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STUDIES ON THE NEPHROTOXICITY OF SILICON

TWO VOLUMES

VOLUME I

ABDUL-RIDHA A. ABDULLAH

Thesis submitted for the
degree of Doctor of Philosophy
University of Glasgow
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January, 1982.

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DEDICATION

I wish to dedicate this work to my wife, Ilham A. Ali for her forbearance while I toiled in a far country. Also, her patience, understanding and sacrifice encouraged me to carry on with this work.

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I wish to express my sincere gratitude to Dr. J.W. Dobbie for his supervision and unstinted and generous way in which he was ever ready to assist. Without his careful attention and encouragement, this work could never have been completed.

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S U M M A R Y

"The discovery of the newer essential trace elements in the recent years has encouraged reconsideration of elements that were previously thought to be environmental contaminants. Silicon is one of these elements and it occupies a unique position"(Carlisle, 1975).

Until recently it was generally accepted that the main source of exposure to silicon in man was the occupational inhalation of silica dust. Contrary to previously held belief silicates have been shown to be absorbed from the alimentary tract and that the kidney is the main organ of excretion of dietary absorbed silicon. In man renal functional impairment is accompanied by a fall in urinary excretion of silicon and a rise in serum silicon (Dobbie et al 1981).

For the past 50 years there have been several reports on the nephrotoxicity of a variety of silicon compounds administered to experimental animals by several different routes. Moreover, there have been claims that acute or chronic exposure to siliceous dusts or prolonged intake of drinking water with a high silica content resulted in renal lesions in man.

Secure in the belief that silicon compounds were non-absorbable and non-toxic there has been an increasing use of silicates by the food and pharmaceutical industries as anticaking, antifoaming or hygroscopic agents.

Thus recent evidence that silicates are absorbed and that the kidney is the main route of excretion raises a natural concern for the liberal ingestion of silicates by man in

in view of the reports of nephrotoxicity in the experimented animal.

Whereas, the few previous investigations of silicon nephrotoxicity have been rather limited in nature and inconclusive as to the nature of the pathogenetic mechanism, this investigation has attempted a study in depth while bringing to bear on the problem many new and different investigative techniques.

This investigation has examined the renal effects of intraperitoneal injection of four silicon compounds to which man may be exposed. The intraperitoneal route was chosen since it was found to be the most convenient and reliable method, whereby the kidney could be exposed to different levels of serum silicon. A series of acute experiments in rats determined that high serum silicon levels resulted in a tubulo-interstitial lesion, which was most pronounced in and around the distal tubule and collecting ducts. Extensive electron microscopic examination of the lesions revealed that the damage was manifest in gross swelling and oedema of the epithelial cells and their cytoplasmic contents. A marked acute inflammatory response in which eosinophils were prominent accompanied the lesions. Significant glomerular pathology did not result from acute silicon nephrotoxicity. All four silicon compounds tested produced a similar degree of renal damage.

Three different, but complementary techniques were then used to demonstrate the concentration and localisation of silicon in the kidney in the acute experimental model.

Atomic absorption spectroscopic (A.A.S.) measurement of silicon in ashed samples showed an early and rapid rise in renal tissue silicon concentrations in contrast to a slower accumulation in other organs. The rarely studied radioactive isotope of silicon, ^{31}Si which was specially made in an atomic reactor for this investigation, confirmed the A.A.S. findings while additionally providing autoradiographic studies which showed silicon concentration in the distal tubule. Ultrastructural localisation of silicon in the experimental model was achieved by electron probe micro-analysis. Significant silicon peaks were located only over the epithelial cell membrane of the distal tubule where maximal cytological damage was known to occur. Contrary to expectation silicon was not found within any of the epithelial lysosomes or indeed within the cell itself.

The acute experimental model, evolved in the rat, when applied under identical experimental conditions to the fresh water fish, did not result in any renal lesion. This was attributed to the absence of the loop of Henle and to the relative inability of the fish to concentrate urine. The evidence thus collected from acute experimentation considered in toto, indicated that high serum silicon perfusing the mammalial kidney produced significant tubular damage. The high lumenal concentration of urinary silicon which, by inference, occurred in the distal nephron of the rat but not in the fish accounted for the major site of silicon nephrotoxicity. The known affinity of silicon compounds for biological membranes, the unique localisation of silicon over the cell membrane, and the

ultrastructural evidence of cytoplasmic and organelle oedema, together suggested the hypothesis that silicon nephrotoxicity was due to membrane damage and alteration in permeability at that site in the body where silicon concentration was at a maximum.

The data obtained from the acute experiments were used to design a series of investigations concerning the long term effects of silicon on the rat kidney. The remote consequence of a short heavy exposure to silicon was determined. The effect of repeated weekly intraperitoneal injections of silicon was assessed over a nine month period (chronic silicon nephrotoxicity) by sequential morphological and biochemical techniques. These experiments showed that either short intense or prolonged low dosage exposure to silicon resulted in a chronic tubulo-interstitial nephropathy arising from tubular damage. As in the acute experiments glomerular lesions were not evident on light microscopy. Electron microscopy however, showed significant ultrastructural changes in the glomerular epithelial cells and subendothelial electron dense deposits in some of the animals.

An immunofluorescence study designed to characterise the nature of the glomerular deposits failed to reproduce the electron dense deposits while the picture was further obscured by the occurrence of glomerular positivity for anti-rat total immunoglobulins and C_3 in both the experimental and control animals. Nevertheless the most signficant finding in this study was the discovery of strong positive staining for total immunoglobulins and C_3 of the inflammatory cells only in the lumen of the distal tubule.

Electron microscopy revealed that these cells were predominantly degranulated polymorphonuclear leucocytes. Thus there appeared to be an interface in the lumen of the distal tubule where an immunogenic event was taking place.

Despite considerable research effort controversy remains over the pathogenesis of analgesic nephropathy. What may in fact be a significant oversight by previous investigators is the fact that silica and silicates are frequently used as bulking and hygroscopic agents in analgesic preparations. Thus a series of experiments were evolved to test the role of silica as a cofactor in analgesic nephropathy. Three agents, sodium salicylate, para-aminophenol and gasil 200 were individually tested using a sliding scale of decreasing dosage designed to determine the threshold at which discernible renal lesions occurred following a single intraperitoneal injection of the agent. Groups of rats were then subjected to weekly injections of the agents, singly and in various combinations for four months. It was found that neither agent given singly produced any renal lesion while sodium salicylate in combination with para-aminophenol caused significant tubular damage. When all three agents were given together the tubular damage was more pronounced and a chronic inflammatory infiltrate expanded the interstitium. These experiments thus demonstrated apparent additive or synergistic effects of the combination of analgesic agents at minimal dosage and demonstrated the accentuation of the renal lesions by incorporation of silica.

The investigations were concluded by an examination of the urinary excretion of silicon in normal healthy controls, workers exposed to high atmospheric siliceous dust and patients with analgesic nephropathy. Failure of compliance to protocol frustrated meaningful interpretation of the results in both instances.

C H A P T E R I

SILICON - THE ELEMENT AND ITS ROLE IN LIFE

GENERAL INTRODUCTION

The role of silicon as a trace element in biological systems has, in comparison with other less abundant terrestial elements, been largely neglected. Information on silicon in a biological context is widely scattered throughout the scientific literature. This chapter presents a review of the chemistry, geochemistry, bio-geochemistry and toxicology of silicon and its compounds with a special emphasis on currently available knowledge of their effect on the mammalian kidney.

1 BASIC PHYSICAL AND CHEMICAL PROPERTIES

Silicon, which is sixth in cosmic abundance, is after oxygen the most abundant element in earth's lithosphere where it constitutes 25.8% of the crust. It is estimated that it forms 0.002% of human body (Geigy Scientific Tables, Seventh Edition, 1970).

It is an electropositive tetravalent element with atomic weight of 28.086 amu density of 2.33 g/ml and atomic number of 14; it is in the same group of the periodic table as carbon (Geigy Scientific Tables, Seventh Edition, 1970). Silicon electron distribution and subshells are shown below:

K .... $1S^2$

L .... $2S^2$ $2P^6$

M .... $3S^2$ $3P^2$

Si^{+4} has an Atomic radius of 0.41 A^o.

Silicon possesses two kinds of isotopes:

A Natural Isotopes (Table 1)

Isotopes	Abundance (per cent)	Mass (a.m.u.)
^{28}Si	92.21	27.97693
^{29}Si	4.70	28.97644
^{30}Si	3.09	29.97376

Table 1: Showing the natural silicon isotopes, their abundance and mass.

B Artificial Isotopes (Table 2)

Isotope	Half-life
^{27}Si	40.0 sec
^{31}Si	2.65 h
^{32}Si	700 yr

Table 2: Artificial silicon isotopes and their half-lives. (Geigy Scientific Tables, Seventh Edition, 1970 and Treatise on Analytical Chemistry, 1962).

2 INORGANIC SILICON COMPOUNDS

These include:

A Crystalline Silica (SiO_2): always referred to as silicon dioxide. This is a misnomer because silica does not exist as a molecule which contains one silicon and two oxygen atoms, but as a large molecule in which silicon is bounded tetrahedrally to four oxygen atoms and each oxygen to two silicon atoms, (the SiO_4 tetrahedron). The structure of the tetrahedron is shown below (Moller, 1953).

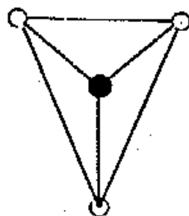


Diagram 1: The SiO_4 tetrahedron structure where the four open circles represent the oxygen atoms while the solid circle in the centre is the silicon atom. The lines represent the side of tetrahedron and not bonds.

Examples of Crystalline Silica are

(a) Quartz: the most common mineral in the lithosphere, is found in most rocks such as sandstone and granite. Quartz is a hard, hexagonal and colourless crystal.

(b) Cristobalite, cubical crystal.

(c) Tridymite, hexagonal crystal.

B Amorphous Silica: these include asbestos, mica, kaolin, opal and diatomaceous earth.

C Silicates: there are a wide variety of compounds ranging from relatively simple molecules through long chains, branching chains, rings and complex polymers (Diagram 2). Silicon is often replaced by aluminium which gives a negative charge to the structure and creates the need for another cation. Silicon anions may occur as discrete anions such as orthosilicate (SiO_4^{4-}) or ions in which a few tetrahedra are linked together in chains, rings or sheets of indeterminate areas (Wells, 1962).

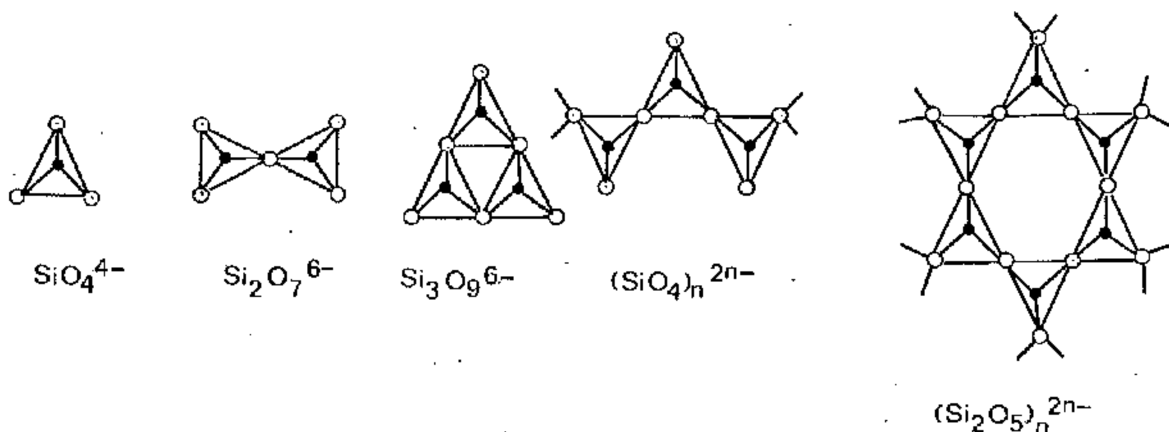
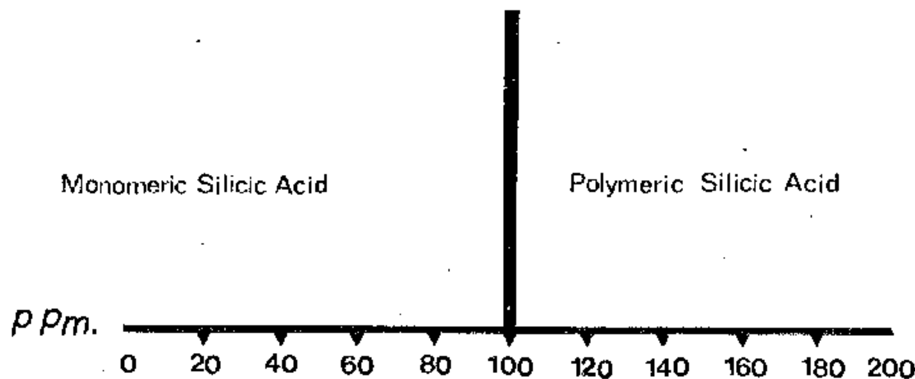


Diagram 2: Represents different structural forms of silicates.

D <u>Physical Chemistry of Inorganic Silicon Compounds</u>:

silicon as the native element does not occur naturally in the earth's crust. When silica or silicates are in contact with water, there is invariably some hydrolysis of Si - O - Si bonds and silicic acid is liberated in small quantities into the aqueous phase. At near neutral pH, the solubility of silicic acid is of the order of 100 ppm and at concentrations below this, it exists as the monomer $Si\ (oH)_4$ (Iler 1955). At concentrations above 100 ppm, monosilicic acid tends to polymerise forming colloidal silica (Diagram 3). Above pH9, silicic acid begins to ionise and its solubility rises sharply. The presence of dissolved organic material also serves to increase the solubility of silica, while the solubility of silicic acid is reduced by the presence of cations such as calcium, aluminium and iron (Iler, 1955).



<u>Diagram 3</u>: The relationship between silicon concentration and polymerisation.

The solubility of silicate depends on the ratio of silicate to alkali metal. Water soluble silicates have a

ratio of around two, as the ratio rises above two, the silicates become insoluble.

After this brief description of the inorganic silicon compounds and silicates, the most common varieties to which man may be exposed include the following:

(a) Sand: used in agriculture, glass, alkali silicate such as water glass, abrasives, insulation, material, silica gel and colloidal silica.
(b) Quartz: used in electronics.
(c) Talc: powder, filler, electronic insulation.
(d) Clay: agriculture, bricks, refractories, pottery.
(e) Shale: bricks, light weight block, cement.
(f) Mica: electronic insulation.
(g) Granite: building, binder, filler.
(h) Feldspar: glass, porcelain.
(i) Chryostile: asbestos, insulation.
(j) Kyanite and mulite - refractories.

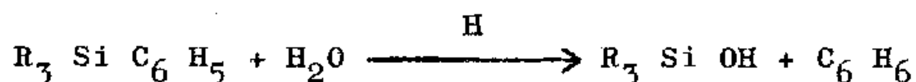
(Treatise on Analytical Chemistry, 1962).

On the other hand, the inorganic silicon compounds which are in common use as biological agents are the antacid magnesium trisilicate ($Mg_2 Si_3 O_8$), silica dust as an insecticide, and sodium hexa fluorosilicate ($Na_2 Si F_6$) which is employed as an insecticide instead of arsenite (Mutch, 1936 a, b and c, Brown, 1951 and Marcovitch, 1928 respectively).

3 ORGANO SILICON COMPOUNDS

These compounds do not exist in nature, and are entirely

man-made. They can, however, be fairly readily prepared in the laboratory. The most striking chemical characteristic of these compounds is their tendency to form silicon oxygen bonds. It is well known that silicon carbon bond is relatively stable, but in terms of chemical non-reactivity the most stable of all silicon bonds is the silicon oxygen bonds (Eaborn, 1960). There are three important groups of organo silicon compounds containing silicon oxygen bonds, silanols (R_3 Si OH), disiloxanes (R_3 Si O Si R_3) and aloxysilanes (R_3 Si O R). The silicon carbon compounds which contain the silicon - alkyl carbon bond are readily hydrolysed in the presence of an acid to yield silanol.

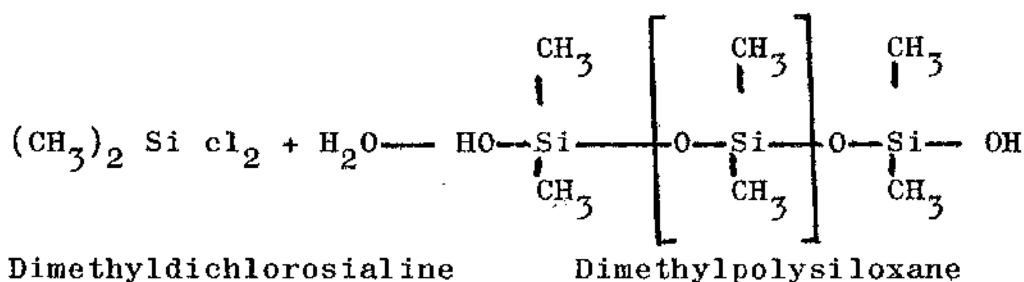


$$R_3\ Si\ C_6\ H_5 + H_2O \xrightarrow{H} R_3\ Si\ OH + C_6\ H_6$$

Other organo silicon compounds include, the silicon halogen compounds, usually referred to as organo silicon halides (R_3 SiX), silicon hydrogen compounds referred to as organo silicon hydrides (R_3 SiH), silicon nitrogen compounds known as silazanes or silylamines - (R_3 Si NH_2) and silicon sulphur compounds named silathianes.

From these organo silicon compounds, several important polymers are readily prepared. The preparation of these polymers depend on Siloxane. Dimethylpolysiloxane is the most common polysiloxane in use. Polysiloxane possess a wide variety of structural possibilities, thus many recent technological developments depend on this polymer. It is prepared by the hydrolysis of dimethyldichlorosialine (page 25). Other polymers include the silicon resins,

silicon rubbers and bouncing putty. These polymers are heat stable and are widely employed as anti-foaming agents, lubricants, insulators, etc. One of their most important properties is their lack of biological activity which has made them attractive and extremely useful materials in the medical world.



4 SILICON IN BIOLOGY

In the biological world it was found that silica and silicates are important nutrient materials, particularly in primitive organisms and plants. Silicon is extracted from the medium they inhabit and is deposited in the form of amorphous silica. It has been demonstrated that Navicula pelliculosa (fresh water diatom) has a cell wall composed of silica (Silicalemma). The relationship between the organic constituents of the cell wall and silicon has been studied by (Reimann et al, 1966). It was noted that if this fresh water diatom was grown in a silica-free medium inhibition of cytokinesis and cell division occurred after mitosis while the addition of silicon facilitated cell wall formation, cell separation and division (Combs et al, 1967). In addition, certain fungi and bacteria, e.g. Bacillus siliceous are capable of breaking down aluminosilicates in the soil. These organisms can be successfully cultured in a granite bowl or in a powdered

glass medium (Tesic and Todorovic, 1959). The role of other bacteria in degradation of silicates and their uptake has been studied by Heinen (1963).

In plants, silicon was also found to be an essential element in the formation of the cuticular double layer as well as in the structure of the silicon cellulose layer which limits water evaporation and serves as a barrier against pathogenic fungi and insects. Silicon deficient plants are thus more liable to water loss by transpiration and become more susceptible to fungal and insect attack (Allison, 1968).

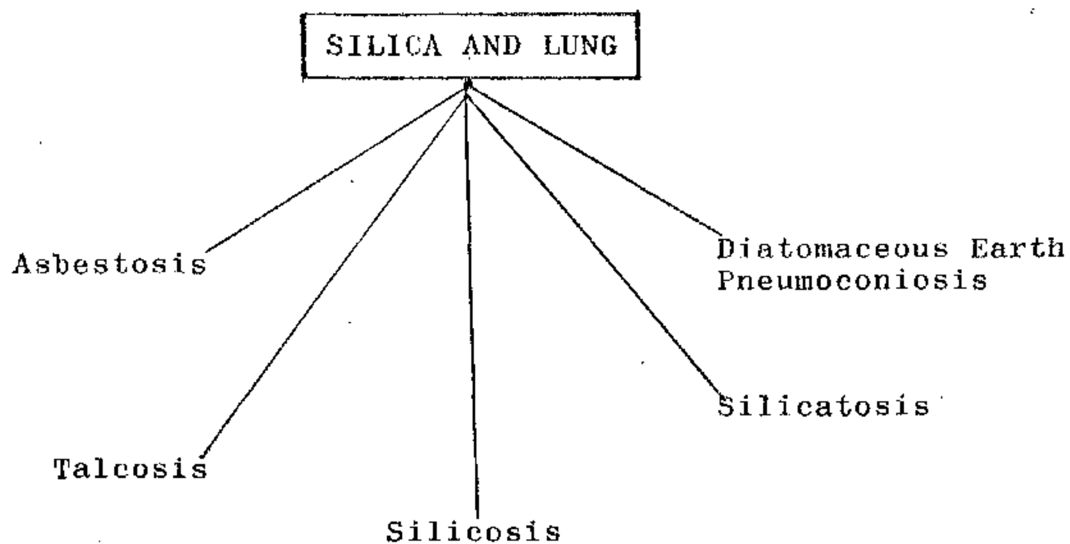
Concerning the evolution of life in the sea, it is known that the concentration of silicon is low (0.01 - 7 ppm) and thus insufficient to permit polymerisation of mono to polysolicic acid (Iler 1955). The effect of silicon on fish kidney is discussed later.

5 TOXICITY OF SILICON COMPOUNDS ON BIOLOGICAL SYSTEMS

Silicon compounds have been incriminated in the pathogenesis of several organ disorders.

A <u>The Lung</u>: Pneumoconiosis: is a well known general term for lung disease resulting from inhalation of siliceous dust particles. The effect of inhalation of different kinds of silicon compounds is summarised in (Diagram 4)

(a) Asbestosis: is a lung disease which results from inhalation of fine rod-like particles (20 - 200 µ long and less than 3 µ wide). The disease is characterised by a foreign body granulomatous reaction around the



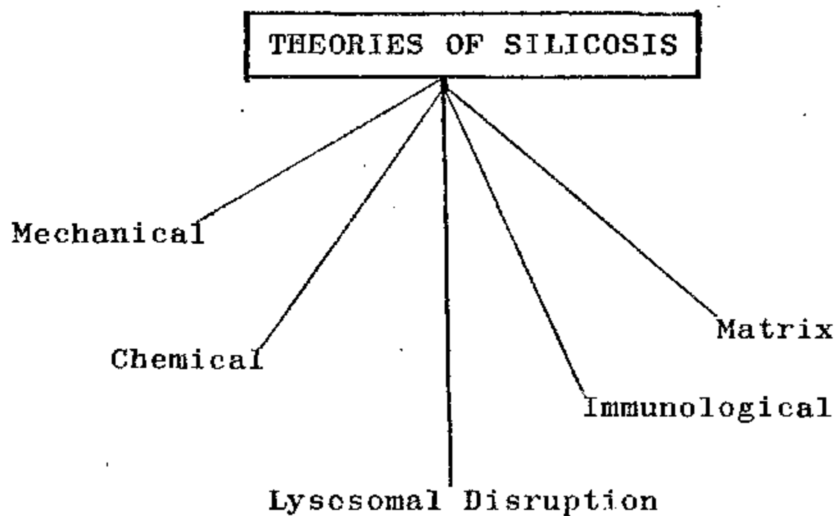


Diagram 4: Showing the effect of silica on the lung and theories of silicosis.

asbestos fibre in pulmonary alveoli. Unlike silicosis, asbestosis cannot be produced in other tissues by injecting asbestos fibres.

(b) Talcosis: a condition similar to asbestosis is caused by the inhalation of talc powder (fibres of magnesium and calcium silicate).

(c) Silicatosis and Diatomaceous Earth Pneumoconiosis: are occupational lung diseases which are caused by the inhalation of silicates or diatomaceous earth. In general, they do not result in such severe pulmonary lesions as those found in asbestosis.

(d) Silicosis: is a chronic, fibrosing disorder of the lung produced by prolonged action of micro-crystalline silica (particles less than 3 μ in diameter). One of the earliest descriptions of pulmonary disease produced by dust is given by Agricola in his Treatise on Mining (1556), while its occurrence in stone cutters was described by Van Dimerbroeck (1672) and Ramazzini (1713) - Quoted by Zaidi (1969). Silicosis is a progressive lung disease which usually requires 3 - 12 years to develop and can be classified according to its stage or phase of development. In the last phase, radiology of the silicotic lung shows massive fibrosis and conglomeration of nodules.

Theories on the Pathogenesis of Silicosis

i) Mechanical Theory: according to this theory, pathogenicity of dust was secondary to trauma caused by sharp silica particles. However, it was shown that silicon carbide, which has the same characteristics

of silica (hard and sharp), was not fibrogenic (Gardner, 1923).

ii) Chemical Theory: it was postulated that the pathogenesis of fibrosis in silicosis was the continuous liberation of silicic acid from the surface of dust particles and the degree of pathogenicity was then related to the rate of release of silicic acid into solution (King, 1947). It was believed that local release of silicic acid caused denaturation of protein.

iii) Immunological Theory: evidence of an immune mechanism in the pathogenesis of silicosis has been advanced by several workers (Vigliani and Pernis, 1963). This theory arose out of the detection of increased amounts of globulins in serum, lymphatics and lung tissue.

iv) Matrix Theory: this theory was based on the fact that quartz has the ability to combine with the amino acids and replace the matrix normally provided by nucleic acids which is necessary for protein bio-synthesis (Seifert 1958 and 1964).

v) Lysosomal Theory: The Cytoxic Effect of Silicon on Macrophages and its Relation to Silicosis.

Most recent work on the cytotoxicity of silicon has concerned its effect on the macrophages. It now seems most likely that the continuous destruction of macrophages by siliceous particles is the primary stimulus towards the slowly progressive fibrosis of the silicotic nodule. Marks and Nagel Shmidt (1959) demonstrated that dehydrogenase activity was reduced in macrophage cell culture incubated with silica,

while Munder et al (1966) found that the lysosomal enzyme, phospholiase A, was activated when macrophages were exposed to silica. This enzyme catalyses the reaction lecithin $\longrightarrow$ lysolecithin and accordingly, the increased amount of lysolecithin indicated the degree of macrophage damage induced by silica. In addition, Harington (1963) believed in a two staged process involved in fibrosis induced by silica. The first stage is the damage to macrophages, while the second is the liberation of a material possessing fibrogenic activity. This material was thought to be lipid in nature. However, the most interesting work in this field is that of Allison et al (1966) who used mouse peritoneal macrophages as a model to study the effect of silicon on their lysosomal system. They found, by phase contrast cinemicrography, electron microscopy and histochemistry, that macrophages incubated with silica were readily shown to take up silica particles into their cytoplasm which resulted in the formation of secondary lysosomes (primary macrophage lysosome + phagosome). After twenty-four hours most of the macrophages died, while silica particles and lysosomal enzymes were found free in the culture media. This experiment was repeated with diamond, aluminium coated silica and silica in the presence of a protective agent such as poly-vinyl-pyridin-N oxide (PVPNO) and showed no evidence of damage to the macrophage. From these findings, the authors concluded that macrophage damage was due to the interaction between the silica particles and the

secondary lysosomal membranes. The mechanism of membrane damage by crystaline silica was thought to be due to the formation of soluble silica acid on the surface of the particles. The silicic acid then acting as a hydrogen donor became bound to the membrane through the phosphate ester groups on the phospholipids (Nasha et al, 1966, Allison, 1968 a and b and Allison, 1971) or to membrane protein (Summerton et al, 1977). The rigid particles thus bound to the membranes ultimately resulted in their rupture.

The toxic effect of silica on the macrophage has also been advanced in the explanation of the potentiation of tuberculous infection in silicotic lungs. In laboratory experimentation, the multiplication of a virulent strain of mycobacterium tuberculosis in cultures of mouse peritoneal macrophages was markedly increased by the addition of a subtoxic dose of quartz. The silica particles and the tuberculous bacilli were located in the same secondary lysosome. The silica, due to its interaction on the lysosomal membrane, facilitate the passage of these bacilli into the cytoplasm where they easily multiplied (Allison and Hart, 1968).

These theories as they apply to the interpretation of silicon nephrotoxicity are considered in greater detail in subsequent chapters.

B <u>The Kidney</u>: the renal disorders associated with inhalation, ingestion, or even parenteral administration of silicon compounds are reviewed under the following headings: experimental silicon nephrotoxicity, and silicon and human kidney.

(a) Experimenal Silicon Nephrotoxicity: the effect of acute or chronic administration of different silicon compounds in the experimental animals has been studied by a number of workers. Gye and Purdy (1922 a and b) demonstrated the acute effect of intraperitoneal and intravenous injections of silica sol (orthosilicic acid - Si $(OH)_4$) on different organs, including the kidney. It was concluded that the production of petechial haemorrhages with extensive degeneration of liver and kidney were readily obtained. In the kidney, the greatest damage was thought to occur in the glomeruli where they found dilatation of capillary loops, serum exudation into the capsular space with capsular epithelial swelling and desquamation. Bacterial colonies were located inside the glomeruli. Red blood cells were seen in the capsular space and in the tubular lumena (red blood casts). The tubular epithelium, although focally affected, did not show the same degree of involvement as the glomeruli. A mild degree of "fatty degeneration" was seen in the cells of collecting tubules. The tubules were generally widely dilated and filled with casts.

On the other hand, the effect after 48 hours of intra-peritoneal injection of silica gel (metasilicic acid - H_2SiO_3) was studied by Policard and Collet (1954), Policard et al (1957) and Policard et al (1960). In contrast, these workers found that a tubular lesion predominated and glomerular changes were minimal. Acute tubular necrosis was established by 48 hours. Electron microscopy demonstrated "granulation" of glomerular basement membrane and fusion of epithelial foot processes. The proximal tubules were oedematous and vacuolated with disruption of the brush border. Electron dense intramitochondrial deposits were noted in the tubular cells.

Following the chronic experimental administration of silica sol, Gye and Purdy (1922 b) found that renal lesions were more prominent than those in the liver. The renal capsule was thickened, and strands of fibrous tissue corresponding in position with the surface depressions passed to the medulla. There was peritubular and periglomerular fibrosis. Occasionally, the process of fibrosis extended to the tuft and the whole glomerulus was sclerosed. In these experiments it was commented that the tubular epithelium was not affected and authors reached the conclusion that the process was that of an early interstitial nephritis.

Contrary evidence presented by Newberne and Wilson (1970) focused mainly on the tubular damage by silicon compounds. Oral preparations of silicon dioxide,

aluminium silicate, sodium silicate and magnesium trisilicate were fed for a period of four weeks to dogs. Renal damage was found in animals receiving sodium silicate and magnesium trisilicate. No lesions were detected in the kidney of animals receiving dioxide and aluminium silicate. The basic nature of renal lesions was the same and varied only in severity and distribution. Tubular epithelial swelling with or without degenerative changes was observed. Interstitial cell infiltration, tubular dilatation and tubular collapse were present in affected areas of the kidney. Although some of the tubules were partially blocked due to swelling, proliferation or regeneration of the epithelium, the tubular lesion seemed to be reactive and not secondary to mechanical blockage. The glomeruli did not exhibit any marked pathological alteration. They concluded that the lesion was one of irritation to tubular epithelium followed by degenerative and regenerative changes while these alterations were accompanied by an interstitial inflammatory cellular infiltrate.

(b) Silicon and Human Kidney: renal lesion associated with industrial exposure to silicon compounds can be divided into three different categories:

i) Renal lesion associated with the classical form of pulmonary silicosis.

ii) Renal lesion associated with acute form of pulmonary silicosis.

iii) Renal lesion associated with heavy silica

exposure, but undetectable pulmonary involvement (negative chest X-ray).

These will now be considered in some detail.

Renal Lesions Associated with Classical Pulmonary Silicosis

In 1951 Saita and Zavaglia, in the examination of twenty patients suffering from pulmonary silicosis, found albuminuria in four, renal concentration defect in nine and azotemia in eight. In contrast, Capezzuto (1963), who studied fifteen cases with advanced pulmonary silicosis, found neither changes in the glomerular filtration rate, proteinuria nor abnormal urinary sediment.

Kolev et al (1970), however, demonstrated histopathological evidence of renal involvement in forty-five patients who had died from late pulmonary silicosis. 51% of the examined kidneys showed glomerular, interstitial and tubular lesions. The glomerular lesions consisted of thickening of the basement membrane, focal and segmental proliferation of endothelial and mesangial cells, synechiae and focal sclerosis. The interstitium was fibrotic. There was no cellular infiltrate of any kind. In the tubules the most marked lesions were found in the proximal convoluted tubule which showed cellular atrophy, dilated lumina filled with proteinaceous material and thickened basal lamina. No lesions were detected in the loop of Henle or distal convoluted

tubules. In the collecting tubules, the main changes were dilatation and cast formation. They suggested that during the course of the pulmonary silicosis, autoantibodies against altered tissue components and macrophages might be formed, this mechanism giving rise to antigen-antibody complexes which, filtering through the glomeruli, result in the glomerular lesion they describe.

<u>Renal Lesions Associated with Acute Form of Pulmonary Silicosis</u>

Acute silicosis has been considered as special entity characterised by short exposure to silica dust, rapid onset of symptoms and progressive course uninfluenced by treatment. This form of pulmonary silicosis was originally described by Middleton (1929). Early reports described the presence of numerous, small nodules embedded in fibrous matrix, compared with large nodules found in classical silicosis (Gardner, 1933). Histological changes closely resembling alveolar proteinosis have been found in a group of these patients (Buechner and AnSari, 1969).

Xipell et al (1977) presented a case of alveolar proteinosis associated with silicosis in which there was evidence of a renal lesion manifested as proteinuria, elevated serum urea and creatinine. Furthermore, Giles et al (1978) described marked proteinuria, hypoalbuminaemia, and raised serum creatinine in a patient with acute pulmonary silicoproteinosis. Urine analysis revealed occasional white and red cell

casts and refractile fat bodies.

In their case, Xipell, et al (1977) described a focal glomerulonephritis. In a more detailed histological presentation, Giles et al (1978) presented similar findings with focal segmental hypercellularity of the glomerular tuft. In addition, they noted an increased number of glomerular epithelial cells which showed nuclear vacuolation, prominent nucleoli and mitotic figures. The proximal tubules exhibited a hydropic degeneration whereas other tubules were dilated. The electron microscopic finding disclosed extensive foot process fusion overlying normal glomerular basement membrane. No immune complexes were observed, but there was accumulation of an electron dense material within the epithelial cell cytoplasm in close apposition to the glomerular basement membrane. The cytoplasm of both epithelial and endothelial cells contained dense granular membrane bound structures which they thought could represent an altered lysosome. Occasionally, myelin figures and microtubular structures were seen in some endothelial cells.

Using immunofluorescence technique, Xipell et al (1977) demonstrated positive focal granular staining of glomerular peripheral capillary loops with IgM, IgA and complement, while Giles et al (1978) reported the presence of positive focal segmental glomerular staining with IgM and C_3 along the glomerular basement membrane together with positive mesangial

deposits. In addition, Giles et al (1978) analysed renal tissue from their case and found the silicon content to be markedly elevated.

Renal Lesion Associated with Heavy Silicon Exposure

Undetected Pulmonary Involvement (negative chest X-ray)

In two separate cases reported by Saldanha et al (1975) and Hauglustaine et al (1980), renal abnormality has been shown in two patients both with occupational exposure to heavy silica dust, but with negative pulmonary radiological findings on chest X-ray. The renal lesions were diagnosed as glomerular and tubulo-interstitial nephritis in the case reported by Saldanha et al (1975) while the renal lesions in the case of Hauglustaine et al (1980) were diagnosed as mild focal segmental proliferative glomerulonephritis. Both patients presented with proteinuria and mild hypertension.

Detailed histopathological description was given in both patients by the two groups of workers. Saldanha et al (1975) described the glomerulopathy as a focal mesangial proliferative lesion with extra capillary wall thickening. There were no adhesions, crescents nor inflammatory cell infiltration. Increased periglomerular connective tissue was noted. The tubular lesions were confined to the proximal convoluted tubules, included widespread vacuolation, increased osmiophilic droplet formation much

exfoliation and apparent necrosis. The distal tubules had only minor changes. The small arterioles showed mild intimal proliferation.

Electron microscopic examination confirmed the mild to moderate glomerular lesion with extensive foot process fusion, and thickened basement membrane due to the presence of low density, granular sub-endothelial deposits. Oedema of the endothelial cell cytoplasm was occasionally noted. Peripheral extension of mesangial cell cytoplasm and matrix was also seen. The proximal convoluted tubules showed epithelial cell desquamation; the lining cells being filled with large vacuoles of low density cytosomes, some of which contained aggregates of an electron dense osmiophilic material. The vacuoles were located mainly in the basal part of the cells or in the supranuclear region, only a few being located in the apical part. An additional finding was occasional cytoplasmic multilamellated bodies (myelin figures). Further changes included an electron dense, membrane enclosed bodies similar to the lysosomes. The basal infoldings and the inter-cellular spaces were wider than normal. The rough endoplasmic reticulum was intact, but the mito-chondria were denser than normal with increased intracristal spaces. Tubular basement membrane was of normal thickness. The interstitium was expanded and oedematous. The distal convoluted tubules and the loop of Henle were intact.

On the other hand, Hauglustaine et al (1980) gave a histological description of mild focal proliferative glomerulinephritis with segmental distribution. According to them, the tubular lesion was confined to cellular hypertrophy. However, they were in agreement with Saldanha et al (1975) over the main electron microscopic findings in that they also found extensive foot process fusion, increased mesangial matrix, mesangial cell interposition and sub-endothelial deposits. Additional changes found by Hauglustaine et al (1980) were sub-epithelial dense deposits and dense granular deposits in the visceral epithelial cells. The ultrastructural changes in the proximal tubules were of similar nature to those recorded by Saldanha et al (1975) including the vacuolation and increased mito-chondrial density. Increased pinocytotic activity near the brush border was an additional observation.

The immunofluorescence findings were different for both groups; Saldanha et al (1975) found only positivity to anti human IgM which was focal and granular. Hauglustaine et al (1980) found insignificant staining for IgG, IgA, IgM, CI_q, C_4, albumin and fibrinogen, but strong positive staining for C_3 in the intima of the blood vessels.

The silicon content of kidney tissue was found to be 200 µg/g (dry weight) by Saldanha et al (1975) and 150 µg/g (dry weight) by Hauglustaine et al (1980). These readings compared with a control range of

14.2 ± 13.3 (S.D.) and 27.0 ± 21.6 (S.D.) ug/g respectively. These findings were considered by both groups to be significant in attributing the lesion to the effect of silicon and hence favouring a diagnosis of silicon nephropathy.

(c) Endemic Nephropathy: silicon has been propounded as an aetiological factor of an important renal disorder called Endemic Nephropathy.

In 1956 reports from twenty villages along the River Iskar (West Bulgaria) first brought to medical attention the existence of one of the most unusual kidney diseases. A year later (1957), a similar malady was reported along the bend of the River Danube and adjacent to it in the Rumanian Benta and Oletania, some 50 to 80 kilometres from the Vratsa region in Bulgaria, and in the Kolubara and Morova districts in Yugoslavia. It was concluded at the planning conference on Endemic Nephropathy of South Eastern Europe (1964) that the Bulgarian, Rumanian and Yugoslavian nephropathies were the same disease.

Endemic nephropathy is exclusively found among farmers who live in rural areas. It usually becomes manifest between 30 - 50 years of age with a slightly higher incidence in women. In Yugoslavia there is an increased incidence of asymptomatic proteinuria among children who live in the endemic area. Endemic nephropathy is an interstitial non-inflammatory renal disease with extensive damage to the tubular

epithelium and only late in its development does one find a secondary glomerular lesion. These changes terminate in marked renal contraction. In general, it is a slowly progressive disorder characterised by a prolonged course leading to uraemia and death.

The clinical picture in adults is invariably dominated by signs and symptoms of uraemia due to late diagnosis of this illness. In addition, there is normochromic or hypochromic anaemia with some features of haemolysis. There is also yellow discolouration of the skin of the palms and feet, thought to be a specific sign for this disease (Xanthochromia). There is mild proteinuria, (usually around 1 g/24 hours) and scanty urinary deposits which include RBC, WBC and granular casts. There is reduced urinary concentration with urine specific gravity around 1012. On plain X-ray of abdomen markedly contracted kidneys can readily be seen. Two important negative signs are features of this disease, absence of any sign of fluid or salt retention and absence of hypertension in 85% of cases. (Planning Conference on Endemic Nephropathy of South Eastern Europe, 1964).

In these patients the kidney is reduced in size, the weight may be as low as 50 g the surface is smooth and the capsule strips easily. The kidney contraction in particular involves the outer cortex. The pathological process can be divided into three stages. In the first stage, there is damage to the tubular epithelial cells. The tubules soon undergo atrophy

and thickening of their basement membranes. Although there is increased interstitial connective tissue, no cellular infiltration has been detected.

In the second stage, there is increased fibrosis and further tubular atrophy. The first sign of glomerular change appears at this stage in the form of focal glomerulitis and focal thickening of the basement membrane.

In the third stage, there is marked fibrosis especially in the outer cortex with complete absence of the tubules and many fibrotic glomeruli. Although the medulla shows the same type of lesion, the histological integrity of the inner zone is better preserved (Planning Conference on Endemic Nephropathy of South Eastern Europe, 1964). The ultra structural pattern of this disease is characterised by an atypical association of tubular, interstitial and glomerular changes. If these changes are considered separately, it would be rather difficult to give any specific pathological conclusion (Jurukova and Dinev, 1972). The electron microscopic changes consist of an increased number of nuclei in the intercapillary spaces, sliding of mesangial cells between endothelial lining and basement membrane, increased mesangial matrix deposition and irregular thickening of capillary basement membrane next to hypertrophied mesangium. There is nodular thickening of capillary basement membrane alternating with less thickened or even intact membrane zones. In addition,

there is an osmiophilic matricial aggregate in the mitochondria of the proximal tubules. The same histological lesion described above has been demonstrated in small mammals living in the same endemic regions (Marches and Rotaru, 1972).

The aetiology of endemic nephropathy is unknown. Although it occurs more frequently in certain families, it is believed to be casually associated with some aspects of rural living. There is neither an intimate correlation between infection and this disease, nor a relationship between it and any of the trace element or heavy metals such as cadmium and uranium. In addition, no incriminating evidence has been found regarding insecticides or chemicals used in agriculture or industrial processes (Planning Conference on Endemic Nephropathy of South Eastern Europe, 1964). Further reviews of the aetiology of endemic nephropathy have been presented by Danilovic et al (1972) and Jezic (1972).

Perhaps the most interesting of all possible aetiologic factors is the drinking water. Epidemiologically, the disease is mainly located in the low lying, flooded areas close to certain rivers, whereas, its incidence is much lower in the mountains. The endemic areas are found mainly in regions characterised by high silicate geochemical structures, where conditions predispose to deposition and accumulation of eroded material along river banks. Thus, it was suggested that silicic acid which may be polymerised

during renal excretion, causing denaturation of kidney proteins, gave rise to the pathological changes of endemic nephropathy (Markovic and Arambasic, 1968).

Experimental induction of lesions similar to endemic nephropathy has been achieved by Roscoulesco and Nicolesco (1972) Markovic and Arambasic (1968) and Markovic et al (1972) using ground quartz suspension in the drinking water of guinea pigs.

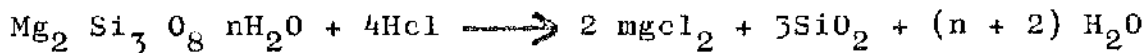
A final intriguing feature of this interesting nephropathy is the high incidence of upper urinary tract tumours. Polyps, papillocarcinomas and carcinomas in the renal pelvis, ureter and urinary bladder have been reported in one third of patients dying from this disease Sindjic, et al (1972), Markovic (1972 a and b).

6 ABSORPTION AND EXCRETION OF SILICA

Since 1936 when the hydrated magnesium trisilicate was first shown to be an active agent in the treatment of hyperchlorhydria, many silicate containing drugs have been offered for unlimited sale and have gained wide popularity (Herman and Goldberg 1960).

Magnesium trisilicate was believed to be an inert medication being neither absorbed from the gastro-intestinal tract nor causing any alteration in the normal physiology of the human body. The hydrated silicates of magnesium have both antacid and adsorbant properties. These characteristics are more evident in the trisilicate and negligible in tetrasilicate and talc preparations

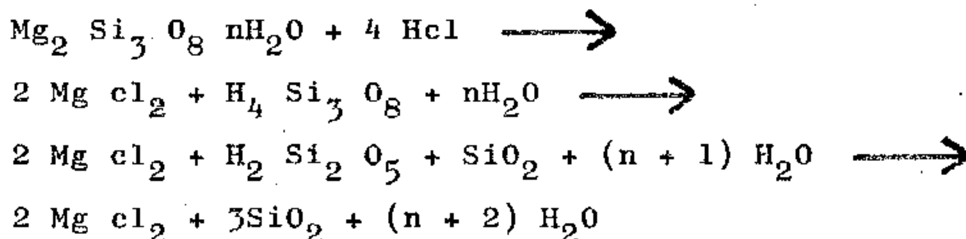
(Mutch, 1936 c). In the stomach magnesium trisilicate reacts with the hydrochloric acid according to the equation.



$$Mg_2\ Si_3\ O_8\ nH_2O + 4Hcl \longrightarrow 2\ mgcl_2 + 3SiO_2 + (n + 2)\ H_2O$$

In the presence of hydrochloric acid, magnesium trisilicate assumes a colloidal gel state. Silica liberated from this reaction is an active adsorptive agent. Both magnesium trisilicate and liberated silica (SiO_2) were not thought to be absorbed from the gastro-intestinal tract and therefore were believed to be incapable of producing alkalosis (Kraemer and Aaron, 1940 and Mutch, 1936 c).

However, Page et al (1941) proved that silica was indeed absorbed from the gastro-intestinal tract and was excreted by the kidneys. The urinary excretion rate of silica depended on the dietary silicon content and was greatly increased after the ingestion of magnesium trisilicate. No fixed renal threshold was demonstrated. The above authors suggested that the reaction between magnesium trisilicate and hydrochloric acid proceeds through three steps.



$$Mg_2\ Si_3\ O_8\ nH_2O + 4\ Hcl \longrightarrow$$
$$2\ Mg\ cl_2 + H_4\ Si_3\ O_8 + nH_2O \longrightarrow$$
$$2\ Mg\ cl_2 + H_2\ Si_2\ O_5 + SiO_2 + (n + 1)\ H_2O \longrightarrow$$
$$2\ Mg\ cl_2 + 3SiO_2 + (n + 2)\ H_2O$$

In the intestine, some of the di or tri silicic acid reacts with sodium to form soluble silicate ($Na_2\ SiO_3$).

This compound is absorbed from the gastro-intestinal tract and excreted in the urine.

In the Renal Unit, University Department of Medicine, Glasgow Royal Infirmary, Dobbie et al (1976) have, for the past five years, been engaged in studies on the absorption and urinary excretion of silicon in man. Investigations of the silicon content of biological fluids was considered to be exceptionally difficult using standard analytical chemical methods. However, they successfully measured silicon in biological fluid using atomic absorption spectroscopy and this has opened up entirely new possibilities in the study of silicon in man.

Some 8% of ingested silicate is absorbed from the alimentary tract and several studies have shown that the kidney is the main excretory organ for dietary absorbed silicon. In health, serum silicon is maintained within a narrow range (0.3 - 0.8 µg/ml), while Dobbie et al (1976 and 1978) have also demonstrated that with increasing renal impairment there is retention within the body of dietary silicon which gives rise to a uraemic hypersilicaemia. Thus, elevated serum silicon levels (3 - 4 times basal level) are encountered in patients with chronic renal failure and in patients treated by haemodialysis (Dobbie et al, 1976 and Dobbie et al, 1978 a and b).

7 SILICA UROLITHIASIS

The renal excretion of silica raises the possibility of its deposition in the urinary tract. This has indeed been studied both in animals and man. However, in animals,

insoluble siliceous deposits were readily seen in the kidney of guinea pigs after oral or intraperitoneal administration of sodium metasilicate - $Na_2 SiO_3 - 5H_2O$ (Settle and Sauer 1960). Histologically, these were found to be lightly stained, homogeneous masses in the renal tubular lumena. Also, silica has been shown to be responsible for urolithiasis in beef cattle fed on dry fodder which has a high silica content (Whiting et al, 1958 and Forman et al, 1959). These calculi were found to contain opaline silica as the main constituent, with varying amounts of calcium oxalate and calcium carbonate.

In man, urinary silica calculi (opaline silica - SiO_2 nH_2O) due to prolonged use of antacids (magnesium trisilicate) have been described (Herman and Goldberg, 1960 and Joekes et al, 1973). In a survey of 800 patients with urinary calculi, Lagergren found five cases in which the stones were composed mainly of silica. All of these patients with siliceous calculi had been on magnesium trisilicate for 3 - 5 years (Lagergren 1962).

The suggested mechanism of development of these urinary siliceous deposits and calculi is thought to be as follows. After the ingestion of insoluble silica, depolymerisation to soluble absorbable silica takes place in the intestine. During renal excretion, as tubular concentration of silicon rises, there is an increasing tendency for silica to polymerise. This is promoted by the occurrence of a fall in pH and thus these factors combine to produce insoluble deposits of silica within the

tubular lumena. As silicon is of comparatively low atomic weight (28 AMU) these deposits and the calculi which may thereby result are usually radiolucent.

8 SUMMARY

This review has shown that silicon is involved in many life processes. Many disparate groups have worked in specific areas of interest involving the interaction of silicon and biology. What is lacking is an overview of its role in normal biological processes and of its potential toxicity to life. This thesis attempts to make a contribution to one of its possible toxic effects and by investigating this aspect with a wide variety of techniques, shed some new light on the role of silicon in biology.

C H A P T E R II

ACUTE EXPERIMENTAL SILICON NEPHROTOXICITY

INTRODUCTION

In view of the fact that there is an ever increasing exposure of man to natural and synthetic silicon compounds and in the knowledge that the toxicology of these substances has been little studied, it would appear that an investigation of silicon nephrotoxicity is long overdue.

This investigation was designed to test the acute nephrotoxicity of a variety of amorphous silicate compounds to which man is currently exposed.

MATERIALS AND METHODS

The acute effects of the intraperitoneal administration of different kinds of silicon compounds on the rat kidney were studied. These compounds were:

(1) Gasil 23 D: is a synthetic, amorphous silica (SiO_2) compound with an average particle size of 1.5 µ (Coulter Counter). It has a surface of around 350 m^2/g and a pore volume of 1.3 ml/g giving a mean pore diameter of 150 X^o.

(2) Gasil 200: is a synthetic amorphous silica (SiO_2) compound with an average particle size of 4 - 5 µ and a surface area of about 800 m^2/g with corresponding low pore diameter.

Both Gasil 23 D and Gasil 200 were suppled by Joseph Crosfield and Sons Ltd., Warrington, Lancashire.

(3) Silica Gel: is a suspension of silicic acid prepared by dissolving sodium metasilicate ($Na_2\ SiO_3\ 5H_2O$) in warm distilled water, then rapidly adding 2M hydrochloric acid with continuous pH monitoring until a precipitate is formed, at which point the pH was adjusted to between 8 and 8.5.

(4) Magnesium Trisilicate: B.P. ($Mg_2\ Si_3\ O_8\ H_2O$).

Methods of Sterilisation

Gasil 23 D, Gasil 200, silica gel and magnesium trisilicate were placed in separate petri dishes, each containing 100 mg of silica compound and sterilised by ultraviolet light or irradiation (Cobalt 60 Unit, Institute of Radiotherapeutics, Belvidere Hospital, Glasgow). The sterile powder was then dissolved in 2 ml sterile normal saline (0.9% Nacl.). Aliquots were tested in the Bacteriology Department, Glasgow Royal Infirmary to confirm the efficacy of the sterilisation procedures.

Animal Data

All animals used in this study were female Sprague-Dawley rats (8 weeks of age and 200 - 250 g in weight). The sixty rats used were divided into five groups of twelve.

Group 1: received a single intraperitoneal injection of 100 mg (in 2 ml sterile saline) of gasil 23 D.

Group 2: received a single intraperitoneal injection of 100 mg (in 2 ml sterile saline) of gasil 200.

Group 3: received a single intraperitoneal injection of 100 mg (in 2 ml sterile saline) of silica gel.

Group 4: received a single intraperitoneal injection of 100 mg (in 2 ml sterile saline) of magnesium trisilicate.

Group 5: received a single intraperitoneal injection of 2 ml normal saline (0.9% Nacl.) and served as the control group.

Sampling

Four rats from each group were sacrificed at day 4, 21 and 35. Kidneys of all the animals were bisected, fixed in 10% phosphate buffered formalin, processed and embedded in paraffin wax. Sections were prepared from the whole face of the bisected kidney to show cortex, medulla, papilla, renal pelvis and upper ureter. The sections were stained by haematoxylin and eosin and examined by light microscopy.

For electron microscopic examination, small blocks of kidney were fixed in 2% phosphate buffered gluteraldehyde and post fixed in 1% osmic acid. After processing and embedding in emix resin, ultrathin sections were cut, mounted on copper grids, stained by urinyl acetate and lead citrate and examined by Phillips 200 electron microscope.

During this study, the sampling for electron microscopy was extensive and a total of 400 electron micrographs were examined.

RESULTS

1 LIGHT MICROSCOPY

A Gasil 23 D

Four Days Effect: Macroscopically, the kidneys were enlarged and pale, their surfaces were smooth and the capsules stripped easily. The principal microscopic findings consisted of lesions mainly confined to the tubules (Figure 1), the only glomerular abnormality being slight dilatation of Bowmen's space. The proximal tubular epithelium was found to be swollen, the cytoplasm was vacuolated, while many of the nuclei showed pyknosis (Figure 2).

The most striking lesions were found in the distal nephron (Figure 3). Many of the collecting tubules showed marked dilatation, their lumena containing an unstained granular material, plugs of desquamated epithelial cells, or acute inflammatory cells. The nuclear changes were swelling, vacuolation, pyknosis, Karyorrhexis and karyolysis. Mitotic figures in the lining epithelium were frequent, while focal ductal epithelial proliferation was also seen. The lesions extended proximally to affect the distal convoluted tubules which showed dilation with flattened epithelial cells. Peritubular inflammatory

cell cuffing, predominantly polymorph nuclear leucocytes was a striking feature.

The cortical and medullary interstitium was oedematous and lightly infiltrated with both acute and chronic inflammatory cells. Eosinophils were focally prominent. The blood vessels appeared normal, there being no evidence of arteritis.

Twenty-one Days Effect: Macroscopically, the kidneys were smaller than normal, the surface was irregularly pitted and the capsules stripped with difficulty.

Microscopically, there was a definite zonation of the lesions which were most marked in the subcapsular and corticomedullary regions (Figure 4 and 5). Although there was dilatation of the distal tubules, it was not as extensive as that seen after four days. The subcapsular lesions included distal tubular damage manifested as focal areas of dilatation surrounded by chronic inflammatory cellular infiltrate and fibrosis (Figure 5). Some of these dilated tubules contained cellular casts (inflamm-atory cells and desquamated epithelial cells), while in others there were hyaline casts. The collecting ducts in the outer cortex were dilated and lined with flattened epithelium. The epithelial lining showed nuclear degenerative changes. These tubules were surrounded by

chronic inflammatory cell infiltrate and fibrosis. The proximal tubules showed only minor changes. Grossly dilated collecting tubules, some in fact showing cyst formation were seen in the subcapsular and corticomedullary zones (Figure 6). In both subcapsular and corticomedullary regions there was focal interstitial inflammatory cell infiltration. The glomeruli were intact but some appeared slightly smaller than normal due to early periglomerular fibrosis.

In the medulla, the dilated collecting ducts still showed significant damage to the lining epithelium. Giant cell formation was occasionally found in the adjacent interstitium (Figures 7 and 8). Early focal necrosis was seen in the loops of Henle. The transitional epithelium was intact. No vascular changes or evidence of arteritis were found.

