OCHRATOXICOSIS IN THE GOAT

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THESIS

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D E C L A R A T I O N

I hereby declare that this thesis entitled OCHRATOXICOSIS IN THE GOAT is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Place : Mannuthy,
Date : 20-1-1983.

C E R T I F I C A T E

Certified that this thesis, entitled OCHRATOXICOSIS IN THE GOAT is a record of research work done independently by Smt.K.I. Maryamma 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

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During the past 20 years a wealth of information has accumulated on toxigenic fungi, their toxins and the resulting toxicoses. The impact of the discovery of aflatoxicosis as a disease entity with a wide spectrum of deleterious effects in human beings and animals has resulted in the intensification of research on other mycotoxicoses also and their cause and effect relationship. Reports on the carcinogenic and teratogenic effects of some of these mycotoxins have added a new dimension to this problem. The range of food and feed stuffs potentially affected by toxigenic fungi is wide and the number of species of fungi which are capable of producing toxins are also large. The fact that more than 100 mycotoxins of varied chemical structure and organotropisms have been identified shows the enormity of the problem. There is now an organized effort devoted to understanding and hopefully, controlling mycotoxicoses.

Mycotoxicosis was encountered for the first time in Kerala in 1964 when aflatoxicosis was diagnosed in ducks and pigs. Since then a variety of mycotoxic conditions have been identified in this State affecting different

classes of Livestock. The warm and humid climatic conditions existing in Kerala are ideally suited for fungal multiplication and toxin production in feed stuff. Toxigenic fungi grow not only in grains but also on grasses, hay and straw. Improper post-harvest technology and poor storing conditions are the main reasons for fungal contamination. One important aspect that has to be considered is the presence of toxins in food in sub-lethal or negligible doses which over a period of time cripple the production performance of an animal. Synergistic action and potentiation also play significant roles in the manifestation of non-specific disease syndromes. Apart from the natural occurrence of many of these mycotoxins in grains and fodder, their residues or metabolites occur in milk and other body tissues of animals which further poses the problem of mycotoxicoses in human beings.

Ochratoxins are a group of mycotoxins having varied toxicity in animals. Several species of the genus Aspergillus and Penicillium have been found to produce ochratoxins. These fungi grow on a wide range of cereals, fruits, oil seeds and prepared foods under various environmental conditions. Ochratoxins have the general structure

L-β-phenylalanine linked by an amide bond to dihydro-isocoumarin. They are mainly nephrotoxic and in higher concentrations are hepatotoxic. The most toxic member of this group is ochratoxin A which has a molecular formula of $C_{20}H_{18}Cl\ N\ O_6$. The pathological effects are severely manifested in monogastric animals and chicken. Besides, ochratoxin A was also found to be teratogenic in rats, hamsters and mice (Hore and Galtier, 1974; Hood et al., 1978). Synergism of ochratoxin A with sterculic acid had carcinogenic effect in rainbow trout (Doster et al., 1978). Residues of ochratoxin A have been detected in tissues of pig and poultry. The human disease known as Endemic Balkan Nephropathy has been associated with ochratoxin.

Like most other mycotoxins, ochratoxins are also heat-stable and survive processing operations. Autoclaving upto 3 hours destroys about 33 to 37.5% of ochratoxins in cereals (Trenk et al., 1971).

It was found that many feed samples which were obtained from different parts of Kerala, when screened, had varying levels of ochratoxin (personal observation). Furthur, there is paucity of information on the various aspects of pathological manifestation of ochratoxicosis

in ruminants. So, this study was undertaken to assess the pathological effects of ochratoxin in the ruminant using goat as the experimental animal. The experiments were designed to study

i) the production of ochratoxin by Aspergillus ochraceus and Aspergillus sulphureus in rice and wheat,

ii) the clinico-pathological manifestation in goats when ochratoxin was administered by different routes and in varying dose schedules,

iii) the synergistic effect of aflatoxin and ochratoxin, and

iv) the ultrastructural basis of cellular damage in ochratoxicosis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The role of mycotoxin as instrumental for the development of renal lesions in swine was suspected by early workers in renal pathology and it goes to the credit of the Danish pathologist Larsen (1923) to discover a peculiar type of kidney disease in swine in the Danish slaughter houses. Natural cases of chronic renal lesions as well as the experimental disease produced by feeding mouldy rye resembled each other closely and so it was thought that the renal lesions that had been encountered resulted from mould toxicosis. Attempts were made to reproduce the renal disease using the isolated filamentous fungi of the Penicillium species (Larsen, 1936). Since these trials did not succeed, it was thought that some bacteria which were concomitantly present in mouldy cereal might have been the toxigenic agent. However, cases of porcine nephropathies encountered in large numbers in Danish slaughter houses were designated as "mould nephrosis".

Ochratoxins

Ochratoxin producing fungi.

In a study of toxigenic fungi, van der Merwe et al. (1965 b) found that feed which were inoculated with three

strains of A. ochraceus isolated from grains by Scott (1965) were toxic to ducklings, mice and rats. Extraction of the toxic principle from one of the strains was done and the chromatographically purified toxin induced fatty changes in hepatic parenchymal cells of ducklings. This toxic principle was designated ochratoxin A. Subsequently, ochratoxins B and C, along with ochratoxin A were extracted and characterised from strains of A. ochraceus Wilh (van der Merwe et al., 1965 a).

Staron et al. (1965) isolated two strains of A. ochraceus from mouldy barley and hay that caused death of fattening lambs and heifers. Later studies proved that six other species of the A. ochraceus group were capable of producing ochratoxins. Wheat inoculated with A. sulphureus yukawa (MRRL 3520) and A. sulphureus (Fres) Thom and Church (MRRL 4077) proved to contain considerable amount of ochratoxin (Lai et al., 1968). A. sclerotiuh, A. alliaceus, A. ostianus and A. petrakii also were capable of producing ochratoxin A and B (Ciegler, 1972; Desseltine et al., 1972). Damzel et al. (1976) isolated eleven strains of A. ochraceus all of which were active ochratoxin producers.

Though ochratoxin A was originally considered as a metabolite of the A. ochraceus group, it was later isolat

from several species of Penicillium also (van Walbeek et al., 1969; Ciegler et al.,1972). The most important and prevalent among these is P. viridicatum. van Walbeek et al. (1969) isolated a strain of P. viridicatum which was found to be a good ochratoxin producer, from the surface growth on 'packed ham'. Ochratoxin A was isolated as a natural contaminant of corn for the first time by Shotwell et al. (1969) and the particular sample was heavily infested with Penicillium species. Scott et al. (1970) reported that the fungal species responsible for ochratoxin production in wheat was P. viridicatum. Natural contamination of most of the cereal grains by ochratoxin A was found to be due to this species of Penicillium (Scott et al., 1972). Other species of Penicillium like P. palitans, P. commune, P. variabili, P. purpurescens, and P. cyclopium also were found to elaborate ochratoxin as their metabolite (Ciegler, 1972).

In a survey of foods for the presence of fungal strains in Sweden, Josefsson et al. (1975) isolated Aspergillus and Penicillium species in varying numbers in samples of rye and wheat flour. The fluorescent strains of both species were found to produce ochratoxin. Torrey and Marth (1977) isolated three strains of fungi which were found to

be capable of producing ochratoxin A from samples of refrigerated and non-refrigerated foods. The isolation and identification of ochratoxin A from fodder barley contaminated with P. verrucosum var. verrucosum in Czechoslovakia was reported (Vesela et al., 1970). Lillehoj and Goransson (1980) found that four strains of P. purpurescens, one of P. verruculosum and one of A. ochraceus species produced ochratoxin in barley.

Ochratoxin in food materials.

Corn samples, cereal products and even heated grains were found to contain ochratoxins (Shotwell et al., 1970; Trenk et al., 1971; Scott et al., 1972). Ochratoxin A was detected in rice, wheat, maize, crushed barley, rye, and oats collected from commercial food processing plants and stores of different parts of the World (Krogh et al., 1974; Harwig and Munro, 1975; Uchiyama et al., 1976; Prior, 1976; Muzic et al., 1976; Juszkiewicz et al., 1976; Clarke and Niles, 1977; Styrett, 1977; Sugimoto et al., 1977). Moderate to high levels of ochratoxin A were detected in maize samples, barley and oats collected from endemic areas of Balkan nephropathy and porcine nephropathy (Balzer et al., 1977; Krogh, 1977). Contamination of flour supplied for human consumption, and animal feed stuffs with Ochratoxin A were reported (Richardson et al.,

1978; Funnel, 1979). In India, Rao et al. (1979) found ochratoxin A in 11 out of 180 food grain samples tested. Different levels of ochratoxin A were found in grains, corn, forage and maize used in poultry feed factories (Prior, 1981; Devi and Polasa, 1982).

Factors influencing ochratoxin production.

A. ochraceus is somewhat xerophytic in nature and can grow at about 80 per cent relative humidity (Christensen, 1962). Under optimal environmental conditions ochratoxin production in significant quantities occurs at 7 to 14 days of growth in the substrate. The maximum temperature at which Penicillium fungi can produce ochratoxin was reported to be -2°C (Mislivec and Fulte, 1979). Growth of fungi in shredded wheat at 21° to 23°C for a period of 19 to 21 days produced good yields of ochratoxin, as shown by Schindler and Nesnera (1970). Frank et al. (1971) found that the optimal temperature for ochratoxin production by A. ochraceus ATCC-3174 was 23°C. The optimal time varied from 7 to 14 days depending on the substrate. Production of ochratoxin in considerable quantities occurred at 25°C after 10 or 12 days' growth in medium containing two per cent yeast extract and four per cent sucrose (Sansing et al., 1973). Lindenfelser and Ziegler (1975) designed

a solid substrate fermenter for the production of ochratoxin in large amounts. The highest yields of ochratoxin A occurred in maltose containing culture media from A. ochraceus in comparison to other carbohydrate containing media (Engel, 1976). Balzer et al. (1977) observed that contamination of maize grain in store houses by Aspergillus and Penicillium species was favoured by certain temperatures and moisture. Usually there was bacterial fermentation before contamination by fungi. Hesseltine (1977) produced ochratoxin A employing solid state of fermentation in wheat, rice and oats. Lillehoj et al. (1978) used a number of species of Aspergillus and Penicillium for production of ochratoxin A in several media. Maximum yield of 350 µg/ml of ochratoxin was obtained by the A. sulphureus isolates in the modified Czapek medium after 11 days of static incubation at 28°C.

Detection and isolation of toxin.

Since the development of a rapid method for detection and identification of ochratoxins by Steyn and Vorwe (1966), several workers have tried different procedures for extraction, purification, isolation and quantification of ochratoxins (Nesheim, 1969; Galtier, 1974; Levi, 1975; Hald and Krogh, 1975; Hagan and Pietjen, 1975; Roberts and

Patterson, 1975). A radioimmunoassay technique employing ^{125}I-labelled ochratoxin A as radioactive antigen was evolved by Aalund et al. (1975). Screening methods for more than one mycotoxin were described by Holaday (1976) and Takeda et al. (1975). A spectroscopic procedure, using carboxypeptidase A for the quantitative measurement of ochratoxin A was evolved by Hult and Gatenbeck (1976). A method using the differences in kinetic parameters of the enzymatic hydrolysis of the two compounds was suggested for analysis of mixtures of ochratoxins using carboxypeptidase A (Hult et al., 1977). Procedures like direct thin layer chromatographic densitometric identification, immunofluorescence microscopical detection, spectrophotofluorimetric detection and high pressure liquid chromatography were described (Reimerdes, 1977; Alling, 1977 a; Engstrom et al., 1977; Czerwiecki, 1978; Hunt et al., 1978; Balzer et al., 1978; Peterson and Ziegler, 1978; Josefsson and Moller, 1979). In a multimycotoxin detection method, Gimeno (1979) used solvents of different pH for extraction of toxins. Screening methods for simultaneous determination of 13-14 mycotoxins including ochratoxin A were described by Gorst-Allman and Steyn (1979) and Takeda et al. (1979). Osborne (1979) demonstrated a reversed phase high pressure liquid chromatography for the detection and quantification

of ochratoxin A in flour and bakery products. Hunt et al. (1979) described a sensitive method of detection of ochratoxin A in kidney using enzymic digestion, dialysis and high pressure liquid chromatography. Schwalgnardt et al. (1980) also employed a high pressure liquid chromatographic method for rapid detection of ochratoxin in substrate. Hult et al. (1980) demonstrated a method of evaluation of ochratoxin in the feed of pigs by estimating the toxin content in blood.

Physical and chemical properties of ochratoxins.

The physical and chemical properties of ochratoxins A, B and C were studied and structural formulae worked out by van der Merwe et al. (1965 b). Ochratoxin A was found to be a colourless crystalline compound, the pure toxin having a melting point of 169°C. It was found to be sparingly soluble in water, but soluble in polar organic solvents and dilute aqueous sodium bicarbonate. Ochratoxin B was characterized as the dechloro analogue of ochratoxin A which often co-occur in cultures along with ochratoxin A. Ochratoxin C was chemically characterized as the ethyl ester of ochratoxin A. Acid and enzymatic hydrolysis of ochratoxin A, yielded L-β-phenylalanine and the isocoumarin acid-ochratoxin alpha (van der Merwe et al., 1965 a).

Ochratoxin A was found to be a stable compound and could be stored in ethanol solution in refrigerator for over a year without loss of toxicity (Chu and Hsu, 1970). The 4-hydroxy ochratoxin A also was later isolated from a strain of P. viridicatum (Hutchinson et al., 1971). Ochratoxin A was found to bind bovine serum albumin (Chu, 1971). Survival of 12.5 to 17 per cent of ochratoxin A in the absence of water and 26 to 35 per cent in the presence of water during autoclaving for three hours has been reported by Trenk et al. (1971). It was observed that decomposition of ochratoxin A occurred on exposure to fluorescent light for several days (Neely and West, 1972). Cleavage of ochratoxin A into nontoxic ochratoxin alpha and phenylalanine occurred when mixed with the contents of rumen, reticulum and omasum of the cow (Hult et al., 1976). Galtier and Alvinerie (1976) treated ochratoxin A with the centrifugal pellet of rat caecal contents containing microbial flora and got ochratoxin alpha as the hydrolysed product. It was also demonstrated that the toxin could be hydrolysed by the rumen fluid of cows and sheep. Esterification into the toxic ochratoxin A also was found possible.

Toxicity in Animals

Ruminants.

Reports on ochratoxicosis in ruminants are few. In

an investigation of mortality in lambs, Staron et al. (1963) isolated toxic strains of A. ochraceus from barley and hay which had been fed to these lambs. Munro et al. (1973) administered ochratoxin A intravenously to pregnant ewes at the dose level of 1 mg per kg body weight. The animals died in less than 24 hours without aborting and the cause of death was found to be either pulmonary congestion with oedema or massive hepatic necrosis. It was found that very little ochratoxin A penetrated the ruminant placenta since no ochratoxin was found in the ewe's amniotic fluid. Foetal tissue levels of toxin were 1/4000 to 1/1000 the levels in the maternal blood. Ochratoxin extracted from mouldy hay caused centrilobular and periportal fatty degeneration of the liver and severe nephrosis in kids (Shadni et al., 1974). Hult et al. (1976) reported on the degradation of ochratoxin A by ruminants. Ochratoxin A was incubated with the contents from the bovine stomach compartments and it was found that the toxin was cleaved into non-toxic ochratoxin alpha and phenylalanine by the contents from all but the abomasum.

Rioelin et al. (1973) induced ochratoxicosis in two calves by giving single dose of 11 mg per kg and 25 mg

per kg body weight of ochratoxin A. Similarly three cows which were three to six months pregnant were dosed with 0.2, 0.75 and 1.66 mg ochratoxin per kg body weight for four to five days. A fourth cow from the same group received 13.5 mg ochratoxin per kg as a single dose. The cow that received single massive dose of ochratoxin A had difficulty in rising, diarrhoea, anorexia and abrupt cessation of milk production, all commencing one day after dosing. On the day next to dosing, 650 mg of ochratoxin A and 4500 mg of ochratoxin alpha were detected in the milk. Thereafter, only ochratoxin alpha could be detected. Complete recovery occurred after four days but milk production never increased above one-third normal during that lactation period. Cows treated at doses of 0.2, 0.75 and 1.66 mg/kg for four to five days remained clinically normal and delivered normal calves. The cow given 1.66 mg/kg daily for four days had ochratoxin alpha in the milk on days one through six but traces of ochratoxin A were detected only on days three, four and five. All cows had traces of ochratoxin alpha in milk and urine. Goats given oral ochratoxin A at the rate of 3 mg/kg developed watery diarrhoea, became dehydrated and died on the fifth day. Centrilobular cloudy swelling of the hepatocytes was the only histological

lesion. All of the experimental goats had a decline in lymphocyte count and an increase in neutrophils during the period of administration of toxin. Serum glutamate-oxaloacetic transaminase (SGOT) activity increased in all cases and serum alkaline phosphatase (ALP) activity declined in animals given ochratoxin at 3 and 2 mg/kg level. An increase in serum ALP was observed at 1 mg/kg dosage level. Shreeve et al. (1979) detected ochratoxin A in the kidneys of a cow fed ochratoxin contaminated ration. Multiple small grey spots on the kidney were the gross lesions while microscopical examination revealed sub-acute interstitial nephritis. Spontaneous occurrence of ochratoxicosis which caused mortality in 63 cattle was reported from Iowa (Lloyd and Stahr, 1980).

Swine.

The cases of 'nephropathy' (Larsen, 1923) and 'mould nephropathy' (Larsen et al., 1962) could in retrospect be considered as due to ochratoxin toxicity. Madsen et al. (1965) administered mouldy barley to pigs and reproduced the symptoms like polydipsia, polyuria, gastroenteritis and retardation of growth. Renal damage characterised by atrophy of the proximal renal tubules, thickening of the tubular basement membrane, hyalinization of glomeruli

and cortical cyst formation was observed in pigs due to ochratoxin ingestion (Buckley, 1971; Elling and Moller, 1973). Changes in renal function and structure and other pathomorphological changes induced by purified ochratoxin A and ochratoxin A contaminated feed were described in this species (Szczech et al., 1973 c; Hyldgaard-Jensen, 1973; Szczech et al., 1974 b).

Pattersen et al. (1976) fed a mixture of ochratoxin A and B to pigs daily for eight days during early pregnancy. It was found that compared with ochratoxin A, ochratoxin B was poorly absorbed and preferentially hydrolysed to B-ochratoxin in the intestinal tract. Ochratoxin A was excreted as unchanged toxin in faeces and urine. Ochratoxin did not cross the placenta to the foetal tissues. The epidemiology of mycotoxic porcine nephropathy showed similarities with that of endemic Balkan nephropathy of man (Krogh et al., 1976 a). Contamination of foodstuffs with ochratoxin A was found to be more frequent in parts of Yugoslavia where Balkan nephropathy was prevalent than in disease free zones (Krogh et al., 1977). The morphological aspects of mycotoxic porcine nephropathy were described and the relationship of changes with those of endemic Balkan nephropathy was indicated by Elling (1977 b). Histological

changes similar to those observed in experimental ochratoxicosis were present in most of the kidneys in which ochratoxin residues were present (Ludqvist et al., 1977). Nephropathy and high concentration of ochratoxin A in kidneys were observed in fattening pigs from two Swedish farms. Whole blood and plasma of pigs also contained varying amounts of ochratoxin residues. The source of toxin was traced to rain-damaged barley which supported growth of the ochratoxin producing fungus, P. verrucosum var-verrucosum. Compounded feed also was found to be contaminated with ochratoxin (Ludqvist et al., 1978). Causal associations of spontaneously occurring nephropathy with mycotoxins have been reviewed, and it was reported that field cases of perirenal oedema were present in piglets, the kidneys of which contained ochratoxin A residues (Krogh, 1973; Krogh et al., 1977).

Alterations in pathomorphological aspects and enzyme activity of renal tubular cells of pigs due to ochratoxin A induced nephropathy were elucidated by histochemical techniques. The activities of reduced nicotinamide adenine dinucleotide phosphate (NADPH) - tetrazolium reductase, lactate dehydrogenase (LDH), glucose - 6 - phosphate dehydrogenase and non-specific alkaline phosphatase were reduced focally corresponding to the areas

with focal tubular atrophy and the degree of reduction was roughly parallel to the degree of atrophy (Elling, 1979).

Dogs.

At levels of 0.2 to 3.0 mg/kg body weight, ochratoxin caused anorexia, weight loss, emesis, tenesmus, passage of blood-stained mucus from the rectum, high rectal temperature and tonsillitis in Beagle dogs (Szczech et al., 1973 a). Pathological changes in urine including high concentration of protein, glucose, isocitric dehydrogenase (ICDH), leucine aminopeptidase (LAP), glutamate pyruvic transaminase (GPT), LDH, GOT and ALP were noticed in dogs in ochratoxicosis. Gross and histopathological changes in tissues and ultrastructural changes in kidneys also were described (Szczech et al., 1973 b, 1974 a, 1974 b).

The synergistic effects of ochratoxin A and citrinin in Beagle dogs were experimentally studied (Kitchen et al., 1977 a,b,c). Clinical signs of toxicosis included anorexia, tenesmus, weight loss, prostration and death when ochratoxin and citrinin were given together. The urinary concentrations of enzymes GOT and LDH were increased. Serum concentrations of sodium and potassium chloride decreased in dogs given high doses of ochratoxin or citrinin.

Cellular and granular casts, ketones, proteins and glucose were present in urine when both toxins were combined or with 10 mg/kg level of citrinin alone. Renal lesions in dogs given ochratoxin were degeneration and necrosis with desquamation of tubular epithelial cells, primarily in the straight segment of the proximal tubules. When both toxins were combined, degeneration and necrosis occurred in proximal and distal convoluted tubules and in their segments and collecting ducts. Dogs given ochratoxin A had necrosis of lymphoid tissues in the spleen, tonsil, thymus, peripheral lymph nodes and lymph nodules of the ileum, colon and rectum. There was ulceration of the mucosa of the intestine in dogs given 10 mg/kg of citrinin and 0.2 mg/kg ochratoxin together. Cytoplasmic vacuolation, myelin figure formation and disarray of organelles were the predominant ultrastructural changes noticed in the animals given 10 mg/kg citrinin alone or 0.2 mg/kg ochratoxin A and 10 mg/kg citrinin. The vacuolar lesions were limited to the proximal tubules in dogs given only ochratoxin A. Myelin figures were in proximal epithelial cells of dogs given ochratoxin A alone or in combination with citrinin.

Poultry.

Mild fatty infiltration in the liver was the important

lesion in ochratoxicosis of ducklings (Theron et al., 1965). Acute nephrosis, hepatic degeneration and visceral gout were observed in chicken in experimental ochratoxicosis (Doupnik and Peckham, 1969; Peckham et al., 1972). Clinical signs and lesions in liver, kidney and intestines due to ochratoxin poisoning of chicks were described by several workers (Choudhary et al., 1971; Chu and Chang, 1971; Huff et al., 1974; Huff et al., 1975; Elling et al., 1975). Huff and Hamilton (1975) reported a longer mean survival time in birds exposed to 43°C and 45% relative humidity in comparison to those exposed to 4°C and 90% relative humidity when 4 - 8 µg/g ochratoxin was fed to chicks. Comparative LD_{50} values of oral ochratoxicosis in chicks, turkeys and Japanese quail have been recorded (Prior et al., 1976; Galtier et al., 1976). Renal lesions and reduction in renal function due to ochratoxicosis of chicken have been described (Krogh et al., 1976 b; Svendsen and Skadhauge, 1976). Huff et al. (1977) reported an increase in the tibial diameter and decreased bone strength in chicken due to ochratoxicosis. Analysis of blood samples from hens fed ochratoxin at 0.5 and 5 ppm levels in ration revealed a decrease in the haematocrit value and leukopenia at the 5 ppm level (Rupic et al., 1977). Elevation in concentration of ALP in serum, increase in prothrombin time, decreased total serum proteins, decline in egg production, reduction in weight gain, and decrease

in glycogen mobilization were described in ochratoxicosis of White Leghorn and broiler chicks (Liker et al., 1978; Prior and Sisodia, 1978; Bitay et al., 1979; Huff et al., 1979; Warren and Hamilton, 1981). Reduced leukocyte counts, impairement in the phagocytic activity of heterophils and decrease in the collagen content of large intestines due to ochratoxicosis in broiler chickens were reported (Chang et al., 1979; Chang and Hamilton, 1980; Warren and Hamilton, 1980). The synergistic action of ochratoxin and aflatoxin in broiler chickens with regard to the liver lipid content and enlargement of crop and gizzard was studied (Huff and Doerr, 1980, 1981). Chang (1982) demonstrated impairment of phagocytosis by monocytes from fowls intoxicated with ochratoxin A.

Pathological effects due to ochratoxicosis were also reported in Japanese quail and turkey poults (Doster et al., 1973; Chang et al., 1981).

Laboratory animals.

Among laboratory animals, rats were extensively used in toxicity studies with ochratoxin (Theron et al., 1966; Purchase and Theron, 1968; Purchase, 1971). The oral LD_{50} value for young rats was estimated to be about 20 to 22 mg/kg. Alteration in cell glycogen metabolism was found in the liver

of rats with ochratoxicosis (Munro et al., 1973; Suzuki and Satoh, 1973). Retardation of growth, reduced feed intake, raised blood urea nitrogen (BUN) level, increased kidney weights and degenerative changes involving the entire renal tubules were observed (Munro et al., 1974). Ochratoxicosis in rats was embryotoxic and teratogenic (Still et al., 1971; Hore and Galtier, 1974; Brown et al., 1976; Arora and Frolen, 1981 and Mayura et al., 1982).

Hayes et al. (1977) computed the oral LD_{50} value of ochratoxin A for 24 hour old rats and found that it was very low when compared to adult rats and the LD_{50} level was approximately 16 times lower when ochratoxin A and rubratoxin B were administered together. Haley and Galtier (1977) observed variations in certain biochemical parameters in orally induced ochratoxicosis of adult male rats. Pharmacokinetic study of ochratoxin A in rats revealed binding of the toxin to serum albumin and the toxin was converted to the less toxic ochratoxin alpha in the rat caecum (Galtier, 1977). The oral LD_{50} of ochratoxin A for male rats was calculated as 23 mg/kg and severe catarrhal or erosive enteritis involving the duodenum and jejunum occurred within 4 hours after dosing. There was necrosis and desquamation of epithelium of the proximal convoluted tubules. Necrosis of cells in the germinal centre of the spleen and lymph

nodes was the other important lesion noticed (Kanisawa et al., 1977). Paul et al. (1979) reported a reduction in steroidogenesis by testicular tissue during ochratoxicosis. Dilatation of seminiferous tubular epithelium, cytolysis, vascular thrombosis and hyperplasia of interstitial tissue were the lesions observed (More and Camguilhen, 1979).

Intraperitoneal injection of ochratoxin A in female rats caused arrest of oestrus cycle in the dioestrus phase and fall in the activity of enzymes which are involved in ovarian steroidogenesis. Ovarian cholesterol and ascorbic acid levels increased following treatment with ochratoxin A (Gupta et al., 1980). The physiopathology of haemorrhagic syndrome related to ochratoxin intoxication was studied in rats by Galtier (1979).

It was found that mice were more resistant to ochratoxin A than rats (Galtier, 1974). In addition to causation of lesions in various organs, ochratoxin is teratogenic and carcinogenic in mice (Carlton et al., 1970; Hayes et al., 1973; Sansing et al., 1976; Zimmerman et al., 1977; Hood et al., 1978; Kanisawa and Suzuki, 1978; Gupta et al., 1979; Szczech and Hood, 1979). In guinea pigs it was found that the lesions were confined mostly to proximal convoluted tubules of the kidney. There was significant reduction of

betaglobulin (Richard et al., 1975; Thacker and Carlton, 1977; Carlton and Szczech, 1978). Hayes and Hood (1976) reported on the teratogenicity of ochratoxin A in guinea pigs.

Biological systems for assay of toxin.

Brown (1969) used brine shrimp larvae for biological assay of mycotoxins and found that the toxicity of partially purified ochratoxin was five times lesser than that of aflatoxin B1. Zebra fish larvae also were found to be sensitive to ochratoxin A (Abdel and Scott, 1980). Biological detection of ochratoxin using chick embryo and cell culture was described by Gedek (1972). Cardeilhac et al. (1972) used tracheal organ cultures for the bioassay of minute quantities of mycotoxins including ochratoxin A. Inhibition of phenylalanyl-t RNA synthetase from Bacillus subtilis by ochratoxin A was reported by Konrad and Roschenthaler (1977).

Hacking et al. (1977) developed brine shrimp and chick embryo assay for mycotoxins including ochratoxin A and the dose-response curves were worked out. Ochratoxin A caused autolysis of Bacillus subtilis when added to the bacterial culture at less than 12 microgram/ml concentration. Inhibition of protein and cell wall synthesis was effected

at concentration < 10 µg/ml (Singer and Roschenthaler, 1973). Ochratoxin A inhibited protein synthesis in Bacillus stearothermophilus (Bunge et al., 1973). Inhibition of protein synthesis was observed in Streptococcus faecalis by ochratoxin A (Heller and Roschenthaler, 1973). In vitro inhibition of yeast phenylalanyl-t RNA synthetase by ochratoxin A was observed (Creppy et al., 1973). Lafont and Lafont (1973) worked out the LD_{50} of ochratoxin A for chick embryo as 1.1 µg/egg when inoculated through air space. Antimicrobial activity of ochratoxin A was observed using Bacillus thuringiensis Berliner by Boutibonnes (1973). Prior (1973) reported that ochratoxin A, F_2 toxin and aflatoxin B, increased mortality of brine shrimp larvae. Out of several mycotoxins tested on brine shrimp, only fusarenon - X and ochratoxin A showed synergistic effect (Tanaka et al., 1973).

Antitoxic agents.

O-methoxycinnamaldehyde from cinnamon inhibited growth of A. ochraceus and A versicolor at the level of 200 µg/ml. Ochratoxin A production by the fungus was inhibited at 25 µg/ml concentration (Horozumi, 1973).

The antitoxic effect of phenylalanine against ochratoxin was investigated by Creppy et al. (1973). Simultaneous injection of 0.8 mg ochratoxin and 1 mg of phenylalanine per mouse gave absolute protection while the same dose of ochratoxin alone proved 100% lethal to mice.

MATERIALS AND METHODS

MATERIALS AND METHODS

Production of ochratoxin

A comparative assessment, under static and shake cultures, was made on the ochratoxin producing ability of two species of Aspergillus viz., A. ochraceus and A. sulphureus grown in moistened, shredded, broken wheat or polished rice.

Fungal strains and culture.

A. ochraceus IMI 132329 supplied by the Commonwealth Mycological Institute and a strain of A. sulphureus received from the Central Food and Technological Research Institute, Mysore, were the species used. The method used by Nesheim (1969) was adopted with certain modifications for the preparation of ochratoxin.

Placed 100 g of shredded broken wheat in a three-litre capacity conical flask and soaked it with 60 ml of tap water for two hours. The flask with the substrate was autoclaved (15 lbs pressure per sq. inch; 20 min) at 120°C. When cooled, the flask was inoculated with fungal spores from 13 day old slants of A. ochraceus. Six such flasks were prepared. The flasks were incubated in the dark at room temperature (about 28°C) for 13 d and shaken once a

day to break up the mycelial mass; the flasks were then taken for extraction of toxin.

Extraction of toxin.

Chloroform (750 ml) was added to each flask through a long stem funnel inserted beside the cotton plug and the flask was then heated on a steam bath until chloroform vapours escaped through the plug. The matted growth was broken up with a spatula and the contents were agitated briefly by swirling. The chloroform was decanted and filtered through Whatman No.4 paper on a Buchner funnel. Re-extracted the culture with two 450 ml portions of chlorofor and the extracts were filtered. The filter residue was washed with 100 ml methanol and the combined extracts were evaporated to dryness in the steam bath. The crude extracts from the different containers were pooled and the final volume made to 150 ml with chloroform. Aliquots of 50 ml from this were added to two litres of hexane and swirled vigorously. Removed the precipitate formed by filtration through Whatman No.1 paper. The second and third 50 ml portions of the pooled extract solutions were, in turn, added to hexane filtrate and the precipitate that formed was removed by filtration. The collected precipitate was partially dried overnight at room temperature and then to constant weight at 34°C.

The hexane precipitate was dissolved in 500 ml chloroform, filtered and extracted once with 200 ml and twice with 100 ml portions of 0.5 % sodium bicarbonate. The bicarbonate extract containing the ochratoxin was back extracted once with 50 ml chloroform before acidification with 300 ml 1 N H_2SO_4. The remaining ochratoxin was then extracted from the aqueous layer with 100 ml portions of chloroform. The combined chloroform extract was washed with 70 ml water after mixing well and this was repeated four times. The extract was evaporated to dryness in a steam bath. The residue was dissolved in 60 ml chloroform, which was added slowly and with stirring to one litre hexane. The precipitate which formed was collected and dried at 34°C.

Quantification of toxin.

