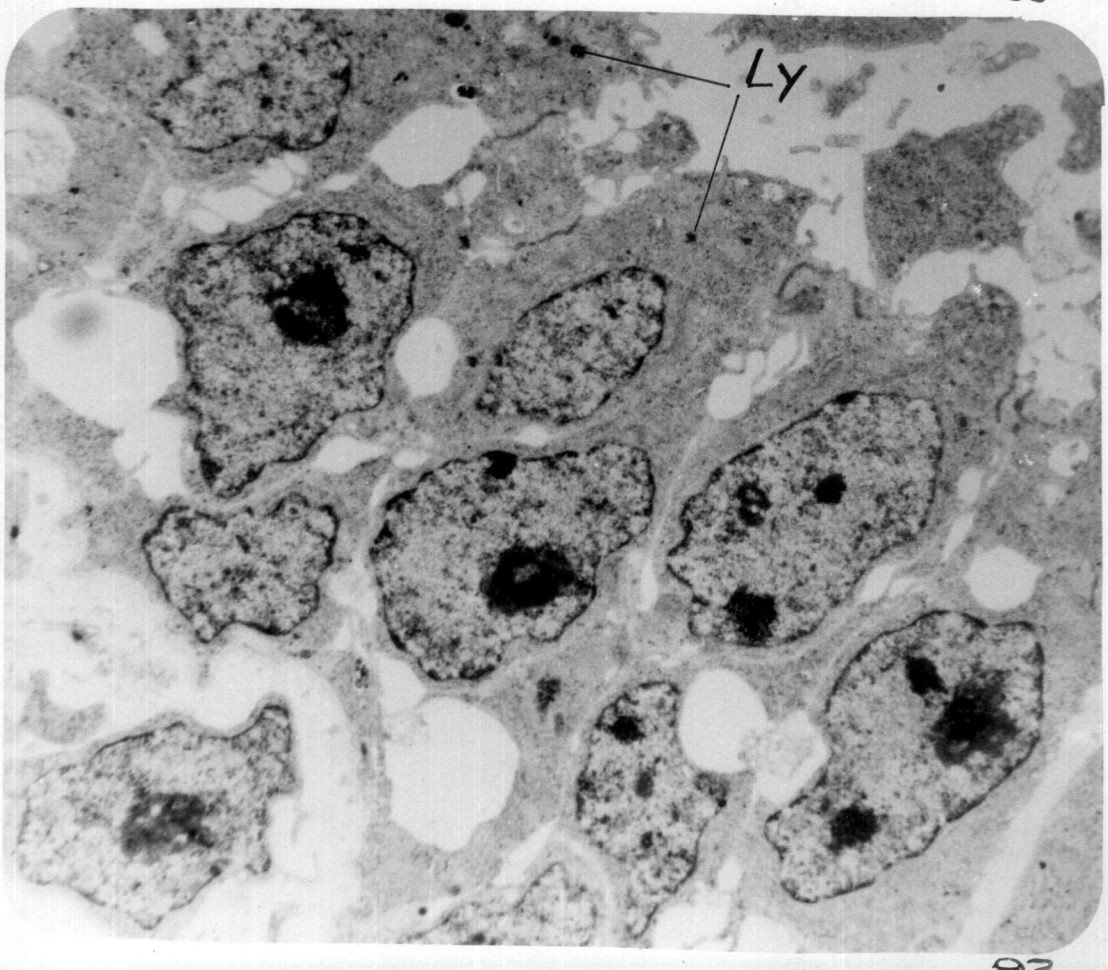
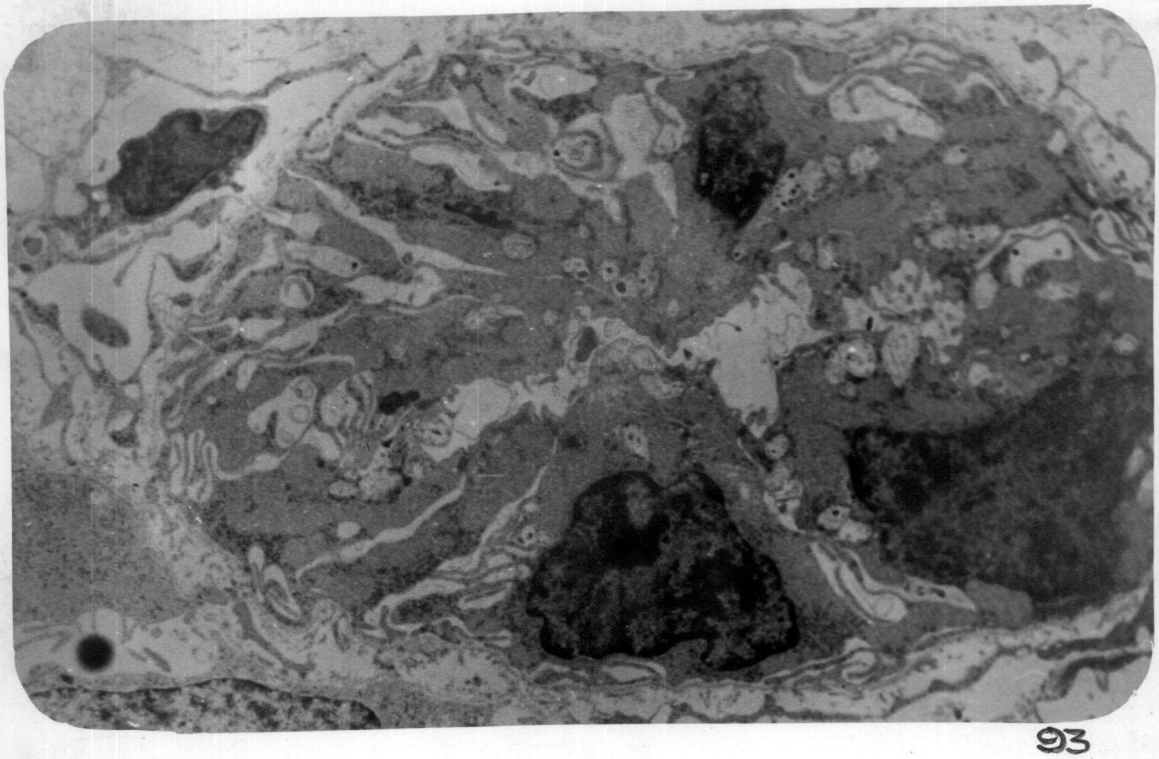