Thirty-five Days Effect: Macroscopically and microscopically, the renal lesion was similar to that seen at twenty-one days and only differed in the degree of subcortical scarring and renal contraction which was noticeably more marked at this stage.

B <u>Gasil 200</u>

Four Days Effect: Macroscopically, the kidneys were enlarged and pale, the surfaces were smooth and regular

while the capsules stripped easily.

Histologically, the lesion in the majority of the animals was identical to that induced by gasil 23 D after four days. However, a few animals showed a different pattern of damage consisting of widespread necrosis throughout the nephron, being particularly marked in the proximal tubules where the lumena were filled with proteinaceous material (Figures 9, 10 & 11). This material was also noted in the glomerular Bowman's space. In the distal part of the nephron, the large collecting ducts near the papilla contained amorphous, non-birefringent granular, unstained material (Figures 12, 13 & 14). In addition, there were plugs of desquamated cells and large numbers of acute inflammatory cells both in and around the duct. In collecting ducts the cellular damage was more marked at corticomedullary junction, the nuclei showing degenerative changes including vacuolation and pyknosis (Figure 15). In the interstitium, the acute inflammatory cells were strikingly less abundant. Vessels were normal.

Twenty-one Days Effect: This stage was characterised by either complete recovery without abnormal macro and microscopic findings, or a very mild lesion consisting of subcapsular and corticomedullary chronic interstitial inflammatory cell infiltrate with minimal tubular damage.

Thirty-five Days Effect: No marked abnormal macro or microscopic findings were seen in the kidneys examined after this period.

C Silica Gel

Four Days Effect: Kidneys were enlarged and pale. Surfaces were smooth and regular. Capsules stripped easily.

Microscopically, approximately half the animals showed the classical histological lesion produced by the other silicon compounds. However, the lesions were, in general, rather more severe, particularly the damage seen in the proximal tubules, loops of Henle and the collecting ducts. The congested interstitium showed marked infiltration with acute inflammatory cells. The severity of the lesion is reflected in the fact that the remainder of the animals showed either focal or widespread tubular necrosis involving all parts of the tubules and collecting duct system (Figure 16).

Twenty-one Days Effect: After this period, kidneys showed slightly irregular surfaces. Size and colour were normal. Microscopically, damage was seen mainly in the collecting ducts (Figure 17). However, in subcapsular areas there were foci of xanthomatous change affecting mainly the proximal tubules.

Thirty-five Days Effect: Macroscopically, there was slight irregularity of the outer surfaces. The kidneys, however, were of normal size and colour.

Microscopically, there were subcapsular foci of xanthomatous changes mainly affecting the proximal tubules, while deep in the papillae hard interstitial deposits and marked epithelial damage in the collecting ducts were observed (Figures 18, 19 & 20),

D Magnesium Trisilicate

Four Days Effect: Macroscopically, some kidneys were normal in size and shape while others were enlarged and pale. Capsules stripped easily.

Microscopicallv, the lesions were rather variable. They varied from minor proximal tubular changes to severe acute tubular necrosis or even focal cortical necrosis (Figure 21). In severe lesions proteinaceous material filled the tubules and Bowman's spaces. In the medulla, changes located in the collecting ducts included epithelial cell damage and cast formation (Figure 22). The interstitium was oedematous and infiltrated with acute inflammatory cells.

Twenty-one Days Effect: Macroscopically, the kidneys were normal in size, shape and colour. Microscopically,

there were either no changes or occasional xanthomatous foci affecting the proximal tubules, while in the distal part of nephron cytoplasmic vacuolation and cast formation were seen (Figure 23).

Thirty-five Days Effect: The findings were similar to those seen at twenty-one days.

2 ULTRASTRUCTURAL CHANGES

Renal ultrastructural changes induced by the different silicon compounds under test were basically similar and accordingly they are described together.

A Glomerular Changes

Although a great many samples were examined, surprisingly few ultrastructural abnormalities were observed in the glomeruli (Figure 24). The occasional abnormality when found was located in the glomerular visceral epithelial cells (Podocytes). The changes included cytoplasmic oedema, increased vacuolation and mitochondrial swelling (Figure 25). Microvillous projections and increased numbers of phagolysosomes were an infrequent but definite finding (Figure 26). No abnormality of number or structure of either endothelial or mesangial cells was observed. The basement membrane and mesangial matrix were normal.

The most significant ultrastructural pathology was found

in the tubules and collecting ducts. A detailed account of the ultrastructural changes in the different parts of the nephron is given below.

B <u>Proximal Tubular Changes</u>

The most striking feature was cellular oedema with dilution, dispersion and disorganisation of organelles (Figures 27,28,29,30 & 31). Occasionally, the brush border was damaged or disrupted (Figure 28). Due to marked alteration or even disappearance of the basal infoldings, identification of damaged proximal tubule was difficult and could only be made with confidence when the brush border or its remnants were found (Figure 32). Variable degenerative nuclear changes were observed from dilatation of perinuclear space, chromatin margination through to nuclear pyknosis (Figures 30 & 32). The commonest mitochondrial abnormality was that of swelling (Figures 29 & 33). Other mitochondrial changes included pyknosis (Figure 34), mitochondrial cytolysosomal formation - osmophilic laminated or whorled membranes seen within the mitochondria (Figure 35), and mitochondrial intracisternal sequestration - where mitochondria were found to be incorporated within endo-plasmic reticulum (Figure 35). The cisternal systems showed vesiculation and dilatation with evidence of degranulation (Figures 32 & 36).

Atrophy and disappearance of the Golgi apparatus was found especially in severely damaged cells (Figure 30). The increased numbers of lysosomal structures, which contained numerous electron dense profiles (Figures 31 & 34), and cytoplasmic multilamellated bodies (Figures 35 & 37) were a prominent feature.

C Distal Tubules

Distal tubular lesions were generally more extensive and intense than those seen in the proximal tubules (Figures 38, 39 & 40). Damage to cell organelles and structures paralleled that seen in the proximal tubules. There were in addition other features, particularly the widespread cellular desquamation and disruption of basal infoldings (Figures 41,42 & 43). The thick and thin loops of Henle also showed marked lesions consisting of extensive mitochondrial swelling and dilatation of the endoplasmic cisternae (Figures 44,45 & 46).

D Collecting Ducts

Severe ultrastructural damage extended into the collecting system. Again the changes were mainly those of cellular oedema, vacuolation, disruption and degeneration of cytoplasmic structures - endoplasmic reticulum and mitochondria (Figure 47).

E <u>Blood Vessel Changes</u>

No striking ultrastructural changes were found in the blood vessels (Figure 48).

DISCUSSION

In the earlier part of this Century, the toxic effect of silica on organs and tissues other than lungs stimulated a certain interest and curiosity. Since then, silicon nephrotoxicity has been studied using different silicon compounds (Silica sol., magnesium trisilicate, sodium silicate etc.), and different routes of administration (oral, intraperitoneal and intravenous).

Magnesium trisilicate was first introduced to modern medicine as an antacid by Mutch (1936 a, b and c). He believed that it was an inert substance which was neither absorbed nor capable of producing alkalosis. On the contrary, Page et al (1940) and more recently Dobbie et al (1976) demonstrated significant urinary excretion of silicon after the ingestion of magnesium trisilicate.

Experimentally, Newborne and Wilson (1970) reported renal damage in dogs fed sodium silicate and magnesium trisilicate for four weeks. Macroscopically, the lesions appeared as focal areas of subcapsular haemorrhage suggesting cortical infarcts which was confirmed microscopically. Although the severity of the lesions varied widely from animal to animal, the distal part of nephron was found to be mainly involved. The tubular epithelium was found to be swollen with or without degenerative

changes. A variable degree of tubular dilatation, collapse and interstitial inflammatory cell infiltrate were also noted. Deposits of a crystaline material were demonstrated in the damaged tubular epithelium. The glomeruli were normal.

Settle and Sauer (1960) observed the development of siliceous deposits in the renal tubules of guinea pigs after oral and intraperitoneal administration of large quantities of soluble silica (sodium metasilicate). Twenty-four hours after intraperitoneal injection of silica, they found the kidneys to be pale, greatly enlarged and were of a firmer consistency on sectioning. They believed that, following administration of silica, urinary silicon concentration rose to a level at which polymerisation occurred and siliceous deposits appeared. The only histological description given was that of lightly stained, homogeneous deposits, thought to be siliceous, found in the kidney tubules.

Gye and Purdy (1922 a) demonstrated that guinea pigs and mice died after 4 - 7 days after the intraperitoneal injection of silica sol $Si(OH)_4$. The peritoneum was inflamed and the intestines were matted together by fibrinous material. Their description of the histological details was scanty. Gye and Purdy (1922 b) also

investigated the acute effect of intravenous injection of silica sol on rabbits. They observed degenerative changes in liver and kidney. In liver, it was mainly necrosis, while in the kidney, they described a widespread glomerular lesion, consisting of marked dilatation of glomerular capillaries and exudate into the urinary spaces. The parietal epithelium was thought to be damaged and in severe cases, they claimed complete necrosis of the glomerular tuft. The tubular damage was described as fatty degeneration, dilatation and cast formation.

However, little knowledge was gained from the work of Gye and Purdy (1922 a and b) as they did not describe the histological changes in any detail after the intraperitoneal injection of silica. In contrast to their findings, marked peritoneal reaction was not encountered in this study. On the other hand, the widespread glomerular lesions described by them after the intravenous injection of silica were not seen in this study where silicon compounds were administered intraperitoneally. Furthermore, their description of tubular fatty changes without confirmation by fat stains raises the suspicion that they were, in fact, describing hydropic vacuolar degenerative changes in the tubules which, in this study, was the hallmark of silicon nephrotoxicity.

However, it is apparent that the methodology of silica administration varied considerably and generally the histological examination and interpretation were superficial. In comparison, this study on acute experimental silicon nephrotoxicity covered four silicon compounds which are in common use and to which man may be exposed. These were injected intraperitoneally to by-pass the intestinal barrier and to facilitate dosage control. Following the injection, on day four, the kidneys showed focal tubulointerstitial lesions located mainly in the corticomedullary areas with the maximum involvement in the distal part of nephron. The proximal tubules, although showing definite changes, were much less affected than the distal tubules and collecting ducts. The striking features of this acute study was the absence of lesions in the glomeruli, both at light and electron microscopic levels. The significance of this in relation to overall silicon nephrotoxicity is considered in greater depth in conjunction with further data detailed in Chapters 3, 4 and 5. The interstitium showed infiltration with polymorphonuclear leucocytes while eosinophils were prominent in the acute stage only. In addition, acute tubular necrosis was occasionally demonstrated. On days twenty-one and thirty-five, the kidneys showed either complete recovery or evidence of mild chronic tubulo-

interstitial nephritis. Amorphous granular deposits were frequently seen in the interstitium and within lumena of damaged tubules. The glomeruli were normal.

The findings of this study are in agreement with those of Newberne and Wilson (1978) with respect to the main site of silica nephrotoxicity - the distal tubules and collecting ducts. One important divergence, however, is that acute tubular necrosis and not cortical necrosis was the most severe lesion encountered in this study. The demonstration of tubular deposits, thought by Settle and Sauer (1960) to be siliceous, were also observed in this study where amorphous, granular deposits were noted in the interstitium around damaged tubules as well as in the tubular lumena.

This study has shown in greater depth and with what is believed to be greater precision the site and extent of the lesion in acute silicon nephrotoxicity. However, the extensive ultrastructural analysis of the cytological changes carried out was done in the hope that it might furnish some insight into the mechanism of silicon toxicity.

The ultrastructural findings in the renal tissue following acute silicon nephrotoxicity, confirmed and amplified the light microscopic changes of tubular lesions which mainly

involved the distal part of nephron. Changes in proximal tubules were, in the main, less marked than in the remainder of the nephron, although in some animals, the severity of the lesions was comparable to that found in the distal part of nephron.

Before discussing these changes, it is important to review the effect of silicon on cellular membrane systems, because silicon membrane interaction may be the basic mechanism involved in the development of these lesions. The first contact of silicon is with plasma cell membrane possibly followed by its contact with intracellular organelle membranes. The effect of silicon on macrophage lysosomal membranes was studied by Allison et al (1966), Allison and Hart (1968) and Allison (1971). They proposed silica lipid interaction, while Summerton et al (1977) supported the theory of membrane damage, but proposed silica-protein interaction. On the other hand, Nash et al (1966) concluded that toxic effect of silicon was due to the capacity of polymeric silicic acid to react as a hydrogen donor with active groups such as phosphate ester groups of phospholipids or to a lesser extent with secondary amide groups of protein. These reactions are capable of producing substantial damage in biological membranes, such as increasing membrane permeability.

Ultrastructural changes in the cytoplasmic organelles were a constant feature of acute silicon nephrotoxicity. The widespread mitochondrial changes encountered in this study are believed to be beyond the normal process of involution and dissolution of these organelles. Mitochondrial swelling, which was the commonest abnormality, is possibily due to entry of water into the organelle. Hydropic mitochondria following silicon administration might be due to silicon induced membrane permeability changes and a state of intramitochondrial electrolyte disequilibrium. Since the permeability of the inner and outer mitochondrial membrane is know to be different, the early stage showed only a mild degree of swelling. There was dilution of the matrix material and peripheral displacement, shortening and reduction in number of the cristae. The intramitochondrial granules tended to disappear at an early stage of the swelling. Generally, there are two known types of mitochondrial swelling which could possibly be involved in the pathogenesis of mitochondrial hydrops in acute silicon nephrotoxicity; passive swelling due to the osmolarity change in the medium, or active swelling, depending on electrolyte transport. Silicon membrane interaction might induce and explain both kinds of swelling. Swelling, is usually initiated actively and finishes

with passive flooding of organelles. The osmotic change in the external environment is an important factor in hydrops, but as far as swollen and non-swollen organelles were found in the same cell, it was suggested (Rouiler, 1950) that chemical derangement inside the organelle itself leads to membrane permeability changes.

Mitochondrial pyknosis was occasionally met during this examination and was associated with severe cellular damage or even cell necrosis following silicon administration. Pyknotic mitochondria were first noted by Watchestein and Besen (1964) who described this organelle regression in the renal tissue damaged by coagulative necrosis induced by DL-Serine. Pyknosis was also demonstrated in rat liver after starvation (Rouiler, 1957), carcinogenic diets (Rouiler and Simon, 1962), human liver in cases of viral hepatitis (Pavel et al, 1971) and in experimentally induced renal tumours in hamster (Mannweiler and Barnhard, (1957).

In addition, silicon was also shown to induce an osmiophilic laminated or whorled membrane in the mitochondria which was a stage in dissolution of these organelles and subsequent incorporation into lysosomal bodies containing the so-called multilamellated bodies. While the sequestration of mitochondria within endoplasmic

reticulum, a process leading to cytolysomal formation, was also occasionally encountered.

The endoplasmic reticulum vesiculation and dilatation was a constant feature in renal tubular epithelium following silicon administration. These changes might be artifactual due to the fixation, but when dilated, vesiculated organelles were present along with normal structures in the same cell, it seems more likely that these changes are secondary to a pathological process. The content of the dilated endoplasmic reticulum appeared more electron lucent than normal, this probably being due to dilution effect.

The swollen mitochondria and the dilated endoplasmic reticulum constitute the change known as cloudy swelling. This phenomenon was first described by Virchow who defined it as intracellular water accumulation due to toxic damage. He believed it was the primary cause of cell death (Cameron, 1952).

Although cytolysosomal formation is regarded as a normal mechanism by which old or damaged organelles are cleared from cells, the increased numbers in this study is considered to be an abnormal finding. Increased numbers of cytolysosomal bodies have been considered to be an indication of sublethal cellular injury due to any toxic

agent such as x-rays, carcinogens, antimetabolites, hypoxia and viruses (de Duve and Wattiax, 1966). This study has shown that these changes are readily induced by silicon toxicity.

The frequent intracytoplasmic multilamellated bodies which were found in this investigation were an additional factor supporting the hypothesis of significant cellular injury induced by silicon administration. Similar changes are known to occur in a variety of other pathological conditions such as Fabry's disease (Rae et al, 1967), Gaucher disease (Jordon, 1964) and drug induced myelin figures (Hruban et al, 1965). However, it must be borne in mind that these changes were believed by others to be due to gluteraldehyde fixation since lipids are not well fixed with gluteraldehyde (Ericsson and Biberfeld, 1967).

Light and electron microscopic evidence showed that silicon compounds cause significant renal tubular damage. It was not possible to determine in this experiment whether cell damage resulted from silicon interaction with the plasmalemma and subsequent changes in its permeability giving rise to cell oedema, or whether the silica membrane interaction extended into the cytplasmic organelles.

SUMMARY AND CONCLUSIONS

Intraperitoneal injection of 100 mg of four different silicon compounds each produced significant renal damage in the rat. In all of the compounds tested the lesions were histologically similar and predominantly affected the tubule. The lesions were most severe in the distal nephron. At twenty-one and thirty-five days after injection, the acute changes had passed, leaving a mild, focal, chronic tubulo-interstitial nephritis.

The ultrastructural findings in acute silicon nephrotoxicity amplified those of marked oedema of the tubular epithelial cells and an acute inflammatory reaction, recognised by light microscopy. Despite extensive examination of the changes in the fine structure of the damaged kidneys, conclusive evidence as to the mode of action of silicon was not obtained. Nevertheless the ultrastructural findings did suggest that silicon nephrotoxicity might be due to damage to membrane systems with resultant cellular oedema. It was thus concluded that the detection of silicon within the renal parenchyma, and if possible, the accurate localisation of silicon on or within the cell organelles might possibily reveal the pathogenetic mechanism which renders silicon toxic to the distal nephron.

C H A P T E R III

DETECTION AND MEASUREMENT OF SILICON IN BIOLOGICAL FLUIDS AND TISSUES

GENERAL INTRODUCTION

The detection and measurement of silicon in biological fluids and tissues was an extremely important aspect of the investigation of silicon nephrotoxicity. Thus, considerable time and effort were expended in the development and evaluation of methods whereby one could monitor serum silicon levels and silicon content of tissues in the experimental animal

Thus the detection and or measurement of silicon was studied using the following methods:

1 Flame atomic absorption spectroscopy.
2 Electron probe microanalysis of ultrathin sections of kidney.
3 Radioactive isotope studies (^{31}Si), including autoradiography.

ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTION

Analytical chemical methods of measuring silicon in biological fluids are known to be difficult and fraught with interference. Dobbie et al (1976) has successfully applied atomic absorption spectroscopy to the measurement of silicon in biological fluids in man. Such techniques were, therefore, applied and assessed in the monitoring of serum silicon levels in the experimental animal given intraperitoneal injections of gasil 200. Tissue silicon levels were likewise measured using ashing as an intermediate step. Thus, the aim of this part of the investigation was the determination of the range of silicon concentration to which the kidneys of the animals were exposed under experimental conditions.

MATERIAL AND METHODS

Instrumentation

Analysis was performed on a Perkin-Elmer atomic absorption spectrophotometer, Model 103, with a Perkin-Elmer, Model 56 recorder (Perkin-Elmer Ltd., Buconsfield, Bucks., E.K.). An intensitron silicon hollow cathode lamp, nitrous oxide burner and nitrous oxide acetylene flame were used. Optimum operating conditions were established using instrument settings as follows:

Wave length	2516	A
Slit width	2	A
Lamp current	15	mA
Acetylene flow rate	9.5	L/min
Nitrous oxide flow rate ..	16.6	L/Min

Silicon standard for atomic absorption (1 mg/ml., 35.6 mmol/l., B.D.H. Chemicals, Poole, U.K.) appropriately diluted with deionized water was used for preparation of working standards. All specimens were centrifuged before analysis, to remove particulate matter. Polystyrene containers were used for storage of specimens and standards. Serum was analysed by direct aspiration of samples or by the method of standard additions.

Materials: Twenty-two, eight week old female Sprague-Dawley rats were divided into three groups:

Group 1: ten rats received a single intraperitoneal injection of 100 mg gasil 2oo suspended in 2 ml of 0.9% Nacl (PH 5.3).

Group 2: ten rats received a single intraperitoneal injection of 100 mg gasil 200 suspended in 2 ml 0.9% NaCl. The PH was adjusted to between 10 - 11 with a few drops of 2.5N NaOH.

Group 3: two rats received a single intraperitoneal injection of 2 ml 0.9% Nacl, and were used as controls.

One rat from Group 1 and one from Group 2 were anaesthetised with ether after 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hours respectively. Blood was aspirated by cardiac puncture for serum silicon estimation. The rats were then sacrificed and the whole kidney and spleen and sample blocks of the liver were washed several times in deionized water to remove any excess unabsorbed silicon contaminating their surface. The control group was treated in the same manner and all tissue processed as follows:

1 Samples were placed in separate, weighed Zirconium crucibles. Tissue wet weights were recorded.
2 They were then transferred to a 110°C oven and kept there until a fixed dry weight was achieved.
3 Samples were then placed in a muffle furnace (400°C) and left overnight.
4 The dry ash was then dissolved in 10 ml 40% HNO_3 (analar HNO_3 diluted with 40 - 100 ml deionized water).
5 Suspensions were centrifuged and examined by atomic absorption spectroscopy.

RESULTS

1 CONTROL ANIMALS

Values obtained from normal control animals are listed below:

	Serum Silicon	Kidney Silicon	Liver Silicon	Spleen Silicon
Animal 1	0.8	12.23	15.69	15.58
Animal 2	0.7	12.29	15.75	13.88

Table 3: Showing serum silicon concentration in controls (µg/ml), and dry tissue silicon levels (µg/g).

The serum silicon levels in the control animals are within the normal range established in the laboratory for Sprague-Dawley rats:

($\bar{X}$ = 0.67 : SD $\pm$ 0.13 : n = 11)

2 TESTED ANIMALS

A Serum Silicon:

(a) Rats receiving intraperitoneal injections of gasil 200 at pH 5.3 (Figures 49):

One hour after injection, the serum silicon had risen to 10.4 µg/ml., representing a 14 fold increase above basal

levels. A peak of 37.8 µg/ml was reached at 48 hours; representing a 50 fold increase above basal levels.

(b) Rats receiving intraperitoneal injection of gasil 200 at pH 10 - 11 (Figure 49):

One hour after injection the serum silicon had risen to 14.5 µg/ml, representing a 19 fold increase above basal levels. At this pH, an earlier peak of 36 µg/ml was reached at 24 hours representing a 48 fold increase above basal levels. At 48 hours the serum silicon had fallen to 12 µg/ml.

B <u>Silicon Levels in Ashed Organs</u>:

(a) Rats receiving intraperitoneal injections of gasil 200 at pH 5.3 (Figure 50).

i) Kidney: One hour after injection, the kidney silicon level had risen to 79.3 µg/g, representing a 6 fold increase above basal level. A peak of 118.2 µg/g was reached at 5 hours; representing a 10 fold increase above basal levels. At 48 hours the kidney silicon level had fallen to 42.4 µg/g.

ii) Liver: One hour after the injection, the liver silicon level had risen to 0.36 µg/g representing a 2 fold increase above basal level. A peak of 146 µg/g was reached at 12 hours,

representing a 9 fold increase above basal levels. At 48 hours the liver silicon level had only fallen to 128 µg/g.

iii) Spleen: One hour after the injection, the spleen silicon level had risen to 40 µg/g; representing a 3 fold increase above basal level. A peak of 160.8 µg/g was reached at 8 hours; representing an 11 fold increase above basal levels. At 48 hours, the spleen silicon level had fallen to 109 µg/g.

(b) Rats receiving intraperitoneal injection of gasil 200 at pH 10 - 11 (Figure 51):

i) Kidney: One hour after the injection, the kidney silicon level had risen to 100.2 µg/g; representing an 8 fold increase above normal. A peak of 122.7 µg/g was reached at 6 hours; representing a 10 fold increase above basal levels. At 48 hours, the kidney silicon level had fallen to 89 µg/ml.

ii) Liver: One hour after the injection, the liver silicon level had risen to 43.2 µg/g; representing a 3 fold increase above normal. A peak of 113.7 µg/g was reached at 10 hours; representing a 7 fold increase above basal levels. At 48 hours, the liver silicon level had fallen to 96.7 µg/g.

iii) Spleen: One hour after the injection, the spleen silicon level had risen to 38.7 µg/g; representing a 3 fold increase above normal. A peak of 205.4 µg/g was reached at 8 hours; representing a 14 fold increase above normal basal levels. At 48 hours, the spleen silicon level had fallen to a 111.7 µg/g.

DISCUSSION

Experimentally, Holt and Yate (1954) measured the silicon content of kidney and liver after the intraperitoneal injection of radioactive silicon (^{31}Si). They compared the effect of varying the pH and concentration and concluded that the the tissue content of silicon was greatly reduced by lowering the pH or by increasing the concentration of the injected silica. These changes were believed to be due to the effect of low pH or high concentration on the polymerisation of silica. In all circumstances they found renal silicon levels to be greater than that of the liver.

High kidney silicon values were found in the documented cases of silicon nephrotoxicity in man. In their case, Saldanha et al (1975) found an ashed kidney silicon concentration of 200 µg/g, while values of 264 and 150 µg/g were found by Giles et al (1978) and Hauglustaine et al (1980) respectively.

This investigation has shown that immediately after intraperitoneal injection of silica, the kidney showed the highest concentration of silicon. The likeliest explanation of this phenomenon is that the small monomeric silica molecules (oligo silicic acid) are rapidly absorbed from the peritoneum and equally rapidly excreted by the

kidney. The higher renal silicon levels found when alkaline silica was used (silica containing a large proportion of soluble monomeric molecules) tends to support this explanation. Kidney silicon levels continued to rise until a peak concentration was observed at 6 hours. At this point, there was little difference between the values obtained for the acid and alkaline silica. By 6 hours the silicon content of the spleen was greater than that of the kidney. After 6 hours whereas the silicon levels in the kidney continued to fall, the levels in spleen and liver rose to peak at 8 and 12 hours resepectively. Thereafter, the silicon content of spleen and liver fell slowly until at 48 hours the levels in all organs were much closer although the silicon content of the kidney remained lower than that in the other two organs.

Analysis of the findings suggest that, after the injection of gasil 200 at such concentrations (100 mg) and pH (5.3 and 10 - 11), part of it reacts with water to form silicic acid of low molecular weight (oligo silicic acid), which was absorbed rapidly. Due to the ability of this monomer to pass the glomeruli, silicon began to rise in the kidney. With passage of time, polymerisation occurred to form large molecules which did not pass the glomeruli, but which steadily accumulated in other tissues. A

further possible explanation of the findings is that the kidney, being a highly vascular organ and the main organ of excretion (Dobbie et al, 1976), that the first phase represents the period of maximum concentration and excretion of silicon by the kidney, while other organs, being incapable of active excretion as is the kidney, showed a steady accumulation.

From this investigation it is apparent that under the experimental conditions the kidney was exposed to very high serum silicon concentrations (50 times normal). If one assumes that the all or most of the silica passes in the ultrafiltrate into the tubules, concentration of urine in the distal part of the nephron must result in very high concentration of intralumenal silica. No micropuncture studies on the behaviour of silicon in the renal tubules have yet been attempted, an exercise which, with current technology, is an extremely unlikely possibility on account of the difficulty of measuring silicon in small samples. Thus, it is not known whether silicon in tubular urine is re-absorbed or even re-excreted in the distal tubules. However, it may be that silicon passes in the ultrafiltrate and is increasingly concentrated as it passes down the tubules.

This investigation would have furnished important information on the renal handling of silicon in the rat if one had timed measurements of urinary silicon throughout the procedure. However, this was not technically possible because of many difficulties. For flame atomic absorption at least 1 ml of urine is required while different forms of collection proved to be open to gross contamination from omnipresent siliceous dust and other silica containing substances.

SUMMARY AND CONCLUSIONS

Intraperitoneal injection of 100 mg of amorphous silica (gasil 200) results in very high concentrations of serum silicon and a rapid increase in the levels of silicon in renal tissue. A slower rise but higher peak tissue levels were found in spleen and liver.

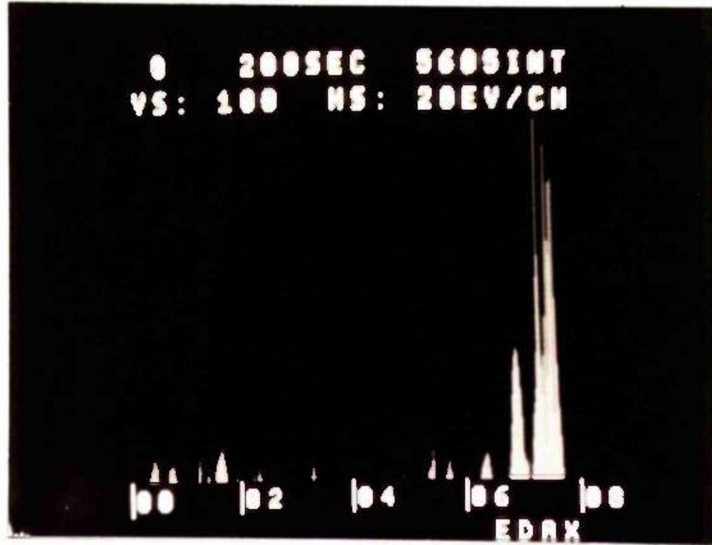
These findings indicated that in this experimental procedure the kidney is exposed to high serum silicon levels. They did not, however, furnish any information on the distribution or behaviour of silicon within the kidney.

ELECTRON PROBE MICROANALYSIS: DETERMINATION AND LOCALISATION OF SILICA IN KIDNEY ULTRATHIN SECTIONS:

INTRODUCTION

Studies using atomic absorption spectroscopy provided both useful and interesting information on absorption and the exposure of the kidney to high serum silicon concentrations. Many questions, however, remained unanswered and thus a further approach, using a different technique was attempted.

A relatively new technique, energy dispersive analysis of x-ray (EDAX), also known as electron probe microanalysis (EPMA), has the capability of demonstrating and localising tissue silicon (Furnahashi et al, 1975). Indeed, this approach has been successfully applied to a study of the localisation of silicon in plant tissue where it is believed to be an essential trace element. Despite difficulties in obtaining access to such a system and necessitating spending a short period at the School of Plant Biology in North Wales where the technique was pioneered, electron proble microanalysis of kidney sections from the experimental animals was attempted with some success.



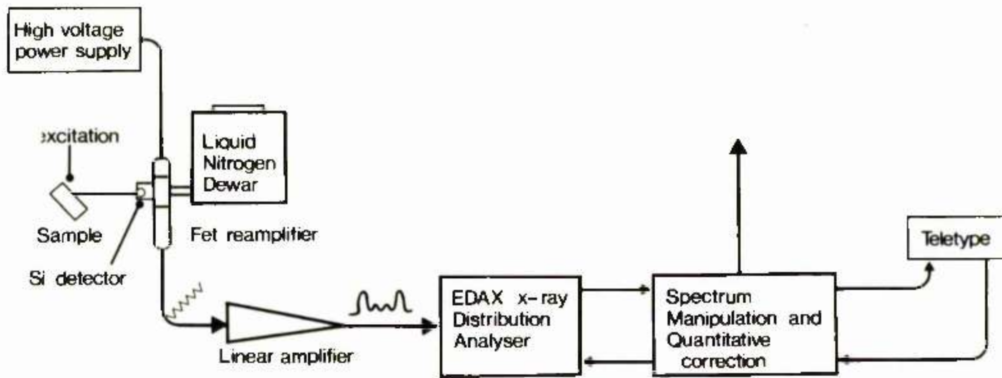


Diagram 5: Block Diagram of Edax System.

MATERIAL AND METHODS

Instrumentation

As shown in diagram 5 x-rays from the specimen enter a wafer of pure silicon, carefully treated with lithium so that there are no impurities or irregularities in the crystal structure that can trap the electrons. As the x-rays enter the silicon, each one loses its energy by creating photoelectrons which ionize the silicon atoms. Since each ionization takes 3.8 electron volts of energy, the final result is a number of ionized silicon atoms equal to x-ray energy divided by 3.8. The electrons are then collected in less than a micro - second by an applied bias voltage and integrated by a field effect-transistor pre-amplifier. The output of this pre-amplifier is a series of steps, with a height of each step proportional to the energy of the corresponding x-ray. The pulses are measured and counted by a special computer called a multichannel analyser. Since the time is proportional to pulse height and thus to x-ray energy, the result is the build up of a spectrum of counts versus x-ray energy. This spectrum is presented for viewing by using a video display. The energies at which peaks occur in the spectrum are readily identified with the elements that produced the x-rays, thus giving elemental

analysis of the specimen. The size of the peaks can also be used to determine quantitatively the amount of various elements.

Materials

Eleven, eight weeks of age, female Sprague-Dawley rats were used in this study. They were divided into two groups.

Group 1: Eight animals, each received 100 mg gasil 200 dissolved in 2 ml 0.9% Nacl as a single intraperitoneal injection.

Group 2: Three animals each received 2 ml 0.9% Nacl as a single intraperitoneal injection and used as control.

After ½, 1, 1½, 2, 4, 6, 24 and 48 hours one rat from Group 1 was anaesthetised and blood sample was taken. The concentration of silicon in serum was measured by atomic absoprtion spectroscopy. A similar procedure was used with animals of Group 2. Small blocks from all animals after 48 hours were processed for the electron probe microanalysis by the following schedule:

1 Overnight fixation in 2% phosphate buffered gluteraldehyde.
2 Dehydration through graded alcohols.

3 Two changes in propylene oxide.

4 Propylene/Epon-1:1.

5 Propylene/Epon-1:3.

6 Embedded in Epon 812.

100 nm sections were cut on LKB ultramicrotome IV, mounted on copper or nickel grids and examined under Phillips 301-EDAX. The electron proble microanalysis was operated under the following conditions.

Specimens were angled at 24°C toward the detector of EDAX 707 energy dispersive analyser and examined using a spot size of 32 nm with an accelerating voltage of 80 KV for 200 seconds. The silicon peak was detected at 1.739 on the horizontal scale.

RESULTS

1 SERUM SILICON LEVELS (μg/ml)

The level of silicon in the serum of tested animals at the point of tissue sampling together with the levels found in the normal controls are given in the table below:

Time	½ h	1 h	1½ h	2 h	4 h	6 h	24 h	48	Control
Serum Silicon μg/ml	*	0.5	17.5	12.9	12	15.8	*	38.0	1 - 0.7 2 - 0.5 3 - 0.7

Table 4: Serum silicon level of tested and control rats.

* No analysis: difficult blood aspiration.

2 ELECTRON PROBE MICROANALYSIS

A Glomeruli (Figures 52, 53 & 54).

The glomerular analysis was carried out in the following regions - capillary space, basement membrane, foot processes, urinary space, cytoplasmic and intracellular organelles of the podocytes, endothelial and mesangial cells.

(a) Capillary Space: Small peaks were found in the controls, while larger peaks appeared between 4 and 24 hours.

(b) Basement Membrane: There was no detectable silicon in this region in the controls. In the tested animals a small peak appeared around 4 hours and this increased in size up to 24 hours diminishing thereafter.

(c) Foot Processes: Small peaks were present in both control and tested animals at all times.

(d) Urinary Space: Silicon was not detected in this region in either controls or in the early period in tested animals. However, small peaks appeared around 24 hours in the tested rats.

(e) Glomerular Cells (endothelial and mesangial cells): Silicon was not detected in either cytoplasm or organelles in control and tested animals.

B <u>Tubules</u> (Figures 55, 56, 57 & 58).

Analysis was performed on the proximal, distal and collecting tubules in all samples and at all time periods.

Large silicon peaks were found only over the cell membrane at the region of intercellular contact of the tubular epithelial cells and over the lumenal surfaces in distal and collecting tubules. These peaks were specifically located over the external surfaces of the plasma membranes.

No silicon was detected on the cytoplasmic or inner aspect of the cell membranes. Neither was any silicon detected within the cell cytoplasm nor organelles. It can be confidently reported that although a large number of lysosomal structures were examined, none revealed the presence of silicon at the level of detection of the microanalyser, neither was there any silicon present in the tubular lumen. Silicon was detected only at 2, 4 and 6 hours after injection. None was demonstrated either before or after this interval.

DISCUSSION

Since the development of EDAX, the prospect of using this system in the prosecution of element detection and analysis at the sub-cellular level has stimulated much interest in the biological sciences and has to date provided valuable information. Not only in medicine where silicon has been located by electron probe micro-analysis in growing bone (Carlisle, 1969 and 1970), but also in other disciplines where the detection of silicon and its relation to different parts of living cell has been of considerable interest (Schafer and Chandler, 1970).

In the School of Plant Biology, University of North Wales in Bangor, where this study was primarily performed, there is an increasing interest in studying this element, using EPMA technique to detect silicon in plant samples (Sangster and Parry, 1976, Parry and Winslow, 1977 and Bennet and Parry, 1980).

During this experiment, attention was focused on the detection and localisation of this element in the renal tissue after the intraperitoneal injection of gasil 200.

In the glomeruli, the silicon peaks were very small, there being little difference between control and tested samples. It seems likely that in the glomerulus silicon

is an element of passage, where it rapidly passes through in the blood or through the basement membrane into the urinary space. The lack of tissue concentration in the glomerulus is, therefore, of significant relevance in view of the absence of glomerular lesions in acute silicon nephrotoxicity. The constant, small, but readily detectable amounts of silicon within the foot processes found in both controls and tested animals suggest that it might therefore be involved in some structural function.

The most unexpected and also the most significant finding of this investigation was the localisation of silicon in the outer surface of the cell membrane, specifically between epithelial cells in the distal and collecting tubules. It is noteworthy that this correlates exactly with the site of maximal pathological change in acute silicon nephrotoxicity. Considering the preoccupation in lysosomal changes by previous workers in the field of silicon cytoxicity, it was surprising and intriguing to find that silicon was not detectable in any of the lysosomal structures, indeed silicon was not found within the cell.

The localisation of silicon over the external surface of the cell could be considered as circumstantial evidence that a membrane silicon interaction had occurred. The

resultant swelling of the cells and disorganisation of the intracellular organelles might therefore be attributable to a change in the permeability of the cell membrane.

The absence of silicon in the lumen of the distal tubules and collecting ducts was unexpected. It is known from human studies that in man following ingestion of silicates there is a massive rise in the urinary concentration of silicon. It is most likely, however, that lumenal silicon is eluted by the tissue processing necessary for obtaining thin sections. The fact that the highest levels of silicon detected in the kidney was in the distal tubule and that this was located over the plasmalemma is possibly indirect evidence that the silicon is strongly bound to the membrane in view of the total absence of silicon in the lumen.

SUMMARY AND CONCLUSIONS

Elemental silicon has been readily detected in the kidney, in ultrathin sections of animals receiving single intraperitoneal injection of gasil 200. Contrary to expectation silicon was detected only on the external surfaces of the cell membrane in the distal and collecting tubules. Silicon was not found within the cells. Thus, these findings suggest that silicon toxicity may be due to the binding of silicon to the plasma cell membrane which results in its damage or alteration of its permeability.

RADIOACTIVE ISOTOPE STUDIES WITH ^{31}Si

INTRODUCTION

The radioisotope, ^{31}Si, was prepared in order to provide another method by which the renal uptake and localisation of silica might be made.

As ^{31}Si has a half-life of only 2.6 hours it must be freshly made in a nuclear reactor and any biological investigation carried out immediately on site. Because of these constraints it is not surprising that the use of isotopic silicon in biological systems has been little studied.

PREPARATION AND TESTING OF ISOTOPE

The isotope was made at the Scottish Universities Research and Reactor centre at East Kilbride. Since this isotope has seldom ever been made, the methodology of its preparation and testing had to be devised and designed on theoretical grounds and its quality and freedom from contamination assessed by trial and error. A step-wise accretion of knowledge and expertise was obtained by making some ten different preparations of isotope. Initially 300 mg of gasil 200 was irradiated in polythene vials for 4 hours in the central vertical stringer of the reactor at a neutron flux of $\sim 4 \times 10^{12} n\ cm^{-2}\ sec^{-1}$. The active material was removed from the reactor and left for 25 minutes to allow prior decay of aluminium activity from the containing can. Trace amounts of manganese and sodium which were natural contaminants of the silica,

rendered radioactive by the irradiation, were then removed by chemical means - elution, precipitation and washing in excess saline solution. This step, however, resulted in considerable prolongation of the time required for preparation of "clean isotope" and resulted in the loss of up to two half-lives of the freshly made isotope. Injection of this preparation, made up in 2 ml of sterile saline, intraperitoneally in the rat gave very faint, sparse activity in autoradiographs of the kidney developed after 24 hours exposure. Determined efforts to reduce the time of chemical cleaning and the curtailing of the dwell-time of the isotope in the rat failed to enhance the activity seen in the autoradiographs although organ counting revealed appreciable activity in the kidney. A further disadvantage of scrupulous cleaning of the isotope was the strong possibility that the decontaminating procedures had altered the physicochemical characteristics of the amorphous silica (gasil 200). For these reasons it was decided to ignore the trace contaminants and to immediately inject into the animal pure irradiated gasil 200, hence conserving a much greater proportion of the active life of ^{31}Si.

MATERIAL AND METHODS

The definitive procedure was established by testing ten isotope preparations in fifteen rats. Isotopic silica: 300 mg of gasil 200 was irradiated for 4 hours in the reactor. The material was then suspended in 6 ml sterile 0.9% saline. 2 ml of the preparation was then injected intraperitoneally (100 mg gasil) into three female Sprague-Dawley rats. 1½ to 2 hours later the animals were anaesthetised. Blood samples were taken and the animals sacrificed. Both kidneys, part of liver and spleen were dissected and cleaned several times with distilled water before measurement by γ counter of the total and percentage activity.

AUTORADIOGRAPHY

Small sample blocks from the kidneys of the three animals were frozen onto chucks with a dry ice/acetone mixture. Sections of 12 μ were cut on the cryostat and mounted on gelatin coated slides. Half of the slides were coated with coarse nuclear research emulsion and half with fine emulsion (Ilford LTD Basildon). The emulsion was prepared by dilution of 25 ml with 25 ml distilled water at 50°C in a water bath. The slides were left to dry and packed in lightproof boxes for 24 hours. They were then developed for 5 minutes in D-19 developer and fixed with amfix for 5 minutes. The slides were washed and counter stained with 1% neutral red.

RESULTS

ISOTOPE

^{31}Si had the following characteristics:

Half-life	2.6 hours	
B emission	1.47 MeV	(100%)
X ray	1.26 MeV	(0.07%)

ORGAN ACTIVITY

As measured by γ counter (microcuries) is presented in Table 5 as total activity and percentage of total activity for the organs - kidney, liver, spleen and blood.

AUTORADIOGRAPHY

Microscopic examination of the slides showed that 1½ to 2 hours after injection of isotopic gasil 200, radioactivity was detectable throughout cortex and medulla. Maximal activity was located in the corticomedullary region while examination at high power showed that the activity was present mainly in the lumen of the distal tubule and collecting duct. Activity was also apparent on the surface of tubular epithelial cells in this region and possibily in peritubular capillaries.

	Total activity (microcuries)	Kidney		Liver		Spleen		Blood	
		weight/g	% total activity	weight/g	% total activity	weight/g	% total activity	weight/g	% total activity
Rat 1	170.7	2.375	1.25	4.451	1.37	0.827	0.23	3.255	1.92
Rat 2	151.7	1.778	1.21	4.203	1.21	0.598	0.16	3.161	1.64
Rat 3	152.6	2.596	1.43	3.799	1.11	0.846	0.24	3.312	2.04

Table 5: Demonstrating the radioactive study in three different rats.

DISCUSSION

The preparation of ^{31}Si proved to be difficult and fraught with problems. The short half-life of the radioisotope demanded the utmost speed in any chemical cleaning or manipulation of the preparation. Clean isotope was prepared and when injected into test animals still had sufficient activity to allow measurement of whole organ uptake. However, the main aim of this investigation was the determination of the intrarenal distribution of the gasil 200 by autoradiography and clean isotope gave only very faint positivity. Nevertheless the distribution as predicted was in the lumen of the distal tubule and collecting ducts.

The simpler and more rapid method of injecting irradiated gasil 200 immediately after preparation did give parallel results to those obtained with the clean isotope and showed concentration of the isotope in the lumen of the distal nephron. An important finding was the apparent absence of uptake by cells either, epithelial or interstitial. This was in keeping with the results obtained by electron probe micro-analysis which showed that cellular, uptake of silicon either did not occur or was of a very low order.

Because of the possible chemical differences between clean and contaminated ^{31}Si and the rapid fall of radioactivity, organ uptake, although measured, did not add significantly to our understanding of the fate and distribution of injected silica.

SUMMARY AND CONCLUSIONS

The radioisotope ^{31}Si was prepared by irradiation in an atomic reactor of amorphous silica powder. (gasil 200). Trace amounts of radioactive sodium ^{24}Na, and manganese, ^{52}Mn contaminated the preparation. This was removed by chemical means to give a "clean" isotope. Because of the short half-life the usefulness of this isotope was limited and animal injection gave only very faint activity and imperfect localisation. Injection of untreated irradiated gasil 200 obviated the time loss in cleaning the isotope and resulted in good localisation of activity in the lumena of distal tubules and collecting ducts. There was no activity over cells.

C H A P T E R IV

CHRONIC EXPERIMENTAL SILICON NEPHROTOXICITY

INTRODUCTION

The purpose of the following investigation was the evaluation of the pathological and biochemical effect of repeated weekly intraperitoneal administration of the silicon compound, gasil 200, on the rat kidney over an extended period (9 months).

The intention was also to:

1 Demonstrate the evolution stage by stage of the renal lesions after silicon injection.
2 Detect any histological relationship between experimentally induced silicon nephrotoxicity and any other known or suspected silicon associated renal disorders such as chronic interstitial nephritis of the Balkans and documented cases of silicon nephrotoxicity.

MATERIAL AND METHODS

The silicon compound used in this study was gasil 200. It was sterilised by irradiation and made ready for intraperitoneal injection by dissolving the required dose in 2 ml 0.9% NaCl. Animals used were female Sprague-Dawley rats, eight weeks old and 200 - 250 g in weight. Three experiments were carried out.

Experiment 1

In order to determine the appropriate dose to be used in the main experiment (Experiment 2), fourteen rats in groups of two, received single intraperitoneal injections of the following doses of gasil 200 - 5, 10, 20, 40, 60, 80 and 100 mg. All animals were sacrificed after twenty-four hours. Kidney samples were fixed in 10% phosphate buffered formalin, processed and embedded in wax, 5 µ sections were cut, stained with haematoxylin and eosin, and examined under the light microscope.

Experiment 2 (Chronic silicon nephrotoxicity)

From information gained from Experiment 1, two doses were chosen for this study - 20 and 60 mg gasil 200. Fifty rats were divided into three groups.

Group 1 Twenty rats receiving weekly intraperitoneal injections of 20 mg gasil 200.

Group 2 Twenty rats receiving weekly intraperitoneal injections of 60 mg gasil 200.

Group 3 Ten rats receiving weekly intraperitoneal injections of 2 ml 0.9% NaCl, served as controls.

SAMPLING

Each month two rats were sampled from Group 1 and 2 and one rat from Group 3. The animals were anaesthetised by ether and blood samples collected by direct cardiac puncture. Samples were centrifuged and the serum evaluated for serum silicon (μg/ml) by atomic absorption spectroscopy, while serum urea, creatinine, electrolytes (Na, K, Cl), carbon dioxide content and total protein were measured by routine clinical biochemical techniques (Biochemistry Department, Glasgow Royal Infirmary). Animals were then sacrificed. Samples of renal tissue were processed for:

1 LIGHT MICROSCOPY

After fixation in 10% phosphate buffered formalin, the samples were then routinely processed for light microscopy, stained with haematoxyline and eosin (H & E), periodic acid shift (PAS) and martius yellow, crystal scarlet, soluble blue (MSB). Random sample blocks from myocardium, liver, spleen, pancreas, lung, peritoneum and intestine were processed and examined in the same manner.

2 ELECTRON MICROSCOPY

Kidney blocks from all animals were fixed in 2% phosphate buffered gluteraldehyde, post-fixed with 1% osmic acid and embedded in emix resin; ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate and examined under the Phillips 200 electron microscope. 500 electron micrographs were assessed in this part of the examination.

3 ELECTRON PROBE MICROANALYSIS

Blocks of renal cortex were obtained from animals sacrificed after 8 months. These were prepared and examined by electron probe microanalysis in the manner described in Chapter 3.

Experiment 3 (The long term effects of a short period of daily intraperitoneal administration of silicon).

Fifteen rats were used in this experiment as follows:

Group 1 Ten rats received daily intraperitoneal injections of 60 mg sterile gasil 200 for seven consecutive days.

Group 2 Five rats received daily intraperitoneal injections of 2 ml 0.9% NaCl for seven consecutive days.

The animals were then left undisturbed, on normal diet, for 100 days, after which they were anaesthetised, and blood samples collected by cardiac puncture for serum silicon and biochemistry as in Experiment 2. Kidney samples were processed for light microscopy as in Experiment 2.

RESULTS

1 EXPERIMENT 1

No discernible histological lesion was detected at 10 mg, while mild dilatation of distal tubules and collecting duct was found with a 20 mg dose. At a dose of 40 mg, there was increased dilatation of the distal part of the nephron and cytoplasmic vacuolation in the proximal tubules. At 60 mg, the only additional finding was a light inflammatory infiltrate in the interstitium. The lesions showed increasing severity at 80 and 100 mg gasil 200.

2 EXPERIMENT 2

A Light Microscopy

First Month

Sixty mg Dose Effects: Macroscopically, the kidneys were enlarged, pale, and the capsule stripped easily. Microscopically, the lesions were those of focal cortico-medullary and subcapsular dilatation of the distal tubules and collecting ducts with acute interstitial inflammatory cellular infiltration. The dilated tubules were filled with granular cast, acute inflammatory and desquamated epithelial cells. The epithelial lining of affected tubules was either flattened or swollen due to

cytoplasmic vacuolation. A variety of degenerative changes, predominantly vacuolation, were seen in the nuclei. Acute inflammatory cells, in which eosinophils were prominent, expanded the interstitium, surrounding and infiltrating the tubular structures. The proximal tubules showed only minor changes comprising cytoplasmic vacuolation or hydropic degeneration. On the other hand, the glomeruli were normal and no changes were detected in the blood vessels.

In the medulla, the collecting ducts and the thick loops of Henle were focally dilated and filled with acute inflammatory and desquamated epithelial cells. The epithelial lining was oedematous with cytoplasmic and nuclear vacuolation. Around damaged tubules there was marked peritubular cuffing with acute inflammatory cells. The medullary interstitium was also infiltrated with acute inflammatory cells and here, as in the cortex, eosinophils figured prominently. However, near the papillae, the interstitium contained an amorphous, granular material which appeared to be derived from damaged collecting ducts. The papillae were otherwise intact and did not show any signfiicant evidence of necrosis. The transitional epithelium showed only a very light infiltrate of acute inflammatory cells. Due

to overlapping of these microscopic findings with those seen in subsequent months (2 and 3), they are illustrated in Figures 61, 62, 63 & 64.

Twenty mg Dose Effects: Kidneys were normal on gross and microscopic examinations.

Second Month

Sixty mg Dose Effects: Macroscopical changes were similar to the sixty mg gasil 200 after one month. The lesions were, in general, histologically similar. However, at this stage, incipient peritubular and periglomerular fibrosis was in evidence.

In the medulla, in contrast, lesions were in most instances more severe, consisting of peritubular cellular cuffing. No specific changes were seen in the transitional epithelium. The sixty mg gasil 200 changes at this stage are illustrated in Figures 62, 63 & 64.

Twenty mg Dose Effects: The macro and microscopic changes were similar to those encountered after one month with sixty mg gasil 200, with the exception that eosinophils were absent.

Third Month

Sixty mg Dose Effects: Macroscopically, the kidneys were again large, pale and the capsules stripped easily.

Microscopically, the lesions were more extensive than those encountered in the first and second months. Islands of relatively normal tissue were surrounded by irregular areas of damaged renal parenchyma. Dilatation of the distal tubules and collecting ducts were particularly pronounced. At this stage, mononuclear cells began to appear in the inflammatory infiltrate. By now, the advancing parenchymal damage rendered identification of specific parts of the tubule increasingly difficult, particularly the proximal parts, although the dilated and distorted distal tubule and collecting ducts could easily be recognised.

In the medulla, the lesions were likewise more advanced. The transitional epithelium was as yet only lightly infiltrated with acute inflammatory cells. Lesions of this nature are demonstrated in figures 61,65 & 66.

Twenty mg dose effect: No evidence of progression was observed.

Fourth Month

Sixty mg Dose Effect: The macro and microscopic appearances were identical to those found at three months.

Twenty mg Dose Effects: No new findings were observed on macroscopic examination, whilst microscopically the changes were identical to those seen at the second and third months. However, eosinophils now began to be observed in the inflammatory infiltrate.

Fifth, Sixth and Seventh Months

Due to the similarity of these lesions, the changes are described together.

Sixty mg Dose Effects: Macroscopically, the kidneys were slightly contracted, irregular in shape, dark brown in colour, and the capsules stripped with difficulty. Microscopically, severe lesions of grossly dilated tubules (distal and collecting) filled with hyaline casts, desquamated epithelial cells and occasionally polymorphonuclear cells. The epithelial lining was thin and showed cytoplasmic and nuclear damage. There was generalised tubular loss, while the surviving tubules were surrounded by fibrous tissue (peritubular fibrosis). Although scattered foci of polymorphonuclear cells were observed, the interstitium was basically infiltrated with mononuclear cells. Bowman's capsule was thickened, while some glomeruli showed marked periglomerular fibrosis. Evidence of intra-renal obstruction was shown by dilatation of the urinary space. Changes at this stage are shown in

Figures 66 & 67. The papillae were examined and found to be oedematous (Figure 68).

Twenty mg Dose Effect: Macroscopically, the kidneys were normal in size and shape. They were now dark brown in colour. Their capsules stripped easily. Microscopically, classical lesions of tubular dilatation and interstitial infiltration with polymorphonuclear and mononuclear inflammatory cells were encountered. The glomeruli showed thickening of Bowman's capsules and some widening of Bowman's spaces.

Eighth and Ninth Months

During this period, there was steady increase in the severity of the lesions.

Sixty mg Dose Effect: Macroscopically, the kidneys were grossly distorted and often globular in appearance (Figure 69). They were brown in colour and smaller in size. The cut surface showed multiple small cysts in a markedly contracted cortex (Figure 70). The capsules were firmly adherent to the outer cortex. Microscopically, severe tubular dilatation and cyst formation were predominated and extended from the corticomedullary zone to the periphery of the kidney. The cysts were lined by a thin flattened epithelium. They were either fluid-filled or occupied by hyaline cast-like material. The interstitium

was widened due to increased fibrosis and infiltration with mononuclear cells and foci of polymorphonuclear cells and eosinophils. The peritubular fibrosis was found to have extended to the medulla (Figure 71). Due to the existence of the cystic dilated tubules, the glomeruli were pushed towards the periphery of the cortex. The Bowman's capsules were thickened and the urinary spaces markedly dilated. The papillae at this stage showed gross oedema. The papillary ducts were severely dilated, inflamed and lined by transitional epithelium deep into the medullary region. These lesions are illustrated in Figures 72 & 73.

Twenty mg Dose Effect: Macroscopically, the kidneys were smaller in size, irregular in shape, brown in colour, and the capsule adhered to the surface of the kidney. Microscopically, the changes were similar to those of 60 mg gasil 200 at the fifth, sixth and seventh months.

SUMMARY OF LIGHT MICROSCOPIC FINDINGS OF EXPERIMENT 2

Light microscopy charted the development and location of the renal lesions of the nine months study. The salient findings can be summarised as follows:

The lesions involved mainly the distal segment of the nephron (distal tubules and collecting ducts). They appeared earlier and were more extensive in the 60 mg weekly injected animals than those receiving 20 mg.

Three discernible stages in the development of the lesion were observed.

Stage 1 Dilatation and degenerative changes in the epithelium of the distal tubule and collecting ducts. Acute interstitial inflammatory infiltrate, in which eosinophils were prominent.

