HISTOCHEMICAL - BIOCHEMICAL INVESTIGATION OF THE KIDNEY OF MALLARD *(ANAS PLATYRHYNCHOS DOMESTICUS)* EXPOSED TO MINING EFFLUENTS.

THESIS

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ZOOLOGY

BY

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M.Sc. (ZOOLOGY)

UNDER THE GUIDANCE OF

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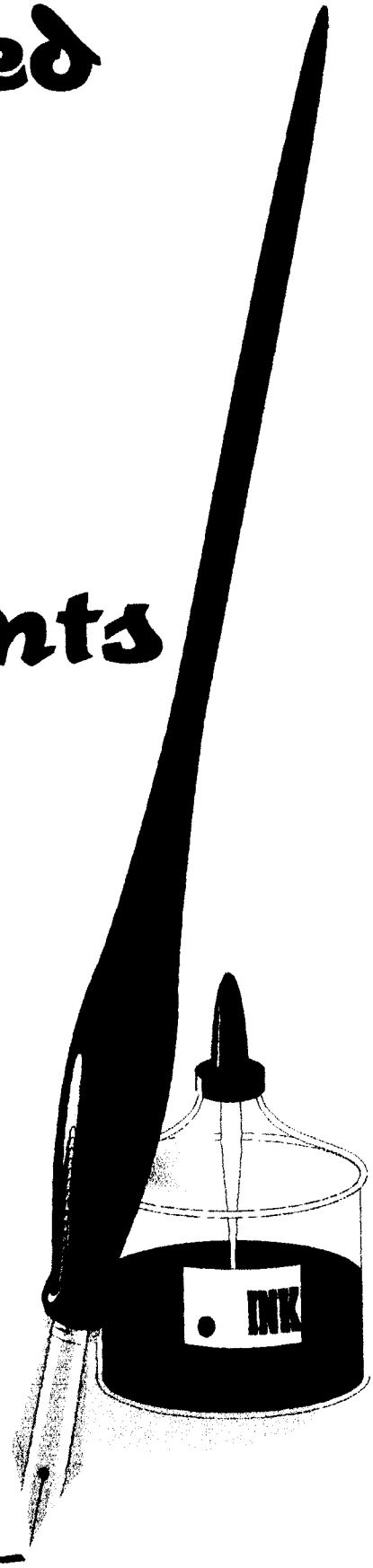
Dedicated

to

My Parents



STATEMENT

As required under the University ordinance 0.413, I state that the present thesis entitled **"HISTOCHEMICAL - BIOCHEMICAL INVESTIGATION OF THE KIDNEY OF MALLARD (*ANAS PLATYRHYNCHOS DOMESTICUS*) EXPOSED TO THE MINING EFFLUENTS"**, is my original contribution and that the same has not been submitted on any previous occasion. To the best of my knowledge, the present study is the first comprehensive study of its kind from the area mentioned.

The literature concerning the problem investigated has been cited. Due acknowledgements have been made wherever facilities have been availed of.

V. S. Bandiwdekar

Vinit S. Bandiwdekar

Certificate

This is to certify that the thesis entitled "**HISTOCHEMICAL-BIOCHEMICAL INVESTIGATION OF THE KIDNEY OF MALLARD (*ANAS PLATYRHYNCHOS DOMESTICUS*) EXPOSED TO THE MINING EFFLUENTS**", submitted by Shri Vinit Shashikant Bandiwdekar for the award of the Degree of Doctor of Philosophy in Zoology is based on the results of laboratory experiments carried out by him under my supervision. The thesis or any part thereof has not previously been submitted for any other degree or diploma.

14-5-96

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PREFACE

Goa rates highly in most tourist's itenery. It's wealth of natural beauty has been tapped and capitalized by the tourism industry. But this pretty picture - postcard perfectness is under threat from the second most important industry of Goa - Mining.

Goa is endowed with mineral wealth which has been the backbone of the Goan export economy. The economy and the environment, thus are pitted against each other. The capital oriented market supports the mining industry, whereas, the environmental lobbyists create a hue and cry about the ravage and pillage of the countryside.

Mining operations are conducted in order to retrieve the mineral deposits, to convert them into economically viable resources. The economic consideration has always been a priority concern with the environmental issue being relegated to the backseat. Thus, the mining industry continued its operations and proposed with no undue hinderances from environmental issues impinging upon their agenda. The stockpiles of dust and slag heaps stands mute testimony to the horrendous rigours borne by the environment.

Mining is an agency of pollution. Awareness regarding restoration of mine does not exist in majority of the mining areas leading to well - known environmental hazards like air pollution, noise pollution, water pollution, not to mention the inevitable soil pollution. Depending on the nature of ore being extracted, the mining practices, etc. waste areas result in having varied climate affecting the flora and fauna of the said area.

The flowing off of stored mine heaps in the form of runoff water, alter the natural habitats of the flora and fauna thereby leading to their depletion or even extinction in some cases. The faunal instinctual life styles prevent them from improvising necessary conditions for their survival in the circumstances. They cannot change the weather in their environment, hence extinction is a concrete

reality in the case of many species. Thus, surveillance and monitoring of the environment as well as the assessment of the change gains central importance.

Biomonitors are usually selected to complement physical monitoring, but may in some instance, provide the only available means of monitoring. For a biomonitor to be useful, it must respond in a sensitive way to changes in the variable for which it is a proxy measure. Environmental biological monitoring is concerned with determining changes in the physiological, anatomical and numerical state due to environmental stress, for example by correlating levels of chemicals in environmental media with concentrations found in living tissue. The importance of biological monitoring lies in the use of organisms to establish the integrated, collective impact of environmental stresses upon animals. Hence, it is obvious that an indicator species must possess characteristics that truly reflect the environmental stress to which it is being subjected. Back in '67 it was observed that bird of prey numbers were declining and breeding success was impaired by breakage of thin - shelled eggs that led to elucidation of the DDT pollution problem. In this case, birds of prey acted as bio - indicators.

Birds occupy a top position in the food chain. Hence, any change in their number of species can definitely give a warning signal to man. Studies on heavy metal pollution effect on birds is also very rare in India. Therefore, in the present investigation, the histological and biochemical changes due to mining effluents on Mallard (*Anas platyrhynchos domesticus*) kidney are studied.

The thesis is divided into seven chapters. The first chapter gives a historical perspective of mining pollution and briefly reviews the work done on the toxic actions of mining effluents on the birds. It also describes the understanding of the problem, parameters for the proposed study and gives the details of the research plan. The experimental techniques and experimental set ups are described in the second chapter. The third chapter describes the histopathology of the kidney. This chapter is divided into 2 sections. The first

describes the ultra structure, histochemistry and bioassay of kidney, where as the second describes the effect of mining effluents on urine.

The fourth chapter gives the retention pattern of the heavy metals from the effluents in kidney and serum and also the pattern of elimination of heavy metals through urine. The fifth chapter presents the mining effluent effects on blood. This chapter is divided into 2 sections. The first describes the haematological observations, whereas the second, the alterations in serum due to mining effluents. The sixth chapter discusses the results at full length and the seventh chapter presents the summary of the work done also suggesting scope for further work.

The thesis is concluded by Bibliography.

CONTENTS

CHAPTER - 1

INTRODUCTION

HISTORICAL PERSPECTIVE OF MINING POLLUTION :

Mining is one of the most common activities of ancient and modern world. Its references are found even in Vedas. Goa is rich in mining deposits and therefore, mining is an important part of the State's economy.

The State of Goa is rich in minerals such as iron ore, manganese ore, bauxite, silica, sand, high magnesia limestone and clay (Swaminathan, 1982). Southern parts of Goa has substantial reserves of bauxite, and aluminous laterite. Presently, the major extractive industries of Goa are iron and manganese mining. Mining is the dominant industry of Goa as today mining operations in the state, are mainly export oriented. Mining is an important activity for Goa since it is a significant foreign exchange earner not only for the state, but also for the country. It contributes to about 10% of total economy (Sardesai, 1985) with iron ore being the most predominant in terms of both production and export (Veeresh, 1989 ; Ganihar, 1990). Natural factors like the presence of a coast line, a very good natural harbour at Marmugao and a number of navigable perennial rivers and streams under the economic exploitation of the deposits make it not merely feasible, but also considerably easier and less expensive. Marmugao, the second biggest port in India handles more than 10 million tonnes of iron ore for export annually. In the process of development, intensive use of natural resources has adversely affected the environment, leading to ecological imbalances.

Deposits of iron and manganese ore occur in an arcuate belt stretching over a length of about 95 km in N - S direction. Width of the belt varies from 3.5 - 7.0 km. Based on iron concentration, iron ore deposits can be broadly divided into 3 zones. The North zone dominant in iron ore comprises about 60% of total iron ore reserves of Goa ; the Central zone comprises of iron ore and ferruginous manganese ore deposits ; the South zone comprises mainly of ferruginous manganese ore deposits. The manganese content increases from

North to South. The present reserves of iron ore are around 1400 million tonnes and is expected to last for another 30 - 40 years (i.e by the year 2020 to 2030) and hence the reject material will be over 5 billion tonnes, excluding the present, already existing 1 billion tonnes.

Untawale (1994) expressed that mining industry in Goa caused immense ecological damage due to improper planning. Land overburdened with reject dumps and mining on the whole, drastically changes the microclimatic conditions which leads to degradation of flora and fauna (Paliwal, 1989). Reports on disposal of waste rock affecting landscape and environment (Subramanyam, 1986) ; ground water depletion (D' Souza and Nayak , 1994) ; well water depletion (Swaminathan, 1982), are well documented.

Pollution may be considered as "the introduction by man into the environment of substances or energy liable to cause hazards to human health and harm to living resources and ecological systems"(Holdgate, 1979). Mining also causes various types of pollution. Noise pollution due to use of about 1000 - 5000 kg explosives for blasting per mine per month (D'Souza, 1991) causes irritation to people due to ground vibrations (Paliwal, 1989) as well as cracks in houses and has deafning effect (Desai, 1990). Noise produced by mining is reported to be of 85 dbs.

Water pollution is caused due to erosion and washoff/runoff from the mined out area and waste dumps (Chaudhari, 1994). In Goa, ore is found below water table and to extract this ore, water is pumped out from the pits in turbid conditions. This water also creates environmental disturbances in the surrounding areas. The rainy season witnesses flowing of red, muddy water from the sides of overburden dumps as well as low-grade ore dumps, forming gullies. The dump surface then becomes hard due to encrustation - a feature common to iron ore rich lateritic soils. Torrential rains rip this encrusted surface causing sheet erosion, rill erosion and later gully erosion, reaching the roads. The run-off carrying silt from the rejection dumps flows down freely into the

surroundings. The tributaries which pass through the mining zone contain very high concentration of total suspended matter (Nayak, 1993). Rain water carries huge quantities of silt and slimes into agricultural land which gets hardened on drying (Swaminathan, 1982).

Soil, the most important source of nutrients for sustenance of life, is least capable of reclaiming itself (Coleman, 1979). As a result of exploitation of useful metals, ground left behind is of a seriously affected quality, deficient in nutrients, unable to support normal ecosystem of the area, and even toxic as in some cases. Excavations as a part of mining activity further degrades the environment. Heavy rainfall during monsoons further add to the misery of soil erosion and pollution of environment in and around the mines. Soil pollution takes place at the actual site of digging inside the mines and secondly, at the paddy fields or agricultural lands located at the foot-hills.

Mining activities like blasting operations, loading of dumpers, vehicular movement etc. all raise collosal amount of dust into the atmosphere making them easily air - borne, as the roads inside as well as outside the mines are not tarred. (Desai, 1990), due to which visibility is also hampered.

High concentration of iron and less concentration of plant nutrients make them not suitable for plant growth (Shetty, *et.al.*, 1987). Reject material is also poor in organic matter content (Veeresh, 1989). Morphological and reproductive characters of plants grown on mine reject dumps were affected. (Torne and Gaonkar, 1989 ; Veeresh, 1989 ; Nyabuto, 1989 ; and Coelho, 1990). Inhibition in germination and reduction in root length in germinating seeds is also reported. (Desai, 1990).

Mine rejects are poor in microbial population because of low content of organic matter (Torne and Gaonkar ; 1989). Reduction in nitrogen fixers ammonifiers, cellulolytic bacteria, phosphorus solublizing bacteria due to toxic effect of mine material are reported (D 'Souza and Nayak, 1994). Diversity and respiration rates of microorganisms in mine soils were found to be lower than

that found in adjacent native soils (Shirey and Sextone, 1989). Low activity is seen in mine soil microbes, as compared to one present in undisturbed area (D'Souza 1993).

Mining activity brings up the soil not suitable for growth of fauna (Detwyler, 1971). Mining leads to migration of animals, exposing them to a different environment that is often beyond their tolerance capacities (Colinvaux, 1973 ; Mac Mohan, 1983), resulting into extinction of most of them. This being the most significant effect of mining on the fauna (Ganihar, 1990).

Iron was found harmful to fishes even in low concentrations as ferric hydroxide clogs up the gills (Hiremath and Shetty, 1987). Even bright flood lights during night at the jetty and continous movement of barges during mining season keeps fish away from the coast. The muddy runoff from the mines into coastal waters and pollution thereof during monsoon is also responsible for fish decline.

Mining mars the precious environment, not only at the actual place of excavation, but also at the place where wastes are dumped. After extraction of iron - rich ore, the rejected ore is dumped on the non-operational surface of the mine in the hope of finding a market in future with the advancement in technology. Dumps are also " stored with a hope" to extract iron economically from a low grade ore. Such wastes pose serious environmental hazards in the form of suspended particulate matter in the atmosphere and suspended and / or dissolved solids in the run-off water as well as in the infiltration water. The run-off of such contaminated water are observed especially during torrential rains which rip off the dumps causing seepage of ores into the running waters. Tributaries of the rivers and streams from nearby mining areas passing through such reject dumps or mining areas usually exhibit a high concentration of total suspended matter (Nayak, 1993).

Brown *et. al.*, (1984) have shown that stream flows may be greater afte mining. Reduced infiltration occurs due to higher bulk density of mine spoil

(Smith *et. al.*, 1971). Greater volumes of run-off can cause greater erosion. Collier *et. al.*, (1970) measured sediment loads of 8.7 t Km^{-2} year $^{-1}$ from mined areas. Decreases in stream pH (Collier *et. al.*, 1970), increases in stream pH (Dyer and Curtis, 1977), and increases in stream sulphate and magnesium contents (Biesecker and George, 1966 ; Curtis, 1973) resulting from surface mining have been reported.

High total acidity and carbon dioxide of runoff water is likely to acidify the receiving water body (Desai, 1990). Collier *et. al.*, (1970) reported decrease in stream pH due to receiving runoff from mines. Differing from this, Brown, *et. al.*, (1984) are of the opinion that the products of oxidation and associated soil chemical reactions, being washed from the soils, cause a steady decrease in pH. High values of phosphates, sulphates, nitrates and hardness indicate salt hazards in the contaminants receiving water bodies (Desai, 1990). Increase in stream sulphate and magnesium contents due to surface mining is reported by Biesecker and George (1966) and Curtis (1973). The same salt hazard is reflected in rise in electrical conductivity of runoff (Desai, 1990). Steep stock piling slopes of the iron ore reject dumps and the possibility of acid formation by salt forming materials due to oxidation during monsoons in the rejects could result in large amount of contaminated runoff.

SURVEY OF LITERATURE :

Mine rejects contain heavy metals. Heavy metals are known to be toxic (Bryan, 1979). Heavy metal input to the environment is largely a result of human activities (Lantzy and Mackenzie, 1979). In recent years, concern about the long term effects of such environmental contaminants has increased (Hutchinson and Meema, 1987). Heavy metal pollution due to mining has an insiduous effect and does not attract enough attention or comment, unless

catastrophic events like the Minamata case or the Itai - Itai disease in Japan, take place. These were caused by metal poisoning of mercury and cadmium respectively. Metals in aquatic environment can be termed as " Conservative pollutants" which once added to the environment are there forever and cannot be broken down to harmless substances by bacterial action like many organic pollutants (Sengupta and Kureishy, 1989). Hence, it becomes imperative to study the toxic effects of such heavy metals in water, which in its turn affects life in general.

On the aquatic front, work is usually carried out on fish as monitors of metals (Leah *et. al.*, 1991). Research on marine mammalian decline due to susceptibility to diseases is well documented (Law *et. al.*, 1991 ; Marcovecchio *et. al.*, 1990). Here, birds offer particular advantage as heavy metal pollution indicator as they feed at the upper trophic level. Most studies of heavy metals reveal that Cd is concentrated in the kidney, lead in bone, mercury in liver and kidney and zinc, copper and iron in liver. In birds, heavy metals are retained in blood, kidney, liver, bones and feathers. They may also accumulate in various other parts or organs of the body, ultimately leading to the decline in bird populations, as it is known that aquatic birds usually feed in the surroundings or in the same aquatic habitat in which they live. The metals entering the body through contaminated food would ultimately be absorbed and partly retained in the organs for either elimination or detoxification and the excessive retentions of these metals could be severely toxic.

i) MORTALITY DUE TO HEAVY METALS :

Mercury has been identified as one of the metals causing mortality of some bird species. Especially seegivorous birds of Scandinavia were reported to be dying due to methyl mercury contamination (Berg *et. al.*, 1966), while Lindberg (1984) reported heavy mortality of falcons (*Falco rusticglus*) due to

mercury poisoning. Similar observations were made by Spalding *et. al.,* (1994) for white herons of southern Florida.

Birkhead (1982), Lindberg (1984), Deuel (1985), Sanderson and Bellrose (1986), Blus *et. al.,* (1989) have observed lead toxicity due to ingestion of spent shots in mute swan (*Cygnus olor*), trumpeter swans and other aquatic birds. Also lead induced embryonic mortalities in aquatic birds. Similarly, selenium induced mortality in aquatic birds is reported by Ohlendorf *et. al.,* (1986), and they have reported embryonic mortality as well as abnormalities in the developing aquatic birds, inhabiting the waterbodies contaminated with selenium from irrigation drainwater.

ii) POLLUTION TOXICITY :

There are several reports of avian mortality caused by pesticide and oil pollution. Cade *et. al.,* (1971) reported DDT induced decline in prequine pollutions of Alaskan and South American habitats and have correlated increase in the death rate to the increase in DDT usage. Numbers of birds killed through major oil pollution incidents are generally high, but depend on a range of circumstances. Some 30,000 birds are estimated to have died as a result of the Torrey Canyon disaster off the Scilly Isles in March 1967, in which 120,000 t of oil were spilled (Bourne, 1976). Cooke *et. al.,* (1982) has described the contamination of eggs of European birds due to pesticides. White *et. al.,* (1983) have observed significant amount of DDE, dieldrin and toxaphene in shore birds. Similarly, Karlin *et. al.,* (1985) have found the residues of DDT and PCBs (Polychlorinated biphenyls) in the eggs of herring gulls, while Rickard and Fitzner (1985) detected minerals and metals in eggs of Canadian goose inhabiting contaminated waters. Risebrough *et. al.,* (1986) observed the thinning of mallard duck eggs due to DDE. Mercury, selenium, cadmium and organochloride induced changes in the eggs of Hawaiian sea birds are reported by Ohlendorf and Harrison (1986). Honda *et. al.,* (1986 a,

b) are of the opinion that mercury found in the eggs of birds is quite negligible in comparison with the amount retained by the body.

The marine wedge tailed birds exposed to the crude oil exhibited reduction in reproduction (Fry *et. al.*, 1986). Fox and Weseloh (1987) found phosphorus pesticides toxic to the birds inhabiting lakes. Burger and Gochfeld (1988) have suggested the use of avian eggs for monitoring the contaminants in the freshwaters. Diamond (1989) observed the decline in the population of piscivorous birds of Ontario and attributed the population decline to the reduced fecundity caused by acidic deposits in the water. Schrank *et. al.*, (1990) noted the suppression of skin response to the phytohemagglutinin by Dexamethasone and Gilbertson (1990) described the sensitivity of baldeagles *(Haliaectus leucocephalus)* and osprey *(Pandian haliaetus)* to the accumulations of DDT and PCBs.

In Briton, predatory birds were found dead due to the depositions of organochlorine and metal residues in the liver (Newton, 1991). The mallard ducklings (*Anas platyrhynchos*) fed on dieldrin spiked food, exhibited significant effects on the survival, growth and behaviours (Nebekar *et. al.*, 1992), while Weseloh *et. al.*, (1994) observed the accumulation of organochlorine in the bodies of white pekin ducks *(Anas platyrhynchos domesticus).*

iii) RETENTION OF METALS :

Heavy metals, particularly mercury, cadmium, lead, zinc have prompted many investigators to document the retentions and toxic actions into the bird tissues, especially after the episodes of pollution hazards in Japan, Sweden, and Iraq (Borg *et. al.*, 1969 ; Fujiki *et. al.*, 1972 ; Bakir *et. al.*, 1973 ; Friberg *et. al.*, 1974). Bryan (1979) reported the metal pollutions as extremely toxic. The increased human activities and the interference in the environment have contributed to the contamination of water bodies by the heavy metals (Hutchinson and Meema, 1987). The metals tend to accumulate in animals to

much higher levels than are found in the environment. Therefore, more attention has been given to the birds for detecting environmental contamination through the studies of metal retentions as they feed at the upper trophic levels of food web. Many researchers are now using aquatic birds (freshwater and marine) for studying retentions and for monitoring environmental contaminations (Jensen *et. al.*, 1972 ; Heinz, 1974 ; Munoz *et. al.*, 1976 ; Bijleveled *et. al.*, 1979 ; Osborn *et. al.*, 1979 ; Jarvinen and Väisänen, 1979 ; Stoneburner *et. al.*, 1980 ; Miller *et. al.*, 1980 ; Hacker *et al.*, (1981, 82) ; Evans and Moon, 1981 ; Hutton, 1981 ; Denheld, 1981 ; Reid and Hacker, 1982 ; Birkhead, 1982 ; Osborn *et. al.*, 1983 ; Custer and Mulhern, 1983 ; Eriksson, 1984 ; Goede and deBruin, 1984; Fossi *et. al.*, 1984 ; Goede, 1985 ; Honda *et. al.*, 1986 a, b; Szefer and Falandysz, 1986 ; Berg *et. al.*, 1987 ; Becker, 1989 ; Scharenberg, 1989 ; Lee *et. al.*, 1989 ; Honda *et. al.*, 1990, Lock *et. al.*, 1992 ; Denneman and DouBen, 1993 ; Heinz, and Fitzgerald, 1993. Sunlof *et. al.*, 1994 ; Stewart *et. al.*, 1994 ; Carpene *et. al.*, 1995). However, some workers (Murton *et. al.*, 1978 and Muirhead & Furness, 1988) are of the opinion that some birds may have high metal burdens for reasons of natural accumulation or detoxification processes unrelated to pollution. But Prasad Rao *et. al.*, (1989) gave the convincing evidence of heavy metal induced toxicity in the mallards.

Earlier it was felt that the retentions of metals by birds would be age and sex dependent. But Furness *et. al.*, (1990a), and Thompson *et. al.*, (1991) showed that there could be individual variation in metal distribution and accumulation but such variations are not related to the age and sex. There could be variations in the retentions of metals in different tissues (Lock *et. al.*, 1992, Kozulin and Pavluschick, 1993 ; Weseloh *et. al.*, 1994 ; Bakre and Sharma, 1995), as plumages tend to store more mercury in the form of methylmercury than other parts (Thompson and Furness, 1989). By contrast levels of cadmium in the kidney seem to increase through adult life in some

species but such a situation might not be for others (Furness and Hutton, 1979; Hutton, 1981), hence a sweeping generalisation of retention patterns cannot be made. Besides, female birds tend to eliminate metals into the eggs, but the amount done away this way is so low that the metal burden of males and females do not vary much (Furness *et. al.*, 1990 a and Thompson *et. al.*, 1991).

iv) MERCURY AND SELENIUM :

Feathers retain mercury in dose dependent fashion (Lewis and Furness, 1991, Scheuhammer, 1987). The accumulation of mercury in feathers correspond to the order of renewal (Braune, 1987 ; Braune and Gaskin, 1987a, b ; Furness *et. al.*, 1986a ; Lewis and Furness , 1991). Heinz (1974) observed mercury level in eggs of mallards to the tune of 6 - 9 µg. Stoneburner *et. al.*, (1980) found higher concentrations of mercury in feathers, eggs, and blood. Sell (1977) and Fo cardi *et. al.*, (1988) have observed that mercury and selenium are transferred to eggs, selenium levels are reported to be slightly higher than mercury levels with concentrations reaching 2.9 µg in herring gull (Leonzio *et. al.*, 1986). Heinz and Fitzgerald (1993) noted selenium poisoning in mallards. The selenium was found in blood. The selenium poisoning leads to mottling of the liver, emptying of the gizzard and accumulation of yellowish fluid around a few organs (Heinz and Fitzgerald, 1993). But Goede (1993) found selenium in eggs and parental blood of a Dutch marine wader and observed that it may not cause a potential threat to the reproduction of birds.

v) CADMIUM AND LEAD :

There are several reports of accumulation of cadmium in the bird kidneys. Levels in other tissues are very low. Scheuhammer (1987) is of a view that recent acute exposure of bird to the cadmium may lead to liver either equaling or exceeding the cadmium level of kidney. Cadmium levels for feather range from zero to 27 µg but very few studies suggest a correlation between

retentions of cadmium in feathers and other soft tissues. Sell (1975) reported that very little cadmium is transferred to eggs, regardless the amount consumed. Many pelagic birds show very high level of cadmium in kidneys but the eggs contain less than 0.7 µg/g fresh weight of egg (Anderlini *et. al.*, 1972 ; Furness and Hutton, 1979 ; Honda *et. al.*, 1986c ; Osborn *et. al.*, 1979 ; Renzoni *et. al.*, 1986). Therefore, the low levels of cadmium in the eggs need not be considered as no intake of cadmium.

Tansy and Roth (1970) showed that feral pigeons took the lead from the atmosphere and from ingested food and grit, and that in samples that were known to be free of lead shot. Pigeons from the urban area had significantly higher levels of lead in bone, feather, nail, liver and kidney than those from the rural areas but the blood levels of lead were same. Prasad Rao *et. al.*, (1989) have reported accumulation of cadmium and lead in the kidney.

vi) OTHER METALS :

Relatively few researchers have studied the levels of other metals in soft tissues, bones, eggs, feathers or blood in detail. Goede (1985) has described the levels of zinc and arsenic in feathers. Hahn (1991) is of the opinion that most of the metal retentions shown by the bird are atmospheric in origin than dietary in origin. Nyholm (1981) and Nyholm and Myhrberg (1977) found retention of aluminium in eggs. Kozulin and Pavluschick (1993) analyzed concentrations of zinc, chromium, cadmium, iron, nickel, manganese, copper from pectoral muscles (major), liver, kidney, oil glands and observed that only nickel concentrations in liver and kidney were significantly higher in birds. Denneman and DouBen (1993) reported variations in the concentrations of As, Se, Fe and Zn in feathers of Barn owls according to their positions in the wing and further observed that metals are stored during growth at the end of the feathers. Weseloh *et. al.* (1994) reported lesser retention of iron, zinc and copper than cadmium in the muscles.

vii) TOXICITY OF METALS TO KIDNEY AND BLOOD :

Nicholson and Osborn (1983) reported kidney lesions in pelagic sea birds with high tissue levels of cadmium and mercury. Muirhead and Furness (1988) have observed kidney lesions in sea birds from Gough Island and the kidney lesions could be correlated to the concentrations of mercury, cadmium, copper and zinc, especially the cadmium levels in the kidney were very high suggesting the role of cadmium in producing renal lesions. Ultrastructural evidence of kidney damage caused to ducks exposed to methyl mercury, lead and cadmium in combination include crystallization of granules in the juxtaglomerular cells due to Cd and Pb ; change in podocytes with fusion of secondary processes and no pedicel differentiation as an impact of Cd, Pb and in proximal tubule, lysosomal bodies and membrane bound vaculoes and large number of secondary lysosomes as well as swollen mitochondria in PT cells (Prasad Rao *et. al.*, 1989). In another significant study (Prasad Rao *et. al.*, 1989), the severity in histopathological changes in kidneys of pekin ducks exposed to methyl mercury chloride, lead acetate and cadmium chloride singly or in combination for 13 weeks, had no significant effect on kidney metallothionein levels or on essential elements in kidney tissues when compared with single metal treatment groups.

Tansy and Roth (1970) observed that if the birds can be caught safely, sampling of blood is comparatively easy and does not require the bird killing, but further opined that this is not practised beyond the studies of lead contamination. Birkhead (1983) opined that blood lead levels, haematocrit and enzyme assays can be used to measure the individual exposure to lead. Mautino and Bell (1987) have reported lead induced impairment of heme synthesis and elevation or excretion of intermediates in mallards. Trust *et. al.*, (1990) found that ingested lead by mallards (*Anas platyrhynchos*) affect antibody production, lymphocyte stimulation and lower hemagglutination titers.

Interactive effects of boron and selenium include increase in haematocrit, haemoglobin and plasma protein concentrations (Hoffman *et. al.*, 1991). Lead poisoning of whooper swans (*Cygnus cygnus*) indicated hypoplasia of bone marrow with increased numbers of polychromatic erythroblasts (Ochiai *et. al.*, 1992). It was further suggested that these changes result from excessive breakdown of erythrocytes, inhibition of hemesynthesis, and impaired erythropoiesis caused by lead shot.

UNDERSTANDING OF THE PROBLEM :

a) BIRDS AS MONITORS OF ENVIRONMENTAL CHANGES :

Like all ecosystems, fresh water and their catchments are subject to natural and man induced changes through physical, chemical and energetic processes. These modifications, in turn, have many biological repercussions and may alter conditions away from those that we value. The assessment of change is thus of central importance. Surveillance and monitoring in fresh water provide some of the most firmly established of all the examples of environmental assessment (Kolkwitz and Marsson, 1909 ; Hynes, 1960 ; Hellawell, 1986). Biological themes have figured prominently in assessments of water quality. Some of the heavy metal contaminants may be scarcely soluble in water, but nevertheless are ingested or incorporated in the organisms while adsorbed to organic matter. It has long been recognized that, although they are chemical in nature, biologically damaging pollutants should be detected using biological, rather than solely chemical criteria (Hynes, 1960). Biological indicators may have the added advantage of integrating exposure to total pollution over a span of time, whereas intermittent chemical sampling may miss short - lived and episodic bouts of pollution. Living organisms also have intrinsic importance, for example to conservation, economics or subsistence. Thus, not only does biological monitoring aid in the assessment of change, it

also permits some understanding of the wider consequences of pollution for biological resources.

For the most part, biological assessments of freshwater quality have involved wholly aquatic organisms such as algae, insects and fish (Hellawell, 1986 ; Bowker and Muir, 1981). In all of these, measurement can involve lengthy lab procedures. By contrast, birds are conspicuous and relatively easily assessed on both running or standing water for the detrimental changes if any in them, even by physical examinations. The realization that birds are integral components of fresh water ecosystems has also grown (Ormerod and Tyler, 1991a). Together, these features have prompted several authors to suggest a potential role for birds in assessing the status of fresh water ecosystems (Diamond and Filion, 1987).

The advantages of birds as monitors as postulated below are generally the raison d 'etre for undertaking the study of toxic actions of pollutants on birds or for monitoring environmental pollution :-

1) Birds are easy to identify and their classification and systematics are well established. Hence, there is little risk of monitoring being confounded by uncertainties regarding the indentities of, or relationships between, the species being studied.
2) Birds are particularly well known organisms, with much research carried out into their ecology and behaviour, and this background knowledge of biology enhances their usefulness as biomonitors, especially by reducing the risk of misinterpretations.
3) Birds tend to be high in the food chain, making them particularly suitable as monitors of any signal that accumulates through the chain.
4) Their long life span makes them suitable for expressing integrated effects of environmental stresses over time.
5) Mobility of birds allow monitoring over a broad spatial scale.

6) One of the most compelling reasons for using birds as biomonitors is quite pragmatic and they are relatively easy to study.

7) Long standing wide interest in birds provide surveillance data that can rather be adapted to fulfill a monitoring role.

8) Birds are also useful in habitat monitoring [Morrison, 1986 ; Järvinen and Väisänen, 1979 ; Saunders *et. al.*, 1985] ; Weather forecasting [Elkins, 1983 ; Fisher, 1952 ; Jouventin and Weimerskirch, 1990] ; and monitoring climate change (Austin *et. al.*, 1993 ; Hudson, 1990 ; Marquiss and Newton, 1990 ; Botkin *et. al.*, 1991]. Transequatorial migrant birds are employed as monitors of the tropics (Winstanley *et. al.*, 1974 ; den Held, 1981 ; Cavé, 1983 ; Kanyamibwa *et. al.*, 1990; Marchant, 1992 ; and Morton, 1992] Thus, birds are the most suitable choice for assessing the environmental pollution toxicity.

b) CHOICE OF THE ANIMAL :

The animal was selected, based on the following points :

1) Although widescale research has been done on the toxic actions of environmental pollutants on fishes and a few bird species, very little is known on the actions of iron mining pollutants on the aquatic birds like mallards (*Anas platyrhynchos domesticus).*

2) As mallards are aquatic any contamination of water is likely to affect them.

3) Besides, Goa is witnessing significant depletion of mallards from the water bodies receiving mining effluents.

4) The mallards are easily available throughout the experimental period, especially from the local supplier.

5) Mallards quickly acclimate to the laboratory conditions.

6) They tolerate experimental stress.

7) They are important birds, as in Goa, duck meat and duck eggs are consumed by some communities.

The reasons mentioned above made *Anas platyrhynchos domesticus,* most suitable for the present investigation. The sex cycles and hormonal variations in females is likely to influence the response of the animals, hence only the males were used for experiments. The males chosen were of same age, and body weight (900 gm to 1.00 Kg) so as to have precise result comparison procured at different time intervals, with different concentrations of mining effluents.

c) PARAMETERS FOR STUDY :

Birds may acquire heavy metals from preening contaminated material off their feathers, and by taking in food or water containing industrial or mining effluent, polluted agricultural runoff or air pollution fallout. All forms of mining activities affect the aquatic environment. Although, precise effect will vary with the type of waste discharged, there is usually an increase in the total dissolved solids in the mine drainage waters. pH of the receiving waters is also brought down which leads even to dissolution of some metals in water (Trivedy, 1990). Mine drainage changes physico - chemical characteristics of water, adversely affects planktons and primary productivity. It also kills fishes and other avifauna.

During monsoon, dumped mine rejects mix with rain water, come down as runoffs and contaminate surrounding waters, thus affecting the avifauna present therein. Heavy metals usually found in the mining effluents are Cu, Pb, Zn, Cd, Hg, Mo, As, Ni, Cr, Mn, and Sn (Desai, 1990). The Pb, Cd, Hg induce severe damage to the kidney of fishes and mammals (Sahu, 1990). Available literature depicts heavy metal effect on avifauna, especially on mallards to be restricted to concentrations of metals in the muscles and liver only. Although studies on *Anas platyrhynchos* were carried out to monitor organochlorine and

other pollutant levels (Chapdelaine *et. al.*, 1987) and radionuclide contamination monitoring (George *et. al.*, 1991 ; Halford *et. al.*, 1981 ; Baeza *et. al.*, 1988), there is hardly any data available on mining effect apart from reproductive dysfunctions due to heavy metals (Heinz, 1974). Hence, the present study is undertaken to study the effects of mining effluents on *Anas platyrhnchos domesticus* and how the mining stress would influence the renal architecture and renal clearance. Besides, the damage caused by heavy metals from mining effluents to the formed elements of blood of the mallard is not known. There are very few reports on the effects of lead contamination along with few other metals on formed elements of birds (Scheuhammer, 1989; Tansy and Roth, 1970) ; hence, it was decided to see if the runoffs /mining effluents containing heavy metals influence the formed elements of the blood and also the serum proper.

Mallards are known to spend most of their time in water. They drink water very often, too. As such, the kidney of birds would get exposed to the toxic constituents present in the water. Hence, such exposed kidney is likely to develop nephretis (Sahu, 1990). Therefore, attention to urine and kidney is focussed for studying effects on glomeruli, proximal and distal convoluted tubules, collecting tubules, changes in renal function, and protein levels. Also, retention of heavy metals in the kidney and blood are given attention.

d) PROPOSED PLAN OF RESEARCH :

Keeping in view the aforementioned review and non availability of any information on the effect of mining effluents on birds with respect to histology, histochemistry and biochemistry of kidney it was decided to investigate : the histology of kidney including ultrastructure, histochemistry of enzymes such as acid phosphatase, alkaline phosphatase, esterase (non specific) from the kidney, bioassays related to acid phosphatase, alkaline phosphatase, non specific esterase activities, proteins, creatinine, uric acid, urea, electrolytes

like Na^+, K^+ Ca^{++}, chlorides from kidney, urine and serum. Haemotological studies like total RBC, total WBC, differential count, platelet count, Hb% were also planned. It was also thought proper, to study heavy metals from the runoff water along with the hydrological parameters like carbon dioxide, dissolved oxygen, sulphates, nitrates, inorganic phosphates, organic phosphates, total phosphates, acidity, alkalinity, dissolved solids, suspended solids, total solids, conductivity and redox potential. Retention pattern of heavy metals from the mining effluents in the kidney and serum and renal clearance of these heavy metals through urine was also undertaken.

CHAPTER - II

MATERIALS

AND

METHODS

The mallards were brought to the laboratory and kept for fifteen days in the cages (length - 6.5 ft ; height - 1 mt. ; width - 1 mt.), so as to allow them to acclimate to the laboratory conditions and after that they were used for the experimental purposes. The animals were weighed prior to treatment of mining effluents (M.E.). Well fed adult male mallards were allowed to drink various concentrations of mining effluents adlibitum and they were sacrificed after the specific intervals.The animals were allowed to drink freely the effluent water in various concentrations so that the effluents with its contaminants enter the body in the most natural way, as could have occured in their natural habitat. There is hardly any information available on the effect of mining effluents on birds so it was decided to treat the animals with 0.01, 0.1, 1.0, 5, 10, 50, 100% concentrations of mining effluent. The concentration effects were studied for the following selected time intervals : 6, 12, 24, 48, 72, 96, 120, and for 0.01% 720 and 1080 hours. The effective toxicity (ET50) was ascertained by exposing the animals to the varying concentrations of mining effluents for varying periods (24 hours to 200 hours) beyond 24 hrs exposure, as at the end of 24 hr the ET 50 was not found for any concentration. Therefore, the experiment was repeated for five times for exalting ET50 for 24 to 200 hours. For evaluating the M.E. toxicity for various periods and concentrations, five sets were used, each set was comprised of seven animals.

Besides this, at the end of desired time intervals the kidneys were investigated for histological, histochemical and biochemical alterations if any, along with blood, serum and urine.

The fresh as well as 6 month's old, mining ore rejects were collected from a stretch of 1km and then were mixed and stored in plastic drums. All the samples were stored in well sealed drums to avoid, oxidation and were used within a week of their collection. Then the iron ore reject samples were packed in boxes (3M x 3M x 25 cm), equipped with run off collecting devices. Each box held 200 kg of Iron ore reject. Five replications of each material were used.

The boxes were put on 3% slope and subjected to 10 cm rain by means of rain simulator (Morin *et. al.* 1967) for 75 days with an interval of 15 days between two rainfalls. The rain simulator had a frame (size = 25.5 x 25.5 inches) of tubing (0.25 inch diameter) with 307 pores (0.01 mm diameter) on the surface measured. The rainfall from simulator was caused by the gravitational force. The glass distilled water was used for rainfall simulations. Soon after each rain the boxes were placed on benches, to drain and dry. Only the runoffs were collected from the collecting device and no leachate was collected. For all the experiments undertaken on mallards the first runoff was used, the subsequent runoffs were, collected after every fortnight and were subsampled for water analyses, such as heavy metals Dissolved oxygen, Carbon dioxide, total solids, total dissolved solids, phenophthalein acidity, total alkalinity, organic phosphorus, total phosphorus, inorganic phosphorus sulphates, nitrates, hardness, Electrical conductivity, Redox potential, pH and sediment load.

I] HYDROLOGICAL STUDIES (WATER ANALYSIS)

Methods as described by Trivedy in Methods of water analysis published by Environ. media Publications, Karad, India.

A] CONDUCTIVITY :

a] Principle :

Electrical conductance is the ability of a substance to conduct the electric current. In water, it is the property caused by the presence of various ionic species.

Conductivity is generally measured with the help of a conductivity meter having a conductance cell containing electrodes of Platinum coated

with Pt.black or carbon. These electrodes are mounted rigidly and placed parallely at a fixed distance. Conductance, when measured between the electrodes having a surface area of 1 cm^2 and placed at a distance of 1 cm is called electrical conductivity and is the property of the water sample, rather, than the measuring system. The term specific conductance is also used in place of electrical conductance. The unit of specific conductivity measurement is µMho.

The ionization of the solutes depends on the temperature, conventionally the results are reported at 25°C.

b] Procedure :

1] Well stirred mining effluent was taken into a beaker.

2] The conductance Cell containing platinum electrodes coated with Pt. black were placed into the beaker containing the effluents.

3] The instrument was switched on and the reading was noted.

4] The instrument which was used, for this experiment was Systronics - 304 conductivity meter, which gave direct reading of conductivity in µMho.

5] Cell constant of the conductance cell was 1 as was stated by the manufacturer.

6] The temperature of the sample was noted and was multiplied by the correction factor for respective temperature.

7] The correction factor for 30°C was 0.092 and 29°C was 0.93.

c] Calculations :

Conductivity in µMho = Observed conductance x Cell constant x temperature factor at 25°C/29°C/30°C

B] TOTAL SOLIDS (T. S) :

a] Principle :

Total solids are determined as the residue left after evaporation of the unfiltered sample.

b] Procedure :

1] Well stirred mining effluent was taken into a beaker.

2] Evaporating porcelain dish of suitable size and of known weight was taken, in which 500 ml of stirred effluent was poured.

3] The unfiltered effluent was evaporated to dryness in a water bath.

4] The final weight of the dish after evaporation of the sample was noted.

c] Calculations :

$$\text{Total Solids gm/L} = \frac{A - B \times 1000}{V}$$

Where A = Final weight of the dish in grams.

B = Initial weight of the dish in grams

V = Volume of sample taken in ml.

TOTAL DISSOLVED SOLIDS (TDS) :

a] Principle :

Total dissolved solids are determined as the residue left after evaporation of the filtered sample.

b] Procedure :

1] Well mixed sample was taken in a beaker.

2] Around 500 ml of sample was filtered.

3] Filtered sample was then taken in an evaporating dish of suitable size and known weight.

4] 500ml of filtered sample was then evaporated to dryness.

5] The weight of the evaporating dish was noted after evaporation.

c] Calculations :

$$\text{TDS, gm / L} = \frac{A - B \times 1000}{V}$$

Where A = Final weight of the dish in grams.

B = Initial weight of the dish in grams.

V = Volume of sample taken in ml.

TOTAL SUSPENDED SOLIDS (TSS) :

Total suspended solids were determined as the difference between the total solids and total dissolved solids.

Calculations :

Total suspended solids (TSS) = TS - TDS

Where TS = Total Solids

TDS = Total dissolved solids.

C] pH :

a] Principle :

pH is the negative log 10 of the hydrogen ion concentration in a solution. For measurement of accurate pH, electrometric methods are employed using the hydrogen ion sensitive electrodes.

b] Apparatus :

For determining the pH of the mining effluents Elico pH meter was used. The accuracy of the pH meter was 0.01.

The pH meter consisted of two electrodes, an indicator glass electrode, and a calomel reference electrode. The pH meter also had a temperature compensation system to avoid the differences arising due to the different temperatures.

c] Procedure :

1] The instrument was switched on and allowed to stabilize.

2] Buffer tablets of 7pH, 4 pH and 9.2 pH were dissolved in 100ml of distilled water for the respective pH.

3] The instrument was then set to a range of 4pH - 7 pH with the respective buffers.

4] Then the electrodes were placed in the well mixed sample for the detection of the pH.

5] The reading displayed from the instrument directly gave the pH value of the respective sample.

D] REDOX POTENTIAL :

a] Principle :

The potential developed on a platinum electrode in the solution due to the substances present in their oxidized or reduced states, is called as redox potential.

In measuring redox potential, a platinum electrode is used as indicator and calomel as reference electrode.

b] Apparatus :

Elico pH meter having facility to measure the potential in millivolts, with a reference calomel and a platinum electrode was used to estimate the redox potential.

c] Procedure :

1] The instrument was switched on 30 minutes before use.

2] Both the electrodes were then placed in effluent sample.

3] The switch was set at millivolts and direct reading of the samples were noted from the scale.

4] The reading were then converted into volts.

E] TOTAL ALKALINITY :

a] Principle :

Total alkalinity is the measure of the capacity of the water to neutralize a strong acid. The alkalinity in the waters is generally imparted by the salts of carbonates, bicarbonates, phosphates, nitrates, borates,

silicates etc. together with the hydroxyl ions in free state. However, most of the waters are rich in carbonates and bicarbonates with little concentration of other alkalinity imparting ions.

Total alkalinity, carbonates and bicarbonates were estimated by titrating the sample with strong acid (HCl), first to pH 8.3 using phenolphthalein as an indicator and then further to pH between 4.2 and 5.4 with methyl orange indicator. In the first case the value is called as phenolphthalein alkalinity (PA) and in second case it is total alkalinity (TA).

b] Reagents :

i] Hydrochloric acid, 0.1N.

ii] Methyl orange indicator : 0.05%. Dissolve 0.05g of methylorange in 100ml of D. W.

iii] Phenolphthalein indicator : 0.5g of phenolphthalein was dissolved in 50 ml of 95% ethanol and 50ml of distilled water was added. 0.05 N CO_2 free NaOH solution was added dropwise, until the solution turned faintly pink.

c] Procedure :

1] 100ml of digested sample was taken in an erlenmeyer flask and 2 drops of phenolphthalein indicator was added to it.

2] If the solution remained colourless, PA = 0 and total alkalinity was determined as described in step 4.

3] If the colour changed to pink after addition of phenolphthalein, titration was carried out by 0.1N HCl until the colour disappeared at the end point. This was phenolphthalein alkalinity (PA).

4] To the same sample 2 - 3 drops of methyl orange were added and titration was carried out further, until the yellow colour changed to pink at the end point. This was denoted as Total Alkalinity (TA).

d] Calculations :

$$\text{PA a Ca } CO_3, \text{ Mg/l} = \frac{(A \times \text{Normality of HCl}) \times 1000 \times 50}{\text{ml of sample}}$$

$$\text{TA as } CaCO_3 \text{ mg/l} = \frac{(B \times \text{Normality of HCl}) \times 1000 \times 50}{\text{ml of sample}}$$

Where A = ml of HCl used with only phenolphthalein

B = ml of total HCl used with phenolphthalein and methylorange

PA = Phenolphthalein alkalinity.

TA = Total alkalinity.

F] ACIDITY :

a] Principle :

Acidity of the water is its capacity to neutralize a strong base to a fixed pH. It is caused by the presence of strong mineral acids, weak acids and hydrolyzing salts of strong acid. In natural unpolluted fresh waters, the acidity is mostly due to the presence of free CO_2 in the form of carbonic acid.

Acidity was determined by titrating the sample with a strong base such as NaOH using methyl orange or phenolphthalein as an indicator. If the sample has strong mineral acids and their salts, then it was titrated

first to pH 3.7 using methyl orange as an indicator. This is called as methyl orange acidity. If the sample is titrated directly to pH 8.3 using phenolphalein, the resultant value is the total acidity.

b] Reagents :

i] Sodium hydroxide - 0.05 N.

ii] Methyl orange indicator - 0.05 %.

iii] Phenolphthalein indicator.

c] Procedure :

1] 100ml of filtered colourless effluent sample was taken in conical flask to which 2-3 drops of methyl orange indicator were added.

2] If the solution turned yellow, the methyl orange acidity was absent. If the contents turned pink the sample was titrated with 0.05 N NaOH. At the endpoint colour changed from pink to yellow.

3] To the same sample few drops of phenolphthalein indicator were added and then further titrated with NaOH until the contents turned pink.

d] Calculations :

$$\text{Methyl orange acidity mg/l as } CaCO_3 = \frac{A \times N \text{ of NaOH} \times 1000 \times 50}{\text{ml sample}}$$

$$\text{Phenolphthalein acidity mg/l as } CaCO_3 = \frac{B \times N \text{ of NaOH} \times 1000 \times 50}{\text{ml sample}}$$

$$\text{Total acidity to pH 8.3 mg/l as } CaCO_3 = \frac{(A+B) \times N \text{ of NaOH} \times 1000 \times 50}{\text{ml sample}}$$

Where, A = Volume of NaOH used with methyl orange titrating the sample to pH 3.7.

B = Volume of NaOH used with phenolphthalein in titrating the sample from pH 3.7 to pH 8.3.

G] CARBON DIOXIDE :

a] Principle :

Free CO_2 was determined by titrating the sample using a strong alkali such as carbonate free NaOH to pH 8.3. At this pH all the free CO_2 is converted into bicarbonates.

b] Reagents :

i] Sodium hydroxide ; 0.05 N.

ii] Phenolphthalein indicator.

c] Procedure :

1] 100ml of filtered effluent sample was taken in a conical flask and to it a few drops of phenolphthalein indicator were added.

2] If the effluent sample turned pink, free CO_2 was considered to be absent. If the sample remained colourless it was titrated against 0.05N NaOH. At the end a pink colour appeared.

d] Calculation :

$$\text{Free } CO_2, \text{ mg/l} = \frac{(\text{ml} \times \text{N}) \text{ of NaOH} \times 1000 \times 44}{\text{ml Sample}}$$

H] DISSOLVED OXYGEN :

Winkler's Iodometric method

a] Principle :

The manganous sulphate reacts with the alkali (KOH or NaOH) to form a white precipitate of manganous hydroxide which in the presence of oxygen, gets oxidized to a brown coloured compound. In the strong acid medium, manganic ions are reduced by iodide ions which get converted to iodine equivalent to the original concentration of oxygen in the sample. The iodine can be titrated against thiosulphate using starch as an indicator.

b] Reagents :

i) Sodium thiosulphate 0.025N

ii) Alkaline potassium iodide solution

Dissolved 100 g of KOH and 50 g of KI in 200 ml of boiled distilled water.

iii) Manganous sulphate solution.

Dissolved 100gm of $MnSO_4 \cdot 4H_2O$ in 200 ml of boiled distilled water and filtered.

iv) Starch solution :

Dissolved 1g of starch in 100 ml of warm distilled water and added a few drops of formaldehyde solution were added.

v) Concentrated sulphuric acid.

c] Procedure :

1] Sample was filled in glass stoppered bottle (BOD Bottle) of known volume (100ml). Care was taken to avoid any kind of bubbling and trapping of air bubbles in the bottle after placing the stopper.

2] 1ml of each of Mn SO_4 and alkaline KI solution were added well below the surface from the walls, to ensure better mixing of reagents using two different pipettes, for the two reagents.

3] After adding the reagents a precipitate was formed.

4] 1.2 ml of concentrated H_2SO_4 was then added into the bottle and was then added into the bottle and was shaken well to dissolve the formed precipitate.

5) The whole contents from the bottle was taken into a conical flask for titration. Bubbling was prevented to avoid further mixing of oxygen.

6) Titration was carried out against sodium thiosulphate solution using starch as an indicator. At the end point the initial dark blue solution changed to a colourless solution. The burette reading was then noted down.

d] Calculations :

When whole content has been titrated :

$$\text{Diss. oxygen, mg/l} = \frac{(\text{ml} \times \text{N}) \text{ of titrant} \times 8 \times 1000}{V1 - V}$$

Where, V1 = Volume of sample bottle after placing the stopper

V = Volume of $MnSO_4$ and KI added.

i] NITRATE :

Brucine method

a] Principle :

Nitrate and brucine react to produce a yellow colour, the intensity of which can be measured at 410 nm. The reaction is highly dependent upon the heat generated during the test.

b] Reagents :

i) Brucine - Sulfanilic acid solution :

Dissolved 1g brucine sulphate in 0.1g of sulfanilic acid in about 70 ml of hot distilled water. After addition of 3 ml conc. HCl, the volume was made up to 100ml. The pink colour developed slowly and did not affect the sensitivity.

ii) Sulphuric acid solution :

500 ml conc H_2SO_4 was added in 125 ml distilled water and cooled.

iii) Sodium chloride solution :

300 g Nacl was dissolved in distilled water and diluted to 1 litre.

iv) Sodium arsenite solution :

5.0 g $NaASO_2$ was dissolved in distilled water to make 1 litre of solution.

v) Standard nitrate solution (1mg/ml) :

0.722 g of KNO_3 was dissolved in distilled water and the volume was made up to 1 litre. This solution contained 100 mg/ml and then it was diluted to 100 times to prepare a solution having 1 mg/ml (10 - 1000ml).

c] Procedure :

1) Sample was made chlorine free by adding 1-2 drops of arsenite solution as chlorine interferes with the nitrate determination.
2) 10ml of sample was taken in 50ml test tube.
3) The test tube was placed in cool water bath and to it 2ml of Nacl solution was added.
4) 10ml of H_2SO_4 was then added to the mixture.
5) Next, 0.5 ml Brucine reagent was added and mixed thoroughly.
6) The test tube was then placed in boiling water bath, for exactly 20 minutes.
7) After 20 minutes the test tube was cooled and reading was recorded at 410 nm using spectrophotometer.
8) Concentration of N03-N was calculated from standard curve.
9) Standard curve was plotted by preparing different aliquots of standard and following the procedure as mentioned above for the sample. The concentration of standard ranged from 0.1 to 1.0 mg/ml.

J] INORGANIC PHOSPHORUS :

a] Principle :

The phosphates in water react with ammonium molybdate and form a complex heteropoly acid (molybdophosphoric acid), which gets reduced to a complex of blue colour in the presence of $SnCl_2$. The absorption of light by this blue colour can be measured at 600nm to calculate the concentration of phosphates.

b] Reagents :

i) Ammonium molybdate solution :

1) 25.0 g of ammonium molybdate was dissolved in 175 ml of distilled water.

2) Then 280 ml of concentrated H_2SO_4 was added to 400 ml of distilled water and cooled. The two solutions (1) and (2) were mixed and diluted to 1 litre.

ii) Stannous chloride solution :

2.5 g of stannous chloride was dissolved in 100ml glycerol by heating on a water bath for rapid dissolution.

iii) Standard phosphate solution :

4.388 g of dried anhydrous dipotassium hydrogen phosphate (K2HP04) was dissolved in distilled water and the volume was made up to 1 litre. This solution was diluted 100 times (10 - 1000ml). This was standard phosphate solution containing 10 mg P/l (1ml = 0.01mg P).

c] Procedure :

1) 50 ml of filtered clear sample was taken in a clean erlenmeyer flask.

2) 2 ml of ammonium molybdate followed by 5 drops of $SnCl_2$ solution was added to the sample

3) The blue colour developed was read at 690nm on a spectrophotometer using a blank with the same amount of the chemicals. Readings were taken exactly after five minutes of the addition of the last reagent.

4) The concentration of the sample was found out with the help of standard curve.

d] Preparation of standard curve :

1) Various dilutions at various intervals of 0.1mg P/l from the standard phosphate solution were made according to the table shown below.

2) 50ml of each dilution was taken and to it 2ml of ammonium molybdate and five drops of $SnCl_2$ solution were added.

3) The developed blue colour was read at 690nm and a graph of absorbance v/s concentration was plotted.

Table: Preparation of various dilutions of phosphorus for the standard curve.

ml of standard solution	Diluted to (ml)	Concentration of PO_4 - P, mg/l
5.0	50	1.0
4.5	50	0.9
4.0	50	0.8
3.5	50	0.7
3.0	50	0.6
2.5	50	0.5
2.0	50	0.4
1.5	50	0.3
1.0	50	0.2
0.5	50	0.1

TOTAL PHOSPHORUS :

a] Principle :

All the forms of phosphorus, whether dissolved or particulate were converted to inorganic forms by digestion and oxidation of the sample. H_2SO_4 - HNO_3 technique was employed for digestion of the sample. The phosphates thus released were determined Spectrophotometrically.

b] Reagents :

i) Concentrated sulphuric acid.

ii) Copper sulphate solution 10%.

iii) Potassium sulphate - solid.

iv) Sodium hydroxide 1N.

v) Sodium hydroxide 5N.

vi) Phenolphthalein indicator.

vii) Ammonium molybdate solution.

viii) Stannous chloride solution.

ix) Nitric acid concentrated.

c] Procedure :

1) Digestion of sample :

25ml of the sample was taken in Kjeldahl flask of 100ml. To it 1ml of conc. H_2SO_4 and 5ml of conc HNO_3 were added. The sample was digested on a heater until the volume was nearly 1ml and heating was further continued until the solution became colourless after complete removal of HNO_3.

2) Estimation of total phosphorous :

i) 20 ml of distilled water and 1 drop phenolphthalein were added to the digest. The acid was neutralized by titrating it with 1N NaoH. The solution turned pink at the end point.

ii) Phosphorus content was determined as described for inorganic phosphorous. The pink colour of phenolphhthalein disappeared after addition of ammounium molybdate.

iii) A separate standard curve was prepared for total phosphorus after the digestion of various dilutions of the standard solution because the chemicals used in digestion might affect the intensity of the developed colour.

d] Calculation :

Concentration of phosphorus was obtained from standard curve and multiplied by the dilution factor.

ORGANIC PHOSPHORUS :

Organic phosphorus was obtained as a difference between total phosphorus and inorganic phosphorus of the sample.

Organic phosphorus mg / l = Total phosphorus - Inorganic phosphorus

K] SULPHATE :

Turbidimetric method

a] Principle :

Sulphate ion is precipitated in the form of barium sulphate by barium chloride in hydrochloric acid medium. The concentration of the sulphate can be determined from the absorbance of the light by barium sulphate and then comparing it with a standard curve.

b] Reagents :

a) Conditioning reagent :

75g of Nacl, 30ml conc. HCl, 100ml 95% ethyl alcohol were mixed in 300ml distilled water, 50ml glycerol was added to this solution and mixed thoroughly.

b) Barium chloride : Crystals of $BaCl_2$.

c) Standard sulphate solution :

0.1479g of anhydrous Na_2SO_4 was dissolved in distilled water to make 1 litre of solution. This solution contained 100 mg/l of sulphate.

c] Procedure :

1) 100ml of effluent sample or a suitable aliquot diluted to 100ml in 250 ml erelenmeyer flask and to which 5.0ml of conditioning reagent was added.
2) Samples were stirred and during stirring a spoonful of $BaCl_2$ crystals were added.
3) The readings were taken on spectrophotometer at 420nm and concentrations of sulphate was found out from the standard curve.

4) Standard graph was prepared by employing same procedure as described above, for 0.0 - 40.0 mg/l at the interval of 5 mg/l.

L] HARDNESS :

EDTA Method

a] Principle :

Hardness is generally caused by the calcium and magnesium ions present in water. Polyvalent ions of some of the other metals like strontium, iron, aluminium, zinc and manganese etc. are also capable of precipitating the soap and thus contributing to the hardness. However, the concentration of these ions is very low in natural waters. Therefore, hardness is generally measured as concentration of only calcium and magnesium, as calcium carbonates are far higher in quantities over other hardness producing ions.

Calcium and magnesium form a complex of wine red colour with Erichrome Black T at pH of 10.0 $\pm$ 0.1. The EDTA has got a stronger affinity towards Ca^{++} and Mg^{++} and therefore by addition EDTA, the former complex is broken down and a new complex of blue colour is formed.

b] Reagents :

i) EDTA solution 0.01M

3.723 g of disodium salt of EDTA was dissolved in distilled water to prepare 1 litre of solution.

ii) Buffer solution

1) 16.9 g ammonium chloride (NH_4Cl) was dissolved in 143ml of concentrated ammonium hydroxide (NH_4OH)
2) 1.179 g of disodium EDTA and 0.780 of $MgSO_4.7H_2O$ were dissolved in 50 ml distilled water. Both (1) and (2) solutions were mixed and diluted to 250ml with distilled water.

iii] Eriochrome Black T indicator - 0.40 g of Eriochrome Black T was mixed with 100 gm NaCl (A. R.) and then ground.

iv] Sodium Sulphide Solution. 5.0 g of $Na_2S.9H_2O$ or 3.7 g $Na_2S.5\ H_2O$ was dissolved in 100 ml of distilled water. The bottle was tightly closed to prevent oxidation.

c] Procedure :

i] 50 ml of sample was taken in a conical flask.

ii] 1ml of buffer was added to the solution.

iii] As the sample had higher amount of heavy metals 1ml of Na_2S solution was added to it.

iv] 100mg of Eriochrome Black T indicator was added to the solution. The solution turned wine red.

v] The contents were then titrated against EDTA Solution.

vi] At the endpoint colour changed from wine red to blue.

d] Calculations :

$$\text{Hardness as mg/l } CaCO_3 = \frac{\text{ml EDTA used} \times 1000}{\text{ml sample.}}$$

II) METHOD OF BIRD URINE COLLECTION :

Eric Skadhauge (1965)

The polythene tubes were used for urine collection. The bird was dressed in a special operation jacket and was kept astride on the operation table. Then using 2% Xylocaine jelly, the bird's anus was anaesthetized and then the urodeum and proctodeum were exposed with the help of clamps and strings as well as with artery forceps. The apertures of the ureter were then exposed and the polysterene tubes were stitched on them. Then a long polythene tube was joined to the stitched polysterene tube and its other end was kept in the test tube. Before this, the bird was allowed to stand on its feet and was supported from below as and when required. The urine samples were collected and stored in a refrigerator at 4°C. Such samples were used after one hour of its collection. The urine samples were collected just 20 minutes before the desired time interval so as to avoid dehydration in the animal.

III] ELECTRON MICROSCOPY :

Benjamin F. Trump and Raymond T. Jones (1978)

a] Requirement :

i] 3% Gluteraldehyde in 0.1M Cacodylate buffer pH 7.4.

ii] 3% Gluteraldehyde in 0.1 M Cacodylate buffer pH 7.4 containing 1% Tannic acid.

iii] 1 M Cacodylate buffer (stock).

iv] 0.2 M Calcium chloride.

v] 0.1 M Cacodylate buffer.

vi] 1% Osmium tetroxide in Cacodylate buffer (working solution. pH 7.4 and 0.2 M).

vii] 50%, 70%, 90% and absolute ethanol.

viii] Propylene : Araldite A mixture (1: 1).

ix] Araldite B Solution.

x] 1% Toluidine blue.

xi] 12.5% Alcoholic uranyl acetate.

xii] Lead Citrate.

b] Primary fixation :

The specimen was fixed with 3% gluteraldehyde in 0.1M. Cacodylate buffer buffer, pH 7.4, containing 1% tannic acid for 2 hrs at 4° C. Subsequent washes were given to the specimen after 2 hours with 0.1 M Cacodylate buffer, pH 7.4.

c] Post fixation :

The specimen was then post fixed with 1% Osmium tetroxide in cacodylate buffer at 4°C for 2 hrs.

d] Dehydration :

After postfixation the specimen was passed through a series of graded alcohol of increasing concentrations as indicated below :

50% ethanol in water	: 10 minutes at 4°C
70% ethanol in water	: 15 minutes at R. T.
90% ethanol in water	: 2 changes of 10 minutes each at R. T.
100% ethanol	: 3 changes of 20 minutes each at R. T.

e] Infiltration and embedding :

The specimen was treated with following reagents step wise as follows :

Propylene oxide	: 2 changes of 10 minutes each at R. T
Propylene	: Araldite : 1 hour at R. T.
Araldite A	: Overnight in a dessicator at R. T.
Araldite B	: 1 hours at R. T.

The specimen was then embedded in BEEM capsules with fresh araldite B. The capsules were then kept in the oven maintained at 60°C for 48 hours.

f] Sectioning and Staining :

The block is freed from the capsule and trimmed. Semi thin sections, 1 micron thickness were cut on an LKB ultratome V with glass knives prepared on an LKB 780 OB knife maker. The sections were taken on a glass slide and dried on a hot plate (60°C) and were subsequently stained with 1% toluidine blue (20 - 30 seconds) and observed under the light microscope.

The selected area for ultra thin sections were marked out. The blocks were further trimmed and ultrathin sections 600 - 900 A° thick corresponding to pale gold colour of the sections were cut. The sections were collected on 200 mesh copper grids and dried under the lamp.

The grids carrying the sections were stained with 12.5% alcoholic uranyl acetate for 1 hour and were washed in distilled water and followed by staining with lead citrate for 10 minutes.

After drying all grids were observed under JEOL - 100S Electron Microscope at 80 KV.

As outlined in the introducing chapter the studies on acid phosphatase, alkaline phosphatase, non specific esterase's, total proteins urea, uric acid, creatinine, electrolytes, calcium and Hematological parameters such as Total WBC, RBC, differential count, platelet count, Hemoglobin % ; were decided upon, to find out the effect of mining effluents on kidney. Such a study will be incomplete if only the variations in the activities of these enzymes, proteins, urea, uric acid, creatinine, electrolytes, calcium and Hematological parameters are studied quantitatively in exact mathematical units. Such quantitative studies will not be able to give any information on histological changes in the kidney structure and kidney enzymes.

It was, hence decided to apply both bioassay, cytochemical and ultrastructural study techniques to the kidney so as to make such a study comprehensive. Though the bioassay techniques give the information on the variation in the enzyme activities total proteins, urea, acric acid, calcium and electrolytes in exact mathematical terms, the cytochemical techniques throw light on morphological, structural changes in the tissue and cellular sites where the variations in the enzyme activities take place. In recent years the histochemical techniques, have also been employed to get some idea about the variations in the activities of the enzymes under varying physiological conditions. In some of the recent studies, variations in the intensities of staining for enzymes under ideal conditions of histochemical technique are generally taken as reflections of the differences in the activities of the enzyme. At present the stage of development of histochemistry and its use as a tool in understanding physiological phenomena in the cells and tissues, comparative studies with biochemical and histochemical techniques seem desirable ;

especially while dealing with the variations in the concentrations or activity of cellular chemical constituents. With this view quantitative histochemical techniques have been devised and applied to the study of enzymes such as amino peptidases, amidases, Esterases, alkaline phosphatases, mono-esterases, lactic dehydrogenases, etc (Glick, 1957 ; Gomori, 1952 ; Glenner, 1957 ; Pears, 1968 ; and Glenner, 1963). But most of these techniques involve employment of complex instruments which may not be easily accessible to a histochemist. Conditions necessary for reliable histochemical quantification such as critical thinness of the section, attainment of steady state of condition of enzyme reaction, avoiding diffusion artifacts etc must be maintained ideally in the absence of which misleading results are obtained

Hence histochemists working within limitations have to depend upon visual comparison of the coloured end product of a histochemical reaction for his assessment of enzyme activity. In static tissues, such an assessment is difficult for want of a reference with which comparisons can be made.

But in physiologically varying tissues and tissues under mining stress, such as those selected in the present study, such a comparison is easy and some idea regarding the alterations in the enzyme activity can be obtained. Various studies reported from some laboratories (Varute, 1970 ; 1972) and those reported from other laboratories (Hayashi and Fishman, 1961 ; 1962 : Fishman and Goldman, 1965 ; Fishman and Baker, 1956) in which a comparison of the enzyme activities from bioassay techniques and Variations in the intensities of staining for enzymes have been made, support the aforementioned conclusion.

Thus, keeping the conditions such as fixation, substrate concentration, pH of the incubation medium, incubation intervals and the concentration of azodye and the coupling interval constant, if any

variation occurs in the intensities of staining they can very well be taken as variations in the enzyme activities.

Thus, in the present study bioassay, histochemical and ultra structural techniques have been employed. The bioassays give a reliable data on the disturbance in the enzyme activities ; Histochemistry on the enzyme localization and also some indications of the variations the enzyme activities in histological and cellular sites where the enzymes are localised where as, Electron microscopy technique gives an insight into alterations in the cellular structure at the ultra structural level.

IU] HISTOCHEMICAL TECHNIQUES :

A] FIXATION :

Localization of esterases, alkaline phosphatases and acid phosphatases was initially studied in case of all the tissues under investigation by employing following fixatives.

i] 5% formalin in 1% aqueous calcium chloride

ii] Chloral hydrate formalin (Formaldehyde 20 ml distilled water 80 ml, Chloral hydrate 100 mg].

(Fishman and Baker , 1956 ; Baker *et al.*, 1958).

iii] Cold absolute acetone for 18 to 24 hours.

iv] Fresh frozen sections without fixation

v] Calcium acetate formaldehyde [20ml of formaldehyde 80 ml of distilled water 2 grams of calcium acetate].

It was found that cellular localisation of esterases, acid phosphatases, alkaline phosphatases was affected by the nature of fixatives. The fresh frozen material showed no loss of enzyme activity but

diffusion artifacts were markedly present. Calcium formalin fixed sections showed less enzyme activity than that observed in fresh frozen material, but diffusion artifacts were negligible. Of the various fixatives, chloral hydrate formalin (CHF) and calcium acetate formaldehyde (CAF) followed by gum sucrose treatment was found to give best enzyme preservation with minimum enzyme inactivation and diffusion of the end product of the enzyme action.

These observations agreed with those of Pearson and Gorse ((1959), Holt and Withers (1958), Pearson and Defendi (1959) and Desai (1983). All the further work was done by fixing the tissues in chloral hydrate formalin at 4°C exactly for 24 hours and this fixation time was kept constant throughout the course of the entire work. Fixed tissues were blotted carefully on a filter paper and transferred to Holts 0.88 M Sucrose solution containing 1% gum acacia. This was also previously chilled to 4°C (Holt 1959). The tissue initially floated at the surface of gum sucrose solution but after some time, sank to the bottom of the tube. The tissues were kept in Holt's gum sucrose solution for exactly 24 hours at 4°C. At the end of 24 hours the tissues were carefully blotted and sections were prepared at 5 μ on Lip Shaw Cryotome. The sections were received in distilled water previously cooled to 4°C and only free floating sections were selected for further treatment.

B] ACID PHOSPHATASE :

Standard coupling Azo - dye technique for Acid phosphatase.
Davis and Ornstein (1959)

1] Solutions : The required solutions were prepared as follows :

a] 0.1 M Vernol acetate buffer - pH 5.0

b] Substrate Solution 20 mg of Na - α napthyl phosphate was dissolved in 20 ml 0.1M Vernol acetate buffer pH 5.0. To this were added 1.5 gm of polyvinyl pyrolidone, and 20 mg of Fast garnet GBC Salt I (ILtd) and the resultant mixture was mixed well and filtered.

2] Technique :

Sections were transferred into incubation cup containing the substrate solution . The cups were then kept at 37°C for incubation . In this technique also optimal incubation timing was decided by trials. It was seen that most of the tissue gave satisfactory results with an incubation period of 2 to 5 minutes.

3] Incubated sections were washed in cold distilled water for two minutes. As the reaction product was formed the sites of acid phosphatase activity showed reddish colour. These sections were taken on clean albuminized slides and blotted with Whatman No. 1 Filter paper with face down and then mounted in polyvinyl pyrolidone.

C] ALKALINE PHOSPHATASE :

Modified Coupling Azo - Dye method for Alkaline phosphatase : Burstone (1962)

1] Solutions : The necessary solutions were prepared as mentioned below :

a] 0.1 M Tris buffer pH 10.

b] Substrate solution :

20 mg of Na - α napthyl phosphate was dissolved in 20 ml 0.1M Tris buffer. To this was added 20 mg of stable diazotate of Fast blue and the mixture was mixed well and filtered.

2] Technique :

Sections were transferred into incubation cup containing substrate solution and then the incubation cups were kept at 37°C. The tissue gave satisfactory results with an incubation period of 3 to 7 minutes.

3] Incubated and stained sections were washed with cold distilled water. The stained sections showed reddish brown sites for alkaline phosphatase activity. These sections were taken on albuminized slides and then mounted in polyvinyl pyrolidone.

D] ESTERASE (NON SPECIFIC E.C.3.1.1)

Localisation was studied by employing the following techniques :

I] Indoxyl acetate method for esterase :

(Pearson and Grose, 1959 ; Holt and Wither, 1958 ; Pearson and Defendi, 1957)

i] Solutions :

All dry reagents were weighed out on an analytical balance as appropriate.

a] 0.1 M Tris buffer pH 7.2

b] 0.05 M Potassium Ferrocyanide.

c] 0.05 M Potassium Ferricyanide.

d] Substrate Solution : 5 Bromo indoxyl acetate 10 mg was dissolved in 2ml ethanol, to this were added 15 ml Tris buffer, 5 ml potassium ferrocyanide, 5 ml potassium ferricyanide and the resultant mixture was filtered.

ii] Technique :

Sections were transferred into the incubation cup containing substrate solution and then the incubation cups were kept at 37°C. The incubation period to give maximum staining was selected by trials at periods varying from 10 to 15 minutes. While selecting optimum incubation timing the biochemical data of the enzyme activity of the tissue was also taken into consideration. The selected optimum incubation period was kept constant throughout the entire work. For the tissue employed in the present study the optimum incubation timing was found to be 15 minutes which was kept unchanged.

iii] Incubated sections were washed in cold distilled water for one minute. The reaction product formed, was seen as blue precipitate of indigo. The sections were taken on clean albuminized slides and blotted with Whatman No. 1 Filter paper with face down and then mounted in polyvinyl pyrolidone.

V] HISTOPATHOLOGY

HAEMATOXYLIN - EOSIN STAINING METHODS FOR HISTOLOGY

1] The paraffin embedding was done with the routine procedure and the paraffin sections of 5μ were cut and were dewaxed and hydrated as usual.

2] The tissue was stained in Haematoxylin Harris mixture for 3 to 5 minutes and washed under tap water.

3] Differentiated (a few seconds) in one percent HCl in 70% alcohol until the section was turned brown.

4] Then the tissues were stained in 0.5% eosin in 70% alcohol for 2 to 3 minutes after upgrading them upto 70% alcohol stage. Then the sections were transferred directly to 95% alcohol to which a few drops of eosin was added.

5] Then the tissues were transferred through two further changes of 95% alcohol and then transferred to absolute alcohol and subsequently immersed in Xylene and mounting was done in DPX.

V]ENZYME ASSAY :

1] ACID PHOSPHATASE :

[orthophosphoric monoester phosphohydrolase E.C.3.1 3.2]

P. Nitrophenol phosphate (Merck, batch No. 20896) was purchased from Merck and all other chemicals prepared were of analar grade.

i) Bioassay method :

Biochemical assay of acid phosphatases was carried out according to the method described by Linhardt and Walter (1965).

A] FOLLOWING REAGENTS WERE PREPARED :

i) 0.05 M, p - nitrophenol phosphate in sodium citrate buffer (0.05 M, pH 4.8) Stored at 4°C

ii) 0.1 N NaOH.

B] PREPARATION OF SAMPLE :

The homogenation of the tissue was carried out in a glass mortor. The usual method of homogenation was modified. The glass mortor and pestle were all washed, rinsed in distilled water, dried and kept in the ice box of refrigerator for nearly five hours before homogenation. Homogenation carried out in such chilled glass mortor and pestle has two advantages.

1] during homogenation no loss of enzyme activity occurs as temperature due to friction of the mortor and pestle does not exceed beyond 12°C at the end of homogenation.

2] during homogenation when the tissue is first crushed at the bottom of the mortar, it instantanously freezes and then gradually thaws, which helps in breaking the lysosomes, where this enzyme is partly localized. When the tissue was thawing, two ml of chilled sodium citrate buffer (0.05M, pH 4.8) was added and homogenation was carried out to completion. When a perfectly uniform suspension was formed, the homogenate was transferred to a calibrated flask and further dilution was done by adding the necessary quantity of the sodium citrate buffer (0.05 M, pH 4.8). Care was taken to see that the final concentration of

tissue homogenate was about 1% (W/V) or even less. Throughout the work period, the concentration of homogenate for a particular tissue was kept practically constant.

C] ENZYME REACTION :

For each assay, three test tubes were taken and into test tube was added 1.0 ml substrate buffer solution and 0.2 ml aliquot of well suspended tissue homogenate. This mixture was allowed to equilibrate for 5 to 10 minutes. A single control test tube contained 1.0ml substrate buffer solution (0.05 M, pH 4.8). The tubes were shaken gently to mix aliquots properly. These were then stoppered with cork stoppers and incubation was carried out in water bath previously adjusted to 37.5°C for 30 minutes. This incubation interval was kept constant throughout the course of the entire work.

D] COLOUR PRODUCTION AND SPECTROPHOTOMETRIC MEASUREMENT :

At the end of the incubation, the reactions were terminated by adding 4 ml of 0.1 N NaOH. and tubes were centrifuged for 5 minutes at 2000 RPM. The optical density was read at 400 nm on 305 Systronics spectrophotometer using control as a reference.

E] CALCULATIONS :

The optical density was converted to micromoles of p - nitrophenol from the formula suggested by Linhardt and Walter (1965) with an assay volume of 5.2 ml, 0.2 ml serum and an incubation period of 30 minutes ; a unit of acid phosphase activity corresponds to an optical density of 0.362 at 400 mμ and is equivalent to 2.76. Factor 2.76 x optical

density of assayed mixture was phosphatase activity for 0.2 ml extracts, which was then calculated for 1gm of the tissue.

FORMULA FOR TISSUE SAMPLE :

$$\frac{\text{Optical density} \times 2.76 \times \text{dilution}}{0.2 \times \text{wt in gram}} = \text{Acid phosphatase in p nitrophenol}$$

FORMULA FOR SERUM AND URINE :

$$\frac{\text{Optical density} \times 2.76 \times 100}{0.2} = \text{p - nitrophenol } \mu\text{mol /100 ml}$$

2] ESTERASE (Non specific E. C. 3.1.1)

p - Nitrophenol assay of esterase was carried out according to the method described by Glenner (1957, 1963).

A] FOLLOWING REAGENTS WERE PREPARED :

i) 0.001 M p - nitro phenol acetate :

63 mg p - nitrophenol acetate in 10ml ethanol (stock solution). The working substrate solution was prepared by diluting 0.1ml of the stock solution to 10 ml.

ii) Phosphate buffer (pH 7.0, 0.66 M).

B] PREPARATION OF SAMPLE :

Homogenation of the tissues was carried out in a manner similar to that described for the bioassay of acid phosphatase. Only in place of

sodium citrate buffer, phosphate buffer (0.66 M, pH 7) was employed for the preparation of the homogenate and for dilution. Rest of the conditions were kept unchanged.

C] ENZYME REACTION :

For each assay, 3 test tubes were taken and to each of which were added 5 ml of ice cold water, 2 ml of phosphate buffer (0.66 M, pH 7), 1ml enzyme sample or serum, as the case may be and 2 ml working substrate solution. Tubes were shaken vigorously and centrifuged for 5 min at 2000 RPM. Reading of assayed tubes were taken at zero hours as a reference for control. Again tubes were vigorously shaken and kept for 20 minutes incubation at 15°C. Assayed tubes were again centrifuged and readings were taken at 400 nm. Both control and incubated mixtures were measured against distilled water as blank. Reading at zero hours was treated as control.

D] CALCULATIONS :

The optical density was converted into micromoles of P-nitrophenol from the calibrated nitrophenol standard curve. The esterase activity was expressed in p- nitrophenol µmol/gm units in case of tissue sample and per 100 ml in case of serum or urine, p - nitrophenol µmoles were calculated using the following formula.

$$\frac{\text{µmoles p - nitrophenol from graph x dilution}}{\text{Wt. in gram}} = \text{µmoles p - nitrophenol / gm for kidney.}$$

$$\frac{\text{µmoles p - nitrophenol from graph x dilution}}{\text{ml of sample}} = \text{µmoles p - nitrophenol / 100 ml for serum and urine.}$$

3] ALKALINE PHOSPHATASE : [E.C.3.1.3.1]

p - Nitrophenol phosphate assay of Alkaline phosphatase was carried out according to the method described by E. J. King and M. D. Armstrong 1934.

A] THE REAGENTS REQUIRED WERE :

i] 0.05 M glycine buffer

ii] 0.02 N NaOH.

B] PREPARATION OF SAMPLE :

Homogenation of the tissue was carried out in a manner similar to that described for the bioassay of acid phosphatase. Only in place of sodium citrate buffer, glycine buffer (0.05 M, pH 10.5) was used for preparation of the homogenate and for dilution. Rest of the conditions were kept unchanged.

C] ENZYME REACTION :

For each assay three test tubes were taken and into each test tube was added 1.0 ml substrate buffer solution and 0.1 ml aliquot of well suspended tissue homogenate or serum or as the case may be. This mixture was allowed to equilibrate for 5 - 10 minutes. A single control test tube contained 1.0 ml substrate buffer solution (0.05 M, pH 10.5). The tubes were shaken gently to mix aliquots properly and incubation was carried out in a water bath, previously adjusted to 37.5°C, for 30 minutes. This incubation interval was kept constant through out the course of the entire work.

D] COLOUR PRODUCTION AND SPECTROPHOTOMETRIC MEASUREMENT :

At the end of the incubation period, the reactions were terminated by adding 10 ml of 0.02 N NaOH and tubes were centrifuged for 5 minutes at 2000 RPM. The optical density was read at 400 nm on 305 Systronics spectrophotometer, where control was used to serve as a reference.

E] CALCULATIONS :

The optical density was converted into milli micromoles of p - nitrophenol from calibrated nitrophenol standard curve. The alkaline phosphatase activity was expressed in p - nitrophenol mμ moles/gm units in case of tissue sample and per 100ml in case of serum and urine. p - nitrophenol mμmoles units were calculated using following formula suggested by E. J. King and M.D. Armstrong.

mμ moles p - nitrophenol units x 20 will give alkaline phosphatase units i.e mmoles units.

VI]OTHER BIOASSAYS :

1] CREATININE :

Auto Span Kit was used for creatnine analysis.

Mitchell (1973) Kammerat (1978) and Bowers (1980)

a) Principle :

Creatinine reacts with picric acid in an alkaline medium to form an orange coloured complex. The rate of formation of this complex is

measured by noting the change in absorbance at 505 nm in a selected interval of time and is proportional to the concentration of creatinine.

The reaction time and the concentration of picric acid and sodium hydroxide have been optimized to avoid interference from ketoacids.

b] Reagents :

Reagent 1	--	Picrate Reagent i.e picric acid surfactant
Reagent 2	--	Sodium Hydroxide
Reagent 3	--	Creatinine standard - 2mg/dL creatinine

c] Preparation of working reagents :

1] Equal volumes of picrate reagent and sodium hydroxide was combined and mixed by swirling gently for 2 minutes to prepare working creatinine Reagent.

2] Reagent 3 creatinine standard is ready for use as supplied.

d] Reagent Stability :

i] Unopened reagents 1 and 2 are stable at room temperature until the expiry date.

ii] Working creatinine Reagent is stable for 7 days at 2 -8°C.

iii] Reagent 3, Creatinine standard is stable at 2 -8°C until the expiry date.

e] Preparation of sample :

1] Freshly separated serum, free from hemolysis, was collected after sacrificing the animal.

2] Urine sample was collected just 20 minutes before the desired time interval and was diluted in the proportion 1:10 $^{V}/_{V}$ with de-ionised water and then used for analysis.

3] Homogenation of tissue was carried out in a manner similar to that described for the bioassay of acid phosphatases. Only in place of sodium citrate buffer, chilled distilled water was employed for the preparation of the homogenate and for dilution. Rest of the conditions were kept unchanged.

f] Equipment :

Creatinine estimation was carried out on automated analyzer, "Seac CH - 100".

g] Programme :

For creatinine analysis the automated analyzer was programmed by using following parameters :-

1] Mode	:	Fixed time
2] Wave length	:	505 nm
3] Temperature	:	37°C
4] Optical pathlength	:	1 cm
5] Blank	:	glass distilled water
6] Working reagent volume	:	1000 µ L
7] Concentration of standard	:	2 mg/dL
8] Delay	:	30 seconds
9] Interval	:	90 seconds
10] Maximum absorbance limit	:	1.5
11] Linearity	:	upto 20 mg/dL
12] Units	:	mg / dL

All the samples and working regents which were stored at 2 - 8°C were brought to room temperature before use. Care was also taken to maintain constant temperature (37 ± 0.5°C) throughout the assay.

h] Procedure :

1] The analyzer was programmed by using parameters stated above.

2] It was ensured that the working creatinine reagent was allowed to stand at room temperature for at least 10 minutes before use.

3] The analyzer was brought to zero with distilled water .

4] 50 µL of creatinine standard was added to 1.0 ml working creatinine reagent. This was mixed briefly and aspirated. The value was noted.

5] 50 µL of serum sample was added to 1.0 ml working creatinine reagent. Mixed briefly and aspirated. The value of which was noted.

6] Step '5' was repeated for remaining samples (urine and kidney extract) while also noting the values.

7] It was recommended that the first absorbance (S 1 for standard and T1 for tests) be taken 30 seconds after adding the standard or sample to the working creatinine reagent. i.e delay of 30 seconds. The second absorbance reading (S2 for standard and T2 for tests) was taken 90 seconds after the first reading (i.e the interval should be 90 seconds).

i] Calculations.

$$\text{1] Serum mg/dL} = \frac{T2 - T1}{S2 - S1} \times 2.0$$

$$\text{2] Urine Creatinine mg/dL} = \frac{T2 - T1}{S2 - S1} \times 20$$

$$\text{3] Kidney creatinine mg/100mg of wet tissue} = \frac{T2 - T1}{S2 - S1} \times 20$$

2] UREA :

Autozyme urea - a reagent set was used to determine urea by urease enzymatic method. Autozyme is a 2 reagent system using two step procedure.

Dumas (1971) and Webster (1977).

a] Principle :

Urease splits urea into ammonia and carbon dioxide. Ammonia released in this reaction reacts with hypochlorite and phenolic chromogen to produce green colour. The absorbance of this green colour at 600 nm is directly proportional to the concentration of urea in specimen.

$NH_2 - CO - NH_2 + \underline{\text{Urease}} \longrightarrow 2NH_3 + CO_2$

NH_3 + Hypochlorite + Phenolic Chromogen ------> Green colour.

b] Reagents :

a] Phosphate buffer pH 7.0	--	35 m Moles/L
b] Urease	--	15 Ku/L
c] Phenolic Chromogen	--	2 m Moles/L
d] Hypochlorite	--	4 m Moles/L
e] Urea Standard	--	40 mg %

c] Reagent Stability and Storage :

1] Enzyme and chromogen solution are stable for 30 days when stored in container at 2 - 8°C.

2] Chromogen bottle was kept closed when not in use to minimise the loss of chlorine from the reagent.

d] Preparation of sample :

Similar procedure was followed for sample preparation as described in creatinine bioassay for serum, urine and kidney extract.

e] Equipment :

Urea analysis was carried out on automated analyser Seac - CH - 100.

f] Programme :

For urea estimation the automated analyzer was programmed by using following parameters :-

1] Reaction Type	--	Endpoint
2] Reaction time	--	8 minutes
3] Reaction Temperature	--	37°C
4] Wave length	--	600 nm

Only required amount of Enzyme solution and chromogen solution were brought to room temperature.

g] Procedure :

The assay was performed as mentioned below.

Five test tubes were kept ready for use.

1] In the first test tube, instead of sample, 1.0 ml enzyme solution was added and then mixed and incubated for three minutes at 37°C. After incubation 1 ml chromogen solution was added.

2] In the second test tube was added 0.01 ml standard + 1.0 ml enzyme solution and then mixed and incubated for 3 minutes at 37°C. After incubation 1.0 ml of chromogen solution was added.

3] In the third, fourth and fifth test tubes, were added 0.01 ml Urine, Kidney, Serum samples respectively + 1.0 ml enzyme solution. This was then mixed and incubated for 3 minutes at 37°C. In the mixture so incubated, 1.0 ml of chromogen was added.

4] After mixing and incubating the assay mixtures of 1 - 3 at 37°C for 5 minutes, absorbance was measured against Blank at 600 nm. The final colour was found to be stable for 2 hours.

h] Calculation :

$$\text{Urea mg \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 40$$

3] URIC ACID :

Uric acid was determined by using Autozyme Uric Acid Kit which is based on enzymatic method using Uricase and Peroxidase. Autozyme uric acid is a single reagent system using one step procedure.

Thefeld (1973) and Fossati (1980).

a] Principle :

Uricase converts uric acid into allantoin and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogen to form red coloured compound, which has a

maximum absorbance at 520 nm. The concentration of the red coloured compound is proportional to the amount of uric acid in specimen.

Uric acid + H_2O + **Uricase** ------> Allantoin + H_2O_2

H_2O_2 + Phenolic chromogen + **Peroxiadase** ------> red coloured compound.

b] Components and Concentration of working solution :

1]	i]	Phosphate buffer	152 m Mol/L.
	ii]	Peroxidase	140 1µ/L
	iii]	Uricase	25 1µ/L
	iv]	Chromogen	2.5 µMol/L
2]		Standard Uric acid =	0.006 %

c] Reagent Storage and Stability :

1] The Reagent Kits were stored at 2 - 8°C and were stable upto expiry date.

2] The working solution was always stored at 4°C in the amber glass enzyme bottles as to prevent auto oxidation of chromogen system by light and air.

d] Specimen collection and preservation :

Procedure for collection of serum, kidney extract and urine was similar to that described for creatinine.

e] Equipment :

Seac CH - 100 automated analysis.

F] Programme :

The parameters used for automated analyzer in the analysis of uric acid were:

a] Reaction type - End point

b] Reaction type - 15 minutes at room temperature.

c] Wave length - 520 nm.

The required amount of working solutions were brought to room temperature before use.

g] Procedure :

Five test tubes were kept ready for use.

1] In the first test tube only 1 ml of working solution was added.

2] In the second test tube, 0.025 ml of standard uric acid + 1.0 ml of working solution was added.

3] In test tube no 3, 4 and 5, 0.025 ml of serum, kidney extract and urine were added respectively + 1.0 ml of working solution in each test tube.

4] The mixture was then incubated for 15 minutes at room temperature (above 25°C) and after completion of the incubation, the absorbance of assay mixture was measured against blank at 520 nm. The final colour was found to be stable for 30 minutes, if not exposed to direct light.

h] Calculations :

$$\text{Uric acid mg \% for Serum and Urine} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6$$

$$\text{Uric acid mg / 100 mg of wet tissue} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 6$$

4] CALCIUM :

Auto Span Reagents were used for estimation of calcium. OCPC, End Point Colorimetry Single Reagent Chemistry method was used for calcium estimation.

Kessler (1964), Morin (1974) and Lorentz (1982).

a] Principle :

At alkaline pH, calcium binds with orthocresolphthalein complexone (OCPC) to form a bluish purple complex. The intensity of the colour so formed is proportional to calcium concentration and is measured at 575 nm.

Interference from magnesium is overcome by the presence of 8 - hydroxyquinoline in Reagent 1, which binds free magnesium ions.

b] Reagents :

Reagent 1] OCPC Reagent	OCPC ; Hydrochloric Acid; 8 Hydroxyquinoline.
Reagent 2] AMP Buffer	AMP buffer, pH 10.4.
Reagent 3] Calcium Standard	10mg/dl - calcium carbonate; Hydrochloric acid.

c] Preparation of working Reagent :

i] Just prior to use, Reagent 1 - OCPC Reagent and Reagent 2 - AMP Buffer were mixed in equal volume i. e. 1 part of OCPC reagent + 1 part AMP buffer. This mixture was labelled as working calcium reagent. Working calcium reagent was prepared fresh as and when required.

ii] Reagent 3 - Calcium standard was ready for use as supplied.

d] Reagent stability :

All opened reagents were found to be stable at 2 - 8°C until the expiry date.

e] Sample or Sepecimen Collection :

i] Unhemolyzed serum was collected with minimal delay.

ii] Fresh urine was collected just 20 minutes before the stipulated time interval. The urine samples were collected in diluted hydrochloric acid (1ml /litre urine), so as to prevent precipitation of calcium salts.

iii] Kidney extract :- Homogenation of the tissue was carried out in a manner, similar to that described for the bioassay of acid phosphatase, only in place of sodium citrate buffer, chilled deionized. 18mohm/cm quality reagent grade I water was used for the preparation of the homgenate and for dilution. Rest of the conditions were kept unaltered.

f] Equipment :

Calcium estimation with Autospan Kit was carried out on Seac C H - 100 automated analyzer.

g] Programme :

For estimation of calcium with the auto analyzer, the assay parameters were programmed as below :

a] Mode	--	Endpoint
b] Wavelength	--	578 nm
c] Temperature	--	37°C
d] Optical path length	--	1 cm
e] Blank	--	Reagent blank
f] Sample volume	--	20 ul
g] Working reagent volume	--	1000 ul
h] Incubation	--	5 minutes
i] Maximum absorbance limit	--	2.0
j] Linearity	--	2.15 mg/dl
k] Stability of colour	--	2 hours
l] Units	--	mg /dL

h] Procedure :

Five test tubes were kept ready for analysis.

1] In the first test tube no sample was added but 1.0 ml of working calcium reagent was added.

2] In the second test tube, 20 µl of calcium standard was added + 1.0 ml of working calcium reagent.

3] In test tubes 3, 4, and 5, 20 µl of samples serum urine and kidney extract respectively were added and then 1.0 ml of working calcium reagent was added in each test tube.

4] All the test tubes contents were mixed well and incubated at 37°C for 5 minutes.

5] The analyzer was programmed using the parameters given above.

6] The analyzer was brought to zero with Reagent Blank.

7] The standard was aspirated and the reading was noted.

8] Test samples were aspirated and readings were noted.

i] Calculation :

Calcium concentration in mg/dL was calculated by using following formula.

$$\text{a] Serum Calcium mg/dL} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 10$$

$$\text{b] Urinary Calcium mg/dL} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 10 \times \text{dilution factor}$$

$$\text{c] Kidney calcium mg/100mg of wet tissue} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 10 \times \text{dilution factor}$$

5] CHLORIDE :

Autospan Reagent Kit was used for estimation of chloride. Thio cyanate, Endpoint Calorimetry, Single Reagent Chemistry method was applied for determining calcium from the samples.

Zall (1665) and Shoenfeld (1956).

a] Principle :

Chloride ions react with a solution containing ferric, mecuric nitrate and thyocyanate ions in equilibrium to form yellow brown ferric thyocyanate. Absorbance measured. at 505 nm is proportional to the concentration of chloride in the specimen.

$$6\ Cl^- + 2\ Fe^{+++} + 3\ Hg(SCN)_2 \text{ -------> } 3\ HgCl_2 + 2\ F_2(SCN)_3$$

b] Reagents :

Reagent 1 ----> Chloride reagent ----> Mercuric nitrate Nitric acid, Ferric Nitrate ; Thiocyanate

Reagent 2 ---> Chloride standard ----> Hydrochloric acid 100 mEq/L

c] Preparation of working reagents :

Both Reagent 1 and Reagent 2 are ready for use as supplied.

d] Reagent Stability :

Reagent 1 is stable at room temperature (15 -30°C) until the expiry date.

Reagent 2 chloride standard is stable at 2 - 8°C until the expiry date.

e] Specimen Collection :

1] Unhemolysed serum was collected with minimum delay after sacrificing the animal.

2] Urine was collected exactly 20 minutes before the desired time point under study. Urine was then diluted two times with deionized water. The results were then multiplied by two, to get the actual values.

f] Equipment :

Seac - CH - 100 Autoanalyzer

g] Programme :

For analysis for chloride from the samples the automated analyzer was programmed by using following parameters.

a] Blank	-	Reagent blank
b] Sample volume	-	100µl or 5 µl
c] Reagent volume	-	1000 µl
d] Concentration of standard	-	100m Eq/L.
e] Incubation	-	3 minutes at RT or 37°C
f] Max absorbance limit	-	2.0
g] Linearity	-	70 - 140 m Eq/L
h] Stability of colour	-	30 minutes
i] Units	-	m Eq/L

If the samples contained more than 140 mEq/L chloride, 1 : 2 dilution with deionized water was carried out and then the results of the diluted samples were multiplyed by 2.

h] Procedure :

Five test tubes were kept ready for analysis of chloride.

1] First test tube was labelled as blank as it contained only 1.0 ml chloride reagent and no sample.

2] Second test tube contained 10 µl of chloride standard + 1.0 ml chloride reagent.

3] Test tubes 3, 4, and 5 contained 1.0 ml of serum, urine, and kidney extract respectively with 1.0 ml chloride reagent in each test tube.

4] All the contents of the test tubes were mixed well and incubated at 37°C for 3 minutes.

5] Analyzer was programmed using the afore mentioned parameters.

6] Blanking of the auto analyzer was done with Reagent blank.

7] Standard sample was aspirated and reading was noted.

8] Test samples were aspirated and readings were noted.

Calculations :

Chlorides from the samples were calculated by using following formula :-

$$\text{Serum Chloride mEq/L} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 100$$

$$\text{Urine chloride mEq/L} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 100 \times \text{dilution factor}$$

$$\text{Kidney extract, chloride mEq /100mg wet weight of tissue} = \frac{\text{Absorbance Test}}{\text{Absorbance standard}} \times 100 \times \text{dilution factor}$$

6] PROTEIN :

Proteins were estimated by Lowery's method

Lowery *et. al.*, 1951.

a] Following reagents were prepared :

i] Standard protein solution : 13 mg Bovine albumin was dissolved in 250 ml distilled water made alkaline.

ii] Lowery's A solution : 2.0% Sodium Carbonate solution in 0.01 M Sodium hydroxide.

iii] Lowery's B_1 solution : 2.0 % Sodium Citrate Solution in distilled water.

iv] Lowery's B_2 Solution : 1.0% Copper Sulphate Solution in distilled water.

v] Lowery's C Solution : 1.0 ml of Lowry's B_1 + 1.0 ml of Lowry's B_2 + 100ml Lowry's A (used within fifteen minutes of its preparation)

vi] Folin's phenol reagent : Market available form was used (s.d. fine-chem. Ltd. Prod. No. 29058).

B] Procedure :

Seven test tubes were kept ready for use.

1] In the first test tube no sample was added but 1.5 ml distilled water and 3.0 ml of Lowery's C solution were added and after 15 minutes 0.5 ml of Folin's phenol reagent was added and then was kept aside undisturbed for an hour.

2] In the second and third test tubes were added 0.2 ml and 0.4 ml of standard protein solution respectively, followed by 1.3 ml and 1.1 ml of distilled water respectively. Then 3 ml of Lowery's C solution was added in each test tube and after 15 minutes, 0.5 ml of Folin's phenol reagent was added. Then they were kept aside undisturbed for an hour.

3] In the test tubes numbered 4, 5, 6 and 7, 0.2, 0.4, 0.6 & 0.8 ml of samples were added respectively followed by 1.3 ml, 1.1 ml , 0.9 ml and 0.7 ml of distilled water respectively and then 3 ml of Lowery's C solution was added in each test tube. This was allowed to stand for 15 minutes. Then 0.5 ml of Folin's phenol reagent was added in each tube. The mixture was allowed to stand for an hour.

4] After one hour the readings were taken on Spectrophotometer at 660nm.

5] The protein value was calculated as shown in the calculations.

c] Preparation of sample :

a] Unhemolysed serum was collected with minimal delay.

b] Fresh urine was collected just 20 minutes before the alloted time interval. The urine samples were deuricated by treating the filtered urine with equal volume of uricase solution (1%) at 37°C for an hour and then the urine sample was centrifuged for ten minutes at 5000 rpm. The supernatent was used for protein estimation.

c] Homogenation of tissue was carried out in manner similar to that described for the bioassay of acid phosphatase. In place of sodium citrate buffer, chilled deionized water was used for the preparation of the homogenate and for dilution. Rest of the conditions were kept unchanged.

d] Calculations :

$$\text{Amount of protein} = \frac{\text{O.D of sample}}{\text{O. D. of standard}} \times \text{known protein}$$

Then, the values for total volume of samples were calculated.

7] ESTIMATION OF Na^+ AND K^+

Estimation of Na^+ and K^+ from Serum and Urine was carried out by Flame photometer Mc Intyre (1958)

a] Preparation of standard for Na^+ and K^+ analysis in serum and urine:

Analytical reagents / chemicals were used. The analytical grade NaCl and KCl was used for standard solutions. NaCl and KCl were dried up for 2 to 3 hours at about 100°C before use. Before weighing, the chemicals were allowed to cool to room temperature in a descicator.

b] Preparation of stock solutions :

Stock A : 1000 mEqu Na^+ / Litre - 58 gm of dry NaCl was weighed and made up to one litre in a volumetric flask with 18 mohm / cm quality reagent grade I water.

Stock B : 1000 mEqu K^+/Litre - 7.46 gm dry KCl was first weighed and dissolved in water and then made up to one litre in a volumetric flask with 18 mohm / cm quality reagent grade I water.

c] Preparation of Na^+ Standards to cover the range 120 - 160 mEqu/litre :

Table

mEqu Na^+ / Litre	120	130	140	150	160
ml Stock A	60	65	70	75	80
ml Stock B	25	25	25	25	25

All the solutions were made upto 500 ml with 18 mohm / cm quality reagent grade I water. These solutions cover the range usually found in serum/plasma sodium.

d] Preparation of K+ standards to cover the range 3 - 8 mEq/Litre

Table

mEqu K^+/ litre	3	4	5	6	7	8
ml Stock A	70	70	70	70	70	70
ml Stock B	15	20	25	30	35	40

All the solutions were made upto 500 ml with 18 mohm / cm quality reagent grade I water. These solutions cover the range usually found in serum / plasma potassium.

e] Working standard for serum / plasma potassium

The standard prepared according to the above tables were diluted in the same way as the serum / plasma samples before use in the flame photometer.

A convenient dilution for most photometers is 1: 100 dilutions for Na^+ and 1:20 dilutions for K^+

f] Standards for urine Na^+ and K^+ :

1] Standard solutions containing 10 mEq K^+/ lit and 10 mEq Na^+ / Lit were prepared by diluting 5 ml of stock A and 50 ml of stock B together to 500 ml in a volumetric flask with 18 mohm / cm quality reagent grade I water. This solution was labelled as stock urine standard.

2] Preparation of working standards :

Dilutions of the the stock urine standard were done as follows :

ml Stock Urine Std	Dilution	Conc mEq / lit Na+ / K+
2.5	100	0.25
5.0	100	0.50
7.5	100	0.75
10.0	100	1.00
15.0	100	1.50

3] These diluted standards were automised directly into the flame without further dilutions. The flame photometer was so adjusted that 1.5 mEq/L standard gave a reading close to the top scale i.e about 100. Setting water to zero, readings for all standards were taken.

A calibration graph was prepared from these readings. Urine samples were diluted (1 - 100) to give flame photometer readings within the range of standards. The urine values (as mEq/L) were obtained by calibration graph by dilution factor.

The following formula was used to report the results as mEq / 24 hours.

$$\frac{\text{mEq / lit}}{1000} \times \text{24 hr urine volume} = \text{m Eq / 24 hours urine.}$$

VII] HEMATOLOGY :

A] HAEMOGLOBIN :

International comittee for standardization in haematology (1980) Sahli (acid hematin) method was adapyed for the estimation of haemoglobin.

a] Principle :

When blood is added to 0.1N hydrochloric acid haemoglobin is converted to brown coloured acid haematin. The resulting colour after dilution is compared with standard brown glass reference blocks of a Sahli haemoglobinometer.

b] Specimen :

Capillary blood or thoroughly mixed anti coagulated venous blood was taken for determination of haemoglobin.

Requirement :

1] Sahli haemoglobinometer - It consists of :

i] A standard brown glass mounted on a comparator.

ii] A graduated tube.

iii] Hb - pipette (0.02 ml).

2] 0.1 N hydrochloric acid.

3] Distilled water.

4] Pasteur pipettes.

d] Procedure :

1] By using Pasteur pipette 0.1N hydrochloric acid was taken into graduated tube upto lowest mark (20% mark).

2] 20 µl blood was drawn in the Hb - pipette without any bubbles and excess blood on the sides of pipette was wiped by using a dry piece of cotton.

3] The drawn blood was then transferred to the acid in the graduated tube. The pipette was well rinsed and the reaction mixture was mixed well and allowed to stand for atleast 10 minutes.

4] The solution was then diluted with distilled water by adding few drops at a time carefully and by mixing the reaction mixture, until the colour matches with the glass plate in the comparator.

5] Matching was done against natural light. The level of the third was noted at its lower meniscus and the reading corresponding to this level on the scale was recorded in g/dl.

B] TOTAL ERYTHROCYTE COUNT BY HAEMOCYTOMETRY.

International comittee for standardization in haematology (1980)

a] Principle :

The blood specimen is diluted 1 : 200 with RBC diluting fluid and cells are counted under high power (40 X objective) by using a counting chamber. The number of cells in undiluted blood are calculated and reported as the number of red cells per mm^3 of whole blood.

b] Requirements :

1] Microscope.

2] Improved Neubauer chamber which has an area of 9 sq.mm and a depth of 0.1 mm.

3] RBC pipette which has a large bulb, containing a red glass bead. It has three marks 0.5, 1.0 and 101. Blood was drawn to 0.5 mark and then diluting fluid was drawn upto the mark 101. The dilution of blood was 1:200.

4] RBC diluting fluid :

It is prepared as follows :

i] Sodium Citrate : 3.0 g

ii] Formalin : 1.0 ml

iii] Distilled water to : 100ml

RBC diluting fluid is isotonic with blood hence haemolysis does not take place. Formalin from the diluting fluid acts as a preservative and checks bacterial and fungal growth and sodium citrate prevents coagulation of blood and provides correct osmotic pressure.

c] Procedure :

1] Double citrated blood was mixed carefully by swirling the bulb.

2] Blood was then quickly drawn in RBC pipette up to 0.5 mark and excess blood outside the pipette was wiped carefully using cotton.

3] Then diluting fluid was drawn upto 101 mark in RBC pipette.

4] Then pipette was rotated rapidly by keeping it horizontal during mixing.

5] After five minutes, by discarding few drops from the pipette and holding it slightly inclined small volume of the fluid was introduced under coverslip which was placed on the counting chamber.

6] The cells were allowed to settle for 2 to 3 minutes.

7] The counting chamber was then placed on the stage of the microscope.

8] Low power (10 X) objective was used and light was adjusted. Then large square in the centre with 25 small squares was located. Under high power (40 X) objective the red blood cells in the four corner squares and in the centre square were counted.

d] Calculation :

Total red blood cells /mm^3 were calculated as follows -

$$\text{Total RBC / } mm^3 = \frac{\text{Number of red cells counted x dilution}}{\text{Area counted x Depth of fluid.}}$$

Where 1] Dilution = 1:200 (i.e 200)

$$\text{2] Area counted} = \frac{80}{400} = \frac{1}{5} \text{ Sq. mm}$$

Since cells were counted in 5 bigger squares and such square is further divided in to 16 small square.

$$\text{Each small square} = \frac{1}{400} \text{ Sq. mm}$$

$$\text{Hence, area of (5 x 16) = 80 such areas} = \frac{80}{400} = \frac{1}{5} \text{ Sq.mm.}$$

$$\text{3] Depth of fluid} = \frac{1}{10} \text{ mm.}$$

4] Number of red cells counted = N

$$\text{Hence Total red blood cells / mm}^3 = \frac{N \times 200}{\frac{1}{5} \times \frac{1}{10}} = N \times 200 \times 50$$

$$= N \times 10{,}000$$

C] TOTAL LEUCOCYTE COUNT BY HAEMOCYTOMETRY :

Van Assendelft and England (1981)

a] Principle :

The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leucocytes. The blood specimen is diluted 1:20 in WBC pipette with the diluting fluid and the cells are counted under low power of the microscope by using counting chamber. The number of cells in undiluted blood are reported per mm ^{3} (µl) of whole blood.

d] Requirements :

1] Microscope.

2] Improved Neubauer Chamber.

3] WBC pipette :

It is smaller than the red cell pipette and its bulb contains a white glass bead. It has three marks 0.5, 1.0 and 11. The blood was drawn up to 0.5 mark and then diluting fluid was drawn upto 11 mark. The dilution of blood was 1 : 20.

4] WBC diluting fluid : - It was prepared as follows :

i] Glacial acetic acid : 2.0 ml.

ii] 1% (W/V) gentian Violet : 1.0ml.

ii] Distilled water : 97 ml.

c] Specimen :

Fresh blood was collected in EDTA bulbs and was used for total WBC count.

d] Procedure :

1] Blood was drawn upto 0.5 mark of a W . B. C pipette.

2] Excess blood outside the pipette was wiped carefully by using cotton. Then diluting fluid was drawn in, up to 11 mark.

3] The contents in the pipette were mixed and after five minutes by discarding few drops, the counting chamber was filled and the cells were allowed to settle for two to three minutes.

4] 'W' marked area having 16 small squares from the counting chamber were then focused by turning objective to low power (10 X).

5] Cells from four 'W' marked corner Squares were counted.

e] Calculations :

The Total WBC were calculated as follows :

$$\text{Number of white cells / mm}^3 \text{ of whole blood} = \frac{\text{Number of white cells counted x Dilution}}{\text{Area counted x Depth of fluid.}}$$

Dilution = 20

Area counted = 4 x 1 Sq. mm = 4 Sq. mm^2

Depth of fluid = 0.1 mm (constant)

$$\text{Hence number of white cells per } mm^3 \text{ of whole blood} = \frac{\text{No of cells counted} \times 20}{4 \times 0.1}$$

$$= \text{No of cells counted} \times 50$$

D] STUDY OF BLOOD SMEAR FOR DIFFERENTIAL LEUCOCYTE COUNT AND CELL MORPHOLOGY :

Macgregor, Scott and Loh (1940)

a] Introduction :

Differential Count is the per cent distribution of various white cells in the peripheral blood. It is determined from a blood smear stained with a polychromatic stain and after examination of the stained smear by using oil immersion objective (total magnification 1000 X). The number of each type of white cell is then expressed as a percentage of the total number of cells. The stained blood smear also helps to study abnormal morphology of Leucocytes and red cells. Study of blood smear helps in the diagnosis of various anemias, leukaemias and detection of blood parasites. Three major steps involved in differential count are (i) preparation of smear (ii) staining of smear and (iii) microscopic examination of the stained smear.

b] Principle :

The polychromic staining solutions (Wright, Leishman, Giemsa) contain methylene blue and eosin. These basic and acidic dyes induce multiple colours when applied to cells. Methanol acts as fixative and also as a solvent. The fixative does not allow any further change in the cells

and makes them adhere to the glass slide. The basic component of white cells (i.e cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic. The acidic components (e.g nucleus with nucleic acid) take blue to purple shades by the basic dye and they are called basophilic. The neutral components of the cell are stained by both the dyes.

c] Procedure :

I] A thin smear was prepared by spreading a small drop of blood evenly on a slide. The smear was prepared as follows :

1] A clean dry slide was taken.

2] A small drop of blood was transferred near the edge of the slide.

3] The spreader was placed at an angle 30° to 35°. The spreader was pulled back until it touched the drop of blood. The blood was allowed to run along the edge of the spreader.

4] The spreader was pushed forward to the end of the slide with a smooth movement.

5] The blood smear was then dried at room temperature to preserve the quality of the film.

II] Fixing the Smear :

The methanol present in the stain fixes the smear. If the staining is to be done later, the blood smear must be fixed with methanol for 2 to 3 minutes to prevent distortion of cell.

III] Staining the film :

a] The smear was covered with the staining solution by adding 10 - 15 drops on the smear for exactly one minute.

b] Equal number of the drops of buffer solution were added. The reaction mixture was mixed adequately by blowing on it through a pipette.

c] A gap was then given before 10 minutes, washing the smear under tap water.

d] The slide was then allowed to dry on the draining rack.

IV] Examination of film :

1] The stained smear was first examined under low power. In an ideal smear three zones were seen i] Thick area (head) ii] body and iii] thin end of the smear (tail).

2] The portion slightly before the tail end was chosen where the red cells are beginning to overlap.

3] The film was then examined under high power (oil immersion) from one field to the next. The type of leucocyte seen in each field was recorded.

4] 500 leucocytes were counted to have high degree of accuracy.

E] DETERMINATION OF PLATELET COUNT :

Bain (1985)

a] Principle :

The blood specimen is diluted 1 : 200 with platelet diluting fluid and cells are counted under high power (40 X objective) by using a counting chamber. The number of cells in undiluted blood are calculated and reported as the number of platelets per mm^3 of whole blood.

b] Requirement :

a] Microscope

b] Improved Neubauer counting chamber.

c] RBC pipette

d] Platelet diluting fluid

It is prepared as follows :

i] Procaine hydrochloride	: 3.0g.
ii] Sodium Chloride	: 10.0 g.
iii] Distilled water	: 100 ml.

Filter it through Whatman No. 44 filter paper and store in a clean and dry plastic container.

c] Specimen :

EDTA anticoagulated blood was taken. A plastic syringe was preferred for blood collection since platelets adhere to glass surface.

d] Procedure :

1] The blood specimen was mixed carefully.

2] By using RBC pipette blood was drawn upto 0.5 mark.

3] Excess blood on the outside of the pipette was wiped carefully by cotton.

4] Then the diluting fluid was drawn upto mark 101 i.e. blood was diluted 1:200 times.

5] The contents from the bulb was throughly mixed.

6] After 5 minutes first drop was discarded and then a small drop was transferred on one side of the counting chamber.

7] The filled mounted counting chamber was placed in a petridish with a moist filter paper. The filled chamber was allowed to stay undisturbed for 15 minutes. This permits the platelets to settle and also prevents evaporation of diluting fluid in the chamber.

8] The counting chamber was placed carefully on the stage of the microscope. The red cell counting area was focussed under low power magnification. The view was then moved to the corner square of the red cell area and was then changed to high power objectives.

9] The platelets appeared like highly refractile particles when observed under reduced light source by adjusting the diaphragm of the microscope.

10] Platelets in all the 25 small squares having an area equivalent to 1 Sq.mm were counted and expressed as platelets per mm^3.

e] Calculations :

$$\text{Platelets per } mm^3 = \frac{\text{Number of platelets counted x Dilution}}{\text{Volume of fluid}}$$

Where :

i] Dilution = 200

ii] Volume of fluid = $1 \times 0.1 = 0.1\ mm^3$

iii] Platelets per mm^3 $= \frac{\text{Number of platelets} \times 200}{0.1}$

$=$ Number of platelets x 2000.

F] DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE :

Wintrobe method : Wintrobe (1974)

a] Introduction :

When anticoagulated blood is allowed to stand undisturbed for a period of time, the erythrocytes tend to sink to the bottom. Two layers are formed, the upper plasma layer and the lower red blood cell layer. The rate at which the red cells fall is known as the erythrocyte sedimentation rate (E.S.R.). The first is the stage of aggregation when the red cells form rouleaux. This is followed by the stage of sedimentation in which the falling of the cells takes place. The larger the aggregation in first stage, the faster the rate of fall.

The changed levels of plasma proteins such as fibrinogen and globulins which accompany most of the acute and chronic infections tend to increase rouleaux formation. E.S.R. is therefore increased in any condition causing an increase in fibrinogen (cause of tissue breakdown).

b] Requirement :

i] Wintrobe tube.

ii] Wintrobe tube stand

iii] Pasteur pipette or 2 ml syringe with needle.

iv] Timer or watch.

c] Specimen :

3 ml of fresh EDTA anticoagulated blood was used for E.S.R. estimation.

d] Procedure :

1] Blood was mixed carefully.

2] By using pasteur pipetter wintrobe tube was filled with the specimen upto zero mark.

3] The tube was placed exactly in vertical position and the timer was set for one hour.

4] At the end of one hour the level of erythrocyte column in terms of mm. was noted.

5] The reading noted gave E.S. R. in mm. directly for the sample.

IX] HEAVY METAL ANALYSIS :

Inductive Coupled Plasma Method (Wallace and Barrett, 1981)

Heavy metal (Zn, Mg, Cr, Sr, Pb, Mn, Fe, Cu, Cd, and Ni) analysis was carried out on **Inductive Couple Plasma Machine (Perkin Elmer - Plasma 400)**. Aldrich Single Element Standards for heavy metal anlaysis were used for the preparation of standards. 18 mohm per cm, quality reagent grade I, water from Barrstead Water Purification System, was employed. Highly purified argon fuel was used to run the machine.

Unhaemolysed serum was diluted 1:20 (v/v) times and later aspirated for heavy metal analysis and the dilution factor was considered for the calculations. For heavy metal analysis from urine, fresh urine was collected 20 minutes prior to the specific time interval and then centrifuged at 2000 rpm for 10 minutes. The supernatent was used for the analysis of heavy metals.

Heavy metals from the kidney was analysed by using 100 mg of homogenised tissue in 10 ml of 18 mohm per cm, quality reagent grade I water, to make 1% solution. Homogenation of the kidney tissue was carried out in a

glass pestle and mortar. The glass pestle and mortar were rinsed in 18 mohm per cm, quality grade I water. The homogenised tissue was centrifuged at 5000 rpm. The supernatent was separated and then it was used for heavy metal analysis.

I) GLOMERULAR FILTRATION RATE (GFR) :

Determination of glomerular filtration rate was carried out by method described by Desai and Varute (1990-91).

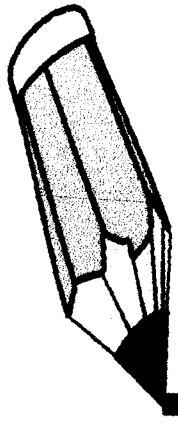
The experiments were performed on mallards weighing nearly 1Kg. Priming doses of inulin equivalent to 50 mg/100 ml and para-amino hippurate (PAH) equivalent to 5 mg/ kg were administered intra-venously atleast 45 minutes before the start of urine collection. After providing sufficient time for equilibration, three clearance periods of 20 minutes were run consecutively and then mallards were fed with different concentraions of mining effluents for different periods. The concentration of inulin were analysed from blood plasma and urine sample by Anthrone method and GFR was calculated by using the formula given below :

$$\text{GFR ml/kg/min} = \frac{\text{Inulin concentration in urine X Rate of urine flow}}{\text{Plasma concentration of inulin}}$$

CHAPTER III

SECTION - A

KIDNEY



PHYSICOCHEMICAL CHARACTERISTICS OF RUNOFF WATERS :

The samples of fresh iron ore rejects were subjected to 10 cm of rain by means of rain simulator. The runoff collected was then used for heavy metal detections and for hydrological studies. The heavy metals in the runoff are compiled in Table 'A' and the physicochemical characteristics of water are given in Table 'B'.

The runoff showed the presence of Barium, Cadmium Cobalt, Chromium, Copper, Iron, Magnesium, Manganese, Nickel, Lead, Strontium, and Zinc in various concentrations. Iron concentration (1910.5 ± 4.2 µg/l) was found to be the highest of all, followed by manganese (857.0 ± 1.37 µg/l); cadmium (710.0 µg/l) and barium (650.0 ± 1.0 µg/l). The concentrations of chromium, cobalt, copper, magnesium,nickel, lead, strontium, and zinc were equivalent to 28.8 ± 0.59, 37.0 ± 0.38, 14.6 ± 1.0, 214.0 ± 2.8, 11.9 ± 0.89, 5.4 ± 0.5, 11.3 ± 0.71 and 81.4 ± 1.0 µg/l respectively.

Oxygen and carbondioxide concentration of mining runoffs was equivalent to 15.8 ± 1.02 and 7.3 ± 0.28 mg/l respectively. The phenolphthalein acidity, total alkalinity, inorganic phosphorus, total phosphorus, organic phosphorus and sulphate concentrations of runoffs was equivalent to 9.70 ± 0.57, 14.0 ± 1.01, 3.073 ± 0.37, 23.217 ± 1.28, 20.144 ± 1.72 and 146.15 ± 3.0 mg/l respectively.

The mining effluents also showed the presence of total solids, total dissolved solids, nitrates and hardness, equivalent to 1.91 ± 0.01, 0.089 ± 0.001, 1.371 ± 0.13 and 8.713 ± 0.27 gm/l respectively. The runoff was acidic (pH 6.1) and the specific conductance and redox potentials were equivalent to 51.0 ± 3.0 µMho and 0.2176 ± 0.001 volts respectively.

TABLE A : HEAVY METALS FROM MINING EFFLUENTS :

	Metal	Concentration
1.	Ba	650.0 ± 1.0 µg/L
2.	Cd	710.0 ± 2.0 µg/L
3.	Co	22.80 ± 0.59 µg/L
4.	Cr	37.0 ± 0.38 µg/L
5.	Cu	14.6 ± 1.0 µg/L
6.	Fe	1910.5 ± 4.20 µg/L
7.	Mg	214.0 ± 2.80 µg/L
8.	Mn	857.0 ± 1.37 µg/L
9.	Ni	11.90 ± 0.89 µg/L
10.	Pb	5.40 ± 0.50 µg/L
11.	Sr	11.30 ± .71 µg/L
12.	Zn	81.40 ± 1.0 µg/L

TABLE B : PHYSICOCHEMICAL CHARACTERISTIC OF RUNOFF WATER:

Sr. No.	Parameters		Concentration
1.	Carbon dioxide	(mg/L)	7.30 ± 0.28
2.	Dissolved Oxygen	(mg/L)	15.80 ± 1.02
3.	Phenolphthalein acidity	(mg/L)	9.70 ± 0.57
4.	Total alkalinity	(mg/L)	14.0 ± 1.01
5.	Inorganic phosphorus	(mg/L)	3.073 ± 0.37
6.	Total phosphorus	(mg/L)	23.217 ± 1.28
7.	Organic phosphorus	(mg/L)	20.144 ± 1.72
8.	Sulphate	(mg/L)	146.15 ± 3.0
9.	Total solids	(gm/L)	1.91 ± 0.01
10.	Total dissolved solids	(gm/L)	0.089 ± 0.001
11.	Nitrate	(gm/L)	1.371 ± 0.13
12.	Hardness	(gm/L)	8.713 ± 0.27
13.	Specific conductance	(µMho)	51.0 ± 3.0
14.	Redox potential	(volts)	0.217 ± 0.001
15.	pH		6.10 ± 0.01

I] HISTOLOGY OF THE BIRD KIDNEY :

Inorder to facilitate better understanding of the morphological changes induced by mining effluents in the kidney, it is felt desirable to give a brief account of the normal histology of the bird kidney before mentioning the morphological changes appearing in the kidney after the exposure to the mining effluents.The histological features of the duck kidney are as follows :

a] Gross morphology :

The kidneys of birds are paired structures lying in pelvic skeletal depression along the backbone. They comprise about 1% of the body weight being somewhat larger in small birds (Johnson, 1968) ; and in species having functional salt glands (Hughes, 1970). Each kidney is usually composed of three lobes (cranial, middle and caudal), although this location is more or less distinct and the relative size of the lobes varies considerably among species (Johnson, 1968). The ureter lies along the ventral surface of the kidney and receives major branches from each lobe. Major circulatory connections have been studied. The arterial blood supply is from the renal artery which supplies the cranial lobe, and the branches of the sciatic artery to the middle and caudal lobe (Siller and Hindle, 1969). The femoral artery may also branch to the kidney (Sturkie, 1965). Avian kidney receives venous blood from the legs, tail and mesenteries via the renal portal system and renal veins provide for the drainage to the vena cava.

b] Nephrons :

The kidney of the normal bird comprises numerous functional excretory units termed as nephrons. Birds possess two basic types of nephrons, including cortical nephrons composed of glomerulus, proximal and distal

convoluted tubules and less numerous juxta medullary or looped nephrons in which the proximal and distal tubules are seperated by a loop of Henle which extends into the medulla.

The glomeruli are small and contain few capillary loops. The cells of the proximal tubules have a brush border to the luminal surface (Sperber, 1960), similar to those of mammals and vertebrates in general.

The basal (capillary facing) surfaces of these cells exhibit numerous small projections similar to those reported for gecko (Roberts and Schmidt - Nielson, 1966) rather than the extensive folding into the mitochondria containing compartments typical of mammals.

The cells of the avian distal tubule also differ from those of the mammals in that they have selectively flat basal membranes (Sperber, 1960 ; Poulson, 1968). Henle's loop in bird also differs in several respects from that of mammals. There are no abrupt transitions between thin and thick segments. Instead, the proximal tubule generally narrows, loses its brush border and continues into the medulla with no change in staining properties (Poulson, 1968). The cells of the thin limb of bird are higher than the squamous epithelium of the mammalian thin limb (Sperber, 1960). As in the short looped nephrons of mammals, the turn of the loop occurs in the ascènding (thick) limb. The thick limb resembles that of mammals in that, the cells are characterized by striations due to parallel mitochondria (Sperber, 1960) and the general association of this cell type with active Na^+ transport is consistent with the probable role of the thick limb as counter current multiplier system.

The nephrons of birds like those of mammals have a region of attachment of the afferent glomerular arteriole and a loop of the distal convoluted tubule at Bowman's capsule. Cells in the arteriole called "Juxta glomerular cells" exhibit characteristic granules thought to contain renin. The adjacent cells of the distal convoluted tubule possess large prominent nuclei

and form the "Macula densa". Juxtaglomerular cells and macula densa together form the juxta glomerular apparatus (Taylor *et. al.*, 1970).

Each nephron consists of a renal capsule, a coiled uriniferous tubule and interstitial haematopoietic tissue. The renal capsule is made up of a glomerulus and Bowman's capsule. Glomerulus is a network of blood capillaries connecting afferent arterioles with efferent arterioles. Glomerular walls are very thin and consists principally of squamoid endothelial cells.

The Bowman's capsule is cupshaped, double-walled structure of which the inner one closely surrounds the glomerulus. This inner wall is composed of visceral epithelium and closely adheres to the capillaries of the glomerulus. The cells of the visceral epithelium are squamoid in nature. The outer wall is known as parietal layer. The latter is thicker than the inner-wall as it is formed mainly of cuboidal cells. A space termed as Bowman's space separates the inner wall from the outer one.

Bowman's capsule is actually the proximal terminal end of the uriniferous tubule and is followed by a tubular neck. Other regions of the tubule are a proximal tubule and a distal tubule. The cells of the collecting tubules are cuboidal and possess centrally placed nuclei. The brush border is absent. Apical mucous granules are prominent in these cells. The proximal tubules bear thin microprocesses called microvilli at the luminal side which form the brush border. The distal tubules do not possess the brush border and their lumen is wide.

The duck kidney contains two types of nephrons : those with no loops of Henle, they are of reptilian type and are located exclusively in the cortex and those of the mammalian type, with long or intermediate length loops, located in the medulla (Braun and Dantzler, 1972).

III] HISTOPATHOLOGICAL ALTERATIONS :

General :

The routine haematoxylin - eosin staining techniques employed to the kidney sections of the ducks exposed to various concentrations of mining effluents (M.E.) for different time intervals revealed histopathological changes in the renal capsule (Glomeruli + Bowman's capsule), proximal tubules, distal tubules and collecting tubules. The histopathological changes occurring in the kidney showed concentration dependency and time dependency, especially the higher concentrations of mining effluents had profound influence on the kidney which showed progressive damage as the time passed. Similarly, the lower concentrations did exhibit time dependent progress in the renal alterations upto certain extent. When the ducks were sacrificed for various time intervals, between thirty minutes of exposure to M.E. and five hours of M.E. exposure no detectable damage to the kidneys was observed, only in a few cases the early necrotic changes like slight swelling of glomeruli, marginal increase in the lumen diameter of the tubules, occasional marginal swelling of the tubule cells were seen but the end of six hours onwards, the renal form changes were detectable and also were quite significant and these changes were not only dose dependent but also progressive.

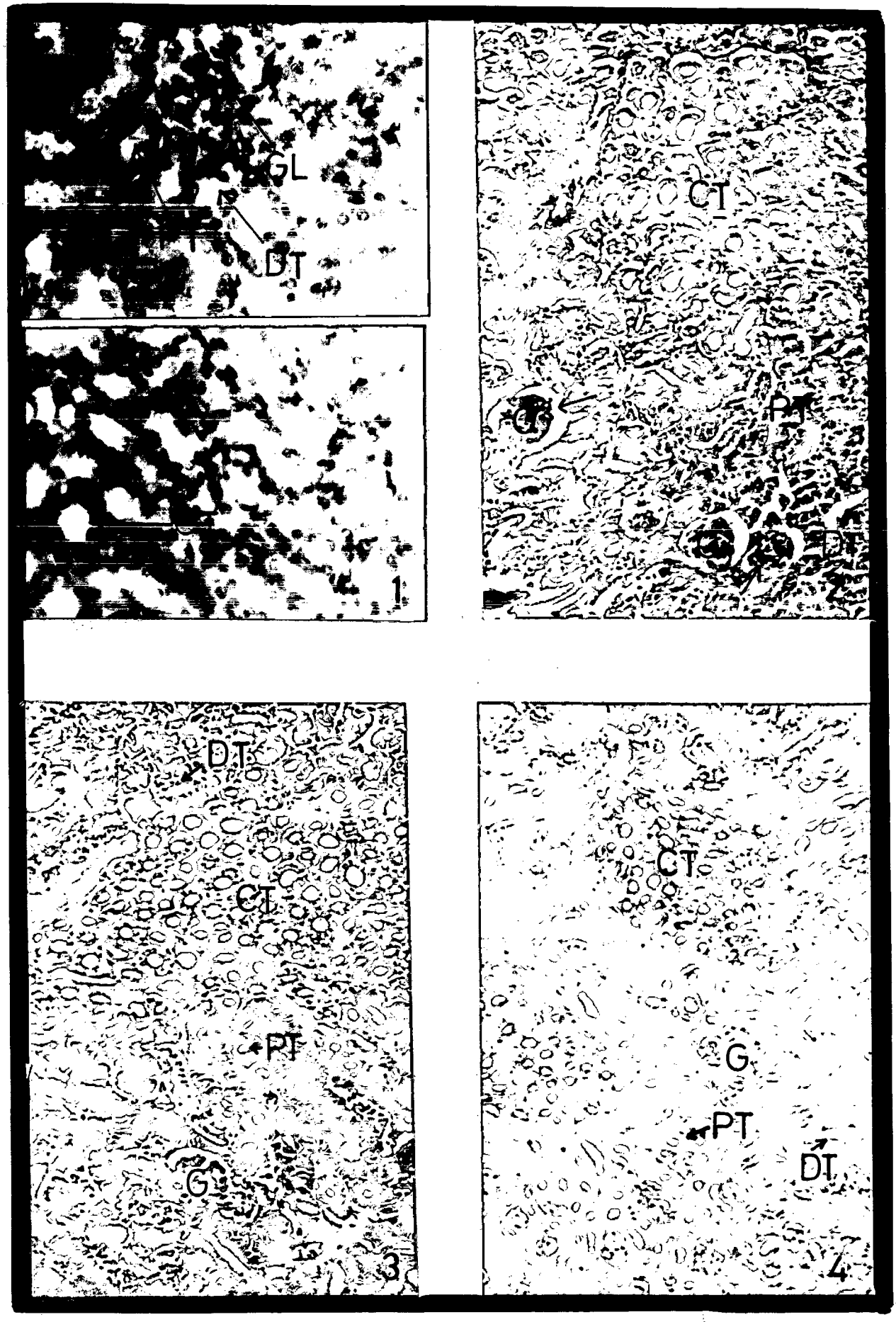
ALTERATIONS IN THE RENAL CAPSULE :

For the sake of convenience the changes in the renal capsule are clubbed together under glomeruli and described as glomerular changes.

GLOMERULI :

The glomerular changes in general were begining with the swelling of the glomeruli and then progressing towards distortion which included disruption

PLATE NO 1



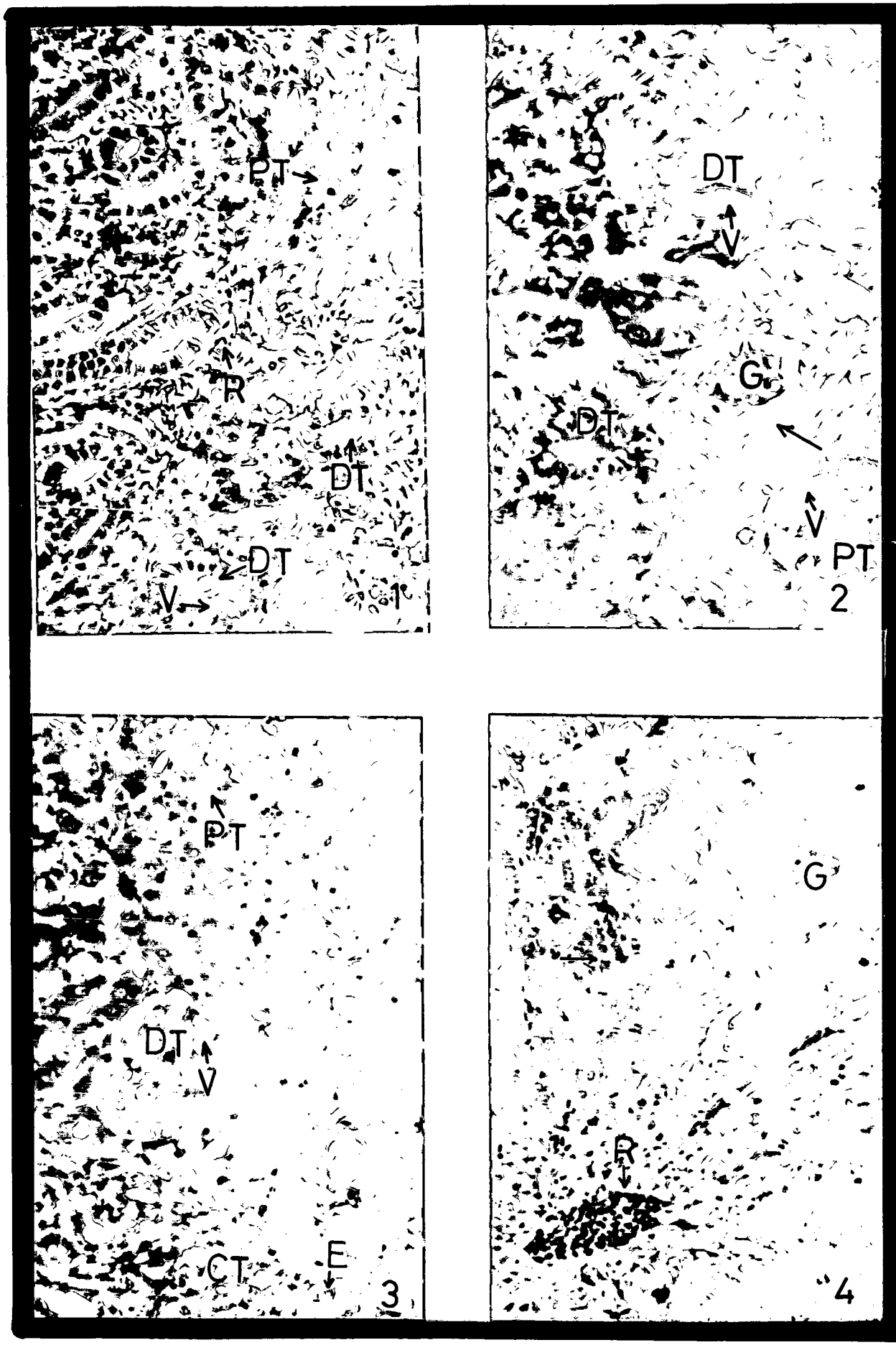
of glomerular walls, vacuolisation or development of empty spaces, release of glomerular exudents into the Bowman's space, breakage of capillaries leading to the bleeding and escape of blood cells. The changes in Bowman's capsule were like, widening of Bowman's space, disruption of Bowman's walls - both inner and outer, change in the shape of Bowman's capsule caused either by acute swelling or shrinkage and sometimes the breakage of glomerulus as well as Bowman's capsule was observed. The intensity of the necrotic changes depended upon the concentration and the duration of exposure to the mining effluents. The number of renal capsules getting affected depended upon the concentration of the mining effluents.

The ducks exposed to **0.01% M.E.** did not show any significant change in the renal capsule up to the end of twenty nine days but showed slight swelling of glomeruli. At the end of seven hundred and twenty hours about 10% glomeruli showed necrotic glomeruli. The main features of glomerular damage were change in shape of the glomeruli, widening of Bowman's space, vacuolisation of the glomeruli and in a few cases, slight widening of Bowman's space **(Plate 1; Fig.2).**

The **0.01%** concentration of **M.E.** induced change in the shape and slight vacuolisation of the glomeruli **(Plate 1: Fig 3)** at the end of one thousand and eighty hours. About 15% corticular glomeruli exhibited damage.

The **0.1% M.E.** induced necrosis of about 15% glomeruli at the end of six hours and the exudents were seen in the Bowman's space **(Plate 1. Fig 4).** Then, up to twenty four hours there was neither further damage to the glomeruli nor there was increase in the number of affected glomeruli. But at the end of twenty four hours there was increase in the severity of the damage. The glomeruli showed the change in shape, vacuolisation, disruption of Bowman's wall, widening of Bowman's space and exudents were seen in the Bowman's space **(Plate 2 : Fig 2).** About 18 percent glomeruli were affected. At the end

PLATE NO 2



CAPTIONS TO THE FIGURES

PLATE 2 :

Fig. 1 : 0.1% M. E. - 12 hours. (20 x 5)

Note the RBCs (R) in the interstitium of the damaged tubules, vacuolisation of the proximal tubule (PT), distortion and vacuolisation (V) of the distal tubule (DT).

Fig 2 : 0.1% M. E. - 24 hours. (40 x 5)

Note the change in the shape of the glomerulus (G), disruption of Bowman's wall (arrow), widening of Bowman's space, vacuolisation (V) of the distal tubules (DT) with disruption of the luminal border, disruption of proximal tubule (PT) with the loss of basal membrane and vacuolisation (V).