A weighed quantity (one mg) of the precipitate was dissolved in 10 ml of benzene for estimation of ochratoxin concentration by thin layer chromatography. Dilution of one in thousand was made from the above solution using benzene. Similar dilution was made for ochratoxin standard (1 mg crystalline toxin in 10 ml benzene) (Makor Chemicals, Israel) also. Chromatographic plates were coated with silica gel SLC-76 to get a uniform spread thickness of 0.25

and dried for two hours at 80°C. Spotted 1,2,3,4,5 µl of the diluted extract on one half of the chromato plate and similar quantities of the standard ochratoxin correspondingly on the other half. In another chromato plate 6,7,8,9 and 10 µl quantities of the diluted extract and identical quantities of the standard were spotted in a similar pattern. The spotted plates were developed with glacial acetic acid - benzene (10 : 90 v/v) by a 30 min run, dried in air for 30 min and then scanned under long-wave ultraviolet illumination. The minimum observable fluorescent spot was located for the 'test' (4 µl ≡ 0.003/ µg. The experiment was repeated with polished rice in a similar way. Both the sets of experiments were also repeated with A. sulphureus. The continuous snake culture method, as has been adopted for the production of aflatoxin (Shotwell et al., 1966) was also employed for preparation of ochratoxin. One hundred grams of broken good quality shredded wheat were placed in six erlenmeyer flasks (500 ml capacity). Moistened the grains with 80 ml distilled water and allowed two hours for the grains to get soaked, shaking the flasks occasionally to ensure uniform distribution of moisture around the grains. The flasks were then autoclaved at 15 lbs pressure per sq. inch for 20 min. and then allowed to cool. A. ochraceus grown in Saboraud's dextrose agar for 13 days was seeded into the grains of each flask

separately. For this, a unit volume (5 ml) of distilled water was added to the slants and mixed to form a suspension; one ml of the suspension was poured into the flask. The flasks were fixed on a New Brunswick shaker which was set in continuous rotary motion at 40 rpm. After every 24 hr., the flasks were dismantled, shaken to break up the clumped grains. Added two ml of distilled water to each flask for the first three days. Clumps of grains that formed were periodically broken up by shaking and by use of a sterile spatula. At the end of 15 days the flasks were dismantled from the shaker and extraction of toxin was done as described for static cultures varying the quantity of chloroform to 350 ml for each extraction. The amount of toxin produced by the strain of fungus was quantified adopting the same procedure applied for static culture.

The experiment on continuous shake culture was repeated with polished rice as substrate.

The entire procedure was repeated with cultures of A. sulphureus.

Experimental Study of Ochratoxicosis in Goats

Thirty-four clinically healthy Saanen - Malabari cross-bred goats (18 males and 16 females) of 1 to 3 months age

were used for the experiment. The animals were selected from the farm stock of the All India Coordinated Project, and from known local households. They were kept under observation for a week before the commencement of the experiment during which period they were screened for common parasitic diseases and other ailments. The ration schedule followed in the goat farm was adopted for these animals. Feed ingredients, chemical composition and feeding schedule are given in tables 1, 2 and 3. Random samples of concentrates and milk were regularly checked for the presence of aflatoxin and ochratoxin by employing standard methods.

Ochratoxin employed in the present study was the purified toxin prepared in this laboratory as described earlier. Crystalline aflatoxin received from Makor Chemicals, Israel, was used to study the synergistic effects of ochratoxin and aflatoxin in goats.

Propylene glycol was used as the vehicle for the toxin in the various studies.

The data pertaining to haematological and biochemical results were subjected to statistical analysis according to Snedecor and Cochran (1967), and $P < 0.05$ was considered to indicate significant difference.

The animals were divided into four batches and each

batch was allotted for toxicity studies.

1. Oral route
2. Intraperitoneal route (i/p)
3. Intravenous route (i/v)
4. Synergistic effects of ochratoxin and aflatoxin.

Oral route.

Ten goats were employed for the study. They were divided into two groups, six in one group and four in the second group. Short term toxicity studies were carried out in the first group and long term toxicity studies in the second group. Ochratoxin was fed individually along with milk to animals.

Short term toxicity

Six goats varying in age between one to two months formed the experimental subjects. They were divided into two subgroups. Two animals (subgroup I) received ochratoxin per os at the rate of 2.5 mg/kg body weight for 6 days. Ochratoxin at the rate of 1 mg/kg body weight was fed to two animals (subgroup II) for 45 days. For each subgroup one animal was kept as control. The animals were sacrificed

at the end of the experimental periods for autopsy and histopathological examination.

Table 1
Ingredients of feed used during the experiment.

Ingredients	Parts in hundred
De-oiled groundnut cake	27
Horsegram	30
Wheat	20
Unsalted dried fish	10
Ricebran	10
Mineral mixture	2
Salt	1
Vitaolend A, B_2, D_3 (Glaxo)	25 g/100 kg feed

Table 2

Percentage chemical composition (on dry matter basis)

Dry matter units	89.4
Crude protein	19.1
Ether extract	3.2
Crude fibre	4.2
Nitrogen-free extract	63.3
Total ash	10.2
Calcium	1.1
Phosphorus	1.03

Table 3

Feeding schedule of goats

Age (months)	Milk (ml)	Concentrates (g)	Roughage (leaves) (g)
1 - 2	500	100	250
2 - 3	400	150	400
3 - 6	-	300	750
6 - 12	-	400	1500

Long term toxicity (Group III)

Ochratoxin was fed at the rate of 0.5 mg/kg body weight to two goats aged three months for a period of eight months at the end of which they were sacrificed for gross and histopathological study. Two goats reared on normal ration formed the control group.

Intraperitoneal route (Groups IV, V and VI).

Seven, 1 to 2 months old kids were used for this study. Toxin was given intraperitoneally at the rate of 2.5 mg/kg body weight to one animal and at the rate of 1 mg/kg body weight to two animals. Two goats were given ochratoxin at the rate of 0.5 mg/kg body weight. The control group consisted of two animals. These animals were observed for a period of 4 days. They were then sacrificed for autopsy and histopathological examinations.

Intravenous route (Groups VII, VIII and IX)

Nine goats, aged 1 - 2 months, were selected for studying

the effect of toxin by i/v route. Two goats received 2.5 mg of toxin/kg body weight. Another goat on normal ration formed the control. The animals were observed for a day and sacrificed the next day for detailed autopsy examination. Ochratoxin at the rate of one mg/kg body weight was administered by i/v route to two goats. One goat was used as the control. These animals were kept under observation for a period of one week at the end of which they were sacrificed for autopsy and histopathological examination. Two animals were given ochratoxin at the rate of 0.5 mg/kg body weight by i/v route. One animal was kept as the control. The animals were observed for three days and were sacrificed on the fourth day.

Synergistic effect of Ochratoxin and Aflatoxin.

Eight, 1 - 2 months old, goats were employed for studying the combined effect of ochratoxin and aflatoxin. Two goats were given by the i/p route ochratoxin at the rate of 1 mg/kg body weight and aflatoxin at the rate of 0.5 mg/kg body weight. Two others received ochratoxin alone by i/p route at the rate of 1 mg/kg body weight. A third group of two animals were given i/p aflatoxin at the rate of 0.5 mg/kg body weight. Two animals served as negative controls.

The animals were sacrificed after a period of observation for two days for pathological studies.

Laboratory procedures

Haematological studies.

Blood samples were collected from all animals before commencement of the experiment, 48 hours after the administration of toxin, and thereafter at weekly intervals upto the twelfth week of experiment and at fortnightly intervals subsequently. Blood samples were also collected from all animals before sacrificing.

Procedures described by Schalm (1965) were followed for determination of total erythrocyte count, packed cell volume, haemoglobin, erythrocyte sedimentation, total and differential leukocyte counts and blood coagulation time.

The biuret assay method of Inchiosa (1964) was adopted for the estimation of total serum proteins and the procedures described by Coles (1967) was followed for determining blood urea nitrogen level. Creatinine estimation was done by the method of Folin and Wu as described by McNeely (1980). The photometric procedure of Henry (1952) was followed for determining the values for icterus index.

Serum enzyme studies.

Enzyme kits, supplied by DeCruz Corporation, Bombay, were used for estimation of ALP, ACP, GOT, GPT and LDH activities in serum.

Urinalysis.

Urine samples, collected prior to administration of toxin, 48 hours after administration of toxin and at weekly intervals, were tested for pH, glucose, protein, ketone bodies, urobilinogen, bilirubin and blood using Multistix reagent strips manufactured by Miles India Ltd. The test results were periodically cross-checked by standard laboratory techniques. Microscopical examination of urine sediments was carried out adopting standard technique (McNeely, 1980).

Morbid Anatomy.

All the experimental as well as the control animals were subjected to detailed autopsy examination on termination of the experimental period. Animals in extremis were sacrificed before death for the purpose of post-mortem examination and collection of tissues for histo-pathological and histo-chemical studies.

Histo-pathology.

Tissues were collected for histo-pathological examination at autopsy. Paraffin sections, cut at 5 - 6 micron thickness, were stained routinely with hematoxylin and eosin. PAS and Van Gieson's methods and reticulum stains were employed in selected sections.

Histo-chemical study.

The techniques described by Pearse (1963) were employed for demonstration of ALP and ACP in paraffin sections.

Ultrastructural study.

Tissues were collected from liver and kidney of the test animals under group IV for ultrastructural studies. The tissues were fixed in 3 per cent glutaraldehyde buffered with cacodylate. The glutaraldehyde fixed tissues were post fixed in osmium tetroxide buffered with S-collidin (Sabatini et al., 1963). The material was embedded in Epon. Sections 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 301 at 60 KV.

RESULTS

RESULTS

Production of Ochratoxin

The quantity of toxin produced by the two species of Aspergillus - A. ochraceus and A. sulphureus in static and shake cultures using rice and wheat as substrates is given in table 4. The amount of toxin produced in shake cultures was more than that produced in static cultures. Wheat was found to act as a better substrate than rice since both A. ochraceus as well as A. sulphureus produced higher amounts of toxin in wheat. Comparatively A. ochraceus was found to be a better toxin producer than A. sulphureus.

Experimental Study of Ochratoxicosis in Goats

Oral administration of toxin.

Group I

Two animals (Group I A) were given ochratoxin at 2.5 mg/kg body weight for 6 days. Gr.I.B. was the control animal. From the second day, the test animals became listless and inactive. These symptoms progressively increased in intensity and the animals appeared very weak when they were sacrificed on the sixth day.

Clinical Pathology

Haematology

Mean values are shown in table 5. Significant reduction

Table 4

Quantity of Ochratoxin A (mg) harvested under different culture systems per 100 g substrate.

Substrate	A. sulphureus		A. ochraceus	
	Static culture	Shake culture	Static culture	Shake culture
Rice	180	195	139	225
Wheat	195	210	204	231

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Haemogram - Group I Mean values

Parameters	Group I A					Group I B				
	Period of observation (days)									
	Before expt.	1	2	3	6	Before expt.	1	2	3	6
Total erythrocytes ($10^6/mm^3$)	16.47	16.21	11.50	11.40	10.20	17.75	17.25	17.80	17.75	17.70
Haemoglobin (g/dl)	8.70	8.70	7.30	7.00*	6.00*	9.00	9.00	9.20	9.00	9.00
Packed cell volume (%)	29.00	26.50	20.50	19.50*	17.30*	30.00	30.00	30.00	30.00	30.00
Total leukocytes ($10^3/mm^3$)	13.15	17.70	14.05	13.50	13.00	17.20	17.10	17.20	17.20	17.10
Neutrophil (%)	42.00	42.00	55.00	59.50	66.00	40.00	39.00	40.00	40.00	40.00
Neutrophil absolute count ($10^3/mm^3$)	7.65	7.55	6.33	7.12	7.96*	6.83	6.66	6.83	6.83	6.84
Lymphocyte (%)	57.50	55.00	47.00	43.50	33.00	57.00	55.00	60.00	56.00	57.00
Lymphocyte absolute count ($10^3/mm^3$)	10.37	9.80	6.63	6.40	5.03*	9.80	9.74	10.32	9.97	9.74
Blood coagulation time (min.)	4.25	4.50	4.50	6.25	10.30	4.50	4.50	4.50	4.50	4.50

* $P < 0.05$

was observed in the packed cell volume. Changes in total leukocyte count, erythrocyte sedimentation rate, and absolute count of neutrophils were not significant. Significant increase in blood coagulation time was noticed on the third day.

Blood chemistry

Mean values are shown in table 6. The alteration in total serum proteins, BUN, creatinine and icterus index were not significant. There was significant rise in SGPT and SGOT on the sixth day. Changes in serum levels of Na, Cl and HCO3 were not significant.

Urinalysis

Changes were observed in the reaction and constituents of urine by the fifth day. Reaction became acidic in Gr.I (1) and neutral in Gr.IA (2). Protein was present in moderate amount and low levels of ketones were detected. Moderate to large amounts of bilirubin and blood were present. Towards the terminal stages, small amount of urobilinogen could be detected. Microscopical examination of urinary sediment revealed presence of moderate numbers of renal epithelial cells, squamous epithelial cells and transitional cells and a few polymorphs. Few numbers of hyaline and granular casts also

Table 6

Blood Chemistry - Mean values

Parameters	Group I A					Group I B				
	Period of observation (days)									
	Before expt.	24 h	2	3	6	Before expt.	24 h	2	3	6
Total serum proteins (g/dl)	5.60	5.60	5.30	4.50	4.50	5.30	5.30	5.30	5.30	5.30
Blood urea nitrogen (mg/dl)	14.30	14.30	15.73	20.60	33.33	13.10	13.10	13.10	13.10	13.10
Creatinine (mg/dl)	1.065	1.22	1.60	2.235	1.65	0.98	0.98	0.96	0.98	0.98
Icterus index (Icterus unit)	1.01	1.05	1.025	1.025	1.375	1.20	1.20	1.20	1.20	1.375
Alkaline phosphatase (B&L units)	9.65	9.65	15.80	13.80	17.80	5.50	5.50	5.50	5.50	6.10
Acid Phosphatase (B&L units)	0.29	0.29	0.42	0.42	0.465	0.43	0.48	0.47	0.47	0.48
Glutamate oxaloacetic transaminase (RF units)	70.00	70.00	102.00	102.00	264.00	63.00	68.00	69.00	69.00	68.00
Glutamate pyruvic transaminase (RF units)	5.00	5.0	10.00	10.00	12.00	2.00	2.0	2.00	2.00	2.00
Lactic dehydrogenase (C/ units)	175.50	175.50	803.50	803.50	379.00	200.00	200.00	200.00	200.00	200.00

$P < 0.05$

were detected. Urine samples of the control animal were negative for pathological constituents and casts.

Patho-anatomy

Autopsy findings

The animals were sacrificed on the sixth day in extremis. Necropsy examination revealed moderate gelatinization of subcutaneous fat. Few petechiae were observed on the ventral part of the abdomen. The pericardial sac contained about ten ml of clear fluid. The left cardiac and diaparagmatic lobes of lungs were moderately congested. There was moderate enlargement of liver. Surface of liver appeared diffusely pale and few foci of petechiae and ecchymoses were seen. Gall bladder contained yellowish green viscid bile.

Moderate swelling of both kidneys was observed. Cortex of both kidneys were pale. Cut surfaces also appeared pale with a few red streaks in the medulla.

Stomach contents were sparse. Circular ulcers with blackened base and raised borders were present near the pillars of the rumen. Mesentery was oedematous. There was hyperaemia of the mucosa of the terminal part of jejunum, caecum and colon. Postmortem lesions of Gr.IA(1) closely resembled

those of Gr.IA(2) but were of a milder degree. No gross lesions were observed in the control animal.

Histo-pathology

Microscopical lesions in the kidneys were prominent. The order of severity among the different parts was as follows: proximal convoluted tubules > Henle's loop > glomeruli > Bowman's capsule > medulla. The majority of the proximal convoluted tubules was found affected. There were degenerative changes in the lining epithelium with loss of brush border (Fig.1). The cytoplasm of these cells was moderately basophilic and the nuclei vesiculated. Few cells were necrotic with aggregation of chromatin of the nuclei and the desquamated cells were present in the lumen of the tubule. Hyaline bodies and PAS positive materials were present in the lumen of these tubules. Some of the tubules were dilated. Granular deposits were present in a few. Increased granularity and vacuolar changes of the cytoplasm were observed in the epithelial cells of the Henle's loop. Lining cells of the distal convoluted tubules and collecting tubules also revealed mild degenerative changes. Proteinaceous material was present in the lumen of some of the collecting tubules. Vacuolar changes were present in the epithelium of some of the glomeruli while frank necrosis of the epithelium and endothelium occurred in some other glomeruli. A few of them were shrunken and reduced very

much in size while haemorrhage was observed in few others. Dilatation of the Bowman's capsule and presence of proteinaceous material in the capsular space were observed in some areas. There was thickening of the Bowman's capsule and the basement membrane of some of the tubules. Moderate depletion of alkaline phosphatase was observed. Degenerative changes ranging from cloudy swelling to fatty change were present in the hepatocytes of the portal areas. Congestion of the central vein, sinusoidal engorgement and haemorrhages in the parenchyma were observed. Individual hepatocytes revealed necrotic changes. The cytoplasm of these cells was very pale. Nuclei showed pyknosis, karyorrhexis and karyolysis. Focal infiltration of the hepatic parenchyma with few polymorphs was seen. Mild to moderate inflammatory reaction with degeneration and necrosis of the lining squamous cells were the lesions in the tongue. Moderate degree of goblet cell hyperplasia was present. Focal ulceration of the epithelium with polymorphonuclear and mononuclear infiltration was observed in the rumen. Abomasal mucosa was oedematous with neutrophilic infiltration. Mild inflammatory changes were observed in the mucosa of the jejunum and colon. There was depletion of lymphocytes in the spleen and in the cortical and paracortical areas of the lymph nodes. Mild follicular degeneration was observed in the spleen. Germinal centres were not prominent

in the follicles of the lymph nodes. In the testis, moderate degeneration of the spermatogonia and presence of eosinophilic material in the lumen of a few tubules were observed. There was lack of differentiation of the spermatids. Degeneration of the germinal epithelium was present in the ovary of the female animal. There was widening of the zona reticularis of the adrenal. In the thyroid, a few follicles were found enlarged. Colloid appeared normal. There was slight reduction in the number of acidophils of the pituitary. Few free erythrocytes were observed in the acini of the pancreas. Lungs were moderately congested. Mucosa of the urinary bladder was hyperaemic.

Tissues of the control animals did not show any histological lesion.

Group II

Animals which received ochratoxin at the rate of one mg/kg body weight daily for a period of 45 days, manifested clinical signs of toxicity by the third week of treatment. General weakness and lethargic disposition were noticed by this time. Progressive emaciation was observed by the sixth week.

Clinical Pathology

Haematology

Mean values are shown in table 7. There was no significant

Table 7

Haemogram - Group II - Mean values

Parameters	Group II A									
	Period of observation (days)									
	Before expt.	1	2	7	14	21	28	35	42	45
Total erythrocytes ($10^6/mm^3$)	15.94	12.50	12.45	12.35	11.90	11.75	11.65	10.00	9.10*	9.00*
Haemoglobin (g/dl)	8.20	8.20	7.30	7.00	6.80	6.20*	5.70*	5.20*	4.80*	4.80*
Packed cell volume(%)	27.90	27.00	23.00	22.90	22.80	22.30	21.50	18.00*	17.50*	17.50*
Total leukocytes ($10^3/mm^3$)	16.65	15.50	15.15	14.35	14.55	13.60	13.50*	12.10*	11.60*	11.60*
Neutrophil (%)	34.50	34.50	41.00	47.00	54.00	56.00	57.50	61.00	61.00	61.50
Neutrophil absolute count ($10^3/mm^3$)	5.75	5.34	6.215	6.931	7.85*	7.712*	7.69*	7.354*	6.944*	7.116*
Lymphocyte (%)	62.50	62.50	56.00	53.00	45.00	42.00	41.50	38.00	39.00	37.00
Lymphocyte absolute count ($10^3/mm^3$)	10.731	9.687	8.24	7.369	6.551*	5.812*	5.615*	4.616*	4.656*	4.312*
Blood coagulation time (min.)	4.25	4.25	4.25	4.25	4.75	5.50	6.25	8.00	9.25	9.25

(conta.....)

Continued Table 7.

Parameters	Group II B									
	Period of observation									
	Before expt.	1	2	7	14	21	28	35	42	45
Total erythrocytes ($10^6/mm^3$)	15.63	15.62	15.46	15.46	15.52	15.72	15.64	15.56	15.70	15.62
Haemoglobin (g/dl)	9.00	9.00	9.00	9.00	9.00	9.20	9.00	9.00	9.00	9.00
Packed cell volume(%)	28.00	28.00	28.00	28.00	28.20	28.00	28.00	28.0	28.00	28.00
Total leukocytes ($10^3/mm^3$)	15.60	15.60	15.20	15.60	15.50	15.60	15.40	15.60	15.40	15.60
Neutrophil (%)	40.00	42.00	42.00	42.00	41.00	42.00	43.00	44.00	43.00	42.00
Neutrophil absolute count ($10^3/mm^3$)	6.24	6.552	6.384	6.552	6.355	6.552	6.665	6.864	6.93	6.552
Lymphocyte (%)	53.00	53.00	55.00	56.00	56.00	53.00	53.00	53.00	53.00	55.00
Lymphocyte absolute count ($10^3/mm^3$)	9.048	9.048	8.36	8.736	8.68	8.335	8.325	8.215	8.462	8.580
Blood coagulation time (min.)	4.50	4.50	4.50	4.50	5.00	4.50	4.50	4.50	4.50	4.50

* $P < 0.05$

reduction in the number of erythrocytes through the 42nd to 45th day in the test animals. The haemoglobin concentration diminished from the 21st day onwards. This reduction was statistically significant. Significant reduction in packed cell volume was observed after five weeks. The total leukocyte number was significantly reduced from the 21st to 45th day. There was significant increase in the absolute neutrophil count and decrease in the absolute lymphocyte count after the first fortnight. Erythrocyte sedimentation rate was not altered in the experimental and control animals. There was increase in blood coagulation time from the initial 4.25 minutes to 9.25 minutes on the 42nd and 45th day.

Blood chemistry

Mean values are shown in table 8. Significant reduction in total serum proteins was observed in the test animals from the 14th day onwards. The rise in blood urea nitrogen on the 45th day and was significant statistically. There was significant rise in icterus index on the 45th day in the test animals. The rise in ALP was significant. There was no significant alteration in the level of ACP. The rise in SGOT was not statistically significant. SGPT level was significantly raised on the 42nd and 45th day.

Table 8

Blood Chemistry - Group II - Mean values

Parameters	Group II A									
	Period of observation (days)									
	Before expt.	24 h	2	7	14	21	28	35	42	45
Total serum proteins (g/dl)	5.30	5.30	5.20	4.20	3.70	3.60	3.50	3.30	2.80	2.90
Blood urea nitrogen (mg/dl)	16.80	16.80	17.75	27.10	29.9*	31.8*	34.55*	35.50	37.40	39.30
Creatinine (mg/dl)	1.90	1.20	1.30	1.55	1.87	2.49	2.96	3.42	3.91	4.03
Icterus index (Icterus units)	1.375	1.375	1.375	1.55	1.625	2.25	2.50	2.525	2.52*	2.50*
Alkaline phosphatase (B.L.B. units)	4.80	4.80	6.40	7.20	7.20	7.20	7.60*	9.00*	10.60*	10.60*
Acid Phosphatase (B.L.B. units)	0.30	0.525	0.525	0.55	0.515	0.46	0.505	0.41	0.525	0.525
Glutamate oxaloacetic transaminase (R.F. units)	67.00	67.00	151.00	81.00	82.00	77.00	67.00	77.00	83.50	82.00
Glutamate pyruvic transaminase (R.F.units)	2.00	2.00	4.00	4.00	4.00	4.00	5.00	6.00*	7.00*	7.00*

(contd.....)

Contd.... Table 8

Parameters	Group II B									
	Period of observation									
	Before expt.	24 h	2	7	14	21	28	35	42	45
Total serum proteins (g/dl)	5.50	5.50	5.60	5.50	5.30	5.50	5.20	5.30	5.50	5.50
Blood urea nitrogen (mg/dl)	14.90	14.90	13.10	13.10	14.90	14.00	14.30	14.90	13.10	14.30
Creatinine (mg/dl)	0.96	0.96	0.96	0.95	0.96	0.95	0.98	0.98	0.96	0.96
Icterus index (Icterus units)	1.90	1.90	1.90	1.90	2.00	2.50	2.50	2.50	2.55	2.50
Alkaline phosphatase (B.L.B. units)	5.00	5.00	5.50	5.50	5.00	5.00	5.50	5.00	5.00	5.00
Acid phosphatase (B.L.B. units)	0.35	0.35	0.37	0.37	0.37	0.35	0.35	0.35	0.35	0.35
Glutamate oxaloacetic transaminase (R.F. units)	66.00	65.00	66.00	65.00	63.00	59.00	65.00	66.00	65.00	66.00
Glutamate pyruvic transaminase (R.F.units)	3.00	3.00	3.00	2.00	2.00	3.00	2.00	3.00	3.00	3.00

* $P < 0.05$

Urinalysis

Urine samples became neutral by the fifth week. Traces of blood and ketone bodies were detected in one of the animals - Gr.II A(2) on the fifth week. Small amounts of bilirubin were observed from the second day onwards. Urine samples of Gr.II A(1) were strongly positive for urobilinogen from the second day.

Microscopical examination of urine sediment revealed presence of moderate numbers of erythrocytes, renal epithelial cells and squamous epithelial cells towards the terminal stages. Polymorphonuclear leukocytes were present at the rate of 1 - 2/HP during this period. Granular and hyaline casts at the rate of 1-2/HP also were noticed by the fifth week of experiment.

Patho-anatomy

Autopsy findings

Morbid changes were more pronounced in Gr.II A(2) than in Gr.II A(1) though the distribution of lesions was identical. Moderate gelatinisation of the subcutaneous fat and few subcutaneous petechiae were seen. About 10 ml of clear serous fluid was present in the pericardium. Surface of liver was moderately pale and on sectioning had a cooked up appearance. The renal capsule was slightly adherent and cortex was

diffusely pale. Reddish linear areas were observed in the medulla of both kidneys. Bladder contained slightly cloudy yellowish urine.

Mucosa of the abomasum was moderately swollen and slightly hyperaemic. Mucosa of the large intestines was moderately hyperaemic.

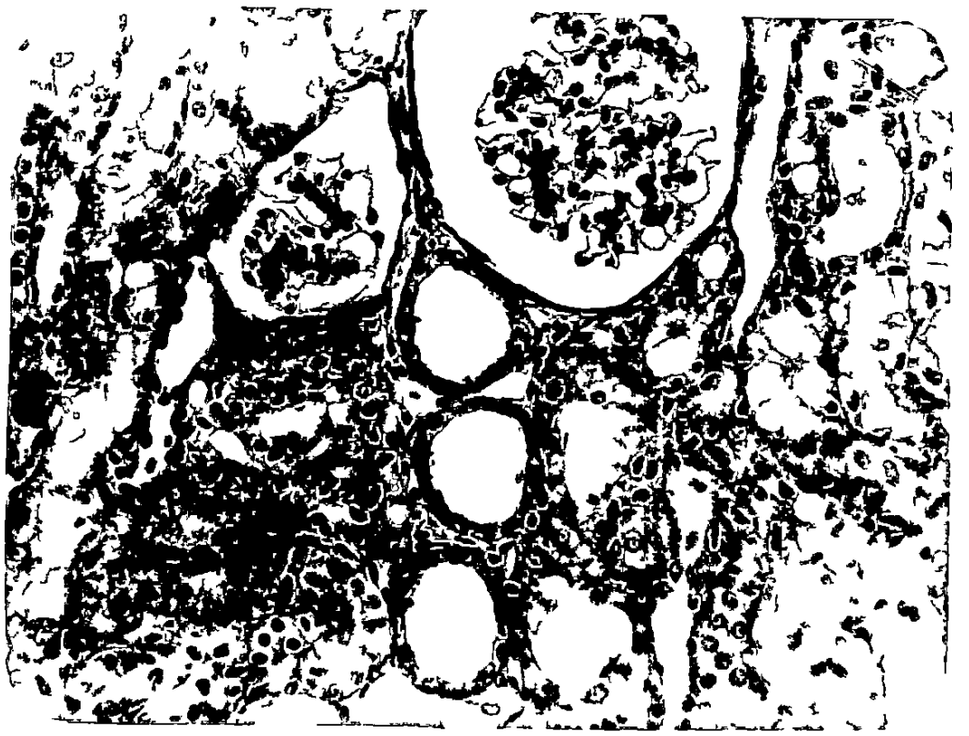
Histo-pathology

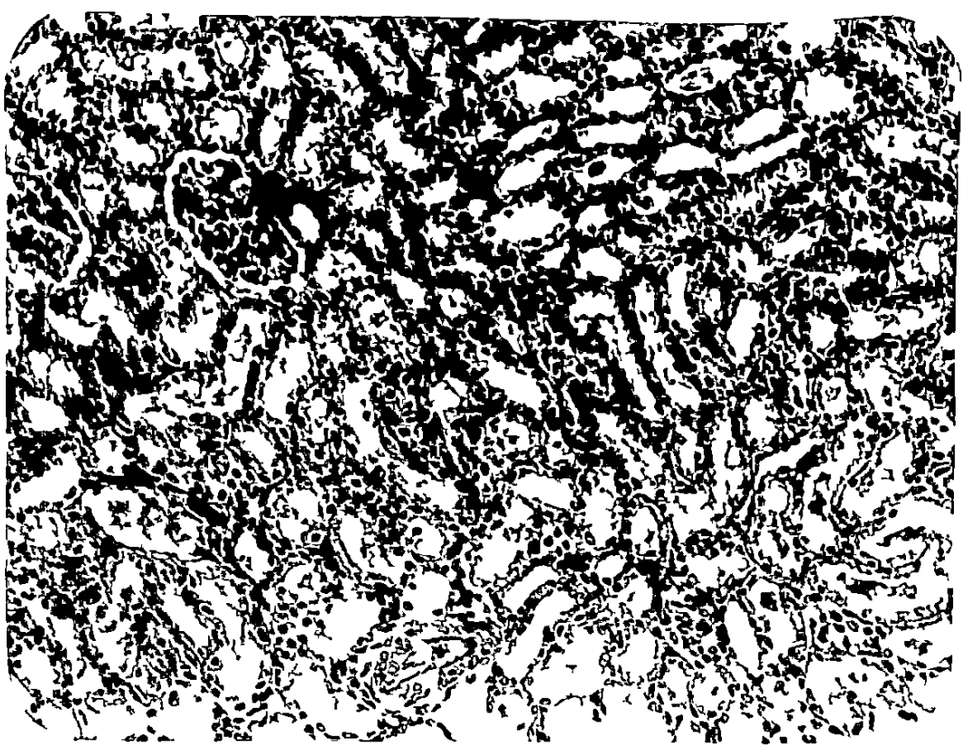
Epithelial cells lining the renal tubules, particularly those of the proximal convoluted tubules revealed moderate degree of degeneration with necrosis of occasional cells. Some of the tubules contained granular eosinophilic debris in their lumen while some others were dilated. PAS positive material was present in the basement membranes and in the lumen of tubules (Fig.2). In focal areas, the interstitial tissue of the tubules was distended with masses of PAS positive material. There was distortion of some of the glomeruli. A few of them were shrunken while others revealed hypercellularity occupying the entire capsular space. Basement membrane of the glomeruli was moderately thickened with presence of PAS positive material. Degeneration of the epithelial cells lining some of the Bowman's capsules and slight thickening of the capsule were observed. The capsular space of several glomeruli contained finely granular eosinophilic debri. In a few tubules the epithelial cells were pushed into the glomerular space simulating tubular epithelial reflux.

Fig.1. Kidney (Gr.I) - Degenerative and necrotic changes in the lining epithelium of the proximal convoluted tubules. Desquamation of cells into the lumen. H & E x 250.

Fig.2. Kidney (Gr.II) - Dilatation of the tubules and thickening of the basement membrane. Loss of brush border in the cells of proximal convoluted tubules. Vacuolar changes in the epithelium of the glomeruli; granular deposit in the Bowman's capsule. PAS x 400.

Fig.3. Liver (Gr.II) - Parenchymatous degeneration, fatty change and necrosis of hepatocytes. PAS x 250.





In the liver, most of the hepatic cells were swollen and had granular cytoplasm indicating parenchymatous degeneration (Fig.3). Fatty changes were more prominent in the centrilobular hepatocytes. Frank necrosis occurred in some of the hepatocytes at the periportal areas. Besides sinusoidal engorgement, there was enlargement of the space of Disse in several locations. Attempted biliary epithelial proliferation was evident.

In the rumen and reticulum, degenerative changes of the lining epithelium were observed. Moderate degree of oedema was seen in the lamina propria. Hyperaemia and submucosal oedema were noticed in the abomasum. In the duodenum, jejunum and ileum necrosis of the lining epithelial cells and focal erosions were observed. There was severe infiltration of the mucosa, and submucosa with lymphoid cells, macrophages and few neutrophils. Goblet cell hyperplasia and subserosal oedema were prominent (Fig.4).