Stage 2 Progressive tubular damage with the appearance and subsequent progression of interstitial fibrosis. Acute and chronic inflammatory cell infiltrate.

Stage 3 Advanced tubular destruction, extensive cyst formation, interstitial fibrosis, light chronic inflammatory cellular infiltrate, glomerular changes secondary to tubular loss and obstruction.

B Ultrastructural Changes

The basic ultrastructural changes in chronic silicon nephrotoxicity were similar in both the 20 and 60 mg gasil 200 dosed animals and only differed in severity and time of development of the lesions. The normal glomerular ultrastructural architecture as found in controls is demonstrated in Figure 74. The sequential ultrastructural changes after weekly injections of silicon (gasil 200) are described month by month.

Month 1

60 mg Dose: The glomeruli showed marked mesangial accumulation of electron dense deposits; these extended into the paramesangial subendothelium (Figure 75). Visceral epithelial cells were swollen but showed no other striking changes. Early tubular changes included proximal tubular damage comprising oedema and increased lysosomal activity.

20 mg Dose: Early glomerular changes at this dose and at this stage were minimal, and consisted of small scattered mesangial deposits (Figure 76) and a slight degree of mesangial cell interposition in the peripheral parts of the capillary loops. The tubular changes were unremarkable.

Month 2

60 mg Dose: Glomeruli showed patchy foot process fusion, while the visceral epithelial cells were found to be swollen and possessed multiple electron dense cytoplasmic multilamellated bodies (Figure 77). The tubules showed severe damage which was mainly located in the distal tubules and collecting ducts. These included widespread cytoplasmic vacuolation and cisternal dilatation. The vacuoles were either electron lucent or contained material of varying electron density and granularity. Moreover, there were increased numbers of lysosomes and cytolysosomes which contained many fragments of cell organelles,

multilamellated membranes and electron dense granules. Mitochondrial swelling and endoplasmic reticulum vesiculation were much in evidence. Severe nuclear damage was occasionally encountered. Generally, there were widened intercellular spaces and thickened basal laminae (Figure 78). Many of the tubules (distal and collecting ducts) were filled with a wide variety of normal or degenerate cells, some of which were ultrastructurally identifiable, others were not. Cells identified were desquamated tubular epithelial cells - some showing nuclear inclusions of indeterminate origin, macrophages and acute inflammatory cells (Figure 79). The interstitium was infiltrated with a variety of acute inflammatory cells in association with an increased number of fibroblasts and excess collagen fibres (Figure 80).

20 mg Dose: Similar, but less frequent ultrastructural changes have been encountered.

<u>Month 3</u>

60 mg Dose: Para mesangial and mesangial electron dense deposits were not in evidence. However, the glomerular visceral epithelial cell damage was more extensive, the cytoplasmic multilamellated bodies and endoplasmic cisternal vesiculation was a prominent feature (Figure 81).

Furthermore, the parietal epithelial cells showed a condensation of granular electron dense material in their cytoplasm (Figure 82). Early periglomerular fibrosis was noted. Tubular damage was identical to that found in the second month, with the exception that residual bodies were found in greater numbers, particularly in the proximal tubules (Figure 83). The acute interstitial inflammatory cellular infiltrates were still encountered.

20 mg Dose: The glomerular ultrastructural lesions were less marked while the tubular damage was similar to the 60 mg dose.

Month 4

60 mg Dose: The glomerular showed more extensive visceral epithelial cell damage of the same ultrastructural description as in Month 3 (Figure 84). The tubular damage was noticeably increased. In the proximal tubules there was a marked increase in the number of vacuoles which contained electron dense material and granules, lysosomes, cytolysosomes (Figure 85) and residual bodies (Figure 86). The most advanced damage in the distal part of the nephron was manifested as total tubular disruption with complete disorganisation of the cytoplasmic organelles, where most of them were incorporated into widespread multilamellated and residual bodies (Figure 87). The tubular basal laminae

were markedly thickened. The loops of Henle were also extensively affected, cellular damage and organelle dissolution being encountered (Figure 88). Interstitial mononuclear cells appeared at this stage, some showing evidence of cytoplasmic damage (Figure 89).

20 mg Dose: Similar, but less extensive ultrastructural changes were found at this period.

Month 5

60 mg Dose: The glomerular damage was more extensive, and for the first time there appeared single membrane-bound lysosomes containing electron dense osmiophilic material. These varied in size, many being large and in some epithelial cells, packed in large numbers into the cytoplasm almost to the exclusion of other organelles (Figures 90 & 91). Patchy foot process fusion was noted. Tubular damage was of similar severity to that found in Month 4, with the exception of increased opening of the intercellular clefts (Figure 92).

20 mg Dose: The glomerular visceral epithelial cells showed a noticeable increase in the lysosomal activity. The tubular lesion was again more extensive. Proximal tubular epithelium was extremely vacuolated, and the cellular cytoplasm possessed many vesicles and vacuoles,

most of which contained electron dense material, homogeneous or granular, while some were transformed to heterophilic cytolysosomes with multilamellated bodies (Figure 93). Distal tubular lesions were also more extensive and showed severe cellular and nuclear damage (Figure 94).

Month 6

60 mg Dose: No progress in the glomerular lesions were found, except a noticeable increase in foot process fusion (Figure 95). The tubular lesions were again identical to the previous months. The interstitial fibrosis was markedly increased showing further progression (Figure 96).

20 mg Dose: Similar ultrastructural changes to those observed at 5 months.

Month 7

60 mg Dose: The glomerular and tubular changes were similar to the previously described lesions (Months 4, 5 & 6). The interstitial ultrastructural changes at this stage, comprised prominent fibrosis and infiltration with mononuclear cells, lymphocytes and plasma cells (Figures 97 & 98).

20 mg Dose: The glomerular lesions showed no progression while the tubular changes were marked and similar to those found at months 5 and 6.

Month 8

60 mg Dose: In addition to the lesions first recognised at the fifth month, there appeared new and interesting changes in the morphology of the visceral epithelial cells. Large irregular cystic spaces were observed in the epithelial cytoplasm. The cysts contained a moderately dispersed, finely granular material not dissimilar to that seen in the urinary spaces or even plasmatic material in normal capillary lumena. A narrow band of condensed cytoplasm hugged the cyst wall, while there was a definite rarefaction of the epithelial cell cytoplasm at other sites. In this lesion, foot process fusion was marked, while margination of cytoplasmic material was likewise condensed at the base of the fused processes. Increased deposition of electron dense material in the mesangium and vesiculation of the mesangial cell cytoplasm were also observed (Figures 99 & 100).

20 mg Dose: No new glomerular or tubular ultrastructural lesions were observed.

Month 9

No ultrastructural studies have been performed at this period.

C **Electron Probe Microanalysis**

Analysis of the glomeruli was carried out in the region of damaged epithelial cells. Surprisingly, no silicon was detected in or around the osmiophilic structures, or in the cyst walls. Small silicon peaks were encountered in the foot processes as in the glomeruli of control and tested animals described in Chapter 3.

D **Serum Biochemistry**

The following is a summary of the salient biochemical results.

(a) Serum Silicon: raised levels were found in both 20 and 60 mg gasil 200 injected animals, higher levels being observed in the 60 mg (Figure 101).

(b) Serum Urea: raised urea levels were recorded in all tested rats, being markedly increased in the 60 mg group (Figure 102).

(c) Serum Creatinine: increased values were observed in both groups (20 and 60 mg) being more pronounced in the 60 mg group (Figure 103).

(d) The serum electrolytes, carbon dioxide content, and total serum protein were within normal limits in both groups of tested rats. The serum protein was evaluated for the first six months only.

3 EXPERIMENT 3

A Macro and Microscopic Findings

Macroscopically, the kidneys were contracted, irregular in shape, with a pitted granular surface and surface cysts. Microscopically, there was widespread damage to the distal tubules and collecting ducts. They were dilated together with multiple cyst formation. There was also interstitial fibrosis and chronic inflammatory cell infiltration, whilst eosinophils were absent. In addition, focal collections of lymphocytes in the cortex were encountered. Islands of surviving proximal tubules surrounded by interstitial fibrosis and chronic inflammatory cells were found. The glomeruli showed expansion of their urinary spaces, occasional disorganisation of capillary architecture (synechia between loops and capsule) and periglomerular fibrosis.

In the medulla, the collecting ducts were notably free of casts, either desquamated cells or amorphous granular material as found in the acute series or in chronic series where weekly injections were used. The ducts were dilated and showed minor epithelial cell damage. The interstitium was lightly infiltrated with chronic inflammatory cells. One notable feature in this series was polypoidal irregularity of the papillary surfaces.

B <u>Serum Biochemistry</u>

(a) Levels of serum silicon, urea and creatinine of tested animals were elevated above those of the control group (Table 6).

(b) Serum electrolytes and carbon dioxide content were within normal limits.

Tested Animals				Control Animals		
	Urea mmol/l	Creatinine µmol/l	Serum Silicon µg/ml	Urea mmol/l	Creatinine µmol/l	Serum Silicon µg/ml
$\bar{X}$	12.41	80.83	1.01	5.88	56	0.52
SD $\pm$	3.76	23.54	0.24	0.92	13.87	0.08
Range	8.2 - 18.5	55 - 120	0.7 - 1.4	5 - 7.4	40 - 75	0.4 - 0.6
Number of Animals	6	6	6	5	5	5

Table 6: Serum urea and creatinine in control and tested rats after seven consecutive intraperitoneal injections of 60 mg gasil 200

DISCUSSION

Whereas the acute experiments, including the tissue localisation of silicon have provided new information on the nature of silicon nephrotoxicity, the series of chronic experiments have attempted to shed some light on the natural history and development of the renal pathology both in the situation where the animal is exposed to silicon over an extended period and also where the animal is allowed to survive after an initial, short but severe period of exposure.

There is undoubted difficulty in correlating the findings of these experiments with previous studies in that the routes of administration, nature of silicate tested, the frequency of dosing and the period of study have varied widely. In general, the periods of exposure in these previous chronic studies have been short and the depth of morphological and biochemical investigations and analysis have been superficial. Likewise, there is difficulty in correlating the findings of the present study with what is known or suspected concerning the effect of silicon compounds, by whatever route, in man. Nevertheless, this investigation can lay claim to have established with greater clarity the development of chronic silicon nephrotoxicity by repeated administration of silicon compound by one route, where the effect on the other body

systems has been minimal.

The long term effects on the kidney of administration of silicon compounds, by whatever route, have been poorly studied in the experimental animal. Using intravenous injections of silica sol, Gye and Purdy (1921 b) merely commented on the findings in the kidney, liver and spleen in four rabbits after a "few" months. Newberne and Wilson (1970) fed dogs several silicates for four weeks, while Marcovic and Arambasic (1968) administered ground quartz in the drinking water of guinea pigs for six months. With the exception of the latter, these studies were characterised by the brevity of the investigation and the paucity of numbers. All were relatively superficial in their histological analysis, none included ultrastructural correlations, while only one (Newberne and Wilson 1970) gave an information of a biochemical nature.

Although the glomerular lesions detected by the light microscopy were minimal, definite glomerular involvement was observed by electron microscopic examination. Progression of the glomerular lesions were found to depend on the stage of development of the overall pathological process. In the third stage, the glomeruli showed marked widening of the Bowman's space and periglomerular fibrosis. These glomerular changes were believed to be secondary to

the extensive tubular damage which constituted the basic lesion in silicon nephrotoxicity. Similar findings have been reported by Marcovic and Arambasic (1968) in the experimental situation (oral administration of quartz) and in human disease (Balkan endemic nephropathy).

At an ultrastructural level the most interesting changes were encountered in the glomerular visceral epithelial cells. Early in the course of this experiment, minor alterations in their fine structure were apparent, while gradually month by month and from stage to stage the lesions progressed and developed. Initially, only cellular oedema with dispersion of organelles was observed. This was followed by extensive cytolysosomal formation and in the last stage a retention within the cell cytoplasm of many large membrane bound structures containing electron dense osmiophilic material. Moreover, large irregular cystic spaces were also noted in the cytoplasm of the affected cells. These visceral epithelial cell changes were most marked in rats injected with 60 mg gasil 200. In addition, patchy epithelial foot processes fusion was occasionally encountered.

From these observations, it seems likely that the glomerular visceral epithelium was subjected to a process causing continual degeneration. Whether these ultra-

structural changes were due to passage of silicon across the glomerular basement membrane and over the surface of the podocyte cell membrane or due to some other mechanism, is not clear. However, the examination of these lysosomal structures by electron probe microanalysis revealed negative results which might indicate total absence of silicon at this site or that silicon was found in such small quantities as to be below the level of detection of the analysis. A small amount of silicon should it be present, might be incorporated within the organelle membrane system producing alteration and damage. However, the early cellular oedema and the late cytoplasmic damage tends to incriminate silicon as having a deleterious effect on the epithelial plasma cell membrane.

In addition to the visceral epithelial damage, the glomeruli showed early electron dense deposits which were both in the paramesangial subendothelium and in the mesangium itself. After one month, these deposits were only infrequently observed but in the last stage the mesangium was markedly widened due to the presence of such deposits, while vesiculation and vacuolation of mesangial cells were observed. The development and nature of these mesangial deposits and their relationship to an immune mechanism, possibly induced by silicon, could not be determined within the scope of this experiment. Thus, a further

investigation, an account of which is contained in the following chapter, was designed in an attempt to shed some light on this interesting finding.

Contrary to reports in the documented cases of human silicon nephropathy regarding glomerular cellular proliferation (Kolev et al, 1970, Saldanha et al, 1975, Giles et al, 1978 and Hauglustaine et al, 1980), no light or ultrastructural evidence of such proliferation was seen during this study. Thus, despite the observed ultrastructural changes in the glomeruli in this chronic experiment, significant glomerular damage was not found in light microscopic examination of large numbers of glomeruli.

This study successfully demonstrated the marked tubular damage. The distal segment of nephron was found to be most vulnerable to this pathological process. Although the light microscopy revealed lesions which were mainly located in the corticomedullary and subcapsular regions, significant medullary changes were also encountered. In general, the lesions were focal, consisting of tubular dilatation, tubular epithelial damage, desquamation and cast formation. These changes were found to be dose related - being more evident in animals injected with 60 mg gasil 200, and slowly progressive towards cyst formation. At an early stage, the tubules were surrounded by acute inflammatory cells, while in second and third

stages, peritubular fibrosis was in evidence which indicates a continuous process of damage to marked tubular loss and increased interstitial fibroblastic reaction.

Ultrastructural observations in the distal tubule and collecting ducts confirmed the light microscopic changes and demonstrated grading of tubular epithelial damage which was either segmental, affecting one part of tubule, or complete, involving all the tubular epithelial cells converting their cytoplasm to a mass of multilamellated and residual bodies. In addition to the tubular epithelial changes, the lumena of the affected tubules were packed with a different kind of cell. Some of these cells showed significant morphological abnormality which might be related to silicon which was presumably present in higher concentration in these regions of nephron. However, the exact reason for such morphological abnormality is not very clear, while part of the interpretation will be dealt with in the following chapter. Further observation in the distal tubule was the progressive thickening of the basal lamina, a finding which was not observed in the control rats.

On the other hand, the proximal tubular changes were less extensive than those found in the distal part of nephron. The light microscopic findings consisted of hydropic degeneration and nuclear vacuolation. These changes were

similar to that reported in Balkan endemic nephropathy - where silicon was also incriminated as possible aetiological factor, and in the documented cases of silicon nephropathy. However, in this study in the third stage, proximal tubular loss was observed and regarded as part of total tubular loss due to progressive tubular destruction and interstitial fibrosis.

Ultrastructurally, the proximal tubular changes were those of widespread vesiculation, vacuolation, cytolysosomal and multilamellated bodies formation. There were progressive thickening of basal lamina while the brush borders were generally intact.

In both proximal and distal tubules, it would appear that the normal process of sequestration of the affected organelles was increased in animals which received silicon, more than in the control animals. The increased number and variety of the lysosomal structures may reflect the severity of the cellular damage induced directly or indirectly by silicon compound in use in this experiment. However in a previous experiment, electron probe microanalysis of kidney tissue sections revealed silicon peaks in the region of the plasma cell membrane which favoured a silicon membrane interaction and partially explained the silicon damage effect on the cells, mainly in distal

segment of nephron. Similar explanation was given by Alison (1971) when he proposed that the rigid structure of the quartz, with many hydrogen bonding groups arranged in a regular and immovable order on its outer surface is the important factor in cell damage, presumably through the formation of multiple bonds which distorts the cellular membrane structure. Moreover, he found that silica particles mixed with serum protein or bronchial wash in a cell medium, failed to produce cell damage. On the contrary, these coated particles must be taken up by cells lysosomes, digest the coat and leave silica particles to react with their membrane to produce cell damage from inside. It was also believed that silica particles can kill macrophages or other target cells by silica plasma membrane interaction when these cells were incubated with larger amounts of silicon (100 mg/ml) in a saline medium.

These previous studies were concerned with silica in its particulate state, but in this study, the soluble form of silica was used (gasil 200), which was injected at a distance from kidney (intraperitoneally) and yet produced marked renal lesions.

There remains to be considered the early and late interstitial inflammatory infiltrate and fibrosis. In the early stage the infiltrate was acute inflammatory in nature,

being mainly polymorphonuclear leucocytes but eosinophils were a prominent feature, particularly in the kidneys of animals receiving the higher dose of gasil 200. In the later stages, chronic inflammatory cells took over, although foci of polymorphs and eosinophils were frequently seen. This was attributed to the repeated intraperitoneal injections of silica. The sequence of inflammatory infiltrates were as might be expected in any situation of recurrent insult. In these experiments giant cells were only occasionally encountered and hence there was no compelling reason to seek explanation of the inflammation in term of foreign body reaction on the part of the renal parenchyma. The prominence of eosinophils, although interesting, remains unexplained. It should be noted, however, that eosinophils were occasionally encountered in the kidney in cases of Balkan nephropathy. More pertinent information on the nature of the inflammatory reaction is provided by a later experiment which is more appropriately dealt with in Chapter 5.

Fibrosis, although a prominent feature in the later stages, was not excessive to the point that one could consider that a manifestation of silicon nephrotoxicity was excessive or exuberant fibrogenesis. In this respect, there appeared to be no analogy with the profuse fibrogenesis encountered in the lung in silicosis.

The overall picture of the effect of repeated administration of silica on the kidney was that of progressive tubulo-interstitial damage with secondary and possibly primary glomerular lesions.

The effect of the damage to the renal parenchyma was reflected in the biochemical findings in those animals. While no correlation was detected between raised serum silicon levels and raised urea and creatinine in both groups of tested rats, this is possibly due to the fluctuation of serum silicon levels as a result of repeated intraperitoneal injections. The normal values of serum electrolytes during the eight month's course indicated the persistence of a certain tubular reserve, in keeping with the histological evidence of the lesions being focal. Previous work of Saldanha et al (1975) also concluded that in an in vitro study, silicon does not inhibit proximal tubular function. The absence of hypoalbuminaemia during the first six months is in line with the absence of any significant foot process fusion. However, as the glomeruli showed definite visceral epithelial damage, shown ultrastructurally to be progressive, serum and urine protein must deserve further investigation.

SUMMARY AND CONCLUSIONS

Weekly intraperitoneal injections of amorphous silica (gasil 200), in two doses (20 and 60 mg) produced in Sprague-Dawley rats, a progressive tubulo-interstitial nephritis predominantly affecting the distal nephron. Nine months administration of silica resulted in renal functional impairment as evidenced by raised serum urea and creatinine levels, animals receiving the higher dosage showing the highest levels.

Electron microscopy demonstrated the development of glomerular lesions comprising degenerative changes in the podocytes and the deposition of electron dense material in the subendothelial paramesangial and mesangial regions. Electron probe microanalysis failed to show any deposit of silicon in the glomeruli of animals subjected to eight months of repeated intraperitoneal injections of gasil 200.

C H A P T E R V

IMMUNOFLUORESCENCE STUDIES

IN SILICON NEPHROTIXICITY:

INTRODUCTION

There has been for some time an interest in a possible immunological component in the pathogenesis of silicon associated disorders such as silicosis. Awareness of this fact together with the demonstration in the glomeruli of electron dense deposits at certain stages in the development of chronic silicon nephrotoxicity stimulated a pilot study on the possible immunological role of silicon in silicon nephrotoxicity.

The design of this study was such that in addition to providing data on the immunological state of the kidney in silicon nephrotoxicity, it also provided further useful biochemical and pathological information on what might be termed subacute silicon nephrotoxicity, to supplement the data obtained from the acute and chronic studies.

MATERIAL AND METHODS

Eighteen female Sprague-Dawley rats, ten weeks of age and 185 - 220 g in weight, were used in this experiment. They were divided into two groups.

Group 1 Ten rats received daily intraperitoneal injections of 60 mg gasil 200 dissolved in 2 ml 0.9% Nacl for seven days.

Group 2 Eight rats received 2 ml 0.9% Nacl intraperitoneally for seven days, as controls.

On day eight, all rats were weighed, anaesthetised and blood collected for serum silicon and biochemistry. Animals were sacrificed and kidney samples taken for the following examination:

1 Light Microscopy Tissue fixed in 10% phosphate buffered formalin, processed and embedded in paraffin. 5 μ sections were stained with haematoxylin and eosin.

2 Electron Microscopy Tissue was fixed in 2% gluteraldehyde, postfixed in 1% osmic acid, processed and embedded in Emix resin, ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope.

3 Immunofluorescence Specimens of renal cortex were frozen in a mixture of CO_2 ice and acetone, and thereafter stored at - 70°C until processing. All tested

and control samples were treated with fluorescein labelled sheep anti-rat total immunoglobulin (Welcome Research Laboratories) and rabbit anti-rat C_3 (prepared in the Department of Immunopathology, Western Infirmary, Glasgow) conjugated with fluorescein labelled sheep anti-rabbit gammaglobulin (Welcome Research Laboratories).

Preparation of Antisera

1 Lyophilised antiserum was dissolved in stated amount of distilled water.

2 To 0.2 g of liver powder, 2 ml of phosphate buffered saline was added and centrifuged at 1000 G for 5 minutes. The supernatant was discarded.

3 Antiserum was added to the washed liver powder and rotated on Matburn for 1 hour at room temperature.

4 They were centrifuged X 2 at 1000 G for 5 minutes.

5 10 μl of antisera aliquots were stored at -20°C.

6 All new antisera were titrated and checked for specifity.

Staining

1 4 μ frozen sections were cut and air dried for 3 minutes at room temperature.

2 They were washed in phosphate buffered saline in a coplin jar on shaker (2 X 10 minutes).

3 Sections were fixed in cold ether alcohol for 10 minutes

and in 95% alcohol for 20 minutes.

4 Sections were washed in phosphate buffered saline.

5 Slides were dried, the sections left moist and placed in a humid box.

6 Antisera were added at correct concentration and incubated for 30 minutes at room temperature.

7 Sections were washed in phosphate buffered saline 3 X 7 minutes.

8 For indirect test steps 7 and 8 were repeated.

9 Sections were mounted in buffered glycerol pH 8.5 and stored in dark at $4^{o}C$.

10 Specimens were examined under blue light.

Buffers

Phosphate Buffered Saline - p.B.s.

8 g Nacl, 0.34 g KH_2PO_4 and 1.21 g of K_2HPO_4 were dissolved in 1 litre of distilled water.

Buffered Glycerol pH 8.5

0.25 ml of 0.1 M tris and 1.7 ml of 0.1 M Hcl were made up to 10 ml in distilled water and 90 ml glycerol was then added.

RESULTS

1 After the completion of the seventh injection, the tested animals demonstrated obvious weakness and weight loss (tested $\bar{X}$ = 159 g SD $\pm$ = 9.4, range = 150 - 175 g) as compared with the control group ($\bar{X}$ = 240 g, SD $\pm$ = 12.5, range = 225 - 265 g).

2 SERUM BIOCHEMISTRY

The serum silicon, urea and creatinine levels of tested and control animals are presented in Table 7.

Animals	Serum Silicon µg/ml	Serum Urea mmol/l	Serum Creatinine µmol/l
Tested n = 10	$\bar{X}$ 22.3 SD $\pm$ 9.9 Range 13.8-36.8	$\bar{X}$ 39.4 SD $\pm$ 18.1 Range 17.2-67.5	$\bar{X}$ 186.4 SD $\pm$ 58.4 Range 110-270
Controls n = 8	$\bar{X}$ 0.58 SD $\pm$ 0.18 Range 0.4-1.0	$\bar{X}$ 5. 5 SD $\pm$ 0.55 Range 4.7-6.0	$\bar{X}$ 44.4 SD $\pm$ 3.2 Range 40-50

Table 7: Showing the serum silicon and biochemistry levels of tested and control rats after seven consecutive intra - peritoneal injections of gasil 200.

3 MACRO AND MICROSCOPIC CHANGES

Macroscopically, the kidneys were markedly enlarged, pale

in colour while the capsules stripped easily. There were no marked macroscopic changes in the liver and spleen.

Microscopically, the lesions were similar to that found in the acute and chronic silicon nephrotoxicity experiments, the distal part of nephron being the main site of damage where there was gross dilatation and widespread early cyst formation. The epithelial lining of affected tubules exhibited a grading of degenerative changes. The lumena of these dilated tubules contained both polymorphonuclear leucocytes and desquamated cells, while frequent deposits of amorphous material and occasional hyaline casts were also seen. Although the proximal tubular epithelium showed minor damage, it was inconspicuous in contrast to that observed in the distal and collecting tubules. The glomeruli and the blood vessels were found to be normal. The interstitium was widely expanded by acute inflammatory cellular infiltrate which consisted of macrophages, plasma cells, lymphocytes, polymorphs and eosinophils.

In addition, there was an early extensive interstitial fibroblastic activity. Giant cells were noted in the interstitium in association with either damaged tubules or deposits of non-birefringent amorphous material. Their frequency was much greater than in either acute (single injection) or chronic experiment. The medullary and

papillary interstitium were heavily infiltrated with the same pattern of inflammatory cells, as seen in the renal cortex. In four of the ten tested animals, the liver showed pathological changes. The lesions which had the appearance of small granulomatoma, consisted of periportal collections of macrophages surrounded, in some instances by a zone of eosinophils. In addition, patchy eosinophilic infiltration without granulomata was found in the liver of one tested rat. The spleen was also examined and no marked microscopic changes were seen. These changes are illustrated in figures 104 & 105.

4 ULTRASTRUCTURAL CHANGES

There were no glomerular lesions, the only finding being the occasional polymorphonuclear leucocytes in the capillary lumena (Figure 106). As described in the previous experiments, the main ultrastructural lesions were noted in the tubules (Figure 107) and interstitium and they were identical to those described in the acute and chronic silicon nephrotoxicity. However, in addition, many of the cells which stuffed the lumen of distal nephron had suffered severe ultrastructural changes consisting of marked cytoplasmic damage and nuclear pyknosis, which rendered their morphological identification extremely difficult (Figure 108). On the other hand, many of the cells were readily

identified as polymorphonuclear leucocytes matching the light microscopic findings. The polymorphonuclear leucocytes in the distal tubular lumena showed striking degranulation.

The interstitium was heavily infiltrated with mononuclear cells and polymorphonuclear leucocytes. The mononuclear cells were of variable size and shape; some with central nuclei were easily recognised as lymphocytes others with well elaborated endoplasmic reticulum were identified as plasma cells, while the large cells with large nuclei and branching cytoplasm which contained much electron dense material were macrophages (Figures 109 & 110). Unlike the lumenal cells, the interstitial inflammatory infiltrate was well preserved and ultrastructurally easily identifiable, moreover there was little evidence of degranulation of polymorphonuclear leucocytes in this site.

5 IMMUNOFLUORESCENCE FINDINGS

Granular immunofluorescence staining for C_3 and total immunoglobulins of moderate intensity was located in the glomeruli of all control and tested rats (Figures 111 & 112). The distribution was mainly mesangial while peripheral capillary linear staining was also occasionally observed. The same degree of intensity was found in a linear pattern along the tubular basal laminae and intima of blood vessels

of the control and tested samples. These were regarded as normal findings in such experimental animals.

The most striking finding, however, was observed in the dilated, damaged distal and collecting tubules of the tested animals; the intratubular cellular casts were uniformly, intensely stained for C_3 and total immunoglobulins (Figures 113 & 114). Immunofluorescence activity was neither demonstrated in the interstitial inflammatory cells (situated just across the tubular wall) nor in the tubular epithelial cells themselves. This finding was not found in any of the control rats.

DISCUSSION

Over the years there has been considerable debate and no little controversy concerning the role of silicon in the pathogenesis of silicosis. One of the many hypothesis advanced has been the effect of silicon on the immunological system.

An interest in the possibility of an immunological component in silicon induced lesions began in 1953, when Caplan demonstrated the association between rheumatoid arthritis and certain unusual radiographic findings of multiple, well defined round opacities in the lungs of coal miners (Caplan, 1953). Later, Caplan et al (1962) found that coal miners with these radiographic changes, but who had no signs nor symptoms of rheumatoid arthritis, had in fact, a high incidence of rheumatoid factor in their serum. These studies were further advanced when it was found that animals experimentally pre-treated with silica by pulmonary insufflation produced an increased antibody response when challenged with a protein antigen (ovalbumin). This effect was thought to be due to the action of silica as an adjuvant to antibody production. Moreover, it was shown that kaolin and coal were, to a lesser extent, also capable of inducing such effects (Pernis and Paroneto, 1962). Indeed, Vigliani and Pernis (1963) observed in silicotic nodules, the presence of abundant deposition of gamma-

globulins, a predominant plasma cell infiltrate, and altered immune serological tests in both the human and experimental situation. They stated, it was their belief, that silicosis as a disease entity was essentially immunological in nature. They argued that the main immunological abnormality was one of locaslised, prolonged stimulation of the immune system which appeared to be directed against a variety of antigens, while quartz particles induced such an alteration as a consequence of continued destruction of silica laden macrophages.

Several investigators have reported on the in vivo effect of silica on the cell mediated immune system. Pearsall and Weiser (1968) found that the intraperitoneal injection of silica 4 hours to three days before grafting leads to prolongation of the survival of skin allografts. The abrogation of resistance to the bone marrow grafts by silica particles was demonstrated by Lotzova and Cudkowicz (1974). While the destruction of macrophage incubated in cell culture with quartz was thought to be due to activation of the complement or complement-like enzymatic system (Vigliani et al, 1961). In addition, Miller and Zarkower (1974) showed the effect of short inhalational exposure of high silica concentration on the tissue involved in the immune responses. They found that silica impaired macrophage function and changed mitogen and

antigen reactivity of T and B lymphocyte population. They concluded that silica inhalation can alter the immunological responsiveness in the spleen, possibly due to the formation of some toxic substance, such as silicic acid, alteration of B cell surface receptors by silica or liberation of unknown toxic substances. In contradiction to those observations and speculations, Schuyler et al (1977) concluded that there was no major evidence of altered cell mediated immunity found in silicotic patients.

Serological examinations have also revealed evidence of disturbed immune function in other forms of pneumoconiosis. Caplan et al (1962) and Pernis et al (1965) have shown an increased incidence of rheumatoid factor in workers with prolonged exposure to asbestosis. Other workers have demonstrated a four-fold increase in the titre of both rheumatoid and antinuclear factors in asbestos workers compared with random samples from the normal population (Turner-Warwick and Parkes, 1970). Similarly, an increased prevalence of circulating antinuclear and rheumatoid factors were found in coal miners and this correlated with the degree of pulmonary fibrosis (Lippmann et al, 1973, Soutar et al, 1974 and Kang et al, 1973). It is worthy of note that a higher incidence of serum antinuclear antibodies were found in silicotic sandblasters, as

compared with other forms of pneumoconiosis (Jones et al, 1976).

Rheumatoid and antinuclear factors are not the only abnormalities detected in pneumoconiotic patients, for Rodnan et al (1967) focused attention on the association of progressive sclerosis (Scleroderma) with coal miners' pneumoconiosis and other forms of silicosis. They point out that the prevalence of progressive systemic sclerosis among coal miners and other men engaged in similar siliceous - dusty trades was higher than that in the general population. They also found that some of these patients ultimately died from renal failure.

In the documented cases of human silicon nephrotoxicity, the immunofluorescence studies on renal tissue were variable in result. However, in the case examined by Saldanha et al (1975), they found only a trace of focal granular glomerular deposition of IgM which did not assume any distinctive pattern. Xipell et al (1977), demonstrated a focal granular staining of peripheral capillary loops of some glomeruli for IgM, IgA and complement. Furthermore, Giles et al (1978) showed focal staining for IgM and C_3 in the mesangium and along the glomerular basement membrane. On the other hand, Hauglustaine et al (1980) described an insignificant amount of IgG. IgA, IgM, CI_q, C_4,

albumen and fibrinogen. There was, however, a strong staining for C_3 in the intima of the blood vessels.

In this study, the glomeruli of control and tested rats showed positive immunofluorescence stain of moderate intensity for C_3 and total immunoglobulins.

In all series of experiments in this study on silicon nephrotoxicity the same species, Sprague-Dawley rats were used. Aware of the development in older animals, particularly males, of glomerular sclerosis, only young females were used. In this experiment, young females were chosen and in order to accelerate the process of silicon nephrotoxicity, a short intensive course of silicon administration was employed. Unfortunately, these precautions were negated in that both controls and tested animals gave a similar pattern of positive fluorescence in the glomeruli. This naturally occurring phenomenon has been noted by other workers on previous occasions. Moreover, the electron microscopy failed to show any evidence of electron dense deposits in short term, subacute experiment. Information salvaged from this study nevertheless was of some significance in that the absence of electron dense deposits perhaps indicates that the late occurrence of deposits in the chronic experiment was more related to ageing changes, possibly in the nature of

glomerulo-sclerosis, common in this strain, and not to direct effect of silicon.

On the other hand, totally unexpected evidence of an immunological event was observed in a specific site in the kidney damaged by exposure to silicon. The interesting and intriguing aspect of this phenomenon is the fact that it occurs only at one location - the polymorphonuclear cells in the distal tubular lumena. Although the significance of this finding is obscure, the fact that similar cells either in the inflamed interstitium, or in the peritubular inflammatory cuffs or even those cells in passage through the tubular wall do not take up the stain. It appears that a local immunological process might be activated inside the tubular lumena giving rise to such phenomenon.

In addition to the immunofluorescence observations, this study was also supplied information on the light and electron microscopic changes of repeated administration of gasil 200. The light microscopic examination revealed renal lesions similar in description and distribution to that observed in the acute and chronic silicon nephrotoxicity where the distal part of nephron was the main site of involvement. It was also readily demonstrated that severe renal histological changes developed after seven

consecutive intraperitoneal injections of 60 mg gasil 200. However, this study has shown an additional interesting finding which included the presence of giant cells in kidney sections and granulomatous reactions in liver sections of tested animals. In the kidney, the giant cells were not associated with any granulomatous reaction while in the liver, the granulomas were not associated with giant cell formation but with eosinophils. The distribution of these granulomas was mainly periductal, perhaps indicating that silicon was excreted in high concentration in the bile producing a certain degree of hepatic damage. It would, therefore, appear that the concentration of circulating silicon under such circumstances - daily intraperitoneal injections of 60 mg gasil 200 - is hepatotoxic as well as nephrotic. These observations are of interest with respect to the findings of Gye and Purdy (1922 b) who found significant hepatic pathology when they achieved high concentrations of circulating silicon with direct intravenous injection of silica sol. No marked damage was encountered in the spleen.

The ultrastructural findings confirmed the light microscopic changes of absence of marked glomerular lesions. On the contrary, the distal tubular epithelial damage, the intralumenal cells, and interstitial inflammatory infiltrate were readily demonstrated. The information

provided by this experiment has unexpectedly proved to be of crucial significance in the formulation of a hypothesis concerning the reasons for the site of maximal renal damage in silicon nephrotoxicity and supplies important clues as to the possible cause of the inflammatory damage at that location.

Concentration of silicon will be maximal in the lumena of the distal tubule and collecting ducts. In the lumena there appears large numbers of polymorphonuclear leucocytes. These polymorphs differ significantly from all other polymorphs in the renal interstitium in that they stain heavily with total immunoglobulin and C_3. Electron microscopy reveals that lumenal polymorphs also show changes unique to that site in that they show massive degranulation and cytoplasmic damage. Thus, there would appear to be an interface in the lumen of the distal tubule where high silicon concentration probably has a specific effect on the polymorphs. It has previously been shown by Policard et al (1959) that silicon has a profound effect on the release of lysosomes (degranulation) from polymorphs in vitro. In this experiment it was also demonstrated that the degranulated polymorphs were heavily coated with total immunoglobulin and C_3. It is not clear whether the effect of silicon on polymorphs is a direct cell membrane interaction and immunogenic activity follows or whether

immunogenic events triggered by silicon precede the de-

granulation.

SUMMARY AND CONCLUSIONS

Rats receiving seven consecutive intraperitoneal injections of 60 mg gasil 200 produced marked histological changes similar in distribution to that seen after single (acute) and weekly (chronic) injections of gasil 200.

Examination by immunofluorescence gave positive glomerular staining for anti-rat C_3 and total immunoglobulin in both dosed and control rats. These findings have been previously reported as occurring in this strain of rat. Electron dense deposits were not detected in the glomeruli of the tested animals. Thus this investigation did not provide any significant data of an immunological occurrence in the glomeruli as a consequence of silicon toxicity.

However, the study showed a unique localisation of positive staining for C_3 and total immunoglobulin only in the cells within the lumen of the distal tubule. These cells, which were predominantly polymorphs, showed on electron microscopy massive degranulation. These findings were taken to represent an immunological event which was happening at a specific interface within the nephron, significantly at the site of maximal structural damage.

C H A P T E R VI

SILICON AND ANALGESIC NEPHROPATHY

INTRODUCTION

Since 1953, when Spulher and Zolinger drew attention to the relationship between the prolonged use of analgesics and chronic interstitial nephritis in man, many clinical studies and much experimental work have been published on this problem. However, despite much endeavour the mechanism of analgesic nephropathy is still poorly understood, for the methodology has varied widely in reliability and in relevance to the human disorder. In addition, in the experimental situation, the role of other factors such as dehydration, bacterial infection, and chemical contamination during the manufacturing process have also been investigated only to provide further diverging results.

Since silicates are incorporated in some analgesic preparations during their manufacture (Gore and Banker, 1979) and since this and other studies have demonstrated the pathogenicity of silicates in producing chronic interstitial nephritis (man and experimental animals), it seemed relevant and useful to design a study in which the role of silicon as a co-factor or adjuvant in the pathogenesis of analgesic nephropathy.

MATERIAL AND METHODS

This study was accomplished by two experiments.

Experiment 1

Acute analgesic renal toxicity: To find the minimum dose which was just capable of producing observable histological change. The analgesic substances tested in this study comprised:

1 Para-aminophenol Hydrochloride (PAP) A phenacetin metabolite which has been previously shown experimentally to be nephrotoxic. (Supplied by Fluka A.G., Buch, S.G., Switzerland).

2 Sodium Salicylate (Supplied by Evans Medical Ltd., Speke, Liverpool).

Twenty, eight weeks old female Sprague-Dawley rats were used. The animals received single intraperitoneal injections of one dose of sterile PAP or sodium salicylate dissolved in 2 ml of sterile distilled water. The doses tested were:

Para-aminophenol HcL - 60, 40, 20 and 10 mg.
Sodium Salicylate - 800, 600, 400, 300, 200 and 150 mg/kg.

Animals were sacrificed after 24 hours, the kidneys were fixed in 10% buffered formalin, processed, stained with haematoxylin and eosin and 5 u sections were

examined by light microscope. Blocks from the kidneys of animals received 10 mg PAP and 200 mg/kg sodium salicylate were fixed in 2% phosphate buffered gluter-aldehyde, postfixed with 1% osmic acid, processed and examined under the electron microscope Philips 200.

RESULTS

Experiment 1

1 LIGHT MICROSCOPIC CHANGES

The histological examination of kidneys revealed the following:

A Para-aminophenol Hcl Treated Animals

In the 60 mg injected animals the kidneys showed widespread acute tubular necrosis, which mainly involved the proximal tubules and was associated with intratubular hyaline cast formation. Patchy, acute tubular necrosis was found in 40 mg PAP injected rats, while only minimal, patchy degenerative foci of proximal tubules were observed in animals which received 20 and 10 mg PAP.

B Sodium Salicylate

Patchy, mid-cortical proximal acute tubular necrosis was encountered in the kidneys of animals which received 800 mg/kg sodium salicylate. Occasional foci of acute tubular necrosis were found in animals injected with 600 and 400 mg/kg while no abnormalities were detected in the kidneys of 300 and 200 mg/kg sodium salicylate injected rats.

2 ELECTRON MICROSCOPY

Sample blocks from the kidneys of animals receiving the lowest dosage of PAP and sodium salicylate were checked by electron microscopy. After 24 hours, animals given 10 mg

PAP showed minimal signs of tubular damage, mainly as organelle swelling and cisternal dilatation. However, whereas no significant ultrastructural changes were encountered in the tubules, swelling of the glomerular endothelial cells were noted in animals receiving 200 mg/kg sodium salicylate.

3 DOSAGE PRODUCING MINIMAL LESIONS

From the results of this experiment, the choice of dose of sodium salicylate was 250 - 300 mg/kg and 10 mg para-aminophenol Hcl.

EXPERIMENT 2

Investigation of the chronic effect of these substances singly and in a variety of combinations was carried out by administering repeated intraperitoneal injections of the following:

1 Single analgesic (PAP Hcl).
2 Single analgesic (sodium salicylate).
3 Combined analgesic (PAP Hcl + sodium salicylate).
4 Combined analgesic (PAP Hcl + salicylate) and silicon (gasil 200).

The choice of dose of sodium salicylate and para-aminophenol Hcl which produced a minimal lesion was determined by the information gained in Experiment 1. The dose of sodium

salicylate was 250 - 300 mg/kg and of para-aminophenol was 10 mg. Likewise the choice of dose of silicon (20 mg) was determined by information obtained in previous experiments (see Chapter 4).

A hundred female Sprague-Dawley weight week old rats which were kept on normal diet and free fluid intake were used. They were divided into five groups of twenty. Each group was injected intraperitoneally with either one or more of the drugs listed above which had been sterilised and dissolved in 2 ml sterile distilled water or 2 ml of sterile 0.9% Nacl in the case of gasil 200 and according to Table 8.

Animals	Number/Group	Drug or Combination Group	Dose
Group 1	20 rats	Para-aminophenol Hcl	10 mg
Group 2	20 rats	Sodium Salicylate	250-300 mg/kg
Group 3	20 rats	Para-aminophenol Hcl and Sodium Salicylate	10 mg + 250-300 mg/kg
Group 4	20 rats	Para-aminophenol Hcl Sodium Salicylate and gasil 200	10 mg 250-300 mg/kg 20 mg
Group 5	20 rats	Distilled water	2 ml

Table 8: Showing the number of rats, drugs and doses used in Experiment 2.

Every month for four months, five rats from each group were anaesthetised, blood samples were taken for estimation of serum electrolytes, urea and creatinine, then sacrificed. Both kidneys and random blocks of liver and spleen were fixed in 10% buffered formalin and processed and examined by light microscopy.

RESULTS

Experiment 2

1 BIOCHEMICAL DATA

Serum electrolytes, urea and creatinine were found to be within the normal range throughout the course of this experiment.

2 HISTOLOGICAL FINDINGS

A Macroscopical Changes

There were no changes in size and shape of the kidneys. The capsules stripped easily.

B Microscopical Changes

Single (PAP and Sodium Salicylate): Over the period of four months, no marked light microscopic changes were observed in the kidney of animals receiving either PAP or sodium salicylate alone.

Combination of PAP and Sodium Salicylate: Definite renal lesions were seen in animals which received a combination of PAP and sodium salicylate. Tubular lesions appeared in the first month consisting of dilatation and degenerative changes in the epithelium. Although the whole nephron was affected, lesions were more severe in the distal and

collecting as compared to the proximal tubules. The dilated tubules located in the cortico-medullary area were lined with flattened epithelium. However, some of the tubules showed swollen epithelium with cytoplasmic and nuclear changes. Desquamated epithelial cells and occasional polymorphs were encountered in the lumena of the dilated tubules - mainly in the medulla. No evidence of acute or chronic interstitial inflammatory infiltrate was detected in animals injected with PAP and sodium salicylate. The glomeruli were found to be normal, vascular lesions were absent, while there was no evidence of any papillary histological changes. These changes are illustrated in Figures 115.

C <u>Combination of PAP, Sodium Salicylate and Silicon</u>

Animals receiving weekly intraperitoneal injections of the three agents showed lesions similar to those found in animals injected with the combination of two analgesics (PAP and sodium salicylate). However, the lesions were of a more consistent nature than in the analgesic combination above, while focal subcapsular tubular derangement was more noticeable. In addition, and more significantly, a chronic interstitial inflammatory infiltrate was found in kidneys of the animals in this series. No evidence of papillary necrosis was noted. These changes are illustrated in Figures 116, 117 & 118.

DISCUSSION

Since 1953, when Spulher and Zolinger reported the relationship between analgesic abuse and chronic interstitial nephritis, there has been an ever increasing interest in the nephrotoxic effects of these compounds. While there is well documented clinical evidence for analgesic induced renal damage, the results of the performed animal experimentation have not, however, been conclusive. According to the duration and classification of the experiment, there are two main types of enquiry:

1 Acute experimental analgesic nephrotoxicity.

2 Subacute and chronic analgesic nephrotoxicity.

In acute experimentation, Green et al (1964) have shown necrosis of the terminal third of the proximal tubules of rat kidney after single intravenous injection of para-aminophenol hydrochloride. It was found that the nephrotoxic property of phenacetin related compounds was associated with the presence in the molecule of amino and hydroxyl groups in para relationship on the benzene ring (Calder et al, 1971). In addition, Funder et al (1972) produced renal tubular ultrastructural changes 1 - 4 hours after the intravenous administration of para-aminophenol hydrochloride, as demonstrated by organelle dilatation, mitochondrial swelling, lysosomal aggregation, intra

cytoplasmic multilamellated bodies and variation in the cytoplasmic density.

Robinson et al (1967) demonstrated massive proximal tubular epithelial cell necrosis in rat kidney 24 hours after a single intraperitoneal injection of sodium salicylate in doses not less than 400 mg/kg body weight. On the other hand, patchy, cortical tubular necrosis was claimed to be found in rat kidney a few hours after the oral administration of 100 mg/kg of acetyl salicylic acid, while tubular regeneration was beginning within 24 hours even when drug intake continued (Arnold et al, 1973).

In subacute and chronic administration, Fordham et al (1965) found that phenacetin resulted in biochemical and histological evidence of renal disease when given by gavage in a dose of 300 - 600 mg/rat/day over a period of four weeks. Ozuer et al (1977) claimed it was possible to induce cortical lesions in rat kidney with 10 - 20 mg/day phenacetin orally for 2 - 6 months. Abrahams et al (1964) have shown that rats fed phenacetin in various combinations with aspirin and caffeine (APC) developed renal lesions after 16 months. The APC fed animals showed lesions consisting of tubular basement membrane thickening, epithelial cell atrophy, mild interstitial inflammatory infiltrate and fibrosis, while tubular dilatation and cast

formation were found 10 months after phenacetin administration. Papillary haemorrhage and necrosis were more prominent in animals treated with APC mixture. Ultrastructurally, Abrahams and Levinson (1970) demonstrated changes in the papillae which consisted of accumulation of platelets in the vasa recta and damage in the epithelial cell lining of the collecting tubules. Similarly, reduction in the number and dimension of vasa recta were found experimentally in the kidneys of rats which received phenacetin or APC (Kincaid-Smith et al, 1968). These authors believed that the vascular changes they demonstrated could be the cause of the necrosis of the papillae in association with high doses of analgesic drugs. Contrary to this belief, normal vessels and glomeruli were found in the kidney of rabbits treated with phenacetin and aspirin for a period of twelve months (Clausen, 1964). The latter was also able to show renal damage predominantly in the distal part of nephron where the cortico-medullary region seemed to be most vulnerable. The damage was characterised by tubular dilatation, epithelial atrophy and deposition of calcium in the tubular lumena. Nanra and Kincaid-Smith (1970), Nanra (1972) supported the view of Abrahams et al (1964) that the incidence of papillary necrosis was more common with aspirin alone or aspirin containing mixtures than with phenacetin (alone). On the

other hand, Nanra and Kincaid-Smith (1973) were capable of demonstrating papillary necrosis and medullary calcification by other anti-inflammatory agents such as paracetamol, phenyl butazone, mefenamic acid, indomethacin, phenazone and propoxyphen. They have postulated that the inhibition of prostaglandin synthesis might be a common pathogenic mechanism. They also commented that water diuresis was an important factor in protecing the kidney from analgesic damage. Conversely, the effect of dehydration as a provoking factor in experimental papillary necrosis has also been demonstrated by Saker and Kincaid-Smith (1969), while an increased incidence of ascending infection was found after E. Coli had been injected into the urinary bladder of rats pre-treated with acetyl salicylic acid over a period of six months (Vivaldi, 1968).

The difficulty in inducing renal lesions mimicing those found in patients abusing analgesics suggested the idea that contaminating factors might be responsible for analgesic nephrotoxicity. Thus, Schnitzer et al (1965) claimed the acetic-4-chloranilide (A_4CA) - a manufactured bye-product contaminating phenacetin, was the offending agent in the haemolytic process produced by what was believed to be phenacetin toxicity. Dobbie (1982) has shown

that daily oral administration of magnesium trisilicate at 60 mg/kg to guinea pigs for four months produced focal tubulo-interstitial nephritis affecting mainly the distal tubules. This is a dosage comparable to what is ingested by patients taking certain compound analgesics (e.g. Askits). He therefore suggested that silicates might be the missing factor in analgesic nephropathy.

In this study, the acute effect of the single intra-peritoneal administration of different doses of para amino-phenol Hcl was studied after 24 hours. Renal lesions were readily seen and they proved to be dose dependent, being severe and widespread in 60 mg doses but mild and focal in 20 mg. Thus, the 10 mg dose PAP Hcl was chosen for this study. Similarly, sodium salicylate was injected intraperitoneally in different doses, variable results were encountered, while the intraperitoneal injection of doses more than 800 mg/kg proved in this experiment to be fatal mainly through internal bleeding. Likewise, difficulties were encountered using a dosage of sodium salicylate of more than 300 mg/kg in combination with the other agents used in this study where most of the animals died early in this experiment. For this reason, the experiment was re-run with a dose of sodium salicylate adjusted to less than 300 mg/kg/week.

As the group of animals receiving repeated intraperitoneal injections of single analgesic drugs were followed from month to month no lesions were found to develop. When however the two analgesic drugs were given together lesions were apparent as early as the first month. These lesions did not progress over the four month period. However the animals which also received small doses of silicon with the combined analgesics showed in addition to the cortico-medullary lesions characteristic of the analgesic combination, subcapsular lesions and an interstitial inflammatory infiltrate of acute and chronic inflammatory cells. Thus by using agents at doses calculated to show no significant lesion on single injection, an incremental effect was obtained by using them in combination.

It is perhaps significant with regard to the possible role of silicates as an adjuvant that although the analgesic combination showed tubular damage interstitial infiltrate only occurred with the addition of silica.

In relation to other experimental work in this field, it is believed that this study has made several relevant contributions to the problem of analgesic nephropathy. In the first instance, renal lesions were produced after only a short period using relatively smaller doses of combined analgesics than those used by most previous workers.

Moreover, this study has demonstrated the ease and efficacy of regular administration by the intraperitoneal route, a route singularly neglected by others.

SUMMARY AND CONCLUSIONS

Intraperitoneal administration of a range of doses of the analgesics sodium salicylate and para-aminophenol HCl determined a dose for each compound which was just below that causing histologically discernible lesions. Administration of each agent on its own over a four month period failed to produce any significant renal lesion. When, however, sodium salicylate and para-aminophenol HCl were given together, significant, though not progressive, renal tubular damage occurred. Addition of small doses of silicon to the combined analgesics produced more extensive tubular damage with super added acute and chronic interstitial inflammatory infiltrate.

These findings suggest that silicates could function as a cofactor in the causation of analgesic nephropathy.

C H A P T E R   VII

THE EFFECT OF

SILICON ON THE FISH KIDNEY

INTRODUCTION

The effect of silicon on rat kidney has already been described, and evidence has been advanced that silicon in certain concentrations is nephrotoxic (Chapters 2 and 4). However, the fact that fish are capable of living both in sea water, where the concentration of silicon is low, and also in fresh water where the concentration can be very high, would seem to indicate either a tolerance to silica or even some fresh water adaptation which protects them from continuous exposure to environmental silicon. It, therefore, seemed appropriate that one should examine the effect on the fish kidney of intraperitoneal injections of silica in doses equivalent to that used in the rat. Furthermore, as fish can be considered as a prototype vertebrate, it seemed reasonable that a study of their reaction to silica would be contributory to the general understanding of the phonomena of silica nephrotoxicity.

MATERIAL AND METHODS

This project was carried out in the Unit of Aquatic Pathobiology, University of Stirling, Scotland.

Three experiments were carried out, each one designed on the results of its predecessor. In all studies, the fish used were rainblow trout (Salmo gairdneri Richardson). 250 - 300 g in weight (equivalent to rat body weight).

Experiment 1

Eighteen fish were divided into two groups which were kept in two separate tanks. The first group consisted of twelve fish which received single intraperitoneal injections of 100 mg sterile gasil 200, dissolved in 2 ml 0.9% Nacl.

The second group consisted of six fish receiving a single intraperitoneal injection of 2 ml 0.9% Nacl and used as control.

Sampling

Four fish from first group and two from the control group were killed by a sharp blow to the head, after four,twenty-one and thirty-five days. Following gross examination of the kidney, dissection of the whole length of this organ was carried out. It was divided transversely into several blocks, each being fixed (10% buffered formalin) and labelled separately. The material was processed through

to paraffin wax, cut at 5 µ and stained with Haematoxylin and Eosin. The sections were examined by light microscopy.

Experiment 2

Fifteen fish were divided into two groups which were kept in separate tanks. The first group, consisting of ten fish, each received weekly intraperitoneal injections of 200 mg sterile gasil 200, dissolved in 2 ml of 0.9% Nacl for five months.

The second group consisting of five fish, each received weekly intraperitoneal injections of 2 ml 0.9% Nacl for five months and served as control.

Sampling

Two fish from the first group and one from the second were killed every month. The kidneys were fixed, processed and examined as in Experiment 1.

Experiment 3

Fifteen fish were divided into two groups. The first group consisting of ten fish, each received 200 mg sterile gasil 200, dissolved in 0.9% Nacl, three times a week for five weeks. Fish of the second group received 2 ml 0.9% Nacl, three times a week for five weeks and served as control.

Sampling

Two fish from the first group and one from the second were sacrificed every week. The kidneys were fixed, processed and examined as in Experiment 1.

RESULTS

The renal histology obtained in these experiments was double checked both in the Department of Aquatic Pathobiology, University of Stirling and here in the Renal Unit, Department of Medicine, Glasgow Royal Infirmary.

Experiment 1

All fish in this experiment survived the period of this study. There were no signs of peritonitis, while the kidneys failed to show any macro or microscopic changes.

Experiment 2 and 3

Some of the tested and control fish died during the course of these experiments. All fish receiving silicon had swollen vents while some showed bleeding from this orifice. There was much fluid in the abdomen as well as signs of peritonitis. These findings were most frequently noted in the tested fish of Experiment 3. However, no macro or microscopic changes were encountered in the kidneys in either experiment.