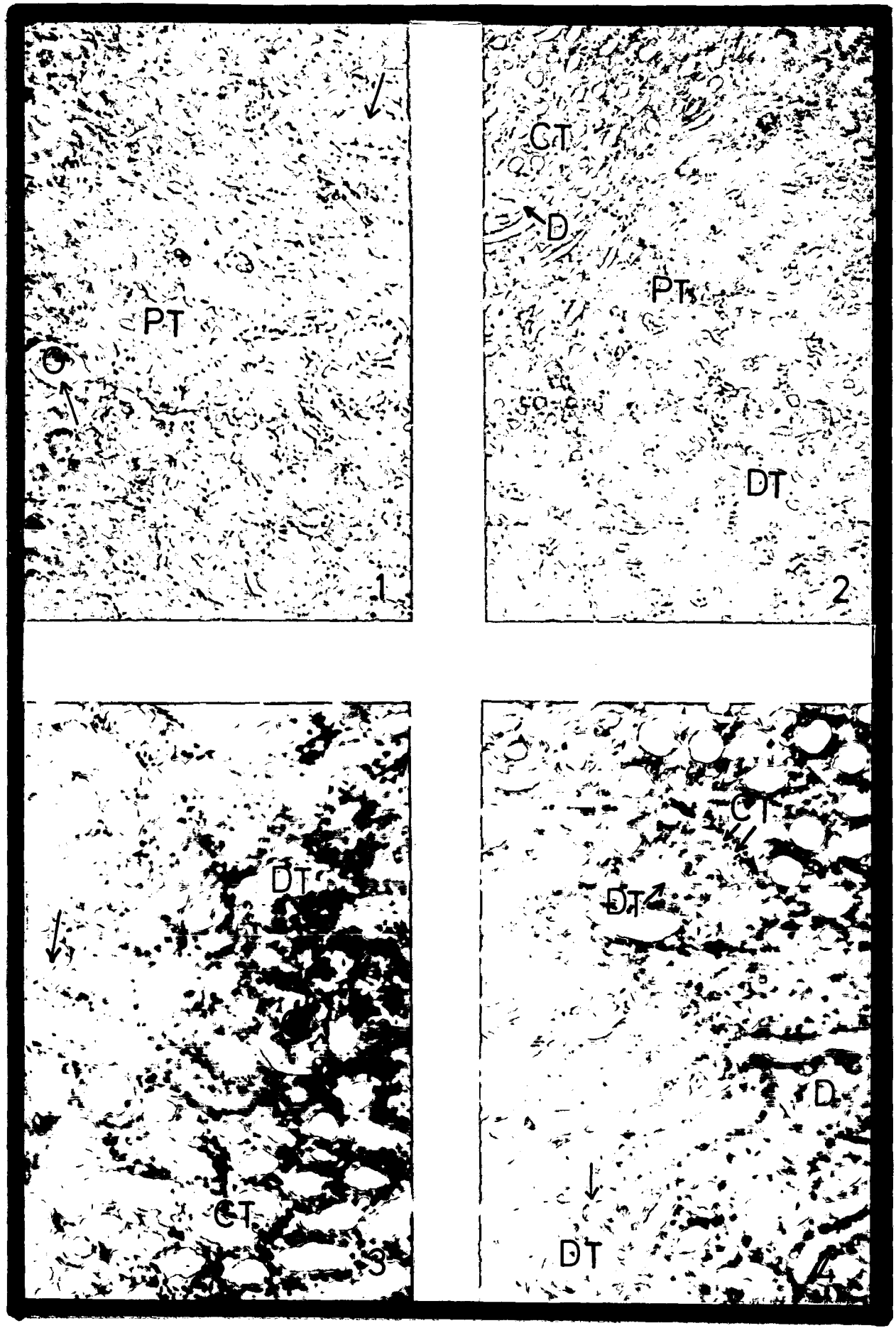
Fig 3 : 0.1% M. E. - 48 hours. (20 x 5)

Note the damaged distal tubule (DT) with vacuolisation (V) and disruption of the basal membrane, damaged proximal tubule (PT), collecting tubules (CT) with exudents (E) in the lumen.

Fig 4 : 0.1% M. E. - 72 hours. (20 x 5)

Note the damaged glomerulus (G) distortion of the tubule (arrow) and RBCs on the distorted tubules.

PLATE NO 3



CAPTIONS TO THE FIGURES

PLATE 3 :

Fig. 1 : 0.1% M. E. - 120 hours. (10 x 5)

Note the broken glomerulus (G) with widening of Bowman's space (arrow), distorted proximal tubule (PT).

Fig. 2 : 1.0% M. E. - 6 hours. (10 x 5)

Note the damaged proximal and distal tubules (PT, DT), and the collecting tubules with the debris (D) in the lumen.

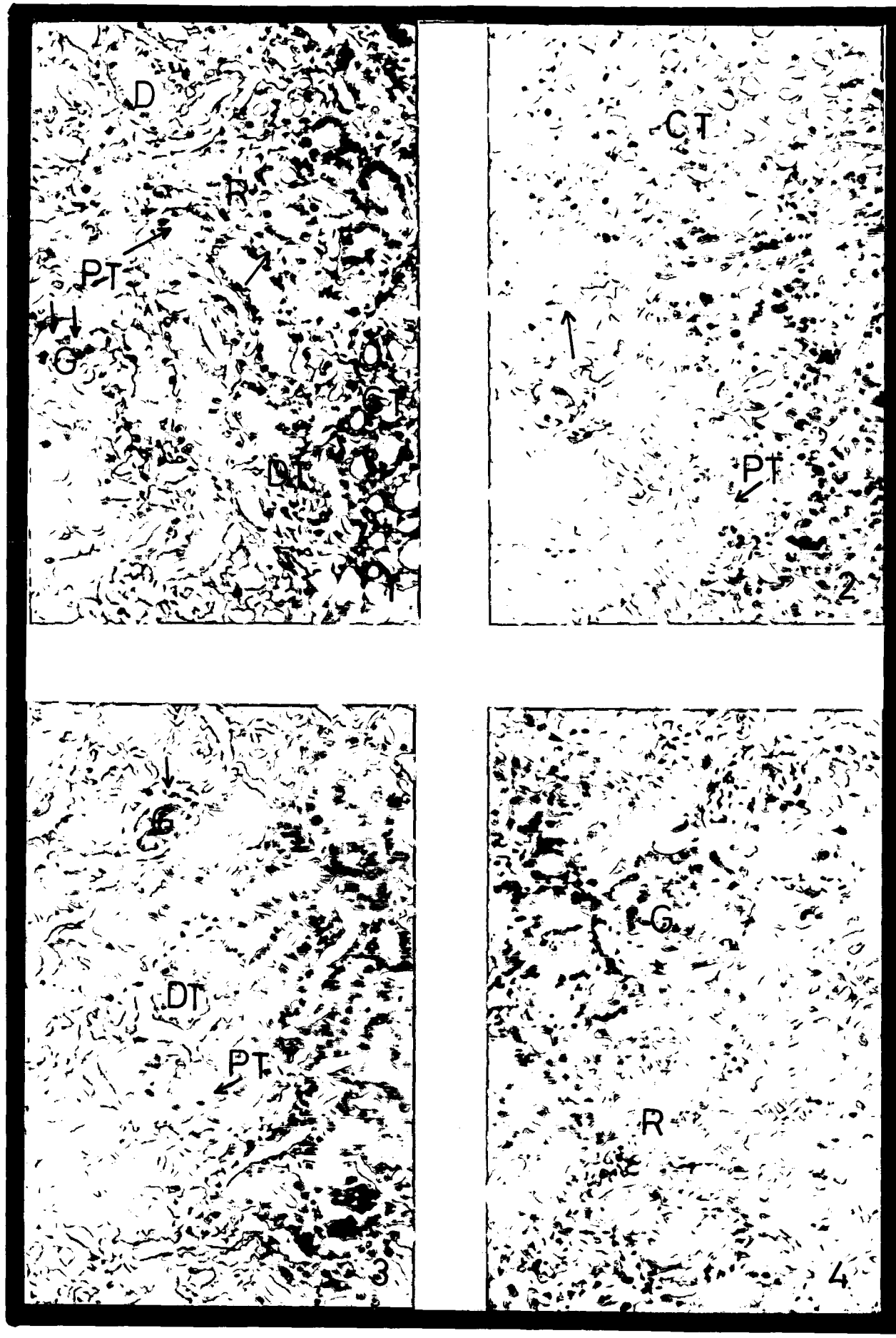
Fig. 3 : 1.0% M. E. - 12 hours. (20 x 5)

Note the damaged collecting tubules (CT) with disruption of the luminal border, damaged glomerulus (G) with exudents in Bowman's space, damaged distal tubule (DT) and vacuolisation (arrow) of the proximal tubule.

Fig. 4 : 1.0% M. E. - 24 hours. (20 x 5)

Note the damaged collecting tubule (CT) with broken luminal border (double arrow), distorted distal tubule with a debris (D) in the lumen. Also note the damaged luminal border (arrow) of distal tubule (D.T.).

PLATE NO 4



CAPTIONS TO THE FIGURES

PLATE 4 :

Fig 1 : 1.0% M. E. - 48 hours. (20 x 5)

Note the RBCs (R) in the interstitial spaces, loss of basal membrane (arrow) of the proximal tubule, damaged glomerulus (G) with exudents (double arrow) in Bowman's space, damaged proximal and distal tubules (PT, DT) and collecting tubules (CT) with the loss of luminal border.

Fig 2 : 1.0% M. E. - 72 hours. (20 x 5)

Note the damaged collecting tubules (CT), distorted distal tubules (arrow) and damaged proximal tubule (PT).

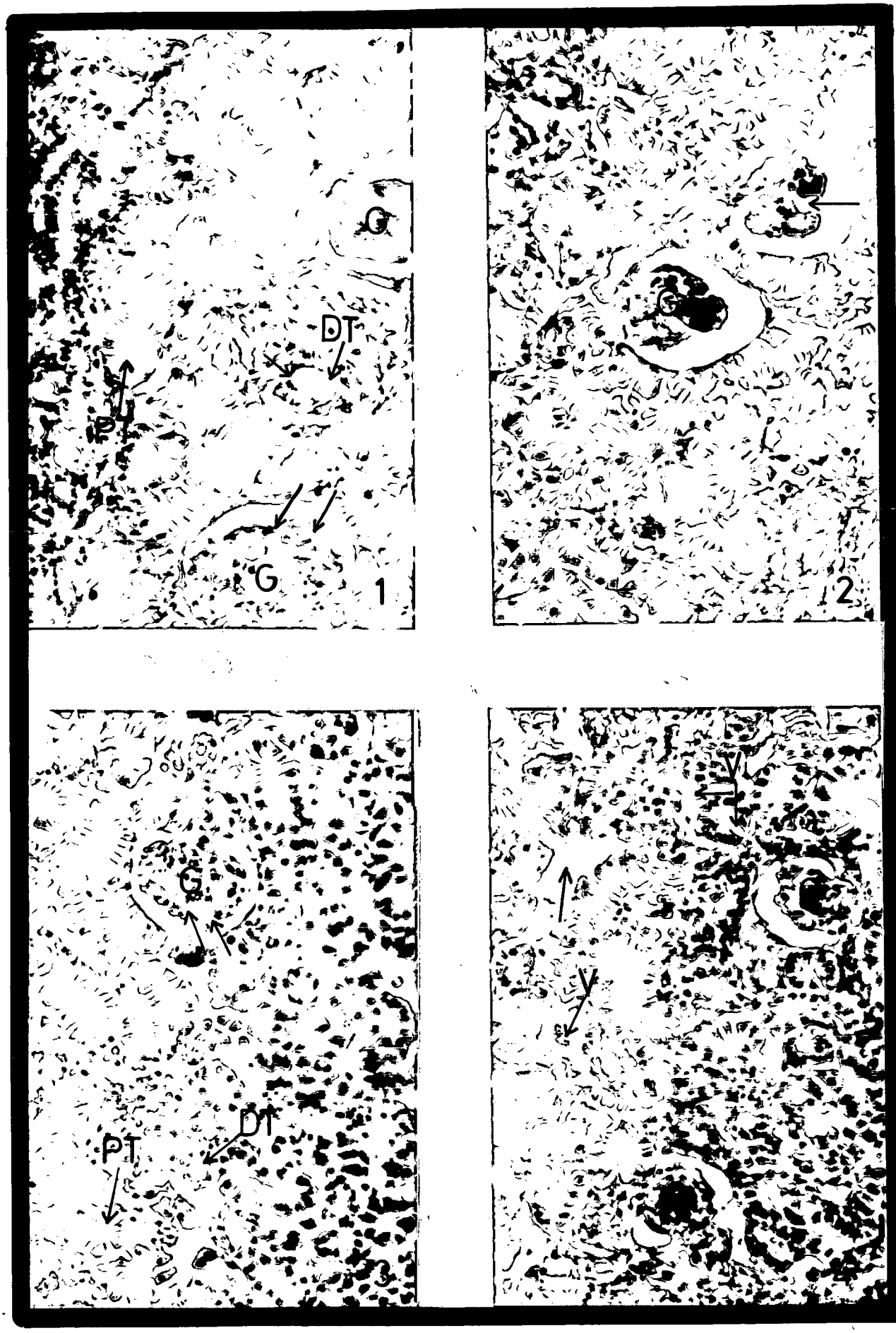
Fig 3 : 1.0% M. E. - 96 hours. (20 x 5)

Note the damaged glomerulus (G) with empty spaces and exudents (arrow) in Bowman's space, damaged distal tubule (DT) and disrupted proximal tubule (PT).

Fig 4 : 1.0% M. E. - 120 hours. (20 x 5)

Note the RBCs (R) in the interstitial spaces, swollen glomerulus (G) with the loss of Bowman's space.

PLATE NO 5



CAPTIONS TO THE FIGURES

PLATE 5 :

Fig 1 : 5.0% M. E. - 6 hours. (20 x 5)

Note the damaged glomerulus (G) with exudents (double arrow) in Bowman's space, the damaged distal tubule (DT) with the loss of luminal border (arrow), damaged proximal tubule (PT) with vacuolisation and disruption of brush border (arrow).

Fig 2 : 5.0% M. E. - 12 hours. (20 x 5)

Note the borken glomerulus (G) with vacuolisation (arrow) and widening of Bowman's space.

Fig 3 : 5.0% M. E. - 24 hours. (20 x 5)

Note the damaged glomerulus with exudents (double arrow) in Bowman's space, damaged distal tubule (DT) and also note the development of empty spaces (arrow) in between the cells of the proximal tubule (PT).

Fig 4 : 5.0% M. E. - 48 hours. (20 x 5)

Note the damaged glomerulus (G) with exudents in Bowman's space, vacuolisation (V) of the tubules and widening of interstitium (arrow) containing exudents.

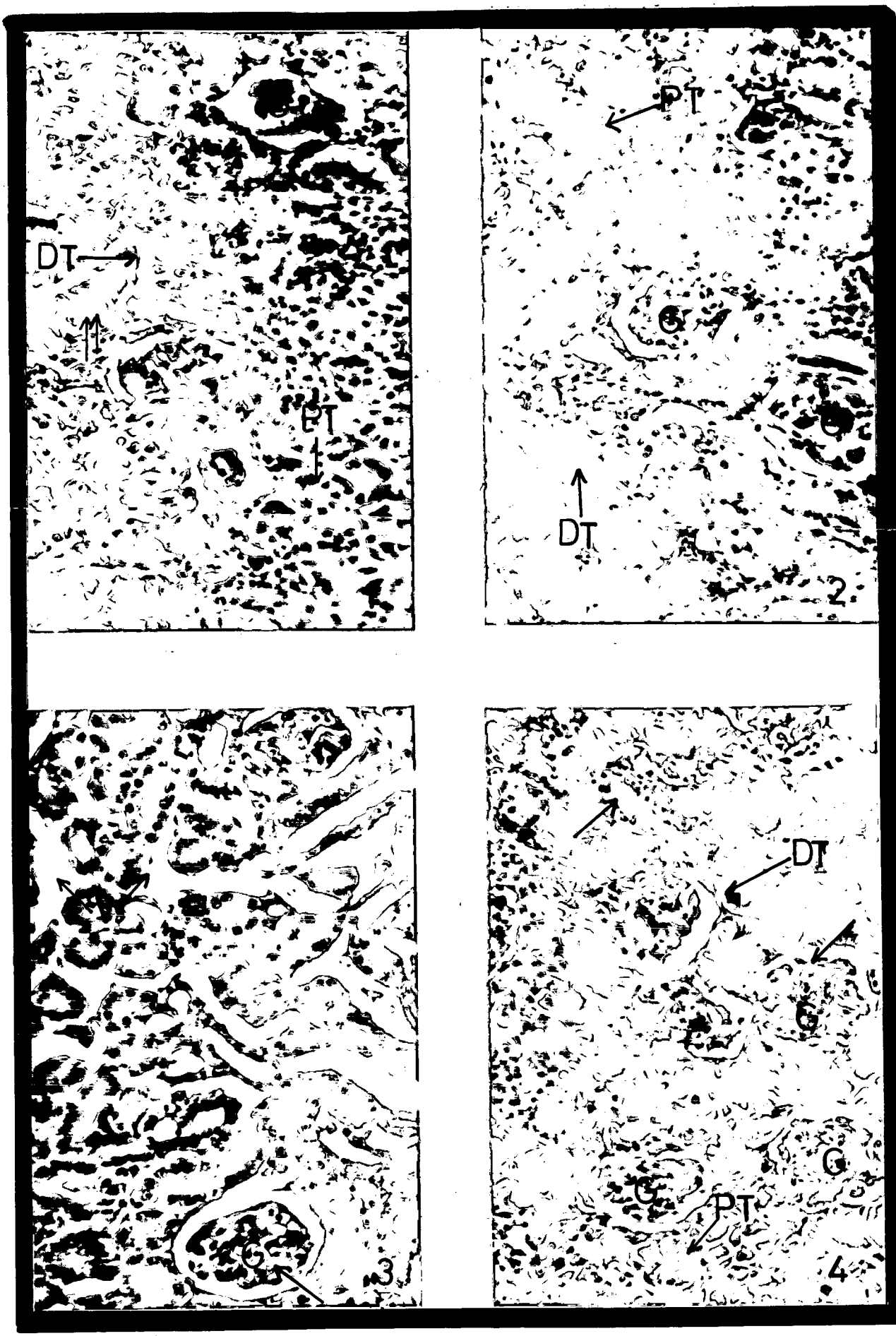
the end of twentyfour and fortyeight hours the glomeruli showed some vacuolisation, widening of Bowman's space with or without exudents **(Plate 5 : Figs 3 and 4)**. There was no further increase in the number of necrotic glomeruli at these hours. At the end of seventytwo hours, some glomeruli were acutely necrotic and exhibited : change in shape, disruption of the glomerular structure and presence of exudents in Bowman's space **(Plate 6 : Fig 1)**. By the end of ninetysix and one twenty hours the glomeruli exhibited : disruption, vacuolisation and exudents in Bowman's space and in few cases damage to the Bowman's wall was seen **(Plate 6 : Figs 2 and 3)**. By the end of both nientysix and one twenty hours, thirtysix percent glomeruli were necrotic.

The **10.0% M.E.** induced damage to about thirty six percent glomeruli at the end of six hours. The glomeruli appeared to be distorted and showed exudents in Bowman's space. In a few cases, the outer wall of Bowman's capsule was disrupted or eroded **(Plate 6 : Fig 4)**. At the end of twelve hours a few glomeruli showed loss of its connection to the afferent and efferent glomerular arterioles **(Plate 7 : Fig 1)** and in few cases, the connection was intact with exudents extending from its sides to the Bowman's space. By the end of fortyeight hours the disruption of the Bowman's wall was seen and the glomeruli in the process of losing its connection with glomerular arterioles were seen and such glomeruli showed the cleft to its side **(Plate 7 : Fig 3)**. There was no increase in the number of necrotic glomeruli at this time interval.

By the end of seventy two hours the glomeruli were acutely damaged with pronounced vacuolisation. The Bowman's inner wall was disrupted and erosion of outer wall was also seen in few instances. Along with the glomerulus the juxta glomerular apparatus was also damaged **(Plate 7 : Fig 4)**. The exudents were seen in Bowman's space.

At the end of ninetysix and seventy two hours about 40% of the glomeruli were necrotic. The change in the shape of glomeruli and distortion of glomerular tuft was seen. The disruption of the Bowman's inner and outer wall

PLATE NO 6



CAPTIONS TO THE FIGURES

PLATE 7 :

Fig 1 : 10.0% M. E. - 12 hours. (20 x 5)

Note the swollen glomeruli (G) and acutely damaged tubules (arrow)

Fig 2 : 10.0% M. E. - 24 hours. (20 x 5)

Note the acutely damaged distal tubule (DT) with debris (-D-) in the lumen, acutely damaged collecting tubules (CT) with disruption of luminal border and accumulation of debris (D) in the lumen.

Fig 3 : 10.0% M. E. - 48 hours. (20 x 5)

Note the swollen and damaged glomerulus (G) with disruption of Bowman's wall (arrow), damaged proximal tubule (PT) and distorted distal tubule (DT) with the debris (D) in the lumen.

Fig 4 : 10.0% M. E. - 72 hours. (20 x 5)

Note acutely damaged glomerulus (G) with exudents in Bowman's space (arrow) acutely vacuolised distal tubule (DT) and distorted collecting tubules (CT) with debris (D) in the lumen.

CAPTIONS TO THE FIGURES

PLATE 6 :

Fig 1 : 5.0% M. E. - 72 hours. (20 x 5)

Note the damaged glomerulus (G) with exudents in Bowman's space, damage distal tubule (DT) with vacuolisation (double arrow), damaged proximal tubule (PT) with the debris (arrow) in the lumen.

Fig 2 : 5.0% M. E. - 96 hours. (20 x 5)

Note the damaged glomerulus (G) with exudents in Bowman's space, damaged proximal and distal tubules (PT, DT) with debris (arrow) in the lumen.

Fig 3 : 5.0% M. E. - 120 hours. (20 x 5)

Note the damaged glomerulus (G) with empty spaces, extreme vacuolisation (V) of the proximal and distal tubules leading to the loss of integrity.

Fig 4 : 10% M. E. - 6 hours. (20 x 5)

Note the distorted glomeruli (G) and exudents (arrow) in Bowman's space, vacuolised distal and proximal tubules (DT, PT).

PLATE NO 7

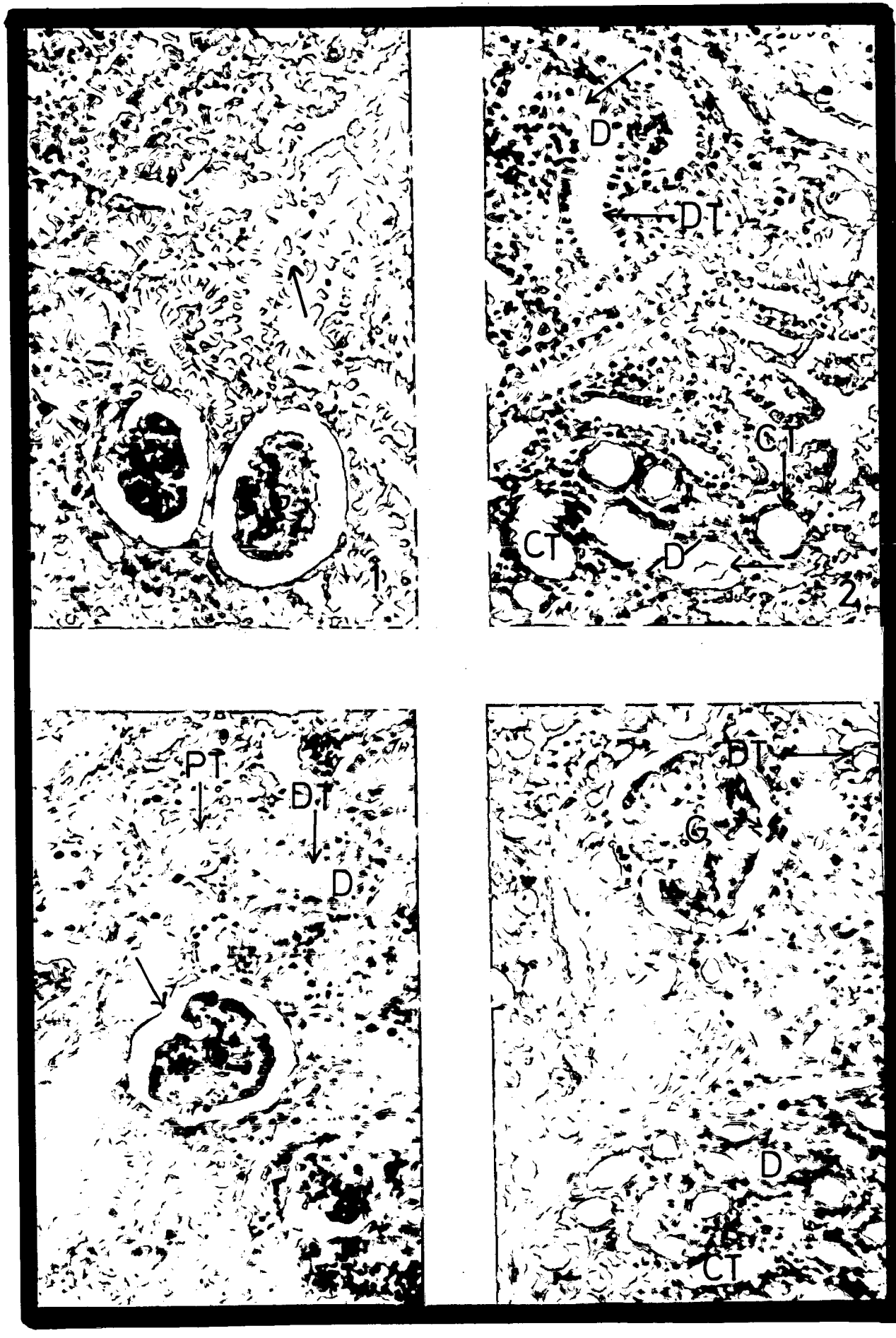
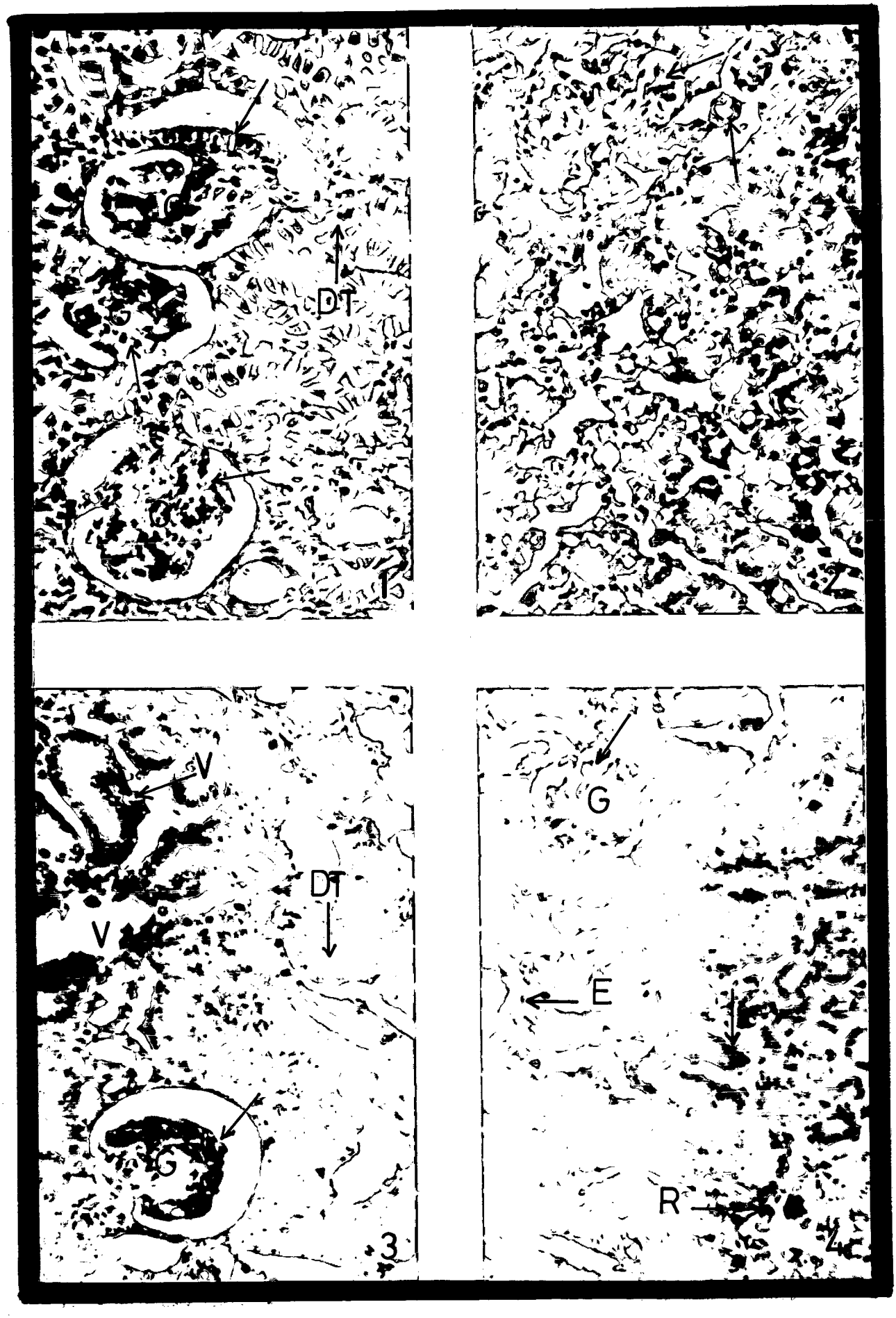


PLATE NO 8



CAPTIONS TO THE FIGURES

PLATE 9 :

Fig 1 : 50.0% M. E. - 24 hours. (20 x 5)

Note the necrotic glomerulus (G) with exudents in the Bowman's space, distorted proximal and distal tubules (PT, DT).

Fig 2 : 50.0% M. E. - 48 hours. (20 x 5)

Note the glomerulus (G) with acute vacuolisation (arrow), displacement of the tubule cells nuclei (N) and the pycnotic nuclei (NP). Also note the loss of integrity of tubules.

Fig 3 : 50.0% M. E. - 72 hours. (20 x 5)

Note swelling of glomerulus (G) and filling of Bowman's space by exudents, acute vacuolisation of tubules (CT), loss of tubule integrity and disruption of tubular lumina.

Fig 4 : 50.0% M. E. - 96 hours. (20 x 5)

Note the damaged collecting tubules (CT), damaged proximal tubules with pycnotic nuclei (NP), the accumulation of cytoplasmic debris (D) in the lumen.

CAPTIONS TO THE FIGURES

PLATE 8 :

Fig 1 : 10.0% M. E. - 96 hours. (20 x 5)

Note the swollen and distorted glomeruli (G) with exudents (arrow) in Bowman's space, acutely distorted distal tubule (DT) with the loss of luminal border.

Fig 2 : 10.0% M. E. - 120 hours. (20 x 5)

Note the acutely damaged tubules with acute vacuolisations (arrow).

Fig 3 : 50.0% M. E. - 6 hours. (20 x 5)

Note widening of Bowman's space (arrow) with exudent, distortion of tubules with vacuolisation (V) also note the disruption of lumen border (DT - arrow).

Fig 4 : 50.0% M. E. - 12 hours. (20 x 5)

Note the distorted glomerulus (G) with exudents (arrow) in the Bowman's space, acute distortion of distal tubule with loss of basal membrane (arrow) the nuclear exudents (E) and RBCs (R) in the interstitium.

of seventy two hours the glomerular damage was seen but there was no increase in the number of affected glomeruli **(Plate 2 : Fig 4)**.

By the end of one twenty hours about twenty percent of glomeruli were found damaged and the main feature was the shrinkage of glomeruli with widening of Bowman's space **(Plate 3 : Fig1)**.

The glomeruli under the influence of **1.0% M.E.** showed reduction in size, presence of exudents in the Bowman's space and vacuolisation **(Plate 3 : Fig3)**. About 20% glomeruli exhibited damage, while about 2-3 percent showed the early necrotic changes such as swelling, slight change of shape and changes in Bowman's space. These changes with glomerular necrosis described above were seen at the end of six, twelve, twenty four and fortyeight hours, especially at the end of fortyeight hours the percentage of the affected glomeruli did not increase, but exudents were seen in the Bowman's space and these exudents sometimes filled the major portions of the Bowman's space **(Plate 4 : Fig 1)**. At the end of ninetysix and one twenty hours, the changes in the shape of the glomeruli, glomerular swelling, vacuolisation and casting of exudents were observed **(Plate 4 : Fig 3 and 4)**. In a few cases, the disruption of Bowman's wall was observed. About twentyeight percent glomeruli were found damaged at the end of ninetysix and one twenty hours.

The **5.0% M.E.** induced damage to about twentyeight to thirty percent renal capsules (glomeruli + Bowman's capsule) at the end of six hours. There was no increase in the number of necrotic glomeruli up to the end of forty eight hours. At the end of six hours a few glomeruli showed change in shape, widening of Bowman's space while others showed swelling with large empty spaces in the glomeruli, disruption of the walls of Bowman's capsule and casting of exudents in the Bowman's space **(Plate 5 : Fig 1)**.

At the end of twelve hours some glomeruli exhibited shrinkage leading to change in shape and significant widening of Bowman's space **(Plate 5 : Fig 2)**. Besides this, the cleaving of glomerulus was also observed in a few cases. By

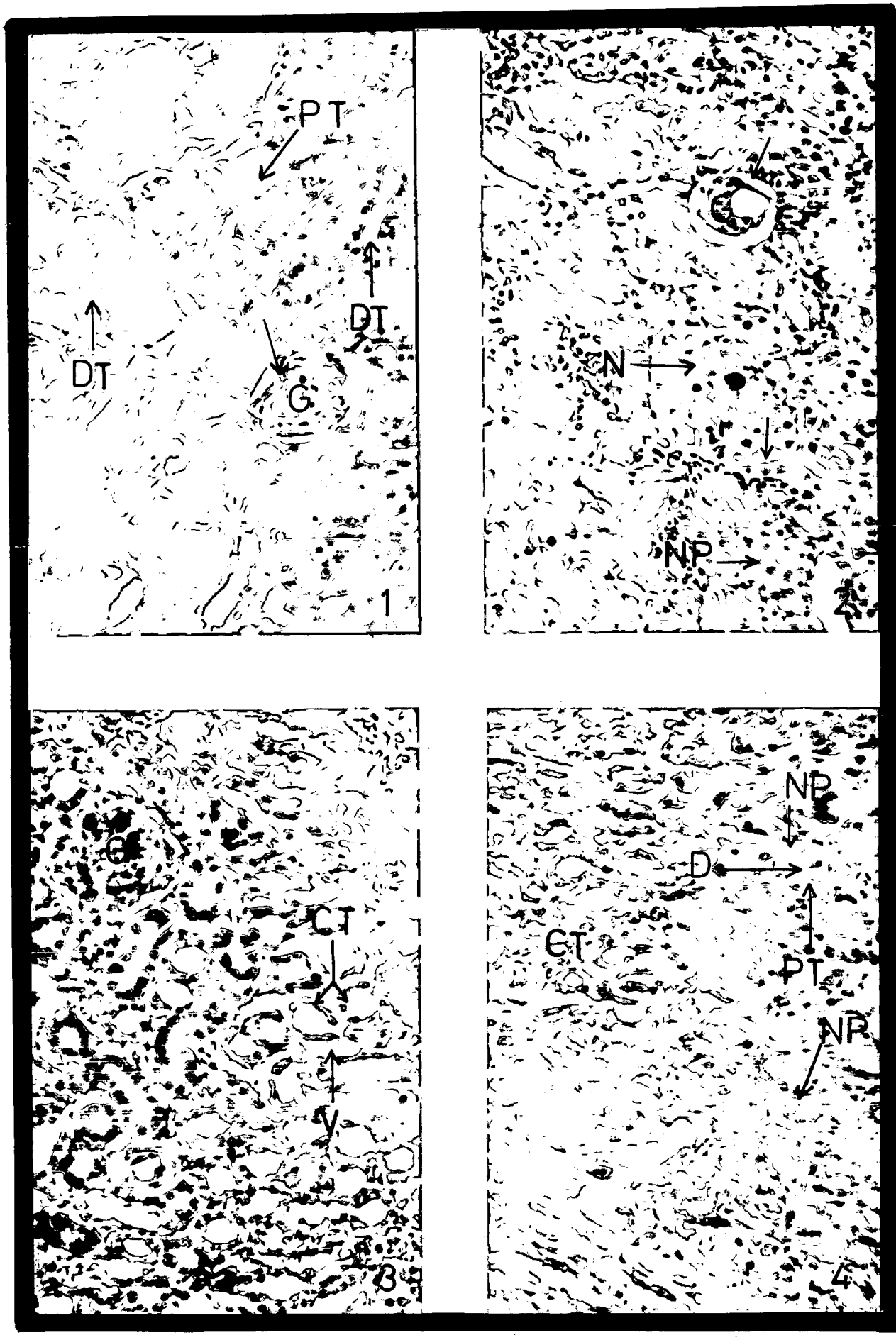
was seen in some cases. Many glomeruli showed the casting of exudents into Bowman's space. The juxta glomerular apparatus appeared to be damaged **(Plate 8 : Fig 1)**.

The **50% M.E.** promoted damage to 45% of the glomeruli at the end of six hours. A few glomeruli with extreme widening of Bowman's space were seen. **(Plate 8 : Fig 3)**. By the end of twelve hours, the glomeruli were distorted with disruption of Bowman's walls and showed exudents in the Bowman's space **(Plate 8 : Fig 4)**.

At the end of twentyfour hours the glomeruli were necrotic and the exudents in the form of nuclei and blood cells were found in the Bowman's space **(Plate 9 : Fig 1)**. There was no further change in the number of affected glomeruli at this time interval, in comparison to that seen at the end of six hours. At the end of fortyeight hours, few glomeruli showed a large empty space in them **(Plate 9 : Fig 2)** with the disruption of Bowman's innerwall and release of exudents in the Bowman's space. By the end of seventytwo hours, few glomeruli showed acute swelling and filling of Bowman's space with exudents **(Plate 9 : Fig 3)**. The disruption of Bowman's wall was also seen. Besides, there was 2% increase in the number of necrotic glomeruli at this time interval.

By the end of the one twenty hours there was 6% increase in the number of necrotic glomeruli. There was significant increase in the Bowman's space and the glomerular shape appeared to be changed. The Bowman's space showed the presence of exudents **(Plate 10: Fig 1)**.

The exposure to **100% M.E.** induced necrosis in about sixty percent glomeruli at the end of six hours. The glomeruli showed acute swelling with blood cells, vacuolisation, release of exudents in Bowman's space and also the macula densa appeared to be damaged **(Plate 10 : Fig 2)** and the necrosis of glomeruli did not progress much up to the end of twentyfour hours, where the glomerular contents invaded the Bowman's space **(Plate 10 : Fig 4)**.


PT
DT
DT
G
1
N
NP
2
G
CT
V
3
NP
D
CT
PT
NP
4

The kidney exhibited presence of about seventy percent necrotic glomeruli at the end of one twenty hours where the macula densa also showed necrosis. The Bowman's space showed the presence of exudents **(Plate 11: Fig.4)**.

THE ALTERATIONS IN PROXIMAL AND DISTAL TUBULES :

The alterations in the proximal and distal tubules were similar when the ducks were exposed to different concentrations of M.E. for various time intervals. Hence, these are described together. For all the concentrations of M.E., there were in-significant changes in the proximal and distal tubules before six hours. Only a very few tubules exhibited slight swelling in one of the tubule cells or sometimes a marginal increase in the size of the tubule indicating swelling. The control animals' kidneys showed normal proximal and distal tubules. The ducks exposed to 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0 percent mining effluents exhibited necrosis in about 15%, 20%, 28-30%, 36%, 45% and 60% proximal and distal tubules at the end of six hours. The necrosis was progressive and involved more tubules as the time progressed. The birds exposed to **0.01% M.E.** did not show any change in the proximal and distal tubules up to the end of twentynine days. At the end of seven hundred and twenty and one thousand and eighty hours, the 10% and 15% tubules respectively were found necrotic.

Both the proximal and distal tubules showed necrotic changes, such as swelling of cells, disruption of tubules, vacuolisation of tubule cells, loss of brush border in proximal tubules and disruption of luminal borders, loss of basal membranes, pycnosis of tubule nuclei, exudation of cytoplasm and nuclei into the interstitium in the bird exposed to **0.01% M.E.** for seven twenty and one thousand and eighty hours **(Plate 1 : Fig 2 and 3)**.

The ducks exposed to **0.1 to 100% M.E.** exhibited pronounced necrosis of both the proximal and distal tubules and most of the necrotic changes

mentioned above for **0.01% M.E.** exposure, were seen but in addition the changes such as sloughing off of cells into the lumen to form the cellular debris, the widening of interstitium, the occurrence of blood cells in the interstitium, acute vacuolisation marked with loss of tubular architecture was observed **(Plate 1 : Figs 2, 3 and 4 ; Plate 2 : Figs, 1, 2, 3 and 4 ; Plate 3 : Figs 1, 2, 3 and 4 ; Plate 6 : Figs 1, 2, 3 and 4 ; Plate 7 : Figs 1, 2, 3 and 4 ; Plate 8 : Figs, 1, 2, 3 and 4 ; Plate 9 : Figs 1, 2, 3 and 4 ; Plate 10 : Figs 1, 2 and 3 ; and Plate 11 : Figs 1, 2, 3 and 4).**

The **0.1% M.E.** induced a slight increase in the number of necrotic tubules at the end of twenty four hours and about eighteen percent tubules were necrotic. All the concentrations **(except 0.1%)** of mining effluents did not increase the number of necrotic tubules from twelve to ninety six hours. By the end of one twenty hours, twenty, twenty eight, thirty six, forty, fiftysix and seventy percent of proximal and distal tubules were necrotic under the influence of 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0 percent mining effluents respectively.

ALTERATION IN THE COLLECTING TUBULES :

Under the influence of 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0 percent M.E. fifteen, twenty, thirty, thirtyfive to forty, fifty and sixty to sixtyfive percent of collecting tubules showed necrosis by the end of six hours. Only the **0.01% M.E.** did not induce any necrosis in the collecting tubules up to the end of twentynine days. By the end of seven twenty and one thousand eighty hours, about 10 and 15 percent tubules showed necrotic changes. The collecting tubules did not show numerical rise in necrosis upto one twenty hours under the influence of mining effluents. But at the end of one twenty hours, 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0 percent M.E. induced necrosis to twenty, thirty, thirtyfive, fortytwo, fiftyfive and seventy percent collecting tubules.

CAPTIONS TO THE FIGURES

PLATE 10 :

Fig 1 : 50.0% M. E. - 120 hours. (20 x 5)

Note the damaged glomeruli (G) with exudents (arrow) in the Bowman's space, the damaged tubules (double arrow)

Fig 2 : 100.0% M. E. - 6 hours. (20 x 5)

Note the swelling of glomerulus (G) with exudents (arrow) in Bowman's space, vacuolisation (V) of the tubules and accumulation of debris (D) in the lumen.

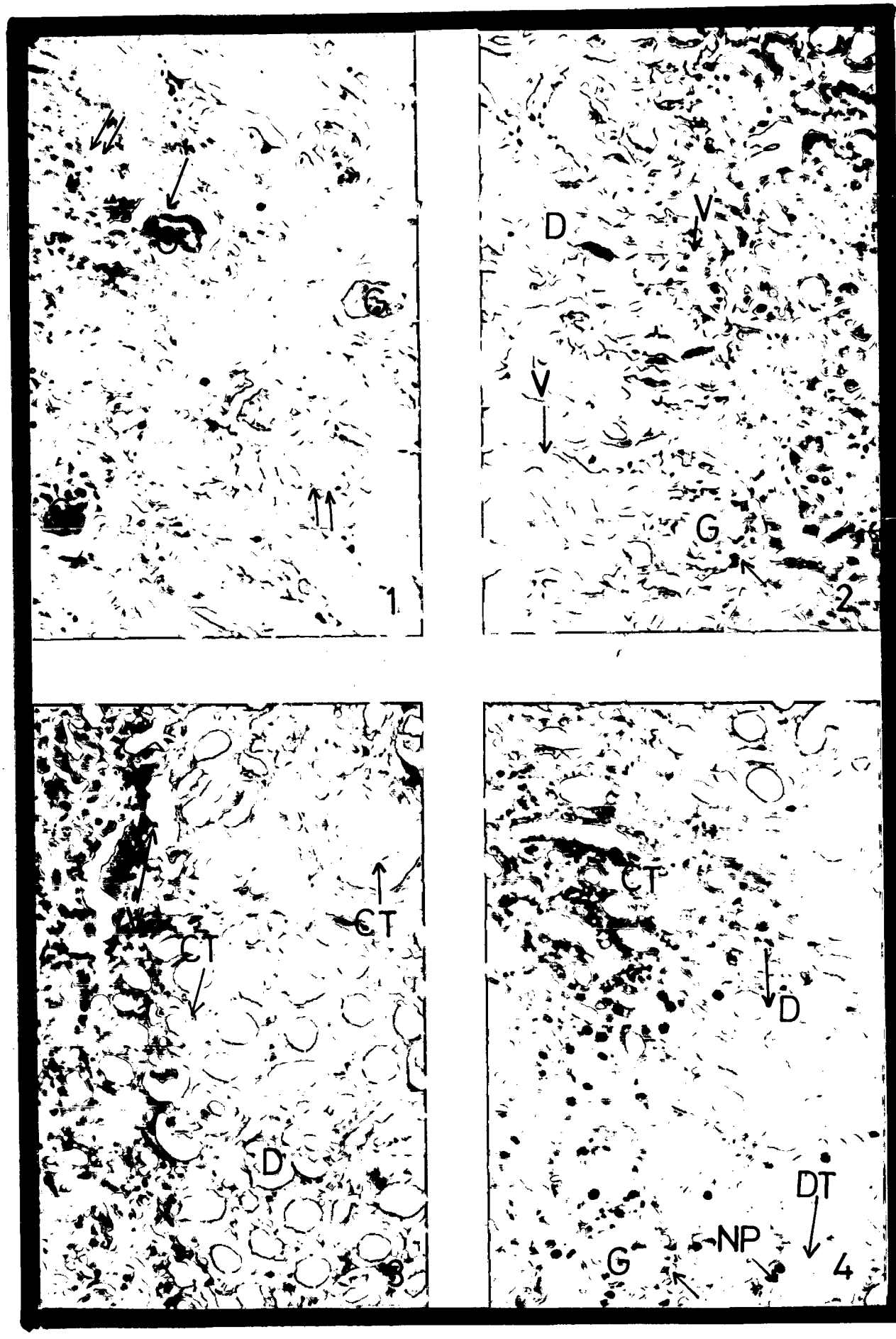
Fig 3 : 100.0% M. E. - 12 hours. (20 x 5)

Note the damaged tubules (CT), acute vacuolisation (V) and accumulation of debris (D) in the lumen.

Fig 4 : 100.0% M.E. - 24 hours. (20 x 5)

Note the damaged collecting tubules (CT), swollen glomerulus (G) with exudents (arrow) in Bowman's space, accumulation of debris (D) in the lumen of distal tubule, pycnotic nuclei (NP).

PLATE NO 10



CAPTIONS TO THE FIGURES

PLATE 11 :

Fig 1 : 100.0% M. E. - 48 hours. (20 x 5)

Note the distorted collecting tubules (CT), proximal tubules (PT) filled with derbis (D) in the lumen, distal tubule (DT) with disrupted luminal border, pycnotic nuclei (NP) in the tubule cells.

Fig 2 : 100% M. E. - 72 hours. (20 x 5)

Note the damaged collecting tubules (CT), distorted proximal tubule (PT) and distal tubule (DT).

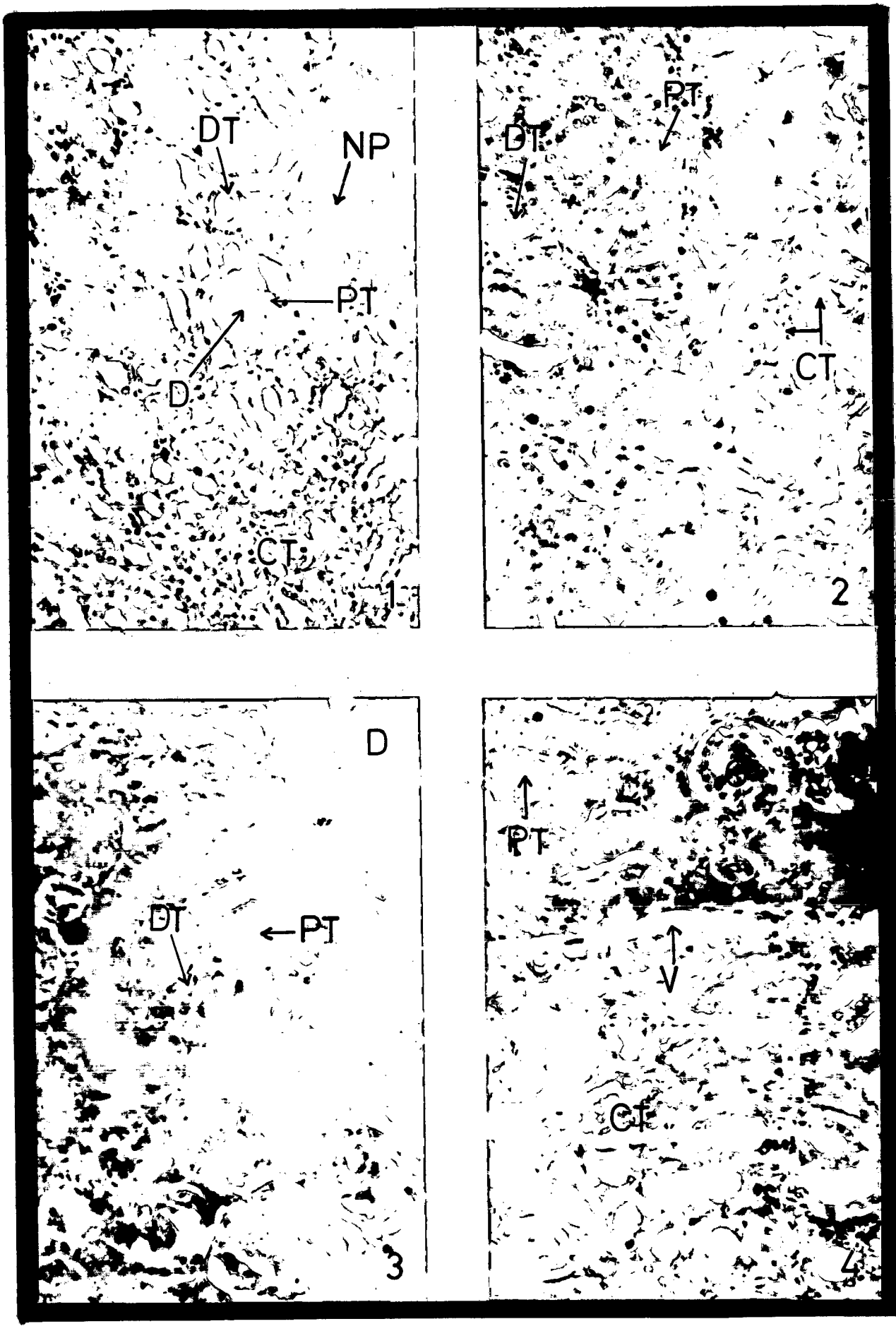
Fig 3 : 100.0% M. E. - 96 hours. (20 x 5)

Note acutely damaged distal tubule (DT) and proximal tubule (PT), debris (D) in the lumen.

Fig 4 : 100.0% M. E. - 120 hours. (20 x 5)

Note acutely damaged collecting tubules, distorted glomerulus (G), damaged proximal tubule (PT), vacuolisation (V) in the collecting tubule.

PLATE NO. 11



II] NORMAL AND ULTRA STRUCTURAL CHANGES IN KIDNEY:

The functional unit of kidney is a nephron, which includes glomerulus and the tubules. The normal glomerulus **(Plate 12 : Fig 1)** is a vascular epithelial organ with tufts of capillaries arranged in the form of lobules and a supporting frame work of extracellular matrix and cells in so-called centrilobular location called the Mesangium. The capillary walls of glomerulus consist of three layers : endothelium, basement membrane and epithelium (podocyte). Foot processes are also present which are separate and are not fused. The clusters of capillaries are freely suspended within the Bowman's capsule from the point of its attachment at the vascular pole. Thus, it has two functional spaces, the capillary lumina and the Bowman's space/cavity or urinary space.

The tubules of the kidney are mainly of three types : Proximal, Distal and Collecting. The proximal tubule **(Plate 12 : Fig 2)** is a heterogenous structure with tall epithelium and the apical membrane is greatly expanded in the form of closely packed microvilli as brush border. The lumen of the proximal tubule is narrow without tissue debris. Normal proximal tubule showed intact mitochondria, intact lysosomal vesicles, no cytoplasmic vacuolisation and no deposition of heavy metal in the cytoplasm and nucleus **(Plate 12 : Fig 2).**

Distal tubules of the kidney of ducks, show normal nucleus with no deposition of heavy metals, intact chromatin material in the nucleus, no deposition of lipid droplets in cytoplasm, wide luminal space with intact luminal border and intact mitochondria in the tubule cells **(Plate 12 : Fig 3).**

The collecting tubules of the kidney are slightly larger in size with tubule cells more in number than the distal tubules. The nucleus is spherical and located more towards the luminal border. The luminal border and tubule basement membrane is intact with intact mitochondria and no lipid droplets in

CAPTIONS TO FIGURES

PLATE 12 :

Fig 1 : Normal glomerulus (1 x 8000)

Note normal mesangial area (M.E.), podocyte (P) foot processes (F), basement membrane (B.M) and Bowman's space (U). capillary lumen (Cp.L), endothelium (En).

Fig 2 : Control Proximal tubules (1 x 5,000)

Note intact brush border (B.B), lumen (L) and mitochondrion (M).

Fig 3 : Control Distal tubule (1 x 3,000)

Note normal nuclei (N), lumen (L) and intact mitochondria.

Fig 4 : Control collecting tubule (1x 2,000)

Note intact luminal border (single arrows), normal nuclei (N), mitochondria (M) and basal membrane (double arrow).

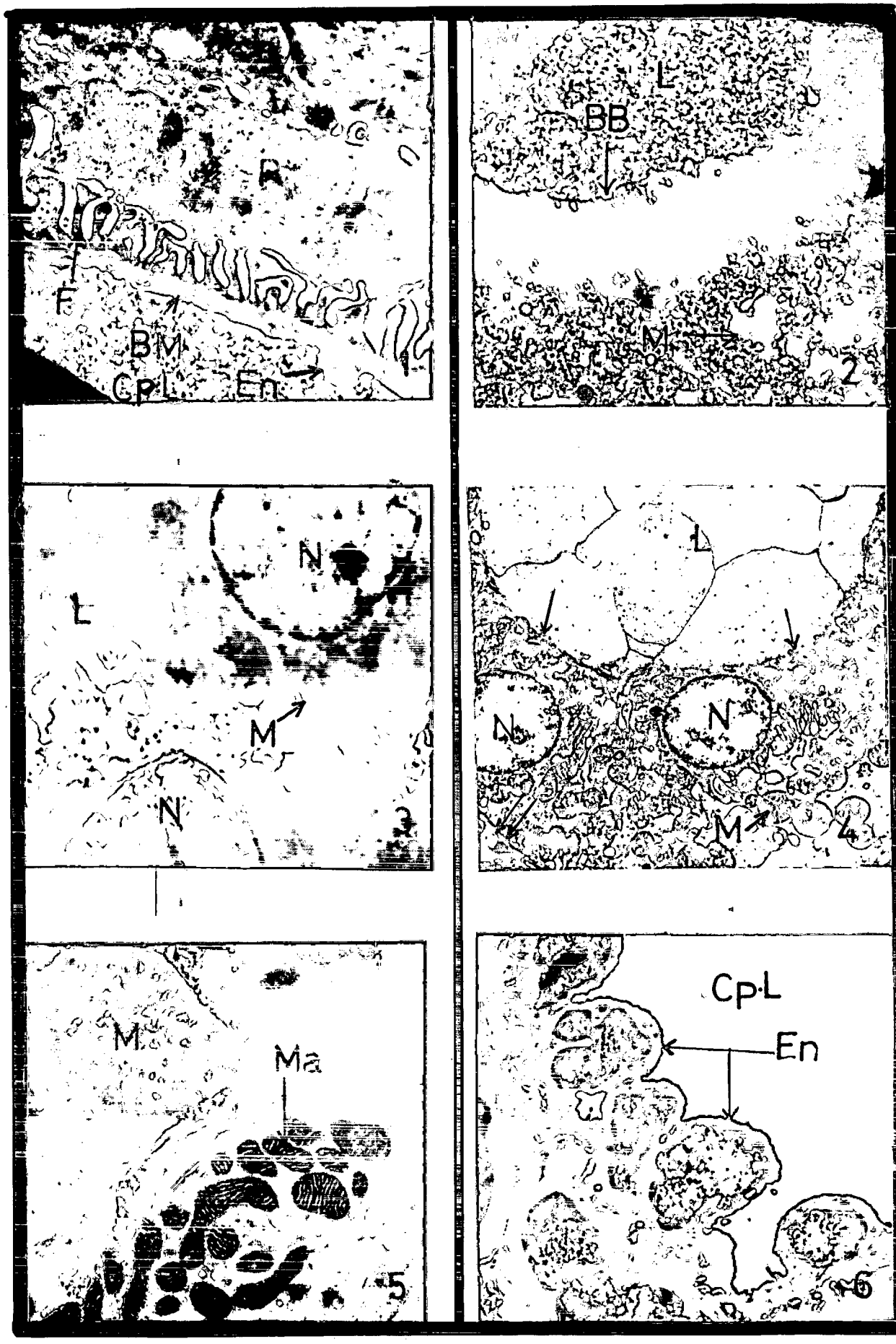
Fig 5 : Control mitochondria (1x 10,000)

Note normal mitochondria (M) and (Ma)

Fig 6 : Normal blood capillary (1 x 4,000)

Note intact capillary endothelium (En), and normal capillary lumen (Cp.L).

PLATE NO 12



CAPTIONS TO FIGURES

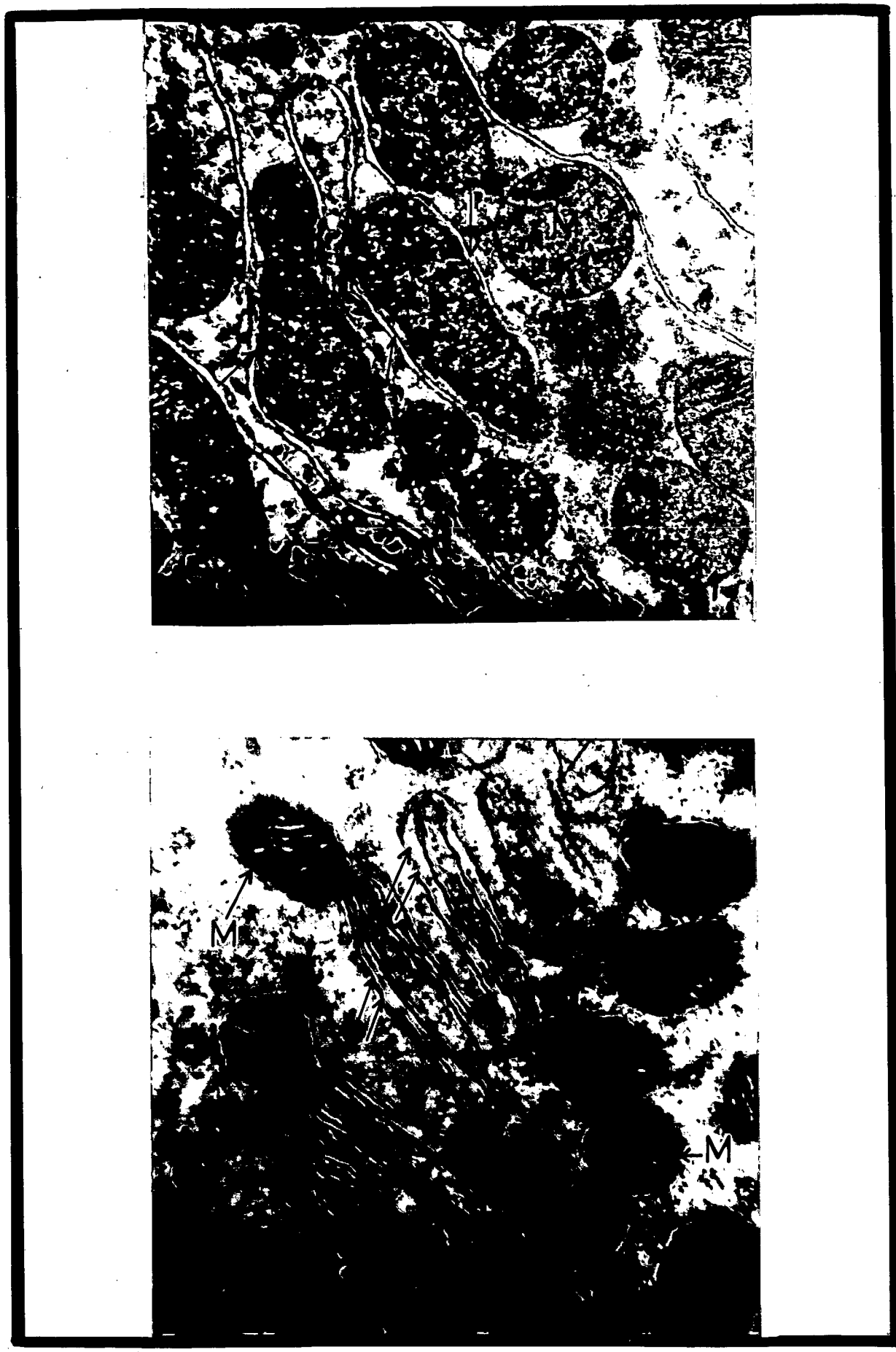
PLATE 13 :

Fig 1 : Mammalian distal tubule (1 x 15,000).

Note spherical and oblong mitochondria (M) with cytoplasmic channels encompassing the mitochondria (arrow).

Fig 2 : Reptilian distal tubule (1 x 30,000).

Note spherical mitochondria (M) also note parallel cristae running from one end to the other (arrow) and cytoplasmic channels found between mitochondrial rows but not covering the mitochondria (double arrows).


M
M

the tubule cytoplasm. The luminal space is slightly larger than distal tubule. **(Plate 12 : Fig 4).**

Under electron microscopy intact mitochondria show prominent cristae without vacuolisation in mitochondrial matrix **(Plate 12 : Fig 5)** and capillary endothelium with undisturbed and undamaged capillary endothelial cells. **(Plate 12 : Fig 6).** In **Plate 12 : Fig 5** the mammalian and reptilian mitochondria are shown. 'Ma' indicates the reptilian mitochondria and 'M' indicates the mammalian mitochondria.

Mammalian and reptilian distal tubules can be indentified by the nature of mitochondria and the position of cytoplasmic channels. In mammalian distal tubules, the mitochondria are spherical and oblong with cytoplasmic channels **(Plate 13 : Fig 1)** encompassing them, where as the reptilian distal tubules show spherical mitochondria with cristae running parallel from one end to the other end. Cytoplasmic channels were flanked by the mitochondria. These did not cover the mitochondria **(Plate 13 : Fig 2).**

A] ULTRA STRUCTURAL ALTERATIONS IN GLOMERULUS UNDER THE INFLUENCE OF MINING EFFLUENTS :

The mallards exposed to **0.01%** mining effluents (M.E.) did not exhibit any significant ultra structural change in glomerulus up to the end of twenty nine days, but at the end of thirty days, significant ultra structural changes in glomerulus were observed. The damaged glomerulus showed fused foot processes, thickening and distortion of basement membrane **(Plate 14 : Fig 1).** Reduction of endothelial cell size followed by endothelial sclerosis, change in the shape of red blood cells, widening of basement membrane, vacuolisation of cytoplasm in podocyte and discontinous basement membrane was also observed **(Plate 14 : Fig 2, Plate 15 : Fig 1 and 2).**

CAPTIONS TO FIGURES

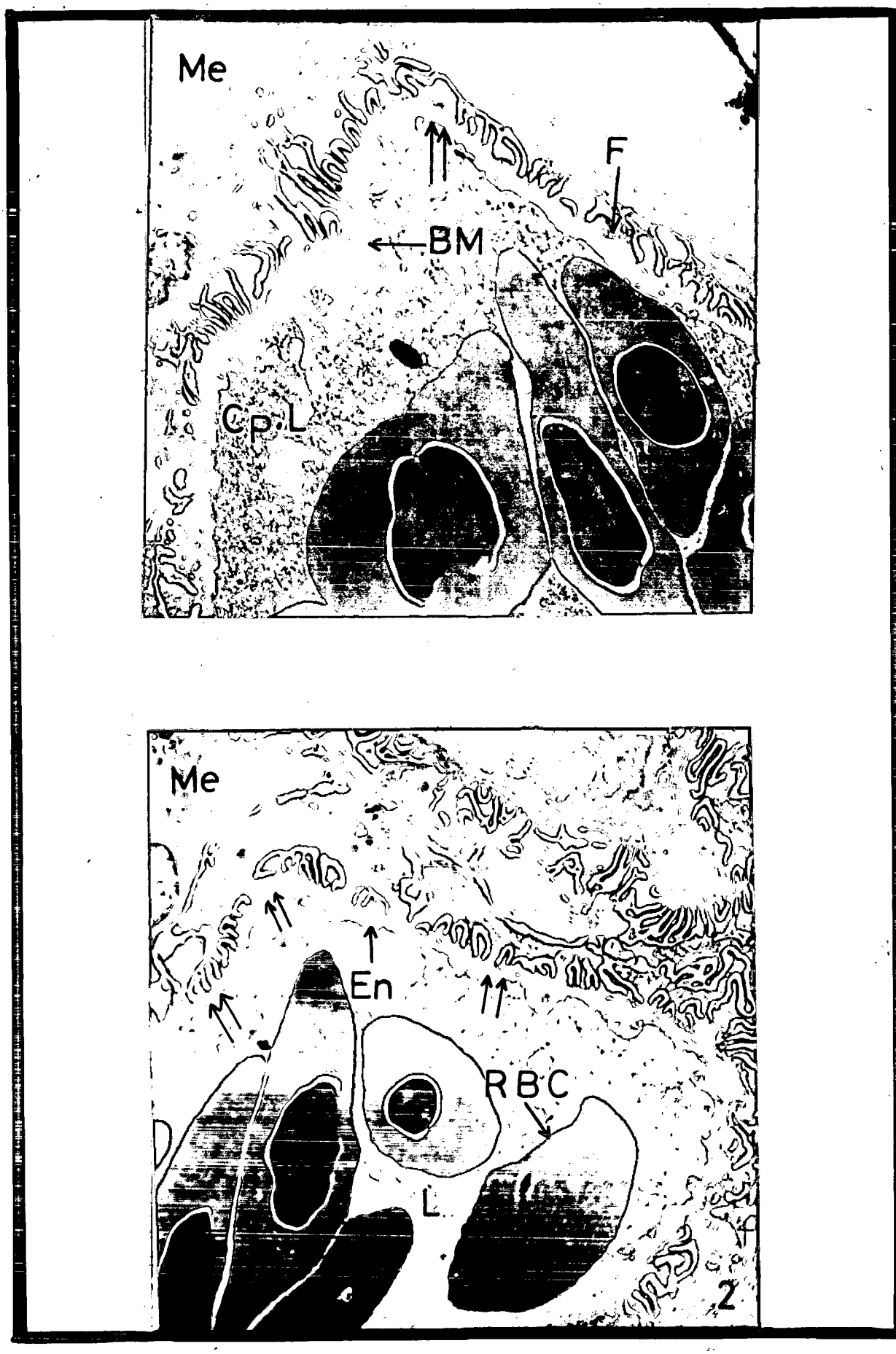
PLATE 14 :

Fig 1 : 0.01% M. E. - 720 hours - Glomerulus - (1 x 5000).

Note the fusion of epithelial foot processes (F), thickening of basement membrane (B.M) and distorted basement membrane (double arrows). Also note normal mesangial (M.E.), capillary lumen (Cp. L).

Fig 2 : 0.01% M. E. - 720 hours - Glomerulus (1 x 4,000).

Note endothelial sclerosis (En) and reduction of endothelial cell size. (double arrow).


Me
F
BM
C.P.L
Me
En
RBC
L
2

CAPTIONS OF FIGURES

PLATE 15 :

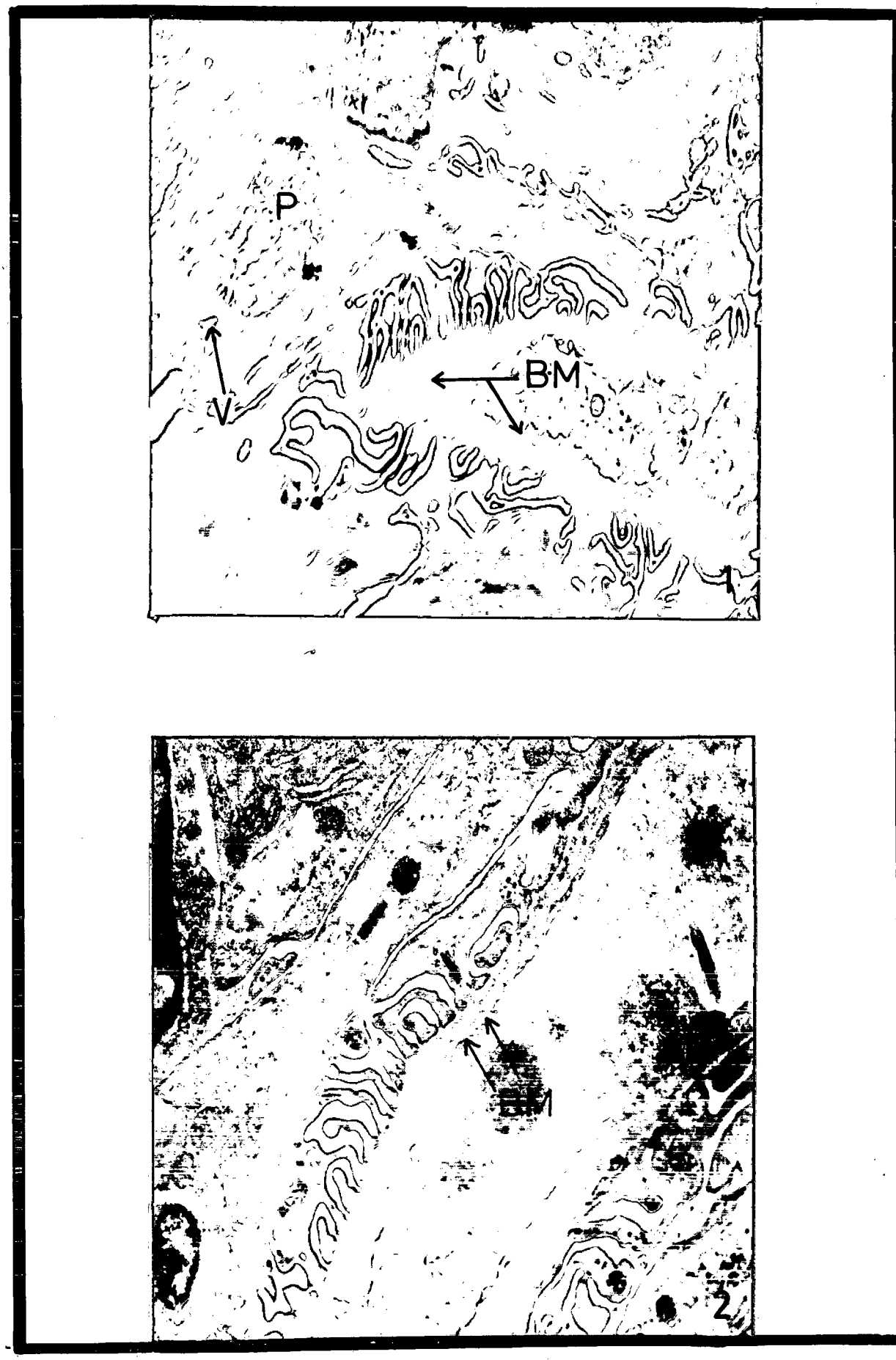
Fig 1 : 0.01% M. E. - 720 hours - Glomerulus (1 x 6,000).

Note irregular basement membrane (B.M), and swollen epithelial cell (podocyte), (P), vacuolisation (v).

Fig 2 : 0.01% M. E. -720 hours - Glomerulus (1 x 10,000).

Note the discontinuous and slightly irregular basement membrane (B.M).

PLATE NO 15



The **0.1% M.E.** at the end of six hours promoted swelling of podocytes, vacuolisation of cytoplasm of podocyte, mitochondrial damage in podocytes **(Plate 19 : Fig 1),** vacuolisation of the foot process, increased Bowman's space, and irregular and distorted basement membrane **(Plate 19 : Fig 2).** 0.1% of M.E. also induced fusion of foot processes, increase in Bowman's space with microvilli in the Bowman's space and smoothening of capillary endothelium followed by endothelial sclerosis **(Plate 20 : Fig 1 and 2)** in glomerulus respectively.

Under the influence of **1.0% M.E.** at the end of six hours ducks showed ultrastructural damage in glomerulus.

Mesangial proliferation also known as diffused proliferative glomerulo - nephritis along with fusion of foot process **(Plate 24 : Fig 1)** was observed in some glomeruli. In some glomeruli damaged basement membrane, increased Bowman's space along with microvilli in the space **(Plate 24 : Fig 2),** endothelial sclerosis with damaged and irregular capillary basal membrane **(Plate 25 : Fig.1)** and mesangial proliferation with damaged endothelium and increased Bowman's space **(Plate 25 : Fig 2)** were observed.

The **5.0% M.E.** induced ultrastructural damage in glomeruli. Focal glomerular sclerosis with complete loss of foot processes fused foot processes and increase in Bowman's space **(Plate 30 : Fig 1)** were observed. Thickening of outer wall of Bowman's capsule, increase in Bowman's space, thickening of basement membrane and smoothening of capillary endothelium **(Plate 30 : Fig.2)** were also observed in some glomeruli.

The ducks exposed to **10.0% M.E.** showed siginifcant alterations in glomeruli. At the end of six hours, complete loss of foot processes, mesangial proliferation thickening of basement membrane, irregularity in basement membrane, vacuolisation of cytoplasm in podocyte and thickening of outerwall of Bowman's capsule followed by increase in Bowman's space **(Plate 34 :**

Fig.1) were observed in many glomeruli. Capillary - Scleroderma along with swelling in podocyte was seen in some gomeruli **(Plate 34 : Fig 2)**.

The mallards exposed to **50.0% M.E.** exhibited maximum ultra structural changes in glomeruli. Focal glomerular sclerosis was observed in many glomeruli. Fusion of foot processes, swelling of podocytes, increase in Bowman's space with occurrence of microvilli in Bowman's space and sclerosis of capillary endothelium was more prominent **(Plate 38 : Fig 1)**. Few glomeruli showed mesangial glomerulonephritis where, mesangial proliferation vacuolisation of cytoplasm in podocyte and capillary endothelial sclerosis were also seen **(Plate 38 : Fig 2)**.

The **100.0% M.E.** also induced ultra structural alterations in glomeruli. Membranous glomerulonephritis, along with fused foot processes, increased Bowman's space, microvilli in Bowman's space and swelling of podocytes **(Plate 41 : Fig 2)** were observed in many glomeruli. Endothelial sclerosis, scleroderma were also observed in some glomeruli **(Plate 41 : Fig 2)**.

B] ULTRA STRUCTURAL CHANGES IN PROXIMAL TUBULES UNDER THE INFLUENCE OF MINING EFFLUENTS (M.E.):

The alterations in the ultrastructure of proximal tubules (PT) were similar when the ducks were exposed to different concentrations of **M.E.** for various time intervals. Hence, these are described together. For all the concentrations of **M.E.** there were insignificant changes in proximal tubules before 6 hours, but at the end of six hours necrosis was progressive and involved more tubules as the time progressed.

The birds exposed to **0.01% M.E.** showed no significant alterations in PT upto the end of twentynine days. But at the end of thirty days (seven hundred and twenty hours), proximal tubules showed disrupted brush border, empty

CAPTIONS TO FIGURES

PLATE 16 :

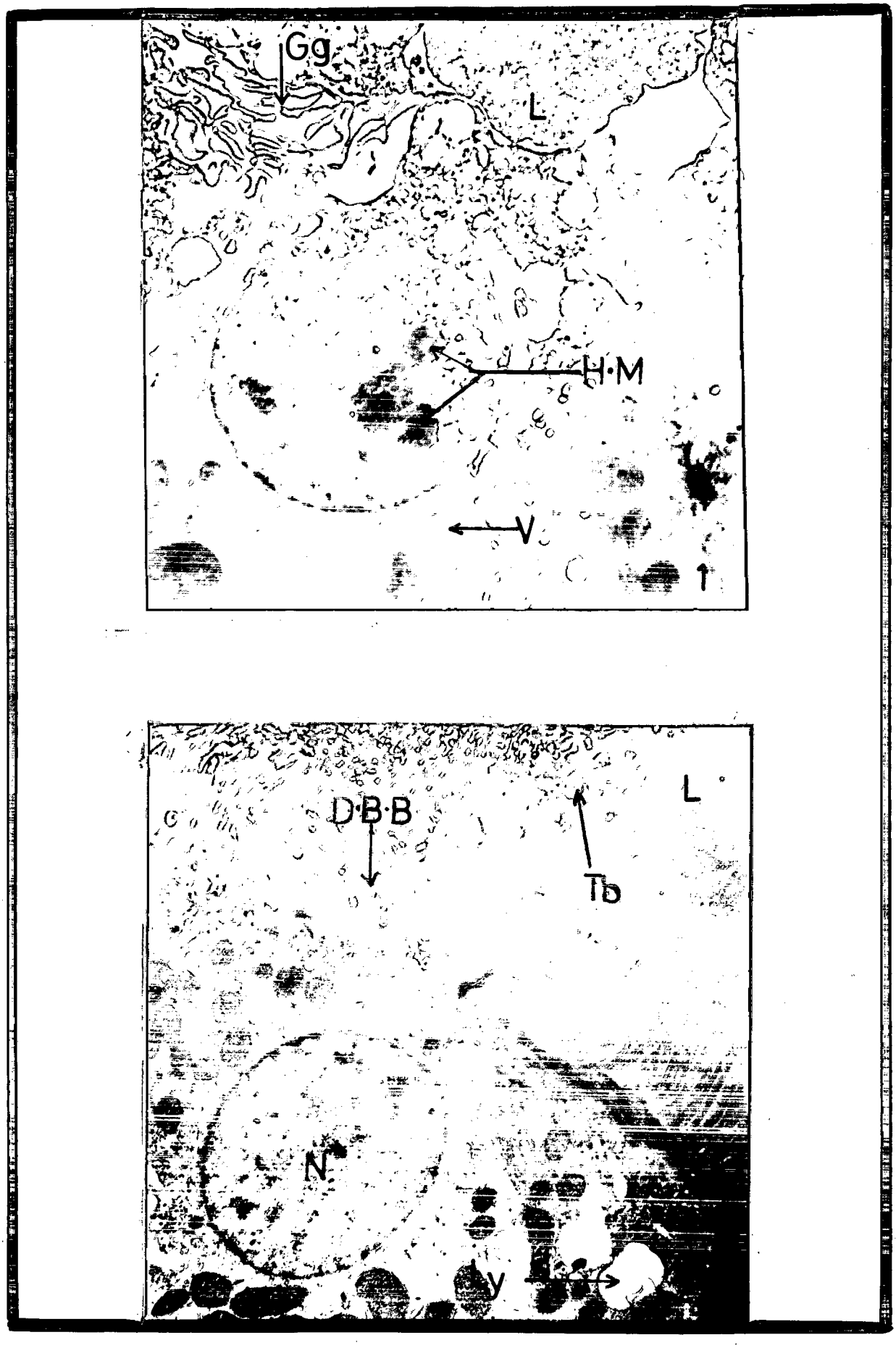
Fig 1 : 0.01% M. E. - 720 hours - Proximal tubule (1 x 6,000).

Note the disrupted brush border (D.B.B), empty lysosomal vesicles (Ly), Nucleus (N) with dispersed chromatin material and tissue debris (Tb) in lumen (L).

Fig 2 : 0.01% M. E. - 720 hours - Distal tubule (1 x 6000).

Note Vacuolisation of cytoplasm (V), swollen Golgi bodies (G) and heavy metal deposition in the nucleus (H.M).

PLATE NO. 16



CAPTIONS TO FIGURES

PLATE 17 :

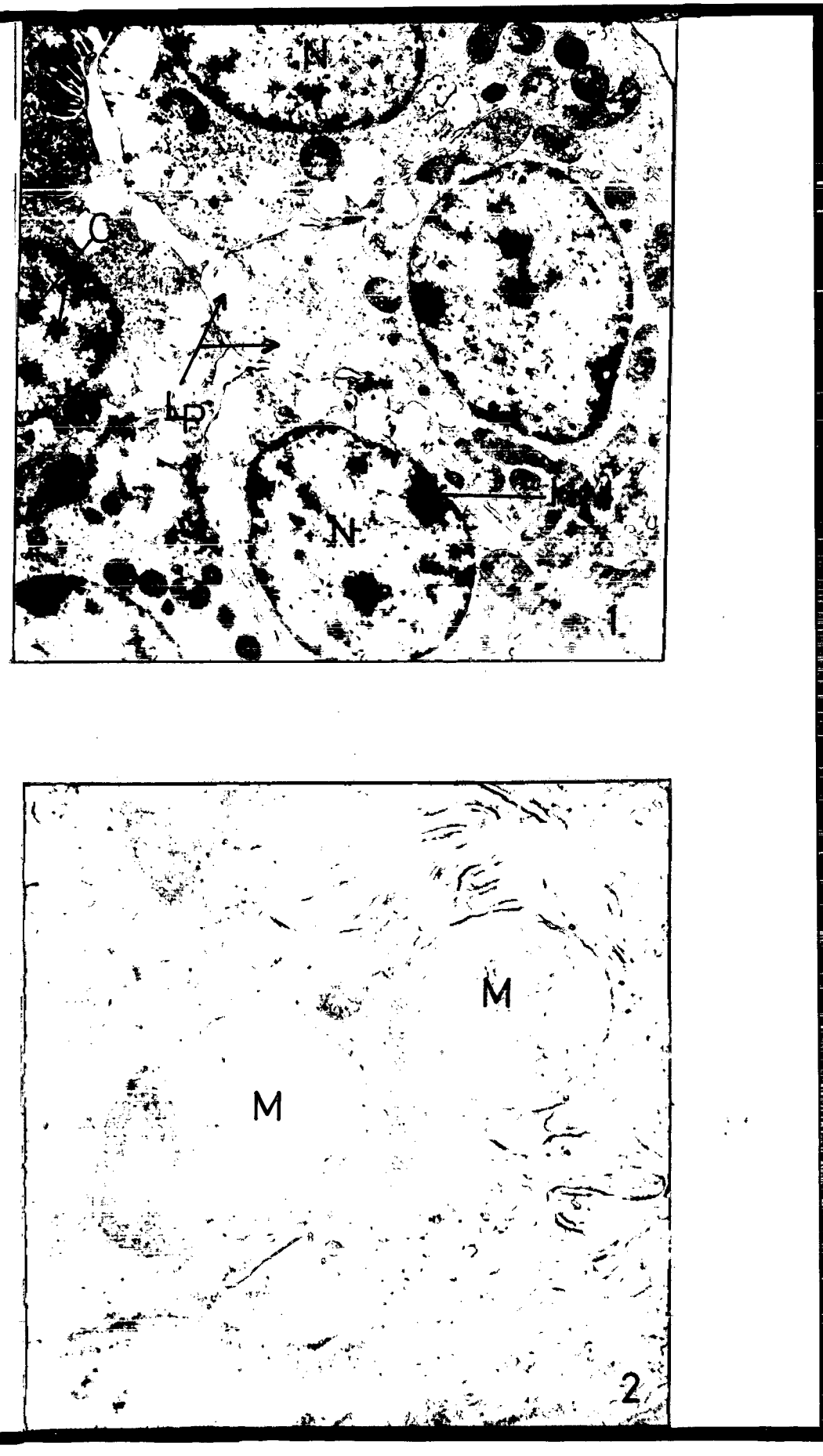
Fig 1 : 0.01% M. E. -720 hours - Distal tubule (1 x 5,000).

Note the lipid droplets in distal tubule (Lp), swelling of tubule with reduction in luminal space (L), pycnotic nucleus (N) with heavy metal depositions (H.M.) and dispersed chromatin (C) in the nucleus.

Fig 2 : 0.01% M. E. - 720 hours - Distial tubule (1 x 20,000).

Note the damaged mitochondria (M) from the Distal tubules.

PLATE NO. 17



CAPTIONS TO FIGURES

PLATE 18 :

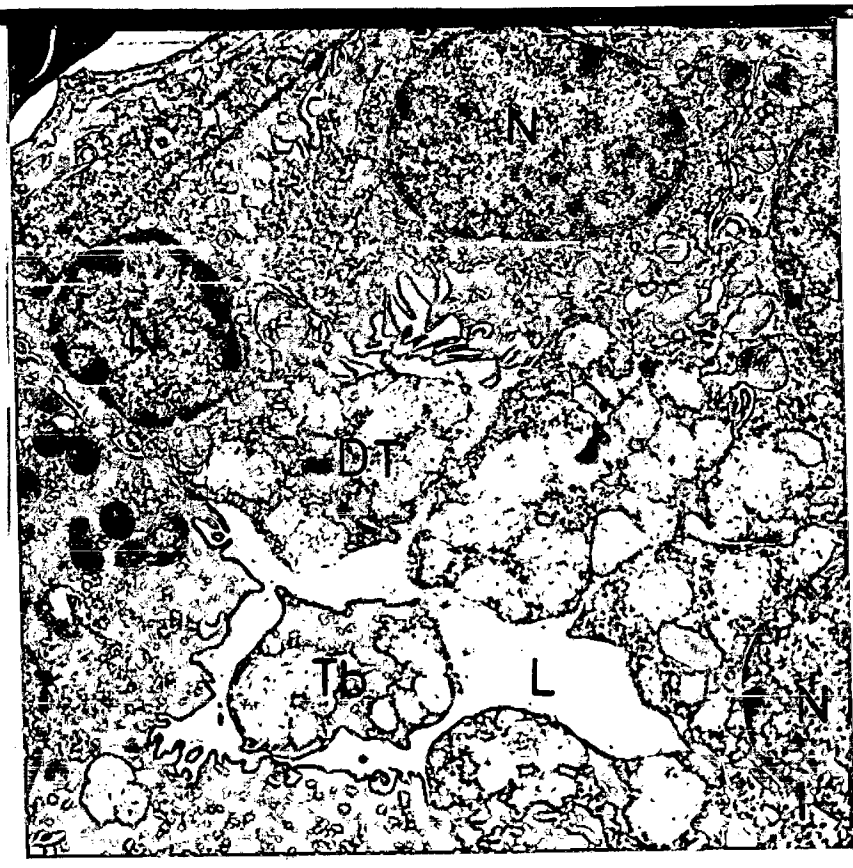
Fig 1 : 0.01% M. E. - 720 hours - Distal tubule (1 x 3,000).

Note the swollen tubule (DT), tissue debris. (Tb) in the lumen (L), nucleus pycnotic and dispersed chromatin.

Fig 2 : 0.01% M. E. - 720 hours - Collecting tubule (1 x 2,000).

Note distorted collecting tubule (C.T.D) mitochondria (M) along with nucleus (N) released in the lumen (L). Also note Red blood cells (RBC) in the lumen (L) and damaged Basal membrane of the tubule (double arrow).

PLATE NO 18



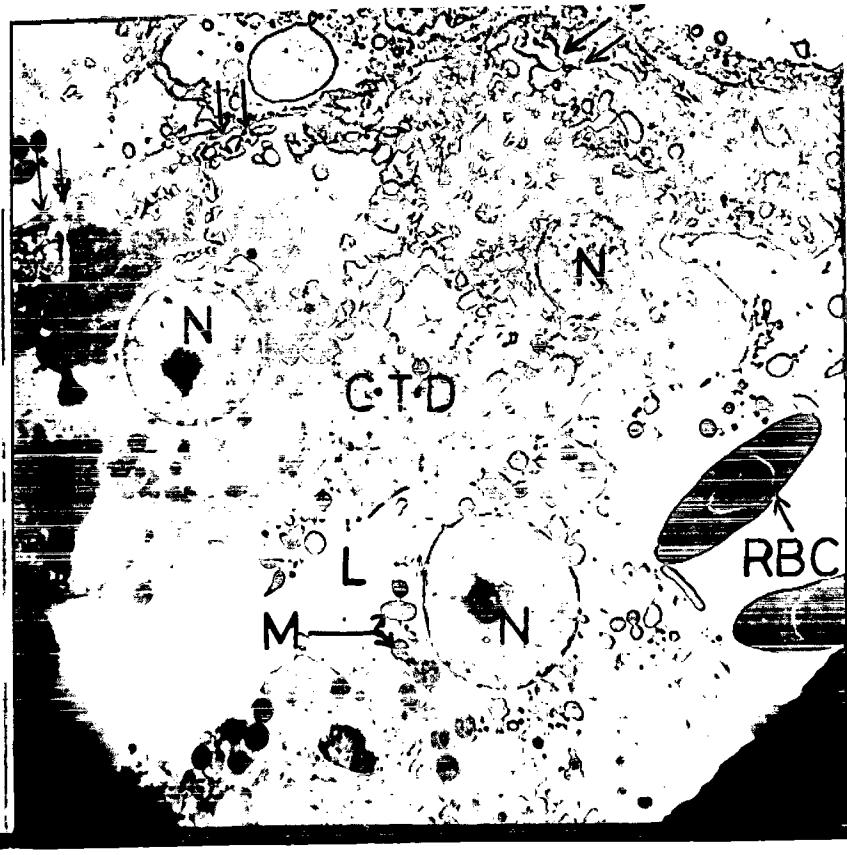
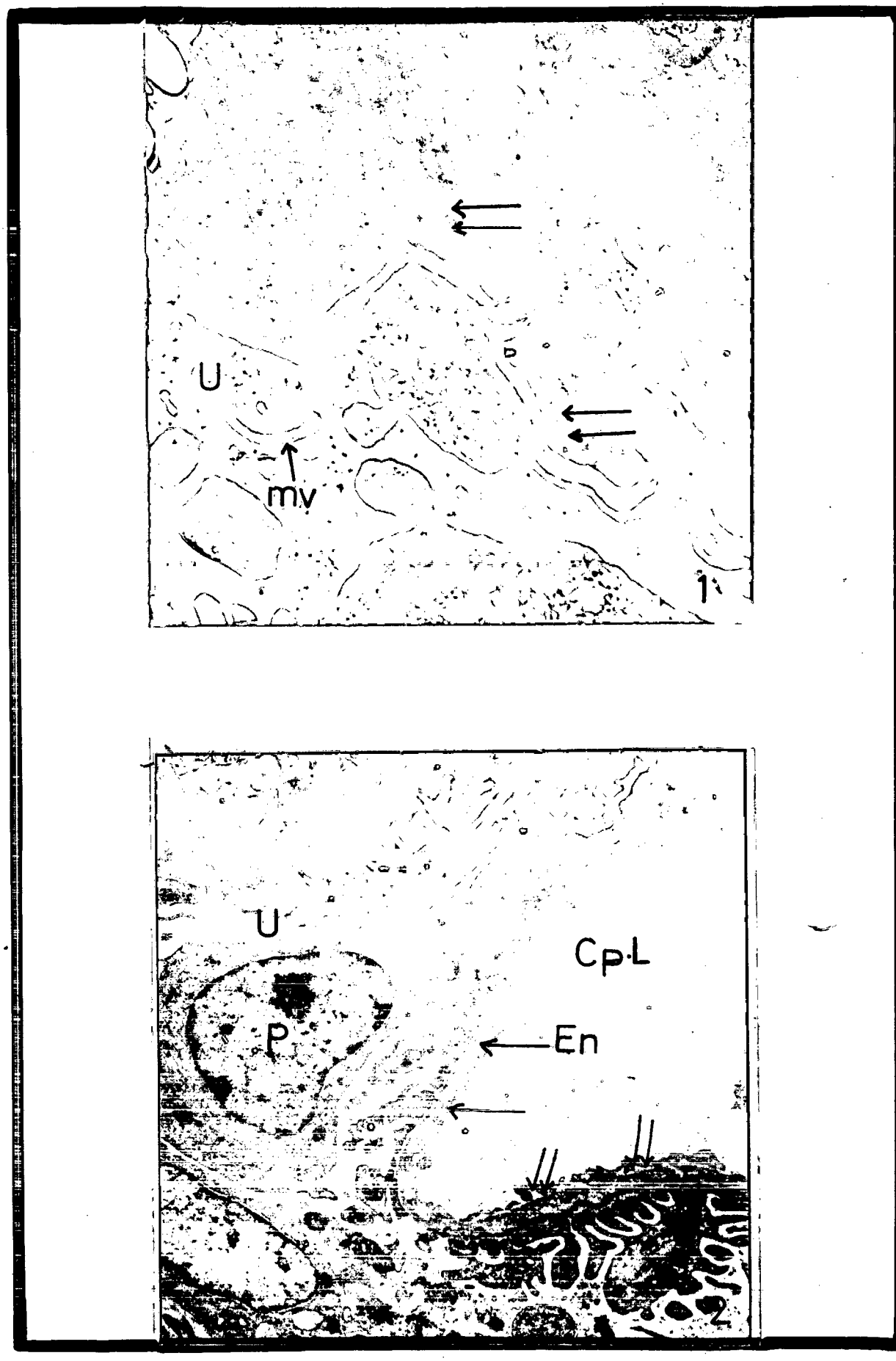


PLATE NO 20



CAPTIONS TO FIGURES

PLATE 21 :

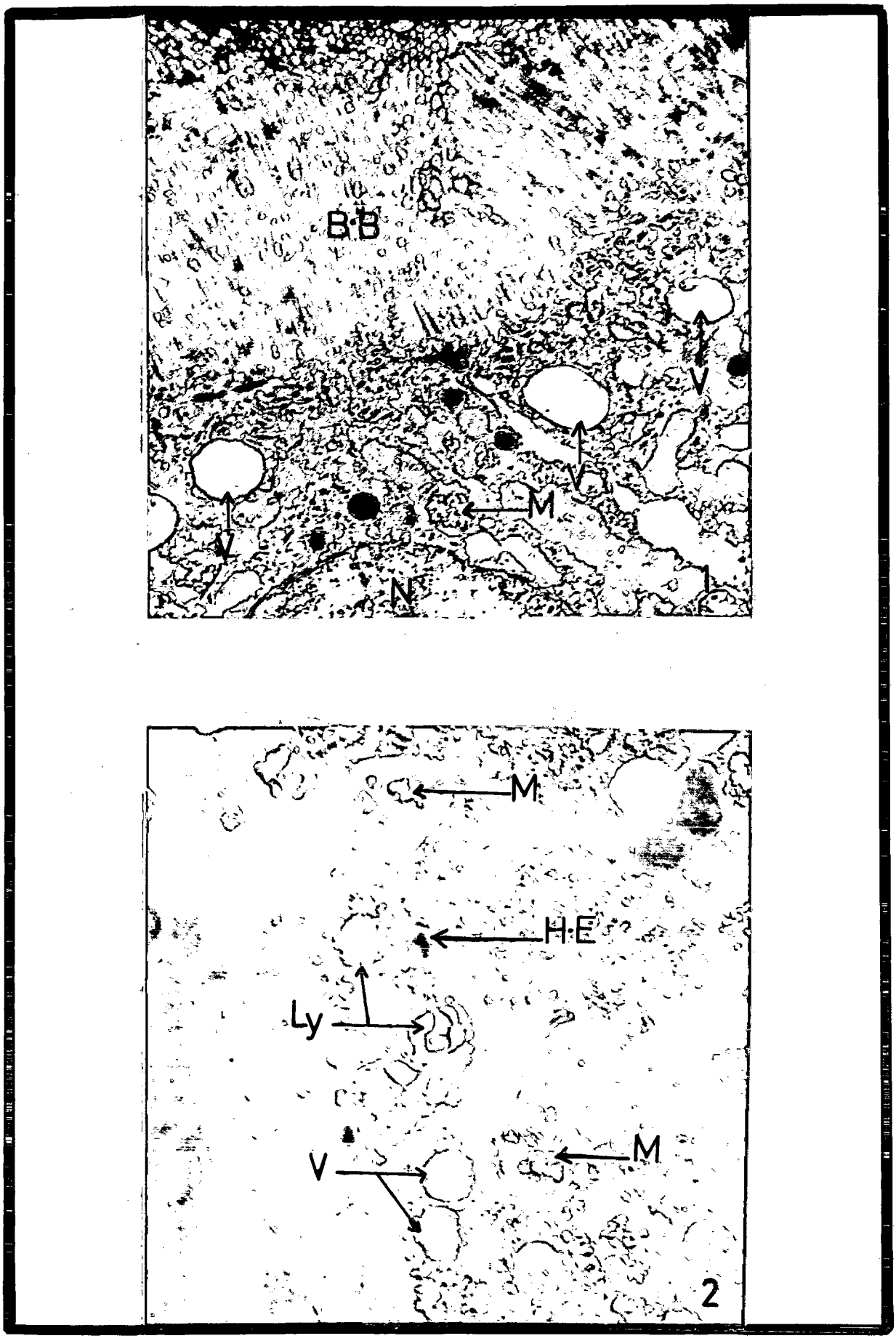
Fig 1 : 0.1% M. E. - 6 hours - Proximal tubule (1 x 5,000).

Note the vacuolisation of cytoplasm (V) and damaged mitochondria (M). Also note brush border (B.B).

Fig 2 : 0.1% M. E. - 6 hours - Proximal tubule (1 x 10,000).

Note the empty lysosomal vesicles (Ly). Vacuolisation of cytoplasm (V) and heavy metal depositions (H.E) in the tubule cytoplasm. Also note damaged mitochondrion. (M)

PLATE NO. 21



CAPTIONS TO FIGURES

PLATE 22 :

Fig 1 : 0.1% M. E. - 6 hours - Distal tubule (1 x 2,000).

Note the distorted and swollen distal tubule (D.D.T), pycnotic nucleus (N), reduced luminal space (L) and deposition of lipid droplets (Lp).

Fig 2 : 0.1% M. E. - 6 hours - Distal tubule (1 x 8,000).

Note the proliferation of Golgi bodies and damaged mitochondrion (M).

CAPTIONS TO FIGURES

PLATE 19 :

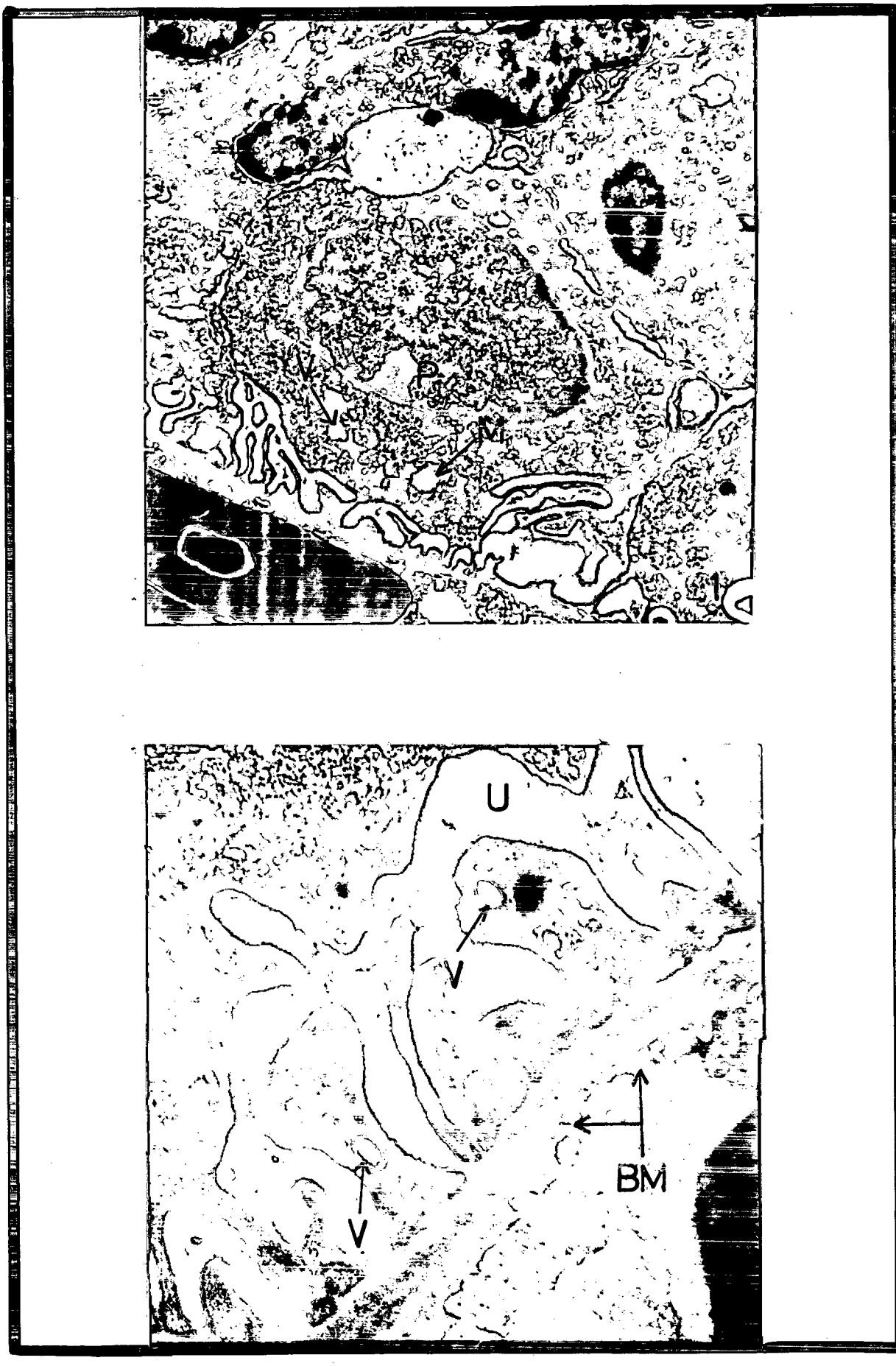
Fig 1 : 0.1% M. E. - 6 hours - Glomerulus - (1 x 6,000).

Note the swollen podocyte (P), vacuolisation of cytoplasm in podocyte (V) and damaged mitochondria (M).

Fig 2 : 0.1% M. E. - 6 hours - Glomerulus (1 x 20,000).

Note the discontinuous and irregular basement membrane (B.M), Increase in Bowman's Space (U), and Vaculisation of foot processes (V).

PLATE NO. 19



CAPTIONS TO FIGURES

PLATE 20 :

Fig 1 : 0.1% M. E. - 6 hours - Glomerulus (1 x 10,000).

Note the focal glomerular sclerosis, complete loss of foot processes (double arrows), increase in Bowman's space (U) and numerous microvilli (mv) in the Bowman's space.

Fig 2 : 0.1% M. E. - 6 hours - Glomerulus (1 x 6,000).

Note smooth and discontinous capillary endothelium (En), and endothelial sclerosis (double arrows).

CAPTIONS TO THE FIGURES

PLATE 1 :

Fig 1 : Control. (10 x 5)

Note the intact glomerulus (GL) ; intact proximal tubule (PT), intact distal tubule (DT) and intact collecting tubule (CT).

Fig 2 : 0.01% M. E. - 720 hours. (10 x 5)

Note the change in the shape of the glomerulus (Arrow). Also note the damaged glomerulus (G) with exudents in Bowman's space. Note the damaged proximal tubules (PT) with the exudation of the nuclei into the empty space. Note the damaged distal tubule (DT) and collecting tubule (CT).

Fig 3 : 0.01% M. E. - 1080 hours. (20 x 5)

Note the damaged glomerulus (G), distorted proximal tubule (PT), damaged distal tubule (DT) and distorted collecting tubule (CT).

Fig 4 : 0.1% M. E. - 6 hours. (10 x 5)

Note the damaged glomerulus (G) with exudents in the Bowman's space, proximal tubule (PT), damaged distal tubule (DT) and loss of architecture of a few collecting tubules (CT).


RBC
D.D.T.
N
L

CAPTIONS TO FIGURES

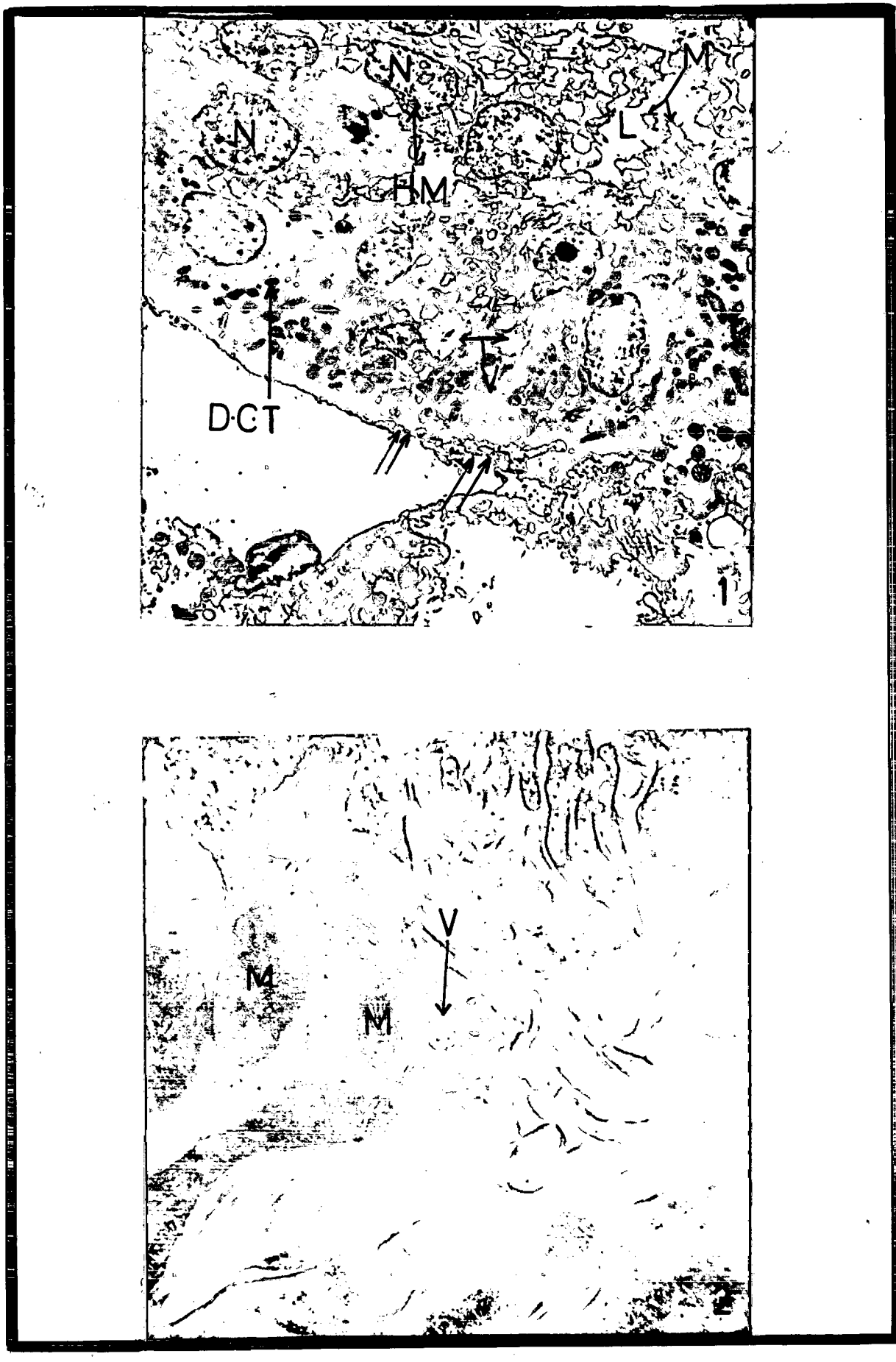
PLATE 23 :

Fig 1 : 0.1% M. E. - 6 hours - Collecting tubule (1 x 2,000).

Note the damaged collecting tubule (D.C.T) pycnotic nucleus (N) with heavy metal depositions (H.M), distorted basal membrane (double arrows), mitochondria (M) in the luminal space and vacuolisation of cytoplasm (V).

Fig 2 : 0.1% M. E. - 6 hours - Collecting tubule (1x 20,000).

Note the vacuolisation in the mitochondrion (V).


N
N
M
L
M
D·C·T
V
1
V
M
M

CAPTIONS OF FIGURES

PLATE 24 :

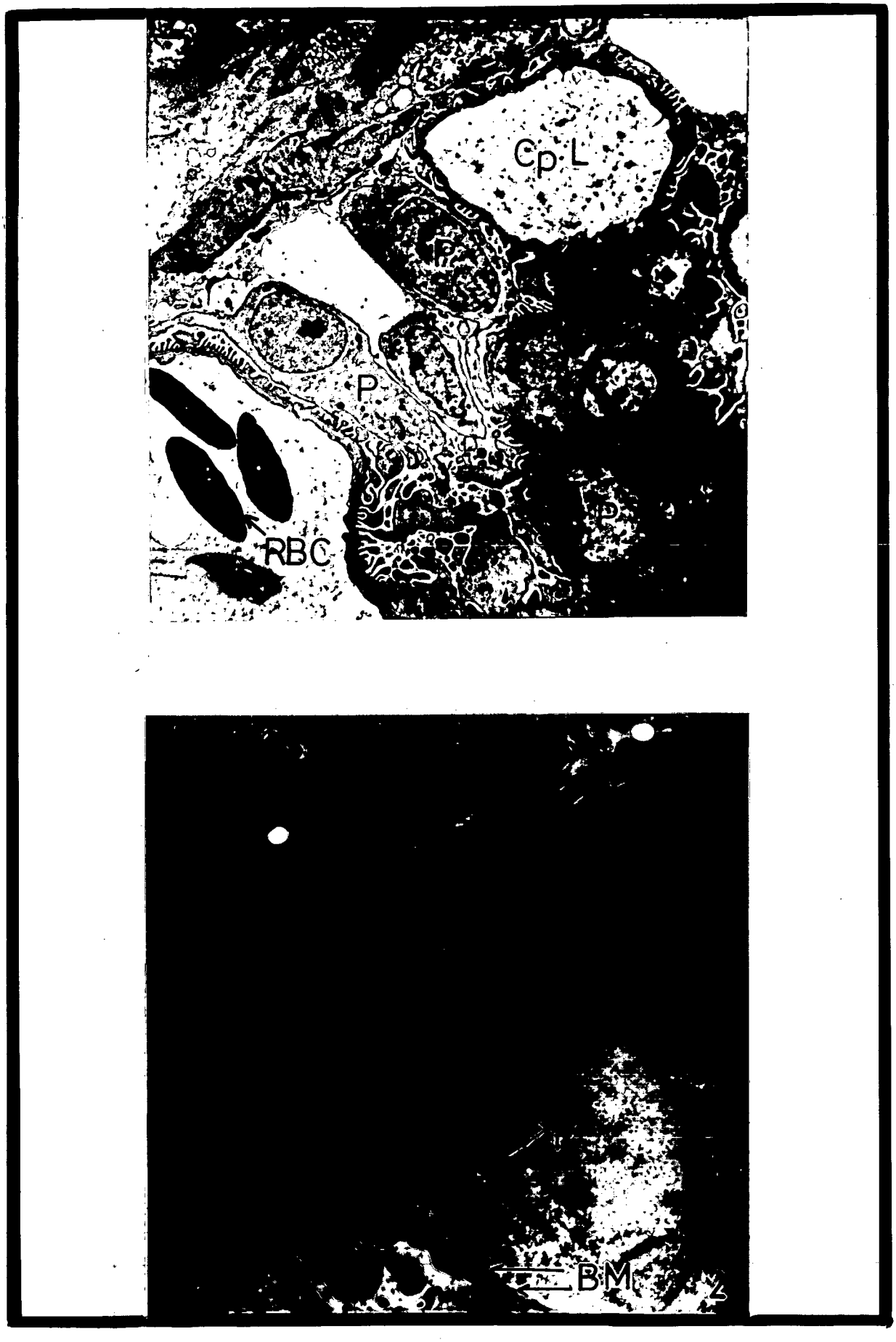
Fig 1 : 1.0% M. E. - 6 hours - Glomerulus (1 x 2,000).

Note the diffused proliferative glomerulonephritis, mesangial proliferation (M.E.P) and complete loss of foot process (double arrows), capillary lumen (Cp.L) and podocyte (P).

Fig 2 : 1% M. E. - 6 hours - Glomerulus (1 x 8,000).

Note the fused foot processes (F), increased Bowman's space (U), microvilli (mv) in the Bowman's space, damaged basement membrane (B.M) and distorted capillary endothelium (En).

PLATE NO 24



CAPTIONS TO FIGURES

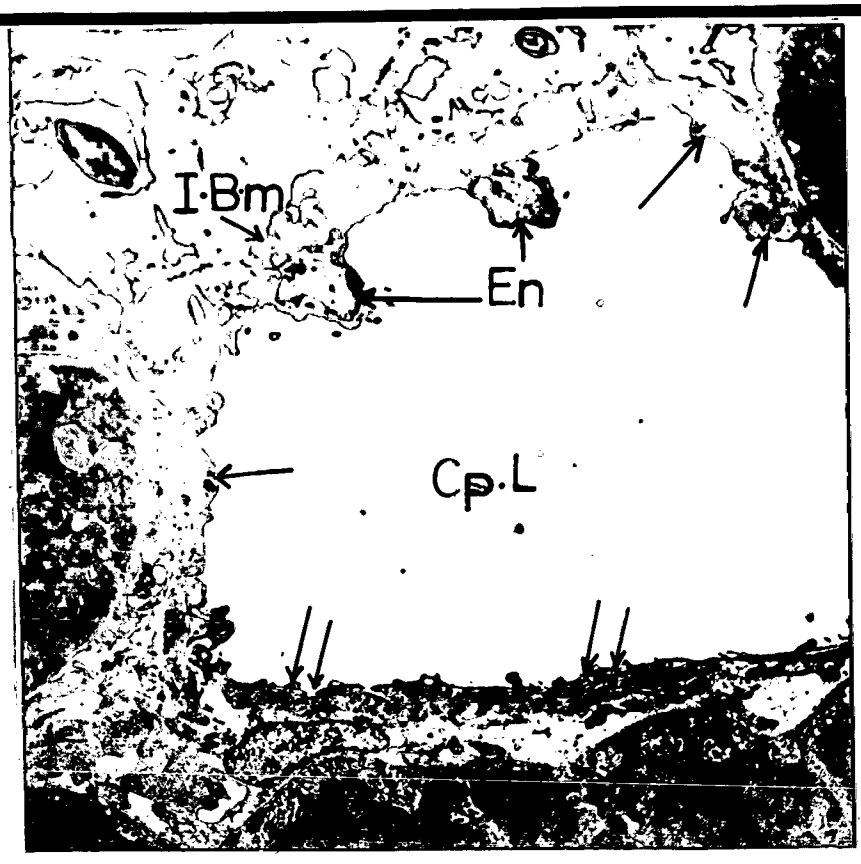
PLATE 25 :

Fig 1 : 1% M. E. - 6 hours - Blood capillary (1 x 2000).

Note the scleroderma of capillary endothelium. Capillary endothelium thickening (arrows), smoothening of capillary endothelium (double arrows) and irregularity of capillary basal membrane (I.B.m).

Fig 2 : 1% M. E. - 6 hours - Glomerulus (1 x 6000).

Note the mesangial proliferation (Me.P), fused foot processes (double arrows), increase in Bowman's space (U) and endothelial sclerosis (En).


I·Bm
En
Cp·L


Ep
RBC
U

CAPTIONS TO FIGURES

PLATE 26 :

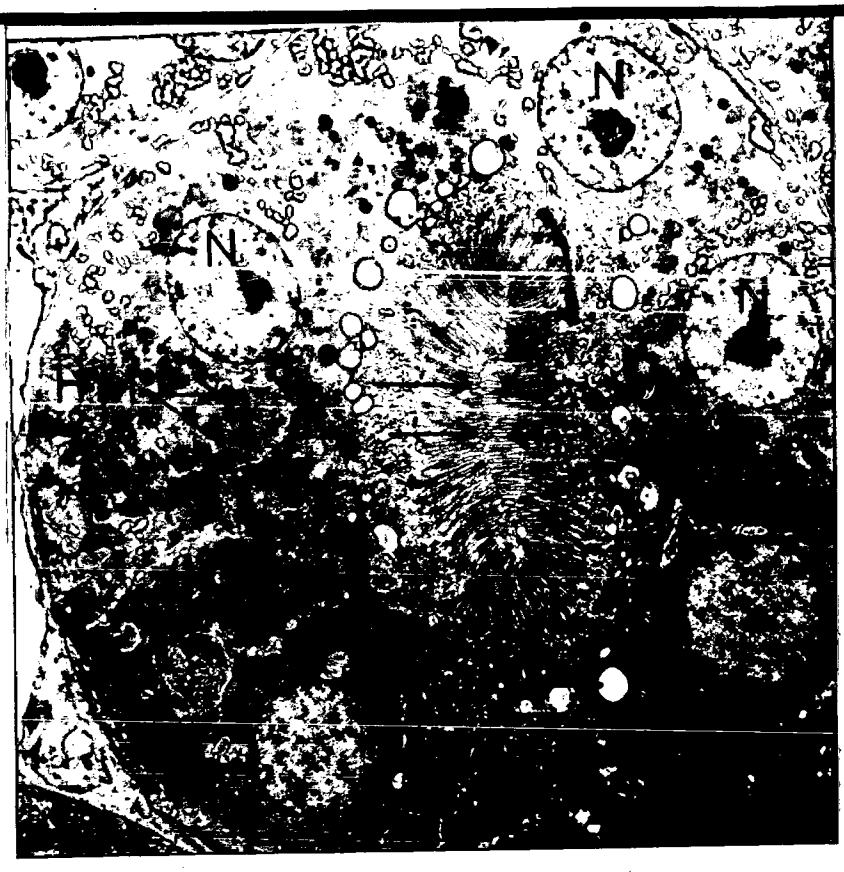
Fig 1 : 1% M. E. - 6 hours - Proximal tubule (1x 2,000).

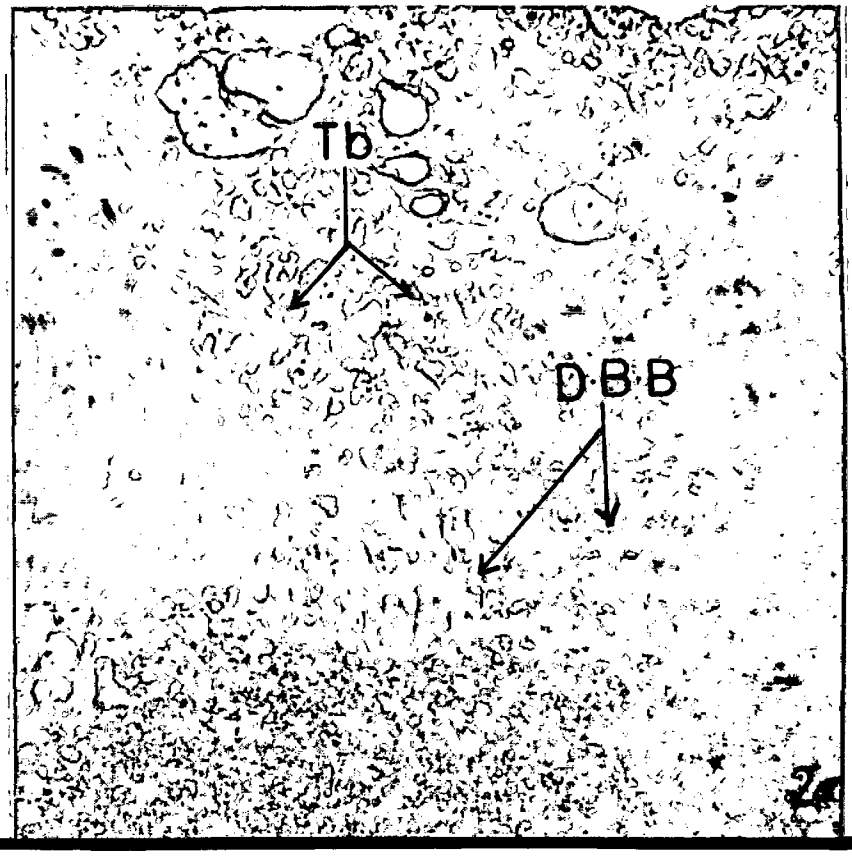
Note the swelling of proximal tubule (P.T), brush boder (double arrows) occupying the whole lumen, heavy metal (H.M.), depositions in cytoplasm, vacuolisation of cytoplasm (V), distortion of chromatin material in the nucleus (N) and empty lysosomal vesicles (Ly).

Fig 2 : 1% M. E. - 6 hours - Proximal tubule (1 x 10,000).

Note the disrupted brush border (D.B.B.) and tissue debris (Tb) in the lumen.

PLATE NO 26





CAPTIONS TO FIGURES

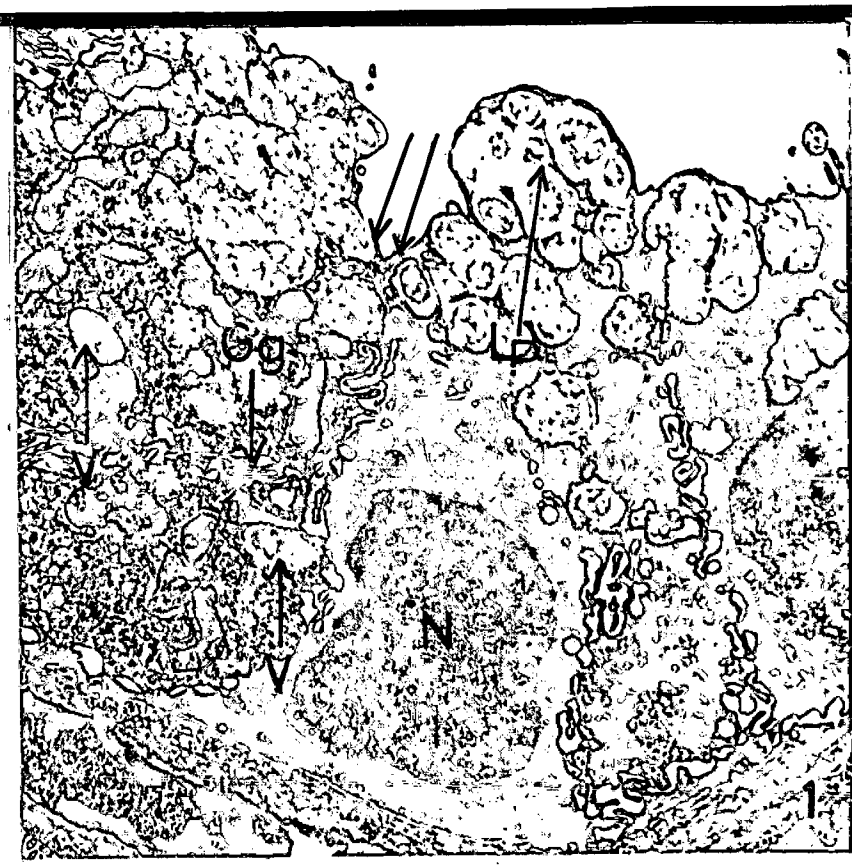
PLATE 27 :

Fig 1 : 1% M. E. - 6 hours - Distal tubule - (1 x 4,000).

Note the lipid (Lp) droplets, vacuolisation of cytoplasm (V), swollen Golgi bodies. Also note autophagic vacuoles (double arrows) and pycnotic nuclei (N).

Fig 2 : 1% M. E. - 6 hours - Distal tubule (1 x 8,000).

Note the seperation of two cells (double arrows). Nucleus with dispersed chromatin (arrow).


N
1


N

CAPTIONS TO FIGURES

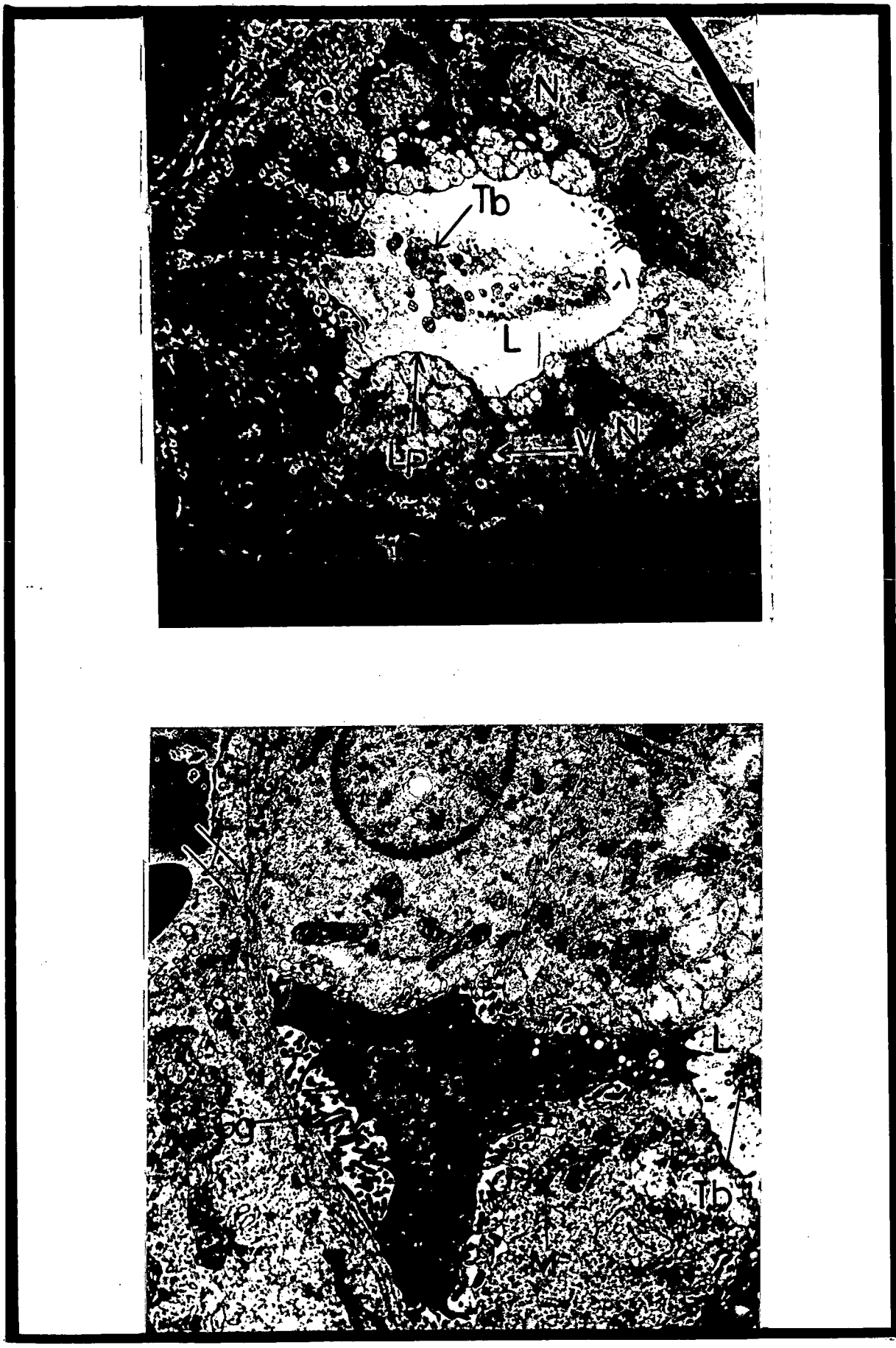
PLATE 28 :

Fig 1 : 1% M. E. - 6 hours - Distal tubule (1 x 2,000).

Note tissue debris (Tb) in the lumen (L), deposition of lipid droplets, pycnotic nucleus (N) and vacuolisation (V) of the cytoplasm.

Fig 2 : Note 1% M. E. - 6 hours - Distial tubule (1 x 3,000).

Note the proliferation of Golgi bodies (Gg), damaged mitochondria (M) and distorted basal membrane (double arrow).


N
Tb
L
lp
V
N
V
L
g
L
Tb

lysosomal vesicles, nucleus with dispersed chromatin material and tissue debris in the lumen **(Plate 16 : Fig 1)**. The ducks exposed to **0.1% to 100%** M.E. exhibited pronounced necrosis of PT and most of the necrotic changes mentioned for **0.01% M.E.** exposure were seen, but in addition, the alterations such as vacuolisation of the cytoplasm, destruction of mitochondria, deposition of heavy metals in the cytoplasm, swelling of PT, shedding of brush border into the lumen, covering the entire lumen and tissue debris along with damaged mitochondria in the lumen were observed **(Plate 21 : Fig 1 and 2, Plate 26 : Fig I and 2, Plate 31 : Fig I and 2, Plate 35 : Fig 1 and 2, Plate 39 : Fig 1 and Plate 42 : Fig 1 and 2).**

The degree of necrosis with different concentrations of M.E. changed at later hours, and the number of damaged tubules increased as the exposure period increased.

C] ULTRA STRUCTURAL ALTERATIONS IN DISTAL TUBULES (DT) :

The birds exposed to **0.01% M.E.** also induced ultrastructural alterations in DT. Mallards did not exhibit any signifcant change in DT before seven hundred and twenty hours (twenty nine days), when exposed to **0.01% M.E.** But at the end of seven hundred and twenty hours (thirty days) the alterations in DT became more significant and prominent. The DT showed necrotic changes such as vacuolisation of cytoplasm, swelling of Golgi bodies, heavy metal deposition in the pycnotic nucleus, deposition of lipids in the cytoplasm, swelling of DT with reduction of luminal space and dispersed chromatin in the nucleus **(Plate 16 : Fig 2, Plate 17 : Fig 1 and 2).** Tissue debris in the lumen along with distorted luminal border was also observed **(Plate 18 : Fig 1).**

When the mallards were introduced to various concentrations of **M.E. (0.1 to 100%)** they showed alterations in DT similar to those described for

0.01% M.E. with variations in the degree of necrosis. The variations in necrosis were such as proliferation of Golgi bodies **(Plate 22 : Fig 2)**, formation of autophagic vacuoles **(Plate 27 : Fig 1)**, separation of tubule cells **(Plate 27: Fig 2)**,occurrence of tissue debris in the tubular lumen. **(Plate 28 : Fig 1, Plate 32 : Fig 1 and 2)**, damaged basal membrane of the tubule **(Plate 28 : Fig 2, Plate 40 : Fig 1)** , heavy deposition of lipid droplets in cytoplasm and heavy metal depositions in nuclei **(Plate 36 : Fig 1 and 2, Plate 39 : Fig 2, Plate 32 : Fig 2)** were also seen in few tubules. Some tubules showed complete distortion of luminal border and damaged mitochondria **(Plate 22 : Fig 1, Plate 43 : Fig 1 and 2)**. Swelling of Golgi bodies was also observed in few tubules **(Plate 28 : Fig 2)**.

All the above mentioned alterations were also observed in later hours involving increase in the number of distal tubules undergoing necrosis and the tubules became acutely necrotic when exposed to the M.E. chronically.

ULTRA STRUCTURAL CHANGES IN COLLECTING TUBULES : (CT)

The ducks exposed to various concentrations of mining effluents **(0.1 to 100%)** showed necrosis of collecting tubules at the end of six hours. Only **0.01% M.E.** did not exhibit any significant change in collecting tubules up to the end of twenty nine days. By the end of seven hundred and twenty hours (thirty days) the tubules showed necrosis. The **0.01% M.E.** induced alterations such as complete distortion of few collecting tubules exudation of nuclei along with tissue debris in the lumen, distortion of basal membrane of the collecting tubules and exudation of red blood cells in the lumen of distorted tubule **(Plate 18 : Fig 2)** were observed.

The mallards when exposed to **0.1 to 100% M.E.** showed alterations in collecting tubules such as, distortion of basal membrane, pycnosis of nuclei

CAPTIONS TO FIGURES

PLATE 29 :

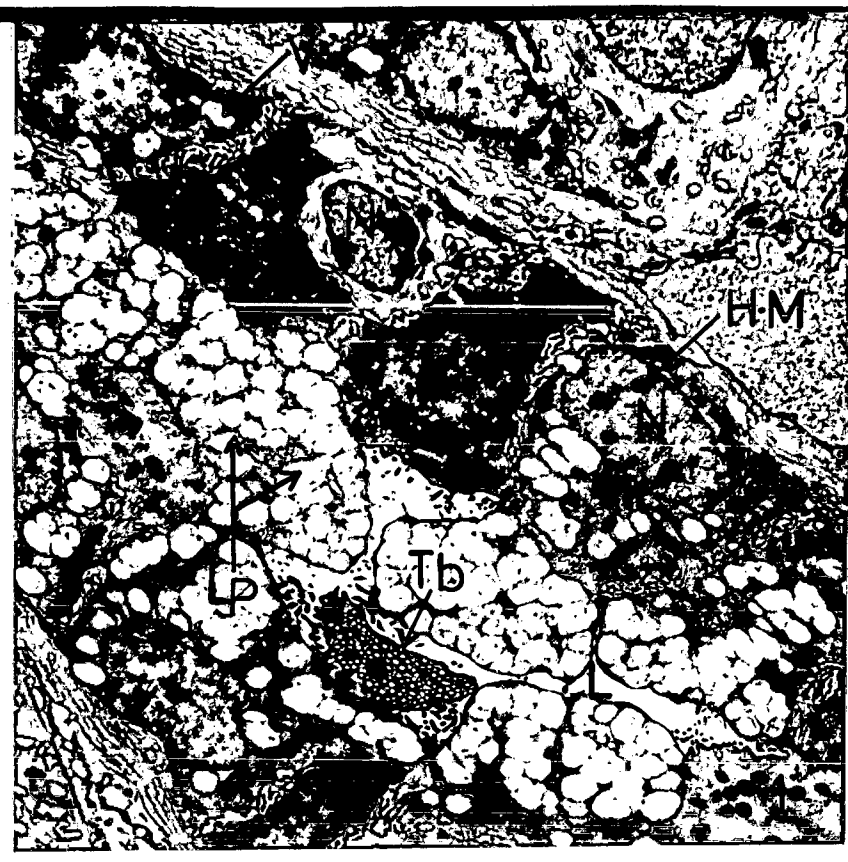
Fig 1 : 1% M. E. - 6 hours - Collecting tubule (1 x 2,000).

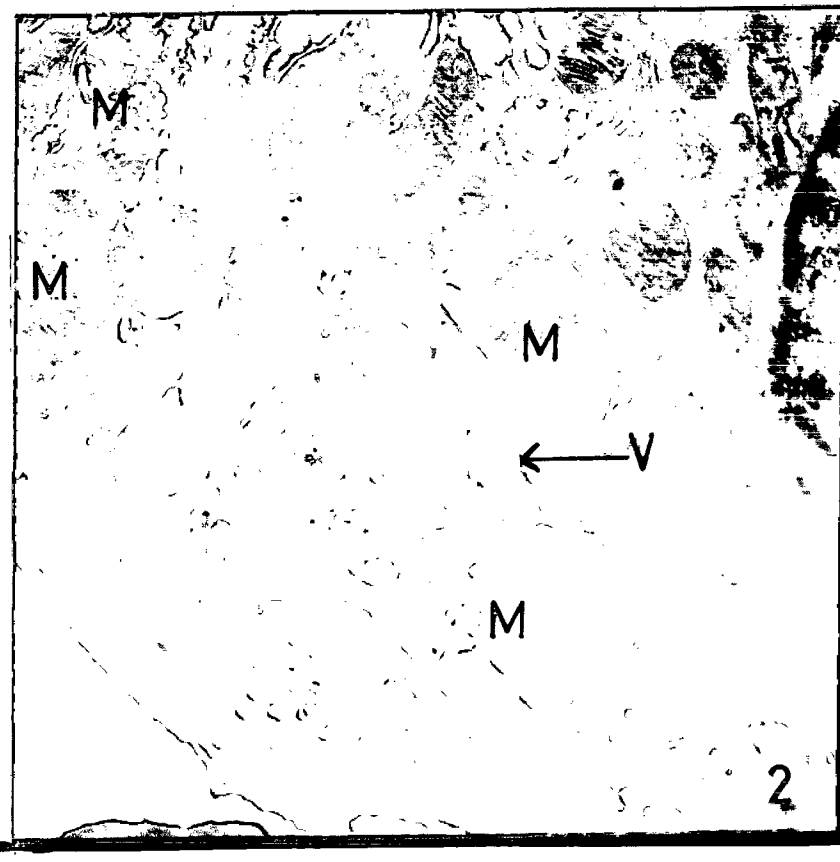
Note the pycnotic nuclei (N), with heavy metal (H.M) depositions. Lipid deposits (Lp) , vacuolisation of cytoplasm (V) and tissue debris (Td) in the lumen (L).

Fig 2 : 1% M. E. - 6 hours - Collecting tubule (1 x 10,000).

Note the damaged mitochondria (M) and cytoplasmic vacuolisation (V).