The salivary glands showed focal areas of degeneration and necrosis of secreting cells. Moderate degree of gliosis was seen in the cerebellum.

In the lymph nodes, no definite germinal centre formation was observed. Areas of lymphoid cell depletion was seen in

the cortical and paracortical areas of lymph nodes (Fig.5). Replacement of the lymphoid cells by reticuloendothelial cells was observed in the cortical region. Oedema and focal areas of depletion of lymphoid cells were seen in the thymus (Fig.6). In the adrenal, moderate hyperplasia of the medullary cells was observed. Other endocrine glands did not reveal any lesion.

Degenerative changes were seen in the germinal epithelium of the seminiferous tubules.

Group III

Animals Gr.III A(1) and Gr.III A(2) which were given ochratoxin at the rate of 0.3 mg/kg body weight for a period of 34 weeks did not show any clinical sign of illness upto the third month of experiment. Listlessness, slow gait and diminished appetite were manifested after this period. These symptoms progressed during the experimental period.

Clinical Pathology

Haematology

Mean values are shown in table 3. Significant reduction was observed in total erythrocyte count, haemoglobin, packed cell volume, total leukocyte count and absolute lymphocyte count. Rise in absolute count of neutrophils was not significant. The increase in blood coagulation time was significant. The erythrocyte sedimentation rate was not altered.

Table 9

Haemogram - Group III - Mean values

Parameters	Group III A									
	Period of observation (days)									
	Before expt.	14 d	28	56	84	112	140	168	196	224
Total erythrocytes (10^6/mm^3)	16.68	14.25	13.06	13.34	13.40*	11.90	11.35*	11.18*	11.02*	10.58*
Haemoglobin (g/dl)	9.90	9.30	9.10	8.80	8.10	7.00*	6.50*	6.20*	5.90*	5.40*
Packed cell volume (%)	31.60	29.00	28.20	28.00	28.00	26.60	24.70	21.20*	20.50*	19.50*
Total leukocytes (10^3/mm^3)	12.90	12.200	12.150	12.10	12.100	12.00	11.650	11.900	11.300	11.400
Neutrophil (%)	26.00	48.50	48.50	48.50	57.50	56.00	58.50	67.00	70.00	72.00
Absolute count neutrophils (10^3/mm^3)	3.393	5.92	5.819	5.971	6.021	6.704	6.803	7.830	8.26	8.148
Lymphocyte (%)	70.50	43.00	43.00	43.50	42.00	42.00	44.50	30.50	27.00	27.00
Absolute count lymphocytes (10^3/mm^3)	9.006	6.04	5.859	5.866	5.124	5.23	5.254	3.662*	3.237*	3.130*
Blood coagulation time	4.00	4.00	4.25	4.50	10.50	10.75	11.25*	11.75*	13.50*	15.00*

(contd....)

(Contd.... Table 3)

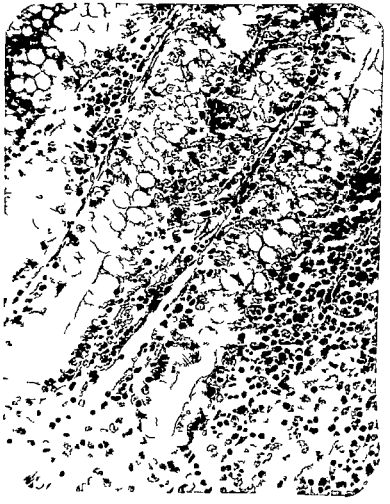
Parameters	Group III B									
	Period of observation (days)									
	Before expt.	14	28	56	84	112	140	168	196	224
Total erythrocytes ($10^6/mm^3$)	16.18	15.34	16.21	16.24	16.29	15.95	16.12	16.21	16.02	16.11
Haemoglobin (g/dl)	9.70	9.90	9.70	9.70	9.70	9.60	9.70	9.70	9.50	9.60
Packed cell volume (%)	32.50	32.50	32.00	32.10	31.90	31.90	32.00	32.30	32.00	32.00
Total leukocytes ($10^3/mm^3$)	12.200	12.100	12.250	12.650	12.700	12.400	12.450	12.250	12.200	12.300
Neutrophil (%)	47.00	45.00	44.00	44.50	47.00	43.00	45.50	44.00	46.00	45.00
Absolute count neutrophils ($10^3/mm^3$)	7.191	5.451	5.533	5.49	5.969	5.332	5.642	5.391	5.608	5.532
Lymphocyte (%)	51.00	53.00	53.50	54.00	51.00	51.00	52.50	53.00	52.50	51.50
Absolute count lymphocytes ($10^3/mm^3$)	5.365	6.404	6.348	6.012	6.477	6.324	6.536	6.491	6.277	6.336
Blood coagulation time	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00

* $P < 0.05$

Fig.4. Ileum (Gr.II) - Goblet cell hyperplasia with infiltration of lymphoid cells, macrophages and neutrophils. H & E x 400.

Fig.5. Lymph node (Gr.II) - Depletion of lymphoid cells in the cortical and paracortical areas. H & E x 250.

Fig.6. Thymus (Gr.II) - Oedema. H & E x 400.



Blood chemistry

Mean values are shown in table 10. The reduction in total serum proteins was significant. There was significant rise in BUN, creatinine and icterus index. Rise in ALP and SGOT was highly significant. Acid phosphatase level rise was significant. Alteration in SGPT was not significant.

Urinalysis

The pH of the urine was lowered from 9 to 7 by the 11th week of experiment. From the 28th week onwards the reaction became acidic with pH 6. Bilirubin appeared in the urine by the 18th week and there was moderate amounts of it in urine throughout the experimental period. Urobilinogen was present in traces in the urine of both test animals from the 20th week onwards. Albumin was noticed by the 15th week. Concentration of albumin increased from the 25th week to the terminal stage of experiment in the test animals. Urine sediments revealed presence of renal epithelial cells and transitional epithelial cells from the 20th week. Squamous epithelial cells were present in the urine throughout the experimental period. Few pus cells were observed from the 26th week.

Patho-anatomy

Autopsy findings

The carcase was emaciated and subcutaneous fat was pale

Table 10

Blood Chemistry - Group III - Mean values

Parameters	Group III A									
	Period of observation (days)									
	Before expt.	14	28	56	84	112	140	168	196	224
Total serum proteins (g/dl)	6.3	5.175	5.50	4.55	4.40	4.15	3.30	3.50	3.10*	2.90*
Blood urea nitrogen (mg/dl)	14.00	16.30	18.70	23.35	25.25	28.90*	29.90*	32.70*	34.50*	37.40*
Creatinine (mg/dl)	0.905	0.915	0.95	1.05	1.17	1.405*	1.35*	1.87*	1.92*	1.98*
Icterus index (Icterus units)	1.35	1.45	1.46	1.43	1.50	1.59	1.62	1.63	1.78	2.19*
Alkaline phosphatase (B.L.B.units)	5.25	5.65	6.50	8.75	9.25	9.75	10.40	12.00*	12.35*	13.65*
Acid phosphatase (B.L.B. units)	0.535	0.65	0.695	0.705	0.705	0.74	0.745*	0.745*	0.70*	0.705*
Glutamate oxaloacetic transaminase (R.F. units)	75.5	117.00	151.00	168.00	208.50*	248.00*	245.00*	263.00*	278.00*	294.00*
Glutamate pyruvic transaminase (R.F. units)	2.50	4.00	4.00	5.00	2.50	6.00	7.50	4.50	7.50	5.50

(contd.....)

(Contd... Table 10)

Parameters	Group III B									
	Period of observation (days)									
	Before expt.	14	28	56	84	112	140	168	196	224
Total serum proteins (g/dl)	6.4	6.25	6.35	6.35	6.55	6.45	6.40	6.55	6.45	6.45
Blood urea nitrogen (mg/dl)	11.41	12.61	12.65	12.15	13.10	13.10	12.80	12.15	12.15	12.80
Creatinine (mg/dl)	0.305	0.315	0.92	0.91	0.91	0.91	0.91	0.91	0.915	0.91
Icterus index (Icterus units)	1.15	1.25	1.15	1.18	1.02	1.00	1.02	1.02	1.10	1.03
Alkaline phosphatase (B.L.B.units)	4.75	4.75	4.90	5.25	5.25	5.25	5.40	5.25	5.00	5.65
Acid phosphatase (B.L.B. units)	0.66	0.605	0.645	0.70	0.65	0.69	0.66	0.71	0.62	0.71
Glutamate oxaloacetic transaminase (R.F.units)	73.00	70.00	71.00	73.00	85.00	75.00	77.00	77.00	74.00	81.00
Glutamate pyruvic transaminase (R.F units)	4.75	2.50	2.50	2.50	3.00	2.50	3.50	4.00	2.50	6.00

* $P \leq 0.05$

yellow with moderate gelatinisation. Superficial lymph nodes were slightly swollen. Liver was enlarged and pale. Gall bladder was distended with greenish bile. Spleen was moderately shrunken. The surface of the kidneys and cortical parenchyma were diffusely pale. Renal fat was yellowish and gelatinised. Rumen mucosa revealed three circumscribed ulcers of about 2 mm diameter with slightly raised borders. The mucosa of the abomasum and intestines was moderately hyperaemic.

Histo-pathology

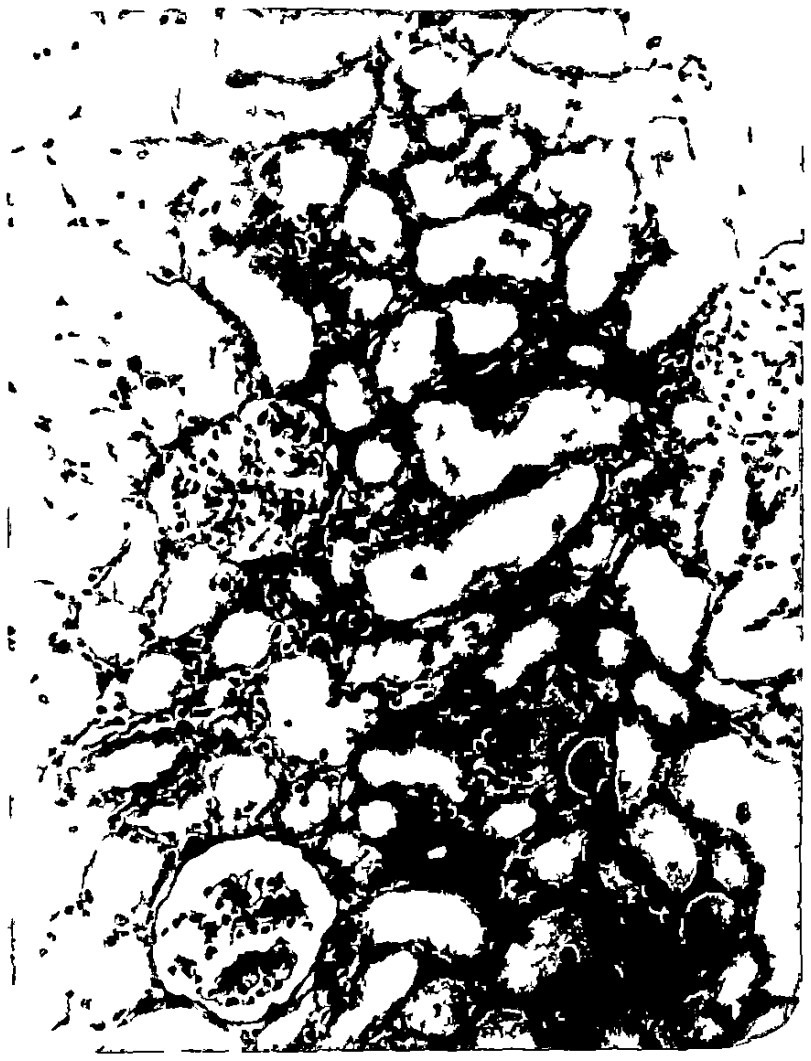
There was degeneration and necrosis of the epithelial cells lining the proximal convoluted tubules of the kidney and to a lesser extent in other segments. Nuclear changes comprising of pyknosis, karyorrhexis and karyolysis were noticed in many cells. Desquamated cells, hyalin casts (Fig. 7) and PAS positive globular bodies were present in the lumen. Some of the collecting tubules were dilated and contained granular casts (Fig. 8). Degeneration and focal necrosis of the endothelium and epithelium of the glomeruli could be seen. There was thickening of the basement membrane and accumulation of fibrin in between the capillary loops. Moderate thickening occurred in the basement membrane of the tubules also (Fig.9).

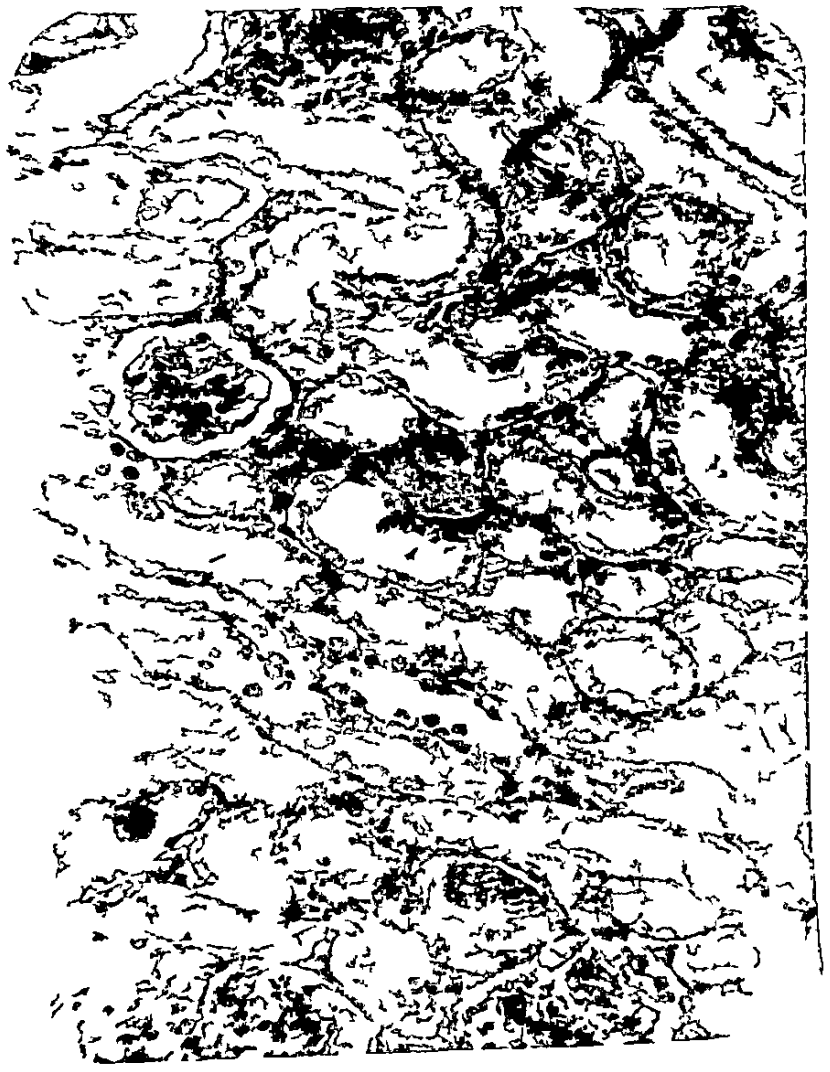
Some of the glomeruli were shrunken. Eosinophilic

Fig.7. Kidney (Gr.III) - Degenerative changes in the epithelium of the tubules some of which contain cellular debri. H & E x 250.

Fig.8. Kidney (Gr.III) - Dilatation of tubules. Denuded epithelium and hyaline bodies in the tubules. H & E x 250.

Fig.9. Kidney (Gr.III) - Thickening of basement membrane. Loss of brush border of the epithelium of proximal convoluted tubules. Degeneration of cells in the glomeruli. PAS x 250.





fibrinous material was observed in the capsular space of the Bowman's capsule. The capsular membrane was thickened and degeneration of epithelial cells could be observed.

In the liver, fatty changes of a few hepatocytes were seen in the portal area. Some of the cells were necrotic. Replacement nodule formation was observed in some foci. Mild biliary epithelial proliferation was seen.

Ulcers were present in the rumen mucosa. These ulcers were infiltrated with mononuclear cells (Fig.10). Vacuolation of epithelium lining the villi of intestines was observed. Numerous eosinophilic granules were seen in the glandular epithelium of the intestines. Moderate depletion of lymphoid cells was seen in the spleen and lymph node. Medulla was oedematous in some lymph nodes.

Intraperitoneal administration of toxin.

Group IV

The test animal Gr.IV A which received ocratoxin at the rate of 2.5 mg/kg body weight was visibly ill from the second day onwards. Appetite was poor and movements were staggering. By the fourth day it was completely off feed and prostrate and was sacrificed.

Clinical Pathology

Haematology

Haematological values are shown in table 11. A marked reduction in the number of erythrocytes was observed.

There was reduction in the values for haemoglobin, packed cell volume and total leukocytes. There was rise in the relative and absolute count of neutrophils on the second and fourth day. Marked reduction in the relative and absolute count of lymphocytes was observed on the third and fourth days while blood coagulation time was found increased. The values of the control animal remained within normal ranges.

Blood Chemistry

The values are given in table 12. There was a reduction in the level of serum protein. Serum creatinine and icterus index were found increased. The levels of ALP, ACP, SGOT and SGPT increased during the experimental period.

Haematological values and biochemical values of blood were within normal ranges in the control animal.

Urinalysis

Moderate to high concentration of protein was detected in the urine on the second and third day. Bilirubin and blood

Table 11

Haemogram - Group IV - Mean values

Parameters	Group IV A					Group IV B				
	Period of observation (days)									
	Before expt.	1	2	3	4	Before expt.	1	2	3	4
Total erythrocytes ($10^6/mm^3$)	16.90	16.90	16.00	10.50	10.11	12.52	12.62	12.42	12.45	12.50
Haemoglobin (g/dl)	9.20	9.20	9.00	8.00	8.00	9.00	9.00	9.00	9.00	9.00
Packed cell volume (%)	25.00	25.00	23.00	22.00	22.00	23.00	28.00	28.00	28.00	23.00
Total leukocytes ($10^3/mm^3$)	16.90	15.50	15.50	11.65	11.500	11.600	11.300	11.600	11.600	11.500
Neutrophil (%)	36.00	36.00	45.00	52.00	58.00	32.00	38.00	37.00	33.00	35.00
Neutrophil absolute count ($10^3/mm^3$)	6.084	5.580	6.975	6.058	66.70	3.712	4.484	4.292	5.280	4.025
Lymphocyte (%)	59.00	52.00	52.00	46.00	40.00	66.00	61.00	62.00	64.00	62.00
Lymphocyte absolute count ($10^3/mm^3$)	9.371	8.060	8.060	5.359	4.600	7.656	7.198	7.192	7.130	7.130
Blood coagulation time (min.)	5.00	5.00	10.00	11.00	12.00	5.00	5.00	5.00	5.00	5.00

* $P < 0.05$

Table 12

Blood Chemistry - Group IV - Mean values

Parameters	Group IV A					Group IV B				
	Period of observation (days)									
	Before expt.	1	2	3	4	Before expt.	1	2	3	4
Total serum proteins (g/dl)	7.50	7.50	5.20	5.00	3.30	5.60	5.50	5.50	5.60	5.60
Blood urea nitrogen (mg/dl)	15.30	15.80	20.00	23.50	30.60	12.00	12.00	12.40	12.40	12.00
Creatinine (mg/dl)	0.96	0.96	1.00	1.25	1.25	1.00	1.00	0.90	0.98	0.98
Icterus index (Icterus units)	1.00	1.00	2.80	3.32	3.82	1.20	1.20	1.30	1.25	1.20
Alkaline phosphatase (B.L.B. units)	4.60	5.00	6.70	10.40	11.30	4.50	5.00	5.00	4.50	4.50
Acid phosphatase (B.L.B. units)	0.00	0.60	0.90	2.03	3.17	0.37	0.35	0.40	0.40	0.37
Glutamate oxaloacetic transaminase (K.F. units)	65.00	72.00	112.00	368.00	332.00	72.00	74.00	69.00	73.00	73.00
Glutamate pyruvic transaminase (K.F. units)	2.00	2.00	5.00	7.00	10.00	3.00	3.00	3.00	3.00	3.00

* $P < 0.05$

were present in small quantities. Microscopical examination of sediment revealed presence of a few erythrocytes, renal epithelial cells and squamous epithelial cells. Hyaline and granular casts were present from the third day. Few numbers of neutrophils and transitional epithelial cells also were present.

Patho-anatomy

Autopsy findings

Carcase was emaciated with gelatinisation of the subcutaneous fat. Subcutaneous tissue was slightly icteric. About 20 ml of pale yellow fluid was present in the peritoneal cavity. Omentum and mesentery were moderately oedematous and vessels congested. Surface of liver was pale and occasional areas of ecchymoses and petechiae were seen. Parenchyma was soft. Gall bladder was distended with bile. There was moderate enlargement of both kidneys. Surface of kidneys was diffusely pale. Cut surfaces revealed pale areas alternating with red streaks. There was gelatinisation and icterus of the renal fat. Urinary bladder contained sparse amount of dark yellow urine. Abomasal mucosa was swollen and hyperaemic. Mucosa of the large intestine was moderately hyperaemic. No gross lesions were observed in the control animal.

Histo-pathology

Microscopical changes were prominent in the tissues of

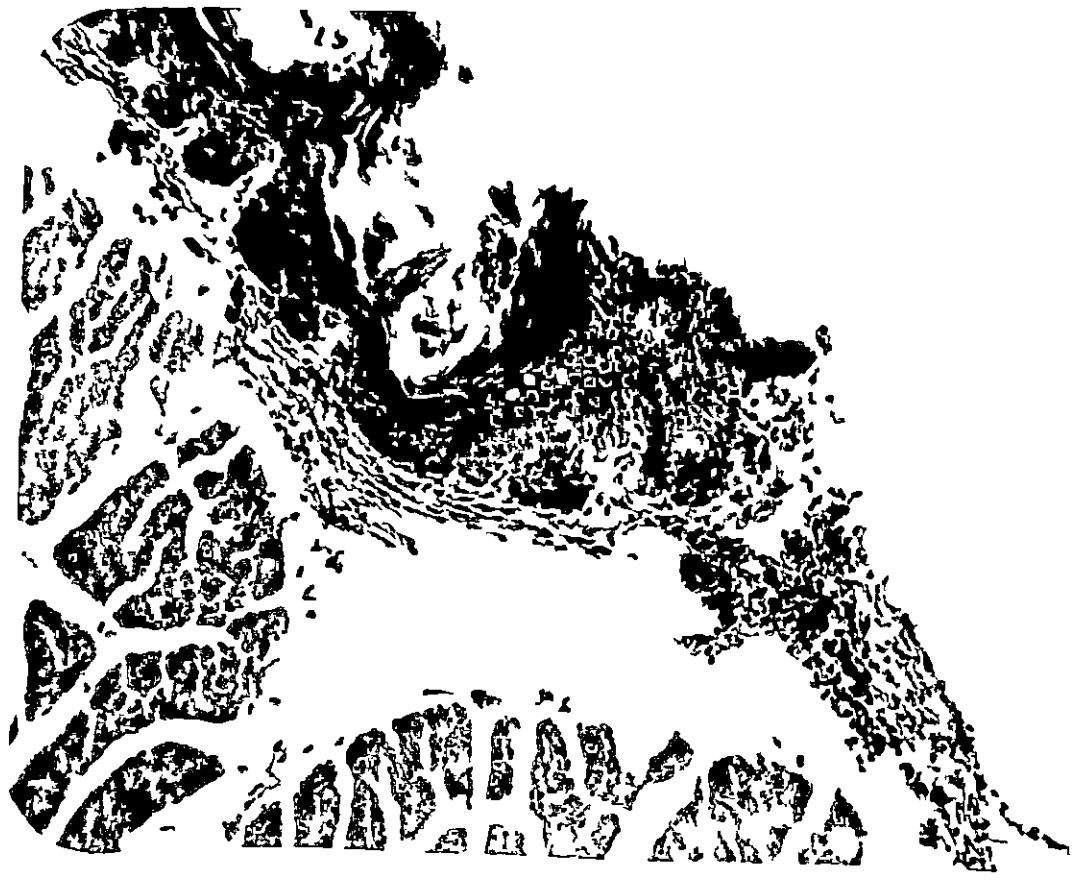
the test animal. In the kidney, cloudy swelling and vacuolar degeneration of the proximal convoluted tubular epithelial cells were prominent lesions. Presence of desquamated cells and granular casts in the lumen of these tubules and those of the collecting tubules was noticed. Several of the tubules were devoid of their lining epithelial cells and were found distended. Hyaline bodies were present in the lumen of some of these tubules. Degeneration and focal necrosis of the epithelium of the Henle's loop and distal convoluted tubules were observed. Several of the glomeruli were shrunken (Fig.11). Eosinophilic granular materials were present in the Bowman's capsule. There was thickening of the basement membrane of the tubules and glomeruli. Free erythrocytes, some of them in the process of hemolysis, were present in the lumen of tubules and in the interstitial tissue. Haemorrhages were severe in the ascending limbs and collecting tubules.

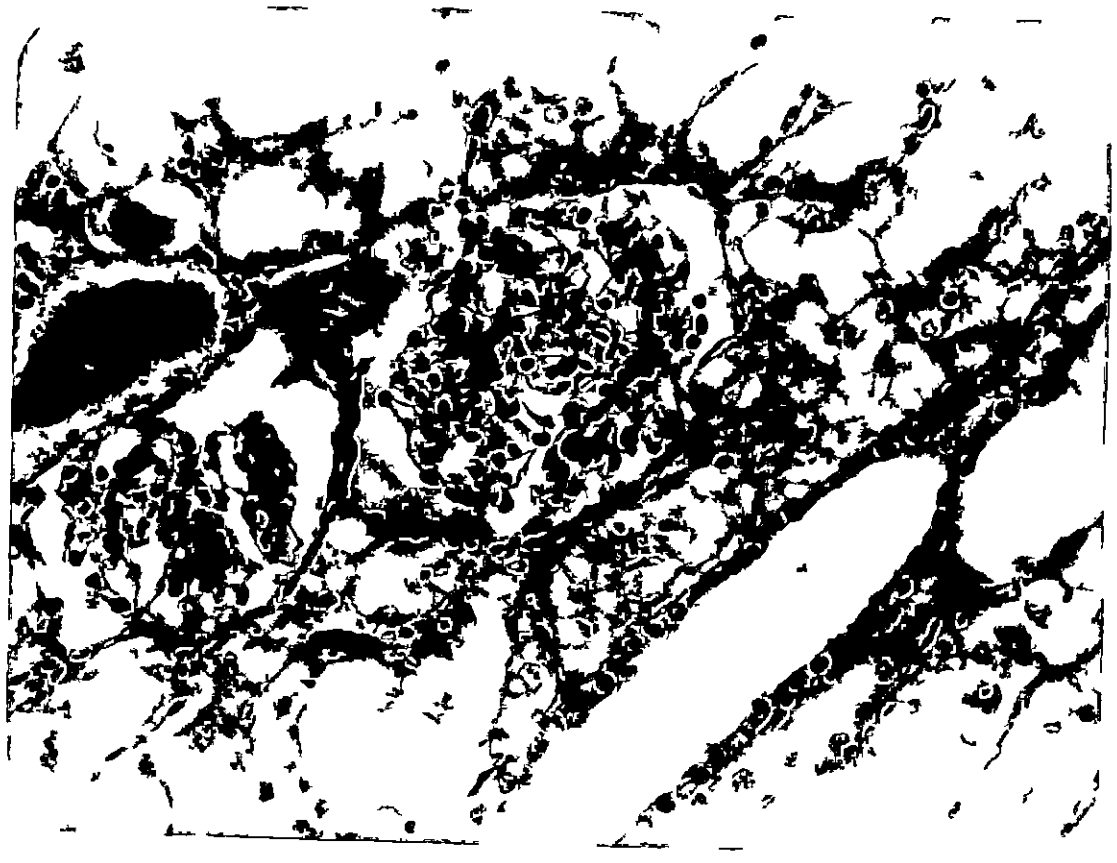
Moderate fatty change was present in the hepatic cells of the portal areas. There was degeneration and frank necrosis of centrilobular hepatocytes and nuclear changes like pyknosis, karyorrhexis and karyolysis were prominent. Some of the cells were in mitotic division. Mallory bodies were present in a few liver cells. Dilatation of the sinusoids and presence of lakes of blood in these areas was observed. When the reticulum staining was employed disruption

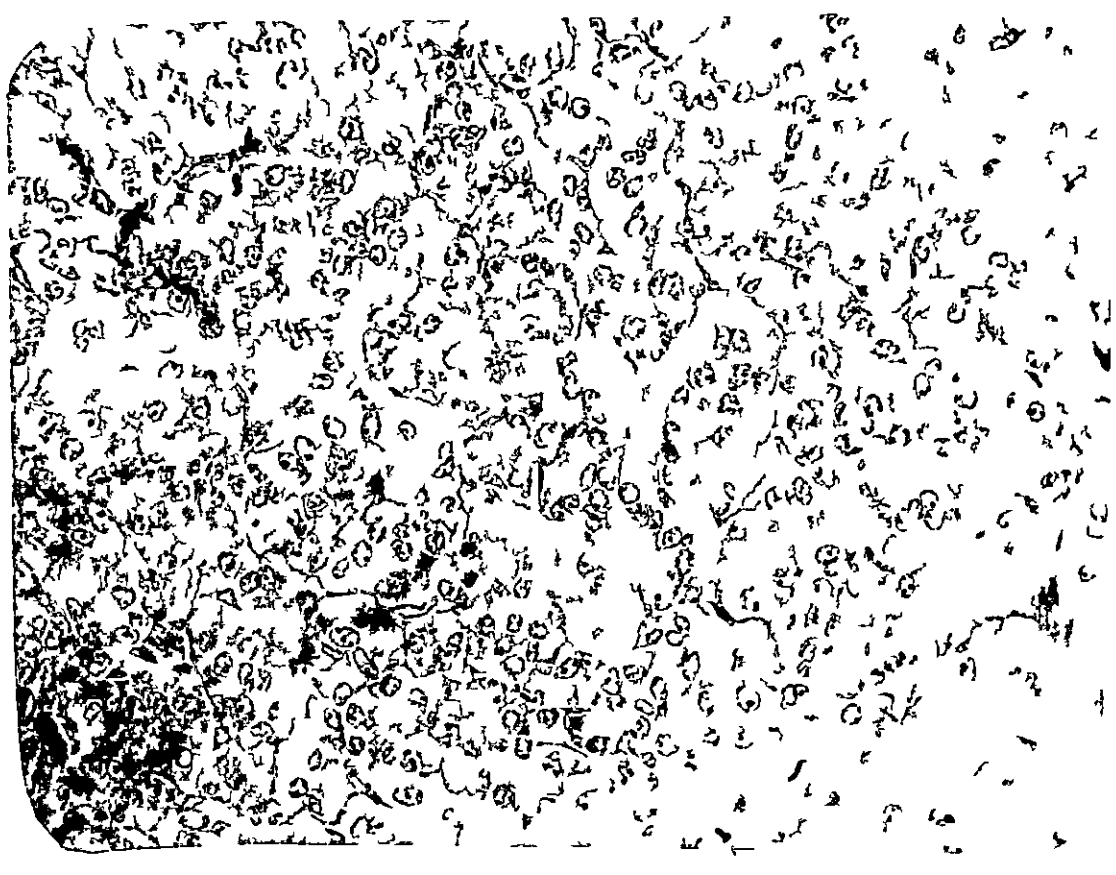
Fig.10. Rumen (Gr.III) - Ulceration. Denudation of epithelial lining and infiltration with mononuclear cells. H & E x 250.

Fig.11. Kidney (Gr.IV) - Shrunken glomerulus. Degeneration of tubular epithelium and hyaline casts in tubules. H & E x 400.

Fig.12. Pituitary (Gr.IV) - Degeneration of acidophils. H & E x 400.







and collapse of the reticular network in focal areas could be noticed. Bile stasis and a mild degree of bile ductular proliferation were the other changes in liver.

Degenerative changes were present in the lining cells of the rumen epithelium. Few small ulcers were observed. Abomasal mucosa was hyperemic and oedematous. Hyperemia and mild epithelial degeneration were observed in the mucosa of small intestines and colon. Increased goblet cell activity was observed in the colon.

In the lymph nodes, there was loss of follicular architecture with depletion of lymphoid cells in the cortical and paracortical areas and replacement with reticuloendothelial cells. Severe oedema was seen in the medulla. Oedema and depletion of lymphoid cells were prominent in the thymus also.

Hyperplastic nodules were present in the adrenal cortex. Some of the acidophils of the pituitary showed granular degeneration (Fig.12).

In the ovary, degeneration and necrosis of large number of granulosa cells and neutrophilic infiltration of the stroma were observed.

Group V

This group received ochratoxin at the rate of one mg/kg

body weight. The animals became slightly inactive and appeared listless from the second day. These clinical signs of illness became slightly more severe by the fourth day.