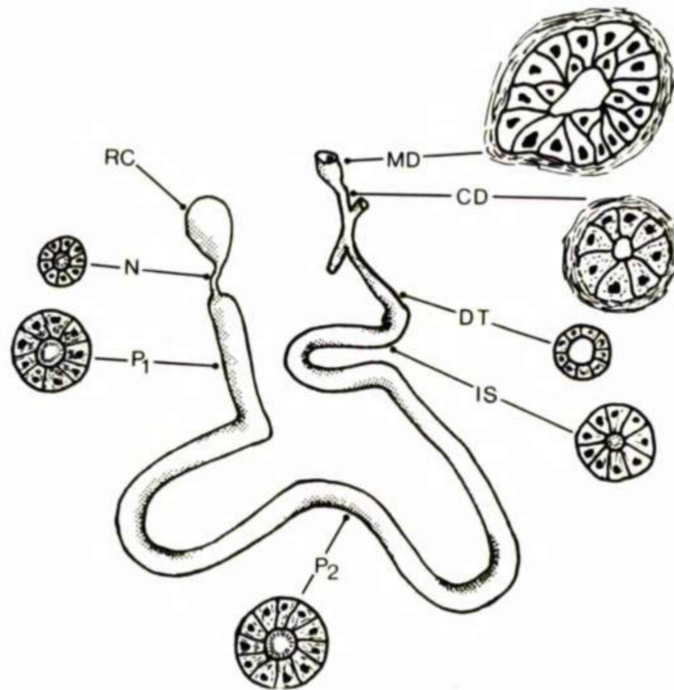


Diagram 6: Schematic drawing of the nephric tubule and duct system in the trout. Distinctions between segments are made on the basis of the characteristic morphology as well as lumenal and tubular diameter. Renal (corpuscle) (RC), neck (N), first portion of proximal segment (P1), second portion of proximal segment (P2). Intermediate segment (IS), distal tubule (DT), collecting duct (CD) and mesonrphric duct (MD) - Anderson and Loewen (1975).

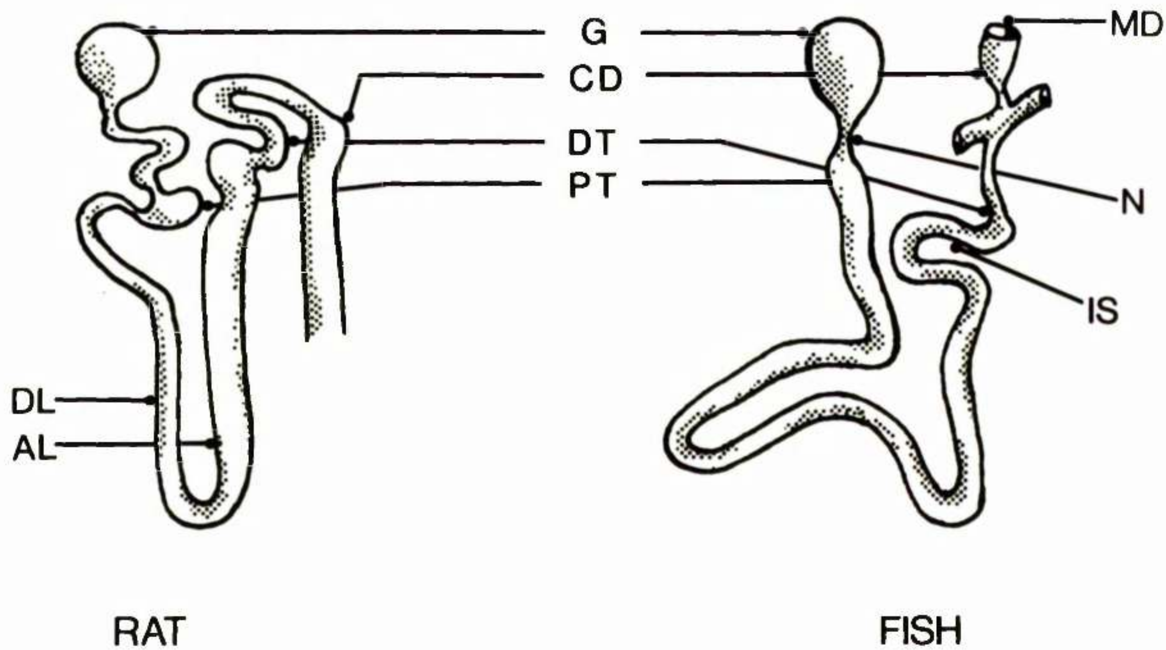


Diagram 7: Schematic diagram of rat and fish nephron. In fish, there is absence of the descending loop (DL) and ascending loop of Henle (AL). Glomerulus (G), proximal tubule (PT), distal tubules (DT) and collecting tubules (CT). In rat there is no neck (N), intermediate segment (IS) or mesonephric duct (MD).

DISCUSSION

In the trout, the kidneys are fused, appearing as a single organ rather than two. They occupy a dorsal retro-peritoneal position along the entire length of the body cavity, bounded by the vertebrae dorsally, the ribs laterally and the swim bladder ventrally. On the basis of morphological difference as well as location, they are divided into cranial or head kidney and a caudal or trunk kidney. The cranial portion is composed mainly of haemopoietic and lvmphoid tissue interspersed with pigment granules. Renal tubules are sparse in the head kidney but rapidly increase in number towards the more caudal regions. Mesonephric ducts emerge from the interior portion to the ventral aspect of the caudal kidney, where they join to form a single duct which terminates ultimately at the anogenital orifice. Inter-renal tissue, homologous to the mammalian adrenal cortex, is scattered along blood vessel walls. Chromaffin tissue, homologous to mammalian adrenal medullary tissue, is found in strips along the dorsal surface of the kidney. The nephron composed of renal corpuscle and tubule is embedded in haemopoietic tissue in the caudal region. The tubules are subdivided into:

1 A short neck segment.

2 The proximal convoluted segment, divided both structurally and functionally into a first and second portion.

3 An intermediate segment, and

4 the distal segment.

The distal segment joins the mesonephric ducts (Anderson and Loewen, 1975). A detailed diagramatic illustration is given in Diagrams 6 & 7.

Considering briefly piscine renal physiology and osmoregulation with respect to habitation in salt and fresh water. In sea water, the blood is hypotonic compared with the external environment, thus the drinking of sea water and subsequent secretion of ions are vital mechanisms for maintaining life in this environment. In fresh water, the blood is hypertonic and homeostatic compensation of the osmotic inflow of water and outflow of ions, must be extremely efficient. In both sea water and fresh water fish, water and salt regulation is achieved by kidney, skin, gills, intestine and bladder (de Ruiter, 1980). However, stenohaline marine fish and Euryhaline fish living in sea water have a low glomerular filtration rate and produce relatively small quantities of isosmotic urine,

while the same fish found in fresh water are more capable of producing high glomerular filtration rates and large volumes of diluted urine (Hickman and Trump, 1969) Henderson et al, 1978).

Although with the development of aqua culture there is increasing interest in fish pathology, as yet little is known concerning renal lesions in fish. However, it is known that fish are susceptible to certain kidney diseases including nephrocalcinosis, observed in trout after, sulfamerazine toxicity (Smith et al, 1973), a diet containing cotton seed (Dunbar and Herman, 1971), diet deficient in magnesium but containing high levels of calcium and phosphorus (Cowey et al, 1977) and after exposure to elevated CO_2 concentration (Smart et al, 1979). Moreover, renal fungal disease characterised by renal granulomatous reaction has been described in Atlantic salomon salmo salar by Richards et al (1980), while hepato-renal syndrome has been described in turbot by Anderson et al (1976).

In the rats, evidence derived from experiments given in Chapter 2 and 4, have shown that silicon was nephrotoxic. The lesions were focal and mainly involved the distal part of nephron. In fish, the kidney is an elongated organ and hence there is a possibility of missing any focally

distributed lesions if sampling is inadequate. Accordingly, the whole fish kidney was processed as described in the Materials and Methods to obviate this problem.

In Experiment 1, the silicon dose and the route of administration was identical to that used in rats (Chapter 2) while sampling was extensive. In fish, in contrast to the finding in rat, there was no demonstrable sign of any renal lesions after four, twenty-one and thirty-five days. From these results, a decision was made to increase the dose of silicon and to give frequent rather than a single intraperitoneal injection (Experiments 2 and 3).

In Experiment 2, where weekly intraperitoneal injections of 200 mg gasil 200, and in Experiment 3 where thrice weekly injections of 200 mg gasil 200 were used, there was no evidence of macro and microscopic renal lesions. It should be noted that a dose of 200 mg gasil 200 proved to be extremely nephrotoxic when injected into rats.

The main difference between the fresh water fish and the mammalian nephron is the length and development of the tubule. A fresh water fish does not have to concentrate urine, indeed because it lives in a hypotonic medium and it must be prepared to excrete large volumes of water via the kidney. Therefore it does not possess the well

developed distal section characteristic of land animals.

Silicon absorbed into the blood from intraperitoneal injection is therefore likely to reach the kidney where it probably passes through in the glomerular filtrate down the shorter tubule with little concentration. Thus the refractoriness of the piscine kidney to the silicon nephrotoxicity demonstrated in rats may be due to the fact that the concentration in the tubules never rises much above serum levels. Hence, if as is suspected, rising concentration of silicon favours increasing polymerisation, then elaboration of the more toxic silicon polymers does not occur in the fish.

It is possible that the gills may excrete some of the silicon load. However, since the gills are concerned mainly with monovalent ions excretion, it is unlikely that it handles silicon. On the other hand, the gut which can excrete divalent ions may be responsible for some of the silicon clearance. In order to determine the exact pathophysiology of silicon handling in fish, rather more complex investigations would be required.

SUMMARY AND CONCLUSIONS

Rainbow trout injected intraperitoneally with large doses of gasil 200 failed to produce renal damage. This may be due to the anatomical absence of the loop of Henle in the fish kidney and the lack of development of the distal nephron as compared to the rat. Thus, the concentration of urine which normally occurs in this region in the rat does not occur in fish and hence high concentrations of silicic acid may therefore not be attained in the distal nephron and collecting ducts after intraperitoneal loading with silica.

CHAPTER VIII

RADIOLOGICAL STUDIES IN EXPERIMENTAL SILICON NEPHROTOXICITY: INTRAVENOUS PYELOGRAPHY AND ANGIOGRAPHY

INTRODUCTION

In the early stages of this investigation, it was considered a possibility that the pathogenesis of silicon nephrotoxicity might in part be mediated by vascular compromise. Although the initial study did not show any evidence of arteritis or other histologically observable anomalies, this did not exclude the possibility that transient vasopasm was an addition factor involved in the pathogenesis. Therefore, an attempt was made to include microangiography in the range of techniques applied to the understanding of silicon nephrotoxicity. In a similar manner, it was considered that intravenous pyelography might furnish more information on renal perfusion in the living animal.

MATERIAL AND METHODS

X-Ray Unit

A reconditioned, portable Newton-Victor x-ray machine with a rheostat incorporated in its circuitry was used. This modification produced an emission of soft x-rays eminently suitable for obtaining high resolution images of small portions of tissue. The machine was checked for both safety and efficiency by the Department of Clinical Physics and Bio-Engineering.

Radio-opaque Media

For intravenous pyelography, Conray 480 radio opaque solution was used.

For angiography, Dobbie's modification of a recipe published by Schlesinger (1957) was used. This is composed of the following ingredients:

1 N caprylic alcohol (dispersing agent).
2 Potassium iodide (36 g).
3 Gelatin (24 g).
4 Bismuth Carbonate (40 g).

The contrast medium was prepared by adding three drops of N Caprylic alcohol to 160 ml of water. The mixture was spun in a blender for 1 minute. The other ingredients were added seriatim with 1 minute mixing after each addition.

The preparation was stored in an air-tight container at $4^{o}C$ and used whenever required. Before injection, 1 ml of 10% neutral formalin was added to 9 ml of the radio-opaque preparation. This was to harden the gelatin within 15 - 20 minutes after injection. The final average particle size was 20 μ.

Films

1 For the plain abdomen and nephrograms, "no screen films" were used. These were supplied and processed by the X-Ray Department, Glasgow Royal Infirmary.

2 For angiography, Ilford line film was used. This is a slow photographic film which gives good contrast and high resolution.

Animals

Female Sprague-Dawley rats were used as follows:

1 Eight, normal control.

2 Group of eight animals which received a single intraperitoneal injection of 100 mg gasil 200 dissolved in 2 ml 0.9% Nacl and examined after four days (acute silicon nephrotoxicity).

3 Group of eight animals which received intraperitoneal injections of 60 mg gasil 200 dissolved in 2 ml 0.9% Nacl for seven consecutive days and examined on day

seventy-eight (chronic silicon nephrotoxicity).

Instrument Setting and Procedure

The x-ray machine was operated at a KV of 40 and a rheostat setting of 180. All examinations were carried out with tube specimen distance of 25 inches.

Prior to examination, animals were anaesthetised with ether, rapidly placed on the examination table and a plain abdominal x-ray performed. A small incision on the ventral aspect of thigh was then made, the femoral vein exposed and 0.5 - 0.7 ml of Conray 480 was rapidly injected. A series of exposures were taken. Optimum exposure time was found to be 15 seconds. The animals were then left quiet to recover. The x-ray films were processed at the Out Patient X-Ray Department, Glasgow Royal Infirmary.

On the following day, the same animals were sacrificed, the inferior vena cava and aorta were exposed and a catheter was introduced into the aorta immediately above the bifurcation. The aorta and the vena cava were ligated below the catheter and above the origin of renal vessels and the kidney was perfused with 100 ml of heparinized 0.9% Nacl.

The radio-opaque media was made ready by mixing 9 ml with 1 ml 10% neutral formalin, it was injected slowly into

the catheter which was already placed in the aorta (arteriogram) or into the vena cava (venogram). The whole block of both kidneys, renal vessels, aorta and vena cava were dissected and immersed in 10% formalin for 24 hours. After 24 hours, the block was placed on the Ilford line film and x-rayed. The exposure time was long (60 - 80 seconds). The negatives were developed in D19, passed through a stop bath of 3% acetic acid and fixed with amfix. Negatives were enlarged up to X 8 with good results.

RESULTS

1 Plain Abdomen X-Ray

Although the resolution of the plain abdomen film was good, the identification of the renal masses was poor.

2 Intravenous Pyelograms

In both control and tested rats, good nephrograms were obtained with ease but the fine details of the intrarenal calyceal system were indistinct. The marked renal enlargement of acute and the contraction in chronic silicon nephrotoxicity was readily demonstrated (Figures 119, 120 & 121).

3 Angiograms

High resolution films were gained from this procedure. Fine details of renal arteries, arterioles and veins were discernible. No significant alterations, however were seen in the arterial architecture in any of the animals exposed to either the acute or chronic silicon administration as compared to the controls (Figure 122).

DISCUSSION

Radiological assessment of the urinary tract in the experimental animal was achieved using simple x-ray devices and simple procedures. Angiography added a new dimension of support to the histological evidence that significant arterial damage was not a feature of silicon nephrotoxicity. However, although it was evident that plain abdomen x-ray films showed little information concerning the size, shape and structural abnormalities of the kidney, these features were readily demonstrated after the intravenous pyelography. The nephrograms of the enlarged kidneys of acute silicon nephrotoxicity and the relatively small, globular kidneys of the chronic silicon nephrotoxicity were clearly imaged. The only disadvantage was the difficulty in showing the calyceal system because of rapid renal clearance of the radio-opaque material and small size of rat renal calyces.

Details of the intra-renal arterial and venous systems of rat kidney were successfully demonstrated. No evidence of marked vascular changes were encountered after silicon administration. As for complete exclusion of vascular spasm, these studies were insufficient to give a definite answer, but what was observed supported the histological findings of negative vascular involvement.

SUMMARY AND CONCLUSION

Radiological studies provided useful information concerning the nature of silicon nephrotoxicity, in that no significant arterial lesions were imaged.

This technique could be successfully employed in other experimental procedures where additional information on the urinary tract of the experimental animal would be helpful.

C H A P T E R IX

URINARY EXCRETION OF SILICON IN SILICATE WORKERS AND IN PATIENTS WITH ANALGESIC NEPHROPATHY

INTRODUCTION

Investigation of the absorption of silica into the blood, either by inhalation or ingestion, has in the past been difficult. The analytical chemical method was long, tedious, subject to many interferences and generally inaccurate.

Recent application of atomic absorption spectroscopy (Dobbie et al, 1976, 1978) to the measurement of silicon in body fluids has opened up new possibilities in studying the uptake and excretion of silicon in man.

It has never been adequately established whether absorption of silica through the large surface area of the lungs occurred with inhalation of high concentrations of amorphous siliceous dust or whether a much greater amount was absorbed through swallowing. Any information relative to this could contribute to our understanding of the association between acute silicosis and renal lesions. Investigation of this problem, however, is fraught with difficulities both in the technical sphere and in the design of a suitable experimental protocol. It is, therefore, not surprising that little has been done in this field.

Likewise, little has been done in the absorption of silicon from silicon-containing drugs. If silicates are of importance (Chapter 6) as a co-factor in the pathogenesis of analgesic nephropathy, then information on the absorption and excretion characteristics of silicon in these patients might be helpful.

Aims of this Study

To determine the pattern of urinary excretion of silicon in:

1 Workers exposed to inhalation of siliceous dusts.

2 Patients with analgesic nephropathy.

MATERIAL AND METHODS

The study of the urinary excretion of silicon was carried out in three different groups which included workers, patients and healthy control individuals.

1 Twenty healthy workers who were engaged in the manufacture of insulating boards in which amorphous silicate was the predominant material (94% silica and 6% cellulose fibre). The workers studied were engaged in the mixing of pure silica and diatomite and were exposed to high atmospheric levels of the siliceous dust in that it was confidentially established that they did not wear the recommended mask. All workers were individually questioned and requested not to take any medicines, especially analgesics including Askit, as well as to abstain from beer for at least 24 hours before starting this test. They were asked to collect urine samples as follows:

A Pre-work urine sample.

B Aliquot from an 8 hour urine collection during work.

C Aliquot from an 8 hour urine collection during holiday (samples were obtained from only four workers).

2 The urinary excretion in patients with analgesic nephropathy was completed by:

A Four female patients with analgesic nephropathy who were regular attenders at the Renal Clinic at Glasgow Royal Infirmary.

B Four healthy female individuals as volunteers. The patients and controls were given single oral doses of magnesium trisilicate at bedtime after the bladder was emptied. Three 24 hour urine collections were made. At the end of the third collection period, blood samples were taken for serum creatinine and creatinine clearance estimations.

All urine samples were checked for urinary silicon concentration using the atomic absorption spectroscopy.

RESULTS

1 URINARY EXCRETION OF SILICON IN WORKERS

The results are separated into four tables.

A Table 9 Shows the findings in subjects who had refrained from drinking as requested.

B Table 10 Gives the findings in the group which, despite requests to the contrary, did drink the night before the test.

C Table 11 Gives an account on urinary silicon concentration and excretion at the end of holiday period, subjects having been away from exposure to silica dust for a period of two weeks.

PRE WORK		8 HOURS WORK PERIOD		
Number	Urine Silicon Concentration µg/ml	Urine Volume ml (8h)	Urine Silicon Concentration µg/ml	8h Urine Silicon Excretion (mg)
1	5.6	240	7.4	1.78
2	5.1	180	11.8	2.12
3	4.8	210	11.7	2.46
4	9.9	250	10.4	2.60
5	6.2	229	10.7	2.45
6	1.0	800	1.9	1.52
7	4.6	515	5.0	2.58
8	5.3	275	7.9	2.17
9	2.7	225	5.1	1.15
	$\bar{X}$ = 5.02		$\bar{X}$ = 7.98	$\bar{X}$ = 2.09
	SD ± = 2.43		SD ± = 3.46	SD ± = 0.509
	Range = 1.0-9.9		Range = 1.9-11.8	Range = 1.15-2.60

Table 9: Showing silicon urinary excretion during 8 hours exposure to industrial silica atmosphere in subjects who did not drink beer prior to test period.

PRE WORK		8 HOURS WORK PERIOD		
Number	Urine Silicon Concentration ug/ml	Urine Volume ml (8h)	Urine Silicon Concentration ug/ml	8h Urine Silicon Excretion (mg)
1	11.2	450	9.4	4.23
2	12.8	220	11.8	2.60
3	13.6	200	10.7	2.14
4	18.8	240	13.3	3.19
5	8.4	325	7.7	2.50
6	24.3	400	24.2	9.68
7	15.8	*	11.2	*
8	32.4	370	16.6	6.14
9	9.4	*	8.8	*
10	43	275	20	5.50
11	28	560	15.2	8.51
	$\bar{X}$ = 19.79 SD $\pm$ = 10.95 Range = 8.4-43		$\bar{X}$ = 13.53 SD $\pm$ = 5.07 Range = 7.7-20	$\bar{X}$ = 4.94 SD $\pm$ = 2.73 Range = 2.14-9.68

* Urine volumes have not been recorded

Table 10: Showing raised silicon urinary excretion due to increase consumption of beer with high silica content. Levels tended to be lower during the 8 hours work period when drinking was not permitted.

Number	8h Work Silicon Concentration $\mu g/ml$	8h Work Silicon Excretion (mg)	8h Holiday Silicon Concentration $\mu g/ml$	8h Holiday Silicon Excretion (mg)
1	7.9	2.17	12.8	3.90
2	5.0	2.58	3.0	2.53
3	20.0	5.50	6.8	4.93
4	1.0	1.9	9.0	10.98

Table 11: Showing the urinary excretion of silicon during work compared with excretion during holiday period. Subject 1 and 4 having drunk large amounts of beer during holiday.

2 URINARY EXCRETION OF SILICON IN PATIENTS WITH ANALGESIC NEPHROPATHY AND CONTROLS AFTER THE INGESTION OF SINGLE ORAL DOSE OF 5 g MAGNESIUM TRISILICATE

A Patients with Analgesic Nephropathy - all of whom had Reduced Creatinine Clearance.

Number	24 Hours Urinary Excretion of Silicon (mg)			Total 3 Days Urinary Excretion of Silicon	Creatinine Clearance
	Day 1	Day 2	Day 3		
1	7.70	41.10	8.40	57.20	48
2	5.38	44.24	14.04	63.66	56
3	5.55	74.7	9.49	89.74	86
4	4.67	25.92	10.84	41.43	86

Table 12: Showing silicon urinary excretion of silicon for three consecutive days after single oral dose of 5 g of magnesium trisilicate to a group of patients with analgesic nephropathy and reduced creatinine clearance.

B Healthy Female Controls

Number	24 Hours Urinary Excretion of Silicon (mg)			Total 3 Day Urinary Excretion ofSilica
	Day 1	Day 2	Day 3	
1	10.9	93.0	18.0	121.9
2	2.2	83.2	9.7	95.7
3	7.2	118.0	16.7	141.9
4	6.4	150.0	70.3	226.7

Table 13: Showing silicon urinary excretion for three consecutive days after the ingestion of single dose of 5 g of magnesium trisilicate in normal healthy females.

DISCUSSION

Jolles and Neurath (1898) first introduced the colorimetric method for the estimation of silicon in soil waters. This method was used by King et al (1938 and 1955) to demonstrate silicon content of urine, blood, liver, lung and kidney tissue. A serious disadvantage of this technique was the inaccuracies caused by interference due to several elements such as phosphorus and iron (Jankowiak and Le Vier, 1971). It was recognised that the chemical estimation of silicon always gave higher results due to the difficulty in preventing phosphorus interference especially when silicon levels are very low. However, these problems encouraged workers in the Department of Medicine, Glasgow Royal Infirmary to measure silicon in biological fluids using the atomic absorption spectroscopy. This method has been applied successfully in the measurement of urinary excretion of silicon in wide range of normal and disease states and proved to be simple, rapid and free from the interferences inherent in the chemical method (Dobbie et al, 1976, 1977, 1978).

It was hoped that this study might have contributed important information on the urinary excretion of silicon in workers exposed to atmospheric levels of amorphous silica either by inhalation or swallowing. Unfortunately, the co-operation of the work force, although, all were keen and interested in the results, was unsatisfactory and resulted in the cessation of other investigations designed to determine the relative amounts of silicon excretion by

different routes (Balance Study). However, Table 9 shows a group of nine workers who had a mean pre-work urinary silicon concentration of 5.02 µg/ml. The mean urinary concentration of silicon in the 8 hours work period for the nine workers was 7.98 ug/ml, while the mean total amount of silicon excreted during 8h work period was 2.09 mg. Although the urinary silicon concentrations in the 8h work samples were higher than the pre-work samples, the concentrations were lower than that found in controls ($\bar{X}$ = 7.75 µg/ml, SD $\pm$ = 3.54) by Dobbie et al (1976). For Table 10 the pre-work urinary silicon concentrations were $\bar{X}$ = 19.79 µg/ml, SD $\pm$=10.9 and a range of 8.4 - 43 ug/ml. The 8h work urinary silicon concentrations were $\bar{X}$ = 13.53 µg/ml, SD $\pm$ = 5.07 and a range of 7.7 - 20 ug/ml, while the 8h work silicon excretion was $\bar{X}$ = 4.94 mg/8h, SD $\pm$ = 2.73 and a range of 2.14 - 9.6 mg/8h. From the behaviour of these values, it would appear that the raised pre-work urinary silicon concentrations in some of the subjects were due to the intake of silicate on the night before starting the test. After questioning this was thought to be due to beer. These high pre-work urinary silicon concentrations decreased during the work period when beer was not drunk. It is known that beer contains high concentrations of silicon. Estimation of silicon in certain beverages yielded the following concentrations: Lager 19.0 µg/ml, Beer 22 ug/ml, Guinness 52 µg/ml, and Whisky 1.5 µg/ml. Table 11 presenting the urinary silicon concentrations and excretion during holiday, which, when compared with 8h work period, it seemed more relevant that

in cases 1, 3 and 4 the increased concentrations and excretions were due to the habitual drink, while case 2 assumed normal pattern. Nevertheless, this excercise has highlighted a hitherto unrecognised factor in the design of such enquiries.

In the second series of this study, the urinary silicon excretion was performed in four female patients with analgesic nephropathy, and four healthy female controls for three consecutive days after the ingestion of single 5 g of magnesium trisilicate. Eighteen patients agreed to take part in this study, but only four satisfactorily completed the protocol. Again, lack of co-operation characterised this study, no doubt due to the underlying psychology of analgesic abusers.

In this study, although the number is small (Tables 12 & 13), it was still shown that the excretion of silica was greater on the second day in both controls and patients - being lower in patients. The total amount of silicon excreted via urine/3 days was found to be lower in patients when compared with controls. The decreased level of silicon excretion could be correlated with the patients' lower creatinine clearance. In one case (No. 4), it seems that the patient did not take the total dose of 5 g magnesium trisilicate, and her urinary silicon concentration and excretion were low.

Similar studies have been previously performed (in healthy controls) by Page et al (1941) using the chemical analysis

for urinary silicon determination, and Dobbie et al (1976) using the atomic absorption spectroscopy. It was concluded by Page et al (1941) that the mean 24 hour excretion of silica in normal individuals was found to be 16.2 mg/24 hours, while the daily oral ingestion of 5 g magnesium trisilicate over a four day period resulted in considerable elevation of 24 hours excretion of silica* (range 118 - 230 MG Sio_2/24 hours). Dobbie et al found a significant rise in the urinary excretion of elemental silicon (3 - 38 fold increase) in a group of fourteen young adults after the ingestion of 5 g magnesium trisilicate.

* Silica = Silicon dioxide.

SUMMARY AND CONCLUSIONS

The urinary excretion of silicon has been investigated in a group of workers who were exposed during working hours to silica dust. Meaningful interpretation of this study was frustrated by the drinking by some of the workers of large amounts of silica - rich beer.

This investigation also included a study of the urinary excretion of silicon in four patients with analgesic nephropathy. When tested with a single oral dose of 5 g magnesium trisilicate, they demonstrated a lower level of urinary silicon excretion than the controls which correlated with their reduced creatinine clearances. No other conclusions were possible.

C H A P T E R   X

GENERAL DISCUSSION

In many respects silicon in a biological context is a frustrating and yet tantalisingly fascinating element. Scattered throughout all of the scientific literature are many anecdotal and often unrelated accounts of studies on the effect or on the role of silicon in biological systems. Only the study on the pathogenetic mechanism of cyrstalline silica in silicosis offers a satisfying and complete account of one quest which has been followed to a conclusion.

It could be claimed that this study has focused on one aspect of silicon in biology more intently than any previous studies. The work of Dobbie on the central role of the kidney in clearing from the body of excess dietary absorbed silicon considered in relation to the previous studies on silicon nephrotoxicity automatically suggested a possible area of fruitful enquiry.

This investigation, in contrast to previous studies, has not simply been a toxicological study on the effect of silicon compounds on the kidney of the experimental animal in which a light microscopic assessment of histological changes in the kidney was the main tool. Rather, it applied many investigative procedures to both the acute and chronic effect of exposure to silicon compounds. The choice of intraperitoneal injection of a stable amorphous silica

ensured a reliable and measurable mode of exposing the kidney to high levels of circulating silicon. The application of the atomic absorption spectroscopy to the measurement of silicon in blood and tissues provided pertinent data on the distribution of silicon in the experimental model not attempted in previous studies. Similarly, sequential morphological assessment by light and electron microscopy has provided information on silicon nephrotoxicity in greater depth than in any of the previous studies.

This study has shown that circulating silicon at levels 50 times normal results in renal damage and that the brunt of the insult falls on the distal nephron. All modes of enquiry (electron probe microanalysis, autoradiography and conversely fish studies) suggest that the concentration of urine in this part of the mammalian nephron results in the exposure of the cell membrane to high concentrations of silicon which may be polymerized. Extensive electron microscopic examinations indicated that cell damage probably resulted from alteration in the permeability of the plasmalemma, particularly in the distal tubules.

In effect, the main conclusion of this investigation was the demonstration that soluble silica is toxic and that its effect was most probably due to damage to cell membrane.

This is an extension or a corollary, to the conclusions of the extensive work carried out in the cytoxicity of crystalline silica during the era of silicosis research. As has been variously stressed in the discussions in this thesis, man is increasingly exposed to natural and synthetic silicates by inhalation and in manufactured food (Neal et al, 1969, Sinclair and Ham, 1971 and Kacprzak, 1977). Thus the significance of this work is most relevant to the stimulation of an awareness of the potential hazards of entry of soluble silicon into body systems.

The investigation of silicon as a co-factor in the pathogenesis of analgesic nephropathy introduces a novel idea into this difficult area of renal disease. The findings suggest that silicates could indeed fit the role of the missing factor so long sought for in this condition.

In uraemic patients there is a hypersilicaemia; the ambient serum silicon levels depend on the amount of silica in the water supply and thus there is a geographical variation in serum silicon in haemodialysed patients. In some regions, this may be up to ten times normal, (Dobbie et al, 1981). The information gained from the present series of investigations is of particular significance to any study of the possible effects of

hypersilicaemia in these patients. Although the kidneys in such patients are no longer of clinical relevance, the effect on other organs of high levels of silicon must cause concern. The finding of hepatic granulomata in some of the animals may be important in this respect.

Thus, the application of the findings of this study to the wider aspect of the human biology and medicine pivot round the cytotoxicity of soluble silicon. By virtue of its essential role in clearing dietary absorbed silica, the mammalian kidney is vulnerable to excessive silicon loading. Nevertheless, the continuous and accelerating development of silicon products in virtually every sphere of human activity, the advent of new forms of treatment which result in abnormal accumulation of trace elements including silicon, all demand a careful reappraisal of the potential biological hazards of soluble silicon as has been attempted in this thesis.

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Figure 1 : Gasil 23 D: 4 Days: Corticomedullary Region:

All parts of the tubules and collecting duct system show degenerative changes with dilatation most prominent in the distal tubules and collecting ducts.

H & E Mag. X 345.

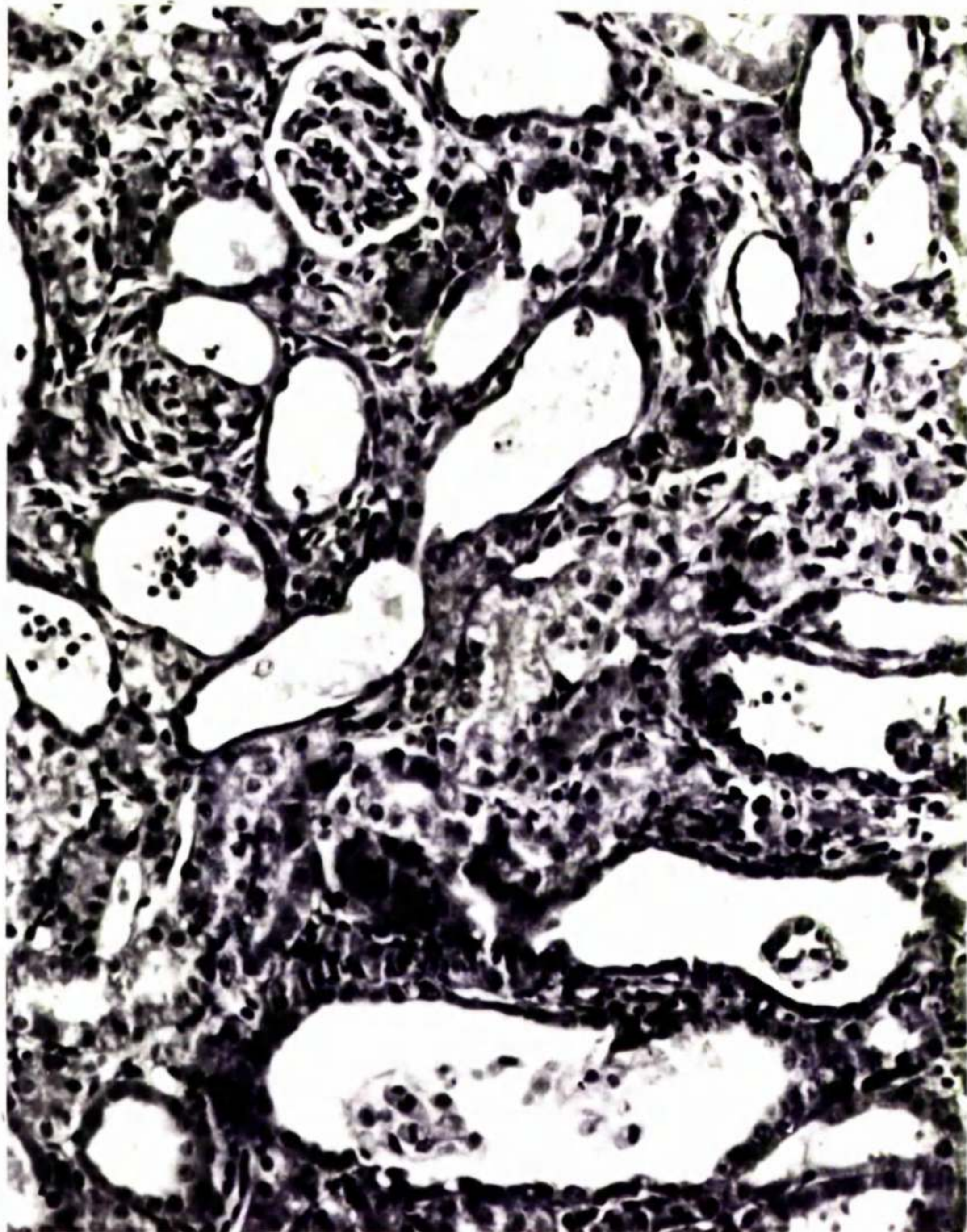


Figure 2 : Gasil 23 D: 4 Days: Cortex

Showing dilatation of the proximal tubules with vacuolation in the epithelial cytoplasm and variation in nuclear morphology. The distal tubules in the centre of the field show markedly flattened epithelium and similar degenerative nuclear changes. The glomerulus is normal.

H & E Mag. X 860.

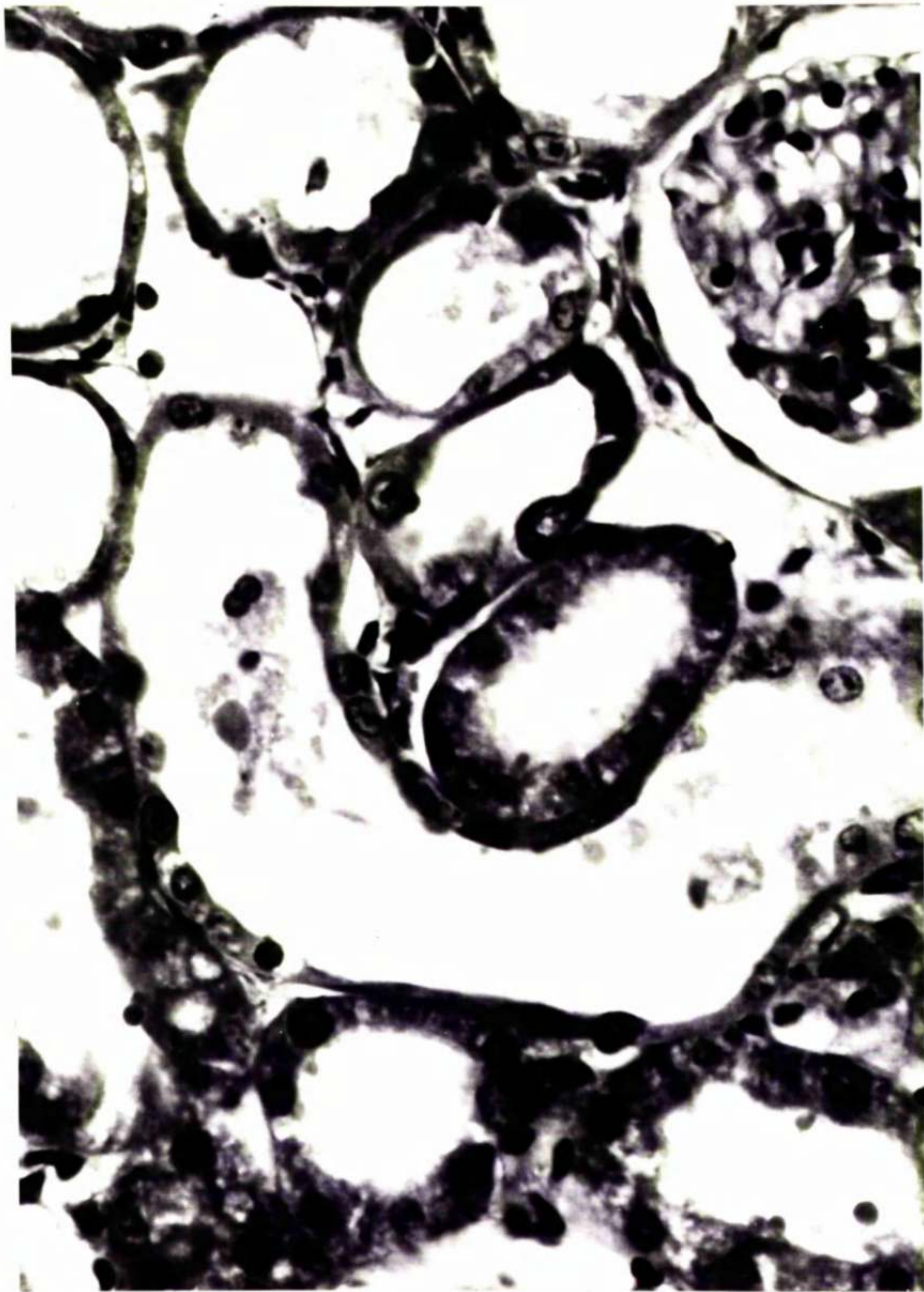


Figure 3 : Gasil 23 D: 4 Days: Medulla

There are marked changes in the collecting ducts which are dilated, contain plugs of desquamated epithelium and acute inflammatory cells. The epithelial lining exhibits a variety of cytoplasmic and nuclear degenerative changes. Similar but lesser damage is noted in the thick and thin loops of Henle, while the interstitium is infiltrated with inflammatory cells.

H & E Mag. X 345.

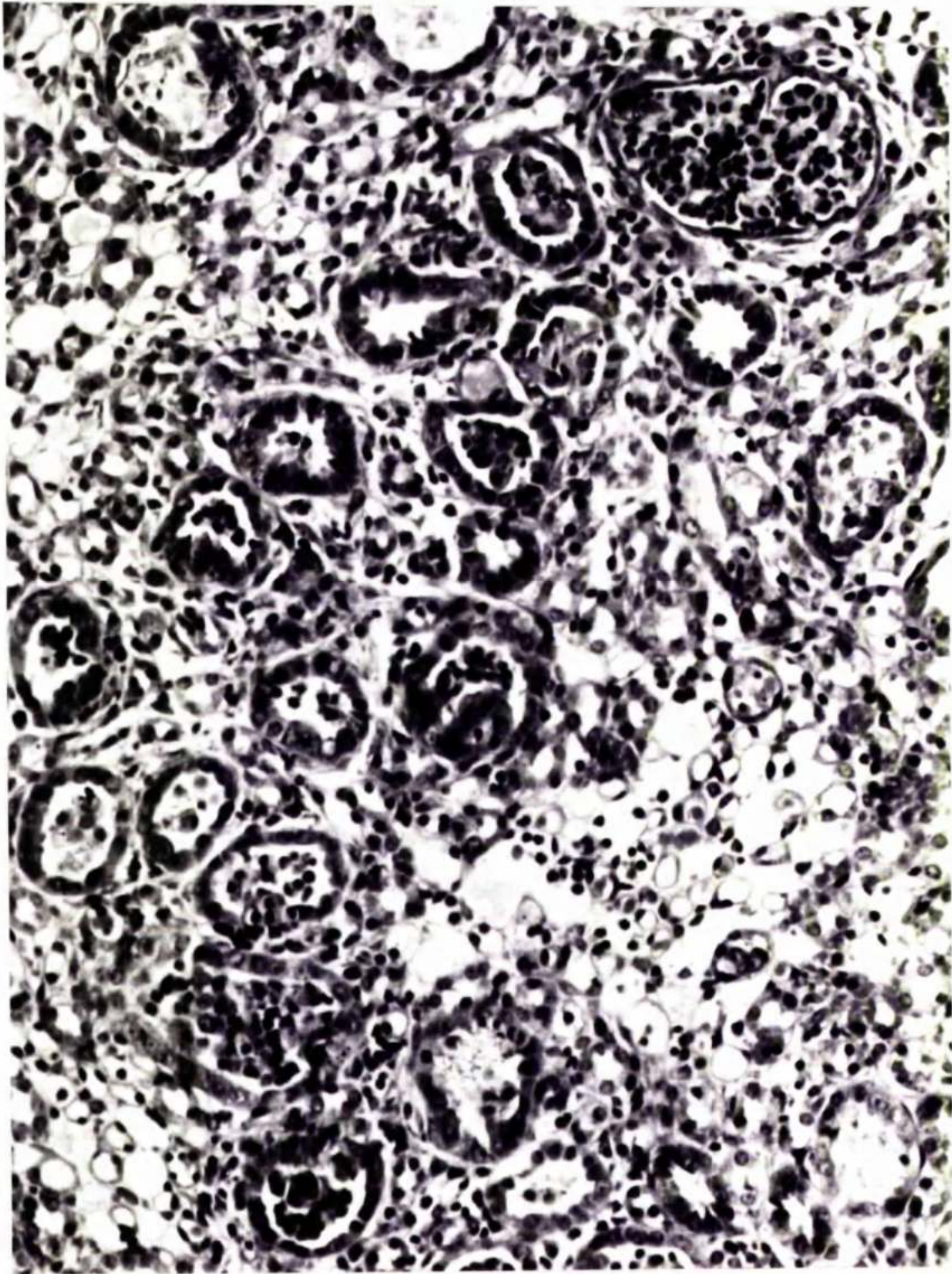
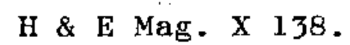


Figure 4 : Gasil 23 D: 21 Days: Cortex:
This Figure shows focal areas of marked interstitial infiltration and fibrosis, tubular loss and dilatation.



H & E Mag. X 138.

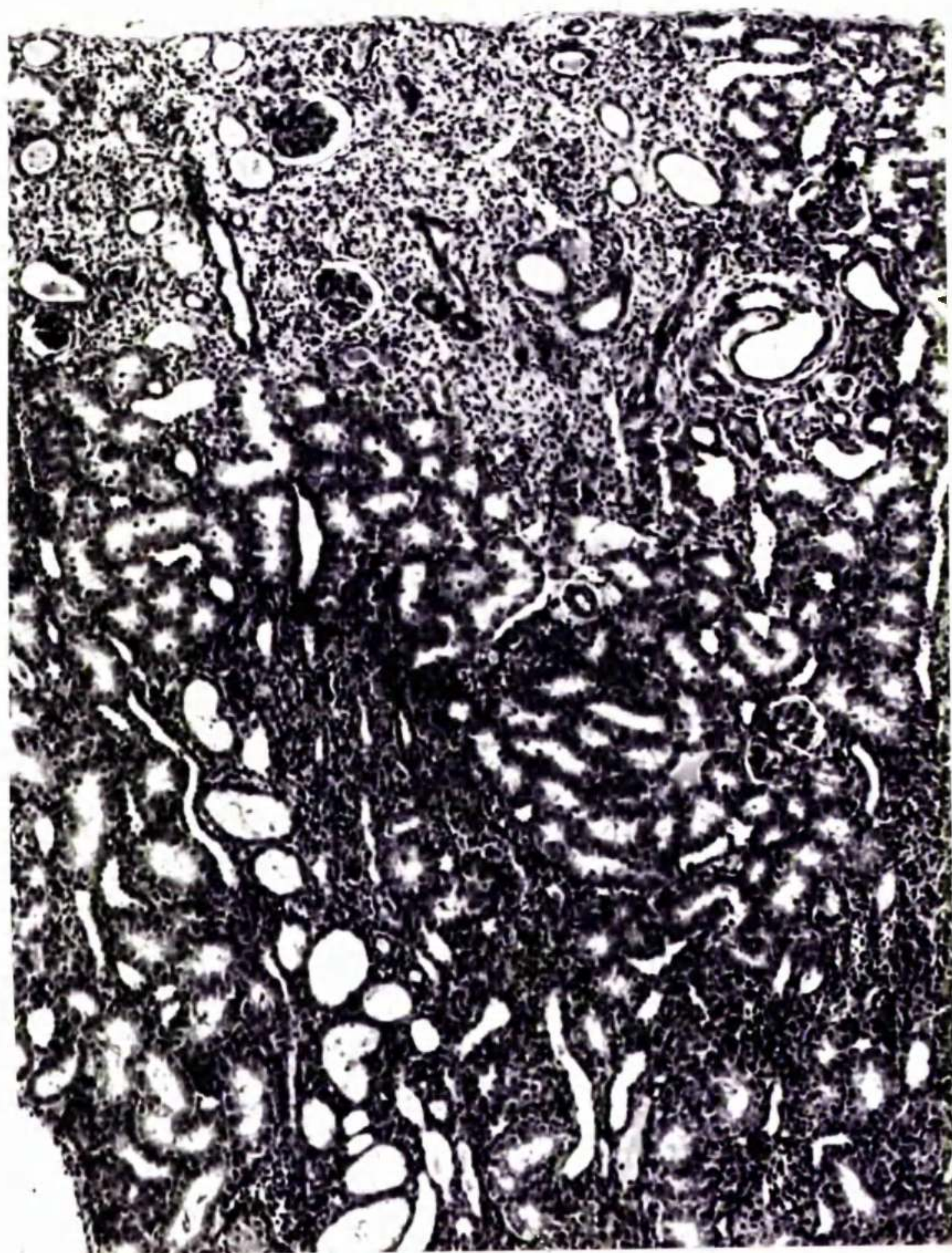


Figure 5 : Gasil 23 D: 21 Days: Subcapsular Region

Showing dilated tubules, some filled with granular debris, peritubular fibrosis and interstitial inflammatory infiltrate.

H & E Mag. X 345.

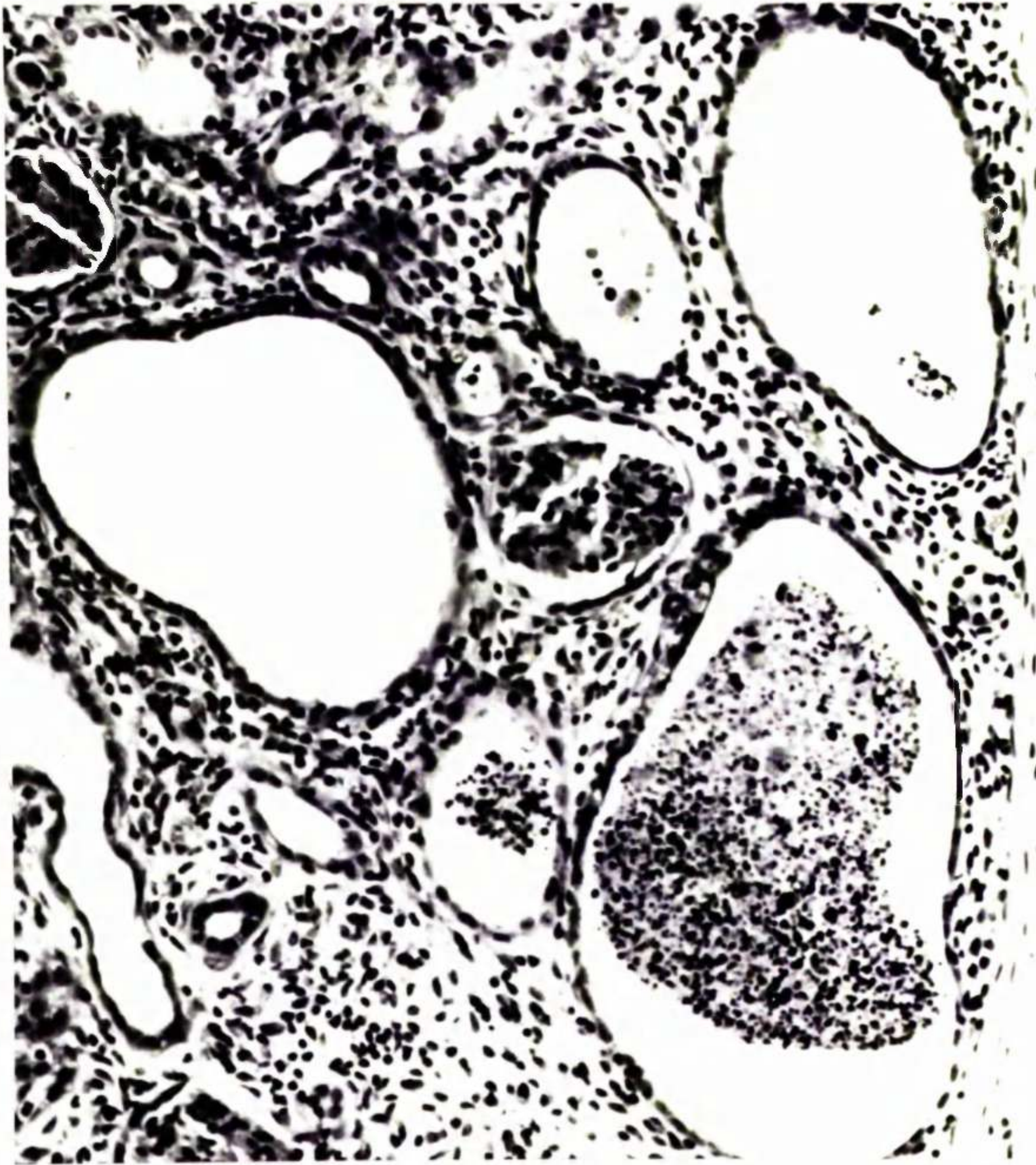


Figure 6 : Gasil 23 D: 21 Days: Subcapsular Region

Subcapsular cortex showing a cyst derived from a damaged tubule with concentrically layered fibrous tissue surrounding it. A deposit of unidentified material lies in the left side of the cyst wall with a reactive inflammatory infiltrate around it.

H & E Mag. X 435.

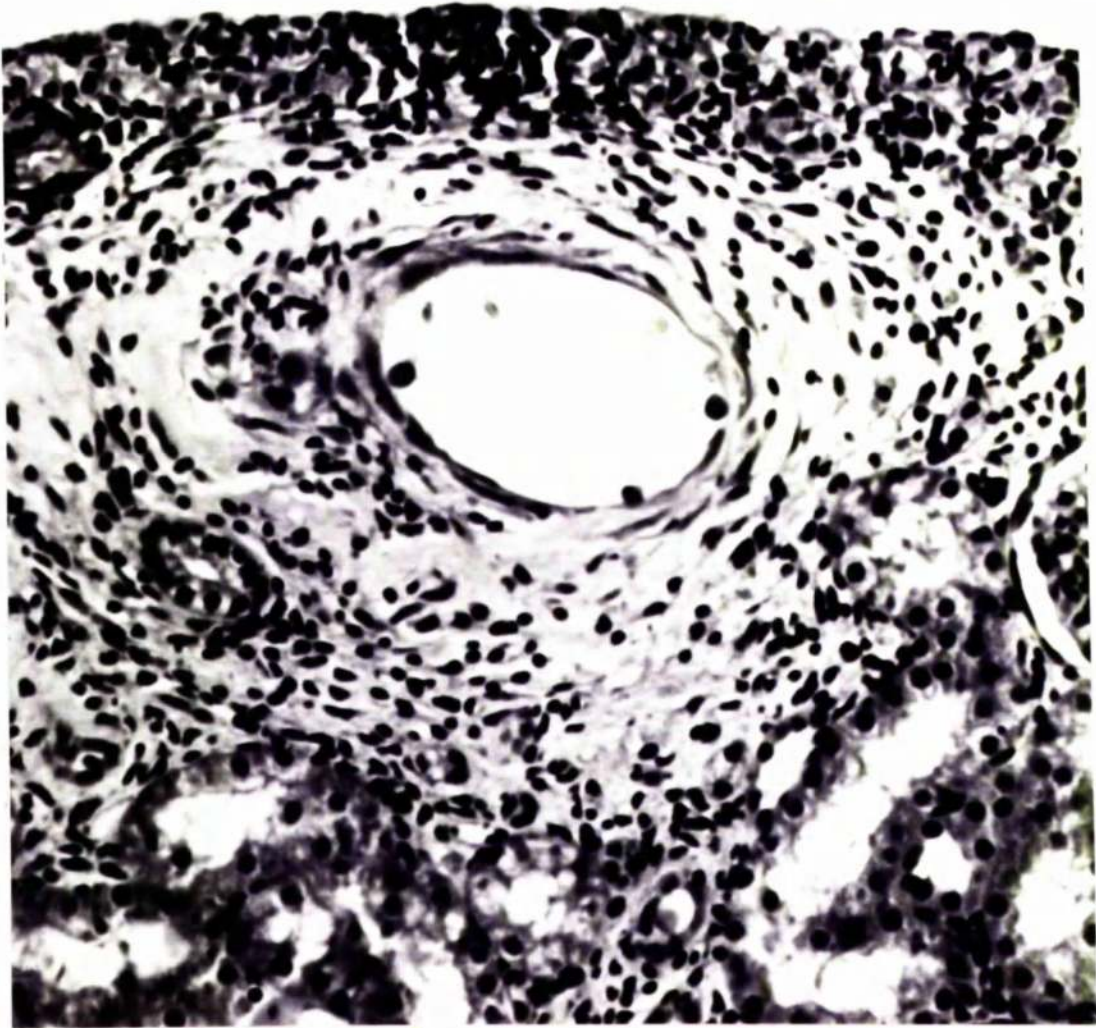


Figure 7 : Gasil 23 D: 21 Days: Medulla

Giant cell formation close to a collecting duct. Large collection of desquamated cells in lumen, while the lining epithelium shows evidence of damage.

H & E Mag. X 345.