PLATE NO 29





CAPTIONS TO FIGURES

PLATE 30 :

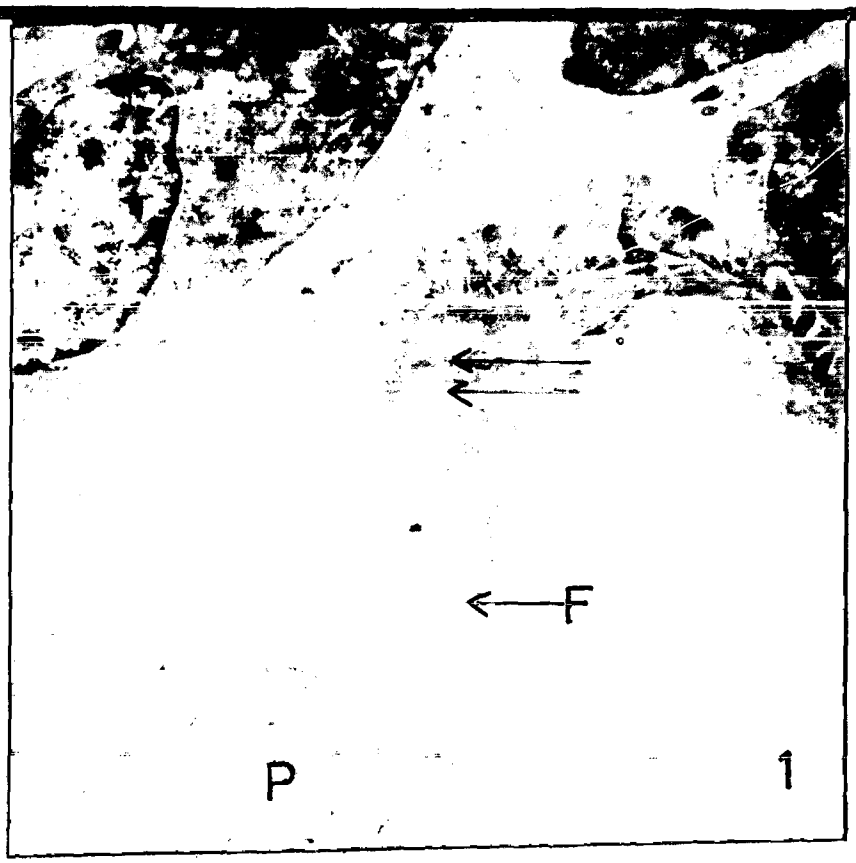
Fig 1 : 5% M. E. - 6 hours - Glomerulus (1 x 6,000).

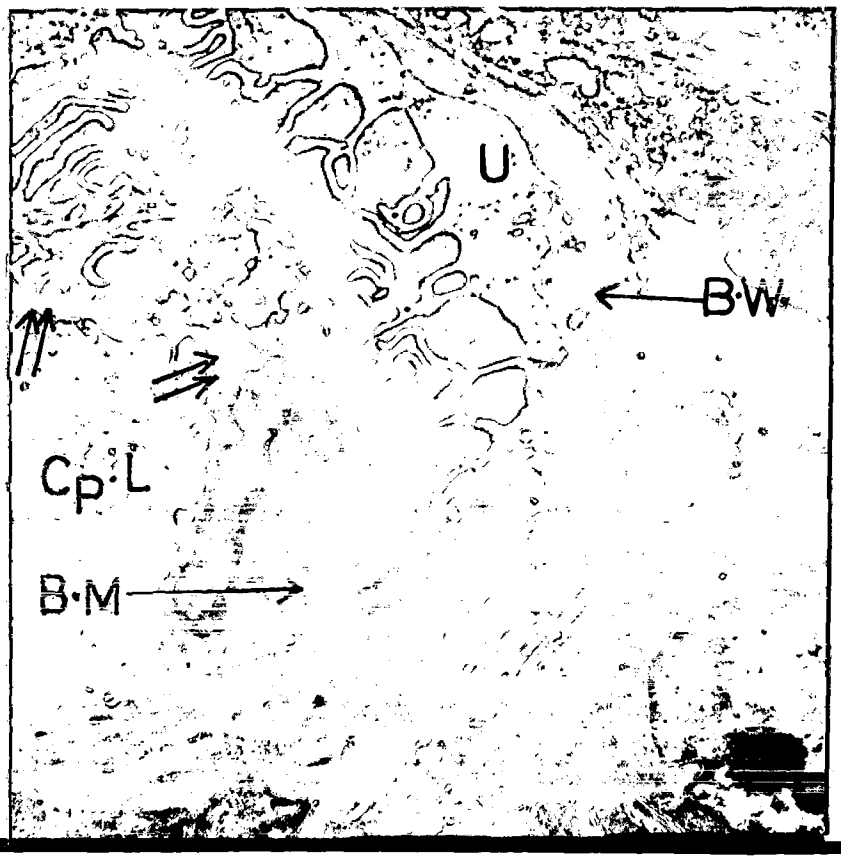
Note the thickening of basement membrane (double arrow), fused foot processes (F) and increased Bowman's space (U).

Fig 2 : 5% M. E. - 6 hours - Glomerulus (1 x 5,000).

Note the increase in the Bowman's space (U), thickening and irregular outer Bowman's capsule membrane (B.W). Thickening of basement membrane (B.M) and smoothening of endothelium (double arrows).

PLATE NO 30





CAPTIONS TO FIGURES

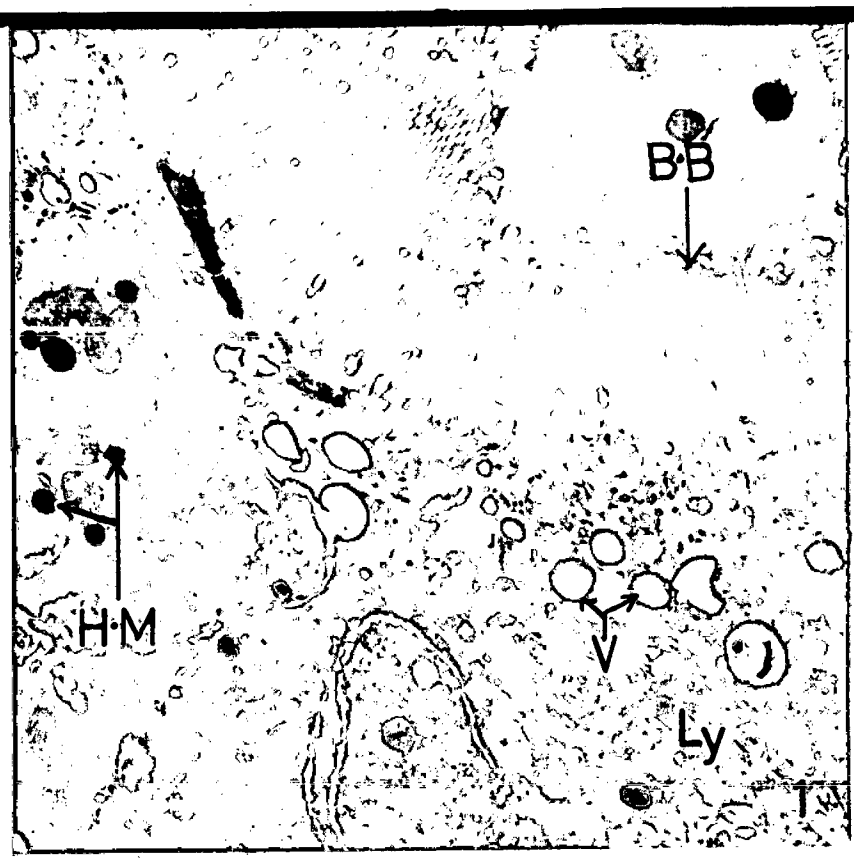
PLATE 31 :

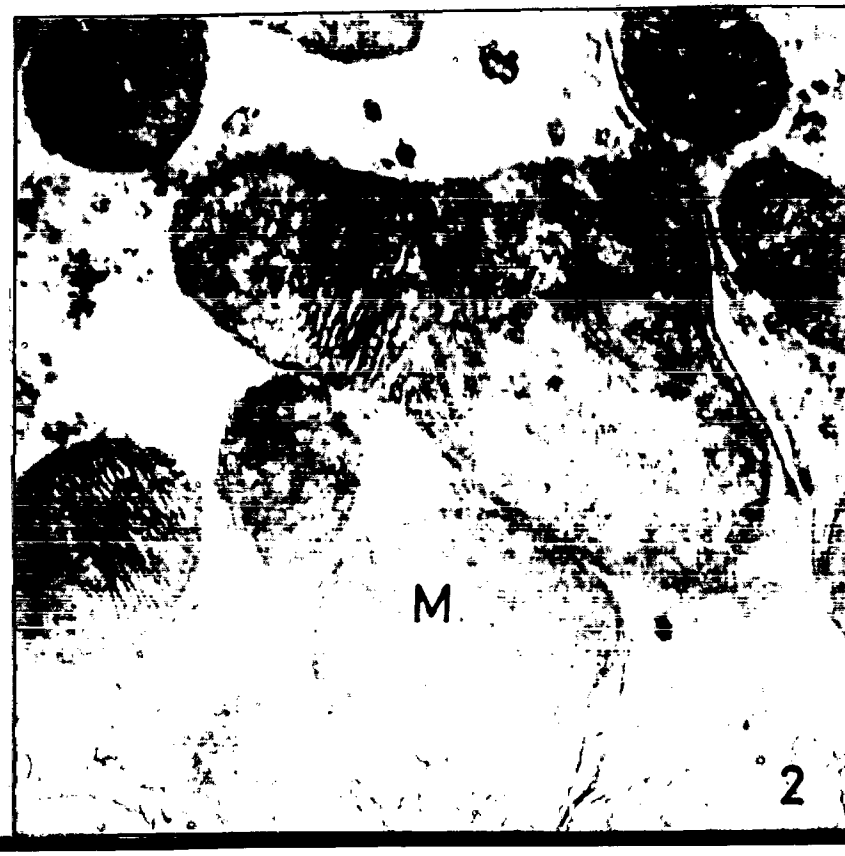
Fig 1 : 5.0% M. E. - 6 hours - Proximal tubule (1 x 5,000).

Note the vacuolisation of cytoplasm (V), empty lysosomal vesicles (Ly) and depositions of heavy metal (H.M).

Fig 2 : 5% M. E. - 6 hours - Proximal tubule (1 x 2500).

Note the damaged mitochondria (M).


B·B
H·M
V
Ly


M
2

CAPTIONS TO FIGURES

PLATE 32 :

Fig 1 : 5% M. E. - 6 hours - Distal tubule (1 x 3,000).

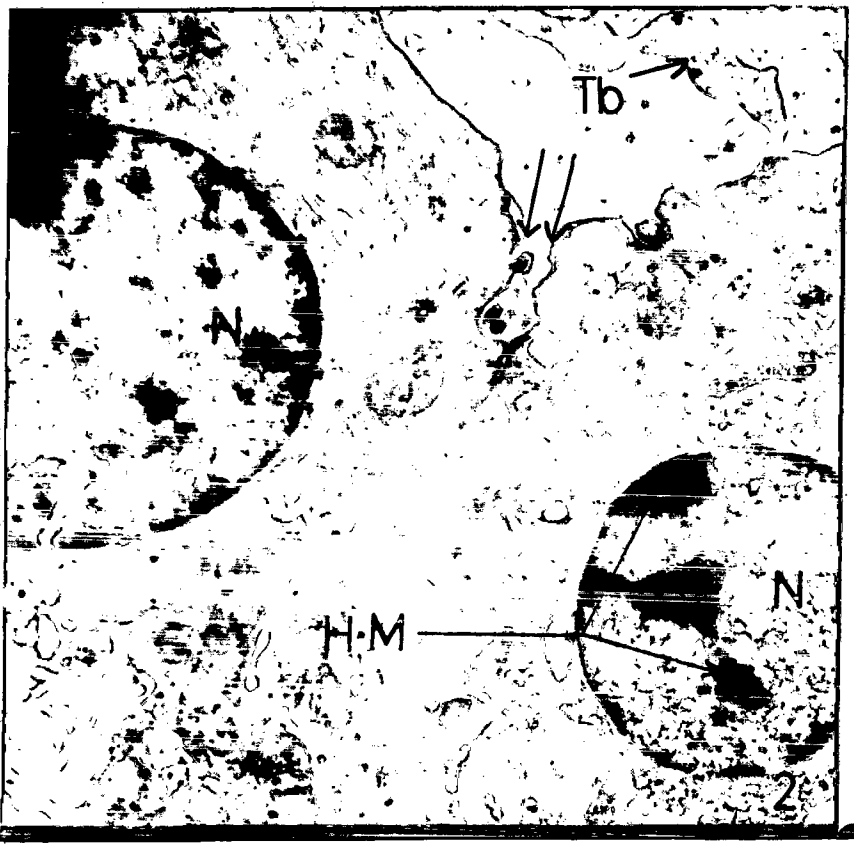
Note swollen distal tubule (DT) with reduced luminal (L) space ; tissue debris in the lumen (Tb).

Fig 2 : 5% M. E. - 6 hours - Distal tubule (1 x 6,000).

Note heavy metal depositions in the nucleus (H.M) and tissue debris in lumen (Tb). Also note autophagic vacuoles (double arrows).

PLATE NO 32

1



2

CAPTIONS TO FIGURES

PLATE 33 :

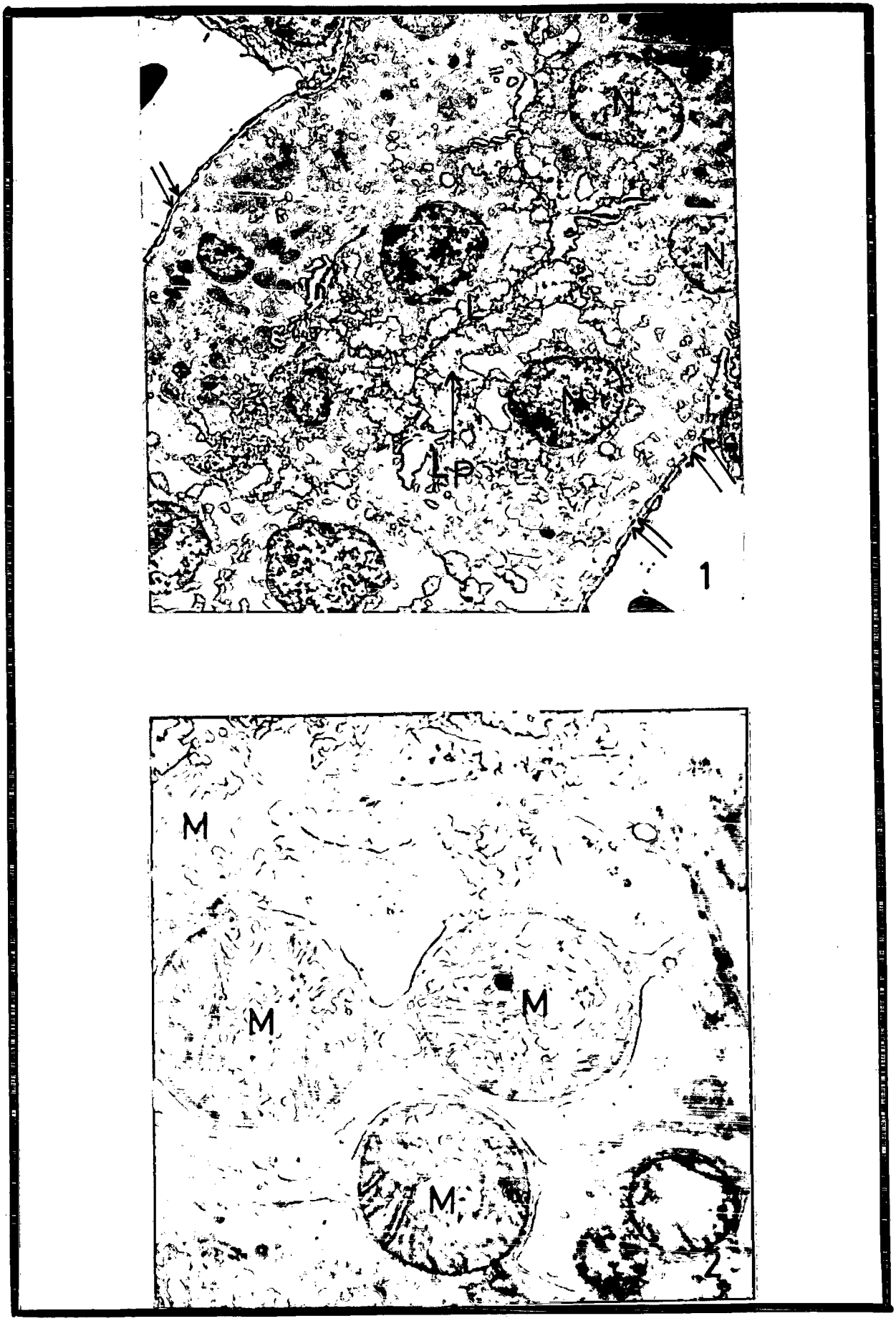
Fig 1 : 5% M. E. - 6 hours - Collecting tubule (1 x 2,300).

Note swollen collecting tubule (CT), reduced luminal space (L) (double arrows) and Pycnotic nucleus (N). Also note large deposits of lipid (Lp).

Fig 2 : 5% M. E. - 6 hours - Collecting tubules (1 x 2,000).

Note damaged mitochondria (M) from collecting tubules.

PLATE NO 33



CAPTIONS TO FIGURES

PLATE 34 :

Fig 1 : 10% M. E. - 6 hours - Glomerulus (1 x 2,000).

Note the thickening of Bowman's capsular outer wall (B.W), increase in Bowman's space (U), irregular and thickened basement membrane (B.M), mesangial proliferation (Me.P). Also note vacuolisation of cytoplasm in podocyte and complete loss of foot processes (double arrows).

Fig 2 : 10% M. E. - 6 hours - Glomerulus (1 x 4,000).

Note the swollen podocyte (P), scleroderma, thickening and slight irregular capillary basement membrane (double arrow). Also note smoothening of capillary endothelium (arrows).


B.W
U
B.M
P
Cp.L
En

CAPTIONS TO FIGURES

PLATE 35 :

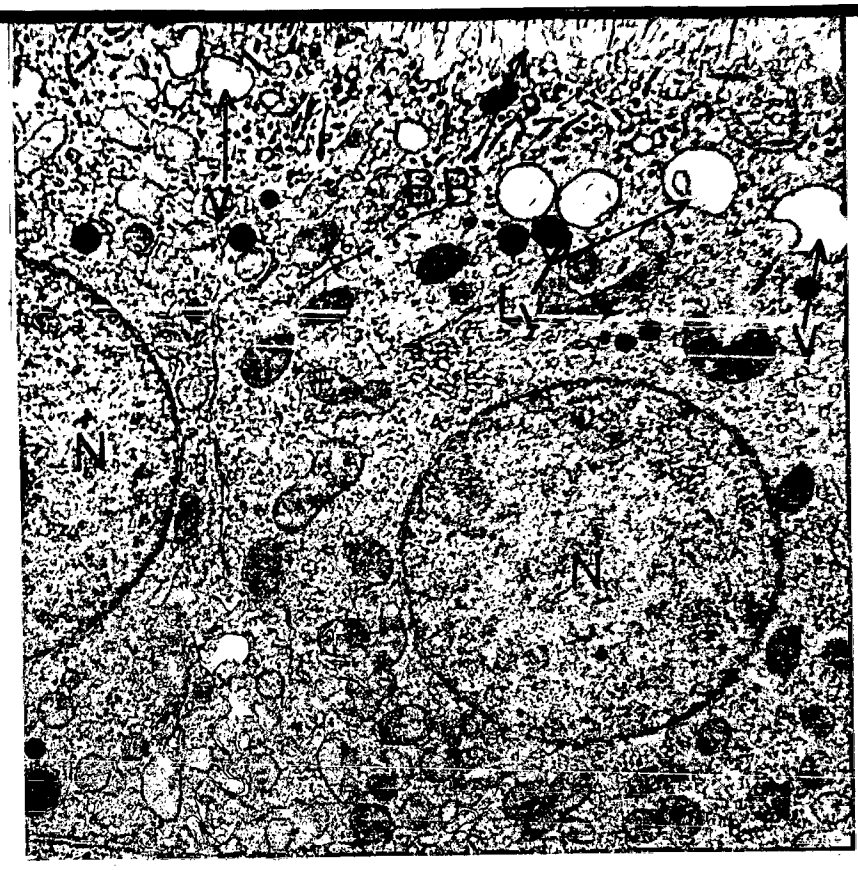
Fig 1 : 10% M. E. - 6 hours - Proximal tubule (1 x 4,000).

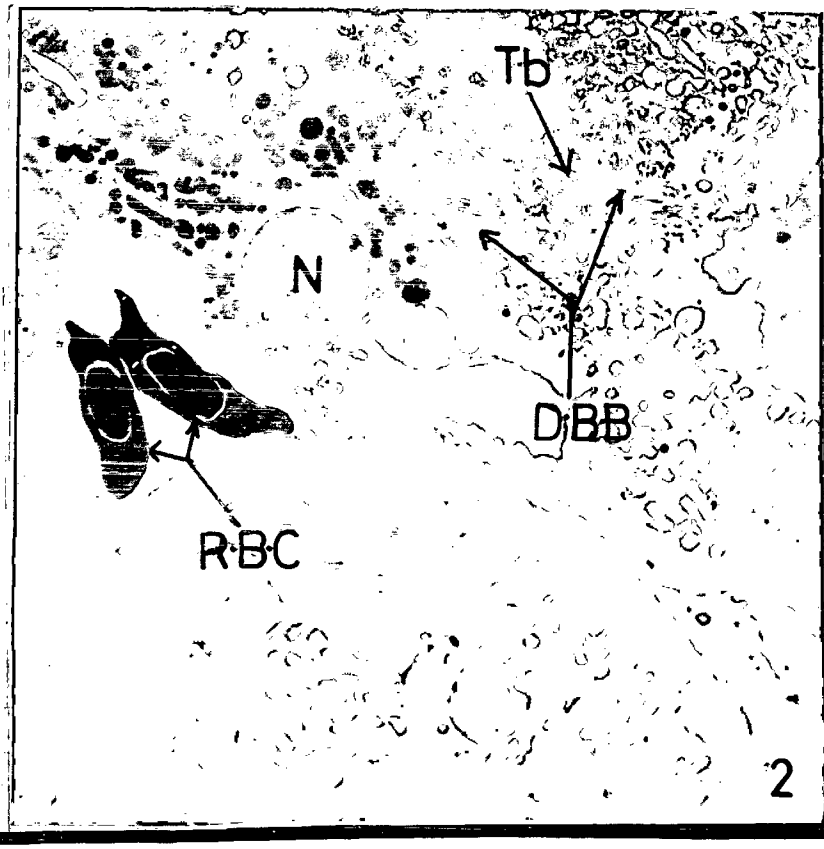
Note the empty lysosomal vesicles (Ly), cytoplasmic vacuolisation (V) and the Nucelus (N) with dispersed chromatin. Also note brush border (B.B).

Fig 2 : 10% M. E. - 6 hours - Proximal tubule (1 x 2,000).

Note the disrupted brush border (D.B.B.), tissue debris in lumen (Tb) and red blood cells in interstitium (RBC).

PLATE NO 35





CAPTIONS TO FIGURES

PLATE 36 :

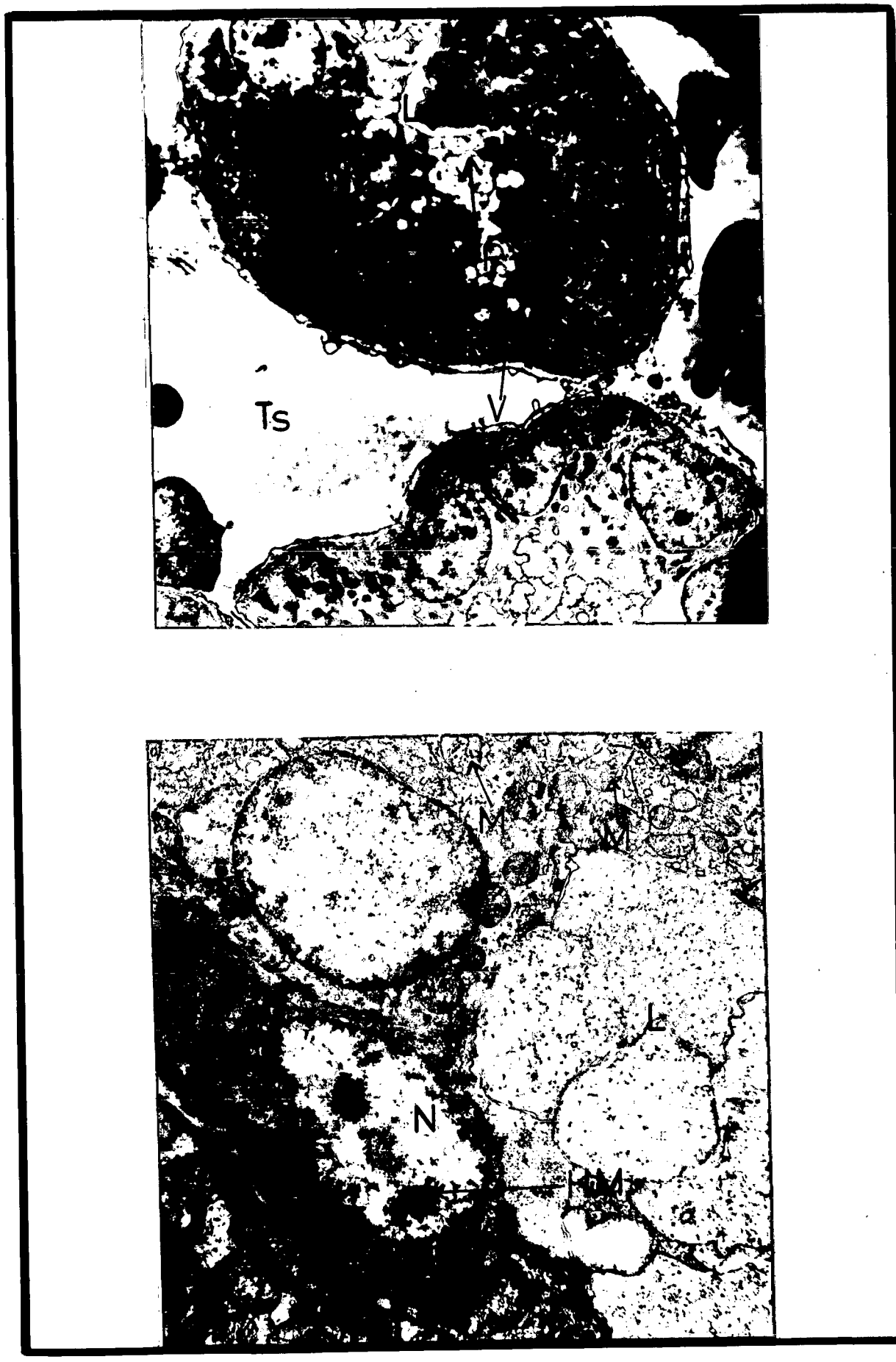
Fig 1 : 10% M. E. - 6 hours - Distal tubule (1 x 2,000).

Note the swollen tubule, lipid deposits (Lp), reduced luminal space (L), pycnotic nucleus (N) and vacuolisation of cytoplasm (V). Also note increase in inter tubular space (Ts).

Fig 2 : 10% M. E. - 6 hours - Distal tubule (1 x 3,000).

Note the damaged mitochondria (M) and pycnotic nucleus (N) with heavy metal deposition (H.M).

PLATE NO 36



CAPTIONS TO FIGURES

PLATE 37 :

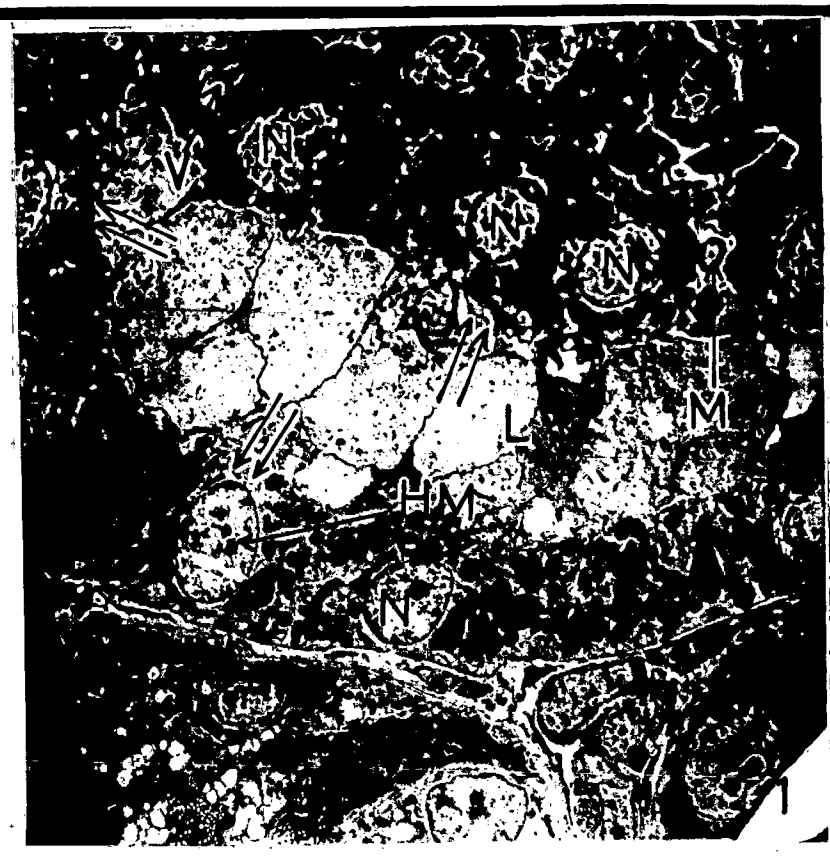
Fig 1 : 10% M. E. - 6 hours - Collecting tubule (1 x 2,000).

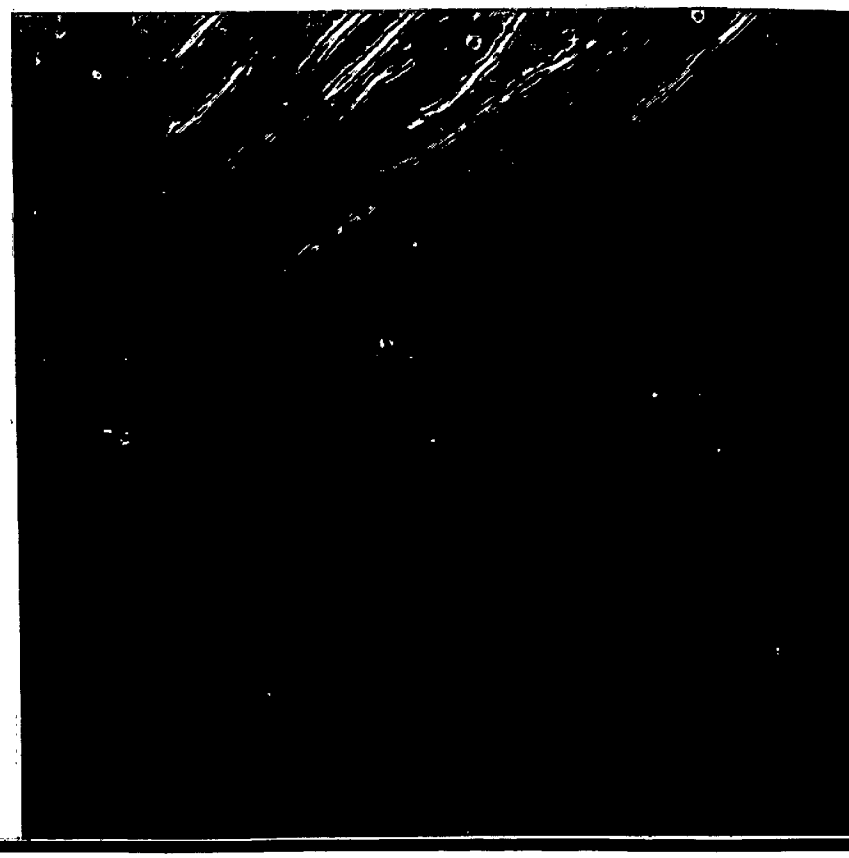
Note the damaged luminal border (double arrows), cytoplasmic vacuolisation (V), pycnotic nucleus (N), with heavy metal (H.M) depositions.

Fig 2 : 10% M. E. - 6 hours - Collecting tubules (1 x 30,000).

Note the damaged mitochondria (M).

PLATE NO 37





CAPTIONS TO FIGURES

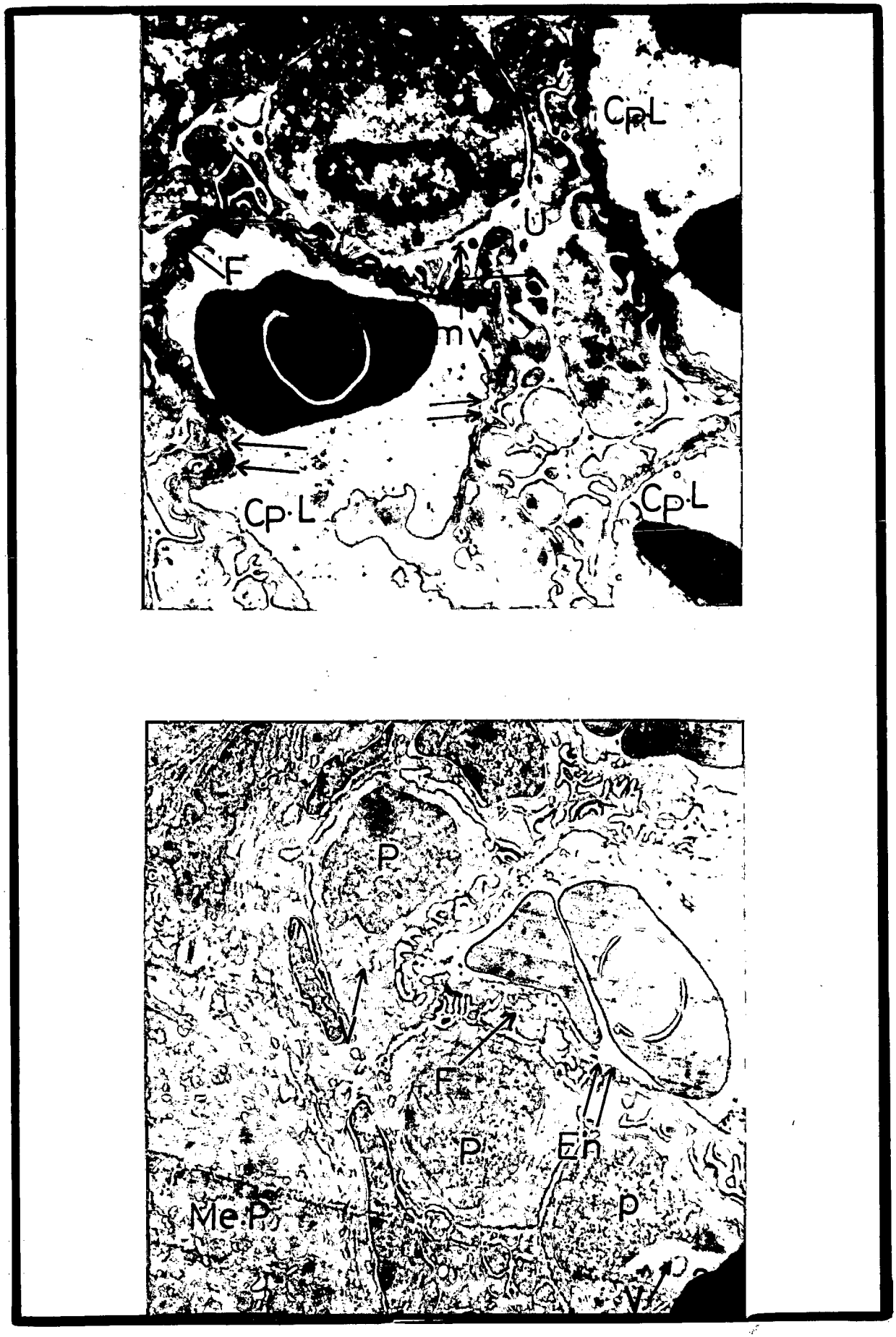
PLATE 38 :

Fig 1 : 50% M. E. - 6 hours - Glomerulus (1 x 4,000).

Note the swollen podocyte (P), fused foot processes, increase in Bowman's space (U), microvilli in Bowman's space (mv) and sclerosis of capillary endothelium (double arrows).

Fig 2 : 50% M. E. - 6 hours - Glomerulus (1 x 4,000).

Note the mesangial proliferation, swollen podocyte (P), fused foot processes (F) and damaged capillary endothelium (En).


Cp·L
U
F
mv
Cp·L
Cp·L
P
F
P
En
Me·P
P

CAPTIONS TO FIGURES

PLATE 39 :

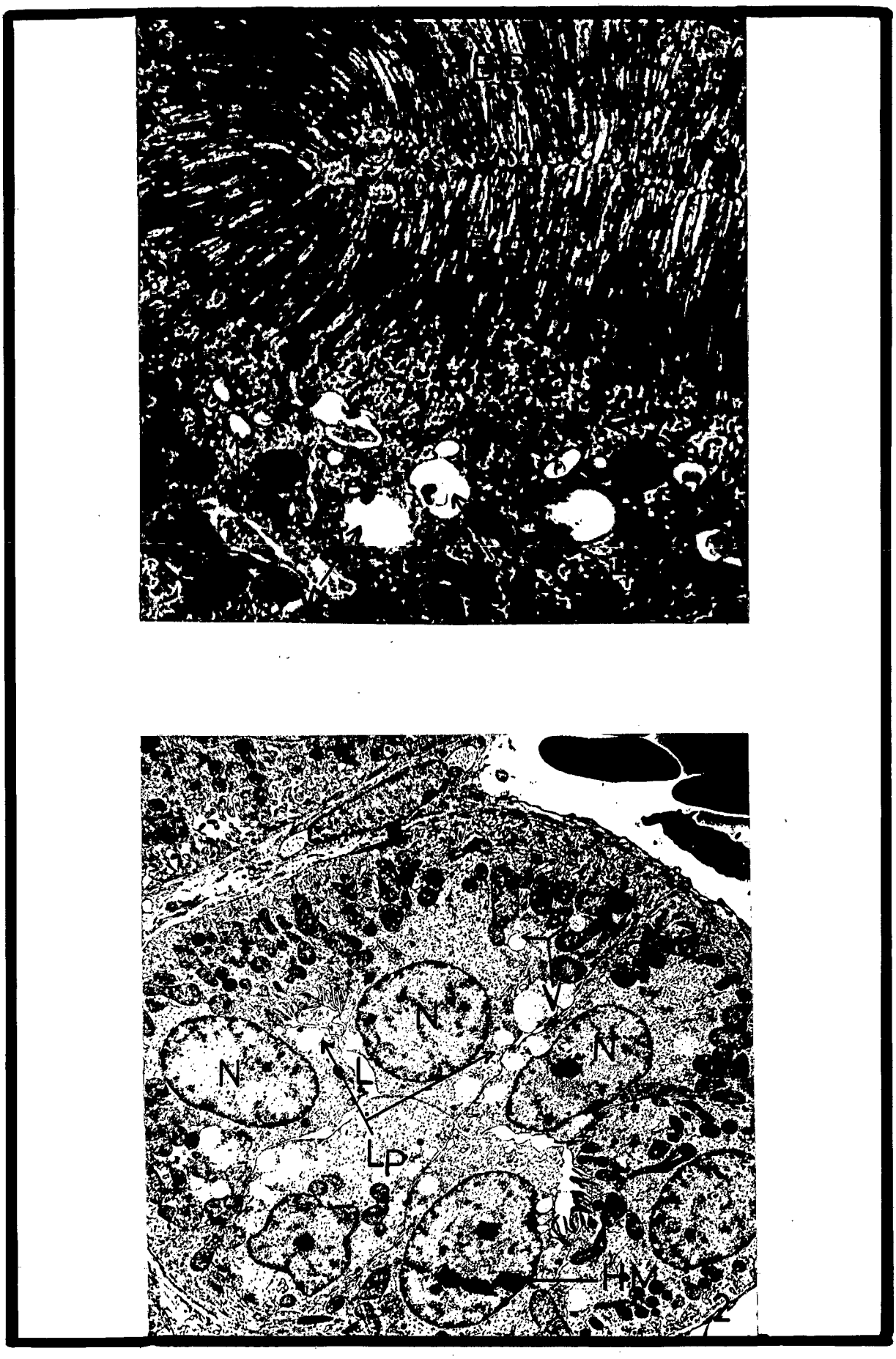
Fig 1 : 50% M. E. - 6 hours - Proximal tubule (1 x 5,000).

Note swelling of proximal tubule, brush border acquiring the entire lumen (double arrows), cytoplasmic vacuolisation (v), empty lysosomal vesicles (Ly) and heavy metal depositions in tubules (H.M).

Fig 2 : 50% M. E. - 6 hours - Distal tubule (1, x 2,000).

Note the lipid droplets (Lp), vacuolisation of cytoplasm, swelling of tubule with reduced luminal space (L) and pycnotic nucleus (N) with heavy metal depositions. (H.M).

PLATE NO 39



CAPTIONS TO FIGURES

PLATE 40 :

Fig 1 : 50% M. E. - 6 hours - Distal tubule (1 x 4,000).

Note damaged basal membrane double arrows, heavy metal (H.M.) deposition in pycnotic nuclei (N) and deposition of lipid droplets (Lp).

Fig 2 : 50% M. E. - 6 hours - Collecting tubule (1 x 4,000).

Note distorted collecting tubule, swollen Golgi bodies (Gg) in the lumen (L), lipid droplets deposition (Lp), discontinous basal membrane (double arrows), pycnotic nucleus (N) with heavy metal (H.M) deposition. Vacuolisation of cytoplasm and damaged mitochondria (M).

CAPTIONS TO FIGURES

PLATE 41 :

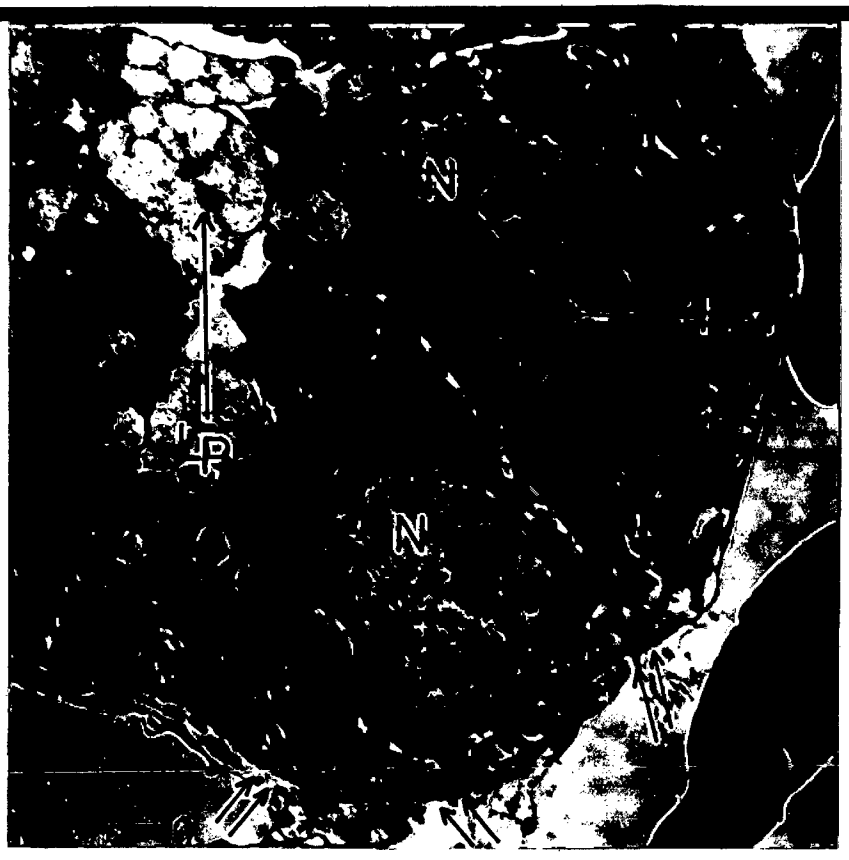
Fig 1 : 100% M. E. - 6 hours - Glomerulus (1 x 3,000).

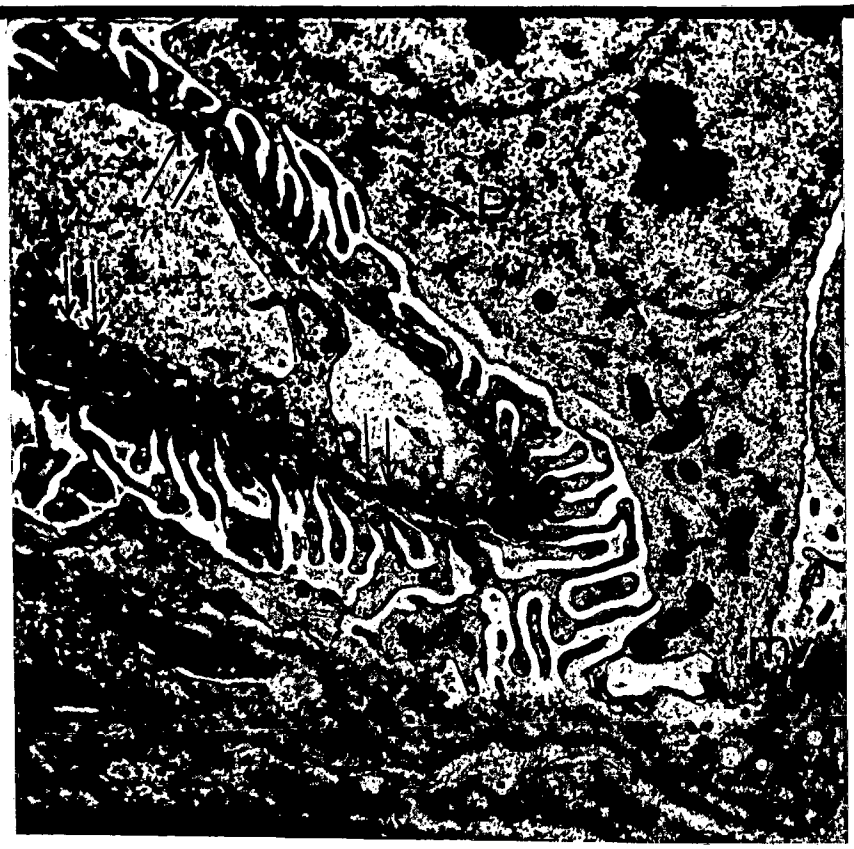
Note the swollen podocyte (P), fused foot processes (F), and irregular and thickened basement membrane (double arrow). Also note microvilli (mv) in Bowman's space.

Fig 2 : 100% M. E. - 6 hours - Glomerulus (1x 4,000).

Note endothelial sclerosis (arrow), increase in Bowman's space (U), and scleroderma of capillary endothelium (double arrows). Also note capillary lumen (CpL).

PLATE NO 40






CpL
2

CAPTIONS TO FIGURES

PLATE 42 :

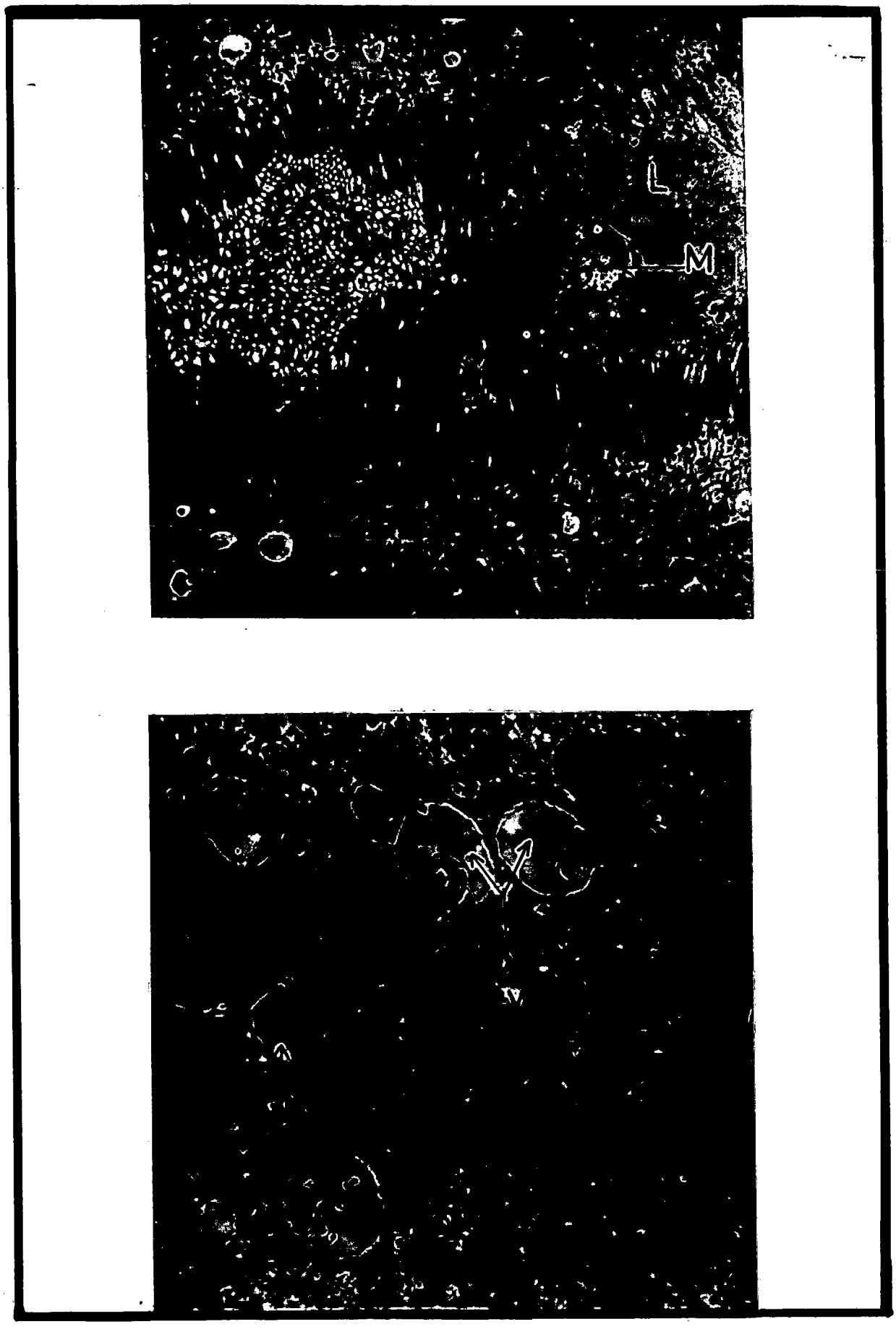
Fig 1 : 100% M. E. - 6 hours - Proximal tubule (1 x 4,000).

Note the disruption of brush border (double arrow), damaged mitochondrion (M) in lumen (L), vacuolisation of cytoplasm (V) and heavy metal deposition (H.M).

Fig 2 : 100% M. E. - 6 hours - Proximal tubule (1 x 10,000).

Note the empty lysosomal vesicles (Ly) and cytoplasmic vacuolisation (V).

PLATE NO 42



CAPTIONS TO FIGURES

PLATE 43 :

Fig 1 : 100% M. E. - 6 hours - Distal tubule (1x 2,000).

Note the pycnotic nucleus (N) with deposition of heavy metal (H.M), reduced luminal space (L) due to swelling, distorted luminal border (double arrows) and tissue debris in the lumen (Tb).

Fig 2 : 100% M. E. - 6 hours - Distal tubule (1 x 25,000).

Note the damaged mitochondrion (M).

PLATE NO 43

CAPTIONS TO FIGURES

PLATE 44 :

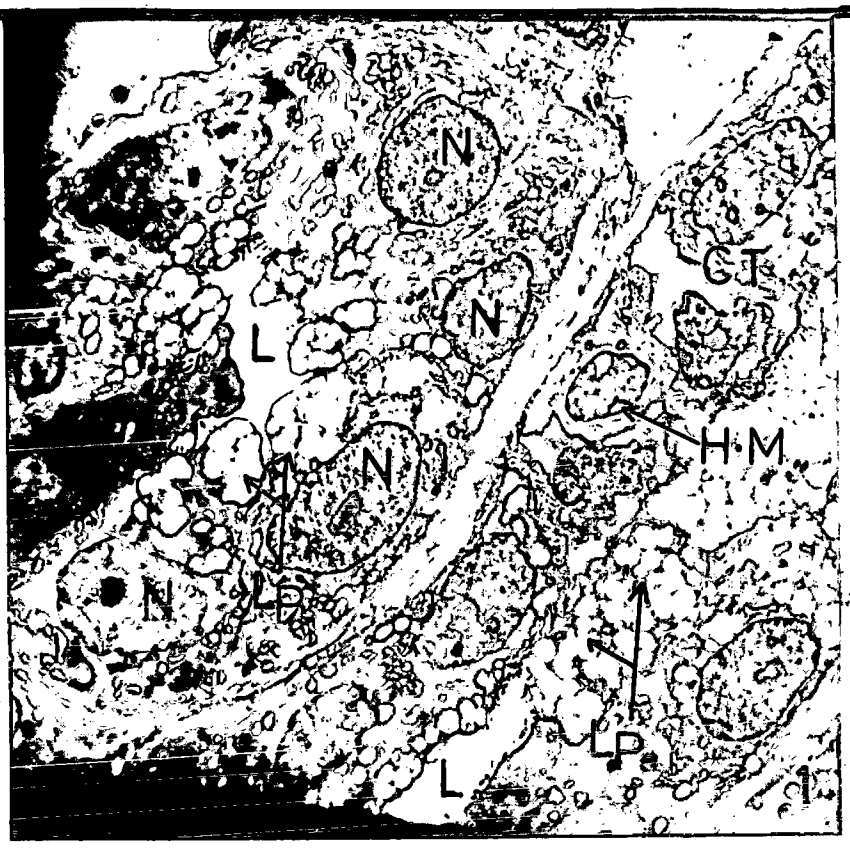
Fig 1 : 100% M. E. - 6 hours - Collecting tubule (1 x 2,000).

Note the heavy deposition of lipid (Lp), pycnotic nuclei (N) with heavy metal deposition and reduced luminal (L) space.

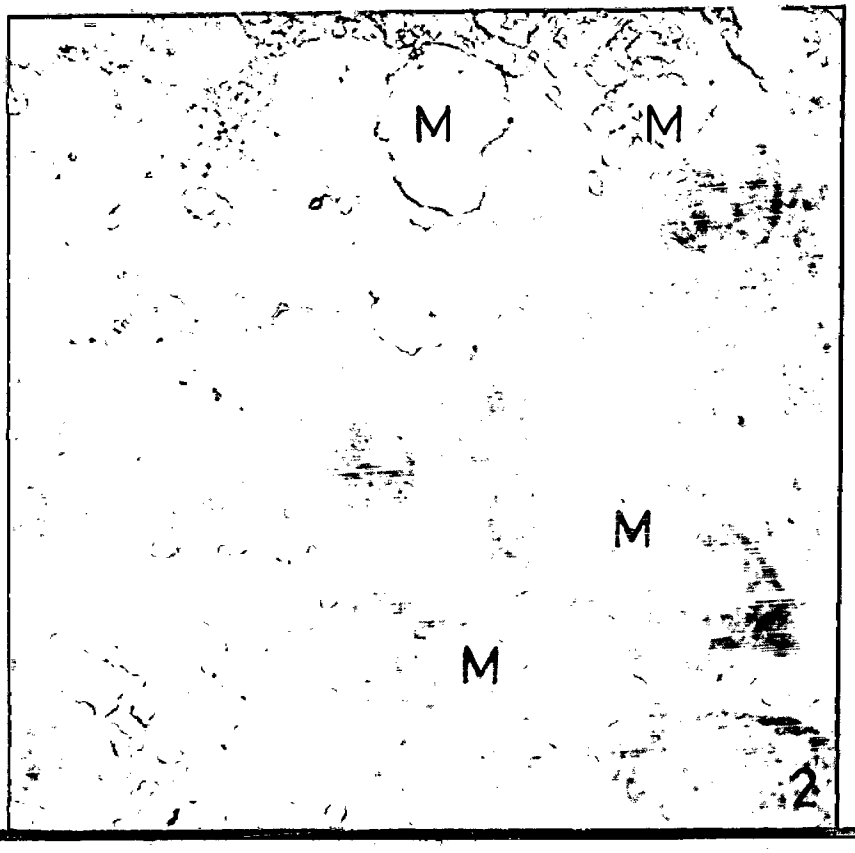
Fig 2 : 100% M. E. - 6 hours - Collecting tubule (1 x 15,000).

Note the damaged mitochondria (M).

PLATE NO 44



1



2

CAPTIONS TO FIGURES

PLATE 45 :

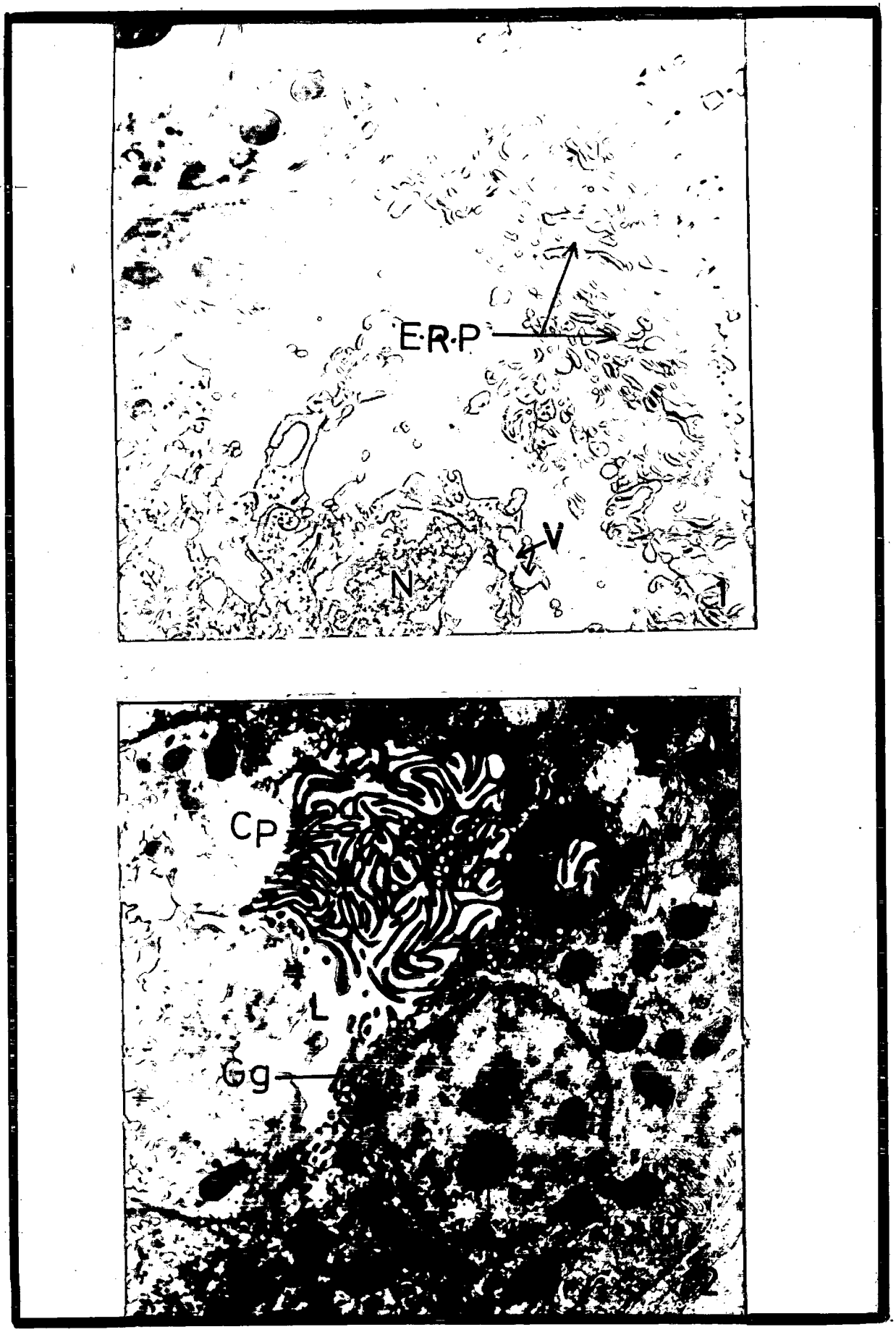
Fig 1 : 100% M. E. - 6 hours - Collecting tubule (1 x 5,000).

Note the Endoplasmic Reticuler proliferation (E.R.P) and vacuolisation of cytoplasm.

Fig 2 : 100% M. E. - 6 hours - Collecting tubules (1 x 5,000).

Note the decrease in luminal space (L), exudation of Golgi bodies in lumen (Gg), vacuolisation of cytoplasm, deposition of heavy metal (H.M.) in nucleus and ciliary processes with E.R. in lumen (Cp). Also note the vacuolisation of the cytoplasm.

PLATE NO 45



deposition of heavy metals in the nuclei **(Plate 23 : Fig 1)**, vacuolisation of mitochondria **(Plate 23 : Fig 2)**, heavy deposition of lipid droplets along with mitochondrial damage **(Plate 29 : Fig 1 and 2, Plate 33 : Fig 1 and 2, Plate 40 : Fig 2)**. Swelling of collecting tubules with reduction in luminal space **(Plate 33 : Fig 1)**, damaged luminal border **(Plate 37 : Fig 1)** and swelling of Golgi bodies **(Plate 40 : Fig 2)** were also observed.

Maximum alterations in collecting tubules were observed with **100% M.E.** where proliferation of endoplasmic recticulum was most prominent, ciliary processes and Golgi profiles were exuded in the lumina of the tubules **(Plate 45 : Fig 1 and 2)**. The degree of necrosis altered when exposure periods were prolonged. The chronic exposures to M.E. promoted increased involvement of the collecting tubules in the process of necrosis.

III] HISTOCHEMICAL ALTERATIONS :

A] ACID PHOSPHATASE :

When different histochemical techniques were employed for the studies of acid phosphatase, the technique of Davies and Ornstein (1959) using α napthyl phosphate as substrate and fast garnet GBC as azodye was found suitable. The studies on acid phosphatase of mallards revealed the presence of two types of renal tubules. One type of renal tubule showed the presence of brush/luminal border and cytoplasmic acid phosphatase and the other showing cytoplasmic acid phosphatase. For the sake of convenience the tubules showing brush/luminal border enzyme activity would be referred as 'BL' type and others showing cytoplasmic enzyme activity as 'Cy' types. It is already established that the avian kidney is composed of mammalian and reptilian tubules. It is very difficult to say whether the mammalian tubules are showing brush/luminal border acid phosphatase or the reptilian ones. It is observed that about sixty percent tubules exhibited brush/luminal border acid phosphatase while about forty percent tubules showed the cytoplasmic acid phosphatase.

The ducks exposed to the mining effluents (M.E.) also showed the acid phosphatase activity bound to the membranes. Thus, the present investigation revealed the presence of three types of acid phosphatase brush/luminal border, cytoplasmic and membrane bound based on the locations. The brush /luminal border acid phosphatase activity in controls was quite high and as a result the azodye staining diffused into lumina, thereby filling them **(Plate 46 : Fig 1)**. A few glomeruli also showed the acid phosphatase activity. In the tubules the lysosomal granular acid phosphatase activity was masked by cytoplasmic staining and this was confirmed by the enzyme activation and inhibition studies.

CAPTIONS TO FIGURES

PLATE 46 :

Fig 1 : Control : Acid phosphatases (A P) - α napthyl - Fast Garnet GBC (Davies & Ornstein, 1959). (10 x 5)

Note selective brush border (PT) and luminal (DT) enzyme activity. Also note diffused cytoplasmic activity with brush/luminal border activity in other tubules (dashed arrows). No enzyme activity in collecting tubules (CT), except in few nuclei.

Fig 2 : 0.01% M. E. - 720 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

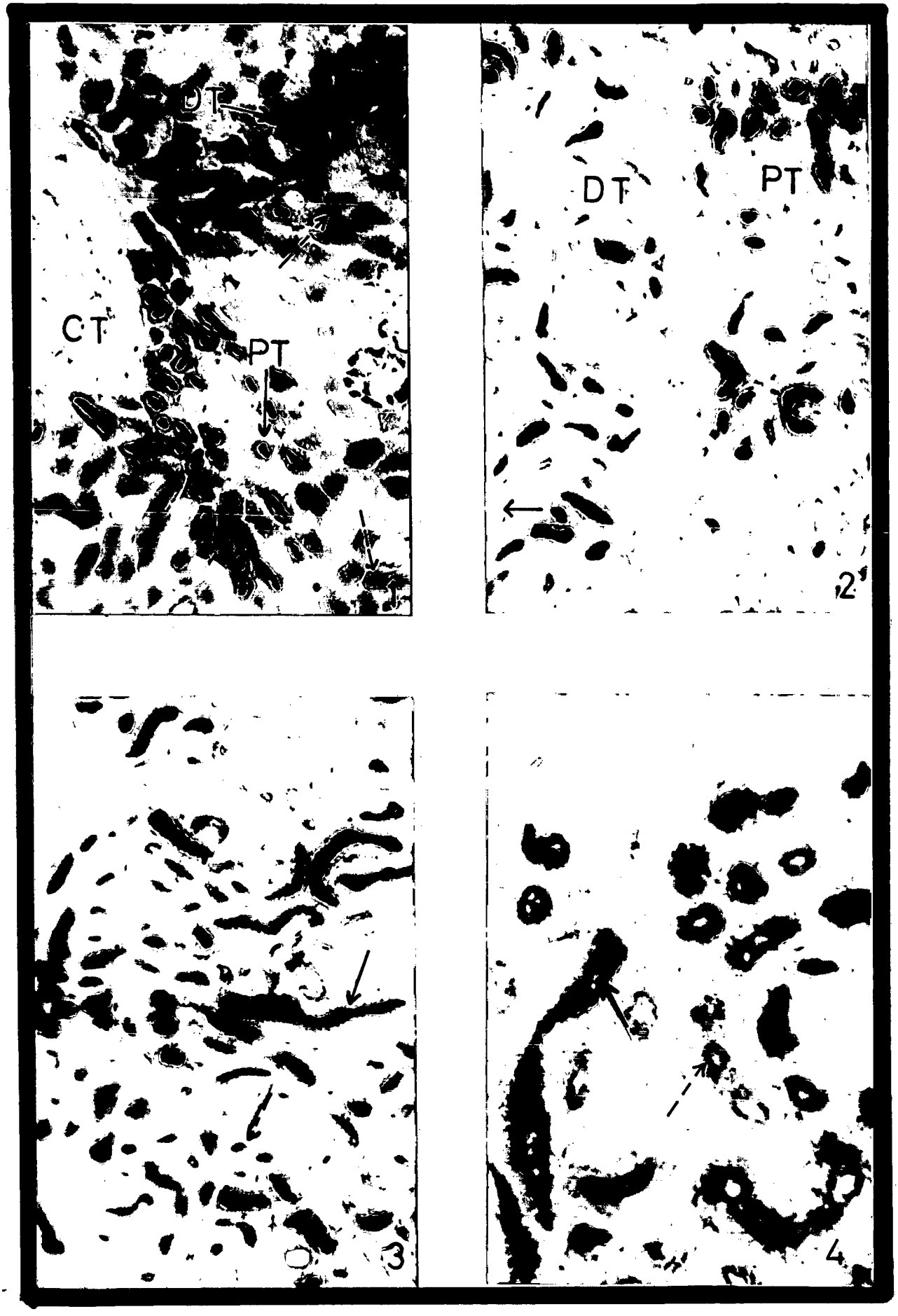
Note increase in the enzyme activity in the brush border/luminal border (PT/DT) of the tubules. Also note occurrence of acid phosphatases in the brush border/luminal border (arrow) of the tubules previously showing cytoplasmic staining.

Fig 3 : 0.01% M. E. - 1080 hours - (A P) - α napthlyl - Fast Garnet GBC. (10 x 5)

Note further increase in the acid phosphatase activity in the brush border/luminal border (arrows) of both the types of tubules identified on the basis of stainability.

Fig 4 : 0.1% M. E. - 6 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note increase in acid phosphatase activity at the brush and luminal borders (solid arrow). Also note the diffused fine granular cytoplasmic and brush/luminal border (dashed arrow) acid phosphatase activity in other tubules.


DT
CT
PT
DT
PT
2
3
4

CAPTIONS TO FIGURES

PLATE 47 :

Fig 1 : 0.1% M. E. - 12 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note reduction in the enzyme activity at the brush/luminal borders (solid arrow) of tubules. Also note the slight reduction in the enzyme activity from the cytoplasm (dashed arrow) and brush / luminal border of the other type of tubules.

Fig 2 : 0.1% M. E. - 48 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note the significant increase in the enzyme activity at the brush/luminal borders (solid arrow) of tubules. Also note increased cytoplasmic enzyme activity in other types of tubules (dashed arrows).

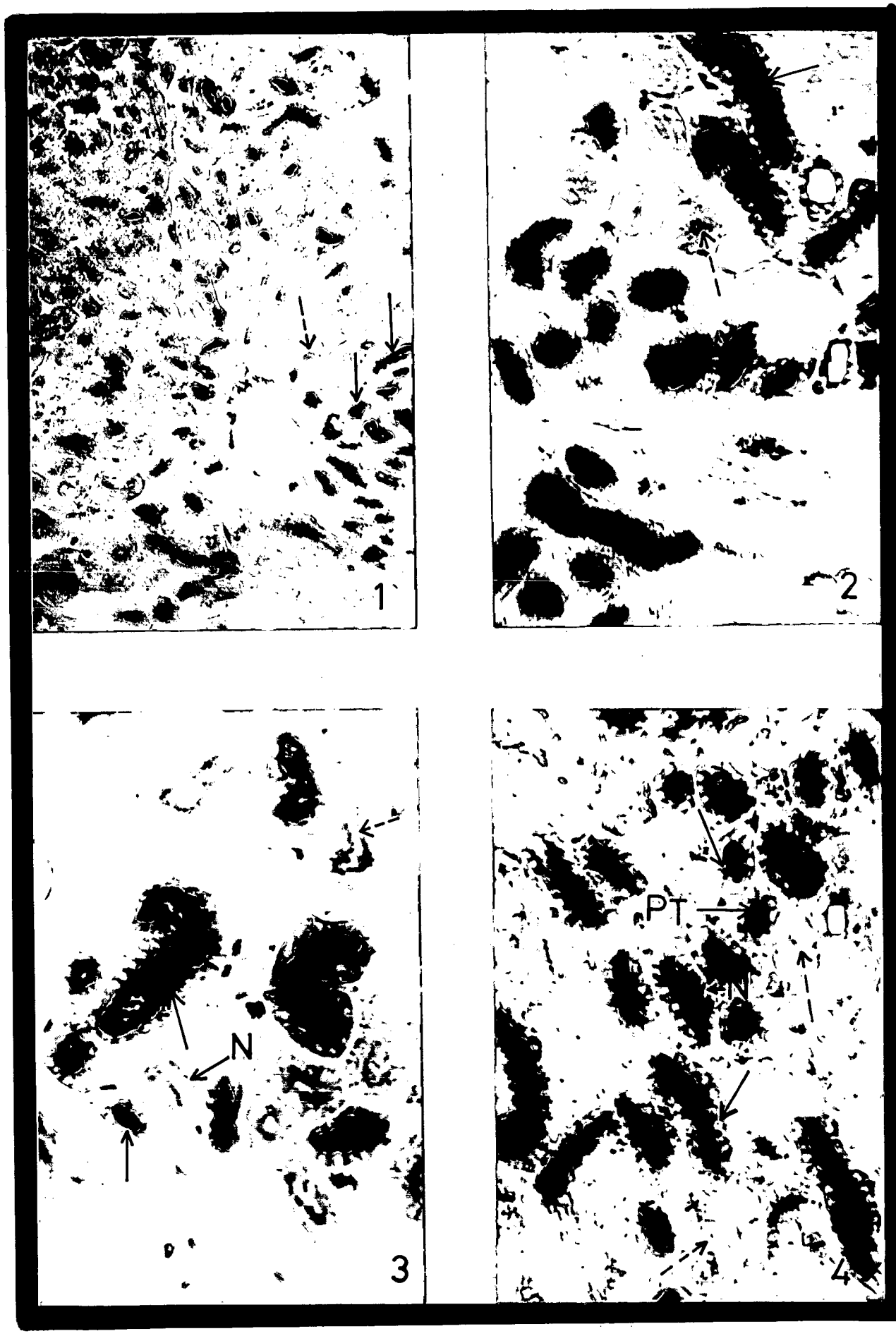
Fig 3 : 0.1% M. E. - 72 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note intense enzyme activity at the brush/luminal (solid arrow) border of few tubules and they also show cytoplasmic enzyme activity. Also note the slight reduction in the cytoplasmic enzyme activity but increase in the brush/luminal (dashed arrow) border enzyme activity of other tubules. Note nuclear (N) enzyme activity.

Fig 4 : 0.1% M. E. - 96 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note profound increase in the enzyme activity of the tubules at brush/luminal (solid arrow) borders and also note diffused enzyme activity with nuclear membrane (N) enzyme activity. Note the other types of tubules with increase in the cytoplasmic activity (dashed arrow).

PLATE NO 47



The mallards exposed to **0.01 % M.E.** did not exhibit any significant change in the histochemistry of acid phosphatase up to the end of twentynine days but by the end of thirty days an increase in the brush/luminal border acid phosphatase was seen and interestingly the 'Cy' tubules previously showing cytoplasmic acid phosphatase showed the appearance of brush/luminal border enzyme activity with the reduction in the cytoplasmic enzyme activity **(Plate 46 : Fig 2)**. At the end of one thousand and eighty hours, both the types of tubules exhibited increase in the brush/luminal border acid phosphatase activity. The glomeruli exhibited slight reduction in the enzyme activity. **(Plate 46 - Fig 3)**.

Under the influence of **0.1% M.E.** at the end of six hours, the tubules (proximal & distal) previously showing brush/luminal border acid phosphatase activity exhibited increase in the enzyme activity. The 'Cy' tubules showing cytoplasmic acid phosphatase activity showed increase in the cytoplasmic enzyme activity but in addition showed insignificant occurrence of brush/luminal border enzyme activity. Even the debris whenever present in the lumina of the tubules exhibited enzyme activity **(Plate 46 : Fig 4)**. At the end of twelve hours there was decrease in the brush/luminal border enzyme activity from all the tubules **(Plate 47 : Fig 1)**. But, at the end of fortyeight hours, there was once again increase in the enzyme activity at the brush/luminal borders of the "BL" tubules. Most of the 'Cy' tubules exhibited increase in the cytoplasmic enzyme activity in them but the 'Cy' type tubules now showed increase in the enzyme activity at the brush/luminal borders and decreased cytoplasmic activity **(Plate 47 : Fig 3)**. In a few tubules also the nuclear membranes showed the acid phosphatase activity.

The **0.1% M.E.** induced profound increase in the brush/luminal border as well as cytoplasmic acid phosphatase activity in 'BL' type tubules while 'Cy' tubules showed loss of brush/luminal border acid phosphatase activity, few of these tubules showed decrease in the cytoplasmic enzyme activity **(Plate 47 :**

CAPTIONS TO FIGURES

PLATE 48 :

Fig 1 : 0.1% M. E. - 120 hours - (A P) - α napthyl-Fast Garnet GBC. (10 x 5)

Note the reduction in the enzyme activity (solid and dashed arrow) in 'BL' type tubules. Also note weak enzyme activity in 'Cy' type tubules (C). Note absence of enzyme activity in collecting tubules (CT) and glomerulus(G).

Fig 2 : 1.0% M. E. - 6 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note increase in the enzyme activity at the brush/luminal (solid arrow) border of the tubules. Also note weak brush/luminal (dashed arrow) border enzyme activity in other types of tubules.

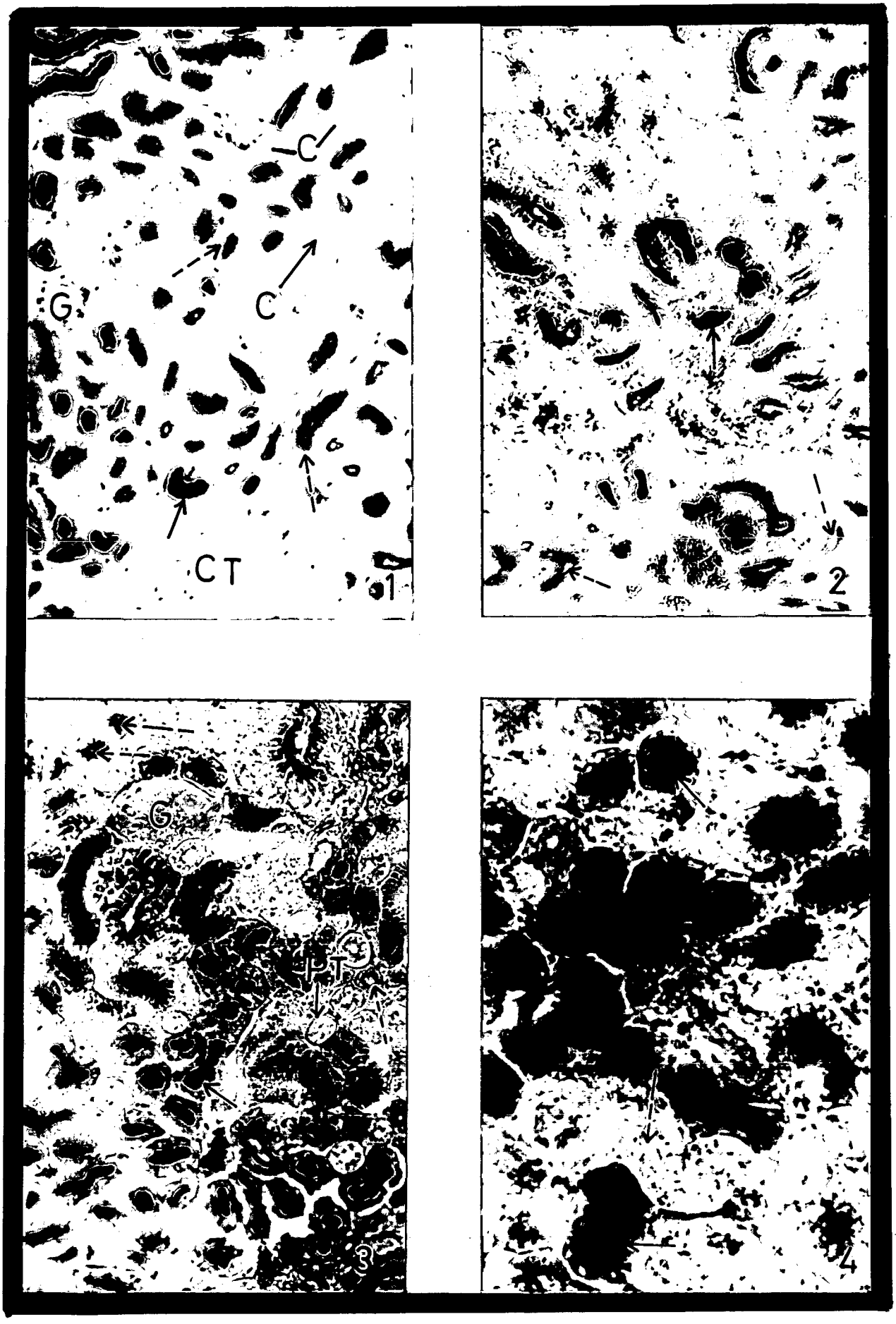
Fig 3 : 1.0% M. E. - 24 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note intense enzyme activity in the brush/luminal (solid arrow) borders of some tubules. Also note slight reduction in the brush/luminal (dashed arrow) border enzyme activity of other types of tubules. Also note the increase in number of tubules showing enzyme activity.

Fig 4 : 1.0% M. E. - 72 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note very intense enzyme activity in brush/luminal (solid arrow) borders. Also note increase in the cytoplasmic enzyme (dashed arrow) activity of the other tubules.

PLATE NO 48



CAPTIONS TO FIGURES

PLATE 49 :

Fig 1 : 1.0% M. E. - 96 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note slight reduction in the enzyme activity at brush/luminal (solid arrow) borders of tubules previously showing very intense enzyme activity. Also note slight reduction in the cytoplasmic enzyme (dashed arrows) activity of other tubules.

Fig 2 : 1.0% M. E. - 120 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note further increase in the acid phosphatase activity at the brush/luminal (solid arrow) borders of the tubules and also cytoplasmic enzyme activity. Also note the return of brush/luminal (dashed arrow) border enzyme activity in 'Cy' type tubules. Glomerulus (G) without changed enzyme activity.

Fig 3 : 5.0% M. E. - 12 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note brush/luminal (solid arrow) border enzyme activity. Also note increased cytoplasmic enzyme (dashed arrow) activity in other types of tubules.

Fig 4 : 5.0% M. E. - 24 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note further increase in the brush/luminal (solid arrow) border enzyme activity. Also note the occurrence of brush/luminal (dashed arrow) border enzyme activity in other tubules.

PLATE NO 49

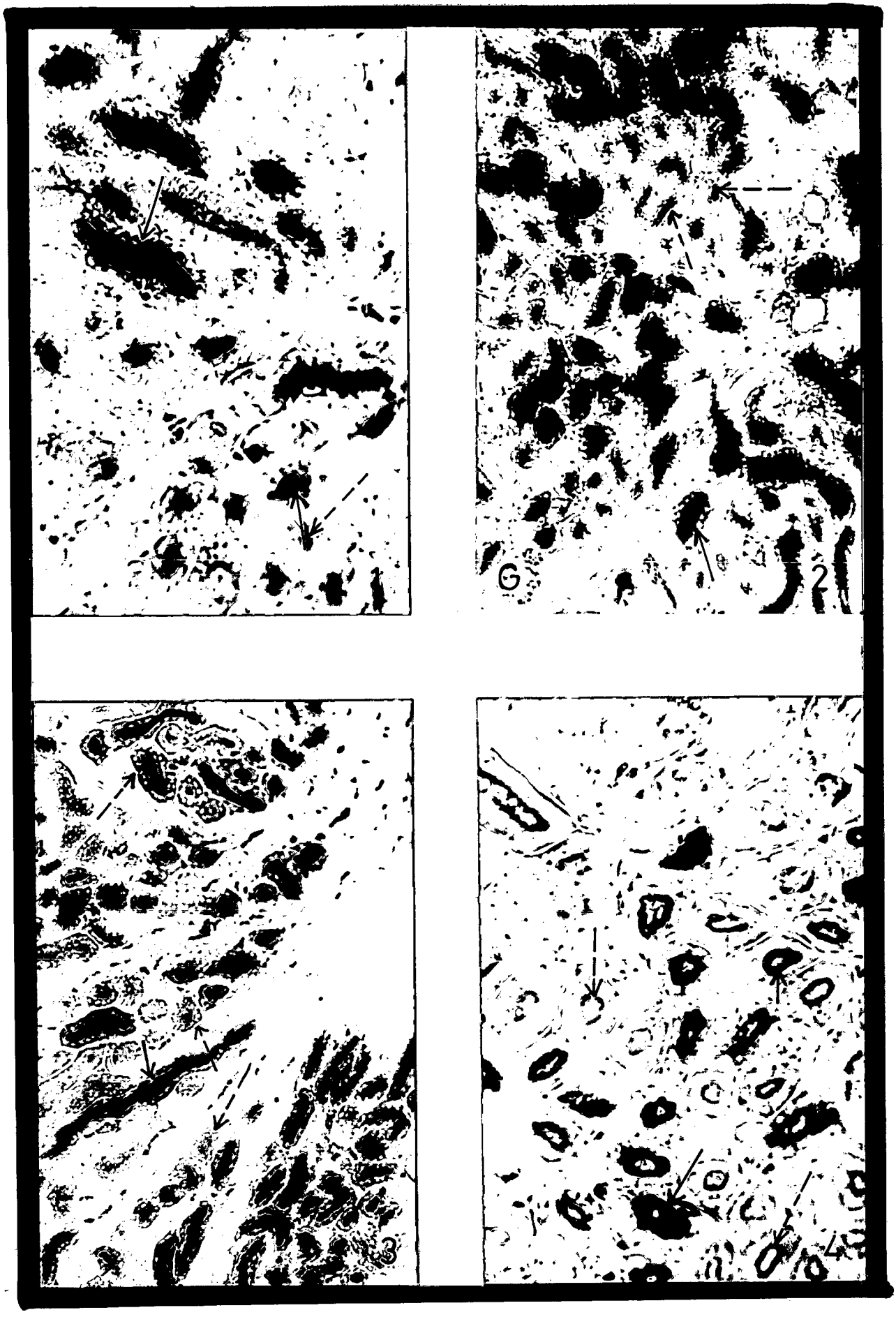
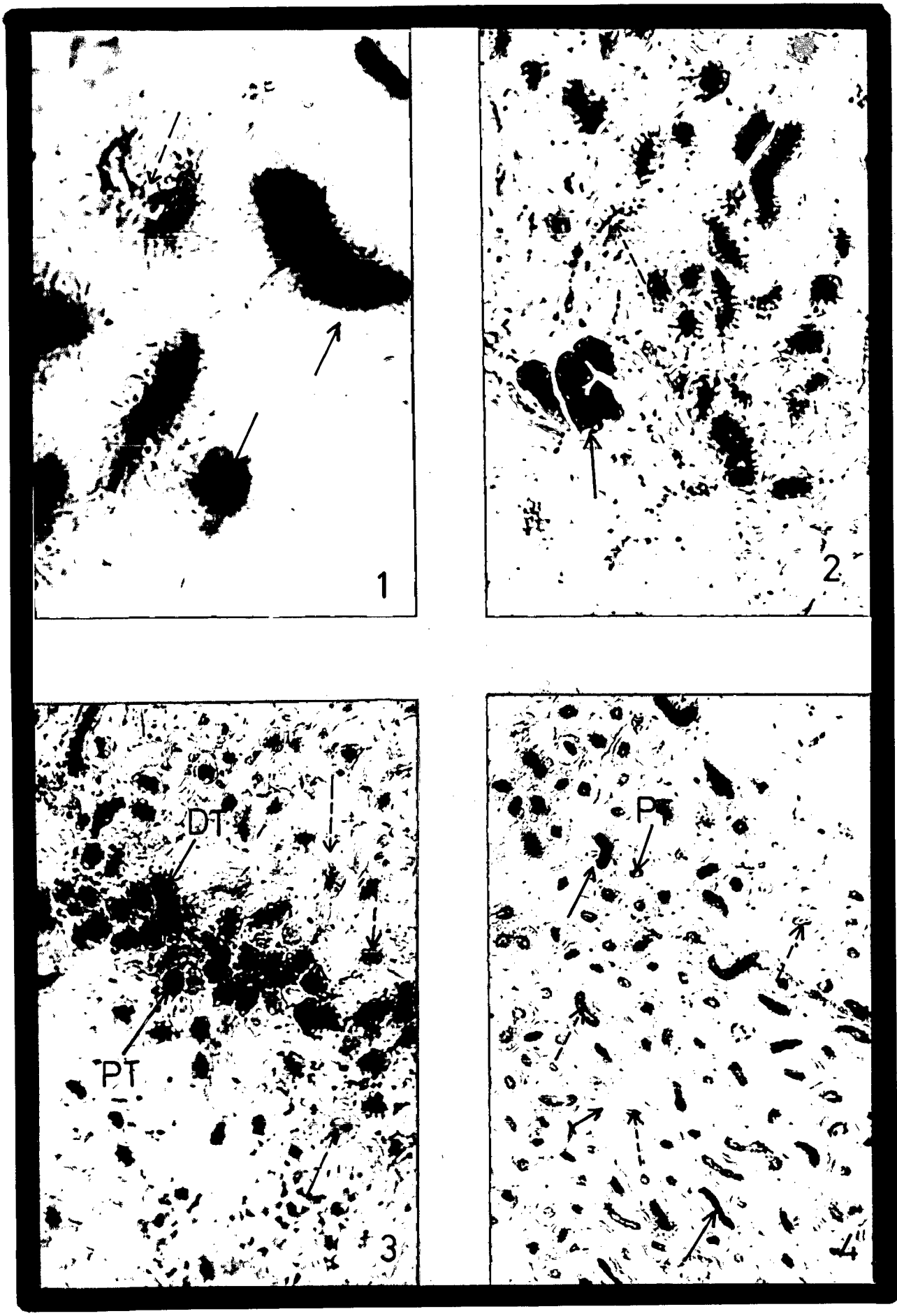


PLATE NO 50



Occasionally the necrotic tubules showed membrane acid phosphatase activity. The collecting tubule nuclei, exudents and basal membranes showed acid phosphatase activity in few cases **(Plate 49 : Fig 3 and 4)**. At the end of fortyeight hours there was no change in the enzyme activity. But at the end of seventytwo hours, there was reduction in the enzyme activity from all the tubules and a weak membrane acid phosphatase activity was seen on the basal membranes of a few necrotic tubules **(Plate 50 : Fig 2)**. Then, at onetwenty hours there was once again increase in the enzyme activity of all the tubules. The glomerulus (G) did not show any significant change in the enzyme activity. In 'Cy' type tubules a very weak brush/luminal border acid phosphatase activity was seen **(Plate 50 : Fig 3)**.

The ducks exposed to **10.0% M.E.** exhibited intense staining in 'BL' type tubules and increased brush/luminal border activity in 'Cy' type tubules (dashed arrow, PT) **(Plate 50 : Fig 4)**. At the end of twelve hours there was a little increase in the acid phosphatase activity of all the tubules but, at the end of twentyfour hours the 'BL' type tubules showed increase at the brush/luminal borders; also cytoplasmic enzyme activity from these tubules increased. The 'Cy' type tubules exhibited increase in the cytoplasmic acid phosphatase activity and in a few tubules the brush/luminal border enzyme activity appeared. The widened interstitium was without the enzyme activity **(Plate 51 : Fig. 1)**.

At the end of fortyeight hours the acid phosphatase activity at the brush/luminal borders of the 'BL' type tubules increased significantly and these tubules showed fine granular and cytoplasmic enzyme activity. The 'Cy' type tubules showed reduction in the cytoplasmic acid phosphatase activity and a very few of these tubules showed brush/luminal border acid phosphatase. In few places the exudents in the interstitium showed enzyme activity **(Plate 51: Fig 2)**. By the end of ninetysix hours the acid phosphatase activity from the 'BL' and 'Cy' type tubules reduced at the brush/luminal borders but cytoplasmic

CAPTIONS TO FIGURES

PLATE 51 :

Fig 1 : 10.0% M. E. - 24 hours - (A P) -α napthyl - Fast Garnet GBC. (20 x 5)

Note increase in the enzyme activity at brush/luminal (solid arrow) border of the tubules which also show cytoplasmic enzyme activity. Also note slight increase in the cytoplasmic enzyme activity of other tubules showing occurrence of brush/luminal (dashed arrow) border activity.

Fig 2 : 10.0% M. E. - 48 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note intense enzyme activity at the brush/luminal (solid arrow) borders of tubules which also show cytoplasmic enzyme activity. Also note the occurrence of brush/luminal (dashed arrow) border enzyme activity in other tubules and also note the increase in the enzyme activity of these tubules.

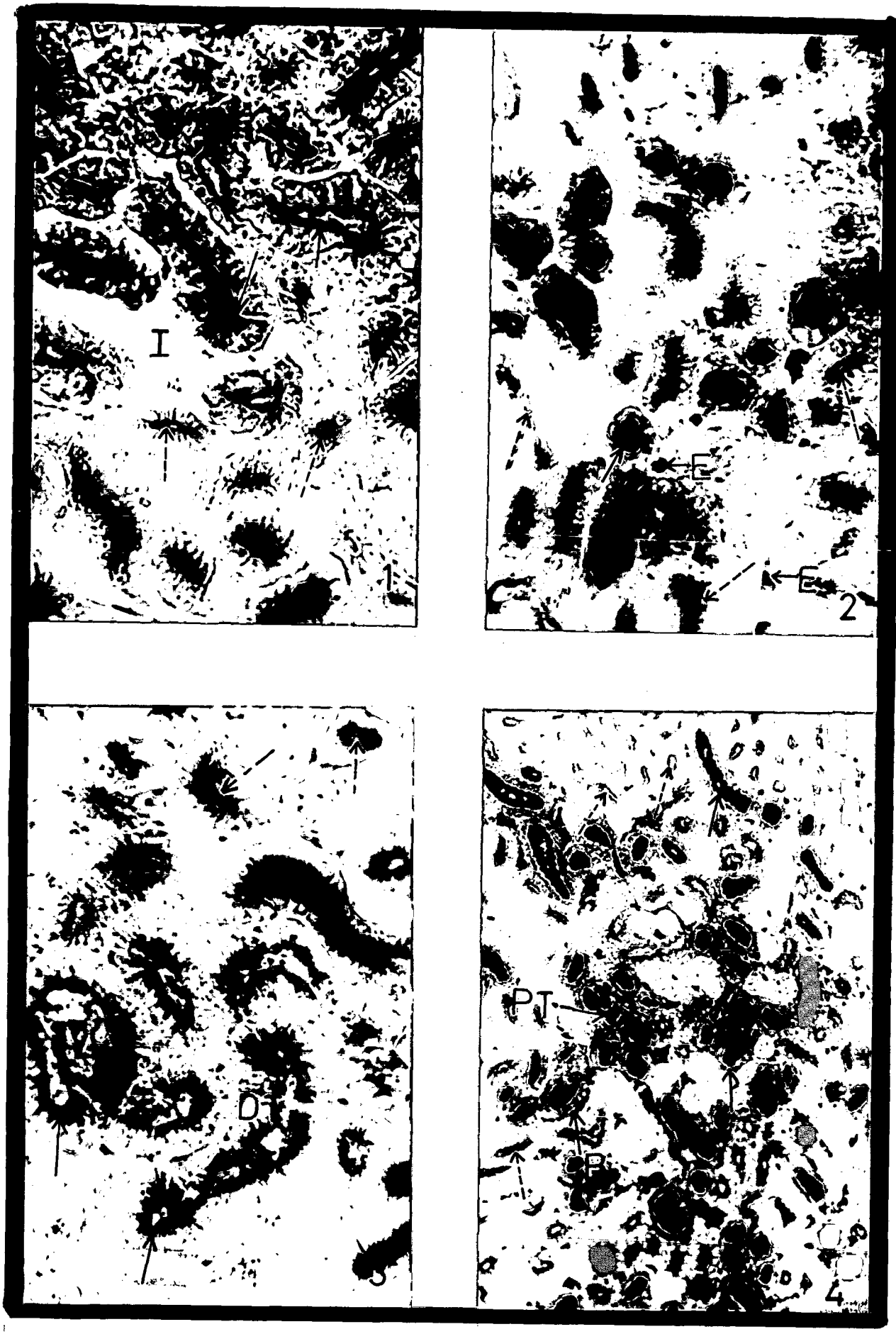
Fig 3 : 10.0% M. E. - 96 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note reduction in the enzyme activity at the brush/luminal (solid arrow) border of the tubules. Also note slight reduction at the brush/luminal (dashed arrow) border enzyme activity of other tubules where now increased cytoplasmic enzyme activity is seen.

Fig 4: 10.0% M. E. - 120 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note increase in the enzyme activity at the brush/luminal (solid arrow) borders of the tubules. Also note increased enzyme activity at the brush/luminal (dashed arrow) borders of the other tubules. Also note occurrence of enzyme activity at the basal membranes (B) of the tubules.

PLATE NO 51



CAPTIONS TO FIGURES

PLATE 52 :

Fig 1 : 50.0% M. E. - 6 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note intense enzyme activity in few tubules (solid arrow). Also note weak cytoplasmic (dashed arrow) enzyme activity.

Fig 2 : 50.0% M. E. - 12 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note the very intense enzyme activity in the tubules (solid arrow). Also note the appearance of enzyme activity in glomerulus (G) and the dense granular enzyme activity in other tubules.

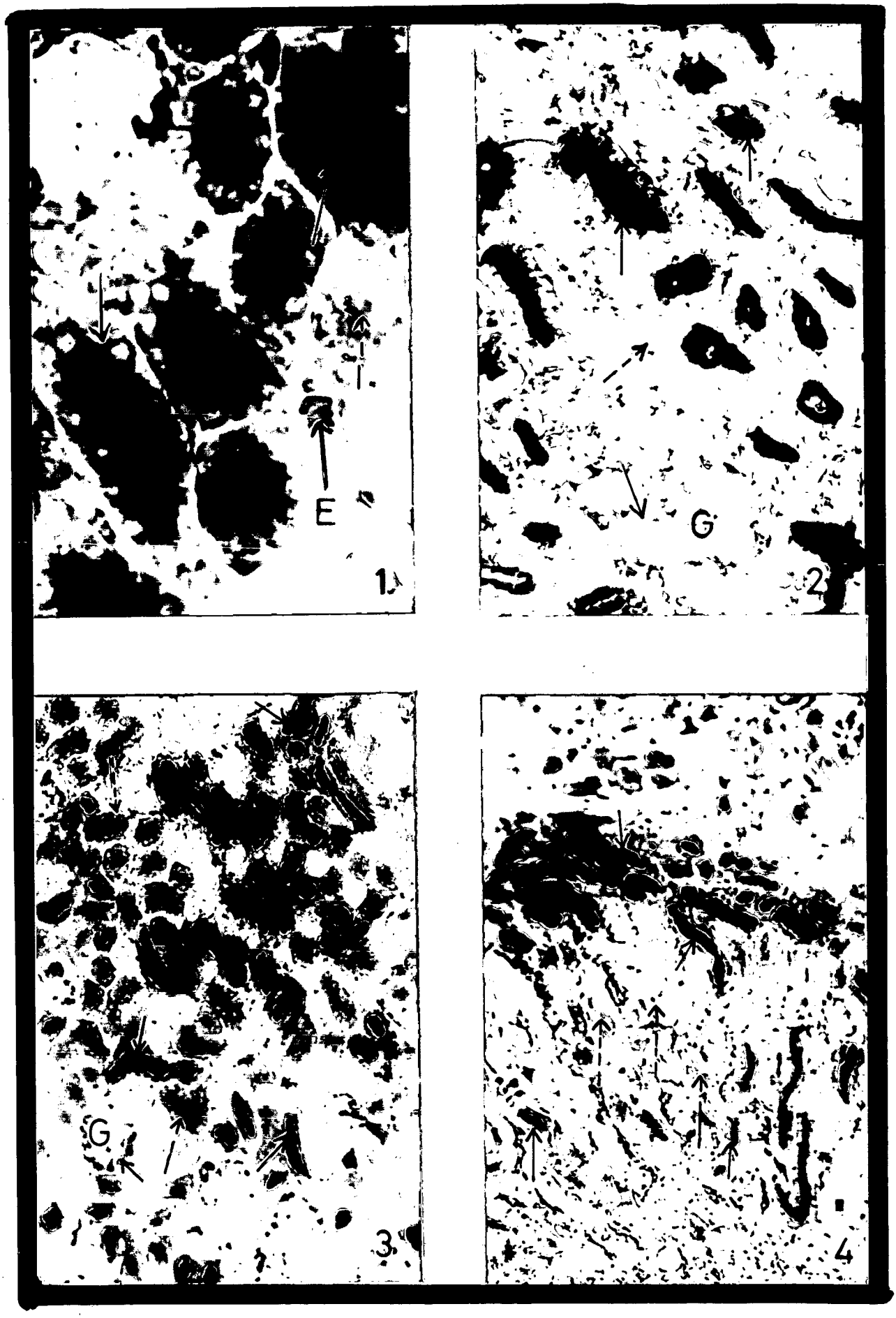
Fig 3 : 50.0% M. E. - 24 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note the slight reduction in the enzyme activity of the tubules (solid arrow). Also note increase in the cytoplasmic enzyme activity of other tubules (dashed arrow). Note increase in glomerular (G) enzyme activity.

Fig 4 : 50.0% M. E. - 48 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note further reduction in the enzyme activity of the tubules previously showing intense activity (solid arrow) Also note reduction in the cytoplasmic enzyme activity of other tubules (dashed arrow) but few of these tubules show brush/luminal border enzyme activity.

PLATE NO 52



acid phosphatase from the 'Cy' tubules increased except for about ten percent tubules where the cytoplasmic enzyme activity reduced. **(Plate 51 : Fig 3)**. Then, at the end of one twenty hours, the acid phosphatase activity at the brush/luminal borders from the 'BL' type tubules increased and the cytoplasm of these tubules showed weak enzyme activity. The 'Cy' type tubules showed slight increase at the brush/luminal borders. A few tubules from both types showed membrane acid phosphatase activity **(Plate 51 : Fig 4)**.

The ducks exposed to **50.0 % M.E.** showed intense brush/luminal border activity in the 'BL' type tubules at the end of six hours while 'Cy' type tubules showed a weak cytoplasmic acid phosphatase activity. Also the cytoplasmic enzyme activity in the 'BL' type tubules was increased a little in few tubules. The exudents in the interstitium exhibited acid phosphatase activity **(Plate 52 : Fig.1)**. By the end of twelve hours there was further increase in the enzyme activity at the brush/luminal borders of the 'BL' type tubules along with the increase in the cytoplasmic acid phosphatase activity. The 'Cy' type tubules showed large granular staining in the tubules and the luminal exudents showed acid phosphatase activity. The necrotic glomeruli showed increase in the enzyme activity **(Plate 52 : Fig 2)**.

At the end of twentyfour hours there was reduction in the enzyme activity at the brush/luminal borders of 'BL' type tubules, while the 'Cy' type tubules showed slight increase in the cytoplasmic activity. The glomeruli showed slight increase in the enzyme activity **(Plate 52 : Fig 3)**. By the end of fortyeight hours the number of 'BL' tubules showing intense activity decreased and these tubules showed the reduced acid phosphatase activity. A few 'Cy' type tubules showed reduction in cytoplasmic enzyme activity with the occurrence of very weak brush/luminal enzyme activity. Some of the other tubules of 'Cy' type exhibited slight increase in the enzyme activity **(Plate 52 : Fig 4)**. At the end of ninetysix hours there was once again increase in the enzyme activity at the brush/luminal borders of the 'BL' type tubules while, the 'Cy' type tubules

CAPTIONS TO FIGURES

PLATE 53 :

Fig 1 : 50.0% M. E. - 96 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note increase in the brush/luminal (solid arrow) border enzyme activity. Also note the increase in the brush/luminal (dashed arrow) border enzyme activity of 'Cy' tubules. Also note the enzyme activity in the exudents (E).

Fig 2 : 50.0% M. E. - 120 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note slight reduction in the enzyme activity of some tubules (solid arrow). Also note reduction in the cytoplasmic enzyme activity of most of the tubules (dashed arrow).

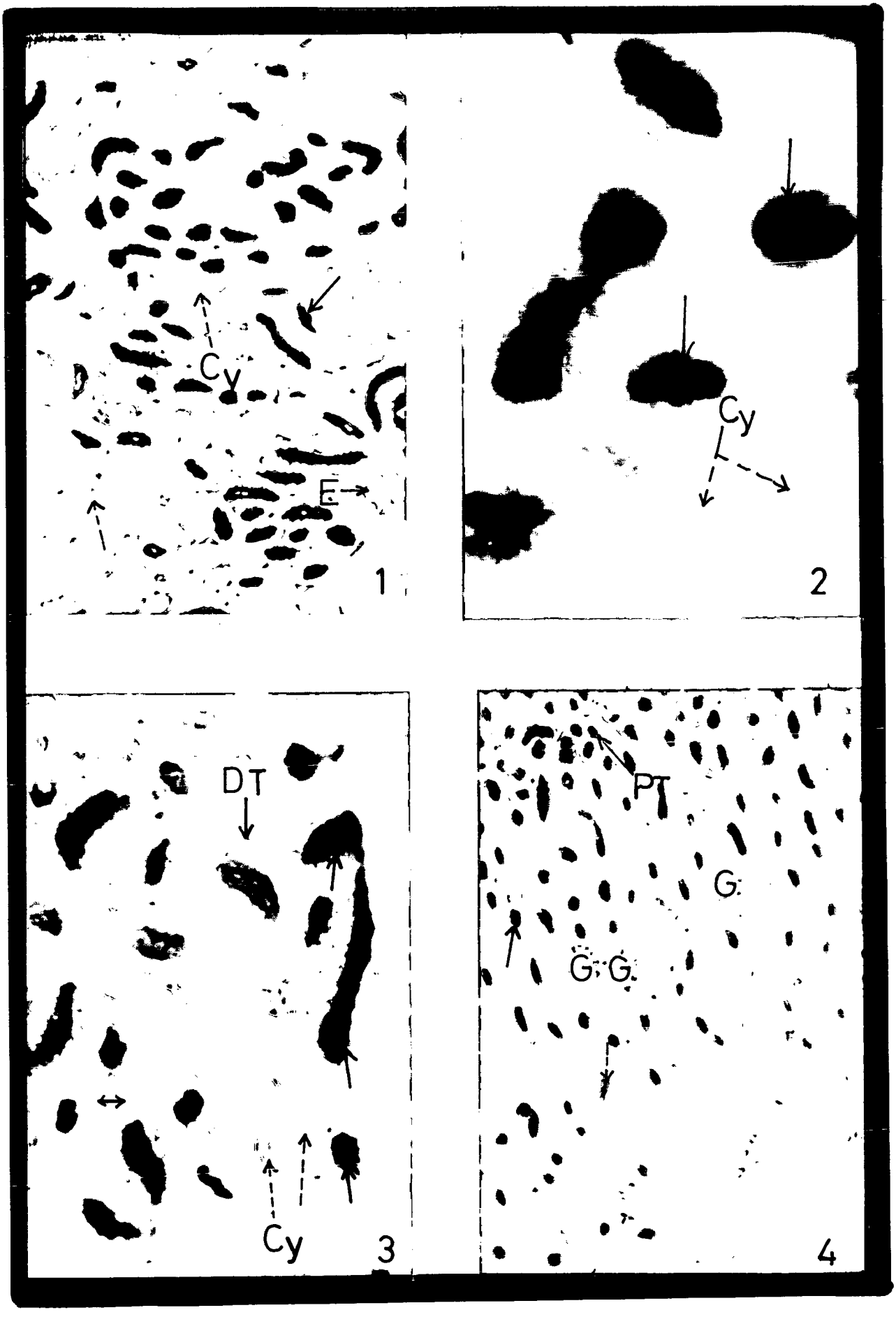
Fig 3 : 100.0% M. E. - 6 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note intense enzyme activity in some tubules (solid arrow) at the brush/luminal border. Also note the increased cytoplasmic activity in general. Note brush/luminal border enzyme activity in 'Cy' tubules (Cy - dashed arrow).

Fig 4 : 100.0% M. E. - 24 hours - (A P) - α napthyl - fast Garnet GBC. (10 x 5)

Note high enzyme activity at the brush/luminal (solid arrow) borders of the tubules. Also note increase in the granular lysosomal staining (dashed arrow).

PLATE NO 53



CAPTIONS TO FIGURES

PLATE 54 :

Fig 1 : 100.0% M. E. - 48 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note high enzyme activity at the brush/luminal (solid arrow) borders of the tubules. Also note the occurrence of granular lysosomal staining (dashed arrow) in some tubules.

Fig 2 : 100.0% M. E. - 72 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note reduction in the acid phosphatase activity from the tubules (solid arrow) and the reduction in the number of tubules showing high brush/luminal border enzyme activity. Note increased enzyme activity in the exudents (E). Other tubules show cytoplasmic granular staining.

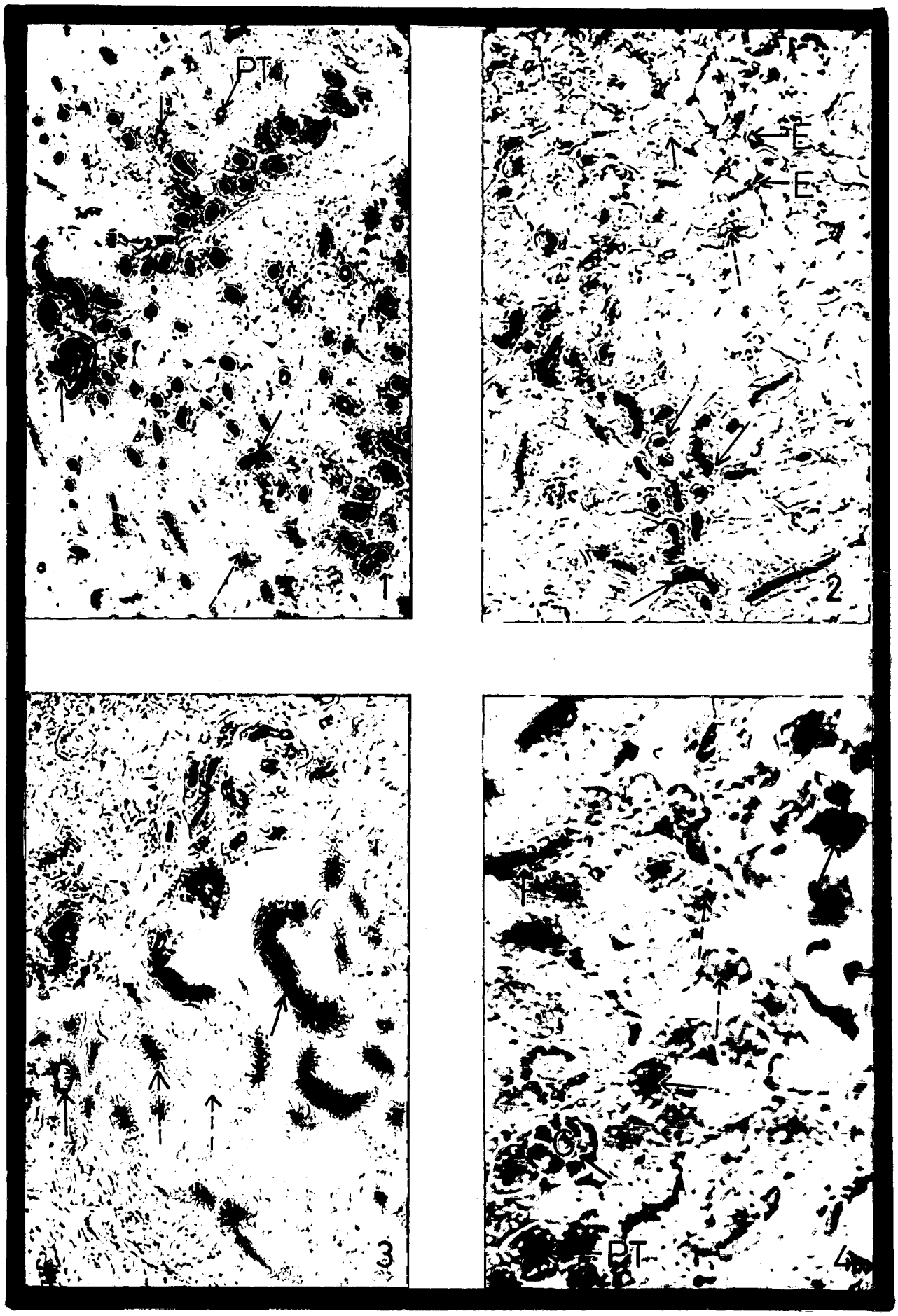
Fig 3 : 100.0% M. E. - 96 hours - (A P) - α napthyl - Fast Garnet GBC. (10 x 5)

Note further reduction in the enzyme activity and reduction in the number of tubule showing brush/luminal border activity (solid arrow). Also note increased cytoplasmic enzyme activity (dashed arrow).

Fig 4 : 100.0% M. E. - 120 hours - (A P) - α napthyl - Fast Garnet GBC. (20 x 5)

Note further reduction in the brush/luminal (solid arrow) border enzyme activity and the tubules showing them. Also note the increased cytoplasmic enzyme activity (dashed arrow).

PLATE NO 54



CAPTIONS TO FIGURES

PLATE 55 :

Fig 1 : Control - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note the moderate granular staining and staining of the nuclear membrane of the distal tubules (D). Also note the two types of tubules - darkly stained (DT) and lightly stained (arrow).

Fig 2 : 0.01% M. E. - 720 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note slight increase and occurrence of the brush/luminal (dashed arrow) border enzyme activity. Also note increase in the lysosomal granular staining (solid arrow) and absence of enzyme activity in interstitium.

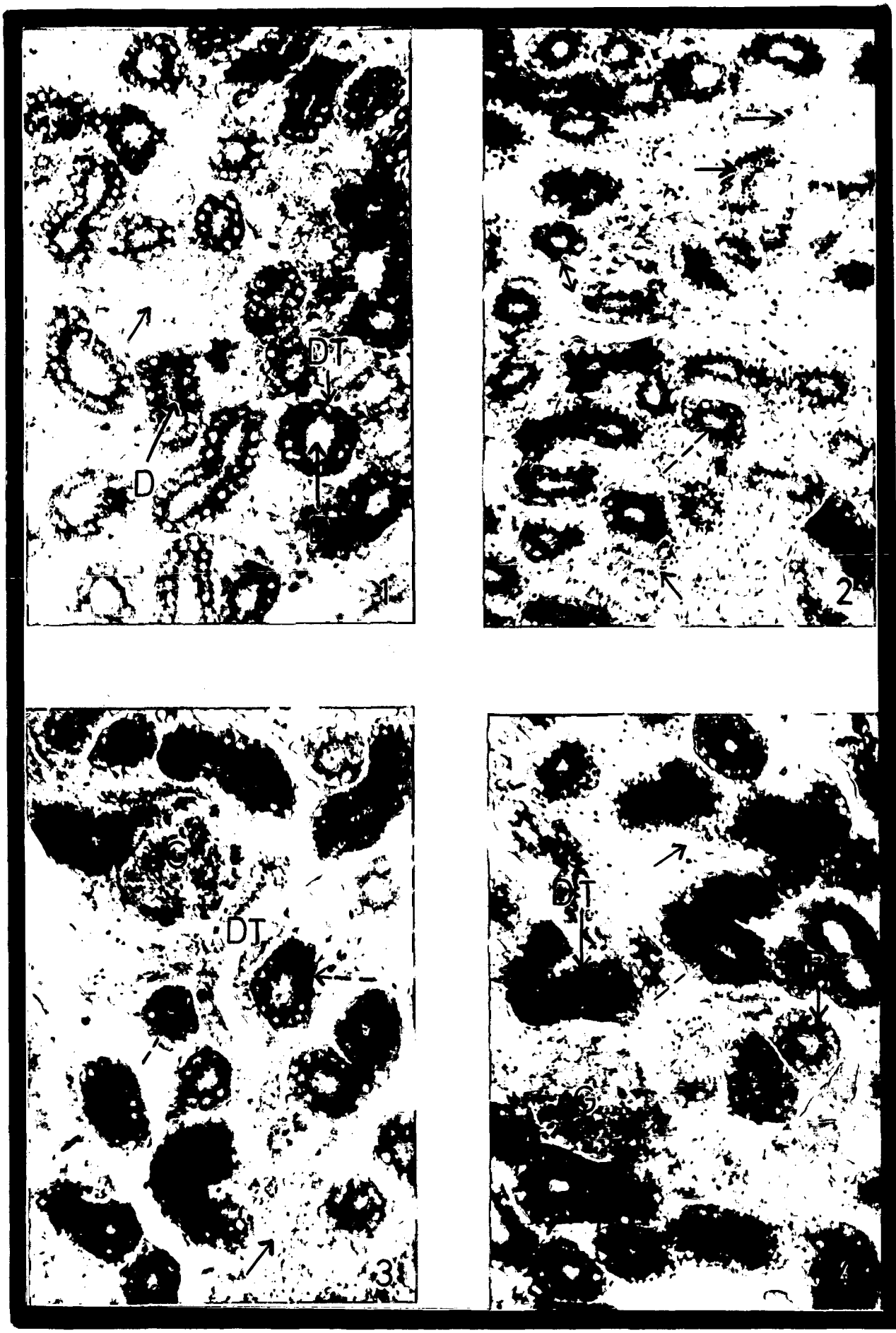
Fig 3 : 0.1% M. E. - 24 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note increase in the enzyme activity of both the types of tubules (solid and dashed arrow). Also note the enzyme activity in glomerulus (G).

Fig 4 : 0.1% M. E. - 48 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note the increased enzyme activity in both the types of tubules (solid and dashed arrow). Also note increased staining of glomerulus (G).

PLATE NO 55



CAPTIONS TO FIGURES

PLATE 56 :

Fig 1 : 0.1% M. E. - 120 hours - Alkaline phosphatase - α napthyl - Fast blue. (40 x 5)

Note further increase in the lysosomal granular staining of both the types of tubules indicating increased enzyme activity (solid and dashed arrow). Also note the enzyme activity in the luminal debris (D).

Fig 2 : 1.0% M. E. - 6 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note the increased enzyme activity in the glomerulus (G), exudents (E) and the necrotic tubules. Also not increased enzyme activity in collecting tubules (CT).

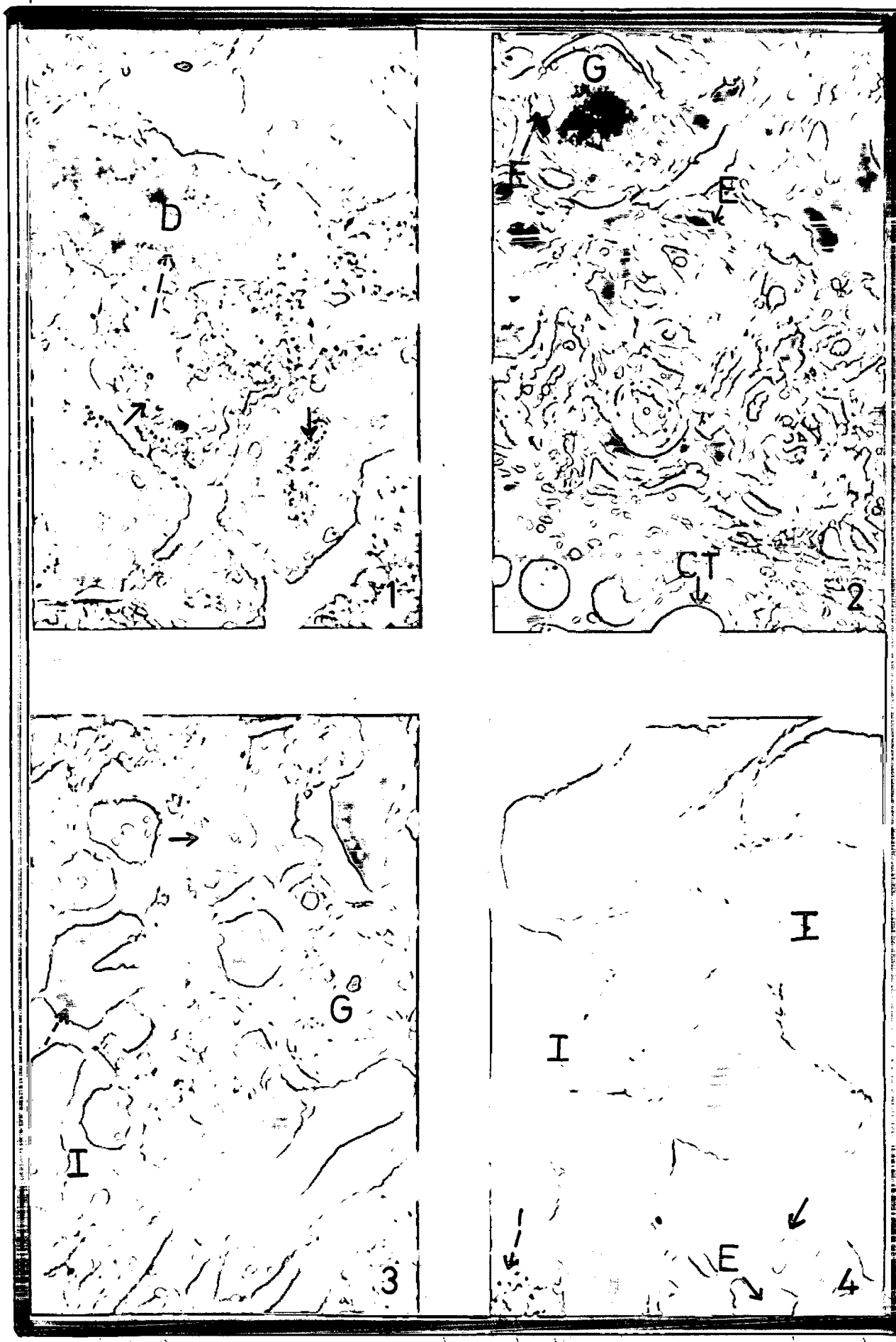
Fig 3 : 1.0% M. E. - 24 hours - Alkaline phosphatase - α napthyl - Fast blue. (40 x 5)

Note the intense enzyme activty in both the types of tubules (solid and dashed arrow), no staining in the interstitium (I). Also note increased glomerular staining.

Fig 4 : 1.0% M. E. - 72 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note increase in the granular lysosomal staining of both the types of tubules (solid and dashed arrow). Insterstitium without staining (I).

PLATE NO 56



activity but the interstitium was without any enzyme activity **(Plate 55 : Fig 3)**. By the end of fortyeight hours there was further increase in the alkaline phosphatase activity in all the tubules. The glomeruli also showed the increased enzyme activity. In a few 'D' tubules increase in brush/luminal border alkaline phosphatase was observed. The interstitium showed absence of enzyme activity **(Plate 55 : Fig 4)**. At the end of seventytwo hours the renal tubules showed decrease in enzyme activity and then a slight increase in the enzyme activity was noticed at the end of ninetysix hours. Then, by the end of one twenty hours there was further increase in the enzyme activity in both 'D' and 'L' type tubules. The increased activity was exhibited by the increased granular lysosomal staining. The interstitium showed absence of enzyme activity **(Plate 56 : Fig 1)**.

The mallards exposed to **1.0% M.E.** promoted increase in the alkaline phosphatase activity at the end of six hours. The glomeruli showed intense lysosomal granular staining along with the 'D' and 'L' type tubules. The collecting tubules also showed increased enzyme activity. The exudents from the interstitium and in the lumina of the tubules showed alkaline phosphatase activity **(Plate 56 : Fig 2)**. By the end of twelve hours the enzyme activity decreased marginally from all the tubules but at the end of twentyfour hours, the alkaline phosphatase activity increased in both the types of tubules, especially 'D' types tubules exhibited intense lysosomal staining. The glomeruli also showed increased enzyme activity but the interstitium showed no enzyme activity. **(Plate 56 : Fig 3)**. Then, at the end of seventytwo hours both the tubules showed intense enzyme activity and the 'D' type tubules showed brush/luminal border alkaline phosphatase along with lysosomal granular enzyme activity. The exudents in the lumen showed enzyme activity. The interstitium did not show enzyme activity **(Plate 56 : Fig. 4)**. By the end of ninetysix hours the alkaline phosphatase activity increased further in both the types of tubules, especially a few 'D' type tubules exhibited very intense

CAPTIONS TO FIGURES

PLATE 57 :

Fig 1 : 1.0% M. E. - 96 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note further increase in the enzyme activity of both the types of tubules. Also note the increase in glomerular staining (G). Interstitium (I) without enzyme activity.

Fig 2 : 5.0% M. E. - 6 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note increased enzyme activity in comparison with the controls. Both the types of tubules show increase in the enzyme activity (solid and dashed arrow) Note the glomerular enzyme activity (G).

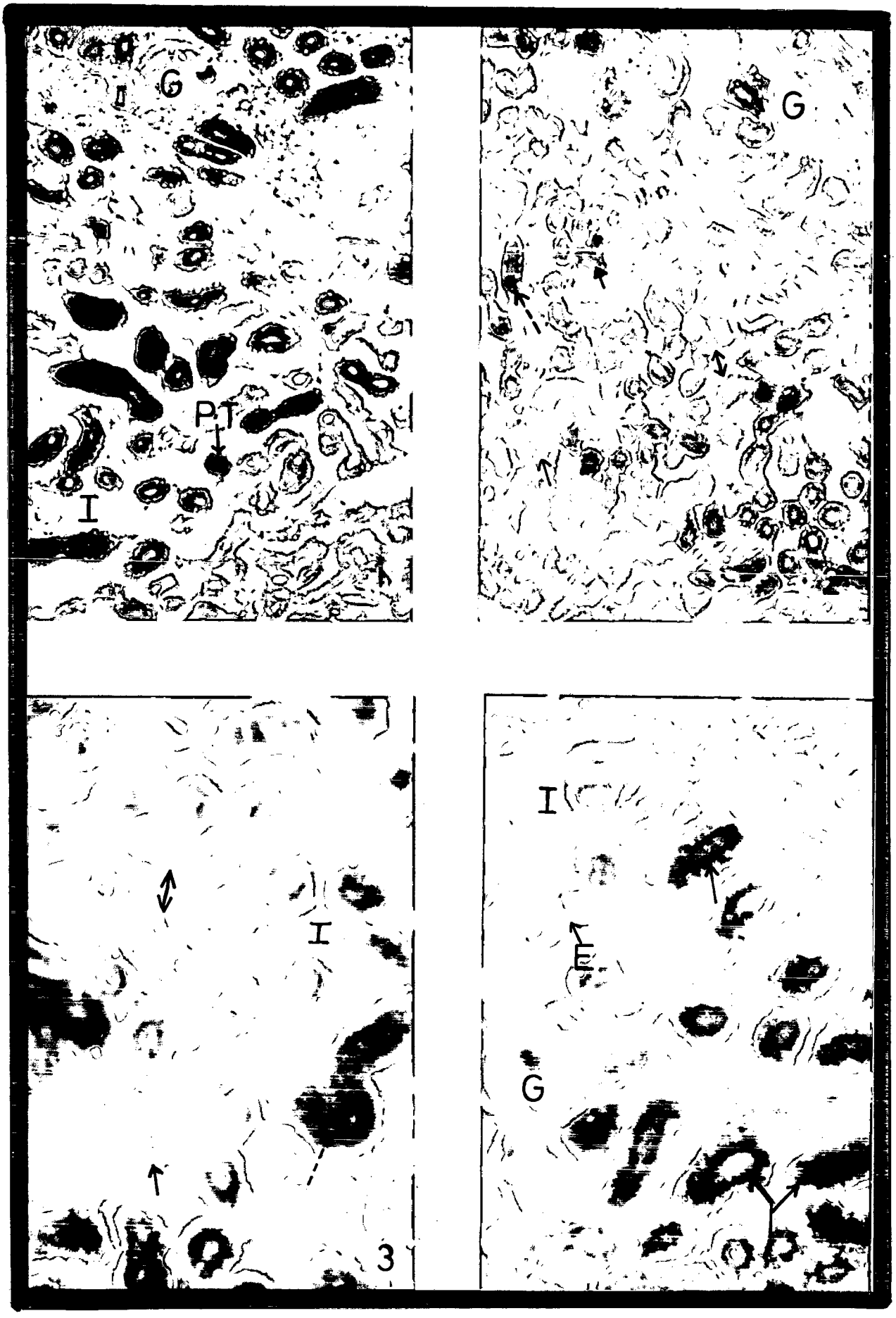
Fig 3 : 5.0% M. E. - 12 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note intense enzyme activity in both types of tubules (solid and dashed arrow). No staining in interstitium (I).

Fig 4 : 5.0% M. E. - 24 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note further increase in the enzyme activity of both the types of tubules. Also note increased enzyme activity in glomerulus (GL). Interstitium (I) without staining, exudents (E) in the interstitium show enzyme activity. The 'D' type tubules show brush/luminal border staining (solid arrow).

PLATE NO 57



enzyme activity and their lumina were filled with the stain. The 'L' type tubules also exhibited increased granular lysosomal staining. The glomeruli showed increased enzyme activity **(Plate 57 : Fig 1)**. The interstitium did not exhibit any enzyme activity. At the end of one twenty hours both the tubules and glomeruli showed further increase in the enzyme activity. The collecting tubules showed marginal increase in the enzyme activity.

Under the influence of **5.0% M.E.** the ducks showed a little increase in the alkaline phosphatase activity in the 'D' and 'L' type tubules. A few 'D' type tubules as well as 'L' tubules showed very weak brush/luminal border alkaline phosphatase activity in addition to the granular lysosomal enzyme activity. The interstitium showed absence of enzyme activity while the glomeruli showed granular lysosomal staining **(Plate 57 : Fig 2)**. By the end of twelve hours there was further increase in the alkaline phosphatase activity in all the renal tubules. The debris in the tubule lumina and the interstitial exudents showed the enzyme activity **(Plate 57 : Fig 3)**, and at the end of twentyfour hours there was further increase in the enzyme activity in all the renal tubules especially the 'D' type tubules showed intense enzyme activity at the brush/luminal borders. Though the interstitium showed no enzyme activity, the exudents showed the enzyme activity. The glomeruli showed increased alkaline phosphatase activity **(Plate 57 : Fig 4)**. By the end of fortyeight hours the alkaline phosphatase activity decrease significantly in the renal tubules and subsequently increased a little at the end of seventytwo hours. The glomeruli showed decrease in the enzyme activity and a few 'D' type tubules showed the appearance brush/luminal border enzyme activity **(Plate 58 : Fig 1)**. Then, subsequently at the end of ninetysix and one twenty hours the alkaline phosphatase activity increased gradually in all the tubules.

When the mallards were introduced to **10% M.E.** both the 'D' and 'L' type tubules exhibited increase in the enzyme activity. Both the types of tubules showed the appearance of brush/luminal border enzyme activity in few cases.

CAPTIONS TO FIGURES

PLATE 58 :

Fig 1 : 5.0% M. E. - 72 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note the tubules showing increased cytoplasmic (solid arrow) enzyme activity and the tubules showing increase in the granular (dashed arrow) lysosomal enzyme activity. Also note the staining in glomeruli (GL) and the brush/luminal border enzyme activity (L).

Fig 2 : 10.0% M. E. - 6 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

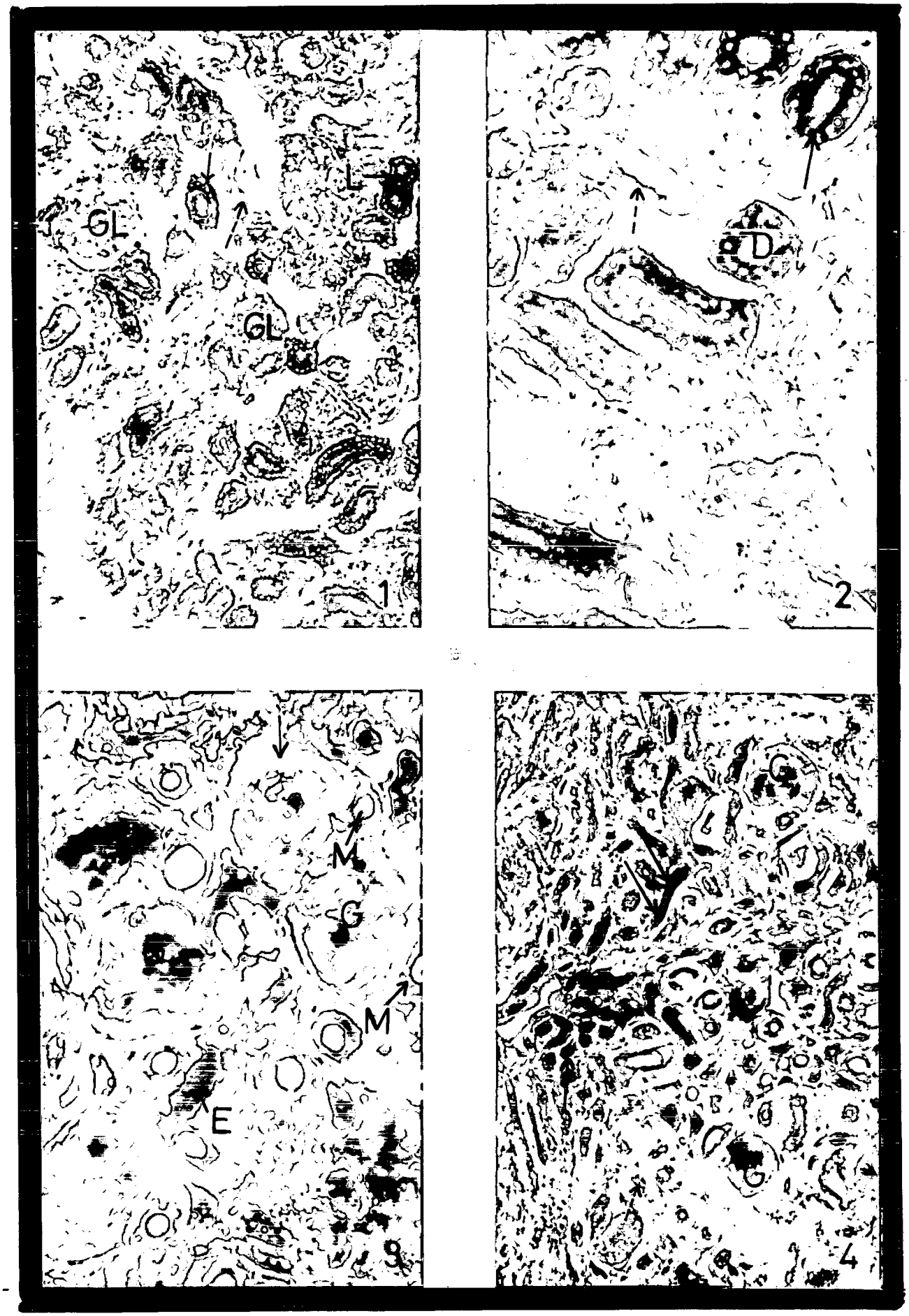
Note the intense granular staining in the tubules (solid arrow) with luminal border staining. Also note the other types of tubules showing less intense granular staining (dashed arrow) with occasional luminal border enzyme activity. Note the enzyme activity in the debris (D).

Fig 3 : 10.0% M. E. - 48 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note the increase in the enzyme activity of all the tubules. Also note the increased staining in the glomerulus (G), enzyme activity in macula densa (M) and in the exudents (E).

Fig 4 : 10.0% M. E. - 120 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note slight decrease in the enzyme activity in both the types of tubules (solid and dashed arrows). Note intense staining in glomerulus (G).


GL
L
GL
1
D
2
M
G
M
E
3
G
4

CAPTIONS TO FIGURES

PLATE 59 :

Fig 1 : 50.0% M. E. - 6 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note the intense staining in the necrotic tubules (solid arrow and dashed arrow). Also note widened interstitium (I) without enzyme activity and debris (D) in the lumen shows enzyme activity.

Fig 2 : 50.0% M. E. - 12 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note acute increase in the enzyme activity of all the tubules (solid arrow and dashed arrow). Also note the dark brush/luminal border staining in the tubules. Glomerulus (G) with increased enzyme activity. Bowman's capsule (B) and macula densa (M) shows enzyme activity.

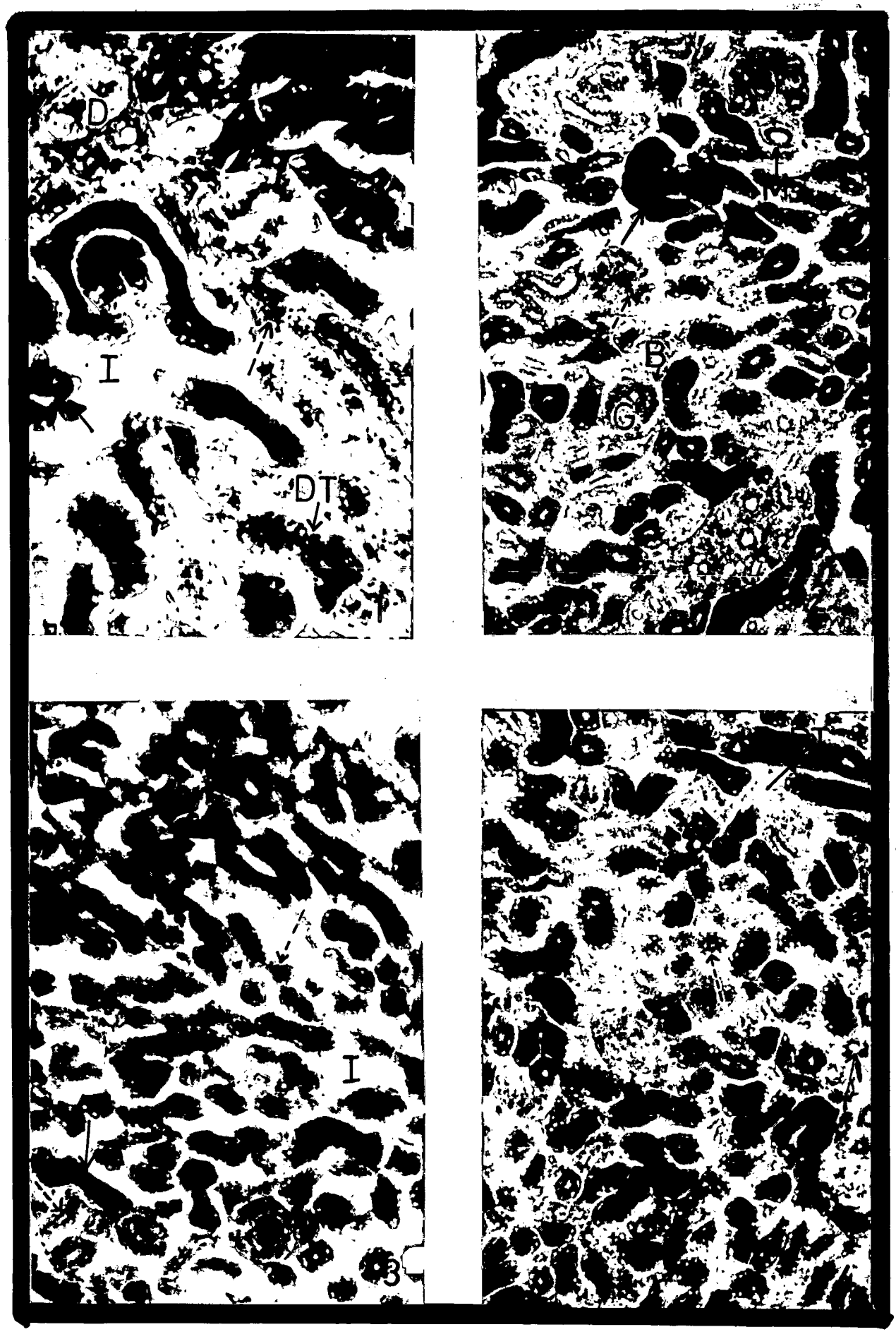
Fig 3 : 50.0% M. E. - 48 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note increase in the enzyme activity of both the types of tubules especially note the appearance of brush/luminal border enzyme activity (solid and dashed arrow). Note the staining in glomerulus (G). Interstitium (I) is without enzyme activity.