92

Fig. 93. Electron micrograph. Kidney (Ochratoxin A and Citrinin). The epithelial cells of the distal convoluted tubules showing destruction of cytoplasmic organelles and plasma membranes. x 12,000.

Fig. 94. Electron micrograph. Kidney (Ochratoxin A and Citrinin). Epithelium of the collecting tubules showing moderate degree of chromatin accumulation. x 14,000



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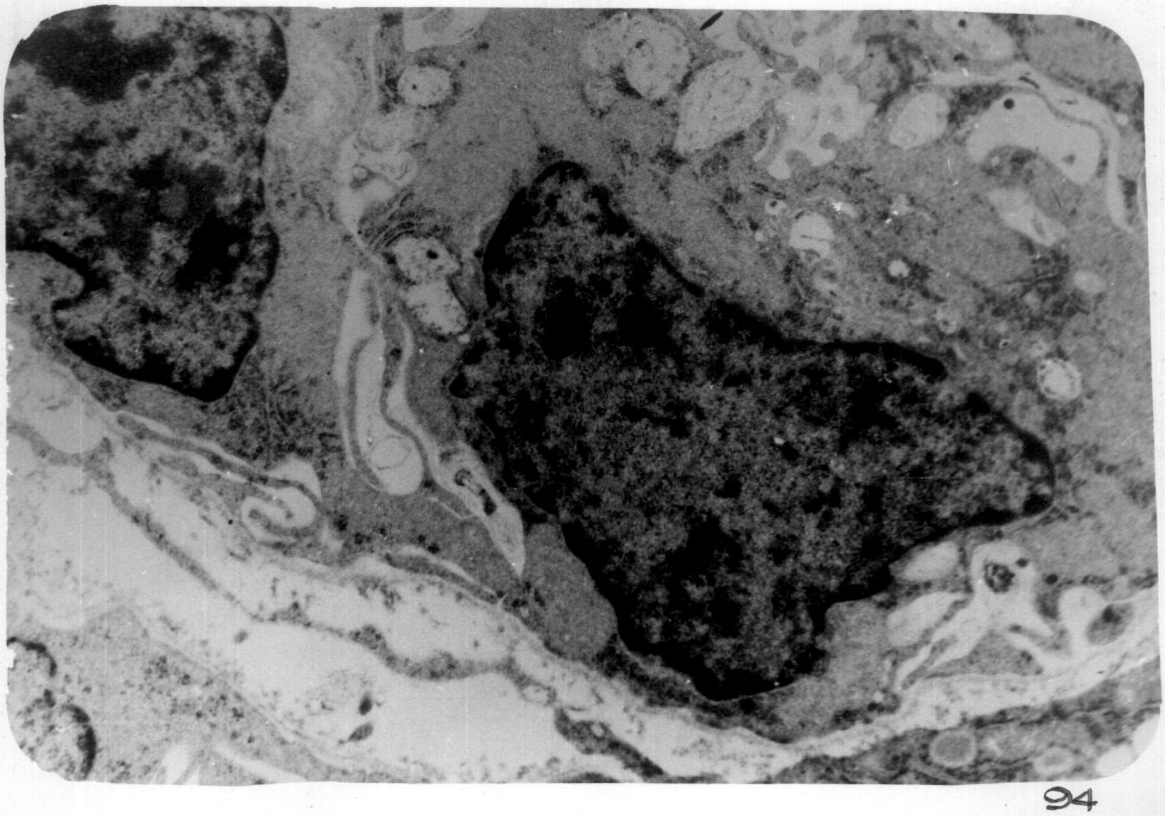
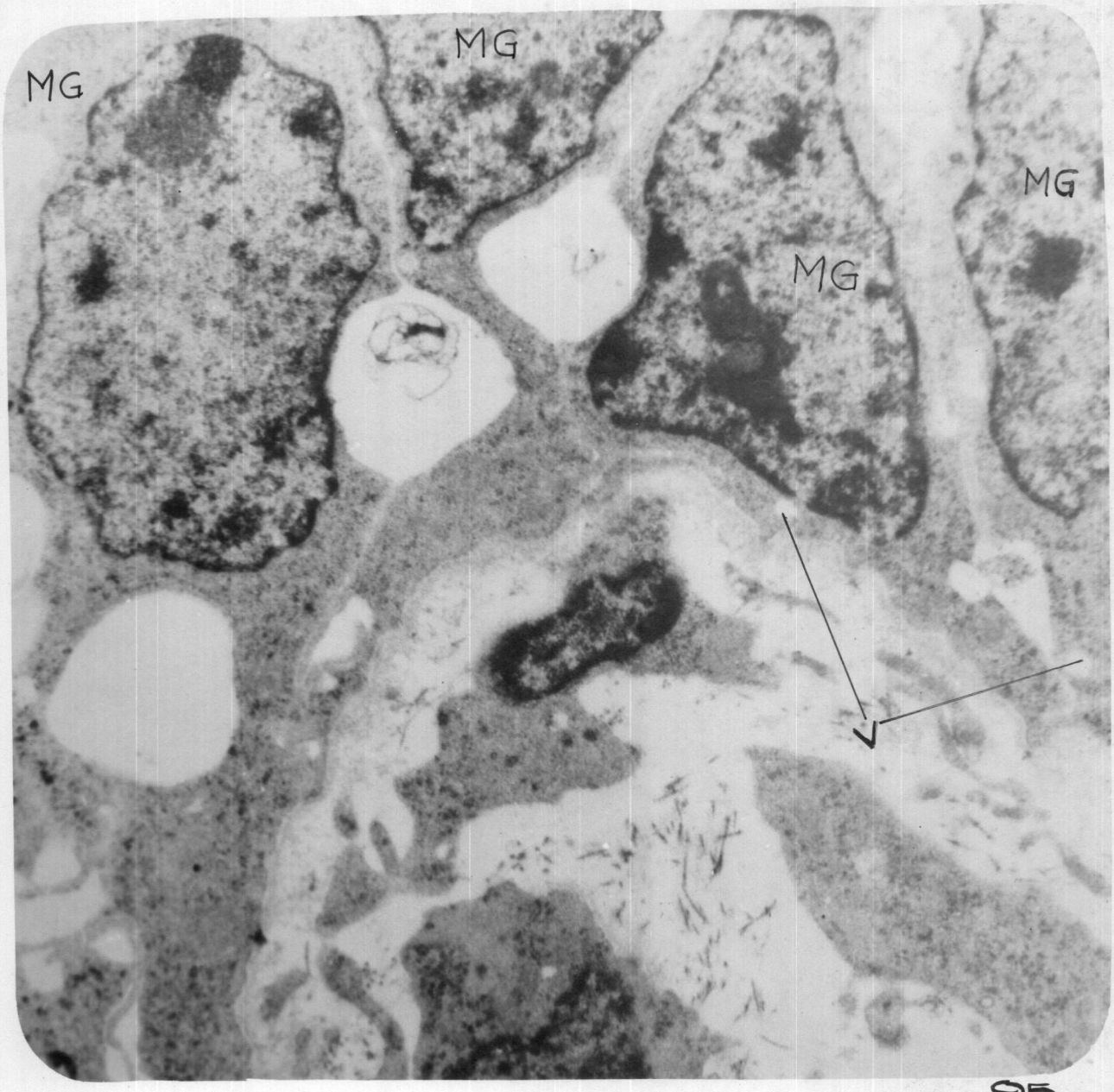