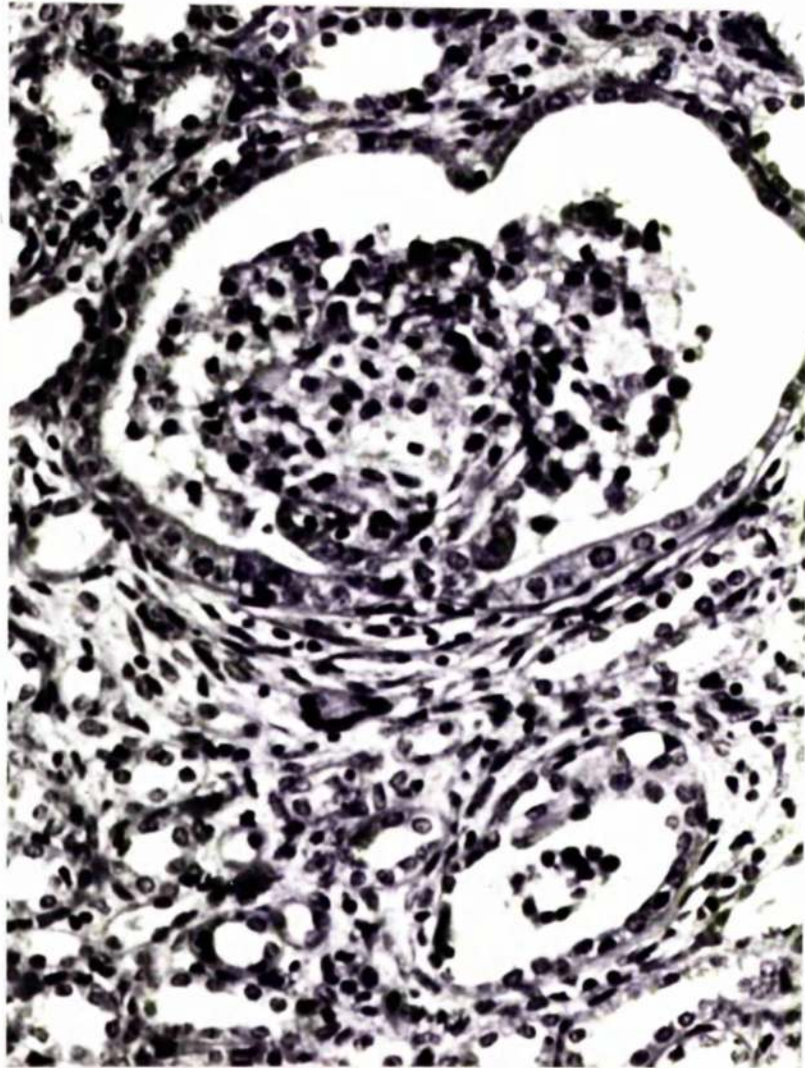


Figure 8 : Gasil 23 D: 21 Days: Collecting Ducts

Collecting duct system still showing epithelial cell changes, particularly variation in nuclear morphology, and cellular casts. Chronic inflammatory cellular infiltrate in interstitium with some fibrosis.

H & E Mag. X 860.

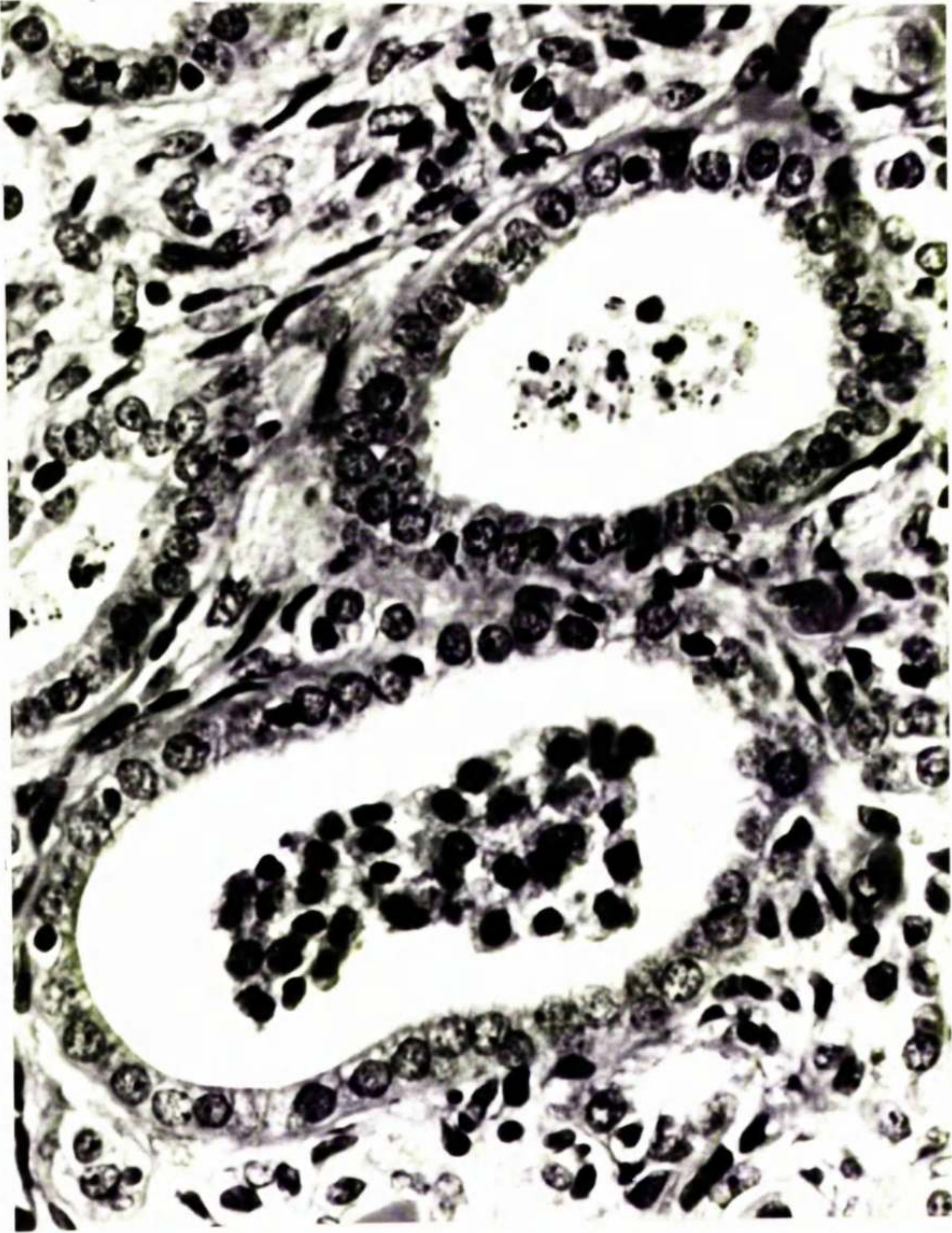


Figure 9 : Gasil 200: 4 Days: Cortex

Difuse tubular necrosis.

H & E Mag. X 345.

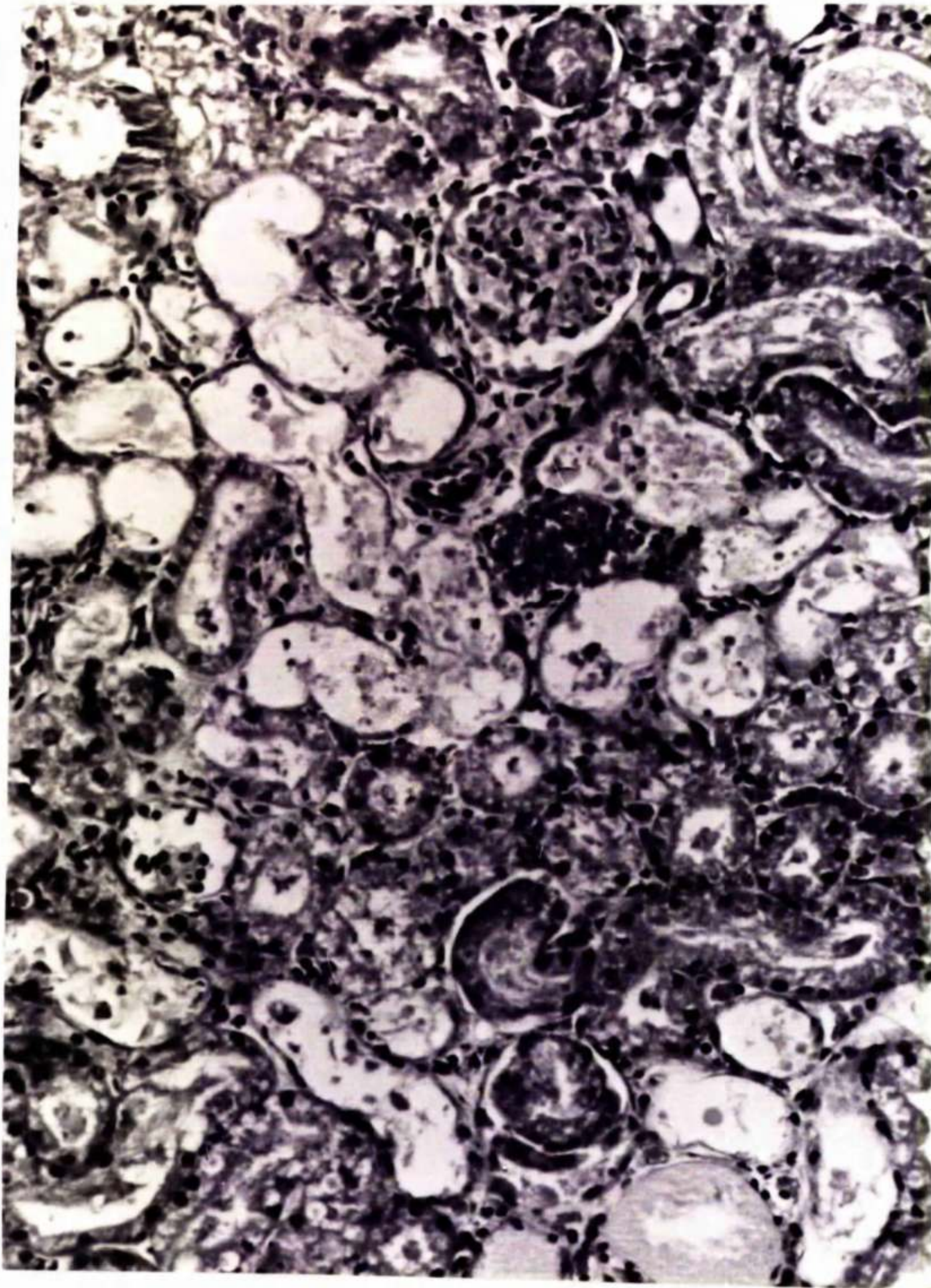


Figure 10 : Gasil 200: 4 Days: Cortex

Wide variation in degree of damage to tubules with necrosis and focal loss of lining epithelium.

H & E Mag. X 860.

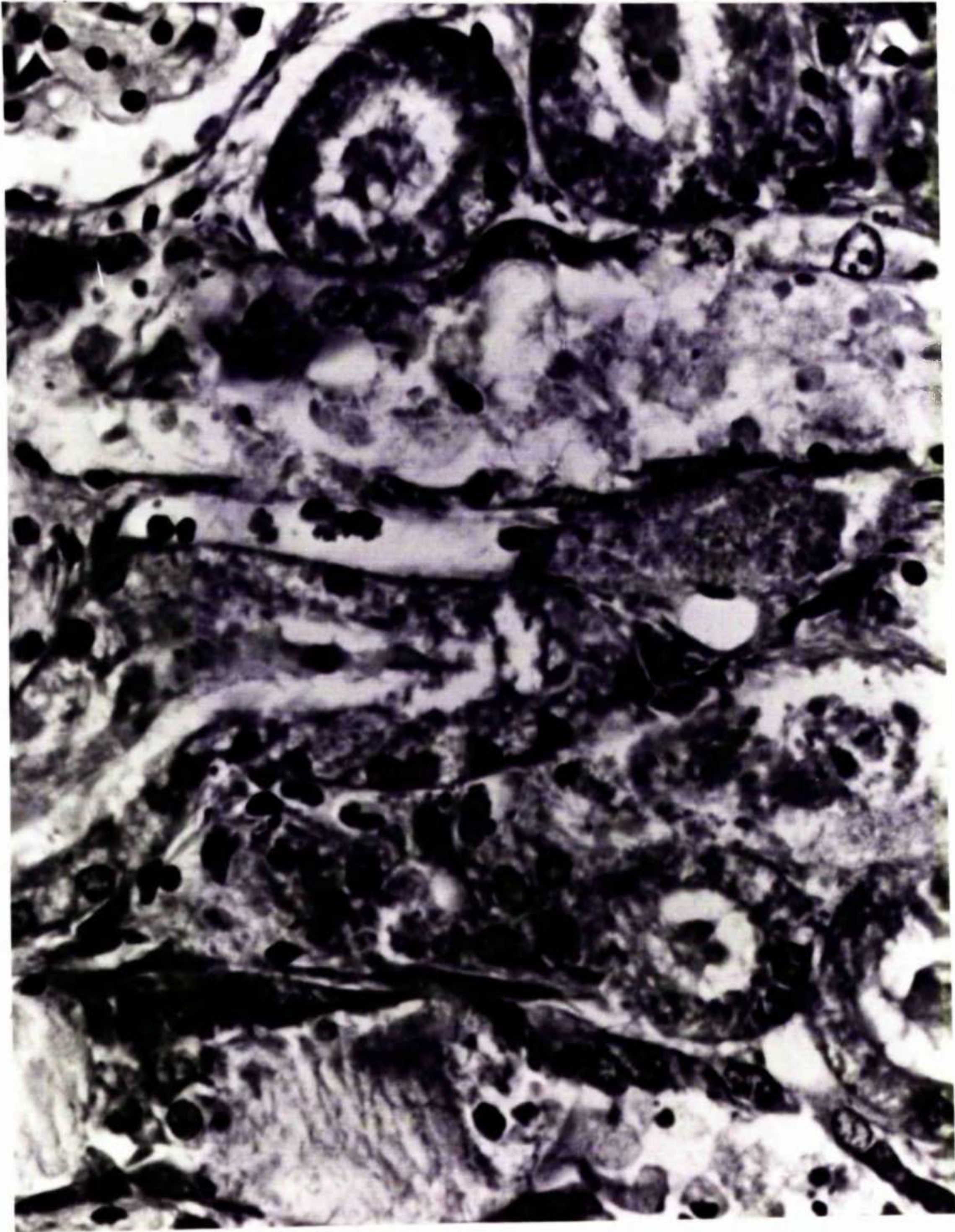


Figure 11 : Gasil 200: 4 Days: Corticomedullary Region

Gradation of damage to tubules through to necrosis and focal tubulorrhexis. A normal arteriole is present in this section.

H & E Mag. X 860.

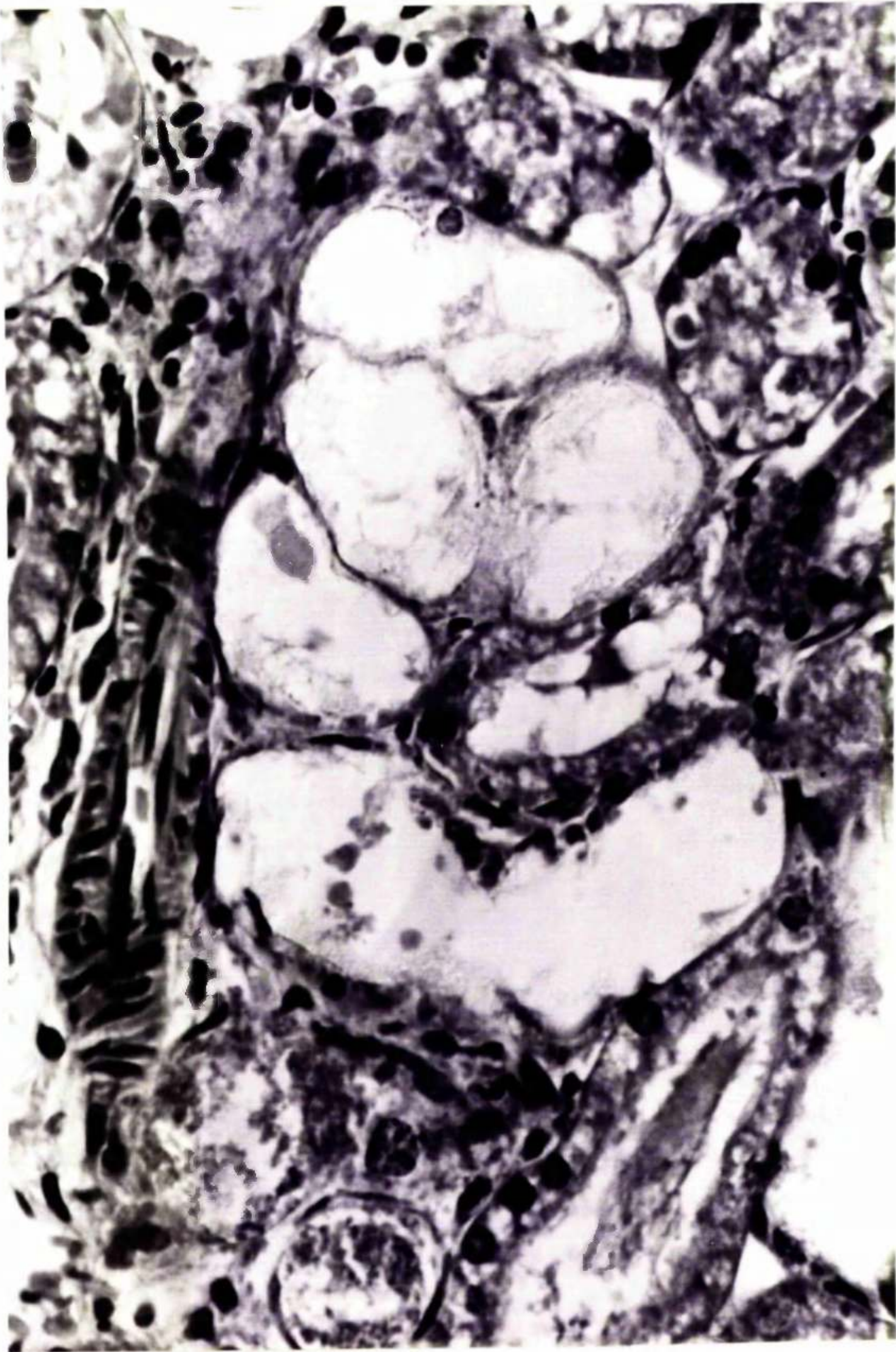


Figure 12 : Gasil 200: 4 Days: Medulla

Peritubular cuffing with acute inflammatory cells. Lumena filled with desquamated epithelial, acute inflammatory cells, and amorphous material.

H & W Mag. X 138C

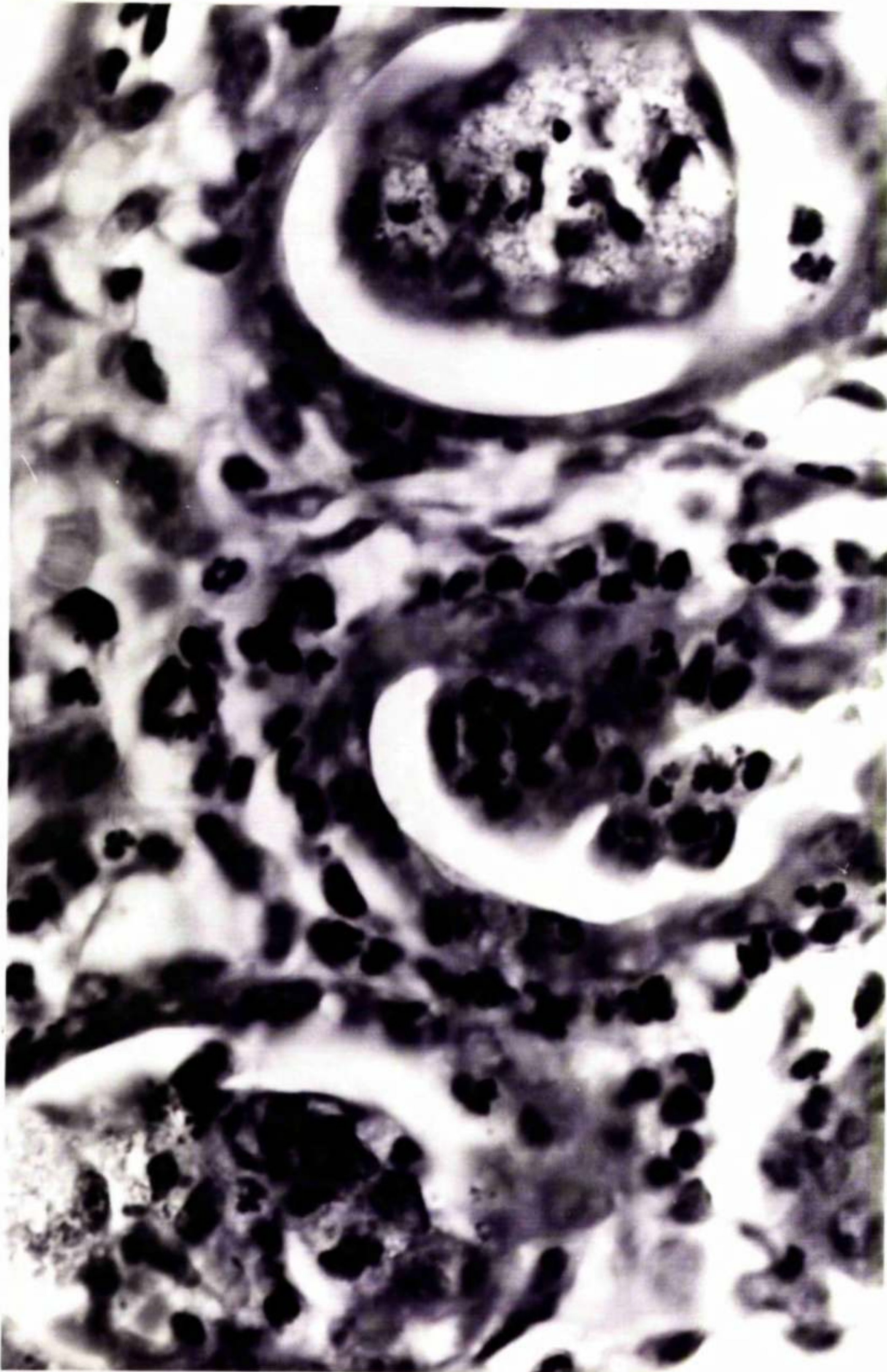


Figure 13 : Gasil 200: 4 Days: Renal Papillae

Variable cellular damage shown in collecting ducts which are both filled and surrounded by acute inflammatory cells. The overlying transitional epithelium shows evidence of cellular disorganisation.

H & E Mag. X 860.

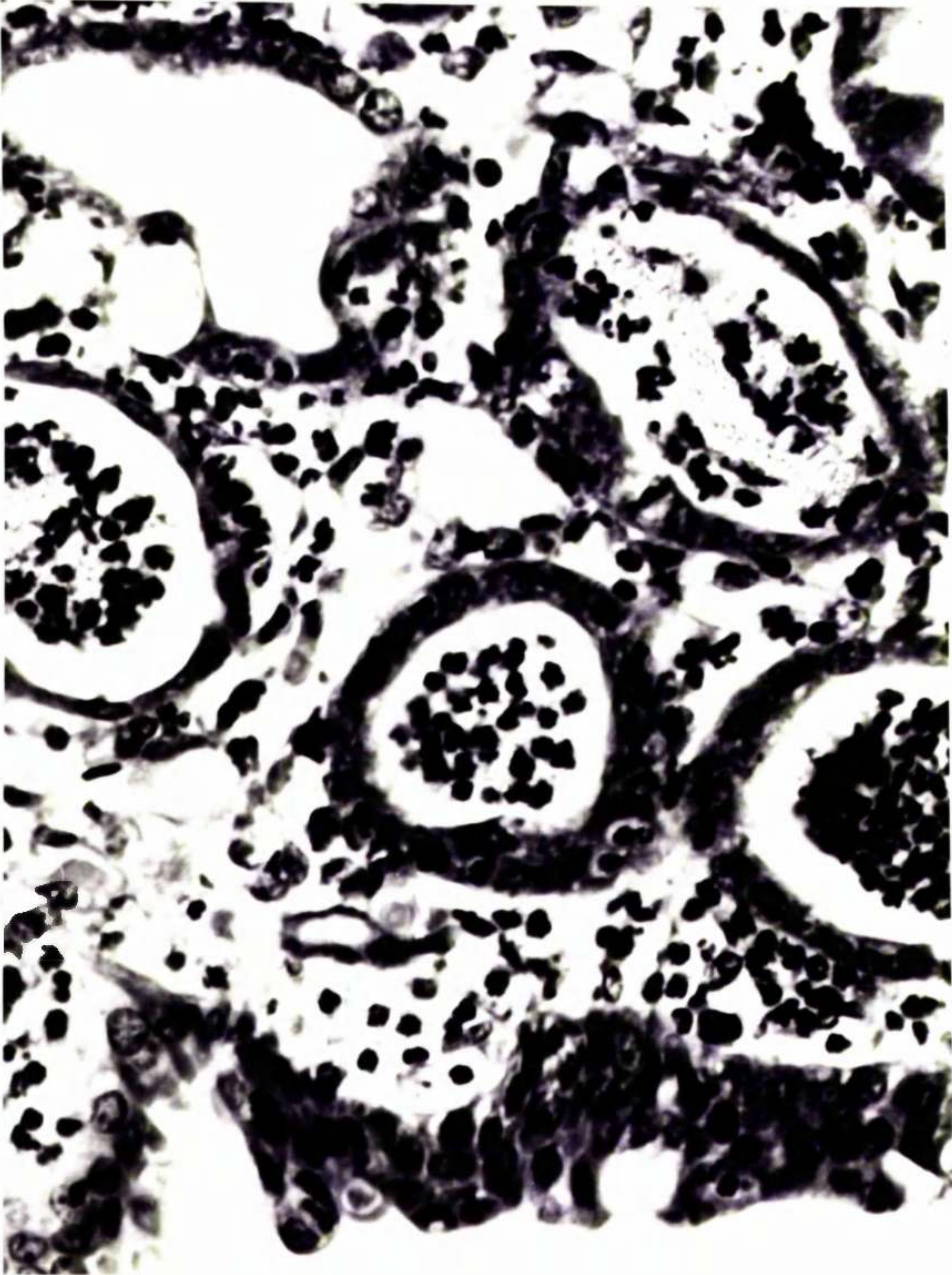


Figure 14 : Gasil 200: 4 Days: Medulla

Dilatation of collecting ducts exhibiting periductal cuffing and plugging with inflammatory cells.

H & E Mag. X 138.

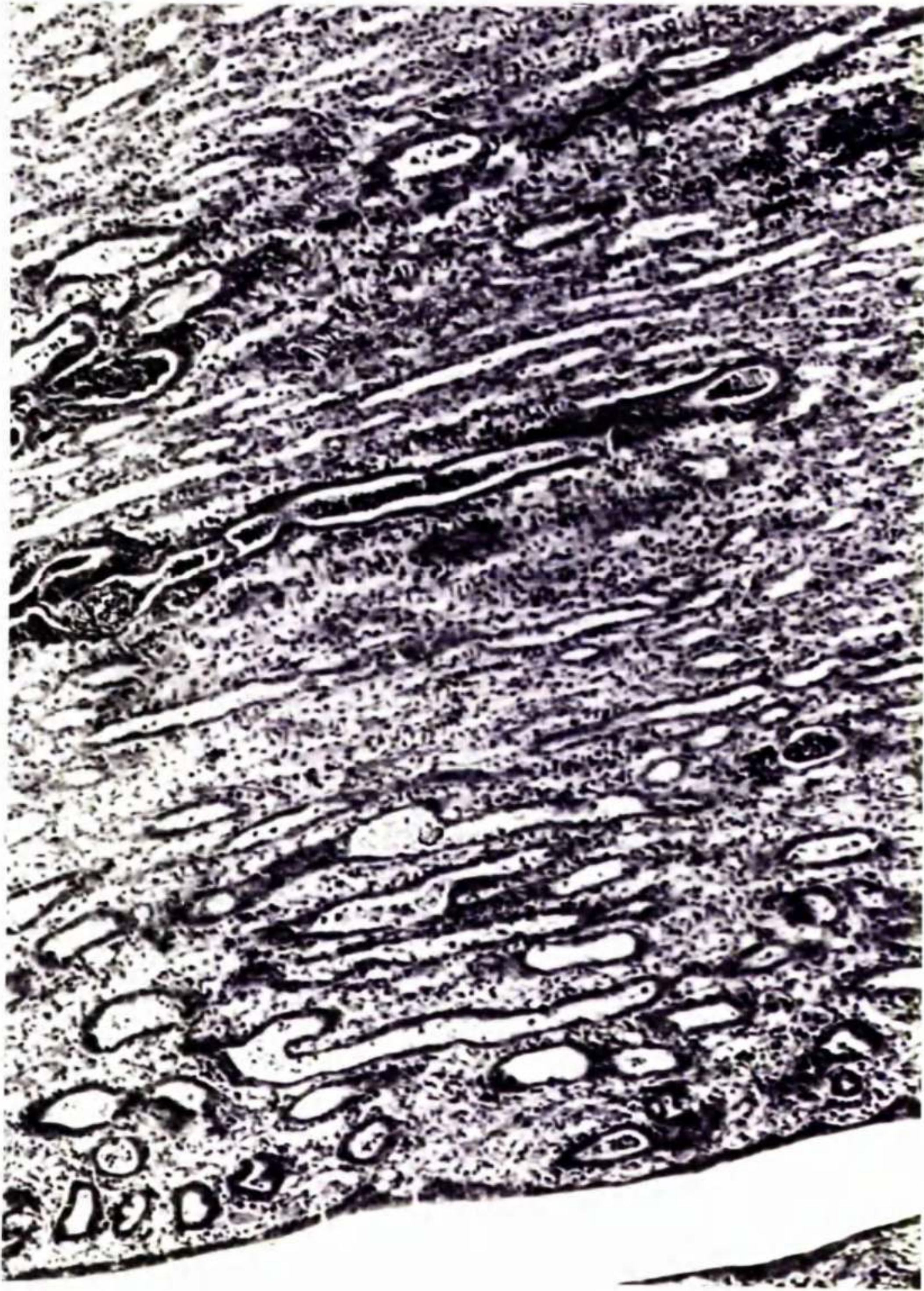


Figure 15 : Gasil 200: 4 Days: Medulla

Collecting ducts showing a wide variety of cellular degenerative changes.

H & E Mag. X 860.

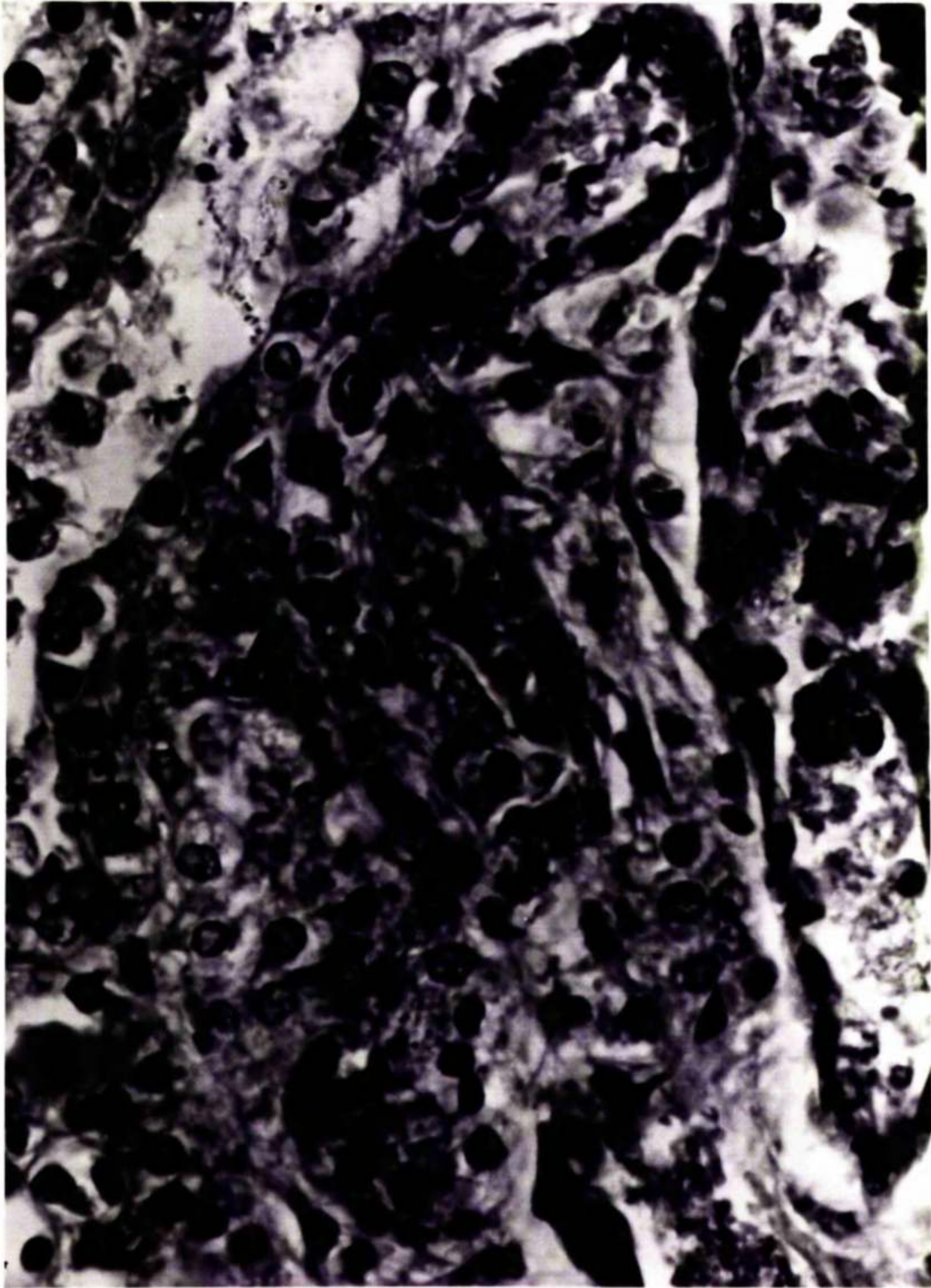


Figure 16 : Silica gel: 4 Days: Cortex

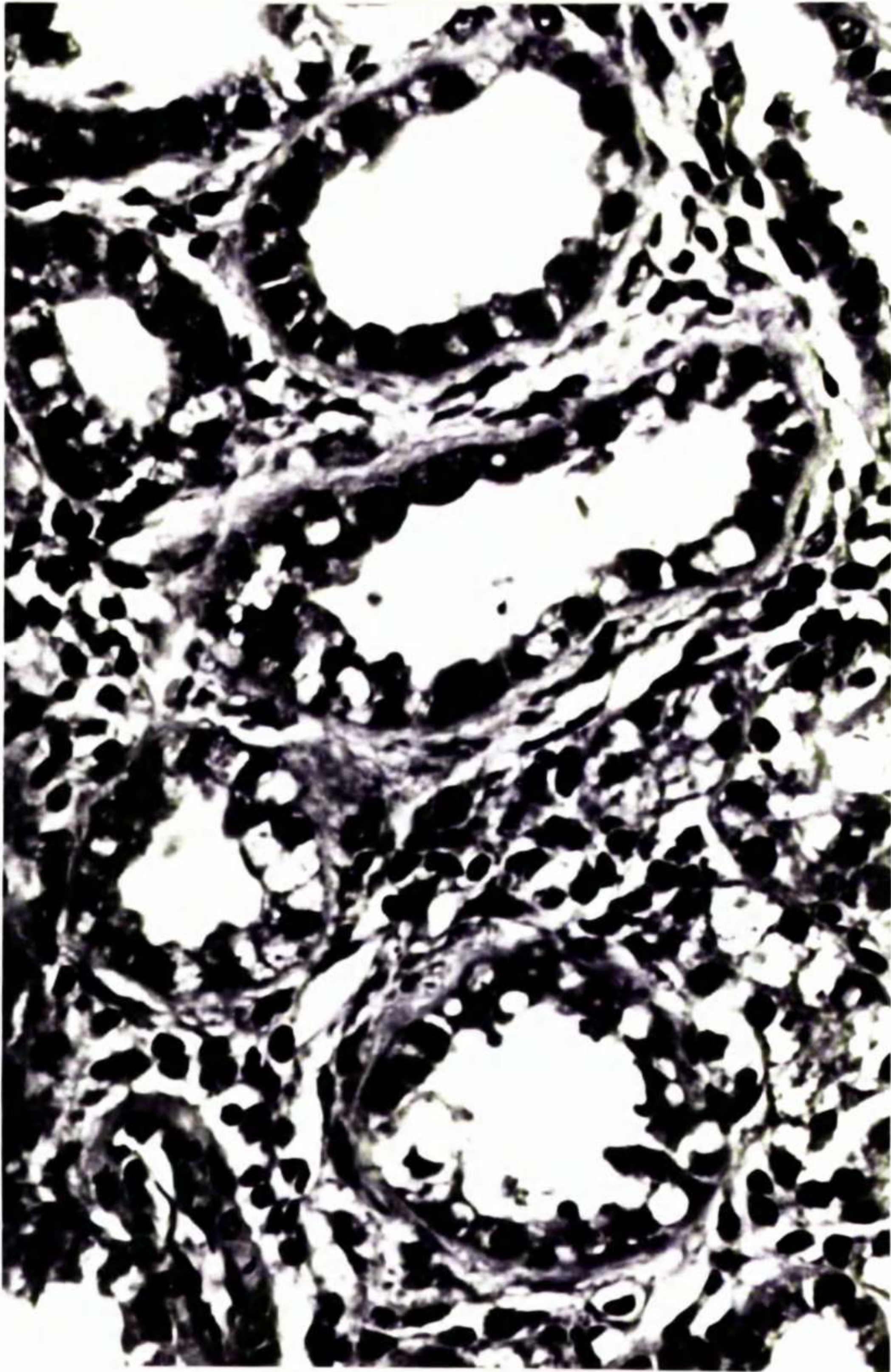
Widespread tubular necrosis.

H & E Mag. X 345.

Figure 17 : Silica gel: 4 Days: Collecting Ducts

A series of collecting ducts with significant epithelial cell damage and vacuolation.

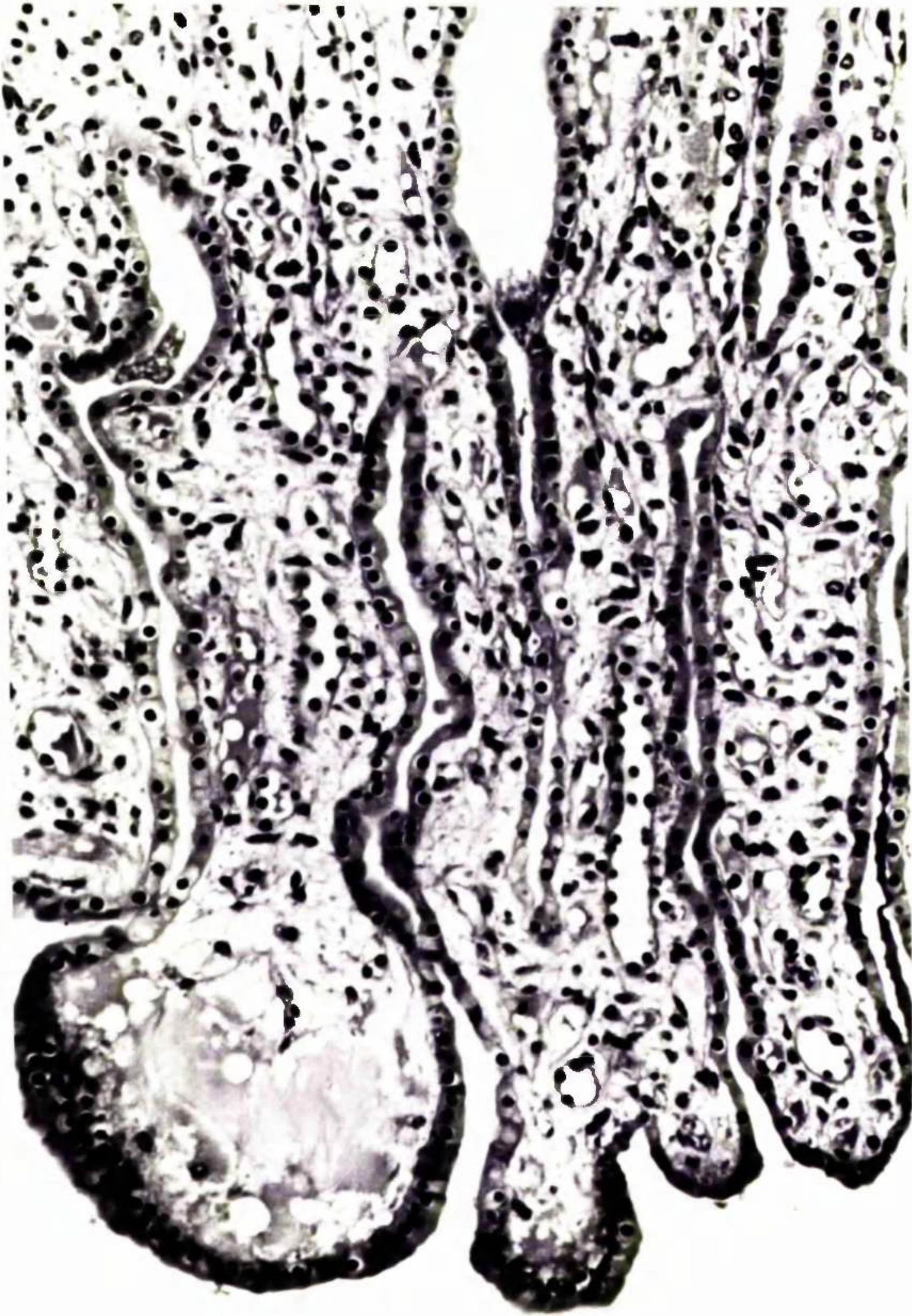
H & E Mag. X 860.

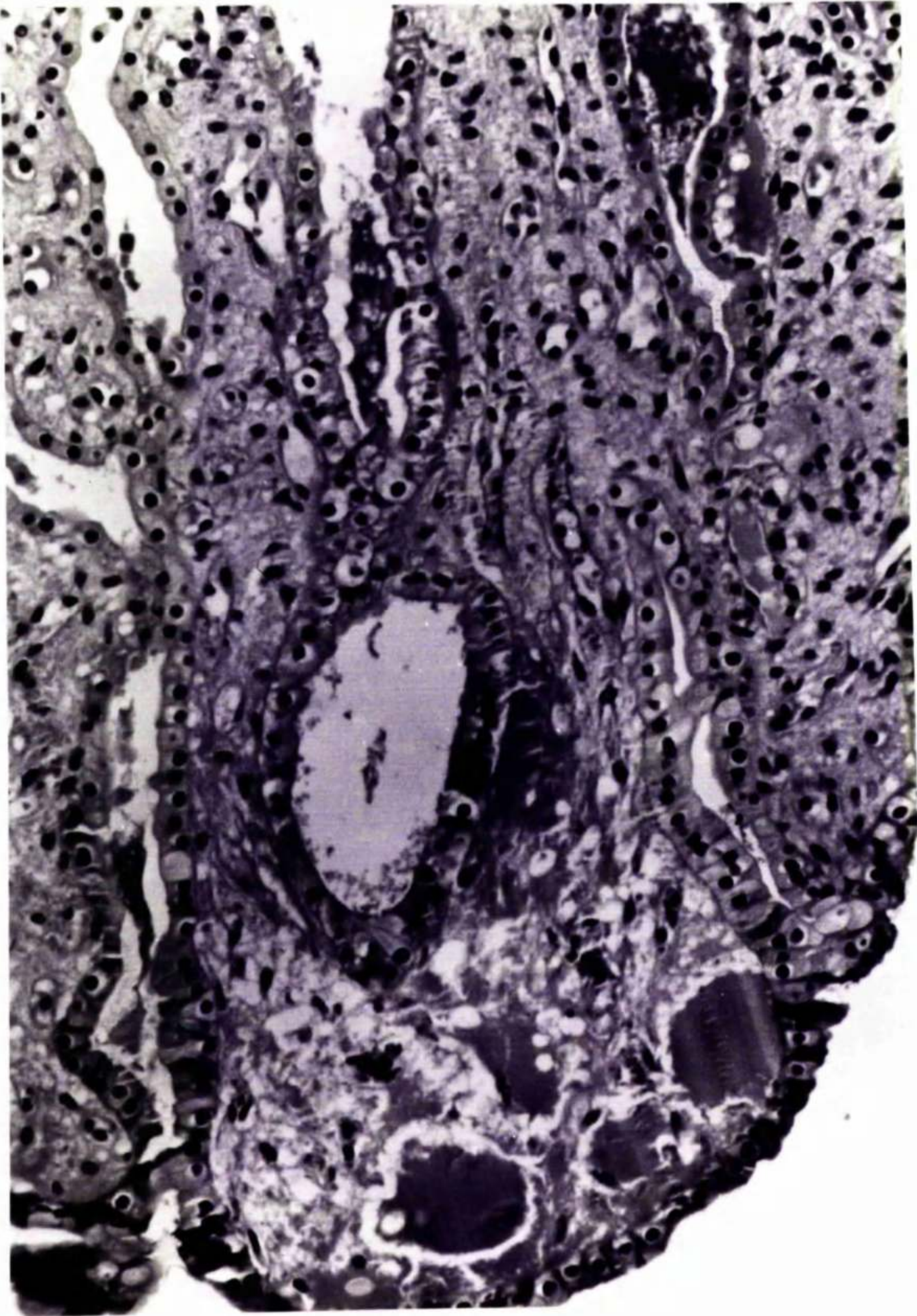


Figures 18, 19 and 20 : Silica gel: 35 Days: Renal Papillae

Collection of hard deposits in interstitium at tips of papillae. Marked cellular changes in collecting duct epithelium with areas of fibrosis.

H & E Mag. X 345.





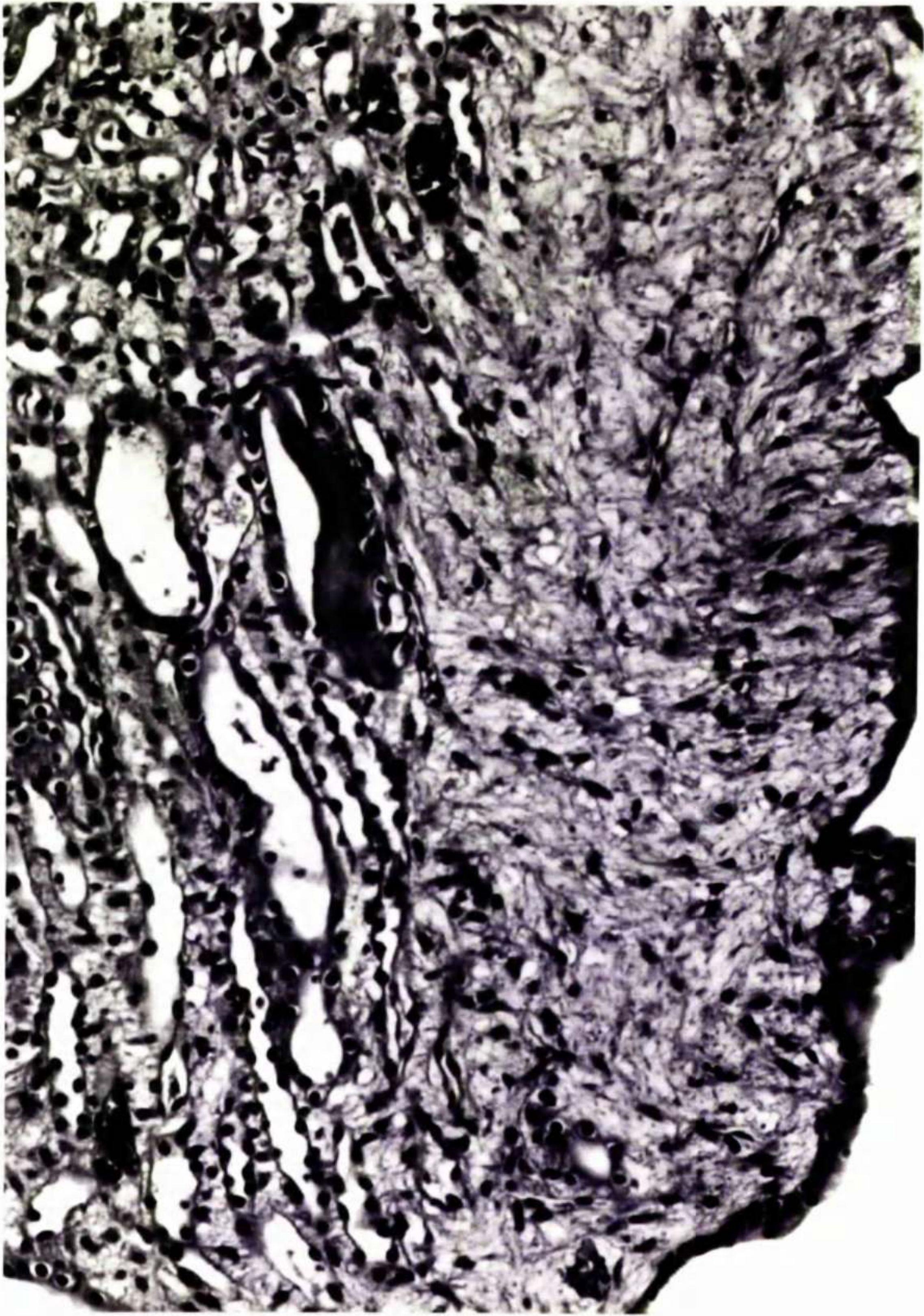


Figure 21 : Magnesium Trisilicate: 4 Days: Cortex

Gross coagulative necrosis of tubules and obvious severe glomerular damage, together constituting focal cortical necrosis.

H & E Mag. X 345.

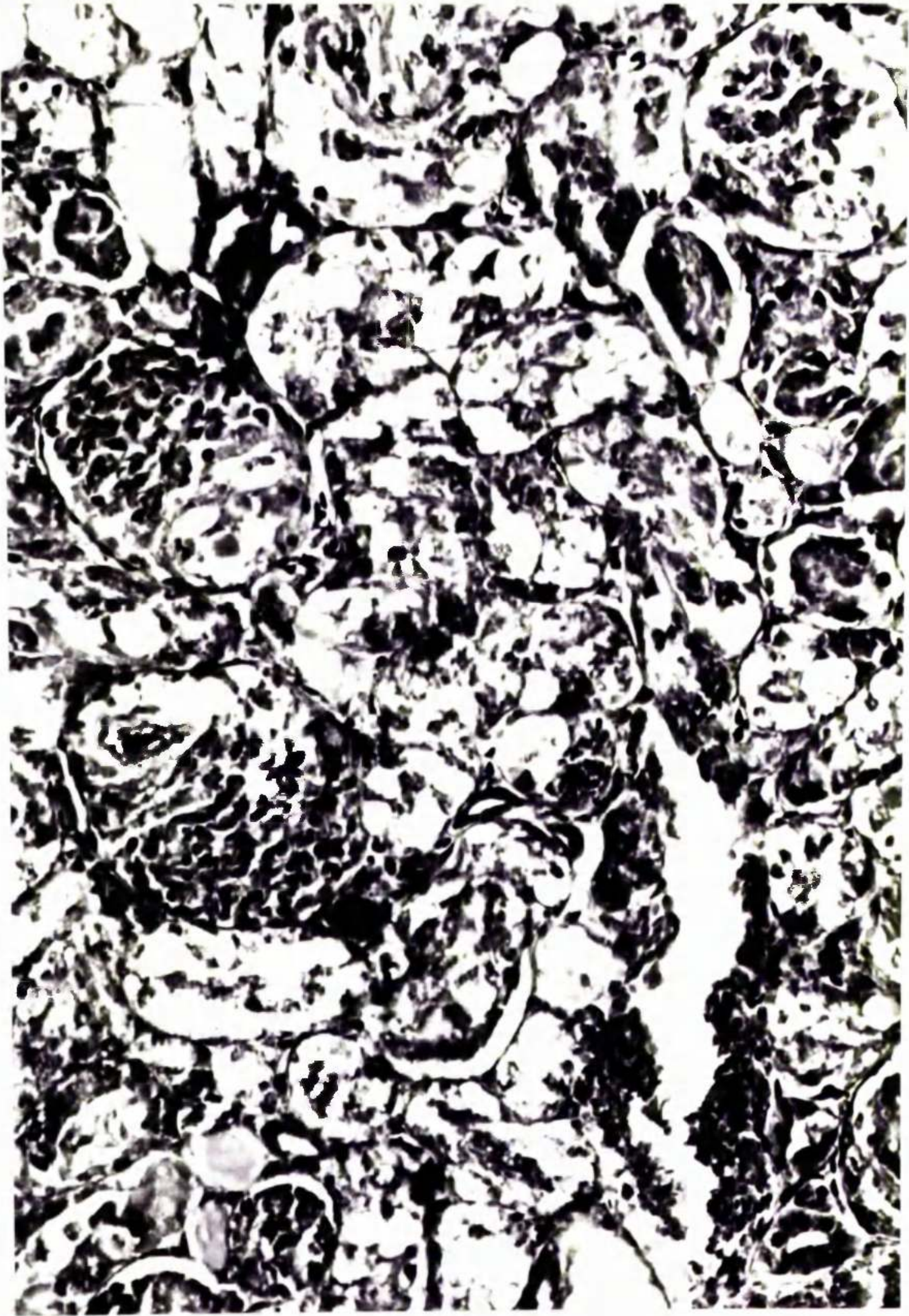


Figure 22 : Magnesium Trisilicate: 4 Days: Renal Papilla

Dilatation and inflammation of collecting ducts
The lumena contain a variety of casts - cellula
and brittle (fractured).

H & E Mag. X 345.

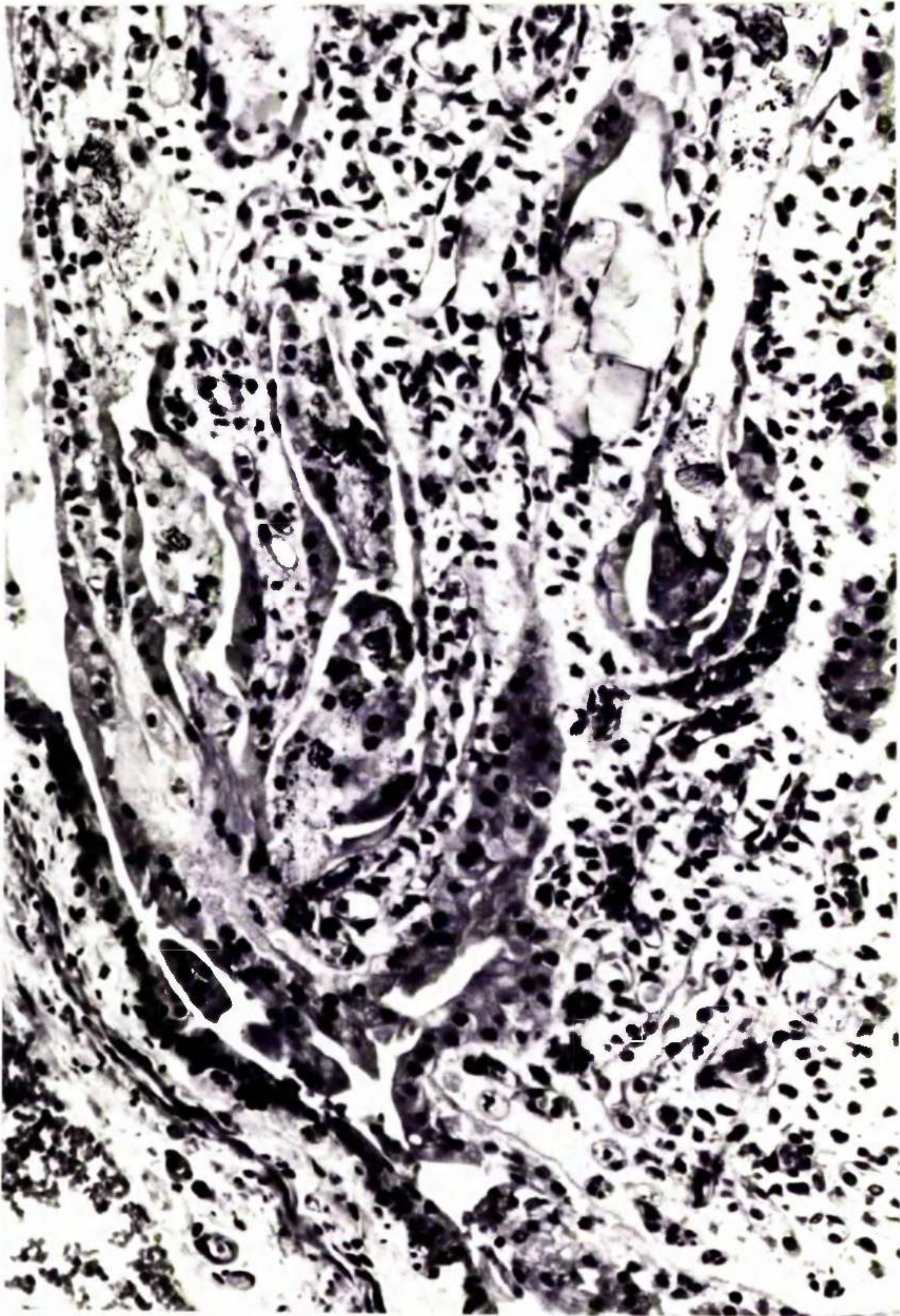


Figure 23 : Magnesium Trisilicate: 21 Days: Cortex

Xanthomatous changed in damages tubules.
Casts in distal tubules which show flattening
of epithelium and cytoplasmic damage.