Fig 4 : 50.0% M.E./120 h - Alkaline phosphatase - Fast blue. (10 x 5)

Note slight decrease in the enzyme activity of both types of tubules (solid arrow and dashed arrow) in comparison to what is obtained at 48 hours.

PLATE NO 59



The exudents in the lumina of the tubules showed enzyme activity. **(Plate 58 : Fig 2)**. By the end of twelve hours the alkaline phosphatase activity increased in all the tubules and then decreased at the end of twentyfour hours. Then at the end of fortyeight hours, there was intense increase in the enzyme activity in all the renal tubules. The macula densa showed intense alkaline phosphatase activity and the exudents in the interstitium showed enzyme activity along with the necrotic glomeruli. In a few cases the Bowman's wall showed membrane alkaline phosphatase activity **(Plate 58 : Fig 3)**. Then the enzyme activity went on decreasing at the subsequent time intervals and at the end of one twenty hours, the alkaline phosphatase activity increased a little in all the renal tubules. The glomeruli showed marginal reduction in the enzyme activity. The debris of the necrotic tubules and the walls of Bowman's capsule showed the enzyme activity **(Plate 58 : Fig 4)**.

The **50% M.E.** promoted sharp increase in the alkaline phosphatase activity at the end of six hours and both the 'D' and 'L' type tubules showed intense enzyme activity. The debris in the lumen of the necrotic tubule showed intense enzyme activity. The interstitium showed absence of enzyme activity **(Plate 59 : Fig 1)**. By the end of twelve hours there was a peak increase in the alkaline phosphatase activity in all the renal tubules. A few 'D' and 'L' type tubules showed brush/luminal border enzyme activity. The macula densa showed intense enzyme activity at the luminal border. The necrotic glomeruli showed intense enzyme activity and the distorted wall of Bowman's capsule showed alkaline phosphatase activity. The debris in the lumen of the few tubules showed enzyme activity and the interstitium was without enzyme activity **(Plate 59 : Fig.2)**. By the end of twenty four hours there was decline in the enzyme activity from all the tubules but at the end of fortyeight hours there was once again increase in the enzyme activity. The brush and luminal borders of a few tubules showed alkaline phosphatase activity. The interstitium shows no enzyme activity **(Plate 59 : Fig 3)**. Then, subsequently the enzyme

CAPTIONS TO FIGURES

PLATE 60 :

Fig 1 : 100.0% M. E. - 6 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note intense brush/luminal border (solid arrow and dashed arrow) staining in both the types of tubules. Also note glomerular staining (G) and interstitium (I) is without staining.

Fig 2 : 100.0% M. E. - 24 hours - Alkaline phosphatase - α napthyl - Fast blue. (10 x 5)

Note decrease in brush/luminal border staining of both the types of tubules and general increase in the cytoplasmic enzyme activity. Also note slight decrease in the glomerular (G) enzyme activity. Note the staining at the luminal border of macula densa (M).

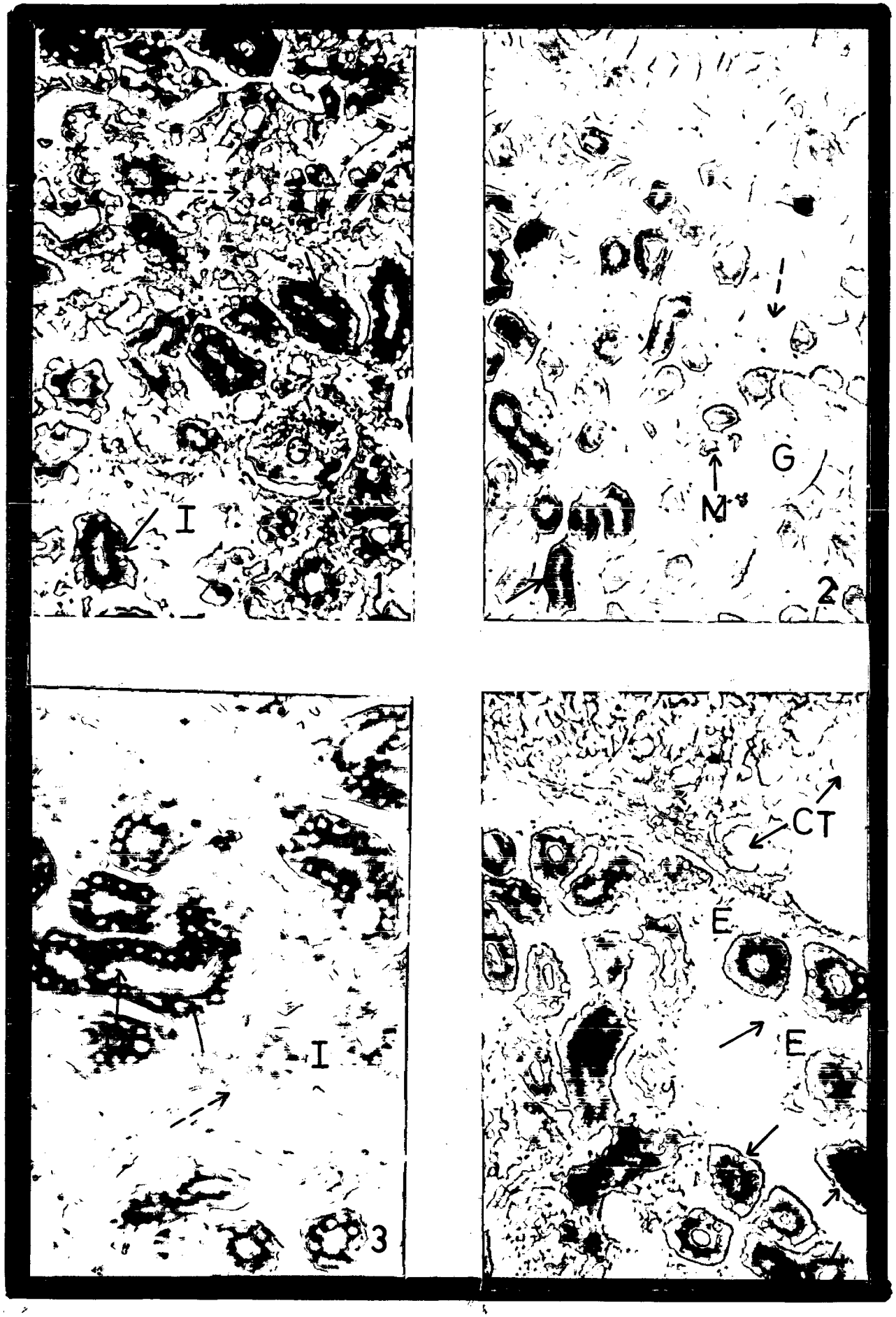
Fig 3 : 100.0% M. E. - 72 hours - Alkaline phosphatase - α napthyl - Fast blue. (40 x 5)

Note significant reduction in the enzyme activity in necrotic tubules. Two types of tubules could be differentiated on the basis of stainability. Also note the staining in the debris (D) and no staining in interstitium (I).

Fig 4 : 100.0% M. E. - 96 hours - Alkaline phosphatase - α napthyl - Fast blue. (20 x 5)

Note intense increase in the enzyme activity of both the types of tubules. The exudents (E) show enzyme activity and the collecting tubules (CT) show increase in the enzyme activity.

PLATE NO 60



activity declined up to the end of ninetysix hours and at the end of one twenty hours, there was once again a little increase in the enzyme activity. The necrotic 'B' type tubules showed brush/luminal border enzyme activity **(Plate 59 : Fig 4)**.

The ducks exposed to **100% M.E.** promoted sharp increase in the enzyme activity in all the renal tubules, especially the 'D' type tubules showed intense brush/luminal border enzyme activity. The necrotic glomeruli showed increase in the enzyme activity. The 'L' type tubules showed increase in the enzyme activity. The 'L' type tubules showed granular lysosomal enzyme activity. The interstitium was without enzyme activity **(Plate 60 : Fig 1)**. By the end of twelve hours there was marginal decline in the enzyme activity in all the tubules and this decline continued up to the end of twentyfour hours. At the end of twentyfour hours the alkaline phosphatase activity from the glomeruli decreased, especially, the brush/luminal border enzyme activity of 'D' type tubules was abolished but the macula densa showed the luminal border enzyme activity **(Plate 60 : Fig 2)**. By the end of fortyeight hours, the alkaline phosphatase activity in all the tubules increased but once again it decreased at the end of seventy two hours, especially, this decrease was pronounced in a few 'D' type tubules. Only a few 'D' tubules showed intense enzyme activity. The 'L' type tubules showed a marginal increase in granular staining. The interstitium remained unstained **(Plate 60 : Fig 3)**. By the end of ninetysix hours, there was once again sharp increase in the alkaline phosphatase activity in all the renal tubules. The 'D' type tubules showed reappearance of brush/luminal border enzyme activity. The collecting tubules showed increase in the alkaline phosphatase activity. The exudents in the interstitium exhibited the enzyme activity. The interstitium was without enzyme activity **(Plate 60 : Fig 4)**.

Then, by the end of one twenty hours, there was once again decrease in the alkaline phosphatase activity of all the tubules.

C] ALTERATIONS IN THE ESTERASE (NONSPECIFIC) ACTIVITY :

Control animals :

The kidney of the control animals showed various staining activites of the nonspecific esterase with the application of different histochemical techniques. But of all the technique tried, the indoxylacetate techniques of Pearson and Grose, 1959 was found most suitable for studying the distribution and localization of the nonspecific esterase activity. It is evident from the histochemical studies in control mallards that there existed two types of renal tubules. The one type, comprised of about sixty percent tubules showing fine granular lysosomal activity and the other comprised of about 40% tubules, without any nonspecific esterase activity. The glomeruli did not exhibit any enzyme activity **(Plate 61 : Fig 1)**. Similarly, sixty percent collecting tubules showed very weak lysosomal staining and the remaining forty percent did not show any enzyme activity.

Pathological changes :

The ducks introduced to **0.01%** mining effluents **(M.E.)** did not show any significant alterations in the nonspecific esterase activity up to the end of twentynine days, but at the end of thirty days (seven twenty hours) the tubules (proximal and distal) previously showing nonspecific esterase activity showed a little increase in the enzyme activity, while from about 40% remaining tubules, about 2-3% tubules showed a very weak non specific esterase activity **(Plate 61 : Fig 2)**. By the end of one thousand and eighty hours (45 days), the sixty percent tubules group showed further increase in the enzyme activity and 2 -

CAPTIONS TO FIGURES

PLATE 61 :

Fig 1 : Control - Esterase (Nonspecific) - Indoxyl actate. (10 x 5)

Note the cytoplasmic esterase activity in some tubules (DT) and also note the other types of tubules (dashed arrow) in the background. The glomerulus (G) shows no staining.

Fig 2 : 0.01% M. E. - 720 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note increase in the cytoplasmic enzyme activity in tubules (DT) and also note the absence of staining (dashed arrow) in other tubules.

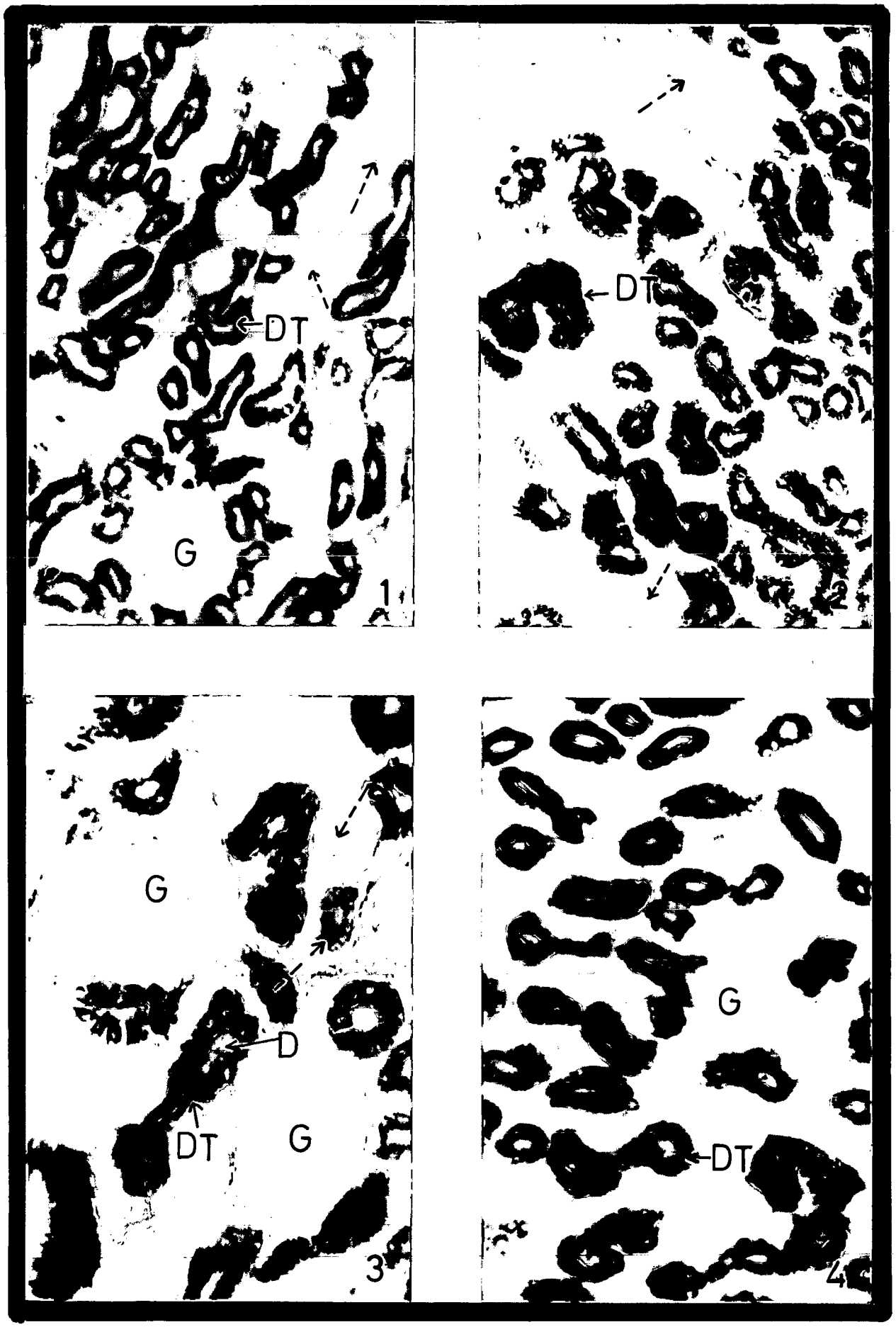
Fig 3 : 0.1% M. E. - 6 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note increase in the esterase activity in the tubules (DT) previously showing staining. Also note weak glomerular enzyme activity (G) . Note very weak staining in other tubules which previously had no enzyme activity.

Fig 4 : 0.1% M. E. - 24 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note intense staining in few tubules and slightly decreased enzyme activity in few tubules (DT). Also note absence of enzyme activity in glomerulus (G) and other tubules.

PLATE NO 61



CAPTIONS TO FIGURES

PLATE 62 :

Fig 1 : 0.1% M. E. - 48 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note slight increase in the esterase activity in the cytoplasm of some tubules (solid arrow). Also note absence of enzyme activity in other tubules (dashed arrow) and collecting tubules (CT).

Fig 2 : 0.1% M. E. - 72 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note slight decrease in the esterase activity of the tubules (solid arrow) previously showing staining and the other tubules show very weak enzyme activity in nuclear membrane. (dashed arrow). Also note membrane esterase of collecting tubules (CT) and weak enzyme activity in glomerulus (G).

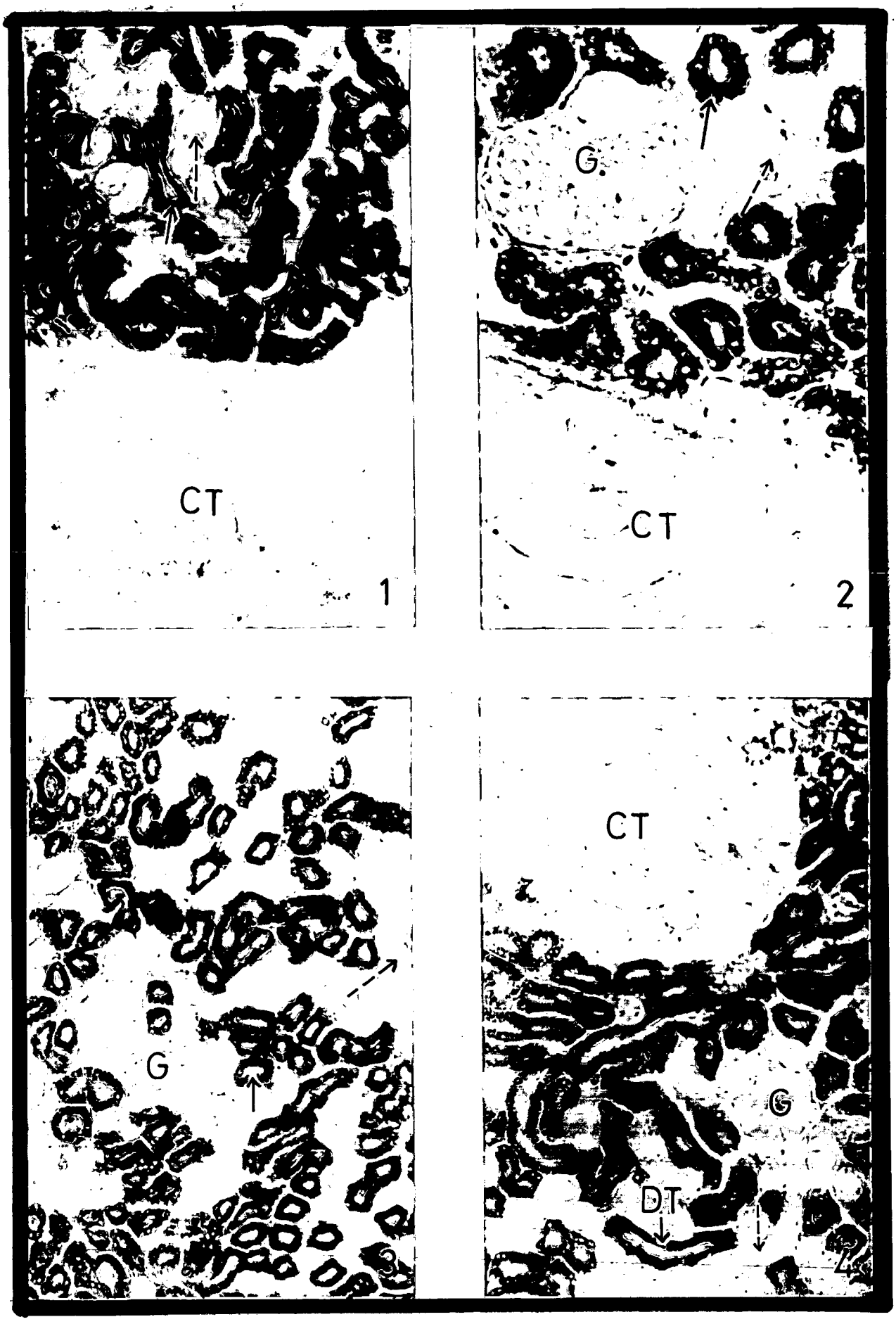
Fig 3 : 1.0 % M. E. - 6 hours - Esterases (Nonspecific) - Indoxyl acetate.

Note increased enzyme activity in the tubules (solid arrow) and absence of enzyme activity in glomerulus (G) and other tubules (dashed arrow).

Fig 4 : 1.0% M. E. - 24 hours - Esterases (Nonspecific) - Indoxyl acetate.

Note slight decrease in the esterase activity of the tubules (DT). Also note absence of enzyme activity in other tubules (dashed arrow), collecting tubules (CT) and glomerulus (G).

PLATE NO 62



3% tubules from the remaining group exhibited further increase in the nonspecific esterase activity.

The ducks subjected to **0.1% M.E.** showed significant increase in the nonspecific esterase activity in about sixty percent tubules but interestingly the remaining forty percent tubules showed a very weak esterase (nonspecific) activity, especially about 2 to 3 percent tubules from this group showed a marginally higher activity. The glomeruli and walls of Bowman's capsule exhibited mild staining **(Plate 61 : Fig 3)**. By the end of twelve hours there was slight decrease in the nonspecific esterase activity in about sixty percent tubules but at the end of twentyfour hours, there was once again a little increase in the enzyme activity in sixty percent tubules, while rest of the tubules showed loss of nonspecific esterase activity. The glomerular staining was abolished **(Plate 61 : Fig 4)**. At the end of forty eight hours the mallards exhibited a slight increase in the non specific esterase activity from the sixty percent tubules while the remaining forty percent did not exhibit any enzyme activity. Similarly, all the collecting tubules did not show any nonspecific esterase activity **(Plate 62 : Fig 1)**. By the end of seventytwo hours the mallards exhibited a slight decrease in the enzyme activity from about sixty percent tubules while, the remaining tubules showed a very weak nuclear membrane nonspecific esterase activity. Similarly, all the collecting tubules showed membrane esterase (nonspecific) activities **(Plate 62 : Fig 2)**. Then at the end of ninetysix hours there was increase in the enzyme activity in about sixty percent tubules along with the occurrence of a weak enzyme activity in the remaining tubules but once at the end of one twenty hours the esterase (nonspecific) activity decreased from all the tubules.

Under the influence of **1.0% M.E.** the ducks showed at the end of six hours a little increase in the nonspecific esterase activity in a little over sixty percent tubules and the activity was fine granular. The remaining tubules as well as glomeruli did not exhibit nonspecific esterase activity. **(Plate 62 : Fig 3)**.

CAPTIONS TO FIGURES

PLATE 63 :

Fig 1 : 1.0% M. E. - 48 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note increase in the enzyme activity in the tubules (solid arrow) and no activity in other tubules (dashed arrow).

Fig 2 : 1.0% M. E. - 96 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

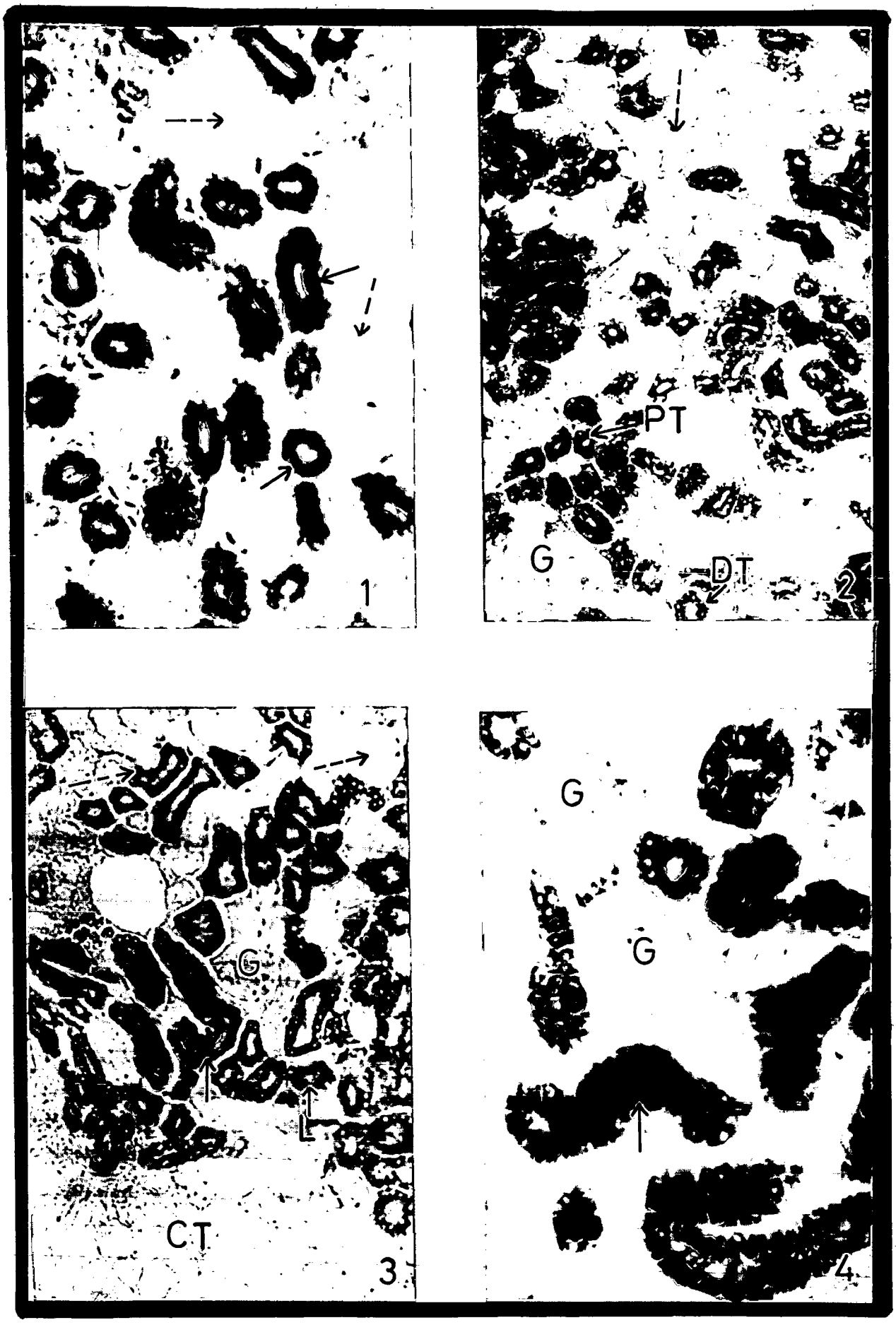
Note reduction in the enzyme activity in proximal and distal tubules (PT, DT) and absence of activity in the other tubules (dashed arrows) and glomerulus (G).

Fig 3 : 5.0% M. E. - 6 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note no significant change in the enzyme activity. Few necrotic tubules show luminal border enzyme activity (↑L). Also note absence of esterase activity in other tubules (dashed arrow), glomerulus (G) and collecting tubules (CT).

Fig 4 : 5.0% M. E. - 24 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note intense staining in the tubules (solid arrow) Necrotic glomeruli (G) show weak enzyme activity.


1
PT
G
DT
2
G
G
L
CT
3
G
4

The collecting tubules did not show enzyme activity. At the end of twelve hours the esterase (nonspecific) activity dropped a little but by the end of twenty four hours the esterase activity decreased in little in comparison to that obtained at the end of six hours. About one percent collecting tubules showed esterase (nonspecific) activity. The glomeruli and other tubules showed absence of enzyme activity **(Plate 62 : Fig 4)**. By the end of fortyeight hours the ducks exhibited sharp increase in the esterase (nonspecific) activity of about sixty percent tubules and occassionally a few tubules from this group showed a weak brush/luminal border activity. The glomeruli and the rest of the tubules did not show enzyme activity **(Plate 63 : Fig 1)**. Then, at the end of seventy two hours the esterase (nonspecific) activity dropped considerably to improve marginally at the end of ninetysix hours but in comparison to that found at the end of fortyeight hours the enzyme activity was low. A little less than sixty percent tubules showed fine granular enzyme activity while the remaining tubules and glomeruli showed absence of esterase (nonspecific) activity **(Plate 63 : Fig 2)**.

The exposure of ducks to **5.0% M.E.** induced no significant increase in the nonspecific esterase activity at the end of six hours, in the sixty percent tubules. The glomeruli and the remaining forty percent tubules as well as collecting tubules exhibited absence of enzyme activity **(Plate 63 : Fig 3)**. At the end of twelve hours the nonspecific esterase activity increased marginally but at the end of twentyfour hours the nonspecific esterase activity increased sharply in about sixty percent tubules and the necrotic tubules from this group showed enzyme activity in the luminal debris. The necrotic glomeruli exhibited the occurrence of esterase (nonspecific) activity and in a few cases the wall of Bowman's capsule showed the membrane enzyme activity. About two percent of the tubules from the remaining group showed a weak nuclear membrane esterase (nonspecific) activity, while the rest of the tubules showed absence of enzyme activity **(Plate 63 : Fig 4)**. A few collecting tubules showed weak

CAPTIONS TO FIGURES

PLATE 64 :

Fig 1 : 5.0% M. E. - 48 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note a slight decrease in the esterase activity from the tubules (solid arrow) and absence of enzyme activity in other tubules (dashed arrow) and glomerulus (G).

Fig 2 : 5.0% M. E. - 120 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note slight increase in the enzyme activity in a few tubules (solid arrow) while the other tubules (DT) are without enzyme activity (dashed arrow).

Fig 3 : 10.0% M. E. - 6 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note increased enzyme activity in proximal and distal tubules (solid arrow). Also note absence of esterase in other tubules (dashed arrow) except for few necrotic tubules.

Fig 4 : 10.0% M. E. - 12 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note reduced enzyme activity in the proximal and distal tubules (solid arrow) and absence of enzyme activity in other tubules (DT, dashed arrow). Also note the occurrence of membrane esterase in the Bowman's wall (G).

glomeruli and other tubules showed absence of enzyme activity **(Plate 66 : Fig.3)**.

Under the influence of **100%** M.E. the ducks exhibited increase in the enzyme activity from sixty percent tubules at the end of six hours, while the glomeruli and remaining forty percent tubules as well as collecting tubules showed absence of esterase activity. Then at the end of twelve hours the nonspecific esterase activity reduced sharply and then subsequently increased at the end of twentyfour hours. A few of the tubules showed very intense enzyme activity and about sixty percent tubules showed very intense of nonspecific esterase activity, while the glomeruli and remaining tubules (38%) showed absence of enzyme activity but about two percent tubules showed weak esterase activity **(Plate 66 : Fig 4)**. By the end of fortyeight hours there was no significant change in the nonspecific esterase activity from the tubules. The glomeruli and collecting tubules showed the absence of enzyme activity. Then, at the end of seventytwo hours the sixty percent tubules showed further increase in the enzyme activity, especially a few of the tubules showed intense staining. The glomeruli, about forty percent tubules and collecting tubules showed no enzyme activity **(Plate 67 : Fig 1)**. By the end of ninetysix hours the ducks exhibited sharp increase in the non specific esterase activity, while at the end of one twenty hours there was no further significant increase in the esterase activity but, about sixty percent tubules exhibited intense fine granular lysosomal staining and the luminal debris showed enzyme activity. The remaining tubules did not show esterase activity **(Plate 67 : Fig 2)**. The glomeruli and the collecting tubules showed absense of esterase (nonspecific) activity.

IV] KIDNEY BIOCHEMICAL STUDIES :

It was observed in general, that the mining effluents of different concentrations affected the activities of enzymes such as acid phosphatase, alkaline phosphatase, nonspecific esterase, and concentrations of total proteins, urea, uric acid , creatinine, calcium and chloride ions from the kidney.

1] ACID PHOSPHATASE :

The alterations in the acid phosphatase activities from the kidney of mallards exposed to different concentrations of mining effluents **(M.E.)** and for the different time intervals are compiled in **Table 1** and these alterations are graphically presented in **Graph 1** in which variations of the enzyme activity are plotted as a function of different M.E. concentrations and various time intervals. The acid phosphatase activity is expressed as µmole units per gram of wet weight of the tissue.

The control animals' kidney exhibited acid phosphatase activity equivalent to 205.62 ± 3.892. The animals exposed to **0.01%** of **M.E.** did not exhibit significant changes in the kidney acid phosphatase activity upto 720 hours, but at the end of 720 hours the activity increased to 399.648 ± 5.478 units and went on increasing upto 575.184 ± 9.328 units at the end of 1080 hours.

The **0.1% M.E.** induced a progressive increase in the enzyme activity up to the end of 96 hours, while at the end of 120 hours it decreased a little **(Graph 1)**. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the kidney acid phosphatase activity was equivalent to 327.06 ± 9.508, 273.24 ± 9.439, 355.212 ± 8.790, 422.004 ± 7.322, 449.88 ± 5.368, 490.728 ± 5.422 and 454.02 ± 4.968 respectively.

Thus, for all the time intervals the acid phosphatase activity remained above that found in the control animals.

The animals exposed to **1.0% M.E.** showed increase in the acid phosphatase activity at the end of six hours but later on exhibited significant fluctuations. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the enzyme activity was equivalent to 364.32 ± 6.858, 332.58 ± 7.948, 510.6 ± 4.443, 487.968 ± 7.176, 569.94 ± 6.348, 557.52 ± 7.645 and 574.08 ± 8.459 units respectively.

The **5.0% M.E.** induced elevation of the acid phosphatase activity at the end of six hours, but decreased at the end of twelve hours and then showed an increase in the activity which remained steady till the end of fortyeight hours. At the end of seventy two hours the enzyme activity decreased once again and kept on increasing gradually up to the end of one twenty hours. Thus, the acid phosphatase activity was found equivalent to 456.228 ± 3.753, 378.948 ± 5.768, 524.676 ± 7.314, 524.400 ± 3.891, 333.132 ± 4.747, 558.348 ± 7.0145 and 588.432 ± 7.120 units at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours respectively.

The mallards exposed to **10.0% M.E.** were found to have increased acid phosphatase activity in the kidney at the end of six hours and the enzyme activity went on increasing up to the end of forty eight hours and then the activity gradually decreased through seventytwo and ninetysix hours. But at the end of one twenty hours the acid phosphatase activity increased once again **(Graph1).** At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the kidney acid phosphatase activity was equivalent to 374.532 ± 7.217, 387.78 ± 8.832, 470.028 ± 6.789, 601.956 ± 6.831, 528.816 ± 7.866, 468.372 ± 6.904 and 738.576 ± 6.072 units respectively.

On exposure to **50.0% M.E.** there was about four fold increase in the kidney acid phosphatase activity at the end of six hours and the activity was

equivalent to 1391.868 ± 12.433 units. The enzyme activity reached to a level of 1609.632 ± 6.348 units at the end of twelve hours and then the enzyme activity kept on decreasing to 1501.992 ± 7.728 and 1290.852 ± 7.010 units for twentyfour and fortyeight hours respectively. Then, once again the enzyme activity steadily increased through seventytwo and ninetysix hours, but it decreased a little at the end of one twenty hours. Thus, the acid phosphatase activity at the end of seventytwo, ninetysix and one twenty hours was equivalent to 1328.802 ± 3.850, 1712.028 ± 8.749 and 1556.088 ± 8.017 units respectively.

The **100.0% M.E.** induced maximum increase in the acid phosphatase activity at the end of six hours and was equivalent to 1830.432 ± 8.390 units. At the end of twelve hours the enzyme activity decreased to 1509.306 ± 9.715 units. But from twentyfour to the end of one twenty hours, the enzyme activity went on decreasing and the maximum decrease in the enzyme activity was observed at the end of one twenty hours. However, for all the time intervals under study, the enzyme activity was above that seen in the kidney of control animals. The kidney exhibited acid phosphatase activity equivalent to 1650.204 ± 1.404, 1646.616 ± 6.541, 874.092 ± 4.981, 798.192 ± 7.486 and 586.5 ± 6.168 units respectively.

TABLE NO. 1 : THE ALTERATIONS IN THE ACID PHOSPHATASE ACTIVITIES OF THE KIDNEY OF MALLARDS EXPOSED TO MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		205.6 ±3.892									
0.01%	M.E.		← No significant change →							399.648 ±5.478	575.184 ±9.328
0.1%	M.E.		327.06 ±9.508	273.24 ±9.439	355.212 ±8.790	422.004 ±7.322	449.88 ±5.368	490.728 ±5.422	454.02 ±4.968	N.D.	N.D.
1%	M.E.		364.32 ±6.858	332.58 ±7.948	510.6 ±4.443	487.968 ±7.176	569.94 ±6.348	557.52 ±7.645	574.08 ±8.459	N.D.	N.D.
5%	M.E.		456.228 ±3.753	378.948 ±5.768	524.676 ±7.314	524.4 ±3.891	333.132 ±4.747	558.348 ±7.014	588.432 ±7.120	N.D.	N.D.
10%	M.E.		374.532 ±7.217	387.78 ±8.832	470.028 ±6.789	601.956 ±6.831	528.816 ±7.866	468.372 ±6.904	738.576 ±6.072	N.D.	N.D.
50%	M.E.		1391.86 ±12.433	1609.63 ±6.348	1501.99 ±7.728	1290.85 ±7.010	1328.80 ±3.850	1712.02 ±8.749	1556.08 ±8.017	N.D.	N.D.
100%	M.E.		1830.43 ±8.390	1509.30 ±9.7152	1650.20 ±1.404	1646.61 ±6.541	874.092 ±4.981	798.192 ±7.486	586.5 ±6.168	N.D.	N.D.

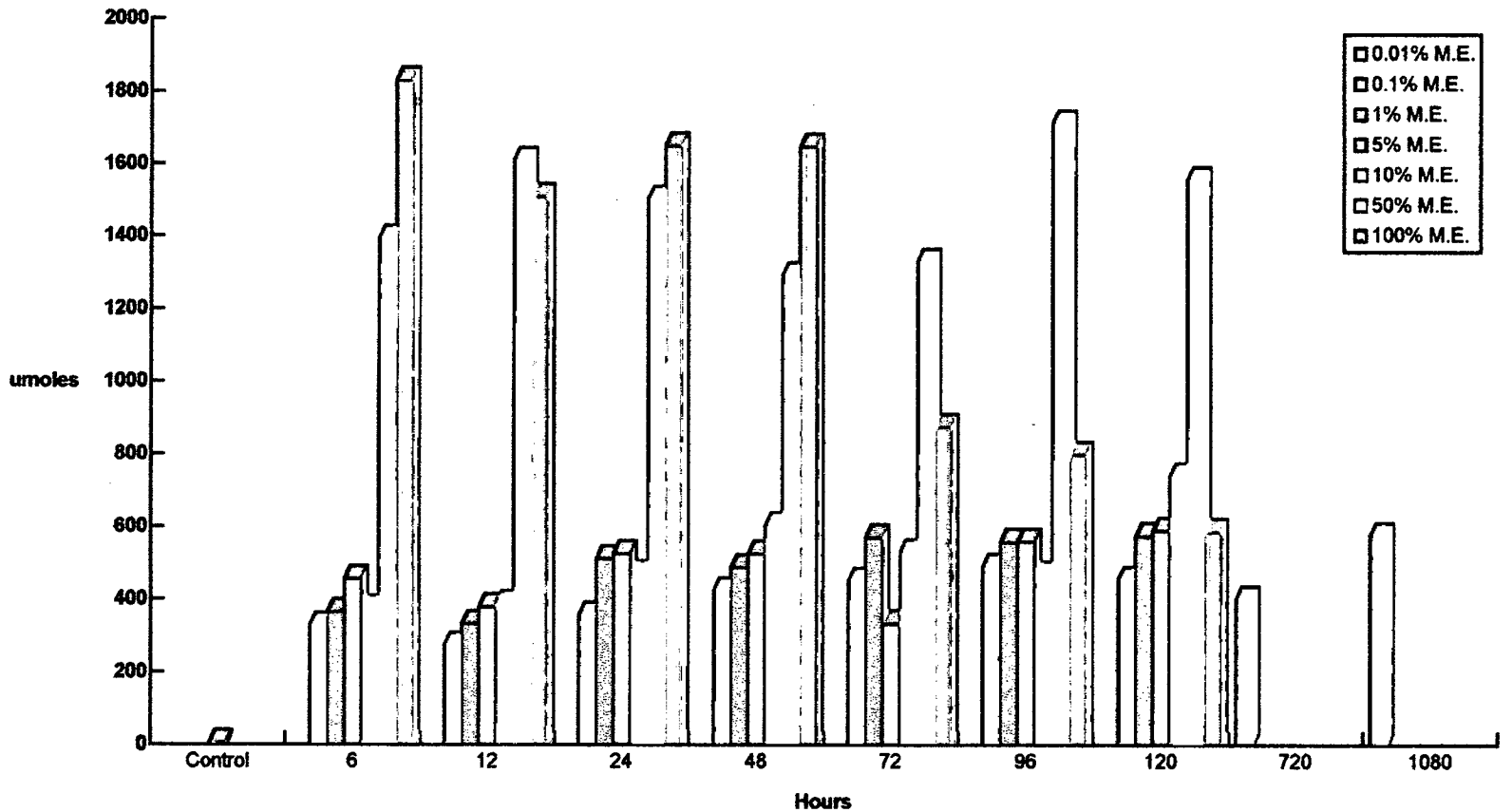
Note : Unit : μmoles/gram wet weight of tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 1 : CHANGES IN ACID PHOSPHATASE ACTIVITY OF KIDNEY OF MALLARD EXPOSED TO MINING EFFLUENTS.

Units : µmoles / g wet weight of tissue



2] ALKALINE PHOSPHATASE :

The changes in the alkaline phosphatase activity of the kidney of mallards exposed for various time intervals to the different concentrations of mining effluents, are given in **Table 2** and are graphically presented in **Graph 2** where the changes in the enzyme activity are presented as a function of various M.E. concentrations and time intervals. The alkaline phosphatase activity is expressed as mmole units per gram wet weight of the tissue.

From **Table 2** it is clear that the alkaline phosphatase activity elevated in a concentration dependent manner for the time interval of six hours except for 5.0%, while such a relation could be seen at the time intervals of twelve and twentyfour hours up to **50% M.E.** concentration. The animals exposed to **0.01% M.E.** did not show any significant change in the kidney alkaline phosphatase activity up to seven twenty hours, but at the end of seven twenty hours it increased slightly, while at the end of one thousand and eighty hours it increased more than one and half fold. The enzyme activity at the end of seven hundred and twenty as well as one thousand and eighty hours was equivalent to 19.75 ± 0.326 and 31.25 ± 0.344 mmole units per gram of wet weight of tissue respectively. Note that the control birds had the enzyme activity eaquivalent to 15.8 ± 1.317 mmole units only.

The animals exposed to **0.1% M.E.** showed a little fluctuation in the enzyme activity. The enzyme activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was eqivalent to 18.5 ± 0.326, 16.75 ± 0.401, 17.5 ± 0.256, 19.25 ± 0.531, 17.25 ± 0.574, 20.75 ± 0.444 and 22.5 ± 0.320 mmole units respectively.

The **1.0% M.E.** induced slight increase in the enzyme activity, but it fluctuated between 20.25 ± 0.354 mmole units and 35.0 ± 0.431 mmole units. The alkaline phosphatase activity observed at the end of six, twelve,

twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 21.75 ± 0.272, 20.25 ± 0.354, 26.0 ± 0.497, 23.0 ± 0.318, 30.5 ± 0.523, 31.25 ± 0.349 and 35.0 ± 0.431 mmole units respectively.

The birds exposed to **5.0% M.E.** exhibited slight increase and fluctuations in the enzyme activity for various time intervals under study. The kidney showed enzyme activity equivalent to 20.75 ± 0.508, 27.0 ± 0.396, 37.5 ± 0.449, 22.5 ± 0.422, 25.25 ± 0.520, 27.0 ± 0.282 and 31.75 ± 0.141 mmole units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The exposure to **10.0% M.E.** induced a significant increase in the alkaline phosphatase activity, but the activity fluctuated between 35.0 ± 0.470 mmole units and 85.0 ± 0.449 mmole units. The maximum increase in the activity was observed at the end of fortyeight hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the enzyme activity was equal to 35.0 ± 0.470, 58.0 ± 0.313, 40.25 ± 0.463, 85.0 ± 0.449, 61.5 ± 0.382, 62.5 ± 0.376 and 74.0 ± 0.433 mmole units respectively.

When the mallards were exposed to **50.0% M.E.**, there was an increase in the enzyme activity in the range of four-fold increase to about ten fold increase in comparison with the enzyme activity seen in the control animals. The maximum increase in the activity was seen at the end of twelve hours. The kidney exhibited alkaline phosphatase activity equivalent to 94.0 ± 0.477, 149.0 ± 0.300, 123.0 ± 0.241, 129.0 ± 0.427, 117.36 ± 0.500, 72.0 ± 0.475 and 100.0 ± 0.515 at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The animals exposed to **100.0% M.E.** did not exhibit rise in the enzyme activity to the extent found when exposed to **50.0% M.E.** However, it was significantly higher than that found for the exposure to 0.01% to 10.0% mining effluents.

The alkaline phosphatase activity observed at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 98.0 ± 0.477, 97.0 ± 0.578, 70.0 ± 0.621, 91.0 ± 0.372, 34.75 ± 0.470, 95.0 ± 0.326 and 85.0 ± 0.479 mmole units respectively.

TABLE NO. 2 : THE ALTERATIONS IN THE ALKALINE PHOSPHATASE ACTIVITY IN THE KIDNEY OF MALLARD EXPOSED TO MINING EFFLUENTS.

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	15.8 ±1.317									
0.01% M.E.		← No significant change →							19.75 ± 0.326	31.25 ±0.3440
0.1% M.E.		18.5 ±0.326	16.75 ±0.401	17.5 ±0.256	19.25 ± 0.531	17.25 ±0.574	20.75 ±0.444	22.5 ± 0.32	N.D.	N.D.
1% M.E.		21.75 ±0.272	20.25 ±0.354	26.0 ± 0.497	23.0 ±0.318	30.5 ±0.523	31.25 ± 0.349	35.0 ± 0.431	N.D.	N.D.
5% M.E.		20.75 ±0.508	27.0 ±0.396	37.5 ±0.449	22.5 ±0.422	25.25 ± 0.52	± 27.0 ± 0.282	31.75 ±0.141	N.D.	N.D.
10% M.E.		35.0 ±0.470	58.0 ±0.313	40.25 ±0.463	85.0 ± 0.449	61.5 ±0.382	62.5 ±0.376	74.0 ±0.433	N.D.	N.D.
50% M.E.		94.0 ±0.477	149.0 ±0.300	123.0 ±0.241	129.0 ±0.427	117.36 ±0.500	72.0 ±0.475	100.00 ± 0.515	N.D.	N.D.
100% M.E.		98.0 ± 0.477	97.0 ± 0.578	70.0 ±0.621	91.0 ±0.372	34.75 ±0.470	95.0 ± 0.326	85.0 ±0.479	N.D.	N.D.

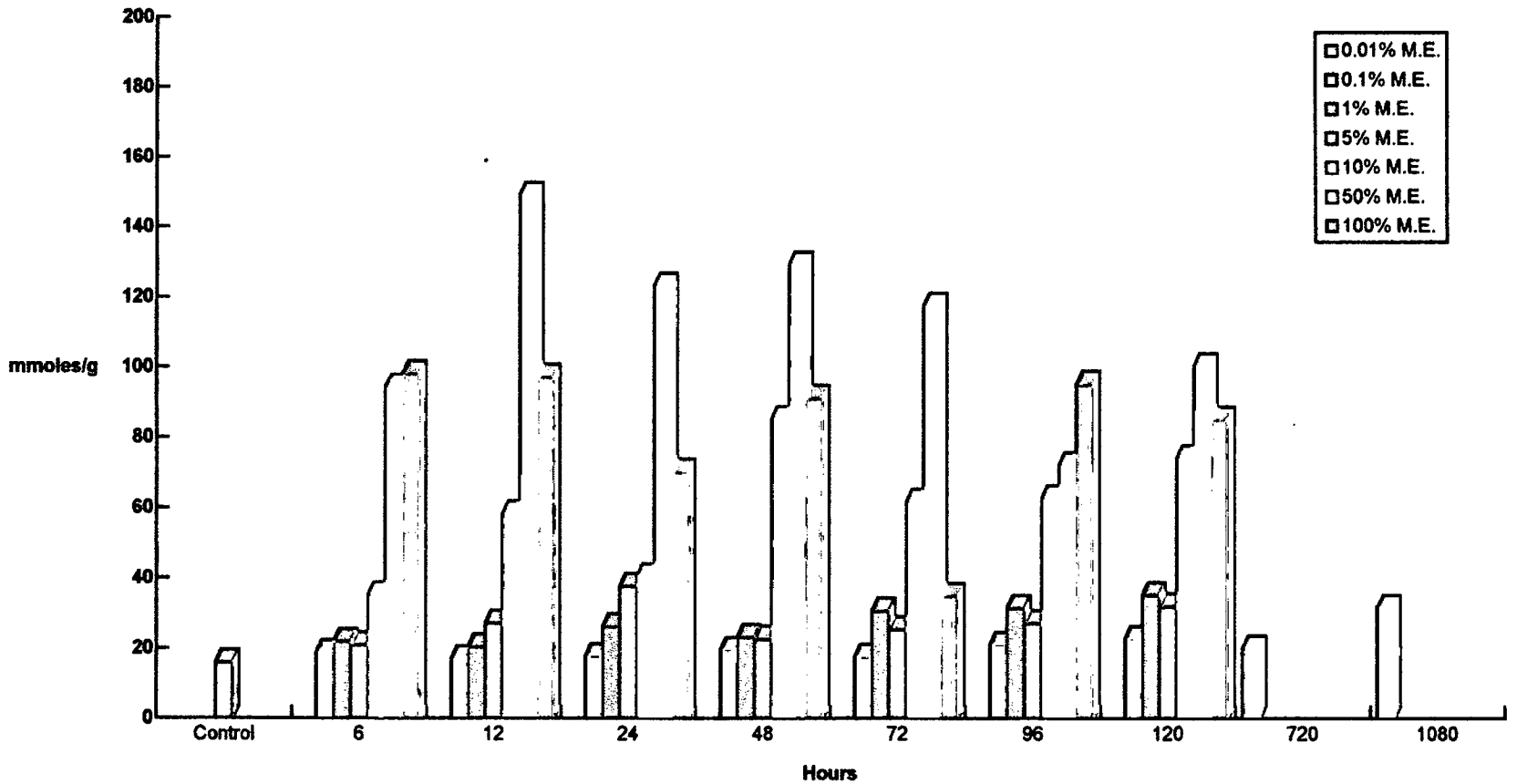
Note : Unit : mmoles/gm wet weight of tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 2 : THE ALTERATIONS IN THE ALKALINE PHOSPHATASE ACTIVITY IN THE KIDNEY OF MALLARD EXPOSED TO MINING EFFLUENTS.

Units : mmoles/gm wet weight of tissue.



3] ESTERASE (NON SPECIFIC) :

The alterations in the esterase (nonspecific) of the kidney of mallard exposed to the mining effluents (M.E.) of different concentrations for various time intervals are tabulated in **Table 3**, while they are graphically shown in **Graph No. 3** wherein they are presented as a function of various concentrations of M.E. over the periods of exposure. From the **Table No. 3** it is obvious that animals exposed to the different concentrations of **M.E.** exhibited wide fluctuations in the enzyme activity and the esterase activity did not show M.E. concentration dependent variations. The control bird showed the nonspecific esterase activity in the kidney to the range of 43.0 ± 0.738 μ mole/units. The animals exposed to **0.01% M.E.** did not show any significant change in the enzyme activity upto seven twenty hours but the enzyme activity increased to 53.0 ± 0.422 and 73.5 ± 0.307 units at the end of seven twenty (30 days) and one thousand and eighty hours (45 days) respectively.

The animals exposed to **0.1% M.E.** showed increase in the enzyme activity at the end of six hours and was equivalent to 61.5 ± 0.213 units. The enzyme activity fluctuated for various time intervals under study and elevated to 75.0 ± 0.162 units at the end of ninetysix hours. At the end of twelve, twentyfour, fortyeight, seventytwo and one twenty hours the enzyme activity was equivalent to 57.5 ± 0.389, 59.5 ± 0.340, 63.0 ± 0.235, 60.5 ± 0.303 and 66.5 ± 0.389 units respectively.

The **1.0% M.E.** induced significant changes in the enzyme activity and it fluctuated between 49.0 ± 0.419 units and 72.5 ± 0.840 units. Interestingly the enzyme activity remained at the same level at the end of twentyfour, ninetysix and one twenty hours. The kidney esterase activity was equivalent to 58.0 ± 0.209, 49.0± 0.419, 54.0 ± 0.922, 72.5 ± 0.840, 50.0 ± 0.440, 54.0 ± 0.310 and 54.0 ± 0.344 at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The mallards treated with **5.0% M.E.** showed increased enzyme activity and the enzyme activity increased progressively from the end of six hours to the end of twentyfour hours and then decreased slightly at the end of fortyeight hours, but at the end of seventytwo and ninetysix hours the enzyme activity increased progressively. The enzyme activity observed at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours was equivalent to 44.0 ± 0.285, 47.5 ± 0.401, 57.5 ± 0.370, 54.0 ± 0.730, 62.0 ± 0.185, 69.0 ± 0.409 and 62.0 ± 0.253 units respectively.

The **10.0% M.E.** induced a minimum increase in the enzyme activity at the end of twentyfour hours and maximum increase in the end of ninetysix hours, while for the rest of the time intervals it fluctuated. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the esterase activity was equivalent to 63.0 ± 0.354, 56.0 ± 0.363, 45.5 ± 0.457, 59.5 ± 0.340, 66.5 ± 0.320, 78.5 ± 0.318 and 77.0 ± 0.307 units respectively.

When the mallards were exposed to **50.0% M.E.** the kidney exhibited very significant increase in the esterase activity. The maximum increase in the enzyme activity was observed at the end of one twenty hours. Generally the enzyme activity remained at or above 60.0 units under the influence of this concentration.

The kidney exhibited enzyme activity equivalent to 61.5 ± 0.215, 63.0 ± 0.392, 60.0 ± 0.248, 80.5 ± 0.185, 70.0 ± 0.422, 78.5 ± 0.365 and 92.5 ± 0.397 units respectively.

Under the influence of **100.0% M.E.** the ducks exhibited the fluctuations in the enzyme activity between 45.5 and 92.5 units. The maximum enzyme activity was observed at the end of one twenty hours. The esterase activity observed at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 62.0 ± 0.271, 45.5 ± 0.445, 65.5 ± 0.414, 68.0 ± 0.449, 77.0 ± 0.545, 90.5 ± 0.463 and 92.5 ± 0.278 units respectively.

TABLE NO. 3 : THE ALTERATIONS IN THE ESTERASE (NON SPECIFIC) ACTIVITY OF THE KIDNEY OF MALLARD EXPOSED TO THE MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		43 ±0.738									
0.01%	M.E.		← No significant change →							53.0 ±0.422	73.5 ±0.307
0.1%	M.E.		61.5 ±0.213	57.5 ±0.389	59.5 ±0.340	63.0 ±0.235	60.5 ±0.303	75.0 ±0.162	66.5 ±0.389	N.D.	N.D.
1%	M.E.		58.0 ±0.209	49.0 ±0.419	54.0 ±0.922	72.5 ±0.840	50.0 ±0.44	54.0 ±0.310	54.0 ±0.334	N.D.	N.D.
5%	M.E.		44.0 ±0.285	47.5 ±0.401	57.5 ±0.370	54.0 ±0.730	62.0 ±0.185	69.0 0.409	62.0 ±0.253	N.D.	N.D.
10%	M.E.		63.0 ±0.354	56.0 ±0.363	45.5 0.457	59.5 ±0.340	66.5 ±0.32	78.5 ±0.318	77.0 ±0.307	N.D.	N.D.
50%	M.E.		61.5 ±0.215	63.0 ±0.392	60.0 ±0.248	80.5 0.185	70.0 ±0.422	78.5 ±0.365	92.5 ±0.397	N.D.	N.D.
100%	M.E.		62.0 ±0.271	45.5 ±0.445	65.5 ±0.414	68.0 0.449	77.0 ±0.545	90.5 ±0.463	92.5 ±0.278	N.D.	N.D.

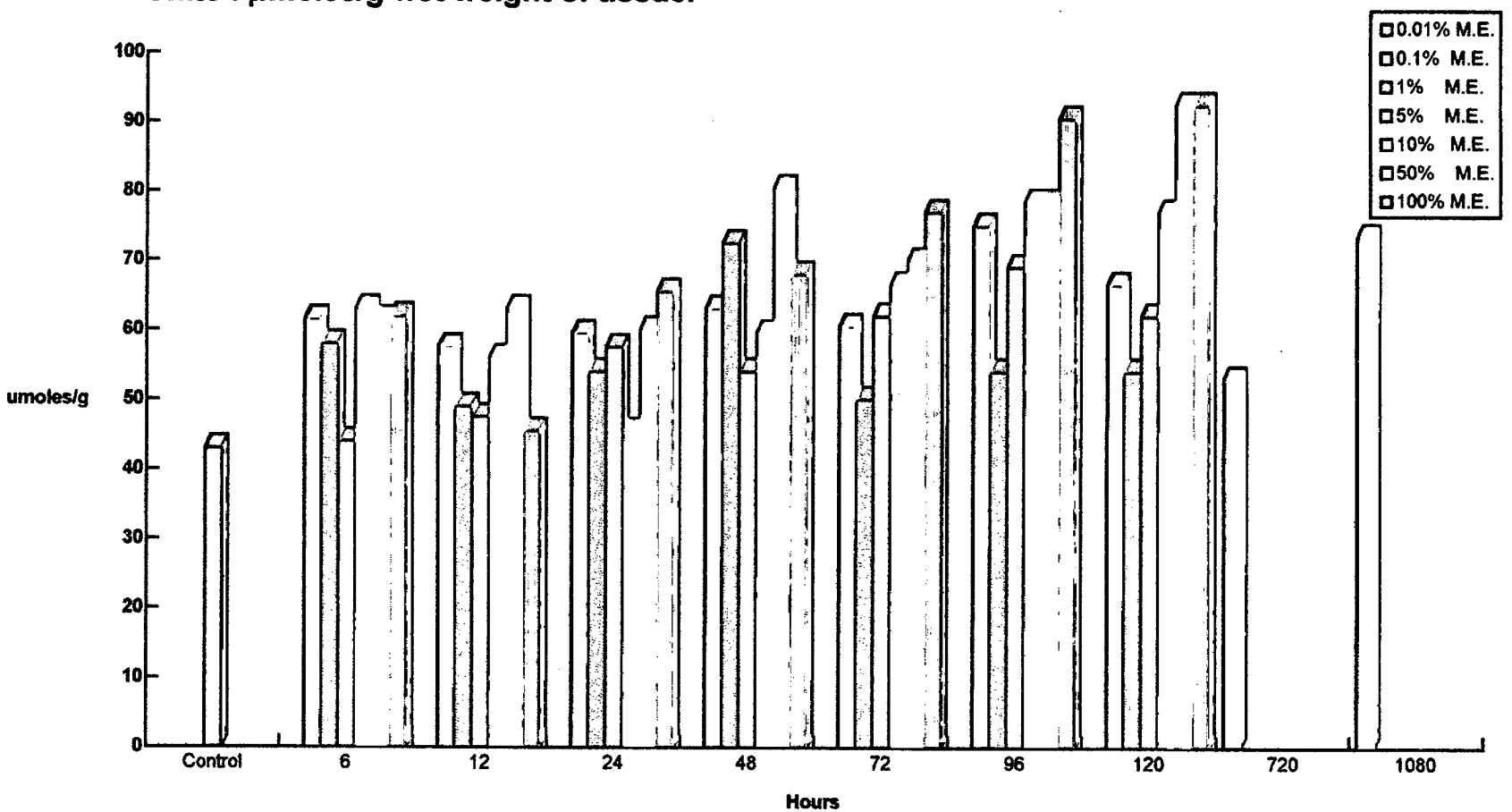
Note : Unit : µmoles/gram wet weight of tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 3 : ALTERATION IN (NON SPECIFIC) ESTERASE OF KIDNEY OF MALLARD EXPOSED TO MINING EFFLUENTS.

Units : µmoles/g wet weight of tissue.



4] TOTAL PROTEINS :

The variations in the total protein contents of the kidney of mallards exposed to the various concentrations of mining effluents **(M.E.)** are compiled in **Table No. 4** and are graphically expressed in **Graph No. 4** where they are shown as a function of M.E. concentrations over different time intervals. The total proteins are expressed as mg/100 mg of the wet weight of the tissue. The control animals showed the total proteins equal to 29.05 ± 0.371 mg. From the **table 4** and **graph 4** it is observed that, under the influence of various concentrations of M.E. the kidney protein level progressively decreased in a concentration dependent manner for the time intervals of six, twelve, seventytwo and one twenty hours.

The mallards exposed to **0.01% M.E.** showed no significant alterations in the total proteins of the kidney up to seven twenty hours but at the end of seven hundred and twenty as well as one thousand and eighty hours, the kidney protein level decreased a little. Thus, at the end of the above referred hours the protein level was equivalent to 28.372 ± 0.310 and 27.90 ± 1.14 mg respectively.

The **0.1% M.E.** induced fluctuations in the protein level from the end of six hours to the end of one hundred and twenty hours. The kidney exhibited the protein cocentration equivalent to 27.392 ± 0.216, 26.893 ± 0.116, 28.293 ± 0.132, 27.09 ± 0.185, 25.391 ± 0.24, 24.837 ± 0.24 and 26.03 ± 1.41 mg at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

When the birds were exposed to **1.0% M.E.** the protein concentration of the kidney showed significant fluctuations for the time intervals under study. The kidney showed the protein contents equivalent to 25.392 ± 0.11, 24.893 ± 0.101, 25.371 ± 0.21, 23.21 ± 0.116, 22.893 ± 0.31, 20.28 ± 0.22 and 22.08 ±

0.223 mg at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **5.0% M.E.** exposure caused progressive decrease in the protein level from the end of six hours to the end of one twenty hours except at the end of fortyeight and ninetysix hours. The protein level at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours were equivalent to 23.789 ± 0.30, 23.0 ± 0.270, 22.342 ± 1.14, 31.87 ± 0.141, 20.32 ± 0.141, 21.27 ± 0.10 and 19.873 ± 0.172 mg respectively.

The birds exposed to **10.0% M.E.** showed time dependent decrease in the protein level of the kidney for all the time intervals under study except for ninetysix hours where a marginal increase in the proteins was found. The protein level found at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 22.381 ± 0.1, 22.09 ± 0.172, 20.82 ± 0.15, 19.890 ± 0.141, 17.368 ± 0.146, 17.890 ± 0.141, and 16.482 ± 0.185 mg respectively.

The **50.0% M.E.** induced very significant decrease in the kidney protein level but the decrease was not dependent upon the exposure period of the mallards to ME. The kidney protein concentration was equivalent to 20.932 ± 0.185, 21.371 ± 0.215, 16.39 ± 0.149, 14.217 ± 0.16, 15.321 ± 0.132, 13.217 ± 0.14 and 13.813 ± 0.27 mg at the end of six, twelve, twentyfour, fortyeight seventytwo, ninetysix and one twenty hours respectively.

The animals treated with **100.0% M.E.** showed maximum reduction in the kidney protein level at the end of one twenty hours, while for the rest of the periods under study it varied between 12.10 and 19.372 mg. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the protein levels was equivalent to 19.372 ± 0.172, 17.21 ± 2.24, 18.37 ± 0.231, 15.32 ± 1.101, 12.10 ± 0.205, 13.37 ± 0.160 and 11.27 ± 0.137 mg respectively.

TABLE NO. 4 : THE ALTERATIONS IN THE TOTAL KIDNEY PROTEINS OF MALLARDS EXPOSED TO MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		29.05 ±0.371									
0.01%	M.E.		← No significant change →							28.372 ±0.310	27.90 ±1.14
0.1%	M.E.		27.392 ±0.216	26.893 ±0.116	28.293 ±0.132	27.09 ±0.185	25.391 ±0.24	24.837 ±0.24	26.03 ±1.41	N.D.	N.D.
1%	M.E.		25.392 ±0.11	24.893 ±0.101	25.371 ±0.21	23.21 ±0.116	22.893 ±0.31	20.28 ±0.22	22.08 ±0.223	N.D.	N.D.
5%	M.E.		23.789 ±0.30	23.0 ±0.270	22.342 ±1.14	31.87 ±0.141	20.32 ±0.141	21.27 ±0.10	19.873 ±0.172	N.D.	N.D.
10%	M.E.		22.381 ±0.1	22.09 ±0.172	20.82 ±0.15	19.890 ±0.141	17.368 ±0.146	17.890 ±0.141	16.482 ±0.185	N.D.	N.D.
50%	M.E.		20.932 ±0.185	21.371 ±0.215	16.39 ±0.149	14.217 ±0.16	15.321 ±0.132	13.217 ±0.14	13.813 ±0.27	N.D.	N.D.
100%	M.E.		19.372 ±0.172	17.21 ±2.24	18.37 ±0.231	15.32 ±1.101	12.10 ±0.205	13.37 ±0.16	11.27 ±0.137	N.D.	N.D.

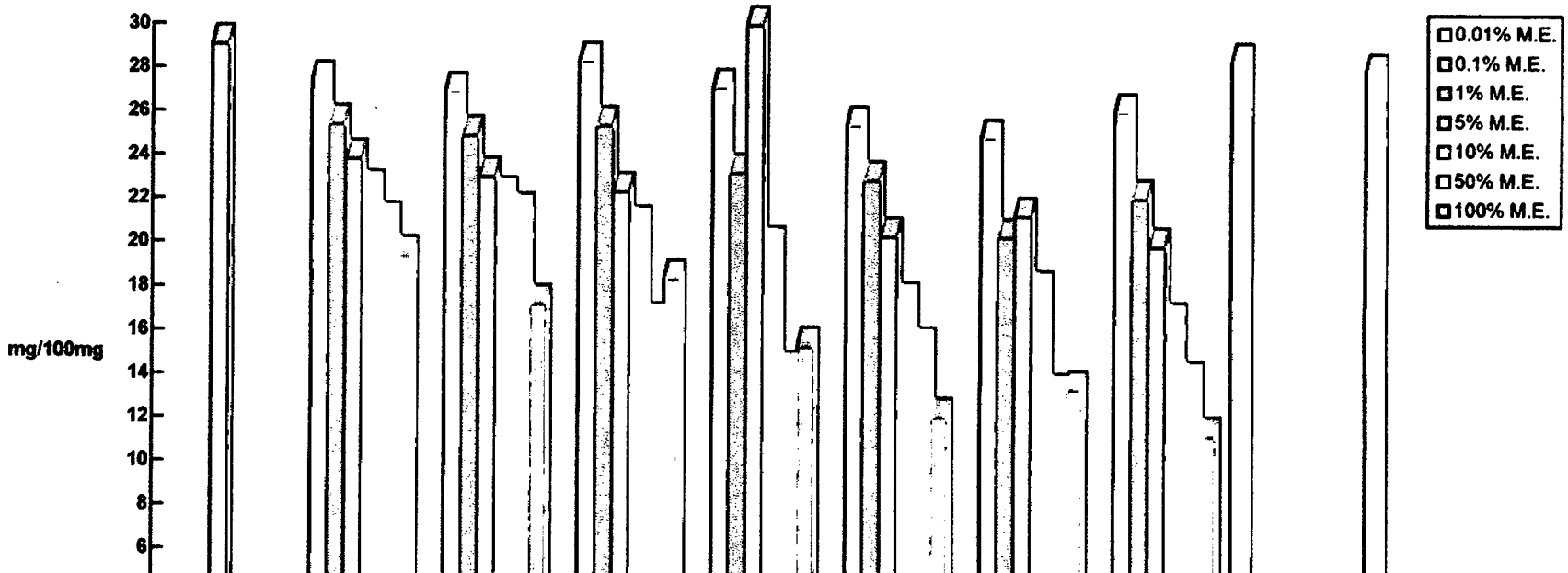
Note : Unit : mg of proteins per 100 mg wet weight of tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 4 : KIDNEY - PROTEINS

Units : mg/100mg wet weight of tissue



5] UREA :

The changes in the urea levels of the kidney of ducks exposed to different concentrations of mining effluents at different time intervals are compiled in **Table No. 5** and are graphically presented in **Graph No. 5** where these changes are given as a function of M.E. concentrations over various time intervals. The control birds showed the urea concentration of kidney equivalent to 1.7 ± 0.315 mg per 100 mg of the wet weight of the tissue.

The birds exposed to **0.01% M.E.** did not exhibit any significant change in the urea levels till the end of twenty nine days but, at the end of thirtieth day (seven hundred and twenty hours) the kidney exhibited an increase in urea level and the urea level kept on increasing till the end of one thousand and eighty hours. Thus, at the end of seven twenty and one thousand and eighty hours the urea level was equivalent to 6.66 ± 1.56 and 9.7 ± 0.982 mg respectively.

The **0.1% M.E.** induced increase in the urea level of the kidney from the end of six hours to the end of fortyeight hours, but at the end of seventytwo hours, the urea level decreased a little and then increased at the end of ninety six and one twenty hours. Thus, at the end of six, twelve, twenty four, forty eight, seventy two, ninetysix and one twenty hours, the urea level was equivalent to 4.5 ± 0.34, 7.0 ± 0.872, 10.0 ± 0.930, 11.0 ± 1.0, 9.0 ± 1.01, 10.3 ± 1.32 and 11.7 ± 1.42 mg respectively.

The mallards exposed to **1.0% M.E.** exhibited steady increase in the urea levels of the kidney from the end of six hours to the end of seventytwo hours but the urea level dropped a little at the end of ninetysix hours and shot up to a high level at the end of one twenty hours. Thus, the kidney urea concentration at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 6.7 ± 0.87, 8.3 ± 1.01, 11.3 ± 1.32, 13.0 ± 1.82, 14.0 ± 2.3, 12.3 ± 0.97 and 16.0 ± 2.37 mg respectively.

When the mallards were exposed to **5.0% M.E.** the kidney urea concentrations showed time dependent increase. The urea concentrations went on increasing from the end of six hours to the end of one twenty hours. Thus at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours the urea concentrations in the kidney was equivalent to 9.3 ± 0.96, 10.1 ± 0.73, 12.7 ± 2.0, 14.7 ± 1.01, 15.7 ± 1.38, 17.0 ± 2.24 and 19.0 ± 2.1 mg respectively.

The **10.0% M.E.** induced fluctuations in the urea levels for the time intervals under study. But the urea levels were quite higher than that found in the control birds. The urea concentrations observed at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours was equivalent to 8.4 ± 0.8, 11.8 ± 0.93, 15.0 ± 0.91, 10.9 ± 2.4, 13.2 ± 1.07, 16.7 ± 1.63 and 15.0 ± 2.0 mg respectively.

The ducks treated with **50.0% M.E.** showed a very high increase in the urea level of the kidney and the increase in the urea concentrations exhibited exposure time dependency up to seventy two hours. The urea concentration in the kidney was equivalent to 10.4 ± 13.7, 12.9 ± 2.7, 1.37 ± 1.16, 16.7 ± 2.1, 18.0 ± 1.37, 15.0 ± 1.98 and 10.0 ± 1.34 mg at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours respectively.

The **100.0% M.E.** exposure induced time depend rise in the kidney urea levels of the ducks up to the end of fortyeight hours and then the urea level declined at the end of seventytwo hours, but once again it went on increasing steadily up to the end of one twenty hours. The highest concentration of urea was observed at the end of one twenty hours only. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the urea concentrations in the kidney was equivalent to 9.8 ± 2.0, 12.7 ± 1.01, 15.3 ± 1.66, 17.4 ± 2.7, 13.0 ± 1.28, 14.2 ± 2.34 and 19.3 ± 1.72 mg respectively.

TABLE NO. 5 : THE CHANGES IN KIDNEY UREA UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		1.7 ±0.315									
0.01%	M.E.		← No significant change →							6.66 ±1.56	9.7 ±0.982
0.1%	M.E.		4.5 ±0.34	7.0 ±0.872	10.0 ±0.93	11.0 ±1.0	9.0 ±1.01	10.3 ±1.32	11.7 ±1.42	N.D.	N.D.
1%	M.E.		6.7 ±0.87	8.3 ±1.01	11.3 ±132	13.0 ±1.82	14.0 ±2.3	12.3 ±0.97	16.0 ±2.37	N.D.	N.D.
5%	M.E.		9.3 ±0.96	10.1 ±0.73	12.7 ±2.0	14.7 ±1.01	15.7 ±1.38	17.0 ±2.24	19.0 ±2.1	N.D.	N.D.
10%	M.E.		8.4 ±0.8	11.8 ±0.93	15.0 ±0.91	10.9 ±2.4	13.2 ±1.07	16.7 ±1.63	15.0 ±2.0	N.D.	N.D.
50%	M.E.		10.4 ±1.37	12.9 ±2.7	13.7 ±1.16	16.7 ±2.1	18.0 ±1.37	15.0 ±1.98	10.0 ±1.34	N.D.	N.D.
100%	M.E.		9.8 ±2.0	12.7 ±1.01	15.3 ±1.66	17.4 ±2.7	13.0 ±1.28	14.2 ±2.34	19.3 ±1.72	N.D.	N.D.

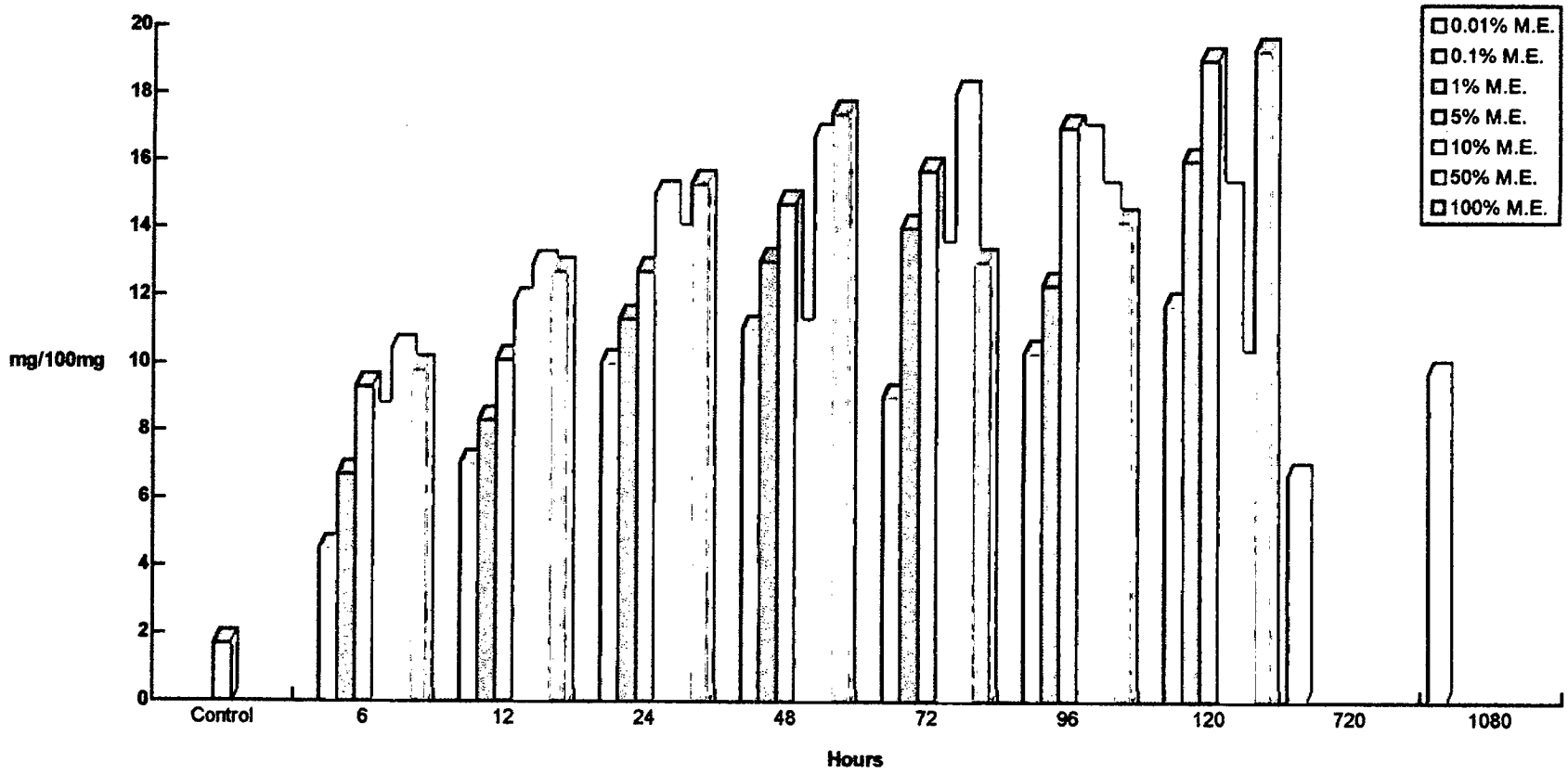
Note : Unit : mg/100mg wet weight of the tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 5 : KIDNEY - UREA

Units : mg/100 mg wet weight of tissue



6] URIC ACID :

The variations in the uric acid concentrations in the kidney of the mallards exposed to the mining effluents of different concentrations at varying time intervals are compiled in the **Table No. 6** and are graphically given in the **Graph No. 6** as a function of M.E. concentrations over various exposure periods. The control birds exhibited uric acid concentration equivalent to 2.3 ± 0.21 mg per 100 mg of the wet weight of the tissue. From the graph No. 6, it is clear that the M.E. induced reduction in the uric acid concentration of the kidney at the end of six hours for 0.1, 1.0, 5.0 and 10.0 percent mining effluents.

The birds exposed to **0.01% of M.E.** did not exhibit any significant change in the uric acid concentration till the begining of seven hundred and twenty hours but at the end of seven hundred and twenty hours the uric acid concentration rose to 7.7± 0.77 mg and continued to rise further to a level of 13.9 ± 1.01 mg at the end of one thousand and eighty hours.

The **0.1% M.E.** induced significant fluctuations in the uric acid concentration. The **0.1% M.E.** induced a significant decrease in the uric acid concentration at the end of six, twelve, and twentyfour hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the kidney uric acid concentration was equivalent to 1.5 ± 0.435, 0.9 ± 0.268, 1.2 ± 0.116, 5.7 ± 0.42, 4.6 ± 0.37, 10.1 ± 0.13 and 9.3 ± 0.27 mg respectively.

The mallards exposed to **1.0% M.E.** exhibited a significant decrease in the uric acid level at the end of six and twentyfour hours, while the maximum increase in the uric acid concentration was observed at the end of twelve hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the uric acid level of the kidney was

equivalent to 1.7 ± 0.13, 14.6 ± 1.18, 1.4 ± 0.18, 2.8 ± 0.158, 7.7 ± 0.4, 9.91 ± 1.01 and 12. 97 ± 1.62 mg respectively.

The **5.0% M.E.** induced a very significant decrease in the kidney uric acid concentrations at all the time intervals under study except fortyeight \hours. The kidney uric acid concentration at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours was equivalent to 0.9 ± 0.034, 0.9 ± 0.029, 0.7 ± 0.038, 2.4 ± 0.113, 1.3 ± 0.05, 1.0 ± 0.02 and 0.8 ± 0.035 mg respectively.

The mallards exposed to **10.0% M.E.** exhibited significant decrease in the uric acid level of the kidney at the end of six, twelve, twentyfour and ninetysix hours, while at the end of fortyeight and one twenty hours it showed over five fold increase in the uric acid concentration. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the kidney uric acid concentration was equivalent to 1.5 ± 0.70, 1.6 ± 0.135, 1.8 ± 0.101, 12.0 ± 1.72, 3.2 ± 0.73, 1.2 ± 0.648 and 11.8 ± 2.2 mg respectively.

The **50.0% M.E.** influenced the uric acid level of the kidney and induced significant fluctuations in the uric acid concentration. The uric acid level observed at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours was equivalent to 2.9 ± 0.316, 2.4 ± 0.77, 3.3 ± 0.57, 4.7 ± 0.305, 7.7 ± 0.8, 3.9 ± 0.477 and 5.3 ± 0.39 mg respectively.

Under the influence of **100.0% M.E.**, the mallards showed a significant increase in the uric acid concentration of the kidney at all the time intervals under study. The uric acid concentration of the kidney was equivalent to 5.7 ± 0.829, 6.9 ± 0.8, 4.9 ± 0.77, 10.37 ± 0.949, 9.8 ± 0.47, 12.0 ± 0.5 and 7.7 ± 1.01 mg at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 6 : THE CHANGES IN THE URIC ACID OF THE KIDNEY UNDER THE INFLUENCE OF MINING EFFLUENTS.

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	2.3 ±0.21									
0.01% M.E.		← No significant change →							7.7 ±0.77	13.9 ±1.01
0.1% M.E.		1.5 ±0.435	0.9 ±0.268	1.2 ±0.116	5.7 ±0.42	4.6 ±0.37	10.1 ±0.13	9.3 ±0.27	N.D.	N.D.
1% M.E.		1.7 ±0.13	14.6 ±1.18	1.4 ±0.18	2.8 ±0.158	7.7 ±0.4	9.91 ±1.01	12.97 ±1.62	N.D.	N.D.
5% M.E.		0.9 ±0.034	0.9 ±0.029	0.7 ±0.038	2.4 ±0.113	1.3 ±0.05	1.0 ±0.020	0.8 ±0.035	N.D.	N.D.
10% M.E.		1.5 ±0.70	1.6 ±0.135	1.8 ±0.101	12.0 ±1.72	3.2 ±0.73	1.2 ±0.648	11.8 ±2.2	N.D.	N.D.
50% M.E.		2.9 ±0.316	2.4 ±0.77	3.3 ±0.57	4.7 ±0.035	7.7 ±0.8	3.9 ±0.477	5.3 ±0.39	N.D.	N.D.
100% M.E.		5.7 ±0.829	6.9 ±0.8	4.9 ±0.77	10.37 ±0.949	9.8 ±0.47	12.0 ±0.5	7.7 ±1.01	N.D.	N.D.

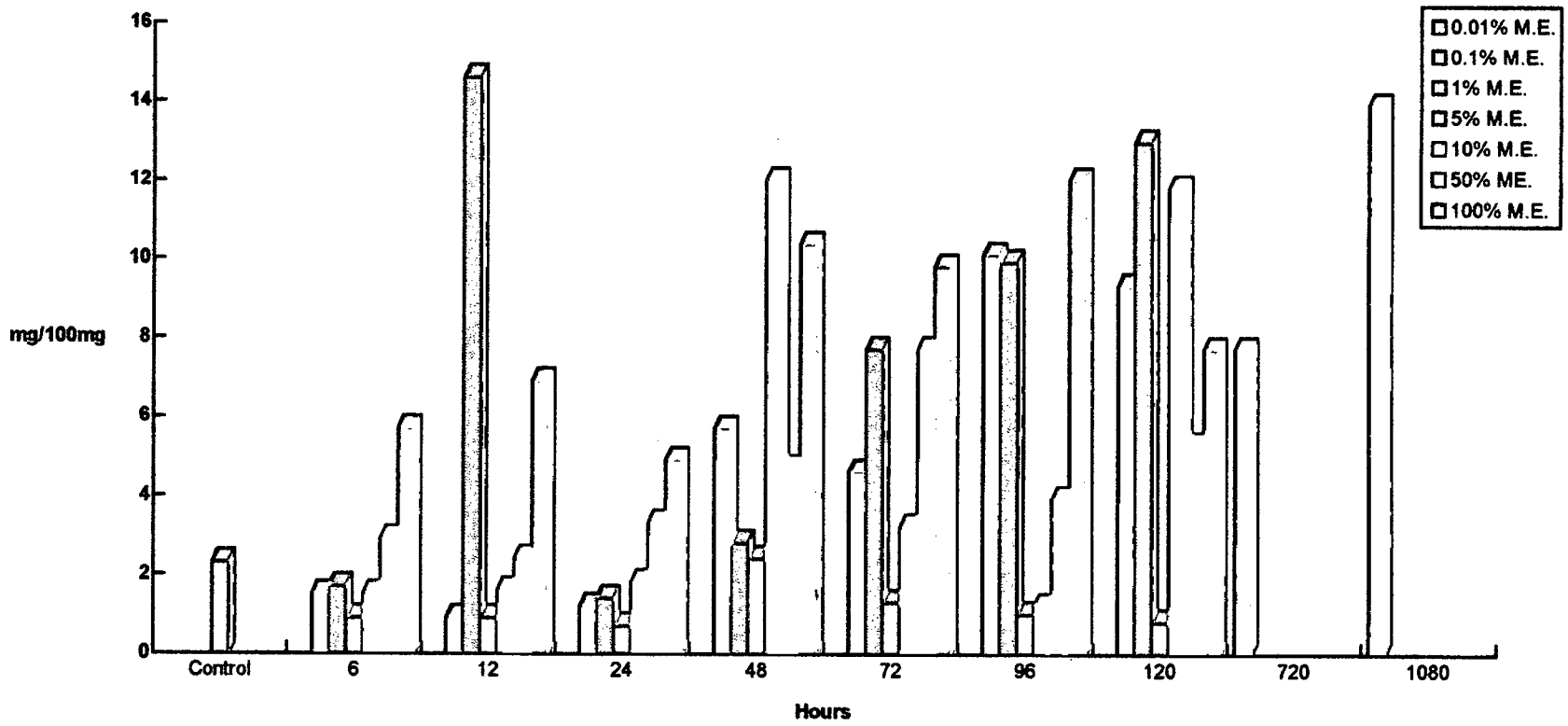
Note : Unit : mg/100mg wet weight of the tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 6 : KIDNEY - URIC ACID.

Units : mg/100 mg wet weight of tissue



7] CREATININE :

The alterations in the creatinine levels of the kidney of mallards exposed to the mining effluents are given in **Table No. 7** and are graphically shown in **Graph No. 7** where they are presented as the function of concentrations of M.E. over the various time intervals. The creatinine concentrations of the kidney are expressed as mg/100 mg wet weight of the tissue. The control animals showed creatinine level equal to 0.17 ± 0.01 mg. From the table it appears that the mining effluents induced significant fluctuations in the concentrations of creatinine.

The mallards exposed to the **0.01% M.E.** did not show any significant change in the creatinine level of the kidney up to seven hundred and twenty hours but, at the end of seven hundred and twenty hours as well as one thousand and eighty hours, the creatinine concentration elevated significantly to 0.345 ± 0.010 and 0.342 ± 0.034 mg respectively in comparison to that found in the control animals.

Under the influence of **0.1% M.E.** the creatinine level of the kidney fluctuated between 0.20 mg and 0.501 mg. The maximum elevation in creatinine for this dose was observed at the end of one hundred and twenty hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the kidney creatinine concentration was equivalent to 0.25 ± 0.010, 0.29 ± 0.054, 0.20 ± 0.037, 0.27 ± 0.029, 0.42 ± 0.040, 0.447 ± 0.038 and 0.501 ± 0.049 mg respectively.

The **1.0% M.E.** induced acute reduction in the kidney creatinine levels at the end of twentyfour hours. A significantly higher level of creatinine was observed at the end of ninetysix hours. Thus, the kidney creatinine concentration was equivalent to 0.28 ± 0.02, 0.20 ± 0.013, 0.09 ± 0.014, 0.285 ± 0.023, 0.203 ± 0.03, 0.302 ± 0.038 and 0.250 ± 0.04 at the end of six, twelve

twentyfour, fortyeight, seventy two, ninetysix and one hundred and twenty hours respectively.

The ducks exposed to **5.0% M.E.**, exhibited acute increase in creatinine concentration at the end of twelve hours, while for the rest of the time intervals the fluctuations in the creatinine levels were observed. The creatinine concentration of the kidney was equivalent to 0.23 ± 0.05, 0.49 ± 0.034, 0.27 ± 0.038, 0.20 ± 0.013, 0.19 ± 0.054, 0.31 ± 0.08 and 0.27 ± 0.035 mg at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninety six and one twenty hours respectively.

The birds under the influence of **10.0% M.E.**, showed wide fluctuations in the creatinine levels of the kidney, but the creatinine concentrations were quite above that observed in the control at all the time intervals. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the kidney creatinine concentration was equivalent to 0.5 ± 0.013, 0.45 ± 0.037, 0.30 ± 0.054, 0.355 ± 0.049, 0.27 ± 0.053, 0.29 ± 0.040 and 0.30 ± 0.035 respectively.

The **50.0% M.E.** induced reduction in the creatinine concentrations of the kidney at the end of twelve hours, while the maximum increase in creatinine concentration was induced at the end of twentyfour hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the kidney creatinine concentration was equivalent to 0.20 ± 0.05, 0.15 ± 0.016, 0.57 ± 0.060, 0.31 ± 0.038, 0.39 ± 0.040, 0.40 ± 0.035 and 0.31 ± 0.020 respectively.

Under the influence of **100.0% M.E.** the ducks exhibited maximum increase in the creatinine concentrations at the end of six hours but the creatinine level decreased at the subsequent time intervals up to the end of twentyfour hours. Then, the creatinine level increased once again at the end of fortyeight hours. After this, the creatinine concentration went on decreasing upto the end of ninetysix hours. The kidney creatinine concentration was

equivalent to 1.1 ± 0.271, 0.80 ± 0.16, 0.73 ± 0.39, 0.96 ± 0.16, 0.83 ± 0.09, 0.34 ± 0.101 and 0.37 ± 0.135 mg at the end of six, twelve, twentyfour, forty eight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 7 : THE CHANGES IN THE CREATININE LEVELS OF KIDNEY UNDER THE INFLUENCE OF MINING EFFLUENTS.

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	0.17 ±0.01									
0.01% M.E.		← No significant change →							0.345 ±0.010	0.342 ±0.034
0.1% M.E.		0.25 ±0.010	0.29 ±0.054	0.20 ±0.037	0.27 ±0.029	0.42 ±0.040	0.447 ±0.038	0.501 ±0.049	N.D.	N.D.
1% M.E.		0.28 ±0.02	0.20 ±0.013	0.09 ±0.014	0.285 ±0.023	0.203 ±0.03	0.302 ±0.038	0.250 ±0.04	N.D.	N.D.
5% M.E.		0.23 ±0.05	0.49 ±0.034	0.27 ±0.038	0.20 ±0.013	0.19 ±0.054	0.31 ±0.08	0.27 ±0.035	N.D.	N.D.
10% M.E.		0.5 ±0.013	0.45 ±0.037	0.30 ±0.054	0.35 ±0.049	0.27 ±0.053	0.29 ±0.040	0.30 ±0.035	N.D.	N.D.
50% M.E.		0.20 ±0.05	0.15 ±0.016	0.57 ±0.060	0.31 ±0.038	0.39 ±0.040	0.40 ±0.035	0.31 ±0.020	N.D.	N.D.
100% M.E.		1.1 ±0.271	0.8 ±0.16	0.73 ±0.039	0.96 ±0.16	0.83 ±0.09	0.34 ±0.101	0.37 ±0.135	N.D.	N.D.

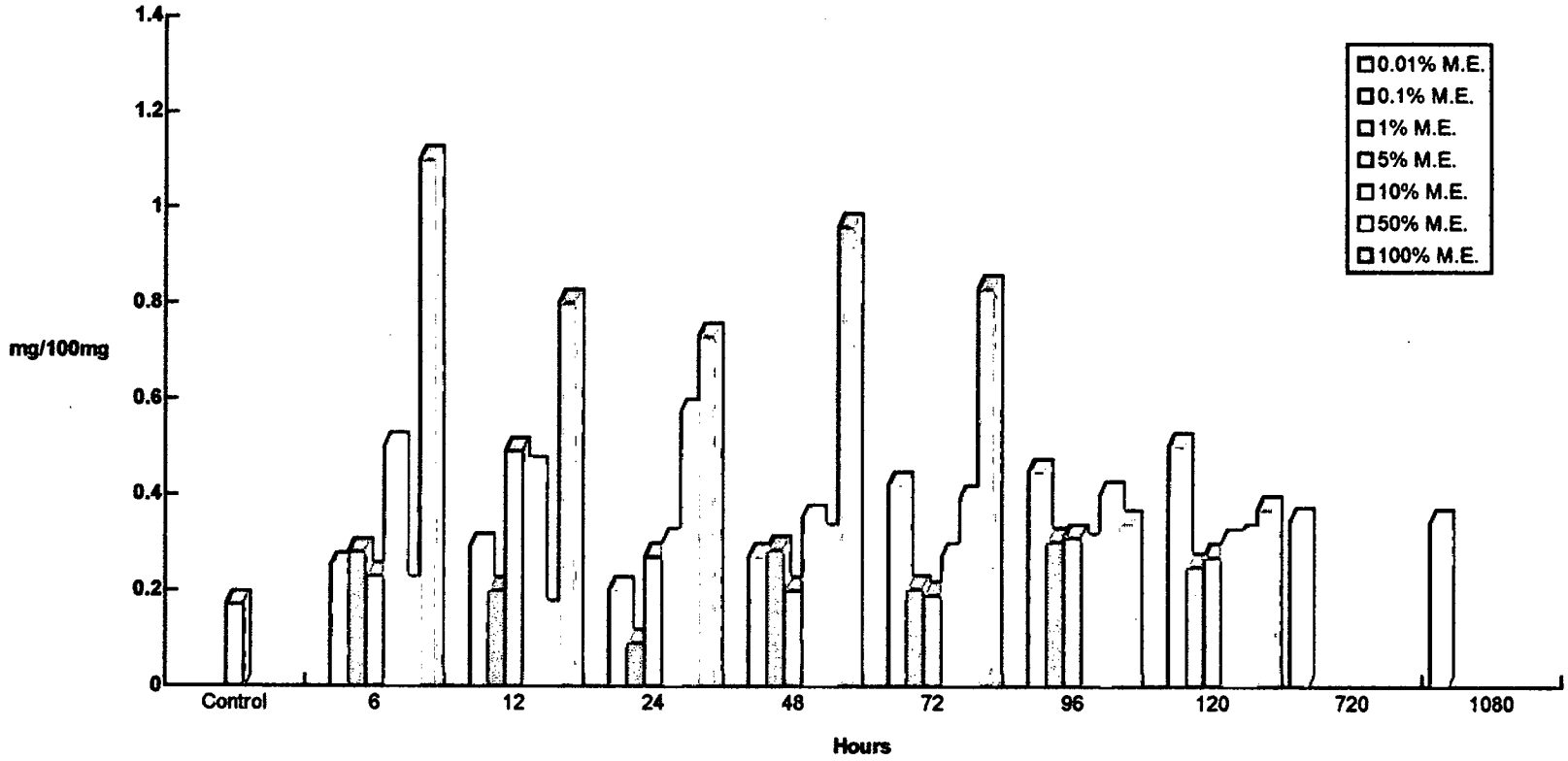
Note : Unit : mg/100mg wet weight of tissue .

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 7 : KIDNEY - CREATININE.

Units : mg/100 mg wet weight of tissue.



8] CALCIUM :

The alterations in the calcium levels of the kidney of mallards exposed to the various concentrations of mining effluents for different time intervals are tabulated in **Table No. 8** and are given graphically in **Graph No. 8** where the calcium changes are expressed as the function of concentration of M.E. over the time intervals under study. The calcium concentrations are expressed as mg/100 mg of the wet weight of the tissue. The control animals showed the calcium level equal to 2.1 ± 0.531 mg/100 mg of wet weight of tissue.

From the **Table No. 8** it appears that the calcium levels of the kidney exhibited concentration dependent increase for the time intervals of six and twentyfour hours. For the time interval of twelve hours the calcium hike exhibited dose dependency for all the concentrations of M.E. except **10%**.

The birds exposed to **0.01% M.E.** did not show any significant change in the calcium levels of the kidney up to seven hundred and twenty hours, but at the end of seven hundred and twenty as well as one thousand and eighty hours the kidney exhibited over two fold increase in the calcium levels. Thus, at the end of seven hundred and twenty and one thousand and eighty hours the calcium concentration of the kidney was equivalent to 4.82 ± 0.31 and 5.38 ± 0.185 mg respectively.

The **0.1% M.E.** induced marginal increase in the calcium level at the end of six and twelve hours, while the highest increase in calcium level was induced at the end of one hundred and twenty hours. The kidney showed the calcium concentration equivalent to 2.25 ± 0.111, 2.74 ± 0.101, 1.825 ± 0.311, 5.14 ± 0.449, 4.12 ± 0.146, 3.94 ± 0.135 and 8.36 ± 0.215 at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

Under the influence of **1.0% M.E.** the kidney of ducks exhibited fluctuations in the calcium levels between 4.1mg and 6.08 mg, but in general, the calcium concentration of the kidney was significantly higher than that found in the control animals. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the kidney calcium concentration was equivalent to 4.28 ± 0.584, 5.3 ± 0.141, 4.64 ± 0.215, 4.84 ± 0.30, 4.1 ± 0.14, 5.6 ± 0.260 and 6.08 ± 0.146 mg respectively.

The ducks exposed to **5.0% M.E.** showed fluctuations in the calcium concentrations of the kidney, but at the end of six, twelve, and twentyfour hours the kidney showed a gradual increase in the calcium level. The calcium level of the kidney at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours was equivalent to 5.3 ± 0.701, 6.52 ± 0.56 , 6.58 ± 0.278, 4.82 ± 0.324, 8.7 ± 0.22, 6.02 ± 0.483 and 6.4 ± 0.303 mg respectively.

The **10.0% M.E.** induced fluctuations in the calcium level of the kidney and the calcium level was higher for all the time intervals under study when compared with that of control. The calcium level appeared to increase gradually from the end of six hours to the end of twentyfour hours. The maximum increase in the calcium was observed at the end of ninetysix hours. The calcium level of the kidney at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours was equivalent to 5.72 ± 0.342, 6.20 ± 0.141, 7.34 ± 0.307, 6.08 ± 0.248, 7.52 ± 0.172, 15.14 ± 0.167 and 6.72 ± 0.278 mg respectively.

The mallards exposed to **50.0% M.E.** exhibited a significant increase in the calcium level of the kidney, but the calcium level was fluctuating between 6.62 to 11.62 mg. The maximum increase in the calcium level was observed at the end of six hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the kidney calcium level was

equivalent to 11.62 ± 0.172, 10.22 ± 0.248, 10.48 ± 0.285, 9.34 ± 0.257, 6.62 ± 0.248, 8.34 ± 0.241 and 6.86 ± 0.101 mg respectively.

The **100.0% M.E.** induced the highest elevation of calcium level at the end of six hours but afterwards the calcium level exhibited wide fluctuations. The calcium concentration of the kidney was equivalent to 21.8 ± 0.493, 18.02 ± 0.177, 20.1 ± 0.532, 3.82 ± 0.146, 4.44 ± 0.344, 6.34 ± 0.205 and 9.42 ± 0.172 mg at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours respectively.

TABLE NO. 8 : THE CHANGES IN THE CALCIUM LEVLES IN KIDNEY UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		2.1 ±0.531									
0.01%	M.E.		← No significant change →							4.82 ±0.31	5.38 ±0.185
0.1%	M.E.		2.25 ±0.111	2.74 ±0.101	1.825 ±0.311	5.14 ±0.449	4.12 ±0.146	3.94 ±0.135	8.36 ±0.215	N.D.	N.D.
1%	M.E.		4.28 ±0.584	5.3 ±0.141	4.64 ±0.215	4.84 ±0.30	4.1 ±0.14	5.6 ±0.260	6.08 ±0.146	N.D.	N.D.
5%	M.E.		5.3 ±0.701	6.52 ±0.56	6.58 ±0.278	4.82 ±0.324	8.7 ±0.22	6.02 ±0.483	6.4 ±0.303	N.D.	N.D.
10%	M.E.		5.72 ±0.342	6.20 ±0.141	7.34 ±0.307	6.08 ±0.248	7.52 ±0.172	15.14 ±0.167	6.72 ±0.278	N.D.	N.D.
50%	M.E.		11.62 ±0.172	10.22 ±0.248	10.48 ±0.285	9.34 ±0.257	6.62 ±0.248	8.34 ±0.241	6.86 ±0.101	N.D.	N.D.
100%	M.E.		21.8 ±0.493	18.02 ±0.177	20.1 ±0.532	3.82 ±0.146	4.44 ±0.344	6.34 ±0.205	9.42 ±0.172	N.D.	N.D.

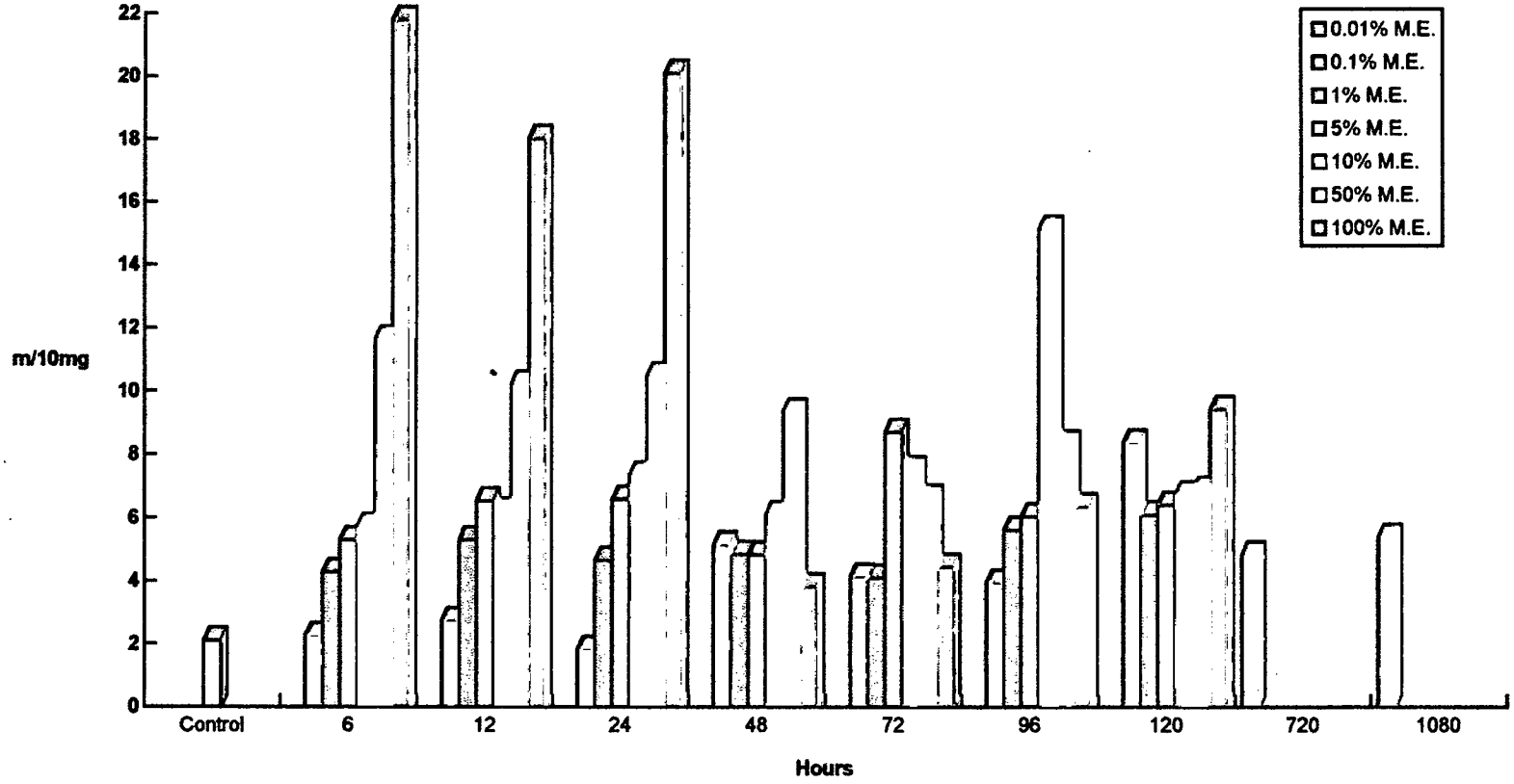
Note : Unit : mg/100mg wet weight of the tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 8 : KIDNEY - CALCIUM

Units : mg/100 mg wet weight of tissue



9] CHLORIDE :

The changes in the chloride contents of the kidney of mallards exposed to the various concentrations of the mining effluents at different time intervals are compiled in **Table No. 9** and are shown graphically in **Graph No. 9** where the changes in the chloride contents are given as a function of concentrations of mining effluents over the different time intervals. The chloride contents are expressed as milliequivalence per 100 mg of the wet weight of the tissue. The control animals showed the chloride contents equivalent to 20.0 ± 1.7 mg /100 mg of the wet weight of the tissue. From the table it appears that the 50.0% and 100.0% M.E. induced over four fold increase in the kidney chloride contents, except a few time intervals.

The birds exposed to **0.01% M.E.** did not show any change in the chloride contents up to seven twenty hours, but at the end of seven twenty and one thousand and eighty hours the kidney chlorides raised to 29.3 ± 0.949 and 38.74 ± 1.10 mEqs respectively.

The **0.1% M.E.** induced significant reductions in the chloride contents but at all the time intervals except six hours. The chloride contents remained below the level found in the control animals. The chloride contents found in the kidney were equivalent to 22.8 ± 2.22, 8.375 ± 0.39, 17.36 ± 0.77, 18.4 ± 0.477, 18.62 ± 0.503, 15.06 ± 0.574 and 14.12 ± 0.305 mEqs at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

When the ducks were exposed to **1.0% M.E.**, the kidney exhibited decrease in chloride contents at all the time intervals under study except at the end of ninetysix hours. The chloride contents of the kidney at the end of six twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours were

equivalent to 17.6 ± 0.316, 16.22 ± 0.416, 17.74 ± 0.241, 7.76 ± 0.69, 5.36 ± 0.349, 41.50 ± 1.0 and 16.3 ± 2.9 mEqs respectively.

The **5.0% M.E.** induced increase in the concentrations of chlorides over that found in the controls, but the chloride values fluctuated between 24.0 and 57.0 mEqs. The chloride contents of the kidney were equivalent to 30.0 ± 1.8, 24.0 ± 0.05, 49.0 ± 1.36, 38.0 ± 2.6, 43.0 ± 1.01, 57.0 ± 2.2 and 37.0 ± 1.09 at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The animals exposed to **10.0% M.E.** showed decrease in the chloride contents at the end of six hours, while the highest concentration was shown at the end of ninetysix hours. The chloride contents fluctuated between 17.0 mEqs and 53 mEqs. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the chloride contents of the kidney were equivalent to 17.0 ± 1.72, 26.0 ± 0.4, 33.0 ± 0.7, 32.0 ± 1.0, 33.0 ± 0.57, 53.0 ± 2.56 and 37.0 ± 1.4 mEqs respectively.

On exposure to **50.0% M.E.** the birds exhibited a remarkable increase in the kidney chloride contents. The chloride contents showed a gradual increase from the end of six hours to the end of twenty four hours. The kidney exhibited chloride contents equivalent to 87.8 ± 1.326, 88.5 ± 0.5, 89.8 ± 1.3, 85.8 ± 1.72, 89.1 ± 1.341, 44.43 ± 0.79 and 42.5 ± 1.239 mEqs at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **100.0% M.E.** induced the peak increase in the chloride contents of the kidney at the end of seventytwo hours and the chloride contents fluctuated between 66.6 and 535.0 mEqs. At the end of six twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the kidney showed chloride contents equivalent to 89.2 ± 1.16, 84.75 ± 0.829, 88.4 ± 1.019, 88.6 ± 0.8, 535 ± 3.86, 66.6 ± 1.85 and 106.6 ± 3.92 mEqs respectively.

TABLE NO. 9 : THE ALTERATIONS IN THE CHLORIDE CONTENTS OF THE KIDNEY UNDER THE INFLUENCE OF MINING EFFLUENTS.

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	20.0 ±1.7									
0.01% M.E.		← No significant change →							29.3 ±0.949	38.74 ±1.10
0.1% M.E.		22.8 ±2.22	8.375 ±0.39	17.36 ±0.77	18.4 ±0.477	18.62 ±0.503	15.06 ±0.574	14.12 ±0.305	N.D.	N.D.
1% M.E.		17.6 ±0.316	16.22 ±0.416	17.74 ±0.241	7.76 ±0.69	5.36 ±0.349	41.50 ±1.0	16.3 ±2.9	N.D.	N.D.
5% M.E.		30.0 ±1.8	24.0 ±0.5	49.0 ±1.36	38.0 ±2.6	43.0 ±1.01	57.0 ±2.2	37.0 ±1.09	N.D.	N.D.
10% M.E.		17.0 ±1.72	26.0 ±0.4	33.0 ±0.7	32.0 ±1.0	33.0 ±0.57	53.0 ±2.56	37.0 ±1.4	N.D.	N.D.
50% M.E.		87.8 ±1.326	88.5 ±0.5	89.8 ±1.3	85.8 ±1.72	89.1 ±1.341	44.43 ±0.79	42.5 ±1.239	N.D.	N.D.
100% M.E.		89.2 ±1.16	84.75 ±0.829	88.4 ±1.019	88.6 ±0.8	535.0 ±3.86	66.6 ±1.85	106.6 ±3.92	N.D.	N.D.

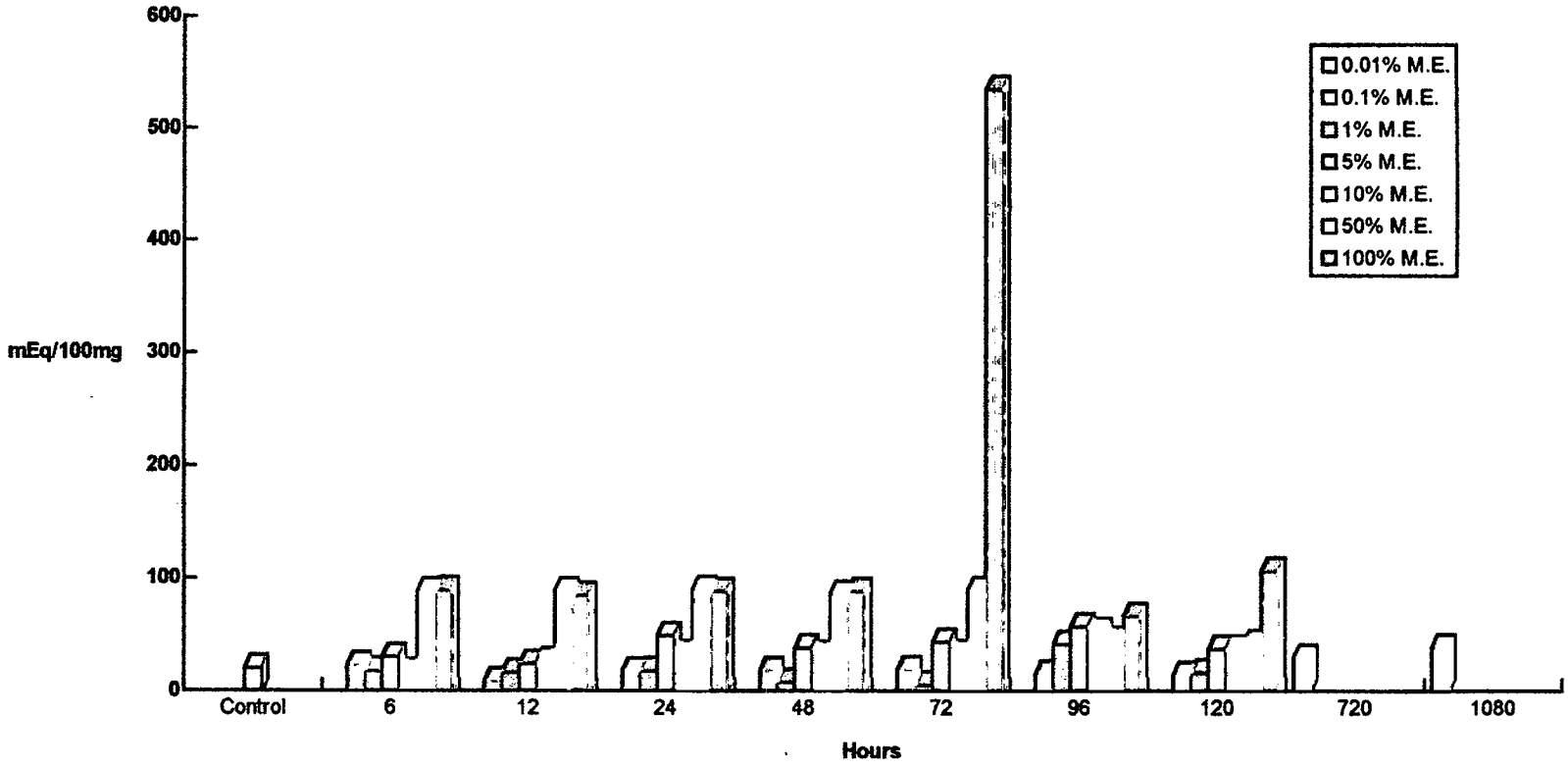
Note : Unit : mEq/100mg wet weight of the tissue.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 9 : KIDNEY - CHLORIDE

Units : mEq/100mg of wet weight tissue.



CHAPTER III

SECTION - B

URINE

A] ACID PHOSPHATASE :

The changes in the renal clearance of acid phosphatase (AP) of the ducks exposed to the different concentrations of mining effluents at different time intervals are compiled in **Table No. 10** and are graphically given in **Graph No. 10.** The activities of the acid phosphatase were calculated on the basis of p-nitrophenol unit (µmol) per hundred millilitres of urine. The control animals did not show any acid phosphatase activity in the urine indicating no renal clearance of the acid phosphatase.

From the table it appears that only at the end of ninetysix hours there was concentration dependent rise in the renal clearance of the acid phosphatase for all concentrations under study, while for the time intervals of twelve and forty eight hours the concentration dependent increase in the renal clearances of AP was observed for **0.1, 1.0, 5.0, 10.0, and 50.0 M.E.** The highest AP activity in the urine was found at the end of fortyeight hours under the influence of **50.0% M.E.**

The birds exposed to **0.01% M.E.** did not show any AP activity in the urine up to seven hundred and twenty hours, but at the end of seven twenty and one thousand and eighty hours the enzyme activity was equivalent to 57.408 ± 5.202 and 142.416 ± 7.314 was observed.

The **0.1% M.E.** induced progressive renal clearance of AP for all the time intervals under study except twentyfour hours. The AP activity in the urine at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 81.42 ± 6.19, 95.496 ± 6.553, 93.288 ± 6.624, 103.224 ± 3.941, 137.724 ± 5.671, 144.348 ± 5.823 and 168.912 ± 6.499 units respectively.

The birds exposed to **1.0% M.E.** showed progressive increase in the renal clearance of AP for all the time intervals under study, except seventy two

hours and one twenty hours, as depicted by the increase in the urinary AP activity. The urinary AP activity was equivalent to 108.744 ± 6.003, 118.404 ± 6.941, 135.792 ± 8.252, 139.104 ± 4.890, 127.65 ±5.299, 160.356 ± 6.669 and 146.832 ± 8.528 units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours.

Under the influence of **5.0% M.E.** the mallards exhibited progressive increase in the renal clearance of AP, as seen by the increased AP activity in the urine, for all the time intervals except fortyeight hours. The urinary AP activity was equivalent to 139.38 ± 5.423, 175.26 ± 5.299, 192.096 ± 6.430, 185.472 ± 7.383, 214.452 ± 6.196, 235.704 ± 9.211 and 253.092 ± 7.383 units respectively.

The ducks exposed to **10.0% M.E.** showed progressive increase in the renal clearance of AP upto the end of seventytwo hours only. The urinary AP activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 176.088 ± 5.796, 209.208 ± 7.866, 231.564 ± 5.878, 264.564 ± 8.142, 302.772 ± 8.790, 280.692 ± 6.207 and 302.772 ±8.293 units respectively.

The exposure to **50.0% M.E.** induced wide fluctuations in the renal clearance of AP but the highest renal clearance of AP was induced at the end of fortyeight hours. The urinary AP activity was equivalent to 167.256 ± 9.850, 332.856 ± 7.111, 137.448 ± 3.750, 651.084 ± 6.948, 369.84 ± 5.789, 533.508 ± 7.014 and 560.28 ± 9.066 units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **100.0% M.E.** induced progressive renal clearance of AP up to the end of fortyeight hours, but at later hours it fluctuated significantly. The urinary AP activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 94.392 ± 4.747, 129.444 ± 4.885, 367.90 ± 5.827, 518.604 ±6.486, 183.54 ± 5.351, 558.624 ± 7.424 and 282.9 ± 6.679 units respectively.

TABLE NO. 10 : THE CHANGES IN THE URINE ACID PHOSPHATASES UNDER THE INFLUENCE OF MINING EFFLUENTS

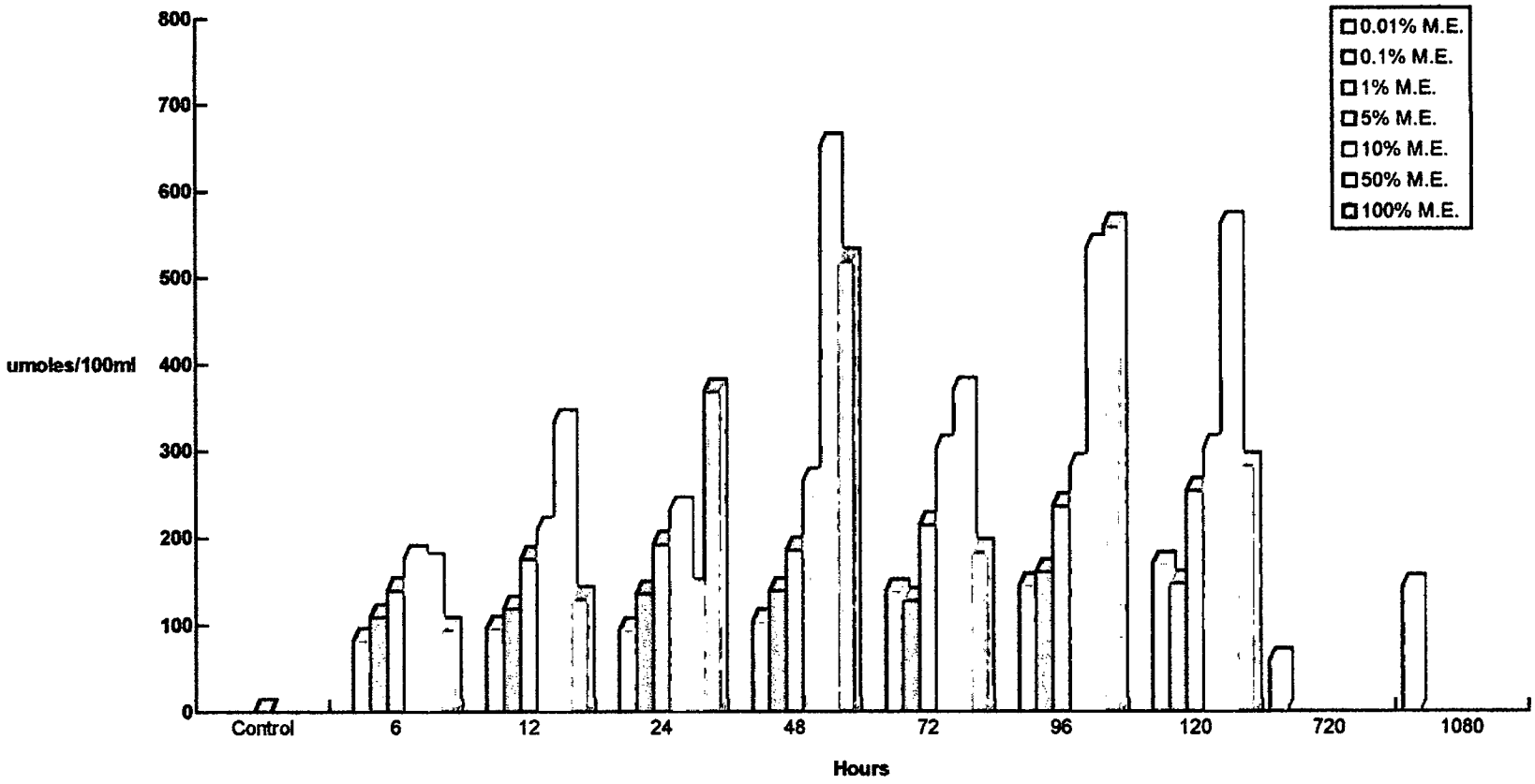
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		0.00 ±0.00									
0.01%	M.E.		← No significant change →							57.408 ±5.202	142.416 ±7.314
0.1%	M.E.		81.42 ±6.19	95.496 ±6.553	93.288 ±6.624	103.224 ±3.941	137.724 ±5.671	144.348 ±5.823	168.912 ±6.499	N.D.	N.D.
1%	M.E.		108.744 ±6.003	118.404 ±6.941	135.792 ±8.252	139.104 ±4.890	127.65 ±5.299	160.356 ±6.669	146.832 ±8.528	N.D.	N.D.
5%	M.E.		139.38 ±5.423	175.26 ±5.299	192.096 ±6.430	185.472 ±7.383	214.452 ±6.196	235.704 ±9.211	253.092 ±7.383	N.D.	N.D.
10%	M.E.		176.088 ±5.796	209.208 ±7.866	231.564 ±5.878	264.564 ±8.142	302.772 ±8.790	280.692 ±6.207	302.772 ±8.293	N.D.	N.D.
50%	M.E.		167.256 9.850	332.856 ±7.111	137.448 ±3.750	651.084 ±6.948	369.84 ±5.789	533.508 7.014	560.28 ±9.066	N.D.	N.D.
100%	M.E.		94.392 ±4.747	129.444 ±4.885	367.90 ±5.827	518.604 ±6.486	183.54 ±5.351	558.624 7.424	282.9 ±6.679	N.D.	N.D.

Note : Unit : p-nitrophinol µmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 10 : URINE - ACID PHOSPHATASE
Units : μmoles/100 ml.



B] ALKALINE PHOSPHATASE :

The renal clearances of alkaline phosphatase (AlP) are tabulated in **Table No. 11** and are given graphically in **Graph No. 11.** The control animals did not show any AlP activity in the urine. The alkaline phosphatase activity is expressed as mmole units per 100 ml of urine. From the graph it is observed that the dose (concentration of M.E.) dependent increase in the renal clearance of AlP was induced in the ducks at the end of twelve and ninetysix hours, while such a relationship was observed at the end of twelve and one twenty hours for **0.1, 1.0, 5.0, 10.0 and 50.0 percent concentrations of M.E.**

The birds exposed to **0.01%** M.E. did not show any AlP activity in the urine up to twentynine days, but at the end of thirty days (seven hundred and twenty hours) the urine showed the AlP activity equivalent to 0.4 ± 0.053 and at the end of one thousand and eighty hours the urinary AlP activity was equivalent to 0.85 ± 0.062 mmole units.

The **0.1%** M.E. induced progressive renal clearance of AlP for all the time intervals under study except twentyfour hours. The AlP urinary activity was equivalent to 0.5 ± 0.040, 0.7 ± 0.034, 0.6 ± 0.04, 0.8 ± 0.048, 0.95 ± 0.066, 1.1 ± 0.082 and 1.3 ± 0.071 units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

On exposure to **1.0 %** M.E. the birds showed progressive urinary clearance of AlP for all the time intervals under study except forty eight hours and in general the renal clearance of AlP was higher than that induced by **0.1%** M.E. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the urinary AlP activity was equivalent to 0.75 ± 0.049, 1.0 ± 0.034, 1.15 ± 0.046, 0.95 ± 0.051, 1.3 ± 0.054, 1.45 ± 0.064 and 1.65 ± 0.020 mmole units respectively.

The **5.0% M.E.** induced significant increase and fluctuations in the renal clearance of AIP. The urinary AIP activity was equivalent to 1.4 ± 0.064, 1.35 ± 0.166, 1.20 ± 0.034, 1.75 ± 0.063, 1.45 ± 0.079, 1.85 ± 0.046 and 2.85 ± 0.112 mmole units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The ducks exposed to **10.0% M.E.** exhibited fluctuations in the renal clearance of AIP and the maximum renal clearance was seen at the end of one twenty hours. The urinary AIP activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 1.35 ± 0.04, 1.40 ± 0.068, 1.85 ± 0.091, 2.10 ± 0.129, 2.90 ± 0.092, 3.10 ± 0.092 and 5.80 ± 0.094 mmole units respectively.

Under the influence of **50.0% M.E.** the ducks exhibited highest renal clearance of AIP at the end of one twenty hours, while at the other time intervals there were fluctuations in the renal clearances. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was eqivalent to 1.50 ± 0.068, 3.50 ± 0.109, 1.30 ± 0.652, 5.10 ± 0.052, 1.30 ± 0.131, 4.15 ± 0.078 and 13.60 ± 0.066 mmole units respectively.

The **100.0% M.E.** exposure of ducks induced fluctuations in the renal clearance of AIP. The urinary AIP activity was equivalent to 5.40 ± 0.119, 3.50 ± 0.085, 4.90 ± 0.123, 10.80 ± 0.091, 2.85 ± 0.120, 4.30 ± 0.149 and 2.40 ± 0.052 mmole units at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

TABLE NO. 11 : THE EFFECT OF MINING EFFLUENTS ON URINE ALKALINE PHOSPHOTASES.

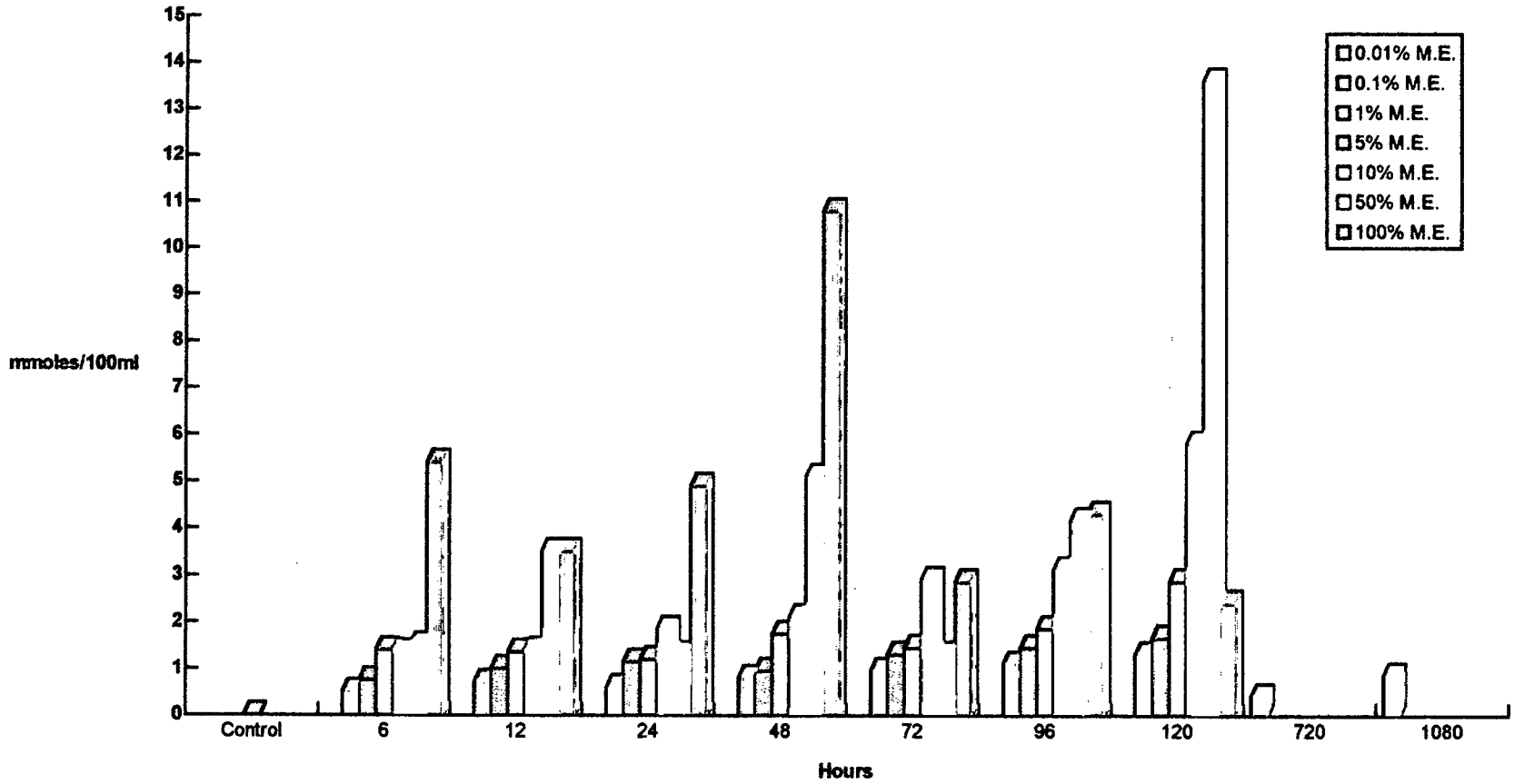
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		0.00 ±0.00									
0:01%	M.E.		← No significant change →							0.4 ±0.053	0.85 ±0.0624
0.1%	M.E.		0.50 ±0.04	0.70 ±0.034	0.60 ±0.04	0.80 ±0.048	0.95 ±0.066	1.10 ±0.082	1.30 ±0.071	N.D.	N.D.
1%	M.E.		0.75 ±0.049	1.0 ±0.034	1.15 ±0.046	0.95 ± 0.051	1.3 0 ± 0.054	1.45 ± 0.064	1.65 ±0.020	N.D.	N.D.
5%	M.E.		1.40 ± 0.064	1.35 ±0.166	1.20 ± 0.034	1.75 ± 0.063	1.45 ± 0.079	1.85 ± 0.046	2.85 ± 0.112	N.D.	N.D.
10%	M.E.		1.35 ± 0.04	1.40 ±0.068	1.85 ± 0.091	2.10 ± 0.129	2.9 ± 0.092	3.10 ± 0.092	5.80 ± 0.094	N.D.	N.D.
50%	M.E.		1.50 ±0.068	3.50 ± 0.109	1.30 ± 0.652	5.10 ± 0.052	1.30 ± 0.131	4.15 ± 0.078	13.6 ± 0.066	N.D.	N.D.
100%	M.E.		5.40 ± 0.119	3.50 ± 0.085	4.90 ± 0.123	10.80 ± 0.091	2.85 ± 0.120	4.30 ± 0.149	2.40 ± 0.052	N.D.	N.D.

Note : Unit : mmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 11 : URINE - ALKALINE PHOSPHATASE
Units : mmoles/100 ml.



C] ESTERASE (NON SPECIFIC) :

The changes in the urine Esterase (nonspecific) activities are compiled in **Table No. 12** and are graphically given in **Graph No. 12.** The Esterase (nonspecific) activity is expressed as μmoles per hundred millilitre of urine. The control birds did not show any renal clearance of Esterase.

From the graph it appears that the urinary excretion of Esterase was dose dependent (M.E. concentration dependent) at the end of twelve, twentyfour, and fortyeight hours. The highest urinary esterase activity was observed at the end of twelve hours under the influence of **100% M.E.** and generally all the M.E. concentrations induced increased urinary Esterase activity.

The birds exposed to **0.01% M.E.** did not show any Esterase activity up to twentynine days but at the end of thirty days (Seventwenty hours). the urinary Esterase activity was equivalent to 100.0 ± 3.098 μmoles and it shot up to 165.0 ± 3.929 μmoles at the end of one thousand and eighty hours.

The **0.1% M.E.** induced progressive increase in the urinary Esterase activity for all the time intervals under study except ninetysix hours. The Esterase activity was equivalent to 205.0 ± 2.059, 270.0 ± 3.611, 315.0 ± 3.059, 345.0 ± 2.059, 370.0 ± 5.851, 315.0 ± 3.261, and 425.0 ± 3.847 units (μmoles) at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours respectively.

The mallards exposed to **1.0% M.E.** exhibited increased urinary Esterase activity indicating increased renal clearance of the enzyme. The urinary enzyme activity showed significant fluctuations and the maximum activity was observed at the end of one twenty hours. The urinary esterase activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty

hours was equivalent to 585.0 ± 3.929, 490.0 ± 2.828, 470.0 ± 2.154, 445.0 ± 3.762, 530.0 ± 7.054, 575.0 ± 3.059 and 620.0 ± 3.006 µmoles respectively.

Under the influence of **5.0% M.E.** the ducks showed some fluctuations in the esterase activity but the enzyme activity did not increase much in comparison to the increase observed under the influence of **1.0% M.E.** The Esterase activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 510.0 ± 2.653, 510.0 ± 2.15, 485.0 ± 3.611, 530.0 ± 2.497, 530.0 ± 5.215, 615.0 ± 6.829 and 645.0 ± 3.249 µmoles respectively.

The **10.0% M.E.** exposure induced rise in the urinary esterase activity but the rise was not dependent upon the exposure duration. Thus, the urinary Esterase activity was equivalent to 575.0 ± 4.578, 600.0 ± 3.544, 510.0 ± 4.923, 630.0 ± 5.810, 645 ± 5.381, 585.0 ± 5.381 and 700.0 ± 4.223 µmoles at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The exposure to **50.0% M.E.** induced further increase in the urinary esterase activity and the maximum activity was found at the end of ninetysix hours. The urinary esterase activity at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 760.0 ± 4.40, 680.0 ± 2.856, 560.0 ± 1.720, 750.0 ± 1.93, 700.0 ± 3.898, 800.0 ± 2.712 and 750.0 ± 2.856 µmoles respectively.

The ducks exposed to **100.0% M.E.** showed many fold increase in the urinary esterase activity. The highest increase in the enzyme activity was observed at the end of twelve hours but the activity decreased very significantly at the end of ninetysix hours. The urinary esterase activity was equivalent to 960.0 ± 3.633, 1560.0 ± 3.006, 680.0 ± 4.45, 1020.0 ± 2.416, 595.0 ± 1.720, 210.0 ± 2.315 and 405.0 ± 5.706 µmoles at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE 12 : THE EFFECT OF MINING EFFLUENTS ON URINE ESTERASES (NONSPECIFIC)

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	0.00 ± 0.00									
0.01% M.E.		← No significant change →							100 ± 3.098	165 ± 3.929
0.1% M.E.		205 ± 2.059	270 ± 3.611	315 ± 3.059	345 ± 2.059	370 ± 5.851	315 ± 3.261	425 ± 3.847	N.D.	N.D.
1% M.E.		585 ± 3.929	490 ± 2.828	470 ± 2.154	445 ± 3.762	530 ± 7.054	575 ± 3.059	620 ±3.006	N.D.	N.D.
5% M.E.		510 ± 2.653	510 ± 2.15	485 ±3.611	530 ± 2.497	530 ± 5.215	615 ± 6.829	645 ± 3.249	N.D.	N.D.
10% M.E.		575 ± 4.578	600 ± 3.544	510 ± 4.923	630 ±5.810	645 ± 5.381	585 ± 5.381	700 ± 4.223	N.D.	N.D.
50% M.E.		760 ± 4.4	680 ± 2.856	560 ± 1.720	750 ± 1.93	700 ± 3.898	800 ± 2.712	750 ± 2.856	N.D.	N.D.
100% M.E.		960 ± 3.633	1560 ± 3.006	680 ± 4.45	1020 ± 2.416	595 ± 1.720	210 ± 2.315	405 ± 5.706	N.D.	N.D.

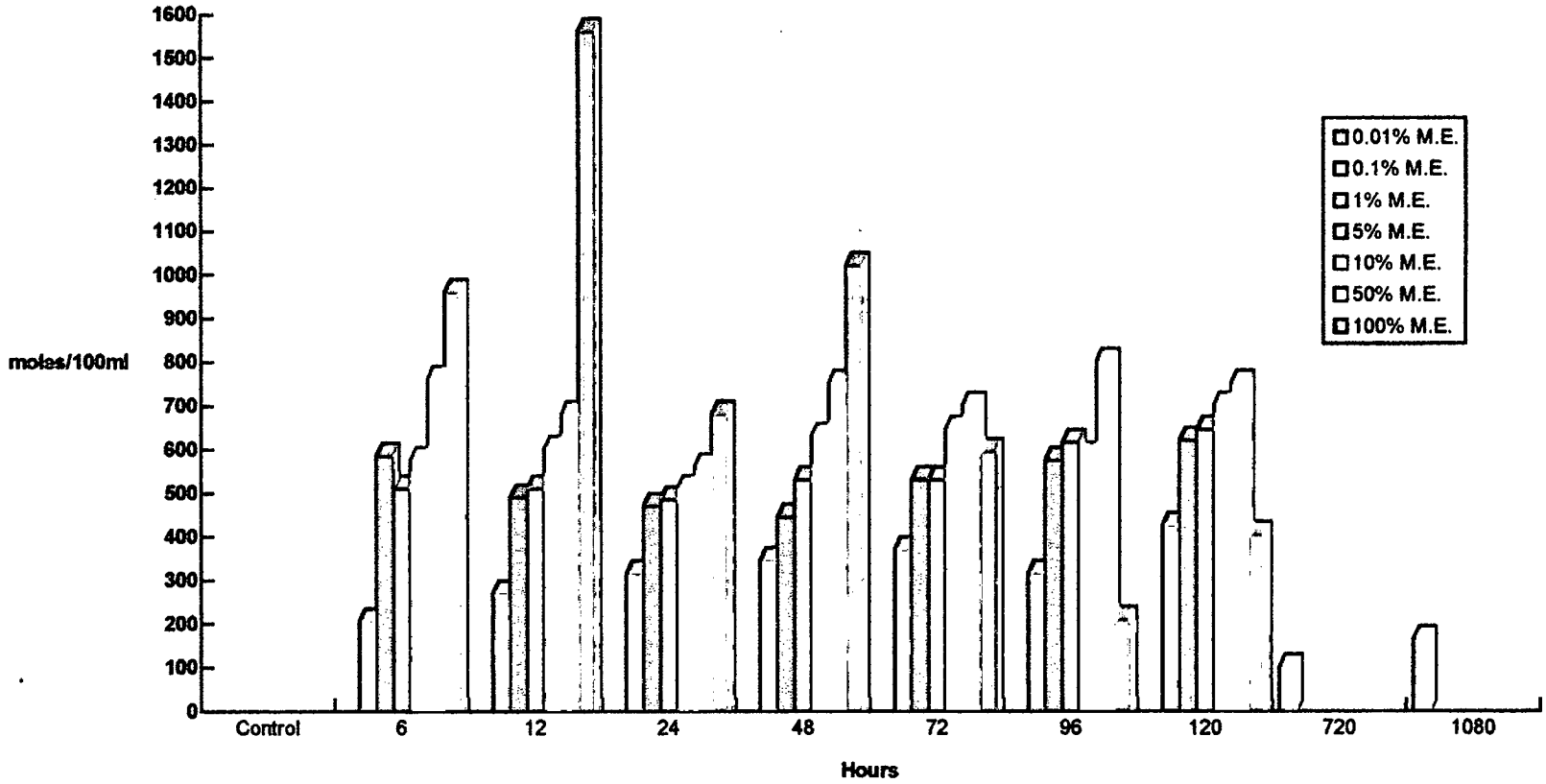
Note : Unit : µmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - P < 0.01.