94

Fig. 95. Electron micrograph. Kidney (Ochratoxin A and Citrinin). Mesangial cells (MG) of the glomerulus showing granular and dense cytoplasm. The plasma membrane is intact. The endoplasmic reticulum is fragmented. Vacuoles (V) present.



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Discussion

5. DISCUSSION

Various factors, exogenous as well as endogenous, have been attributed to cause embryomortality in chicken. Apart from infections, genetic factors, nutritional causes, toxic substances and other physiological causes, it is well known that management and incubating conditions are significant factors in the causation of embryomortality. Variation in incubating temperature can result in a multitude of pathologic manifestation in the embryo. Reports on the incidence of embryomortality and on the occurrence of various abnormalities give varied figures depending on the management conditions.

Omphalitis and associated septicaemic conditions contributed to 5% of the abnormal embryos, while the rest showed other lesions. Spontaneous malformations have been observed by several investigators and an incidence upto 9.5% (Byerly, 1930) was reported. The occurrence of individual types of spontaneous malformations also showed great variation in the reports published (William et al. 1969; Romanoff and Romanoff, 1972). The various factors attributed to causation of malformations are genetic, nutritional including toxicants, and infections. It has been reported that a great majority of congenital malformations are due to genetic factors eventhough many of these hereditary abnormalities could be modified by the influence of environment both physical and chemical.

Inbreeding has been shown to increase embryonic mortality due to malformations (Sittman et al. 1966). It has been reported that many of the genetic defects follow the normal Mendelian pattern of inheritance. In the present investigations the eggs obtained for hatching were from hens which were crossed with cocks of different genetic lines and therefore inbreeding does not seem to operate as a causative factor. Detailed investigation would be required to pinpoint whether other genetic factors were involved in the causation of malformations.

Eventration viscera including herniation of yolk sac was seen in 2380 (43.7%) of the embryos examined. Coleman (1986) reported that ectopic viscera could be due to overheating during 16-20 days of incubation. Haemorrhage and oedema seen on the head and neck in this study could also be attributed to overheating either in the setter or hatcher.

There are numerous reports on the role of nutritional factors especially vitamin and mineral deficiencies in causing malformations. The deficiency of various B group vitamins in the hens diet has been shown to have pathological effect on the developing embryo. Cravans et al. (1944) and Couch et al. (1948) had reported chondrodystrophy and parrot beak in the developing embryo when there was biotin deficiency in the hen's diet. Similarly there are numerous reports about malformations like dwarf type embryos and micromelia caused by riboflavin deficiency in the hen's diet. One thousand four

hundred and seventy three (27.08%) cases in this investigation showed oedema in the region of head and neck, some of them also having oedema in other locations also. Similar changes of oedema of subcutis and muscular haemorrhage were observed in the upper cervical region along with areas of necrosis in the piping muscle of embryos and day old chicks by Soldati and Lodrini (1972). Rigdson et al. (1968) observed oedema and haemorrhage in the piping muscles of embryos. This was more extensively seen in embryos that failed to hatch than in normal chicks. No specific etiology was attributed to this condition. Apart from postural abnormalities and toxicosis, deficiency of vitamin E has been pinpointed as a significant factor for such conditions. Since the brain did not reveal any malacic foci it is unlikely that this could be due to vitamin E deficiency. In the present investigation many abnormalities were encountered which were similar to those described for deficiencies of other vitamins. Even-though it can be considered that the feed is not deficient in vitamins, there is a possibility that individual hens might be relatively deficient because of other factors like improper absorption and utilisation. The role of these vitamins on the growth and metabolism of the differentiating embryonic cell is well known and so it is likely that at least some of these malformations could be the result of vitamin deficiencies in the hen. An experiment, after feeding specific deficient diet in the hen and studying the pattern of embryo

mortality and the nature of malformations, could identify the direct role of the various vitamins.