H & E Mag. X 860.

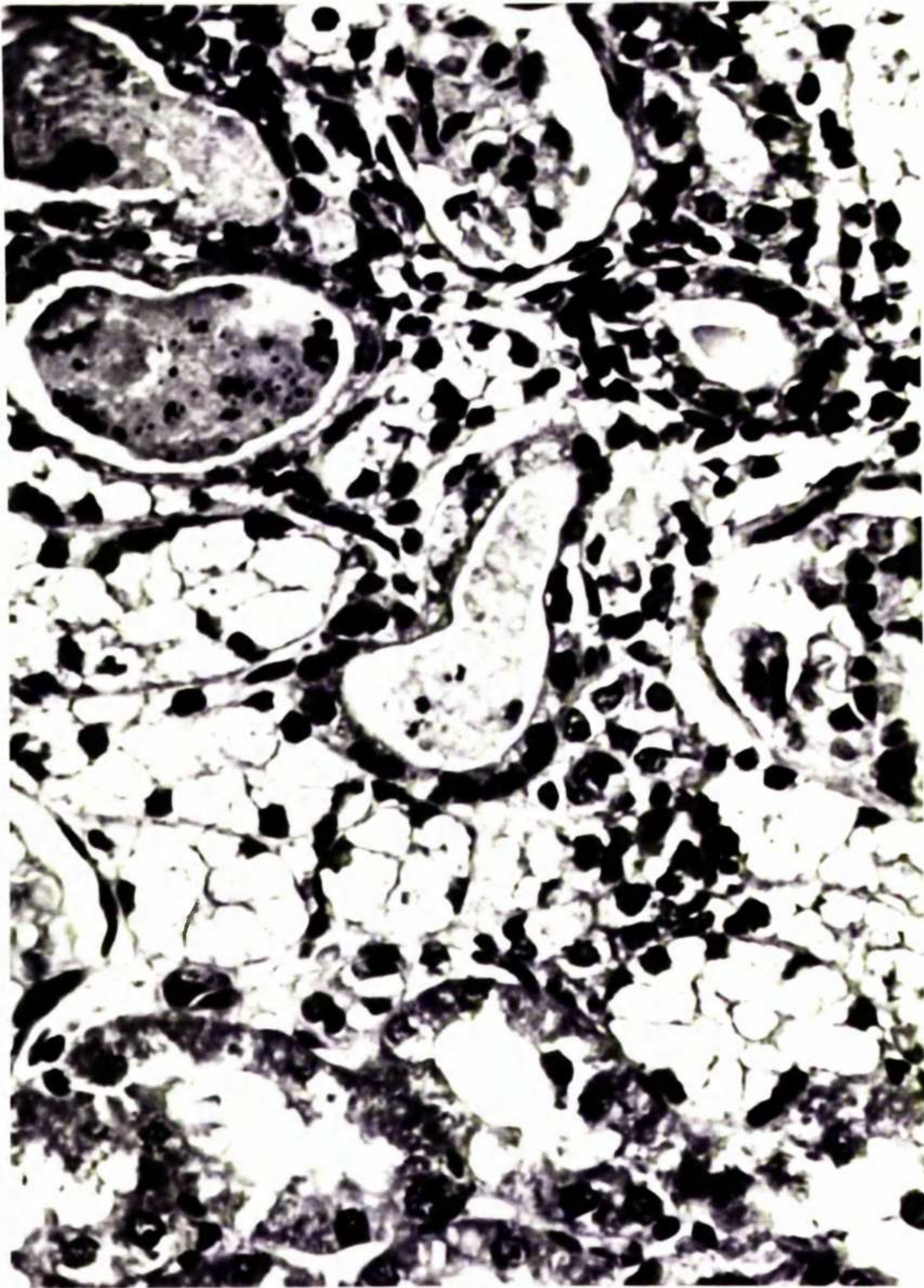


Figure 24 : Gasil 200: 4 Days: Glomerulus

Electron micrograph demonstrating normal glomerular ultrastructure.

X 12696.

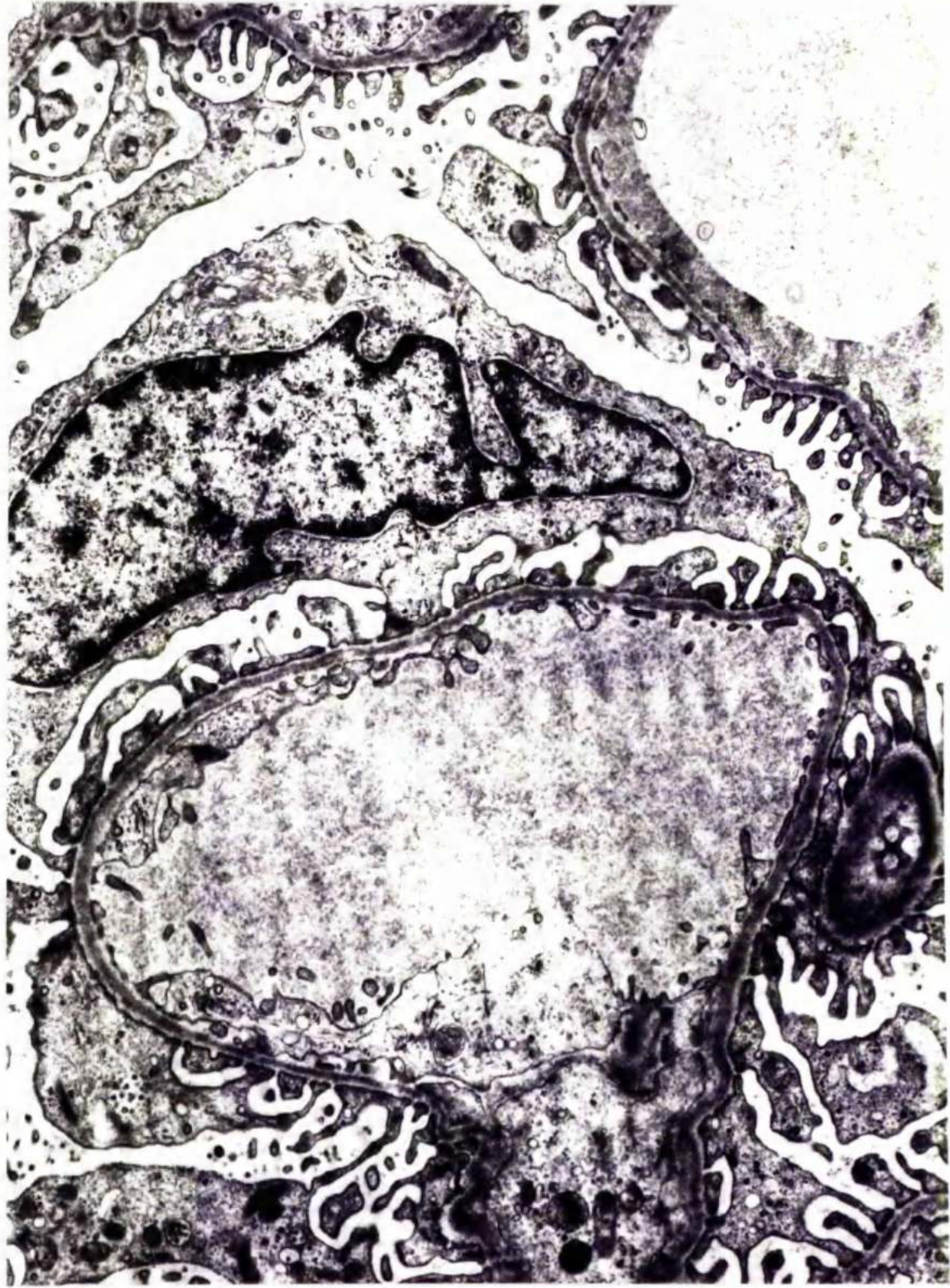


Figure 25 : Gasil 23 D: 4 Days: Glomerulus

Electron micrograph showing glomerular visceral epithelial cell changes - dispersion of cell organelles, mitochondrial swelling and early cytolysosomal formation.

X 35880.

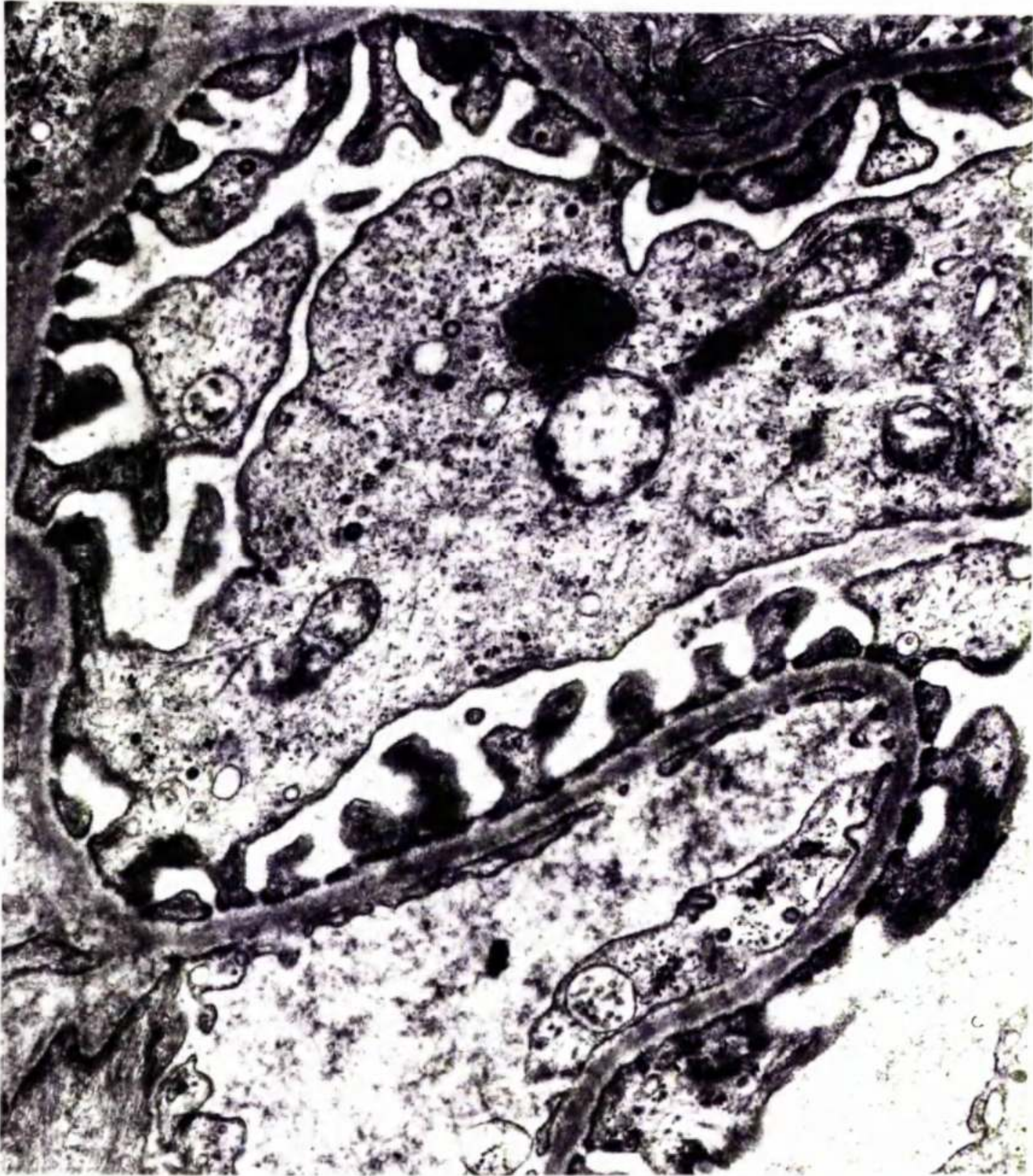


Figure 26 : Gasil 23 D: 21 Days: Glomerulus

Epithelial cell reactivity in the form of microvillous projections. A large phagolysosomal (P) distends and deforms a secondary trabeculum cut in cross section.

X 12696.

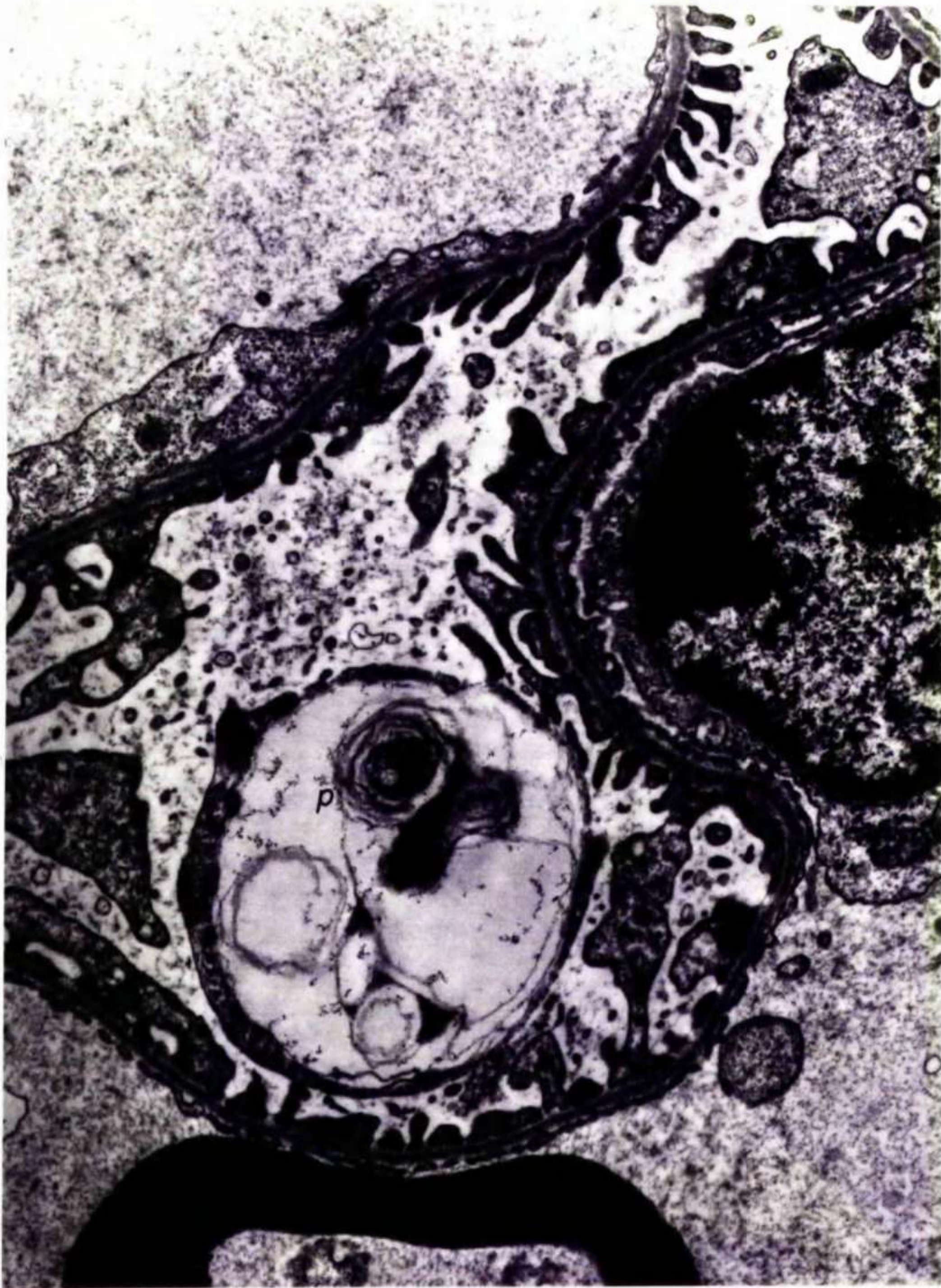

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Figure 27 : Gasil 200: 21 Days: Proximal Tubule

Alternate epithelial cells showing marked apical oedema, disorganisation and dispersal of organelles, with loss of brush border.

X 3864.

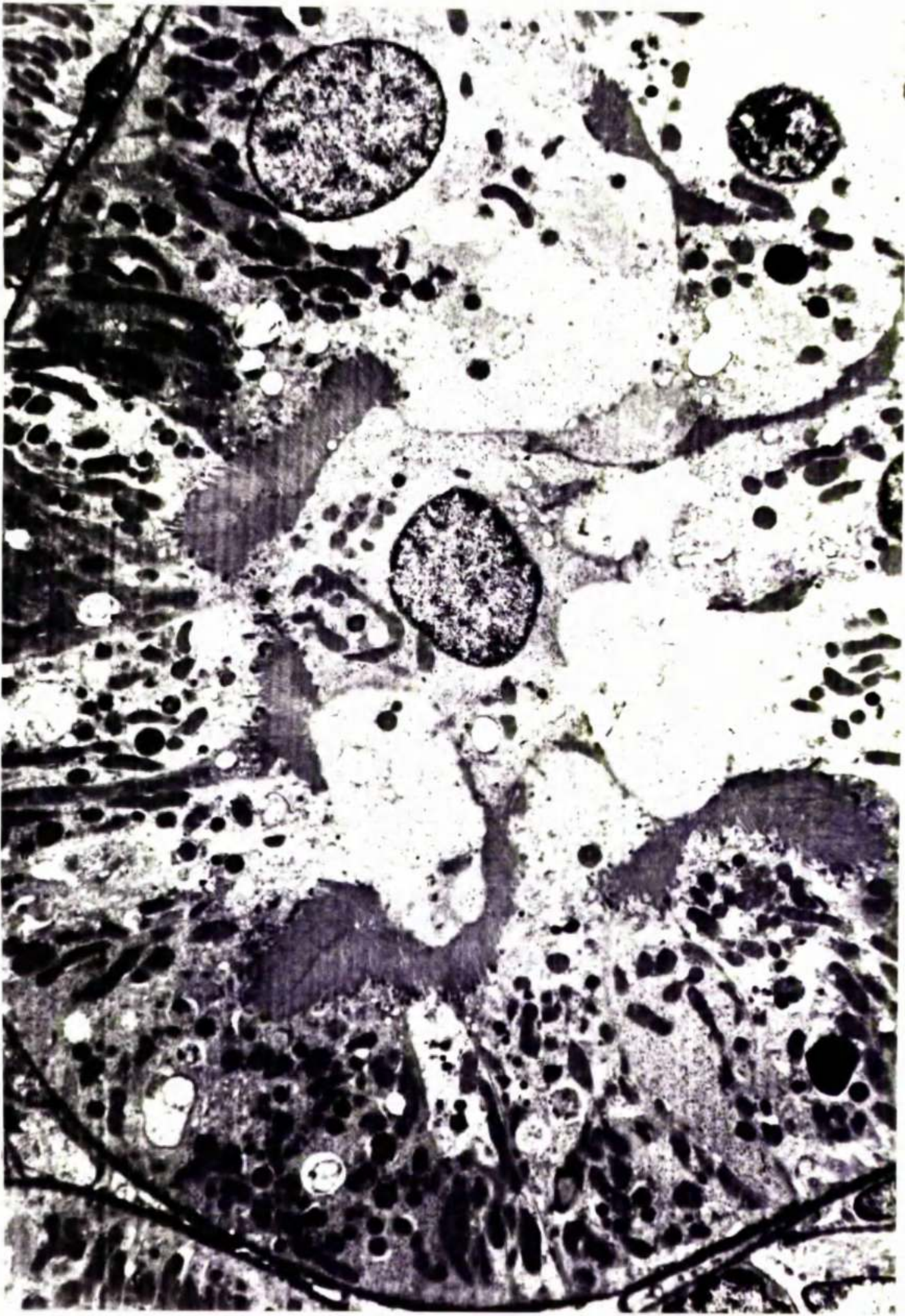

Figure 28 : Gasil 200: 4 Days: Proximal Tubule

Electron micrograph showing disruption of the integrity of the brush border.

X 7728.

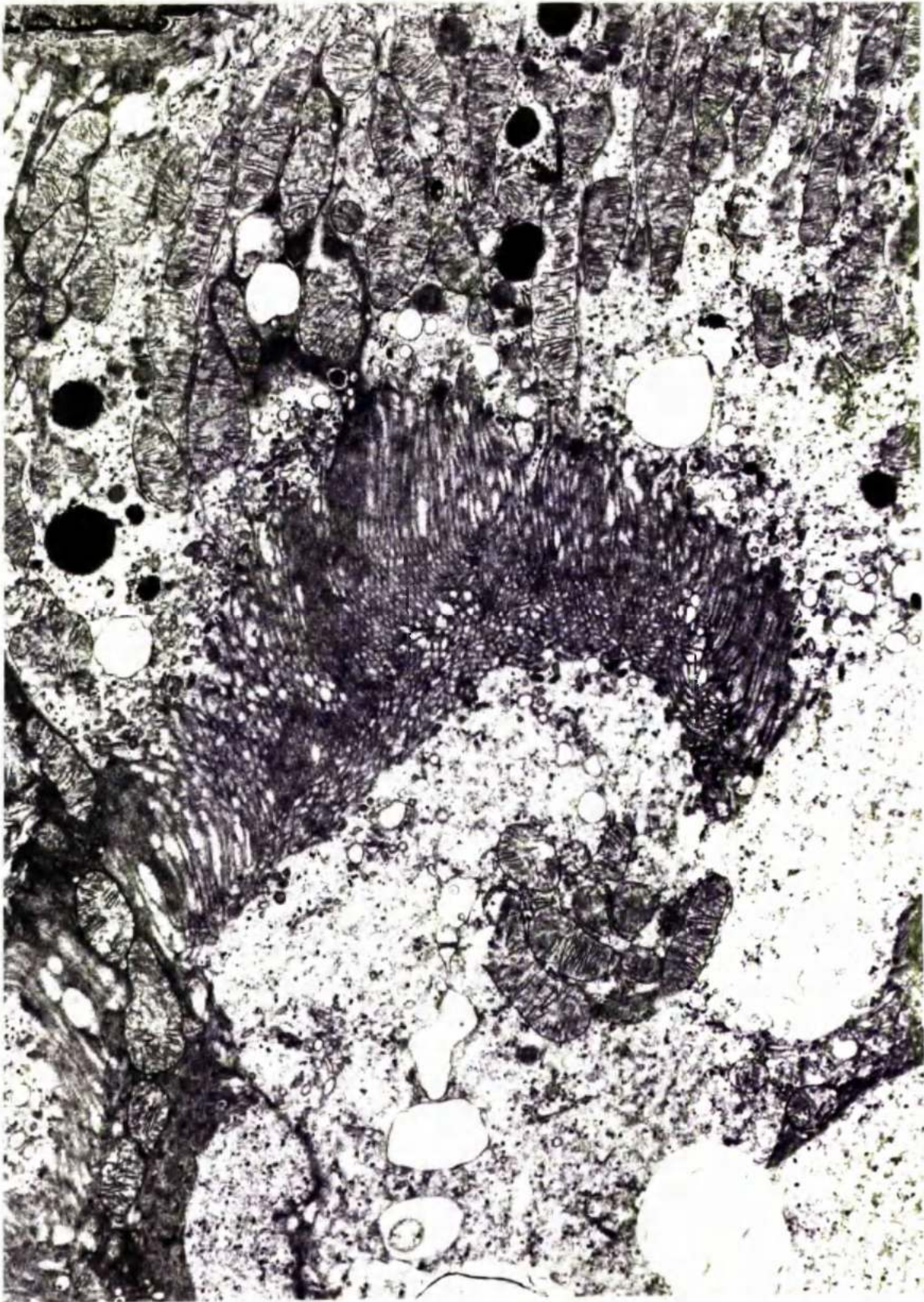

Figure 29 : Gasil 200: 21 Days: Proximal Tubule

Such is the damage and distortion of cell structures - loss of basal infolding, variable non-orientated mitochondrial profiles, mitochondrial swelling, that identification of the nature of this tubular structure is only made possible by the presence of small areas of surviving brush border.

X 7728.

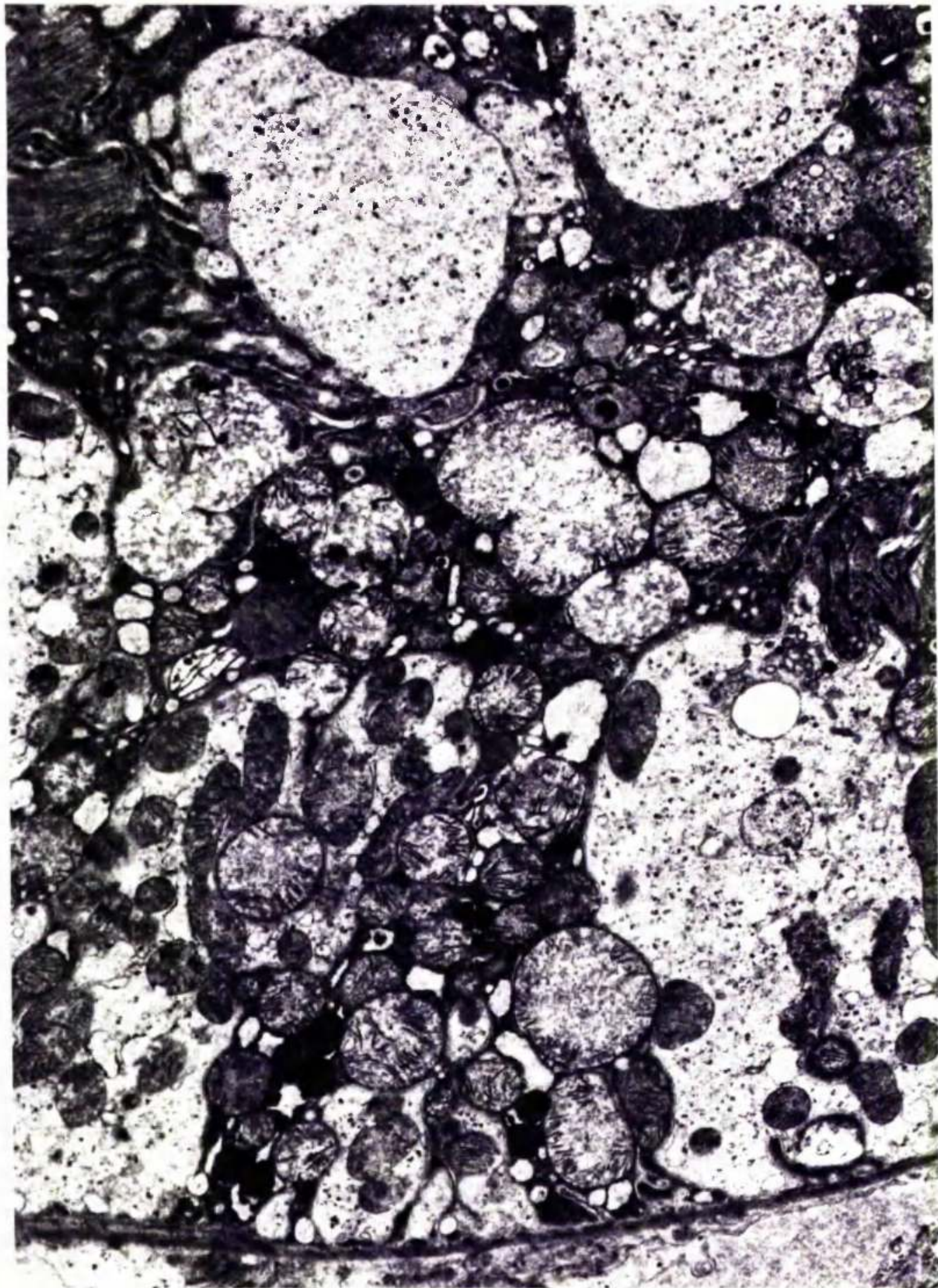

Figure 30 : Gasil 200: 4 Days: Proximal Tubule

Low power electron micrograph showing a gradation of damage to proximal tubular cells, comprising oedema; dilatation of cysternal systems, dispersion and degeneration of organelles, perinuclear vacuolation, nuclear pyknosis and chromatin margination. The brush border, however, is intact.

X 3864

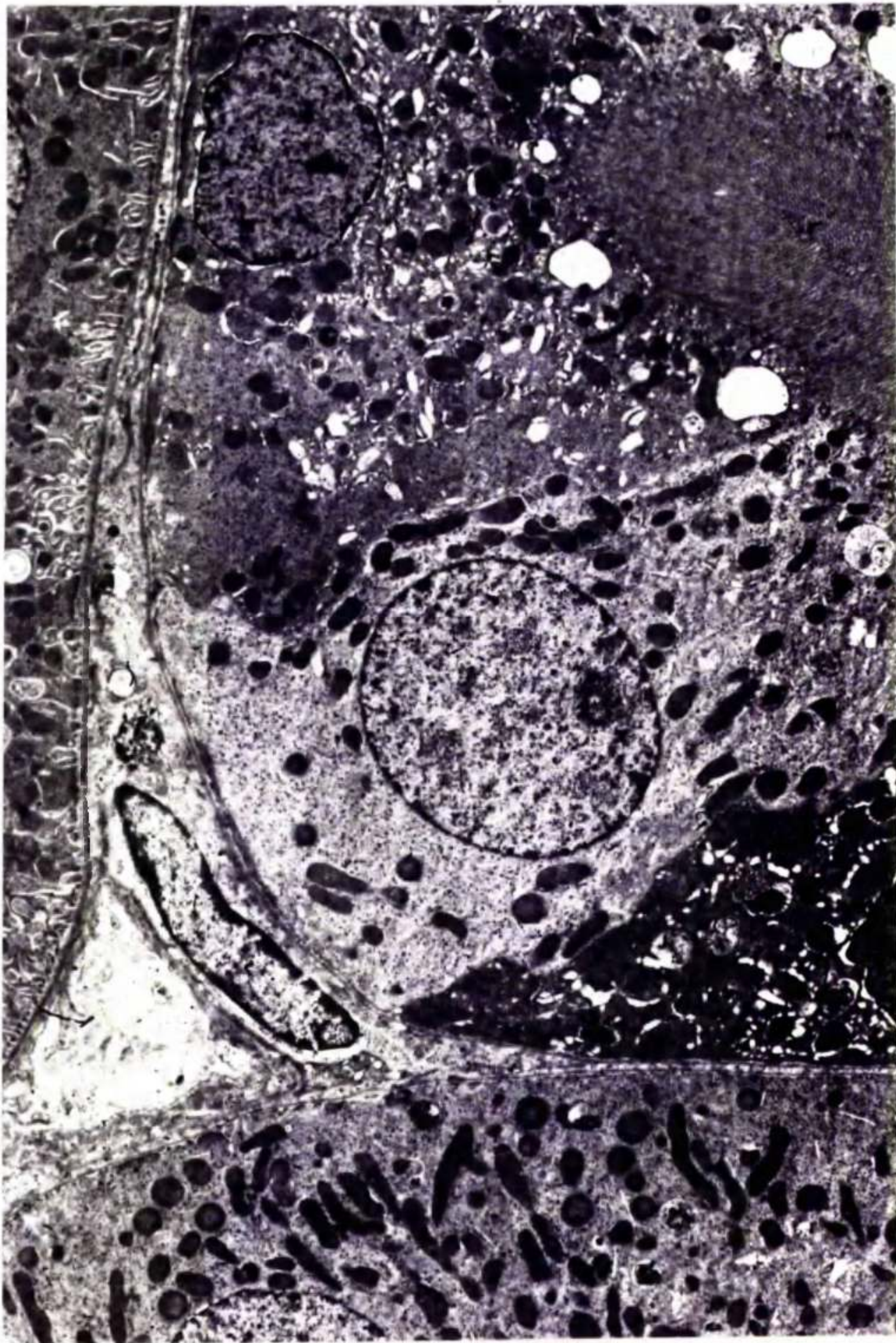

Figure 31 : Gasil 23 D: 35 Days: Proximal Tubule

Numerous membrane bound structures containing granular electron dense material - cyto-lysosomes. Widespread vesiculation is also present.

X 20930.

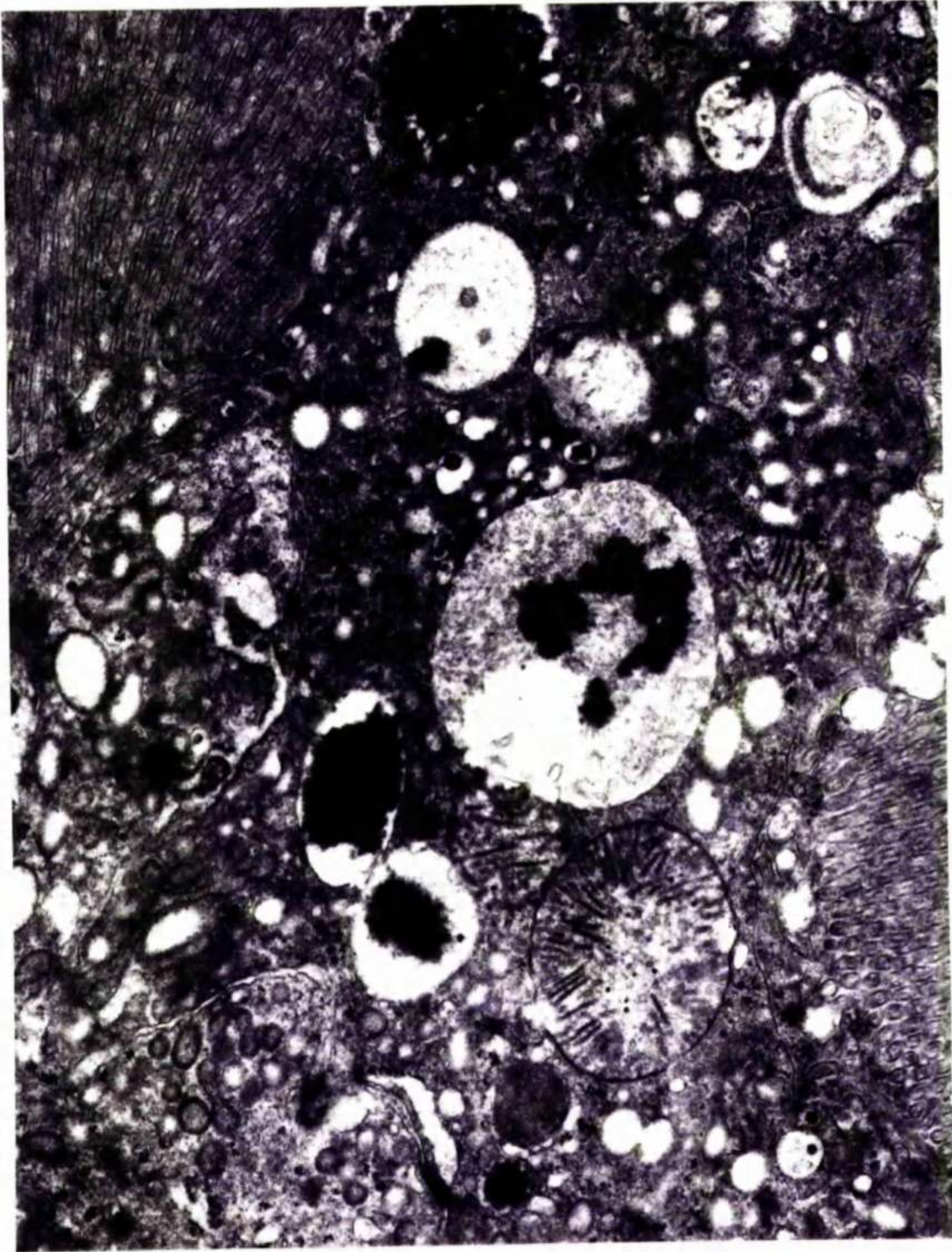

Figure 32 : Gasil 200 : 4 Days: Proximal Tubule

Showing widespread vesiculation and dilatation of endoplasmic reticulum. Single membrane bound structures containing electron dense material are also much in evidence (Cytolysosomes Chromatin margination and vacuolation of perinuclear space is also present. There is loss of the normal pattern of basal infoldings.

X 12696.

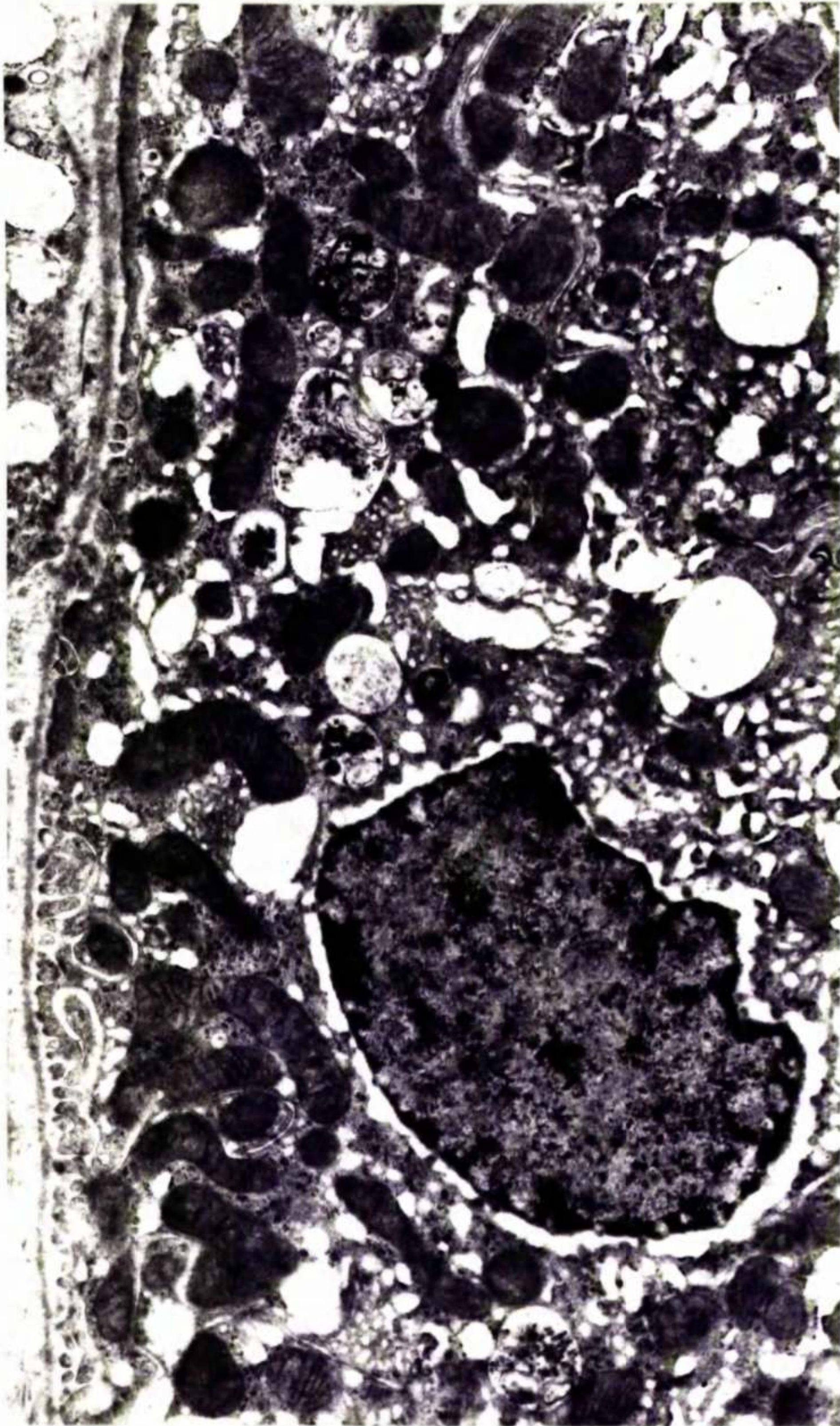

Figure 33 : Gasil 23 D: 4 Days: Mitochondria

Widespread mitochondrial swelling with loss of mitochondrial granules and dispersion of the cristae.

X 20930.

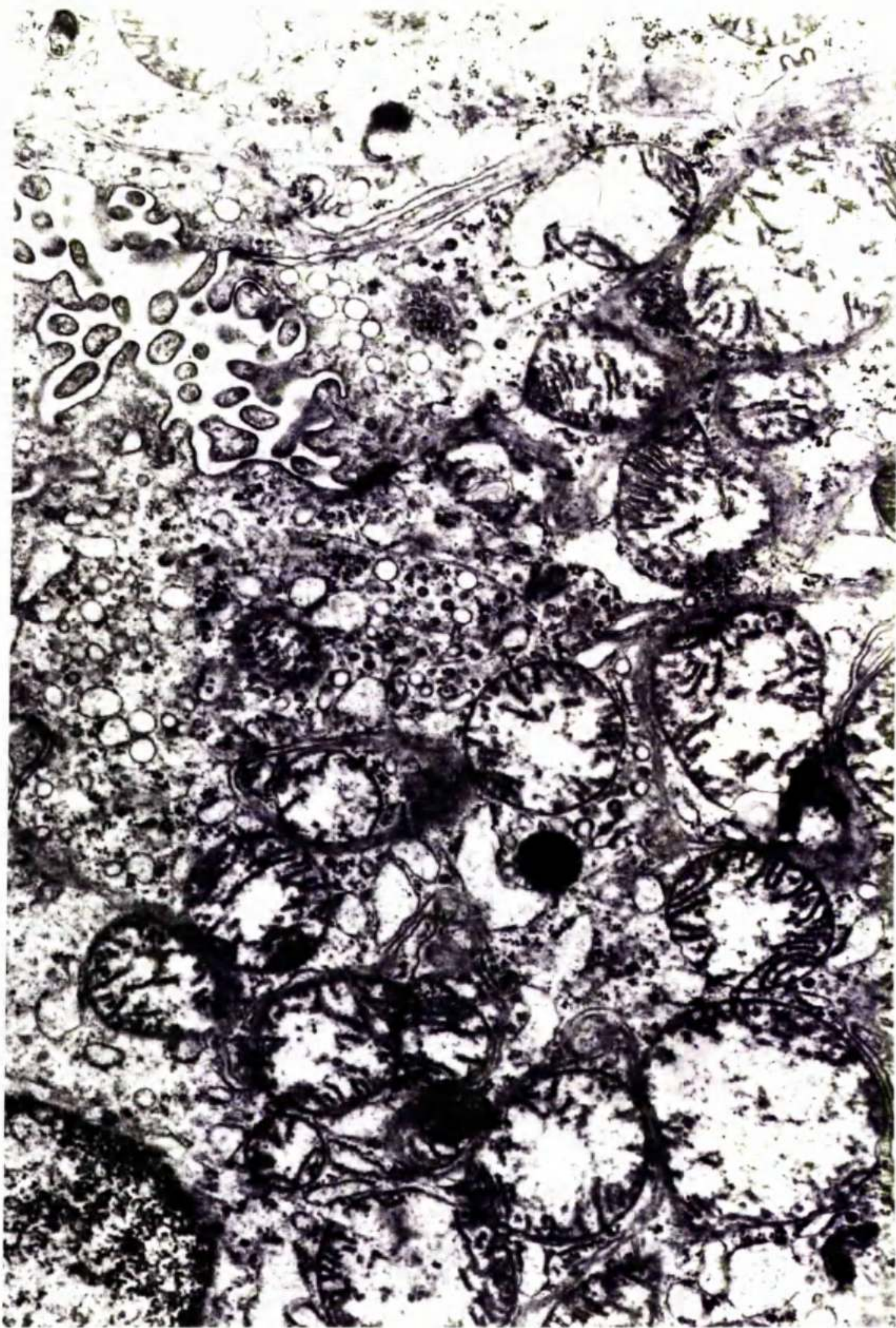

Figure 34 : Gasil 23 D: 4 Days: Lysosomes

Electron micrograph showing secondary lysosomal structures which contain electron dense profiles of indefinite origin. In addition, the increased density and decreased size of the mitochondria are demonstrated.

X 20930.

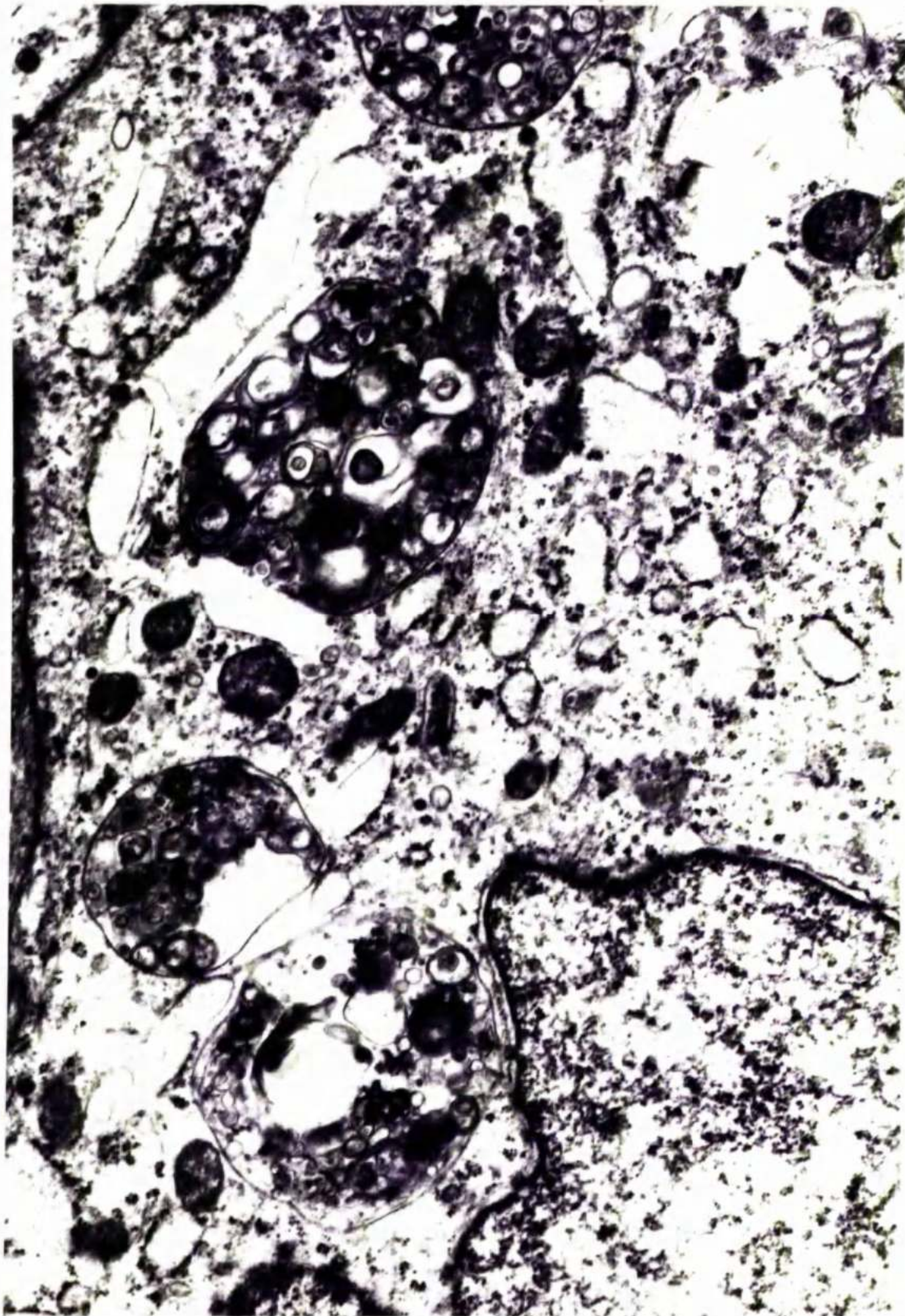

Figure 35 : Gasil 200: 35 Days: Mitochondria

Electron micrograph showing intramitochondrial multilamellated membrane structures and mitochondrial intracisternal sequestration.

X 35880.

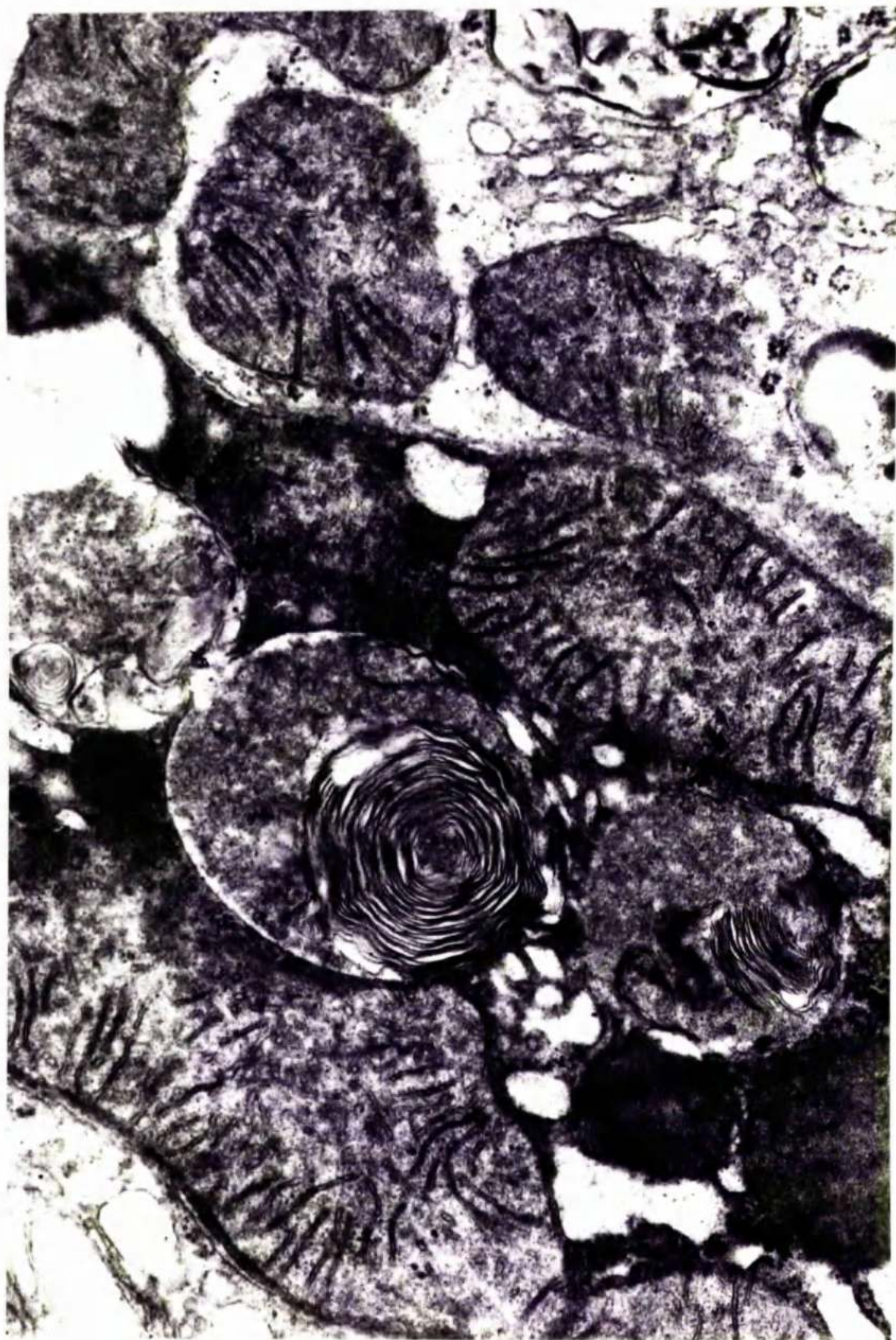

Figure 36 : Silica Gel: 4 Days: Endoplasmic Reticulum

Endoplasmic reticulum vesiculation and dilatation with evidence of degranulation.

X 20930.

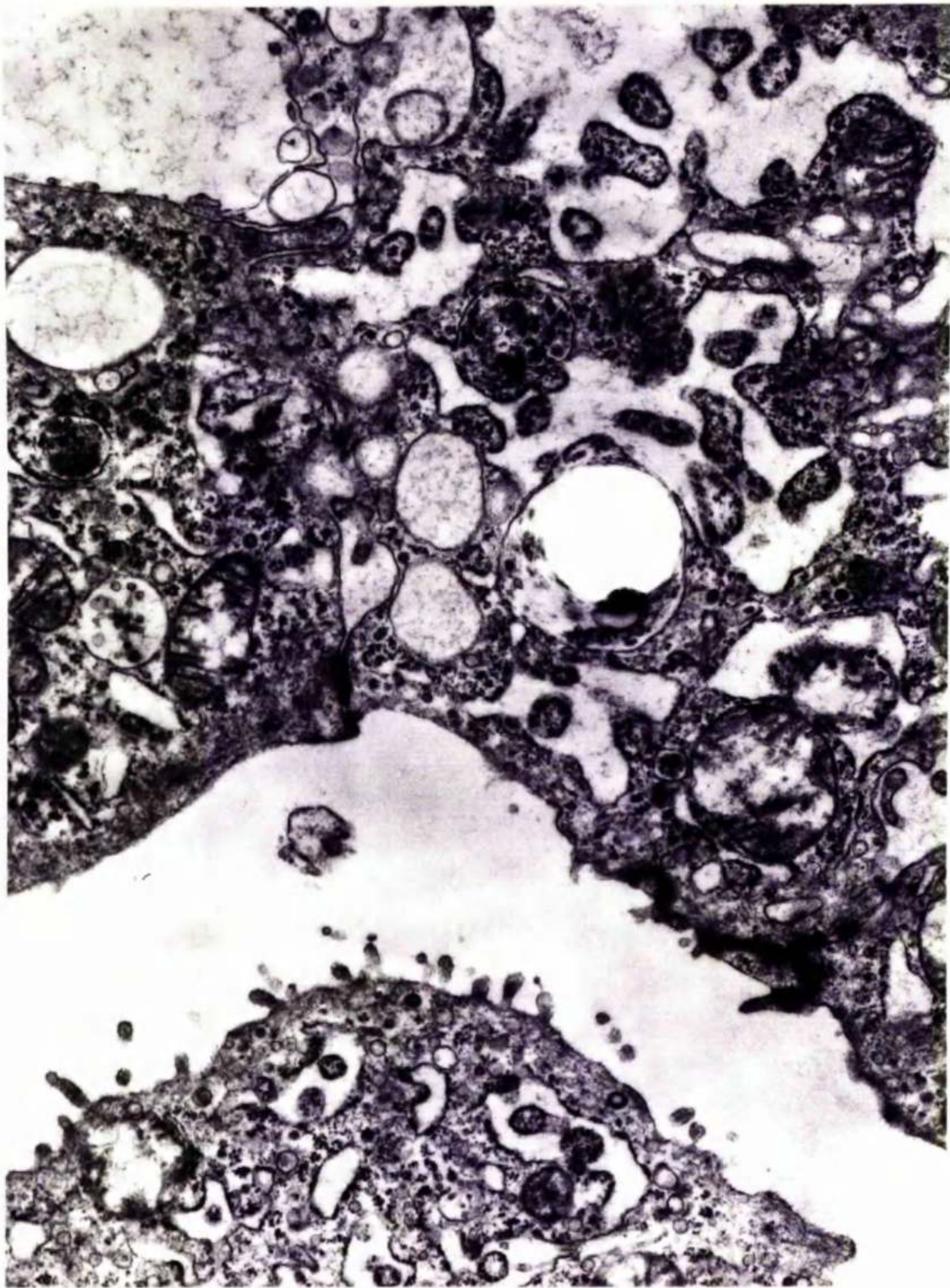

Figure 37 : Gasil 200: 35 Days: Proximal Tubule

Apical portion of epithelial cell whose cytoplasm is filled with multilamellated bodies (myelin figures). There is also scattered free in the cytoplasm electron dense material not bound by any clear membrane.