Clinical Pathology

Haematology

Haematological values are shown in table 13. Significant reduction in the total erythrocyte count was noticed in one of the test animals. Reduction in the haemoglobin concentration was significant only in one animal. Slight reduction in packed cell volume and total leukocyte was noticed. Reduction in the absolute lymphocyte count occurred on the second day. No alteration was observed in the erythrocyte sedimentation rate and blood coagulation time.

Blood chemistry

Mean values are shown in table 14. The reduction in total serum proteins was significant in one of the test animals. There was a significant rise in blood urea nitrogen and creatinine levels. There was a rise in icterus index. There was increase in the levels of serum ALP, ACP, GOT and GPT.

Urinalysis

The reaction of urine became acidic by the second day.

Table 13

Haemogram - Group V - Mean values

Parameters	Group V A				Group V I			
	Period of observation (days)							
	Before expt.	1	2	3	Before expt.	1	2	3
otal erythrocytes ($10^6/mm^3$)	17.09	14.15	14.33	14.00	14.175	13.79	14.085	13.975
aemoglobin (g/dl)	8.10	7.90	7.70*	9.50	9.50	9.50	9.50	9.50
acked cell volume %)	27.00	27.00	26.70	26.70	29.00	29.00	29.00	29.00
otal leukocytes ($10^3/mm^3$)	13.40	13.95	12.25	12.300	11.400	11.90	11.50	11.40
eutrophil (%)	36.50	44.00	45.00	44.00	36.50	41.00	39.50	38.00
eutrophil absolute ount ($10^3/mm^3$)	4.969	6.172	5.6025	5.496	4.152	4.832	4.5395	4.562
ymphocyte %	62.50	55.50	52.50	53.50	61.50	57.00	59.00	60.00
ymphocyte absolute ount ($10^3/mm^3$)	8.297	7.425	6.352	6.436	7.020	6.779	6.788	6.904
lood coagulation ime (min.)	5.00	4.50	5.50	5.00	4.50	4.50	5.00	4.50

* $P < 0.05$

Table 14

Blood Chemistry - Group V - Mean Values

Parameters	Group V A				Group V B			
	Period of observation (days)							
	Before expt.	1	2	3	Before expt.	1	2	3
Total serum proteins (g/dl)	5.40	5.35	5.35	5.30	5.55	5.50	5.50	5.55
Blood urea nitrogen (mg/dl)	12.30	20.30*	24.30*	24.30*	11.90	12.30	12.20	11.90
Creatinine (mg/dl)	1.00	1.56*	1.60*	1.60*	0.93	0.93	0.94	0.98
Icterus index (Icterus units)	1.40	1.96*	3.12*	3.53*	1.30	1.20	1.30	1.25
Alkaline phosphatase (B.L.B. units)	5.50	7.05*	7.70*	8.00*	5.05	5.00	5.25	5.00
Acid phosphatase (B.L.B. units)	0.41	1.23	1.70	2.06	0.36	0.35	0.385	0.375
Glutamate oxaloacetic transaminase (K.F. units)	69.50	107.00	185.00	205.50*	70.00	69.50	67.00	70.00
Glutamate pyruvic transaminase (K.F. units)	3.00	5.50*	6.00*	7.00*	2.50	2.50	2.50	2.50

*P < 0.05

Traces of protein appeared by this time. Traces of bilirubin, blood and ketone were observed in the urine of the test animals during the third and fourth day. Urinary sediment showed, scray numbers of renal epithelial cells and granular casts from the second day onwards.

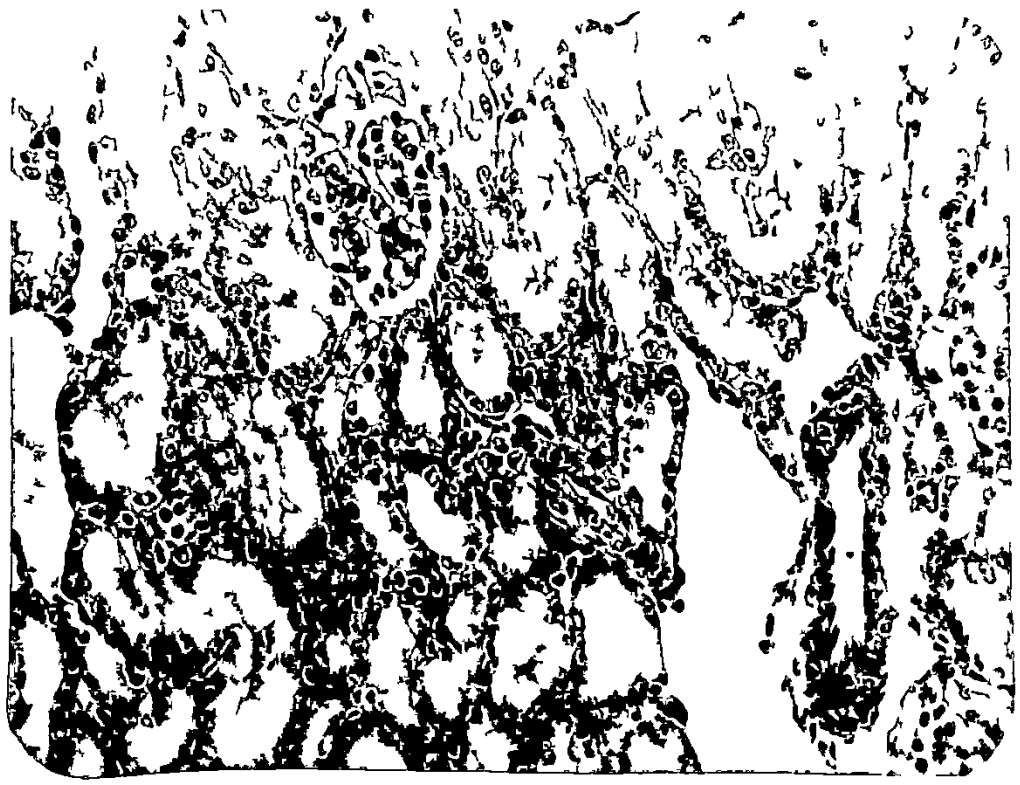
Patho-anatomy.

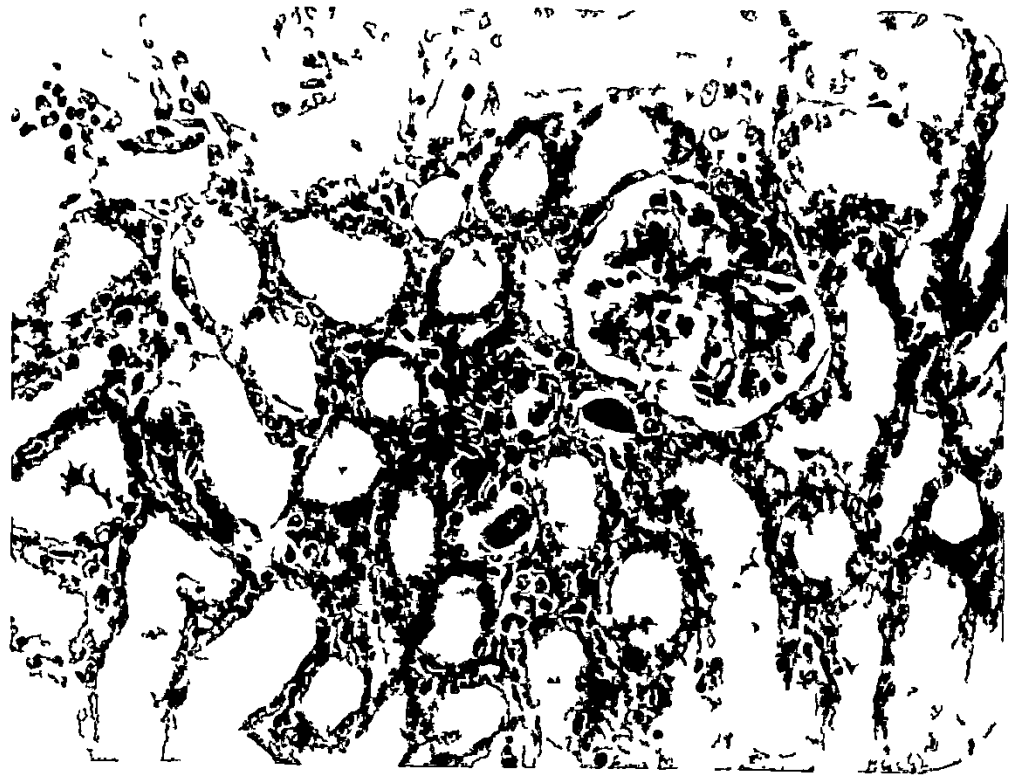
Autopsy findings

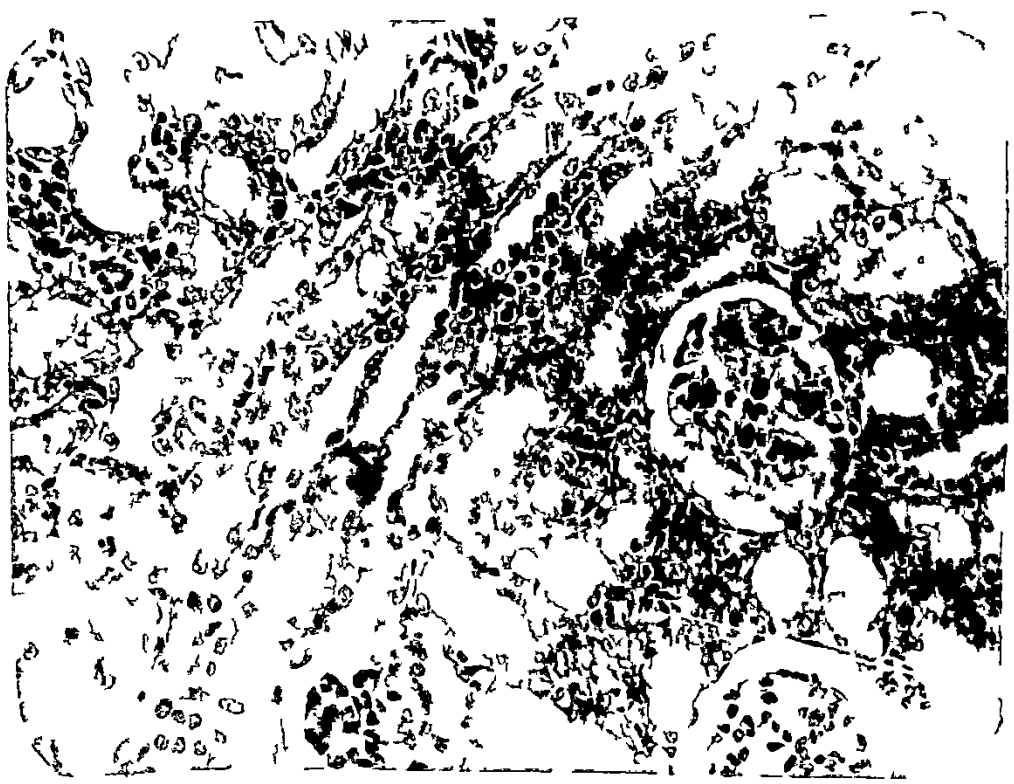
Lesions were observed in the liver, kidneys and gastro-intestinal mucosa. There was slight hepatomegaly and pallor of the liver parenchyma. Sectioned hepatic tissue had a cooked up appearance. Gall bladder was partially distended with yellowish green bile. Both kidneys were slightly swollen. Renal cortex was moderately pale and medullary vessels engorged. Urinary bladder contained sparse amount of dark yellow urine. Gastric and intestinal mucosa were moderately hyperaemic. Perirenal and suocutaneous fat appeared gelatinous. About 20 ml of pale yellow fluid was present in the peritoneal cavity. Omentum and mesentery were moderately oedematous and vessels congested.

Histo-pathology

Degeneration, necrosis and desquamation of the epithelial cells of a large number of proximal convoluted tubules were prominent (Fig.13). Focal tubular atrophy and destruction







leading to dilatation was observed in a few sections. These cysts were filled with casts and cellular debri. Several of the tubules contained deeply eosinophilic homogeneous substance. Varying degree of parenchymatous degeneration and vacuolar changes were present focally in the collecting tubules of the medulla. Few of the epithelial cells of the glomeruli had undergone vacuolar changes (Fig.14). These glomeruli appeared to occupy the entire capsular space while a few others were atrophied. Moderate thickening of the Bowman's capsule was seen. The capsular space beneath the membrane contained cell debri and finely granular material.

In the liver, fatty changes occurred in scattered hepatocytes while most other cells suffered parenchymatous degeneration. Few hepatocytes were necrotic. Central veins were moderately congested. Mild biliary proliferation was evident.

Focal ulcer formation occurred in the rumen of one of the animals. There was degeneration and focal necrosis of the mucosa of the abomasum. Submucosal oedema was noticed. Focal vacuolar degeneration, necrosis and erosions were observed in the lining mucosa of small intestines and colon. These areas showed moderate infiltration of mononuclear cells. In focal areas, collection of lymphoid cells in groups was observed. The necrotic epithelium together with the inflammatory exudate

formed diphtheritic membrane over the mucosa in a few places. Goblet cell hyperplasia was moderate in the colon. Necrosis and desquamation of the glandular epithelium were seen in some sections of intestines. Focal destruction of the epithelium of the villi was noticed.

Well defined lymphoid follicles were absent in the lymph node. Oedema of the medulla and depletion of lymphoid cells in the cortical and paracortical areas were seen. Depletion of lymphoid cells in the splenic corpuscles was a prominent lesion. Widening of the zona fasciculata was the change observed in the adrenals. A few follicles of the thyroid were devoid of the colloid. There were focal areas of hyperplasia of the epithelial cells. Degeneration of acidophils in focal areas was observed in the pituitary. Degeneration and desquamation of seminiferous epithelium was seen in few tubules of the testis.

Macroscopical and microscopical lesions were absent in the controls.

Group VI

Clinical Pathology

Test animals in this group were given ochratoxin at the rate of 0.5 mg/kg body weight by intraperitoneal route. They were found slightly indisposed with poor appetite from second day. The animals were sacrificed on the fourth day.

Haematology

The mean values are shown in table 15. Slight reduction in the number of total erythrocytes was noticed on the third day. The haemoglobin concentration was significantly reduced in the test animals. Significant reduction in the packed cell volume was observed on the fourth day. Reduction in the total white cell count was not significant. The absolute count of lymphocytes was lowered. There was no significant alteration in the blood coagulation time and erythrocyte sedimentation rate during the experimental period in the test animals. Haematological values for the control animal remained within normal range throughout the period of experiment.

Blood chemistry

The mean values are shown in table 16. No significant alteration was observed in the total serum protein concentration in any of the animals. There was an increase in BUN and blood creatinine levels on the final day. The alteration was not statistically significant. The elevation of ALP level was significant. The alteration observed in acid phosphatase level was not significant. GOT level in serum was elevated from the second day onwards. A rise in glutamate pyruvic transaminase level in serum was observed on the second day. These alterations in enzyme levels were not significant.

Table 15

Haematology - Group VI - Mean values

Parameters	Group VI A					Group VI B				
	Period of observation (days)									
	Before expt.	1	2	3	4	Before expt.	1	2	3	4
Total erythrocytes ($10^6/mm^3$)	13.485	13.29	12.41	11.60	11.89	12.52	12.62	12.42	12.49	12.56
Haemoglobin (g/dl)	10.20	10.10	9.70	9.90	9.90*	9.00	9.00	9.00	9.20	9.20
Packed cell volume (%)	29.70	29.30	28.50	28.50	28.40*	28.00	28.20	28.00	28.60	28.00
Total leukocytes ($10^3/mm^3$)	12.200	12.25	10.000	10.350	10.450	11.50	11.00	11.60	11.50	11.00
Neutrophil (%)	35.00	37.50	44.00	46.50	46.00	32.00	38.00	37.00	37.00	36.00
Neutrophil absolute count ($10^3/mm^3$)	4.261	4.552	4.608	4.312	4.808*	3.712	4.484	4.292	4.255	3.960
Lymphocyte (%)	63.00	60.00	54.00	52.00	52.50	65.00	61.00	62.00	62.00	61.00
Lymphocyte absolute count ($10^3/mm^3$)	7.335	7.35	5.429	5.393*	5,436*	7.656	7.198	7.192	7.130	6.710
Blood coagulation time	4.50	4.50	4.50	4.60	4.60	4.50	4.50	4.50	4.50	4.50

* $P < 0.05$

Table 16

Blood Chemistry - Group VI - Mean values

Parameters	Group VI A					Group VI B				
	Period of observation (days)									
	Before expt.	1	2	3	4th	Before expt.	1	2	3	4
Total serum proteins (g/dl)	5.35	5.20	5.15	5.15	5.20	5.60	5.50	5.50	5.60	5.60
Blood urea nitrogen (mg/dl)	11.90	12.60	14.00	13.30	13.85	12.00	12.00	12.40	12.00	12.00
Creatinine (mg/dl)	0.90	1.00	1.60	1.60	1.60	1.00	1.00	0.90	0.98	0.98
Icterus index (Icterus units)	1.10	1.10	1.25	1.10	1.00	1.30	1.20	1.30	1.25	1.25
Alkaline phosphatase (B.L.B. units)	5.50	5.00	7.05	7.50*	7.25*	4.50	5.00	5.00	4.40	4.50
Acid phosphatase (B.L.B. units)	0.36	0.41	1.40	1.40	1.10	0.37	0.35	0.40	0.40	0.40
Glutamate oxalo-acetic transaminase (K.F. units)	66.50	73.00	107.00	107.00	102.50	72.00	74.00	69.00	72.00	72.00
Glutamate pyruvic transaminase (K.F. units)	2.50	3.00	4.00	3.70	3.00	3.00	3.00	3.00	3.00	3.00

* $P < 0.05$

Urinalysis

The pH of urine was lowered from 9 to 8 in the test animals. Traces of bilirubin and urobilinogen were noticed on the third day. Sediment revealed moderate number of squamous epithelial cells, few transitional epithelial cells and stray renal epithelial cells. Urine samples of the control did not show any pathological alteration.

Patho-anatomy

Autopsy findings

Slight and diffuse pallor of the hepatic parenchyma was noticed. Gall-bladder was partially filled with greenish yellow, moderately viscous bile. Surfaces of both kidneys were moderately pale. Light red streaks and spots were present in focal areas in the cortex and medulla. Urinary bladder contained moderate amount of yellow urine. Fore stomachs were partially distended with food. Abomasal and intestinal mucosa were oedematous and moderately hyperaemic.

Histo-pathology

Histological lesions were limited mostly to the epithelial cells lining the proximal convoluted tubules and to a lesser extent in other parts of the tubules. Some of these cells were enlarged and contained granular eosinophilic material

in their cytoplasm. Several tubular lining cells had lost their brush border. Nuclear condensation was observed in some. Granular debris and a few rounded and desquamated cells were present in a few tubules. Stray cells of the Henle's loop and distal convoluted tubules revealed retrogressive changes. Moderate congestion of capillaries and venules was seen in the cortex and medulla. Parenchymatous degeneration of hepatocytes in scattered foci was evident. Mild degree of bile ductular proliferation could be noticed. Hyperaemia of vessels was observed in the abomasum and small intestines. The lamina propria and submucosa showed oedema.

Histological lesions were not evident in the tissue of the control animal.

Intravenous Administration of Toxin.

Group VII

Animals received ochratoxin at the rate of 2.5 mg/kg body weight. They became very weak and were sacrificed in extremis at 24 hr post administration.

Clinical Pathology

Haematology

Mean values are shown in table 17. Slight reduction in erythrocyte count, haemoglobin concentration and total leukocyte

Table 17

Haemogram - Group VII - Mean values

Parameters	Group VII A		Group VII B	
	Period of observation			
	Before expt.	24 h	Before expt.	24 h
Total erythrocytes ($10^6/mm^3$)	13.685	11.24	14.50	14.00
Haemoglobin (g/dl)	8.30	7.40	8.00	8.00
Packed cell volume (%)	24.50	25.00	27.00	26.80
Total leukocytes ($10^3/mm^3$)	15.300	12.500	15.500	15.400
Neutrophil (%)	25.50	53.50	38.00	39.00
Neutrophil - absolute count ($10^3/mm^3$)	3.542	8.256	5.730	7.20
Lymphocyte (%)	76.00	39.00	60.00	59.00
Lymphocyte - absolute count ($10^3/mm^3$)	12.002	5.313	9.300	9.086
Blood coagulation time (min.)	4.25	5.00	4.00	4.00

* $P < 0.05$

count was observed. While the absolute count for neutrophils showed an increase, the lymphocyte count was seen decreased. No alteration was noticed in the erythrocyte sedimentation rate or blood coagulation time.

Blood chemistry

Mean values are given in table 18. There was a reduction in the concentration of total serum proteins by the 24th hour. A rise in blood urea level and icterus index was noticed during the final stage. Slight elevation in creatinine level occurred by the 24th hour. There was marked rise in the level of serum ALP, ACP, GOT and GPT level in the test animals.

Urinalysis

The pH was lowered from 9 to 6 at 24 hr post administration. Urine samples were positive for protein, bilirubin and urobilinogen. Sediment showed erythrocytes, renal epithelial cells, squamous epithelial cells and granular casts. Few numbers of transitional epithelial cells and neutrophils also were seen. Pathological changes were not observed in the urine of control animals.

Patho-anatomy

Autopsy findings

Petechiae and ecchymoses were present in the subcutaneous

Table 18

Blood Chemistry - Group VII - Mean values

Parameters	Group VII A		Group VII B	
	Period of observation (days)			
	Before expt.	24 h	Before expt.	24 h
Total serum proteins (g/dl)	5.40	5.55	5.30	5.30
Blood urea nitrogen (mg/dl)	13.60	21.50	13.10	13.10
Creatinine (mg/dl)	1.10	1.45	1.00	1.00
Icterus index (Icterus index)	1.975	3.075	1.90	1.95
Alkaline phosphatase (B.L.B. units)	4.95	20.50	4.50	5.00
Acid phosphatase (B.L.B. units)	0.47	3.84	0.35	0.37
Glutamate oxaloacetic transaminase (R.F. units)	68.50	442.00	70.00	70.00
Glutamate pyruvic transaminase (R.F. units)	8.00	15.50	5.00	5.00
Lactic dehydrogenase (C.U. units)	174.50	303.50	200.00	200.00

* $P < 0.05$

tissue on the ventral aspect of the abdomen, brisket, flanks and on the liver surface. About 100 ml of clear, pale fluid was present in the abdominal cavity. Liver was moderately enlarged and pale. Hepatic parenchyma was soft and oily. Kidneys were moderately swollen and pale. Moderate quantity of dark yellow urine was present in the urinary bladder. Abomasal mucosa was hyperemic and oedematous. Yellowish fluid contents were present in the lumen of small intestines. Contents of caecum, colon and rectum were semisolid. Mucosa of intestines was moderately hyperaemic and oedematous. There was no morbid change in the carcase of the control.

Histo-pathology

Lesions in the kidneys involved mainly the tubules and glomeruli. Degeneration and necrosis of the epithelial cells were observed in a large number of proximal convoluted tubules. Similar changes also were noticed in some of the Henle's loops, distal convoluted tubules and collecting tubules. Cellular debris and casts were present in some of the tubules. Vacuolation and swelling of the endothelium and epithelium of many glomeruli were observed (Fig.15). These glomeruli appeared to fill the capsular space. In some others proteinaceous materials and cellular debris were present in the capsular space. Lining epithelial cells of the Bowman's capsule revealed

degeneration and necrosis. Degenerated and desquamated epithelial cells were observed in the ducts of Bellini.

Changes in the liver were mainly necrotic in nature. Few cells around the central veins only were preserved. Parenchymatous degeneration and fatty changes were observed in some of the paracentral hepatocytes. In other areas, eosinophilic granularity of the cytoplasm and dispersion of the nuclei were evident. Pyknosis, karyorrhexis and karyolysis of nuclei were all very prominent (Fig.16,17). Hyaline structures surrounded by a halo were seen in some cells. The reticulum framework was found disrupted in many places and collapsed. Sinusoids were dilated and the space of Disse oedematous.

Lymphoid cells in the lymph nodes and spleen revealed varying degrees of necrotic changes. Mild vacuolar changes were seen in scattered epithelial cells lining the papillae of rumen and reticulum. Abomasal mucosa was moderately hyperemic. Mild degenerative changes were seen in the epithelial cells of the intestinal mucosa.

Vacuolar degeneration and necrosis occurred in some of the acinar epithelial cells of the pancreas. Degenerative changes were present in the adrenal medulla and cells of zona fasciculata.

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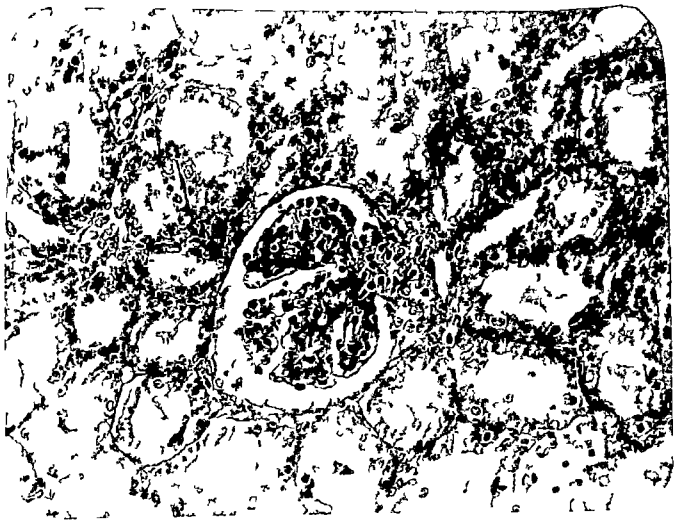
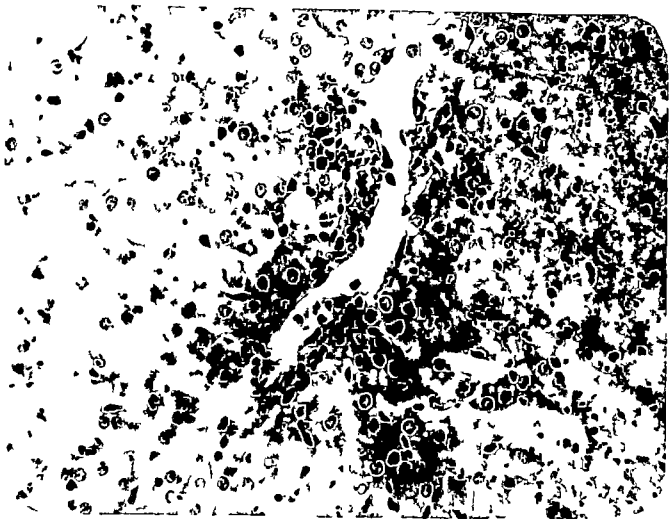
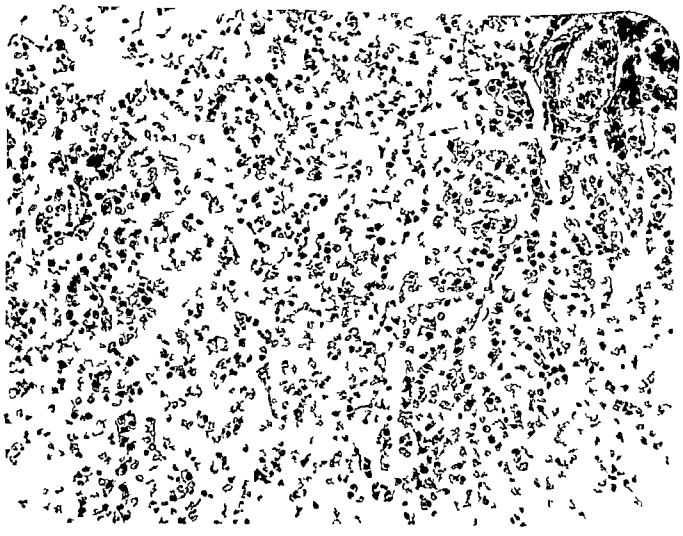






Histological lesions were not present in the organs of the control.

Group VIII

The goats which received ochratoxin i/v at the rate of one mg/kg body weight showed severe anorexia, incoordination and depression during the experimental period of one week.

Clinical Pathology

Haematology

Mean values are shown in table 19. Reduction in erythrocyte count was noticed but this was not significantly different from that of the control. Values for haemoglobin, packed cell volume, total leukocytes and absolute lymphocyte counts showed a reduction during the experimental period. An increase in blood coagulation time and absolute count of neutrophils was noticed on the third day.

Blood chemistry

Mean values are given in table 20. Total serum proteins was found reduced. There was elevation in values for blood urea nitrogen, creatinine and icterus index. The alterations in biochemical parameters were significant statistically.

There was rise in the levels of serum ALP, GOT and GPT. The increase in serum enzyme levels in the test animals was

Table 19

Haemogram - Group VIII - Mean values

Parameters	Group VIII A				Group VIII B			
	Period of observation (days)							
	Before expt.	1	2	3	Before expt.	1	2	3
Total erythrocytes ($10^6/mm^3$)	16.63	16.30	14.07	15.22	15.42	15.42	15.00	15.20
Haemoglobin (g/dl)	10.10	10.00	9.20*	8.50*	9.80	9.80	9.60	9.800
Packed cell volume (%)	30.00	30.00	23.50	25.70	28.00	28.00	28.00	28.20
Total leukocytes ($10^3/mm^3$)	12.20	11.60	10.90	10.60*	12.60	12.90	12.50	12.60
Neutrophil (%)	26.50	31.50	47.00	50.50	41.00	40.00	45.00	42.00
Neutrophil absolute count ($10^3/mm^3$)	3.219	3.810	5.120	5.343*	5.166	5.160	5.625	5.292
Lymphocyte (%)	63.50	67.50	51.50	53.00	53.00	56.00	54.00	57.00
Lymphocyte absolute count ($10^3/mm^3$)	8.273	7.833	5.613	5.606*	7.308	7.224	6.750	7.182
Blood coagulation time (min)	4.75	5.00	6.00	6.25	4.50	5.00	4.50	4.50

* $P < 0.05$

Table 20
Blood Chemistry - Group VIII - Mean values

Parameters	Group VIII A				Group VIII B			
	Period of observation (days)							
	Before expt.	1	2	3	Before expt.	1	2	3
Total serum proteins (g/dl)	5.80	4.35	3.55	3.50*	5.30	5.30	5.20	5.30
Blood urea nitrogen (mg/dl)	11.90	12.80	24.30*	29.00*	12.00	12.00	13.10	12.60
Creatinine (mg/dl)	1.02	1.04	1.52	1.19*	0.96	0.96	0.98	0.96
Icterus Index (Icterus units)	1.175	2.00	3.00	3.80*	1.40	1.40	1.36	1.40
Alkaline phosphatase (B.L.B. units)	4.75	9.60	12.25*	14.70*	4.00	4.50	4.50	4.50
Acid phosphatase (B.L.B. units)	0.425	1.01	2.32*	2.70*	0.47	0.40	0.45	0.40
Glutamate oxaloacetic transaminase (R.F. units)	66.00	110.00	153.00*	185.00*	60.00	60.00	62.00	62.00
Glutamate pyruvic transaminase (R.F. units)	2.50	5.50	9.00*	10.00*	2.00	2.00	2.00	2.00

* $P < 0.05$

significant statistically.

Urinalysis

The reaction of urine turned acidic on the second day of treatment. Urine samples contained low levels of protein from the second day. Moderate amount of bilirubin and slight quantity of blood were present on the second and third day. Microscopical examination of urine sediment revealed presence of moderate numbers of erythrocytes, squamous epithelial cells, renal epithelial cells and granular casts.

Patho-anatomy

Autopsy findings

Carcases of both the test animals were emaciated and there was gelatinisation of subcutaneous fat. Petechiae and ecchymotic patches were present in the subcutis of the brisket and shoulders. Liver was pale, friable and had subcapsular haemorrhages. Gall bladder was moderately oedematous and distended with yellowish green bile. Kidneys were moderately engorged and congested. Sparse amount of yellow urine was present in the urinary bladder. Mucosa of the abomasum, small intestines and colon was moderately hyperemic and oedematous.

Histo-pathology

In the kidney, large number of proximal convoluted tubules revealed degenerated epithelium. The brush borders

were blurred in most cases and were absent in others. Some of the epithelial cells desquamated into the tubular lumen. Hyaline bodies were present in a few tubules. Some of the lining epithelial cells had eosinophilic, granular cytoplasm, pyknotic nuclei and ruptured cell membranes. Degenerative changes were also evident in a few epithelial cells of the collecting tubules and Henle's loop. Granularity of cytoplasm and occasional vacuolation were seen in the endothelium and epithelium of the glomeruli. The glomerular tufts in many cases appeared to be enlarged occupying the entire capsular space. Collapse of a few glomeruli could be seen.

In the liver, most of the hepatocytes had undergone parenchymatous degeneration. Fatty changes were observed in a few hepatocytes of the periportal area. Sinusoidal engorgement and necrotic changes of a few hepatocytes in the paracentral area were the other changes observed. Mild degree of bile duct proliferation was evident.