Similarly, deficiency of minerals was found associated with embryo abnormalities. Manganese deficiency was reported to cause chondrodystrophy like parrot beak, micromelia while zinc deficiency resulted in conditions like ectromelia and microphthalmia (Blamburg et al. 1960). As mentioned earlier it is not possible from this study to come to a conclusion whether deficiency of minerals had played any role in causing developmental abnormalities. Long term experiments with the induction of individual mineral deficiency in hens would be required to specifically assign mineral deficiency in the causation of different types of malformations.

Poor hatchery management including possible contamination of the hatching eggs can be considered as a major etiological factor in causing embryo and early chick mortality. The lesions of omphalitis and septicaemia are definite indicators of infections of the embryo. This infection could have been caused either vertically from the hen or exogenously by contamination. Various microorganisms like Salmonella, E. coli, Staphylococcus, Micrococcus, Klebsiella, Pseudomonas and Mycoplasma have been incriminated in causing embryomortality (Chute and Cole, 1954; Orajaka and Mohan, 1985). In order to ascertain the types of bacteria associated with embryomortality 150 embryos which had suspected lesions of microbial infections were subjected to bacteriological

examinations. A total of 118 bacterial isolates was made and 26 samples showed the presence of more than one type of organism. The organisms isolated were Staphylococcus, Coryneforms, Bacillus, Micrococcus, Aerococcus, Coliforms, Proteus, Pseudomonas, Alkaligenus and Aerogenes. This finding is in agreement with those reported earlier by other workers on the role of bacteria in causing embryo mortality. In addition to the external contamination, Harry (1957) reported that death of embryos could result from infection of the yolk by a number of bacteria present in the alimentary tract, and on the skin of hen. Bacteria with enzyme systems capable of disrupting and degrading the yolk lipoproteins multiply extensively when present in the yolk and also allow proliferation of other species of bacteria present. The histopathological examination of tissues from various organs confirmed the septicaemic nature of the infections. This underlines the necessity of hygienic handling of hatching eggs. Some of these embryos if allowed to hatch would result in weaklings which would be susceptible to other infections or which would result in poor growth and production performance. No attempt was made in this study to isolate viral agents. It is well known that many viruses like Infectious bronchitis, Inclusion body hepatitis, Infectious laryngiotracheitis, New Castle disease and Avian encephalomyelitis viruses caused severe lesions, growth retardation and mortality of the chicken embryo. Histopathological examination of the embryos did not

show specific lesions to pinpoint any viral infection. However this requires further detailed investigation.

Experimentally many chemical agents and hormones have also been found to cause pathological lesions including severe teratological defects. These factors do not operate in the present condition.

The importance of mycotoxins in the causation of pathological lesions in the adult birds and their likely storage in the yolk focuses the necessity of identifying the mycotoxins as specific factors in causing mortality, teratological defects, pathological changes and growth retardation. It has been proved that ochratoxin A is teratogenic to the mammalian embryo (Arora, 1982) and that toxins like aflatoxin and citrinin are also likely to play significant role in causing embryonic lesions (Wyllie and Morehouse, 1978). The feed given to the hens occasionally contained varying quantities of mycotoxins like aflatoxins and ochratoxin (Maryamma, 1986) and the lesions in the embryos suggest the possible involvement of mycotoxins in at least causing some of these. In order to assess the pathological alterations and cytological changes, the embryos were experimentally inoculated with Ochratoxin A, citrinin and a combination of ochratoxin A and citrinin. Oxytetracycline was administered since many similar antibiotics are feed additives and have been reported to have a teratological effect.

The essential histological changes seen in embryos inoculated with oxytetracyclines were degeneration in various organs including gastrointestinal tract along with oedema and osteodystrophia. A basic interference in the process of ossification was noticed. Rolle and Bevelander (1966) reported that the toxicity of oxytetracycline is influenced by the route of administration. Gentry (1958) found that oxytetracycline is five or six times more toxic when inoculated at 10 days into the air cell as when injected into yolk sac. Oxytetracyclines are actively transported into the cells and have an inhibitory effect on protein biosynthesis after binding to 30 s ribosomal particles(Prat, 1973). It was also reported that this binding apparently resulted in an inhibition of translocation of amino acid-laden transfer-RNA (comprising the growing peptide chain) from the donor. Cessation of protein biosynthesis could result in inhibition of cell multiplication but it was found that 80 s ribosomes of eukaryotes were much less sensitive to the effect of tetracyclines (Prat, 1973). Tetracyclines have also been considered as irritants causing enterocolitis, stomatitis, glossitis and pharyngitis (Evans, 1968) and the present study confirms the lesions on the alimentary tract. Renal and hepatic damage has also been reported in tetracycline toxicity (Prat, 1973). Apart from the degenerative changes the significant alteration seen in the present study was the marked disturbance in bone formation. Eventhough it has been reported that protein

synthesis is not as markedly inhibited in higher animals as in bacteria there are indications in this study that matrix formation by chondroblast and osteoblast is interfered with. This might have contributed to the dystrophic changes as well as to the disturbed and delayed calcification. Tetracycline feed supplementation is a practice in many countries. At sub-therapeutic levels of tetracycline administration, it has been reported that there was increase of weight gain in birds over controls (Prat, 1973). The mechanism by which this take place in entirely not certain but is often considered to result from suppression of intestinal bacteria which might divert part of the bird's energy from growth to the combat of infections and its products, even when overt symptoms of infection may not be observable. Mitscher (1978) reported that there existed a finite possibility of tetracycline residues in eggs which might interfere with hatching and in causing embryopathies.