GRAPH NO. 12 : URINE - ESTERASE (NON SPECIFIC)

Units : µmoles/100 ml.



D] PROTEINS :

The **Table No. 13** shows the variations in the protein contents of the urine of mallards exposed to the different concentrations of the mining effluents for various time intervals and the same is graphically given in **Graph No.13.** The proteins from the urine are expressed as gram per litre of the urine. The urine of the control animals did not show any proteins. From the table it is obvious that the birds under the influence of mining effluents promoted the renal clearance of proteins but renal clearance of proteins was neither dose nor time dependent.

The **0.01% M.E.** did not induce renal clearance of proteins upto twentynine days exposure, but at the end of thirty days (seven twenty hours) the renal clearance was equivalent to 3.30 ± 0.13 g/L and it elevated to 4.6 ± 0.21 g/L at the end of one thousand and eighty hours.

The animals exposed to **0.1% M.E.** showed fluctuations in the renal clearance of proteins and the urine exhibited decrease in proteins clearance than that observed under the influence of **0.01% M.E.** The urine proteins at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours amounted to 2.68 ± 0.116, 2.96 ± 0.101, 2.94 ± 0.10, 2.48 ± 0.172, 2.82 ± 0.14, 2.96 ± 0.101 and 2.70 ± 0.228 grams per litre respectively.

Under the influence of **1.0% M.E.** the renal clearance of proteins fluctuated between 2.16 g and 3.72 g per litre of urine. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of the proteins was equivalent to 3.175 ± 0.147, 2.16 ± 0.101, 3.72 ± 0.213, 2.64 ± 0.215, 2.38 ± 0.23, 3.38 ± 0.172 and 3.64 ± 0.30 grams per litre respectively.

The ducks exposed to **5.0% M.E.** showed the renal clearance of proteins less than 3.4 g. The urine showed presence of 2.70 ± 0.244, 2.48 ± 0.16, 2.32

± 0.172, 2.40 ± 0.178, 2.46 ± 0.185, 3.40 ± 0.282 and 2.54 ± 0.162 grams of proteins per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

Under the influence of **10.0% M.E.** the renal clearance of proteins varied between 2.54 g and 2.74 grams. The urine showed the presence of 2.68 ± 0.17, 2.68 ± 0.130, 2.74 ± 0.135, 2.54 ± 0.257, 2.62 ± 0.172, 2.72 ± 0.16 and 2.56 ± 0.215 grams of proteins per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours respectively.

The **50.0% M.E.** treatment to the ducks promoted increase in the renal clearance of proteins. The maximum clearance was observed at the end of ninetysix hours, while minimum clearance was seen at the end of twentyfour hours. Thus, the renal clearance of proteins amounted to 3.22 ± 0.172, 3.96 ± 0.101, 2.96 ± 0.101, 4.16 ± 0.185, 3.2 ± 0.14, 4.28 ± 0.172 and 3.6 ± 0.129 grams per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **100.0% M.E.** induced significant fluctuations in the protein contents of the urine and the highest quantity was observed at the end of twentyfour hours. The urine protein level was equivalent to 3.20 ± 0.228, 3.54 ± 0.21, 4.46 ± 0.162, 3.94 ± 0.101, 3.98 ± 0.172, 2.36 ± 1.132 and 2.41 ± 0.100 grams per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

TABLE NO. 13 : URINE PROTEIN LEVELS UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		0									
0.01%	M.E.		← No significant change →							3.30 ±0.13	4.60 ±0.21
0.1%	M.E.		2.68 ±0.116	2.96 ±0.101	2.94 ±0.10	2.48 ±0.172	2.82 ±0.14	2.96 ±0.101	2.70 ±0.228	N.D.	N.D.
1%	M.E.		3.175 ±0.147	2.16 ±0.101	3.72 ±0.213	2.64 ±0.215	2.38 ±0.23	3.38 ±0.172	3.64 ±0.300	N.D.	N.D.
5%	M.E.		2.70 ±0.244	2.48 ±0.16	2.32 ±0.172	2.40 ±0.178	2.46 ±0.185	3.40 ±0.282	2.54 ±0.162	N.D.	N.D.
10%	M.E.		2.68 ±0.17	2.68 ±0.130	2.74 ±0.135	2.54 ±0.257	2.62 ±0.172	2.72 ±0.16	2.56 ±0.215	N.D.	N.D.
50%	M.E.		3.22 ±0.172	3.96 ±0.101	2.96 ±0.101	4.16 ±0.185	3.20 ±0.14	4.28 ±0.172	3.60 ±0.129	N.D.	N.D.
100%	M.E.		3.20 ±0.228	3.54 ±0.21	4.46 ±0.162	3.94 ±0.101	3.98 ±0.172	2.36 ±1.132	2.41 ±0.100	N.D.	N.D.

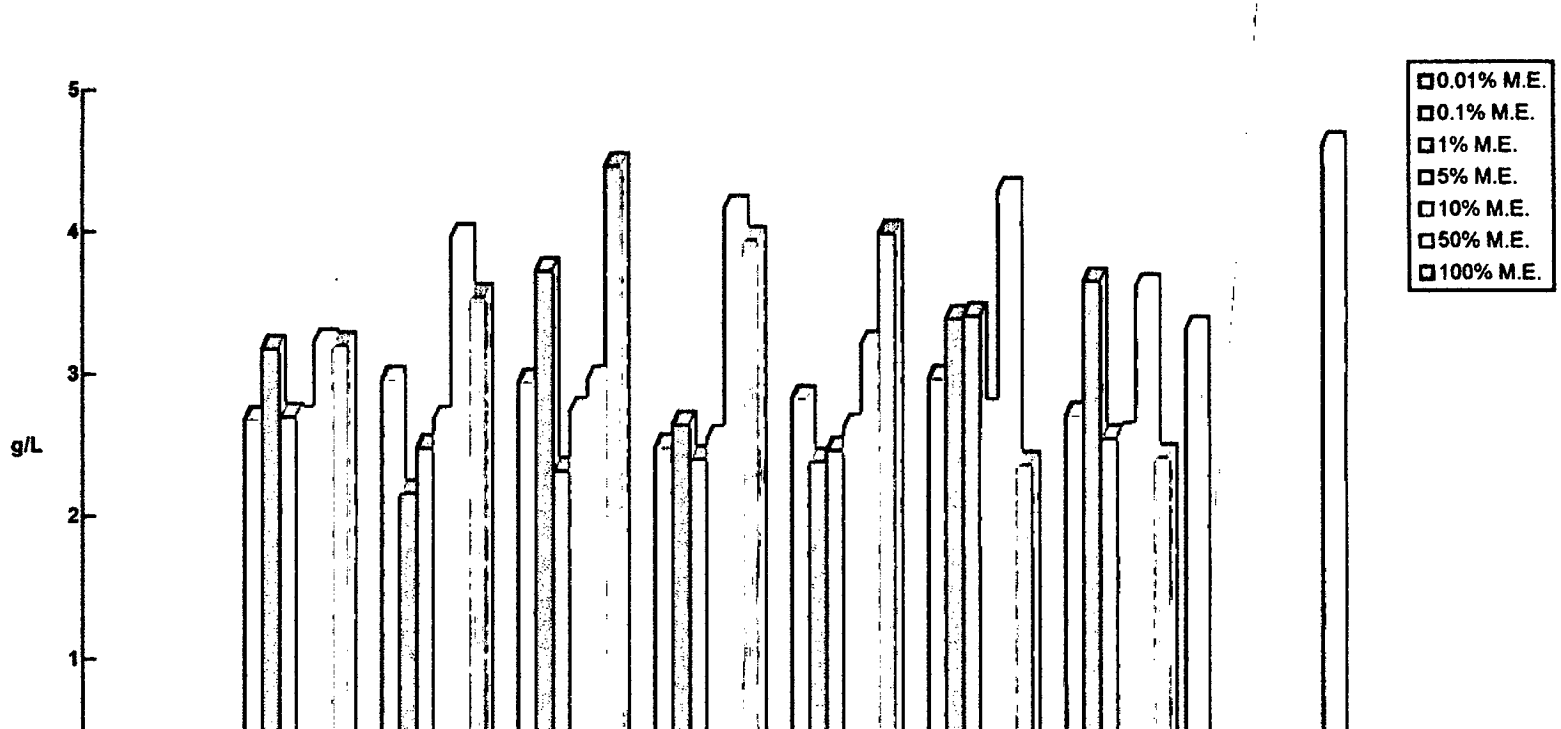
Note : Unit : Urine protein in g/L.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 13 : URINE - PROTEINS

Units : g/L



E] UREA :

The changes in the urinary clearance of urea under the influence of different concentrations of mining effluents at various time intervals are compiled in **Table No. 14** and are graphically presented in **Graph No. 14.** The urea from the urine is expressed as milligrams of urea per hundred millilitre of urine. The control birds showed 16.70 ± 2.07 mg of urea in the urine.

From the graph it appears that the renal clearance of urea was profusely influenced by the mining effluents and the renal clearance of urea showed dose (concentration of M.E.) dependency at the end of twelve hours but such a relationship was not observed for any other time interval under investigation. The maximum renal clearance of urea was observed at the end of one twenty hours under the influence of **100% M.E.**

The ducks exposed to **0.01% M.E.** did not exhibit any significant change in the renal clearance of urea up to the end of twenty nine days, but at the end of thirty days (sevenhundred and twenty hours) the urea level increased to 19.8 ± 3.10 mg/litre while at the end of one thousand and eighty hours the urea level raised to 32.9 ± 4.90 mg/litre.

The **0.1% M.E.** exposure promoted fluctuations in the renal clearance of urea. The maximum clearance was observed at the end of one twenty hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the urea concentration in the urine was equivalent to 17.0 ± 5.5, 20.1 ± 3.0, 19.3 ± 1.9, 24.0 ± 2.7, 21.23 ± 2.9, 22.0 ± 3.5 and 27.0 ± 4.10 mg/litre respectively.

The birds exposed to **1.0% M.E.** showed steady increase in the urea clearance up to the end of fortyeight hours, but at the later hours the urea clearance showed fluctuations. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the renal

clearance of urea was equivalent to 19.0 ± 4.9, 21.0 ± 5.1, 23.0 ± 3.9, 24.0 ± 1.40, 19.7 ± 2.0, 26.90 ± 2.2 and 25.0 ± 2.9 mg/litre respectively.

The **5.0% M.E.** influenced the renal clearance of urea significantly and the maximum clearance was found at the end of ninetysix hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of urea was equivalent to 21.3 ± 4.2, 23.0 ± 3.01, 27.70 ± 4.20, 20.20 ± 1.7, 29.0 ± 1.9, 30.3 ± 2.7 and 29.7 ± 2.9 mg/litre respectively.

Under the influence of **10.0% M.E.** the ducks showed maximum renal clearance of urea at the end of one twenty hours and the urea concentration of the urine varied between 18.3 mg and 39.0 mg. The urea contents of the urine were equivalent to 18.3 ± 3.7, 23.5 ± 2.7, 27.0 ± 3.2, 30.2 ± 5.4, 37.0 ± 3.8, 29.3 ± 2.2 and 39.0 ±1.01 mg/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **50.0% M.E.** induced significant alterations in the renal clearance of urea and the urea clearance showed time dependent elevation up to the end of seventy two hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the renal clearance of urea was equivalent to 20.70 ± 2.1, 24.0 ± 5.4, 33.70 ± 5.1, 35.90 ± 3.8, 48.90 ± 4.0, 40.20 ± 2.10 and 42.30 ± 2.56 mg/litre respectively.

The ducks exposed to **100.0% M.E.** promoted successive increase in the renal clearance of urea for all the time intervals under study except ninetysix hours. The maximum urea concentration was found at the end of one twenty hours.

Thus, the renal clearance of urea at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours was equivalent to 27.3 ± 2.1, 29.0 ± 2.2, 34.0 ± 3.0, 37.9 ± 3.2, 49.7 ± 4.8, 47.0 ± 5.7 and 53.70 ± 3.0 mg/litre respectively.

TABLE NO. 14 : THE CHANGES IN THE UREA EXCRETION OF MALLARDS UNDER THE INFLUENCE OF MINING EFFLUENTS.

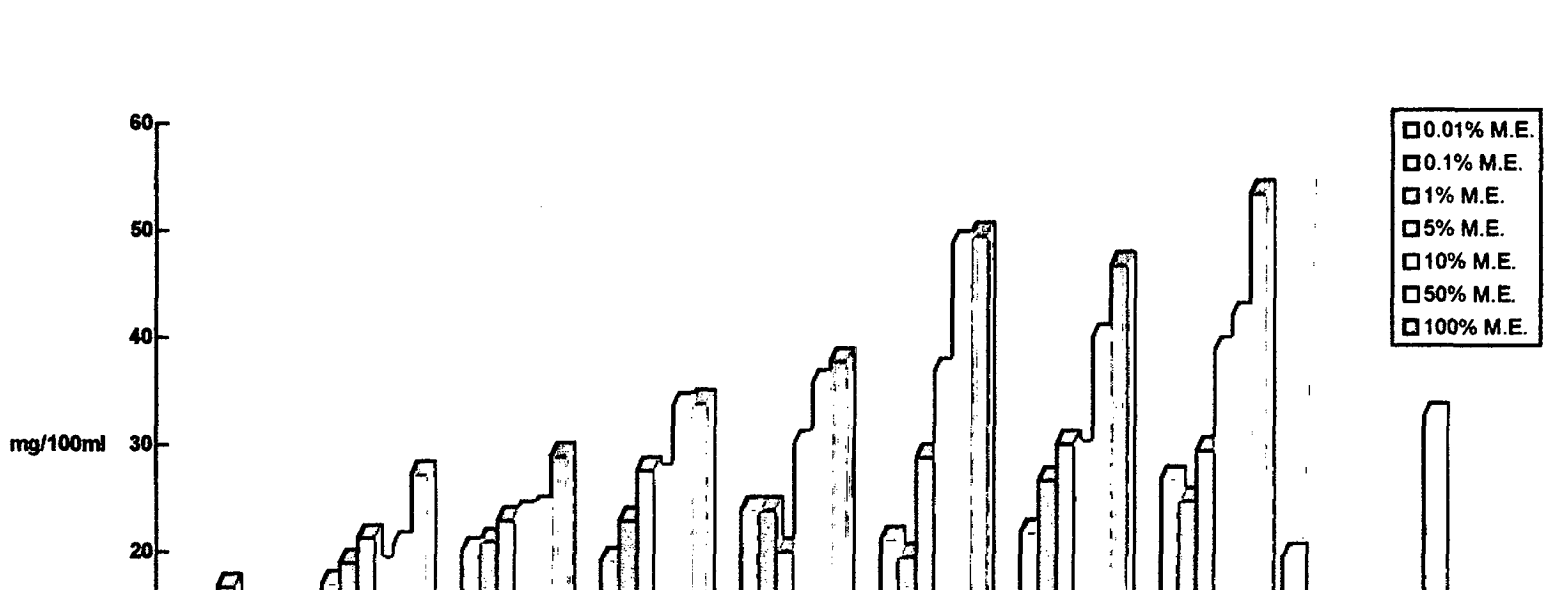
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		16.70 ±2.07									
0.01%	M.E.		← No significant change →							19.80 ±3.10	32.90 ±4.90
0.1%	M.E.		17.0 ±5.50	20.10 ±3.0	19.30 ±1.90	24.0 ±2.70	21.23 ±2.90	22.0 ±3.50	27.0 ±4.10	N.D.	N.D.
1%	M.E.		19.0 ±4.90	21.0 ±5.10	23.0 ±3.90	24.0 ±1.40	19.70 ±2.0	26.90 ±2.20	25.0 ±2.90	N.D.	N.D.
5%	M.E.		21.30 ±4.20	23.0 ±3.01	27.70 ±4.20	20.20 ±1.70	29.0 ±1.90	30.30 ±2.70	29.70 ±2.90	N.D.	N.D.
10%	M.E.		18.30 ±3.70	23.50 ±2.70	27.0 ±3.20	30.20 ±5.40	37.0 ±3.80	29.30 ±2.20	39.0 ±1.01	N.D.	N.D.
50%	M.E.		20.70 ±2.10	24.0 ±5.40	33.70 ±5.10	35.90 ±3.80	48.90 ±4.0	40.20 ±2.10	42.30 ±2.56	N.D.	N.D.
100%	M.E.		27.30 ±2.10	29.0 ±2.20	34.0 ±3.0	37.90 ±3.20	49.70 ±4.80	47.0 ±5.70	53.70 ±3.0	N.D.	N.D.

Note : Unit : Urea in mg/100ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 14 : URINE - UREA
Units : mg/100 ml.



F] URIC ACID :

The changes in the uric acid contents of the urine of mallards exposed to various concentrations of mining effluents at different time intervals are tabulated in **Table No.15** and are graphically expressed in **Graph No. 15.** The birds are uricotelic, therefore normally the ducks excrete 19.0 ± 2.3 milligrams of uric acid per dL of urine. The uric acid in the urine is expressed as milligrams per dL of the urine.

It is evident from the table that the mining effluents promoted wide but significant fluctuations in uric acid excretion. The highest concentration of uric acid was observed at the end of ninetysix hours under the influence of **5% M.E.** while the lowest excretion of uric acid was seen at the end of fortyeight hours under the influence of **10.0% M.E.**

The mallards exposed to **0.01% M.E.** did not exhibit any significant change in the uric acid clearance up to the end of twentynine days, but at the end of thirty days (Seven twenty hours) there was slight increase in the uric acid level and it went on increasing up to the end of one thousand and eighty hours. Thus, the uric acid level at the end of seven twenty and one thousand and eighty hours was equivalent to 23.70 ± 1.7 and 39.732 ± 2.57 mg/dL respectively.

The **0.1% M.E.** induced fluctuations in the renal clearance of uric acid. The maximum clearance of uric acid was observed at the end of seventytwo hours, while the minimum was observed at the end of ninetysix hours. The renal clearance of uric acid was equivalent to 20.1 ± 2.85, 22.3 ± 3.0, 24.1 ± 1.41, 19.78 ± 2.0, 25.1 ± 2.315, 18.3 ± 1.85 and 20.9 ± 1.62 mg/dL at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The birds exposed to **1.0% M.E.** showed fluctuations in the uric acid clearance and the minimum clearance was observed at the end of twelve hours while the maximum clearance was observed at the end of ninetysix hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of uric acid was equivalent to 25.0 ± 3.2, 12.0 ± 2.2, 26.279 ± 1.01, 20.87 ± 3.12, 24.0 ± 2.4, 29.0 ± 2.6 and 20.837 ± 1.6 mg/dL respectively.

The ducks treated with **5.0% M.E.** showed wide fluctuations in the uric acid clearance. The highest renal clearance of uric acid was observed at the end of ninetysix hours, while the lowest clearance was observed at the end of six hours. The urinary uric acid concentrations were equivalent to 19.2 ± 2.4, 28.3 ± 2.8, 76.4 ± 3.1, 25.1 ± 2.56, 23.9 ± 4.8, 87.0 ± 5.3 and 31.5 ± 1.01 mg/dL at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **10.0% M.E.** promoted maximum renal clearance at the end of six hours and then the uric acid levels went on decreasing significantly till the end of fortyeight hours. The renal clearance of uric acid at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 43.5 ± 2.059, 33.2 ± 1.01, 24.8 ± 1.72, 11.5 ± 2.39, 35.9 ± 1.41, 25.2 ± 1.36 and 19.0 ± 2.2 mg/dL respectively.

The **50.0% M.E.** influenced the uric acid clearance significantly. It induced wide fluctuations in the urinary uric acid levels. The uric acid level at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 29.7 ± 4.4, 37.0 ± 3.521, 30.9 ± 4.8, 41.73 ± 3.95, 64.7 ± 2.1, 47.0 ± 3.1 and 53.0 ± 3.52 mg/dL respectively.

The ducks exposed to **100.0% M.E.** exhibited significant fluctuations in the renal clearance of uric acid. The maximum renal clearance of uric acid was observed at the end of seventytwo hours, while the minimum clearance was seen at the end of twelve hours. Thus, at the end of six, twelve, twentyfour,

fortyeight, seventytwo, ninety-six and one hundred and twenty hours the renal clearance of uric acid was equivalent to 38.0 ± 2.39, 32.0 ± 2.87, 47.897 ± 1.7, 43.289 ± 2.607, 73.0 ± 3.92, 57.0 ± 3.5 and 69.0 ± 4.31 mg/dL respectively.

TABLE NO. 15 : THE EFFECT OF MINING EFFLUENTS ON URINE - URIC ACID LEVELS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		19.0 ±2.3									
0.01%	M.E.		← No significant change →							23.70 ±1.70	39.732 ±2.57
0.1%	M.E.		20.1 ±2.85	22.3 ±3.0	24.1 ±1.41	19.78 ±2.0	25.10 ±2.315	18.30 ±1.85	20.9 ±1.62	N.D.	N.D.
1%	M.E.		25.0 ±3.2	12.0 ±2.2	26.279 ±1.01	20.872 ±3.12	24.0 ±2.4	29.0 ±2.6	20.837 ±1.6	N.D.	N.D.
5%	M.E.		19.2 ±2.4	28.3 ±2.8	76.4 ±3.1	25.1 ±2.56	23.9 ±4.8	87.0 ±5.3	31.5 ±1.01	N.D.	N.D.
10%	M.E.		43.5 ±2.059	33.2 ±1.01	24.8 ±1.72	11.5 ±2.39	35.9 ±1.41	25.2 ±1.36	19.0 ±2.2	N.D.	N.D.
50%	M.E.		29.7 ±4.4	37.0 ±3.521	30.90 ±4.8	41.73 ±3.95	64.70 ±2.1	47.0 ±3.1	53.0 ±3.52	N.D.	N.D.
100%	M.E.		38.0 ±2.39	32.0 ±2.87	47.897 ±1.7	43.289 ±2.607	73.0 ±3.92	57.0 ±3.5	69.0 ±4.31	N.D.	N.D.

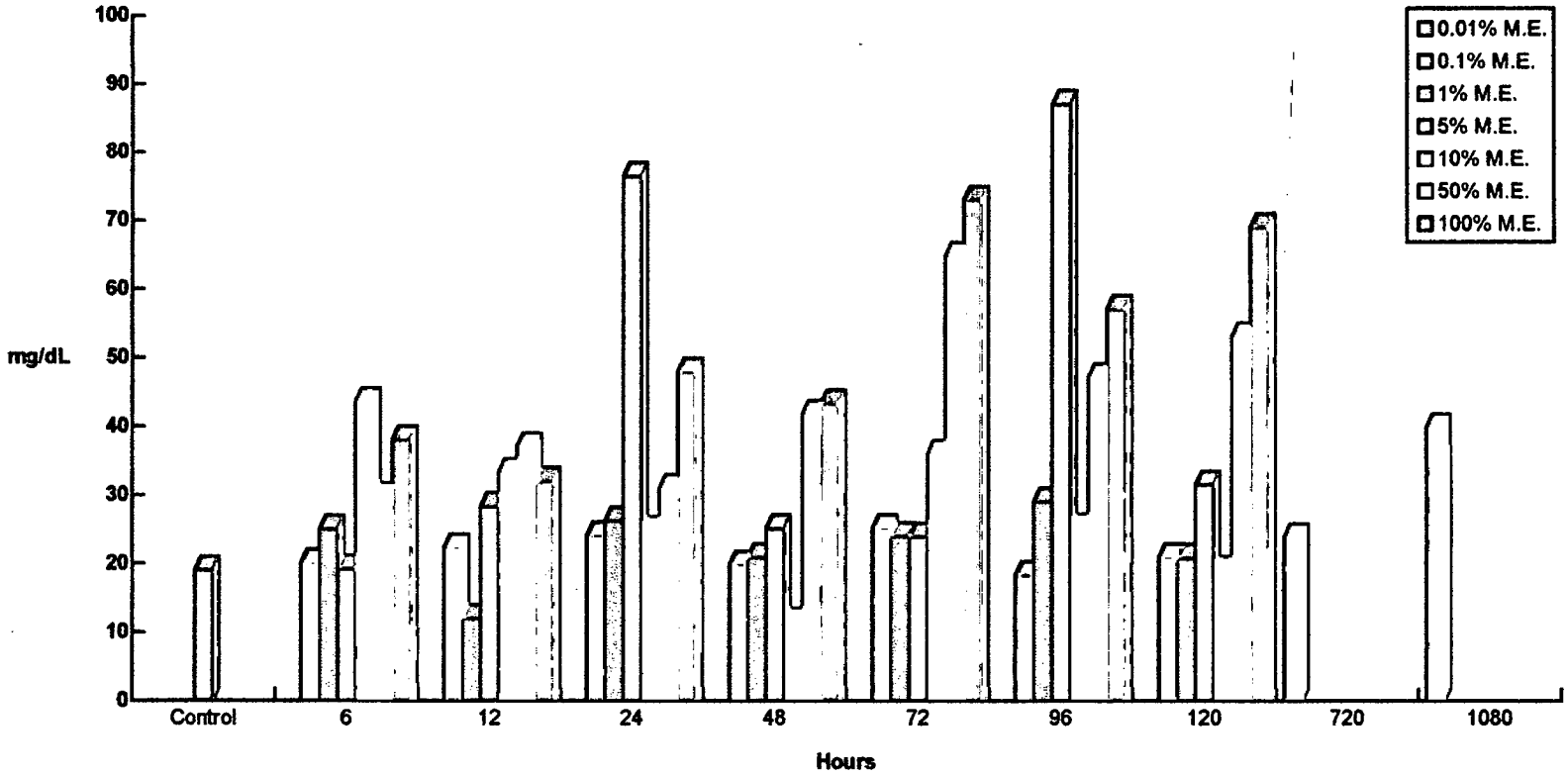
Note : Unit : Uric acid in mg/dl.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 15 : URINE - URIC ACID

Units : mg/dL of the urine.



G] CREATININE :

The **Table No. 16** shows the alterations in the urine creatinine level of the ducks exposed to mining effluents in different concentrations at different time intervals while these changes are graphically given in **Graph No. 16.** The creatinine concentrations of the urine are expressed as milligrams per litre of the urine. The control birds showed the renal clearance of creatinine equal to 0.08 ± 0.01 mg/L.

From the graph it is evident that the renal clearance of creatinine was dose dependent at the end of six and twelve hours, while at the rest of the time intervals it did not exhibit such relationship. The mallards exposed to **0.01%** M.E. did not exhibit any significant change in the urine creatinine levels up to the end of twentynine days but at the end of thirty days there was significant increase in the creatinine clearance which further increased at the end of one thousand and eighty hours. Thus, at the end of seven twenty and one thousand eighty hours the urine craeatinine level was equivalent to 0.248 ± 0.038 and 0.731 ± 0.051 milligrams per litre of urine.

The ducks exposed to **0.1% of M.E.** showed significant fluctuations in the renal clearance of creatinine and the maximum clearance was observed at the end of one twenty hours, while the minimum clearance was seen at the end of fortyeight hours. The renal clearance of creatinine was equivalent to 0.37 ± 0.010, 0.43 ± 0.040, 0.34 ± 0.030, 0.25 ± 0.078, 0.73 ± 0.060, 0.84 ± 0.050 and 1.32 ± 0.021 mg/L at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours respectively.

The **1.0% M.E.** promoted maximum clearance of creatinine at the end of one twenty hours, while the minimum clearance was seen at the end of twenty four hours. But in general the renal clearance of creatinine was at least four fold higher than that observed for the control ones. The creatinine clearance at

the end of one twenty hours was about twenty fold more than that found for the controls. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the urine creatinine values were equivalent to 0.43 ± 0.029, 0.890 ± 0.14, 0.37 ± 0.037, 0.587 ± 0.11, 1.237 ± 0.115, 0.789 ± 0.020, 1.602 ± 0.0344 mg/L respectively.

Under the influence of **5.0% M.E.** the ducks showed the progressive increase in the renal clearance of creatinine upto the end of twentyfour hours, while at the rest of the time intervals it showed significant fluctuations. The increase in the renal clearance of creatinine at the end of seventy two hour was over twentynine fold. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the renal clearance of creatinine was equivalent to 0.80 ± 0.03, 0.92 ± 0.042, 1.9 ± 0.035, 1.57 ± 0.101, 2.38 ± 0.01, 1.80 ± 0.231 and 1.30 ± 0.135 mg/L respectively.

The birds exposed to **10.0% M.E.** showed wide fluctuations in the renal clearance of creatinine. The maximum clearance of creatinine was observed at the end of ninetysix hours. The creatinine clearance was equivalent to 1.10 ± 0.07, 1.0 ± 0.020, 0.7 ± 0.016, 1.20 ± 0.08, 1.50 ± 0.060, 2.10 ± 0.01 and 1.20 ± 0.010 mg/L at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

Under the influence of **50.0% M.E.** the renal clearance of creatinine increased significantly, especially at the end of ninetysix, hours, it was fortyfold more than that observed in the control ones. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of creatinine was equivalent to 1.60 ± 0.18, 1.80 ± 0.16, 2.10 ± 0.10, 2.8 ± 0.101, 3.10 ± 0.271, 3.20 ± 0.37 and 2.78 ± 0.256 mg/L respectively.

The **100.0% M.E.** induced fluctuations in the renal clearance of creatinine and the maximum creatinine clearance was observed at the end of seventytwo hours, while the minimum clearance was observed at the end of six hours. The creatinine values of the urine were equivalent to 1.90 ± 0.162, 2.70

± 0.116, 2.0 ± 0.13, 2.10 ± 0.426, 3.0 ± 0.162, 2.80 ± 0.377 and 2.0 ± 0.120 mg/L at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 16 : EFFECT OF MINING EFFLUENT ON THE URINE CREATININE LEVEL.

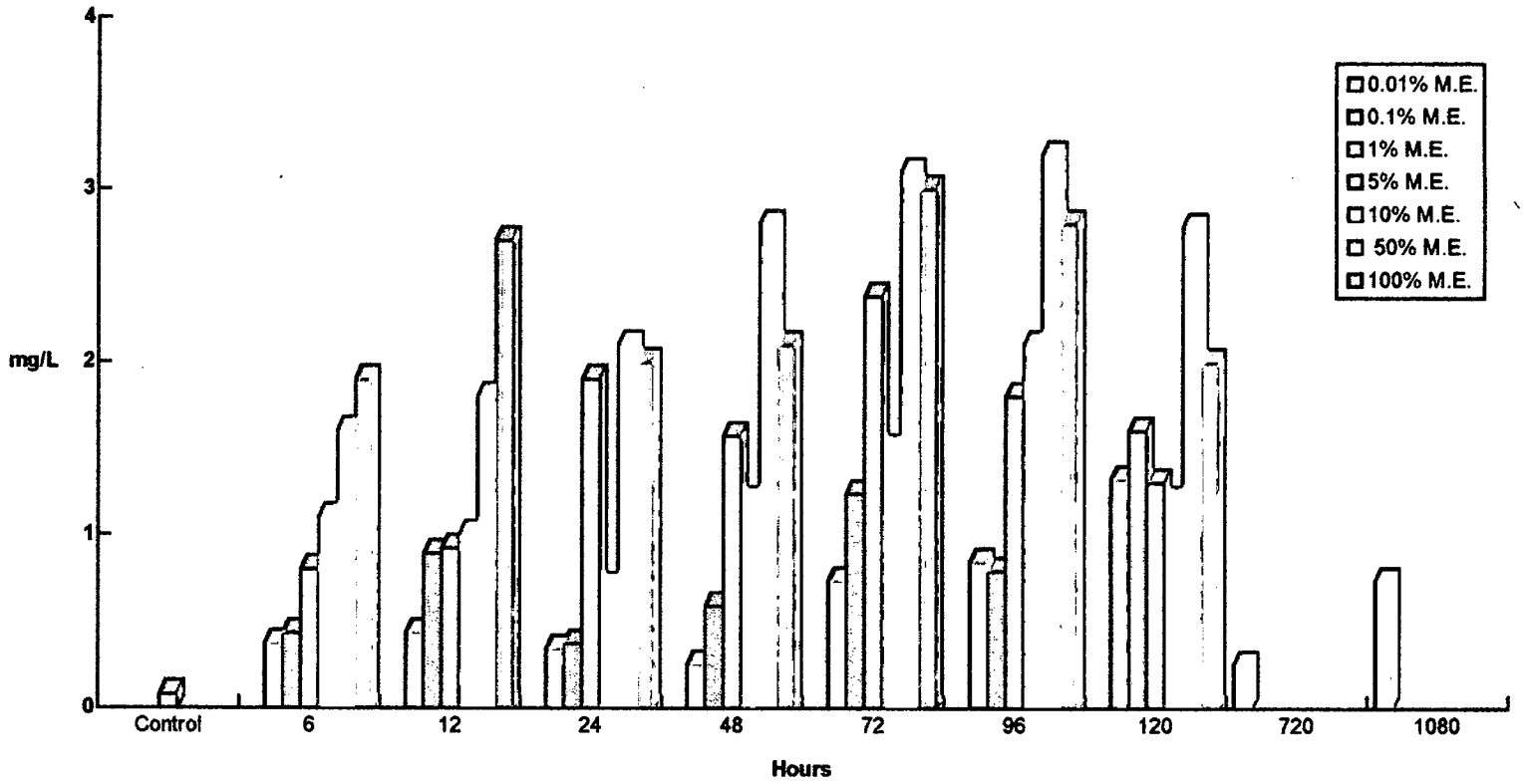
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		0.08 ±0.01									
0.01%	M.E.		← No significant change →							0.248 ±0.038	0.731 ±0.051
0.1%	M.E.		0.37 ±0.010	0.43 ±0.04	0.34 ±0.03	0.25 ±0.078	0.73 ±0.06	0.84 ±0.05	1.32 ±0.021	N.D.	N.D.
1%	M.E.		0.43 ±0.029	0.890 ±0.14	0.37 ±0.037	0.587 ±0.11	1.237 ±0.115	0.789 ±0.020	1.602 ±0.344	N.D.	N.D.
5%	M.E.		0.80 ±0.03	0.92 ±0.042	1.90 ±0.035	1.57 ±0.101	2.38 ±0.1	1.80 ±0.231	1.30 ±0.135	N.D.	N.D.
10%	M.E.		1.10 ±0.07	1.0 ±0.020	0.70 ±0.016	1.20 ±0.08	1.50 ±0.06	2.10 ±0.01	1.20 ±0.010	N.D.	N.D.
50%	M.E.		1.60 ±0.18	1.80 ±0.16	2.10 ±0.1	2.80 ±0.101	3.10 ±0.271	3.20 ±0.37	2.78 ±0.256	N.D.	N.D.
100%	M.E.		1.90 ±0.162	2.70 ±0.116	2.0 ±0.13	2.10 ±0.426	3.0 ±0.162	2.80 ±0.377	2.0 ±0.12	N.D.	N.D.

Note : Unit : Creatinine in mg/litre.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 16 : URINE - CREATININE
Units : mg/L



H] CALCIUM :

The changes in the renal clearance of calcium under the influence of mining effluents of various concentrations at different time intervals are tabulated in **Table No. 17** and graphically presented in **Graph No. 17.** The renal calcium clearance is expressed as milligrams of calcium per dL of urine. The control birds showed the renal clearance i.e. urine calcium level equivalent to 1.4 ± 0.236 mg per dL.

From the table and graph it is seen that the highest renal calcium clearance was induced by **100% M.E.** at the end of twenty four hours, while the lowest calcium clearance was induced by **1.0% M.E.** at the end of six hours. However, all the concentrations of M.E. induced renal calcium clearance higher than that of control ones.

The animals exposed to **0.01% M.E.** did not exhibit any significant change in the calcium clearance up to the end of twentynine days, but at the end of thirty days (seven twenty hours) and one thousand eighty hours the calcium clearance rose over four and six times the normal levels respectively. Thus, at the end of seven twenty and one thousand and eighty hours, the urine calcium level was equivalent to 5.79 ± 0.40 and 9.32 ± 0.363 milligrams/dL.

The **0.1% M.E.** induced fluctuations in the calcium clearance. The maximum urine calcium level was found at the end of one twenty hours, while the lowest was observed at the end of seventytwo hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the urine calcium level was equivalent to 2.7 ± 0.14, 2.70 ± 0.10, 4.60 ± 0.178, 3.44 ± 0.185, 2.66 ± 0.185, 5.64 ± 0.215 and 7.56 ± 0.215 milligrams/dL respectively.

Under the influence of **1.0% M.E.** the renal calcium clearance gradually increased upto the end of twentyfour hours but dropped at the end of fortyeight

and seventytwo hours. Then once again, the calcium clearance raised over five times the normal level. The maximum calcium clearance was observed at the end of one twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventy two, ninety six and one twenty hours, the renal calcium clearance was equivalent to 2.62 ± 0.386, 3.30 ± 0.260, 4.22 ± 0.278, 2.88 ± 0.116, 2.70 ± 0.228 , 7.32 ± 0.925 and 8.12 ± 0.203 milligrams/dL respectively.

The mallards exposed to **5.0% M.E.** exhibited fluctuations in the calcium clearance. The renal clearance of calcium was over seven fold at the end of seventy two hours, to that observed in the controls. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the urine calcium level was equivalent to 8.78 ± 0.43, 8.947 ± 0.227, 7.92 ± 0.318, 4.7± 0.209, 10.06 ± 0.287, 9.24 ± 0.272 and 7.48 ± 0.172 milligrams/dL respectively.

Under the influence of **10.0% M.E.** the ducks showed significant changes in the renal clearance of calcium. The maximum calcium clearance was observed at the end of one twenty hours, while the minimum was observed at the end of twelve hours. The renal clearance of calcium was equivalent to 7.58 ± 0.213, 5.20 ± 0.140, 7.58 ± 0.074, 6.8 ± 0.240, 8.28 ± 0.667, 8.02 ± 0.172 and 18.52 ± 0.256 milligrams/dL at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **50.0% M.E.** promoted wide fluctuations in the renal clearance of calcium and the maximum calcium clearance was induced at the end of twenty-four hours after the steady increase in the clearance through six and twelve hours. The calcium level in the urine at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 11.48 ± 0.172, 12.10 ± 0.340, 13.06 ± 0.241, 7.06 ± 0.287, 8.08 ± 0.292, 6.86 ± 0.101 and 6.2 ± 0.140 milligrams/dL respectively.

The birds exposed to **100.0% M.E.** exhibited very high clearance of calcium through urine and over fifteen fold increase in calcium clearance was

observed at the end of twentyfour hours, while a little less than four fold increase in urine calcium level was found at the end of seventytwo hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the urine calcium was equivalent to 20.92 ± 0.457, 18.80 ± 0.283, 22.08 ± 0.271, 9.26 ± 0.210, 5.56, ± 0.215, 9.42 ± 0.172 and 8.50 ± 0.260 milligrams/dL respectively.

TABLE NO. 17 : EFFECTS OF MINING EFFLUENTS ON THE RENAL CLEARANCE OF CALCIUM.

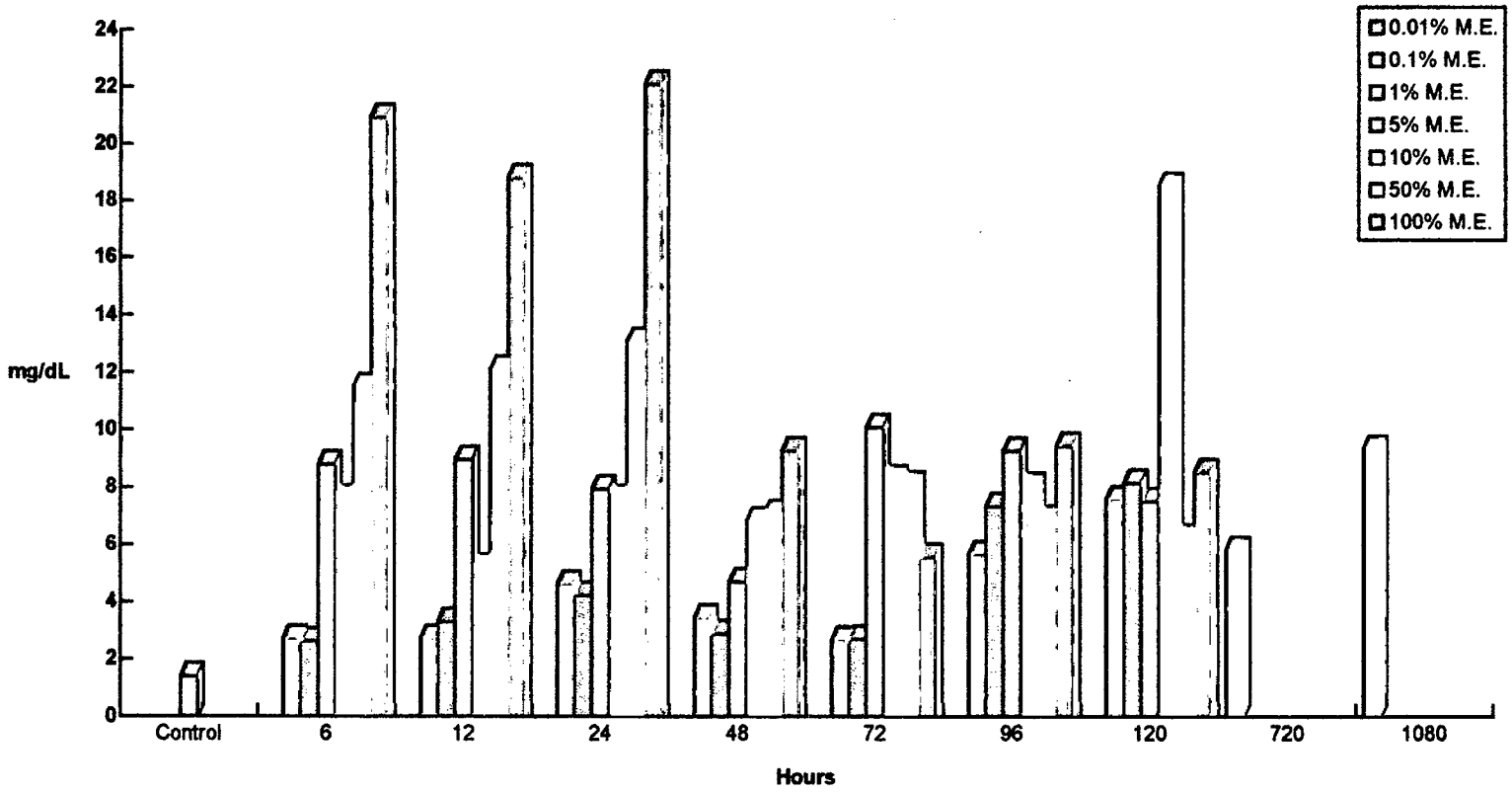
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		1.40 ±0.238									
0.01%	M.E.		← No significant change →							5.797 ±0.40	9.32 ± 0.363
0.1%	M.E.		2.70 ±0.14	2.70 ±0.10	4.60 ±0.178	3.44 ±0.185	2.66 ±0.185	5.64 ±0.215	7.56 ±0.215	N.D.	N.D.
1%	M.E.		2.62 ±0.386	3.30 ±0.260	4.22 ±0.278	2.88 ±0.116	2.70 ±0.228	7.32 ±0.925	8.12 ±0.203	N.D.	N.D.
5%	M.E.		8.78 ±0.43	8.947 ±0.227	7.92 ±0.318	4.70 ±0.209	10.06 ±0.287	9.24 ±0.272	7.48 ±0.172	N.D.	N.D.
10%	M.E.		7.58 ±0.213	5.20 ±0.14	7.58 ±0.074	6.80 ±0.24	8 28 ±0.667	8.02 ±0.172	18.52 ±0.256	N.D.	N.D.
50%	M.E.		11.48 ±0.172	12.10 ±0.340	13.06 ±0.241	7.06 ±0.287	8.08 ±0.292	6.86 ±0.101	6.20 ±0.14	N.D.	N.D.
100%	M.E.		20.92 ±0.457	18.80 ±0.282	22.08 ±0.271	9.26 ±0.21	5.56 ±0.215	9.42 ±0.172	8.50 ±0.260	N.D.	N.D.

Note : Unit : Calcium in mg/dL.

N.D. : Not Determined.

All alterations are statistically significant - P < 0.01.

GRAPH NO. 17 URINE - CALCIUM
Units : mg/dL



I] CHLORIDE :

The alterations in the chloride contents of the urine under the influence of mining effluents in different concentrations at various time intervals are compiled in **Table No. 18** and are graphically given in **Graph No. 18.** The chlorides from the urine are expressed as milliequivalence per litre of the urine. The control animals exhibited 30.0 ± 1.010 milliequivalence of chlorides in the urine.

From the graph it appears that the mining effluents promoted increases and wide fluctuations in the chloride clearances. The renal clearance of chlorides exhibited M.E. concentration dependency at the end of ninetysix hours and partially at the end of one twenty hours. The **100% M.E.** induced the highest clearance of chlorides at the end of seventytwo hours. At the end of seventwenty and one thousand and eighty hours the renal clearance of chlorides was equivalent to 54.74 ± 2.48 and 47.68 ± 0.4 mEq/litre respectively.

The mallards exposed to **0.01% M.E.** did not show any change in the renal clearance of chlorides up to the end of twentynine days but at the end of thirty days there was a very significant increase in the chloride clearance. Then at the end of one thousand and eighty hours there was slight decrease in the chloride clearance as compared with the clearance observed at the end of seven twenty hours.

Under the influence of **0.1% M.E.** there was an increase in the chloride clearance at the end of six hours and subsequently there was decrease in the urine chloride levels up to the end of fortyeight hours. Then, at the end of seventy two hours there was abrupt increase in the chloride clearance. Thus, the chloride clearance was equivalent to 56.80 ± 0.758, 46.78 ± 1.148, 43.12 ± 0.70, 42. 34 ± 0.76, 74.10 ± 1.206, 28.42 ± 1.64 and 39.12 ± 2.678

milliequivalence at the end of six, twelve, twentyfour, fortyeight seventytwo, ninetysix and one twenty hours respectively.

The **1.0 % M.E.** induced maximum decrease in the chloride clearance at the end of one twenty hours while a marginal increase was promoted at the end of six and twelve hours. A significant increase in the renal clearance of chlorides was observed at the end of ninetysix hours. The chloride clearance at the end of six, twelve, twentyfour, fortyeight seventytwo, ninetysix and one twenty hours was equivalent to 32.70 ± 1.47, 30.82 ± 0.577, 19.80 ± 0.54, 26.20 ± 1.720, 26.05 ± 0.722, 54.26 ± 0.97 and 12.04 ± 2.3 milliequivalence/litre respectively.

The exposure of mallards to **5.0% M.E.** promoted time dependent increase in the chloride clearance up to the end of seventy two hours but subsequently it decreased sharply at the end of ninetysix and one twenty hours. Thus, the renal clearance of chlorides was equivalent to 97.0 ± 0.205, 100.0 ± 0.583, 142.0 ± 0.612, 149.0 ± 1.3, 151.0 ± 2.48, 96.0 ± 1.148 and 68.0 ± 2.17 milliequivalence/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

On exposure to **10.0% M.E.** the birds showed fluctuations in the chloride clearance. The chloride clearance raised over five fold at the end of six hours and then subsequently went on decreasing upto the end of twentyfour hours. The urine showed the chloride values equivalent to 169.0 ± 3.1, 157.0 ± 1.34, 146.0 ± 5.1, 159.0 ± 2.1, 149.0 ± 1.36, 163.0 ± 0.50 and 170.0 ± 0.416 milliequivalence/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **50.0% M.E.** induced very high chloride clearance at the end of one hundred and twenty hours and the chloride level fluctuated between 44.66 and 265.0 milliequivalence per litre. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of chloride was

equivalent to 85.0 ± 3.03, 80.0 ± 1.6, 74.0 ± 3.4, 85.0 ± 1.26, 44.66 ± 0.763, 174.80 ± 2.56 and 265.0 ± 2.3 milliequivalence/litre respectively.

The ducks treated with **100.0% M.E.** showed very high chloride clearance for all the time intervals under study except fortyeight hours. Initially, the chloride clearance increased sharply at the end of six hours and subsequently continued to increase up to the end of twentyfour hours, but at the end of 48 hours, the chloride clearance dropped sharply but then increased abruptly at the end of seventytwo hours to a peak level. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the renal clearance of chlorides was equivalent to 177.25 ± 1.78, 221.50 ± 2.17, 266.0 ± 2.54, 88.15 + 1.44, 896.0 ± 1.01, 370.0 ± 5.03 and 576.6 ± 4.31 milliequivalence/litre respectively.

TABLE NO. 18 : EFFECT OF MINING EFFLUENTS ON THE URINE CHLORIDES.

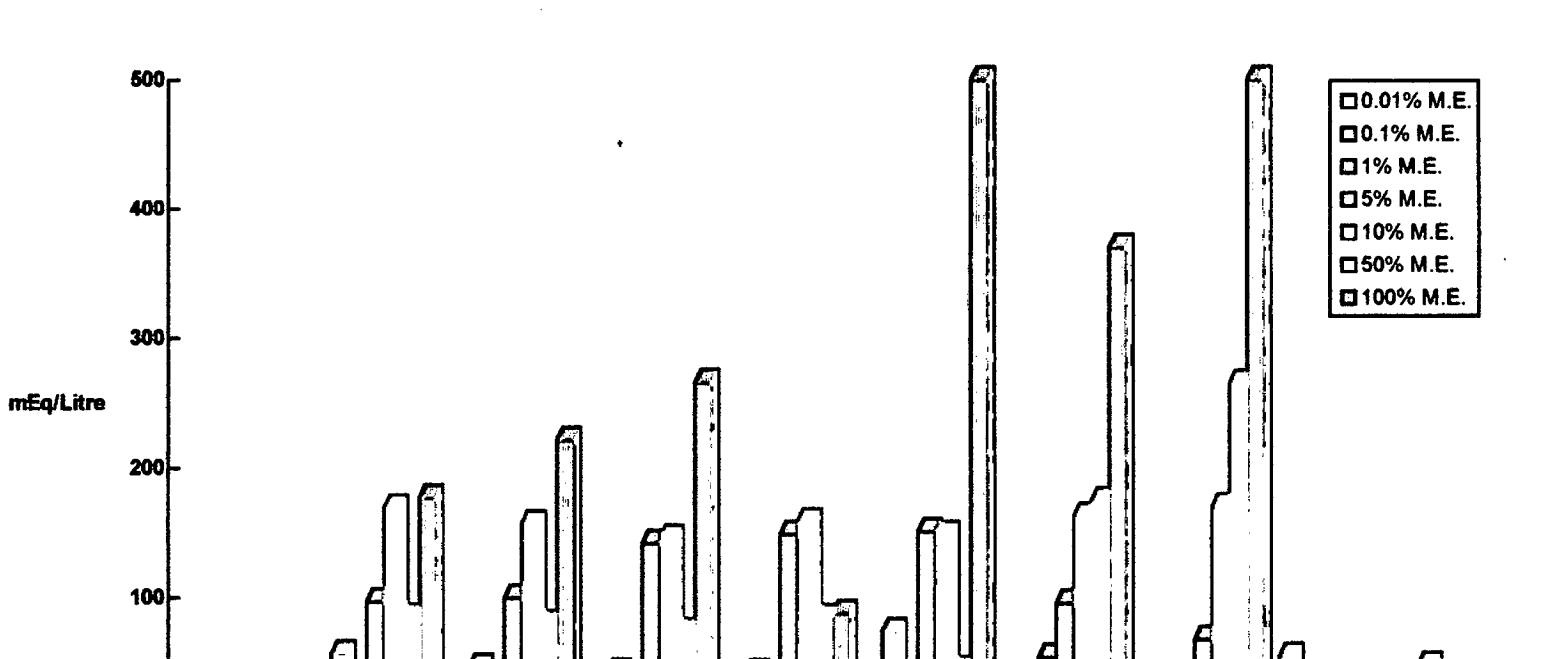
		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	30.0 ±1.01									
0.01% M.E.		← No significant change →							54.74 ±2.48	47.68 ±0.4
0.1% M.E.		56.80 ±0.758	46.78 ±1.148	43.12 ±0.70	42.34 ±0.76	74.10 ±1.206	28.42 ±1.64	39.12 ±2.678	N.D.	N.D.
1% M.E.		32.70 ±1.47	30.82 ±0.577	19.80 ±0.54	26.20 ±1.720	26.05 ±0.722	54.26 ±0.97	12.04 ±2.3	N.D.	N.D.
5% M.E.		97 ±0.205	100 ±0.583	142 ±0.612	149 ±1.3	151 ±2.48	96 ±1.148	68 ±2.17	N.D.	N.D.
10% M.E.		169 ±3.1	157 ±1.34	146 ±5.1	159 ±2.1	149 ±1.36	163 ±0.50	170 ±0.416	N.D.	N.D.
50% M.E.		85.0 ±3.03	80.0 ±1.6	74.0 ±3.4	85.0 ±1.26	44.66 ±0.763	174.80 ±2.56	265.0 ±2.3	N.D.	N.D.
100% M.E.		177.25 ±1.78	221.5 ±2.17	266.0 ±2.54	88.15 ±1.44	896.0 ±1.01	370.0 ±5.03	576.6 ±4.31	N.D.	N.D.

Note : Unit : Chlorides in mEqs/L.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 18 : URINE - CHLORIDE
Units - mEq/Litre



J] ALTERATIONS IN URINE ELECTROLYTES :

a] SODIUM :

The variations in the renal clearance of sodium under the influence of mining effluents are compiled in **Table No. 19** and are given graphically in **Graph No. 19.** The sodium clearance is expressed as milliequivalence per litre of the urine. The control animals exhibited renal clearance of Na^+ equivalent to 62.0 ± 4.1 milliequivalence/litre.

From the graph it is evident that the mining effluents had a pronounced influence on the Na^+ clearance and generally at all the time intervals there was increased Na^+ clearance. The M.E. induced wide fluctuations in Na^+ clearance and the highest renal clearance of Na^+ was promoted at the end of seventytwo hours under the influence of **100.0% M.E.,** while the minimum of 68.2 milliequivalence/litre clearance was induced at the end of twentyfour hours under the influence of **0.1% M.E.**

The animals exposed to **0.01% M.E.** did not show any significant change in the Na^+ clearance up to the end of twenty nine days but at the end of the thirtydays the renal clearance of Na^+ increased to 72.4 ± 1.62 milliequivalence/litre and went on increasing to 91.40 ± 1.74 milliequivalence/litre at the end of one thousand and eighty hours.

The **0.1% M.E.** promoted significant fluctuations in the renal clearance of sodium but in general the Na^+ clearance was higher than that observed in the controls. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the Na^+ clearance was equivalent to 69.40 ± 1.01, 79.0 ± 0.63, 68.20 ± 1.72, 80.0 ± 4.4, 83.60 ± 3.87, 72.60 ± 1.62 and 73.20 ± 2.315 milliequivalence/litre respectively.

Under the influence of **1.0% M.E.** the ducks showed variations in the Na^+ clearance and the Na^+ clearance was equivalent to 87.0 ± 1.41, 74.4 ±

2.8, 86.20 ± 1.7, 93.20 ± 2.1 68.80 ± 2.3, 87.0 ± 1.4 and 73.0 ± 1.6 milliequivalence/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The ducks exposed to **5.0% M.E.** showed sharp increase in Na^+ clearance at the end of six hours but, later on, it declined gradually up to the end of fortyeight hours. Then, at the end of six, twelve, twentyfour, fortyeight , seventytwo, ninetysix and onetwenty hours the Na+ clearance was equivalent to 93.20 ± 2.1, 85.40 ± 2.24, 80.80 ± 2.22, 70.80 ± 1.4, 85.0 ± 3.0, 91.40 ± 6.0 and 79.0 ± 1.4 milliequivalence/litre respectively.

The treatment of **10.0% M.E.** promoted significant fluctuations in the renal clearance of Na^+. The maximum Na^+ clearance was observed at the end of seventytwo hours. The renal clearance of Na^+ was equivalent to 73.20 ± 2.1, 79.20 ± 1.93, 73.20 ± 2.1, 84.20 ± 2.13, 88.80 ± 1.166, 79.20 ± 2.03 and 83.80 ± 2.48 milliequivalence/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The ducks exposed to **50.0% M.E.** showed high clearance of Na^+ but there were fluctuations in the Na^+ clearance for different time intervals. The renal clearance of Na^+ at the end of six, twelve, twentyfour, fortyeight seventytwo, ninetysix and one twenty hours was equivalent to 73.0 ± 2.0, 86.0 ± 2.4, 77.20 ± 3.70, 94.20 ± 1.16, 99.20 ± 1.70, 93.20 ± 2.5 and 91.40 ± 4.6 milliequivalence/litre respectively.

The **100.0% M.E.** induced highest renal clearance of Na+ at the end of seventy two hours and at all the time intervals under investigation the Na+ clearance was about three fold more than that observed in the control animals. Thus at the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix, and one twenty hours the renal clearance of Na^+ was equivalent to 91.80 ± 1.70, 88.80 ± 2.85, 100.60 ± 1.85, 91.40 ± 1.62, 101.20 ± 3.10, 99.40 ± 1.01 and 100.60 ± 1.35 milliequivalence/litre respectively.

TABLE NO. 19 : RENAL CLEARANCE OF SODIUM UNDER THE INFLUENCE OF MINING EFFLUENTS.

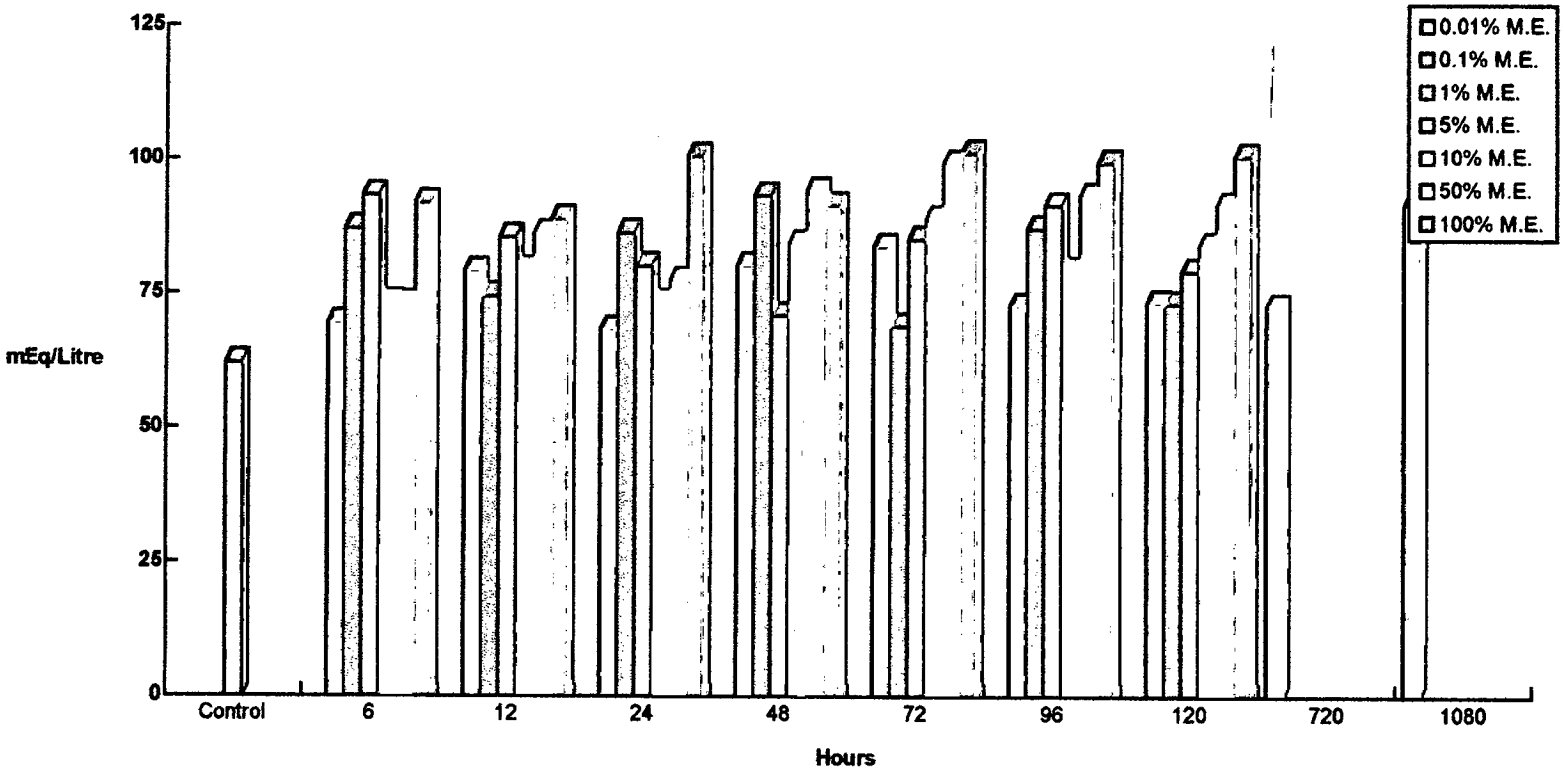
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		62.0 ±4.1									
0.01%	M.E.		← No significant change →							72.4 ±1.62	91.40 ±1.74
0.1%	M.E.		69.40 ±1.01	79.0 ±0.63	68.20 ±1.72	80.0 ±4.4	83.60 ±3.87	72.60 ±1.62	73.20 ±2.315	N.D.	N.D.
1%	M.E.		87.0 ±1.41	74.40 ±2.8	86.20 ±1.7	93.20 ±2.1	68.80 ±2.3	87.0 ±1.4	73.0 ±1.6	N.D.	N.D.
5%	M.E.		93.20 ±2.1	85.40 ±2.24	80.80 ±2.22	70.80 ±1.4	85.0 ±3.0	91.40 ±6.0	79.0 ±1.4	N.D.	N.D.
10%	M.E.		73.20 ±2.1	79.20 ±1.93	73.20 ±2.1	84.20 ±2.13	88.80 ±1.166	79.20 ±2.03	83.80 ±2.48	N.D.	N.D.
50%	M.E.		73.0 ±2.0	86.0 ±2.4	77.20 ±3.7	94.20 ±1.16	99.20 ±1.7	93.20 ±2.5	91.40 ±4.6	N.D.	N.D.
100%	M.E.		91.80 ±1.7	88.80 ±2.85	100.60 ±1.85	91.40 ±1.62	101.20 ±3.1	99.40 ±1.01	100.60 ±1.35	N.D.	N.D.

Note : Unit : Sodium in mEq/Litre.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 19 : URINE - SODIUM
Units : mEq/L.



b] POTASSIUM :

The changes in the renal clearance of K^+ under the influence of mining effluents are compiled in **Table No. 20** and are graphically presented in **Graph No.20.** The renal clearance of K^+ is expressed in milliequivalence (mEq) per litre of the urine. The control animals showed K^+ clearance equivalent to 4.20 ± 0.18 milliequivalence per litre.

From the graph it appears that the renal clearance of K^+ was dose dependent i.e. concentration dependent at the end of fortyeight hours and **100% M.E.** induced significantly higher renal clearance of K^+.

When the ducks were exposed to **0.01% M.E.** there was no significant change in the renal clearance of potassium up to the end of twentynine days but at the end of thirty days (seven twenty hours) there was an increase in K^+ clearance which further increased at the end of one thousand and eighty hours. Thus, the K^+ clearance was equivalent to 5.60 ± 0.20 and 8.46 ± 0.101 milliequivalence per litre at the end of seven twenty and one thousand eighty hours respectiveiy.

The **0.1% M.E.** induced fluctuations in the renal clearance of K^+ and a relatively higher clearance was observed at the end of ninetysix hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the renal clearance of K^+ was equivalent to 6.72 ± 0.07, 5.60 ± 0.20, 7.14 ± 0.16, 5.80 ± 0.158, 5.0 ± 0.06, 7.225 ± 0.129 and 5.38 ± 0.09 milliequivalence per litre respectively.

The **1.0% M.E.** promoted significant variations in the K^+ clearance. The renal clearance of K^+ at the end of six, twelve, twentyfour fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 7.34 ± 0.101, 5.78 ± 0.116, 7.22 ± 0.11, 6.16 ± 0.10, 8.28 ± 0.11, 5.0 ± 0.141 and 6.84 ± 0.101 milliequivalence per litre respectively.

Under the influence of **5.0% M.E.** there was significant rise in K^+ clearance at the end of six and twelve hours but subsequently the K^+ clearance went on decreasing up to the end of ninetysix hours. Thus, the K^+ clearance was equivalent to 8.36 ± 0.135, 9.46 ± 0.185, 8.0 ± 0.167, 6.58 ± 0.203, 7.64 ± 0.21, 5.62 ± 0.172 and 7.48 ± 0.22 milliequivalence per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The ducks subjected to **10.0% M.E.** exhibited high K^+ clearance but the K^+ clearance was fluctuating between 7.20 and 9.82 milliequivalence per litre. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of K^+ was equivalent to 7.20 ± 0.132, 8.40 ± 0.248, 7.50 ± 0.30, 9.16 ± 0.546, 9.82 ± 0.172, 9.08 ± 0.450 and 9.44 ± 0.17 milliequivalence per litre respectively.

The **50.0% M.E.** promoted very high clearance of K^+ and the maximum clearance was observed at the end of twelve hours, while the minimum clearance was observed at the end of one twenty hours. Thus, the renal clearance of K^+ was equivalent to 12.98 ± 0.13, 14.92 ± 0.17, 12.04 ± 0.162, 9.21 ± 0.134, 12.46 ± 0.23 , 8.76 ± 0.12 and 5.44 ± 0.20 milliequivalence per litreat the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The mallard subjected to **100.0% M.E.** showed the highest renal clearance of K^+ at the end of six hours and the minimum at the end of one twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the renal clearance of K+ was equivalent to 17.86 ± 6.30, 11.34 ± 0.215, 14.98 ± 0.222, 13.14 ± 0.307, 17.0 ± 0.316, 12.0 ± 0.66 and 10.66 ± 0.162 milliequivalence per litre respectively.

TABLE NO. 20 : THE VARIATION IN POTASSSIUM CLEARANCE UNDER THE INFLUENCE OF MINING EFFLUENTS.

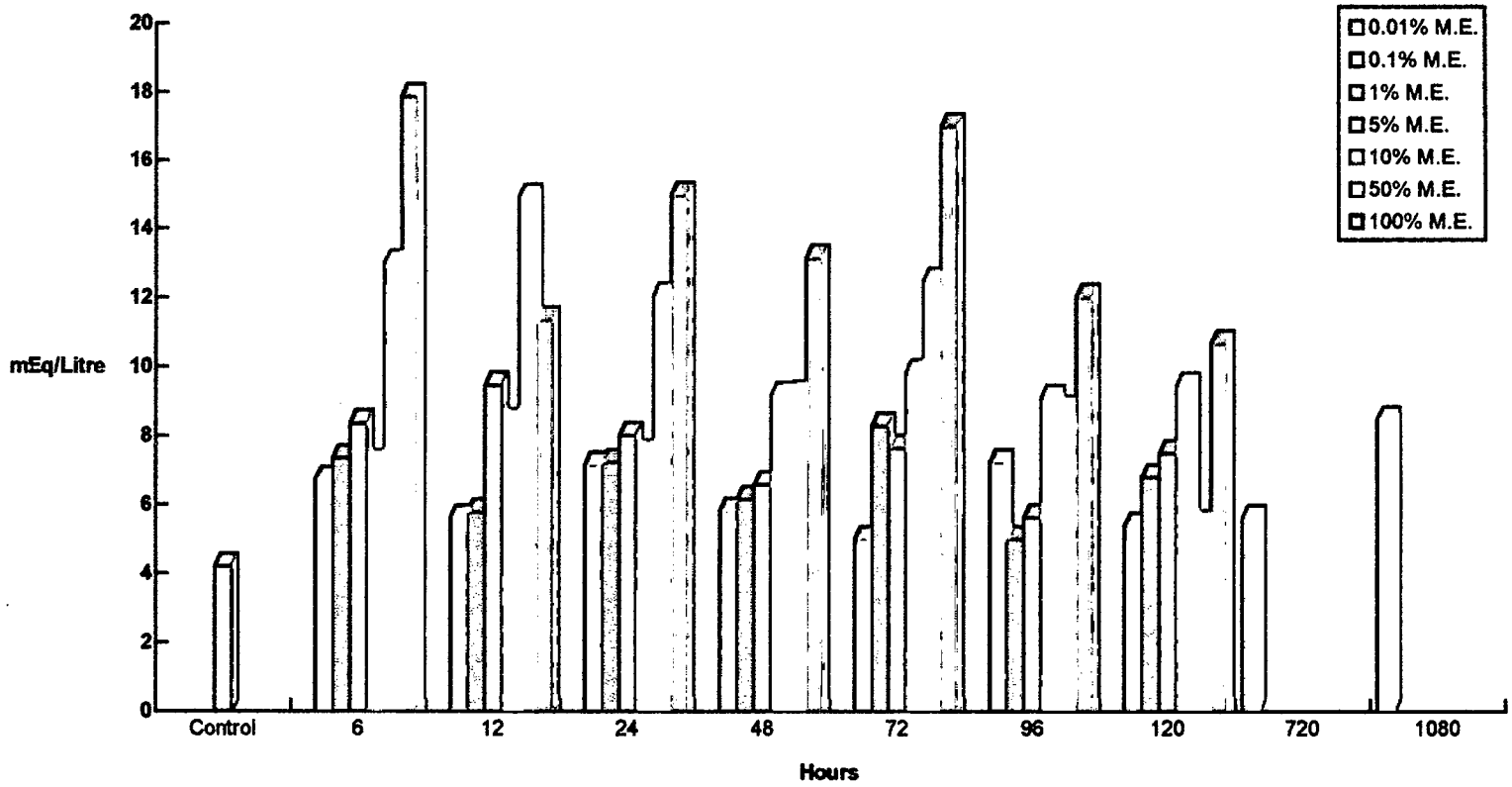
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		4.20 ±0.18									
0.01%	M.E.		← No significant change →							5.6 ±0.20	8.46 ±0.101
0.1%	M.E.		6.72 ±0.07	5.60 ±0.2	7.14 ±0.16	5.80 ±0.158	5.0 ±0.06	7.225 ±0.129	5.38 ±0.09	N.D.	N.D.
1%	M.E.		7.34 ±0.101	5.78 ±0.116	7.22 ±0.11	6.16 ±0.10	8.28 ±0.11	5.0 ±0.141	6.84 ±0.101	N.D.	N.D.
5%	M.E.		8.36 ±0.135	9.46 ±0.185	8.0 ±0.167	6.58 ±0.203	7.64 ±0.21	5.62 ±0.172	7.48 ±0.22	N.D.	N.D.
10%	M.E.		7.20 ±0.132	8.40 ±0.248	7.50 ±0.3	9.16 ±0.546	9.82 ±0.172	9.08 ±0.45	9.44 ±0.17	N.D.	N.D.
50%	M.E.		12.98 ±0.13	14.92 ±0.17	12.04 ±0.162	9.21 ±0.134	12.46 ±0.23	8.76 ±0.12	5.44 ±0.2	N.D.	N.D.
100%	M.E.		17.86 ±6.30	11.34 ±0.215	14.98 ±0.222	13.14 ±0.307	17.0 ±0.316	12.0 ±0.66	10.66 ±0.162	N.D.	N.D.

Note : Unit : Potassium in mEqs / Litre.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 20 : URINE - POTASSIUM
Units : mEq/L



c] CHANGES IN URINE FLOW :

The changes in the urine flow are given in **Table No 20-A** and are graphically presented in the **Graph No 20-A.** The rates of urine flow are expressed as millilitres of urine per Kilogram body weight per minute. The control ducks exhibited urine flow equivalent to 0.166 ± 0.001 ml/kg/min.

From the table and the graph it appears that almost all the concentrations of mining effluents (**M.E.**) induced concentration dependent and exposure period dependent changes in the urine flow barring a few exceptions. The very low concentration like **0.01% M.E.** did not induce any change in the rate of urine flow up to the end of twenty nine days but at the end of thirty days the rate of urine flow decreased significantly and kept on decreasing up to the end of fortyfive days. The rate of urine flow at the end of thirty and fortyfive days was equivalent to 0.137 ± 0.02 and 0.132 ± 0.03 ml/kg/min. The **0.1% M.E.** induced progressive decrease in the rate of urine flow. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the urine flow was equivalent to 0.165 ± 0.03, 0.159 ± 0.04, 0.154 ± 0.03, 0.150 ± 0.04, 0.150 ± 0.05, 0.143 ± 0.01 and 0.140 ± 0.01 millilitre/kg/min respectively. The **1.0% M.E.** also induced reduction in the urine flow in the progressive manner from the end of six hours to the end of one hundred and twenty hours. By the end of six, twelve, twentyfour, fortyeight, seventy two, ninety six and one hundred and twenty hours the rate of urine flow was equivalent to 0.162 ±0.03, 0.157 ± 0.01, 0.155 ± 0.03, 0.149 ± 0.04, 0.147 ± 0.05, 0.145 ± 0.01 and 0.139 ± 0.02 ml/kg/min respectively.

The **5.0% M.E.** induced reduction in the rate of urine flow. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the rate of urine flow was equivalent to 0.162 ± 0.001, 0.159 ±

0.03, 0.154 ± 0.02, 0.147 ± 0.03, 0.139 ± 0.05, 0.140 ± 0.01 and 0.131 ± 0.02 ml/kg/min respectively.

Under the influence of **10.0% M.E.** the ducks exhibited reduction in the rate of urine flow. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the rate of urine flow was equivalent to 0.160 ± 0.01, 0.158 ± 0.03, 0.149 ± 0.04, 0.150 ± 0.02, 0.153 ± 0.01, 0.139 ± 0.03 and 0.129 ± 0.06 ml/kg/min respectively.

The ducks subjected to **50.0% M.E.** showed sharp reduction in the urine flow. By the end of six, twelve, twentyfour, forty eight, seventy two, ninetysix and one hundred and twenty hours the rate of urine flow was equivalent to 0.158 ± 0.01, 0.155 ± 0.001, 0.143 ± 0.03, 0.142 ± 0.01, 0.132 ± 0.01, 0.121 ± 0.02 and 0.118 ± 0.002 ml/kg/min respectively.

The exposure of mallards to **100.0% M.E.** promoted reduction in the rate of urine flow. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the rate of urine flow was equivalent to 0.167 ± 0.001, 0.143 ± 0.003, 0.139 ± 0.004, 0.140 ± 0.001, 0.123 ± 0.01, 0.108 ± 0.02 and 0.101 ± 0.001 ml/kg/min respectively. All the reductions in the rate of urine flow were statistically significant ($P < 0.01$).

TABLE NO. 20A : CHANGES IN URINE FLOW UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		0.001 ±0.1669									
0.01%	M.E.		← No significant change →							0.137 ± 0.02	0.132 ± 0.03
0.10%	M.E.		0.165 ±0.03	0.159 ± 0.04	0.154 0.03	0.150 ±0.04	0.150 ±0.05	0.143 ±0.01	0.140 ±0.01	N.D.	N.D.
1%	M.E.		0.162 ±0.03	0.157 ±0.01	0.155 ±0.03	0.149 ±0.04	0.147 ±0.05	0.145 ±0.01	0.139 ±0.02	N.D.	N.D.
5%	M.E.		0.162 ±0.001	0.159 ±0.03	0.154 ±0.02	0.147 ±0.03	0.139 ±0.05	0.140 ±0.01	0.131 ±0.02	N.D.	N.D.
10%	M.E.		0.160 ±0.01	0.158 ±0.03	0.149 ±0.04	0.150 ±0.02	0.153 ±0.01	0.139 ±0.03	0.129 ±0.06	N.D.	N.D.
50%	M.E.		0.158 ±0.01	0.155 ±0.001	0.143 ±0.03	0.142 ±0.01	0.132 ±0.01	0.121 ±0.02	0.118 ±0.002	N.D.	N.D.
100%	M.E.		0.167 ±0.001	0.143 ±0.003	0.139 ±0.004	0.140 ±0.001	0.123 ±0.01	0.108 ±0.02	0.101 ±0.001	N.D.	N.D.

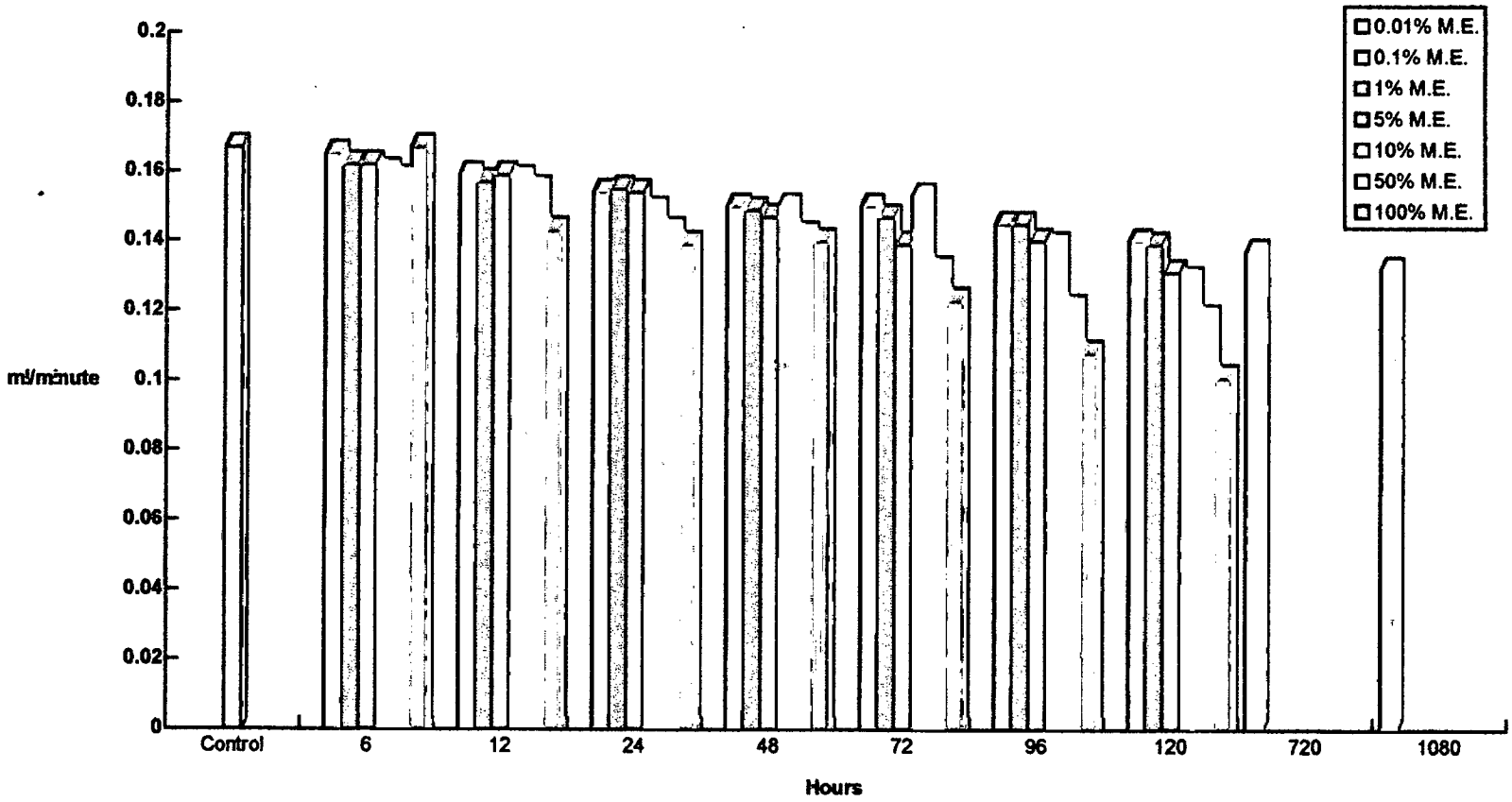
Note : Unit : ml/min.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 20 A : RATE OF URINE FORMATION.

Units : ml/minute



1.937 ± 0.3, 1.954 ± 0.4, 1.845 ± 0.1, 1.837 ± 0.7 and 1.828 ± 0.3 ml/kg/min respectively.

The exposure of ducks to **10.0% M.E.** introduced reductions in GFR at the various time intervals and the GFR was equivalent to 1.978 ± 0.31, 1.872 ± 0.21, 1.910 ± 0.17, 1.832 ± 0.189 , 1.891 ±0.193, 1.871 ± 0.17 and 1.798 ± 0.189 ml/kg/min at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one hundred and twenty hours respectively.

On exposing the ducks to **50.0% M.E.** the GFR reduced significantly and then went on declining progressively as the exposure period increased. At the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one hundred and twenty hours the GFR was equivalent to 1.931 ± 0.2, 1.892 ± 0.3, 1.876 ± 0.17, 1.831 ± 0.18, 1.801 ± 0.2, 1.793 ± 0.31 and 1.741 ± 0.178 ml/kg/min respectively.

The **100.0% M.E.** induced sharp reductions in the GFR. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the GFR was equivalent to 1.890 ± 0.3, 1.738 ± 0.178, 1.701 ± 0.21, 1.639 ± 0.231, 1.648 ± 0.1, 1.732 ± 0.12 and 1.579 ± 0.17 ml/kg/min respectively.

All the reductions in GFR were statistically significant ($P < 0.01$).

TABLE NO. 20B : CHANGES IN GLOMERULAR FILTRATION RATE (GFR) UNDER THE INFLUENCE OF MINING EFFLUENTS.

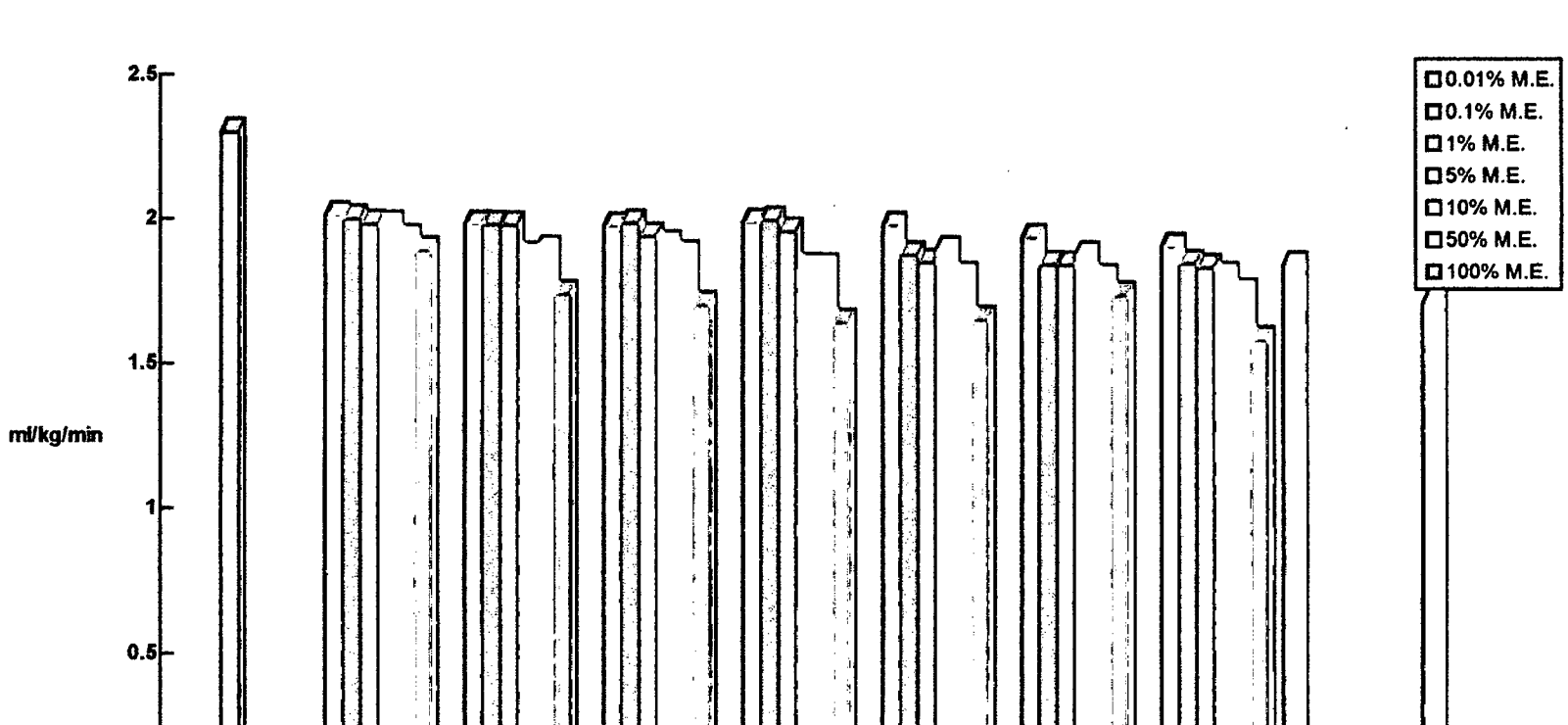
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		2.30 ±0.127									
0.01%	M.E.		← No significant change →							1.837 ±0.01	1.701 ±0.09
0.10%	M.E.		2.01 ±0.1	1.98 ±0.3	1.973 ±0.01	1.985 ±0.173	1.975 ±0.2	1.932 ±0.09	1.901 ±0.132	N.D.	N.D.
1%	M.E.		1.999 ±0.1	1.978 ±0.009	1.983 ±0.1	1.993 ±0.2	1.872 ±0.31	1.838 ±0.27	1.842 ±0.21	N.D.	N.D.
5%	M.E.		1.981 ±0.1	1.976 ±0.2	1.937 ±0.3	1.954 ±0.4	1.845 ±0.1	1.837 0.7	1.828 ±0.3	N.D.	N.D.
10%	M.E.		1.978 ±0.31	1.872 ±0.21	1.910 ±0.17	1.832 ±0.189	1.891 ±0.193	1.871 ±0.17	1.798 ±0.189	N.D.	N.D.
50%	M.E.		1.931 ±0.2	1.892 ±0.3	1.876 ±0.17	1.831 ±0.18	1.801 ±0.2	1.793 ±0.31	1.741 ±0.178	N.D.	N.D.
100%	M.E.		1.89 ±0.3	1.738 ±0.178	1.701 ±0.21	1.639 ±0.231	1.648 ±0.1	1.732 ±0.12	1.579 ±0.17	N.D.	N.D.

Note : Unit : ml/kg/min.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 20 B : GLOMERULAR FILTRATION RATE IN URINE.
Units : ml/kg/min.



Chapter - iv

Heavy Metal Retention

RETENTION PATTERNS OF HEAVY METALS :

Generally, in the natural environment the heavy metals are found as contaminants of water and food resources of the animals. These heavy metals are the products of several human activities and the increased human interference in the environment. The aquatic birds get into their bodies these heavy metals through the food and water they take in. The body, then, tries to detoxify these metals or retain them in someparts and attempts to eliminate them. The birds appear to have developed an unique route of elimination of these metals through the feathers. The birds have been found to retain the heavy metals into their bones, blood, liver, kidney, muscles, feathers etc. The mining is a source of metal contaminants for the aquatic bodies and the mining effluents are likely to carry these contaminants to the waterbodies. In the present investigation the mallards were found to retain several heavy metals in different concentrations in the serum, and kidney and even the birds exhibited renal clearance of the heavy metals.

A] RETENTION IN KIDNEYS :

The alterations in the retentions of heavy metals are compiled in **table No. 22.0, 22.1, 22.2 and 22.3** and the retention values are expressed as micrograms (μg) per gram wet weight of tissue. The mallards *(Anas platyrhychos domesticus)* exposed to mining effluents **(M.E.)** exhibited retention of cadmium, copper, chromium, iron, lead, magnesium, manganese, nickel and zinc. The control animals exhibited chromium, iron, magnesium, manganese and zinc in the kidneys in concentrations equivalent to 0.01 ± 0.0001, 0.10 ± 0.0001, 0.02 ± 0.0001, 0.09 ± 0.001 and 0.08 ± 0.001 μg / g respectively.

TABLE A] : NORMAL VALUES FOR HEAVY METAL RETENTION AND RENAL CLEARANCE.

Control	Cd	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Kidney µg/gm Ww	Nil	0.01 ±0.0001	Nil	0.10 ± 0.0001	0.02 ± 0.0001	0.09 ± 0.001	Nil	Nil	0.08 ± 0.001
Serum µgms/Litre	1.00 ± 0.01	1.00 ± 0.10	2.00 ± 0.5	45.00 ± 2.00	5.00 ± 1.00	132.00 ± 1.00	Nil	Nil	19.00 ± 7.00
Renal clearance µgms/Litre	Nil	Nil	Nil	4.00 ± 0.1	Nil	2.00 ± 1.00	Nil	Nil	3.00 ± 0.1.

TABLE 22.0: RENTENTION OF HEAVY METALS IN THE DUCKS EXPOSED TO 0.01% MINING EFFLUENTS (M.E.).

0.01% M.E. :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
30 Days	0.49 ± 0.001	0.07 ±0.002	0.02 ± 0.0001	0.27 ± 0.001	0.13 ± 0.003	0.20 ± 0.001	0.30 ± 0.002	0.10 ± 0.002	0.19 ± 0.001
45 Days	0.87 ± 0.003	0.09 ± 0.001	0.23 ± 0.002	0.89 ± 0.003	0.37 ± 0.002	0.19 ± 0.002	0.09 ± 0.001	0.21 ± 0.003	1.02 ± 0.001

When the ducks were exposed to **0.01 % M.E.**, the retentions of the heavy metals and the alterations in their retentions up to the end of twentynine day were not significant but at the end of thirty days (seven hundred and twenty days) the kidney exhibited maximum retention of cadmium. The kidney concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 0.49 ±0.001, 0.07 ± 0.002, 0.02 ± 0.0001, 0.27 ± 0.001, 0.13 ± 0.003, 0.20 ± 0.001, 0.30 ± 0.002, 0.10 ± 0.002 and 0.19 ± 0.001 micrograms respectively. By the end of fortyfive days (one thousand and eighty hours) the kidney showed maximum retention of iron. The kidney concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 0.87 ± 0.003, 0.09 ± 0.001, 0.23 ± 0.002, 0.89 ± 0.003, 0.37 ± 0.002, 0.19 ± 0.002, 0.09 ± 0.001, 0.21 ± 0.003 and 1.02 ± 0.001 micrograms respectively **(Table 22.0).**

The animals exposed to **0.1% M.E.** showed maximum retention of iron and mininium of cadmium and lead at the end of six hours. At the end of six hours, the cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc concentrations in the kidney were equivalent to 0.01 ± 0.0001, 0.13 ± 0.001, 0.09 ± 0.003, 3.21 ± 0.02, 0.01 ± 0.0001 0.09 ± 0.003, 1.31 ± 0.002, 0.2 ± 0.001 and 0.2 ± 0.001 micrograms respectively.

By the end of twelve hours the kidney showed maximum retention of iron followed by manganese and cadmium. The retention of cadmium chromium, copper, iron, magnesium, manganese, nickel and zinc were equivalent to 1.27 ± 0.02, 0.14 ± 0.002, 0.03 ± 0.001, 2.41 ± 0.004, 0.03 ± 0.01, 2.13 ± 0.002, 0.05 ± 0.01 and 0.1 ± 0.0001 micrograms respectively.

At the end of twentyfour hours the kidney showed maximum retention of cadmium followed by iron and manganese. Thus, the kidney concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel and zinc were equivalent to 2.10 ± 0.12, 0.173 ± 0.002, 0.04 ± 0.001, 1.73 ± 0.021, 0.00,

0.13 ± 0.001, 1.73 ± 0.02, 0.07 ± 0.01 and 0.3 ± 0.001 micrograms respectively.

Then by the end of fortyeight hours the iron retention was maximum followed by cadmium. The levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel and zinc were equivalent to 3.21 ± 0.05, 0.12 ± 0.012, 0.09 ± 0.021, 5.97 ± 0.14, 0.07 ± 0.001, 0.71 ± 0.015, 0.08 ± 0.002 and 0.38 ± 0.002 micrograms respectively.

At the end of seventy two hours the iron retention was maximum and was followed by cadmium. The kidney concentrations of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 2.81 ± 0.03, 0.09 ± 0.003, 0.10 ± 0.0001, 3.2 ± 0.001, 0.00, 0.09 ± 0.003, 0.59 ± 0.002, 0.09 ± 0.001 and 0.17 ± 0.001 micrograms respectively.

The kidney retention of iron was maximum at the end of ninety six hours. The retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.93 ± 0.04, 0.10 ± 0.002, 0.09 ±0.002, 3.92 ± 0.040, 0.02 ± 0.0021, 0.05 ± 0.002, 0.69 ± 0.021, 0.06 ± 0.001 and 0.25 ± 0.002 micrograms respectively.

By the end of one twenty hours the retention of iron was maximum. Thus, the kidney concentrations of cadmium, chromium, copper, iron, lead, mangnesium, manganese, nickel and zinc were equivalent to 1.72 ± 0.024, 0.139 ± 0.0001, 0.13 ± 0.0012, 7.26 ± 0.043, 0.00, 0.08 ± 0.001, 1.03 ± 0.014, 0.08 ± 0.002 and 0.31 ± 0.0012 micrograms respectively **(Table 22.1)**.

The ducks subjected to **1.0% M.E.** showed maximum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc at the end of six/seventytwo, six, ninetysix, one twenty hours, fortyeight/ seventytwo, one twenty hours, six hours, ninetysix and one twenty hours respectively. By the end of six hours, the kidney retention of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc was equivalent to 3.1 ± 0.003, 1.0 ± 0.0012, 0.03± 0.002, 3.8 ± 0.016, 0.10 ± 0.001,

TABLE : 22.1 RETENTION OF HEAVY METALS IN KIDNEY UNDER INFLUENCE OF 0.1 and 1.0% M.E.

0.1% M.E. :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	0.01 ± 0.0001	0.13 ± 0.001	0.09 ± 0.003	3.21 ± 0.02	0.09 ± 0.003	1.31 ± 0.002	0.02 ± 0.0001	0.01 ±0.0001	0.2 ±0.001
12 hours	1.27 ± 0.02	0.14 ± 0.002	0.03 ± 0.001	2.41 ± 0.004	0.03 ± 0.01	2.13 ± 0.002	0.05 ± 0.01	Nil	0.1 ±0.0001
24 hours	2.10 ± 0.12	0.173 ± 0.002	0.04 ± 0.001	1.73 ± 0.021	0.13 ± 0.001	1.73 ± 0.02	0.07 ± 0.01	Nil	0.3 ± 0.001
48 hours	3.21 ± 0.05	0.12 ± 0.012	0.09 ± 0.021	5.97 ± 0.14	0.07 ± 0.001	0.71 ± 0.015	0.08 ± 0.002	Nil	0.38 ± 0.002
72 hours	2.81 ± 0.03	0.09 ± 0.003	0.10 ± 0.0001	3.2 ± 0.001	0.09 ± 0.003	0.59 ± 0.002	0.09 ± 0.001	Nil	0.17 ± 0.001
96 hours	1.93 ± 0.04	0.10 ± 0.002	0.09 ± 0.002	3.92 ± 0.040	0.05 ± 0.002	0.69 ± 0.021	0.06 ± 0.001	0.02 ± 0.0021	0.25 ± 0.002
120 hours	1.72 ± 0.024	0.139 ± 0.0001	0.13 ± 0.0012	7.26 ± 0.043	0.08 ± 0.001	1.03 ± 0.014	0.08 ± 0.002	Nil	0.31 ± 0.0012

1.0% M.E. :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	3.1 ± 0.003	1.00 ± 0.0012	0.03 ± 0.002	3.8 ± 0.016	3.2 ± 0.002	0.9 ± 0.002	0.07 ± 0.004	0.1 ± 0.001	1.73 ± 0.002
12 hours	2.17 ± 0.02	0.7 ± 0.016	Nil	4.7 ± 0.004	2.9 ± 0.05	0.30 ± 0.001	0.20 ± 0.006	0.1 ± 0.001	1.67 ± 0.002
24 hours	2.10 ± 0.008	0.4 ± 0.005	Nil	5.20 ± 0.051	2.70 ± 0.044	0.4 ± 0.002	0.090 ± 0.001	0.100 ± 0.003	1.43 ± 0.022
48 hours	2.39 ± 0.016	0.73 ± 0.03	Nil	5.3 ± 0.035	2.9 ± 0.06	0.57 ± 0.003	0.05 ± 0.021	0.20 ± 0.001	1.57 ± 0.004
72 hours	3.10 ± 0.041	0.18 ± 0.002	0.4 ± 0.03	4.10 ± 0.10	1.9 ± 0.066	0.71 ± 0.004	0.07 ± 0.001	0.20 ± 0.013	1.80 ± 0.06
96 hours	2.17 ± 0.003	0.07 ± 0.002	0.42 ± 0.004	6.3 ± 0.019	3.0 ± 0.012	0.81 ± 0.004	0.53 ± 0.001	0.04 ± 0.001	1.73 ± 0.002
120 hour	1.73 ± 0.031	0.09 ± 0.001	0.03 ± 0.001	7.20 ± 0.06	4.30 ± 0.006	0.71 ± 0.010	0.070 ± 0.005	0.07 ± 0.002	1.90 ± 0.002

3.2 ± 0.002, 0.9 ± 0.002, 0.07 ± 0.004 and 1.73 ± 0.002 micrograms respectively.

At the end of twelve hours the kidney concentrations of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 2.17 ± 0.02, 0.7 ± 0.016, 0.00 , 4.7 ± 0.004, 0.1 ± 0.001, 2.9 ± 0.05, 0.30 ± 0.001, 0.20 ± 0.006 and 1.67 ± 0.002 micrograms respectively. Then by the end of twentyfour hours the kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 2.10 ± 0.008, 0.40 ± 0.005, 0.00, 5.20 ± 0.051, 0.10 ± 0.003, 2.70 ± 0.044, 0.40 ± 0.002, 0.090 ± 0.001 and 1.43 ± 0.022 micrograms respectively.

The kidney at the end of fortyeight hours, retained cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc, in concentrations equivalent to 2.39 ± 0.016, 0.73 ± 0.03, 5.30 ± 0.035, 0.20 ± 0.001, 2.90 ± 0.06, 0.57 ± 0.003, 0.05 ± 0.021 and 1.57 ± 0.004 micrograms respectively.

By the end of seventytwo hours the kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 3.10 ± 0.041, 0.18 ± 0.002, 0.4 ± 0.03, 4.10 ± 0.10, 0.20 ± 0.013, 1.90 ± 0.066, 0.71 ± 0.004, 0.07 ± 0.001 and 1.80 ± 0.06 micrograms respectively.

The kidney retentions at the end of ninetysix hours, of cadmium, chromium, copper, iron, lead, mangnesium, manganese, nickel and zinc were equivalent to 2.17 ± 0.003, 0.070 ± 0.002, 0.42 ± 0.004, 6.3 ± 0.019, 0.04 ± 0.001, 3.0 ± 0.012, 0.81 ± 0.004, 0.53 ± 0.001 and 1.73 ± 0.002 micrograms respectively.

At the end of one twenty hours, the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.73 ± 0.031, 0.09 ± 0.001, 0.030 ± 0.001, 7.20 ± 0.06, 0.070 ± 0.002, 4.30 ±

TABLE 22.2: RETENTION OF HEAVY METALS IN THE KIDNEY UNDER THE INFLUENCE OF 5.0% AND 10.0% M.E.

5.0% M. E :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	3.10 ± 0.012	1.7 ± 0.014	0.5 ± 0.002	6.5 ± 0.4	3.3 ± 0.012	0.3 ± 0.001	1.2 ± 0.021	0.6 ± 0.026	6.2 ± 0.024
12 hours	1.37 ± 0.003	1.3 ± 0.004	1.0 ± 0.001	7.6 ± 0.007	4.2 ± 0.016	0.5 ± 0.014	1.0 ± 0.002	0.9 ± 0.013	12.8 ± 0.044
24 hours	1.81 ± 0.003	1.0 ± 0.06	0.7 ± 0.002	6.9 ± 0.006	2.1 ± 0.10	0.400 ± 0.010	1.2 ± 0.04	0.60 ± 0.014	6.6 ± 0.014
48 hours	1.71 ± 0.013	1.3 ± 0.003	0.6 ± 0.004	6.6 ± 0.012	3.0 ± 0.04	0.5 ± 0.002	1.1 ± 0.003	0.9 ± 0.003	7.0 ± 0.061
72 hours	1.71 ± 0.07	1.41 ± 0.041	0.6 ± 0.004	7.9 ± 0.004	4.2 ± 0.021	0.3 ± 0.002	0.9 ± 0.005	1.3 ± 0.001	8.7 ± 0.024
96 hours	2.31 ± 0.003	1.31 ± 0.029	0.8 ± 0.006	8.5 ± 0.013	3.3 ± 0.013	0.5 ± 0.001	1.3 ± 0.004	1.1 ± 0.005	10.8 ± 0.03
120 hours	1.37 ± 0.001	1.10 ± 0.04	0.7 ± 0.003	5.8 ± 0.055	3.9 ± 0.066	0.4 ± 0.001	0.7 ± 0.006	0.8 ± 0.004	0.8 ± 0.006

10.0% M.E.

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	1.34 ± 0.008	2.7 ± 0.002	0.3 ± 0.004	8.8 ± 0.04	9.3 ± 0.004	0.370 ± 0.002	0.9 ± 0.002	0.3 ± 0.001	3.9 ± 0.026
12 hours	2.17 ± 0.008	1.7 ± 0.040	0.5 ± 0.007	6.31 ± 0.032	13.0 ± 0.012	0.80 ± 0.006	1.2 ± 0.013	0.7 ± 0.001	4.7 ± 0.042
24 hours	1.98 ± 0.002	1.2 ± 0.001	0.5 ± 0.002	4.1 ± 0.002	9.8 ± 0.088	0.91 ± 0.001	1.37 ± 0.011	0.8 ± 0.032	4.0 ± 0.016
48 hours	1.63 ± 0.030	1.9 ± 0.014	0.7 ± 0.012	3.1 ± 0.021	7.3 ± 0.07	1.01 ± 0.010	1.47 ± 0.04	0.5 ± 0.002	5.9 ± 0.061
72 hours	2.81 ± 0.014	1.8 ± 0.002	0.8 ± 0.003	2.8 ± 0.06	6.4 ± 0.006	0.63 ± 0.014	1.9 ± 0.040	0.7 ± 0.022	3.1 ± 0.11
96 hours	1.76 ± 0.003	1.7 ± 0.001	0.7 ± 0.001	8.9 ± 0.006	5.7 ± 0.004	0.71 ± 0.006	1.73 ± 0.003	0.9 ± 0.11	3.9 ± 0.14
120 hours	3.10 ± 0.013	9.0 ± 0.034	0.6 ± 0.001	1.3 ± 0.003	7.9 ± 0.061	0.87 ± 0.005	0.9 ± 0.008	0.6 ± 0.004	6.2 ± 0.066

0.006, 0.71 ± 0.010, 0.070 ± 0.005 and 1.90 ± 0.002 micrograms respectively **(Table 22.1)**.

Under the influence of **5.0%** M.E. the maximum kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were found at the end of six, six, twelve, ninetysix, seventytwo, twelve/seventytwo, twelve/fortyeight/ninetysix, ninetysix and twelve hours respectively.

By the end of six hours, the kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 3.10 ± 0.012, 1.7 ± 0.014, 0.50 ± 0.002, 6.50 ± 0.4, 0.60 ± 0.026, 3.30 ± 0.012, 0.30 ± 0.001, 1.20 ± 0.021 and 6.20 ± 0.024 micrograms respectively. The kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc at the end of twelve hours, were equivalent to 1.37 ± 0.003, 1.30 ± 0.004, 1.0 ± 0.001, 7.60 ± 0.007, 0.90 ± 0.013, 4.20 ± 0.016, 0.50 ± 0.014, 1.0 ± 0.002 and 12.80 ± 0.044 micrograms respectively.

By the end of twenty four hours, the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.81 ± 0.003, 1.0 ± 0.06, 0.70 ± 0.002, 6.9 ± 0.006, 0.60 ± 0.014, 2.10 ± 0.10, 0.40 ± 0.010, 1.20 ± 0.04 and 6.60 ± 0.014 micrograms respectively. The kidney retentions at the end of fortyeight hours, of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.71 ± 0.013, 1.30 ± 0.003, 0.60 ± 0.004, 6.60 ± 0.012, 0.90 ± 0.003, 3.0 ± 0.04, 0.50 ± 0.002, 1.10 ± 0.003 and 7.0 ± 0.061 micrograms respectively.

The seventy two hours exposure to **5%** M.E. induced retentions of cadmium, chromium, copper, iron, lead, magnesium, maganese, nickel and zinc in the kidney of ducks in concentrations equivalent to 1.71 ± 0.07, 1.41 ± 0.041, 0.60 ± 0.004, 7.90 ± 0.004, 1.30 ± 0.001, 4.20 ± 0.021, 0.30 ± 0.002, 0.90 ± 0.005 and 8.70 ± 0.024 micrograms respectively.

By the end of ninetysix hours the kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 2.31 ± 0.003, 1.31 ± 0.029, 0.80 ± 0.006, 8.50 ± 0.013, 1.10 ± 0.005, 3.30 ± 0.013, 0.50 ± 0.001, 1.30 ± 0.004 and 10.80 ± 0.03 micrograms respectively.

The one hundred and twentyhours exposure of ducks to **5.0% M.E.** promoted retentions in the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 1.37 ± 0.001, 1.10 ± 0.04, 0.70 ± 0.003, 5.80 ± 0.055, 0.80 ± 0.004, 3.90 ± 0.066, 0.40 ± 0.001, 0.70 ± 0.006 and 0.80 ± 0.006 micrograms respectively.

The **10% M.E.** induced maximum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc at the end of onetwenty, onetwenty, seventytwo, ninetysix, ninetysix, twelve, fortyeight, seventytwo and one twenty hours respectively.

By the end of six hours the kidney retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.34 ± 0.008, 2.70 ± 0.002, 0.30 ± 0.004, 8.80 ± 0.04, 0.30 ± 0.001, 9.30 ± 0.004, 0.37 ± 0.002, 0.90 ± 0.002 and 3.90 ± 0.026 micrograms respectively. At the end of twelve hours the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent 2.17 ± 0.008, 1.70 ± 0.040, 0.50 ± 0.007, 6.31 ± 0.032, 0.70 ± 0.001, 13.0 ± 0.012, 0.80 ± 0.006, 1.20 ± 0.013 and 4.70 ± 0.042 micrograms respectively.

The twentyfour hour exposure of ducks to **10% M.E.** promoted retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 1.98 ± 0.002, 1.20 ± 0.001, 0.50 ± 0.002, 4.10 ± 0.002, 0.80 ± 0.032, 9.80 ± 0.088, 0.91 ± 0.001, 1.37 ± 0.011 and 4.00 ± 0.016 micrograms respectively. By the end of forty eight hours, the retention in the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.63 ± 0.030, 1.90 ± 0.014, 0.70 ± 0.012,

3.10 ± 0.021, 0.50 ± 0.002, 7.30 ± 0.07, 1.01 ± 0.010, 1.47 ± 0.04 and 5.90 ± 0.061micrograms respectively.

The ducks exposed for seventytwo hours to **10.0% M.E.** exhibited kindney concentrations of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc equivalent to 2.81 ± 0.014, 1.80 ± 0.002, 0.80 ± 0.003, 2.80 ± 0.06, 0.70 ± 0.022, 6.40 ± 0.006, 0.63 ± 0.014, 1.90 ± 0.040 and 3.10 ± 0.11 micrograms respectively. The ninetysix hours exposure of ducks to **10.0% M.E.** caused retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 1.76 ± 0.003, 1.70 ± 0.001, 0.70 ± 0.001, 8.9 ± 0.006, 0.90 ± 0.11, 5.70 ± 0.004, 0.71 ± 0.006, 1.73 ± 0.003 and 3.90 ± 0.14 micrograms respectively.

By the end of one twenty hours the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 3.10 ± 0.013, 9.0 ± 0.034, 0.60 ± 0.001, 1.3 ± 0.003, 0.60 ± 0.004, 7.90 ± 0.061, 0.87 ± 0.005, 0.9 ± 0.008 and 6.20 ± 0.066 micrograms respectively.

The **50.0% M.E.** induced retentions of heavy metals into the kidney of ducks. The maximum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were observed at the end of fortyeight, twelve, one twenty, twelve, seventytwo, fortyeight, twelve, twentyfour and one twenty hours respectively. By the end of six hours the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 2.91 ± 0.012, 3.10 ± 0.021, 0.60 ± 0.003, 6.50 ± 0.014, 0.80 ± 0.001, 1.30 ± 0.03, 0.30 ± 0.002, 1.30 ± 0.001 and 5.80 ± 0.001 micrograms respectively. The twelve hour exposure promoted retention of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 2.51 ± 0.005, 3.70 ± 0.004, 0.80 ± 0.003, 9.30 ± 0.14, 1.0 ± 0.032, 0.70 ± 0.002, 19.10 ± 0.12, 2.10 ± 0.04 and 2.45 ± 0.014 micrograms respectively. At the end of twentyfour hours exposure of ducks to **50% M.E.**, the kidney concentrations of cadmium, chromium, copper,

iron, lead, magnesium, manganese, nickel and zinc were equivalent to 3.10 ± 0.001, 3.40 ± 0.004, 0.60 ± 0.004, 4.30 ± 0.04, 0.30 ± 0.013, 1.70 ± 0.001, 0.40 ± 0.001, 3.70 ± 0.034 and 6.20 ± 0.024 micrograms respectively.

The fortyeight hours exposure of ducks to **50.0% M.E.** induced retention of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 4.30 ± 0.004, 2.90 ± 0.005, 0.50 ± 0.007, 6.30 ± 0.019, 0.90 ± 0.008, 2.10 ± 0.016, 0.70 ± 0.003, 3.50 ± 0.025 and 8.10 ± 0.04 micrograms respectively. By the end of seventytwo hours, the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 3.70 ± 0.014, 2.70 ± 0.02, 0.90 ± 0.001, 5.60 ± 0.06, 1.30 ± 0.030, 0.90 ± 0.001, 0.40 ± 0.012, 3.20 ± 0.002 and 9.10 ± 0.004 micrograms respectively. On exposure for ninetysix hours to **50% M.E.** the ducks exhibited retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 0.30 ± 0.003, 2.10 ± 0.006, 0.90 ± 0.002, 4.90 ± 0.006, 0.70 ± 0.003, 1.0 ± 0.008, 0.40 ± 0.003, 2.40 ± 0.010 and 7.10 ± 0.04 micrograms respectively. By the end of one hundred and twenty hours exposure the ducks retained in kidney cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 2.20 ± 0.08, 2.30 ± 0.01, 1.10 ± 0.10, 5.90 ± 0.06, 0.80 ± 0.02, 1.70 ± 0.003, 0.90 ± 0.001, 1.90 ± 0.06 and 12.0 ± 0.04 micrograms respectively.

The ducks subjected to **100% M.E.** showed wide varations in the retentions of the heavy metals. The maximum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel, and zinc were found at the end of twelve, ninetysix, onetwenty, onetwenty, ninetysix hours, twentyfour, twentyfour, twentyfour and one twenty hours respectively. By the end of six hours the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc are equivalent to 2.10 ± 0.014, 3.0 ± 0.014, 3.13 ± 0.072, 21.40 ± 0.10, 1.60 ± 0.024, 0.90 ± 0.001, 13.20 ± 0.026,

TABLE 22.3 : RETENTION OF HEAVY METALS IN THE KIDNEY UNDER THE INFLUENCE OF 50% AND 100%M.E.

50.0% M.E. :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	2.91 ± 0.012	3.1 ± 0.021	0.6 ± 0.003	6.5 ± 0.014	1.3 ± 0.03	0.3 ± 0.002	1.3 ± 0.001	0.8 ± 0.001	5.8 ± 0.001
12 hours	2.51 ± 0.005	3.7 ± 0.004	0.8 ± 0.003	9.30 ± 0.14	0.7 ± 0.002	19.1 ± 0.12	2.1 ± 0.04	1.0 ± 0.032	2.45 ± 0.014
24 hours	3.1 ± 0.001	3.4 ± 0.004	0.6 ± 0.004	4.3 ± 0.04	1.7 ± 0.001	0.4 ± 0.001	3.7 ± 0.034	0.3 ± 0.013	6.2 ± 0.024
48 hours	4.3 ± 0.004	2.9 ± 0.005	0.5 ± 0.007	6.3 ± 0.019	2.1 ± 0.016	0.7 ± 0.003	3.5 ± 0.025	0.9 ± 0.008	8.1 ± 0.04
72 hours	3.7 ± 0.014	2.7 ± 0.02	0.9 ± 0.001	5.6 ± 0.06	0.9 ± 0.001	0.4 ± 0.012	3.2 ± 0.002	1.3 ± 0.030	9.1 ± 0.004
96 hours	0.3 ± 0.003	2.1 ± 0.006	0.9 ± 0.002	4.9 ± 0.006	1.0 ± 0.008	0.4 ± 0.003	2.4 ± 0.010	0.7 ±0.003	7.1 ± 0.04
120 hours	2.2 ± 0.08	2.3 ± 0.01	1.1 ± 0.10	5.9 ± 0.06	1.7 ± 0.003	0.9 ± 0.001	1.9 ± 0.06	0.8 ± 0.02	12.0 ±0.04

100.0% M.E. :

Time	Cd µg/gm Ww	Cr µg/gm Ww	Cu µg/gm Ww	Fe µg/gm Ww	Mg µg/gm Ww	Mn µg/gm Ww	Ni µg/gm Ww	Pb µg/gm Ww	Zn µg/gm Ww
6 hours	2.1 ± 0.014	3.0 ± 0.014	3.13 ± 0.072	21.4 ± 0.10	0.9 ± 0.001	13.2 ± 0.026	2.3 ± 0.06	1.6 ± 0.024	20.2 ± 0.041
12 hours	3.2 ± 0.12	3.72 ± 0.014	1.7 ± 0.013	14.3 ± 0.096	0.7 ± 0.003	8.9 ± 0.16	3.4 ± 0.064	2.3 ± 0.008	23.7 ± 0.015
24 hours	1.31 ± 0.06	4.31 ± 0.032	1.21 ± 0.044	20.0 ± 0.009	1.03 ± 0.056	17.0 ± 0.008	4.5 ± 0.005	1.9 ± 0.016	9.7 ± 0.014
48 hours	2.7 ± 0.06	4.7 ± 0.035	1.05 ± 0.04	8.9 ± 0.006	0.89 ± 0.014	3.7 ± 0.005	2.1 ± 0.011	0.3 ± 0.001	8.3 ± 0.032
72 hours	1.9 ± 0.06	4.9 ± 0.016	2.3 ± 0.016	7.4 ± 0.012	0.3 ± 0.014	5.7 ± 0.061	3.1 ± 0.054	2.7 ± 0.001	9.8 ± 0.055
96 hours	2.9 ± 0.008	9.3 ± 0.008	3.4 ± 0.066	7.0 ± 0.15	0.9 ± 0.003	7.3 ± 0.12	1.32 ± 0.016	4.1 ± 0.030	23.7 ± 0.032
120 hours	3.12 ± 0.014	5.8 ± 0.004	3.82 ± 0.05	24.1 ± 0.14	0.52 ± 0.003	9.7 ± 0.037	0.9 ± 0.001	0.8 ± 0.048	28.4 ± 0.055

2.30 ± 0.06 and 20.20 ± 0.041 micrograms respectively. On exposure to **100% M.E.** for twelve hours the ducks exhibited retentions into the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 3.20 ± 0.12, 3.72 ± 0.014, 1.70 ± 0.013, 14.3 ± 0.096, 2.30 ± 0.008, 0.70 ± 0.003, 8.90 ± 0.16, 3.40 ± 0.064 and 23.70 ± 0.015 micrograms respectively.

At the end of twentyfour hours the retentions in the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 1.31 ± 0.06, 4.31 ± 0.032, 1.21 ± 0.044, 20.0 ± 0.009, 1.90 ± 0.016, 1.03 ± 0.056, 17.0 ± 0.008, 4.50 ± 0.005 and 9.70 ± 0.014 micrograms respectively. The ducks exposed for fortyeight hours to **100% M.E.** showed retentions into the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 2.70 ± 0.06, 4.70 ± 0.035, 1.05 ± 0.04, 8.90 ± 0.006, 0.30 ± 0.001, 0.89 ± 0.014, 3.70 ± 0.005, 2.10 ± 0.011 and 8.30 ± 0.032 micrograms respectively.

The seventytwo hours exposure of ducks to **100% M.E.** promoted retentions into the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 1.90 ± 0.06, 4.90 ± 0.016, 2.30 ± 0.016, 7.40 ± 0.012, 2.70 ± 0.001, 0.30 ± 0.014, 5.70 ± 0.061, 3.10 ± 0.054 and 9.80 ± 0.055 micrograms respectively. By the end of ninetysix hours the retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc into the kidneys were equivalent to 2.90 ± 0.008, 9.30 ± 0.008, 3.40 ± 0.066, 7.00 ± 0.15, 4.10 ± 0.030, 0.90 ± 0.003, 7.30 ± 0.12, 1.32 ± 0.016 and 23.70 ± 0.032 micrograms respectively. On exposure for one hundred and twenty hours the ducks exhibited retentions into the kidney of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in concentrations equivalent to 3.12 ± 0.014, 5.80 ± 0.004, 3.82 ± 0.05, 24.10 ± 0.14, 0.80 ± 0.048, 0.52 ± 0.003, 9.70 ± 0.037, 0.90 ± 0.001 and 28.40 ± 0.055 micrograms respectively.

B] RETENTION IN SERUM :

The changes in the retentions of heavy metals in the serum are compiled in **Table No. 23.0, 23.1, 23.2 and 23.3.** The retention values are expressed as micrograms (μg) per litre of the serum. The ducks ***(Anas platyrhynchos domesticus)*** exposed to mining effluents **(M.E.)** exhibited retention of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc. The control animals exhibited cadmium, chromium, copper, iron, magnesium, manganese and zinc 1.0 ± 0.01, 1.0 ± 0.1, 2.0 ± 0.5, 45.0 ± 2.0, 5.0 ± 1, 1320.0 ± 1.0, and 19.0 ± 7.0 micrograms respectively.

On exposure to **0.01% M.E.** the retentions of heavy metals and their changes in concentrations at different time intervals up to twentynine days were satistically non significant but at the end of thirty days (seven hundred and twenty hours) the serum retained zinc maximally and the concentrations of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 81.00 ± 2.0, 9.0 ± 1.00, 9.00 ± 1.00, 89.00 ± 1.00, 31.00 ± 1.00, 37.00 ± 1.00, 37.00 ± 7.00, 7.00 ± 2.00 and 93.00 ± 1.00 micrograms respectively **(Table 23.0)**. By the end of fortyfive days (one thousand and eighty hours) the serum levels of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 271.00 ± 1.00, 13.70 ± 2.00, 83.00 ± 2.00, 109.00 ± 1.00, 51.00 ± 2.00, 59.00 ± 2.00, 49.00 ± 3.00, 10.00 ± 2.00 and 271.00 ± 2.00 micrograms respectively.

When the ducks were exposed to **0.1% M.E. (Table 23.1)** the maximum serum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were observed at the end of ninetysix, seventytwo, ninetysix, one twenty, one twenty, twentyfour, one twenty, twentyfour and twentyfour hours respectively.

TABLE 23.0 : RENTENTION OF HEAVY METALS IN THE SERUM OF DUCKS EXPOSED TO 0.01% M.E.

0.01% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
30 Days	81.00 ± 2.00	9.00 ± 1.00	9.00 ± 1.00	89.00 ± 1.00	37.00 ± 1.00	37.00 ± 7.00	7.00 ± 2.00	31.00 ± 1.00	93.00 ± 1.00
45 Days	271.00 ± 1.00	13.70 ± 2.00	83.00 ± 2.00	109.00 ± 1.00	59.00 ± 2.00	49.00 ± 3.00	10.00 ± 2.00	51.00 ± 2.00	271.00 ± 2.00

TABLE 23.1 : RETENTION OF HEAVY METALS IN THE SERUM OF DUCKS EXPOSED TO 0.1% AND 1% M.E.

0.1% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	73.10 ± 1.00	43.00 ± 2.00	51.00 ± 1.00	731.00 ± 1.00	17.00 ± 2.00	Nil	8.00 ± 2.00	0.00 ± 0.00	70.00 ± 1.00
12 hours	321.00 ± 4.00	37.00 ± 5.00	53.00 ± 4.00	531.00 ± 3.00	28.00 ± 2.00	Nil	7.00 ± 1.00	1.00 ± 0.00	50.00 ± 2.00
24 hours	327.00 ± 20.00	39.10 ± 0.10	42.10 ± 1.00	421.00 ± 40.00	43.00 ± 2.00	Nil	9.00 ± 1.00	2.00 ± 0.10	81.00 ± 3.00
48 hours	427.00 ± 2.00	29.70 ± 2.00	23.00 ± 7.00	793.00 ± 32.00	42.00 ± 2.00	Nil	7.00 ± 0.10	1.00 ± 0.00	61.00 ± 3.00
72 hours	531.00 ± 11.00	47.00 ± 1.00	57.00 ± 1.00	871.00 ± 24.00	31.00 ± 1.00	Nil	5.00 ± 1.00	2.00 ± 0.10	39.00 ± 2.00
96 hours	621.00 ± 5.00	23.00 ± 0.10	59.00 ± 2.00	832.00 ± 32.00	40.00 ± 2.00	Nil	3.00 ± 1.00	0.00 ± 0.00	49.00 ± 2.10
120 hours	381.00 ± 3.00	41.00 ± 0.10	48.00 ± 2.30	887.00 ± 1.60	39.00 ± 2.00	27.00 ± 2.40	2.00 ± 1.00	3.00 ± 0.10	57.00 ± 1.20

1% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6-hours	1320.00 ± 3.00	71.00 ± 1.20	41.00 ± 5.00	7200.00 ± 6.00	735.00 ± 4.00	Nil	34.00 ± 2.00	7.00 ± 2.4	21.00 ± 10.00
12 hours	1249.00 ± 1.50	31.00 ± 1.00	50.00 ± 13.00	3271.00 ± 12.00	576.00 ± 4.00	Nil	55.00 ± 3.00	20.00 ± 4.00	271.00 ± 12.00
24 hours	1349.00 ± 4.00	29.00 ± 3.00	43.00 ± 11.00	2712.00 ± 12.00	430.00 ± 4.00	Nil	34.00 ± 2.00	0.00 ± 0.00	193.00 ± 13.00
48 hours	2219.00 ± 21.00	53.00 ± 4.00	40.00 ± 3.00	1371.00 ± 32.00	493.00 ± 8.00	Nil	93.00 ± 16.00	30.00 ± 0.10	214.00 ± 12.00
72 hours	2317.00 ± 18.00	9.00 ± 1.00	45.00 ± 4.00	1978.00 ± 66.00	517.00 ± 11.00	Nil	7.90 ± 2.90	40.00 ± 3.00	197.00 ± 4.00
96 hours	1790.00 ± 30.00	17.00 ± 6.00	52.00 ± 2.00	2017.00 ± 6.00	632.00 ± 3.00	Nil	8.10 ± 4.00	9.00 ± 1.20	231.00 ± 14.00
120 hours	1891.00 ± 21.00	39.00 ± 2.00	63.00 ± 3.00	1386.00 ± 26.00	710.00 ± 2.00	Nil	91.00 ± 4.10	20.00 ± 2.00	271.00 ± 1.30

On exposure for six hours to **0.1% M.E.**, the ducks showed retentions in serum of cadmium, chromium, copper, iron, lead, magnesium manganese, nickel and zinc equivalent to 73.10 ± 1.00, 43.00 ± 2.00,51.00 ± 1.00, 731.00 ± 1.00, Nil, 17.00 ± 2.00., Nil, 8.0 ± 2.00 and 70.00 ± 1.00 micrograms respectively. By the end of twelve hours exposure the serum concentrations of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc were equivalent to 321.00 ± 4.00. 37.00 ± 5.00, 53.00 ± 4.00, 531.00 ± 3.00, 1.00 ± 0.00, 28.00 ± 2.00, Nil, 7.00 ± 1.00 and 50.00 ± 2.00 micrograms respectively. The exposure of ducks to **0.1% M.E.** for twentyfour hours induced maximum retention of iron and the serum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel, and zinc were equivalent to 327.00 ± 20.00, 39.10 ± 0.10, 42.10 ± 1.00, 421.00 ± 40.00, 2.00 ± 0.10, 43.00 ± 2.00, Nil, 9.00 ± 1.00 and 81.00 ± 3.00 micrograms respectively. The exposure of ducks to **0.1% M.E.** for fortyeight hours induced increase in iron retention. The serum retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel, and zinc were equivalent to 427.00 ± 2.00, 29.70 ± 2.00, 23.00 ± 7.00, 793.00 ± 32.00, 1.00 ± 0.00, 42.00 ± 2.00, Nil, 7.00 ± 0.10 and 61.00 ± 3.00 micrograms per litre respectively.

By the end of seventytwo hours, the serum retentions of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 531.00 ± 11.00, 47.00 ± 1.00, 57.00 ± 1.00, 871.00 ± 24.00, 31.00 ± 1.00, Nil, 5.00 ± 1.00, 2.00 ± 0.10 and 39.00 ± 2.00 micrograms per litre respectively. The ninetysix hours exposure of ducks to **0.1% M.E.** induced higher retention of iron and cadmium. The serum concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 621.00 ± 5.00, 23.00 ± 0.10, 59.00 ± 2.00, 832.00 ± 32.00, 40.00 ± 2.00, Nil, 3.00 ± 1.00, Nil and 49.00 ± 2.10 micrograms per litre respectively. After the exposure of ducks to **0.1% M.E.** for one twenty hours, the serum concentrations of cadmium, chromium, copper, iron, magnesium, manganese,

nickel, lead and zinc were equivalent to 381.00 ± 3.00, 41.00 ± 0.10, 48.00 ± 2.30, 887.00 ± 1.6, 39.00 ± 2.00, 27.00 ± 2.40, 2.00 ± 1.00, 3.00 ± 0.10 and 57.00 ± 1.20 micrograms per litre respectively.

When the ducks were exposed to **1.0% ME** the maximum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were observed at the end of seventy two, six, onetwenty, six, six, fortyeight, seventytwo and twelve/one twenty hours respectively. At the end of six hours the serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1320.00 ± 3.00, 71.00 ± 1.20, 41.00 ± 5.00, 720.00 ± 6.00, 735.00 ± 4.00, 34.00 ± 2.00, 7.00 ± 2.4 and 21.00 ± 10.00 micrograms per litre respectively. The twelve hours exposure of ducks to **1.0% M.E.** promoted maximum retention of iron into the serum. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1249.00 ± 1.50, 31.00 ± 1.00, 50.00 ± 13.00, 3271.00 ± 12.00, 576.00 ± 4.00, 55.00 ± 3.00, 20.00 ± 4.00 and 271.00 ± 12.00 micrograms per litre respectively. The twenty four hours exposure of ducks to **1.0% M.E.** induced higher retentions of iron and cadmium in to the serum. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, and zinc were equivalent to 1349.00 ± 4.00, 29.00 ± 3.00, 43.00 ± 11.00, 2712.00 ± 12.00, 430.00 ± 4.00, 34.00 ± 2.00 and 193.00 ± 13.00 micrograms per litre respectively. By the end of forty eight hours the cadmium retention in the serum was higher than other metals. Thus, the serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2219 ± 21.00, 53.00 ± 4.00, 40.00 ± 3.00, 1371.00 ± 32.00, 493.00 ± 8.00, 93.00 ± 16.00, 30.00 ± 0.10 and 214.00 ± 12.00 micrograms per litre respectively.

The seventytwo hours exposure of ducks to **1.0%M.E.** caused maximum retention of cadmium. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2317.00 ±

18.00, 9.00 ± 1.00, 45.00 ± 4.00, 1978.00 ± 66.00, 517.00 ± 11.00, 7.90 ± 2.90, 40.00 ± 3.00 and 197.00 ± 4.00 micrograms per litre respectively. At the end of ninetysix hours the serum retention of iron was maximum followed by cadmium. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1790.00 ± 30.00, 17.00 ± 6.00, 52.00 ± 2.00, 2017.00 ± 6.00, 632.00 ± 3.00, 8.10 ± 4.00, 9.00 ± 1.20 and 231.00 ± 14.00 micrograms per litre respectively. The one hundred and twenty hours exposure of ducks to **1.0% M.E.** promoted higher retention of cadmium followed by iron and magnesium. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1891.00 ± 21.00, 39.00 ± 2.00, 63.00 ± 3.00, 1386.00 ± 26.0, 710.00 ± 2.00, 91.00 ± 4.10, 20.00 ± 2.00 and 271.00 ± 1.30 micrograms per litre respectively.

The ducks subjected to **5.0% M.E.** exhibited significantly higher retentions of cadmium iron, magnesium, and zinc into the serum. The highest serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were observed at the end of six, six, seventytwo, onetwenty, onetwenty, seventytwo, fortyeight and seventytwo hours respectively.

At the end of the six hours zinc, iron, cadmium and magnesium retentions in the serum were significantly higher. Thus, the serum retentions of cadmium, chromium, copper, iron, magnesium, nickel , lead and zinc were equivalent to 2131.00 ± 13.00, 390.00 ± 1.10, 210.00 ± 1.00, 3220.00 ± 20.00, 1270.00 ± 13.00, 270.0 ± 4.00, 140.00 ± 2.00 and 5320.00 ± 14.00. micrograms per litre respectively. The twelve hours subjection of ducks to **5% M.E.** promoted maximum retention of zinc into the serum followed by iron and cadmium. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2013 ± 10.00, 280.00 ±6.00, 140.00 ± 4.00, 2800.00 ± 39.00, 1030.00 ± 4.00, 290.00 ± 2.00, 35.00 ± 1.50 and 5915.00 ± 43.00 micrograms per litre respectively. By the end of twentyfour hours expsoure to **5.0% M.E.** the ducks exhibited higher levels of zinc, iron,

TABLE 23.2 : RETENTION OF HEAVY METALS IN THE SERUM OF DUCKS EXPOSED TO 5.0% AND 10.0% M.E.

5% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	2131.00 ± 13.00	390.00 ± 1.100	210.00 ± 1.00	3220.00 ± 20.00	1270.00 ± 13.00	Nil	270.00 ± 4.00	140.00 ± 2.00	5320.00 ± 14.00
12 hours	2013.00 ± 10.00	280.00 ± 6.00	140.00 ± 4.00	2800.00 ± 39.00	1030.00 ± 4.00	Nil	290.00 ± 2.00	35.00 ± 1.500	5915.00 ± 43.00
24 hours	1371.00 ± 33.00	270.00 ± 10.00	140.00 ± 13.00	2800.00 ± 21.00	1480.00 ± 14.00	Nil	370.00 ± 3.50	70.00 ± 1.30	7280 ± 1.40
48 hours	1013.00 ± 1.60	310.00 ± 0.60	140.00 ± 1.00	1890.00 ± 4.00	1390.00 ± 1.40	Nil	380.00 ± 32.00	350.00 ± 13.00	3640 ± 1.90
72 hours	1371.00 ± 31.00	290.00 ± 2.00	245.00 ± 12.00	3990.00 ± 14.00	2380.00 ± 32.00	Nil	470.00 ± 31.00	105.00 ± 1.00	7630.00 ± 6.00
96 hours	983.00 ± 3.00	210.00 ± 1.00	175.00 ± 4.00	1960.00 ± 3.00	2430.00 ± 42.00	Nil	170.00 ± 1.00	35.00 ± 1.400	4900.00 ± 9.00
120 hours	1217.00 ± 6.00	190.00 ± 8.00	105.00 ± 5.00	5740.00 ± 7.00	2470.00 ± 30.00	Nil	97.00 ± 1.00	70.00 ± 1.00	4585.00 ± 16.00

10% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	3170.00 ± 70.00	1473.00 ± 4.00	130.00 ± 1.00	3271.00 ± 10.00	2130.00 ± 34.00	Nil	1030.00 ± 13.00	110.00 ± 10.00	1930.00 ± 3.00
12 hours	2100.00 ± 10.00	89.00 ± 24.00	271.00 ± 4.20	2141.00 ± 4.00	1730.00 ± 6.00	Nil	37.00 ± 3.00	90.00 ± 4.00	2710.00 ± 4.10
24 hours	2197.00 ± 27.00	37.00 ± 7.00	171.00 ± 4.30	3171.00 ± 4.00	1293.00 ±12.00	Nil	390.00 ± 19.00	170.00 ± 7.00	1370.00 ± 1.40
48 hours	2790.00 ± 19.00	310.00 ± 4.00	184.00 ± 8.00	2101.00 ± 12.00	1401.00 ± 32.00	Nil	400.00 ± 32.00	200.00 ± 2.00	1520.00 ± 15.00
72 hours	2376.00 ± 32.00	380.00 ± 40.00	137.00 ± 3.00	1710.00 ± 14.00	2137.00 ± 14.00	Nil	510.00 ± 15.00	170.00 ± 3.00	990.00 ± 4.00
96 hours	2147.00 ± 1.70	290.00 ± 1.90	141.00 ± 6.00	2103.00 ± 1.20	2108.00 ± 1.70	Nil	300.00 ± 3.90	193.00 ± 2.00	1030.00 ± 11.00
120 hours	1987.00 ± 4.10	170.00 ± 4.10	151.00 ± 3.10	1871.00 ± 8.10	2173.00 ± 3.70	Nil	470.00 ± 2.20	171.00 ± 3.00	2370.00 ± 6.00

magnesium and cadmium. Thus, the serum retention of cadmium, chromium, copper , iron, magnesium, nickel, lead and zinc were equivalent to 1371.00 ± 33.00, 270.00 ± 10.00, 140.00 ± 1.00, 2800.0 ± 21.00, 1480.00 ± 14.00, 370.00 ± 3.50, 70 ± 1.30 and 7280 ± 1.40 micrograms per litre respectively. The ducks exposed to **5.0% M.E.** for fortyeight hours exhibited higher retentions of cadmium, iron, magnesium and zinc into the serum but zinc retention was highest of all these metals. The serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead, and zinc were equivalent to 1013.00 ± 1.60, 310.00 ± 0.6, 140.00 ± 1.00, 1890.0 ± 4.00, 1390.0 ± 1.40, 380.00 ± 32.00, 350.00 ± 13.00 and 3640.00 ± 1.90 micrograms per litre respectively. The seventy two hours treatment/exposure of ducks with **5.0% M.E.** promoted highest serum retention of zinc, followed by iron, magnesium and cadmium. The serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1371.00 ± 31.00, 290.00 ± 2.00, 245.00 ± 12.00, 3990.00 ± 14.00, 2380.00 ± 32.00, 470.00 ± 31.00, 105.00 ± 1.00 and 7630.00 ± 6.00 micrograms per litre respectively. By the end of ninetysix hours exposure to **5.0% M.E.** the serum showed higher retentions of zinc, magnesium and iron. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 983.00 ± 3.00, 210.00 ± 1.00, 175.00 ± 4.00, 1960.00 ± 3.00, 2430.00 ± 42.00, 170.00 ± 1.00, 35.00 ± 1.400 and 4900.00 ± 9.00 micrograms per litre respectively. At the end of one twenty hours exposure to **5.0% M.E.** the serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1217.00 ± 6.00, 190.00 ± 8.00, 105.00 ± 5.00, 5740.00 ± 7.00, 2470.00 ± 30.00, 97.00 ± 1.00, 70.00 ± 1.00 and 4585.00 ± 16.00 micrograms per litre respectively **(Table 23.2)**.

The mallards subjected to **10% M.E.** exhibited maximum retention of cadmium, chromium, copper, iron, magnesium, nickel, lead, and zinc at the end of six, six, twelve, six, onetwenty, six, fortyeight and twelve hours respectively.

By the end of six hours the serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 3170.00 ± 70.00, 1473.00 ± 4.00, 130.00 ± 1.00, 3271.00 ± 10.00, 2130.00 ± 34.00, 1030.00 ± 13.00, 110.00 ± 10.00 and 1930.00 ± 3.00 micrograms per litre respectively. Thus, at the end of six hours the cadmium and iron retentions were very high.

The animals exposed to **10.0% M.E.** for twelve hours showed high retentions of cadmium, iron and zinc in the serum. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2100.00 ± 10.00, 89.00 ± 24.00, 271.00 ± 4.20, 2141.00 ± 4.00 1730.00 ± 6.00, 37.00 ± 3.00, 90.00 ± 4.00 and 2710.00 ± 4.10 micrograms per litre respectively.