X 20930.

Figure 38 : Gasil 23 D: 4 Days: Distal Tubule

Apical portion of distal tubule showing lumenal surface. There is gross dilatation and vesiculation of cisternal systems, mitochondrial swelling and damage is also noted.

X 12690.

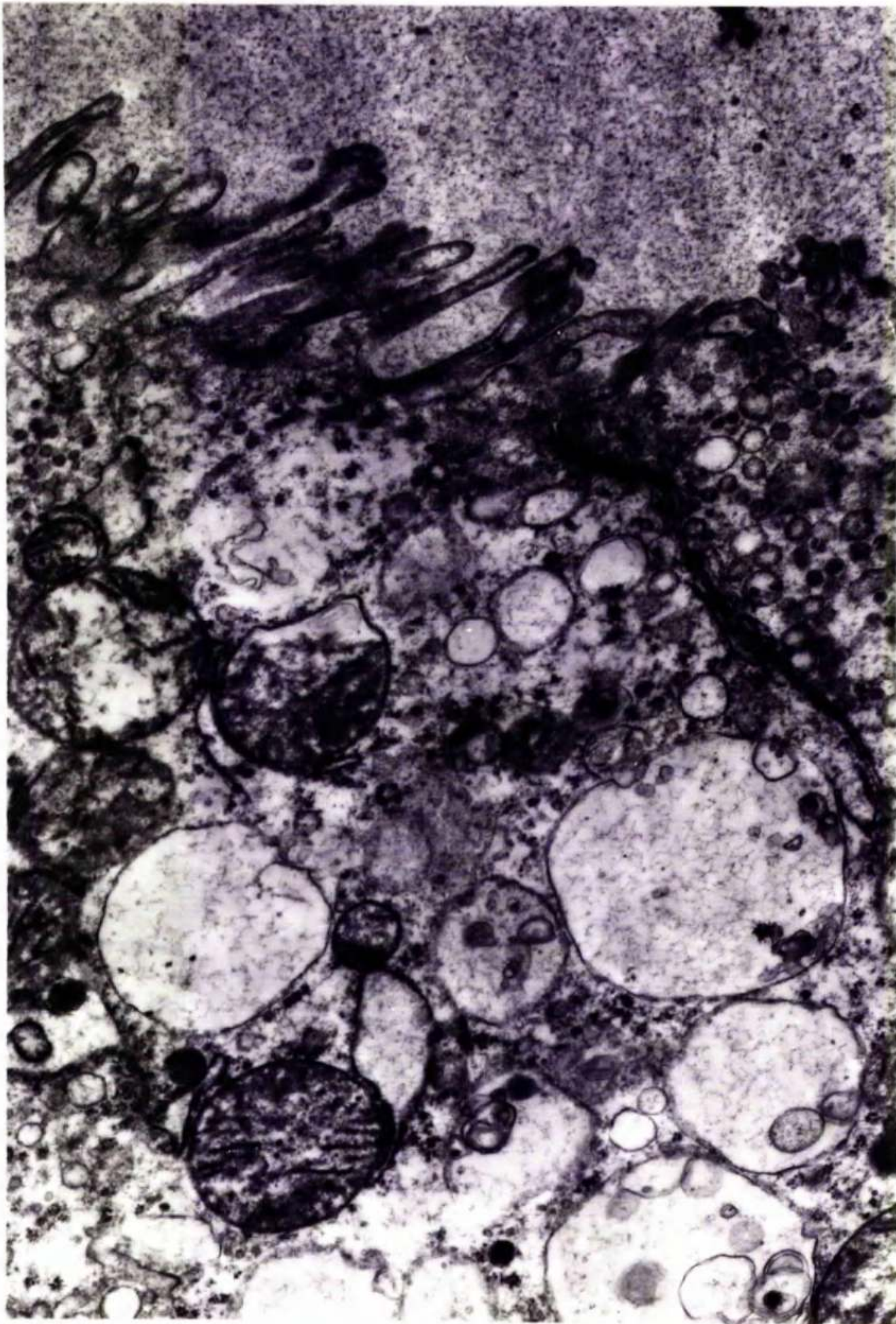

Figure 39 : Gasil 200: 21 Days: Distal Tubule

Swollen epithelial cells, showing dilution and dispersion of cytoplasmic organelles with disappearance of endoplasmic reticulum. The mitochondria show increased density.

X 7728.

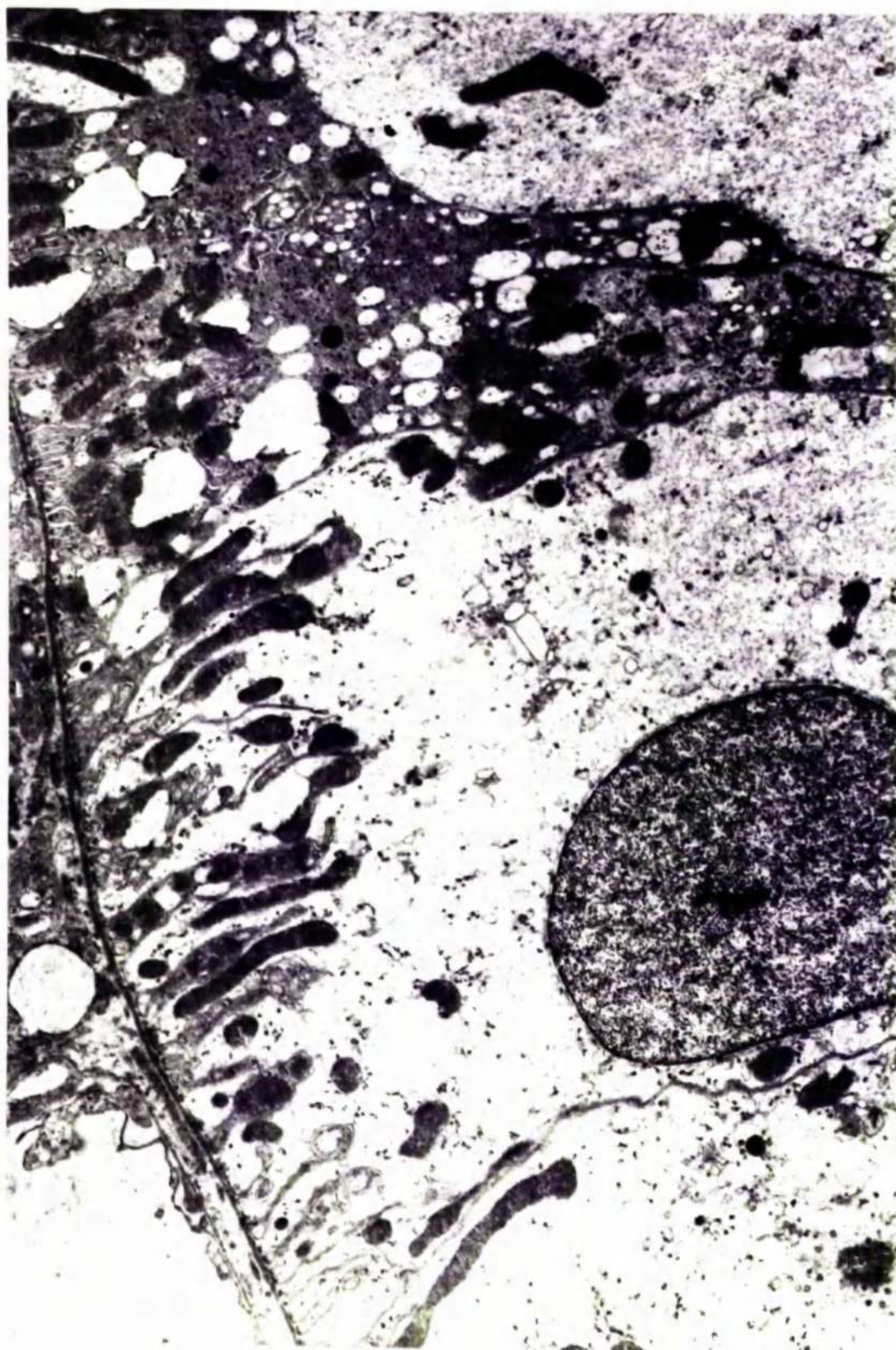

Figure 40 : Magnesium Trisilicate: 4 Days: Distal Tubule

Distal tubule lined by swollen epithelial cells: Atrophic golgi apparatus (G) is observed.

X 7728.

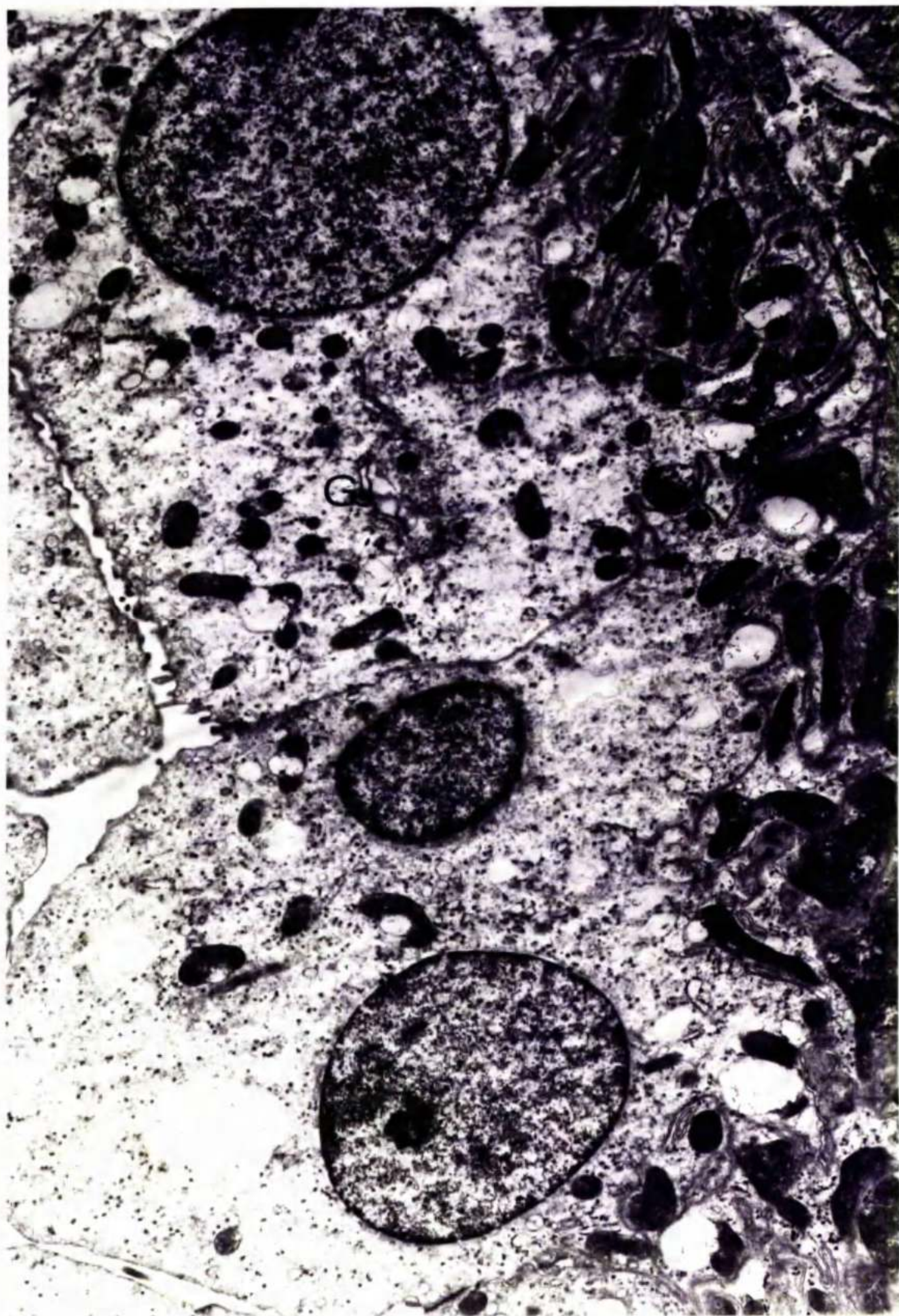
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Figure 41 : Gasil 200: 21 Days: Distal Tubule

Portion of distal tubule whose dilated lumen is filled with desquamated epithelial cells.

X 3864.

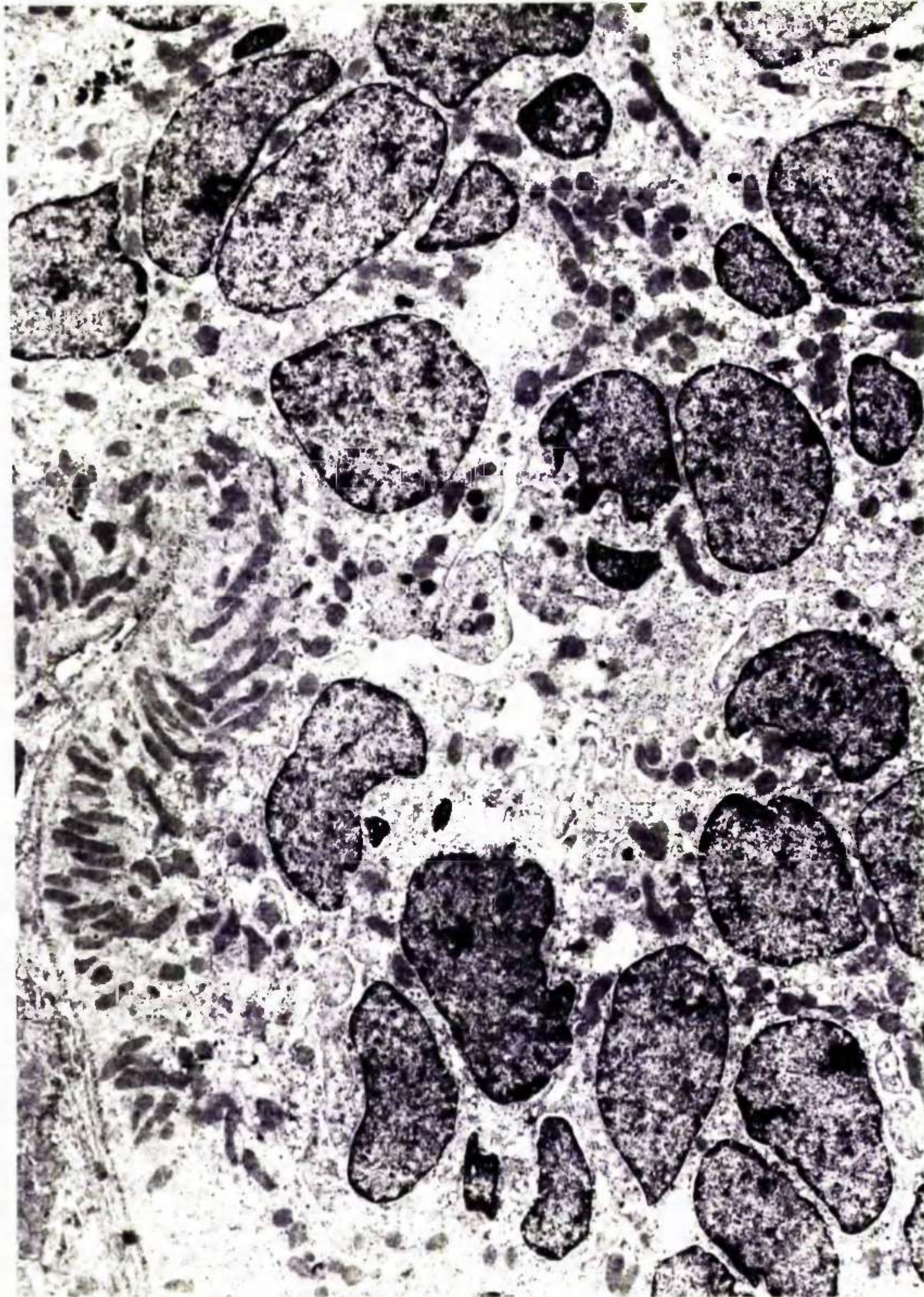


Figure 42 : Gasil 200: 35 Days: Distal Tubule

Showing dilated distal tubule. The main features are those of cellular swelling, loss of peg-like villi from the lumenal surface, loss of basal infolding, disorganisation of organelles with mitochondrial pyknosis and increased numbers of lysosomes.

X 3864.

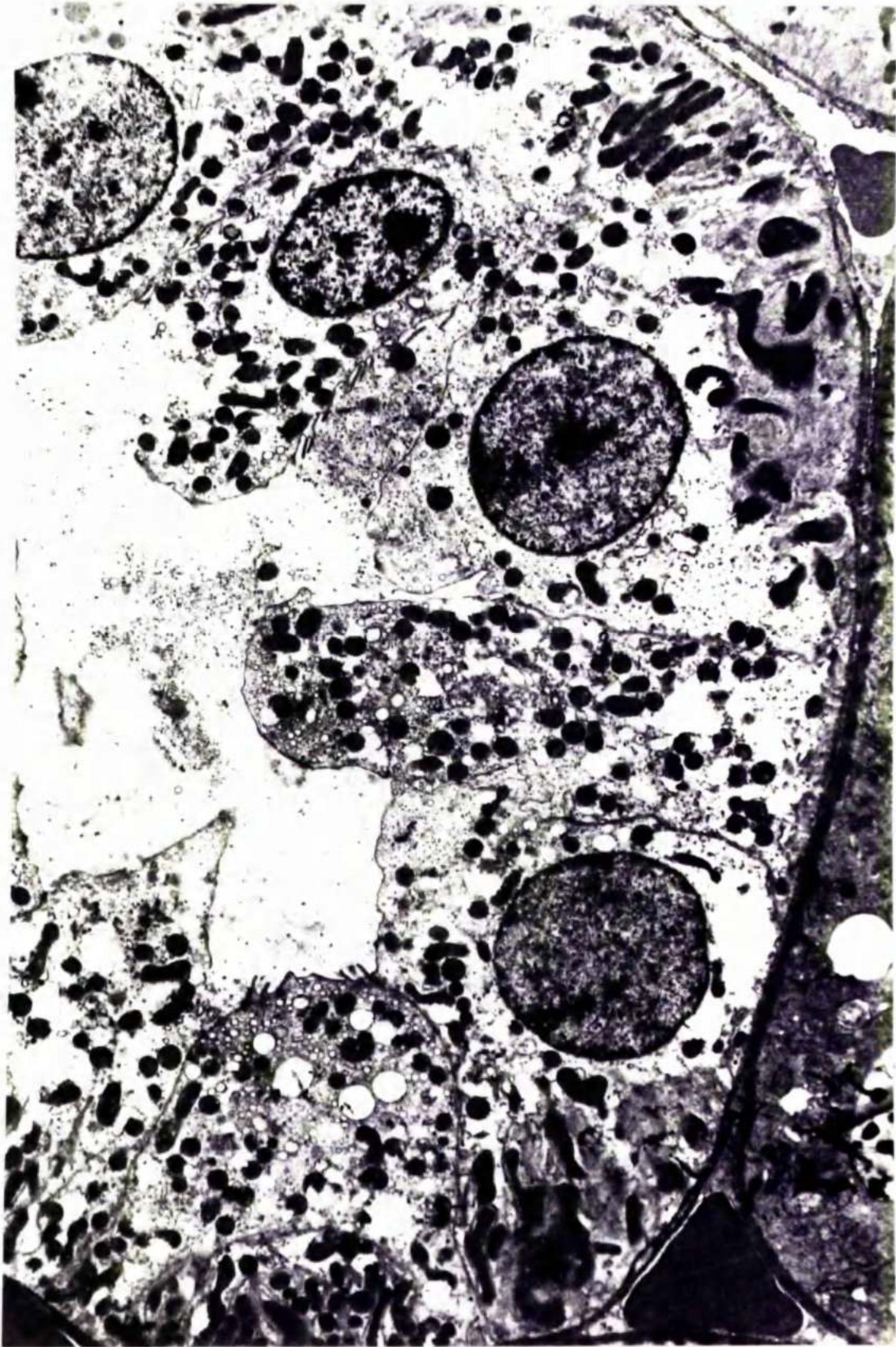


Figure 43 : Magnesium Trisilicate: 4 Days: Distal Tubule

High power details of cytoplasmic oedema demonstrating disruption of basal infolding in distal tubule.

X 20930

Figure 44 : Silica Gel: 35 Days: Thick loop of Henle

Widespread cytoplasmic vacuolation in thick loop.

X3864.

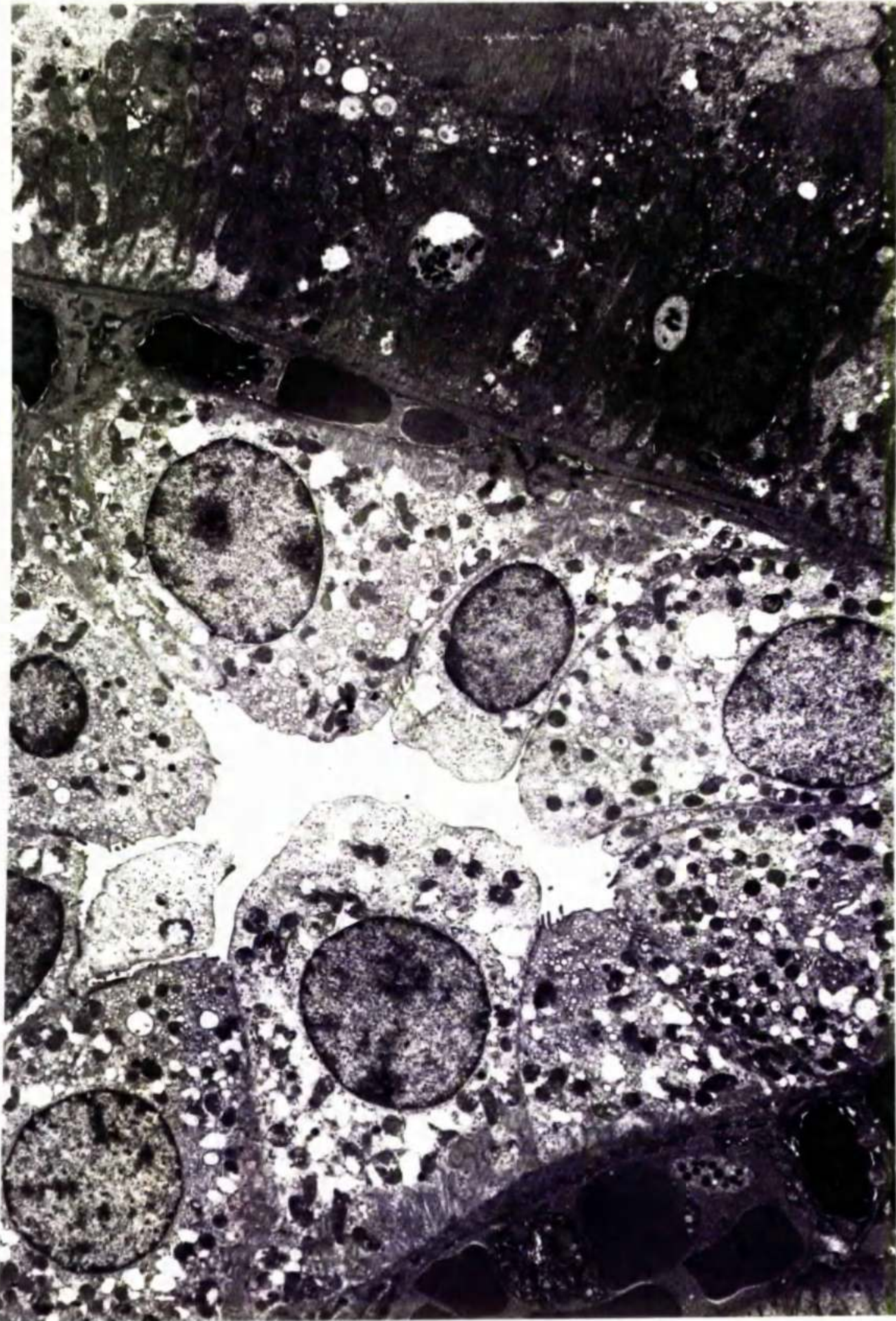


Figure 45 : Silica Gel: 4 Days: Thick loop of Henle

Thick loop showing marked swelling and vacuolation of cytoplasmic organelles.

X 7728.

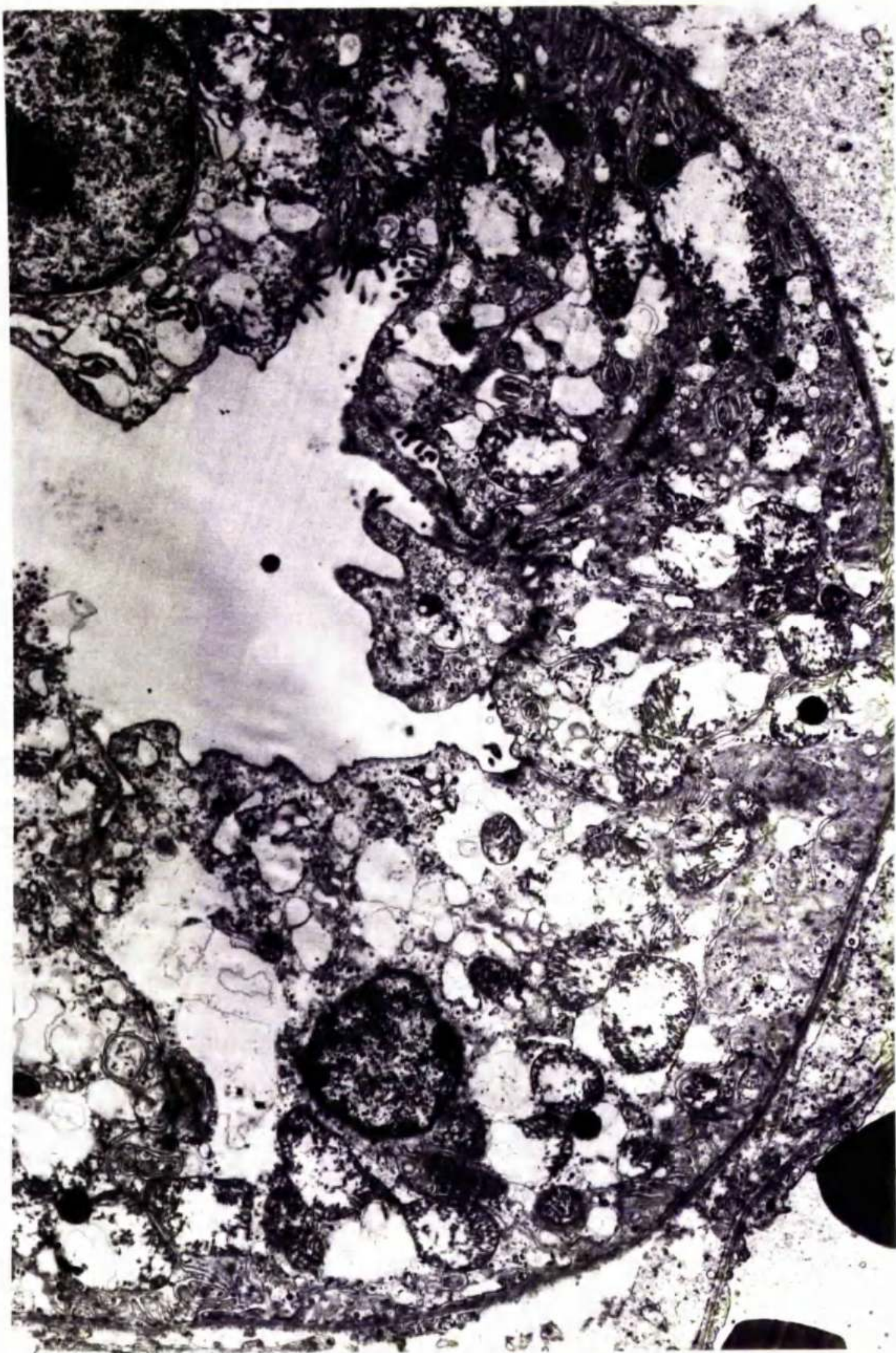


Figure 46 : Silica Gel: 4 Days: Thin loop of Henle

Marked degenerative changes with cytoplasmic vacuolation in the thin loop.

X 7728.

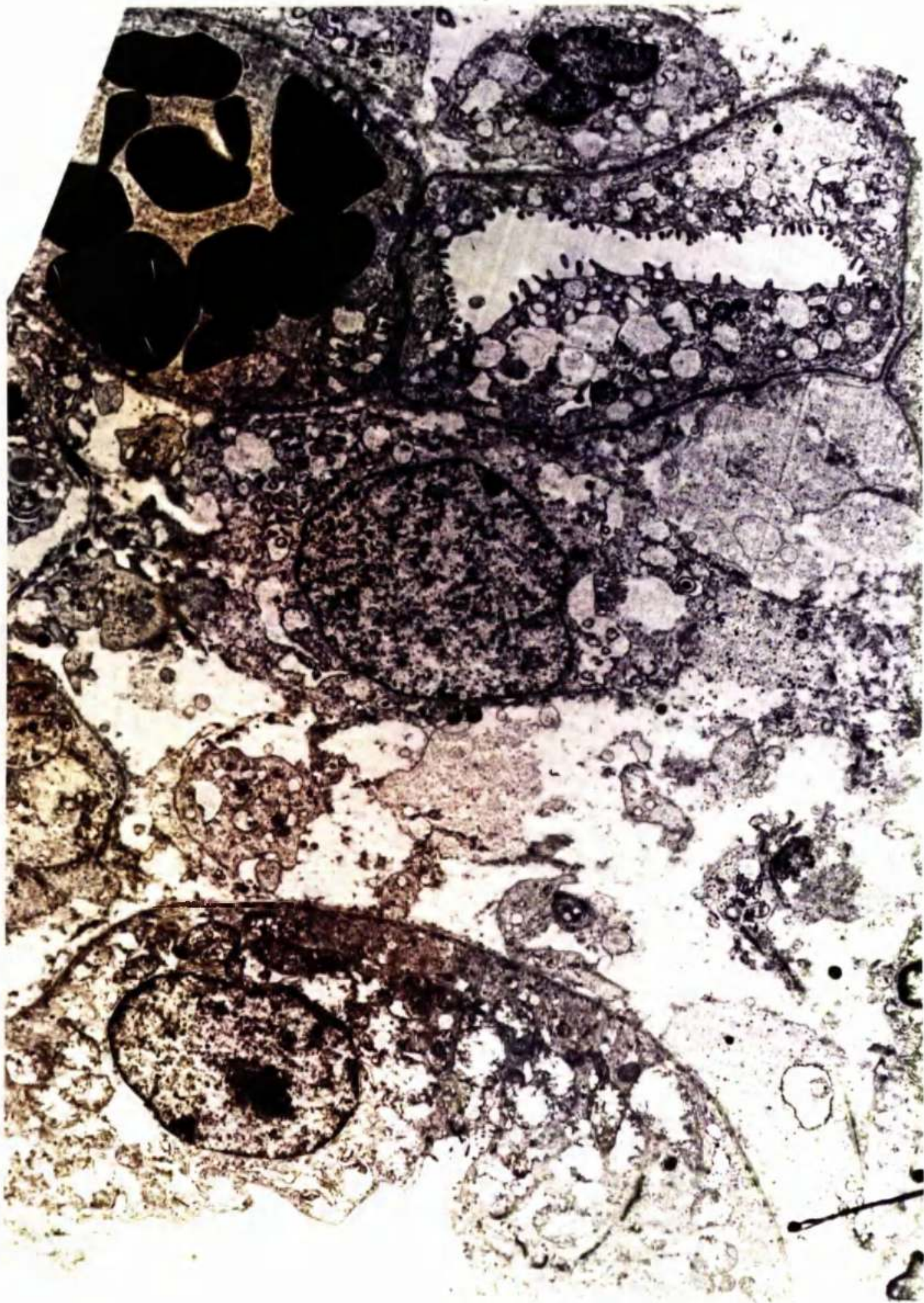


Figure 47 : Silica Gel: 4 Days: Collecting Duct

Marked nuclear and cytoplasmic degenerative changes in a portion of collecting duct.

X 7728.

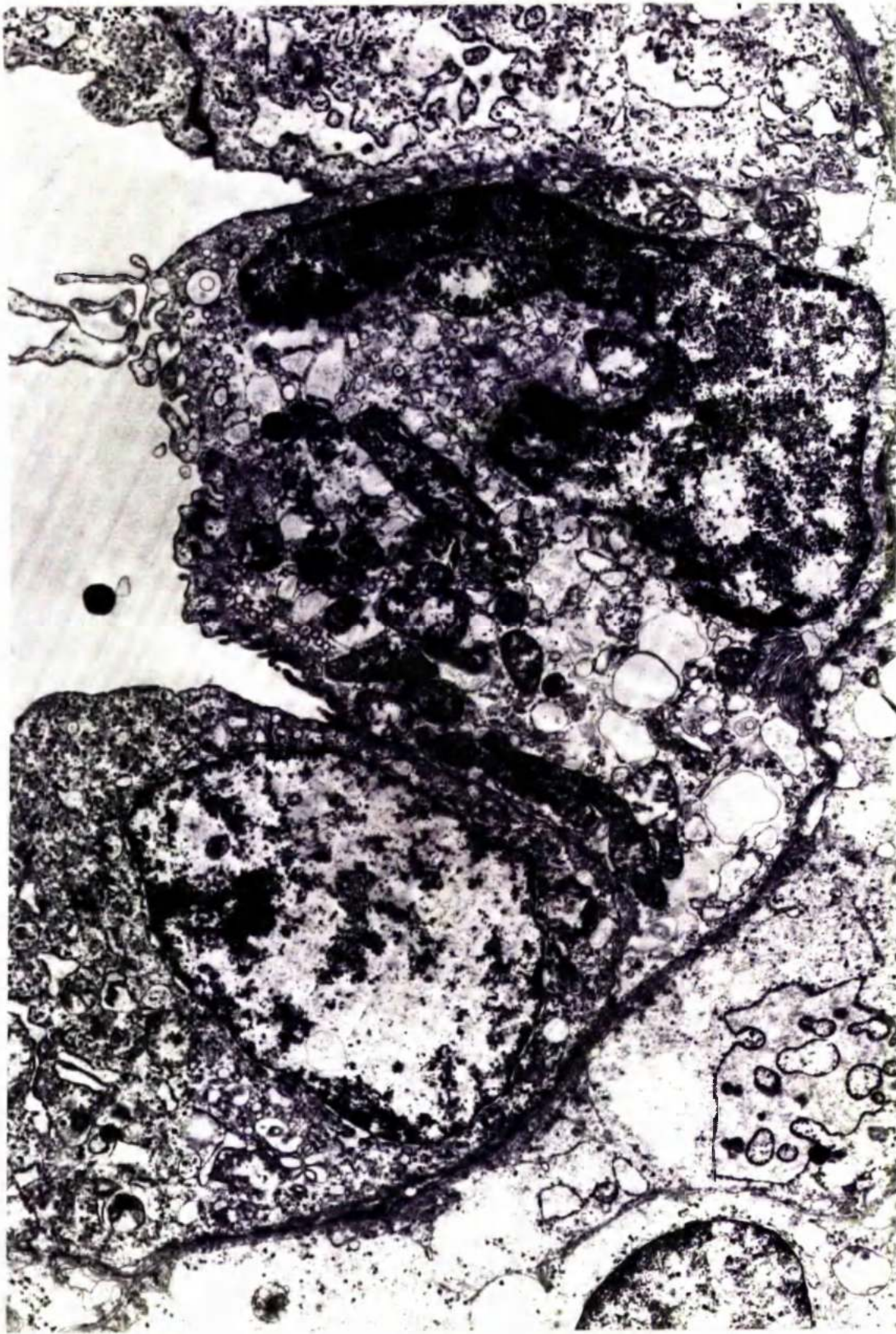


Figure 48 : Gasil 200: 35 Days: Arteriole

This electron micrograph demonstrates a normal arteriolar ultrastructural pattern.

X 5520.

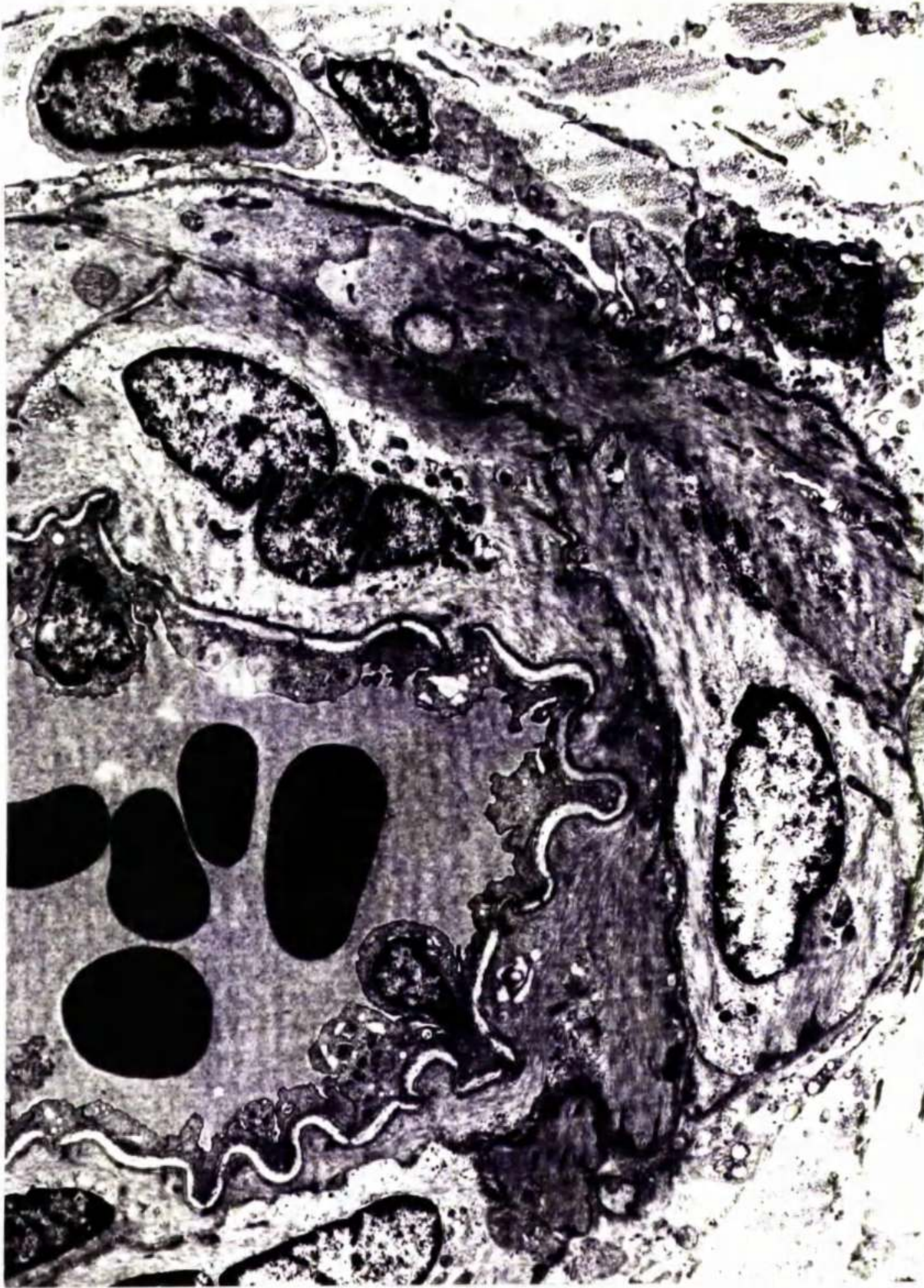


Figure 49 : Serum Silicon Levels (μg/ml) After a single intraperitoneal injection of 100 mg gasil 200 at pH 5.3 and 10 - 11 followed over a 48 hour period. Both peak around 6 - 8 hours, the alkaline silica being slightly higher. A second peak occurs with alkaline silica at 24 hours, the levels falling thereafter. After the initial peak and fall, the low pH silica rises steadily to 48 hours. The broken line represents the serum silicon levels in control animals. Note that throughout the period studied, the serum silicon in the tested animals remained at least 10 times that of the controls.

● pH 5.3

o pH 10 - 11

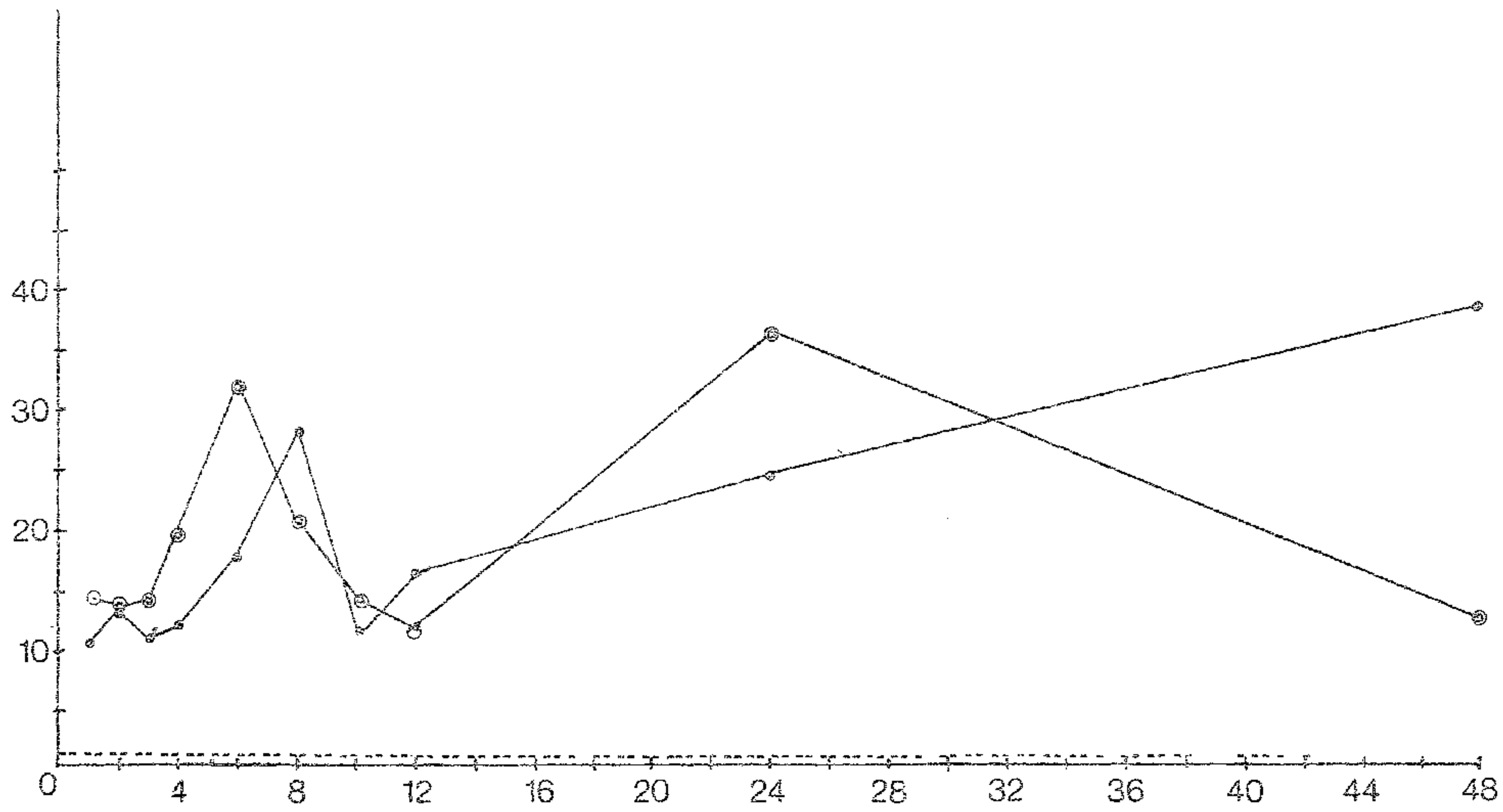

40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
44
48

Figure 50 : Ashed tissue levels of silicon (µg/g) for Kidney, Liver and Spleen in animals after a single intraperitoneal injection of 100 mg gasil 200 at pH 5.3, followed over a 48 hour period.

● Kidney

o Liver

■ Spleen

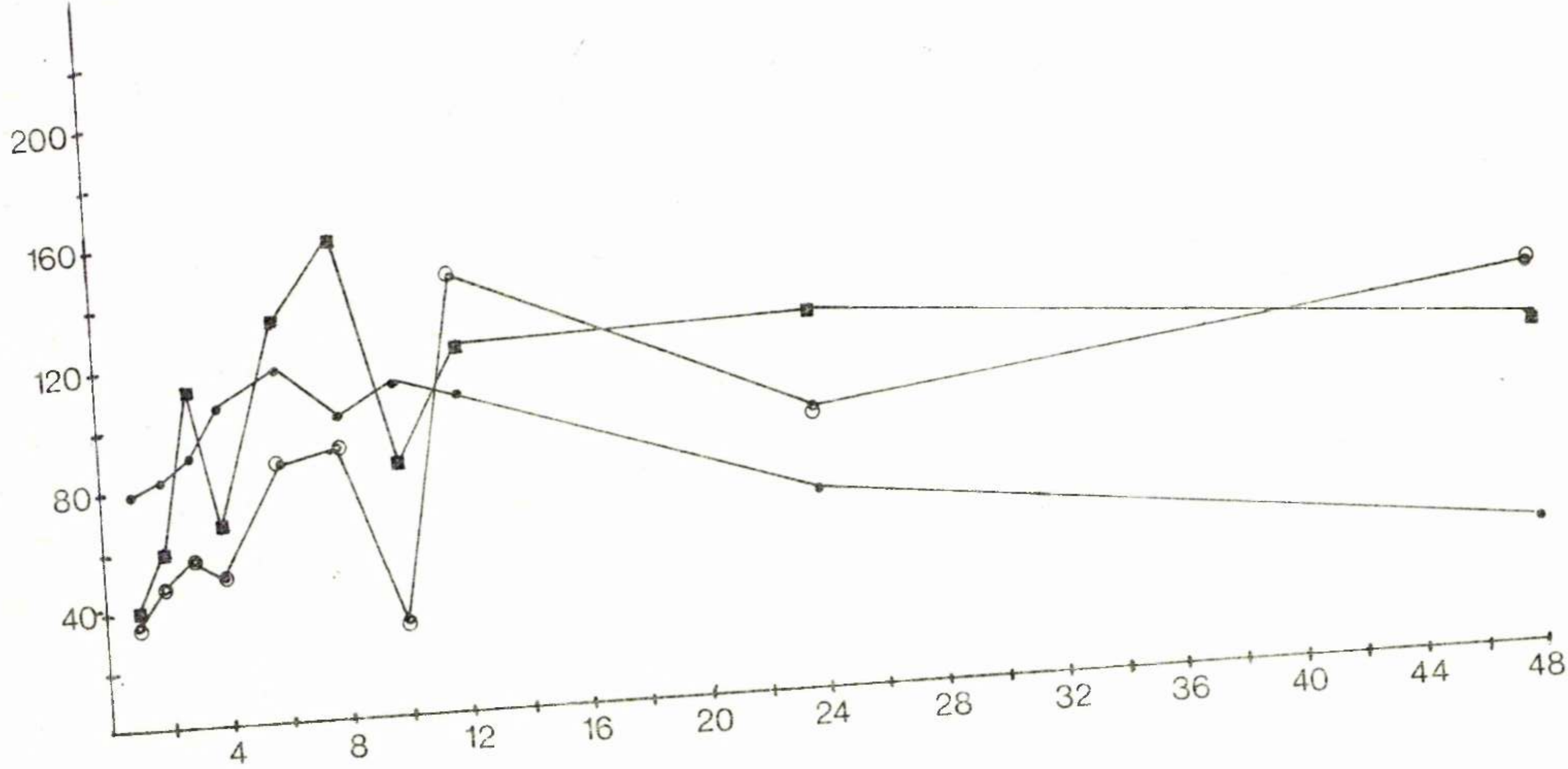
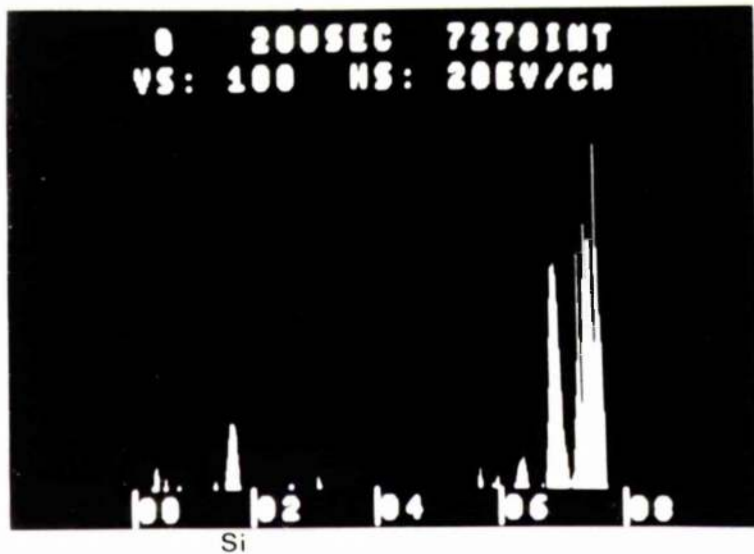

200
160
120
80
40
4
8
12
16
20
24
28
32
36
40
44
48

Figure 52: Electron probe micro-analysis of control glomeruli.

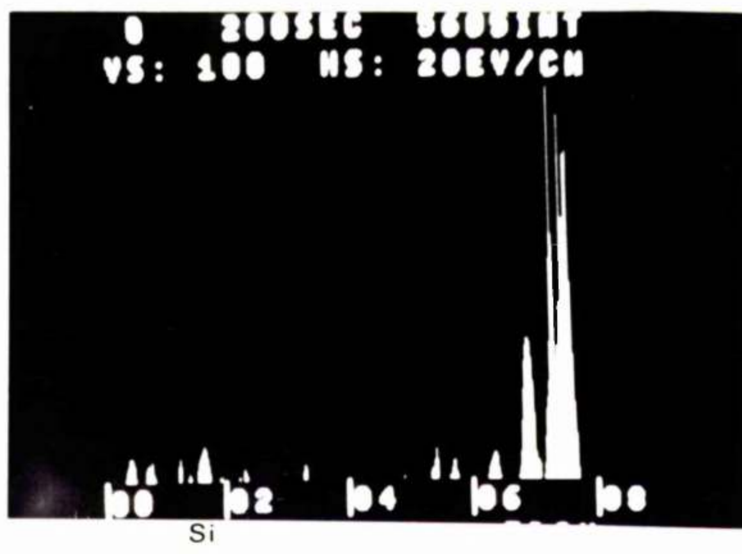
A) Foot process.

B) Basement membrane.

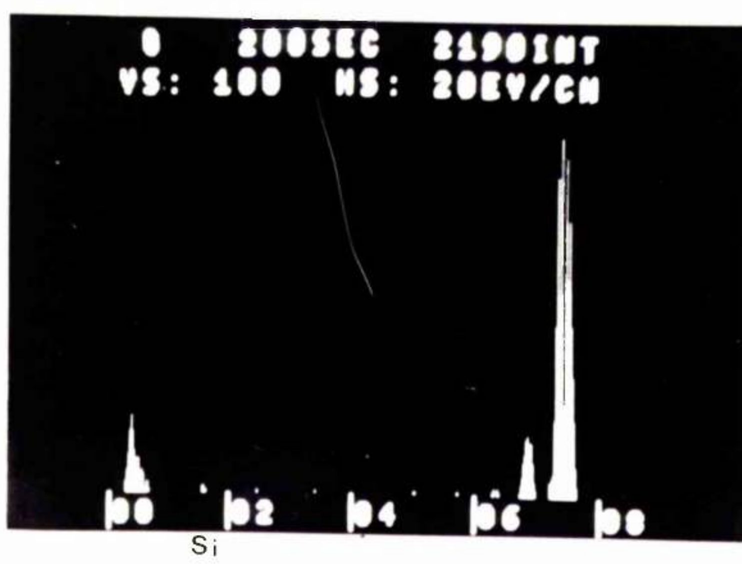
C) Capillary space.



A



B



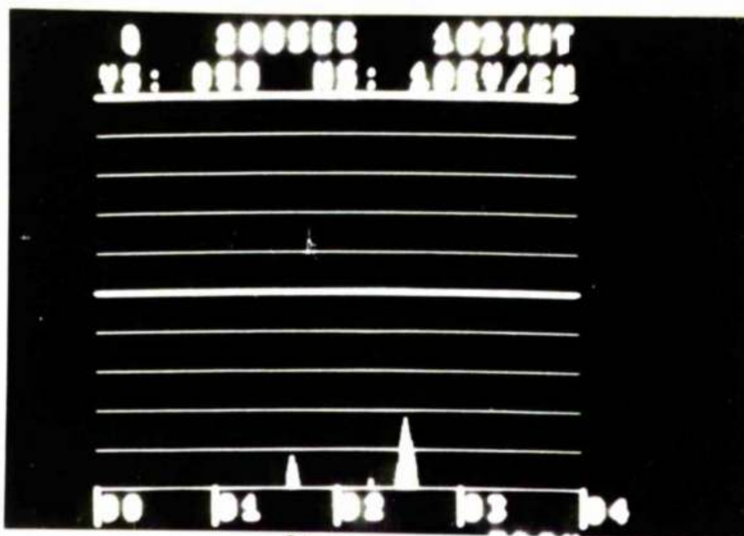
C

Figure 53: Electron proble micro-analysis of glomeruli in tested animals 4 hours after a single intraperitoneal injection of 100 mg gasil 200.

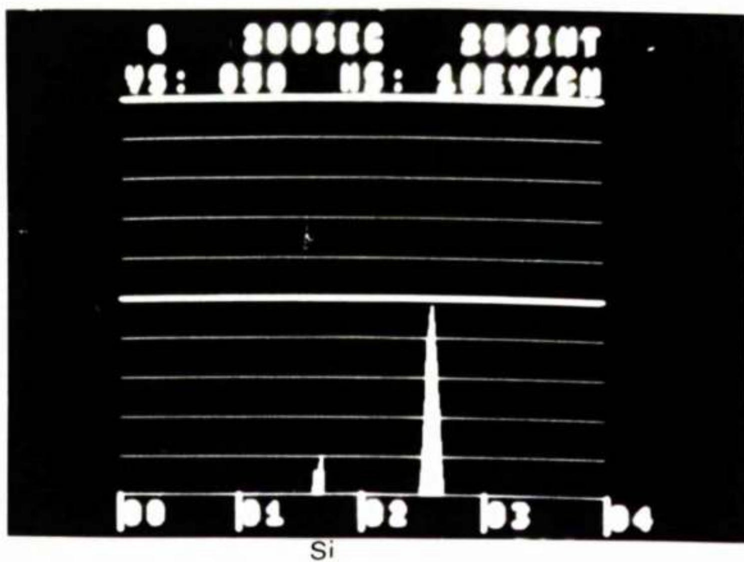
A) Foot process.

B) Basement membrane.