Ochratoxin A is a known inhibitor of mitochondrial transport systems (Meisher and Chan, 1974) and so,many of the structural alteration seen in this study could very well be correlated with this metabolic damage. Further, direct damage to the membrane systems could itself result in a seriesof structural defects. Necrosis of renal tubular and periportal hepatic cells have been reported in many species (Purchase and Theron, 1968; Maryamma, 1983). The important structural alterations seen in this study were imperfect bone formation

with defective ossification, oedema and degeneration of the components of the developing brain and eye and degenerative and necrotic changes most specifically in the developing kidney and liver. Potent teratogenic activity of ochratoxin A has been demonstrated in hamsters, rats, mice and chicken. Arora (1982) had elucidated the pathogenesis of malformations induced in mice by ochratoxin A. Malformations of the head, jaws, tail and limb was reported with an incidence of 40-45% hamster foetuses (Wyllie and Morehouse, 1978). Repeated doses produced a high dose-dependent incidence of heart and skeletal abnormalities. In the present study apart from the degenerative and necrotic changes, chondrodystrophic changes were also noticed after ochratoxin A administration. The malformations reported by Gilani et al. (1978) in chicken embryo after ochratoxin A administration were microophthalmia, exencephaly, everted viscera and reduced body size. All these manifestations were recorded in the present study also. Detailed investigation would be required to attribute the level and route of toxin administration and the age of embryo to specific teratological defects. In the present study, degeneration and necrosis of mesenchymal cells were noticed in very early stages which might probably be related to inhibition of phosphorylation and stopping of mitochondrial respiration. Apart from frank necrosis during development, which could result in morphological defects, disturbed differentiation could result in imperfect chondrogenesis, osteogenesis, ossification and even calcification.

Degenerative changes were noticed in the cellular components of the developing eye and brain. The degenerative changes during the early stages have resulted in disappearance of portion of the membrane extending from the pupillary borders of the lens by means of the collagenous fibers that stretch from lens to ciliary processes. The ciliary body acts as a structure physically, supporting the lens. When there is degeneration of oedema this function is interfered with. The ganglionic layer of the retina has also been found to be defective. The oedema and degeneration of the neural components along with defective bone formation had caused many teratological defects in the head. Arora (1982) had described changes in the brain and eye of mouse foetus.

From the available literature it is seen that no study has been conducted on the ultrastructural aspects of the toxicity of Ochratoxin A, citrinin and of citrinin and ochratoxin A together in the chicken embryo. The ultrastructural changes noticed in the chick embryonic kidney and liver after ochratoxin A administration were similar to those described earlier in chicks and in other species. There was increase in lipid droplet which could be very well correlated with impairment of protein synthesis. In a normal cell a lipid acceptor protein is produced by rough endoplasmic reticulum. This in combination with triglycerides are released into blood. It is likely that the damaged endoplasmic reticulum depressed the synthesis of lipid-acceptor protein

which resulted in accumulation of lipids in the cell. The changes in the nuclei and endoplasmic reticulum could contribute for this. The changes were comparatively more severe in the kidney than in the liver. The fact that ultrastructural alterations were more intense in differentiated cells than in undifferentiated cells suggests that target sites could be those where metabolic functions are well delienated or developed. The changes in the glomeruli were mainly characterized by the fenestrated nature of the endothelial surface of the basement membrane and damage to the foot processes of podocytes. These changes in the chick embryo were identical to those described by Maryamma (1983) in goats after experimental administration of ochratoxin. She suggested that ochratoxin damages the membranes of the mitochondria impairing enzyme activities with consequent disturbance of cellular functions. The fusion of the foot processes along with fragmentation of the basement membrane might result in glomerular destruction and would facilitate passage of large macro molecule. In a lethaly injured cell the damage to the mitochondria was not selective but general representation of overall cellular damage (Maryamma, 1983). Elling (1979) demonstrated reduction in the activity of NADH tetrazolium reductase and succinate dehydrogenase in the epithelial cells of the proximal convoluted tubules in experimental ochratoxicosis in pigs. Reduction in the activity of these enzymes may cause decreased function of tricarboxylic acid cycle of the respiratory chain resulting in oxidative phosphorylation.

This may cause disturbance in energy production which may result in the histological lesions seen.

The proliferation of smooth endoplasmic reticulum usually observed in the hepatic cells when exposed to toxins was not observed in the present study of embryos exposed to ochratoxin. This might be due to the embryonic nature of hepatic cells where organellar functional specificity would not have developed or due to alternate metabolic excretory pathway. In the liver it was found that there was increase in the perichromatin granules in the nucleus. Increase in perichromatin granules has been suggested as an indicator of impairment of protein synthesis (Derenzeni and Moyne, 1978). Increase in the number of cytolysosomes is a general indicator of sublethal injury to cell. Nucleolar fragmentation could be due to metabolic derangement due to ATP deficiency or inhibition of RNA synthesis secondary to metabolic disturbance (Shenosuka et al. 1970). Mallory bodies as described by Maryamma (1983) in the liver of goats after administration of ochratoxin was not observed in this investigation.

Ochratoxin A has been shown to impair the development and histogenesis of lymphoid organs, liver, spleen, kidney, adrenal, lungs, cardiac muscle etc. in the mouse foetus. This is in agreement with results obtained in this study where the embryo, in general, was reduced in size and histologically, the organs appeared hypoplastic and showed poor cellular differentiation. The degenerative changes seen both in the

epithelial and lymphoid components of the developing bursa of Fabricius and thymus have great significance since those embryos which were exposed to ochratoxin A would be immunodeficient even if they hatch out.