On exposure to **10.0% M.E.** for twentyfour hours, the serum retained cadmium, iron, magnesium and zinc at a significantly higher level. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nicke, lead and zinc were equivalent to 2197.00 ± 27.00, 37.00 ± 7.00, 171.00 ± 4.30, 3171.00 ± 4.00, 1293.00 ± 12.00, 390.00 ± 19.00, 170.00 ± 7.00 and 1370.00 ± 1.40 micrograms per litre respectively.

The ducks subjected to **10.0% M.E.** for fortyeight hours showed higher retentions of cadmium iron, magnesium and zinc into the serum. The serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2790.00 ± 19.00, 310.00 ± 4.00, 184.00 ± 8.00 , 2101.00 ± 12.00, 1401.00 ± 32.00, 400.00 ± 32.00, 200.00 ± 2.00 and 1520.00 ± 15.00 micrograms per litre respectively.

The seventytwo hours exposure of ducks to **10% M.E.** induced higher retentions of cadmium and magnesium in to the serum. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2376.00 ± 32.00, 380.00 ± 40.00, 137.00 ± 3.00,

1710.00 ± 14.00, 2137.00 ± 14.00, 510.00 ± 15.00, 170.00 ± 3.00 and 990.00 ± 4.00 micrograms per litre respectively.

On exposing animals to **10% M.E.** for ninetysix hours, the serum showed higher retentions of cadmium, iron and magnesium. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel , lead and zinc were equivalent to 2147.00 ± 1.70, 290.00 ± 1.90, 141.00 ± 6.00, 2103.00 ± 1.20, 2108.00 ± 1.70, 300.00 ± 3.90, 193.00 ± 2.00 and 1030.00 ± 11.00 micrograms per litre respectively.

The one hundred and twenty hours exposure of ducks to **10.0% M.E.** caused higher retentions of magnesium and zinc into the serum. The serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1987.00 ± 4.10, 170.00 ± 4.10, 151.00 ± 3.10, 1871.00 ± 8.10, 2173.00 ± 3.70, 470.00 ± 2.20, 171.00 ± 3.00 and 2370.00 ± 6.00 micrograms per litre respectively **(Table 23.2)**.

When the ducks were exposed to **50% M.E.** the maximum serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were observed at the end of twelve, six/ninetysix, twentyfour, ninetysix, six, one twenty, twentyfour and fortyeight hours respectively. At the end of six hours the serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2370.00 ± 1.00, 2170.00 ± 2.00, 175.00 ± 1.50, 2450.00 ± 2.50, 1700.0 ± 2.00, 1370.00 ± 1.40, 35.00 ± 3.30 and 5075.00 ± 1.70 micrograms per litre respectively.

The twelve hours exposure of ducks to **50% M.E.** induced higher retention of cadmium and zinc in the serum. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 3127.00 ± 12.30, 1390.00 ± 3.40, 175.0 ± 5.00, 875.00 ± 5.10, 1380.00 ± 7.00, 830.00 ± 7.00, 35.00 ± 1.50 and 2450.0 ± 41.00 micrograms per litre respectively.

TABLE 23.3 : RETENTION OF HEAVY METALS IN THE SERUM OF DUCKS EXPOSED TO 50% AND 100% M.E.

50% M. E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	2370.00 ± 1.00	2170.00 ± 2.00	175.00 ± 1.50	2450.00 ± 2.50	1700.00 ± 2.00	Nil	1370.00 ± 1.40	35.00 ± 3.30	5075.00 ± 1.70
12 hours	3127.00 ± 12.30	1390.0 ±3.40	175.0 ± 5.0	875.00 ± 5.1	1380.00 ± 7.00	Nil	830.00 ± 7.00	35.00 ± 1.50	2450.0 ± 41.00
24 hours	1290 ± 90.00	1840.00 ± 14.00	245.00 ± 42.00	1925.00 ± 1.10	1520.00 ± 1.50	Nil	970.00 ± 6.00	140.00 ± 1.40	2240.00 ± 31.00
48 hours	1470.00 ± 30.00	1790.00 ± 90.00	210.00 ± 6.00	2520.00 ± 16.00	1570.00 ± 14.00	Nil	730.00 ± 32.00	70.00 ± 2.1	7560.00 ±14.00
72 hours	1570.00 ± 71.00	1930.0 ± 30.00	175.00 ± 6.200	2590.00 ±140.00	1590.00 ± 90.00	Nil	1270.00 ±1.4	70.00 ± 10.00	3780.00 ± 4.00
96 hours	2390.00 ± 3.90	2170.00 ± 10.00	105.00 ± 2.00	3010.00 ± 6.00	990.00 ± 6.00	Nil	1030.00 ± 1.00	20.00 ± 1.00	7350.00 ± 3.00
120 hours	2870.00 ± 40.00	2060.00 ± 22.00	175.00 ± 30.00	2555.00 ± 16.00	890.00 ± 32.00	Nil	1890.00 ± 16.00	70.00 ± 2.00	3080.00 ± 6.00

100% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	5310.00 ± 18.00	3207.00 ± 24.00	2913.00 ± 64.00	9120.00 ± 30.00	930.00 ± 18.00	3012.00 ± 4.00	1820.00 ± 61.00	1307.00 ± 16.00	8732.00 ± 38.00
12 hours	4270.00 ± 27.00	2102.00 ± 12.00	3100.00 ± 80.00	6320.00 ± 22.00	870.00 ± 70.00	4310.00 ±110.00	1720.00 ± 12.00	80.00 ± 13.00	3020.00 ± 2.00
24 hours	6370.00 ± 91.00	2371.00 ± 71.00	3270.00 ± 16.00	7100.00 ± 37.00	1030.00 ± 13.00	5010.00 ± 32.00	1420.00 ± 14.00	1710.00 ± 62.00	5230.00 ± 51.00
48 hours	3890.00 ± 80.00	2540.00 ± 60.00	4100.00 ± 56.00	5130.00 ±12.00	1070.00 ± 50.00	2710.00 ± 50.00	1370.00 ± 30.00	890.00 ± 80.00	6230.00 ±13.00
72 hours	2130.00 ± 60.00	2917.00 ± 18.00	1320.00 ± 13.00	7020.00 ± 31.00	980.00 ± 6.00	3210.00 ± 15.00	1390.00 ± 8.00	2010.00 ± 20.00	4370.00 ± 18.00
96 hours	4320.00 ± 70.00	4031.00 ± 51.00	1890.00 ±16.00	3730.00 ±125.00	191.00 ± 11.00	4150.00 ±15.00	930.00 ± 4.00	930.00 ± 41.00	8030.00 ± 19.00
120 hours	5570.00 ± 88.00	5201.00 ± 42.00	2370.00 ±112.00	8720.00 ±130.00	820.00 ± 14.00	5170.00 ± 90.00	1020.00 ± 2.00	1320.00 ±110.00	8930.00 ±156.00

The subjection of ducks to **50% M.E.** for twentyfour hours promoted higher serum retentions of zinc and iron. The serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1290.0 ± 90.00, 1840.00 ± 14.00, 245.00 ± 42.00, 1925.00 ± 1.10, 1520.00 ± 1.50, 970.00 ± 6.00, 140.00 ± 1.40 and 2240.00 ± 31.00 micrograms per litre respectively.

The ducks treated with **50% M.E.** for fortyeight hours exhibited high retention of zinc into the serum followed by iron. The serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1470.00 ± 30.00, 1790.00 ± 90.00, 210.00 ± 6.00, 2520.00 ± 16.00, 1570.00 ± 14.00, 730.00 ± 32.00, 70.00 ± 2.10, 7560.00 ± 14.00 micrograms per litre respectively.

At the end of seventytwo hours treatment with **50% M.E.**, the serum levels of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 1570.00 ± 71.00, 1930.00 ± 30.00, 175.00 ± 6.20 2590.00 ± 140.00, 1590.00 ± 90.00, 1270.00 ± 1.40, 70.00 ± 10.00 and 3780.00 ± 4.00 micrograms per litre respectively.

The ninetysix hours exposure of ducks to **50% M.E.** promoted very high retentions of zinc and iron. The serum concentrations of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc were equivalent to 2390.00 ± 3.90, 2170.00 ± 10.00, 105.00 ± 2.00, 3010.00 ± 6.00, 990.00 ± 6.00, 1030.00 ± 1.00, 20.00 ± 1.00 and 7350.00 ± 3.00 micrograms per litre respectively.

The one twenty hours treatment of ducks with **50% M.E.** induced serum retentions of cadmium, chromium, copper, iron, magnesium, nickel, lead and zinc equivalent to 2870.00 ± 40.00, 2060.00 ± 22.00, 175.00 ± 30.00, 2555.00 ± 16.00, 890.00 ± 32.00, 1890.00 ± 16.00, 70.00 ± 2.00 and 3080.00 ± 6.00 micrograms per litre respectively **(Table 23.3)**.

The ducks exposed to **100% M.E.** exhibited highest serum retention of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and

zinc at the end of twentyfour, one twenty, fortyeight, six, fortyeight, one twenty, six, seventytwo and onetwenty hours respectively. At the end of six hours the serum concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 5310.00 ± 18.00, 3207.00 ± 24.00, 2913.00 ± 64.00, 9120.00 ± 30.0, 930.00 ± 18.00, 3012.00 ± 4.00 1820.00 ± 61.00, 1307.00 ± 16.00 and 8732.00 ± 38.00 micrograms litre respectively.

The exposure of ducks to **100% M.E.** for twelve hours promoted high serum retentions of iron, manganese, cadmium, copper and zinc. The serum concentrations of cadmium, chromium, copper iron, magnesium, manganese, nickel, lead and zinc were equivalent to 4270.00 ± 27.00, 2102.00 ± 12.00, 3100.00 ± 80.00, 6320.00 ± 22.00, 870.00 ± 70.00, 4310.00 ± 110.00, 1720.00 ± 12.00, 80.00 ± 13.00 and 3020.00 ± 2.00 micrograms per litre respectively. The ducks subjected to **100% M.E.** for twentyfour hours showed high serum retentions of iron, manganese, zinc, cadmium, copper and chromium. Thus, the serum retentions of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 6370.00 ± 91.00, 2371.00 ± 71.00, 3270.0 ± 16.00, 7100.0 ± 37.00, 1030.00 ± 13.00, 5010.00 ± 32.00, 1420.00 ± 14.00, 1710.00 ± 62.00 and 5230.00 ± 51.00 micrograms per litre respectively.

The fortyeight hours exposure of ducks to **100% M.E.** induced higher serum retentions of zinc, iron, manganese, copper, cadmium, and chromium. The serum levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 3890.00 ± 80.00, 2540.00 ± 60.00, 41000.00 ± 56.00, 5130.00 ± 12.00, 1070.0 ± 50.00, 2710.00 ± 50.00, 1370.0 ± 30.00, 890.00 ± 80.00 and 6230.0 ± 13.0 micrograms per litre respectively.

The ducks subjected to **100% M.E.** for seventytwo hours showed high serum retentions of iron, zinc, manganese, chromium, cadmium and lead.

Thus, the serum levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 2130.00 ± 60.00, 2917.00 ± 18.00, 1320.00 ± 13.00, 7020.00 ± 31.00, 980.00 ± 6.00, 3210.00 ± 15.00, 1390.00 ± 8.00, 2010.00 ± 20.00 and 4370.00 ± 18.00 micrograms per litre respectively.

On exposing the bird to **100% M.E.** for ninetysix hours the serum showed very high retentions for zinc, cadmium, chromium, manganese, and iron. The serum concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 4320.00 ± 70.00 , 4031.00 ± 51.00, 1890.00 ± 16.00, 3730.00 ± 125.00, 191.00 ± 11.00, 4150.00 ± 15.00, 930.00 ± 4.00, 930.00 ± 41.00 and 8030.00 ± 19.00 micrograms per litre respectively.

The one hundred and twenty hours treatment of **100% M.E.** promoted very high serum retentions of cadmium, chromium, iron, manganese and zinc. The serum retentions of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 5570.00 ± 88.00, 5201.00 ± 42.00, 2370 ± 112.00, 8720.00 ± 130.00, 820.00 ± 14.00, 5170.00 ± 90.00, 1020.00 ± 2.00, 1320.00 ± 110.00 and 8930.00 ± 156.00 micrograms per litre respectively **(Table No. 23.3).**

C] THE RENAL CLEARANCE OF HEAVY METALS :

The alterations in the renal clearance of heavy metals through urine are compiled in Table No. **24.0, 24.1, 24.2 and 24.3.** The renal clearance values are expressed as micrograms of the metal per litre of urine. The urine of control animlas exhibited presence of iron, manganese and zinc in concentrations equivalent to 4.00 ± 0.1, 2.00 ± 1.00 and 3.0 ± 0.1 micrograms per litre respectively. The other metals were not present in all the urine samples collected at the time intervals under study. The ducks ***(Anas platyrhynchos domesticus)*** exposed to a very low concentration of **0.01% M.E.** did not exhibit any change in the renal clearance pattern up to the end of twentynine days but at the end of thirty days (seven hundred and twenty hours) the urine samples showed the cadmium, chromium, copper, iorn, magnesium, manganese, nickel, lead and zinc concentrations equivalent to 32.0 ± 2.2, 8.0 ± 1.0, 7.0 ± 2.0 , 37.2 ± 2.0, 10.0 ± 1.0, 10.0 ± 1.0, 12.0 ± 1.0, 8.0 ± 0.30 and 32.0 ± 2.0 micrograms per litre respectively. By the end of fortyfive days (one thousand and eighty hours) the renal clearance of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc was equivalent to 73.0 ± 0.2, 8.0 ± 0.4, 17.0 ± 0.2, 30.0 ± 1.0, 21.0 ± 0.4, 9.0 ± 0.1, 82.0 ± 4.0, 12.0 ± 1.0 and 97.0 ± 1.0 micrograms per litre respectively **(Table 24.0)**.

The ducks exposed to **0.1% M.E.** showed wide fluctuations in the renal clearance of heavy metals at different time intervals. The renal clearance at the end of six hours was equivalent to 70.0 ± 0.2, 30.0± 1.0, 71.0 ± 1.0, 3.0 ± 0.10, 68.0 ± 1.0, 1.0 ± 0.01, and 30.0 ± 1.0 micrograms per litre for chromium, copper, iron, magnesium, manganese, nickel and zinc respectively.

Thus it appeared that the chromium, iron and manganese clearance i.e elimination through urine was quite high.

TABLE 24.0 : RENAL CLEARANCE OF HEAVY METALS IN DUCKS EXPOSED TO 0.01% M.E.

0.01% M. E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
30 Days	32.00 ± 2.2	8.00 ± 1.00	7.00 ± 2.00	37.2 ± 2.0	10.00 ± 1.00	10.00 ± 1.00	12.00 ± 1.00	8.00 ± 0.30	32.00 ± 2.00
45 Days	73.00 ± 0.20	8.00 ± 0.40	17.00 ± 0.20	30.00 ± 1.00	21.00 ± 0.4	9.00 ± 0.10	82.00 ± 4.00	12.00 ± 1.00	97.00 ± 1.00

TABLE 24.1 : RENAL CLEARANCE OF HEAVY METALS IN DUCKS EXPOSED TO 0.1% AND 1% ME.

0.1% M. E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	Nil	70.00 ± 0.20	30.00 ± 1.00	71.00 ± 1.00	3.00 ±0.10	68.00 ± 1.00	1.00 ± 0.01	Nil	30.00 ± 1.00
12 hours	13.00 ± 0.20	3.00 ± 0.01	20.00 ± 1.00	71.00 ± 1.00	2.00 ± 0.01	56.00 ± 3.00	Nil	Nil	37.00 ± 2.00
24 hours	32.00 ± 2.00	4.00 ± 0.01	10.00 ± 0.01	81.00 ± 1.00	1.00 ± 0.01	49.00 ± 5.00	2.00 ± 0.01	1.00 ± 0.01	42.00 ± 4.00
48 hours	42.00 ± 2.00	Nil	37.00 ± 1.00	71.00 ± 4.00	23.00 ± 0.01	21.00 ± 1.00	1.00 ± 0.01	2.00 ± 0.01	31.00 ± 2.00
72 hours	51.00 ± 2.40	Nil	28.00 ± 2.00	87.00 ± 2.00	24.00 ± 2.00	9.00 ± 1.00	1.00 ± 0.01	1.00 ± 0.01	25.00 ± 2.00
96 hours	42.00 ± 8.00	7.00 ± 0.11	29.00 ± 0.11	91.00 ± 2.00	32.00 ± 0.01	32.00 ± 0.11	Nil	Nil	17.00 ± 1.20
120 hours	51.00 ± 2.00	8.00 ± 1.20	37.00 ± 2.00	82.00 ± 1.00	37.00 ± 4.00	27.00 ± 1.00	2.00 ± 0.01	1.00 ± 0.01	27.00 ± 2.00

1.0% M.E.

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	40.00 ± 1.40	50.00 ± 1.00	Nil	90.00 ± 4.00	70.00 ± 1.50	40.00 ± 3.00	8.00 ± 1.30	4.00 ± 0.1	120.00 ± 4.00
12 hours	45.00 ± 3.00	70.00 ± 1.00	9.00 ± 1.00	137.00 ± 4.00	50.00 ± 1.20	20.00 ± 3.00	3.00 ± 1.10	9.00 ± 2.00	150.00 ± 1.10
24 hours	57.00 ± 1.60	20.00 ± 0.02	20.00 ± 1.40	131.00 ± 2.00	90.00 ± 4.00	10.00 ± 0.01	4.00 ± 0.02	Nil	170.00 ± 1.20
48 hours	60.00 ± 1.00	27.00 ± 3.00	30.00 ± 0.26	130.00 ± 2.00	50.00 ± 6.00	30.00 ± 3.10	7.00 ± 0.20	10.00 ± 1.10	80.00 ± 2.40
72 hours	70.00 ± 1.70	Nil	30.00 ± 3.00	150.00 ± 2.00	70.00 ± 1.00	40.00 ± 2.00	2.00 ± 0.01	10.00 ± 1.00	90.00 ± 1.20
96 hours	57.00 ± 4.00	92.00 ± 1.00	35.00 ± 3.00	173.00 ± 2.00	80.00 ± 2.00	310.00 ± 1.200	3.00 ± 0.01	Nil	110.00 ± 1.00
120 hours	75.00 ± 2.00	72.00 ± 6.00	45.00 ± 1.00	182.00 ± 4.00	90.00 ± 1.00	40.00 ± 2.00	9.00 ± 2.00	3.00 ± 0.01	140.00 ± 4.00

The twelve hour exposure of ducks to **0.1% M.E.** induced high renal clearance of iron and manganese. The renal clearance of cadmium, chromium, copper, iron, magnesium, manganese and zinc was equivalent to 13.0 ± 0.2, 3.0 ± 0.01, 20.0 ± 1.0, 71.0 ± 1.0, 2.0 ± 0.01, 56.0 ± 3.0 and 37.0 ± 2.0 micrograms per litre respectively. On exposure to **0.1% M.E.** for twentyfour hours the ducks showed high renal clearance of iron, manganese, zinc and cadmium. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 32.0 ± 2.0, 4.0 ± 0.01, 10.0 ± 0.01, 81.0 ± 1.0, 1.0 ± 0.01, 49.0 ± 5.0, 2.0 ± 0.01, 1.0 ± 0.01 and 42.0 ± 4.0 micrograms per litre respectively. By the end of forty eight hours the renal clearances of iron, cadmium, copper, manganese and zinc were higher than those of other metals. Thus, the renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 42.0 ± 2.0, 0.00, 37.0 ± 1.0, 71.0 ± 4.0, 23.0 ± 0.01, 21.0 ± 1.0, 1.0 ± 0.01, 2.0 ± 0.01 and 31.0 ± 2.0 micrograms (per litre) respectively.

The ducks exposed to **0.1% M.E.** for seventytwo hours showed significantly higher renal clearance of iron and cadmium. The renal clearances of cadmium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 51.0 ± 2.4, 28.0 ± 2.0, 87.0 ± 2.0, 24.0 ± 2.0, 9.0 ±1.0, 1.0 ± 0.01, 1.0 ± 0.01 and 25.0 ± 2.0 micrograms per litre respectively.

Exposure to **0.1% M.E.** for ninetysix hours induced significant renal clearances of iron, cadmium, magnesium, manganese and copper. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese and zinc were equivaletn to 42.0 ± 8.0, 7.0 ± 0.11, 29.0 ± 0.11, 91.0 ± 2.0, 32.0 ± 0.1, 32.0 ± 0.11, and 17.0 ± 1.2 micrograms respectively.

By the end of one twenty hours exposure to **0.1% M.E.** the urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 51.0 ± 2.0, 8.0 ± 1.2, 37.0 ± 2.0, 82.0 ±

1.0, 37.0 ± 4.0, 27.0 ± 1.0, 2.0 ± 0.01, 1.0 ± 0.01 and 27.0 ± 2.0 micrograms respectively.

When the ducks were exposed to **1.0% M.E.** the maximum renal clearances of chromium, lead and zinc were observed at the end of ninetysix, seventytwo/fortyeight and twentyfour hours respectively. While cadmium, copper, iron, magnesium, nickel clearances were highest at the end of one twenty hours and manganese at ninetysix hours exposure. The ducks exposed for six hours to **1.0% M.E.** showed renal clearances of cadmium, chromium, iron, magnesium, manganese, nickel, lead and zinc equivalent to 40.0 ± 1.4, 50.0 ± 1.0, 90.0 ± 4.0, 70.0 ± 1.5, 40.0 ± 3.0, 8.0 ± 1.3, 4.0 ± 0.1, and 120.0 ± 4.0 micrograms respectively. There was no renal clearance of copper at this time interval. On exposure for twelve hours to **1.0% M.E.** the renal clearances for zinc and iron were very high. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 45.0 ± 3.0, 70.0 ± 1.0, 9.0 ± 1.0, 137.0 ± 4.0, 50.0 ± 1.2, 20.0 ± 3.0, 3.0 ± 1.0, 9.0 ± 2.0 and 150.0 ± 1.1 micrograms respectively.

The subjection of ducks to **1.0% M.E.** for twentyfour hours promoted maximum renal clearance of zinc and iron followed by magnesium and cadmium. The renal clearances of cadmium, chromium, copper iron, magnesium, manganese, nickel, and zinc were equivalent to 57.0 ± 1.6, 20.0 ± 0.02, 20.0 ± 1.4, 131.0 ± 2.0, 90.0 ± 4.0, 10.0 ± 0.01, 4.0 ± 0.02 and 170.0 ± 1.20 micrograms respectively. There was no renal clearance of lead at this time interval.

The fortyeight hours exposure of ducks to **1.0% M.E.** caused higher renal clearances of iron, zinc, cadmium and magnesium. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 60.0 ± 1.0, 27.0 ± 3.0, 30.0 ± 0.26, 130.0 ± 2.0, 50.0 ± 6.0, 30.0 ± 3.1, 7.0 ± 0.2, 10.0 ± 1.10 and 80.0 ± 2.4 micrograms respectively. By the end of seventytwo hours exposure to **1.0% M.E.** the renal clearances of

iron, zinc, cadmium, and magnesium were quite high. The urine concentrations of cadmium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 70.0 ± 1.7, 30.0 ± 3.0, 150.0 ± 2.0, 70.0 ± 1.0, 40.0 ± 2.0, 2.0 ± 0.01, 10.0 ± 1.0 and 90.0 ± 1.2 micrograms respectively. There was no renal clearance of chromium.

The ducks exposed to **1.0% M.E.** for ninetysix hours showed a very high renal clearance of manganese, iron and zinc while there was no renal clearance of lead. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, and zinc were equivalent to 57.0 ± 4.0, 92.0 ± 1.0, 35.0 ± 3.0, 173.0 ± 2.0, 80.0 ± 2.0, 310.0 ± 1.2, 3.0 ± 0.01 and 110.0 ± 1.0 micrograms respectively.

The one hundred and twenty hours exposure of ducks to **1.0% M.E.** promoted maximum renal clearance of iron and zinc. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 75.0 ± 2.0, 72.0 ± 6.0, 45.0 ± 1.0, 182.0 ± 4.0, 90.0 ± 1.0, 40.0 ± 2.0, 9.0 ± 2.0, 3.0 ± 0.01 ad 140.0 ± 4.0 micrograms respectively.

When the mallards were subjected to **5.0% M.E.** the maximum renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were observed at the end of seventytwo, ninetysix, seventytwo/twenty four, seventytwo, forty eight, one twenty, six, twentyfour / fortyeight and ninetysix hours respectively. At the end of six hours exposure the urine concentrations of cadmium, chromium, copper, magnesium, nickel, lead and zinc were equivalent to 30.0 ± 1.0, 20.0 ± 1.0, 20.0 ± 1.0, 30.0 ± 3.0, 70.0 ± 1.2, 10.0 ± 0.50 and 310.0 ± 1.5 micrograms respectively.

The ducks exposed to **5%M.E.** for twelve hours did not show renal clearance of chromium and iron while the renal clearances of cadmium and zinc very high. The renal clearances of cadmium, copper, magnesium, manganese, nickel, lead and zinc were equivalent to 70.0 ± 4.0, 20.0 ± 3.0, 50.0 ± 5.0, 10.0 ± 0.01, 30.0 ± 1.0, 3.0 ± 0.1 and 250.0 ± 2.2 micrograms

TABLE 24.2 : RENAL CLEARANCE OF HEAVY METALS IN DUCKS EXPOSED TO 5% AND 10% M.E.

5.0 % M. E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	30.00 ± 1.00	20.00 ± 1.00	20.00 ± 1.00	Nil	30.00 ± 3.00	Nil	70.00 ± 1.20	10.00 ± 0.5	310.00 ± 1.50
12 hours	70.00 ± 4.00	Nil	20.00 ± 3.00	Nil	50.00 ± 5.00	10.00 ± 0.01	30.00 ± 1.00	3.00 ± 0.1	250.00 ± 2.2
24 hours	90.00 ± 1.00	Nil	50.00 ± 1.40	20.00 ± 1.00	60.00 ± 3.00	10.00 ± 1.00	20.00 ± 6.00	50.00 ± 4.00	410.00 ± 1.20
48 hours	100.00 ± 0.40	Nil	20.00 ± 1.2	Nil	700.00 ± 1.10	10.00 ± 0.10	40.00 ± 0.40	50.00 ± 2.00	260.00 ± 1.00
72 hours	130.00 ± 1.00	Nil	50.00 ± 4.00	1130.00 ± 3.10	90.00 ± 1.40	10.00 ± 0.10	20.00 ± 1.90	2.00 ± 0.06	65.00 ± 0.60
96 hours	90.00 ± 6.00	89.00 ± 4.00	10.00 ± 0.1	Nil	80.00 ± 3.00	10.00 ± 1.00	40.00 ± 1.00	30.00 ± 3.00	460.00 ± 4.00
120 hours	91.00 ± 6.00	20.00 ± 1.30	40.00 ± 1.40	40.00 ± 1.10	70.00 ± 4.10	50.00 ± 3.00	30.00 ± 1.00	30.00 ± 1.20	410.00 ± 1.40

10% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	80.00 ± 2.20	Nil	20.00 ± 4.00	10.00 ± 0.10	80.00 ± 2.00	70.00 ± 2.00	30.00 ± 1.10	20.00 ± 1.00	290.00 ± 1.90
12 hours	30.00 ± 1.00	10.00 ± 0.10	30.00 ± 2.00	190.00 ± 1.20	70.00 ± 4.10	20.00 ± 0.10	40.00 ± 3.00	10.00 ± 0.10	320.00 ± 6.00
24 hours	70.00 ± 3.7	30.00 ± 1.10	40.00 ± 1.40	210.00 ± 1.10	90.00 ± 2.10	20.00 ± 4.00	70.00 ± 1.70	20.00 ± 1.00	190.00 ± 2.00
48 hours	50.00 ± 2.00	40.00 ± 2.00	50.00 ± 2.00	100.00 ± 1.00	70.00 ± 2.00	10.00 ± 0.10	20.00 ± 0.10	70.00 ± 2.00	310.00 ± 1.30
72 hours	60.00 ± 3.00	70.00 ± 3.7	60.00 ± 1.00	170.00 ± 7.00	80.00 ± 1.7	30.00 ± 0.60	30.00 ± 0.30	30.00 ± 1.30	220.00 ± 2.00
96 hours	50.00 ± 4.40	50.00 ± 1.00	30.00 ± 1.30	170.00 ± 1.10	50.00 ± 4.00	40.00 ± 1.50	20.00 ± 0.60	80.00 ± 8.00	320.00 ± 4.20
120 hours	70.00 ± 3.40	30.00 ± 4.00	20.00 ± 0.30	230.00 ± 6.00	90.00 ± 1.00	21.00 ± 1.00	40.00 ± 1.00	90.00 ± 2.00	390.00 ± 1.90

respectively. The twenty four hours exposure of ducks to **5% M.E.** induced very high renal clearance of zinc. There was no renal clearance of chromium. The urine concentrations of cadmium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 90.0 ± 1.0, 50.0 ± 1.4, 20.0 ± 1.0, 60.0 ± 3.0, 10.0 ± 1.0, 20.0 ± 6.0, 50.0 ± 4.0 and 410.0 ± 1.2 micrograms respectively.

On exposure to **5.0 % M.E.** for fortyeight hours, the ducks exhibited the highest renal clearance of magnesium. The renal clearance of zinc was high but there was no renal clearance of chromium and iron. The urine levels of cadmium, copper, magnesium, manganese, nickel, lead and zinc were equivalent to 100.0 ± 0.40, 20.0 ± 1.2, 700.0 ±1.10, 10.0 ± 0.1, 40.0 ± 0.4, 50.0 ± 2.0, and 260.0 ± 1.0 micrograms respectively.

The seventytwo hours exposure to **5% M.E.** promoted highest renal clearance of iron but there was no renal clearance of chromium. The renal clearances of cadmium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 130.0 ± 1.0, 50.0 ± 4.0, 1130.0 ± 3.1, 90.0 ± 1.4, 10.0 ± 0.1, 20.0 ± 1.9, 2.0 ± 0.06 and 65.0 ± 0.6 micrograms respectively.

On exposing the ducks to **5.0% M.E.** for ninetysix hours, there was induction of highest renal clearance of zinc while there was no clearance of iron. The renal clearances of cadmium, chromium and magnesium were high. Thus, the renal clearances of cadmium, chromium, copper, magnesium, manganese, nickel, lead and zinc were equivalent to 90.0 ± 6.0, 89.0 ± 4.0, 10.0 ± 0.1, 80.0 ± 3.0, 10.0 ± 1.0, 40.0 ± 1.0, 30.0 ± 3.0 and 460.0 ± 4.0 micrograms respectively. By the end of one twentyhours of exposure the renal clearance of zinc was maximum in comparison with other metal clearances. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 91.0 ± 6.0, 20.0 ± 1.3, 40.0 ± 1.4, 40.0 ± 1.1, 70.0 ± 4.1, 50.0 ± 3.0, 30.0 ± 1.0, 30.0 ± 1.2 and 410.0 ± 1.4 micrograms respectively.

When the mallards were subjected to **10.0% M.E.** the maximum renal clearances of cadmium and zinc were observed at the end of six hours, and by the end of twentyfour hours the iron and zinc clearances were maximum. But the renal clearances of iron and zinc were highest at the end of seventytwo hours while the iron, magnesium, lead and zinc clearances were highest at the end of one twenty hours. By the end of six hours the renal clearances of cadmium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 80.0 ± 2.2, 20.0 ± 4.0, 10.0 ± 0.1, 80.0 ± 2.0, 70.0 ± 2.0, 30.0 ± 1.1, 20.0 ± 1.0 and 290.0 ± 1.9 micrograms respectively. There was no renal clearance of chromium at this time interval. The twelve hours exposure of ducks to **10.0% M.E.** caused high renal clearance of zinc and iron. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 30.0 ± 1.0, 10.0 ± 0.1, 30.0 ± 2.0, 190.0 ± 1.2, 70.0 ± 4.1, 20.0 ± 0.1, 40.0 ± 3.0, 10.0 ± 0.1 and 320.0 ± 6.0 micrograms respectively.

The twentyfour hour exposure of ducks to **10.0% M.E.** caused very high renal clearance of iron and zinc. The urine concentrations of cadmium chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 70.0 ± 3.7, 30.0 ± 1.1 40.0 ± 1.4, 210.0 ± 1.1, 90.0 ± 2.1, 20.0 ± 4.0, 70.0 ± 1.7, 20.0 ± 1.0 and 190.0 ± 2.0 micrograms respectively. On exposing ducks to **10.0% M.E.** for fortyeight hours, the higher renal clearances of zinc and iron were observed. The renal clearance of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc was equivalent to 50.0 ± 2.0, 40.0 ± 2.0, 50.0 ± 2.0, 100.0 ± 1.0, 70.0 ± 2.0, 10.0 ± 0.1, 20.0 ± 0.1, 70.0 ± 2.0 and 310.0 ± 1.3 micrograms respectively. The seventytwo hours exposure to **10.0% M.E.** induced high renal clearances of cadmium, chromium, copper, iron, magnesium and zinc. Thus the renal clearances of cadmium, chromium, copper iron, magnesium, manganese, nickel, lead and

zinc were equivalent to 60.0 ± 3.0, 70.0 ± 3.7, 60.0 ± 1.0, 170.0 ± 7.0, 80.0 ± 1.7, 30.0 ± 0.6, 30.0 ± 0.3, 30.0 ± 1.3 and 220.0 ± 2.0 micrograms respectively.

The ninetysix hours exposure to **10% M.E.** promoted very high renal clearances of zinc and iron. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 50.0 ± 4.4, 50.0 ± 1.0, 30.0 ± 1.3, 170.0 ± 1.1, 50.0 ± 4.0, 40.0 ± 1.5, 20.0 ± 0.6, 80.0 ± 8.0 and 320.0 ± 4.2 micrograms respectively.

By the end of one hundred and twenty hours exposure to **10% M.E.**, the highest renal clearance of zinc and iron were observed. The urine levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 70.0 ± 3.4, 30.0 ± 4.0, 20.0 ± 0.3, 230.0 ± 6.0, 90.0 ± 1.0, 21.0 ± 1.0, 40.0 ± 1.0, 90.0 ± 2.0 and 390.0 ± 1.9 micrograms respectively.

The ducks subjected to **50.0% M.E.** exhibited the highest renal clearances of cadmium, chromium, manganese, nickel, lead and zinc at the end of ninetysix, six / one twenty, fortyeight, ninetysix, one twenty and twentyfour hours respectively. Similarly the copper, iron and magnesium clearances were very high at the end of twentyfour hours. By the end of six hours exposure the renal clearances of cadmium, chromium, copper, magnesium, nickel and zinc were equivalent to 30.0 ± 2.0, 70.0 ± 3.0, 20.0 ± 1.4, 70.0 ± 2.0, 30.0 ± 1.1 and 430.0 ± 3.0 micrograms respectively. The ducks did not show any renal clearance of iron, manganese and lead at this time interval.

The twelve hour exposure of ducks to **50.0% M.E.** promoted high renal clearance of magnesium and zinc. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 30.0 ± 3.0, 30.0 ± 1.3, 10.0 ± 0.1, 40.0 ± 1.3, 90.0 ± 2.0, 50.0 ± 4.0, 50.0 ± 4.0, 30.0 ± 1.3 and 460.0 ± 2.6 micrograms per litre respectively. On exposing ducks for twentyfour hours to **50.0% M.E.** the highest renal clearances of zinc and iron were observed. The urine levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were

Table 24.3 : RENAL CLEARANCE OF HEAVY METALS IN DUCKS EXPOSED TO 50% AND 100% M.E.

50% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	30.00 ± 2.00	70.00 ± 3.00	20.00 ± 1.40	Nil	70.00 ± 2.00	Nil	30.00 ± 1.10	Nil	430.00 ± 3.00
12 hours	30.00 ± 3.00	30.00 ± 1.30	10.00 ± 0.10	40.00 ± 1.30	90.00 ± 2.00	50.00 ± 4.00	50.00 ± 4.00	30.00 ± 1.30	460.00 ± 2.60
24 hours	40.00 ±2.00	20.00 ± 1.00	70.00 ± 3.00	550.00 ± 14.00	100.00 ± 1.00	10.00 ± 2.00	60.00 ± 2.00	20.00 ± 1.00	630.00 ± 23.0
48 hours	50.00 ± 2.00	40.00 ± 2.00	20.00 ± 1.00	120.00 ± 1.00	90.00 ± 3.00	80.00 ± 6.00	40.00 ± 1.00	10.00 ± 1.00	300.00 ± 3.00
72 hours	40.00 ± 1.60	50.00 ± 2.00	30.00 ± 3.00	Nil	30.00 ± 4.00	10.00 ± 1.00	70.00 ± 3.00	30.00 ± 1.00	320.00 ± 2.40
96 hours	80.00 ± 3.00	60.00 ± 1.00	30.00 ± 6.00	110.00 ± 1.00	60.00 ± 3.00	10.00 ± 0.10	80.00 ± 4.00	30.00 ± 1.00	460.00 ± 3.00
120 hours	70.00 ± 2.00	70.00 ± 3.00	30.00 ± 1.00	Nil	43.00 ± 1.00	Nil	30.00 ± 1.00	40.00 ± 2.00	240.00 ± 4.50

100% M.E. :

Time	Cd µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mg µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Zn µg/l
6 hours	13.00 ± 1.20	90.00 ± 3.00	80.00 ± 2.20	90.00 ± 1.20	30.00 ± 1.00	70.00 ± 3.00	90.00 ± 2.00	20.00 ± 1.00	1310.00 ± 26.00
12 hours	90.00 ± 6.00	103.00 ± 2.00	70.00 ± 4.00	120.00 ± 1.00	70.00 ± 3.00	50.00 ± 1.00	30.00 ± 1.10	30.00 ± 1.10	830.00 ± 22.00
24 hours	83.00 ± 2.00	80.00 ± 6.00	30.00 ± 1.30	230.00 ± 14.0	20.00 ± 0.50	130.00 ± 0.40	20.00 ± 4.00	70.00 ± 6.00	3010.00 ± 52.0
48 hours	97.00 ± 3.20	100.00 ± 0.10	80.00 ± 6.00	570.00 ± 5.70	50.00 ± 3.20	101.00 ± 1.00	10.00 ± 0.10	20.00 ± 3.20	2010.00 ± 40.00
72 hours	73.00 ± 1.80	92.00 5.2	100.00 ± 3.90	530.00 ± 3.60	80.00 ± 1.60	80.00 ± 3.20	40.00 ±5.20	80.00 ± 5.20	1370.00 ± 13.00
96 hours	101.00 ± 1.00	71.00 ± 2.00	90.00 ± 3.00	570.00 ± 1.20	30.00 ± 1.00	102.00 ± 0.20	50.00 ± 4.00	30.00 ± 9.00	2910.00 ± 52.00
120 hours	93.00 ± 1.00	97.00 ± 1.30	130.00 ± 4.00	670.00 ± 2.00	20.00 ± 1.00	230.00 ± 2.00	70.00 ± 3.00	300.00 ± 1.00	390.00 ± 4.00

equivalent to 40.0 ± 2.0, 20.0 ± 1.0, 70.0 ± 3.0, 550.0 ± 14.0, 100.0 ± 1.0, 10.0 ± 2.0, 60.0 ± 2.0, 20.0 ± 1.0 and 630.0 ± 23.0 micrograms per litre of urine respectively. By the end of fortyeight hours exposure to **50% M.E.**, high renal clearances of iron and zinc were seen. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 50.0 ± 2.0, 40.0 ± 2.0, 20.0 ± 1.0, 120.0 ± 1.0, 90.0 ± 3.0, 80.0 ± 6.0, 40.0 ± 1.0, 10.0 ± 1.0 and 300.0 ± 3.0 micrograms per litre respectively.

The ducks exposed to **50.0 % M.E.** for seventytwo hours showed very high renal clearance of zinc but here was no clearance of iron. The renal clearances of cadmium, chromium, copper, magnesium, manganese, nickel, lead and zinc were equivalent to 40.0 ± 1.6, 50 .0 ± 2.0, 30.0 ± 3.0, 30.0 ± 4.0, 10.0 ± 1.0, 70.0 ± 3.0, 30.0 ± 1.0 and 320.0 ± 2.4 micrograms respectively.

The ninetysix hours exposure of ducks to **50% M.E.** promoted very high renal clearance of zinc and iron. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 80.0 ± 3.0, 60.0 ± 1.0, 30.0 ± 6.0, 110.0 ± 1.0, 60.0 ± 3.0, 10.0 ± 0.1, 80.0 ± 4.0, 30.0 ± 1.0 and 460.0 ± 3.0 micrograms per litre of urine respectively.

By the end of one hundred and twenty hours the renal clearances of cadmium, chromium, copper, magnesium, nickel, lead and zinc were equivalent to 70.0 ± 2.0, 70.0 ± 3.0, 30.0 ± 1.0, 43.0 ± 1.0, 30.0± 1.0, 40.0 ± 2.0 and 240.0 ± 4.5 micrograms respectively. There was no renal clearance of iron and manganese though there was very high renal clearance of zinc.

When the ducks were exposed to **100% M.E.** the renal clearance of zinc was quite high throughout the study period. The highest renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were observed at the end of ninetysix, twelve, one twenty, one twenty, seventytwo, one twenty, six, one twenty and twentyfour hours respectively. By the end of six hours the renal clearances of cadmium, chromium, copper, iron,

magnesium, manganese, nickel, lead and zinc were equivalent to 13.0 ± 1.2, 90.0 ± 3.0, 80.0 ± 2.2, 90.0 ± 1.2, 30.0 ± 1.0, 70.0 ± 3.0, 90.0 ± 2.0, 20.0 ± 1.0 and 1310.0 ± 26.0 micrograms respectively. The exposure of ducks for twelve hours to **100% M.E.** promoted high renal clearances of chromium, iron and zinc. The urine levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 90.0 ± 6.0, 103.0 ± 2.0, 70.0 ± 4.0, 120.0 ± 1.0, 70.0 ± 3.0, 50.0 ± 1.0, 30.0 ± 1.1, 30.0 ± 1.1 and 830.0 ± 22 micrograms respectively. By the end of twentyfour hours exposure to **100% M.E.** the highest renal clearance of zinc was observed. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 83.0 ± 2.0, 80.0 ± 6.0, 30.0 ± 1.3, 230.0 ± 14.0, 20.0 ± 0.50, 130.0 ± 0.4, 20.0 ± 4.0, 70.0 ± 6.0 and 3010.0 ± 52.0 micrograms per litre respectively.

The fortyeight hour exposure of ducks to **100.0 % M.E.** caused very high renal clearances of iron and zinc. The renal clearances of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 97.0 ± 3.2, 100.0 ± 0.1, 80.0 ± 6.0, 570.0 ± 5.7, 50.0 ± 3.2, 101.0 ± 1.0, 10.0 ± 0.1, 20.0 ± 3.2 and 2010.0 ± 40.0 micrograms respectively. On exposure to seventytwo hours to **100% M.E.**, the ducks exhibited very high renal clearances of iron and zinc. The urine concentrations of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 73.0 ± 1.8, 92.0 ± 5.2, 100.0 ± 3.9, 530.0 ± 3.6, 80.0 ± 1.6, 80.0 ± 3.2, 40.0 ± 5.2, 80.0 ± 5.2 and 1370.0 ± 13.0 micrograms respectively. The ducks exposed for ninetysix hours to **100.0% M.E.** exhibited very high renal clearances of zinc and iron. Similarly the renal clearances of cadmium, chromium, copper, iron, manganese, nickel, lead, magnesium, manganese, nickel, lead and zinc were equivalent to 101.0 ± 1.0, 71.0 ± 2.0, 90.0 ± 3.0, 570.0 ± 12.0, 30.0 ± 1.0, 102.0 ± 0.2, 50.0 ± 4.0, 30.0 ± 9.0 and 2910.0 ± 52.0 micrograms respectively. The one hundred and twenty hour exposure to

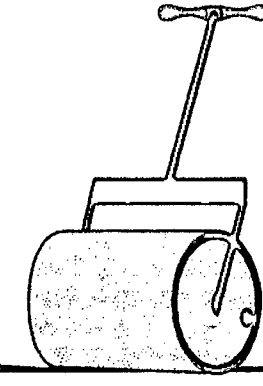
100.0% M.E. induced high renal clearances of copper, iron, manganese, lead and zinc. The renal clearance of iron was the highest.

The urine levels of cadmium, chromium, copper, iron, magnesium, manganese, nickel, lead and zinc were equivalent to 93.0 ± 1.0, 97.0 ± 1.3, 130.0 ± 4.0, 670.0 ± 2.0, 20.0 ± 1.0, 230.0 ± 2.0, 70.0 ± 3.0, 300.0 ± 1.0 and 390.0 ± 4.0 micrograms respectively.

CHAPTER-V

SECTION - A

HAEMATOLOGY



The mallards exposed to mining effluents **(M.E.)** showed various alterations in the concentrations of haemoglobin, RBC Counts WBC counts, Differential counts, blood platelet counts, Erythroyte sedimentation rates and in addition brought about significant changes in the serum constituents levels depending upon the concentrations of mining effluents and the durations of exposures to M.E.

I) ALTERATIONS IN HAEMOGLOBIN :

The changes in the haemoglobin (Hb) concentrations of blood under the influence of mining effluents (M.E.) at various time intervals are given in **Table No.24.0** and are graphically presented in **Graph No. 24.** The Hb concentrations are expressed as grams percent. The control animals showed Hb concentration equivalent to 15.3 ± 1.2 grams percent. The **100% M.E.** induced maximum reduction in Hb concentration at the end of twentyfour hours. All the concentrations of the mining effluents promoted reduction in the haemoglobin concentration.

The ducks exposed to **0.01% M.E.** did not show any significant change in the Hb level up to the end of twentynine days but at the end of thirty days (seven hundred and twenty hours), the Hb concentration dropped to 12.54± 0.272 grams and at the end of fortyfive days (one thousand and eighty hours) it further reduced to 11.94 ± 0.135 grams percent.

On exposure to **0.1% M.E.** the ducks showed fluctuations in the Hb concentrations. By the end of six, twelve, twentyfour, fortyeight , seventytwo, ninetysix and one twenty hours, the Hb concentrations were equivalent to 11.0 ± 0.583, 13.02 ± 0.213, 10.60 ± 0.389, 13.96 ± 0.205, 12.0 ± 0.141, 12.20 ± 0.424 and 11.80 ± 0.141 grams respectively.

Under the influence of **1.0%** M.E. the Hb concentrations of the ducks fluctuated between 11.26 gms to 15.02 gms. The Hb concentrations observed at the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one

twenty hours were equivalent to 15.02 ±0.354, 11.98 ± 0.132, 11.26 ± 0.326, 12.0 ± 0.282, 13.98 ± 0.213, 14.02 ± 0.312 and 12.12 ± 0.348 grams respectively.

The ducks subjected to **5% M.E.** showed reduction in Hb concentrations at all the time intervals under study but, the reduction was not uniform. The Hb concentrations of blood were equivalent to 13.02 ± 0.481, 13.14 ± 0.417, 12.46 ± 0.361, 11.26 ± 0.338, 11.60 ± 0.393, 14.04 ± 0.287 and 11.44 ± 0.344 gram percent at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one hundred and twenty hours repectively.

The **10.0% M.E.** induced decrease in the Hb concentrations of the mallards for all the time intervals under study and the degree of decrement was neither M.E. concentration dependent nor exposure time dependent. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, one hundred and twenty hours, the Hb concentrations of the blood were equivalent to 11.40 ± 0.236, 12.02 ± 0.16, 12.0 ± 0.616, 11.92 ± 0.172, ± 12.44 ± 0.32, 14.16 ± 0.392 and 12.96 ± 0.241 grams percent respectively.

The exposure to **50.0% M.E.** promoted decrease in the Hb concentration but the decrement did not reflect any relevance to the high M.E. concentration. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, one twenty hours, the Hb concentrations of the blood were equivalent to 13.98 ± 0.132, 13.60 ± 0.346, 13.10 ± 0.678, 14.0 ± 0.141, 13.36 ± 0.50, 12.36 ± 0.257 and 12.34 ± 0.265 gram percent respectively.

The **100% M.E.** caused maximum decrease in the Hb concentration at the end of twentyfour hours but Hb concentration increased a little subsequently through fortyeight and seventytwo hours. Thus, the Hb concentrations of the ducks were equivalent to 14.46 ± 0.205, 13.90 ± 0.340, 9.98 ± 0.494, 12.0 ± 0.141, 12.50 ± 0.109, 11.84 ± 0.162 and 12.60 ± 0.303 grams percent at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 24.0 : ALTERATIONS IN HAEMOGLOBIN CONCENTRATIONS OF DUCKS EXPOSED TO MINING EFFLUENTS.

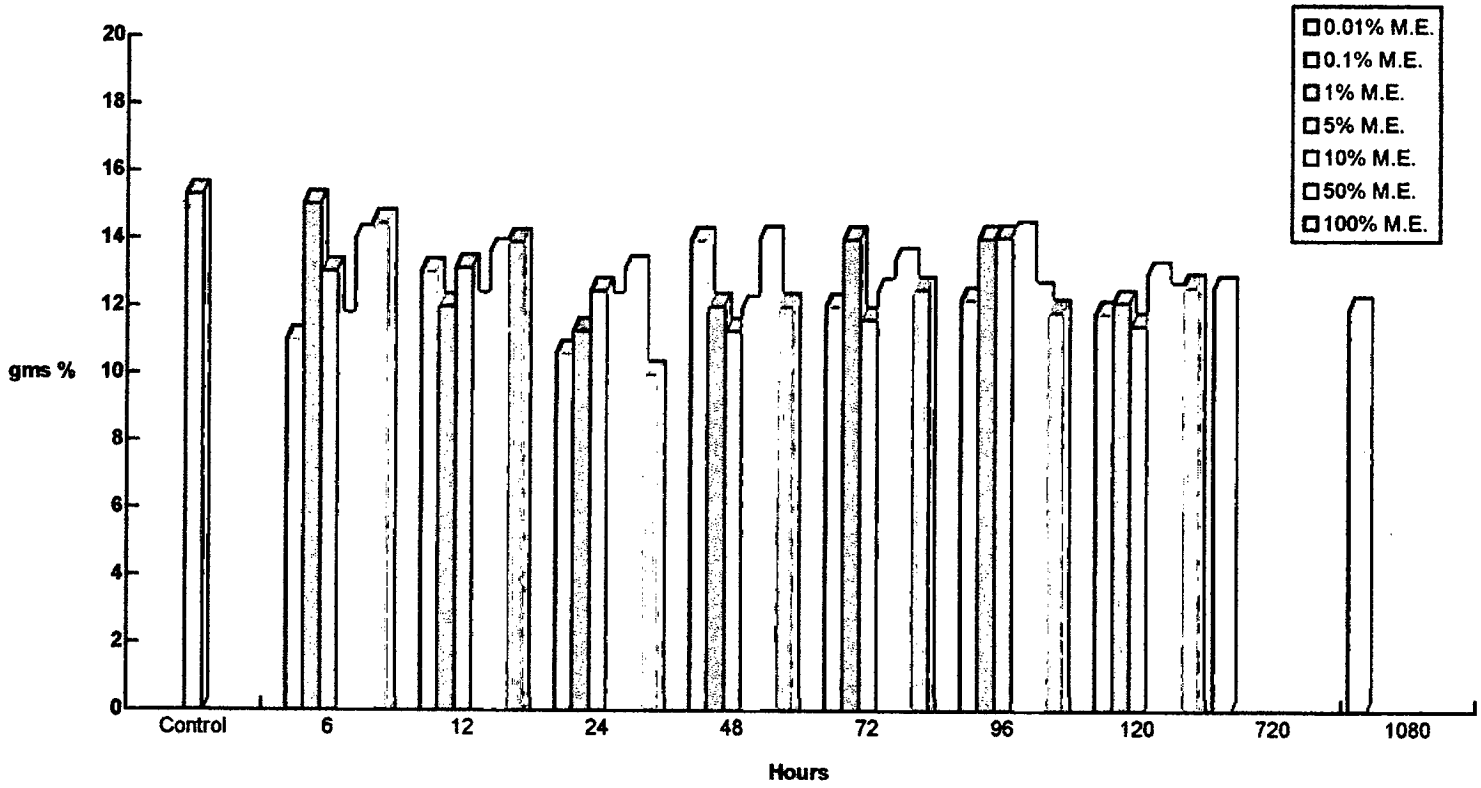
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		15.30 ±1.20									
0.01%	M.E.		← No significant change →							12.54 ±0.2727	11.94 ±0.1356
0.1%	M.E.		11.0 ±0.583	13.02 ±0.213	10.60 ±0.389	13.96 ±0.205	12.0 ±0.141	12.20 ±0.424	11.80 ±0.141	N.D.	N.D.
1%	M.E.		15.02 ±0.354	11.98 ±0.132	11.26 ±0.326	12.0 ±0.282	13.98 ±0.213	14.02 ±0.312	12.12 ±0.348	N.D.	N.D.
5%	M.E.		13.02 ±0.481	13.14 ±0.417	12.46 ±0.361	11.26 ±0.338	11.60 ±0.393	14.04 ±0.287	11.44 ±0.344	N.D.	N.D.
10%	M.E.		11.40 ±0.236	12.02 ±0.16	12.0 ±0.616	11.92 ±0.172	12.44 ±0.32	14.16 ±0.392	12.96 ±0.241	N.D.	N.D.
50%	M.E.		13.98 ±0.132	13.6 ±0.346	13.10 ±0.678	14.0 ±0.141	13.36 ±0.500	12.36 ±0.257	12.34 ±0.265	N.D.	N.D.
100%	M.E.		14.46 ±0.205	13.90 ±0.340	9.98 ±0.494	12.0 ±0.141	12.50 ±0.109	11.84 ±0.162	12.60 ±0.303	N.D.	N.D.

Note : Unit : Haemoglobin in gms %.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 24.0 : ALTERATIONS IN HAEMOGLOBIN CONCENTRATIONS OF DUCKS EXPOSED TO MINING EFFLUENTS.
Units : gms %.



II] ALTERATION IN RBC. COUNTS :

The Changes in the total red blood corpuscels (RBC) counts are compiled in **Table No.25** and Graphically presented in **Graph No. 25.** The RBC counts are expressed as millions per cubic millimeter. The control ducks showed the RBC count equivalent to 5.3 ± 0.017 millions per cubic millimeter. From the table and graph it appears that under the influence of mining effluents (M.E.) the ducks exhibited reduction in erythropoises as evidenced by the reduced total RBC counts. But the M.E. induced fluctuations in the RBC counts, though the total RBC count was less than that observed in the Control. Under the influence of only **5.0 %** M.E. at the end of ninetysix hours a significant recovery in the total RBC count was observed. The **100%** M.E. induced the maximum reduction in the total RBC. count at the end of twentyfour hours only.

When the ducks were exposed to **0.01%** M.E. there was no significant change in the total RBC counts up to the end of twentynine days but at the end of sevenhundred and twenty hours (thirty days) there was reduction in the total RBC count which continued to decrease up to the end of fortyfive days (one thousand and eighty hours). By the end of seven twenty and onethousand and eighty hours the total RBC counts were equivalent to 4.304 ± 0.010 and 3.948 ± 0.043 millions per cubic millimeter respectively.

The ducks subjected to **0.1%** M.E. exhibited sharp decrease in the total RBC counts at the end of six and twenty four hours.

The total RBC count was equivalent to 3.688 ± 0.139, 4.28 ± 0.102, 3.59 ± 0.285, 4.626 ± 0.053, 4.016 ± 0.049, 4.16 ± 0.115 and 4.024 ± 0.050 millions per cubic millimeter at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **1.0% M.E.** induced pronounced reductions in the total RBC counts at the end of twelve and twentyfour hours. At the end of six, twelve, twentfour, forty eight, seventytwo, ninetysix and one twenty hours the total RBC count was equivalent to 4.92 ± 0.136, 3.924 ± 0.149, 3.614 ± 0.270, 4.05 ± 0.157, 4.232 ± 0.154, 4.76 ± 0.086 and 4.008 ± 0.109 millions per cubic millimeter respectively.

Under the influence of **5.0% M.E.** the marked decrease and recovery in the total RBC count was observed at the end of seventytwo hours and ninetysix hours respectively. But at the end of one twenty hours there was once again sharp decrease in the total RBC count. The total RBC count at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 4.36 ± 0.115, 4.326 ± 0.243, 4.19 ± 0.185, 4.526 ± 0.060, 3.776 ± 0.053, 5.20 ± 0.070 and 3.948 ± 0.038 millions per cubic millimeter respectively.

The ducks exposed to **10.0% M.E.** showed profound decrease in the total RBC count at the end of six hours and a significant recovery in the total RBC count was observed at the end of ninetysix hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the total RBC count was equivalent to 3.904 ± 0.034, 4.008 ± 0.038, 3.962 ± 0.035, 3.98 ± 0.035, 4.38 ± 0.029, 4.88 ± 0.213 and 4.04 ± 0.185 millions per cubic millimeter respectively.

The exposure of ducks to **50% M.E.** promoted decrease in the total number of erythrocytes and though the erythrocyte count was low it flucutated between 4.2 and 4.69 millions per cubic millimeter. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the total RBC count was equivalent to 4.69 ± 0.037, 4.578 ± 0.054, 4.664 ± 0.037, 4.632 ± 0.029, 4.62 ± 0.031, 4.30 ± 0.013 and 4.20 ± 0.059 millions per cubic millimeter respectively.

The ducks subjected to **100% M.E.** showed pronounced decrease in the total number of erythrocytes at the end of twentyfour hours. The total RBC count at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 4.346 ± 0.020, 4.28 ± 0.016, 3.118 ± 0.013, 4.158 ± 0.035, 4.416 ± 0.010, 3.55 ± 0.040 and 4.184 ± 0.010 millions per cubic millimeter respectively.

TABLE NO : 25 : THE ALTERATIONS IN TOTAL R.B.C COUNTS UNDER THE INFLUENCE OF MINING EFFLUENTS.

		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	5.30 ± 0.017									
0.01% M.E.	← No significant change →								4.304 ±0.010	3.948 ±0.043
0.1% M.E.		3.688 ±0.139	4.28 ±0.102	3.59 ±0.285	4.626 ±0.053	4.016 ±0.049	4.16 ±0.115	4.024 ±0.050	N.D.	N.D.
1% M.E.		4.92 ±0.136	3.924 ±0.149	3.614 ±0.270	4.05 ±0.157	4.232 ±0.154	4.76 ±0.086	4.008 ±0.109	N.D.	N.D.
5% M.E.		4.36 ±0.115	4.326 ±0.243	4.19 ±0.185	4.526 ±0.060	3.776 ±0.053	5.20 ±0.070	3.948 ±0.038	N.D.	N.D.
10% M.E.		3.904 ±0.034	4.008 ±0.038	3.962 ±0.035	3.98 ±0.035	4.38 ±0.029	4.88 ±0.213	4.04 ±0.185	N.D.	N.D.
50% M.E.		4.69 ±0.037	4.578 ±0.054	4.664 ±0.037	4.632 ±0.029	4.62 ±0.031	4.30 ±0.013	4.20 ±0.059	N.D.	N.D.
100% M.E.		4.346 ±0.020	4.28 ±0.016	3.118 ±0.013	4.158 ±0.035	4.416 ±0.010	3.55 ±0.040	4.184 ±0.010	N.D.	N.D.

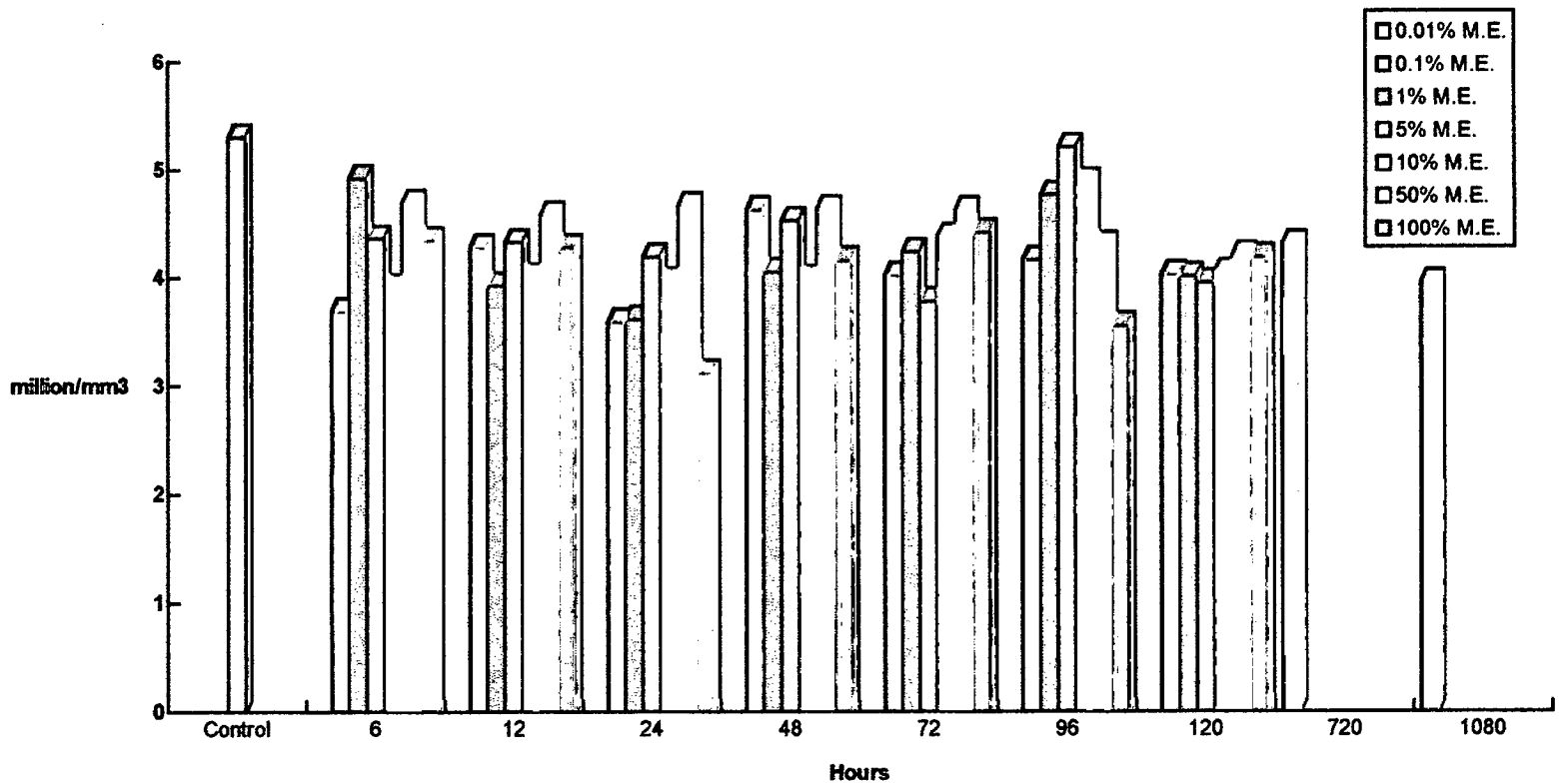
Note.: Unit : Million /mm^3.

N.D. : Not Determined.

All alterations are statistically significant - P < 0.01.

GRAPH NO. 25 : THE ALTERATIONS IN TOTAL RBC COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : million/mm^3.



III) ALTERATIONS IN WBC COUNTS :

The alterations in the total leucocytes (WBC) counts are given in **Table No.26** and are graphically presented in **Graph No. 26.** The total WBC counts are expressed as the number per cubic millimeter. The control animals had total leucocyte count equivalent to 6800 ± 20.0 cells per cubic millimeter. From the table and the Graph it appears that the mining effluents (M.E.) induced increase in the number of leuocytes but the increase was neither M.E. concentration dependent nor exposure period dependent. The maximum increase in the total number of leucocytes was observed at the end of twentyfour hours under the influence of 50 percent mining effluents (M.E.). Though, the M.E. induced increase in leucocyte numbers over the normal range, there were wide fluctuations in the WBC counts for different time intervals.

When the ducks were expsoed to **0.01% M.E.** there were no significant changes in the total leucocyte counts till the end of twentynine days. But at the end of seven hundred and twenty hours (thirty days) there was sharp increase in the leucocyte numbers which increased very marginally (nonsignificantly) at the end of fortyfive days (one thousand and eighty hours). Thus at the end of seven hundred and twenty hours and one thousand and eighty hours the total WBC count was equivalent to 9213 ± 31.24 and 9268 ± 34.81 cells per cubic millimeter.

On exposure to **0.1% M.E.**, the total WBC count of ducks raised to a high level at the end of ninetysix hours and then dropped subsequently. But in general the **0.1% M.E.** induced increase in the number of leucocytes. Thus, the total WBC count at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 10309.0 ± 35.06, 10251.0 ± 53.64, 11805.0 ± 16.41, 9199.0 ± 15.09, 9211.0 ± 43.08, 12904.0 ± 31.5 and 8927.0 ± 40.05 cells per cubic millimeter respectively.

The ducks treated with **1.0% M.E.** showed fluctuations in the total WBC count for various time intervals under study and at the end of twelve hours exposure, there was a pronounced increase in the number of leucocytes. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the total WBC count was equivalent to 9197.0 ± 12.70, 12301.0 ± 12.56, 10323.0 ± 29.75, 10310.0 ± 28.47, 9924.0 ± 36.77, 9424.0 ± 26.747 and 8598.0 ± 19.27 cells per cubic millimeter respectively.

The animals subjected to **5.0% M.E.** exhibited significant increase in the total WBC count at all the time intervals in comparison with that observed in the controls. By the end of six and twelve hours there was prominient increase in the total number of leucocytes and then the leucocyte numbers exhibited considerable fluctuations. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and onetwenty hours, the total WBC count was equivalent to 10599.0 ± 16.49, 10800 ± 42.85, 9205 ± 18.75, 10147 ± 32.13, 9841 ± 48.10, 8614 ± 31.90 and 9219 ± 32.25 cells percubic millimeter respectively.

The exposure to **10.0% M.E.** promoted sharp increase in the leucocyte number and the leucocyte population remained over eleven thousand from the end of six hours to the end of fortyeight hours. The total W.B.C. count at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 11605.0 ± 15.5, 11828.0 ± 30.8, 11916.0 ± 33.5, 11225.0 ± 29.74, 10640.0 ± 81.51, 10617.0 ± 31.53 and 9200.0 ± 26.40 cells per cubic millimeter respectively.

Under the influence of **50.0% M.E.**, the ducks exhibited the highest number of leucocytes at the end of twentyfour hours and the leucocyte population remained over eleven thousand for all the time intervals under study. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the leucocyte count was

equivalent to 11219.0 ± 36.80, 12909.0 ± 29.11, 20868.0 ± 42.91, 14617.0 ± 38.48, 11719.0 ± 37.90, 15218.0 ± 37.83 and 15623.0 ± 33.60 cells per cubic millimeter respectively.

The **100%** M.E. induced increase in leucocyte population but the increase was not as high as found under the influence of 50% M.E. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the total W.B.C. count was equivalent to 9836.0 ± 182.16, 9868.0 ± 39.043, 10752.0 ± 39.69, 10887.0 ± 45.305, 8800.0 ± 316.2, 10694.0 ± 54.921 and 11914.0 ± 71.03 cells per cubic millimeter respectively.

TABLE NO. 26 : THE CHANGES IN TOTAL WBC COUNTS UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		6800 ± 20									
0.01%	M.E.		← No significant change →							9213 ±31.24	9268 ±34.81
0.1%	M.E.		10309 ±35.06	10251 ±53.64	11805 ±16.41	9199 ±15.09	9211 ±43.08	12904 ±31.5	8927 ±40.05	N.D.	N.D.
1%	M.E.		9197 ±12.70	12301 ±12.56	10323 ±29.75	10310 ±28.47	9924 ±36.77	9424 ±26.747	8598 ±19.27	N.D.	N.D.
5%	M.E.		10599 ±16.49	10800 ±42.85	9205 ±18.75	10147 ±32.13	9841 48.10	8614 ±31.90	9219 ±32.25	N.D.	N.D.
10%	M.E.		11605 ±15.5	11828 ± 30.8	11916 ±33.5	11225 ±29.74	10640 ±81.51	10617 ±31.53	9200 ±26.40	N.D.	N.D.
50%	M.E.		11219 ±36.80	12909 ±29.110	20868 ±42.91	14617 ±38.48	11719 ±37.90	15218 ±37.83	15623 ±33.60	N.D.	N.D.
100%	M.E.		9836 ±182.16	9868 ±39.043	10752 ±39.69	10887 ±45.305	8800 ±316.2	10694 ±54.921	11914 ±71.03	N.D.	N.D.

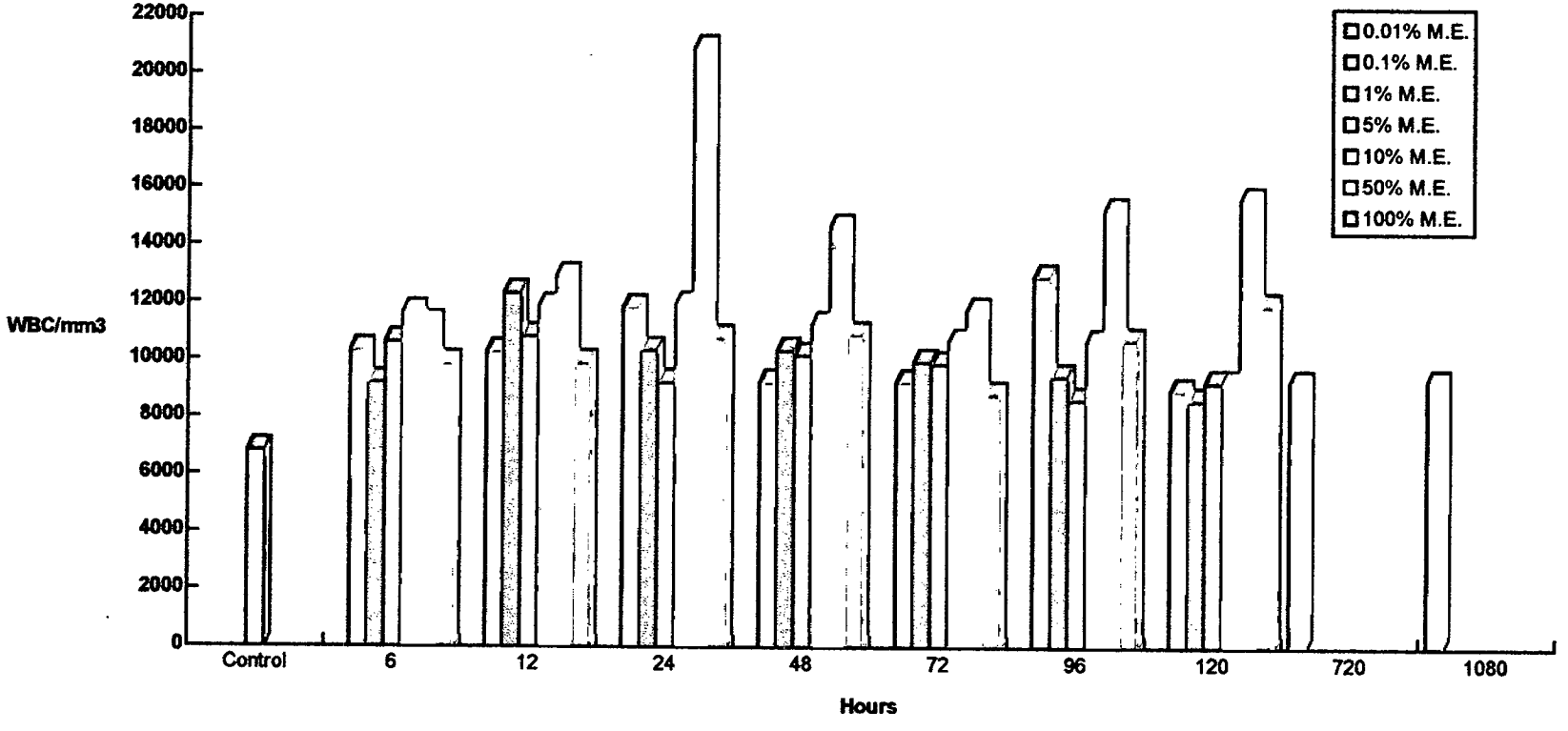
Note : Unit : WBC Count /mm^3.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 26 : THE CHANGES IN TOTAL LEUCOCYTE COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : Total WBC / mm^3



IV) ALTERATIONS IN THE LEUCOCYTE DIFFERENTIAL COUNT :

The alterations in the differential counts of leucocytes are given in **Table No.27** and are graphically presented in **Graph No. 27.1, 27.2 and 27.3.** The differential count envisages the occurrences of different types of leucocytes in specific percentages. Normally in the animals blood the leucocytes such as neutrophils, lymphocytes, eosinophils, basophils and monocytes are found in specific ranges. The occurrences of these individual leucocyte types are expressed as percents of total number of leucocytes. The control birds exhibited the presence of neutrophils, lymphocytes and eosinophils while the basophils and monocytes were absent. The proportions of neutrophils, lymphocytes and eosinophils were equivalent to 38.0 ± 2.08%, 60.0 ± 2.0% and 2.0 ± 0.87% respectively.

A) THE CHANGES IN NEUTROPHIL COUNT :

The exposure to **0.01% M.E.** did not produce any significant change in the neutrophil count up to the end of twentynine days but at the end of thirty days (seven hundred & twenty hours) there was significant increase in the neutrophil count which further increased by the end of fortyfive days (one thousand and eighty hours). Thus, at the end of thirty and forty five days exposure to **0.01% M.E.**, the neutorphil percentage was equivalent to 50.0 ± 1.72 and 60.0 ± 2.5 percent respectively.

The **0.1% M.E.** induced fluctuations in the neutrophil counts and the maximum increase in the neutrophil count was observed at the end of fortyeight hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the neutrophil count was equivalent to 41.0 ± 2.4, 53.0 ± 2.5, 40.0 ± 2.05, 69.0 ± 2.4, 60.0 ± 1.7, 40.0 ± 2.1 and 44.0 ± 3.1 percent respectively.

Under the influence of **1.0% M.E.** there was significant reduction in the neutrophil count at the end of twelve and ninetysix hours while at the end of seventytwo hours there was sharp rise in the neutrophil number. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the neutrophil count was equivalent to 45.0 ± 2.6, 31.0 ± 1.7, 50.0 ± 3.6, 42.0 ± 2.1, 55.0 ± 2.6, 32.0 ± 3.7 and 47.0 ± 2.1 percent respectively.

The animals subjected to **5.0% M.E.** showed increase in the neutrophil percentage at the end of six hours and the neutrophil count went on increasing up to the end of twelve hours and subsequently the neutrophil count dropped gradually for a period from fortyeight hours to ninetysix hours. Then, once again the neutrophil count rose prominently. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the neutrophil count was equivalent to 54.0 ± 3.2, 61.0 ± 1.62, 48.0 ± 1.01, 41.0 ± 2.4, 40.0 ± 3.8, 40.0 ± 2.1 and 59.0 ± 1.8 percent respectively.

The exposure of birds to **10% M.E.** induced successive rise in the neutrophil count from the end of twentyfour hours to onetwenty hours. Initially at the end of six and twelve hours exposure, the bird showed marginal increase in the neutrophil count.

Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the neutrophil count was equivalent to 40.0 ± 2.0, 42.0 ± 2.13, 39.0 ± 1.72, 40.0 ± 2.13, 48.0 ± 2.6, 55.0 ± 2.6 and 64.0 ± 2.87 percent respectively.

On exposure to **50.0% M.E.** , the ducks exhibited sharp decrease in the neutrophil count at the end of twentyfour and fortyeight hours. The neutrophil count remained low up to seventytwo hours and then increased at the end of ninetysix hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and onetwenty hours the neutrophil count was equivalent

to 33.0 ± 2.607, 35.0 ± 2.82, 22.0 ± 3.26, 22.0 ± 2.99, 25.0 ± 3.1, 45.0 ± 3.4, and 40.0 ± 1.95 percent respectively.

The **100% M.E.** promoted maximum increase in the neutrophil count at the end of one twenty hours and at the end of seventytwo hours the neutrophil count was marginally lower than that observed in the control animals. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the neutrophil count was equivalent to 46.0 ± 1.32, 43.0 ± 1.326, 46.0 ± 2.28, 43.0 ± 2.56, 37.0 ± 1.624, 39.0 ± 2.1 and 71.0 ± 1.78 percent respectively.

TABLE NO. 27.0 : ALTERATIONS IN THE NEUTROPHIL COUNTS UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	M.E.	38% ±2.08									
0.01%	M.E.		← No significant change →							50% ±1.72	60% ±2.5
0.1%	M.E.		41% ±2.4	53% ±2.5	40% ±2.05	69% ±2.4	60% ±1.7	40% ±2.1	44% ±3.1	N.D.	N.D.
1%	M.E.		45% ±2.6	31% ±1.7	50% ±3.6	42% ±2.1	55% ±2.6	32% ±3.7	47% ±2.1	N.D.	N.D.
5%	M.E.		54% ±3.2	61% 1.62	48% ±1.01	41% ±2.4	40% ±3.8	40% ±2.1	59% ±1.8	N.D.	N.D.
10%	M.E.		40% ±2.0	42% ±2.13	39% ±1.72	40% ±2.13	48% ±2.6	55% ±2.6	64% ±2.87	N.D.	N.D.
50%	M.E.		33% ±2.607	35% ±2.82	22% ±3.26	22% ±2.99	25% ±3.1	45% ±3.4	40% ±1.95	N.D.	N.D.
100%	M.E.		46% ±1.32	43% ±1.326	46% ±2.28	43% ±2.56	37% ±1.624	39% ±2.1	71% ±1.78	N.D.	N.D.

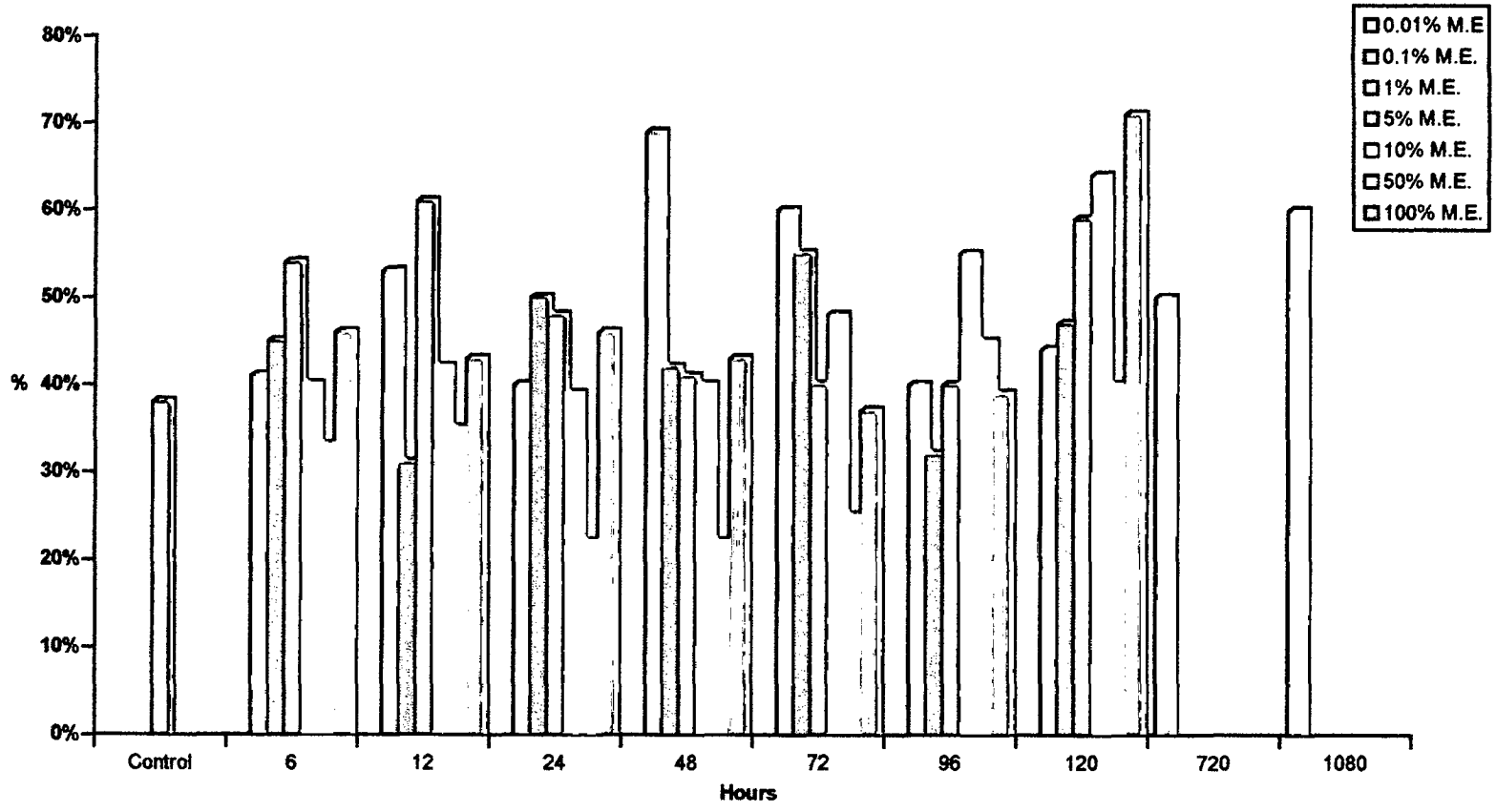
Note : Unit : Neutrophils in %.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 27 : ALTERATIONS IN THE NEUTROPHIL COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : Neutrophils in %



B) THE CHANGES IN LYMPHOCYTE COUNT :

The control animals had sixty percent lymphocytes in the blood among the leucocytes. The exposure of ducks to **0.01% M.E.** could not alter the lymphocyte count up to the end of twentynine days but at the end of thirty days the lymphocyte count reduced significantly and kept on decreasing up to the end of fortyfive days. Thus, at the end of thirty days (seven hundred and twenty hours) and fortyfive days (one thousand and eighty hours), the lymphocyte count was equivalent to 41.0 ± 2.2 and 32.0 ± 1.4 percent respectively.

Under the influence of **0.1% M.E.**, there was, in general reduction in the lymphocyte count. The acute reduction in the lymphocyte number was observed at the end of twelve hours and then the lymphocyte count increased gradually from twentyfour hours to ninetysix hours but it remained below that observed in control bird. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and onetwenty hours, the lymphocyte count was equivalent to 32.0 ± 2.7, 25.0 ± 2.6, 27.0 ± 1.9, 30.0 ± 1.72, 30.0 ± 1.6, 43.0 ± 3.6 and 34.0 ± 2.4 percent respectively.

The exposure to **1.0% M.E.** caused decrease in lymphocyte count. The relatively high decrease in lymphocyte count was observed at the end of seventy two hours and then it recorded significant elevation at the end of ninetysix hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the lymphocyte count was equivalent to 42.0 ± 2.5, 38.0 ± 1.6, 34.0 ± 3.31, 41.0 ± 1.8, 32.0 ± 1.8, 56.0 ± 2.4 and 44.0 ± 2.8 percent respectively.

The **5.0% M.E.** promoted acute decline in lymphocyte number at the end of twelve hours and a significant recovery in the lymphocyte percentage was observed at the end of ninetysix hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the lymphocyte count

was equivalent to 29.0 ± 2.7, 27.0 ± 1.41, 40.0 ± 1.4, 39.0 ± 1.6, 37.0 ± 1.01, 54.0 ± 3.0 and 30.0 ± 1.42 percent respectively.

The ducks treated with **10.0% M.E.** exhibited reduction in the lymphocyte count and it fluctuated between thirty percent and thirty eight percent. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the lymphocyte count was equivalent to 36.0 ± 1.93, 33.0 ± 2.9, 36.0 ± 2.4, 38.0 ± 2.2, 37.0 ± 2.0, 30.0 ± 1.9 and 38.0 ± 1.7 percent respectively.

The **50% M.E.** induced maximum increase in the lymphocyte count the end of twentfour hours while at the rest of the time intervals under study there was reduction in the lymphocyte number. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the lymphocyte count was equivalent to 43.0 ± 2.8, 44.0 ± 2.03, 74.0 ± 2.31, 47.0 ± 1.72, 55.0 ± 2.4, 40.0 ± 3.16 and 42.0 ± 2.8 percent respectively.

The ducks exposed to **100% M.E.** showed acute reduction in the lymphocyte count at the end of one twenty hours and the lymphocyte count remained lower to that observed in the control animals at all the time intervals under study. By the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix, and one twenty hours the lymphocyte count was equivalent to 50.0 ± 1.41, 55.0 ± 1.6, 42.0 ± 1.4, 47.0 ± 2.13, 56.0 ± 1.9, 53.0 ± 2.13 and 21.0 ± 1.93 percent respectively.

TABLE NO. 27.1 : ALTERATIONS IN THE LYMPHOCYTE COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	M.E.	60% ±2.0									
0.01%	M.E.		← No significant change →							41% ±2.2	32% ±1.4
0.1%	M.E.		32% ±2.7	25% ±2.6	27% ±1.9	30% ±1.72	30% ±1.6	43% ±3.6	34% ±2.4	N.D.	N.D.
1%	M.E.		42% ±2.5	38% ±1.6	34% ±3.31	41% ±1.8	32% ±1.8	56% ±2.4	44% ±2.8	N.D.	N.D.
5%	M.E.		29% ±2.7	27% ±1.41	40% ±1.4	39% ±1.6	37% 1.01	54% ±3.0	30% ±1.42	N.D.	N.D.
10%	M.E.		36% ±1.93	33% ±2.9	36% ±2.4	38% ±2.2	37% ±2.0	30% ±1.9	38% ±1.7	N.D.	N.D.
50%	M.E.		43% ±2.8	44% ±2.03	74% ±2.31	47% ±1.72	55% ±2.4	40% ±3.16	42% ±2.8	N.D.	N.D.
100%	M.E.		50% ±1.414	55% ±1.6	42% ±1.4	47% ±2.13	56% ±1.9	53% ±2.13	21% ±1.93	N.D.	N.D.

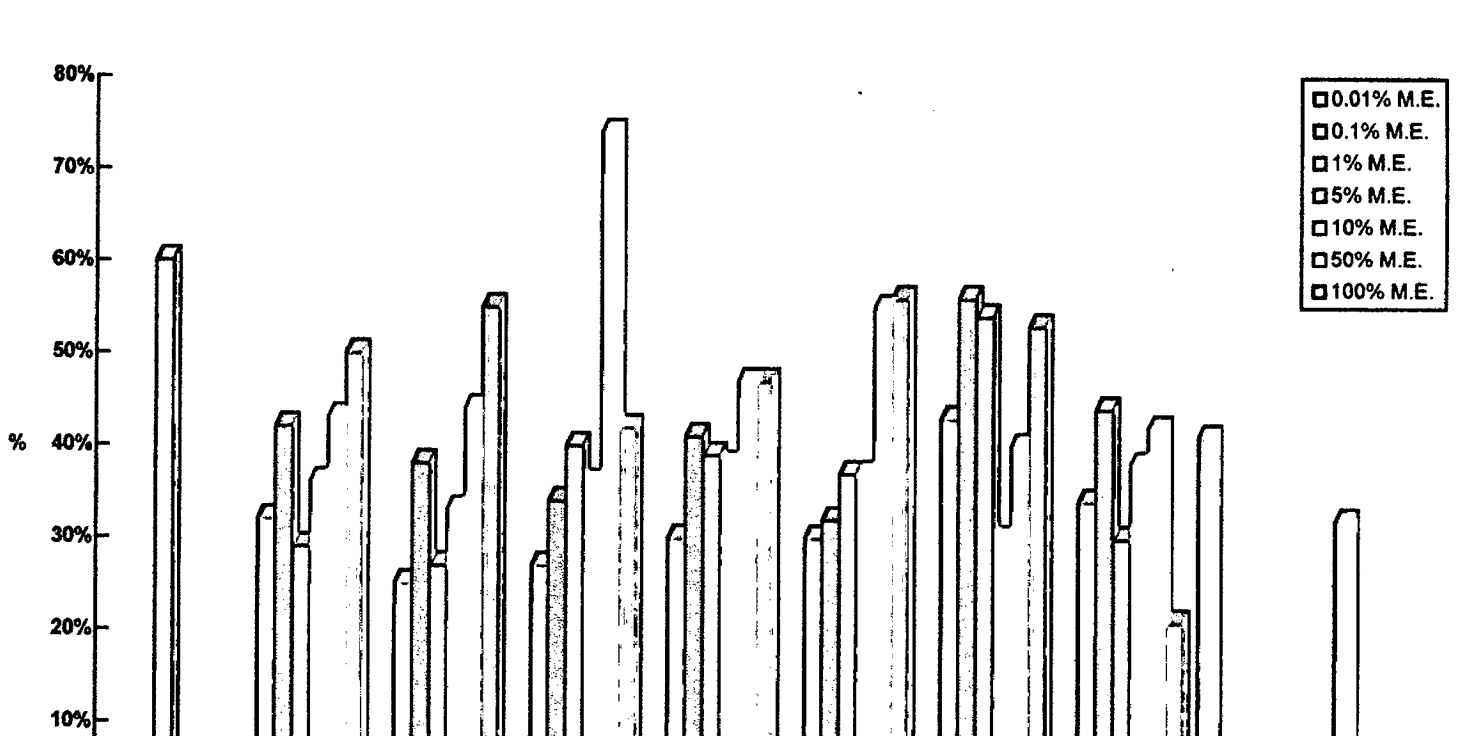
Note : Unit : Lymphocytes in %.

N.D. : Not Determined.

All alterations are statistically significant - P < 0.01.

GRAPH NO. 27.1 : ALTERATIONS IN LYMPHOCYTE COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : Lymphocytes in %



C) THE ALTERATIONS IN THE EOSINOPHIL COUNT :

The control animals exhibited just 2.0% eosinophils in the blood. The **0.01% M.E.** did not induce any significant change in the eosinophil count up to the end of twentynine days but at the end of thirty and fortyfive days the eosinophil number increased significantly. The eosinophil count was 10.0 ± 2.0 percent at the end of thirty and fortyfive days.

The ducks exposed to **0.1% M.E.** exhibited wide fluctuations in the eosinophil count. The acute increase in eosinophil count was observed at the end of twentyfour hours. By the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one twenty hours the eosinophil count was equivalent to 20.0 ± 1.01, 16.0 ± 1.74, 31.0 ± 1.7, 9.0 ± 1.01, 11.0 ± 1.6, 16.0 ± 1.2 and 20.0 ± 1.3 percent respectively.

Under the influence of **1.0% M.E.** the ducks showed an acute eosinophilia at the end of twelve hours and then gradually the eosinophil count decreased up to the end of seventytwo hours in comparison with what was observed at the end of twelve hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the eosinophil count was equivalent to 12.0 ± 1.4, 29.0 ± 1.6, 14.0 ± 2.7, 12.0 ± 1.4, 10.0 ± 0.7, 12.0 ± 2.5 and 8.0 ± 1.01 percent respectively.

The ducks subjected to **5.0% M.E.** showed the eosinophil count in the range of nine to twenty two percent. At all the time intervals under study, the eosinophil count was higher than that observed for controls. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the eosinophil count was equivalent to 14.0 ± 2.1, 12.0 ± 1.41, 9.0 ± 1.7, 11.0 ± 1.72, 22.0 ± 1.3, 9.0 ± 1.6 and 10.0 ± 1.72 percent respectively.

The exposure to **10.0% M.E.** promoted pronounced eosinophilia. At the end of six, twelve and twentyfour hours the eosinophil count was about twelve

fold higher than that observed in the controls. The eosinophil count was equivalent to 24.0 ± 2.2, 25.0 ± 3.3, 25.0 ± 1.42, 21.0 ± 1.8, 15.0 ± 2.7, 15.0 ± 1.3 and 8.0 ± 1.0 percent at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

Under the influence of **50.0% M.E.** acute eosinophilia was observed at the end of fortyeight hours and in general the eosinophil count was higher than that observed in the control animals. The eosinophil count was equivalent to 22.0 ± 3.0, 21.0 ± 1.72, 7.0 ± 2.0, 29.0 ± 3.0, 17.0 ± 1.8, 16.0 ± 1.41 and 18.0 ± 0.74 percent at the end of six, twelve twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **100.0 % M.E.** induced relatively less increase in eosinophil counts as compared to that produced by the other concentrations of M.E. At the end of six, twelve, twentyfour fortyeight, seventytwo, ninetysix, and one twenty hours, the eosinophil count was equivalent to 4.0 ± 0.748, 2.0 ± 0.748, 12.0 ± 1.9, 11.0 ± 1.41, 10.0 ± 1.16, 9.0 ± 1.32 and 8.0 ± 1.01 percent respectively.

TABLE NO. 27.2: ALTERATIONS IN THE EOSINOPHIL COUNTS UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	M.E.	2% ±0.87									
0.01%	M.E.		← No significant change →							10% ±2.0	10% ±2.0
0.1%	M.E.		20% ±1.01	16% ±1.74	31% ±1.7	9% ±1.01	11% ±1.6	16% ±1.2	20% ±1.3	N.D.	N.D.
1%	M.E.		12% ±1.4	29% ±1.6	14% ±2.7	12% ±1.4	10% ±0.7	12% ±2.5	8% ±1.01	N.D.	N.D.
5%	M.E.		14% ±2.1	12% ±1.41	9% ±1.7	11% ±1.72	22% ±1.3	9% ±1.6	10% ±1.72	N.D.	N.D.
10%	M.E.		24% ±2.2	25% ±3.3	25% ±1.42	21% ±1.8	15% ±2.7	15% ±1.3	8% ±1.0	N.D.	N.D.
50%	M.E.		22% ±3.0	21% ±1.72	7% ±2.0	29% ±3.0	17% ±1.8	16% ±1.41	18% ±0.74	N.D.	N.D.
100%	M.E.		4% ±0.748	2% ±0.748	12% ±1.9	11% ±1.41	10% ±1.16	9% ±1.32	8% ±1.01	N.D.	N.D.

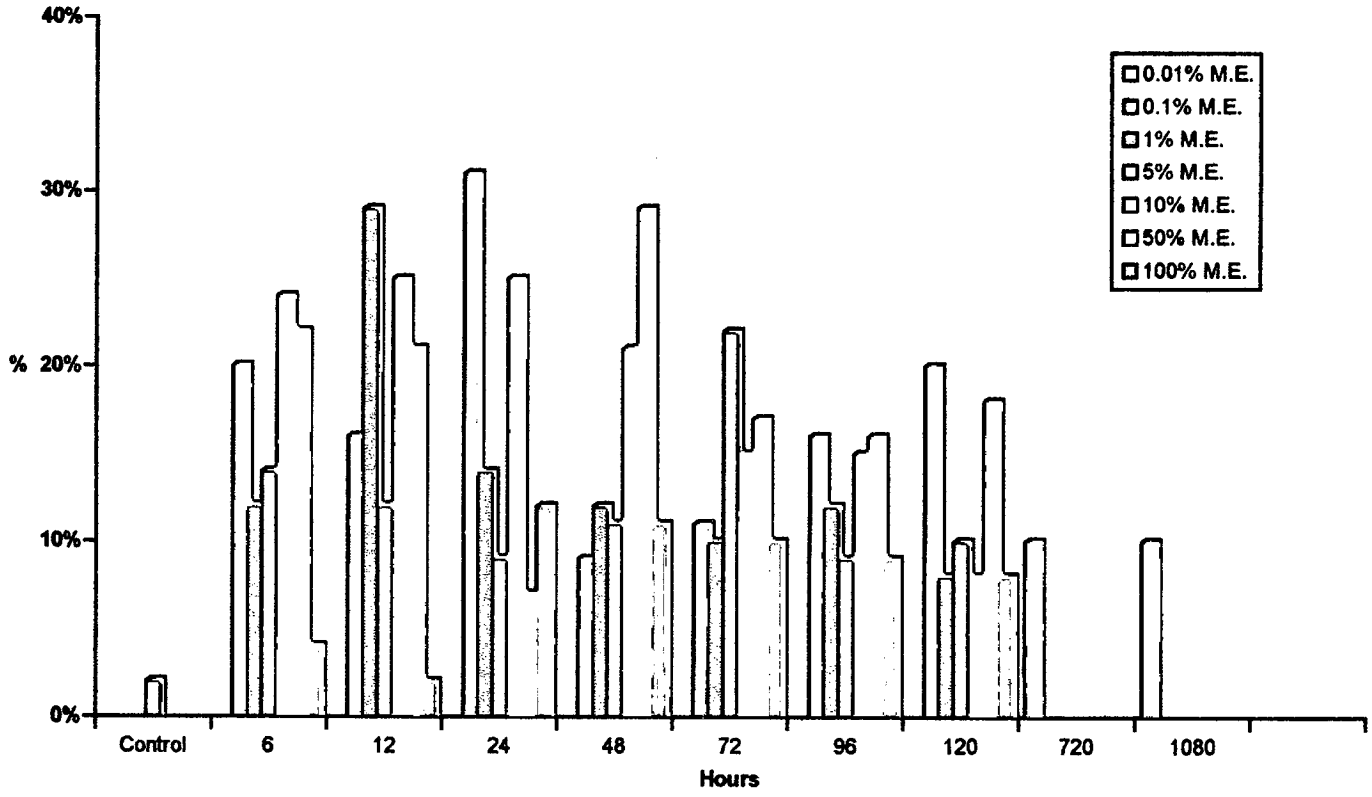
Note : Unit : Eosinophils in %.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 27.2 : ALTERATIONS IN THE EOSINOPHIL COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : Eosinophils in %



V) THE ALTERATIONS IN BLOOD PLATELET COUNT :

The changes in the blood platelet count are compiled in **Table No. 28** and are graphically presented in **Graph No. 28.** The blood platelet counts are expressed as cells per cubic millimeter. The control animals had blood platelet count equivalent to 153000 ± 2000 cells per cubic millimeter. From the table and graph it appears that the changes in the blood platelet counts were neither M.E. concentrations dependent nor exposure period dependent. The **1.0%** M.E. induced acute increase in the blood platelet count at the end of six hours and no other concentration of M.E. could induce such as pronounced increase in blood platelet count at any time interval.

When the animals were exposed to **0.01%** M.E. there was no significant change in the blood platelets count up to the end of twentynine days but at the end of thirty days (seven hundred and twenty hours) there was pronounced increase in the platelet count which showed progressive increase up to the end of fortyfive days (one thousand and eighty hours) . Then, at the end of thirty and fortyfive days the blood platelet count was equivalent to 260,000 ± 1000 and 275,000 ± 2000 cells per cubic millimeter respectively.

The birds exposed to **0.1%** M.E. showed increase in the blood platelet counts but the platelet count fluctuated for different periods. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the blood platelet count was equivalent to 226, 000 ± 3000, 260,000 ± 1230, 202,000 ± 984, 230,000 ± 1000, 215,000 ± 1400, 219,000 ± 1000 and 206,000 ± 1000 cells per cubic millimeter respectively.

Under the influence of **1.0%** M.E. the ducks showed highest increase in the blood platelet number at the end of six hours and then the platelet count went on decreasing gradually up to the end of fortyeight hours. Then the platelet count kept on increasing up to the end of ninetysix hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty

hours, the blood platelet count was equivalent to 360,000 ± 2000, 285,000 ± 592, 282,000 ± 973 and 216,000 ± 2010, 295000 ± 1200, 298000 ± 2700 and 265000 ± 2000 cells percubic millimeter respectively.

The ducks subjected to **5.0% M.E.** exhibited increase in the number of blood platelets at the end of six hours and then it kept on increasing up to the end of twentyfour hours. The platelet count fluctuated during the subsequent time intervals. The blood platelet count was equivalent to 208,000 ± 1000, 210,000 ± 792, 260,000 ± 1000, 190,000 ± 1500, 220,000 ± 2500, 215000 ± 2000 and 240,000 ± 1340 cells per cubic millimeter at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **10.0% M.E.** induced fluctuations in the blood platelet count. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the blood platelet count was equivalent to 216,000± 2000, 219,000 ± 1500, 217,000 ± 889, 230,000 ± 1400, 225,000 ± 1000, 185,000 ± 1000 and 190,000 ± 1930 cells per cubic millimeter respectively.

On exposure to **50% M.E.** the ducks exhibited acute increase in the blood platelet numbers and the platelet count marginally fluctuated from the end of six hours to the end of fortyeight hours. By the end of seventytwo hours the platelet count dropped a little but improved subsequently. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the blood platelet count was equivalent to 295,000 ± 1670, 298,000 ± 1800, 296,000 ± 1000, 299,000 ± 757, 268,000 ± 1397, 300,000 ± 1300 and 295,000 ± 1500 cells per cubic millimeter respectively.

The ducks treated with **100.0 % M.E.** showed elevation of blood platelet count for all the time intervals as compared with the blood platelet count observed for the control birds. The blood platelet number exhibited fluctuations but remained almost same for six, ninetysix and one twenty hours. Thus, the blood platelet count was equivalent to 250,000 ± 1000, 245,000 ± 1500,

216,000 ± 892, 265,000 ± 937, 292,000 ± 1000, 250,000 ± 2,000 and 250,000 ± 796 cells percubic millimeter at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

TABLE NO. 28 : ALTERATIONS IN THE PLATELET COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		153,000 ± 2,000									
0.01%	M.E.		← No significant change →							260,000 ±1,000	275,000 ±2,000
0.1%	M.E.		226,000 ±3,000	260,000 ±1,230	202,000 ±984	230,000 ±1,000	215,000 ±1,400	219,000 ±1000	206,000 ±1000	N.D.	N.D.
1%	M.E.		360,000 ±2,000	285,000 ±592	282,000 ±973	216,000 ±2,010	295,000 ±1,200	298,000 ±2,700	265,000 ±2,000	N.D.	N.D.
5%	M.E.		208,000 ±1,000	210,000 ±792	260,000 ±1,000	190,000 ±1,500	220,000 ±2,500	215,000 ±2,000	240,000 ±1,340	N.D.	N.D.
10%	M.E.		216,000 ±2,000	219,000 ±1,500	217,000 ±889	230,000 ±1,400	225,000 ±1,000	185,000 ±1,000	190,000 ±1,930	N.D.	N.D.
50%	M.E.		295,000 ±1,670	298,000 ±1,800	296,000 ±1,000	299,000 ±757	268,000 ±1,397	300,000 ±1,300	295,000 ±1,500	N.D.	N.D.
100%	M.E.		250,000 ±1,000	245,000 ±1,500	216,000 ±892	265,000 ±937	292,000 ±1,000	250,000 ±2,000	250,000 ±796	N.D.	N.D.

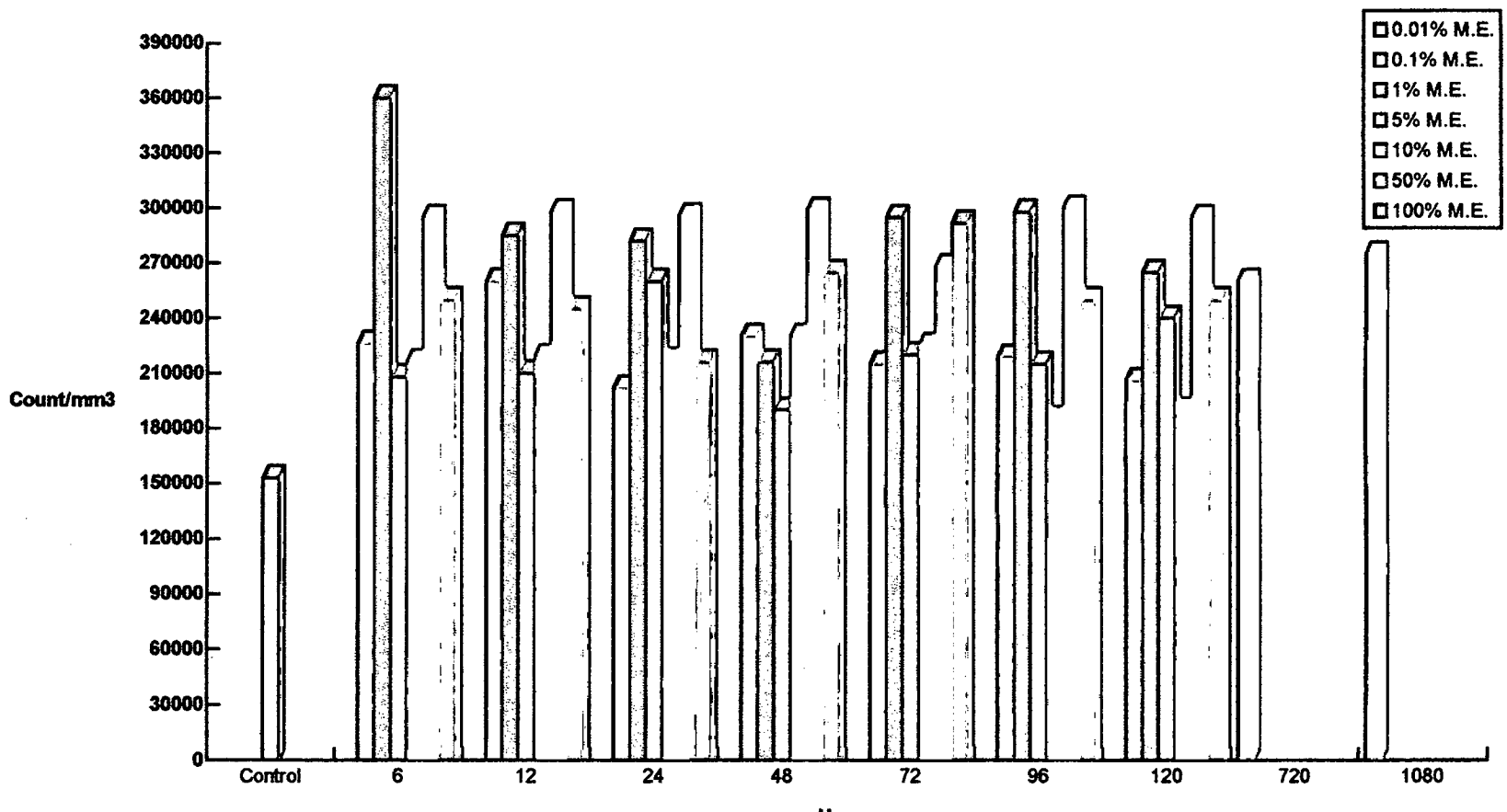
Note : Unit : Platelet Counts / mm^3.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 28 : ALTERATIONS IN THE PLATELET COUNT UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : Platelet Count / mm^3



VI) THE ALTERATIONS IN THE ERYTHROCYTE SEDIMENTATION RATE (E.S.R). :

The alterations in the Erythrocyte Sedimentation Rate (E.S.R) are given in **Table No. 29** and are graphically expressed in **Graph No. 29.** The E.S.R. is expressed as millimeter per hour. The control birds had E.S.R. equivalent to 2.0 ± 0.00 millimeter per hour.

From the table and graph it appears that the changes in the E.S.R. were neither M.E. concentration dependent nor exposure period dependent. The **0.1 and 1.0% M.E.** reduced the E.S.R. by the end of six hours. The maximum increase in E.S.R. was observed under the influence of **5.0 and 100.0% M.E.** at the end of nientysix and one twenty hours respectively.

The exposure of mallards to **0.01% M.E.** did not change the E.S.R. up to the end of twentynine days but at the end of thirty days (seven hundred and twenty hours) the E.S.R. increased marginally but the fortyfive days exposure promoted over two fold increase in E.S.R. if compared with what was obtained for the control.

Under the influence of **0.1% M.E.** the ducks exhibited fifty percent reduction in E.S.R. for six and twelve hours exposures. The ducks showed acute increase in E.S.R. under the influence of **0.1% M.E.** at the end of twentyfour and seventytwo hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the E.S.R. was equivalent to 2.0 ± 0.00, 2.0 ± 0.00, 10.0 ± 2.0, 5.0 ± 1.0, 10.0 ± 2.0, 3.0 ± 0.00 and 8.0 ± 1.0 millimeter per hour respectively.

The ducks subjected to **1.0% M.E.** exhibited fifty percent reduction in E.S.R. at the end of six hours and then showed pronounced and steady increase in E.S.R. from the end of twelve hours to the end of forty eight hours. Then, onceagain the E.S.R. decreased sharply at the end of seventytwo and

ninetysix hours. The E.S.R. was equivalent to 2.0 ± 0.00, 8.0 ± 2.0, 10.0 ± 0.00, 12.0 ± 2.0, 5.0 ± 0.00, 3.00 ± 0.00 and 8.0 ± 1.0 millimeter per hour at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **5.0% M.E.** promoted sharp increase in E.S.R. at the end of six and twelve hours but the highest rise in E.S.R.was observed at the end of ninetysix hours. The E.S.R. observed at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours was equivalent to 12.0 ± 1.0, 14.0 ± 1.0, 5.0 ± 0.00, 10.0 ± 1.0, 5.0 ± 0.00, 16.0 ± 2.0 and 10.0 ± 1.0 millimeter per hour, respectively.

The exposure of ducks to **10.0% M.E.** caused increase in E.S.R. The E.S.R. increased predominantly at the end of six, twelve, twentyfour and fortyeight hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the E.S.R. was equivalent to 10.0 ± 1.0, 12.0 ± 1.0, 11.0 ± 1.0, 15.0 ± 1.0, 10.0 ± 1.0, 8.0 ± 0.00 and 9.0 ± 0.00 millimeter per hour, respectively.

The ducks treated with **50.0% M.E.** showed time dependent increase in E.S.R. from the end of six hours to the end of fortyeight hours but the E.S.R. decreased slightly by the end of seventy two hours. Then, onceagain the E.S.R. increased a little at the end of ninetysix hours and remained unchanged up to the end of one twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the E.S.R. was equivalent to 8.0 ± 1.0, 9.0 ± 1.0, 12.0 ± 1.10, 15.0 ± 1.0, 10.0 ± 1.0, 12.0 ± 1.0 and 12.0 ± 1.0 millimeters (per hour), respectively.

The **100.0% M.E.** induced very acute increase in E.S.R. at the end of twentyfour and one twenty hours. For the rest of the time intervals the E.S.R. remained above what was obtained for the control animals . Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty

hours the E.S.R. was equivalent to 10.0 ± 1.0, 10.0 ± 2.0, 15.0 ± 1.0, 12.0 ± 1.0, 10.0 ± 1.0, 8.0 ± 1.0 and 16.0 ± 2.0 millimeters (per hour) respectively.

TABLE NO. 29 : ALTERATIONS IN THE ERYTHROCYTE SEDIMENTATION RATE (ESR) UNDER THE INFLUENCE OF MINING EFFLUENTS.

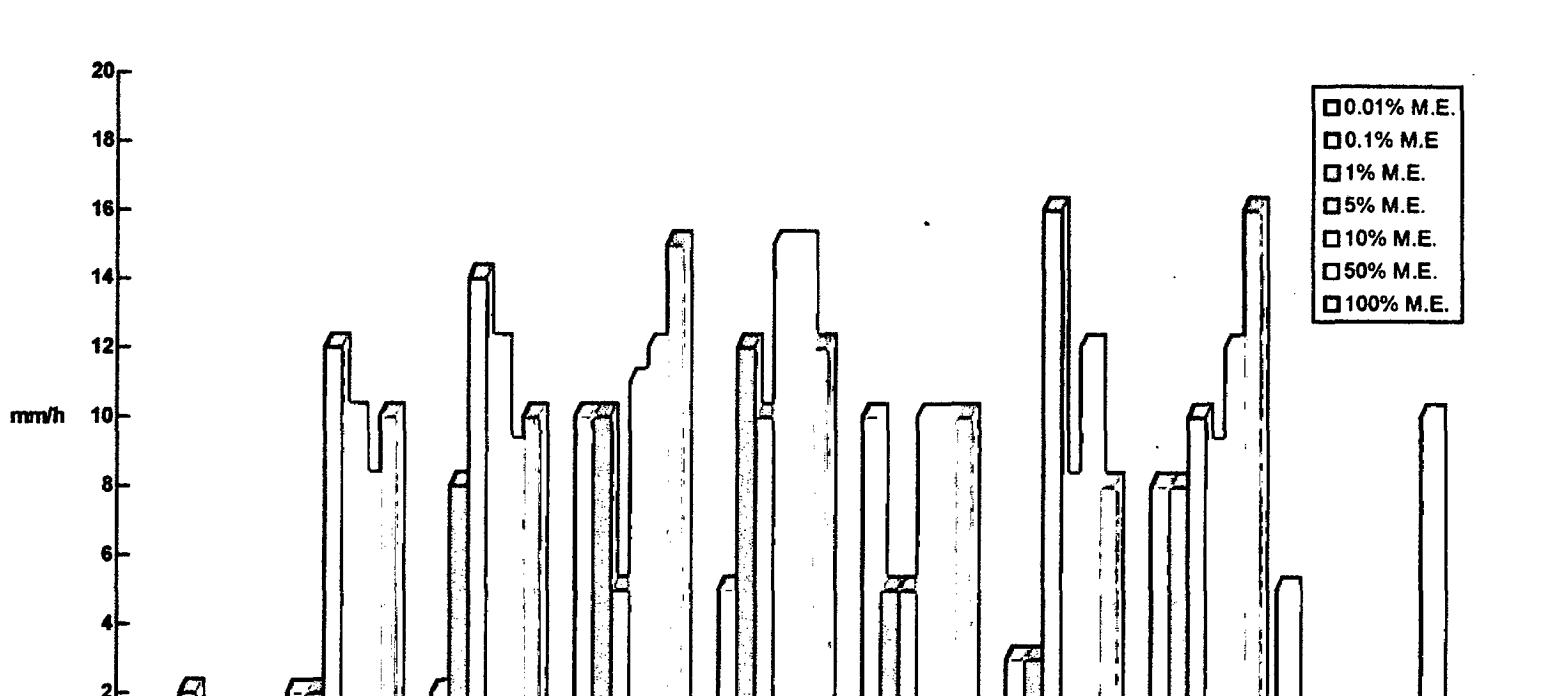
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		2.0 ±0.00									
0.01%	M.E.		← No significant change →							5 ±1.0	10 ±1.0
0.1%	M.E.		2 ±0.00	2 ±0.00	10 ±2.0	5 ±1.0	10 ±2.0	3 ±0.00	8 ±1.0	N.D..	N.D..
1%	M.E.		2 ±0.00	8 ±2.0	10 ±0.00	12 ±2.0	5 ±0.00	3 ±0.00	8 ±1.00	N.D..	N.D..
5%	M.E.		12 ±1.00	14 ±1.00	5 ±0.00	10 ±1.0	5 ±0.00	16 ±2.0	10 ±1.0	N.D..	N.D..
10%	M.E.		10 ±1.0	12 ±1.0	11 ±1.0	15 ±1.0	10 ±1.0	8 ±0.00	9 ±0.00	N.D..	N.D..
50%	M.E.		8 ±1.0	9 ±1.0	12 ±1.10	15 ±1.0	10 ±1.0	12 ±1.0	12 ±1.0	N.D..	N.D..
100%	M.E.		10 ±1.0	10 ±2.0	15 ±1.0	12 ±1.0	10 ±1.0	8 ±1.0	16 ±2.0	N.D..	N.D..

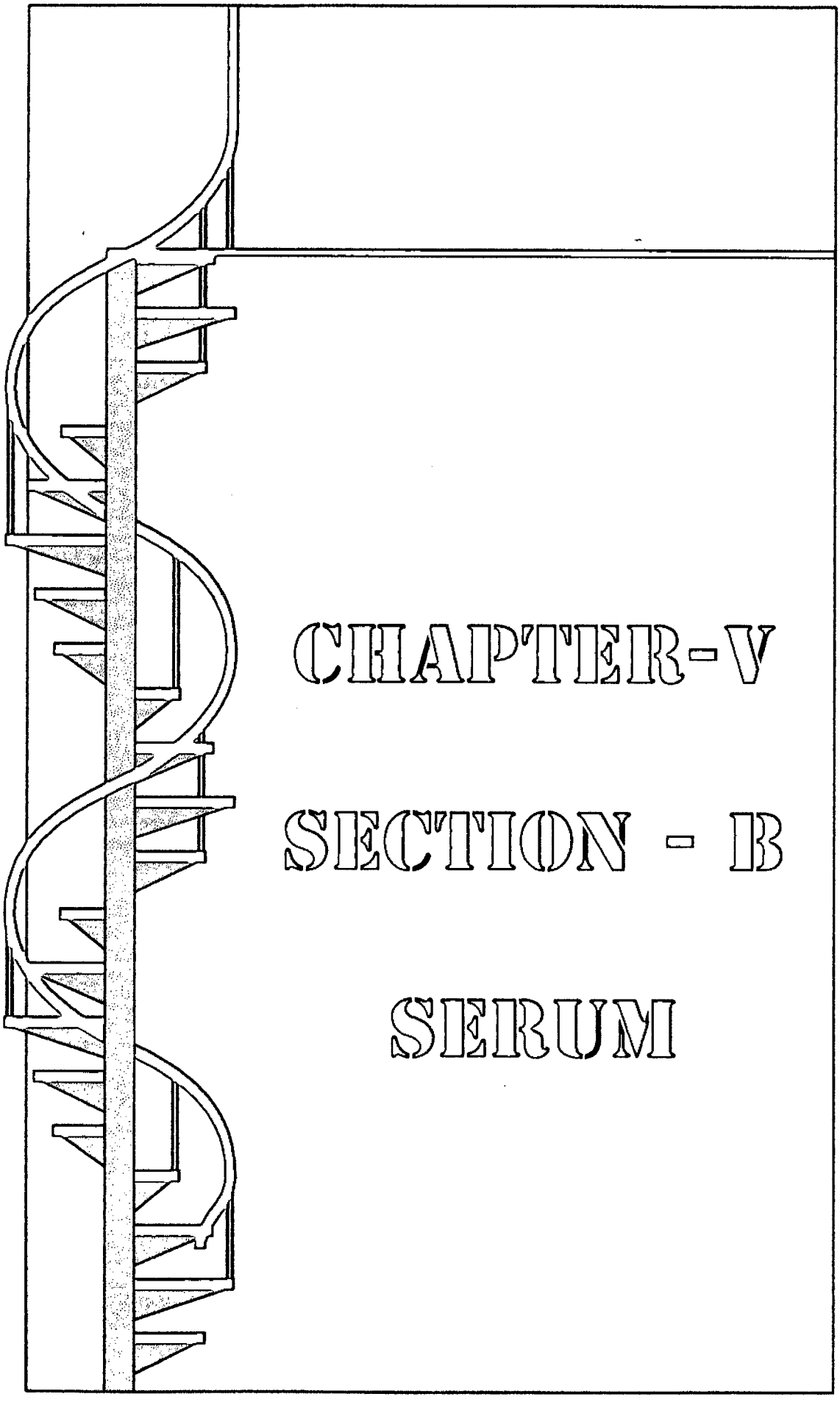
Note : Unit : mm/hour.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 29 : CHANGES IN E. S. R. UNDER THE INFLUENCE OF MINING EFFLUENTS
Units : mm/h





CHAPTER-V

SECTION - B

SERUM

1) PROTEINS :

The alterations in the serum protein levels are compiled in **Table No. 30** and are graphically presented in **Graph No. 30**. The serum protein level (Total proteins) is expressed as grams per litre of serum. The control birds showed the serum protein concentration equivalent to 3.4 ± 0.31 grams per litre. From the graph and table it appears that all the concentrations of mining effluents (M.E.) at all the time intervals (except **1.0% M.E.** at the end of seventytwo hours) induced rise in the serum protein level. But the rise in the concentrations of the serum proteins was neither M.E. concentration dependent nor exposure time dependent.

The exposure of ducks to **0.01% M.E.** did not induce any significant change in the serum protein levels up to the end of twenty nine days but at the end of thirty days there was significant increase in the serum protein concentrations which continued to increase up to the end of fortyfive days. Thus the serum protein concentrations were equivalent to 4.3 ± 0.2 and 6.6 ± 0.17 grams per litre at the end of thirty and fortyfive days respectively.

Under the influence of **0.1% M.E.** the ducks showed the rise in the concentrations of the serum proteins at the end of six hours and it continued to rise up to the end of twenty four hours and then subsequently decreased gradually up to the end of ninety six hours but at the end of one twenty hours the serum protein concentrations increased once again. The serum protein concentrations were equivalent to 4.62 ± 0.193, 4.9 ± 0.141, 6.26 ± 0.149, 5.36 ± 0.30, 5.20 ± 0.141, 4.80 ± 0.263 and 5.32 ± 0.231 grams per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The ducks subjected to **1.0%** M.E. showed maximum reduction in the serum protein concentrations at the end of seventy two hours while a sharp increase in the serum protein level was shown at the end of one twenty hours. The serum protein level at the end of six, twelve, twentyfour, fortyeight,

seventytwo, ninetysix and one twenty hours was equivalent to 5.68 ± 0.172, 4.30 ± 0.2, 5.32 ± 0.213, 5.10 ± 0.178, 3.32 ± 0.231, 6.10 ± 0.389 and 6.70 ± 0.275 grams per litre, respectively.

The exposure to **5.0%** M.E. promoted increase in the serum protein concentrations but the rise in serum protein levels had no correlation with the exposure period. The serum protein concentrations were equivalent to 5.18 ± 0.172, 5.10 ± 0.140, 5.98 ±0.132, 5.24 ±0.162, 5.54 ± 0.101, 6.56 ± 0.185 and 4.16 ± 0.215 grams per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The **10.0% M.E.** induced fluctuations in the serum protein concentrations but in general, the serum levels of the proteins were higher than those observed in the controled animals. The serum protein concentrations were equivalent to 5.28 ± 0.116, 5.58 ± 0.132, 5.82 ± 0.271, 4.5 ± 0.141, 5.66 ± 0.220, 5.10 ± 0.141 and 5.60 ± 0.140 grams per litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

On exposure to **50.0%** M.E. there was acute rise in the serum protein levels but the rise in serum protein concentrations had no correlation with the exposure period. Infact, the serum protein levels exhibited fluctuations. Thus at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the serum concentrations of the proteins were equivalent to 5.80 ± 0.089, 5.95 ± 0.182, 5.88 ± 0.1, 6.62 ± 0.17, 6.22 ± 0.172, 6.84 ± 0.108 and 5.82 ± 0.074 grams per litre, respectively.

The exposure to **100%** M.E. caused maximum increase in the serum protein concentrations at the end of fortyeight hours and at the rest of the time intervals, the serum protein levels remained above that found in the control. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the serum protein concentrations were equivalent to 5.88 ± 0.097, 6.44 ± 0.241, 6.52 ± 0.213, 7.10 ± 0.209, 6.94 ± 0.10, 6.20 ± 0.167 and 5.76 ± 0.137 grams per litre, respectively.

TABLE NO. 30 : ALTERATIONS IN SERUM PROTEINS INDUCED BY MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	M.E.	3.4 ±0.31									
0.01%	M.E.		← No significant change →							4.3 ±0.2	6.6 ±0.17
0.1%	M.E.		4.62 ±0.193	4.90 ±0.141	6.26 ±0.149	5.36 ±0.300	5.20 ±0.141	4.80 ±0.263	5.32 ±0.231	N.D.	N.D.
1%	M.E.		5.68 ±0.172	4.30 ±0.2	5.32 ±0.213	5.10 ±0.178	3.32 ±0.231	6.10 ±0.389	6.70 ±0.275	N.D.	N.D.
5%	M.E.		5.18 ±0.172	5.10 ±0.14	5.98 ±0.132	5.24 ±0.162	5.54 ±0.101	6.56 ±0.185	4.16 ±0.215	N.D.	N.D.
10%	M.E.		5.28 ±0.116	5.58 ±0.132	5.82 ±0.271	4.50 ±0.141	5.66 ±0.22	5.10 ±0.141	5.60 ±0.14	N.D.	N.D.
50%	M.E.		5.80 ±0.089	5.95 ±0.182	5.88 ±0.1	6.62 ±0.17	6.22 ±0.172	6.84 ±0.108	5.82 ±0.074	N.D.	N.D.
100%	M.E.		5.88 ±0.097	6.44 ±0.241	6.52 ±0.213	7.10 ±0.209	6.94 ±0.10	6.20 ±0.167	5.76 ±0.137	N.D.	N.D.

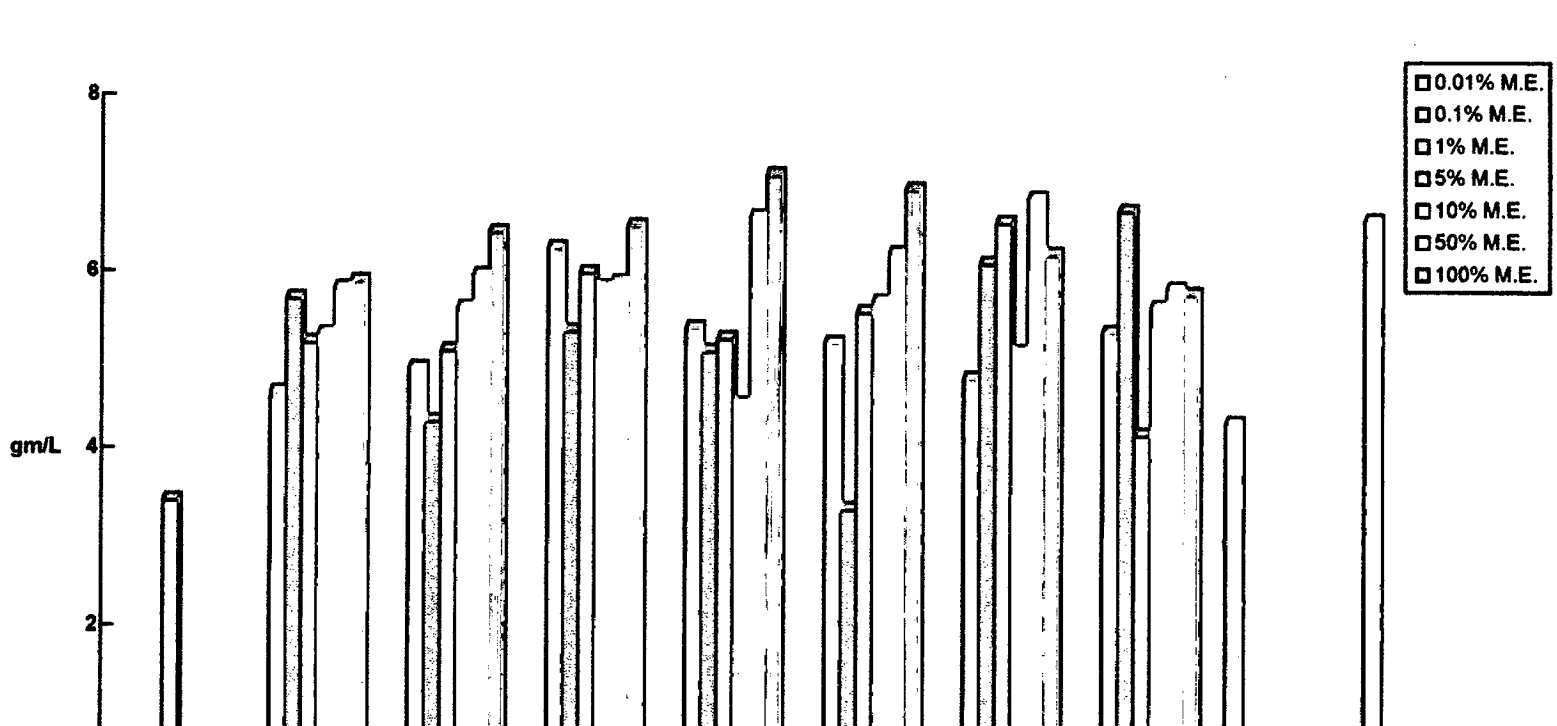
Note : Unit : Serum protein in gm/L.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 30 : ALTERATIONS IN SERUM PROTEIN LEVELS UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : gm/L



2) ACID PHOSPHATASE ACTIVITY :

The alterations in the serum acid phosphatase activity are given in **Table No.31** and are graphically expressed in **Graph No. 31**. The serum acid phosphatase activity is expressed as units per hundred millilitre of serum. The control animals showed acid phosphatase activity equivalent to 310.50 ± 3.08 units per hundred millilitre.

From the table and the graph it appears that the serum acid phosphatase activity showed M.E. concentration dependent (from **0.1 to 50% M.E.**) increase for fortyeight hours time interval, while for the **M.E.** concentrations like **10.0** and **50.0** percent it exhibited exposure time dependent rise from the end of six hours to the end of forty eighty hours.

When the ducks were exposed to **0.01% M.E.** the serum acid phosphatase activity did not exhibit any significant change up to the end of twenty nine days but it increased significantly at the end of thirty days (seven hundred and twenty hours) and continued to increase up to the end of fortyfive days (one thousand and eighty hours). Thus, at the end of thirty and fortyfive days the acid phosphatase activity was equivalent to 411.24 ± 13.772 and 678.96 ± 8.638 units per hundred milli litre of serum respectively.

The exposure to ducks to **0.1% M.E.** induced marginal increase in the acid phosphatase activity at the end of six and twelve hours but subsequently it increased significantly and fluctuated considerably. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the acid phosphatase activity was equivalent to 387.78 ± 6.693, 324.576 ± 10.948, 422.556 ± 1.642, 485.208 ± 11.495, 545.10 ±13.399, 570.492 ± 10.225 and 584.016 ± 13.358 units per hundred millilitre of serum respectively.

The **1.0% M.E.** induced sharp increase in the acid phosphatase activity at the end of six hours and the enzyme activity decreased a little by the end of twelve hours. Then, the acid phosphatase activity increased significantly at

the end of twentyfour hours and subsequently went on increasing steadily up to the end of one twenty hours. Thus, at the end of six, twelve twentyfour fortyeight, seventy two, ninetysix and one twenty hours the acid phosphatase activity was equivalent to 451.26 ± 11.440, 438.012 ± 10.984, 582.084 ± 12.985, 589.812 ± 12.834, 673.164 ± 9.494, 707.664 ± 1.656 and 712.908 ± 1.756 units per hundred millilitre of serum respectively.

The exposure of ducks to **5.0% M.E.** promoted acute decrease and increase in the acid phosphatase activity at the end of seventy two and one twenty hours respectively. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the serum acid phosphatase activity was equivalent to 592.848 ± 1.464, 521.916 ± 6.669, 708.216 ± 8.917, 678.96 ± 9.399, 70.704 ± 11.914, 759.276 ± 1.435 and 837.66 ± 1.711 units per hundred milli litre of serum, respectively.

The ducks treated with **10.0% M.E.** exhibited sharp increase in the serum acid phosphatase activity at the end of six hours and subsequently the enzyme activity increased steadily up to the end of fortyeight hours. The maximum increase in the enzyme activity was observed at the end of one twenty hours. Thus, the acid phosphatase activity was equivalent to 538.752 ± 7.384, 594.54 ± 10.764, 674.82 ± 68.379, 834.072 ± 12.513, 815.304 ± 59.450, 730.02 ± 5.682 and 994.704 ± 10.192 units per hundred millilitre of serum at the end of six, twelve, twentyfour, fortyeight, seventytwo ninetysix and one twenty hours respectively.

The ducks subjected to **50% M.E.** exhibited increase in the serum acid phosphatase activity at the end of six hours and the enzyme activity went on increasing up to the end of fortyeight hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the acid phosphatase activity was equivalent to 387.78 ± 7.521, 523.848 ± 11.298, 664.608 ± 9.729, 909.42 ± 7.645, 374.256 ± 10.294, 452.916 ± 5.682 and 304.428 ± 9.894 units per hundred millilitre of serum respectively.

The 100% M.E. exposure caused reduction in the acid phosphatase activity at all the time intervals under study except six hours. At the end of six hours the serum acid phosphatase activity raised sharply. The maximum reduction in the acid phosphatase activity was observed at the end of twentyfour hours. The serum acid phosphatase activity was equivalent to 597.264 ± 6.720, 302.772 ± 10.488, 150.42 ± 1.386, 166.98 ± 4.14, 154.836 ± 9.494, 269.376 ± 12.42 and 208.656 ± 8.914 units per hundred millilitre of serum at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

TABLE NO. 31 : ALTERATIONS IN THE SERUM ACID PHOSPHATASE ACTIVITY UNDER THE INFLUENCE OF MINING EFFLUENTS.

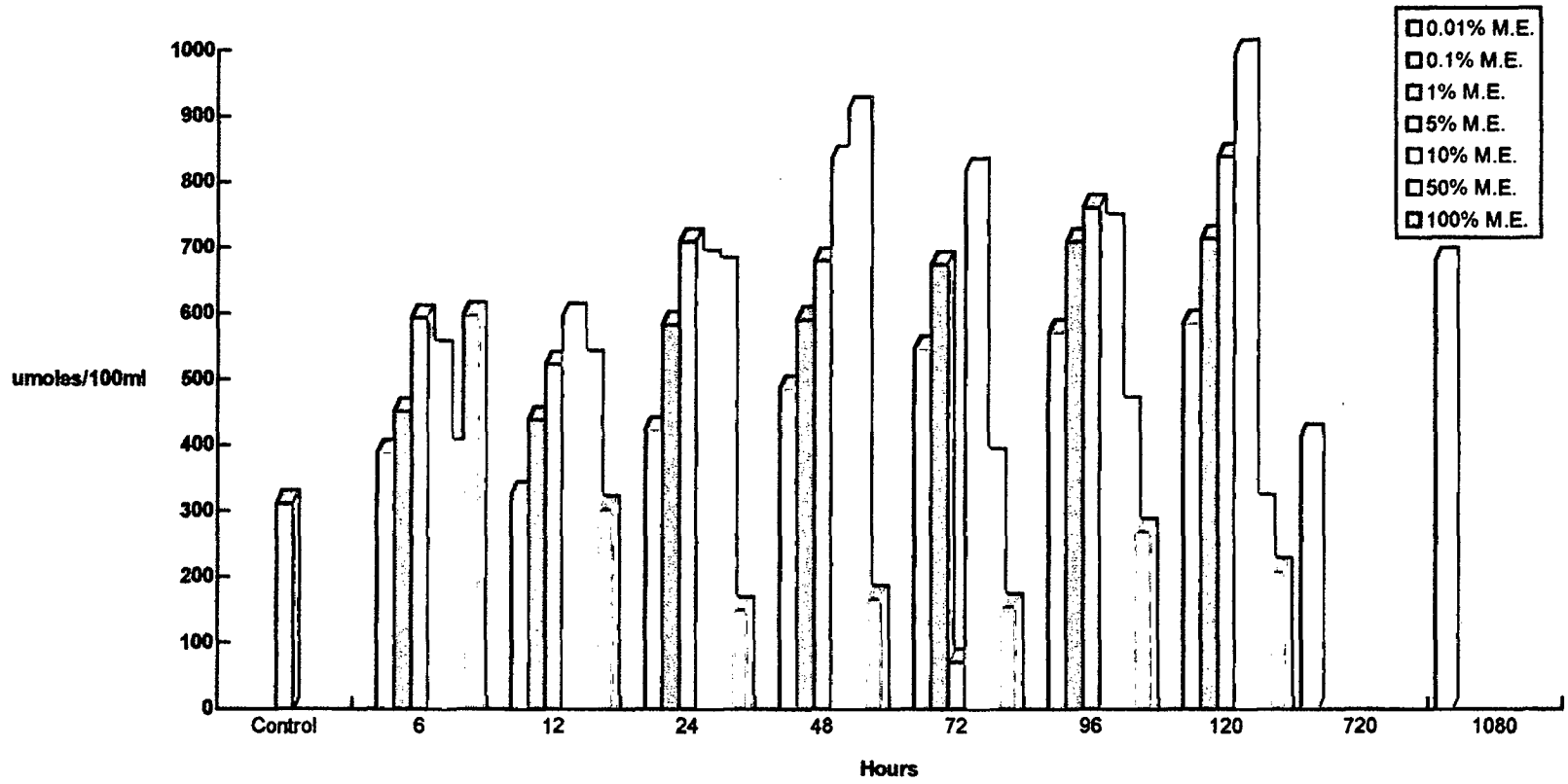
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		310.5 ± 3.08									
0.01%	M.E.		← No significant change →							411.24 ±13.772	678.96 ±8.638
0.10%	M.E.		387.78 ±6.693	324.576 ±10.948	422.556 ± 1.642	485.208 ±11.495	545.10 ±13.399	570.492 ±10.225	584.016 ±13.358	N.D.	N.D.
1%	M.E.		451.26 ±11.440	438.012 ±10.984	582.084 ±12.985	589.812 ±12.834	673.164 ± 9.494	707.664 ± 1.656	712.908 ± 1.756	N.D.	N.D.
5%	M.E.		592.848 ±1.464	521.916 ±6.669	708.216 ±8.917	678.96 ±9.399	70.704 ±11.914	759.276 ±1.435	837.66 ±1.711	N.D.	N.D.
10%	M.E.		538.752 ±7.384	594.54 ±10.764	674.82 ±68.379	834.072 ±12.513	815.304 ±59.450	730.02 ±5.682	994.704 ±10.192	N.D.	N.D.
50%	M.E.		387.78 ±7.521	523.848 ±11.298	664.608 ±9.729	909.42 ±7.645	374.256 ±10.294	452.916 ±5.682	304.428 ±9.894	N.D.	N.D.
100%	M.E.		597.264 ±6.720	302.772 ±10.488	150.42 ±1.386	166.98 ±4.14	154.836 ±9.494	269.376 ±12.42	208.656 ±8.914	N.D.	N.D.

Note : Unit : µmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 31 : SERUM - ACID PHOSPHATASE.
Units : µmoles/100 ml.