Vacuolation of a few epithelial cells lining the papillae of rumen and reticulum was observed. Mucosa of abomasum and intestines was moderately hyperemic. Focal areas of epithelial damage with mild degree of mononuclear cell infiltration were seen in the ileum and jejunum. Other

organs and tissues did not reveal any histological lesion of significance.

Group IX

Animals which received ochratoxin at the rate of 0.5 mg/kg body weight did not show any marked clinical symptoms except slight weakness on the day next to the administration of toxin. There was also reduction in feed intake for the first two days after toxin administration. The animals remained weak and listless upto the seventh day when they were sacrificed. The control did not show clinical signs of illness.

Clinical Pathology

Haematology

Mean values are shown in table 21. Slight reduction in the values for total erythrocytes and haemoglobin was noticed on the 7th day which was found to be significant. The reduction in packed cell volume in the test group was not significant. There was a reduction in total leukocytes and absolute lymphocyte values while the total neutrophil number increased.

There was no alteration in the erythrocyte sedimentation rate. Slight increase in blood coagulation time was noticed from the second day in the test animals.

Blood chemistry

Mean values are shown in table 22. Slight reduction in

Table 21

Haematology - Group IX - Mean values

Parameters	Group IX A					Group IX B				
	Period of observation (days)									
	Before expt.	1	2	3	7	Before expt.	1	2	3	7
Total erythrocytes ($10^6/mm^3$)	16.50	16.10	14.35*	14.45*	14.80*	12.52	12.25	12.00	12.50	12.60
Haemoglobin (g/dl)	10.30	9.80	9.40*	9.80*	9.80*	9.60	9.60	9.60	9.80	9.80
Packed cell volume (%)	30.10	29.70	28.00	28.00	28.00	28.00	28.00	27.60	27.60	27.40
Total leukocytes ($10^3/mm^3$)	12.35	11.15*	11.55*	11.50*	11.50*	12.20	11.30	11.00	12.20	12.00
Neutrophil %	32.00	34.500	44.00	46.50	51.50	25.00	25.00	33.00	33.00	40.00
Neutrophil absolute count ($10^3/mm^3$)	3.219	3.810	5.120*	5.343*	5.922	3.156	5.160	5.625	5.292	4.800
Lymphocyte (%)	65.00	63.00	54.00	54.00	52.50	25.00	25.00	38.00	38.00	40.00
Lymphocyte absolute count ($10^3/mm^3$)	8.273	7.333	5.615*	5.606*	6.300	7.308	7.224	6.750	7.182	4.300
Blood coagulation time (min.)	4.50	4.50	5.00	5.25*	5.00	4.50	4.50	4.50	4.50	4.50

* $P < 0.05$

Table 22

Blood Chemistry - Group IX - Mean values

Parameters	Group IX A					Group IX B				
	Period of observation (days)									
	Before expt.	24 h	2	3	7	Before expt.	24 h	2	3	7d
Total serum proteins (g/dl)	6.60	6.40	6.25	6.30	6.45	6.30	6.30	6.20	6.30	6.30
Blood urea nitrogen (mg/dl)	12.40	13.00	12.45	22.45*	25.35*	11.20	13.10	12.80	12.30	11.20
Creatinine (mg/dl)	1.005	1.005	1.24	1.24	1.38	0.94	0.94	0.95	0.95	0.94
Icterus index (Icterus units)	1.30	1.60	1.90	1.90	1.30	1.40	1.40	1.40	1.40	1.40
Alkaline phosphatase (B.L.B. units)	4.50	6.50	9.35*	9.35*	5.25	5.50	5.50	5.80	5.80	5.50
Acid phosphatase (B.L.B. units)	0.47	0.42	1.77	1.77	1.25	0.37	0.47	0.54	0.54	0.48
Glutamate oxaloacetic transaminase (R.F. units)	78.00	143.00	226.50*	226.50*	117.00	60.00	62.00	62.00	62.00	62.00
Glutamate pyruvic transaminase (R.F. units)	5.00	5.00	10.00*	10.00*	7.50	3.00	3.00	3.00	3.00	3.00

* $P < 0.05$

the total serum proteins was observed in the test animals. The blood creatinine level was found increased. The erythrocyte sedimentation rate was not altered. There was significant rise in serum ALP level while it was found that the rise in ACP level was not significant.

The levels of SGOT and SGPT were found increased from the second day.

Urinalysis

The alkaline urine turned neutral to acidic by the 24th hour of experiment and traces of protein, bilirubin and urobilinogen appeared from this period onwards in the test animals. Microscopical examination of urine sediment revealed presence of a few erythrocytes, renal epithelial cells and hyaline casts.

Patho-anatomy

Autopsy findings

There was slight enlargement and softness of the liver. The gall bladder was partially distended with greenish yellow bile. Surface of the kidneys were moderately pale. Few red streaks were present on the cut surface of the cortex and medulla. Moderate amount of urine was present in the urinary bladder. Severe hyperemia and oedema of the abomasal and intestinal mucosa were observed.

Histo-pathology

Mild degenerative changes were observed in the epithelial

cells of theproximal convoluted tubules of kidney. Some of these cells had lost their brush border. The epithelial cells were swollen with granular eosinophilic cytoplasm. Few tubules contained eosinophilic granular material in their lumen. Changes were not observed in other parts of the nephron. Mild degenerative changes were present in the periportal hepatocytes. Occasionally vacuolar changes were seen in these cells. Congestion of vessels and oedema were seen in the mucosa of abomasum and intestines.

Synergistic effect of ochratoxin and aflatoxin.

Group X

This group was given ochratoxin at the rate of one mg/kg body weight and aflatoxin at the rate of 0.5 mg/kg body weight by intraperitoneal injection. The animals became visibly ill by the end of the first day. They went off-feed, appeared weak and the gait was staggering. By the second day they could not stand up and was completely prostrate and were sacrificed 48 hrs post administration of toxin.

Clinical Pathology

Haematology

Mean values are given in table 23. Moderate reduction in erythrocyte count, packed cell volume and haemoglobin concentration was observed.

Table 23

Haemogram - Group A - Mean Values

Parameters	Group A		
	Period of observation (days)		
	Before expt.	1	2
Total erythrocytes ($10^6/mm^3$)	18.00	12.22	10.30
Haemoglobin (g/dl)	9.70	8.25	7.80*
Packed cell volume (%)	28.40	25.00	21.00*
Total leukocytes ($10^3/mm^3$)	12.400	13.075	12.350*
Neutrophil (%)	31.00	37.00	38.00
Neutrophil absolute count ($10^3/mm^3$)	2.872	4.383	4.571*
Lymphocyte (%)	68.50	62.00	61.00
Lymphocyte absolute count ($10^3/mm^3$)	3.862	0.795	0.753*
Blood coagulation time (min.)	5.00	5.75	3.25*

* $P < 0.05$

There was a significant rise in the absolute count of neutrophils while there was marked reduction in the lymphocyte absolute count. In addition, the blood coagulation time was also increased.

Blood chemistry

Mean values are shown in table 24. There was a slight reduction in concentration of total serum proteins and an increase in blood urea nitrogen and creatinine levels. There was marked increase in icterus index during the period of observation. Group 4 was significantly different from other groups.

The levels of serum enzymes, ALP, ACP, GOT and GPT were found elevated.

Urinalysis

The reaction of urine turned acidic with pH6 by the 24th hour and 5.25 on the second day. Traces to moderate amount of protein was detected from the 24th hour. Bilirubin, urobilinogen and blood were present in moderate quantities on the second day. Microscopical examination of urine sediment on the first and second day revealed presence of moderate numbers of erythrocytes, renal epithelial cells, squamous epithelial cells and a few transitional epithelial cells, hyaline and granular casts.

Table 24

Blood Chemistry - Group X - Mean values

Parameters	Group X		
	Period of observation (days)		
	Before expt.	1	2
Total serum proteins (g/dl)	5.45	4.30	4.25
Blood urea nitrogen (mg/dl)	13.10	36.45	42.50**
Creatinine (mg/dl)	1.04	1.31	3.12*
Icterus index (Icterus units)	1.35	8.83	15.80
Alkaline phosphatase (B.L.B. units)	5.05	20.00	20.50*
Acid phosphatase (B.L.B. unit)	0.36	2.24	3.19
Glutamate oxaloacetic transaminase (R.F. units)	71.00	254.00	410.00
Glutamate pyruvic transaminase (R.F. units)	3.50	6.50	24.50*

* $P < 0.05$

Patho-anatomy

Autopsy findings

Gross pathological lesions were more pronounced in Gr.X (1) in comparison to Gr. X(2). Changes were similar in nature and only differed in severity. Both the animals were emaciated. Icteric discolouration and gelatinisation of subcutaneous fat were prominent lesions. Petechiae and ecchymoses were present in the subcutis of the neck, brisket, axilla, flanks and ventral abdomen. About 20 ml of slightly icteric clear fluid was present in the pericardium. Similar extravasation was observed also in the peritoneal cavity. Liver was moderately enlarged, pale and extremely friable. Petechiae and ecchymoses were present all over the surface; more on the dorsal surface. Parenchyma when cut revealed pale areas with red centres and streaks. Greenish yellow viscid bile was noticed in the gall bladder. Wall of the gall bladder was swollen. The spleen was slightly engorged. Both the kidneys were swollen and congested. Linear areas of pallor were observed in the cortex and medulla. Mucosa of abomasum and intestines was moderately hyperaemic. Moderate amount of yellowish, mucus and mixed semisolid material was present in the lumen of intestines.

Histo-pathology

In the kidney some of the glomeruli were found shrunken.

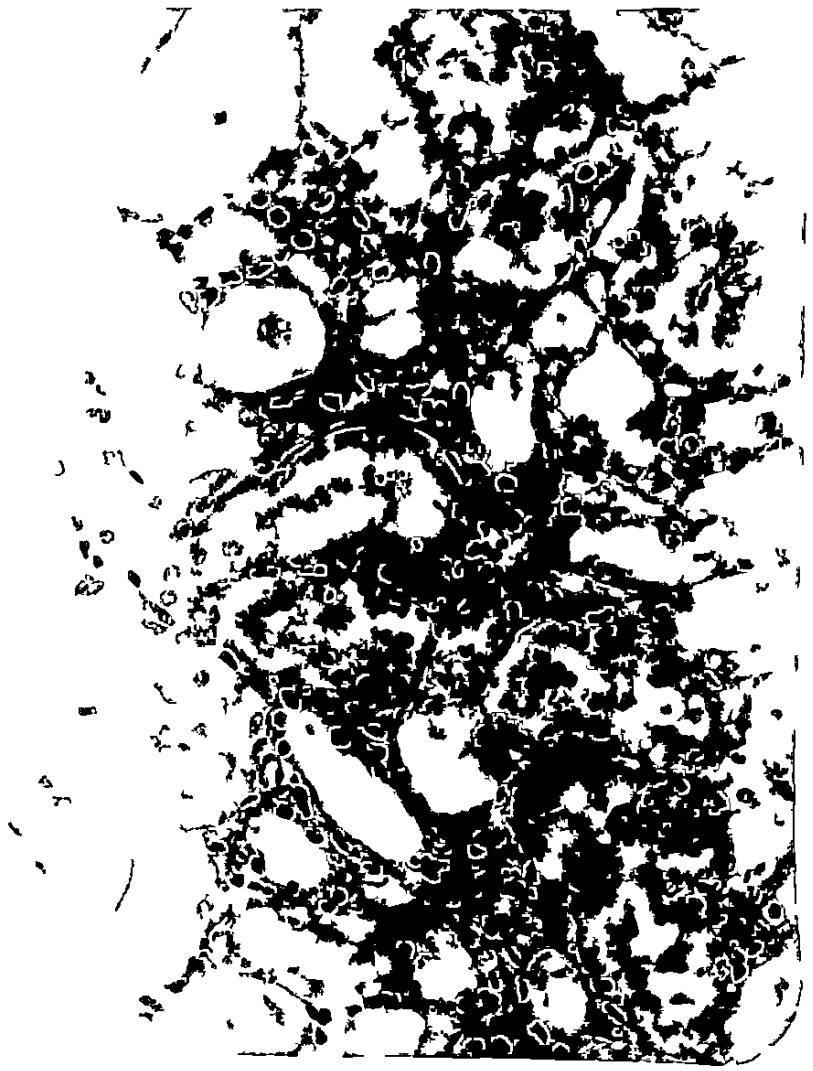
Many glomeruli had PAS positive material present among the capillary loops (Fig.18). The capsular membrane was thickened. Lining epithelial cells were degenerated, necrosed and desquamated. Masses of eosinophilic debri were seen in the capsular space. Almost all the proximal convoluted tubules revealed evidence of severe damage. There was loss of brush border, vacuolation of lining epithelial cells and necrosis of majority of the cells. Granular casts, eosinophilic debri and a few erythrocytes were present in the lumen of these tubules. Haemorrhages occurred in the interstitial space. Degenerative and necrotic changes were found in the tubules along with areas of haemorrhage (Fig.19). Epithelial cells lining the Henle's loop were vacuolated and some of them necrotic. PAS positive globular material was present in the lumen. A few cells of the distal convoluted tubules and collecting tubules revealed varying degree of degeneration and necrosis. Massive haemorrhages were observed in the medulla.

In the liver, severe and extensive necrosis of hepatocytes were prominent (Fig.20). Only a few cells around the central veins in some foci escaped the damage. Large number of degenerated hepatocytes contained fat droplets. Central veins were engorged with blood and haemorrhages were seen in the parenchyma. Nuclear changes like pyknosis, karyorrhexis and karyolysis were prominent in large number of hepatocytes. Fragments of chromatin material were seen lying in the destroyed parenchyma. Some of the hepatocytes

Fig.19. Kidney (Gr.X) - Degenerative changes in the tubular epithelium, cellular debri and hyalin structures in the lumen. H & E x 250.

Fig.20. Liver (Gr.X) - Extensive degenerative and necrotic changes in hepatocytes. H & E x 400.

Fig.21. Adrenal (Gr.X) - Degenerative changes of cells in the medulla. H & E x 250.



contained Mallory bodies. Moderate biliary hyperplasia was present.

Severe reduction of lymphoid cells was observed in the cortical and paracortical areas of lymph nodes. The number of follicles was few and reticuloendothelial hyperplasia was evident. Medulla showed oedematous changes. Depletion of lymphoid cells was also seen in the spleen and thymus. Vacuolar changes were observed in the cells of the superficial epithelial layer of rumen and reticulum. Congestion of vessels and moderate oedema of lamina propria were the lesions in the abomasum. In the intestines, the cells of the mucosa showed focal degeneration and denudation forming erosions. The lumen of the intestine revealed the desquamated epithelial cells. Goblet cell hyperplasia and mononuclear infiltration of the mucosa and lamina propria were also observed. Depletion of lymphoid cells was observed in the Payer's patches.

In the adrenals, degeneration and necrosis of cells in the medulla were prominent lesions (Fig.21). Some of these cells desquamated into the lumen. Haemorrhages also were observed in the medulla and in some areas of the zona fasciculata. In one animal hyperplastic nodules were present in the cortex. Slight oedema was observed in the pituitary.

In the thyroid, epithelial cells lining many of the

follicles were degenerated and these cells desquamated into the lumen. Only few of the follicles contained colloid. Focal collections of basophilic granules were found in the interstitial space. Focal degeneration and necrosis of the acinar cells were seen in the pancreas (Fig.22). The epithelium of the ductular membrane showed degeneration and desquamation.

Aflatoxin controls

Group XI

The animals which were given crystalline aflatoxin by the intraperitoneal route at the rate of 0.5 mg/kg body weight became slightly inactive and listless on the second day.

Clinical Pathology

Haematology

Mean values are shown in table 25. There was slight reduction in the values of the total erythrocyte, haemoglobin concentration and packed cell volume. The total leukocyte number and the absolute neutrophilic and lymphocytic counts showed slight increase. The blood coagulation time was also increased.

Blood chemistry

Mean values are shown in table 26. Slight reduction in

Table 25

Haemogram - Group XI - Mean values

Parameters	Group XI		
	Period of observation		
	Before expt.	1	2
Total erythrocytes ($10^6/mm^3$)	12.55	12.52	11.83
Haemoglobin (g/dl)	9.30	8.70	8.60
Packed cell volume (%)	26.50	25.90	25.80
Total leukocytes ($10^3/mm^3$)	15.150	16.800	16.000
Neutrophil (%)	43.50	45.00	45.00
Neutrophil absolute count ($10^3/mm^3$)	6.583	7.560	7.200*
Lymphocyte (%)	54.50	53.50	53.00
Lymphocyte absolute count ($10^3/mm^3$)	8.255	8.985	8.480
Blood coagulation time (min.)	4.75	4.75	7.50

* $P < 0.05$

Table 26

Blood Chemistry - Group XI - Mean values

Parameters	Group XI		
	Period of observation (days)		
	Before expt.	1	2
Total serum proteins (g/dl)	4.90	4.75	4.60
Blood urea nitrogen (mg/dl)	12.10	14.00	15.85
Creatinine (mg/dl)	0.95	0.98	0.90
Icterus index (Icterus units)	1.78	1.83	2.00
Alkaline phosphatase (B.L.B.units)	4.55	5.30	5.85
Acid phosphatase (B.L.B. units)	0.65	1.50	1.825
Glutamate oxaloacetic transaminase (R.F. units)	66.50	103.00	275.00
Glutamate pyruvic transaminase (R.F. units)	2.00	5.00	7.50

serum protein level occurred. An increase in blood urea nitrogen level and icterus index was observed while creatinine values showed a marginal increase. There was elevation of serum ALP, ACP, GOT and GPT values.

Urinalysis

The pH was lowered from 9 to 8. Pathological constituents were not detected in the urine. Microscopical examination of sediment revealed only few squamous epithelial cells.

Patho-anatomy

Autopsy findings

There was slight enlargement of liver; the hepatic parenchyma had a dull appearance and was slightly friable. Focal areas of pallor was observed on the surface of the kidneys. Abomasal mucosa was moderately hyperaemic. No other gross lesions were detected.

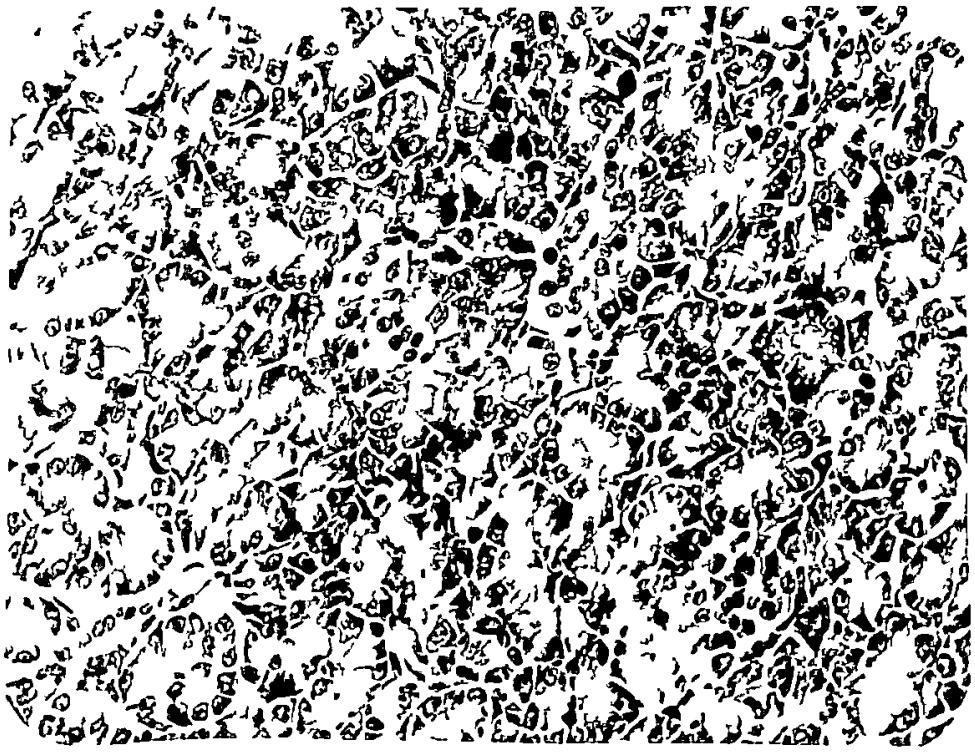
Histo-pathology

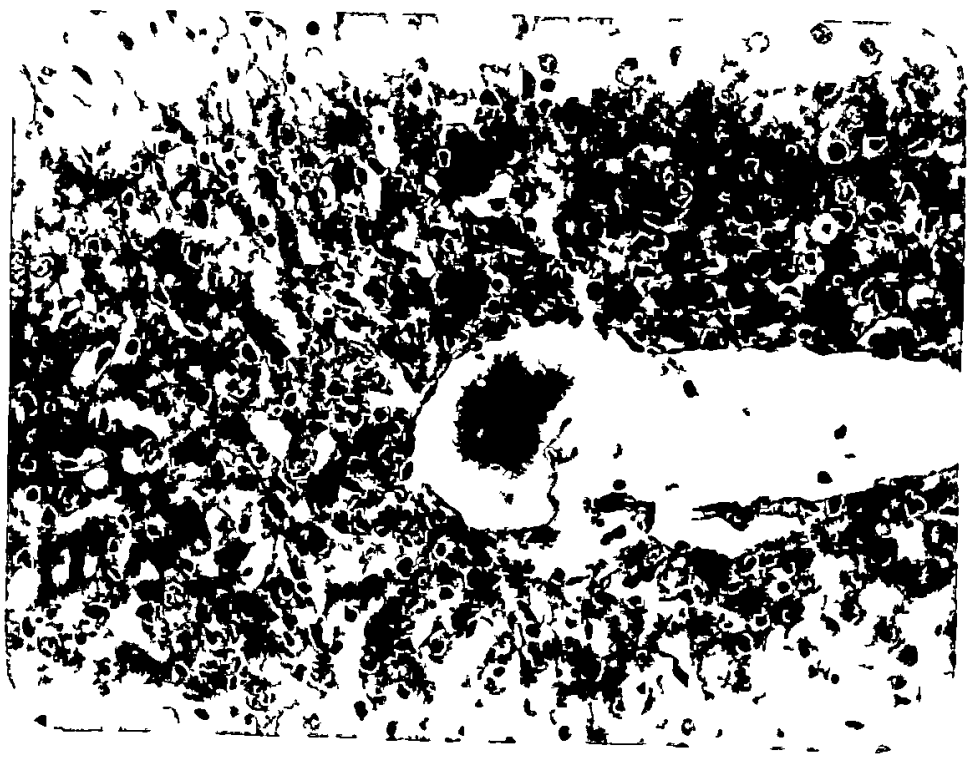
Histological lesions were observed mainly in the liver and kidney. Cloudy swelling and fatty change occurred in a few hepatocytes in the centrilobular location. There was widening of the sinusoids and necrosis of occasional hepatocytes (Fig.25). In the kidney, degeneration of tubular epithelium was observed. Some of the cells desquamated

Fig.22. Pancreas (Gr.X) - Parenchymatous degeneration of acinar cells. H & E x 400.

Fig.23. Liver (Gr.XI) - Degeneration and necrosis of hepatocytes. H & E x 250.

Fig.24. Kidney (Gr.XII) - Parenchymatous degeneration of tubular epithelium and swelling of glomerulus. H & E x 250.





into the lumen of the tubules. Vacuolation of the glomerular epithelium also was observed in a few foci. Congestion of vessels and occasional haemorrhages in the interstitial tissue could be seen. Engorgement of vessels were observed in the abomasal mucosa. Moderate degree of lymphoid depletion was evident in the spleen.

Ochratoxin controls and negative controls

Group XII

Gr. XII A(1) and Gr.XII A (2) were employed to study the effect of intraperitoneal administration of ochratoxin at the rate of one mg/kg body weight for the observation period of two days. Group XII B formed the negative control. The animals given ochratoxin became inactive and dull. Appetite was poor.

Clinical Pathology

Haematology

Mean values are shown in table 27. No significant reduction occurred in the total erythrocyte count during the period of observation. The concentration of haemoglobin and packed cell volume was slightly decreased.

Only slight increase in number was observed in the total leukocyte count during the period of experiment. There was

Table 27

Haemogram - Group XII - Mean values

Parameters	Group XII A			Group XII B		
	Period of observation (days)					
	Before expt.	1	2	Before expt.	1	2
Total erythrocytes (10^6 x mm^3)	14.66	14.50	14.16	16.06	15.75	15.96
Haemoglobin (g/dl)	8.80	8.70	8.10	9.00	8.90	9.10
Packed cell volume(%)	23.50	23.40	27.30	29.00	29.00	29.00
Total leukocytes ($10^3/mm^3$)	14.300	14.500	14.500	14.800	14.950	14.900
Neutrophil (%)	40.00	45.50	51.00	37.50	37.50	38.00
Neutrophil absolute count (10^3 / mm^3)	5.760	6.630	7.425	5.545	5.725	5.651
Lymphocyte (%)	58.00	52.50	43.00	61.00	62.00	62.00
Lymphocyte absolute count (10^3 / mm^3)	8.250	7.575	6.135	9.023	9.300	9.249
Blood coagulation time (min.)	4.75	4.75	4.75	4.75	4.75	4.75

a rise in absolute count of neutrophils while the lymphocyte absolute count showed decreased values. No alteration was observed in blood coagulation time and erythrocyte sedimentation rate.

Blood chemistry

Mean values are shown in table 23. There was no significant alteration in serum proteins. An increase in BUN level and creatinine levels was observed in the test group. No alteration occurred in the controls. Only slight variation of icterus index was observed in the test animals. There was rise in the values of serum ALP, ACP and GOT. Though a transient elevation occurred in SGPT by the 24th hour, the level declined to the pre-experimental value by the second day.

Bar graphs depicting the comparative values of ALP, GOT, GPT, icterus index, BUN and creatinine in groups X, XI & XII are presented in figures 39 - 44.

Urinalysis

The alkaline urine turned neutral by the 24th hour in the case of Gr.XII A(1) while it became acidic by this time in the case of Gr.XII A(2). Traces of protein, bilirubin and urobilinogen were observed. A few renal epithelial cells, squamous

Table 28

Blood Chemistry - Group XII - Mean values

Parameters	Group XII A			Group XII B		
	Period of observation (days)					
	Before expt.	1	2	Before expt.	1	2
Total serum proteins (g/dl)	5.50	5.45	5.20	5.35	5.35	5.35
Blood urea nitrogen (mg/dl)	11.90	21.50	22.40	12.00	12.20	12.00
Creatinine (mg/dl)	0.99	1.56	1.60	0.97	0.97	0.97
Icterus index (Icterus units)	1.35	1.50	2.06	1.25	1.25	1.20
Alkaline phosphatase (B.L.B. units)	5.50	7.25	7.45	4.55	4.45	4.55
Acid phosphatase (B.L.B. units)	0.36	1.23	1.73	0.41	0.385	0.40
Glutamate oxaloacetic transaminase (R.F. units)	69.00	105.00	177.00	66.00	65.00	66.50
Glutamate pyruvic transaminase (R.F. units)	2.50	5.50	2.50	2.50	3.00	2.50

epithelial cells and hyaline casts were present. Stray numbers of neutrophils and transitional epithelial cells also were observed.

Patho-anatomy

Autopsy findings

Gross lesions were confined to liver, kidney and gastro-intestinal mucosa. Slightly swollen liver and distended gall bladder with slightly oedematous wall were seen. The surface and sectioned areas of renal cortical parenchyma showed focal areas of pallor. The mucosa of abomasum and small intestines was moderately hyperaemic.

Histo-pathology

Microscopical alterations in the kidney were mainly confined to the proximal convoluted tubules though minor changes were observed in other parts of the nephron and glomeruli. Loss of brush border, granularity and vacuolation of epithelial cells lining the proximal convoluted tubules were seen. Some of the cells desquamate into the tubular lumen (Fig.24). Hyaline droplets were observed in the lumen of few tubules. Parenchymatous degeneration was observed in some epithelial cells of the Henle's loop. Vacuolar degeneration was observed in the epithelial cells of a few glomeruli. Some of the glomeruli exhibited hyper-

cellularity and occupied the entire capsular space. Congestion and small haemorrhages were present in the medulla. Parenchymatous degeneration of hepatocytes in focal areas was observed in the sections of liver. A few cells around the central veins in some foci showed fatty degeneration. Moderate congestion of hepatic vessels and necrosis of hepatocytes in scattered areas were seen.

Mild degenerative changes were observed in the lining epithelial cells of the rumen and reticulum. Blood vessels of the abomasum were congested and mucosa moderately oedematous. Vacuolar changes of the lining epithelium of the villi were seen in the mucosa of the small intestines. The blood vessels were congested.

Pathological alterations were not noticed in the negative controls.

Ultrastructural changes

Group VII

Kidneys

Extensive necrobiotic changes were discerned in the cells of the different parts of the nephron and the renal corpuscle. The changes were intense, more especially in the proximal convoluted tubule.

The epithelial cells of the proximal convoluted tubules exhibited varying degree of changes, many of them with extensive organellar disorientation and destruction (Fig.25). The microvilli which were long and regularly arranged in the normal cells became disoriented and disrupted or appeared fused. While they had only minimal changes of fragmentation in some locations, in others they were completely lost and the cells appeared denuded. The apical canaliculi were found to be numerous in some cells. In the apical cytoplasm, vacuoles which were clear and of varying sizes were noticed; some of them containing electroniucent material and/or electron dense myelinoid bodies. Mitochondria which were concentrated towards the basal portion of the cell showed complete loss of morphological identity or had only mild degree of disorganization. There were diverse variations of form and internal structure. Increase in the mitochondrial matrix density with complete loss of cristae were seen in cells showing advanced picture of cell damage. Irregularly shaped floccular densities were seen in mitochondrial matrix. In some cells there was a modest increase in the size of the mitochondria and dilution of matrix. The cristae, however, appeared displaced to the periphery and showed varying degree of disorientation, shortening and reduction in numbers (Fig.26). In some, the matrix was homogenous while in others there were multiple electron-

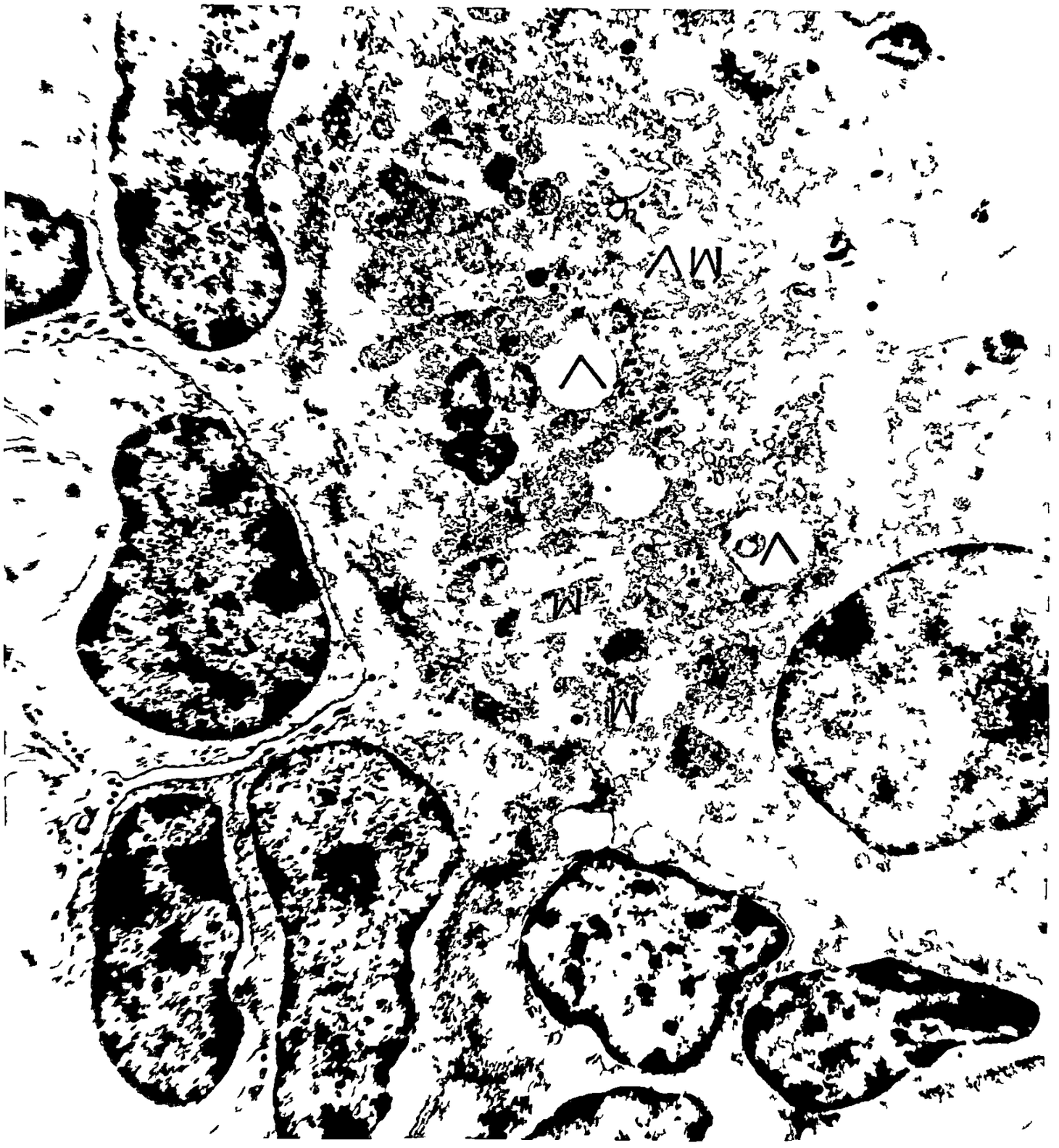

MV
V
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Fig.26. Mitochondria in the epithelial cells of the PCT. There is swelling of the matrix and varying degree of disorientation of cristae with loss of intramitochondrial granules. A myelinoid structure is seen within the matrix. X 1,00,000.

lucent foci. Frank cavitation was also seen. Breaks in the mitochondrial - limiting membrane were also encountered. The intramitochondrial dense granules had disappeared in many cases. Transformation and incorporation of damaged mitochondria into cytolysosomes were seen. Such processes were indicated by the presence of dense lamellated or whorled membranes and lysosomal bodies containing myelin figures (Fig.27) Sequestration of mitochondria with or without other organelles was also noticed. Associated with these changes varying grades of dilatation, fragmentation and dissolution of endoplasmic reticulum had occurred. Structures of the golgi complex had undergone destruction. A few free ribosomes were found free in the cytoplasm. The basement membranes of these cells with advanced organellar changes showed loss of homogeneity and fragmentation. The intensity of nuclear changes showed great variation. In some the nuclear morphology was completely lost with a washed out appearance while in others chromatin clumping, redistribution of the granular and fibrillar components, nucleolar fragmentation and disruption of nuclear membranes were observed.