The pathological alterations in the embryo after administration of citrinin were mainly characterized by degenerative changes in the kidney and liver, more severely in the kidney. There was no apparent disturbance in bone formation unlike in the embryos exposed to ochratoxin. Chondrogenesis, osteoblastic proliferation and calcification were found to be normal. But generalized oedema was a characteristic feature in citrinin toxicity. In addition to the degenerative changes seen in the kidney and liver the epithelial cells of the ciliary body also showed mild degenerative changes between the conjunctival vascular layer and the sclerotic coat. It is not possible to say whether this alteration was specific or only a manifestation of the degenerative changes as seen in other organs. Similarly it could not be ascertained whether process of calcification seen in the kidneys and liver after citrinin administration was specific or not. It was reported that in rats fed toxic cultures of P. citrinin, degeneration and dilatation of the tubules were accompanied by mineralization of debris within the lumen at the cortico medullary junction (Wyllie and Morehouse, 1978). It has also been reported that the most striking pathological changes caused by citrinin in experimental animals is kidney damage. But in

the present investigation apart from renal damage, degenerative/necrotic changes were also observed in other organs in the chick embryo. Ultrastructural examination revealed that pathological alterations were severe in the tubular cells of the kidney. In some cells the organellar changes indicated complete necrosis while in others there was only moderate changes with swollen mitochondria. In general, the changes in the nuclei were not pronounced and the intensity of damage was more in the membrane structures. In the kidney also the ultrastructural changes in the glomeruli were not as pronounced as in the tubular epithelium. The podocyte foot processes showed only a slight disorientation. In the liver the ultrastructural changes were very minimal eventhoughina few cells, ring shaped nucleoli with desegregation of nucleolonema and granular components was noticed. The segregation of filamentous components from the granular components is considered reversible (Ghadially, 1982). Simard and Bernard (1966) postulated that nucleolar segregation probably reflects DNA binding and inhibition DNA dependent RNA synthesis because of the loss of template activity of DNA. The intensity of pathological alteration after simultaneous administration of ochratoxin and citrinin was more than what was seen when ochratoxin and citrinin were administered separately. The quantity of citrinin and ochratoxin administered was half as that when the toxins were administered individually. In the liver in addition to the degenerative changes seen when

ochratoxin A and citrinin was administered there appeared to be damage to the reticular fibres resulting in foci of haemorrhage. Even with the lower dose of ochratoxin disturbance in bone development was also seen indicating clearly that even small doses might cause disturbance in chondrogenesis, osteogenesis and calcification. It is likely that the toxicity of ochratoxin A was synergestically affected by the simultaneous administration of citrinin. The early topographical and cellular alterations induced by these agents may well be the initiating events leading to the pathological manifestations noticed.

The response of the chick embryo to the inoculation of avian influenza virus was general, with vascular, cellular and degenerative reactions. Eventhough there was generalized congestion, emigration of heterophils was not a characteristic feature. The virus might have caused severe vascular injury especially to the endothelium as revealed by oedema in many locations including the brain. Occasionally haemorrhage was also noticed. It was very evident that the virus had marked cytotoxic action since there was degeneration and necrosis of cells of the parenchymatous organs. In the heart the myocardial fibres were found fragmented and there was myolysis. The myocardial fibres were found separated by severe oedema. It could not be clearly ascertained whether the degenerative changes of the neurons of the brain were primarily due to the action of the virus or secondarily due to the severe oedema that was manifested.

The cellular reactions were mainly mononuclear and by light microscopy it was found difficult to identify whether they are lymphoid cells or macrophages or other undifferentiated cells of the hemoblastic type. Eventhough there was severe congestion when examined at 18 hours, emigration and infiltration of the heterophils was not noticed. It was not evident from this study whether this failure of emigration was due to the functional incompetence of the cells because of the early stage of the embryo or due to the inability of the embryo to respond to the chemical mediators to initiate emigration or due to the inability of the embryo to elaborate the chemical mediators themselves. It should be worth-while to study this aspect to clarify to what age the chicken embryo can respond to different antigens by initiating cellular emigration and antibody production.

This preliminary study has indicated numerous areas for further investigations. Some of these areas would be (i) the level of mycotoxins present in the eggs when the hens are exposed to feed containing mycotoxins, (ii) the actual time of initial exposure by a toxicant and its level which would have a teratogenic effect, (iii) the sub-cellular mechanisms which would initiate morbid processes in the embryo and (iv) the factors associated with cellular response and antibody production in the chick embryo after antigenic stimulation.

Summary

6. SUMMARY

This study was conducted to assess the nature of embryo-mortality in hatcheries, to find out the possible causes and to investigate the pathoanatomical features of embryopathies. A total of 5440 embryos (out of 32,700 eggs set) which failed to hatch on the 21st day was studied pathomorphologically. Out of these, 150 embryos which showed signs of infection were subjected to bacteriological investigation. In order to ascertain the role of various agents in causing mortality and malformations in the embryo, experimental studies were conducted by inoculating Oxytetracycline, Ochratoxin A, Citrinin, a combination of Ochratoxin A and Citrinin and avian influenza virus. Ultrastructural studies were conducted to elucidate the subcellular changes in the liver and kidneys of the embryo after administration of Ochratoxin A, Citrinin and a combination of Ochratoxin A and Citrinin.