3] ALKALINE PHOSPHATASE :

The changes in the serum alkaline phosphatase activity under the influence of mining effluents are compiled in **Table No. 32** and are graphically presented in **Graph No. 32.** The alkaline phosphatase activity is expressed as mmol units per hundred millilitre of serum. The control animals had the serum alkaline phosphatase (AIP) activity equivalent to 3.16 ± 0.537 mmoles per hundred millilitre of serum. From the table and graph it appears that the increase in serum alkaline phosphatase activity was concentration dependent for **0.1 to 10% M.E.** concentrations at the end of six and twentyfour hours only. The maximum increase in the AIP activity was observed at the end of six hours under the influence of **50% M.E.** while the highest reduction in the AIP activity was observed at the end of one twenty hours under the influence of **100% M.E.**

The ducks exposed to **0.01% M.E.** did not show significant changes in the serum AIP activity up to the end of twenty nine days but at the end of thirty days (seven hundred and twenty hours) the serum AIP activity increased marginally and continued to increase further up to the end of fortyfive days. Thus, the serum AIP activity was equivalent to 4.0 ± 0.120 and 6.80 ± 0.136 mmoles per hundred millilitre at the end of thirty and fortyfive days.

Under the influence of **0.1% M.E.** the serum AIP activity exhibited exposure time dependent increase. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the serum AIP activity was equivalent to 3.85 ± 0.068, 4.0 ± 0.073, 4.15 ± 0.109, 4.30 ± 0.092, 4.60 ± 0.100, 5.0 ± 0.048 and 5.55 ± 0.098 mmoles per hundred millilitre of serum respectively.

The exposure of ducks to **1.0% M.E.** promoted steady increase in the serum AIP activity at all the time intervals under study except at the end of forty eight hours. The acute increase in the serum AIP activity was observed at the end of one twenty hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the serum AIP activity was

equivalent to 5.10 ± 0.034, 5.25 ± 0.077, 6.50 ± 0.089, 5.45 ± 0.084, 7.20 ± 0.051, 7.40 ± 0.114 and 8.35 ± 0.125 mmoles per hundred millilitre serum respectively.

On exposing the ducks to **5.0% M.E.** the serum AIP activity increased at the end of six hours and continued to increase further up to the end of twenty four hours, but the serum AIP activity dropped significantly by the end of forty eight hours. Then, subsequently the AIP activity showed steady rise up to the end of ninetysix hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one hundred and twenty hours, the serum AIP activity was equivalent to 5.45 ± 0.104, 8.55 ± 0.108, 9.05 ± 0.086, 5.20 ± 0.121, 5.75 ± 0.056, 6.50 ± 0.071 and 6.45 ± 0.068 mmoles per hundred millilitre of serum respectively.

The **10.0% M.E.** induced fluctuations in the serum AIP activity at different time intervals. A very high AIP activity was observed at the end of fortyeight hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the serum AIP activity was equivalent to 7.70 ± 0.090, 13.0 ± 0.103, 10.0 ± 0.103, 20.40 ± 0.107, 14.80 ± 0.128, 15.40 ± 0.086 and 17.0 ± 0.117 mmoles per hundred millilitre of serum respectively.

When the ducks were introduced to **50.0% M.E.** the highest serum AIP activity was observed at the end of six hours. The AIP activity decreased gradually from the end of fortyeight hours to the end of one hundred and twenty hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, one hundred and twenty hours, the serum AIP activity was equivalent to 23.60 ± 0.145, 7.0 ± 0.087, 16.80 ± 0.092, 12.90 ± 0.106, 10.20 ± 0.107, 5.45 ± 0.062 and 4.35 ± 0.103 mmoles per hundred millilitres of serum respectively.

The exposure of ducks to **100% M.E.** caused progressive reduction in the serum AIP activity from the end of twelve hours to the end of forty eight hours after the initial rise in AIP activity at the end of six hours. The maximum

reduction in the serum AIP was observed at the end of one hundred and twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, one hundred and twenty hours, the serum AIP activity was equivalent to 17.70 ± 0.154, 10.20 ± 0.196, 8.25 ± 0.137, 7.90 ± 0.075, 10.20 ± 0.808, 4.30 ± 0.104 and 2.60 ± 0.0876 mmoles per hundred millilitres of serum respectively.

TABLE NO. 32: ALTERATIONS IN THE SERUM ALKALINE PHOSPHATASES ACTIVITY UNDER THE INFLUENCE OF MINING EFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		3.16 ± 0.537									
0.01%	M.E.		← No significant change →							4.0 ±0.120	6.80 ±0.136
0.10%	M.E.		3.85 ±0.068	4.0 ±0.073	4.15 ±0.109	4.30 ±0.092	4.60 ±0.100	5.0 ±0.048	5.55 ±0.098	N.D.	N.D.
1%	M.E.		5.10 ±0.034	5.25 ±0.077	6.50 ±0.089	5.45 ±0.084	7.20 ±0.051	7.40 ±0.114	8.35 ±0.125	N.D.	N.D.
5%	M.E.		5.45 ±0.104	8.55 ±0.108	9.05 ±0.086	5.20 ±0.121	5.75 ±0.056	6.50 ±0.071	6.45 ±0.068	N.D.	N.D.
10%	M.E.		7.70 ±0.090	13.0 ±0.103	10.0 ±0.103	20.40 ±0.107	14.80 ±0.128	15.40 ±0.086	17.0 ±0.117	N.D.	N.D.
50%	M.E.		23.60 ±0.145	7.0 ±0.087	16.80 ±0.092	12.90 ±0.106	10.20 ±0.107	5.45 ±0.062	4.35 ±0.103	N.D.	N.D.
100%	M.E.		17.70 ±0.154	10.20 ±0.196	8.25 ±0.137	7.90 ±0.075	10.20 ±0.808	4.30 ±0.104	2.60 ±0.0876	N.D.	N.D.

Note : Unit : mmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 32 : SERUM - ALKALINE PHOSPHATASE.
Units : mmoles/100 ml.

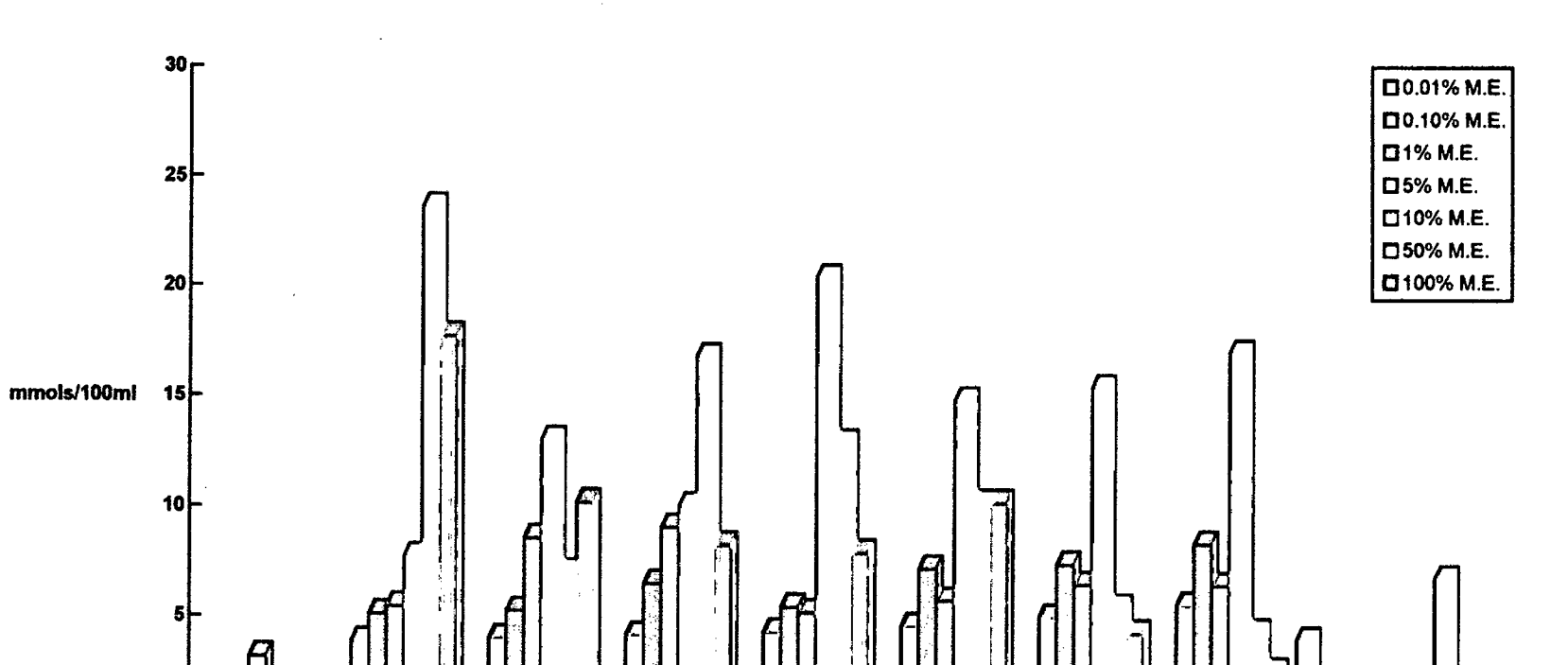
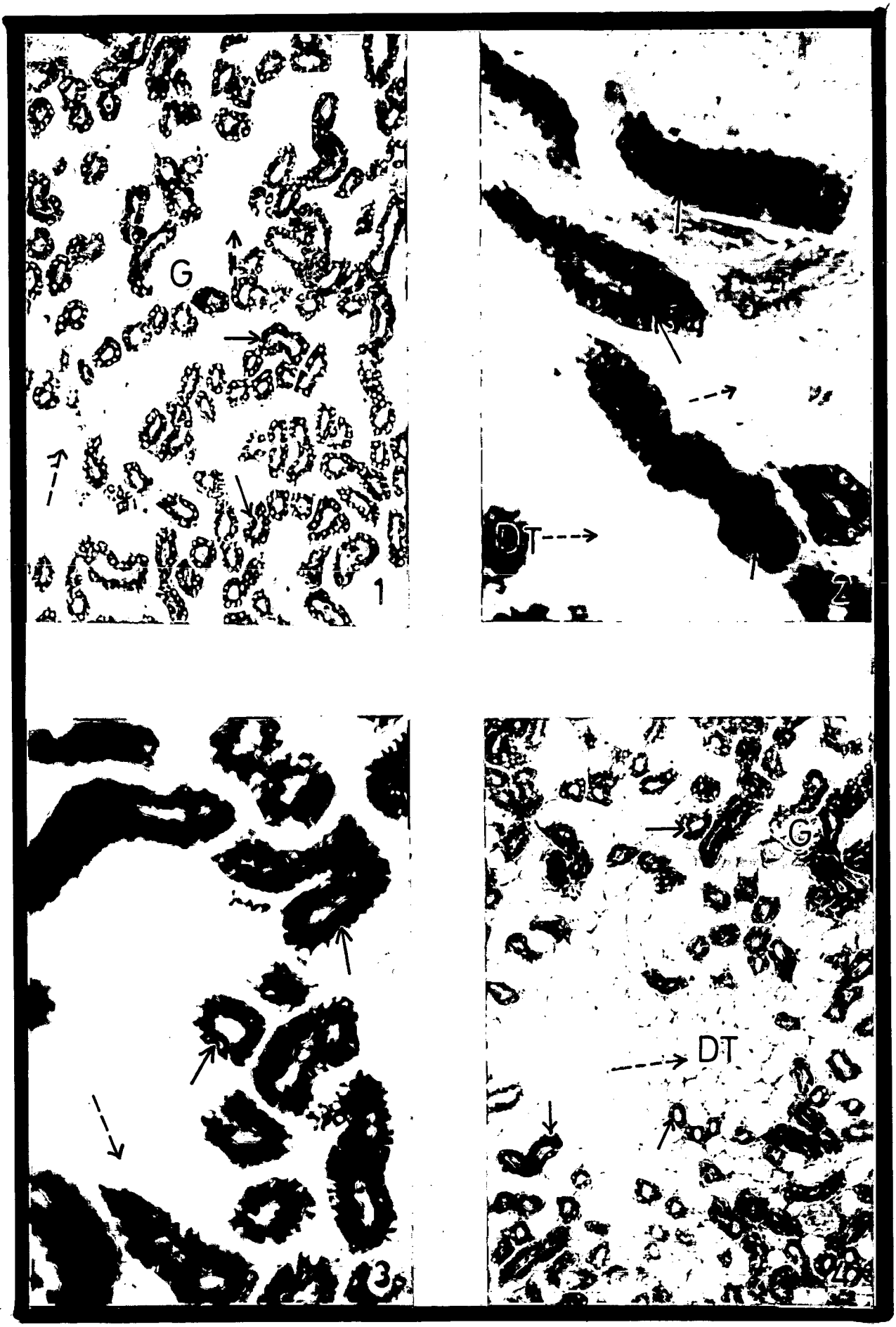


PLATE NO 64



enzyme activity. By the end of fortyeight hours the duck showed a marginal decrease in the nonspecific esterase activity while the glomeruli and the other tubules did not exhibit any esterase (nonspecific) activity. In a few cases the walls of Bowman's capsule showed a very weak enzyme activity **(Plate 64 : Fig 1)**. Subsequently, at the end of seventytwo and ninetysix hours the ducks showed gradual increase in the enzyme activity in about sixty percent tubules, but by the end of one twenty hours the ducks showed increase in the enzyme activity in comparison to that seen at the end of fortyeight hours and showed marginally less than that observed at ninetysix hours. The other forty percent tubules showed the absence of enzyme activity **(Plate 64 : Fig 2)**. The glomeruli and collecting tubules also showed absence of esterase (nonspecific) activity.

Under the influence of **10.0% M.E.** the ducks showed a sharp increase in the enzyme activity in about sixty percent tubules and a few of these tubules showed the brush/luminal border esterase (nonspecific) activity. About thirty eight percent tubules showed absence of enzyme activity, while about two percent tubules showed weak, membrane bound esterase activity **(Plate 64 : Fig 3)**. By the end of twelve hours the sixty percent tubules showed slight reduction in the enzyme activity while about thirty eight percent tubules showed absence of enzyme activity and around two percent tubules exhibited weak, membrane bound, esterase activity. The wall of Bowman's capsule showed mild esterase activity **(Plate 64 : Fig 4)**. At the end of twentyfour hours the ducks exhibited further decrease in the nonspecific esterase activity in about sixty percent tubules (proximal and distal). These tubules occasionally showed weak, membrane bound, esterase activity. The other tubules did not show any activity **(Plate 65 : Fig 1)**. The glomeruli and collecting tubules showed absence of enzyme activity. By the end of fortyeight hours the nonspecific esterase activity from about sixty percent tubules increased sharply while the remaining tubules showed absence of enzyme activity. The glomeruli did not

CAPTIONS TO FIGURES

PLATE 65 :

Fig 1 : 10.0% M. E. - 24 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note further reduction in the enzyme activity from the tubules and the occurrence of membrane esterase at the nucleus (solid arrow). Also note the absence of enzyme activity from other tubules (dashed arrow).

Fig 2 : 10.0% M. E. - 48 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note increase in esterase activity in the tubules (solid arrow) and absence of activity in other tubules (dashed arrow) as well as in glomerulus (GL). Also note weak esterase in the luminal debris. (D).

Fig 3 : 10.0% M. E. - 72 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note further increase in the esterase activity (solid arrow) and absence of esterase activity in other tubules.

Fig 4 : 10.0% M. E. - 120 hours - Esterase (Nonspecific) - Indoxylacetate. (20 x 5)

Note intense staining in the tubules (DT, solid arrow) and absence of enzyme activity in other tubules (dashed arrow).

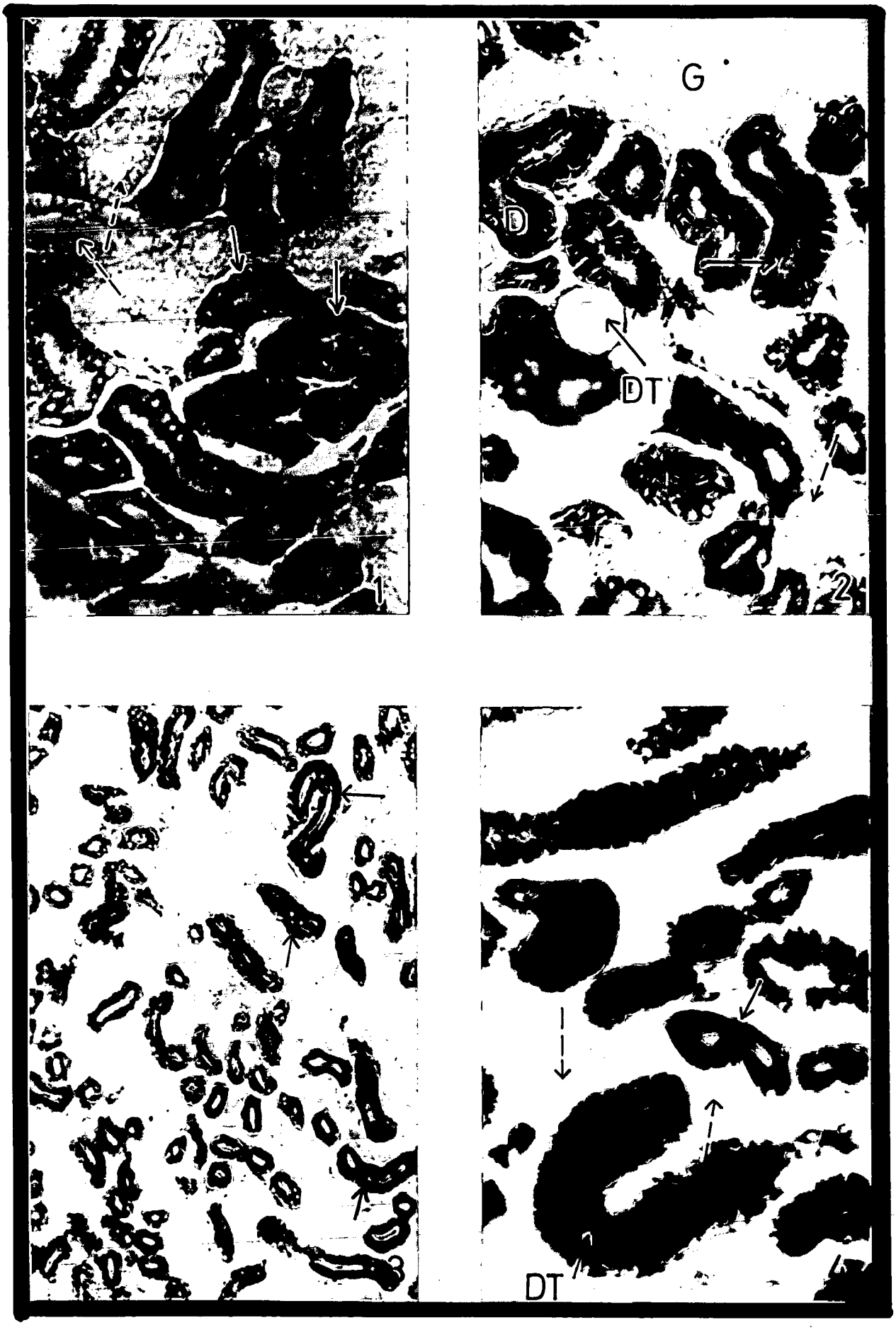

G
D
DT
1
2
3
DT

exhibit any enzyme activity but the debris in the lumen showed weak enzyme activity. The interstitium was without esterase activity **(Plate 65 : Fig 2)**. By the end of seventytwo hours the ducks exhibited further increase in the nonspecific esterase activity from about sixty percent tubules, while the remaining forty percent tubules showed absence of enzyme activity **(Plate 65 : Fig 3)**. The glomeruli and collecting tubules showed no enzyme activity. At the end of ninetysix hours, the ducks showed increase in the esterase activity from about sixty percent tubules while at the end of one twenty hours there was no significant change in the enzyme activity if compared to that obtained at ninetysix hours. Some of the tubules from the sixty percent group showed very intense enzyme activity and the luminal debris showed weak enzyme activity. The other tubules (about forty percent) did not show any enzyme activity. The interstitium was without any enzyme activity **(Plate 65 : Fig 4)**.

When the mallards were exposed to **50% M.E.** a few tubules showed sharp increase in the enzyme activity and in some cases they showed brush/luminal border esterase activity. The other tubules, glomeruli and collecting tubules showed absence of esterase (nonspecific) activity. The interstitium showed no enzyme activity **(Plate 66 : Fig 1)**. Subsequently, the ducks showed gradual reduction in the enzyme activity at the end of twelve and twentyfour hours. But by the end of fortyeight hours the ducks showed increase in the esterase activity in about sixty percent tubules, while the remaining tubules, glomeruli and collecting tubules exhibited absence of enzyme activity. The luminal debris showed esterase activity **(Plate 66 : Fig 2)**. Then, at the end of seventy two hours, once again the nonspecific esterase activity reduced sharply from the sixty percent tubules and increased by the end of ninetysix hours. By the end of one twenty hours the ducks showed very intense enzyme activity in about sixty percent tubules and a few of these tubules showed brush/luminal border esterase (nonspecific) activity. The

CAPTIONS TO FIGURES

PLATE 66 :

Fig 1 : 50.0% M. E. - 6 hours - Esterase (Nonspecific) - Indoxyl acetate. (20 x 5)

Note intense staining in few tubules (solid arrow) and reduced esterase activity in some tubules (DT). Also note the absence of enzyme activity in other tubules (dashed arrow). The collecting tubules (CT) show absence of enzyme activity. Glomerulus (G) shows no enzyme activity.

Fig 2 : 50.0% M. E. - 48 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note increase in the enzyme activity in some tubules (solid arrow). Also note the absence of esterase activity in other tubules (dashed arrow) and collecting tubules (CT).

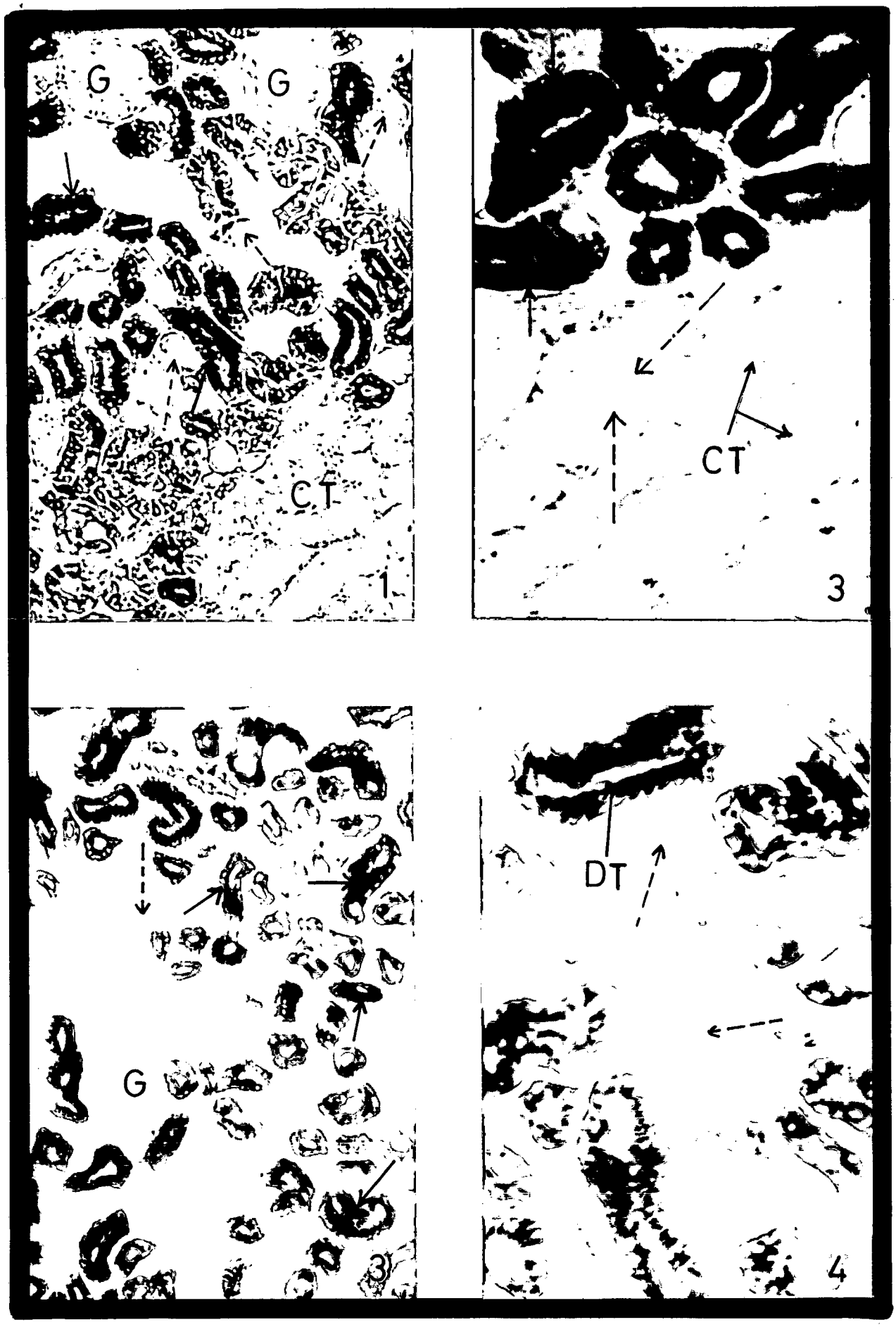
Fig 3 : 50.0% M. E. - 120 hours - Esterase(Nonspecific) _ Indoxyl acetate. (10 x 5)

Note intense staining in the tubules (solid arrow) and absence of enzyme activity in other tubules (dashed arrow) and glomerulus (G).

Fig 4 : 100.0% M. E. - 24 hours - Esterase (Nonspecific) - Indoxyl acetate.
(20 x 5)

Note increase in the esterase activity in some tubules (DT) and absence of enzyme activity in other tubules (dashed arrow).

PLATE NO 66



CAPTIONS TO FIGURES

PLATE 67 :

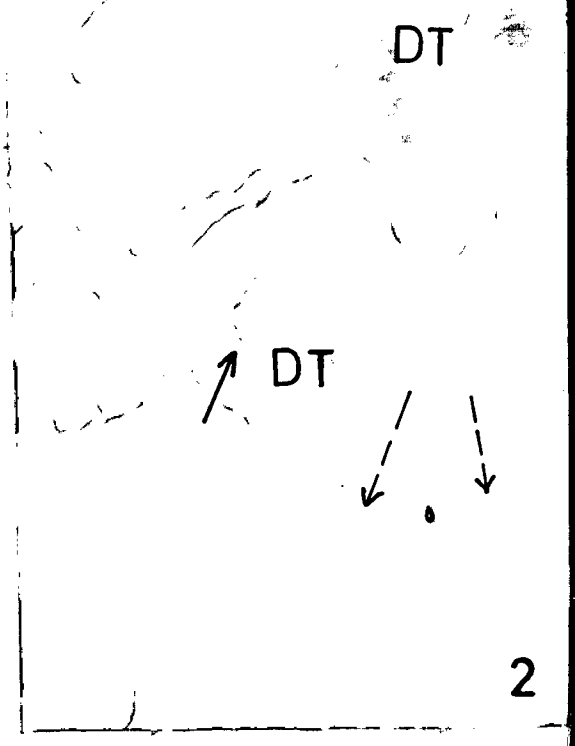
Fig 1 : 100.0% M. E. - 72 hours - Esterase (Nonspecific) - Indoxyl acetate. (10 x 5)

Note further increase in the enzyme activity and absence of esterase activity in glomerulus (G) and collecting tubules (CT).

Fig 2 : 100.0% M. E. - 120 hours - Esterase (Nonspecific) - Indoxyl acetate. (40 x 5)

Note intense staining in the tubules (DT) and the esterase activity in the luminal debris. Also note absence of enzyme activity in other tubules (dashed arrow).

PLATE NO 67



The ultrastructural changes induced by M.E. in the proximal tubules are like disruption of brush border, emptying of lysosomal vesicles, pycnosis of tubule nuclei, accumulation of cytoplasmic and nuclear debris in the lumina, vacuolisation of tubule cytoplasm, destruction of mitochondria, deposition of heavy metals in the tubule cytoplasm. The intensity of necrotic alterations appear to depend upon the exposure period and the concentrations of M.E. The necrotic changes in the proximal tubules may be due to the deposition of heavy metals, activation of lysosomes and lysosomal enzymes which promote degenerative changes and in addition the depletion of energy due to the destruction of mitochondria may activate lysosomes to promote degeneration of tubule structure. Prasad Rao *et. al.,* (1989) have reported accumulation of lipid droplets, lysosomal bodies and membrane bound vacuoles in methyl mercury treated ducks while lead exposed birds exhibit large number of secondary lysosomes and swollen mitochondria in proximal tubules. These observations support the findings in the present investigation up to some extent but the ultrastructural changes observed under the influence of M.E. are more pronounced. The ducks do not show any ultra structural change probably till the heavy metal retentions reach a threshold level to promote any toxic action.

The ultrastructural changes in the distal tubules are like vacuolisation of cytoplasm, swelling of Golgi bodies, heavy metal depositions, pycnosis of nuclei, lipid depositions in the cytoplasm, swelling of distal tubules, reduction in lumina depositions of tissue debris in the lumina, proliferations of Golgi bodies, formation of autophagic vacuoles, widening of intercellular space, disruption of basal membrane, heavy metal depositions in the nuclei of tubule cells, destruction of mitochondria, etc. These ultrastructural changes are observed at the end of six hours and at later hours the degree of necrosis increases depending upon the exposure period and the concentrations of M.E. Prasad Rao *et. al.,* (1989) report the lipid depositions in the proximal tubules of ducks exposed to heavy metals like methyl mercury, lead and cadmium but in the

present study the lipid depositions occur in the cytoplasm of distal tubule cells. The ultra structural changes observed in the present investigations under the influence of various concentrations of M.E. are quite pronounced and have not been reported thus far. The presence of heavy metal depositions in the tubule cell cytoplasm and nucleoplasm clearly indicate the possibility of heavy metals' involvement in inducing necrotic changes.

The changes in the ultrastructure of collecting tubules under the influence of M.E. are quite pronounced and are like distortion of basal membranes, pycnosis of nuclei, depositions of heavy metals in the nuclei, vacuolisation of mitochondria, heavy deposition of lipid droplets, disruption of luminal borders, swelling of Golgi bodies, proliferation of endoplasmic reticulum, ciliary processes, Golgi profiles. The 100.0% M.E. promote acute proliferations of E.R., ciliary processes and Golgi profiles into the lumina.

The degree of acuteness of the ultrastructural changes depend upon the exposure period and the concentration of M.E. The mitochondrial and endoplasmic reticular damages along with the destruction of Golgi could lead to severe alterations/disruptions in the metabolism of the tubules and such alterations may develop energy crisis. The destruction of mitochondria may lead to shortage of energy which may activate lysosomes leading to the degenerations of the tissue.

Some researchers have reported heavy metal promoted renal lesions to the fishes (Saxena and Parashari, 1981 ; Thiyagarajah *et. al.*, 1989). These observations support the involvement of heavy metals in nephrotoxicity. Thus, in the present investigation the heavy metals could be associated with the nephrotoxicity observed in the ducks.

C) HISTOCHEMICAL CHANGES :

I) ACID PHOSPHATASE :

The studies on acid phosphatase of the kidneys of control ducks revealed the presence of two types of renal tubules. One type shows the presence of brush/luminal border and cytoplasmic acid phosphatase activity while the other type shows only cytoplasmic activity. Nearly sixty percent tubules bear brush/luminal border as well as cytoplasmic acid phosphatase activity while, forty percent tubules contain only cytoplasmic activity. It is very difficult to adjudge on the basis of acid phosphatase activity,if the tubules are reptilian type or mammalian type. On exposure to mining effluents the ducks show the acid phosphatase activity bound to the membranes in addition to brush/luminal border and cytoplasmic acid phosphatase activity.

Therefore, the present investigation reveals the presence of three types of acid phosphatases based on the locations, a brush/luminal border, cytoplasmic and membrane bound. The kidney sections of the controls show brush/luminal border acid phosphatase activity and a few glomeruli show acid phosphatase activity. These glomeruli could be mammalian type as mammalian glomeruli are known to bear acid phosphatase activity (Farquhar et al., 1961, Dingle and Fell, 1969).

The ducks exposed to 0.01% M.E. bear the increased acid phosphatase activity at the end of thirty days which increases further up to the end of fortyfive days. The increase in acid phosphatase activity indicates activations of lysosomes and hence the increase in the degenerative changes.

By the end of six hours the acid phosphatase activity increases as the M.E. concentration increases, indicating higher the concentrations of M.E., higher is the enzyme activity. As the degree of necrosis increases with the M.E. concentration naturally the proportionate rise in acid phosphatase activity

is justified as the acid phosphatase is a lysosomal enzyme and the lysosomes are responsible for autophagy viz-a-viz degenerations of tissues.

The 0.1, 5.0, and 100.0% M.E. concentrations induce decrease in the acid phosphatase activity at the end of twelve hours while 1.0, 10.0 and 50.0 percent concentrations of M.E. promote increase in the acid phosphatase activity from the brush/luminal borders. The 50.0% M.E. induce cytoplasmic granular lysosomal staining.

The M.E. concentrations of 1.0, 5.0, 10.0 and 100.0 percent induce increase in acid phosphatase activity at the end of twenty four hours while 50% M.E. promote decrease in brush/luminal border enzyme activity but at the same time cause increase in glomerular and cytoplasmic acid phosphatase activity. The 5.0% M.E. induce decrease in the cytoplasmic enzyme activity. By the end of fortyeight hours, the 0.1, 1.0 and 10.0 percent M.E. induce increase in the enzyme activity while 50 percent M.E. promote decrease in brush/luminal border acid phosphatase activity and increase in cytoplasmic enzyme activity. The 100% M.E. cause decrease in the cytoplasmic acid phosphatase activity. At the end of seventy two hours the 0.1 and 1.0 percent M.E. promote increase in acid phosphatase activity at the brush/luminal borders and also increase the brush/luminal border enzyme activity in the tubules previously showing cytoplasmic enzyme activity. The 100 percent M.E. induce decrease in brush/luminal border activity but at the same time increase in the cytoplasmic acid phosphatase activity.

The M.E. concentrations of 0.1 and 50.0 percent induce increase in brush/luminal border acid phosphatase activity at the end of ninetysix hours while 1.0, 5.0, 10.0 and 100 percent M.E. promote decrease in the acid phosphatase activity but at the same time 10.0% M.E. induce increase in the cytoplasmic granular enzyme activity. By the end of one hundred and twenty hours the 0.1, 50.0 and 100% M.E. cause reduction in the acid phosphatase

activity. The 1.0 and 5.0% M.E. concentrations induce increase in the enzyme activity.

It is interesting to note that under the influence of M.E. the acid phosphatase activity change the locations/sites and appears at the membranes such as Bowman's wall, basement membrane and nuclear membranes. Even the cytoplasmic and nuclear debris in the lumina show acid phosphatase activity.

Acid phosphatase is a lysosomal enzyme and generally the lysosomes of the cells of the proximal tubules are responsible for the absorption of proteins and other high molecular weight substances from the glomerular filtrate which come to the lumina of the tubules (Maunsbach, 1973). The proximal and distal tubule lysosomes are responsible for the degradation of cytoplasmic components including mitochondria-like structures or organelle complexes (Straus and Oliver, 1955, Sellers, 1955 ; Squire *et. al.,* 1962 ; Pitts, 1963 ; Hamburger *et. al.*, 1968 ; Rhodin, 1954 ; Novikoff, 1959 ; Miller, 1960, 1962 ; Miller and Palade, 1964 ; Ericsson, 1964 ; Trump and Bulger, 1965 as cited by Dingle and Fell, 1969). During the necrosis of the glomeruli all the above mentioned components are released and then they come to the renal tubules. The renal tubules also receive injury and under such pathological conditions, to carry out the usual physiological functions the lysosomal acid phosphatase may be increasing up to certain extent but in the period of acute necrosis the enzyme activity reduces as it gets excreted through urine and passes down the concentration gradient to the extracellular spaces and blood (Merill *et. al.,* 1956)

The lysosomes of intercapillary cells are responsible for sweeping and unclogging the basement membrane (Farquhar . ; 1961; Farquhar and Palade, 1962 as cited by Dingle and Fell, 1969) and the degradation of plasma proteins which have passed through glomerular basement membrane (Dixon *et. al.,* 1950 ; Michael *et. al.,* 1977). The acid phosphatases appearing in the

glomeruli may probably have some role in the degradation of proteins or absorbed residues. The rise in acid phosphatase activity may be for dealing with the degradable debris and the loss may be due to its excretion through urine or release into the blood and extracellular spaces. The luminal debris may be showing enzyme activity of cytoplasmic origin or may be released from the brush/luminal borders for the degradation of debris. The appearance of membrane acid phosphatases in Bowman's capsule may be for degrading the exudents in the Bowman's space as well as for degradation of damaged membrane components. Similarly, the nuclear membrane acid phosphatase may be responsible for the degradation of nucleoplasmic debris.

II) ALKALINE PHOSPHATASE :

The duck kidney bears the typical granular lysosomal alkaline phosphatase activity. The staining reactions of the kidney sections reveal the two types of tubules : one darkly stained and the other lightly stained. The darkly staining proximal and distal tubules show moderate granular alkaline phosphatase activity with occasional weak brush/luminal border enzyme activity as well as membrane alkaline phosphatase activity at the nuclear membranes. The light staining tubules bear just the fine granular (lysosomal) enzyme activity.

The 0.01% M.E. promote increase in the alkaline phosphatase (AIP) activity at the end of thirty and fortyfive days when the necrosis is progressing. The AIP activity from all the renal tubules increases at the end of six hours in the M.E. concentration dependent manner i.e the rise in AIP activity was proportionate to the increase in M.E. concentration. The histopathological studies have revealed that the degree of necrosis increases as the M.E. concentration rises. Therefore, the rise in AIP activity may be for degrading the degenerative products/tissue debris as AIP is a lysosomal enzyme and the lysosomes are responsible for the degradation of cytoplasmic components

including mitochondria or organelle complexes (Dingle and Fell, 1969). All the concentrations of M.E. induce increase in AIP activity at all the time intervals under study except at a few occasions. Especially, 1.0 and 100.0% M.E. at the end of 12 hours, 10.0, 50.0 and 100.0% M.E. at the end of twenty four hours, 5.0% M.E. at the end of fortyeight hours ; 10.0, 50.0 and 100.0 % M.E. at the end of seventy two hours ; 10.0% at the end of ninetysix hours and 100.0% M.E. at the end of one hundred and twenty hours, induce reduction in the AIP activity. The glomerular AIP activity appears to increase according to the degree of necrosis. The exudents exhibit increased AIP activity and the enzyme activity may be cytoplasmic in origin or probably secreted from the brush/luminal borders. A pronounced increase in the granular lysosomal staining observed in the glomeruli and the cytoplasm can be taken as the activation of lysosomes, thereby promoting the release of AIP. Maunsbach (1973) has postulated that the glomerular filtrates under necrotic conditions contain proteins as well as high molecular weight substances which come to the tubules' lumina and the lysosomes of the cells of the tubules, especially of proximal tubules are responsible for the absorption of proteins and high molecular weight substances. In the present study the rise in lysosomal staining from the cytoplasm of both the proximal and distal tubules as well as elevated brush/luminal border, AIP activity may probably be responsible for the absorption of material from the tubules' lumen as suggested by Maunsbach (1973). Besides, the rise in the lysosomal staining may be for sweeping and unclogging of the basement membranes (Farquhar *et al.*, 1961 ; Farquhar and Palade, 1962). The reduction in the AIP activity at a few time intervals under the influence of some concentrations of M.E. may be due to the acute necrosis of the proximal and distal tubules causing exudation of tissue debris into the lumen and excretion of AIP through urine as well as passing down the concentration gradient to the extracellular spaces and blood (Merill *et. al.*, 1956). The appearance of AIP at the membranes may be due to the loss of

cytoplasmic AIP as these membrane AIPs may be useful for degrading the exudents from the Bowman's space or nucleoplasm or interstitium or even for degradation of damaged membrane proteins.

It is interesting to note that the collecting tubules did not show acid phosphatase activity but do exhibit the alkaline phosphatase activity and the alkaline phosphatase being a lysosomal enzyme may be considered responsible for the degradation of the tissue debris/products of necrosis. The AIP in the luminal exudents may be of cytoplasmic origin as well as from the brush/luminal border release. The membrane AIP of collecting tubules may be playing the role ascribed to it for proximal and distal tubules under necrosis. The enzyme activation/inhibition studies confirm that AIP is a true lysosomal enzyme.

III) NONSPECIFIC ESTERASE :

The histochemical studies of esterase (nonspecific) with indoxyl acetate method reveal the presence of two types of renal tubules on the basis of distribution of esterase activity, in the kidney of control and experimental ducks. The first group comprising of about sixty percent tubules bear the fine granular lysosomal activity and the other group comprising of about forty percent tubules are without any esterase activity. The sixty percent collecting tubules bear very weak lysosomal enzyme activity and the remaining have no esterase activity. The glomeruli do not have any esterase activity.

The 0.01% M.E. treatment induces increase in the esterase (nonspecific) activity at the end of thirty and forty five days. This increase in esterase activity shows its association with progression of necrosis. The histochemical studies of esterase (nonspecific) clearly reveal the increase of esterase activity in association with the rise in concentrations of M.E., especially in the 60 percent of proximal and distal tubules at the end of six

hours and the enzyme activity is mostly lysosomal (granular) but in a few occasions brush luminal/border and membrane esterases are present.

The 0.1% M.E. induces appearances of esterases in the second group of tubules where normally no enzyme activity is seen. This concentration of M.E. also promotes occurrence of glomerular and membrane esterases in Bowman's wall in a few cases. But the collecting tubules, glomeruli and forty percent proximal and distal tubules bear no esterase activity under the influence of M.E. In general, mostly all the concentrations of M.E. induce rise in the esterase activity from sixty percent tubules for all time intervals except a few reductions observed under the influence of a few M.E. concentrations for a few time intervals. These inclines and declines in the esterase activity appear to depend upon the degree of necrosis and the particular stage of necrosis (early or late). In a very few occasions the collecting tubules show the esterase activity in the form of membrane bound enzyme. Thus, it appears that the esterase does not contribute to the necrosis or degradation of necrotic products of collecting tubules. The exudents in the lumina of the renal tubules contain esterase which may be of cytoplasmic or luminal border in origin and may be considered as responsible for the degradation of the debris or being eliminated with debris. The rise in nonspecific esterase activity from the proximal tubules may be considered essential for absorption of material from the lumen (Maunsbach, 1973), as damaged glomeruli are likely to pass on the proteins and other high molecular weight substances to the renal tubules. Besides, the rise in non - specific esterase from the necrotic proximal and distal tubules may be considered essential for degradation of cytoplasmic components including mitochondria and organelle complexes (Straus and Oliver, 1955 ; Sellers, 1955 ; Squire *et.al.,* 1962; Pitts, 1963 ; Hamburger *et. al.*, 1968 ; Rhodin, 1954; Novikoff, 1959 ; Miller, 1960, 1962 ; Miller and Palade, 1964 ; Ericsson, 1964 ; Trump and Bulger, 1965 as cited by Dingle and Fell, 1969). The reduction in the esterase activity from the renal tubules may probably be due to the

excretion of estrases through urine and its passing down the concentration gradient to the extracellular spaces and blood (Merill *et. al.,* 1956). The appearance of esterase in glomeruli may be required for unclogging of basement membrane as suggested by Farquhar . . . (1961) and Farquhar & Palade (1962). Besides, the lysosomal esterase activity from glomeruli be considered essential for the degradation of plasma proteins which are passing through glomerular basement membrane (Dixon *et. al.,* 1950; Michael *et. al.,* 1977). The appearance of membrane esterase in Bowman's wall could be for degradation of exudents from the Bowman's space or for the degradation of membrane proteins released during membrane disruptions. Similarly, the membrane esterase from the collecting tubules be considered essential for degradation of tissue debris in the lumina or for the degradation of exudents in the interstitium or intercellular spaces.

Thus, the histochemical studies of AP, AIP, and nonspecific esterase reveal that the degradation of necrotic contents of the collecting tubules is entrusted to the alkaline phosphatase and to a little extent to other two enzymes as and when they appear either in the cytoplasm or membranes.

BIOCHEMICAL STUDIES :

I) ACID PHOSPHATASE :

The acid phosphatase activity increases in association with the increase in the concentrations of mining effluents at the end of six hours except for 10.0% M.E. The 0.01 and 0.1% M.E. induce progressive increase in the acid phosphatase activity as the exposure period increases but 1.0, 5.0, 10.0, 50.0% M.E. induce reduction in the acid phosphatase activity at the end of a few time intervals, though otherwise these M.E. concentrations induce progressive increase in the acid phosphatase activity in association with the

increase in the degree of necrosis. Particularly at the end of one hundred and twenty hours, the 0.1, 1.0, 5.0, 10.0 and 50.0 percent M.E. induce increase in the acid phosphatase activity beyond the level present at the end of six hours. The 100.0% M.E. induces maximum increase in the acid phosphatase activity at the end of six hours and the activity keeps on decreasing from the end of seventy two hours to the end of one hundred and twenty hours. The rise and falls in the acid phosphatase activity appear to be correlated with the progress and state of necrotic activity of the kidney.

Slater and Greenbaum (1963) reported that the increase in acid phosphatase (AP) activity of the kidney is propotional to the degree of necrosis and the AP activity does not increase before the onset of renal injury. Therefore, in the present investigation the AP activity does not increase under the influence of 0.01% M.E. up to the end of twentynine days, as, till this time, there is no onset of renal injury but in the later periods the acid phosphatase activity increase in proportion with the progress of necrosis. Under the influence of other concentrations of M.E. the AP activity increases in proportion with the degree of necrosis induced by M.E. for a specific exposure period.

The other researchers have also reported this enzyme in necrosis in other tissues. Becker and Barron (1961) have reported an increase in AP activity in the injured neurons. Gould and Holt (1961), Kawai (1963), Holtzman and Novikoff (1965) and Friede (1966) have reported increase in the AP activity in the axons undergoing changes subsequent to injury. Dianzani (1963) have reported an increase in AP activity subsequent to the liver injury : The relationship between the elevation of AP activity and necrosis is well established and a vast literature is available on this aspect. (Lysosomes in Biology and Pathology Vol. 1 to 5 edited by Dingle and Fell Dean. Vol I - 1969, Vol II - 69, Vol III - 1973, Vol. IV - 1975 and Vol. V - 1976).

In the present investigation the enhancement of AP activity under the influence of : 0.1% M.E. from the end of six to the end of one hundred and

twenty hours ; 1.0% M.E. for six, twentyfour, seventy two and one hundred and twenty hours ; 5.0% M.E. from six, twenty four, fortyeight, ninetysix and one twenty hours ; 10.0% M.E. at the end of six, twelve, twenty four, fortyeight, ninetysix and one twenty hours ; 50% M.E. at the end of six, twelve, nientysix and 100% M.E. at the end of six hours, follow the degree of necrosis. By the time the kidney exhibits maximum tubular necrosis, the AP activity increases far above the control level and above the level observed at the end of six hours (except for 100% M.E.) as the exposure period of one twenty hours approaches. The histological studies have revealed the progression of necrosis and involvement of more tubules in necrosis as the exposure period increases. These observations indicate the relationship between the progress of necrosis and progressive increase of AP activity. The AP activity increases in renal nephritis to destroy or to break down the damaged cell constituents (Sztriha et. al., 1975). Dingle and Fell (1969) have reported the scavanging role of AP in liver necrosis. Kobayashi *et. al.,* (1971) have reported high level of AP activity in diseased tissue. The elevation of AP activity in the damaged portion of the kidney is an important factor in the metabolism of necrotic kidney. The same may be true in the present case as the rise in the acid phosphatase runs parallel to the rise in the necrosis of the kidney.

It may possibly be that during necrosis some of the lysosomes get lost or the lysosomal enzymes from the cytoplasm may get passed on to the lumen along with the cytoplasmic debris. Histochemical studies support this as the luminal debris show the lysosomal staining. Hence, this may be the reason of decrease in the AP activity at a few time intervals. In the rat proximal tubules cytosomes and cytosegresomes are found and both these bodies are responsible for high specific AP activity, at least in part (Straus, 1956). The renal damage may probably be responsible for the loss of such bodies which may atleast partly be related to the decrease in AP activity, especially for very large M.E. concentrations which cause acute necrosis. This may also happen

for the M.E. concentrations above 1.0% M.E. when they induce acute necrosis at a few time intervals, may be in a few tubules. But it needs further investigation to throw adequate light on this aspect. The higher renal clearance of AP also supports the above suggestion. Merill *et. al.,* (1956) put up a hypothesis of passing down of enzymes against the concentration gradient into the extracellular space and blood. In the present studies the reduction in the AP activity may be due to such a phenomeon as suggested by Merill *et. al.,* (1956). In the present investigation the serum shows an increase in AP activity and this increase can be correlated to the decrease in the kidney AP up to certain extent. Thus, the decrease in AP activity in the kidney may be due to its renal clearance and passing down in to the serum as well as into the extracellular spaces. But these correlations are by no means constant as it depends upon the degree of necrosis, passing of the enzyme down the concentration gradient into the extracellular space and blood. The intermittent fluctuations in the enzyme activity can be attributed to the lysosomes which become very active after the cytoplasmic exudation which take with them some of the lysosomes to the debris.

II) ALKALINE PHOSPHATASE :

The alkaline phosphatase (AIP) activity increases in association with the increase in the concentrations of mining effluents (from 0.1 to 50%) at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninety six and one hundred and twenty hours barring a few time intervals/instances. The alkaline phosphatase activity fluctuates for some time intervals but remains above that obtained for the control. The fluctuations in the AIP activity correlate partly with the degree of necrosis, number of renal tubules involved in necrosis and partly with the renal clearance of AIP as well as its passing down the concentration gradient in to the extracellular space and blood, (Merill *et. al.,* 1956). But a perfect stable correlation in the fluctuations of AIP activity with the

aforementioned factors cannot be obtained. There is some correlation between the degree of necrosis and the enhancement of the AIP activity. The higher concentrations of M.E. induce acute necrosis and hence under such conditions enhancement of AIP activity is obtained. The AIP activity does not increase before the onset of injury. Therefore, these observations are in agreement with the observations of Slater and Greenbaum (1963) for acid phosphatase. Here, one must remember that like AP, AIP is also a lysosomal enzyme. Under the influence of 0.01% M.E. the AIP activity does not increase upto the end of twentynine days as the necrosis is not set on. But once the necrosis sets on, the AIP activity increases gradually. The other concentrations of M.E. also do not induce AIP enhancement up to the beginning of six hours period as the necrosis does not begin. The enhancement in AIP activity in proportion to the degree of necrosis may be justifiable as such a correlation has been obtained by many researchers with various necrotic tissues for AP activity (Becker and Barron, 1961 ; Gould and Holt, 1961 ; Kawai, 1963 ; Holtzman and Novikoff, 1965 ; Friede, 1966 ; Dianzani, 1963 and Dingle and Fell, 1969).

III) NON SPECIFIC ESTERASE :

The renal nonspecific esterase activity does not increase before the onset of necrosis but soon after the begining of necrosis, a slight increase in the esterase activity is seen and when the necrosis progresses, then only significant increase in the esterase activity is seen. Almost all the concentrations of M.E. except 5.0% induce significant increase in the esterase activity at the end of six hours. Histopathological studies have revealed that as the exposure period increases, the necrosis progresses and more number of tubules are involved in the necrosis. In the present studies also it is observed that, as the necrosis progresses and more number of tubules are becoming necrotic, the esterase activity increases up to certain extent, especially at the end of one twenty hours when more tubules are necrotic, the enhancement in

esterase activity over that level seen at six hours, is obtained. The fluctuations in the enzyme activity may probably be due to the passing of esterases down the concentration gradient into the extracellular space and blood (Merill *et. al.,* 1956) and partly may be due to the renal clearance of the enzyme through urine as well as due to the passing of cytosomes and cytosegresomes in to the debris (Straus, 1956). The elevation of serum esterase activity supports Merill *et. al.,* (1956) hypothesis and the elevation of esterase activity in proportion to the increase in necrosis can be considered as in agreement with the observation of some researches for AP in kidney and other tissues (Sztriha *et. al.,* 1975 ; Dingle and Fell, 1969 ; Kobayashi *et. al.,* 1971 ; Becker and Barron, 1961 ; Gould & Holt, 1961 ; Kawai, 1963 ; Holtzman & Novikoff, 1965 ; and Friede, 1966), as esterase is also lysosomal enzyme.

IV) PROTEINS :

The present investigation shows that the mining effluents (M.E.) reduce the kidney protein levels. Under the influence of all the M.E. concentrations the protein concentrations decrease and the decrease in the protein level appears to be dependent upon the degree of necrosis which is dependent upon the M.E. concentration as well as exposure periods. For very low concentrations of M.E. there are no changes in protein levels which indicates that there is no renal damage. The histopathological observations also show that the very low concentrations of M.E. do not induce renal necrosis unless the animals are exposed to M.E. for a longer period. In the present investigation the non alterations of the amount of proteins in the kidney up to the end of twenty nine days under the influence of 0.01% M.E. clearly shows that there is no renal damage. But after thirty days exposure the kidney shows reduction in the amount of proteins indicating the onset of necrosis.

Therefore, the reductions in the amount of proteins may be due to necrosis. Under the influence of larger M.E. concentrations, the kidney tubules

undergo acute necrosis and the tubules lose their integrity and many tubule cells are damaged and sloughed off. Their cytoplasmic constituents get broken down by the action of lysosomal enzymes. Besides these, cytoplasmic debris is exuded in the lumina or interstitial spaces and the proteins pass down the urine and also escape into the circulation. The increase in serum protein levels and urine protein levels substantiate this. Thus, the necrosis of renal tubules, loss of proteins through urine and its passing down the concentration gradient in to the extracellular spaces as well as blood may be responsible for the reduction of protein levels.

The recovery in protein levels at a few time intervals may be due to the attempts of the kidney to regenerate the lost cells/tissue. Such incidents of recovery of protein levels appear to occur under the influence of low M.E. concentrations especially for longer exposure periods but such recovery is induced only for 0.1, 1.0 & 5.0% M.E. concentrations at one or two time intervals. Probably at these time intervals the protein synthesis might be stepped up but it needs further investigation to throw more light on this aspect.

V) UREA :

The studies on the kidney urea levels show that the mining effluents profoundly influence the urea levels and very significantly increase the urea concentrations. Mining effluents to a large extent, induce exposure period and concentration dependent rise in the kidney urea concentrations and in a few occasions the urea levels decline slightly but the urea never comes to the control level. These observations suggest that the M.E. promote enhancement of urea synthesis though the birds are basically uricotelic. Under the influence of M.E. the kidney undergoes necrosis and the lysosomal enzymes may be promoting degradation of proteins. These excess of proteins or their degraded products vis-a-vis the amino acid enter the circulations as evidenced by the rises in serum protein levels, and these may be taken to the liver for

deamination and transamination. During such processes, more ammonia may be getting produced which eventually may be detoxified by its conversion into urea and this excess urea may be passed on to the kidney for elimination. This hypothesis is supported by the obervations of urea elevations in the serum, kidney and urine. But it needs further investigation to throw more light in it. It may be of great interest to know how exactly the protein metabolism is stepped up It may be of interest to find out if M.E. promote rise in the Argenine levels and if the enzymes involved in the ornithine cycle escalate their activities. The slight decline in the urea level at a few time intervals may be due to the passing of urea into the urine and blood.

VI) URIC ACID :

The studies of the kidney uric acid levels indicate that very low concentrations of M.E. (0.01%) induce elevation of uric acid concentration if the animals are exposed to M.E. for a very long period. Similarly, 0.1 and 1.0% M.E. promote successive rise in uric acid concentrations from the end of fortyeight hours to the end of one twenty hours, while the 0.1, 1.0, 5.0 and 10.0% M.E. concentrations induce reduction (except at the end of twelve hours for 1.0%) in the uric acid level up to end of twenty four hours. The higher concentrations of M.E. induce elevations of uric acid levels. Though a perfect relationship between the kidney concentrations and the renal clearance is not established, some correlation is seen. When the kidney concentrations of uric acids are high under the influence of 0.1% M.E. at the end of fortyeight, seventy two and ninety six hours, the urine concentrations of uric acid are low. Similar situation is observed for 10.0% M.E. at the end of fortyeight and one twenty hours. But the lower concentrations of kidney uric acid and high renal clearance of uric acid are induced by : 0.1% and 1.0% M.E. at the end of six, twelve, twentyfour hours ; 5.0% M.E. at the end of all the time intervals under investigation ; 10.0% M.E. at the end of six, twelve, twentyfour and ninetysix

hours ; 50.0% M.E. at the end of twelve hours. Interestingly, another type of correlation between kidney uric acid level and renal clearance (high kidney concentration and high renal clearance) is found under the influence of : 0.01% M.E. at the end of thirty and fortyfive days ; 0.1% M.E. at the end of one twenty hours ; 1.0% M.E. at the end of fortyeight, seventytwo, ninetysix and one twenty hours ; 10.0% M.E. at the end of seventytwo hours ; 50.0% M.E. at the end of six, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours and by 100% M.E. at the end of all the time intervals under investigation.

Therefore, the reduction in the kidney uric acid level could be partly due to the necrosis of the kidney leading to the exudations of cytoplasmic debris and passing out of uric acid through urine. The birds' kidneys are known to produce nearly twice as much uric acid as the liver (Chou, 1972) and hence, reduction of uric acid synthesis under necrotic conditions may partly be responsible for the decline in uric acid level. The increase in the uric acid concentrations may be due to the increased purine metabolism leading to uric acid synthesis by the kidney under the influence of mining effluents. Similarly, the liver uric acid synthesis may be increased under the influence of M.E. and the synthesised uric acid may be passed on to the renal tubules through blood for its elimination. This may partly elevate the kidney uric acid concentrations. But this needs further investigation to throw more light on this aspect.

VII) CREATININE :

The present investigation indicates that the mining effluents (M.E.) of all the concentrations tested induce increase in the creatinine concentrations of the kidney at all the time intervals under investigation (except 1.0% M.E. at the end of twentyfour and seventytwo hours). The relatively low concentrations of M.E. induce elevation of creatinine amounts when birds are exposed to them for longer periods while higher concentrations can induce sharp rise in creatinine as early as completion of six hours. The M.E. promote fluctuations in

the creatinine levels between the end of six hours and one hundred and twenty hours. In general the creatinine level appears to be high.

Creatinine represents the waste products of creatine metabolism and it arises in the body from the spontaneous break down of creatine phosphate. It serves practically no apparent function in the body. The creatinine level increases due to excessive tissue destruction releasing creatinine or due to failure of creatine being properly phosphorylated (Chatterjee, 1972).

In the present studies the elevation of creatinine in the renal tissue may be due to the tissue destruction promoted by mining effluents. Besides the destruction of renal tissue, the M.E. may be promoting destructions of other tissues in the body as well as may be interferring in the process of phosphorylation of creatine in the muscles which may step up the conversion of creatine to creatinine and this excess of creatinine may be passed on to the kidney for elimination. This may also promote the elevation of creatinine in the kidney up to certain extent. The fluctuations in the kidney creatinine may be related to the fluctuations in the destructions of renal tissues as well as other tissues promoted by the mining effluents. The elevation of kidney tissue creatinine in proportion to the renal necrosis substantiates Chatterjee's (1972) view of relations between the tissue damage and creatinine level. The acute reduction in creatinine level can be interpreted on the basis of its excessive elimination through urine or its passing down the concentration gradient into the extracellular space or blood as suggested by Merill *et. al.*, (1956).

VIII) CALCIUM :

The M.E. of different concentrations induce concentration dependent elevation in the calcium levels of the kidney at the time intervals of six, twelve, and twenty four hours but for the subsequent periods such a relationship is not obtained. Only 0.1% M.E. induce reduction in the renal calcium level at the end of twentyfour hours while, rest of the M.E. concentrations promote increase

in calcium level above that present in the control animals. The mining effluents induce fluctuations in the calcium levels. The rise in the renal calcium level may be due to the necrosis which cause damage to several cytoplasmic organelle thereby releasing the calcium bound to the organelle and proteins. Besides this, the M.E. may be inducing damage to other organs of the animal, thereby releasing more calcium in the blood. This extracalcium may be passed on to the urine for excretion and may lead to the elevation and fluctuations of calcium. In addition if M.E. is inducing release of parathyroid hormone (PTH) then, under the influence of PTH the distal tubular reabsorption of calcium may be promoted which may increase the kidney retention of calcium.

Although the determinants of ionized calcium concentrations on the cytoplasm of cells are largely unknown, the cytosolic, ionized calcium concentration is known to change with changes in membrane calcium fluxes. In some mammalian cells, PTH has been shown to increase cellular calcium (Howard, 1989). In similar studies calcitonin is shown to induce renal cellular calcium content.

Therefore the elevations and fluctuations in the renal calcium contents may also be due to the changes in membrane calcium fluxes, PTH influence, as well as due to calcitonin. It is difficult to say which of the mechanism is contributing more to the elevation of calcium. At present it is safe to propose that the elevation in renal calcium contents may be partly due to necrosis causing release of bound calcium, partly by increased mobilization of calcium, by passing of calcium by blood to kidney by the action of PTH and calcitonin and partly due to the changes in membrane calcium fluxes. The M.E. may also influence another regulatory route of β - adrenergic which may activate phospholipase C and inositol triphosphate or the IP_3 system which increases intracellular calcium. Therefore, this may lead to accumulation of Ca^{++}. Depending upon the increase or decrease of the operation of any one

mechanism, the renal calcium contents may fluctuate and acute decline in a few of these mechanisms may reduce the renal calcium contents.

IX) CHLORIDE :

Under the influence of 0.1 and 1.0% M.E. the renal chloride contents reduce sharply while the higher concentrations of M.E. induce increase in the renal chloride contents. The 100% M.E. induce highest increase in the renal chloride contents. The renal chloride contents undergo some fluctuations as the exposure period increases but a perfect correlation between the M.E. concentration/exposure period and renal chloride contents is not seen. In general the chloride contents of the kidney increase under the influence of mining effluents barring few exceptions.

Several lines of evidence strongly suggest that Na^+ and Cl^- are cotransported across the luminal membranes by a carrier (Ericson and Spring, 1982 ; Frizzell *et. al.,* 1979). The coupled carriers are capable of producing uphill transport of one partner and the other partner moves down hill through a large enough gradient (i.e. secondary active transport). The coupled carrier scheme suggests that Cl^- can be transported uphill into the cell. In the present investigation the M.E. may be promoting the enhancement of these coupled carriers which may then elevate the renal Cl^- contents. Probably under the influence of M.E. this coupled carrier system is activated which may lead to more transport of chloride ions. Now it is known that the second messengers in the sequence of regulatory events within the cell include cAMP and calcium ions. Cyclic AMP may activate a protein kinase that phosphorylates and opens closed chloride channels, thereby increasing the chloride transport. Therefore, mining effluents may be activating cAMP synthesis and activating protein kinases to promote phosphorylation and opening of chloride channels. The M.E. may also influence another regulatory route of β - adrenergic which may activate phospholipase C and inositol triphosphate, or the IP_3 system, which

increases intracellular calcium. A rise in intracellular calcium has been shown to open Cl^- and K^+ channels (Sterling, 1989). In the present investigation the kidney is shown to retain more calcium under the influence of mining effluents and this elevated calcium may open the chloride channels as suggested by Sterling (1989) leading to the elevation of chloride contents of the kidney. But it needs further investigation to throw more light on this aspect. The chloride contents may fluctuate in association with the rise and decline in the chloride transport mediated either by coupled carrier system or by cAMP activation of protein kinase or by raising intracellular calcium levels. In the present context as the calcium level is seen elevated in the kidney the calcium mediated transport of chlorides and its accumulation seems more logical. The reduction in kidney Cl^- contents may be due to its passing down the concentration gradient into blood and its passing down to the urine.

II) SECTION B - URINE ANALYSIS

ENZYMES : I) ACID PHOSPHATASE (AP) :

It is now an established fact that enzymes appear in the urine after the renal insult, leading to the renal injury. Besides, variety of enzymes are eliminated through urine under pathological conditions developed due to renal impairments. In the present investigation the control animals do not excrete any enzyme or protein through urine indicating non existence of any renal damage.

From the present investigation it appears that the mining effluents induce enzyme - urea, a pathological state where enzymes and urea appear in the urine. The various concentrations of M.E. induce concentration dependent rise in the renal clearance of AP at the end of twelve, fortyeight and ninetysix

hours and the 50% M.E. promote the maximum clearance of AP at the end of fortyeight hours. The renal clearances of AP elevate progressively as the exposure periods increase, but at few times the AP activity gets reduced.

Normally, urine does not show presence of acid phosphatase. Only in pathological urines the lysosomal enzymes are known to occur. The variations in the renal clearances of acid phosphatase are dependent upon the degree of necrosis but a perfect correlation between the necrotic state and renal clearance is not evident. The lysosomes from the cytoplasm may come into the lumina along with the cytoplasmic exudents and then may pass into the urine. Thus, the acid phosphatase comes into the urine from the injured/damaged renal tubules. But this may not be the only source. The mining effluents are likely to induce injury to the alimentary tract, especially to the stomach intestine and also to the liver or muscles depending upon the depositions of heavy metals of the M.E. origin into them. The acid phosphatase of these tissue origin is likely to be passed on to the blood during circulation thereby elevating the blood AP. The animal body may tend to dispose off this excess AP which might promote serum protein degradation, through urine. Therefore, the urinary AP may also be due to the contribution of the injured tissues other than the kidney. Therefore, the elevation and the decrement of acid phosphatase from the serum and the injured organs may be affecting the renal clearance of AP This may be the reason for fluctuations in urinary AP.

II) ALKALINE PHOSPHATASE (AIP) :

All the concentrations of mining effluents induce concentration depended elevation in the renal clearance of AIP at the end of twelve and ninetysix hours, while 0.1 to 50% M.E. promote increase in urinary excretion of AIP in accordance with the rise in the M.E. concentration at the end of ninetysix hours. The renal clearance of AIP increases progressively as the exposure period increases except for a few occasions when the urinary excretion drops.

The renal clearance of AIP appears to be dependent upon the degree of necrosis. Thus, the progressive necrosis may be responsible for progressive renal clearance of AIP under the influence of M.E. During necrosis the tissue debris and the lysosomes are swept in to the tubules' lumina and hence the lysosomal enzymes like AIP are seen in the urine. Besides, the contribution of damaged renal tissue, the serum bearing elevated AIP may be contributing the renal clearance of AIP. The serum AIP elevations may be due to the other tissues undergoing necrotic changes under the influence of mining effluents. The decreases in the renal clearance of AIP may be due to the varying degree of necrosis of tissues which vary the serum AIP levels. The serum tends to eliminate AIP only when it rises above the tolerance level and if the AIP rise is not alarmingly high or less than the threshold level, the amount of AIP passing to the urine would naturally drop. The serum may be eliminating AIP to avoid induction of degenerative changes in the serum proteins under the influence of AIP. In short, the occurrence of AIP in the urine is a natural process under the development of pathological conditions in the animal body and may be considered as a protective mechanism and an indication of tissue damage, especially of a renal tissue damage and the amount of AIP in urine i.e. AIP activity in urine may be considered as a measure of renal pathological state.

III) NONSPECIFIC ESTERASE :

The M.E. induce concentration dependent renal clearance of nonspecific esterase at the end of twelve, twentyfour and fortyeight hours and the 100% M.E. promotes highest renal clearance at the end of twelve hours, but in general, all the concentrations of M.E. promote increases in the urinary elimination of nonspecific esterase, though there are at some occasions rise and falls in the renal clearances of esterase.

Like other lysosomal enzymes, the appearances of esterase in the urine can be interpreted on the grounds of necrosis. The degree of necrosis decides

the quantity of esterase to be released in the urine but a perfect correlation in the degree of necrosis in one organ and the renal clearance of esterase is not always obtainable. In the present studies it is evident that the lysosomes from the cytoplasm may be coming to the lumina of the tubules along with the cytoplasmic debris and then may get passed on to the urine. Thus, the esterase may be coming to the urine from the injured nephrons. Besides kidney, the organs like alimentary tract and the liver are likely to get exposed to the mining effluents which may induce necrosis in them depending upon the concentration of M.E and the heavy metals deposited in the organs. Under such necrotic conditions the lysosomal enzyme esterase may be released into the serum and serum esterase may get elevated. This elevation of serum esterase can induce more renal clearance of esterase. The fluctuations or reductions in the renal eliminations of esterase may therefore depend upon the necrotic states of the organs, the elevations of serum esterase and on the passing down of the esterase against the concentration gradient in to the extracellular spaces (Merill *et. al.,* 1956). It is also known that the release of enzymes into the blood and extracellular space correlates with the degree of necrosis or the size of the area of infarction (Metkiewski *et. al.,* 1968, Zelman *et. al.,* 1959). Therefore, the rise or fall in the urine esterase is likely to depend upon the area of renal infraction, the level of serum esterase and the amount of esterase being passed to the extracellular space.

IV) PROTEINS :

The mining effluents appear to induce protein-urea into the ducks as indicated by the occurrence of protein into the urine. It appears that the occurrence of proteins in the urine does not perfectly depend upon the concentrations of M.E. as well as upon the exposure period. The urinary elimination of proteins fluctuate considerably under the influence of mining effluents. Normally the proteins are not excreted through urine and the ducks

do not excrete proteins through the urine as observed in the controls. The low concentrations of M.E. induce relatively higher elimination of proteins at the end of six hours, but when the necrosis extends to many tubules at the end of one twenty hours, they induce pronounced elimination of proteins, but the higher concentrations tend to eliminate more proteins in the early exposure periods as they promote acute necrosis very early and at the later hours, comparatively less proteins are eliminated.

Palmela *et. al.*, (1966) have shown that the kidney is highly unselective in the excretion of protein molecules in acute ischemic renal failure. Karlwilhelm *et. al.*, (1977) have shown that protein-urea exists in nephrotoxic nephritis. Beregi *et. al.*, (1962) have shown that 5-HT induce proteinemia and have shown proteins in Bowman's capsule and basement membrane. Skjorten and Fredrik (1964) have reported fibrin precipitation in glomeruli in cases of bilateral renal necrosis. Mandel and Popper (1971) reported protein-urea in ischemic necrosis. Maiorca *et. al.*, (1967) reported protein-urea with nephrotic syndrome and other chronic kidney diseases with or without uremia. Kashgurian and Hayslett (1977) have mentioned that tubular disorders induce protein-urea.

The protein-urea induction may be due to the failure to reabsorb protein, normally filtered by glomeruli, possibly complicated by a leak of plasma proteins directly into the tubular lumen and failure of their reabsorption (Palmela *et. al.*, 1966). In the present investigation the cytoplasmic debris is found in the lumina of tubules and the tubules also bear damaged brush and luminal border. Under such conditions the tubules would fail to reabsorb the proteins from the debris, as a result the proteins may enter into the urine. The quantity of protein occurrence in the urine may then fluctuate with the degree of necrosis and partial reabsorption of proteins by some tubules. Further Carone *et. al.*, (1979) have observed that mammalian renal tubules play an important role in the metabolism of proteins and peptides. Only the proximal tubules

possess the mechanism of degradation or transport of these substances and reabsorption of metabolic products. Since these tubules undergo necrosis under the influence of M.E., such protein metabolism may be getting disturbed resulting in the excretion of proteins through urine. Besides, the M.E. induced variations in the urine outputs which may partly influence the renal elimination of proteins. The fluctuations in the renal clearances of proteins may also be due to the variations in the serum protein levels, variations in the degree of necrosis of other organs like alimentary canal and liver and also due to the variations in absorptions of the proteins by the intact tubules which have escaped damage.

Prasad Rao *et. al.,* (1989) have also reported changes in the kidney proteins but unfortunately there is no single report on the elimination of proteins through urine by birds under the influence of heavy metals or mining effluents. Desai (1983) has reported renal clearance of proteins by fowls under the conditions of renal necrosis. Thus, the appearance of proteins in the urine may be considered due to the renal impairment or renal necrosis by the M.E.

V) UREA :

The renal clearance of urea is profoundly influenced by the mining effluents and the renal clearance of urea shows M.E. concentration dependency at the end of twelve hours and such a relationship is not introduced by M.E. at any other exposure period under study. But interestingly the mining effluents (M.E.) induce a pathological condition called as polyurea at all the time intervals under study. The M.E. induces a progressive polyurea up to certain extent as at some time intervals the renal clearances drop.

About 40% of the filtered urea is absorbed in the proximal convoluted tubule and when ADH levels are high, water is absorbed from the collecting tubules resulting in the rise in urea concentration. Under the influence of M.E. the kidney undergoes necrosis and depending upon the concentration of M.E.

and the exposure period the necrosis progresses. The proximal tubules are seen to get injured. Therefore, the necrotic proximal tubules may not be able to perform the absorption of urea thereby increasing the urea concentrations in the tubular fluid which may be passed to the collecting tubules. The collecting tubules may be attempting the absorption of water and electrolytes under the influence of ADH which may further increase the urea concentration leading to the excessive release of urea in the urine to develop a condition called polyurea. The drops in the renal clearance of urea may be partly due to the improved absorption of urea by the intact proximal tubules and partly due to the passing of urea in to the extracellular space and blood. The alterations in serum urea levels are also likely to influence the renal clearance of urea.

Brulles *et. al.,* (1969) have reported that increase in GFR increases urea clearance. Park *et. al.,* (1969) have shown the reduction in urea excretion in relation to fall in GFR. Niesel *et. al.,* (1970) have shown the inter dependence of urea, NaCl and KCl excretion. In the present study the renal clearance of urea is high though the rate of urine flow and GFR are reduced under the influence of mining effluents. This is in contrast to the observations of Brulles *et. al.,* (1969) and Park *et. al.,* (1969). Therefore, the reasons for the polyurea may be the necrosis of proximal tubules and absorption of more water by the collecting tubules, as suggested earlier.

VIII) URIC ACID :

The mining effluents (M.E.) induce wide fluctuations in the renal clearance of uric acid. The 5.0% M.E. promotes highest renal clearance of uric acid at the end of ninetysix hours while, 10.0% M.E. promotes lowest renal clearance of uric acid at the end of fortyeight hours. Generally, the higher concentrations of M.E. induce increase in the renal clearance of uric acid but in few-instances the uric acid clearances fall below the control levels. The increase in the renal clearance of the uric acid may be due to the increase in

the kidney uric acid concentrations caused by the probable stepping up of the renal uric acid synthesis. At the same time increase in the synthesis of uric acid by the liver due to increased purine metabolism coupled with rise in serum uric acid level may induce increase in the renal clearance of uric acid under the influence of high M.E. concentration. The fluctuations in the uric acid synthesis and the uric acid levels of the kidney may promote fluctuations in the renal clearance of uric acid.

VI) CREATININE :

The mining effluents induce increase in the renal clearance of creatinine. M.E. induces concentration dependent enhancement in the renal clearance of creatinine at the end of six and twelve hours, but, generally the M.E. concentration induce increase in the renal clearance of creatinine as the exposure period increases but a perfect correlation is not obtained. M.E. induces wide fluctuations in the renal clearances of creatinine.

The enhancement of renal clearance of creatinine may be due to the increase in the renal creatinine level and also due to the increased serum creatinine level. The fluctuations in the renal creatinine clearances may depend upon the fluctuations in the serum creatinine level as well as kidney creatinine levels. Indirectly the renal creatinine level would also depend upon the alterations in the degree of muscular dystrophy brought about by the M.E. and also on the failures of phosphorylations of creatine. As the creatinine represents the waste products of creatine metabolism, the enhancement of creatine metabolism may elevate the renal clearance of creatinine and the fluctuations in creatine metabolism may fluctuate the renal clearance. But it needs further investigation to find out how M.E. induces alterations in the creatine metabolism. But it is clear that M.E. are inducing creatinuria in the ducks. The induction of creatinuria is a pathological symptom, indicating the animals are under stress. Rennick (1967) has shown that creatinine is

secreted by the renal tubules and some of it is absorbed by the tubules. But the damaged tubules would fail to reabsorb the creatinine. As a result the creatinine clearance in urine may significantly increase. Therefore, the renal clearance may be stepped up.

VII) CALCIUM :

The 100% M.E. induces highest renal clearance of calcium at the end of twenty four hours while relatively low but higher than control level, renal clearance is induced by 1.0% M.E. at the end of six hours. However, all the concentrations of M.E. promote enhanced renal clearance of calcium. The M.E. induce wide fluctuations in the renal clearance of calcium.

The enhancement in the renal clearance of calcium may be due to the renal necrosis. The M.E. induces renal necrosis and the degree of necrosis partly depends upon the concentration of M.E. and the exposure period. During necrosis the tissue is damaged, the cytoplasmic debris is exuded in the tubules' lumina and the ultra structural studies have revealed the breakdown of mitochondria, proliferations of Golgi bodies and E.R. into the tubules' lumina. Therefore, the calcium present in the cytoplasmic debris, calcium bound to mitochondria and other organelles may be swept into the tubules from where it may be passed down to the urine thereby increasing the urine calcium level.

Besides this the elevations of serum calcium levels may also induce increase in the renal clearance of calcium. The fluctuations in the renal clearance of calcium may be due to the fluctuations in the serum calcium levels and changes in the degree of necrosis as well as due to the variations in the calcium absorptions by the intact renal tubules which have escaped damage.

VIII) CHLORIDES :

The mining effluents (M.E.) appear to induce enhancement of renal clearance of chlorides as well as wide fluctuations in the urinary

concentrations. The higher concentrations of M.E. appear to induce more renal clearances of chlorides though a perfect correlation between the high concentrations of M.E. and elevated renal clearances are difficult to attain. But at the end of ninetysix hours and partially at the end of one twenty hours a nearly perfect correlation is reached. The 100% M.E. induce the highest clearance of chlorides.

The elevated renal clearances of chlorides could be due to the elevated renal concentrations of chlorides and partly also due to the elevations in chloride contents of the serum. The fluctuations in the chloride contents may be due to the fluctuations in the renal and serum chloride levels. Besides this, the variations in the degree of necrosis would also contribute to the elevations and fluctuations in the urinary chloride contents. During necrosis the cytoplasmic debris is swept into the lumina and the chloride contents of the debris may get passed on to the urine thereby increasing the urinary chloride levels. Besides, the intact renal tubules especially collecting tubules may try to reabsorb some of the chlorides and these reabsorptions of chlorides may partly contribute to the fluctuations in renal clearances of chloride. However, the reabsorption of chlorides by the intact collecting tubules & renal apparatus under elevated serum chlorides and renal chloride levels would be very negligible and even may not take place but under the circumstances, drop in chloride levels of renal tissues and serum levels being not so high, the collecting tubules may absorb more chlorides which may partially change the renal clearance of chlorides. But largely the changes in the renal clearances of chlorides would depend upon the degree of necrosis, accumulation of calcium ions which are known to induce chloride accumulations in renal tissues and elevations or depletions of serum chloride levels.

IX) ELECTROLYTES :

A) Na^+

The mining effluents profoundly influence the renal clearance of Na^+ and M.E. induce elevated renal clearance at all the exposure periods under study. The low concentrations of M.E. induce slightly elevated renal clearance at the end of six and twelve hours while the highest renal clearance of Na^+ is promoted by 100% M.E. at the end of seventy two hours. The M.E. also induce wide fluctuations in the renal clearance of Na^+. Carone *et. al.,* (1979) have observed that the renal disease in animals and in man or in renal disorders with comparable or greater reduction in GFR, Na^+ retention does not occur and fractional excretion of Na^+ in urine is increased in proportion to the reduction in GFR. In the present studies the reduction in GFR and reduced urine outputs are observed under the influence of mining effluents. Therefore, reduction in GFR and urine out put under the influence of M.E. may be inducing failures of Na^+ retentions, thereby promoting more renal clearance of Na^+. Besides this, the damage of the proximal and distal tubules may decrease the absorption of Na^+, which may lead to an increase in the Na^+ excretion. Thus, the tubular necrosis may be responsible for high Na^+ clearance (Kashgurian and Hayslett, 1977). Laurence and Friedler (1966) reported that angiotensin induced vasodilation resulted in marked increase in Na^+ excretion and decrease in Na^+ reabsorption. It would be of immense interest to find out if M.E. induce the angiotensin mediated Na^+ excretion. During necrosis the juxta glomerular apparatus is also getting injured, as a result the angiotensin production may be decreasing which possibly may induce increase in the renal clearance of Na^+. In addition to this, the acute increase in the serum Na^+ levels may also contribute to the elevation of the renal clearance of Na^+. The fluctuations in the renal clearances of Na^+ may be due to the fluctuations in the serum Na^+

levels, variations in the degree of necrosis at different time intervals and also due to the variations in the GFR as well as urine output.

B) K^+ :

The mining effluents (M.E.) induce rise in the renal clearance of K^+. The renal clearance of K^+ is concentration dependent at the end of fortyeight hours and the 100% M.E. promote relatively very high renal clearance of K^+. But in general, though the renal clearance of K^+ is higher than the control, the K^+ clearance fluctuates widely under the influence of M.E. Giebisch and Gerhead (1965) reported that K^+ secretion of the distal tubules is influenced by four factors : the extracellular K^+ concentration, the electrical gradient across the luminal tubular membrane, the strength of the active transport process reabsorbing K^+ and the K^+ permeability of the luminal cell membrane. The renal distal tubules undergo necrosis under the influence of mining effluents and the necrotic tubules lose the luminal borders which then may fail to reabsorb the K^+. Therefore, the renal clearance of K^+ may be getting enhanced. Thus, this may be the cause of higher renal clearance of K^+. Higher the degree of necrosis, higher may be the renal clearance of K^+. The fluctuations in the renal K^+ clearance may be due to the variations in the degree of necrosis and the number of tubules involved in the necrosis. Besides this the elevations in the serum K^+ levels may also promote the increase in the K^+ clearance through urine and the rises and falls in the serum K^+ levels may induce alterations in the renal clearances of K^+. Besides this, the decrease in active transport as a result of ATP depletion due to necrosis and damaged mitochondria, would result in excessive renal clearance of K^+.

X) CHANGES IN URINE FLOW AND GFR :

The mining effluents induce alterations in the urine flow and GFR. The different concentrations of M.E. induce reduction in urine flow and these

reductions are more over proportionate to the M.E. concentrations and exposure period. The rate of urine flow partly depends upon the state of dehydration and partly on the arterial pressure / pressure in glomerular capiliaries (Sturkie, 1976). In the present studies, the mining effluents induce damage to the glomeruli, proximal, distal and collecting tubules. The necrosis of glomeruli would induce decrease in gomerular capillary blood pressure which may reduce the rate of urine flow. Similarly, the renal tubular necrosis may develop inability in the tubules to reabsorb water, especially in the collecting tubules, as a result there would be more loss of water and this loss of water and failure to reabsorb water may eventually induce dehydration. Therefore, after necrosis, the rates of urine flows may drop due to the decreased glomerular filtration and dehydration. Besides this, the macula densa also regulate glomerular capillary pressure through afferent arterioles (Sullivan and Grantham, 1982). In the present studies the macula densa also appears to be damaged, hence it may not be able to regulate the gomerular capillary blood pressure. The fluctuations in the urine flow may be due to the variations in the degree of necrosis at different time intervals for various concentrations of mining effluents.

The mining effluents (M.E.) induce reductions in the Glomerular Flitration Rates (GFR) in proportion to the concentrations of M.E. The M.E. also induces fluctuations in the GFR at some time intervals. The decrease in GFR under the influence of M.E. may be due to the decrease in urine flow and dehydration (Sturkie, 1976 ; Skadhauge and Schmidt - Nielson, 1967). GFR reduction in quail has been attributed to a reduction in the number of filtering reptilian nephrons coupled with drop in urine flow (Sturkie, 1976). In the present investigation the M.E. induces necrosis of both the reptilian and mammalian tubules along with the glomeruli. Therefore, the number of intact filtering nephrons of both the reptilian and mammalian types get reduced which may reduce the GFR . Shoemaker (1967) proposed that GFR in bird reduces

with decrease in urine flow. And this could be the reason for drop in GFR of ducks under the influence of M.E. as M.E. reduces urine flow.

Macula densa cells of the juxta glomerular apparatus sense the rate of fluid flow through the adjacent distal tubules and signal the afferent arteriole to constrict (Sullivan and Grantham, 1982). In the present investigation M.E. induces injury to the macula densa of the juxta glomerular apparatus and the damaged macula densa may fail to sense the rate of fluid flow through the adjacent tubules and hence may fail to signal the afferent arteriole. This may result in the alterations in GFR. Therefore some fluctuations in GFR may be due to the variations in the number of glomeruli and macula densa getting involved in the necrosis. Besides this angiotensin may also be responsible for alterations in GFR up to certain extent as angiotensin is known to be associated with GFR (Sturkie, 1976). But it needs further investigation to find out if M.E. alters the angiotensin levels.

RETENTION PATTERNS :

A] KIDNEY :

The mining effluents induce retentions of cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in the kidney. The ultrastructural studies have also supported the retentions of heavy metals. All the metals are retained maximally at specific time intervals and mostly the heavy metal retentions depend upon the M.E. concentrations and the exposure periods. At the end of six hours cadmium, chromium, iron, and magnesium are retained maximally under the influence of M.E. While at the end of twelve hours cadmium, chromium, copper, iron, magnesium, manganese and zinc are retained significantly high. Twenty four hours exposure promotes maximum retentions of cadmium, iron (for 0.1% M.E. only), magnesium and manganese

IV) ESTERASE (NON SPECIFIC) :

The alterations in the serum nonspecific esterase are compiled in **Table No.33** and are graphically expressed in **Graph No. 33.** The non specific esterase activity is expressed as µmoles per hundred millilitre of serum. The control birds exhibited nonspecific esterase activity equivalent to 560.0 ± 4.10 µmoles per hundred millilitre of serum. From the table and the graph it is obvious that the serum nonspecific esterase activity increased in a mining effluent (M.E.) concentration dependent manner for all concentrations except **5% M.E.**, at the end of six hours. Similarly, the M.E. concentration dependent elevation of esterase (non specific) activity was induced at the end of ninetysix and one hundred and twenty hours by the concentrations ranging from 0.1 to 50.0 percent. Besides, the M.E. concentrations like as 0.1 and 5.0 percent induced elevation of nonspecific esterase activity in relation to the exposure period, up to the end of ninetysix hours.

The ducks exposed to **0.01% mining effluents (M.E.)** did not exhibit any significant alteration in the non specific esterase activity up to the end of twenty nine days. But by end of thirty days the serum nonspecific esterase activity increased a little and continued to increase further up to the end of fortyfive days. Thus, at the end of thirty and fortyfive days the nonspecific esterase activity was equivalent to 630.0 ± 2.135 and 750.0 ± 2.607 µmoles per 100 ml respectively.

Under the influence of **0.1% M.E.** the serum nonspecific esterase activity increased significantly at the end of six hours and then exhibited exposure time dependent elevation up to the end of ninety six hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum nonspecific esterase activity was equivalent to 820.0 ± 1.414, 855.0 ± 3.370, 900.0 ± 6.752, 950.0 ± 2.059, 1020.0 ± 6.887, 1100.0 ± 8.795 and 1090.0 ± 3.878 µmoles per 100 ml.

On exposing the ducks to **1.0%** M.E. the nonspecific esterase activity increased sharply at the end of six hours and then continued to increase up to the end of fortyeight hours and then dropped a little at the end of seventytwo hours. Then, once again the esterase (non specific) activity increased at the end of ninetysix hours and continued to increase up to the end of one hundred and twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the non specific esterase activity was equivalent to 950.0 ± 3.49, 980.0 ± 2.60, 1020.0 ± 3.20, 1230.0 ± 10.0, 1050.0 ± 1.85, 1150.0 ± 2.315 and 1190.0 ± 4.019 µmoles per 100 ml respectively.

The **5%** M.E. induced progressive elevation in the esterase activity from the end of six hours to the end of ninetysix hours indicating exposure time dependency of the hike in the enzyme activity. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum non specific esterase activity was equivalent to 940.0 ± 4.534, 960 ± 6.73, 1040 ± 1.414, 1050 ± 4.242, 1170.0 ± 2.449, 1380.0 ± 2.416 and 1280.0 ± 4.147 µmoles per 100 ml respectively.

The ducks treated with **10.0%** M.E. exhibited almost two folds increase in the esterase activity at all the time intervals under study except twentyfour hours. The non specific esterase activity showed progressive increase from the end of fortyeight hours to one hundred and twenty hours. At the end of one hundred and twenty hours the esterase activity was maximum for this concentration of M.E. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the serum esterase (non specific) activity was equivalent to 1190.0 ± 3.20, 1150.0 ± 4.127, 980.0 ± 4.56, 1190.0 ± 3.033, 1330.0 ± 4.534, 1390.0 ± 2.416 and 1470.0 ± 3.929 µmoles per 100 ml respectively.

On exposure to **50.0%** M. E. the ducks showed acute increase in the non specific esterase activity at the end of six hours and then the enzyme activity

steadily decreased up to the end of twentyfour hours. The maximum increase in the esterase activity was observed at the end of one hundred and twenty hours for this M.E. concentration. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the serum esterase (non specific) was equivalent to 1360.0 ± 3.20, 1290.0 ± 4.36, 1190.0 ± 3.162, 1540.0 ± 5.16, 1320.0 ± 4.04, 1640.0 ± 4.069 and 1670.0 ± 1.414 µmoles per 100 ml respectively.

The **100% M.E.** induced the highest increase in the esterase activity at the end of twelve hours and then subsequently the esterase activity went on decreasing up to the end of ninety six hours. By the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one twenty hours the serum non specific esterase activity was equivalent to 1540.0 ± 4.223, 2040.0 ± 3.929, 1720.0 ± 3.611, 1680.0 ± 3.464, 1420.0 ± 2.856, 1070.0 ± 4.223 and 1300.0 ± 3.26 µmoles per 100 ml respectively.

TABLE NO. 33 : ALTERATIONS IN THE SERUM ESTERASE (NON SPECIFIC) ACTIVITY UNDER THE INFLUENCE OF MINING EFLUENTS.

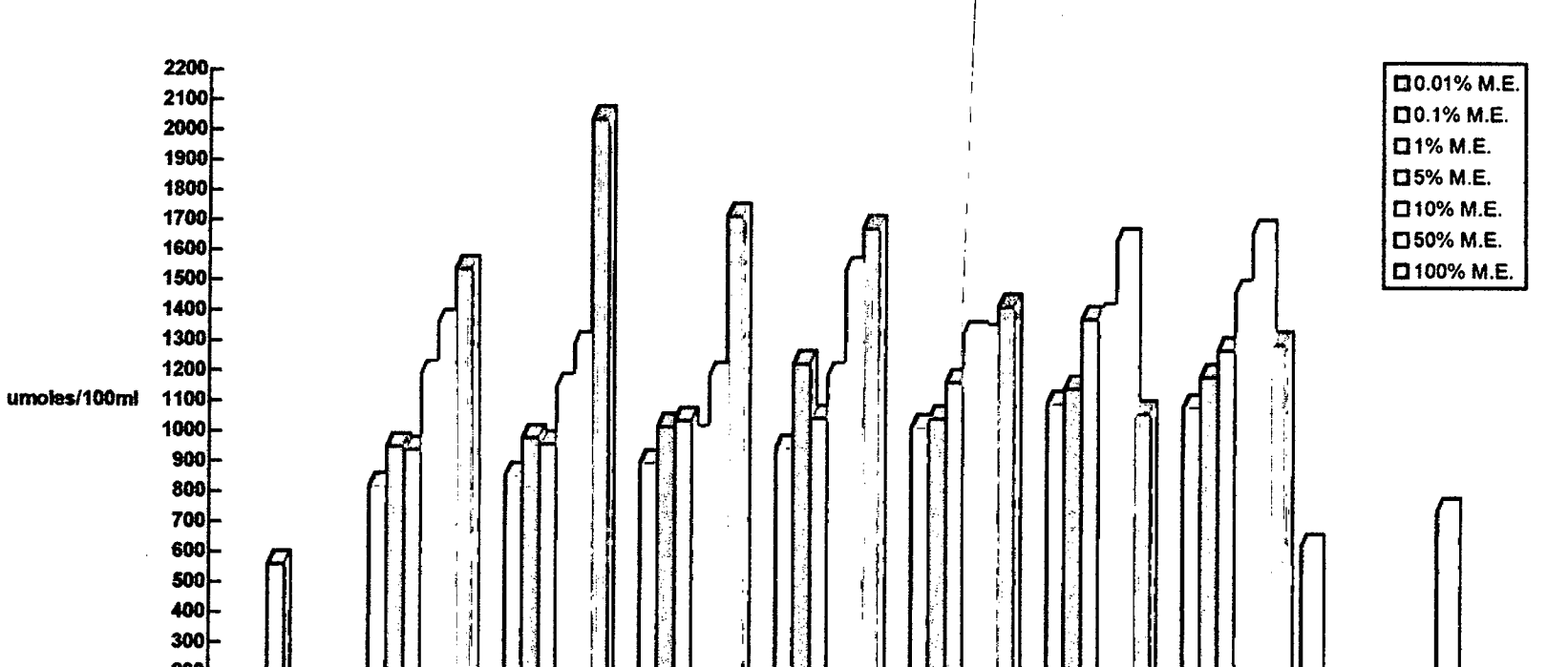
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		560.0 ± 4.10									
0.01%	M.E.		← No significant change →							630 ± 2.135	750 ± 2.607
0.10%	M.E.		820 ± 1.414	855 ± 3.370	900 ± 6.752	950 ± 2.059	1020 ± 6.887	1100 ± 8.795	1090 ± 3.878	N.D.	N.D.
1%	M.E.		950 ± 3.49	980 ± 2.60	1020 ± 3.20	1230 ± 10.0	1050 ± 1.85	1150 ± 2.315	1190 ± 4.019	N.D.	N.D.
5%	M.E.		940 ± 4.534	960 ± 6.73	1040 ± 1.414	1050 ± 4.242	1170 ± 2.449	1380 ± 2.416	1280 ± 4.147	N.D.	N.D.
10%	M.E.		1190 ± 3.20	1150 ± 4.127	980 ± 4.56	1190 ± 3.033	1330 ± 4.534	1390 ± 2.416	1470 ± 3.929	N.D.	N.D.
50%	M.E.		1360 ± 3.20	1290 ± 4.36	1190 ± 3.162	1540 ± 5.16	1320 ± 4.04	1640 ± 4.069	1670 ± 1.414	N.D.	N.D.
100%	M.E.		1540 ± 4.223	2040 ± 3.929	1720 ± 3.611	1680 ± 3.464	1420 ± 2.856	1070 ± 4.223	1300 ± 3.26	N.D.	N.D.

Note : Unit : µmoles/100 ml.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 33 : SERUM - ESTERASE.
Units : µmoles/100ml.



V) ALTERATIONS IN SERUM UREA :

The alterations in the serum urea concentrations are compiled in **Table No.34** and are grahically expressed in **Graph No. 34.** The serum urea concentrations are expressed as milligrams per 100 ml of serum. The control birds showed the urea concentration equivalent to 6.3 ± 1.0 mg per 100 ml. From the table and the graph it appears that all the concentrations of mining effluents (M.E.) induced rise in the serum urea level. At the end of ninety six hours the M.E. concentration dependent rise in the serum urea level was observed. Similarly, the M.E. concentration dependent elevation in serum urea concentration was noticed at the end of six hours except for **50% M.E.** It was observed that the higher concentrations of M.E. induced higher elevation in serum urea at many occasions. It is also seen from the graph that **50% M.E.** promoted exposure period dependent rise in the serum urea level and a similar correlation was caused by **100%** M.E. up to the end of ninetysix hours.

When the ducks were exposed to **0.01% M.E.**, there was no significant change in the serum urea level up to the end of twenty nine days but at the end of thirty days the serum urea level elevated significantly and continued to increase further up to the end of forty five days. Thus, at the end of thirty and forty five days the serum urea concentrations was equivalent to 8.20 ± 0.93 and 11.30 ± 1.27 mg per 100 ml.

The ducks subjected to **0.1%** M.E. showed exposure time dependent increase in the serum urea concentrations up to the end of seventy two hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum urea level was equivalent to 7.0 ± 0.629, 7.50 ± 0.791, 8.90 ± 0.932, 9.50 ± 1.24, 10.0 ± 1.30, 7.90 ± 0.91 and 8.30 ± 0.87 mg per 100 ml respectively.

Under the influence of **1.0%** M.E. the ducks exhibited fluctuations in the serum urea level. The serum urea level fluctuated between 7.90 ± 0.89, and 10.20 ± 1.07 mg per 100 ml. By the end of six, twelve, twenty four, fortyeight,

seventy two,ninetysix and one hundred and twenty hours, the serum urea level was equivalent to 8.90 ± 0.73, 8.50 ± 0.60, 7.90 ± 0.89, 9.0 ± 0.937, 9.30 ± 1.32, 10.20 ± 1.07 and 8.90 ± 1.74 mg per 100 ml respectively.

On exposure to **5.0% M.E.** the serum urea concentrations of the ducks fluctuated and the fluctuations ranged between 9.20 ± 1.60 and 12.20 ± 0.70 mg per 100 ml. The serum urea concentrations were equivalent to 9.20 ± 1.6, 10.30 ± 2.10 , 9.70 ± 1.356, 11.30 ± 1.40, 10.90 ± 1.0, 12.20 ± 0.70 and 11.90 ± 2.0 mg per 100 ml at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

The **10.0% M.E.** induced exposure period dependent rise in serum urea level from the end of six hours to the end of twentyfour hours as well as from the end of seventy two hours to the end of one twenty hours. The serum urea level was equivalent to 9.70 ± 0.90, 10.90 ± 0.70, 11.70 ± 1.80, 11.00 ± 1.10, 12.70 ± 1.60, 13.00 ± 1.01 and 13.70 ± 0.80 mg per 100 ml at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

The **50.0% M.E.** promoted exposure period dependent rise in serum urea concentrations. The acute rise in serum urea level was observed at the end of one twenty hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninety six and one hundred and twenty hours the serum urea level was equivalent to 8.90 ± 1.78, 10.30 ± 1.62, 11.30 ± 1.20, 13.70 ± 0.80, 14.30 ± 2.10, 15.70 ± 1.40 and 15.90 ± 1.10 mg per 100 ml respectively.

The ducks treated with **100% M.E.** showed exposure period dependent elevations in the serum urea level up to the end of ninetysix hours only. The highest inrease in the serum urea level was noticed at the end of nientysix hours only. Thus, by the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the serum urea concentration was equivalent to 10.70 ± 1.16, 11.00 ± 1.01, 12.70 ± 1.62, 13.40 ± 1.35, 14.70 ± 1.70, 16.90 ± 1.85 and 15.40 ± 1.40 mg per 100 ml respectively.

TABLE NO. 34 : ALTERATION IN THE SERUM UREA UNDER THE INFLUENCE OF MINING EFFLUENTS.

			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		6.30 ±1.0									
0.01%	M.E.		← No significant change →							8.20 ±0.93	11.30 ±1.27
0.1%	M.E.		7.0 ±0.629	7.50 ±0.791	8.90 ±0.932	9.50 ±1.24	10.0 ±1.3	7.90 ±0.91	8.30 ±0.87	N.D.	N.D.
1%	M.E.		8.90 ±0.73	8.50 ±0.6	7.90 ±0.89	9.0 ±0.937	9.30 ±1.32	10.20 ±1.07	8.90 ±1.74	N.D.	N.D.
5%	M.E.		9.20 ±1.6	10.30 ±2.1	9.70 ±1.356	11.30 ±1.4	10.90 ±1.0	12.20 ±0.70	11.90 ±2.0	N.D.	N.D.
10%	M.E.		9.70 ±0.9	10.90 ±0.70	11.70 ±1.8	11.0 ±1.1	12.70 ±1.6	13.0 ±1.01	13.70 ±0.8	N.D.	N.D.
50%	M.E.		8.90 ±1.78	10.30 ±1.62	11.30 ±1.2	13.70 ±0.8	14.30 ±2.1	15.70 ±1.4	15.90 ±1.1	N.D.	N.D.
100%	M.E.		10.70 ±1.16	11.0 ±1.01	12.70 ±1.62	13.40 ±1.35	14.70 ±1.7	16.90 ±1.85	15.40 ±1.4	N.D.	N.D.

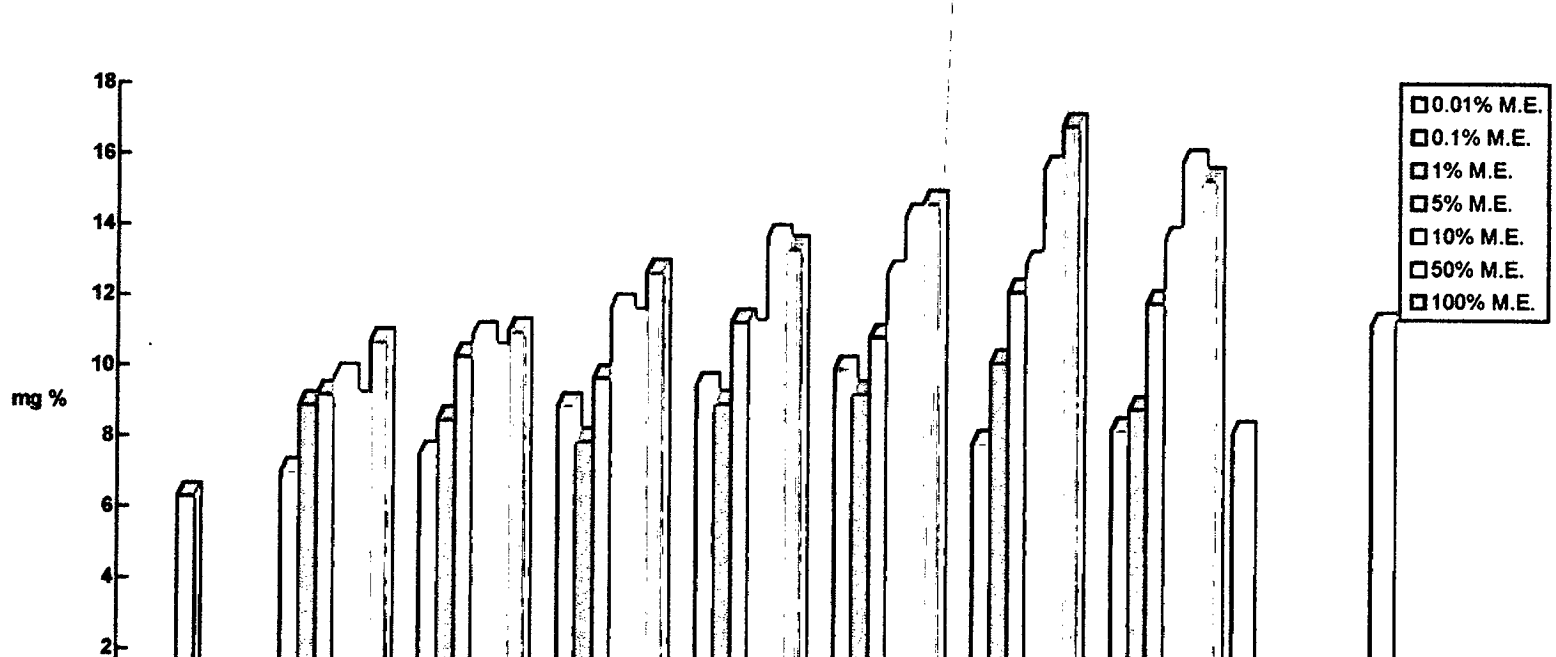
Note : Unit : Urea in mg/dl.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 34 : CHANGES IN THE SERUM UREA LEVEL IN DUCKS UNDER THE INFLUENCE OF MINING EFFLUENTS.

Units : mg %



VI) ALTERATIONS IN URIC ACID :

The alterations in the serum uric acid levels of the ducks under the influence of mining effluents (M.E.) are compiled in **Table No. 35** and are graphically presented in **Graph No. 35.** The serum uric acid levels are expressed as milligrams per hundred millilitre of serum. The control animals showed the serum uric acid concentration equivalent to 9.0± 0.37 mg per 100 ml.

From the graph and the table it is obvious that the mining effluents (M.E.) induced wide and significant fluctuations in the serum uric acid levels. The highest increase in the serum uric acid level was induced by **100% M.E.** at the end of ninety six hours while the highest reduction in the uric acid level was promoted by **5.0%** M.E. at the end of seventy two hours.

When the ducks were exposed to **0.01% M.E** there was no significant change in the serum uric acid concentration up to the end of twentynine days but at the end of thirty days the serum uric acid level reduced significantly and then subsequently increased at the end of forty five days. Thus, the serum uric acid level was equivalent to 6.90 ± 0.80 and 10.90 ± 0.53 mg per 100 ml at the end of thirty and forty five days respectively.

The exposure of ducks to **0.1% M.E.** induced acute reduction in the serum uric acid level at the end of six hours and subsequently the uric acid level steadily increased up to the end of twentyfour hours. But at the end of fortyeight hours the serum uric acid level decreased once again and then showed a steady increase up to the end of one hundred and twenty hours. Thus, at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours the serum uric acid level was equivalent to 3.30 ± 0.24, 5.10 ± 0.39, 5.60 ± 0.43, 3.70 ± 0.11, 4.30 ± 0.17, 4.90 ± 0.25 and 5.50 ± 0.342 mg per 100 ml respectively.

Under the influence of **1.0%** M.E. the ducks exhibited significant fluctuations in the uric acid level and the serum uric acid level fluctuated

between 2.90 ± 0.377 and 8.0 ± 0.271 mg per 100 ml. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum uric acid level was equivalent ot 4.80 ± 0.17, 7.40 ± 0.43, 5.50 ± 0.215, 4.50 ± 0.34, 2.90 ± 0.377, 5.40 ± 0.13, and 8.0 ± 0.271 mg per 100 ml respectively.

The ducks subjected to **5.0% M.E.** exhibited the highest reduction in the uric level at the end of seventy two hours. The serum uric acid level fluctuated between 2.20 ± 0.282 and 5.30 ± 0.13 mg per 100 ml. By the end of six, twelve, twenty four, fortyeight, seventy two, ninetysix and one twenty hours the serum uric acid level was equivalent to 4.0 ± 0.13, 2.80 ± 0.24, 5.30 ± 0.13 4.30 ± 0.37, 2.20 ± 0.282, 4.50 ± 0.24 and 3.00 ± 0.135 mg per 100 ml respectively.

The exposure of ducks to **10.0% M.E.** promoted considerable fluctuations in the serum uric acid level. The serum uric acid level fluctuated between 4.20 ± 0.30 and 10.10 ± 0.17 mg per 100 ml. The serum uric acid level was equivalent to 4.20 ± 0.30, 4.80 ± 0.53, 5.40 ± 0.303, 4.50 ± 0.146, 10.10 ± 0.17, 5.80 ± 0.44 and 6.50 ± 0.70 mg per 100 ml at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours respectively.

The **50.0% M.E.** induced reduction in the serum uric acid level at the end of six hours and subsequently the serum uric acid level increased steadily up to the end of seventy two hours. At the end of twenty hours the serum uric acid level elevated sharply. Thus, the serum uric acid concentration was equivalent to 5.90 ± 0.80, 7.30 ± 0.426, 8.20 ± 1.78, 9.10 ± 1.85, 10. 172 ± 1.41, 8.70 ± 1.01 and 12.732 ± 2.0 mg per 100 ml at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

On exposing the ducks to **100.0% M.E.**, the serum uric acid level fluctuated between 8.40 ± 0.462 and 14.60 ± 1.32 mg per 100 ml. And the **100% M.E.** promoted the highest increase in serum uric acid level at the end of ninety six hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo,

ninetysix and one twenty hours, the serum uric acid level was equivalent to 9.70 ± 0.271, 8.40 ± 0.462, 10.70 ± 1.56, 13.20 ± 1.16, 12.60 ± 1.62, 14.60 ± 1.32 and 9.80 ± 1.01 mg per 100 ml respectively.

TABLE NO. 35 : ALTERATIONS IN THE SERUM URIC ACID LEVEL UNDER THE INFLUENCE OF MINING EFFLUENTS.

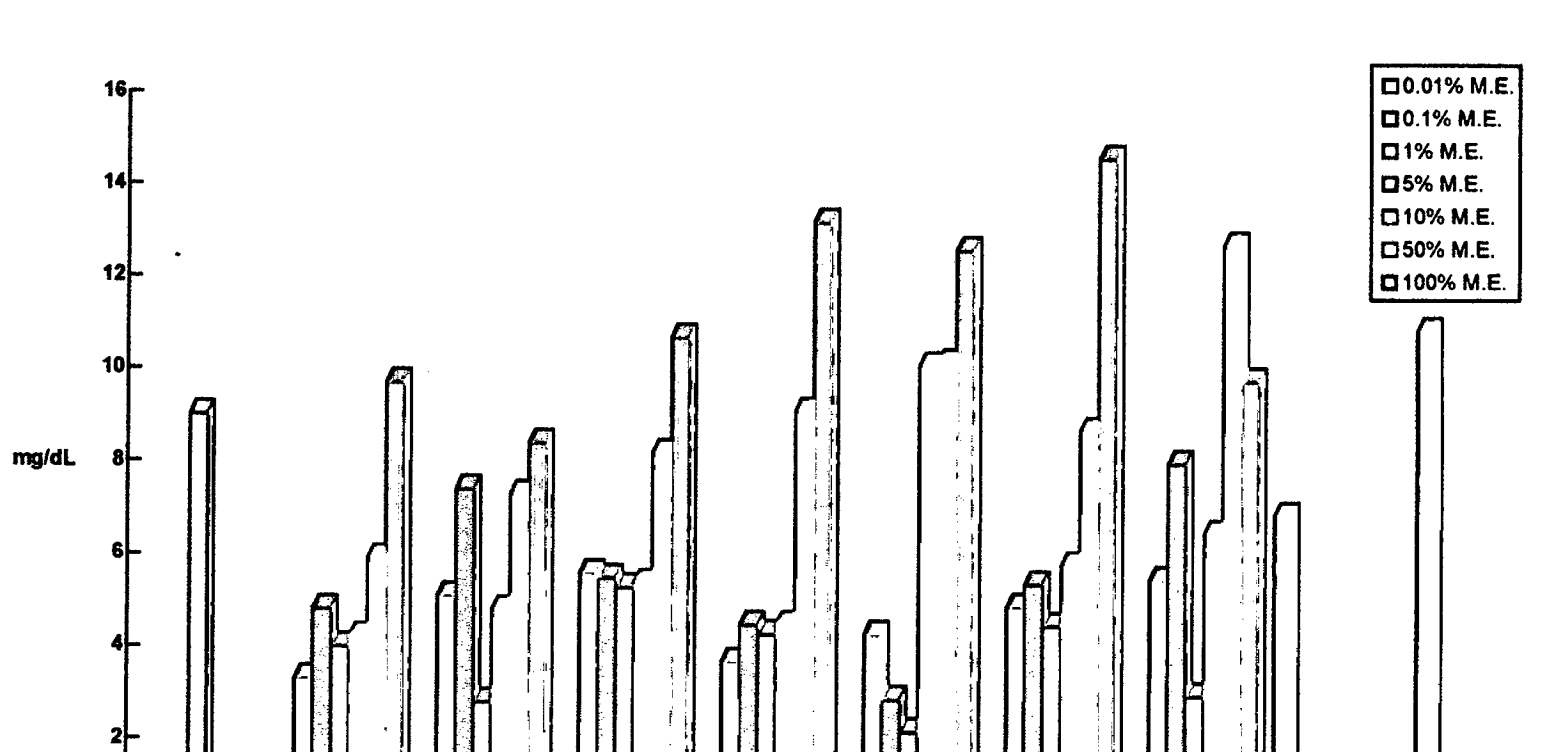
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		9.0 ±0.37									
0.01%	M.E.		← No significant change →							6.9 ±0.80	10.9 ±0.53
0.1%	M.E.		3.30 ±0.24	5.10 ±0.39	5.60 ±0.43	3.70 ±0.11	4.30 ±0.17	4.90 ±0.25	5.50 ±0.342	N.D.	N.D.
1%	M.E.		4.80 ±0.17	7.40 ±0.43	5.50 ±0.215	4.50 ±0.34	2.90 ±0.377	5.40 ±0.13	8.0 ±0.271	N.D.	N.D.
5%	M.E.		4.0 ±0.13	2.80 ±0.24	5.30 ±0.13	4.30 ±0.37	2.20 ±0.282	4.50 ±0.24	3.0 ±0.135	N.D.	N.D.
10%	M.E.		4.20 ±0.3	4.80 ±0.53	5.40 ±0.303	4.50 ±0.146	10.10 ±0.17	5.80 ±0.44	6.50 ±0.70	N.D.	N.D.
50%	M.E.		5.90 ±0.8	7.30 ±0.426	8.20 ±1.78	9.10 ±1.85	10.172 ±1.41	8.70 ±1.01	12.732 ±2.0	N.D.	N.D.
100%	M.E.		9.70 ±0.271	8.40 ±0.462	10.70 ±1.56	13.20 ±1.16	12.60 ±1.62	14.60 ±1.32	9.80 ±1.01	N.D.	N.D.

Note : Unit : Uric acid in mg/dL.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 35 : ALTERATIONS IN THE SERUM URIC ACID LEVEL.
Units : mg/dL.



VII) ALTERATIONS IN THE SERUM CREATININE :

The alterations in the serum creatinine levels are tabulated in **Table No.36** and are graphically expressed in **Graph No. 36.** The serum creatinine concentrations are expressed as milligrams per 100 ml (per decilitre) . The control birds exhibited the serum creatinine concentration equivalent to 0.2 ± 0.02 mg per decilitre.

From the table and the graph it appears that under the influence of mining effluents (M.E.) the ducks showed M.E. concentration dependent increase in the serum creatinine level at the end of six hours. Similarly, the exposure of ducks to M.E for twelve hours promoted increase in the serum creatinine level as the M.E. concentrations increased except for **10% M.E.**

When the ducks were treated with **0.01% M.E.** there was no significant change in the serum creatinine levels up to the end of twentynine days but at the end of thirty days the serum creatinine concentrations increased and continued to increase further up to the end of forty five days. Thus, at the end of thirty and forty five days the serum creatinine level was equivalent to 0.50 ± 0.098 and 0.90 ± 0.215 mg per decilitre respectively.

Under the influence of **0.1% M.E.** the serum creatinine level fluctuated between 0.5 ± 0.141 and 1.375 ± 0.147 mg per decilitre. The maximum increase in the serum creatinine level was observed under the influence of **0.1% M.E.** at the end of one hundred and twenty hours.

By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum creatinine concentration was equivalent to 0.50 ± 0.141, 0.68 ± 0.312, 0.48 ± 0.132, 0.34 ± 0.101, 1.06 ± 0.185, 1.24 ± 0.101 and 1.375 ± 0.147 mg per decilitre respectively.

On exposure to **1.0% M.E.** the ducks exhibited fluctuations in the serum creatinine levels and the serum creatinine level ranged between 0.46 ± 0.135 and 1.84 ± 0.101 mg per decilitre. By the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours the serum creatinine

level was equivalent to 0.60 ± 0.141, 1.06 ± 0.162, 0.46 ± 0.135, 0.86 ± 0.185, 1.42 ± 0.172, 1.04 ± 0.101 and 1.84 ± 0.101 mg per decilitre respectively.

The ducks subjected to **5.0%** M.E. showed increase in serum creatinine level from the end of six hours to the end of twentyfour hours and subsequently the serum creatinine level fluctuated. The maximum serum creatinine level for this M.E. concentration was observed at the end of seventytwo hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours the serum creatinine level was equivalent to 0.96 ± .0.215, 1.36 ± 0.233, 2.08 ± 0.256, 1.76 ± 0.377, 2.52 ± 0.193, 2.02 ± 0.074 and 1.48 ± 0.231 mg per decilitre respectively.

The **10.0%** M.E. induced fluctuations in the serum creatinine level and the creatinine level ranged between 0.84 ± 0.397 and 2.42 ± 0.172 mg per decilitre. This dose of M.E. induced maximum increase in serum creatinine level at the end of ninetysix hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the serum creatinine level was equivalent to 1.48 ± 0.285, 1.24 ± 0.307, 0.84 ± 0.397, 1.46 ± 0.241, 1.74 ± 0.185, 2.42 ± 0.172 and 1.45 ± 0.165 mg per decilitre respectively.

Under the influence of **50%** M.E. the serum creatinine levels increased progressively from the end of six hours to the end of ninety six hours indicating exposure period dependency of creatinine elevation. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours the serum creatinine level was equivalent to 1.74 ± 0.705, 1.94 ± 0.32, 2.56 ± 0.445, 3.02 ± 0.29 3.42 ± 0.074, 3.60 ± 0.141 and 3.02 ± 0.172 mg per decilitre respectively.

The ducks treated with **100%** M.E. exhibited acute rise in the serum creatinine level and the creatinine level fluctuated between 2.23 ± 0.617 and 3.72 ± 0.634 mg per decilitre. The highest increase in the serum creatinine level was observed at the end of seventy two hours and this rise was over twelve fold. The serum creatinine level was equivalent to 2.86 ± 0.24, 3.46 ±

0.382, 2.60 ± 0.648, 2.23 ± 0.617, 3.72 ± 0.634, 3.06 ± 0.730 and 2.25 ± 0.456 mg per decilitre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 36 : ALTERATIONS IN THE SERUM CREATININE UNDER THE INFLUENCE TO MINING EFFLUENT.

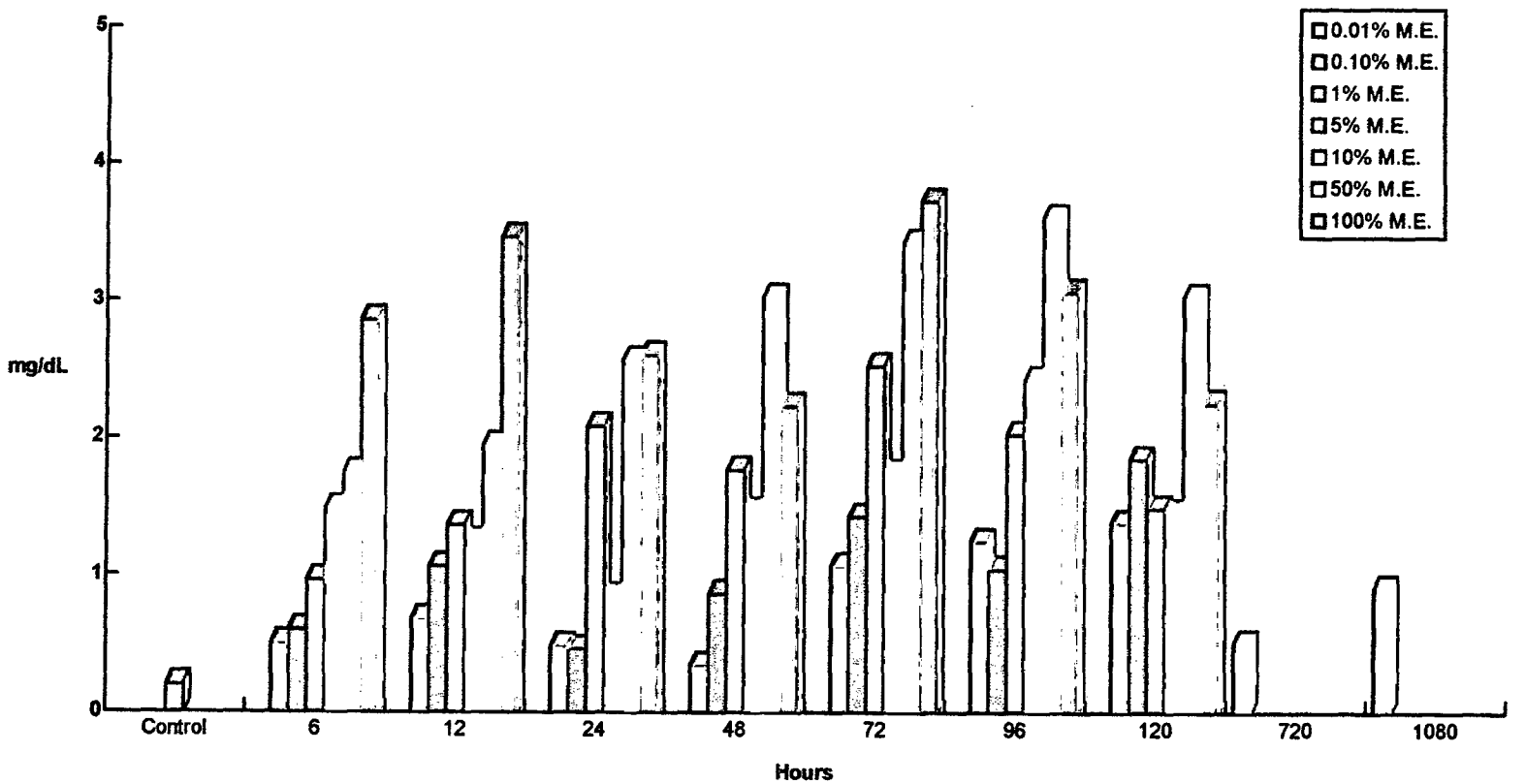
		6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control	0.20 ± 0.02									
0.01% M.E.	← No significant change →								0.50 ±0.098	0.90 ±0.215
0.1% M.E.		0.50 ±0.141	0.68 ±0.312	0.48 ±0.132	0.34 ±0.101	1.06 ±0.185	1.24 ±0.101	1.375 ±0.147	N.D.	N.D.
1% M.E.		0.60 ±0.141	1.06 ±0.162	0.46 ±0.135	0.86 ±0.185	1.42 ±0.172	1.04 ±0.101	1.84 ±0.101	N.D.	N.D.
5% M.E.		0.96 ±0.215	1.36 ±0.233	2.08 ±0.256	1.76 ±0.377	2.52 ±0.193	2.02 ±0.074	1.48 ±0.231	N.D.	N.D.
10% M.E.		1.48 ±0.285	1.24 ±0.307	0.84 ±0.397	1.46 ±0.241	1.74 ±0.185	2.42 ±0.172	1.45 ±0.165	N.D.	N.D.
50% M.E.		1.74 ±0.705	1.94 ±0.32	2.56 ±0.445	3.02 ±0.29	3.42 ±0.074	3.60 ±0.141	3.02 ±0.172	N.D.	N.D.
100% M.E.		2.86 ±0.24	3.46 ±0.382	2.60 ±0.648	2.23 ±0.617	3.72 ±0.634	3.06 ±0.730	2.25 ±0.456	N.D.	N.D.

Note : Unit : Creatinine in mg/dL.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 36 : ALTERATIONS IN THE SERUM CREATININE LEVEL.
Units : mg/dL.



VIII) ALTERATIONS IN SERUM CALCIUM LEVEL :

The changes in the serum calcium level under the influence of mining effluents (M.E.) are given in **Table No. 37** and are graphically presented in **Graph No. 37**. The serum calcium level is expressed as milligrams (mg) per decilitre (100 ml). The control animals had serum calcium level equivalent to 4.30 ± 0.10 mg per decilitre. From the table and graph it appears that all the concentrations of M.E. except **10.0%** promoted concentration dependent elevations in the serum calcium level at the end of six and twelve hours. The **10.0% M.E.** induced highest increase in the serum calcium level at the end of one hundred and twenty hours while **0.1% M.E.** induced reduction in the serum calcium level at the end of six and twelve hours.

The ducks treated with **0.01% M.E.** did not exhibit any significant change in the serum calcium level up to the end of twenty nine days but at the end of thirty days the serum calcium level elevated and continued to elevate up to the end of forty five days. Thus, at the end of thirty and fortyfive days the serum calcium level was equivalent to 8.37 ± 0.172, 13.72 ± 0.312 mg per decilitre respectively.

When the ducks were subjected to **0.1% M.E.**, the serum calcium concentrations dropped marginally at the end of six and twelve hours and then elevated gradually from the end of twentyfour hours to the end of one twenty hours, except for seventy two hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninentysix and one hundred and twenty hours the calcium concentrations were equivalent to 4.02 ± 0.116, 4.02 ± 0.116, 5.94 ± 0.205, 7.04 ± 0.185, 6.64 ± 0.249, 9.70 ± 0.167 and 13.64 ± 0.257 mg per decilitre respectively.

The exposure of ducks to **1.0% M.E.** promoted fluctuations in the serum calcium levels and the calcium level fluctuated between 6.06 ± 0.386 and 12.26 ± 0.185 mg per decilitre. Thus, this dose of M.E. in general caused elevation in the serum calcium concentration. By the end of six, twelve, twentyfour, forty

eight, seventytwo, ninetysix and one twenty hours, the serum calcium level was equivalent to 6.80 ± 0.404, 6.06 ± 0.386, 7.50 ± 0.228, 8.06 ± 0.344, 6.66 ± 0.185, 12.26 ± 0.185 and 11.56 ± 0.508 mg per decilitre respectively.

The **5.0% M.E.** induced elevation in the serum calcium level and the calcium concentrations varied between 6.84 ± 0.30 and 11.0 ± 0.228 mg per decilitre. The serum calcium level was equivalent to 9.0 ± 0.363, 9.88 ± 0.172, 10.28 ± 0.466, 8.14 ± 0.313, 11.0 ± 0.228, 8.98 ± 0.172 and 6.84 ± 0.30 mg per decilitre at the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

Under the influence of **10.0% M.E.** the ducks showed exposure period dependent rise in the serum calcium level. Thus, longer the exposures of ducks to 10% M.E. the higher the concentrations of calcium were observed. This dose induced the highest elevation in serum calcium level at the end of one twenty hours and the serum calcium level was equivalent to 5.50 ± 0.20, 6.62 ± 0.172, 7.68 ± 0.116, 13.30 ± 0.260, 17.60 ± 0.244, 22.325 ± 0.402 and 26.20 ± 0.316 mg per decilitre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

The **50.0% M.E.** caused sharp rise in the serum calcium level and the serum calcium level fluctuated between 10.06 ± 0.257 and 18.56 ± 0.195 mg per decilitre. The serum calcium concentration was equivalent to 15.42 ± 0.365, 10.06 ± 0.257, 10.12 ± 0.376, 11.34 ± 0.392, 13.34 ± 0.257, 18.56 ± 0.195 and 14.72 ± 0.132 mg per decilitre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one hundred and twenty hours respectively.

The exposure of ducks to **100% M.E.** promoted, in general, elevation of serum calcium concentration, especially at the end of six and twentyfour hours the calcium level increased acutely. The serum calcium level varied between 8.68 ± 0.27 and 23.18 ± 1.783 mg per decilitre. The serum calcium level was equivalent to 21.94 ± 0.349, 18.50 ± 0.303, 23.18 ± 1.783, 10.26 ± 0.50, 9.48 ±

0.278, 8.68 ± 0.27 and 15.84 ± 0.135 mg per decilitre at the end of six, twelve, twenty four, fortyeight, seventy two, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 37 : ALTERATIONS IN THE SERUM CALCIUM LEVEL UNDER THE INFLUENCE OF MINING EFFLUENTS.

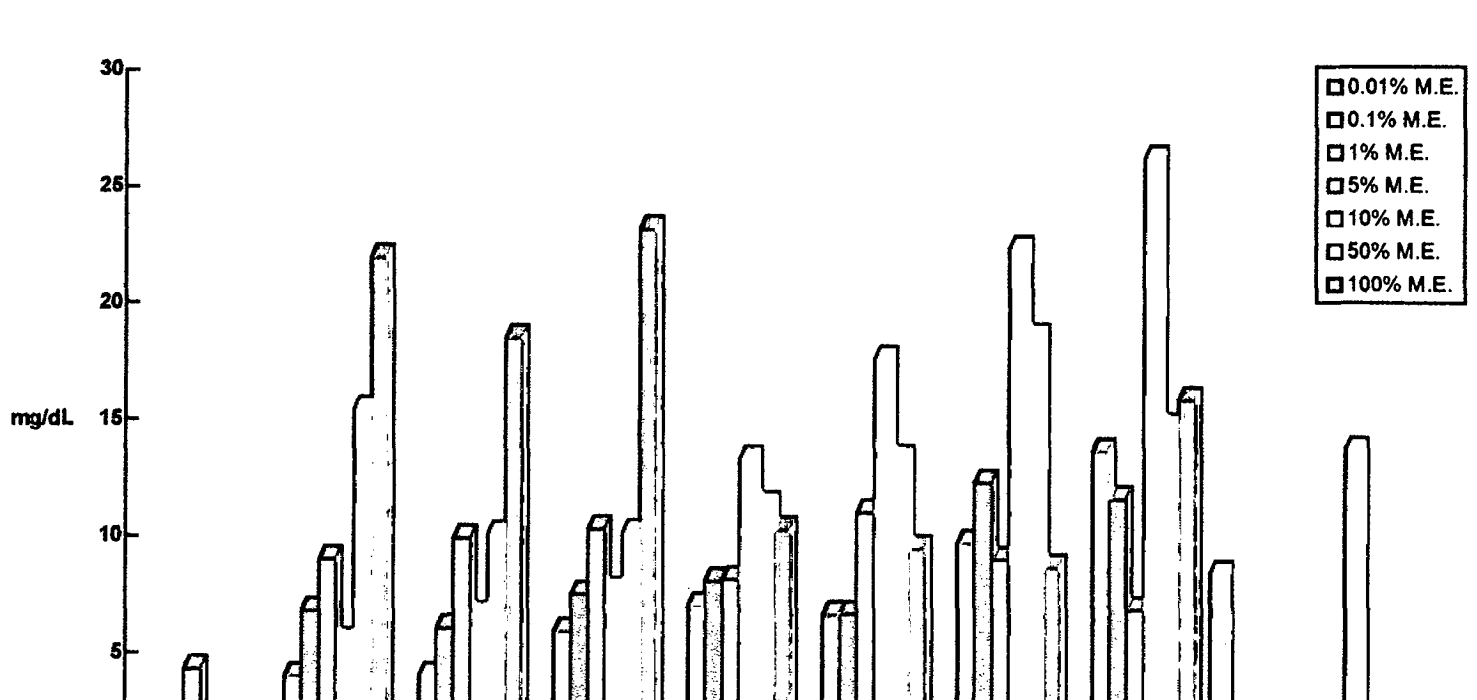
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		4.30 ± 0.1									
0.01%	M.E.		← No significant change →							8.37 ±0.172	13.72 ± 0.312
0.1%	M.E.		4.02 ±0.116	4.02 ±0.116	5.94 ±0.205	7.04 ±0.185	6.64 ±0.249	9.70 ±0.167	13.64 ±0.257	N.D.	N.D.
1%	M.E.		6.80 ±0.404	6.06 ±0.386	7.50 ±0.228	8.06 ±0.344	6.66 ±0.185	12.26 ±0.185	11.56 ±0.508	N.D.	N.D.
5%	M.E.		9.0 ±0.363	9.88 ±0.172	10.28 ±0.466	8.14 ±0.313	11.0 ±0.228	8.98 ±0.172	6.84 ±0.30	N.D.	N.D.
10%	M.E.		5.50 ±0.20	6.62 ±0.172	7.68 ±0.116	13.30 ±0.260	17.60 ±0.244	22.325 ±0.402	26.20 ±0.316	N.D.	N.D.
50%	M.E.		15.42 ±0.365	10.06 ±0.257	10.12 ±0.376	11.34 ±0.392	13.34 ±0.257	18.56 ±0.195	14.72 ±0.132	N.D.	N.D.
100%	M.E.		21.94 ±0.349	18.50 ±0.303	23.18 ±1.783	10.26 ±0.50	9.48 ±0.278	8.68 ±0.27	15.84 ±0.135	N.D.	N.D.

Note : Unit : Calcium in mg/dL.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 37 : ALTERATIONS IN THE SERUM CALCIUM LEVEL.
Units : mg/dL.



IX) ALTERATIONS IN THE SERUM CHLORIDE CONCENTRATIONS :

The alterations in the serum chloride concentrations under the influence of mining effluents (M.E.) are compiled in **Table No. 38** and are graphically expressed in **Graph No. 38**. The chloride concentrations of the serum are expressed as milliequivalance (mEq) per litre. The control animals showed the serum chloride concentrations equivalent to 89.0 ± 2.70 mEq/litre.

From the table and the graph it appears that mining effluents profoundly influenced the serum chloride concentrations, especially the higher concentrations of M.E induced acute increase in the serum chloride levels. The serum chloride levels did not alter in general in a dose (M.E. concentrations) and exposure time dependent manner. The **50% M.E.** induced highest rise in the serum chloride level at the end of six hours while the acute reduction in the chloride concentrations was promoted by **0.1% M.E.** at the end of forty eight hours.

The ducks exposed to **0.01%** M.E. did not show any significant change in the serum chloride level up to the end of twenty nine days but at the end of thirty days the chloride concentrations increased significantly and continued to increase further up to the end of forty five days. By the end of thirty and forty five days, the serum chloride concentrations were equivalent to 110.80 ± 5.10 and 184.60 ± 1.624 mEq per litre respectively.

Under the influence of **0.1%** M.E. the serum chloride level dropped acutely at the end of forty eight hours and increased significantly to a higher level at the end of ninetysix hours. The serum chloride level was equivalent to 126.92 ± 2.10, 121.0 ± 1.41, 118.0 ± 1.632, 52.0 ± 1.41, 77.0 ± 1.341, 127.68 ± 0.80 and 120. 58 ± 1.36 mEq per litre at the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one hundred and twenty hours respectively.

On exposure to **1.0%** M.E. the ducks exhibited fluctuations in the serum chloride concentrations and the serum chloride concentrations varied between

105.0 ± 0.77 and 176.40 ± 4.31 mEq per litre. By the end of one hundred and twenty hours exposure the serum chloride levels elevated acutely. The serum chloride level was equivalent to 115.40 ± 2.059, 105.0 ± 0.77, 165.0 ± 1.6, 108.0 ± 3.521, 137.0 ± 4.4, 128.60 ± 1.496 and 176.40 ± 4.31 mEq per litre at the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

The ducks subjected to **5.0% M.E.** exhibited elevation of serum chloride contents but the serum chloride concentrations varied between 120.0 ± 4.8 and 190.0 ± 3.12 mEq/litre. By the end of six, twelve, twentyfour, fortyeight, seventy two, ninetysix and one twenty hours the serum chloride concentrations were equivalent to 125.0 ± 1.85, 120.0 ± 4.8, 189.0 ± 1.01, 185.0 ± 4.40, 190.0 ± 3.12, 153.0 ± 1.78 and 131.0 ± 1.41 mEq/litre respectively.

When the ducks were introduced to **10.0% M.E.** the serum chloride levels elevated sharply and the maximum chloride contents were seen at the end of fortyeight hours while at the end of one hundred and twenty hours the chloride concentrations lowered a little and remained above that observed in the control. The serum chloride concentration was equivalent to 176.0 ± 2.20, 178.0 ± 1.16, 170.0 ± 3.92, 193.0 ± 2.90, 180.0 ± 1.72, 110.0 ± 1.23 and 100.0 ± 1.30 mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

The **50% M.E.** promoted the highest increase in the serum chloride level at the end of six hours and for almost all time intervals except fortyeight hours, the serum chloride levels were four to five fold higher than that observed in the controls. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours, the serum chloride concentration was equivalent to 485.0 ± 2.607, 452.0 ± 3.95, 443.20, ± 2.39, 137.20 ± 2.40, 391.0 ± 7.50, 443.40 ± 2.87 and 444.60 ± 2.57 mEq/litre respectively.

The exposure of ducks to **100% M.E.** caused over four fold increase in the serum chloride concentration up to the end of fortyeight hours but at the

end of seventy two hours, the serum chloride level dropped below that found in the control animals. Then, once again the chloride concentrations increased progressively for the subsequent exposure periods. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the serum chloride concentrations were equivalent to 443.40 ± 3.10, 439.40 ± 4.49, 445.0 ± 1.09, 443.80 ± 3.18, 78.80 ± 1.72, 110.0 ± 3.10 and 127.20 ± 2.10 mEq/litre respectively.

TABLE NO. 38 : ALTERATIONS IN THE SERUM CHLORIDE CONCENTRATION UNDER THE INFLUENCE OF MINING EFFLUENTS.