The organellar changes seen in the epithelial cells of other parts of the nephron were qualitatively similar to those in the epithelial cells of the proximal convoluted

Fig. 27. Portion of two adjacent epithelial cells of the proximal convoluted tubule. There is extensive organellar destruction. Numerous vacuoles (V), lysosomes (L) seen. Cytolysosome with myelinoid structure (MB). X 30,000.

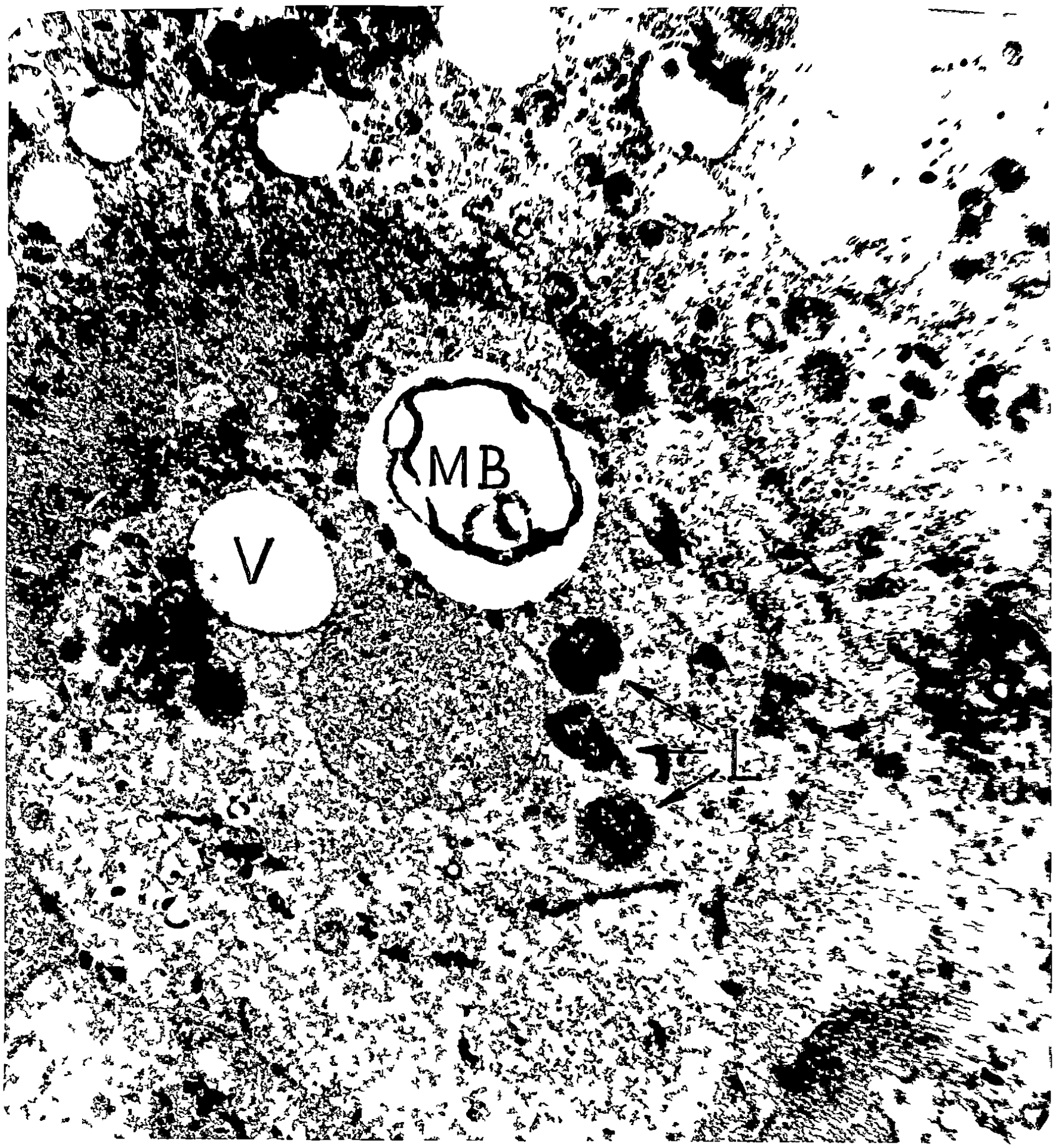

MB
V

Fig. 28. Epithelial cells of distal convoluted tubule. The cells appear washed out. Mitochondria (M) show varying grades of structural alteration. Nucleus showing partial chromatin dissolution. X 15,000.

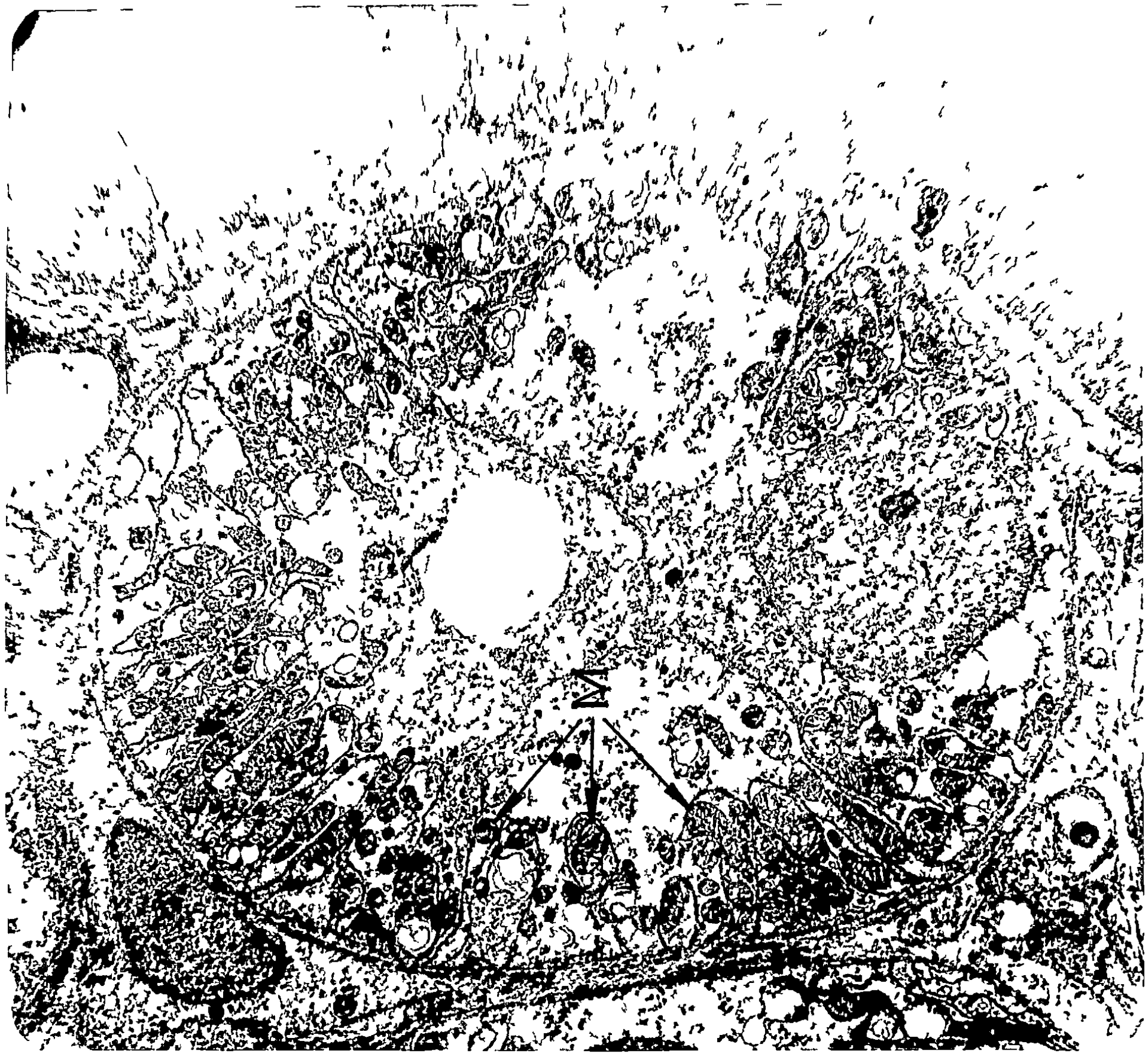
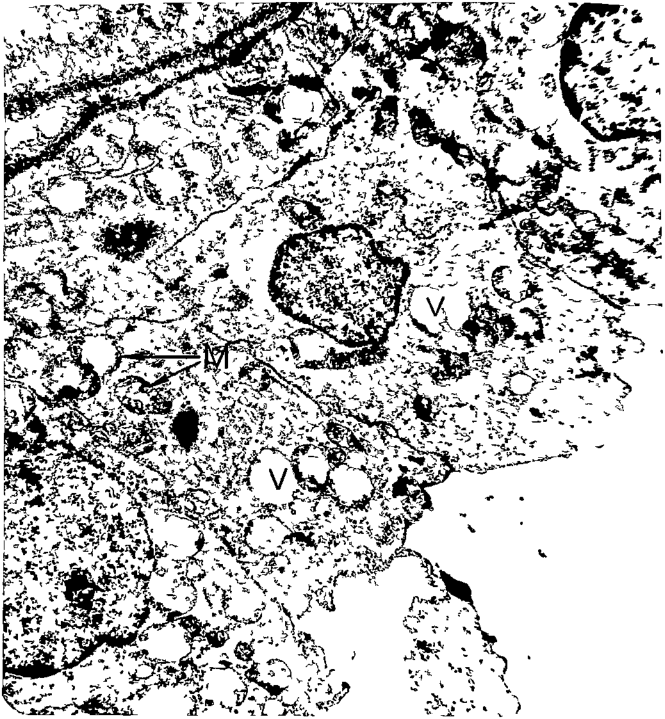

M

Fig.28 A. Epithelial cells in the loop of Henle showing damaged mitochondria (M) and vacuoles (V). Cell junctions show loss of structural cohesion. X 27,000.


V
M
V

tubules but were less severe in intensity. In the distal convoluted tubules the elaborately compartmentalized infoldings of the basal membrane with deep incursions into the cytoplasm could still be seen in many cells while some others had a partially washed up appearance with retention of granular electron dense material in the matrix and a few mitochondriae which depicted bizzare morphologic patterns of disorganized membranes and cristae (Fig.28). Lysosomes and dense bodies were seen in large numbers(Fig.28 A).

The cells of the loop of Henle composed of the straight portion of the proximal tubule, descending limb of Henle and ascending thin limb showed identical changes but of lesser intensity (Figs. 29, 30). Loss of structural cohesion of the cell junctions was noticed. Attenuated or altered desmosomes were quite common. Details of intercellular gap were lacking but the filaments converging upon the plaques were still present in some while in others only some cytoplasmic fuzz was discerned.

In the glomerulus, the nucleus of the podocytes were intact except for moderate chromation clumping (Fig.31). There was some amount of disaggregation of ribosomes from endoplasmic reticulum. Fragmentation and destruction of

Fig.29. Henle's loop - Epithelial cells showing loss of cytoarchitecture. Mitochondria (M) show loss of cristae. Basement membrane intact even though intercellular junctions are attenuated. X 25,000.

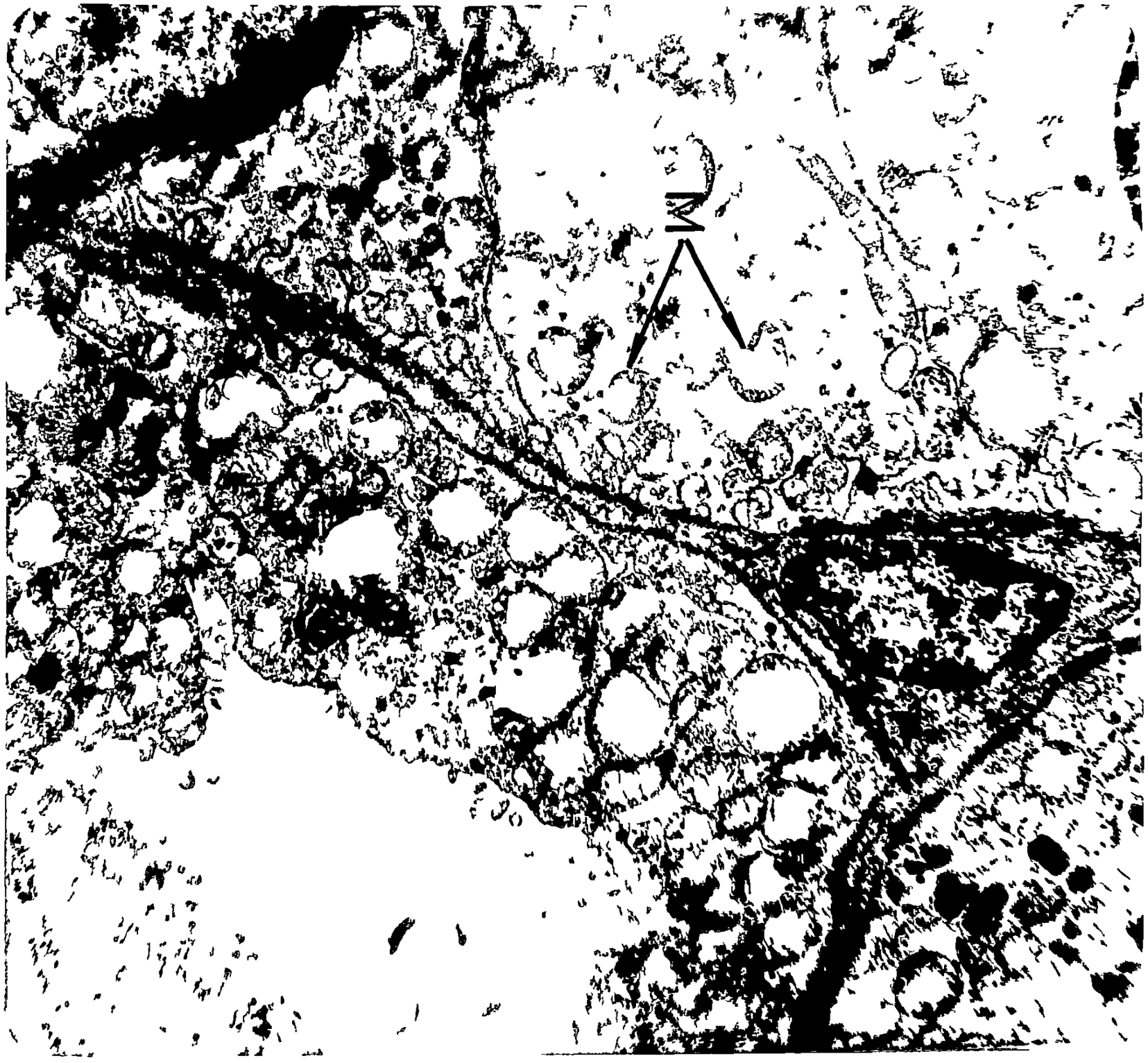

M

Fig.30. Epithelial cells of ascending limb of Henle showing extensive alterations in mitochondria. Nucleus (N) show chromatin clumping and dissolution. Mitochondria (M) show severe damage. Basement membrane intact. X 15,000.

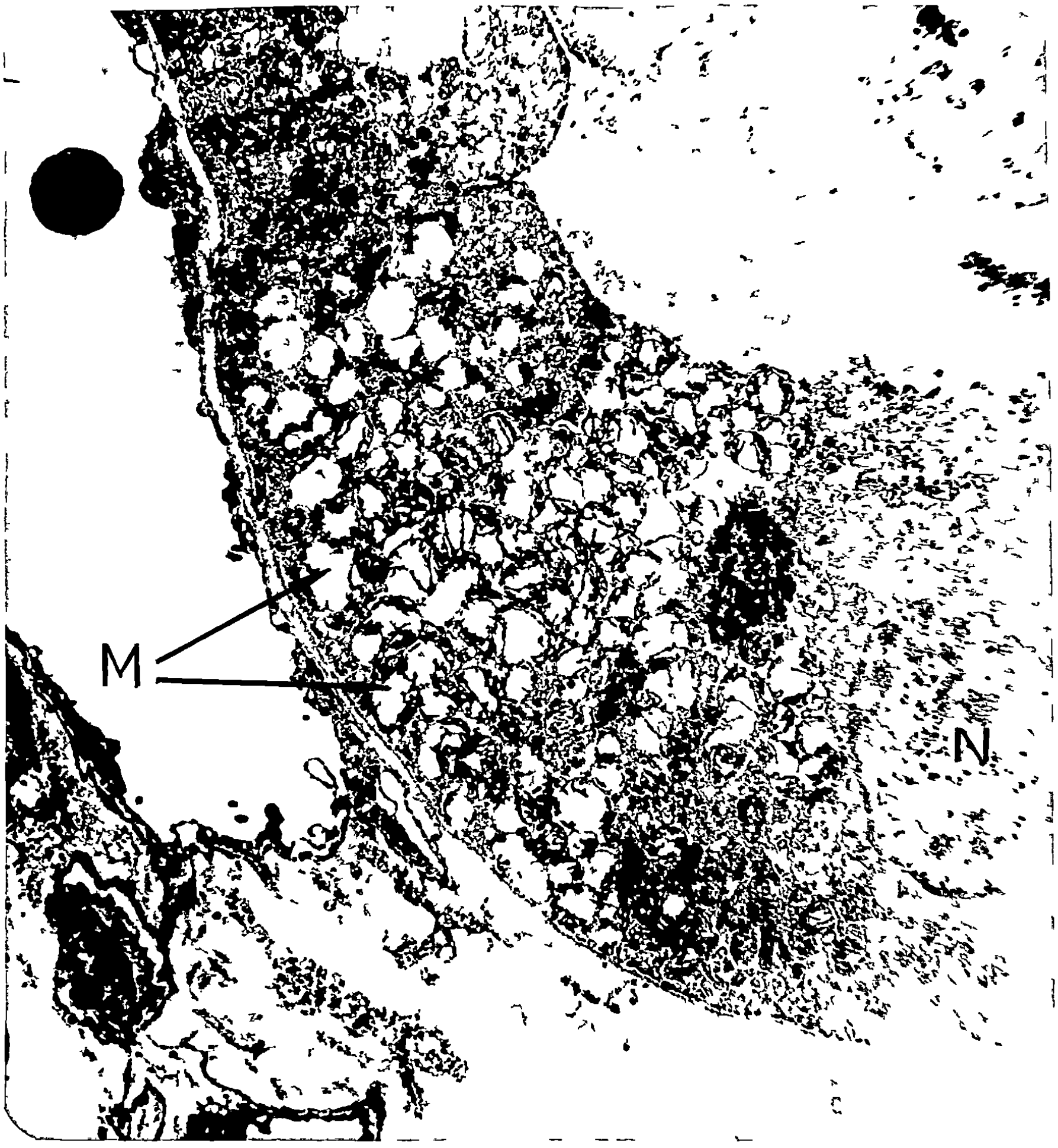
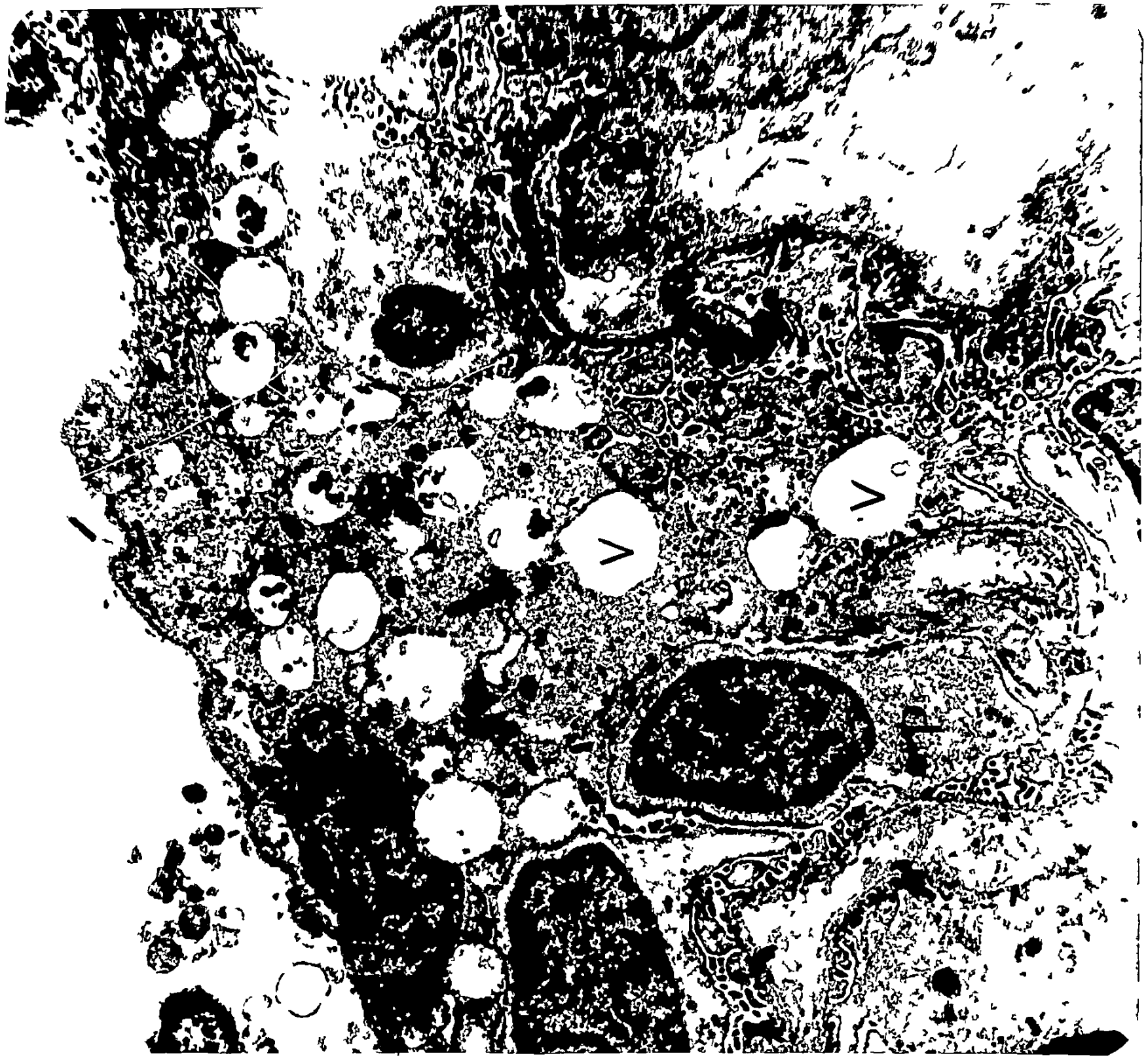

M
N

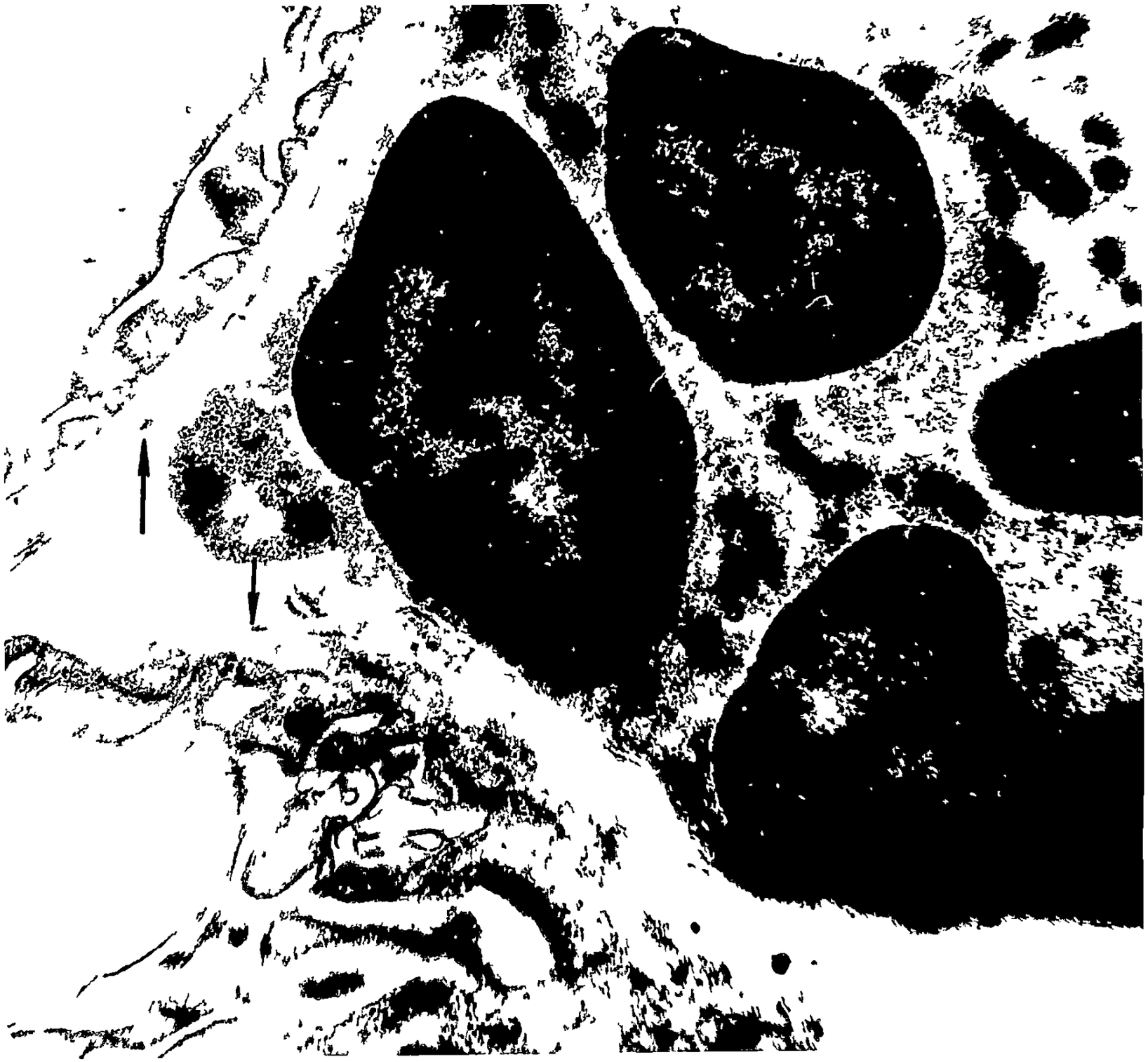
Fig.31. Glomerular region showing mesangial cells and podocytes (P) with foot processes. The cytoplasm of podocytes appear homogeneous with loss of structural details. Chromatin clumping in nucleus. Mesangium show numerous vacuolar (V) structures. X 15,000.



filaments and microtubules were seen. Many of the mitochondria appeared swollen. The mitochondrial matrix appeared homogeneous with increased electron lucency. The foot processes were seen extending to the basal lamina. Normally, the foot processes were seen aligned on the outer surface of the glomerular basal lamina with the fenestrated glomerular capillaries on the inside. In these cases, in some areas there was disruption of the normally regularly spaced arrangement of the foot processes and appearance of much larger cell processes or segments of the podocyte cytoplasm resting on the basal lamina (Fig.32). This gave the appearance of fusion of foot processes even though it is quite likely that this appearance might be due to the swelling and retraction of the foot processes so that the capillary wall was covered by large swollen processes or segments of podocyte cytoplasm. The fenestrae in the endothelial layer appeared very prominent and small breaks and thinning of the basal lamina were very often noticed. In some glomeruli there was extensive destruction of the integrity of the basement membrane along with destruction of the interdigitating whorled and distorted profiles were noticed(Fig.33). The endothelium of the capillaries showed varying grades of organellar changes. Pinocytic vesicles were seen in some cells. The filaments and microtubules

Fig.32. Portion of glomerulus showing podocyte foot processes. There is loss of structural homogeneity. Some of them appear larger and distorted. X 52,000.

Fig.33. Swollen, distorted or damaged foot processes of podocytes. Basal lamina show thinning or breakages. X 60,000.



appeared fragmented in some cells while in others there was complete absence of microtubular structures. On the inside of the basal lamina the fenestrae appeared very large than normal.

The mesangium of the glomeruli consisted of mesangial cells embedded in an unusually abundant mesangial matrix. Irregular often slightly ill defined and rather granular areas of increased electron density were seen in the mesangial matrix. Compared to the epithelial cells of the nephron the mesangial cells did not appear as severely damaged even though the nuclei exhibited caromatin clumping along the nuclear membrane and there was moderate destruction of cytoarchitecture.

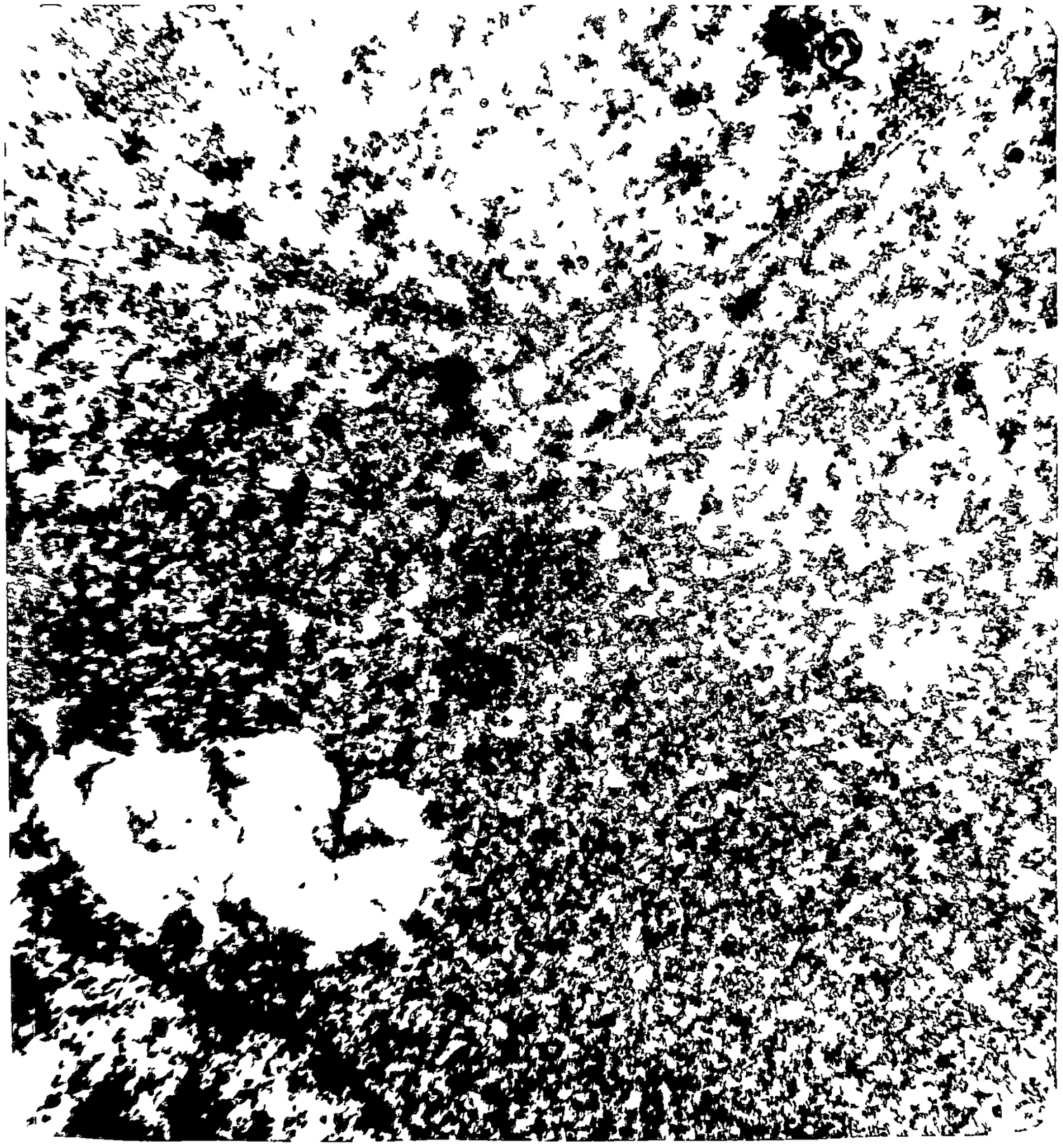
Liver

Some cells showed extensive alterations and destruction of plasma membrane and organelles (Figs.34,35). The plasma membrane showed many configurational changes. In a few cells there were bleb like protrusions of the cytoplasm into the sinusoids.