The various abnormalities noticed were early embryonic death, curled embryos, dead in shell, live sticky embryos, oedema of head and neck, omphalitis and septicaemia, gastroschisis with herniation of yolk sac, coelosoma, dwarfs, curled toe, microphthalmia, anophthalmia, brachycephaly, parrot beak, crossed beak, brachygnathia and streptosomia. A few cases of diprosopus, polymelia, ectromelia, thoracomelia, phocomelia and duplicatus were also encountered.

Sixty per cent of the embryos examined had double or multiple defects. Twenty-seven per cent had oedema in the

region of the head and neck. In these, histologically there was fragmentation and myolysis of fibres. Such changes could be due to postural abnormality, toxicosis or vitamin E deficiency. Thirty-eight per cent of cases because of their poor development indicated early embryonic death, that is before 10 days of incubation. Ten per cent of the embryos were small in size and had a curled appearance. The size of the embryo indicated their death had occurred between 10th and 18th days of incubation. Dwarf embryos constituted 11.3% (Total 615). Out of these 55 showed extreme shortness of legs and 70 cases revealed haemorrhages in the liver, kidney and heart. Histologically there was degeneration and necrosis of these organs. Among these dwarf embryos 22 numbers had generalised oedema.

Five per cent of the cases revealed omphalitis and lesions of general septicaemia. This was seen in embryos where there was herniation of yolk sac as well in those embryos where the yolk was retained within the body. Hundred and fifty of these embryos were subjected to bacteriological examination. A total of 115 bacterial isolates was made and 26 samples showed the presence of more than one type of organism. The organisms isolated were Staphylococcus, Corynebacterium, Bacillus, Micrococcus, Aerococcus, Coliforms, Proteus, Pseudomonas, Alkaligenus and Aeromonas. Contamination of the hatching eggs could be the factor for yolk sac infections and septicaemia.

Fifteen per cent of the embryos were classified as 'dead in shell'. They did not reveal any malformations except

for moderate degree of congestion and degenerative changes in the liver and kidney. Embryos which were classified as 'live sticky' constituted 20%. Coeloscma (7.87%) was characterized by eventration of viscera which varied from loops of intestines to entire viscera seen outside the body.

The abnormalities and deformities of bone and cartilage encountered were beak abnormalities like parrot beak (2.3%), crossed beak (3.69%), brachygnathia (0.28%) and agnathia (0.02%), micromelia (2.5%), polymelia (0.02%), ectromelia (0.02%), curled toe (4.49%), brachycephaly (2.5%) and cranioschisis with encephalocoele (11.03%). In cranioschisis, there was partial failure of the fusion of cranium and herniation of the brain. In such cases brain was malacic with neuronal degeneration. It was suggested that many of these developmental defects could be due to nutritional factors or due to toxicants. In 2430 embryos (45%) various types of malpositions were observed. They were single or multiple.

The other malformations encountered were few in number. When oxytetracycline was given to 8 day-old embryos, only 47.5% survived at 21 days. The rest died during various stages of incubation. The mean average weight of the embryos that survived was significantly lower than that of controls. Degenerative changes were noticed in liver, kidney and digestive organs. Generalised oedema was observed. There was interference in chondrogenesis and osteogenesis and calcification.

It was postulated that these might have contributed to the dystrophic changes in the bones.

In the group of embryos inoculated with Ochratoxin A, 75% died and showed a marked reduction in size and weight. The embryos which were dead at various stages of incubation showed imperfect organogenesis. There was marked degeneration and necrosis of the cells of various developing organs. Aberration of development was seen in the growing bones resulting even in craniaschisis in a few cases. Histopathological lesions were consistently seen in the eye. In the retina, there was detachment or folding of the ganglionic layer. Degeneration of the lymphoid elements of the developing thymus and bursa of Fabricius was also noticed. The ultrastructural changes in the liver and kidneys were mainly characterised by damage to the membrane system. The mitochondria showed changes varying from swelling to severe destruction of the organellar structures. There was fragmentation and degranulation of the rough endoplasmic reticulum. Lysosomes were present. Nuclear changes reflected the degree of injury to the cells. There was moderate destruction of the brush border of the proximal convoluted tubule. The changes in the glomeruli were mainly confined to the basement membrane and podocytes. There was an increase in the fenestrated nature of the endothelial surface of the basement membrane and damage to the foot process of the podocytes. It was suggested that since ochratoxin A was a known inhibitor

of mitochondrial transport systems many of the structural alterations could be correlated to this metabolic change and the resultant disturbed differentiation could result in imperfect organogenesis including osteodystrophic changes.

There was 67.5% mortality when the embryos were inoculated with citrinin. The embryos that survived had significantly lower weight than controls. The embryos which died before 21 days showed general congestion, degeneration and necrosis of various organs. The developing bones were apparently normal. In the brain there was slight oedema of the diencephalon. The embryos that survived upto 21 days also showed degeneration and necrotic changes in the kidney and liver. Calcified foci were also noticed in these organs. In three cases the epithelial cells of the ciliary body of the eye showed slight degeneration between the conjunctivo-vascular layer of the ciliary bodies and sclerotic coat. Slight oedema and degeneration of the brain was also noticed.

The ultrastructural changes in the liver associated with citrinin toxicity were mitochondrial swelling with damaged cristae, ring shaped nucleoli with desegregation of nucleolenoma and granular components, partial lysis of plasma membrane and presence of debri in the bile canaliculi. In the kidney the epithelial cells of the tubules showed the severest form of damage with some cells showing organellar changes indicative of advanced necrobiotic changes. Chromatolysis was partial or complete with occasional lysis of nuclear

membrane. Slight swelling with enlargement of the outer compartment to complete lysis of the cristae was noticed in the mitochondria. In the glomeruli there was mild to severe swelling of mitochondria of podocytes, mesangial cells, endothelial cells and cells lining the Bowman's capsule. There was occasional fusion of podocyte foot-processes.