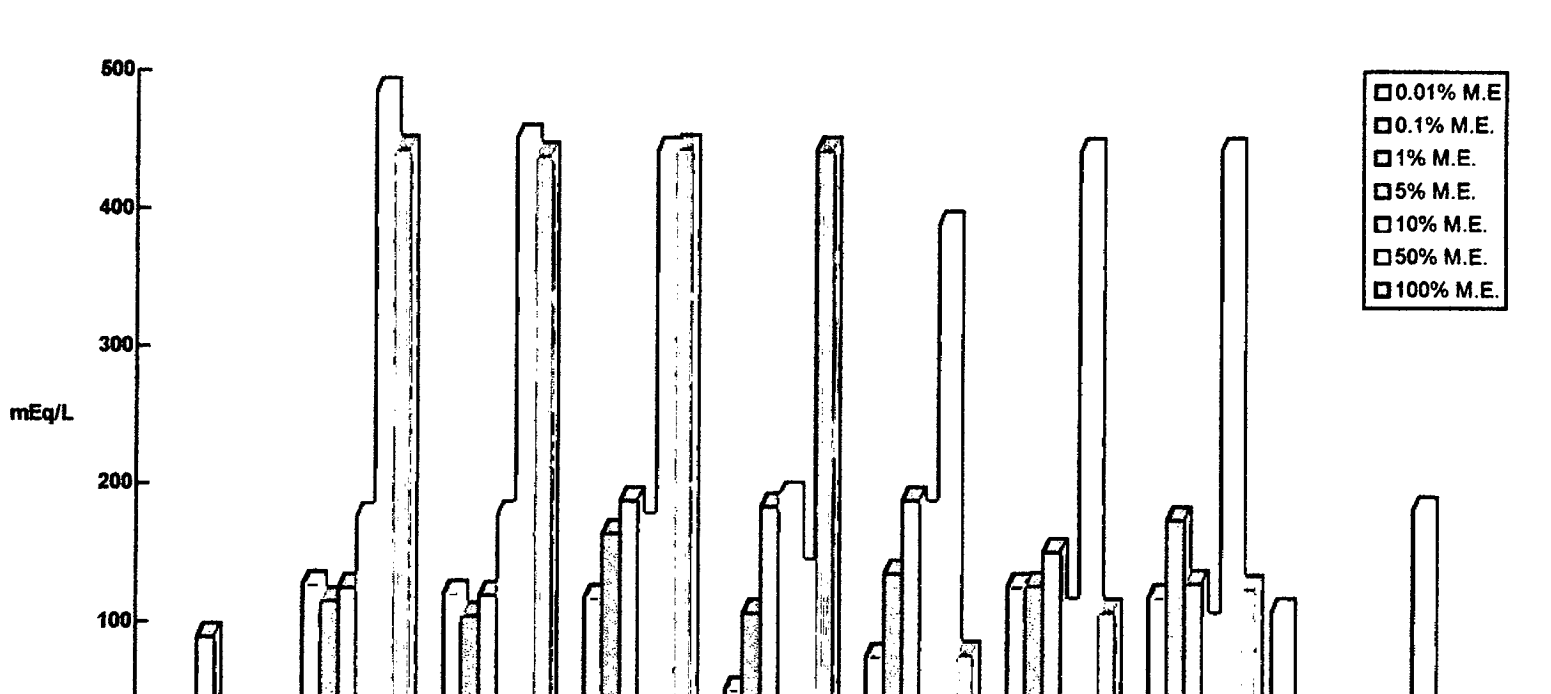
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		89.0 ±2.7									
0.01%	M.E.		← No significant change →							110.80 ±5.1	184.60 ±1.624
0.1%	M.E.		126.92 ±2.10	121.0 ±1.41	118.0 ±1.632	52.0 ±1.41	77.0 ±1.341	127.68 ±0.80	120.58 ±1.36	N.D.	N.D.
1%	M.E.		115.4 ±2.059	105.0 ±0.77	165.0 ±1.6	108.0 ±3.521	137.0 ±4.40	128.60 ±1.496	176.40 ±4.31	N.D.	N.D.
5%	M.E.		125.0 ±1.85	120.0 ±4.80	189.0 ±1.01	185.0 ±4.40	190.0 ±3.12	153.0 ±1.78	131.0 ±1.41	N.D.	N.D.
10%	M.E.		176.0 ±2.20	178.0 ±1.16	170.0 ±3.92	193.0 ±2.90	180.0 ±1.72	110.0 ±1.23	100.0 ±1.30	N.D.	N.D.
50%	M.E.		485.0 ±2.607	452.0 ±3.95	443.20 ±2.39	137.20 ±2.40	391.0 ±7.50	443.40 ±2.87	444.60 ±2.57	N.D.	N.D.
100%	M.E.		443.40 ±3.10	439.40 ±4.49	445.0 ±1.09	443.80 ±3.18	78.80 ±1.72	110.0 ±3.10	127.20 ±2.10	N.D.	N.D.

Note : Unit : Chloride in mEq/Litre.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 38 : ALTERATIONS IN SERUM CHLORIDE LEVEL.
Units : mEq/L.



X) ALTERATIONS IN THE SERUM ELECTROLYTES :

a) Na^+ :

The alterations in the serum sodium concentrations under the influence of mining effluents (M.E.) are given in **Table No. 39** and are graphically presented in **Graph No. 39**. The Na^+ concentrations of the serum are expressed as milliequivalence per litre. The control animals showed serum Na^+ concentration equal to 120.0 ± 3.0 mEq/litre.

From the graph and the table it appears that M.E. induced elevation in the serum Na^+ concentrations at all the time intervals under study and under all the M.E. concentrations. The elevations in the serum Na^+ contents were neither M.E. concentration dependent nor exposure period dependent.

When the ducks were exposed to **0.01% M.E.** the serum Na^+ concentrations did not vary significantly up to the end of twentynine days but at the end of thirty days the Na^+ concentration elevated and continued to increase further up to the end of forty five days. Then, the serum Na^+ concentration was equivalent to 160.0 ± 2.10 and 172.80 ± 1.62 mEq/litre at the end of thirty and forty five days respectively.

The exposure of ducks to **0.1% M.E.** promoted increase in the serum Na^+ concentration at the end of six hours and the Na^+ level continued to increase progressively up to the end of twenty four hour. Subsequently, the serum Na^+ concentrations fluctuated. By the end of six, twelve, twentyfour, fortyeight seventytwo, ninetysix and one hundred and twenty hours, the serum Na^+ concentration was equivalent to 141.0 ± 1.78, 150.80 ± 1.16, 160.60 ± 2.10, 142.20 ± 2.78, 160.60 ± 1.624, 172.0 ± 2.80 and 146.40 ± 2.65 mEq/litre respectively.

Under the influence of **1.0% M.E.** the serum Na^+ concentrations elevated and fluctuated between 144.20 ± 1.72 and 161.0 ± 1.854 mEq/litre. By the end of ninety six hours the serum Na^+ concentration increased by about

thirty three percent. The serum Na^+ concentration was equivalent to 144.20 ± 1.72, 156.40 ± 1.356, 147.0 ± 2.20, 158.60 ± 1.01, 148.80 ± 1.720, 161.60 ± 1.854 and 148.60 ± 1.01mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

When the ducks were introduced to **5.0% M.E.** the serum Na^+ concentration varied in the range of 148.40 ± 1.20 to 179.0 ± 3.40 mEq/litre. By the end of one hundred and twenty hours, the serum Na^+ level stepped up by fortynine percent. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours the serum Na^+ concentration was equivalent to 158.60 ± 1.01, 155.40 ± 3.0, 164.20 ± 3.124, 174.20 ± 3.42, 164.40 ± 3.80, 148.40 ± 1.20 and 179.0 ± 3.40 mEq/litre respectively.

The exposure of ducks to **10.0%** M.E. induced rise in serum Na^+ level at the end of six hours and it continued to increase gradually up to the end of twenty four hours but subsequently the Na^+ concentrations fluctuated considerably. The Na^+ concentration increased maximally by 42.30 percent at the end of ninetysix hours. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix, and one twenty hours, the serum Na^+ concentration was equivalent to 148.0 ± 1.414, 151.40 ± 1.743, 158.60 ± 2.416, 149.0 ± 1.41, 162.40 ± 3.44, 170.80 ± 2.56 and 162.40 ± 3.261 mEq/litre respectively.

The ducks subjected to **50.0%** M.E. exhibited significant variations in the serum Na^+ concentrations but at all the time intervals the Na^+ level remained elevated. The Na^+ concentration elevated to maximally 65.66 percent at the end of ninetysix hours. The serum Na^+ concentration was equivalent to 159.80 ± 1.40, 170.40 ± 2.0, 161.0 ± 1.85, 186.60 ± 4.80, 194.0 ± 3.0, 198.80 ± 2.31 and 181.20 ± 4.40 mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

TABLE NO. 39 : ALTERATIONS IN THE SERUM SODIUM CONCENTRATIONS UNDER THE INFLUENCE OF MINING EFFLUENTS

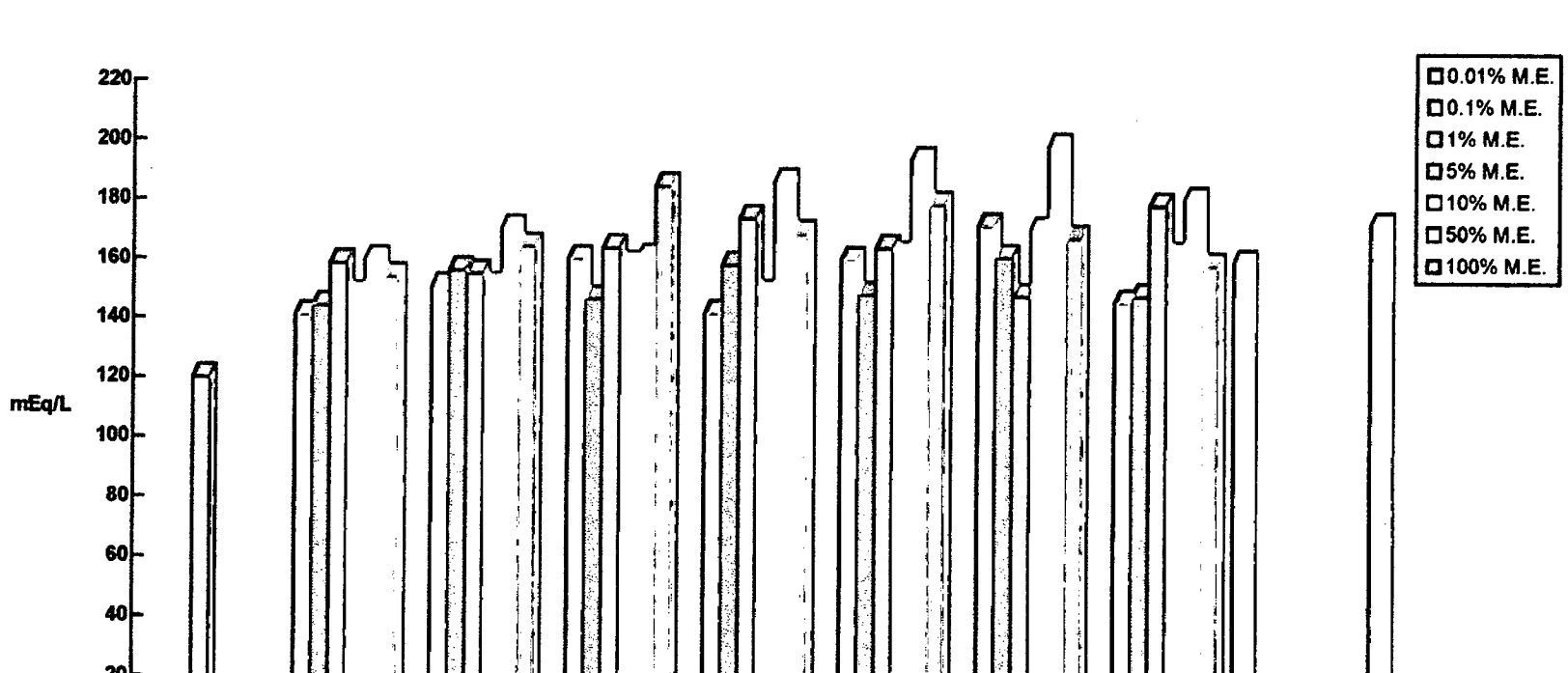
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		120.0 ± 3.0									
0.01%	M.E.		← No significant change →							160.0 ±2.10	172.80 ±1.62
0.1%	M.E.		141.0 ±1.78	150.80 ±1.16	160.60 ±2.10	142.20 ±2.78	160.60 ±1.624	172.0 ±2.8	146.40 ±2.65	N.D.	N.D.
1%	M.E.		144.20 ±1.720	156.40 ±1.356	147.0 ±2.20	158.60 ±1.01	148.80 ±1.720	161.60 ±1.854	148.60 ±1.01	N.D.	N.D.
5%	M.E.		158.60 ±1.01	155.40 ±3.0	164.20 ±3.124	174.20 ±3.42	164.40 ±3.80	148.40 ±1.20	179.0 ±3.40	N.D.	N.D.
10%	M.E.		148.0 ±1.414	151.40 ±1.743	158.60 ±2.416	149.0 ±1.41	162.40 ±3.44	170.8 ±2.56	162.40 ±3.261	N.D.	N.D.
50%	M.E.		159.80 ±1.40	170.40 ±2.0	161.0 ±1.85	186.60 ±4.80	194.0 ±3.0	198.80 ±2.31	181.20 ±4.40	N.D.	N.D.
100%	M.E.		154.40 ±1.80	164.60 ±5.30	185.0 ±3.20	169.20 ±1.320	179.20 ±2.48	168.0 ±1.72	159.0 ±1.414	N.D.	N.D.

Note : Unit : Sodium in mEq/L.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 39 : ALTERATIONS IN SERUM SODIUM LEVEL.
Unit : mEq/L.



b) K^+ :

The changes in the serum K^+ concentrations under the influence of mining effluents (M.E.) are compiled in **Table No. 40** and are graphically given in **Graph No. 40.** The serum K^+ concentration is expressed as milliequivalence (mEq) per litre. The controls exhibited K^+ concentration equivalent to 4.0 ± 0.17 mEq/litre.

From the table and the graph it appears that the various M.E. concentrations induced elevation in the serum K^+ level. The serum K^+ concentrations elevated in a M.E. concentration dependent manner at the end of six, twentyfour and seventytwo hours. The highest rise in K^+ concentration was promoted by **100% M.E.** at the end of seventytwo hours and this increase was over six fold of the control value.

When the ducks were exposed to **0.01% M.E.** the serum K^+ concentrations did not show any significant change up to the end of twentynine days but at the end of thirty days the serum K^+ concentration increased and continued to increase up to the end of forty five days. Thus, at the end of thirty and forty five days the serum K^+ concentration was equivalent to 7.70 ± 0.14 and 10.82 ± 0.11 mEq/litre respectively.

The ducks introduced to **0.1%** M.E. showed fluctuations in the serum K^+ levels. The K^+ concentrations fluctuated between 4.84 ± 0.101 and 8.52 ± 0.132 mEq/litre. The K^+ concentration increased over two fold at the end of six and fortyeight hours. The serum K^+ concentration was equivalent to 8.52 ± 0.132, 7.70 ± 0.14, 6.62 ± 0.16, 8.02 ± 0.116, 7.16 ± 0.24, 5.96 ± 0.13 and 4.84 ± 0.101 mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

Under the influence of **1.0%** M.E. the serum K^+ concentration increased over two fold at the end of six hours and continued to decrease gradually up to the end of twentyfour hours. Then, once again, the serum K^+ concentration increased significantly at the end of fortyeight hours and subsequently went on

decreasing up to the end of one hundred and twenty hours. By the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the K^+ concentration was equivalent to 10.58 ± 0.172, 9.04 ± 0.135, 8.64 ± 0.149, 9.64 ± 0.249, 8.0 ± 0.17, 5.62 ± 0.10 and 4.86 ± 0.135 mEq/litre respectively.

The exposure of ducks to **5.0% M.E.** promoted over three fold increase in serum K^+ concentration at the end of six hours and subsequently the K^+ concentrations went on declining up to the end of fortyeight hours. Then, once again the K^+ concentration elevated over two folds of the control value at the end of seventy two hours and decreased gradually at the end of ninetysix and one hundred and twenty hours. The serum K^+ concentration was equivalent to 12.16. ± 0.12, 11.42 ± 0.172, 10.46 ± 0.162, 9.24 ± 0.135, 10.78 ± 0.116, 8.68 ± 0.25 and 6.62 ± 0.23 mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours respectively.

On exposing the ducks to **10% M.E.** the serum K^+ level elevated 3.83 folds of the control value at the end of six hours and subsequently decreased gradually up to the end of twenty four hours. Then, again, the K^+ concentration increased a little at the end of fortyeight hours but went on declining steadily up to the end of one hundred and twenty hours. By the end of six, twelve, twenty four, fortyeight, seventytwo, ninetysix and one twenty hours the serum K^+ concentration was equivalent to 15.32 ± 0.271, 13.22 ± 0.16, 12.04 ± 0.18, 12.82 ± 0.24, 10.80 ± 0.282, 8.60 ± 0.20 , and 5.64 ± 0.215 mEq/litre respectively.

The **50.0% M.E.** induced significant variations in the serum K^+ levels of the ducks. By the end of twelve hours, the serum K^+ concentration increased over five folds of the control value but the minimum of two folds and little more, increase in K^+ concentration was maintained at the end of one hundred and twenty hours. The serum K^+ concentration was equivalent to 19.16 ± 0.377, 20.72 ± 0.256, 17.10 ± 0.374, 14.56 ± 0.272, 17.10 ± 0.228, 11.04 ± 0.372 and

8.84 ± 0.12 mEq/litre at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one twenty hours respectively.

The exposure of ducks to 100.0% M.E. caused very sharp rise in the serum K^+ levels. By the end of seventy two hours the ducks exhibited highest rise in the serum K^+ concentration. The serum K^+ concentrations ranged between 16.72 ± 0.426 and 27.78 ± 0.16 mEq/litre. At the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours, the serum K^+ concentration was equivalent to 22.78 ± 0.435, 19.80 ± 0.268, 22.22 ± 1.18, 24.32 ± 0.39, 27.78 ± 0.16, 21.52 ± 0.342 and 16.72 ± 0.426 mEq/litre respectively.

TABLE NO. 40 : ALTERATIONS IN THE SERUM POTASSIUM CONCENTRATION IN THE DUCKS UNDER THE INFLUENCE OF MINING EFFLUENTS.

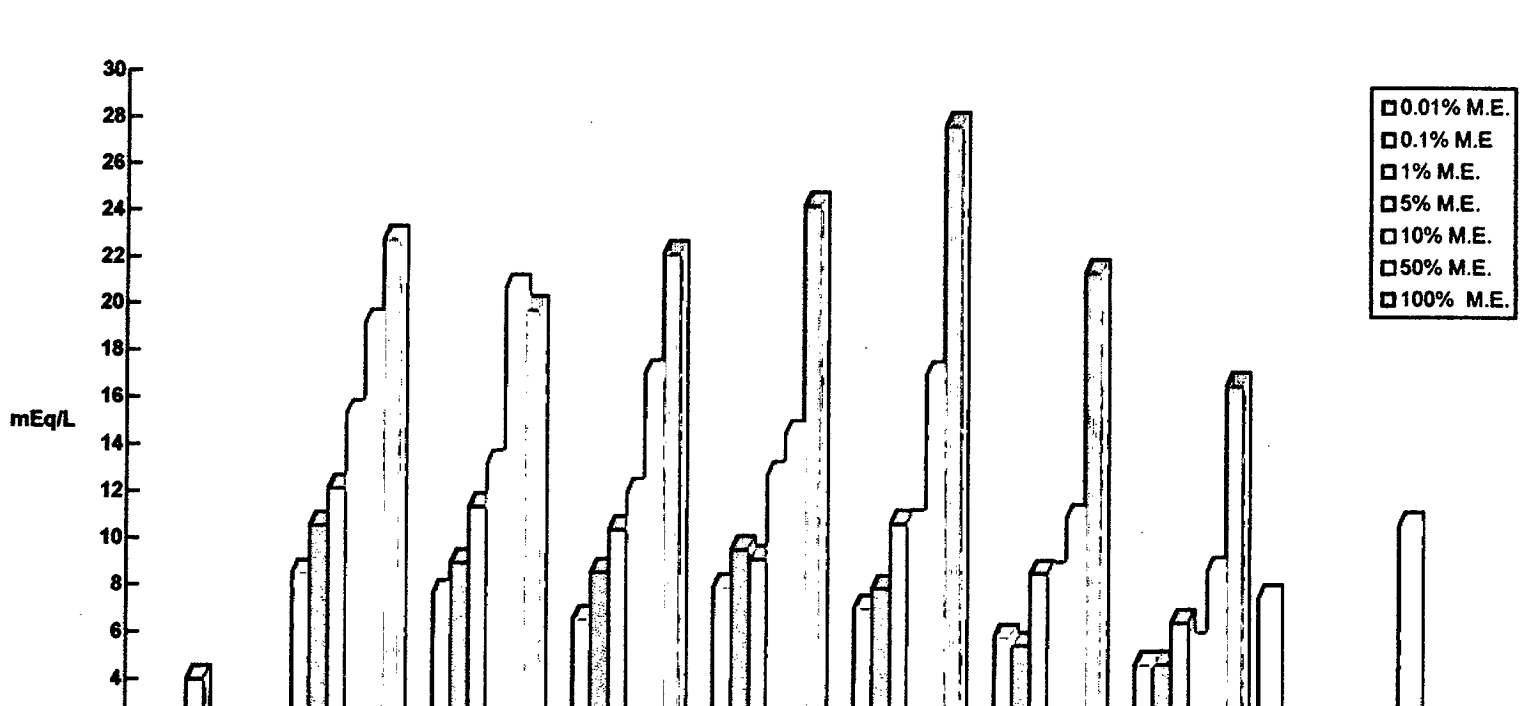
			6 Hours	12 Hours	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours	720 Hours	1080 Hours
Control		4.0 ± 0.17									
0.01%	M.E.		← No significant change →							7.70 ±0.14	10.82 ±0.11
0.1%	M.E.		8.52 ±0.132	7.70 ±0.14	6.62 ±0.16	8.02 ±0.116	7.16 ±0.24	5.96 ±0.13	4.84 ±0.101	N.D.	N.D.
1%	M.E.		10.58 ±0.172	9.04 ±0.135	8.64 ±0.149	9.64 ±0.249	8.0 ±0.17	5.62 ±0.10	4.86 ±0.135	N.D.	N.D.
5%	M.E.		12.16 ±0.12	11.42 ±0.172	10.46 ±0.162	9.24 ±0.135	10.78 ±0.116	8.68 ±0.25	6.62 ±0.23	N.D.	N.D.
10%	M.E.		15.32 ±0.271	13.22 ±0.16	12.04 ±0.18	12.82 ±0.24	10.80 ±0.282	8.60 ±0.20	5.64 ±0.215	N.D.	N.D.
50%	M.E.		19.16 ±0.377	20.72 ±0.256	17.10 ±0.374	14.56 ±0.272	17.10 ±0.228	11.04 ±0.372	8.84 ±0.12	N.D.	N.D.
100%	M.E.		22.78 ±0.435	19.80 ±0.268	22.22 ±1.18	24.32 ±0.39	27.78 ±0.16	21.52 ±0.342	16.72 ±0.426	N.D.	N.D.

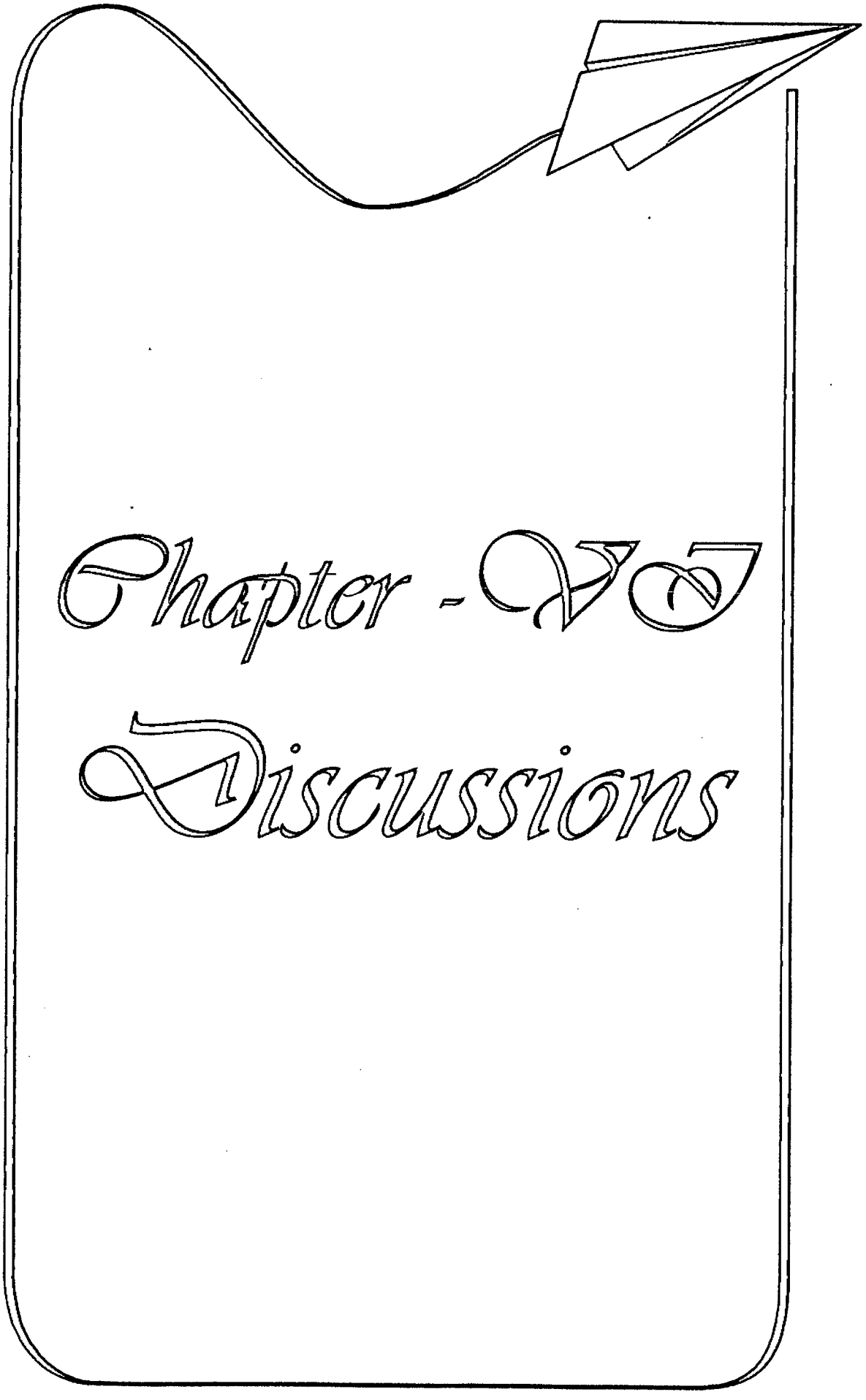
Note : Unit : Serum potassium in mEq / Litre.

N.D. : Not Determined.

All alterations are statistically significant - $P < 0.01$.

GRAPH NO. 40 : ALTERATIONS IN SERUM POTASSIUM LEVEL.
Units : mEq/L.





Chapter - VI

Discussions

The review of the available literature reveals that the birds have been widely used as monitors of environmental deterioration. Many researchers have studied the retentions of heavy metals, insecticides, pesticides and PCBs in the birds. Now it is known that the birds' tissues like kidney, liver, blood, muscles and feathers retain the pollutants mentioned above which enter into the animal's body from the contaminated environment through the food, water or direct physical contact.

Goa has been witnessing the deterioration of the environment due to the extensive mining activities. The stock piled iron ore rejects would add to the pollution of aquatic bodies during rainfall, when the runoffs from the various iron ore reject dumps mix with them. The present investigation confirms that the runoffs from the iron ore rejects dumps could contaminate the receiving water bodies. The runoffs from the simulated iron ore reject dumps exposed to the simulated rainfall contain heavy metals such as barium, cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc. The runoff contains highest concentration of magnesium (2.14 mg/l), followed by iron (1.91 mg/l), manganese (0.857 mg/l), cadmium (0.71 mg/l), barium (0.650 mg/l) and zinc (0.0814 mg/l). Besides this, the physico - chemical characteristics show that the runoffs have high load of total solids, total phosphorus, organic phosphorous, sulphates and nitrates. The large quantities of such runoff if mixed with the natural water body would undoubtedly pollute it : Similar observations on the runoffs of mining origin have been reported by Desai (1990).

There are few reports on the kidney lesions in sea birds produced by high tissue levels of cadmium and mercury and metals like copper and zinc have also been reported to be associated with kidney lesion. Recently Prasad Rao *et. al.*, (1989) reported severity in histopathological changes in kidneys of pekin ducks exposed to methyl mercury chloride, lead acetate and cadmium chloride singly or in combination for 13 weeks.

There is hardly any report on kidney lesions of birds caused by the mining effluents containing heavy metals. In a tropical country like India, the birds especially aquatic and non aquatic are likely to get exposed to the heavy metals through the water they drink. Thus, this could be the most natural route for the heavy metals'/pollutants' entry in to the bird body, besides the entry of these contaminants through food. From the available literature it can be seen that most of the workers have administered the heavy metals intramuscularly or intraperitoneally and some have studied the toxic effects of pollutants in the natural bird populations where in they have ascertained the presence of heavy metals in the animal tissues and have postulated/correlated the kidney damage to the heavy metal load in the animal body.

In the present investigation it is observed that the ducks weighing one kilogram, drink on an average, about 400 ml of water per day throughout the year. This could be due to the hot and humid climate of Goa. Thus, the ducks drinking so much of water a day are likely to receive large quantities of heavy metals and other pollutants. These ducks under such conditions tend to defecate frequently in comparison with other smaller birds.

The present studies have clearly revealed the toxic actions of these M.E. on the kidney.

A) HISTOPATHOLOGY OF THE KIDNEY :

The routine haematoxylin - eosin stained sections of the kidneys taken at the end of six, twelve, twentyfour, fortyeight, seventytwo, ninetysix and one hundred and twenty hours after exposure of ducks to 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0%, mining effluents (M.E.) exhibited M.E. concentration dependent and up to a large extent exposure period dependent necrosis of the glomeruli, proximal, distal and collecting tubules. The ducks exposed to a low M.E. concentration like 0.01% do not show renal damage up to the end of twenty nine days but do show renal necrosis at the end of thirty days indicating that

the prolonged exposures of ducks to very low concentrations of mining effluents could be toxic. Thus, in a large water body if mining effluents mix to such an extent that the water body bears the metal concentrations equivalent to that found in 0.01% M.E. or even a little less, the renal tissue of the aquatic birds getting exposed for a prolonged period of even one year or less would get progressively damaged. On the other hand, if the heavy metal concentrations of the water body keep on rising as the mining effluents keep on mixing in it at every rainfall, the ducks inhabiting such waterbodies would definitely get affected adversely.

From the present investigation it appears that the glomerular changes in general are begining with glomerular swelling and then progress towards distortion which include disruption of the glomerular walls, vacuolisation or development of empty spaces, release of glomerular exudents in to the Bowman's space, breaking of capillaries leading to the bleeding and escape of blood cells into the renal tissue. The changes in the Bowman's capsule are like widening of Bowman's space and disruption of Bowman's inner and outer walls. The number of glomeruli getting affected depend upon the M.E. concentration and exposure period. The M.E concentration like 0.01% can cause damage to about 10.0% glomeruli and the prolonged exposure cause rise in the number of necrotic glomeruli. The severity of glomerular damage and the number of affected glomeruli depend upon the M.E. concentration and exposure period. The higher the M.E. concentration, the higher the number of affected glomeruli as well as higher the degree of necrosis. But it is interesting to note that both the reptilian and mammalian glomeruli get equally affected but some glomeruli escape damage.

The necrotic alterations in proximal and distal tubules are same under the influence of various concentrations of mining effluents. All the concentrations of M.E. fail to produce any necrosis of the renal tubules in the early periods of exposure (less than six hours) but a few tubules exhibit slight

swelling of one of the tubule cells or marginal widening of lumina indicating setting in of early necrotic changes. The 0.1, 1.0, 5.0, 10.0, 50.0 and 100.0% M.E. induces necrosis in about 15%, 20%, 28 - 30%, 36%, 45% and 60% proximal and distal tubules respectively at the end of six hours and the necrosis progresses as the exposure period prolongs. The 0.01% M.E. cannot induce necrosis in the renal tubules for short duration exposures. Only prolonged exposures can promote necrosis of the renal tubules. The prominent features of the necrosis of proximal and distal tubules are : swelling of tubule cells, disruption of tubules, vacuolisation of tubule cytoplasm, loss of brush border/luminal border, loss of basal membranes, pycnosis of the tubule nuclei, widening of interstitium exudation of cytoplasmic and nuclear debris, blood cells into the lumina or interstitium.

Similarly different concentrations of M.E. induce necrosis of collecting tubules depending upon the exposure period and the M.E. concentration. Most of the necrotic changes of collecting tubules are similar to those observed for proximal and distal tubules. The causes of glomerular, proximal, distal and collecting tubule's necrosis could be the toxicity caused by the accumulation of metals in the kidney. Nicholson and Osborn (1983) hold high tissue levels of cadmium and mercury responsible by kidney lesions of pelagic sea birds while Muirhead and Furness (1988) correlate kidney lesions of sea birds to the concentrations of mercury, cadmium, copper and zinc and further they suggest the role of cadmium in producing renal lesion. Prasad Rao *et. al.*, (1989) showed the renal lesions in the pekin ducks exposed to methyl mercury chloride, lead acetate and cadmium chloride, singly or in combination. Pena and Iturri (1993) suggest that cadmium can possibly induce hypertension and reduction in blood volume. Pankakoski *et. al.*, (1993) propose the role of cadmium in nephrotoxicity seen in moles, caused by increased renal concentration of cadmium. Tomera *et. al.*, (1991) opine that the dietary cadmium, as related to the environment, may be partially or totally responsible

for adverse effects in human health and they pertain to elevated mean blood pressure resulting in chronic hypertension. Kodama *et. al.,* (1992) show the renal dysfunction of dogs under the influence of cadmium. Similarly, Dorian *et. al.,* (1992) report chronic, but not acute exposure to inorganic cadmium, produces renal damage and opine that inter organ redistribution of cadmium is responsible for the chronic nephrotoxicity effect of cadmium.

In the present investigation the kidney necrosis could be due to the accumulation of heavy metals in the kidney at different time intervals under the influence of various M.E. concentrations. It is difficult to correlate any single heavy metal to the renal damage and hence the combinations of various metals could be considered responsible for renal necrosis, though a limited contribution of a specific metal's highest accumulation at specific time interval could also be admitted. Even, up to certain extent accumulation of cadmium in kidney could also be inducing nephrotoxicity as suggested by some researchers. Therefore, the metals like Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn together might be influencing the renal damage as and when they are retained by the kidney. The renal necrosis could also be due to the chronic hypertension and altered blood supply as proposed by Tomera *et. al.,* (1991). In the present investigation the ultrastructural studies have revealed the mitochondrial damage which could induce disruptions in the energy supply to the tissues leading to the activation of lysosomal enzymes which might be contributing to the renal damage through autophagy/autolysis. The empty lysosomal vesicles also indicate this possibility.

It would be very interesting to find out which of the heavy metal or some metals in synergistic manner induce hypertension, through their interference in the production of angiotension II. Angiotensin II has been reported to be associated with renal hypertension (Atkinson 1982).

B) NORMAL ULTRASTRUCTURE AND ULTRASTRUTURAL CHANGES :

The electron microscopic studies of the control bird clearly reveal the presence of reptilian and mammalian tubules. The reptilian tubules characteristically bear the oblong or oval mitochondria who bear long cristae which traverse the mitochondrial matrix from one end to other and the cytoplasmic channels are flanked by the mitochondria. These features are clearly noticed in the electron microscopic studies of calotes kidney. The study of rat kidney under electron microscope reveal the renal tubules bearing round mitochondria with cristae leaving minute empty spaces and interestingly the mitochondria are encompassed by the cytoplasmic channels to maintain close associations which might be for transporting nutrients or contents of cytoplasm to the mitochondrion and vice versa. When the duck kidneys are studied electron microscopically, the renal tubules exhibit the characteristics of both the reptilian and mammalian tubules. The reptilian type of mitochondria with their characteristic features of cristae and cytoplasmic channels are seen, indicating that such tubules may be reptilian type. On the other hand, the tubules bearing mammalian type mitochondria with cytoplasmic channels encompassing them or maintaining very close relations, may be considered as mammalian types. Braun and Dantzler (1972) have reported the presence of reptilian and mammalian nephrons in birds on the basis of anatomy of nephrons, especially on the basis of absence or presence of loop of Henle. Desai (1983) reports the presence of reptilian and mammalian nephrons in *Gallus domesticus* on the basis of distribution of mucopolysaccharides. Now, here an ultrastructural evidence is provided for the presence of reptilian and mammalian renal tubules on the basis of mitochondrial characteristics and associations of cytoplasmic channels.

ULTRASTRUCTURAL CHANGES :

The functional unit of kidney is a nephron which includes glomerulus and the tubules. The normal glomerulus exhibits tufts of capillaries arranged in the form of lobules and a framework of extracellular matrix and cells in so called centrilobular location called as mesangium. The capillary walls of glomerulus appear intact with foot processes of podocytes separate from each other. The capillary lumina and Bowman's space appear normal. The microvilli known as brush borders of the proximal tubules appear intact in the normal kidney and the lumina are clear. The tubule cells exhibit normal mitochondria, intact lysosomal vesicles, normal cytoplasm, normal nuclei and no deposition of heavy metals. The chromatin material of the nuclei remain intact. The distal tubules show comparatively wide lumina and the tubule cells show no lipid droplets. The collecting tubules also exhibit intact tubule cells and luminal border.

The low M.E. concentration (0.01%) fail to induce any ultra structural change up to the end of twenty nine days but by the end of thirty days the ultrastructural changes are seen. This could be due to the non accumulation of the threshold level of heavy metals up to the end of twenty nine days and on the thirtieth day, probably the threshold level of heavy metal depositions occur leading to the onset of necrosis.

The ultrastructural changes occurring in the glomeruli are M.E. concentration dependent as well as exposure period dependent, especially the severity and the number of glomeruli involved in necrosis depend upon the two factors mentioned above.

In general, the M.E. induced glomerular changes are like fusing of foot processes, swelling of podocytes, thickening and distorsion of basement membrane, reduction of endothelial cell size, widening of Bowman's space and basement membrane, vacuolisation of the podocyte cytoplasm and disruption

of the basement membrane. In addition, the swelling of mitochondria, mitochondrial damage, endothelial sclerosis, mesangial proliferation (diffused proliferative glomerulonephritis) are seen.

The higher concentrations of M.E. induce severe damage to the glomeruli. Under the influence of 10.0% M.E., the foot processes are completely lost and the capillary - scleroderma gets induced. The 50.0% M.E. induce maximum damage. Many glomeruli exhibit focal glomerular sclerosis and a few show mesangial glomerulonephritis where mesangial proliferation, vacuolisation of podocyte cytoplasm and capillary endothelial sclerosis are seen.

The 100.0% M.E. promote membranous glomerulonephritis along with fused foot processes, increased Bowman's space, microvilli tresspassing in the Bowman's space and scleroderma. These ultrastructural changes may be occurring due to the heavy metal depositions in the renal tissue leading to the toxicity. The swelling and bursting or loss of cristae of mitochondria would affect the cell's energy budget and the degenerative changes may be set in, leading to the death of cells, thereby causing ultrastructural changes and dysfunctioning of glomeruli. Prasad Rao *et. al.*, (1989) have reported marked changes in the podocytes with fusion of secondary processes with no pedicel differentiation under the influence of methyl mercury, cadmium and lead administered in combination to the pekin ducks and they consider the ultrastructural changes due to the metal toxicity. In the present investigation the M.E. contain metals like cadmium, copper, chromium, iron, magnesium, manganese, nickel, lead and zinc and all these metals are retained by the kidney in different concentrations. Therefore, these metals in a synergistic manner could be inducing ultrastructural changes in the glomeruli in a concentration and exposure period dependent manner as has been found by Prasad Rao *et. al.*, (1989) in ducks under the influence of few metals.

(for 100.0% M.E. only), zinc (for 50.0 and 100.0% only). The forty eight hour exposure to M.E. induces maximum retentions of cadmium (for 0.1% and 50.0% M.E.), iron (for 0.1% M.E.), lead (1.0% M.E.), magnesium (50.0% M.E.), manganese (10.0% M.E.). The seventy two hours exposure to M.E. causes maximum retentions of cadmium (1.0% M.E.), copper (10.0% M.E.), iron (0.1% M.E.), lead (1.0, 5.0 and 50.0% M.E.), magnesium (5.0% M.E.) and nickel (10.0% M.E.). The ninetysix hours exposure induces maximum retentions of chromium (100.0% M.E.), copper (1.0% M.E.), iron (0.1% M.E.), lead (10.0% and 100.0% M.E.), manganese (5.0% M.E.) and nickel (1.0% and 5.0% M.E.) and one hundred and twenty hours exposure promotes maximum retentions of cadmium (0.1% M.E.), chromium (10.0% M.E.), copper (50.0% and 100.0% M.E.), iron (0.1 and 100.0% M.E.), magnesium (1.0% M.E.) and zinc (1.0, 10.0, 50.0 and 100% M.E.). Thus zinc from the M.E. gets retained maximally at very long exposures. The very low concentrations of M.E. induce retentions of cadmium and iron only and hence these metals may be major contributors for the renal necrosis. The differences in the retentions of different metals and maximum retentions at specific exposure periods may be explained on the basis of differential mobilisations of the metals in the animal body and also on the basis of differences in the renal eliminations in different quantities as well as the variations in the passing on of metals towards blood.

Generally, the animals tend to eliminate the metals either by excreting them through urine, faeces and feathers or by retaining them in different tissues for detoxification by binding them to some proteins. The kidneys may be attempting to eliminate the metals brought to them, through renal clearances and the excess amount may be retained to avoid the toxic actions. Some metals like zinc, cadmium, copper, magnesium may be getting retained in high concentrations in the kidney after prolonged exposures. These metals may promptly be mobilised to other organs for detoxification and to the feathers in early hours as well as may be eliminated through urine, faeces and feathers.

(Furness *et. al.,* 1986 a ; Braune and Gaskin, 1987 a, b ; Thompson and Furness, 1989 ; Furness *et. al.*, 1990 a ; Thompson *et. al.,* 1991). But once the threshold levels of retention are reached in other organs, these metals may be brought in large quantities to the kidney for elimination. This may result in accumulation of these metals in the kidney. Muirhead and Furness (1988) and Murton *et. al.,* (1978) are of the opinion that some birds may have high metal burdens for reasons of natural accumulation or detoxification processes unrelated to pollution. In the present investigation the ducks exhibit high metal burdens as a result of drinking contaminated water. Thus, in the natural environment or the habitats of the ducks, if the water bodies are contaminated with heavy metals, the ducks may develop high burdens of heavy metals after drinking such a contaminated water or by eating the food contaminated with heavy metals.

The findings in the present investigation of the retentions of heavy metals in the kidneys of ducks and nepthrotoxicity induced by them are well in agreement with the observations of several researchers who analysed the heavy metal burdens in the kidneys and its pathology in the natural populations of birds. Simpson *et. al.,* (1979) reported increase in kidney lead concentration in mute swans (*Cygnus olor*) and found the chronic lead toxicity induced by them. Stoneburner *et. al.,* (1980) found high cadmium and selenium retentions in the kidneys of sooty terns. Hacker *et. al.,* (1981) observed lead and cadmium in the kidneys of herons (*Hydranassa tricolor*). Karlong *et. al.,* (1983) noticed retentions of cadmium, copper, lead and mercury in the kidneys of common eiders (*Somateria mollissima*). Guilio (1993), finds retentions of cadmium, copper, lead and zinc in the kidneys of water fowls. Nicholson *et. al.,* (1983) studied nephrotoxicity of cadmium and mercury in the sea birds. Struger *et. al.,* (1987) reported highest retentions of cadmium in the kidney along with relatively less retentions of lead, mercury in the herring gulls. Honda *et. al.,* (1986) showed renal retentions of cadmium, copper, iron, maganese,

mercury, lead, nickel and zinc in great white Egret (*Egretta alba*). King and Cromartie (1986) detected renal retentions of cadmium, mercury, lead, and selenium in water bird species. Braune and Gaskin (1987) detected mercury in the kidney of Bonaparte's gulls (*Larus - philadelphia*). Muirhead and Furness (1988) reported kidney lesions due to cadmium, copper, mercury and zinc in the sea birds. Prasad Rao *et. al.,* (1989) reported nephrotoxicity and ultrastructural changes in the kidneys of pekin ducks under the influence of cadmium, methyl mercury and lead when these were administered to the ducks in large doses or in combinations. Honda *et. al.,* (1990) detected renal retentions of cadmium, copper, iron, manganese, mercury and zinc in the pelagic sea birds. Lewis and Furness (1991) showed the retentions of mercury in the kidneys of laboratory reared black - headed gull (*Larus ridibundus*) chicks. Elliott *et. al.,* (1992) studied the renal retentions of cadmium, lead and mercury along with other eighteen trace elements in the Atlantic Canadian Seabirds. Hontelez *et. al.,* (1992) observed renal retentions of cadmium and lead in mallards staying in polluted environment. Lock *et. al.,* (1992) reported higher retentions of cadmium and relatively less retentions of copper, mercury and zinc in sea birds. Kozulin and Pavluschick (1993) found kidney retentions of cadmium, chromium, copper, iron, lead, manganese, nickel and zinc in the mallards (*Anas platychynchos*) inhabiting in the natural habitat suspected to be deteriorated. St Louis (1993) found metal accumulations in the kidneys of tree swallows. El - Shabaka *et. al.,* (1993) demonstrated lead nitrate induced destructions in the nephric units of kidneys, aspecially the proximal, distal and collecting tubules. Moreover they found reduction in the glomerular size of such kidneys. Guitart *et. al.,* (1994) showed lead accumulation in the kidney of mallards. Spalding *et. al.,* (1994) showed nephrotoxicity of mercury to the great white herons leading to the death. Stewart *et. al.,* (1994) showed renal retentions of cadmium, copper, mercury and zinc in common guillemots (*Uria aalge*). Bakre and Sharma (1995) have recently reported higher lead

concentrations in the kidneys of sparrows (*Passer domesticus*). Carpene *et. al.*, (1995) demonstrated renal retentions of cadmium, copper, iron and zinc in the water fowls.

Thus, it is observed that many different bird species tend to accumulate heavy metals in their kidneys which then show renal necrosis when the threshold limit of tolerance is reached. It is interesting to note that most of the workers detected the renal retentions of heavy metals and its toxic actions in the natural populations of bird species and very few have treated the bird with the heavy metals in different physiological and non-physiological doses given either intramuscularly or intraperitoneally.

The present investigation also shows the retentions of heavy metals in the mallards exposed to mining effluents through drinking of water contaminated with M.E. and the ducks subsequently show the renal necrosis, the degree of which depends upon the exposure period and M.E. concentrations. Based on the observations of several researchers, it can be concluded that the kidney retains the heavy metals present in the mining effluents and these heavy metals may be responsible for the renal damage. The retentions of heavy metals in the kidney are in well agreement with the findings of several researchers. The present investigation also clearly reveals the toxic actions of the mining effluents.

B) SERUM :

The ducks (*Anas platyrhynchos domesticus*) exposed to mining effluents (M.E.) retain cadmium, chromium, copper, iron, lead, magnesium, manganese, nickel and zinc in the serum. The six hours exposure of birds to M.E. induce maximum serum retention of cadmium (5.0% and10.% M.E.), chromium (5.0%, 10.0%, 50.0% M.E.), iron (1.0%, 10.0% and 100.0% M.E.) magnesium (1.0% and 50.0% M.E.) and nickel (10.0% and 100.0%). The twelve hours exposure promotes maximal serum retentions of cadmium (50.0% M.E.), copper (10.0%

M.E.), and zinc (1.0% and 1.0% M.E.) and no other metals are retained. After twenty four hours exposure the serum retains cadmium (100.0% M.E.), copper (50.0% and 100.0% M.E.), lead (50.0% M.E.), magnesium (0.1% M.E.), and zinc (0.1% M.E.). The forty eight hour exposure of M.E. induces high retentions of lead (5.0 and 10.0% M.E.), magnesium (100.0% M.E.), nickel (1.0% M.E.) and zinc (50% M.E.). At the end of seventy two hours the serum retains, maximally cadmium (1.0% M.E.), chromium (0.1% M.E.), copper (5.0% M.E.), lead (1.0% and 100.0% M.E.), nickel (5.0% M.E.) and zinc (5.0% M.E.). The ninetysix hour exposure to M.E. promote high serum retentions of cadmium (0.1% M.E.), chromium (50.0% M.E.), copper (0.1% M.E.), and Iron (50.0% M.E.). But one hundred and twenty hours exposure of ducks to M.E.. induce maximum serum retentions of chromium (100.0% M.E.), iron (0.1% and 5.0% M.E.), lead (0.1% M.E.), magnesium (5.0% and 10.0% M.E.), manganese (0.1% and 100.0% M.E.), nickel (50.0% M.E.) and zinc (1.0% and 100.0% M.E.). Thus, the mining effluents induce retentions of different metals into the serum in different concentrations at different exposure periods.

The retentions of the heavy metals into the serum may be due to the entry of heavy metals into the circulation through the absorption of heavy metals by the stomach and intestine of birds when they drink the mining effluents. The kidney tends to retain the heavy metals in relatively higher concentrations and some of these metals may be passed on to the blood (Merill *et. al.,* 1956). The fluctuations in the serum retentions of the heavy metals may be due to the differential passing down of heavy metals against the concentration gradient into the extracellular space (Merill *et. al.,* 1956) as well as due to the variations in the release of heavy metals into the circulation by the kidney, liver, muscles and alimentary tract and bones which are also known to retain the heavy metals (Borg *et. al.,* 1969 ; Blus *et. al.,* 1989). The amount of heavy metals released in the serum may also depend upon the degree of necrosis developed in different soft tissues of the body. Several researchers

have reported the accumulation of heavy metals in the blood (Mautino and Bell, 1987; O' Halloran *et. al.*, 1989 ; Trust *et. al.*, 1990 ; Samuel *et. al.*, 1992 ; Henny *et. al.*, 1991 ; Havera *et. al.*, 1992 ; Loranger *et. al.*, 1994) and its toxic actions on the blood constituents. Thus, the occurrence and retention of heavy metals in the duck serum are well in agreement with the observations of several researchers cited above.

II) RENAL CLEARANCE OF HEAVY METALS :

The mining effluents induce significantly high renal clearances of heavy metals but the amounts of heavy metals cleared by the kidney vary for different exposure periods.

The six hours exposure to mining effluents promotes maximum renal clearance of cadmium (10.0% M.E.), chromium (0.1% and 50.0% M.E.), iron (0.1% M.E.), magnesium (10.0% M.E.), manganese (0.1% M.E.) and nickel (5.0% and 100.0% M.E.). The twelve hours exposure of ducks to M.E. induce maximum renal clearance of chromium (100.0% M.E.), iron (0.1% M.E.) and manganese (0.1% M.E.). By the end of twenty four hours exposure to M.E. the ducks show maximum renal clearances of chromium (0.1% M.E.), iron (0.1% M.E.), magnesium (10.0% M.E.), nickel (10.0% M.E.) and zinc (0.1%, 1.0%, 50.0% and 100.0% M.E.). The M.E. induces maximum renal clearances of cadmium (0.1% M.E.), copper (0.1% M.E.), iron, (0.1% M.E.), lead (5.0% M.E.), magnesium (5.0% M.E.), manganese (0.1% and 50.0% M.E.) and zinc (0.1% M.E.) at the end of fortyeight hours.

The exposure of ducks to M.E. for 72 hours induce maximum renal clearance of cadmium (0.1% and 5.0% M.E.), chromium (10.0% M.E.), copper (5.0% and 10.0% M.E.), iron (0.1%, 5.0% M.E.), lead (1.0% M.E.) and magnesium (100.0% M.E.). The maximum renal clearances of cadmium (0.1%, 50% and 100.0% M.E.), chromium (1.0% and 5.0% M.E.), copper (0.1% M.E.),

iron (0.1% M.E.), magnesium (0.1% M.E.), manganese (0.1% M.E.), nickel (50.0% M.E.), and zinc (5.0% M.E.) are induced by mining effluents at the end of ninetysix hours.

The exposures of ducks for one hundred and twenty hours to M.E. induces maximum renal clearances of chromium (50.0% M.E.), copper (1.0% and 100.0%), iron (1.0%, 10.0% and 100.0% M.E.), lead (10.0%, 50.0% and 100.0% M.E.), magnesium (1.0% and 10.0% M.E.), manganese (1.0%, 5.0% and 100.0% M.E.), nickel (1.0% M.E.) and zinc (10.0% M.E.).

Thus, the maximum renal clearances of heavy metals do not exclusively depend upon the concentrations of M.E. and the exposure periods. The renal clearances of heavy metals fluctuate under the influence of mining effluents.

Unfortunately there is not a single reference available till today on the renal clearances of heavy metals by the birds under the influence of pollutants.

In about 37% occasions the high renal clearances of heavy metals are associated with either high kidney retentions or high serum retentions of heavy metals. But at about 63% occasions no such correlation is seen. Under such circumstances the high renal clearances of heavy metals may be due to the progress of necrosis and release of exudents in to the tubules lumina. The fluctuations in the renal clearances of heavy metals may be due to the variations in the degree of necrosis and the variations in the number of tubules getting necrotic, and the release of heavy metals by the tubules across the luminal borders.

HAEMATOLOGICAL ALTERATIONS :

I) ALTERATIONS IN HAEMOGLOBIN CONCENTRATIONS :

All the concentrations of mining effluents influence the Hb levels of the ducks and the 100% M.E. induces maximum reduction in Hb concentration at the end of twenty four hours. The mining effluents induce fluctuations in the Hb

concentrations at different time intervals and all the while, Hb concentrations are maintained at a level below that observed in the control. Mautino and Bell (1987) reported altered heme synthesis in mallards under the influence of lead. Henny *et. al.,* (1991) found no adverse change in the Hb concentrations in Osprey under the influence of lead. Hoffman *et. al.,* (1991) noticed increase in Hb concentrations in mallard ducklings under the influence of barium and selenium. The reductions in the Hb concentrations in ducks exposed to mining effluents which contain heavy metals may be considered due to reduction in heme synthesis as suggested by Mautino and Bell (1987) and Hunter and Webeser (1980) and also it may be due to decrease in RBC counts observed in the present work. The heavy metals present in M.E. may be influencing Hb concentrations due to metal toxicity. Similar observations on the reduction in the Hb concentrations of fishes under the influence of heavy metals have been reported by several researchers (Shandilya and Banerjee, 1989 ; Rao *et. al.,* 1990 ; Mukherjee and Sinha, 1993 ; Gill and Epple, 1993). The fluctuations in Hb concentrations under the influence of M.E. may be due to the fluctuations in the RBC counts, heme synthesis and alterations in the erythropoiesis as well as due to variations in the release of mature RBCs from the erythropoietic material.

II) ALTERATIONS IN RBC COUNT :

The present investigation clearly shows the influence of mining effluents on the erythropoiesis as evidenced by the reduction in total erythrocyte counts. The mining effluents induce reduction in total RBC count and it undergoes fluctuations at various time intervals depending upon the M.E. concentration and the exposure period. The 5.0% M.E. induces significant recovery in the total RBC count at the end of ninetysix hours while the 100% M.E. induces the highest reduction in the total RBC count at the end of twenty four hours.

The reduction in the total RBC count may be due to the supression of the erythropoiesis caused by the mining effluents, especially the heavy metals from the mining effluents may be synergestically affecting the erythropoiesis. Unfortunately, there is hardly any work done on the birds with reference to erythrocyte count alterations induced by heavy metal toxicity. But the alterations observed in the RBC count of ducks under the influence of mining effluents are well in agreement with the findings of several researchers in the fishes, under the influence of heavy metal toxicity. Banerjee and Kumari (1988) reported reduction in total RBC count in Anabas under the influence of zinc, mercury and cadmium. Shandilya and Banerjee (1989) reported erythropenia (decrease in erythropoiesis) in Heteropneustes under the influence of zinc and chromium. Rao *et. al.*, (1990) reported decrease in total erythrocyte count in fishes inhabiting polluted waters of Vishakhapatnam harbour. Allen (1993) reported lead induced reductions in total RBC counts in Oreochromis. Gill and Epple (1993) showed the decrease in total erythrocyte count in Anguilla under the influence of cadmium. Mukherjee and Sinha (1993) showed lead induced decrease in total erythrocyte count in Labeo. Wepener *et. al.*, (1992) opined that the use of haematological methods as indicators of sublethal stress can supply valuable information concerning the physiological reactions to the changing environment. Saravanan and Natarajan (1991) argued that haematological parameter changes under metal-toxication are indicators of metallic stress. Thus the reduction in the total erythrocyte count observed in the ducks under the influence of mining effluents may be considered as an indication of stress and the continuous decrease in erythrocytes may lead to erythropenia. The decrease and fluctuation in RBC count may also be due to the decrease and fluctuations in renal erythropoietic factor responsible for splitting globulin from blood to form erythropoieten which stimulates erythropoiesis. The degree of renal necrosis would disturb the release in renal erythropoietic factor, thereby decreasing or fluctuating

erythropoiesis (Erslev, 1975). The fluctuations in the total erythrocyte counts could be considered due to the rise and falls in the heavy metals from the blood which may promote rise and falls in the heavy metal depositions in bone marrows and may also induce alterations in oxygen supply and also on the degree of renal necrosis & release of renal erythropoietic factor. It would be very interesting to study the effects of mining effluents on the bone marrow with a view to study erythropoiesis. The slight recovery in the RBC count may be considered as an attempt of the animal body to cope up with the reduction in RBCs and O_2 supplies to the tissues.

II) ALTERATIONS IN WBC COUNTS :

The mining effluents (M.E.) induce increase in the total leucocyte counts in rather independent of concentrations and exposure periods. The 50% M.E. induces maximum rise in the total WBC counts at the end of twenty four hours exposure. Though M.E. induces elevations in leucocyte numbers, the leucocyte counts vary for different exposure periods. But in general, the M.E. induces rise in the leucocyte counts.

There is hardly any report on the synergestic actions of heavy metals in the bird leucocyte counts. The elevations in WBC counts are well in agreement with the observations made by several researchers on the actions of heavy metals in the fish leucocyte counts (Shandilya and Banerjee 1989 ; Rao *et. al.*, 1990 ; Gill and Epple, 1993 ; Alkahem, 1994 ; Allen, 1994). The fluctuations in leucocyte counts could be due to the variations in serum heavy metal levels and variations in O_2 supplies as well as due to the variations in depositions of heavy metals in the bone marrow. Menkin (1955) isolated two polypeptides, thermolabile and thermostable in the fractions (protein) of cell exudents and showed that these were the leucocytosis promoting factors. In the present investigation the cellular exudents are released as a result of necrosis and these exudents may contain the leucopoietic factors to promote

leucopoiesis. The rise and fall in the concentration of these factors may induce rise and fall in the WBC count, but it needs further investigation to find out how mining effluents influence bone marrow and promote leucopoiesis. Also, the depositions of heavy metals from the bone marrows need also be studied at length.

IV) ALTERATIONS IN WBC DIFFERENTIAL COUNTS :

The mining effluents (M.E.) induce significant changes in the Neutrophils, Lymphocyte and Eosinophil percentages. The 1.0%, 5.0% and 50.0% M.E. induce decrease in the neutrophil count, especially 5.0% and 50.0% M.E. induce sharp reduction in neutrophils at the end of 24, 48 and 72 hours exposures. The 0.01%, 0.1%, 10.0% and 100% M.E. induce increase in neutrophil counts at some time intervals and at few, the level fluctuates. But interestingly the 0.01%, 0.1%, 10.0% and 100% M.E. induce sharp reductions in lymphocyte counts and at some times, the lymphocyte count was found to be below normal. All the concentrations of M.E. induce eosinophilia to the ducks at many exposure periods and in few instances the eosinophil count drops. Thus, the M.E. induce mark alterations in the differential counts, especially the granulocyte counts are elevated.

There are hardly any reports on the heavy metal induced alterations in the birds' WBC differential counts. Some researchers have relied on the heavy metal assays of blood for detecting retentions. O'Halloran *et. al.,* (1989) reported lead induced haematological disorders. Hoffman *et. al.,* (1991) describe the Barium and Selenite induced changes in hematocrit, Hb and plasma proteins of mallard ducklings. Loranger *et. al.,* (1994) report biochemical profiles in pigeons under the influence of Mn^{++} contamination. But the heavy metal induced alterations in the WBC differential counts have been reported for fishes. Shandilya and Banerjee (1989) reported increase in lymphocytes of fishes exposed to zinc and chromium. Rao *et. al.,* (1990) have

observed increase in lymphocyte count and leucocytosis under the synergistic action of Pb, Cd, Cu, Fe, Zn and Mn in fishes inhabiting polluted waters. Gill & Epple (1993) report significant increase in the granulocytes (Neutrophils & Eosinophils) in Anguilla exposed to cadmium. Alkahem (1994) also reported increase in granulocytes in *Oreochromis sp.* under the influence of nickel.

In the present investigation the increase in granulocytes is observed under the influence of mining effluents which may be considered due to the heavy metals present in the M.E. as observed in fishes. The exudents and inflammatory cells release a neutropoietin factor which promotes rise in neutrophils production when this factor reaches the bone marrow through circulation. The release of neutropoietin may depend on inflammation and necrosis (Chatterjee, 1977). Eosinophil production may be increased under the influence of similar factor, probably for promoting phagocytosis of the tissue debris and products of necrosis from blood.

The mining effluents may be influencing leucopoietic tissues thereby influencing the leucopoiesis, especially M.E. may be influencing the bone marrow through leucopoietic factors and may be influencing the myeloblasts or stem cells responsible for the leucocyte differentiations. The rise in eosinophils can be considered as a response to the sublethal stress of heavy metals. Thus, it appears that the mining effluents are inducing neutrophilia and eosinophilia in the ducks. The fluctuations in the differential counts may be due to the fluctuations in the blood retentions of heavy metals as well as retentions in bone marrow and also due to the degree of necrosis and release of leucopoietic factors.

It needs further investigation to throw more light on the actions of mining effluents on the leucopoietic tissue and differentiation of leucocytes. It would be interesting to find out the exact mechanism of the induction of granulocyte elevations.

V) ALTERATION IN BLOOD PLATELETS :

The mining effluents alter the blood platelet counts independent of M.E. concentrations and exposure periods. The 1.0% M.E. induces acute increase in the blood platelet counts at the end of six hours exposure and other M.E. concentrations fail to promote such a rise in blood platelets. The M.E. concentrations like 0.01%, 0.1%, 5.0%, 10.0%, 50.0% and 100.0% promote increase in the blood platelet counts and it undergoes fluctuations. It is known that the blood platelets are useful for repairs of capillary endothelium, initiation of blood clotting and assisting haemostatic mechanisms. The M.E. are inducing necrosis of kidney, glomerular-capillaries, and may also be causing injuries to other tissues, which result in bleeding. The increase in the production of blood platelets may be in response to the necrosis and bleeding, in order to effectively clot the blood, repair the capillary endothelia of glomeruli. Therefore, the rise and fluctuations in the blood platelet number may be due to the variations in the degree of necrosis and bleeding as well as partly due to the variations in the activation of megakaryocytes from the bone marrow for the formation of blood platelets.

VI) ALTERATION IN ERYTHROCYTE SEDIMENTATION RATE (E.S.R.) :

The mining effluents influence the Erythrocyte Sedimentation Rate (E.S.R.) very significantly. The 0.01%, 5.0%, 50.0% and 100.0% M.E. induce sharp increase in E.S.R., while 0.1% and 1.0% M.E. induce sharp decline in E.S.R. at the begining and then increase and show some fluctuations. The 10.0% M.E. induces steady rise in E.S.R. up to the end of fortyeight hours and then induces decrease.

The E.S.R. accelerates when the plasma bears an increased positive charge. The plasma positive charge increases when cholesterol, lipids, fibrinogen and α - globulin levels from the blood rises. In the present studies,

the renal necrosis have been shown to release tissue exudents and promote heavy lipid depositions in the kidney as evidenced by the light microscopic and ultrastructural studies. The tissue injuries may release lipids, cholesterol in specific and other tissue proteins in to the circulation which may increase the E.S.R. (Chatterjee, 1977). Besides the increases in leucocyte numbers and thrombocyte numbers (blood platelet counts) may also alter the density of formed elements, thereby accelerating E.S.R. The accelerated rates of E.S.R. suggest organic disease rather than functional disorder. Therefore, the accelerations and de-accelerations of E.S.R. may be due to the increase and decrease in the release of substances from the injured tissues which may increase the plasma positive charge and also may alter the densities of the formed elements. Besides, the decreases in E.S.R. are also due to the increases in the nucleoproteins in circulation (Chatterjee 1977). Therefore, the sharp decrease in E.S.R. may also be due to the increase of nucleoproteins from the blood due to the increased breakdown of tissue debris by the lysosomal enzymes. The E.S.R. fluctuations therefore, may be due to the rise and fall in blood lipid, proteins and nucleoproteins depending upon the necrosis and its progress.

BIOASSAY OF SERUM :

I) ALTERATIONS IN PROTEINS :

All the concentrations of mining effluents (M.E.) at all the time intervals (except 1.0% M.E. at the end of seventytwo hours) induce rise in the serum protein levels but the M.E. does not induce concentration dependent or exposure period dependent elevations in the serum proteins.

The M.E. induce renal necrosis and may also be inducing injuries to other organs like alimentary tract, liver, etc. The rise in the serum proteins may

be considered as due to the necrosis of the kidney and other organs and the proteins from the necrotic tissues may be passed down to the blood as per Merill's hypothesis (1956), thereby increasing the serum protein levels. All the M.E. concentrations induce necrosis, therefore, the serum protein levels elevate for all the concentrations. The fluctuations in the serum protein levels, may be occurring due to the variations in the degree of necrosis at different time intervals for different M.E. concentrations which may vary the release of proteins into the serum. Also the variations in the degree of necrosis of other organs may influence the release of proteins in the serum and the variations in the renal clearances of proteins through urine may also induce fluctuations in the serum protein levels. The pronounced release of proteins through the urine may induce sharp decreases in the serum protein levels. Besides the passing of proteins against the concentration gradient in to the extracellular spaces may also induce variations in the serum protein levels.

II) ALTERATIONS IN ACID PHOSPHATASE (AP) :

From 0.1% to 50.0% M.E. concentrations there is induction of concentration dependent elevations in the serum acid phosphatase activity at the end of forty eight hours exposure while 10.0% and 50.0% M.E. concentrations induce exposure period dependent enhancement in the AP activity from the end of six hours to the end of fortyeight hours. The 5.0% M.E. induces the highest reduction in the serum acid phosphatase activity at the end of seventy two hours exposure while 50.0% M.E. induces reduction in AP activity at the end of one hundred and twenty hours. The 100.0% M.E. concentration induces reduction in AP activity below normal level at all the time intervals under study except six hours. The 10.0% M.E. induces highest rise in serum AP activity at the end of one hundred and twenty hours.

The rises in the serum AP activity may be occurring due to the progressive necrosis during which the AP passes down to the serum, thereby increasing serum AP level (Merill *et. al.,* 1956). During necrosis the AP gets released from the injured tissues. Many researchers have shown that the increases in serum AP are due to the damage to the cells of an organ. Rees *et. al.,* (1961) ; Fox *et. al.,* (1962) ; Rees and Shotlander (1963) ; Slater and Greenbaum (1965) have shown that the carbon tetra chloride induced liver necrosis promoted rapid elevations in the serum enzyme activities due to the leakage from damaged tissues. Dingle and Fell (1968) suggested the scavenging action of AP during necrosis. The liver ischemia releases lysosomal enzymes in to the serum (Duvede Beaufay, 1959 ; Bassi and Barnelli - Zazzera, 1964 ; and Kerr, 1965). Baccino *et. al.,* (1965), Alpers and Isselbacher (1967) have reported the release of lysosomal enzymes during liver injury. Clinical studies have shown correlation between the severity of the diseases and the extent of enzyme activities in the serum. Janoff, *et. al.,* (1962) showed elevated levels of acid hydrolases in the circulating blood following inductions of tissue insults. Therefore, on the basis of the findings of above cited researchers, it can be inferred that the serum elevations in AP activity could be due to leakage of AP from the necrotic kidney as well as from the other organs exposed to the mining effluents. The fluctuations in the serum AP may be occurring due to the release of enzyme in the extracellular spaces against the concentration gradient (Merill *et. al.*, 1956) as well as may be due to the renal clearance of AP. The acute falls in AP can be explained on the basis of excessive renal clearances, excessive releases into the extracellular spaces as well as due to the recovery of the necrotic tissue up to certain extent.

III) ALTERATIONS IN ALKALINE PHOSPHATASE (AIP) :

The serum AIP activity gets influenced by mining effluents (M.E.). The 0.1% to 10.0% M.E. induce concentration dependent progressive elevations in

serum AIP at the end of six and twenty four hours. The 50.0% M.E. induces highest elevation in AIP at the end of six hours while the 100.0% M.E. induces highest reductions in the serum AIP at the end of one hundred and twenty hours. The 0.1% M.E. induces exposure period dependent elevation in the serum AIP while rest of the M.E. concentrations promote fluctuations in AIP activity.

The enhancements in the serum AIP activity may be explained on the basis of Merill *et. al.,* hypothesis (1956). The progressive renal necrosis and probable necrosis of other tissues may be promoting the leakages of lysosomal enzymes (AIP) in the serum thereby increasing the serum AIP levels. The reductions in the serum AIP may be occurring due to the renal clearances of AIP and its passing down the concentration gradients into the extracellular spaces as well as due to the recovery of necrotic tissues or due to the completion of necrosis of the affected tubules. If the necrosis of the affected tubules is completed and the lysosomal enzymes eliminated with lysosomal vacuoles getting emptied, the serum AIP activity may get acutely reduced for the want of leakage of AIP to the serum from the damaged tissue.

The fluctuation in AIP could be occurring due to the elimination of AIP through urine and due to the fluctuating releases of AIP from the damaged tissues.

IV) ALTERATIONS IN NONSPECIFIC ESTERASE :

All the concentrations of M.E. except 5.0% induce enhancement in serum nonspecific esterase activity in a concentration dependent manner at the end of six hours. Similarly 0.1% to 50.0% M.E. induce concentration dependent elevations in the serum nonspecific esterase activity at the end of ninetysix and one hundred and twenty hours. Besides, 0.1% and 5.0% M.E. induce exposure period dependent rise in the serum esterase activity. The progressive elevations in the serum esterase activity with reference to increase

in exposure periods can be explained on the basis of progress of necrosis as the exposure period lengthens. During necrosis the esterase (nonspecific) being lysosomal be getting released/leaked from the damaged kidney thereby elevating the serum esterase activity. The concentration dependent enhancement of serum esterase activity could also be occurring due to the passing of enzyme from the progressively necrotic tissues to the serum as suggested by Merill *et. al.,* (1956) and other researchers (Zelman *et. al.,* 1959 ; Alpers and Isselbacher, 1967, Janoff *et. al.,* 1962). The fluctuations in the serum esterase could be taking place due to the renal clearance of esterase, passing down of esterase into the extracellular spaces and infrequent release of esterase from the different organs in different stages of necrosis. The significant reductions in the esterase activity could be occurring due to the increased renal clearances or due to the decrease in leakages/release of enzyme from the necrotic tissue owing to the completion of necrosis or recovery of necrosis.

V) SERUM UREA :

All the concentrations of mining effluents induce elevation of serum urea level and up to certain extent the enhancement in urea concentration is dependent upon the exposure period. The M.E. induces fluctuations in the urea concentrations. The M.E. induces concentration dependent elevations in the serum urea level at the end of ninety six hours exposure. At many instances the higher concentrations of M.E. induce higher elevations in the serum urea levels.

The elevations in the serum urea may be due to M.E. induced urea retentions in the kidney irrespective of the degree of necrosis. Desai (1983) have shown the urea retentions by the necrotic kidney due to elevations in kidney mucopolysaccharides of fowls. The kidney may be releasing the urea to the blood thereby increasing the serum urea levels. Therefore, the degree of

necrosis and the retentions of urea by the kidney may induce rise in serum urea levels due to the passing down of urea to the blood (Merill *et. al.,* 1956). Besides the rise in urea synthesis by liver as a result of increased protein metabolism may also be contributing to the serum urea rise, the fluctuations in the serum urea levels may be occurring due to the renal clearance of urea passing down of urea into the extracellular spaces against concentration gradient (Merill *et. al.,* 1956) as well as due to the rises and falls in the synthesis of urea. The sharp reductions in serum urea levels may be occurring due to the excessive renal clearances of urea.

VI) ALTERATIONS IN SERUM URIC ACID :

The mining effluents (M.E.) induce reductions in serum uric acid levels almost at all the time intervals except few. Though the serum uric acid levels are below normal the uric acid concentrations fluctuate under the influence of M.E. The 100% M.E. induces highest elevation of serum uric acid level at the end of ninetysix hours exposure while, the 50.0% M.E. induces the highest reduction in the serum uric acid levels at the end of seventy two hours.

The reduction in serum uric acid levels may be occurring due to the renal clearance of uric acid as well as due to the tendency of kidneys to retain more uric acid and partly due to the lesser release of uric acid to the serum with more passing down of uric acid to the extracellular spaces. The elevations in the serum uric acid levels could be considered due to the involvement of more tubules in the necrosis promoting more breakdown of nucleoproteins which could enhance the nucleotide metabolism, thereby producing more uric acid, which subsequently gets released into the serum. The fluctuations in the serum uric acid levels could be seen due to the variations in the renal clearances, variations in the passing down of uric acid to the extracellular spaces as well as due to the variations in uric acid synthesis by the kidney and liver.

VII) ALTERATIONS IN THE SERUM CREATININE :

The mining effluents (M.E.) induce concentration-dependent elevations in the serum creatinine level at the end of six hours and twelve hours (except for 10.0% M.E.). The M.E. induces wide fluctuations in the serum creatinine levels but at all the time intervals under the influence of all M.E. concentrations, the serum creatinine levels elevate. These elevations in the serum creatinine level could be occurring due to the increased metabolism of creatine induced by necrosis of kidney and probably of other tissues and muscles. The increased synthesis of creatinine may promote increased release into the serum.

The fluctuations in the serum creatinine may be considered due to the variations in renal clearances, passing down of creatinine to extracellular spaces and variations in the degree of necrosis.

VIII) ALTERATIONS IN SERUM CALCIUM :

All the concentrations of mining effluents (M.E.) except 10.0% induce concentration dependent elevations in the serum calcium levels at the end of six and twelve hours. The 10.0% M.E. induces highest rise in serum Ca^{++} levels at the end of one hundred and twenty hours exposure while the 0.1% M.E. induces acute reduction in the serum calcium levels at the end of six and twelve hours. In general, the serum calcium levels are maintained at a level higher than the normal under the influence of M.E. The M.E. induces significant fluctuations in the serum calcium levels.

The elevations in the serum calcium levels may be occurring due to the necrosis of the renal tubules as well as probably of other organs. In the present investigation the kidney tissue debris have been detected in the tubules lumina and also damages to the cytoplasmic organelle have been detected in the ultrastructural studies. As a result of this, the calcium from the

cytoplasm, calcium bound to the proteins and calcium of mitochondrial, endoplasmic reticular may be getting released into the circulation (Merill *et. al.,* 1956) thereby, elevating the serum calcium level. The fluctuations in the serum calcium levels can be explained on the basis of renal clearances of calcium, accompained by passing down of Ca^{++} into the extracellular spaces as suggested by Merill *et. al.,* (1956) and partly due to the variations in the degree of necrosis. The reductions in calcium levels may be taking place due to the renal clearance and excessive passing down of calcium to the extracellular spaces and also due to the lesser degree of necrosis in early hours due to the relatively low concentrations of M.E.

IX) ALTERATIONS IN SERUM CHLORIDES :

Mining effluents (M.E.) profoundly influence the serum chloride levels, especially the high concentrations of M.E. induce acute enhancement of serum chloride concentrations. The M.E. does not change the serum chloride concentrations exactly in an exposure and concentration dependent manner though the chloride concentrations get elevated at the end of six hours with reference to the rise in M.E. concentration indicating a limited concentration dependency. The 50.0% M.E. induce highest elevation in the serum chloride levels at the end of six hours exposure while, 0.1% M.E. induces acute reduction in the serum chloride level at the end of forty eight hours' exposure.

The elevations in the serum chloride concentrations may be taking place due to the increase in the degree of necrosis of the kidney as well as some other organs exposed to the M.E. In the present investigation the cytoplasmic debris have been seen accumulated in the tubules' lumina and as a result the tissue chlorides/cytoplasmic chlorides may be passed on to the serum as suggested by Merill *et. al.,* (1956), thereby increasing the serum chloride level. In addition the failure of the kidney to reabsorb and retain the chlorides from the lumina of the tubules also help in elevating the serum chloride levels. The

other organs undergoing necrosis may also release the chlorides from them into the general circulation. These extra release of chlorides may contribute significantly to the elevation of serum chlorides.

The fluctuations in the serum chloride levels may be considered due to the variations in renal clearance, partly due to the variations in the passing of chlorides to the extracellular spaces against the concentration gradients and may also be due to the variations in the release of chlorides from the necrotic tissues which may be in different stages of necrosis. The acute reductions in the chloride concentrations may be occurring due to the excessive passing on of chlorides in to the urine as well as into the extracellular spaces.

X) ALTERATIONS IN SERUM ELECTROLYTES :

a) SODIUM :

All the concentrations of M.E. induce elevations in the serum sodium concentrations at all the time intervals under study but the M.E. does not induce concentration dependent or exposure period dependent elevations in the serum Na^+ levels.

The elevations in the serum sodium levels may be occurring due to the release of electrolytes from the necrotic tissues. In the present investigation the cytoplasmic debris have been detected in the tubules lumina and this may promote the release of electrolytes from them to the blood (Merill *et. al.,* 1956), thereby elevating the serum Na^+ level. Besides the organs like alimentary canal and liver are likely to get exposed to the M.E. and may be undergoing necrosis. This may promote the release of Na^+ from the tissue debris into the serum. The fluctuations in the serum Na^+ levels may be occurring due to the variations in the release of Na^+ from the necrotic tissues depending upon the necrotic stage and also may be due to the variations in passing down the

electrolytes into the extracellular spaces and also probably may be due to the alterations in the release of electrolytes from the other necrotic tissues.

The sodium homeostasis is maintained primarily by the balance of intake and the urinary excretion of Na^+ . The regulation of the rate of Na^+ excretion is dependent upon complex control systems which involve several levels of functional organization, which include the interaction of the kidney with other organ systems such as liver, central nervous system and the endocrine system (Desai, 1983). The decrease in the Na^+ may be occurring due to the increased renal clearances of Na^+ or may be passing of Na^+ in excess into the extracellular spaces. But it needs further investigation to know if M.E. are influencing electrolyte homeostasis through its influence or interference in the liver, CNS and endocrine system.

b) POTASSIUM :

The M.E. induces elevations in the serum K^+ concentrations in a M.E. concentration dependent manner at the end of six, twentyfour and seventy two hours. The M.E. concentrations of 0.1% to 50% induce concentration dependent rise in serum K^+ levels at the end of twelve hours exposure. The 100.0% M.E. induces the highest increase in serum K^+ at the end of seventytwo hours. The M.E. induces fluctuations in serum K^+ levels for other exposure periods.

The elevations in the serum K^+ levels could be due to the release of K^+ from the necrotic tissues as suggested for Na^+. The histological studies have revealed the exudations of cytoplasmic debris into the tubules lumina from where the K^+ may be passed on to the blood. The elevations in serum K^+ may depend upon the degree of necrosis of renal tubules and also other organs undergoing necrosis. The fluctuations in K^+ levels could be occurring due to the variations in renal clearance of K^+ , variations in the release of K^+ from the necrotic tissue depending upon the stages of necrosis (early and late)

as well as due to the variations in passing of electrolytes to the extracellular spaces.

The reductions in serum K^+ levels may also be occurring due to the increased renal clearance of K^+ and may also be due to the increased passing of electrolytes to the extracellular space and partly due to the enhanced absorption of K^+ by some renal tubules. Thus it can be concluded that the mining effluents, even of a very low concentrations can be harmful to the aquatic birds exposed to these effluents for a long period. The mining effluents are likely to pass into the animals' body through the water intake and cause damage to the kidney depending upon the degree of contamination, thereby eventually causing death of birds. The kidneys and the blood of ducks retain heavy metals from the mining effluents and induce renal necrosis. The M.E. promote the rupture of mitochondria and proliferations of ER and Golgi bodies, thereby disturbing the renal metabolism which eventually impairs the kidney functioning. Thus the runoffs of the iron ore rejects, when contaminate the waterbody, would influence the aquatic forms and this may be the cause of elimination or decrease in the rapid decline of mallard populations from the mining belts as well as from the areas not far off from the mining belts. Not only this the runoffs when mix with the streams, rivulets and rivers would affect the animals far off from the mining sites.

CHAPTER - VII

SUMMARY

Though mining is the second largest industry after agriculture and is providing man some of the valuable materials like metals, chemicals, fuel for energy, rocks and stones for building houses, mining is also an environmentally damaging activity. Goa is known for its extensive iron ore deposits. The mining activities in Goa are disturbing several thousands of hectares of land. Another major event that attracts attention is the huge stock piling of iron ore rejects. Both the fresh mine spoils and the dumped iron ore rejects/ores could deteriorate the water qualities of the water bodies, especially when the effluents during monsoon reach them. The available data indicate that stream flows may be greater after mining (Brown *et. al.*, 1984). Collier *et. al.*, (1970) have shown that effluents from mining areas increase the sediment load by 9457 tonnes/km^2/year.

Mining pollution can affect birds, especially aquatic, directly through lethal or sublethal stress and indirectly through habitat alterations. Birds may acquire heavy metals from preening contaminated material off their feathers and by taking in food or water containing mining effluents or air pollution fallouts of mining origin. There are several reports of damaging action of heavy metals of industrial and agricultural origin. Especially mercury and lead toxicity has been reported for birds like pheasants, partridge, pigeons and a number of corvids. The other heavy metals like Zn^{++}, Co^{++}, Cu^{++}, Ni^{++}, Mn^{++}, Cd^{++}, Se^{+++}, As^{+++} and Al^{+++} have been reported to be retained and toxic to the birds. Toxic effects of the contaminants from the freshwater system have been reported for piscivorous birds, notably grebes, cormorants, pelicans, herons, gulls terns, ospreys and the aquatic kingfishers. But there is hardly any report on the toxic effects of mining effluents on the birds, especially on the toxic actions of runoffs on the kidney, urine and blood of mallards. Therefore, in the present studies an attempt has been made to study the toxic actions of the mining effluents on mallard (*Anas platyrhynchos domesticus*) kidney.

The iron ore rejects were collected from the mining sites and were subjected to the stimulated rainfall. The runoffs were collected and analysed. The runoffs contain heavy metals like Ba^{++}, Cd^{++}, Cu^{++}, Cr^{++}, Co^{++}, Fe^{+++} Mg^{++}, Mn^{++}, Ni^{++}, Pb^{+++}, Sr^{++}, Zn^{++}.

HISTOPATHOLOGY :

The routine histopathological studies of the kidney revealed the induction of necrosis of the nephrons of both the reptilian and mammalian type. The pathological changes were seen in renal capsule (Glomeruli + Bowman's capsule), proximal tubules, distal tubules and collecting tubules. The histopathological alterations were dependent upon M.E. concentrations and exposure periods. The M.E. concentrations induce in early hours of exposure, the swelling of glomeruli, marginal increase in lumen diameter of the tubules, occasional marginal swelling of the tubule cells, but in the later hours the necrotic changes were quite pronounced. The glomeruli exhibited distortion of glomeruli disruption of Bowman's wall, vacuolisation of glomeruli, release of exudents in the Bowman's space, widening of Bowman's space, breakage of glomerular capillaries leading to bleeding.

The alterations in the proximal and distal convoluted tubules were identical. Both these tubules showed necrotic changes depending upon M.E. concentration and exposure period. The prominent necrotic changes were : swelling of tubule cells, vacuolisation of tubule cells, loss of brush/luminal borders, disruption/loss of basal membranes, pycnosis of tubule nuclei, exudation of cytoplasm and nuclei into the tubule lumen or into the interstitium, widening of interstitium.

The collecting tubules also showed necrotic changes such as swelling of tubules, sloughing off of cellular contents or cells into the lumen, vacuolisation, pycnosis of nuclei, disruption of luminal and basement membranes, and total

loss of integrity. The severity of necrosis of collecting tubules was M.E. concentration and exposure period dependent. These pathological changes appeared to be induced due to the activation of lysosomes, increase of renal blood pressure and depositions of heavy metals, and depletion of energy.

ULTRASTRUCTURAL CHANGES :

The ultrastructural studies of the reptilian, avian and mammalian kidneys helped in identifying the mammalian and reptilian tubules from the bird kidneys. The reptilian kidney bears typical mitochondria with elongated cristae extending from side to side and the mitochondria are elongated/oblong. The cytoplasmic channels run between the rows of mitochondria or the mitochondria flank the cytoplasmic channels. The mammalian kidneys bear round/spherical mitochondria with cristae extending towards the centre and the cytoplasmic channels surround or encompass the mitochondria. The avian kidneys show the tubules bearing either reptilian type mitochondria or mammalian type mitochondria, therefore indicating the reptilian and mammalian architecture of the kidney.

The mining effluents induced ultrastructural changes in glomeruli. The prominent glomerular changes were : fused foot processes, thickening and distortion of basement membrane, reduction of the endothelial cell size followed by endothelial sclerosis, change in the shape of RBC, widening of basement membrane, vacuolisation of the cytoplasm of podocyte, mesengial proliferation, focal glomerular sclerosis.

Ultrastructural changes in proximal tubule :

The ultrastructural changes in the proximal tubules were : disruption of brush border, emptying of lysosomal vesicles,dispersion of nuclear chromatin, exudation of tissue debris in to the lumen, vacuolisation of cytoplasm,

destruction of mitochondria, depositions of heavy metals in the cytoplasm, swelling of P.T., and shedding of brush border.

Ultrastructural changes in Distal tubules :

The ultrastructural changes in the distal tubules were : vacuolisation of cytoplasm, swelling of Golgi bodies, depositions of heavy metals, pycnosis of nuclei, lipid depositions, reduction of luminal space, dispersion of nuclear chromatin, distortion of luminal borders, proliferation of Golgi bodies, formation of autophagic vacuoles, separation of tubule cells, disruption of basal membranes, heavy depositions of lipids, intranuclear depositions of heavy metals.

Ultrastructural changes in Collecting tubules :

The collecting tubules showed ultrastructural changes such as distortion of tubules, exudation of nuclei with tissue debris into the lumen, distortion of basal membrane, exudation of RBCs in the lumen, intranuclear heavy metal depositions, vacuolisation of mitochondria, heavy depositions of lipid droplets, reduction in luminal space, disruption of luminal border, swelling of Golgi bodies, proliferation of endoplasmic reticulum and ciliary processes.

The severity of the ultrastructural changes depended upon the M.E. concentrations and exposure periods. Thus the mitochondrial damage, proliferation of Golgi and E.R. and swelling of Golgi could lead to the severe alteration in the metabolism of kidney cells thereby inducing necrotic changes, especially loss of mitochondria could deprive or disrupt the energy supply.

Histochemical :

The histochemical studies of acid phosphatase, alkaline phosphatase and nonspecific esterase showed the M.E. concentration and exposure period dependent alterations in the enzyme activity and the changes in the

locations/sites of enzyme activity. The histochemical studies have revealed the existence of cytoplasmic, brush/luminal border and membrane bound enzymes. The enzyme activities varied in proportion to the degree of necrosis. The luminal debris exhibited enzyme activity. All the three enzymes being lysosomal be considered responsible for autodigestion/autophagy leading to the necrosis. The lysosomal enzymes have scavenging roles and are also responsible for the degradation of the proteins.

BIOASSAYS :

The bioassays of the kidney showed elevations of the lysosomal enzyme activities. The acid phosphatase, alkaline phosphatase and nonspecific esterase activities elevated in response to the mining effluents and exposure periods. Upto certain extent the elevations in the renal enzyme activities of the lysosomal types were M.E. concentration dependent and exposure period dependent. The lysosomal enzyme activities fluctuated at different time intervals but the increase in the enzyme activities were progressive and appeared to be in tune with the degree of necrosis. The increased enzyme activities were due to activation of lysosomes and they play an important role in the degradation of necrotic debris and degradation of proteins. The fluctuations in the enzyme activities could be due to the passing down of enzymes into the serum and extracellular spaces.

The bioassay of proteins showed progressive decrease in the total proteins of the kidney in a M.E. concentration and exposure period dependent manner. The kidney protein levels fluctuated widely. The decrease in the kidney protein levels is due to the necrosis of tissue and degradation of proteins by the lysosomal enzymes. The fluctuations in the kidney total proteins could be due to the release of proteins to the blood and extracellular spaces.

The kidney urea levels elevated in proportion to the exposure period and the M.E. concentration upto certain extent. The low M.E. concentrations increased kidney urea levels. The kidney urea levels fluctuated at different time intervals. The 100% M.E. induced highest elevation of kidney urea at the end of one twenty hours. The rise in kidney urea could be due to the retention of urea by the kidney and partly also due to the increased protein metabolism or degradation of proteins.

The kidney uric acid levels were influenced by the M.E. M.E. induced reduction in the uric acid concentrations of the kidney at the end of six hours under the influence of 0.1%, 1.0%, 5.0% 10.0% M.E. The 0.1% M.E. induced significant decrease in the uric acid concentrations of the kidney at the end of six, twelve and twenty four hours. The higher concentrations of M.E. induced pronounced increase in uric acid levels. The rise in uric acid levels could be due to increase in the uric acid synthesis by the kidney under the influence of M.E. and the fluctuations in the uric acid levels could be due to the variations in uric acid synthesis, renal clearance and release of uric acid into the serum.

The M.E. induced significant fluctuations in the creatinine concentrations of the kidney. The rise and falls in the kidney creatinine levels could be due to the alterations in creatine metabolism and altered retentions of creatinine by the kidney. The M.E. induced fluctuations in the kidney calcium and chloride levels. These fluctuations could be due to the degree of necrosis and variations in the release of calcium and chlorides by the kidney.

The urine analysis exhibited the occurrences of acid phosphatase, alkaline phosphatase, nonspecific esterase and proteins in the urine showing a pathological state called enzyme urea. Similarly, the urine analysis showed occurrences of urea, uric acid, creatinine, calcium, chlorides and electrolytes. The renal clearances of these substances depended upon the degree of necrosis and are released into the urine due to necrosis of kidney.

The M.E. induced alterations in the urine flow and glomerular filtration rates. Under the influence of mining effluents the urine flow and GFR reduced in proprotion to the M.E. concentrations and exposure periods. The alterations in urine flow and GFR were due to the renal necrosis, altered blood flow to the glomeruli.

RETENTION PATTERNS :

The mallards exposed to the M.E. exhibited kidney retentions of cadmium, copper, chromium, iron, lead, magnesium, manganese, nickel and zinc. The retentions of heavy metals varied in accordance with the concentrations of M.E. and exposure period. The degree of necrosis and retentions exhibited some correlation. The retentions depended upon the release of heavy metals from the kidney to the serum and urine. The serum also appeared to retain the heavy metals retained by the kidney. The serum retentions varied with the degree of necrosis, M.E. concentrations and exposure period. The serum retentions varied due to the variations in kidney retentions, renal necrosis, release of heavy metals from the kidney and due to the variations in the renal clearances. The urine analysis exhibited renal clearances of heavy metals in tune with serum levels of heavy metals, levels of heavy metals in the kidney and the concentrations of M.E. and exposure period. The heavy metals like Cd, Fe and Zn appeared to be retained more followed by Cr, Cu, Mg and Ni but the retentions depended upon the exposure period.

HAEMATOLOGICAL ALTERATIONS :

Under the influence of mining effluents the erythrocyte count reduced and fluctuated though it was less than normal. The 5.0% M.E. induced recovery in total RBC count at the end of ninety six hours. The 100% M.E. induced maximum reduction in the total RBC count at the end of twenty four hours. The

reduction in the total erythrocyte count under the influence of M.E. could be due to the M.E. action on erythropoietic tissue as well as reduction in the production of erythropoietin a substance produced by kidney. The variations in the erythropoietin may induce variation in erythropoiesis. The bleeding during renal glomerular damage can also influence RBC count.

The M.E. induced increase in the total leucocyte count, but the increase was neither M.E. concentration dependent nor exposure period dependent. The 50.0% M.E. induced maximum elevation of WBC count at the end of twenty four hours. The leucocyte number fluctuated under the influence of M.E. The elevation in the leucocyte counts may be due to more release of leucopoietin under the influence of M.E. and it could also be due to the renal necrosis, due to liberation of adrenaline under stress.

The 100% M.E. induced maximum reduction in Hb concentration at the end of twenty four hours. All the concentrations of M.E. promoted reduction in Hb concentration. The reduction in Haemoglobin concentrations could be due to the reduction in the total RBC count and probably due to the interference of M.E. in Hb synthesis, by way of inhibiting one of the enzymes involved in Hb synthesis.

The M.E. induced alterations in the differential counts. M.E. induced reduction in lymphocyte count and elevation in the neutrophils and eosinophils. The elevations in neutrophils and eosinophils varied with the M.E. concentrations and exposure period. The rise in neutrophils could be due to the release of neutropoietin, due to necrosis of renal tubules. The M.E. could not induce concentration and exposure period dependent alteration in blood platelet counts. The 1.0% M.E. induced sharp increase in the blood platelet count at the end of six hours, but the blood platelet number showed fluctuations under the influence of M.E. The blood platelet counts varied may be due to the influence of M.E. on the megakaryocytes. The E.S.R. alterations were neither M.E. concentration dependent nor exposure period dependent. The 0.1% and

1.0% M.E. reduced the ESR by the end of six hours. The maximum increase in ESR was observed under the influence of 5.0% and 100% M.E. at the end of ninety six and one twenty hours respectively. The E.S.R. values fluctuated under the influence of rest of the M.E. concentrations and exposure periods. The elevations in the E.S.R. could be occurring due to the increase in the formed elements like leucocytes and increase in the positive charges of the plasma. The reductions in E.S.R. could be due to the necrosis which releases nucleoproteins and rise in nucleoproteins can reduce the E.S.R.

The bioassay of serum exhibited significant changes in proteins, lysosomal enzymes like acid phosphatase, alkaline phosphatase, non specific esterase, urea levels, uric acid and creatinine concentrations, calcium and chloride levels as well as electrolyte levels. All these serum constituents exhibited elevations and drops under the influence of mining effluents and exhibited considerable fluctuations. The elevations, drops and fluctuations in serum proteins, and enzymes could be occurring due to the variations in the degree of necrosis and due to the necrosis, they are released in to the serum thereby altering the serum levels. All the above referred constituents of the serum exhibit fluctuations in their concentrations due to the necrosis, passing down of these substances into the extracellular spaces and also partly due to their varying release in the urine.

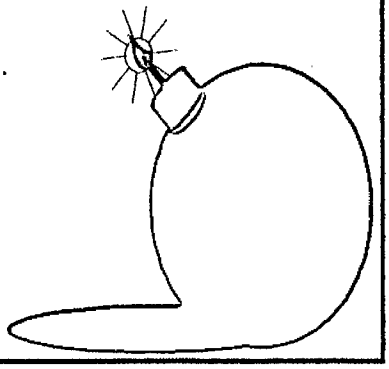
The present study clearly shows that the mining effluents could be toxic to the birds, especially aquatic birds who have very easy access to the water and due to the such a contaminated water the birds may have a high load of heavy metals which can be toxic to the renal apparatus and may result in stress and death of birds. Therefore, mining pollution could be one of the causes of rapid dwindling of duck populations and populations of aquatic birds.

SUGGESTIONS OF FUTURE WORK :

It needs further investigation to study how M.E. influence the erythropoietic tissue. How M.E., if influencing Hb synthesis, brings alterations in Hb synthesis. It would also be of interest to find out how leucocyte counts are altered : what is the mechanism in operation and what role M.E. plays. Even how M.E. influences the bone marrow, remains to be understood.

M.E. has been shown to induce necrosis. It would be of interest to know how the glomerular necrosis is set in. Is it due to the induction of hypertension? What role does the juxta glomerular apparatus play ? Is angiotensin II synthesis stepped up by M.E. ? How M.E. influences uric acid synthesis in the kidney? What is the mechanism of such action ? Are the endocrine glands influenced by the M.E.? M.E. appears to influence the calcium release and Ca^{++} as well as chloride concentration. How is M.E. influencing Ca^{++}chloride regulation ? Does it influence Cyclic AMP synthesis or protein kinases ? Does M.E. induce change in β - adrenergic activation of phospholipase C and inositol ? Does M.E. influence IP_3 system for increase in intracellular Ca^{++} ? Thus, the present investigation has raised some questions which need to be addressed to know the actions of M.E. or heavy metals.

BIBLIOGRAPHY



Alkahem, H. F. 1994 : *J. Uni. Kuwait. Sc.*, 21(2), 243 - 252.

Allen, P. 1993 : *Comp. Biochem. Physiol. C.*, 105 C(2), 213 - 217.

Allen, P. 1994 : *Comp. Biochem. Physiol. C.*, 108 C(1), 117 - 121.

Alpers and Isselbacher 1967 : *Biochem. Biophys. Acta.*, 137, 33.

Anderlini, V.C., Connors, P. G., Risebrough, R. W. and Martin, J. H. 1972 : Blackburg Virginia Polytechnic Institute and State University, 49 - 62.

Atkinson , I. 1982 : *Ren. Physiol.*, 4 (5), 235-44.

Austin, A., Clark, N. A., Greenwood, J. J. D. and Rehfisch, M.M. 1993 : BTO Research Report 99, British Trust for Ornithology, Thetford.

Baccino, F.M.G., A. Rita and Dianzani 1965 : *Enzymology,* 29, 169.

Baeza, A., del Rio M., Miro, C. 1988 : *Health Phys.*, 55, 863-7.

Bain, B. J. 1985 : *Scandinavian Journal of Haematology,* 35, 77.

Bakir, F., Damiuji, S.F., Amin - Zaki, L. 1973 : *Science,* 181, 176 -9.

Bakre, P.P., Sharma, A. 1995 : *Journal of Environmental Biology,* 16 (1), 15 - 17.

Banerjee, V. and Kumari, K. 1988 : *Environ. Ecol.*, 6 (3), 737 - 739.

Bassi, A. and Bernelli - Zazzera 1964 : *Exptl. Mol. Pathol.*, 3,332.

Becker, N. and Barron, K.D. 1961 : *Am. J. Pathol.*, 38, 161.

Becker, P. H. 1989 : *Helgolander Meeresuntersuchungen,* 43, 395 - 405.

Beregi, E., Foldes, I., Jankovics, R. and Csotortok, L. 1962 : *Arhc. Int. Pharmacodyn.*, 138, 268 - 275.

Berg, M., Vanden, Blank, F., Heeremans, C., Wagenaar, 1987 : *Arch. Environ. Cont. Toxicol.*, 16 (2), 149 - 158.

Berg, W., Johnels, A., Sjostrand, B. and Westermark, T. 1966 : *Oikos,* 17, 71 - 83.

Biesecker, J. E. and George, J.R. 1966 : *Circs. U.S. Geol. Surv.*, No. 526, 1 - 27.

Bijleveld, M.F.I. J., Goeldin, P. and Mayol, J. 1979 : *Environ. Conserv.*, 6, 139 - 43.

Birkhead, M. E. 1983 : *J. Zool.*, Lond., 19, 59 - 73.

Birkhead, M.E. 1982 : *J. Zool.*, Lond., 198, 15 - 25.

Blus, L. J., Stround, R. K., Reiswig, B., Mc Encaney, T. 1989 : *Environ. Toxicol. Chem.*, 8 (3), 263 - 271.

Borg, K., Wanntorp, H., Erne, K. and Hanko, E. 1969 : *Viltrevy,* 6, 301 - 79.

Botkin, D.B., Woodby, A. and Nisbet, R.A. 1991 : *Biological Conservation,* 56, 63 - 78.

Bourne, W.R.P. 1976 : In Sea birds and pollution in Marine Pollution (ed. R. Johnston), Academic Press, London, 403 - 502.

Bowers, L. D. 1980 : *Clin. Chem.* 26, 551.

Bowker, D.W. and Muir, H. J. 1981 : *J. Biol. Education.,* 15 (1), 35 - 41.

Braun, E. J. and Dantzler, W.H. 1972 : *Am. J. Physiol.,* 222, 617.

Braune, B.M. 1987 : *Arch. Environ. Contam. Toxicol.,* 16, 217 - 24.

Braune, B.M. and Gaskin, D.E. 1987 b : *Ornis Scand.,* 18, 244 - 50.

Braune, B.M. and Gaskin, D.E. 1987a : *Arch. Environ. Contam. Toxicol.,* 16, 539 - 49.

Brown, K.W., Thomas, J. C., and Launius, K.W. 1984 : *Environ. Pollut.* Ser., B, 8, 119 - 131.

Brulles, A., Gras, J., Margina, N., Toress, N., Brun, C. 1969 : *Clin. Chem., Acta.,* 24 (2), 261-265.

Bryan, G.W. 1979 : *Phil. Trans. Roy. Soc.* Lond., B, 286, 483 - 505.

Burger, J. and Gochfeld, M. 1988 : *Environ. Monitor. Assess.,* 11, 127 - 35.

Burstone, M.S. 1962 : As cited in "Enzyme Histochemistry", by Pearse, 3rd Ed. Vol. I, Acad. Press., New York.

Cade, T. J., Lincer, J. L., White, C.M. 1971 : *Science,* 172, 955 - 7.

Carone Frank, Darryl Peterson, Suzzanni Oparel and Pullman, T. 1979 : *Kid. Int,*16 (3) , 277 -78.

Carpene, E., Serra, R., Isani, G. 1995 : *Journal of wildlife diseases,* 31 (1), 49 - 56.

Cave', A.J. 1983 : *Ardea,* 71, 217 - 24.

Chapdelaine, G., Laporte, P. and Nettleship, D.N. 1987 : *Can. J. Zool.,* 65, 2992 - 6.

Chatterjee, C. C. 1972 : In " Human Physiology," Vol. I, Ed. V, Pub. New Central Book Agency, Calcutta.

Chatterjee, C. C. 1977 : In "Human Physiology", Vol. I, Ed. VII Pub. New Central Book Agency, Calcutta.

Chou, S. T. 1972 : *Can. J. Physiol. Pharmacol.,* 50, 936.

Choudhari, S.K. 1994 : Bulletin No. 27, Indian Bureau of Mines. 37 - 40.

Coelho, F.L. 1990 : As cited in Technical Report submitted to DOE^{n}., 1993.

Coleman, A.M. 1979 : *Architects Journal,* 165, 91 - 134.

Colinvaux, P.A. 1973 : In Introduction to ecology, John Wiley and Sons., 621.

Collier, C.R., Pickering, R. J. and Musser, J.J. 1970 : In "Hydrologic influences of strip mining" ed. by C.R. Collier, R.J. Pickering, and J.J. Musser. Geol. Surv. Prof. Pap. 427 - C, C_1 -

C_9 , C_{31} , C_{45} . Washington, U.S. Government Printing office.

Cooke, A.S., Bell, A. A. and Hass, M. B. 1982 : In Predatory Birds, Pesticides and Pollution. Institute of Terrestrial Ecology, Cambridge.

Curtis, W.R. 1973 : In Ecology and reclamation of devastated lands. Ed. by R. J. Hutnik & G. Davis New York, Gordon & Breach. 145 - 57.

Custer, T.W., and Mulhern, B.L. 1983 : *Bull. Environ. Contam. Toxicol.,* 30 (2), 178 - 185.

D'Souza, J. 1993 : In Effect of mining on microbial ecology and environment in the Bicholim Taluka of Goa and the study of possible, restoration processes in the region. On going project funded by DOEⁿ. Technical Report.

D'Souza, J. and Nayak, G. N. 1994 : Biodiversity in the Western Ghats. An information Kit. WWF - SIDA, 9.

D'Souza, S. 1991 : In Environmental study of mines in Goa, Indian Institute of Ecology and Environment - A report. 1 - 42.

Davies, B.J., and Ornstein, L. 1959 : *J. Histochem. Cytochem.,* 7, 297.

Den Held, J. J. 1981 : *Ardea,* 69, 185 - 91.

Denneman, W.D., and Dou Ben, P.E.T. 1993 : *Environmental Pollution,* 82 (3) : 301 - 310.

Desai, P.V. and Varute, A.T. 1987 : *J. Shivaji University,* India. 23, 311- 318.

Desai, P.V. 1983 : In Histochemical - Biochemical investigation of fowl kidney (*Gallus domesticus*) under the serotonin creatinine sulphate induced hypertension. Ph.D Thesis submitted to Shivaji University, Kolhapur.

Desai, P.V. 1990 : In Impact of mining on environment. Ed. by Trivedy & Sinha, Ashish Pub., New Delhi. 143 - 152.

Desai, R.M. 1990 : State of mining environment in Western Ghats - A report. DOEn, India. 1 - 42.

Detwyler, T.R. 1971 : In Man's impact on environment. Mc Graw Hill Book Company, 1 - 731.

Deuel, B. 1985 : *Calif. Fish Game,* 71 (2), 125 - 128.

Diamond, A.W. 1989 : *Environmental Monitoring and Assessment,* 12, 75 - 82.

Diamond, A.W. and Filion, F. 1987 : ICBP Tech. Publ. 6, International Council for Bird Preservation, Cambridge.

Dianzani, M.U. 1963 : Ciba Found. Symposium. Lysosomes, 335-352.

Dingle, J. D. and Fell, H. 1968 : *J. Cell Sci.,* 24, 25.

Dingle, J.D. and Honor Fell 1969 : In Lysosomes in Biology and Pathology, Vol. 1-6. N.H. Pub. Comp. London.

Dixon, K.C. and Herbertson, B.M. 1950 : *J. Pathol. Bact.,* 62, 335.

Dorian, C., Gattone, V.H - II,

Klaassen, C.D. 1992 : *Toxicology and Applied Pharmacology,* 114 (2), 173 - 181.

Dumans, B.T. 1971 : *Clin. Chem., Acta,* 31, 87.

Duvede Beaufay 1959 : *Biochem. J.,* 73, 610.

Dyer, K. L. and Curtis, W.R. 1977 : USDA for, Serv. Pap. NE - 372, 1 - 13. N.E. Forest Experiment Station.

El - Shabaka, H., Abu - Sinha, G., Al - Henzab, N. 1993 : *Qat. Uni. Sci. J.,* 13 (2), 271 - 275.

Elkins, N. 1983 : In Weather and Bird behaviour, T. K. A.D. Poyser, Calton.

Elliott, J.E., Scheuhammer, A.M., Leighton, F.A., Pearce, P.A. 1992 : *Arch. Environ. Contam. Toxicol.,* 22 (1), 63 - 73.

Ericsson, J.L.E. and Spring 1982 : As cited by Desai In " Histochemical - Biochemical investigation of fowl kidney (G*allus domesticus*), under the influence of serotonin creatinine sulphate induce hypertension", Thesis submitted to Shivaji University, Kolhapur 1983.

Ericsson, J.L.E. 1964 : *Acta Pathol. Microbiol. Scand. Suppl.,* 168.

Eriksson, M.O.G. 1984 : *Ambio,* 13 (4), 260 - 2.

Erslev, A. J. 1975 : *Am. J. Med.,* 58, 25.

Evans, P. R. and Moon, S. J. 1981 : In Environmental and biological aspects (eds. P.J. Say and B.A. Whitton), University of Durham, Durham, 181 - 90.

Farquhar and Palade, G. E. 1962 : *J. Cell. Biol.,* 13, 55.

Farquhar and Palade, G.E. 1967 : *J. Expt. Med.,* 114, 699.

Farquhar 1961 : In Lysosomes in Biology and Pathology by Dingle & Fell. Vol. 1-6.

Fisher, J . 1952 : In The Fulmar, Pub. Collins, London.

Fishman, W. H. and Baker, J.R. 1956 : *J. Histochem. Cytochem.,* 4, 570.

Focardi, S., Fossi, C., Lambertini, M. 1988 : *Environ. Monit. Assess.,* 10, 43 - 50.

Fossati, P. 1980 : *Clin. Chem.,* 26, 227.

Fossi, C., Focardi, S., Leonzio, C., Renzoni, A. 1984 : *Environ. Conserv.,* 11(4), 345 - 350.

Fox, C.F., B.D. Dinman and Frajola 1962 : *Proc. Soc. Expt. Biol. Med.,* 111, 731.

Fox, G.A. and Weseloh, D.V. 1987 : In The value of Birds (eds. A.W. Diamond and F. Filion) ICBP Tech. Publ. 6, International Council for Bird Preservation, Cambridge. 209 - 16.

Friberg, L., Iscator, M., Nordberg, G. and Kjellstorm, J. 1974 : Cadmium in the environment., 2nd ed., CRC Press, New York.

Friede, R.L. 1966 : In Topographic brain chemistry, N.Y., Academic Press.

Frizzell, 1979 : As cited by Desai In " Histochemical - Biochemical investigation of fowl kidney (G*allus domesticus*), under the serotonin creatinine sulphate induced hypertension" Thesis submitted to Shivaji University, Kolhapur 1983.

Fry, D. M., Swenson, J., Addiego, L. A.,Grau, C.R., Kang, A., 1986 : *Arch. Environ. Contam. Toxicol.,* 15 (4), 453 - 463.

Fujiki, M., Tajima, S. and Omori, A. 1972 : *Japan J. Hyg.,* 27, 115 - 17.

Furness, R. W., Muirhead, S.J. and Woodburn, M. 1986a : *Mar. Pollut. Bull.,* 17, 27-30.

Furness, R.W., and Hutton, M. 1979 : *Environ. Pollut.,* 1, 261 - 8.

Furness, R.W., Lewis, S.A. and Mills, J.A. 1990a : *Environ. Pollut.,* 63, 33 - 9.

Ganihar, S. R. 1990 : In Impact of mining on the faunal composition of Goa, Ph.D. thesis submitted to Goa University (unpublished), 1 - 264.

George, L.S., Dallas, C.E., Brisbin, I.L., Jr. and Evans, D.L. 1991 : *Ecotox. and Env. Safety,* 21, 337 - 47.

Giebisch and Gerhead 1965 : *Med. Welt.,* 65 (20), 1091 - 1097.

Gilberton, M. 1990 : *Environ. Monit. Assess.,* 15, 219 - 23.

Gill, T.S., Epple, A. 1993 : *Ecotoxicol. Environ., SAF.* 25 (2), 227 - 235.

Glenner, G. G. 1957 : *J. Histochem. Cytochem.,* 5, 297.

Glenner, G.G. 1963 : J. Histochem. Cytochem., 11, 285.

Glick, D. 1957 : In "Technique of Histochemistry", Interscience Publishers, New York, 43.

Goede, A.A. 1985 : *Environ. Pollut.,* 37, 287 - 309.

Goede, A.A. 1993 : *Arch. Environ. Contam. Toxicol.,* 25 (1), 79 - 84.

Goede, A.A., and de Bruin, M. 1984 : *Environ. Pollut.,* 8, 281 -98.

Gould, R. and Holt, S.G. 1961 : Proc. Anat. Soc. Great Britain and Ireland. London. Taylor and Francis Ltd. P. 45.

Guilio, R.T. - di 1993 : *Diss. Abst. Int. PT. B. Sci. and Eng.,* 44 (1), 265.

Guitart, R., To - Figueras, J., Mateo, R., Bertolero, A., Cerradelo, S., Martinez-Vilalta, A. 1994 : *Arch. Environ. Contam. Toxicol.,* 27 (3), 289 - 293.

Hacker, C.S., Cheney, M. A., Schroder, G.D. 1981 : *Ecotoxicol. Environ. Saf.,* 5 (2), 211 - 244.

Hacker, C.S., Maedqen, J.L., Schroder, G.D., Weir, F.W. 1982 : *Arch. Environ. Contam. Toxicol.*,11 (1), 99 - 102.

Hahn, E. 1991 : *Berichtedes Forschungszentrums, Julich.*

Halford, D.K., Millard, J.B. and Markham, O.D. 1981 : *Health Phys.*, 40, 173 - 81.

Hamburger Richet, Crosnier Funch., Antoine Ducrot, Mery Monter 1968 : In Nephrology, Vol. I, Philadelphia London, Toronto. Saunders Co.

Havera, S. P., Whitton, R.M., Shealy, R.T. 1992 : *J. Wildl. Manage.*, 56 (3), 539 - 545.

Heinz, G. 1974 : *Bull. Environ. Contam. Toxicol.*, 11, 386 - 92.

Heinz, G.H. and Fitzgerald, M.A. 1993 : *Arch. Environ. Contam. Toxicol.*, 25 (1), 90 - 94.

Hellawel, J. M. 1986 : In Biological Surveillance of Rivers, Water Research Centre, Medmenham.

Henny, C.J., Blus, L. J., Hoffman, D.J., Grove, R.A., Hatfield, J.S. 1991 : *Arch. Environ. Contam. Toxicol.*, 21 (3), 415 - 424.

Hiremath, K.G. and Shetty, S.M. 1987 : DOE[n]. Technical report, 21 - 27.

Hoffman, D.J., Sanderson, C. J., Le-Captain, L.J., Cromartie, E., Pendloton,

G.W. 1991 : *Arch. Environ. Contam. Toxicol.,* 20 (2), 288 - 94.

Holdgate, M. W. 1979 : In A perspective of Environmental Pollution, Pub. Cambridge University Press, Cambridge.

Holtzman and Novikoff, A.B. 1965 : *J. Cell. Biol.,* 27, 651.

Honda, K., Lee, D.P. and Tatsukawa, R. 1990 : *Environ. Pollut.,* 65, 209 - 18.

Honda, K., Marcovecchio, J.E., Kan, S., Tatsukawa, R., Ogi, H. 1990 : *Arch. Environ. Contam. Toxicol.,* 19 (5), 704 - 711.

Honda, K., Min, B.Y. and Tatsukawa, R. 1986 b : *Arch. Environ. Contam. Toxicol.,* 15, 185 - 97.

Honda, K., Nasu, T. and Tatsukawa, R. 1986 a : *Environ. Pollut.,* 42, 325 - 34.

Honda, K., Yamamoto, Y., Hidaka, H. and Tatsukawa, R. 1986c : *Mem. Nat. Inst. Polar Res., Special Issue,* 40, 443 - 53.

Hontelez, L.C.M.P., Dungen, H. M., Vanden, Baars, A.J. 1992 : *Arch. Environ. Contam. Toxicol.,* 23 (4) , 453 - 456.

Howard, G. 1989 : Calcium metabolism & Physiology of bone In "Text Book of Physiology" Ed. by Patton,

Fuchs, Hille, Scher, Steiner. Vol. II, 21st Edition. W.B., 1461.

Hudson, R. 1990 : BTO Research Report 47, British Trust for Ornithology, Tring.

Hughes, M.R. 1970 : *Condor,* 72, 164.

Hunter, B. and Wobeser, G. 1980 : *Avian. Dis.,* 24 (1), 169.

Hutchinson, T.C. and Meema, K.M. 1987 : In Effects of Atmospheric Pollutants on Forests, Wetland and Agricultural Ecosystems. Springer - Verlag, New York.

Hutton, M. 1981 : *Environ. Pollut. Serv.,* A, 26, 129 - 45.

Hynes, H. B.N. 1960 : In Biology of Polluted Waters, Liverpool University Press, Liverpool.

International Committee for Standardization in Haematology 1980 : *Journal of Nuclear Medicine,* 21, 793.

Janoff, Weissman, Zweifach and Thomas 1962 : *J. Exptl. Med.,* 116, 451.

Järvinen, O. and Väisanen, R.A 1979 : Oikos, 33, 261-271.

Jensen, S., Johnels, A.G., Olsson, M. and Westermark, T. 1972 : Proc. XVth Int. Ornithol. Congr., Leiden., 455 - 6.

Johnson, O. W. 1968 : *Auk,* 85, 216.

Jouventin, P. and Weimerskirch, H. 1990 : *Nature,* 343, 746 - 8.

Kammcraat, C. 1978 : *Clin. Chim. Acta.,* 84, 119.

Kanyamibwa, S., Schierer, A., Pradel, R. and Le Breton,

J.D. 1990 : *Ibis,* 132, 27 - 35.

Karlin, A., Rantamaeki, P.,
Lemmetyinen, R. 1985 : *ORNIS - FENN.,* 62 (4), 168 - 170.

Karlong, O., Elvestad, K.,
Clausen, B. 1983 : *Nord. Vet. Med.,* 35 (12), 448 - 451.

Karlwilhelln, Kuhn.
Koh. 1977 : *Lab. Inv.*, 36 (4), 375.

Kashgurian, M.,
Hayslett, J. 1977 : *Am. J. Pathol.,* 89, 199.

Kawai, H. 1963 : *Exptl. Neurol.,* 7, 457.

Kerr, J.F. R. 1965 : *J. Pathol. Bacteriol.,* 90, 419.

Kessler, G. 1964 : *Clin. Chem.,* 10, 686.

King, E.J. and Armstrong
M.D. 1934 : *Canad. Med. Assoc. J.,* 31 396.

King, A., and
Cromartie, E. 1986 : *Colonial Waterbirds.* 9 (1), 90 - 94.

Kobayashi, Kaizo, Yumiko,
Kishimoto, and Kiyashi
Shimizu 1971 : *Clin. Chim. Acta.,* 35 (1), 173 - 183.

Kodama, Y., Matsund, K.,
Kawamoto, T., Kayama, F.,
Tsuchiya, K. 1992 : *J. Environ. Sci. Heal. Environ. Sci. & Eng.,* 27 (1) , 165 - 198.

Kolkwitz, R. and
Marsson, M. 1909 : *Int. Rev. ges. Hydrobiol.,* 2 , 125 - 52.

Kozulin, A. and
Pavluschick, T. 1993 : *Acta Ornithologica* (Warsaw), 28 (1) , 56 - 61.

Lantzy, R. J. and

Mackenzie, F.T. 1979 : *Geochimica et Cosmochimica Acta*, 43, 511 - 25.

Laurence and Friedler 1966 : As cited by Desai In "Histochemical - Biochemical Investigation of fowl kidney *(Gallus domesticus),* under the serotonin creatinine sulphate induced hypertension" Thesis submitted to Shivaji University, Kolhapur, 1983.

Law, R. J. Fileman, C.F., Hopkins, A.D . 1991 : *Mar. Pollut. Bull.*, 22, 183 - 91.

Leah, R. T., Evans, S. J., Johnson, M.S. and Collings, S. 1991 : *Mar. Pollut. Bull.,* 22, 172 - 5.

Lee, D.P., Honda, K., Tatsukawa, R. and Won, P. 1989 : *Bull. Environ. Contam. Toxicol.*, 43, 550 - 5.

Leonzio, C., Fossi, C. and Forcardi, S. 1986 : *Bull. Environ. Contam. Toxicol.*, 37, 219 - 25.

Lewis, S.A. and Furness, R.W. 1991 : *Arch. Environ. Contam. Toxicol.*, 21, 316 - 20.

Lindberg, P. 1984 : *Bull. Environ. Contam. Toxicol.*, 12, 227 - 32.

Lock, J. W., Thompson, D.R., Furness R.W., Bartle, J.A. 1992 : *Environ. Pollut.*, 75 (3), 289 - 300.

Loranger, S., Demers, G., Kennedy, G., Forget, E., Zayed, J. 1994 : *Arch. Environ. Contam. Toxicol.*, 27 (3), 311 - 317.

Lorentz, K. 1982 : *Clin. Chim. Acta.,* 126, 327.

Lowery, O.H., Rosenbrough, N.G., Farr, A.L. and Randall, R.G. 1951 : *J. Biol. Chem.,* 193 , 265 - 275.

Mac Mohan, J. A. 1983 : In Ecology, and the human lot, 67th Faculty honor lecture, Utah State Univ. Press., 1- 31.

Macgregor, R.G., Scott Richards, W. and Loh, G.L. 1940 : *Journal of Pathology and Bacteriology,* 51, 337.

Macrovecchio, J.E., Moreno, V.J., Bastida, R.O. 1990 : *Mar. Pollut. Bull.,* 21, 299 - 304.

Maiorca, R. L., Scarpioni, V., Cameli, G. C., Carrara and P. Dall, A. Glio 1967 : *Clin. Chim Acta.,* 16 (2), 253 - 57.

Mandel and Popper 1971 : As cited by Murray, in Patho. Bact., 90(2), 1965.

Marchant, J.H. 1992 : *Ibis,* 134 (Suppl. 1), 113 - 19.

Marquiss, M. and Newton, I. 1990 : In Birds, in The Green-house Effect and Terrestrial Ecosystems of the UK (eds. M.G.R. Cannell and M.D. Hooper) ITE Research Publication, HMSO, London, 38 - 42.

Maunsbach, A. 1973 : In "Kidney - Physiopathology and Lysosomes" as cited by Dingle Vol. 5. - Lysosomes in Biology & Pathology, N. H. Publi. Com. London.

Mautino, M., and Bell, J.U. 1987 : *Bull. Environ. Contam. Toxicol.,* 38 (1), 78 - 85.

Mc Intyre 1958 : As cited In Microanalysis in Medical Biochemistry. Fourth Edition IDP, Wooton, (1964) 71 - 74. Publishers J. & A Churchill Ltd., London.

Menkin, K. 1955 : In " Human Physiology" by Chatterjee, 1977, Vol. I, New Central Book Agency, Calcutta.

Merill, J. M., Lamleystone, Grace, Menceley 1956 : *Am. J. Med.,* 160, 1454.

Metkiewski Eugeniacz, Kosmicki Bogumil 1968 : *Acta., Physiol. Pol.,* 19 (1), 59 - 64.

Michael Kashgurian, John Hayslett, Benjamin H. Spargo 1977 : *Ass. J. Pathol.,* 89, 77.

Miller, D. S., Butler, R.G., Trivelpiece, W.Z., Janes Butler, S., Green, S. Peakall, G., Lambert, G., Peakall, D.B. 1980 : *Biol. Lab.,* 20, 137 - 138.

Miller, F. 1960 : *J. Biophys. Biochem. Cytol.,* 8, 689.

Miller, F. 1962 : In "Acid phosphatase localization in renal protein absorption droplets". ed. S. S. Breeze Jr. Proc. 5th Intern. Congr. Electron Microscopy, Philadelphia. Vol. 2. New York, Academic Press., P. Q -2.

Miller, F. and Palade, G. E. 1964 : *J. Cell Biol.*, 23, 519.

Mitchell, J.R. 1973 : *Clin. Chem.*, 19, 908.

Morin, J., Goldberg, D. and Seginer, I. 1967 : *Trans. Am. Soc. Agri. Engg.*, 10, 74 - 9.

Morin, L.G. 1974 : *Am.J. Clin. Pathol.*, 61, 114

Morrison, M.L. 1986 : *Current Ornithology*, 3, 429 - 51.

Morton, E.S. 1992 : In Ecology and Conservation of Migrant Landbirds (eds. J.M. Hagan and D.W. Johnston), Smithsonian Institution Press, Washington.

Muirhead, S.J. and Furness, R.W. 1988 : *Mar. Pollut. Bull.*, 19, 278 - 83.

Mukherjee, J. K., Sinha, G. M. 1993 : *J. freshwater. Biol.*, 5 (3), 245 - 251.

Munoz, R.V., Jr., Hacker, C.S. and Gesell, T.F. 1976 : *J. Wildl. Dis.*, 12, 139 - 43.

Murton, R. K., Osborn, D. and Ward, P. 1978 : *Ibis*, 120, 106 -7.

Nayak, G.N. 1993 : In Studies on sediment flux of rivers, estuaries and adjoining coastal waters of Goa, West Coast of India. Technical report submitted to DOE^{n}. India. 1 - 69.

Nebekar, A.V., Griffis, W.L., Stutzman, T.W., Schuytema, G.S., Carey, L.A., Scherer, S. M. 1992 : *Environ. Toxicol. Chem.*, 11 (5), 687 - 699.

Newton, I. 1991 : Acta XX Congressus Internationalis Ornithologici, IV, 2487 - 93.

Nicholson, J.K. and Osborn, D. 1983 : *J. Zool.*, Lond., 200, 99 - 118.

Niesel, W. H., Roskenblack, Hanke, P., Specht, N. 1970 : *Pelugers Arch. Eur. J. Pysiol.*, 315 (4), 308 - 320.

Novikoff, A. B. 1959 : *J. Biophys. Biochem., Cytol.*, 6, 136.

Nyabuto, H. N. 1989 : In Taxonomic studies, description, and analysis of vegetation at Pale and Sirigao villages of Goa. M.Sc. thesis, submitted to Goa University. 1 - 337.

Nyholm, N.E.I. 1981 : *Environ. Res.*, 26, 363 - 71.

Nyholm, N.E.I. and Myhrberg, H.E. 1977 : *Oikos*, 29, 336 -41.

O' - Halloran, J., Myers, A. A., Duggan, P.F. 1989 : *J. Zool.*, 218 (4), 627 - 632.

Ochiai, K., Jin, K., Itakura, C., Goryo, M., Yamashita, K., Mizuno, N., Fujinaga, T., Tsuzuki , T. 1992 : *Avian - Dis.* Vol. 36 (2), 313 - 323.

Ohlendorf, H.M. and Harrison, C.S. 1986 : *Environ., Pollut.*, 11, 169 - 91.

Ohlendorf, H.M., Hoffman, D.J., Saiki, M.K., Aldrich, T.W. 1986 : *Sci. Total - Environ.*, 52 (1 - 2), 49 - 63.

Ormerod, S. J. and Tyler, S.J. 1991a : *Freshwat. Biol.*, 25, 105 - 16.

Osborn, D., Every, W.J., Bull K.R. 1983 : *Environ., Pollut. A - Ecol. Biol.*, 31 (4), 261 - 275.

Osborn, D., Harris, M. P. and Nicholson, J.K. 1979 : *Comp. Biochem. Physiol.,* 64 C, 61 - 7.

Paliwal, H.V. 1989 : In National seminar on protection of environment and ecology by mining industries, Vol. II., Federation of Indian Mineral Industries. 427 - 433.

Palmela, R., Mac lean and Robson, J. S. 1966 : *Clin. Sci.,* 30 (1), 91 - 102.

Pankakoski, E., Hyvarinem, H., Jalkanen, M., Koivisto, I. 1993 : *Environ. Poll.,* 80 (1), 9 - 16.

Park, R., Lawrance, Rabinowitz 1969 : *Proc. Soc. Exp. Biol. Med.,* 32 (1), 27 - 29.

Pena, A. and Iturri, S. J. 1993 : *Comp. Pharmacol & Toxicol.,* 106 (2), 315 - 319.

Pitts, R. F. 1963 : In Physiology of the kidney and body fluids. Chicago Year Book Medical Publishers.

Poulson, T.L. 1965 : Science, 148, 389.

Prasada Rao, P.V.V, Jordan, S.A., Bhatnagar, M.K. 1989 : *J. Toxicol. Environ. Health,* 26 (3), 327 - 348.

Prasada Rao, P.V.V., Jordan, S.A., Bhatnagar, M.K. 1989 : *J. Environ. Pathol. Toxicol. Oncol.,* 9 (1), 19 - 44.

Rao, D. P., Bhaskar, B. R., Rao, K.S., Prasad, Y. V. D., Rao N. S., Rao T. V. V. 1990 : *Mar. Environ. Res.,* 30 (3), 217 - 231.

Rees, K. R. and Shotlander, P. 1963 : *Proc. Roy. Soc. London Ser. B,* 157, 517.

Rees, K. R., Sinha, K. P., and Spector, W. G. 1961 : *J. Pathol. Bacterio.,* 81, 107.

Reid, M. and Hacker, C.S. 1982 : *Mar. Pollut. Bull.,*13, 387- 9.

Rennick, B. R., 1967 : *Am. J. Pharmac.,* 57, 149.

Renzoni, A., Focardi, S., Fossi, C. 1986 : *Environ. Pollut.,* 40, 17 - 35.

Rhodin, J. 1954 : In Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. Thesis. Stockholm, Karolinska Institutet.

Rickard, W.H., and Fitzner, R.E. 1985 : *SCI.,* 59 (1), 28 - 32.

Risebrough, R.W., Jarman, W.M., Springer, A.M., Walker, W. II, Hunt, W.G. 1986 : *Environ. Toxicol. Chem.,* 5 (1), 13 - 19.

Roberts, S. and Schmidt - Neilson 1966 : In Textbook of Avian Physiology, Ed. Donald Farner, Academic Press, New York, 1977.

Sahu, K. C. 1990 : In Impact of mining on environment (eds. R.K. Trivedy, M.P. Sinha), Ashish Pub. New Delhi. 63 - 89.

Samuel, M.D., Bowers, E.F., Franson, J. C. 1992 : *J. Wildl. Dis.,* 28 (4), 555 - 561.

Sanderson, G.C. and

Bellrose, F.C. 1986 : In Illinois Natural History Society Survey, Special Publication 4, *Illinois Natural History Society.*

Saravanan, T.S., and Natarajan, M. 1991 : *J. Ecotoxicol. Environ. Monit.,* 1 (4), 265 - 269.

Sardesai, J. B. 1985 : In Earth resources for Goa's development, Technical Report. 424 - 429.

Saunders, D.A., Rowley, I. and Smith, G.T. 1985 : In Birds of Eucalypt Forests and Woodland : Ecology, Conservation, Management (eds. A. Keast, H. F. Recher, H. Ford and D. Saunders) Surrey Bealty, Chipping Norton (New South Wales), 309 - 21.

Saxena, O.P., and Parashari, A. 1981 : *Int. J. Acad. Inchthyol.,* 2 (2), 55 - 59.

Scharenberg, W. 1989 : *J. Orn.,* 130, 25 - 33.

Scheuhammer, A. M. 1987 : *Environ. Pollut.,* 46, 263 - 95

Scheuhammer, A.M. 1989 : *J. Wildl. Manage.,* 53, 759 - 65.

Schrank, C.S., Cook, M.E., Hansen, W.R. 1990 : *P. Environ. Toxicol. Cent.,* 309, 26 (3), 307 - 315.

Sell, J. L. 1977 : *Poult. Sci.,* 56 , 939 - 48.

Sell, J.L. 1975 : *Pollut. Sci.,* 54, 1674 - 8.

Sellers, A. 1955 : In "Lysosomes in Biology and Pathology" as cited by Dingle and Fell. N. H. Pub. Comp. London.

Sengupta, R. and

Kureishy, T.W. 1989 : "State of the Art" pp. 165 - 181. In : Water Pollution (Conservation and Management) Eds. A.K. Sinha, Ram Boojha and P.N. Vishwanathan). Gyanodaya Prakashan, Nainital.

Shandilya, S. and Banerjee, V. 1989 : *Environ. Ecol.* 7 (1) 16 - 23.

Shetty, S.M., Ganihar, S.R. and Hiremath, K.G. 1987 : In Effect of mining on the ecosystem of Sanguem, Bicholim, Sattari, and Quepem talukas, Goa, Technical Report. DOEⁿ 8 - 14.

Shirey, J. J. and Sextone, A.J. 1989 : In FEMS Microbial Ecology. 59 - 70.

Shoemaker, V. H. 1967 : *Am. Zool.*, 7, 736.

Shoenfeld, R.G. 1956 : *Clin. Chem.*, 10, 533.

Siller, W. G. and Hindle, R. M. 1969 : *J. Anat.*, 194, 117.

Simpson, V.R., Hunt, A. E., Funch, M. C. 1979 : *Environ. Pollut.*, 18 (3), 187 - 202.

Skadhauge Erick and Bodil - Schmidt - Nielsen 1967 : *Am. J. Physiol.*, 212 (4), 793 - 798.

Skadhauge, Erick and Bodil - Schmidt - Nielsen 1967 : *Am. J. Physiol.*, 212 (6), 1313 - 18.

Skjorten Fredrik 1964 : *Pathol. Microbiol. Scand.*, 61 (3), 405 - 524.

Slater, T. F. and Greenbaum, W. 1963 : *Biochem. J.*, 96, 484.

Slater, T. F. and Greenbaum, W. 1965 : *Biochem. J.*, 96, 484.

Smith, R.M., Tryon, E.H.

and Tyner, E.H. 1971 : In Soil development on mine spoil. West Virginia Univ. Agric. Exp. Sta. Publ., Morgantown, Agricultural Experiment Station.

Spalding, M.G., Bjork, R.D., Powell, G.V.N., Sundlof, S.F. 1994 : *Journal of wild life management,* 58 (4), 735 - 39.

Sperber, I. 1960 : Excretion, In "Biology and comparative physiology of birds" Ed. A.J. Marshall, New York, Acad., Press. Chap. 12.

Squire, J. R., Hardwicke, J. and Soothill, J. F. 1962 : In D. A. K. Black, ed. Renal disease. Oxford, Blackwell Scientific Publications, 213 - 232.

St. Louis, V. L. Brecbaart, L., Barlow, J. C. Kalverkamp, J. F., 1993 : *Environ. Toxicol. Chem.,* 12 (7), 1203 - 1207.

Sterling, C. E. 1989 : In "The Textbook of Physiology " Vol. II. Ed. Patton *et. al.,* Pub. Saunders Company, Philadelphia, 1056 - 57.

Stewart, F.M., Thompson, D.R., Furness, R.W., Harrison, N. 1994 : *Arch. Environ. Contam. Toxicol.,* 27 (2), 168 - 175.

Stoneburner, D.L., Patty, P.C., Robertson, W.B., Jr. 1980 : *Sci. Total. Environ.,* 14(2), 147 - 152.

Straus, A. 1956 : *J. Biophys. Biochem. Cytol.,* 2, 513.

Straus, W. & Oliver, J. 1955 : *J. Exptl. Med.,* 102, 1.

Struger, J., Elliott, J. E.,

Weseloh, D.V. 1987 : *J. Great Lakes Res.*, 13 (1), 43 - 55.

Sturkie, P. D. 1965 : In Avian Physiology, 2nd Ed. (P.D. Sturkie ed.). Ithaca N. Y : Cornell Univ. Press, Chap. 13.

Sturkie, P.D. 1976 : In "Avian Physiology" 3rd Edn. Ed. Sturkie P.D. Springer - Verlag, New York, 269.

Subramanyam, H. 1986 : Seminar on mining and environmental preservation, *GMOEA* and *DIM*, 34 - 38.

Sullivan, L. P. and Grantham, J. J. 1982 : In " Physiology of the kidney". 2nd Edn. Philadelphia Pub. Lea & Febiger.

Sundlof, S. F., Spalding, M.G., Wentworth, J.D., Steible, C.K. 1994 : *Arch. Environ. Contam. Toxicol.*, 27 (3), 299 - 305.

Swaminathan, M. S. 1982 : In Report of the task force on Eco - Development Plan for Goa, Government of India, New Delhi, 136.

Szefer, P. and Falandysz, J. 1986 : *SCI. Total Environ.*, 53 (3), 193 - 199.

Sztriha, L., Bati, L. and Ormos, J. 1975 : *Br. J. Exp. Pathol.*, 56 (6), 495 - 501.

Tansy, M.F. and Roth, R.P. 1970 : Air Pollut. Control. *Control. Assoc. J.*, 20, 307 - 9.

Taylor, S.H. and Shillingford, J.P. 1959 : *Blue Brit. Heart J.*, 21, 497.

Thefeld, W. 1973 : *Dtsch. Med. Wschr.*, 98, 380- 1.

Thiyagarajah, A., Zwerner, D.E., Hargins, W. J., Jr. 1989 : *J. Environ. Pathol. Toxicol. Oncol.,* 9 (3), 261 - 268.

Thompson, D. R., Hamer, K.C. and Furness, R.W. 1991 : *J. Appl. Ecol.,* 28, 672 - 84.

Thompson, D.R. and Furness, R.W. 1989 : *Mar. Pollut. Bull.,* 20, 577 - 9.

Tomera, J.F., Kukulka, S.P. Ltlfcrd, K., Harakal C. 1991: *Coronary Artery Disease,* 2 (7), 769 - 774.

Torne, S.G. and Gaonkar, R.V. 1989 : Response of plant species to the mining sites situated at Pale and Sirigao, Technical Report, DOEn. 1 - 109.

Trivedy, R.K. 1990 : In Impact of mining on Environment (eds. R.K. Trivedy, and M.P. Sinha), Ashish Pub. New Delhi. 1 - 18.

Trivedy, R.K., and Goel, P.K. 1984 : Chemical and Biological Methods for Water Pollution Studies. Env. Pub. Karad, India.

Trump, B. and Bulger, R.E. 1965 : *Federation Proc.,* 24, 616.

Trump, B.F., and Jones, R.J. 1978 : In Diagnostic Electron Microscopy. Vol. I, Wiley Medical Publication, New York.

Trust, K. A., Miller, M.W., Ringelman, J.K., Orme, I. M. 1990 : *J. Wildl. Dis.,* 26 (3), 316 - 322.

Untawale, G. A. 1994 : In Biodiversity in the western ghats. Information Kit. WWF, SIDA p. G-4-2/2.

Van Assendelft, O.W. and England, J.M. 1981 : In Advances in Haematological Methods : The blood count CRC Press, Boca Raton, Florida.

Veeresh, A.V. 1989 : In Response of plant species to the mining rejects, Ph.D. thesis submitted to Goa University (unpublished). 1- 188.

Wallace, G.F., and Barrett, P. 1981 : In "Analytical Methods Development for Inductively Coupled Plasma Spectrometry", The Perkin - Elmer Corporation, Norwalk, CT.

Webster D. 1977 : *Clin. Chem.*, 23, 663.

Wepener, V., Vuren, J. H. J. - van, Preez, H., H - Du 1992 : *Bull. Eviron. Contam. Toxicol.,* 49 (4), 613 - 619.

Weseloh, D.V.C., Struger, J. Hebert, C. 1994 : *Journal of Great Lakes Research,* 20 (1), 277 - 288.

White, D.H., Mitchell, C.A., Kaiser, T.E. 1983 : *Arch. Environ. Contam. Toxicol.*, 12 (2) 241 - 245.

Winstanley, D., Spencer, R. and Williamson, K. 1974 : *Bird study,* 21, 1 - 41.

Wintrobe, M.M. 1974 : In Clinal Haematology, 7th Edition, Lea, Febiger, Philadelphia, Pennsylvania, 1896.

Zall, D.M. 1665 : *Anal. Chem.*, 28.

Zelman, C., Wang, H. and Appleham, J. 1959 : *Am. J. Med. Sci.*, 237, 323.