There was vesiculation, fragmentation and dissolution of the membranes of the RER. Dilatation of the cisternae were seen in a few locations. Disaggregation of ribosomes and detachment from RR was seen. In a few locations

N

Fig.35. Portion of two hepatic cells showing advanced necrobiotic changes. Remnants of few mitochondria and endoplasmic reticulum seen. There is clumping and redistribution of chromatin in the nucleus. X 70,000.



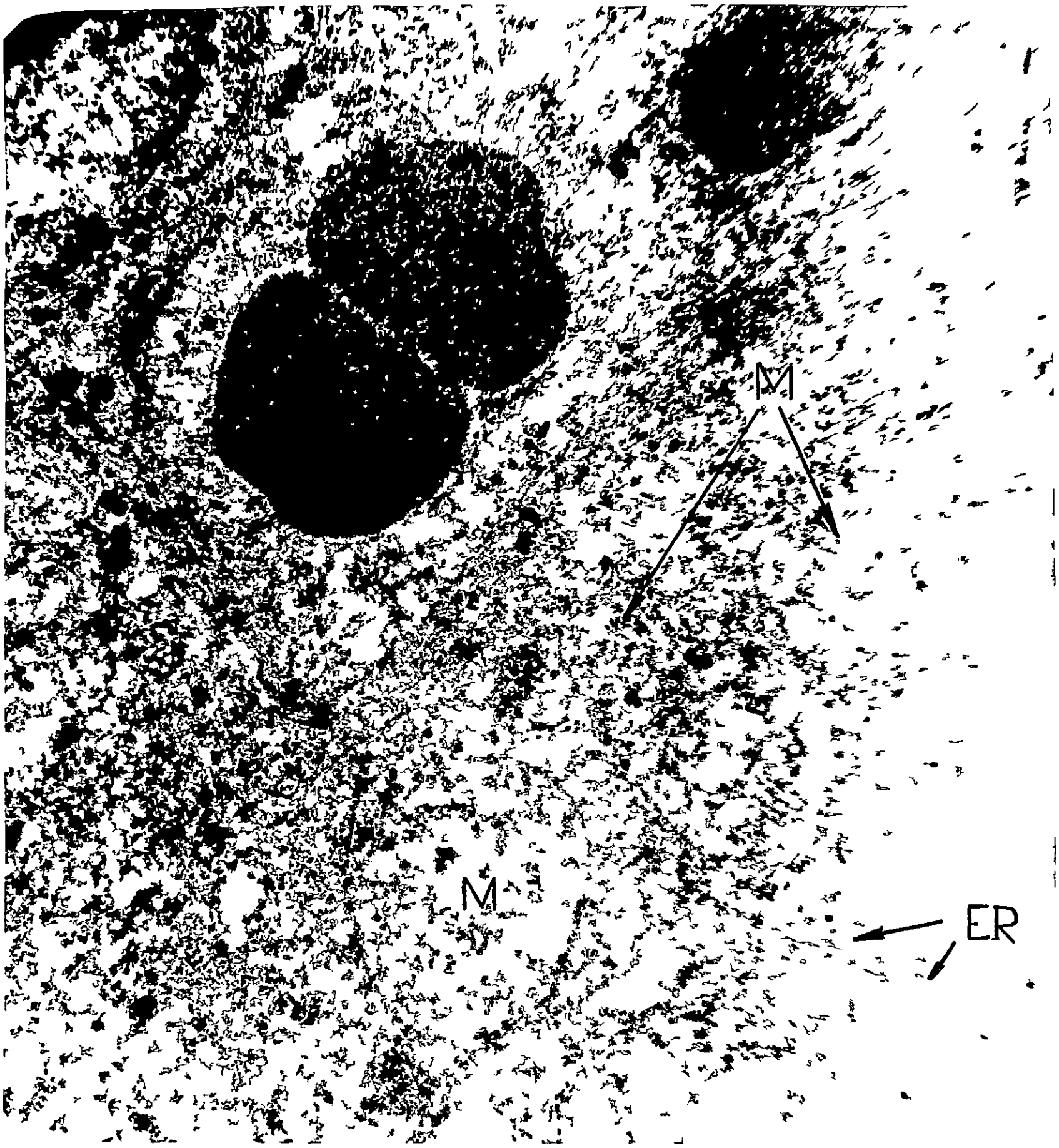
polyribosomes showing a helical configuration were noticed. Dilatation of the cisternae and vesicles of Golgi complex occurred accompanied by vesiculation and dilatation of the ER. In some cells there was complete loss of morphological identity of the Golgi complex with fragmentation. Numerous cytolysosomes, characterised by sequestrated cell organelles and cytomembranes within its substance were seen. The sequestrated material was well preserved and easily identifiable or in various states of breakdown and degradation. In some cases it was difficult to identify whether a lysosome was a cytolysosome or a phagolysosome. Residual bodies containing undigested electron-dense lipidic residues or myelinoid structure were also noticed. In many cells the cytoplasm presented extensive structural changes in the form of destruction of endoplasmic reticulum, microbodies and Golgi complex. It was difficult to identify glycogen granules if they were present.

Various alterations in the mitochondrial morphology were seen in the hepatic cells. There was swelling of the matrix in the inner chamber. The cristae showed varying degree of disorientation, shortening and reduction in numbers. Matrix was pale and had a fluffy appearance. In

Fig.36. Portion of a hepatic cell showing aggregates of fibrils with the morphology of Mallory bodies (ML). Nucleoli condensed. Fragmentation of cristae of mitochondria (m) and presence of electron dense material. X 52,000.


ML
M

Fig.37. Hepatic cell showing lipid droplets in the cytoplasm. Fragmented endoplasmic reticulum (ER) and damaged mitochondria (M). X 52,000.


M
M
ER

around the nucleolus. In cells which appeared lethally injured the chromatin appeared as markedly condensed or had disappeared. The nuclear envelope had also a fragmented appearance. Separation and redistribution of the structural components of the nucleolus were noticed. In some cells there was condensation of the fibrillar portion while in others there was redistribution resulting in distinct granular, fibrillar and amorphous portions. Those which revealed advanced cytoplasmic changes indicative of necrosis also had fragmented nucleolus.

The endothelial cells which were flat and having nuclei which protruded into the sinusoid lumina also showed varying degrees of damage. The small and large fenestrae were still present in some cells. Microvilli on the sinusoidal surface of underlying hepatocytes were retained in a few cells. The cell contacts between endothelial cells consisted of junctional complexes characterised by a slight increase in the electron density of the membrane and adjacent cytoplasm. In general intact organelles were sparse. Kupffer cells also showed changes some of them having abundant and morphologically heterogeneous population of phagocytic vacuoles. The bundles of reticulin fibres present in the space of Disse were found fragmented. The short microvilli which were normally present were absent. The intercellular surface of the ductular cells were irregular and were found separated

Fig.33. Bile canaliculi showing alteration of the canalicular membrane into multi-lamellar folds. x 80,000.

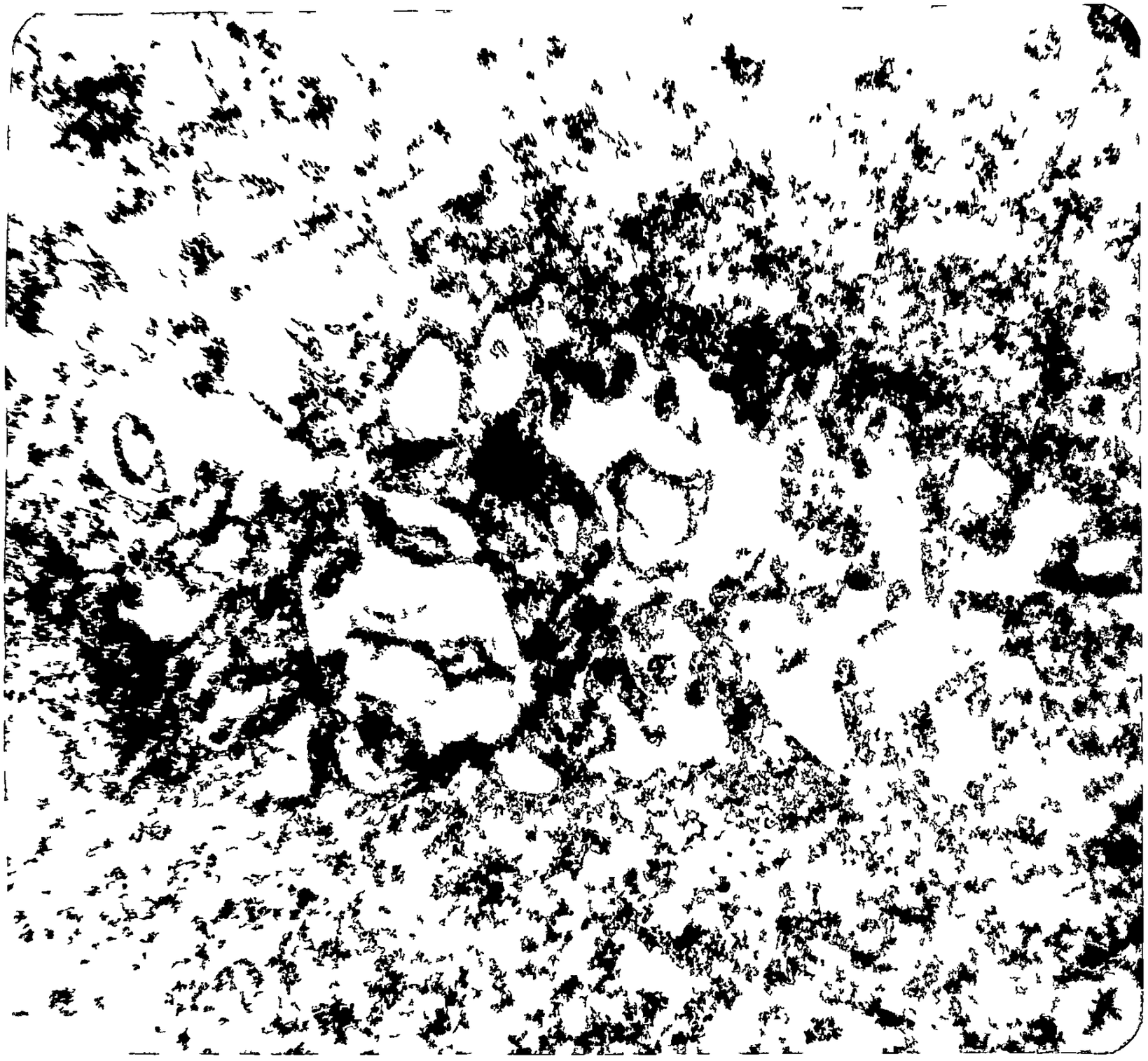


Fig.39 to 44. Bar graphs showing the levels of ALP, GOT, GPT, icterus index, BUN and creatinine in blood in groups X, XI and XII.

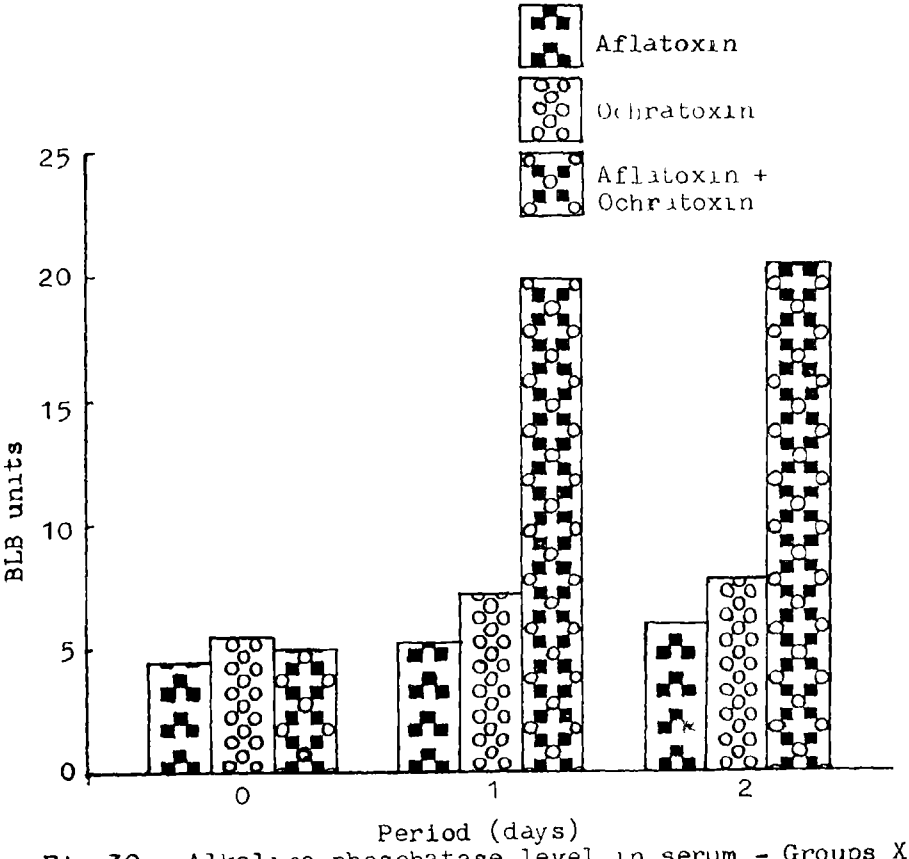


Fig.39. Alkaline phosphatase level in serum - Groups X,XI,XII.

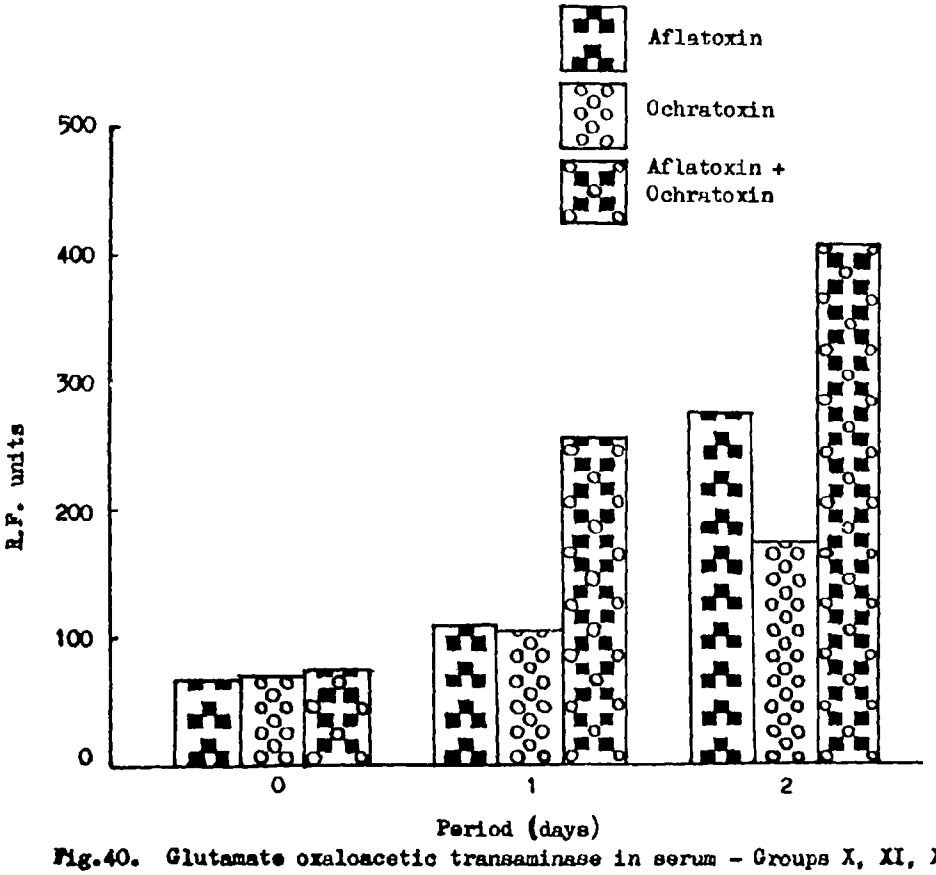


Fig.40. Glutamate oxaloacetic transaminase in serum - Groups X, XI, XII.

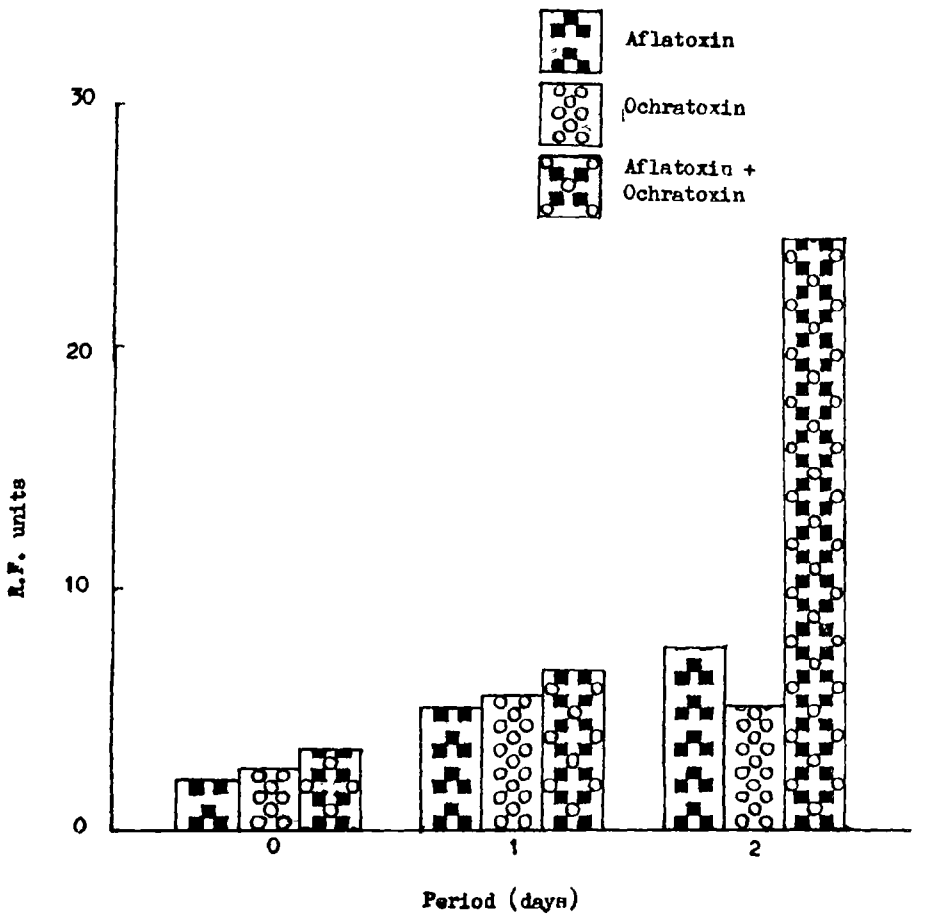


Fig.41. Glutamate pyruvic transaminase level in serum - Groups X, XI, XII.

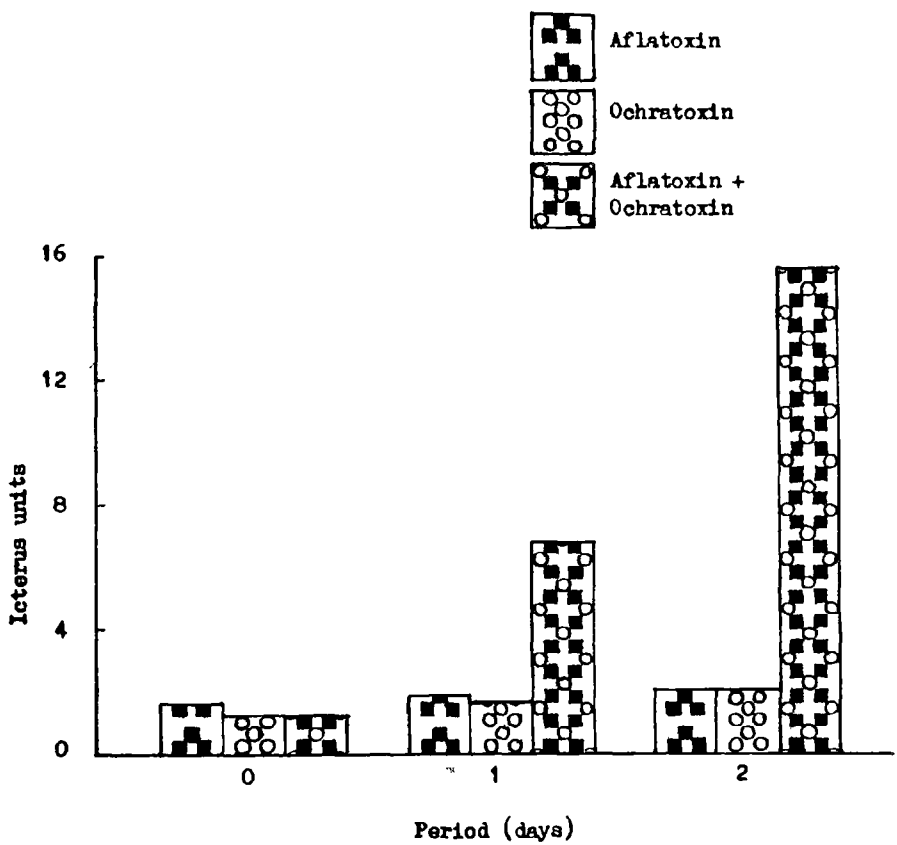


Fig.42. Icterus index - Groups X, XI, XII.

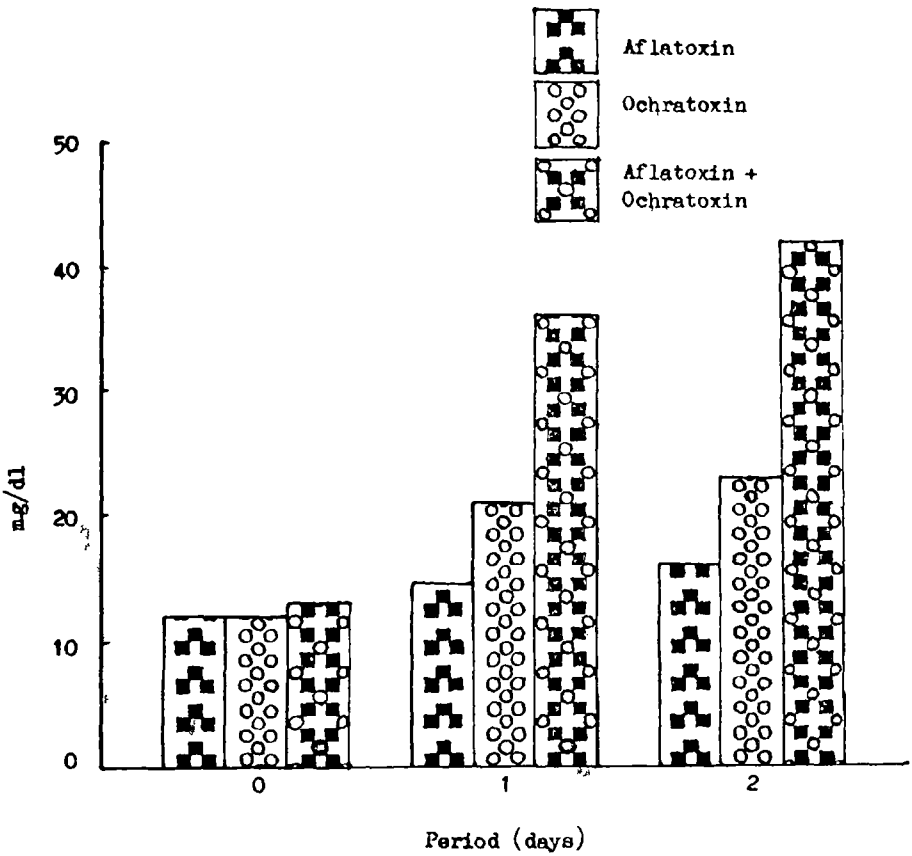


Fig.43. Blood urea nitrogen level - Groups X, XI, XII.

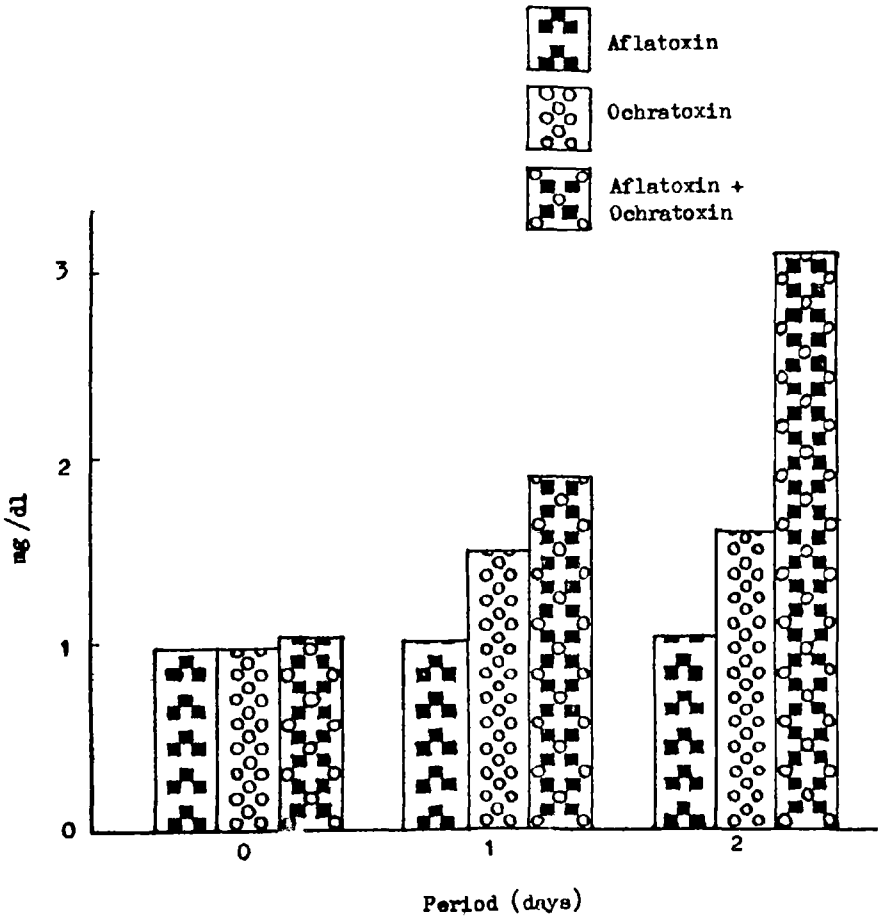


Fig.44. Creatinine level in blood - Groups X,XI, XII.

from each other. The basal surfaces of the cells were detatched from the basement membrane. The endoplasmic reticulum was found fragmented and the mitochondria were swollen. Lysosomes were few. The prominent Golgi complex was found disrupted. Alterations in the tight junctions were found to be a constant occurrence. The lateral cell membrane surface has been found to be increased in length and complexity. A bizarre lamellar transformation of the bile canaliculi was noticed occasionally which resulted from the alteration of the canalicular membrane from microvilli which were transformed by widening and flattening into multi-lamellar folds (Fig.38). The lumina of many canaliculi were filled with granular material and large irregularly shaped membraneous particles. Many of these membraneous structures were pleomorphic and variable in structure, but some components had features similar to microvilli.

DISCUSSION

DISCUSSION

A comparative assessment of production of ochratoxin by Aspergillus ochraceus and Aspergillus sulphureus on wheat and rice, under the static and shake culture methods was made. The strain of A. ochraceus employed was found to be a superior producer of ochratoxin under the static and shake culture in both the substrates than A. sulphureus. Hesseltine et al. (1972) also found that among all the species tested by them A. ochraceus produced the highest yield of ochratoxin in wheat and cracked corn. But, Lillehoj et al. (1973) reported higher yield of ochratoxin A by A. sulphureus in modified Czapek medium after 11 days of static incubation at 28°C. Frenk et al. (1971) reported chopped corn to be the best substrate for A. ochraceus for ochratoxin production. Polished rice and wheat bran yielded only lesser amounts of toxin. In experiments conducted by Hesseltine et al. (1972), wheat was found to be a superior substrate than rice. In the present study higher yield of ochratoxin was obtained in wheat under both static and shake culture techniques than in rice. Schindler and Nesheim (1970) observed maximum production of ochratoxin A in shredded wheat at 22°C after an incubation period of 19 to 21 days.

In the present investigation, the quantity of ochratoxin

produced by the two species of fungi in rice and wheat was higher in shake culture system than in static culture technique. In the experiments conducted by Hesseltine et al. (1972) shake culture technique was employed for production of ochratoxin from wheat, corn and rice to determine the optimal substrate and fermentation period required for maximum yield. The superiority of the shake culture system is that it effectively distributed the inoculum. The homogeneity of the culture system was maintained throughout the fermentation period preventing formation of mycelial mass which was characteristic of static mould culture and which restricted growth to individual wheat kernels to the fermenter atmosphere momentarily during each revolution. Agitation also facilitated heat exchange and prevented localised overheating of substrate. Lindenfelser and Ciegler (1975) have demonstrated that an agitation rate of 15 rpm resulted in the highest ochratoxin yields in wheat during a fermentation period of 12 to 19 days. The yield of ochratoxin was 4000 µg/g under these conditions. At '0' rpm the yield was only 100 to 200 µg/g with no agitation. It is evident then that under conditions of grain storage when the temperature varies between 20° and 30°C, there is enough scope for production of the toxin once the grain gets contaminated

with the fungi even though the conditions may be sub-optimal.

This study was undertaken to understand the pathological effects of ochratoxin in ruminants and the goat was employed as the experimental animal. Graded doses of the toxin were administered by the oral, intraperitoneal and intravenous routes. The intravenous and intraperitoneal routes were employed to avoid the toxin being hydrolysed in the rumen as it is known that ochratoxin A is metabolized in the rumen to ochratoxin alpha which has very little toxic action.

The diagnostic parameters employed in this investigation for assessing the pathobiological effects showed altered values depending on the dose (total quantity of toxin administered), duration and route of administration. In general, there has been depression of haemopoiesis and occurrence of necrobiotic changes in various organs more specifically in the kidney, liver and lymphoid tissues, with consequent clinicopathological manifestations. The fact that oral administration of toxin also effected structural alterations, even though in a milder degree, indicates that atleast a percentage of the toxin ingested is absorbed before being hydrolysed in the rumen or only a part of the toxin is hydrolysed.

The mechanism of action of ochratoxin A may be due to the presence of chlorine moiety in its structure. Chlorine alone may increase toxicity of ochratoxin A by increasing the dissociation of the phenolic hydroxyl group involved in the binding of toxin and serum albumin and possibly the binding of toxin to cellular protein. The toxin when becomes associated with cellular membranes, the free radicals and oxidative deterioration of polyunsaturated fats may potentiate the cell injury. Ochratoxin A has also been found to inhibit oxidative phosphorylation of rat liver mitochondria (Theron et al., 1966). Such inhibition of cellular respiration may initiate impairement of cellular functions in addition to structural alterations. When there is inhibition of oxidative phosphorylation, there is a rapid fall of cellular adenosine triphosphate concentration and corresponding rise in ADP, AMP and inorganic phosphate. Along with these changes the inner compartment of mitochondria lined by the inner limiting membrane undergoes contraction. This may reflect the efflux of ions accompanied by water. As the injury approached the point of irreversibility, flocculent densities considered to represent denaturation of matrical proteins appear within the inner compartment of swollen mitochondria. The appearance of the flocculent densities

correlates with the loss of matrical enzymes and by this time the mitochondria were irreversibly injured (Laiho and Trump, 1975). Mitochondria with damaged and altered membranes, matrical densities and flocculent deposits were consistently found in the present investigation. So it is very evident from this study that toxin damaged the membranes of the mitochondria, impairing enzyme activities with consequent impairment of cellular function. In a lethally injured cell, the damage to the mitochondria was not selective but a general representation of overall cellular damage.