There was 87.5% mortality of embryos in groups inoculated with the combination of Ochratoxin A and Citrinin. There was reduction in weight of embryos that survived upto 21 days and the malformations noticed were cranioschisis, herniation of yolk sac, curled toe and eventration of viscera. General oedema was also observed. The histological changes in the liver, kidney, alimentary organs, thymus and bursa of Fabricius were severe degeneration and necrosis. Interference in the chondrogenesis, osteogenesis, ossification and calcification was noticed in the bones. The ill-developed cranial bones resulted in imperfect fusion resulting in herniation of brain. There was oedema of brain and degeneration of neurons. In the eye there was degeneration of the ganglionic cell layers of the retina and epithelial cells of the ciliary body. The ultrastructural changes seen in the liver and kidney were more severe than what were observed in ochratoxin A and citrinin toxicity individually. Ring shaped and giant mitochondria were observed. There was degranulation of ribosomes. In the Kupffer cells there was increased number of lysosomes. The organellar damage was more severe in the proximal and

distal convoluted tubules than in other parts of the kidney. The basement membrane of the glomeruli was found to have lost its homogeneous nature and there was fusion of foot processes of the podocytes. It was observed that the toxicity of Ochratoxin A and citrinin was synergistically augmented by their simultaneous administration.

The response of the chick embryo to the inoculation of avian influenza virus was characterized by general vascular, cellular and degenerative changes. Eventhough there was general congestion, heterophilic infiltration was not a characteristic feature. It could not be ascertained from this study whether the failure of heterophilic emigration was due to the functional incompetence of these cells because of the early stage of the embryo or due to the inability of the embryo to respond to the chemical mediators or to produce the chemical mediators themselves. The cytotoxic action of the virus was evident by the degenerative and necrotic changes of the parenchymatous organs. The myocardium was fragmented and showed myolysis. There was general oedema. Neuronal degeneration and liquifactive necrosis were seen in the brain.

This study was helped to elucidate the pathomorphology and some aspects and the etiology of embryomortality in chicken. The histological and ultrastructural aspects have helped to identify the cellular and subcellular events associated with mortality and teratological defects in the chicken embryo.

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EMBRYO MORTALITY IN CHICKEN

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ABSTRACT

The nature of embryomortality in hatcheries and the possible causes for embrypathies were studied. Experimental studies were conducted by inoculating Oxytetracycline, Ochratoxin A, Citrinin and a combination of Ochratoxin A and Citrinin and avian influeza virus. Ultrastructural studies were conducted to elucidate the subcellular changes in the liver and kidneys of the embryo after administration of the mycotoxins.

The various abnormalties noticed were early embryonic death, curled embryos, dead in shell, live sticky embryos, oedema of head and neck, omphalitis and septicaemia, gastroschisis with herniation of yolk sac, coelosoma, dwarfs, curled toe, microphthalmia, anophthalmia, brachycephaly, parrot beak, crossed beak, brachygnatha, streptocemia and a few cases of diprosopus, polymelia, ectromelia, thoracomelia, phocomelia and duplicatus. Sixty per cent of these embryos had double or multiple defects.

A total of 115 bacterial isolates was made from a total of 150 embryos and 26 samles showed the presence of more than one type of organism. The organisms isolated were Staphylococcus, Corynebacterium, Bacillus, Micrococcus, Aerococcus, Proteus, Pseudomonas, Alkaligenus, Aeromonas and Coliforms. Contamination of the hatching eggs could be the cause for these infections.

When oxytetracycline was given to 8 day old embryos only 47.5% survived upto 21 days. The mean average weight of the embryos was significantly lower than that of controls. Degenerative changes were noticed in liver, kidney and digestive organs. Generalised oedema was observed. There was interference in chondrogenesis, osteogenesis and calcification.

In the group of embryos inoculated with ochratoxin A 75% died. These embryos showed imperfect organogenesis. There was marked degeneration and necrosis of the cells of various developing organs. The ultrastructural changes in the liver and kidneys were mainly characterised by damage to the membrane systems. The 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. 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.

There was 67.5% mortality in the citrinin treated group. Significant reduction in the weight of the embryos was noticed. There was degeneration and necrosis in the kidney, liver and other organs. Calcified foci were observed in the kidney and liver. The ultrastructural changes in the liver were swelling of mitochondria with damaged cristae and ring shaped nucleoli

with desegregation of nucleolonema and granular components. In the kidney there was severe damage to the epithelial cells of the tubules. Chromatolysis and lysis of nuclear membrane were seen. Slight swelling with enlargement of the outer compartment to complete lysis of the cristae was noticed in the mitochondria. In glomeruli there was swelling of mitochondria of the podocytes, mesangial cells, endothelial cells and cells lining the Bowman's capsule.

In the group which were treated with citrinin and ochratoxin A there was 87.5% mortality and there was reduction in weight of the embryos. The survived embryos showed malformation like cranioschisis, herniation of yolk, curled toe and eventration of viscera. Histologically there was severe degeneration and necrosis of all organs and there was interference in the organogenesis.

The ultrastructural changes were more severe than those observed in ochratoxin A and citrinin toxicity individually. Ring shaped and giant mitochondria were observed. There was degranulation of ribosomes. In the Kupffer cells there was increased number of lysosomes. The organellar damage was more severe in the proximal and distal convoluted tubules. The basement membrane of the glomeruli was found to have lost its homogenous nature and there was fusion of foot-processes of the podocytes.

When the embryos were inoculated with avian influenza viru

there was general vascular, cellular and degenerative changes. Heterophils were not present eventhough there was general congestion.

The cytotoxic action of the virus was evident by the degenerative and necrotic changes of the parenchymatous organs There was fragmentation and lysis of myocardium. Neuronal degeneration and liquifactive necrosis were seen in the brain.