Haematological values were altered in the animals in varying degrees. There was significant reduction of haemoglobin, packed cell volume and absolute lymphocyte count in most of the experimental groups. A lower daily dose of toxin through oral route needed a longer period for manifesting its effect because of the higher quantity of toxin needed for such an effect. Since there was a lag period before these changes were seen, it could very well be presumed that action of the toxin is more severe on the immature cells of the bone marrow than on the mature cells in circulation. However, Ribelin et al. (1978) reported elevated levels of haemoglobin and haematocrit values in caprine ochratoxicosis. This elevated value could very well be correlated to haemo-concentration due to

dehydration of the animals. The lowering of haematological values seen in this study is in agreement with those observed in clinical and experimental cases of ochratoxicosis in chicken, turkeys and mice (Peckham et al., 1972; Chang et al., 1981; Gupta et al., 1979). Significant increase in the blood coagulation time was noticed in Groups I, II & III which were given a total quantity of 120, 405 and 735 mg ochratoxin by the oral route. Such an observation was also noticed by Doerr (1977) in animals which received aflatoxin and ochratoxin together. Prior and Sisodia (1973) have reported increased prothrombin time in ochratoxicosis in chicken.

Reduction of total serum protein was evident from the 14th day onwards in goats that received ochratoxin at the rate of 1 mg/kg body weight per os. In the lower dose group, reduction was noticed only by the 12th week. Goats which received oral dose of the toxin at the level of 2.5 mg/kg did not show any significant alteration in 6 days. This may be due to the short term of toxin induction and small concentration of the toxin that might have reached the general circulation after rumen degradation. Results of the experiments conducted by Creppy et al. (1979) showed that ochratoxin A inhibits an enzyme essential for protein synthesis in eukaryotes. At the

ultrastructural level it has been found in this study that there was disaggregation of ribosomes and fragmentation of rough surface endoplasmic reticulum of hepatocytes. This indicates the impairment of protein synthesis in hepatic cells which is further reflected in the serum. Whether primary effect of the toxin was in the synthesis of RNA, which further impaired specific protein synthesis or due to the structural damage to the endoplasmic reticulum could not be very clearly ascertained from this study. The toxins could alter the chemical composition of the membrane caused by lipid peroxidation which could result in phase transitions within membranes leading to vesicle formation (Kavanau, 1965). This phase separation within the membrane might also result from failure to renew some membrane components due to inhibition of phospholipids and proteins which occurs during the intoxication of ochratoxin.

The reduction in concentration of serum proteins may also be the result of glomerular proteinuria subsequent to increased permeability of glomerular capillary epithelium to macromolecules. The splitting of the glomerular basement membrane, the loss of the foot processes and the occurrence of spaces between the glomerular basement membrane and epithelial cells seen in this study could be the structural

alterations to cause increased permeability to proteins. Renal loss of high molecular weight proteins was also observed in porcine nephropathy also (Krogh et al., 1974).

Significant increase in blood urea nitrogen and blood creatinine was noticed in most of the test animals including those which were given aflatoxin and ochratoxin together. The increase could be correlated to glomerular damage and to a certain extent to liver injury. Peckham et al. (1971) found increased blood uric acid in chicken while Szczech et al.(1973 a) reported high BUN values for pigs. The elevation in BUN and creatinine levels is an indication of the severity of kidney damage resulting in the retention of nitrogenous substances in the general circulation.

Significant rise in icterus index was noticed in animals of Groups II, III, VIII & X. The rise was observed only by the 45th day in Gr.II animals which were fed 1 mg/kg body weight. Administration of toxin at the level of 0.5 mg/kg body weight by intraperitoneal and intravenous routes did not cause rise in icterus index. But noticeable rise was observed in animals which were given 2.5 mg/kg by similar routes. In Group X (combined administration of ochratoxin and aflatoxin), the increase in icterus index was significantly different when compared with the

aflatoxin and ochratoxin controls. An increase in the serum bilirubin concentration was reported by Ribelin et al. (1973) in goats fed ochratoxin at the dosage level of 2 mg/kg body weight. Rise in icterus index is an indication of the hepatic damage caused by the toxin. In the combined toxicity with ochratoxin and aflatoxin, hepatocellular necrosis was more pronounced than when individual toxins were given. Aflatoxin is a known potent hepato-toxin and it is quite natural that severe liver damage resulted when there was combined toxicity. The hepatic cells are damaged to such an extent that they cannot perform their excretory function. In addition the swollen hepatic cells might block the bile canaliculi which result in bilirubin-diglucoronide to be reabsorbed into the blood. Maryamma and Sivadas (1975) observed a similar rise in icterus index in aflatoxicosis of goats.

Serum level of ALP was increased significantly in almost all groups. Rise in ALP level was also observed in combined toxicity of ochratoxin and aflatoxin. Ribelin et al. (1978) observed an increase in serum ALP in the goat dosed orally with ochratoxin A at the level of 1 mg/kg body weight. When higher doses of toxin were given they observed decline in enzyme level. The increase in ALP level in the different treatment groups in the present

experiment is probably related to epithelial damage of the renal tubules and hepatocellular injury. Ochratoxin might have affected membrane permeability of cells containing this enzyme. An increase in the activity of ALP in proportion to the concentration of ochratoxin was noticed in chicks by Liker et al. (1978). Increased urinary excretion of ALP has been reported in dogs. This was correlated with reduction in ALP activity in all the proximal tubules which was demonstrated histochemically in dogs given ochratoxin A. ALP was reduced corresponding to the injury of proximal tubules (Krogh et al., 1976 a). Enzyme histochemistry employed in the present study also showed a reduction in ALP activity in the proximal convoluted tubules and liver cells.

Significant rise in serum SGOT was observed in Gr.III (0.5 mg/kg - oral) animals by the 32nd week. In Gr.V animals rise was evident by the third day and in Gr.VIII and Gr.IX by the seventh day. SGOT level of the goats under combined toxicity was significantly high from the ochratoxin and aflatoxin controls. Animals that received the highest dose of toxin by intravenous and intraperitoneal routes also showed increase in enzyme level. Increased urinary excretion of GOT, LDH and ALP

was reported in pigs and dogs (Szczech et al., 1973 c, 1974 a).

The changes observed in the physical and chemical constituents of urine are consistent with the histopathological changes in the renal tubular epithelium and glomeruli. Berndt and Hayes (1973) observed altered urine osmolality, increased urinary protein excretion, and presence of ketones and glucose in urine of rats given ochratoxin A. It was suggested that increased urinary protein excretion may reflect interference with protein reabsorption. Munro et al. (1974) observed reduced urine volume, decreased pH, increased specific gravity and increased protein in urine in rats. Szczech et al. (1973 c) observed polyuria, glycosuria and proteinuria along with increased urinary excretions of LDH, ICDH and GOT in pigs which were exposed to A. ochraceus or crystalline ochratoxin A. Proteinuria may be due to an increase of glomerular permeability to macromolecules. Granular casts, necrotic renal epithelium and high protein concentration and enzymes had been also reported in urine of dogs (Szczech et al., 1973 a).

Histopathological changes in organs and tissues varied in intensity depending on the route, total quantity of ochratoxin and concentration of ochratoxin administered.

Severity of lesions in organs were in the following descending order : kidney, liver, intestines, stomach, lymph nodes, spleen, thymus, genital organs and endocrines. In the kidneys, the intensity of lesions was more in the proximal convoluted tubule. Henle's loop, distal convoluted tubules and glomeruli were also affected but to a lesser extent. The epithelium of the collecting tubules also showed degenerative and necrotic changes. Changes were more prominent and widespread in the animals that were given the toxin by intravenous and intraperitoneal routes than in animals which were given the same quantity of ochratoxin by the oral route though the difference was mainly in the degree of severity. Ribelin et al.(1978) reported that rumen is a major site of detoxification by hydrolysis for ochratoxin A. This explains the lesser intensity of pathobiological effects seen in the experiments with oral feeding. Elling (1979) demonstrated reduction in the activity of NADH tetrazolium reductase and succinate dehydrogenase in the epithelial cells of the proximal convoluted tubules of pigs in experimental ochratoxicosis. Reduction in activity of these enzymes may cause decreased function of the tricarboxylic acid cycle and of the respiratory chain resulting in reduction of oxidative phosphorylation. In turn, there is reduction

in energy production in these cells which may be the obvious reason for the histological lesions and impairment of function. Occurrence of lesions in a focal pattern may be due to the fact that morphological lesions do not appear until the biochemical alterations have reached a threshold level. In areas where extensive destruction of brush border occurred, reduction in ALP was observed. Changes in the proximal convoluted tubules suggest the primary site of deposition and action of this toxin to be the proximal tubules. This corresponds with the demonstration of ochratoxin A at the site by immunofluorescence technique (Elling, 1977). Chu (1971) demonstrated interaction between ochratoxin A and albumin and suggested that albumin may serve as a transport molecule for ochratoxin. As albumin in the glomerular filtrate is absorbed by the proximal tubule, it could be a mechanism by which ochratoxin reaches the cells of the proximal convoluted tubules. Ochratoxin A may also reach the tubules through the peritubular capillaries. Multi-focal ischaemia in the kidneys of rats given various kinds of nephrotoxic chemicals was demonstrated by Oliver (1953). Both ischaemic and cytotoxic lesions occurred in cases of acute tubular necrosis in man. In case the nephrotoxin causes damage to the tubular epithelium primarily by a

mechanism involving luminal contact, repair and recovery are the most probable sequelae. Regenerative changes were observed only in few tubules in the present study. This was more conspicuous in the lower dose group. Regeneration of tubular epithelium occurred as new cells were produced by the few proximal tubular epithelial cells that survived. These cells used the existing basement membrane for tubular reconstruction. Fibrosis was not a feature in this condition. It can be seen that the factors which favour repair of renal tubules damaged by nephrotoxic chemicals include necrosis that leaves some residual epithelial cells to repopulate denuded basement membranes and the lack of pre-existing renal disease (Cuppage and Tate, 1967).

The granular eosinophilic debri observed in the lumen of the affected tubules represent the desquamated and disintegrated epithelial cells, protein particles and other deposits in the urinary filtrate. The PAS positive globular masses in the lumen, basement membrane of tubules and some of the Bowman's capsule indicate deposition of glycoprotein in these areas. This suggests influence with the reabsorption of glucose and protein by the tubules which get deposited on the basement membranes or form globular masses within the tubules. Eosinophilic granules

were present in the epithelial cells of some of the proximal convoluted tubules in the lower dosage groups. Munro et al. (1974) had observed such eosinophilic granules in the proximal convoluted tubular cells of rats given ochratoxin at the rate of 5.00 ppm in diet. Durant et al. (1964) suggested that eosinophilic granules are due to reabsorbed plasma protein and is a result of the ageing process which was accelerated by ochratoxin A.

Necrosis of renal tubular epithelium, especially in the proximal convoluted tubules, was observed in pigs fed with cultures of A. ostianus that contained ochratoxin A (Szczech et al.,1973 c). In chronic porcine nephropathy, different segments of the nephron and interstitial tissue were affected (Elling, 1977). Necrosis and desquamation of renal tubular epithelium and presence of eosinophilic granular casts in the proximal and distal convoluted tubules observed in the present study have also been reported in experimental ochratoxicosis of dogs (Szczech et al., 1973 a). The difference between severely affected and moderately affected kidneys was the greater number of necrotic and vacuolar cells within a tubule and the greater number of affected tubules; animals given higher doses had more affected tubules with more numerous necrotic and vacuolated cells. Carlton and Szczech (1973)

demonstrated different degree of necrosis of the epithelium lining the proximal convoluted tubules and presence of proteinaceous casts in the lumen of tubules in guinea pigs dosed with ochratoxin at the rate of 5 and 10 mg/kg body weight. They have also described swelling, degeneration and necrosis of the epithelial cells of the proximal convoluted tubules and subsequent occlusion of the lumen in hamsters.

Renal lesions of ochratoxicosis in rats were not confined to the proximal convoluted tubules. Though necrosis of the epithelial cells of the proximal convoluted tubules was the most prominent renal change, depending upon the dosage of ochratoxin A, tubules of the outer zone of the medulla and some collecting tubules also were affected and contained necrotic epithelium (Munro et al., 1973, 1974; Purchase and Theron, 1968).

Shadmi et al. (1974) described severe nephrosis and hepatosis in kids due to feeding of ochratoxin. Renal lesions were mostly confined to the proximal tubules and were less in severity compared to lesions in liver.

Renal fibrosis was not a feature in the present study even though thickening of the basement membrane and moderate dilatation of the renal tubules were noticed. Chronic exposure to low levels of ochratoxin A for larger periods

is apparently required for the development of renal fibrosis. Szczech et al. (1973 c) reported that fibrosis was not a feature of acute ochratoxicosis in young pigs.

Extrarenal lesions observed in the present study were mainly of liver, stomach, intestine, lymph nodes, thymus and spleen. Testes/ovary, thyroid, adrenal and pituitary showed mild lesions in animals exposed to toxin at the level of 2.5 and one mg/kg body weight.

Pathological lesions were observed in the liver of all animals exposed to ochratoxin. The severity of changes was in direct proportion to the total amount of toxin administered. The lesions were more extensive and severe when the toxin was administered intravenously or intraperitoneally than when the oral route was adopted. Degenerative changes ranging from cloudy swelling and fatty change to necrosis of hepatocytes in focal areas were seen in animals which were given the toxin per os. Frank necrosis of hepatocytes, haemorrhages, presence of Mallory bodies, disruption and collapse of reticular network in focal areas, bile stasis and mild degree of bile ductular proliferation were the lesions in the animals which received ochratoxin at higher levels by intraperitoneal and intravenous routes. Similar changes but in a milder degree were observed in other experimental animals.

Mild vacuolar changes were seen in scattered epithelial cells lining the papillae of rumen and reticulum in the higher dose groups. Few ulcers were observed in the rumen of animals which received ochratoxin orally and intraperitoneally. Hyperaemia and oedema of abomasum, varying degree of degenerative and inflammatory changes, necrosis of the lining epithelial cells and goblet cell hyperplasia of intestines were the other lesions in the gastrointestinal tract of experimental animals. All these reactions are due to the action of the toxin on the epithelial cells and consequent reaction of tissues. The oedema noticed in the gastro-intestinal tract and in other organs is due to the injury of the vascular endothelium.

Necrosis of lymphocytes and depletion of lymphocytes from the spleen and lymph nodes and lymphocytopaenia were observed in all animals which were given toxin by the oral route and in the animals given toxin at 2.5 mg/kg body weight level i/v, and i/p and at 1 mg/kg body weight level i/p. Lymph node oedema was observed in the highest dose level group given ochratoxin intraperitoneally. Lymphoid depletion in the thymus was seen in two of the experimental animals. Degeneration of seminiferous epithelium was observed in the testes of the animals that received ochratoxin by oral route at the level of 2.5 mg and 1 mg/kg body weight and in an animal which received 1 mg/kg body weight level of ochratoxin intraperitoneally. This implies that male animals subjected

to ochratoxin at high levels or over prolonged periods may become poor semen producers. Degeneration and necrosis of the germinal epithelium were also observed in the ovary of animals that were given toxin at the rate of 2.5mg/kg body weight per os and by i/v route. This indicates the role of ochratoxin in causing sub-fertility or infertility in goats and needs detailed investigation. Few follicles of the thyroid were found enlarged in this group of animals. Slight reduction in the number of acidophils of the pituitary and focal degeneration of acidophils were noticed in the Gr.V animals (1 mg/kg body weight i/p). Widening of the zona fasciculata of adrenals also was observed in this group. Lesions in the adrenal and pituitary were observed in Group IV (1 mg/kg oral), Group V (2.5 mg/i/p) and Group VII (2.5 mg/i/v) animals. Vacuolar degeneration and necrosis were seen in some of the acinar cells of the pancreas in the animals of the latter group. It is likely that the pathological alterations seen in these organs are a reflection of general process of cellular injury than any specific target action.

The synergistic effect of aflatoxin with ochratoxin is very clearly manifested in the severity of pathological lesions and in the altered values of the levels of some enzymes, icterus index, BUN and creatinine in the blood

(Fig. 39 to 44). The degenerative and necrotic processes in the liver and kidneys were distinctly prominent than those in other groups. The pathological changes involved all the different segments of the nephron. Alterations due to the combined toxicosis were also prominent in the gastrointestinal tract, adrenals, pituitary and thyroid whereas lesions in these organs were not of such severity when either aflatoxin or ochratoxin was given alone. Depletion of lymphoid cells was observed in spleen and thymus. Clinical signs of illness, biochemical alterations in blood, gross and histopathological lesions in organs strongly suggest synergistic action of the toxins.

Synergistic effects of ochratoxin and aflatoxin in body tissues have been demonstrated earlier in broiler chicken. Increase in liver lipid content has been mainly attributed to the toxic action of aflatoxin. The kidney was the most sensitive organ to the combined toxicity of aflatoxin and ochratoxin A in birds (Huff and Doerr, 1980, 1981). Campbell et al. (1981) demonstrated decrease in circulating lymphocytes and increase in heterophils in aflatoxicosis of broilers. Ochratoxicosis decreased eosinophil population. Ochratoxin and aflatoxin together reduced the immune status of chicken. Doerr and Huff (1980, 1981) demonstrated increased prothrombin time, diminution of packed cell volume,

haemoglobin, total serum protein and albumin by combined toxicity in broiler chicken. During combined treatment the uric acid levels were significantly elevated. The elevation in uric acid was more than that observed in birds which received ochratoxin alone. Hati et al. (1981) have reported slight necrosis of hepatic cells and degenerative changes in kidneys of rats which were fed aflatoxin B1 and ochratoxin A together. Such pathological alterations did not occur in animals given either toxin alone. As is evident from the clinico-pathological and pathoanatomical changes combined toxicity causes very severe deleterious effect on the physiopathology of the animals. This brings to focus an important aspect that toxins, when they act together, even if they are present in low doses cause severe damage to cellular structure and function.

Ultrastructural examination of tissues in acute ochratoxicosis revealed extensive organellar and membrane destruction both in liver and kidneys. The plasma membranes of hepatocytes, the epithelial cells of glomeruli and the proximal convoluted tubular cells showed marked configurational changes probably due to the direct action of the toxin. Such a change could also result from secondary hypoxia or anoxia. The vacuoles noticed in the epithelial

cells of proximal convoluted tubules might represent invaginations of the cell surface and might contain ATPase indicating their nonlysosomal property. Trimmer (1975) found such vesicles in isolated hepatocytes when exposed to the toxin, phalloidin. The vesiculation and fragmentation of the endoplasmic reticulum and disaggregation of the ribosomes of the hepatic cells indicate an impairment of protein synthesis. The proliferation of smooth endoplasmic reticulum seen in hepatic cells when exposed to a wide variety of toxic substances was not noticed in the present investigation. A probable factor for this might be the high level of toxin exposure which inflicted severe injury to the cells. Whenever, there is marked hypertrophy of SER, there is a corresponding increase in the activity of some of the oxidases of biotransformation such as NADPH - cytochrome reductase and cytochrome P 450, which are closely linked to or integrated into an electron transport chain of the ER membrane. The enzymatic activity of the biotransformation system affects the intensity and duration of the effect of endogenous and exogenous substances which are metabolized by the liver.

The liver cells contained large number of lipid droplets within the endoplasmic reticulum and as smooth membrane bound vacuoles (liposomes) which coalesced to form large

lipid droplets in the cytoplasmic matrix. The liver plays a key role in regulation of lipid metabolism of the body by synthesizing and transporting relatively large amounts of lipid to the rest of the body. Morphologically, the endoplasmic reticulum is the principal site of lipid synthesis; some of the lipoprotein are transported to the Golgi complex where remodelling, concentration and segregation take place, before they are released into the space of Disse.

Many theories have been advanced to explain the occurrence of liposomes in liver subjected to toxic injury. However, one concept that seems acceptable is that lipid accumulation is secondary to depressed protein synthesis, a point supported in this study by the disorganization of the ER. It is believed that in normal situation a lipid acceptor protein is produced by RER and this combines with triglycerides to form very low density lipoproteins which are released into blood. It is possible that the toxin damaged the RER and depressed the synthesis of this protein but not the synthesis of triglycerides resulting in the accumulation of fat in the liver. The vesiculated and fragmented appearance of ER along with degranulation of ribosomes has also been reported in a variety of conditions of toxicity and deficiencies. Alteration of the chemical

composition of the membrane caused by lipid peroxidation could cause phase transitions within certain membranes which in turn might lead to budding off vesicles. It might also be possible that phase transitions within the membrane might result from failure to renew some membrane components due to inhibition of synthesis of phospholipids and proteins. There is ample biochemical evidence that protein synthesis is in fact impaired by drugs which produce the change. The increase in the perichromatin granules in the nucleus seen in this study also supports this contention. Various studies have indicated that an increase in the number of perichromatin granules may be an indicator of aberration in protein synthesis. It has been postulated that at least some of the perichromatin granules come from nucleolus, because an accumulation of perichromatin granule is seen in the juxta nucleolar region after administration of various toxins including aflatoxin B_1 (Derenzini and Moyne, 1978).

The epithelial cells of the proximal convoluted tubules showed lysosomal structures of various morphologic patterns; most of them being autolysosomes and multivesicular bodies. Myelin figures were also consistently encountered in cells showing necrobiotic changes. Increase in the number of cytolysosomes indicates a sub-lethal intracellular focal

injury caused the mycotoxin. Myelinosomes have been produced in numerous cell types by a variety of drugs. It is quite possible that in ochratoxicosis myelinoid bodies are formed because the toxin became bound to lipidic membranes thus making them difficult to be digested. It could also be that the toxin selectively inhibited lysosomal enzymes. The power of lysosomal hydrolases to degrade lipid is limited and variable and myelinoid bodies persist when the lipids became hydrated. Otherwise the lipid may persist as droplets or electron dense granules.

Fragmentation of the nucleolus was observed in most of the cells. In view of the parallels between structural and biochemical changes it has been suggested that nucleolar fragmentation reflects the metabolic derangement due to ATP deficiency or inhibition of RNA synthesis secondary to a metabolic disturbance (Shinozuka et al., 1970).

As seen in electron microscopy in this investigation the Mallory body is composed of aggregates of fibrils without a limiting membrane. These fibrils could have resulted from a degenerative process or the result from new synthesis and to contain contractile protein. Of the three morphological variants, the types seen in the hepatic cells represent the type I (Denk and Eckerstorfer, 1977) consisting of bundles of fibrils in parallel arrays which measure 9 to 12 nm.

Mallory body filament assembly includes polypeptides of the cytokeratin class of intermediate filaments and also higher molecular weight polypeptides normally found only in the cytokeratins of mature keratocytes of the epidermis (French, 1983). Mallory bodies form and grow in size not as a result of increased protein incorporation, but rather because they are resistant to dissolution.

Fusion of foot processes of podocytes was seen in the glomerulus. This is characterised by a disappearance of the regularly spaced small foot processes and the appearance of much larger irregular cell processes or segments of podocyte cytoplasm resting on the basal lamina. It seems unlikely that this change results from actual fusion of neighbouring foot processes of different podocytes, although images suggesting fusion of the cell membranes of two neighbouring foot processes have been seen on rare occasions. Scanning electron microscopic studies has shown that the appearance of the so-called fusion of foot processes is more likely due to a swelling and retraction of foot processes so that capillary wall is covered by large swollen processes or segments of podocyte cytoplasm (Buss and Lamberts, 1975). It is evident that these changes might be the major responsible factor aiding passage of macromolecular substances through the glomerulus resulting in the various clinicopathological manifestations. Apart from the organellar

changes in the cells, this morphological alteration seen at the ultrastructural level along with the fragmentation of the basement membranes in the glomerulus could be considered as a significant observation in ochratoxicosis in the goat.

The results of this study indicate that pathobiological manifestations are more intense when toxin is administered parenterally. It is quite evident that when orally ingested, higher levels of toxin and a longer duration are required for manifestation of toxic symptoms. Addition of aflatoxins further enhanced and intensified the pathological alterations and clinical symptoms. Ultrastructurally, it is seen that the membrane system in the cells are severely affected by the toxin in addition to alterations of other structures.

Further ultrastructural, histochemical and biochemical studies are needed employing sublethal doses to dilineate the exact structural and functional changes caused by ochratoxin. It is also necessary to find out ways and means to destroy or detoxify the elaborated toxin in the feed so that field cases of ochratoxicosis can be minimised.

SUMMARY

SUMMARY

An experimental study was undertaken to delineate the pathological effects of ochratoxin in goats. Ochratoxin was prepared in the laboratory and a comparative assessment of production of ochratoxin by A. ochraceus and A. sulphureus on wheat and rice under static and shake culture methods was made. A. ochraceus was found to be a better toxin producing strain in both substrates under static and shake culture systems and wheat was found to be a better substrate than rice.

For the toxicity studies, Saanen - Malabari cross-bred goats of 1 to 3 months age were employed. Purified ochratoxin prepared in the laboratory was administered to the animals by oral, intraperitoneal and intravenous routes. Two different dose levels, 2.5 mg/kg body weight, 1 mg/kg body weight and 0.5 mg/kg body weight were adopted for all treatments.

Clinical signs, haematological and biochemical alterations, pathological alterations in urine, morbid anatomy, histopathological lesions and ultrastructural changes in tissues and cells were studied. Crystalline toxins (Makor Chemicals, Israel) were used for the study of synergistic effect of ochratoxin and aflatoxin. The toxins were administered by the i/p route.

Varying degree of clinical manifestation and haematological changes were noticed in animals subjected to ochratoxin administration. Basically the animals became weak and listless and there was reduction of total erythrocyte count, PCV, haemoglobin value, and lymphocyte count. Serum protein level was found lowered. BUN, and creatinine level and coagulation time were high. But the change and the degree of variation depended on the dose, total quantity and route of administration of the toxin and the duration of the experiment. The effect was more severe when Aflatoxin and Ochratoxin were administered simultaneously.

Decline in the concentration of total serum proteins was noticed in 1 mg/kg and 0.5 mg/kg oral dose group from second and 12th week onwards respectively. Reduction in serum protein concentration occurred in the 2.5 mg/kg and 1 mg/kg levels of ochratoxin by the parenteral route. Significant increase in BUN and creatine levels were noticed in most of the test animals. Rise in icterus index was observed in the 2.5 mg/kg dose level groups when the toxin was administered by the parenteral route. In the oral dose group of 1 mg and 0.5 mg/kg, rise in icterus index was observed only after 1½ months and 3 months respectively. Rise in icterus index was pronounced in animals that received ochratoxin and aflatoxin simultaneously. Rise in ALP was

noticed in almost all test groups including those which were given ochratoxin and aflatoxin. Significant rise in SGOT was noticed in the long term toxicity group by the 32nd week and in animals that were given 2.5 mg/kg rate of toxin by parenteral route. Rise in SGPT was significant during the terminal stages in the animals given 2.5 mg and 1 mg/kg rate of ochratoxin by the oral route and in animals employed in combined toxicity studies. Rise in ALP was noticed in all test groups including those which were given ochratoxin and aflatoxin simultaneously. Rise in SGOT was noticed in the long term toxicity. The significant changes in the urine were a lowering of pH, albuminuria, and the presence of epithelial cells and casts.

The important pathological effect of ochratoxicosis was mainly necrobiotic. These changes were observed in varying degrees depending on the total quantity of toxin administered and route of administration. Severity of lesions in organs were in the following descending order: kidney, liver, intestines, stomach, lymph nodes, spleen, thymus, genital organs, endocrines. In the kidneys, the order of intensity of pathological changes was : proximal convoluted tubules, Henle's loop, distal convoluted tubule, glomeruli, collecting tubules. Retrogressive changes of different degrees and necrosis of the lining epithelial cells of tubules

and endothelium and epithelium of glomeruli were the important alterations. In the higher dose groups, degenerative changes in glomeruli and Bowman's capsule including shrinkage of a few glomeruli occurred. Thickening of the basement membrane of the tubules and the Bowman's capsule was also observed. Proteinaeous material was present in the Bowman's capsule in the highest dose groups. Necrosis and desquamation of renal tubular epithelium of the proximal and distal convoluted tubules were associated with the presence of eosinophilic granular casts and PAS positive bodies in the lumen in several sites. Renal changes of degeneration and necrosis were found to be more intense when ochratoxin and aflatoxin were administered simultaneously.

Besides the kidney, pathological lesions were observed in the liver, stomachs, intestines, lymphoid organs, testes, ovary, thyroid, adrenal and pituitary. Important changes noticed in the liver were fatty infiltration, necrosis of hepatocytes and haemorrhage. Bile stasis, mild bile ductular proliferation and focal disruption and collapse of reticulum were noticed in the higher dose groups. The hepatic changes were most severely manifested in the combined toxicity of ochratoxin and aflatoxin. Severe and extensive degeneration and necrosis of hepatocytes sparing only few cells around

the central vein could be noticed. Many of the hepatocytes were heavily infiltrated with fat droplets. Extensive haemorrhages occurred in the parenchyma. Mallory bodies were present in few liver cells. Moderate biliary hyperplasia was seen. Alteration in lymph nodes and spleen were due to necrosis and subsequent depletion of lymphocytes. Lymphoid depletion in thymus was noticed in two animals. In the animals that were given 2.5 mg and 1 mg/kg body weight by oral route, and in the 1 mg/kg body weight dose group by i/p route, degeneration of seminiferous epithelium was evident. Lesions in ovary ranging from focal degeneration to necrosis of germinal epithelium were seen in the highest dose groups by oral and i/v route of administration. Lesions in the endocrines included focal degeneration of acidophils in pituitary, widening of zona fasciculata of adrenals, and focal degeneration and necrosis of pancreatic acini. Patnological alterations in pituitary, adrenal and thyroid were more severe in the animals which were given ochratoxin and aflatoxin combinely by i/p route.

Ultrastructural changes in the kidney and liver of animals which received ochratoxin at the dose level of 2.5 mg/kg body weight (i/v) revealed severe changes in the cell structures. The microvilli of the epithelial cells of proximal convoluted tubules were disoriented, disrupted and

fused. In some locations there was complete loss of microvilli. Vacuoles some of which containing electronlucent material or electron dense myelinoid bodies were present in the apical cytoplasm of the cells. Mitochondrial damage of varying degrees could be seen. Increase in size of mitochondria, changes in densities of matrix, disorientation, shortening and reduction of cristae and breakage in the limiting membrane were the important changes. The damaged mitochondria were incorporated into cytolysosomes in the form of whorled membranes and lysosomal bodies. The endoplasmic reticulum had fragmented in many locations. Clumping of chromatin, redistribution of their granular and fibrillar components, nucleolar fragmentation and disruption of nuclear membrane were the nuclear changes seen. Ultrastructural alterations of lesser severity were noticed in the lining cells of the distal convoluted tubules and Henle's loop. In the glomerular epithelial cells there was moderate chromatin clumping, disaggregation of ribosomes from endoplasmic reticulum and fragmentation and destruction of filaments. Microtubules were seen. Disruption of the regular arrangement of the foot processes simulating fusion of several of them and also their destruction were seen in the podocytes. Destruction of the integrity of the basement membrane was also observed in some glomeruli. In the endothelium of the

capillaries, pinocytic vesicles, fragmentation and even absence of microtubules were noticed. There was moderate destruction of cytoarchitecture of the mesangial cells.

In the liver, some of the hepatocytes showed extensive alterations of the plasma membrane and organelles. Bleb like protrusion of the cytoplasm into the sinusoids were observed in some. Vesiculation, fragmentation and dissolution of the membrane of the RER, disaggregation of ribosomes and helical configuration of polyribosomes were seen. The Golgi complex showed dilatation of cisternae and vesicles with complete loss of morphological identity of some. Large number of cytolysosomes were present. Salient alterations in the morphology of mitochondria were swelling of the matrix, disorientation, shortening and reduction in number of cristae, occasional cavitation of the matrix and breaks in the lining membrane. Mallory bodies and lipid droplets were present. Clumping, condensation and disappearance of chromatin and fragmentation of nucleolus and nuclear membrane were seen. Alterations occurred in the tight junctions and the bile canaliculi presented a bizarre lamellar transformation.

Pathological alterations were more pronounced when ochratoxin was administered by the parenteral route. Oral

administration of toxin also effected structural alterations indicating that at least some fraction of ochratoxin have escaped the process of degradation into the nontoxic ochratoxin alpha. Higher doses of toxin induced changes in kidney and liver with more or less equal intensity. Morphological alterations in other organ systems were milder in nature.

It is surmised that the chlorine moiety in the toxin structure could be mainly responsible for the absorption of toxin by the cellular protein and the association of it with the unit membrane causes release of free radicals and deterioration of polyunsaturated fats, potentiating cellular damage. The impairment of cellular function might be due to inhibition of oxidative enzymes which seems possible from the extensive ultrastructural alterations observed in the mitochondria and ER. Biochemical changes like high BUN and creatinine level were evidently due to the necrobiotic changes in the kidney. Rise in ALP, SGOT and SGPT indicate hepatic and renal injury. Interference in the synthesis of proteins by damaged hepatic cells and escape of protein molecules due to alterations in the podocyte foot processes and basement membranes may account for the reduced serum protein levels. The nature of organellar destruction with configurational changes in cells is indicative of the toxic potency of the mycotoxin on the biological system

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