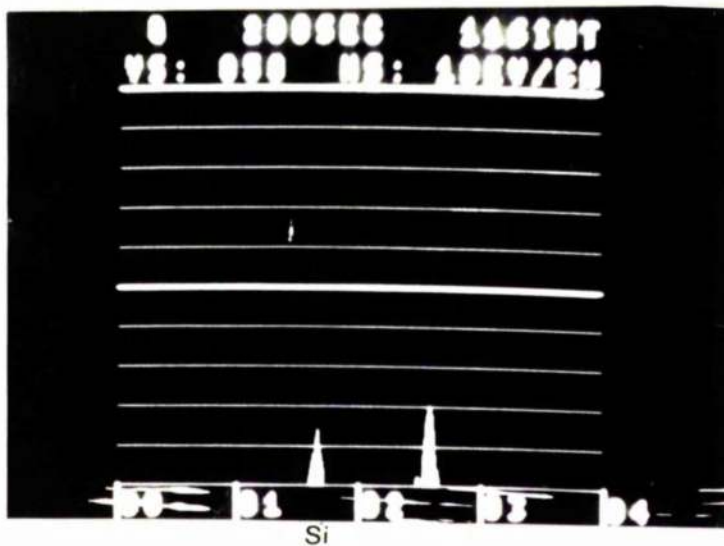
C) Capillary space.



A



B



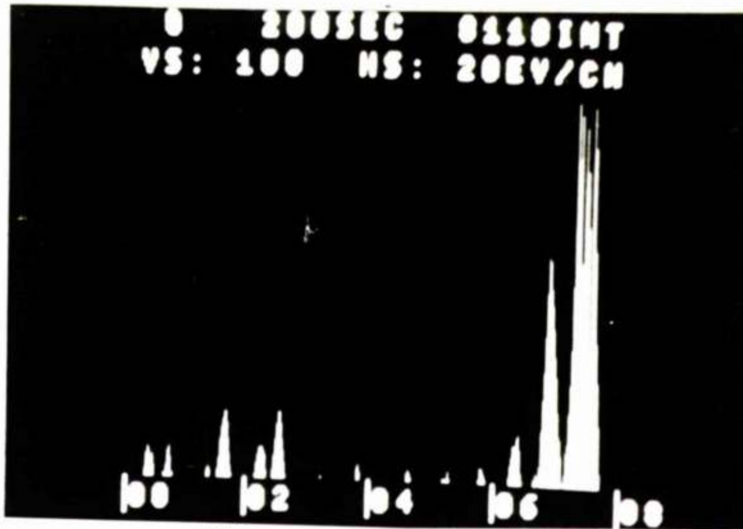
C

Figure 54: Electron probe micro-analysis of glomeruli in tested animals 24 hours afer a single intraperitoneal injection of 100 mg gasil 200.

A) Foot process.

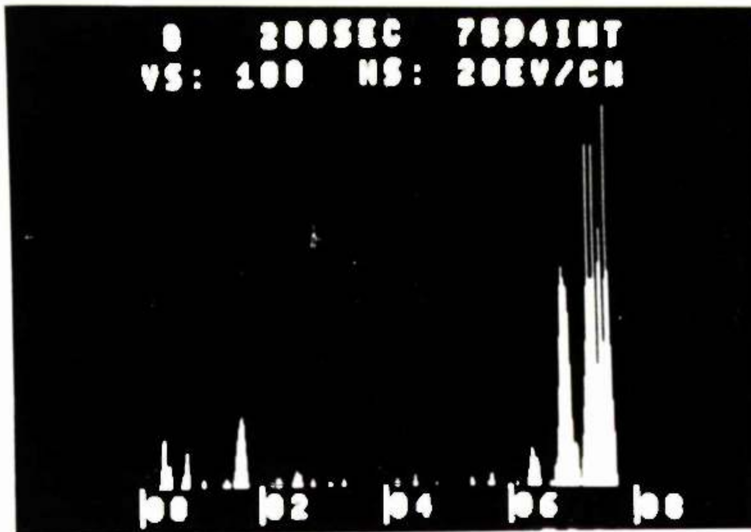
B) Basement membrane.

C) Capillary space.



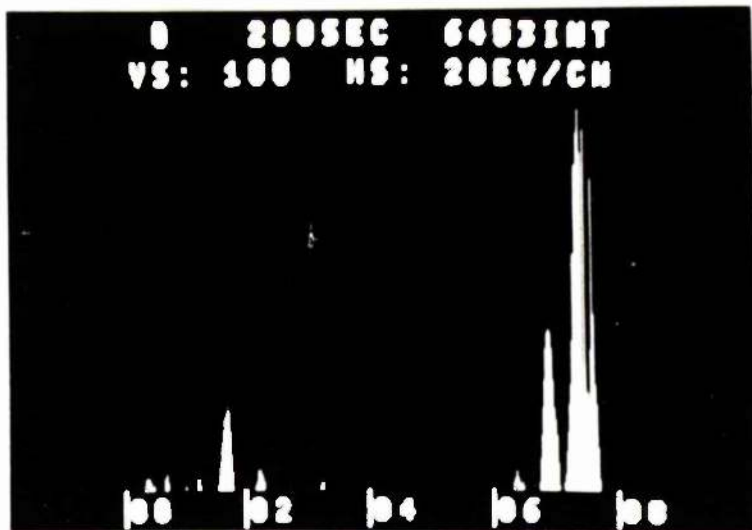
Si

A



Si

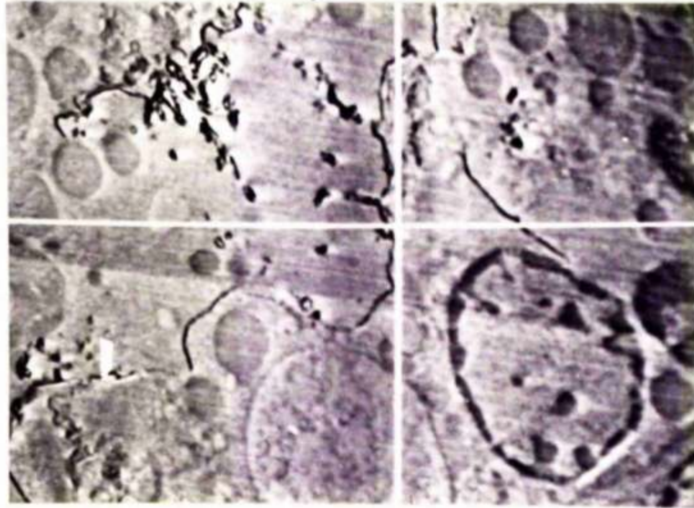
B

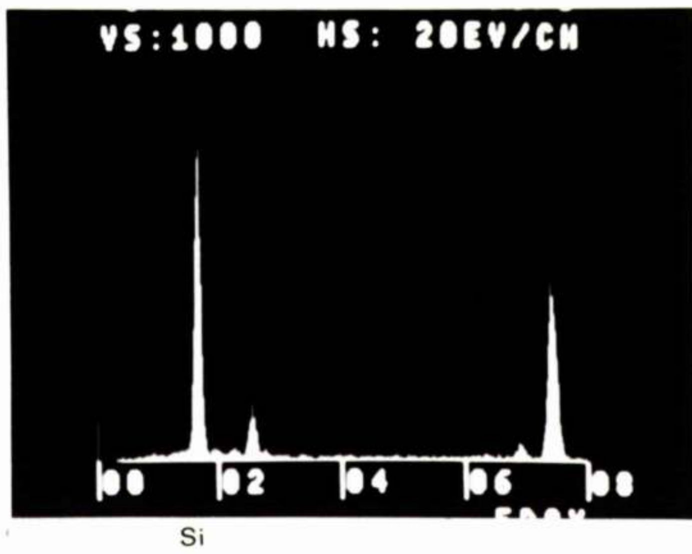


Si

C

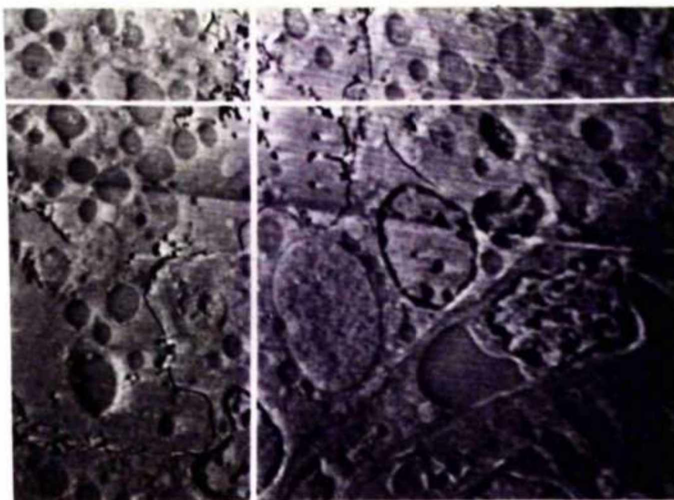
Figure 55: Electron probe micro-analysis of distal tubule in tested animals 4 hours after a single intraperitoneal injection of 100 mg gasil 200. A high silicon peak was detected directly over the cell membrane at the lumenal surface.

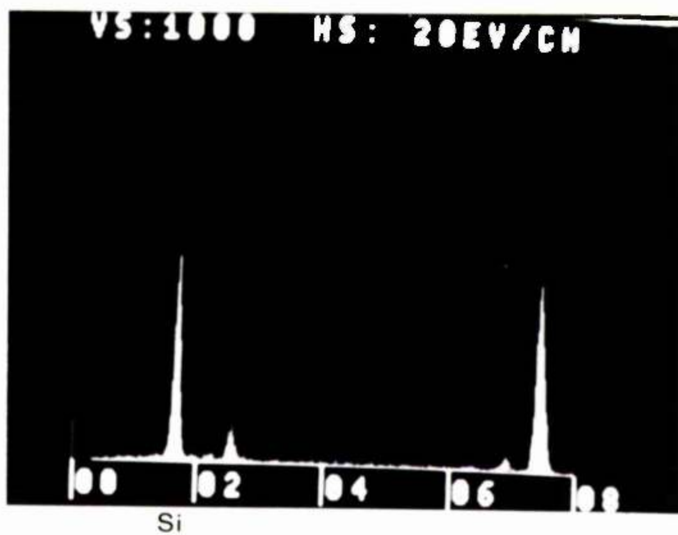




Si

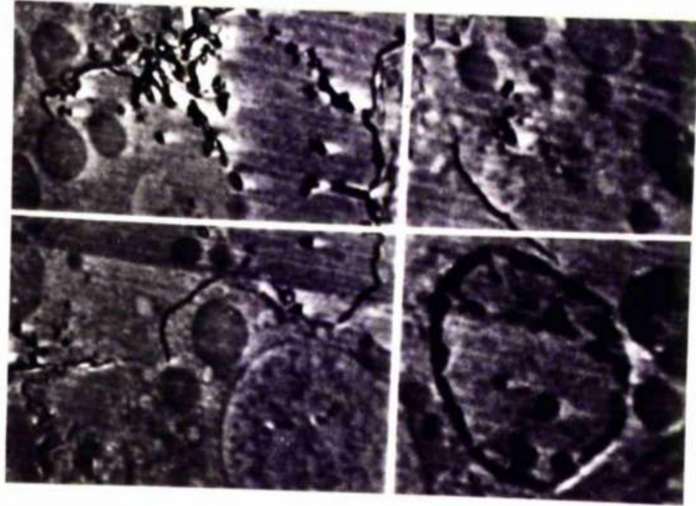
Figure 56: Electron probe micro-analysis of distal tubule in tested animals 4 hours after a sinle intraperitoneal injection of 100 mg gasil 200. A high silicon peak was detected over the membrane of the microvillous projections on the lumenal surface.





Si

Figure 57: Electron probe micro-analysis of distal tubule in tested animals 4 hours after a single intraperitoneal injection of 100 mg gasil 200. No silicon was detected, as shown here, within the cytoplasm of the epithelial cell.



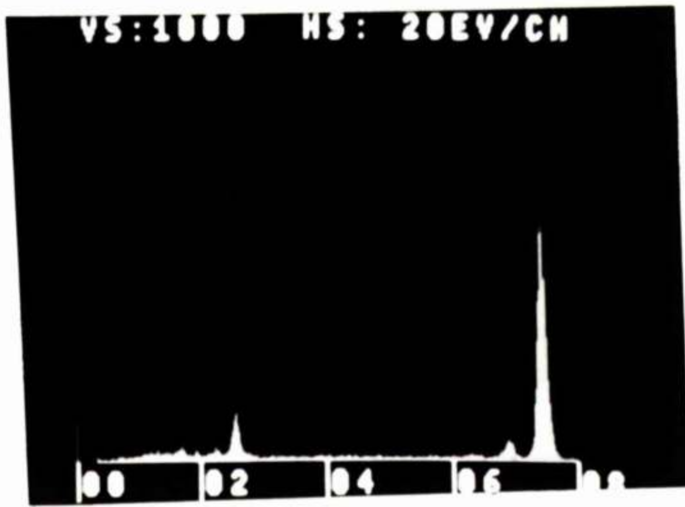
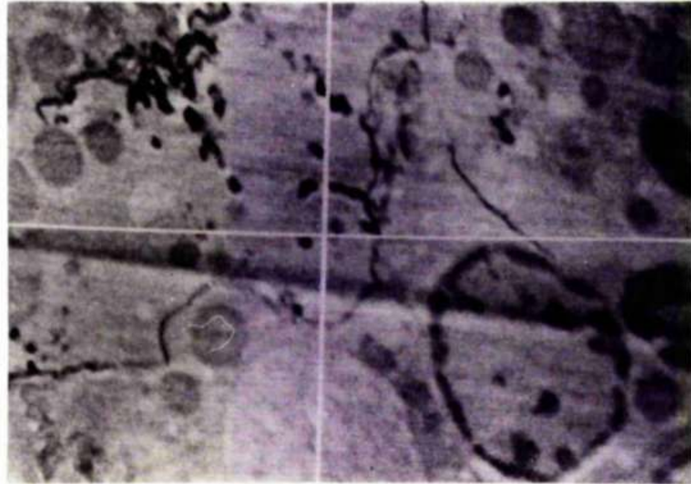

VS:1000 HS: 20EV/CM
00
02
04
06

Figure 58: Electron probe micro-analysis of distal tubule in tested animals 4 hours after a single intraperitoneal injection of 100 mg gasil 200. No silicon was detected, as shown here, within the lumenal cavity.



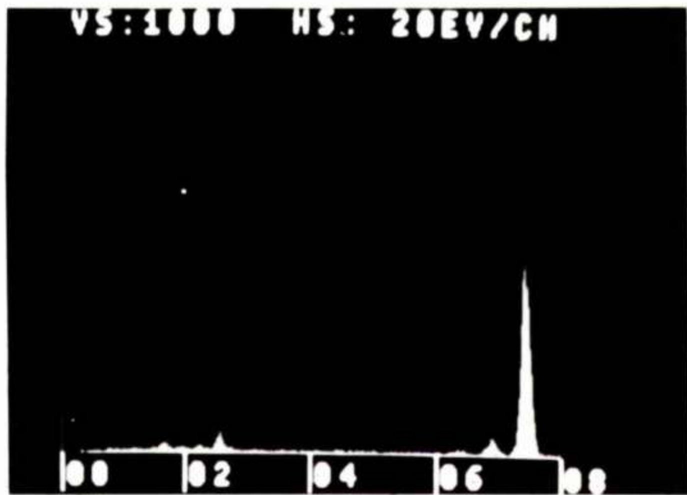

VS:1000 HS: 20EV/CM
00
02
04
06
08

Figure 59: Autoradiograph 3 hours following injection of ^{31}Si showing activity mainly located in the lumena of tubules and possibly capillaries of the corticomedullary region.

X 375

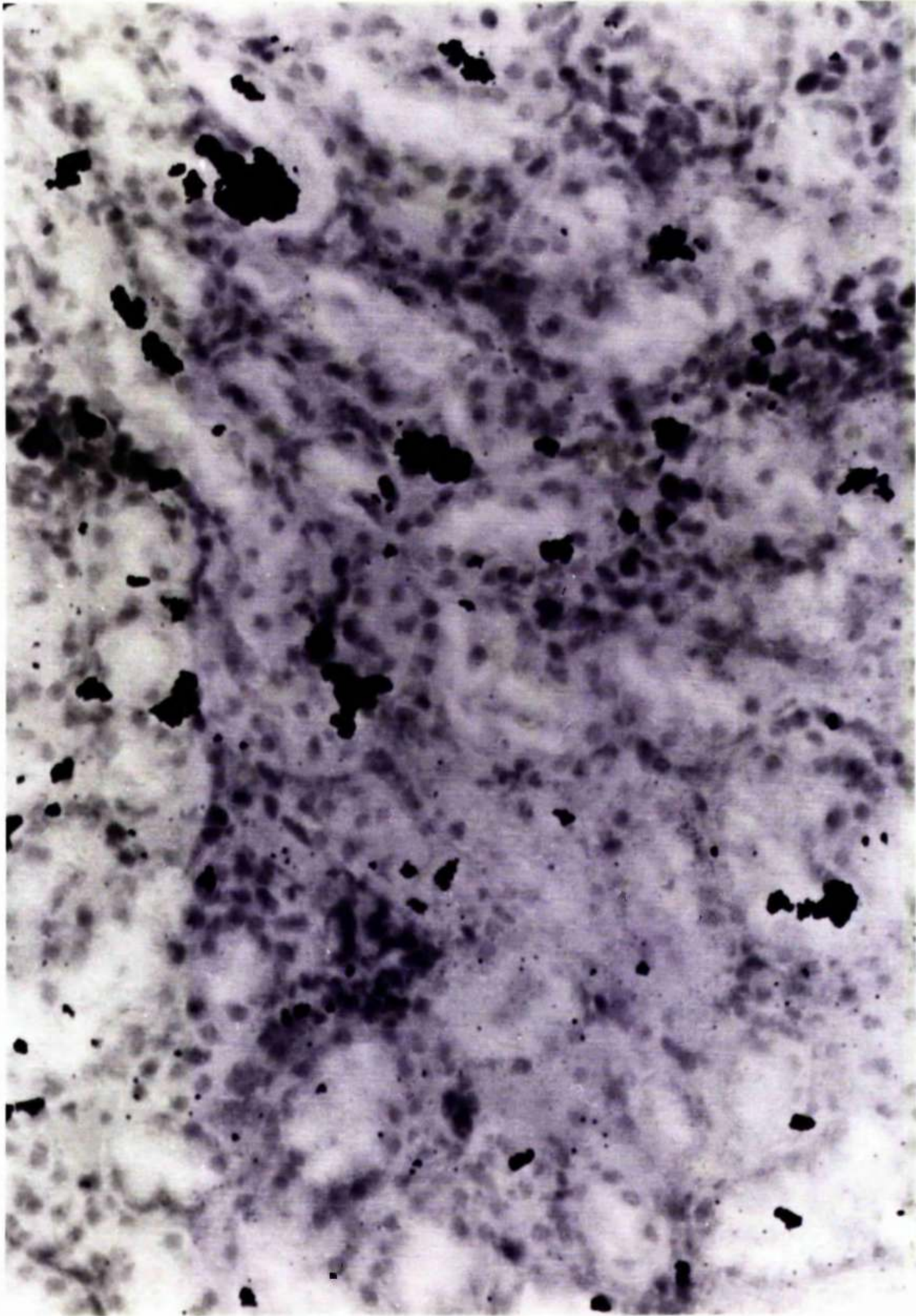


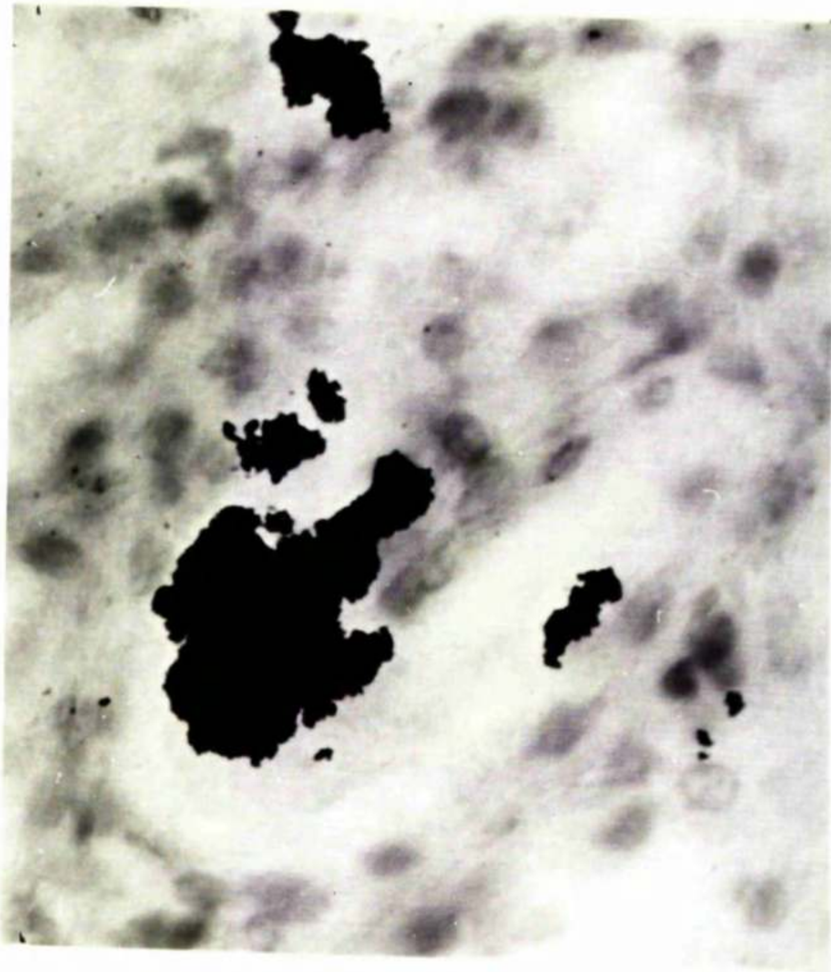
Figure 60: Autoradiographs taken 3 hours following injection of ^{31}Si showing

A) Activity in the lumen of a J-shaped portion of distal tubule.

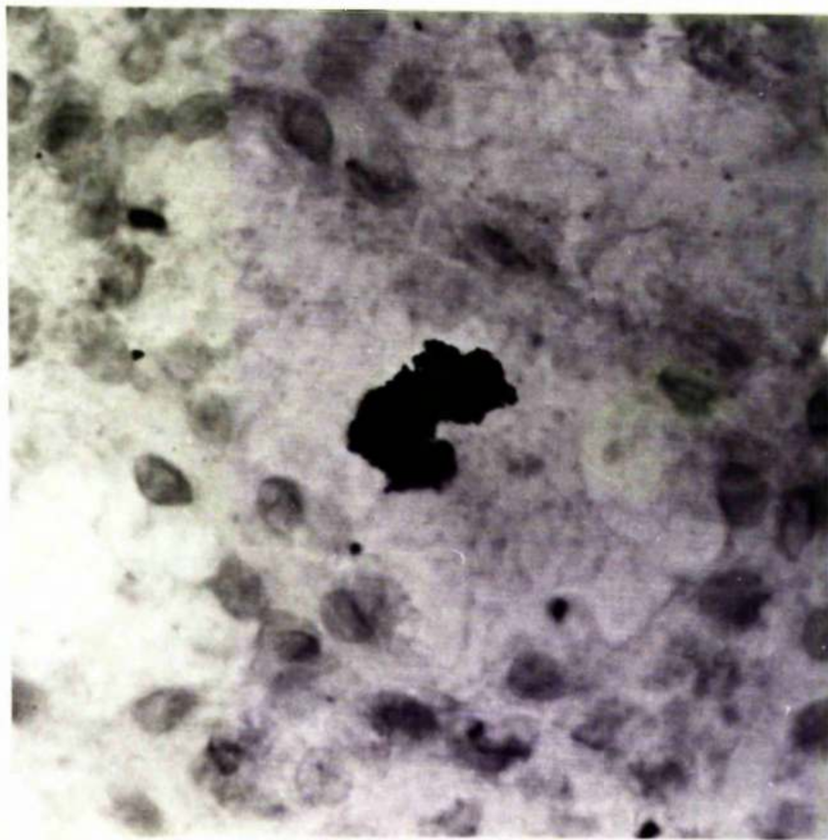
Mag. X 940.

B) Activity mainly in lumen of a collecting duct but also faint activity located over lumenal surface of the epithelial lining.

Mag. X 940



A



B

Figure 61: Gasil 200: 60 mg: 3 Months:

Low power photomicrograph showing irregular outer surface with focal subcapsular and corticomedullary tubular dilatation and interstitial inflammatory infiltrate.

H & E Mag. X 86.

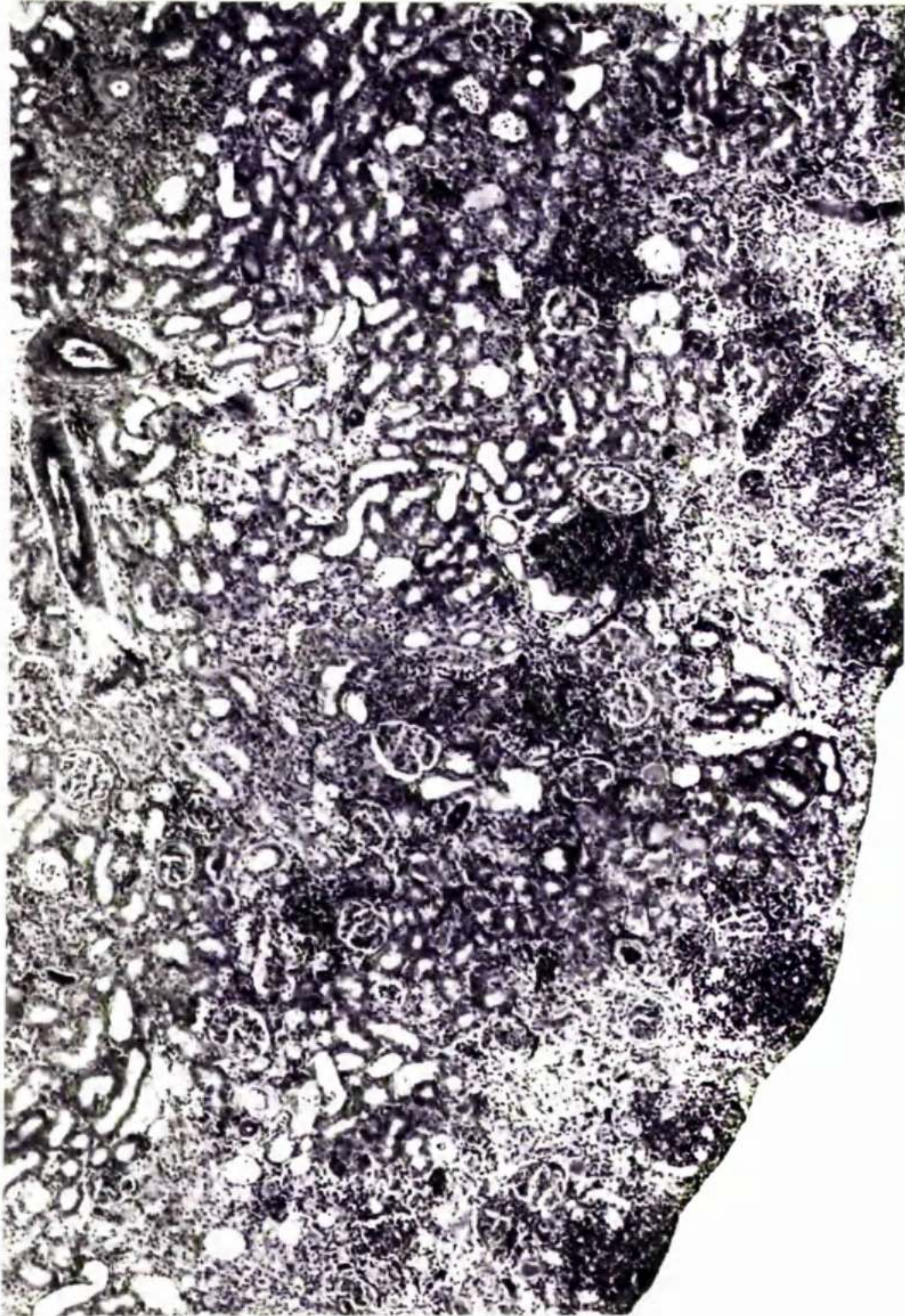


Figure 62: Gasil 200: 60 mg: 2 Months:

Corticomedullary region showing dilatation of the distal tubules which are lined by flattened epithelium. The lumena contain acute inflammatory and desquamated epithelial cells. There is, in addition, a diffuse interstitial inflammatory infiltrate.

H & E Mag. X 345.

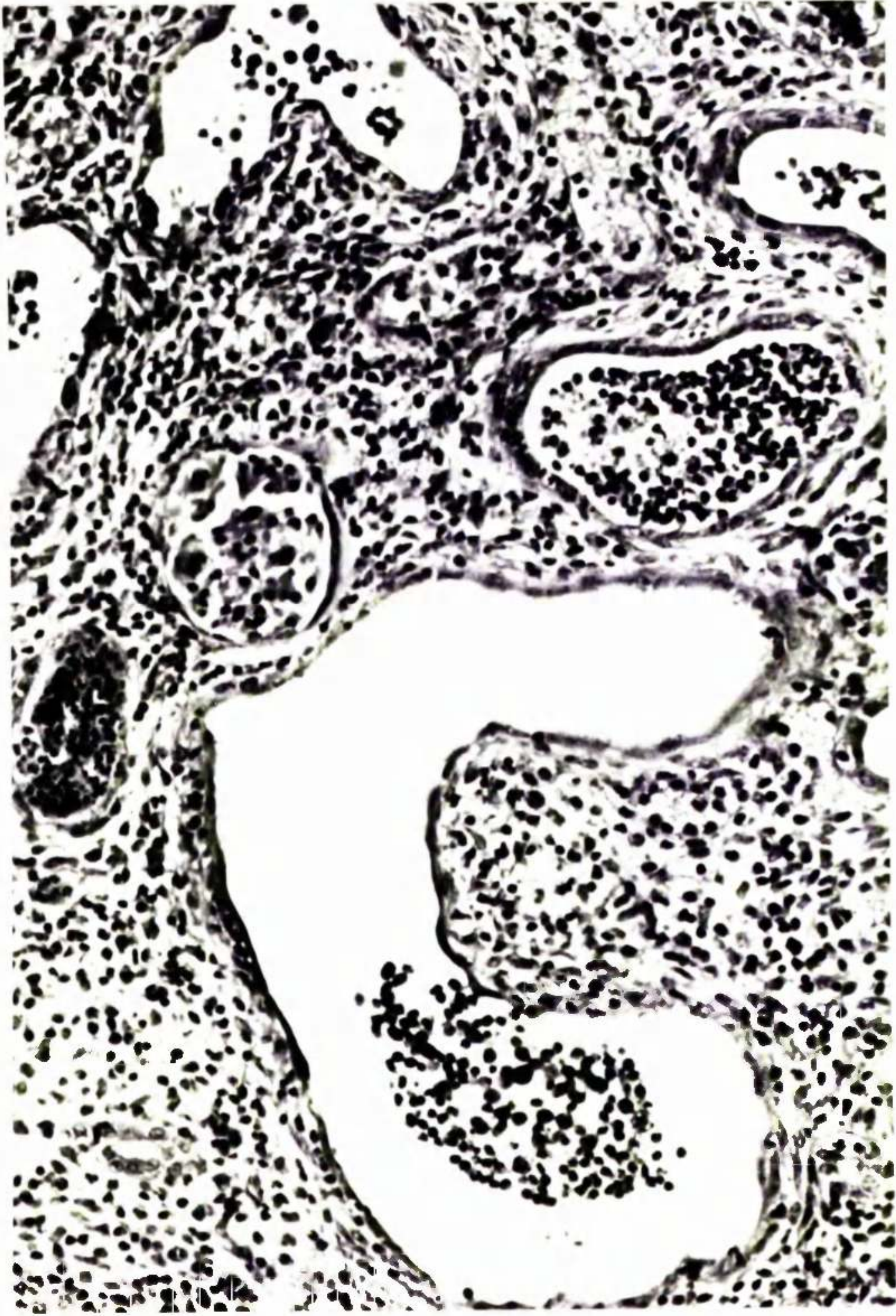


Figure 63: Gasil 200: 60 mg: 3 Months:

Shows corticomedullary region with dilated distal and collecting tubules. The lumena of the affected tubules contain inflammatory and desquamated epithelial cells while the lining epithelium shows swelling or flattening and variable nuclear changes. Early peritubular fibrosis and moderate interstitial inflammatory infiltrate are also in evidence.

H & E Mag. X 345

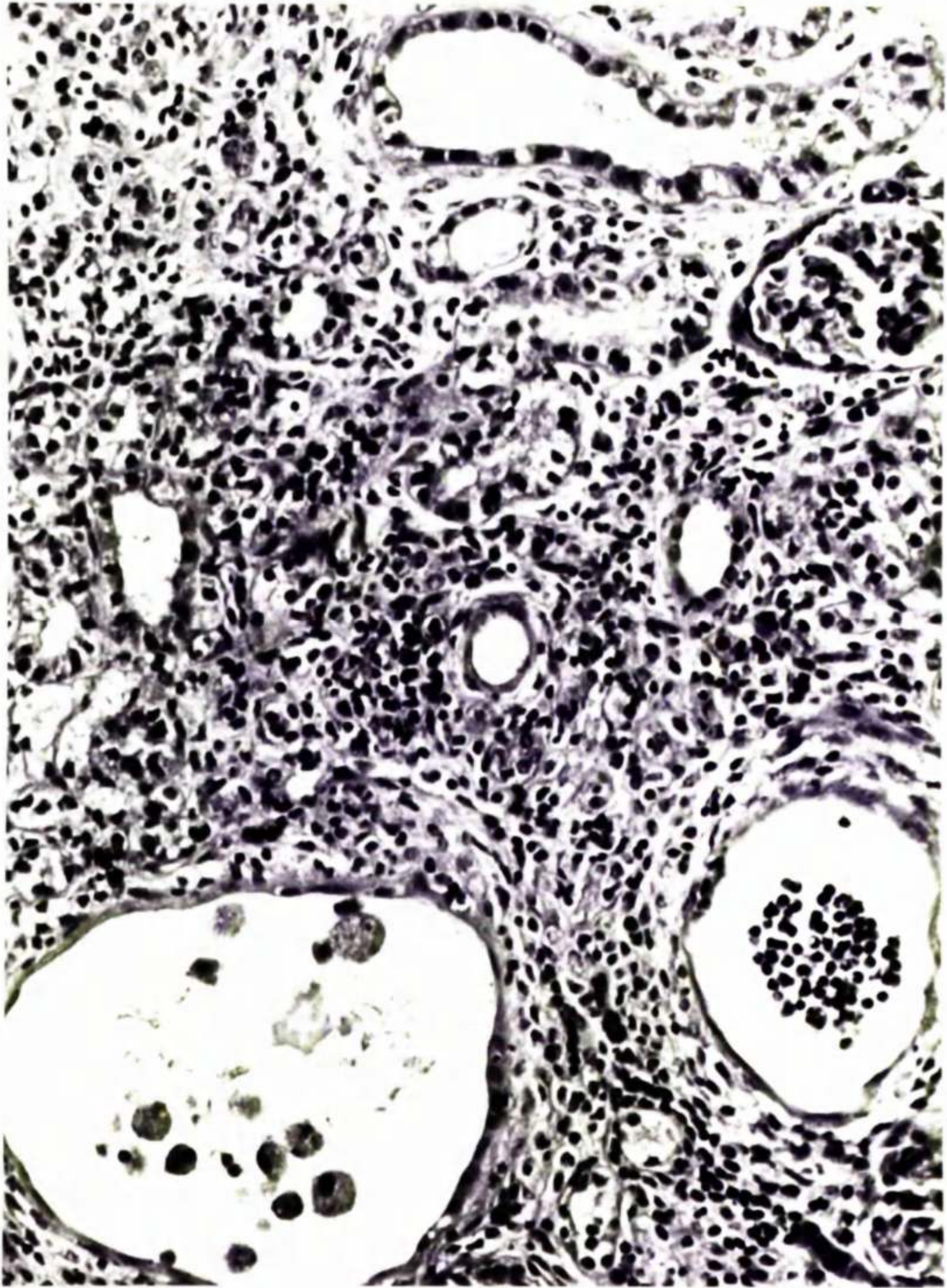


Figure 64: Gasil 200: 60 mg: 2 Months:

Medulla, demonstrating dilated collecting ducts and thick loop of Henle. The dilated ducts are filled with acute inflammatory and desquamated epithelial cells. The epithelial lining is oedematous with cytoplasmic and nuclear vacuolation. There is also an acute interstitial inflammatory infiltrate.

H & E Mag. X 345.

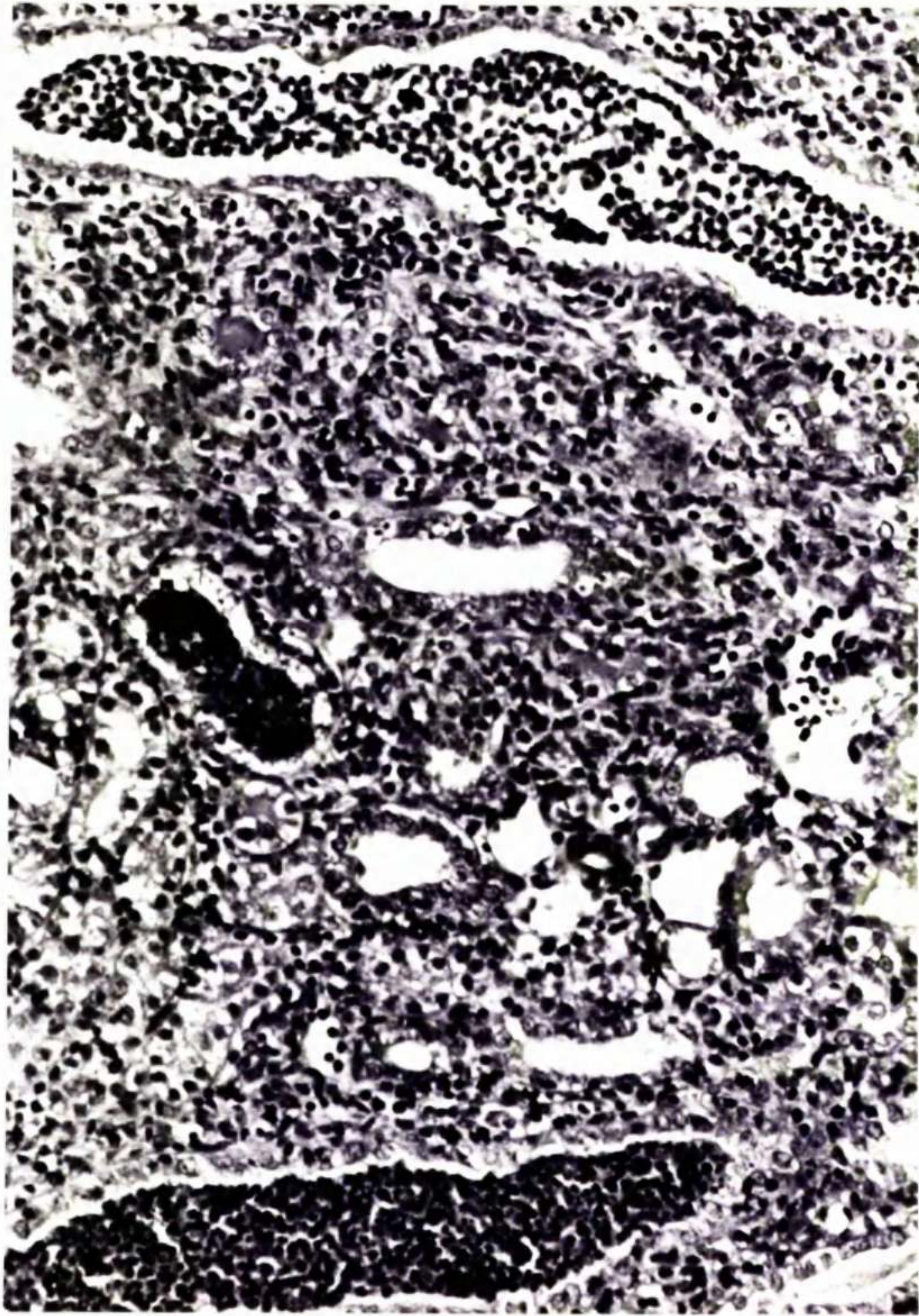


Figure 65: Gasil 200: 60 mg: 3 Months:

Corticomedullary region demonstrating marked tubular damage and destruction - with a widespread inflammatory infiltrate bordering on abscess formation in some areas.

H & E Mag. X 345.

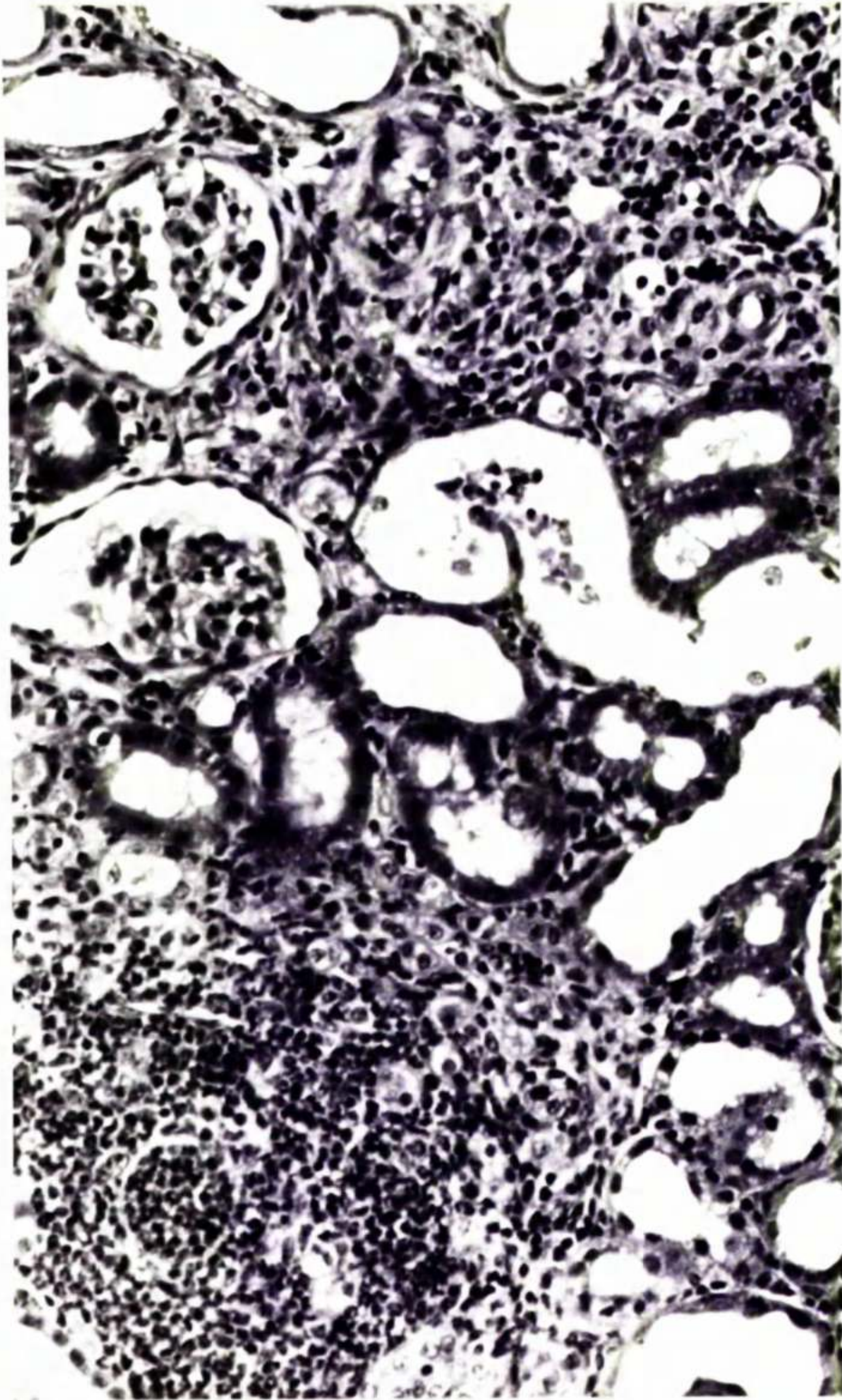


Figure 66: Gasil 200: 60 mg: 3 Months:

Corticomedullary region showing marked peritubular fibrosis and chronic interstitial inflammatory infiltrate.

H & E Mag. X 345

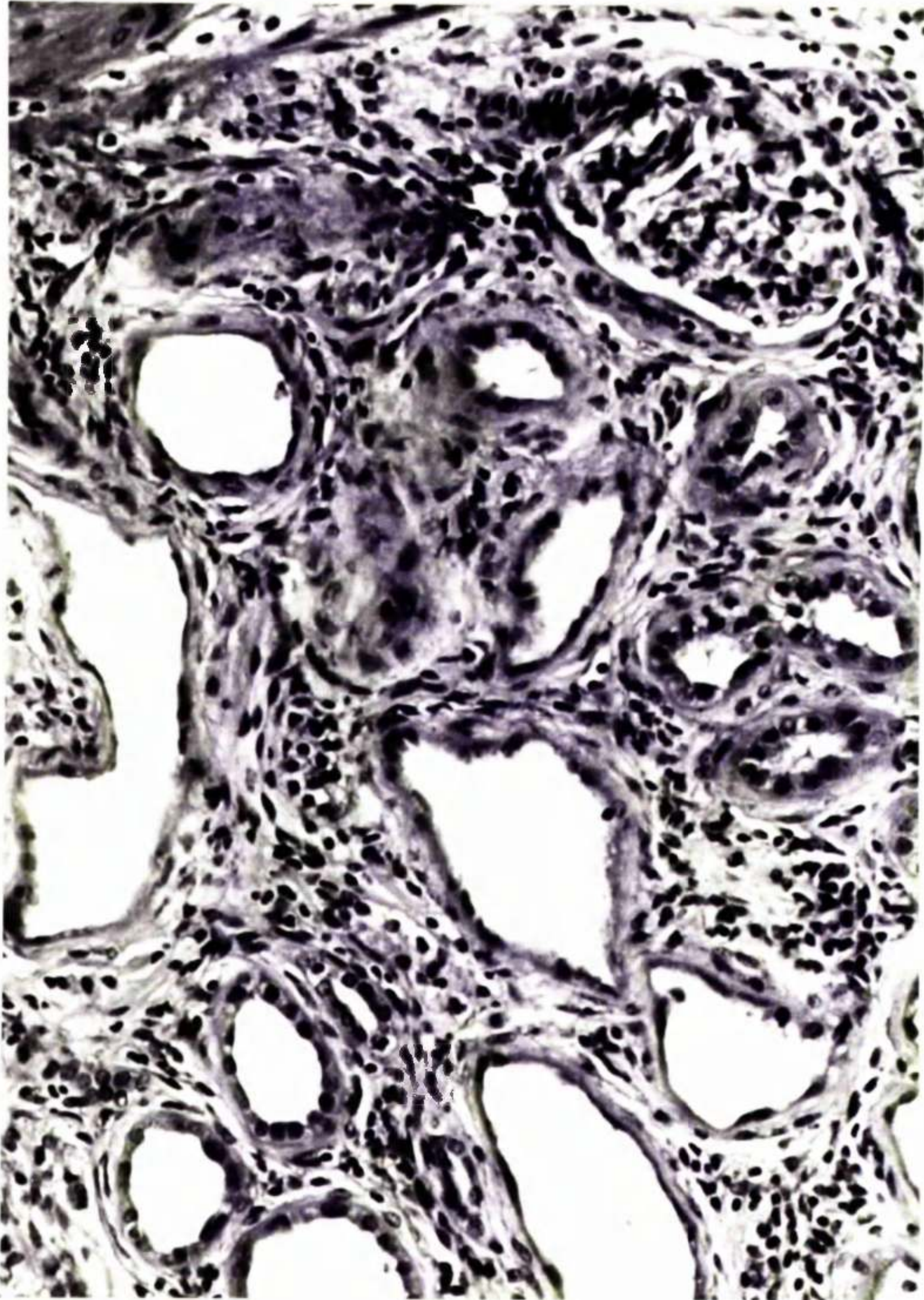


Figure 67: Gasil 200: 60 mg: 5 Months:

Corticomedullary region demonstrating marked tubular dilatation and cast formation, the lining epithelium is flattened. A widespread acute and chronic inflammatory cellular infiltrate is observed. Slight dilatation of Bowman's space and early thickening of their capsules are also in evidence.

H & E Mag. X 138

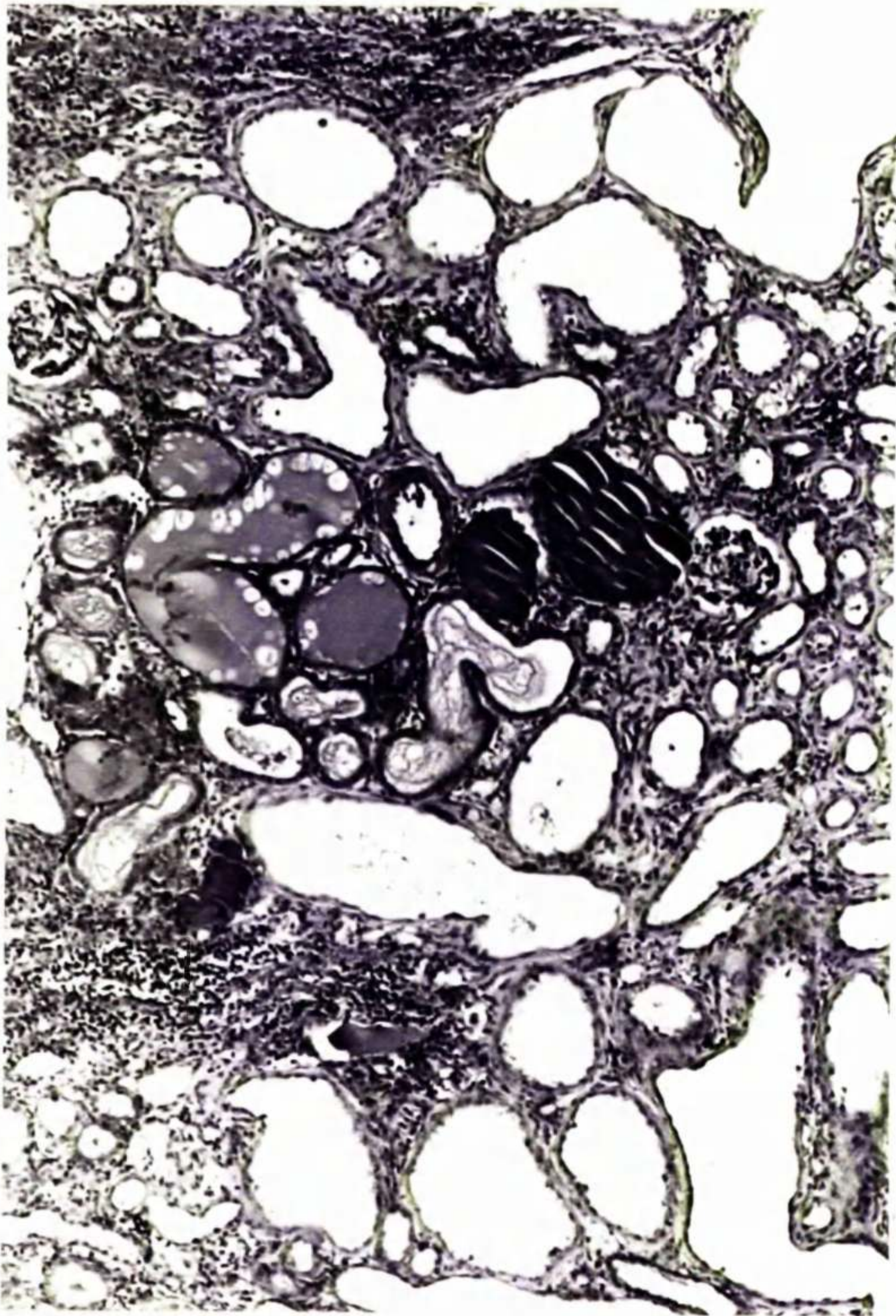


Figure 68: Gasil 200: 60 mg: 5 Months:

Renal papillae demonstrating marked oedema and light interstitial inflammatory infiltrate. Dilatation of the papillary ducts which are lined by transitional epithelium deep into the medullary region.

H & E Mag. X 138.

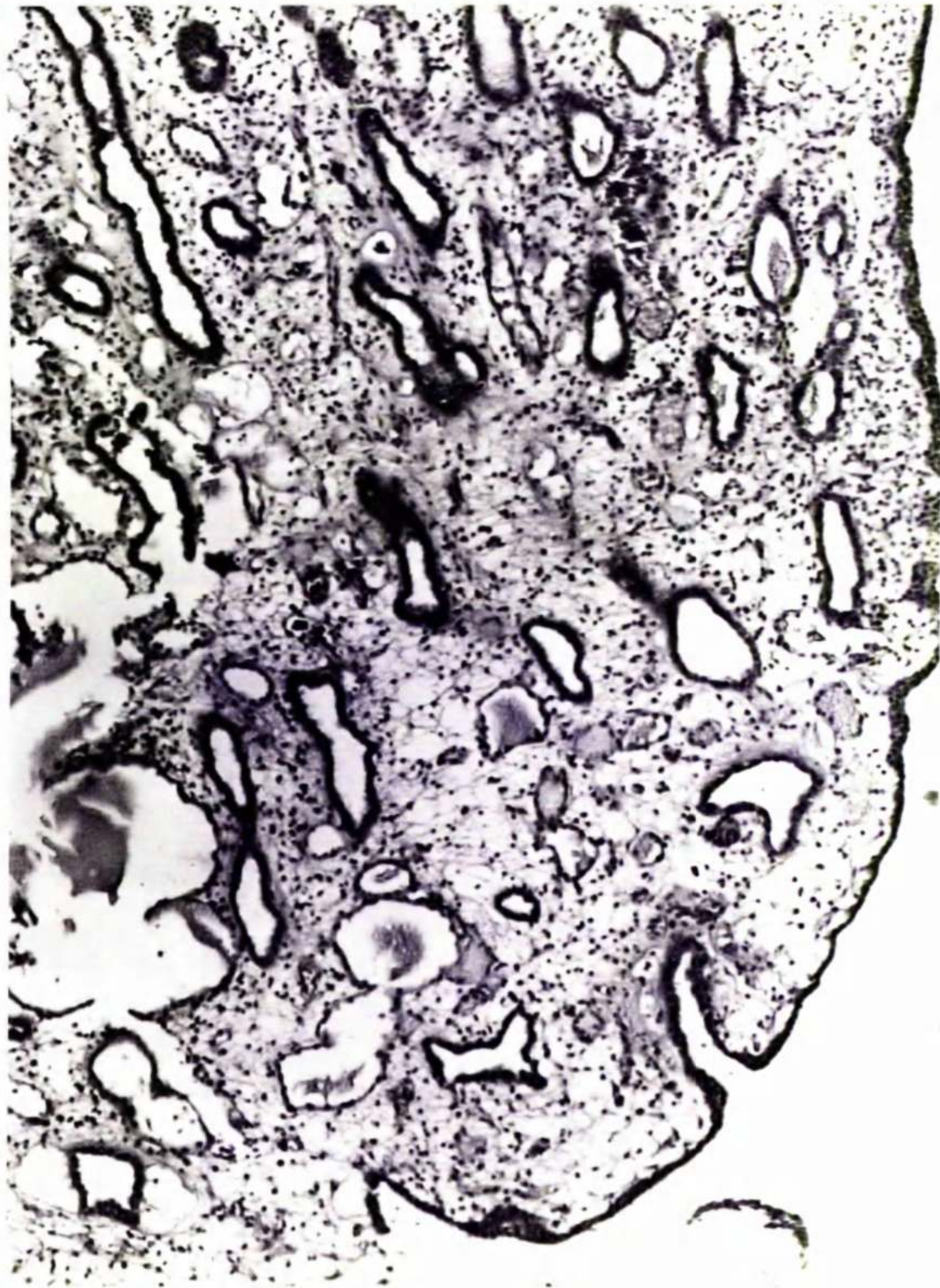


Figure 69: Gasil 200: 60 mg: 9 Months:

Low power photomicrograph of a whole kidney section showing widespread tubular dilatation in both cortex and medulla. The cortex is thinned and its outer surface irregular. There is an overall globular contraction of the kidney.

H & E Mag. X 18.5

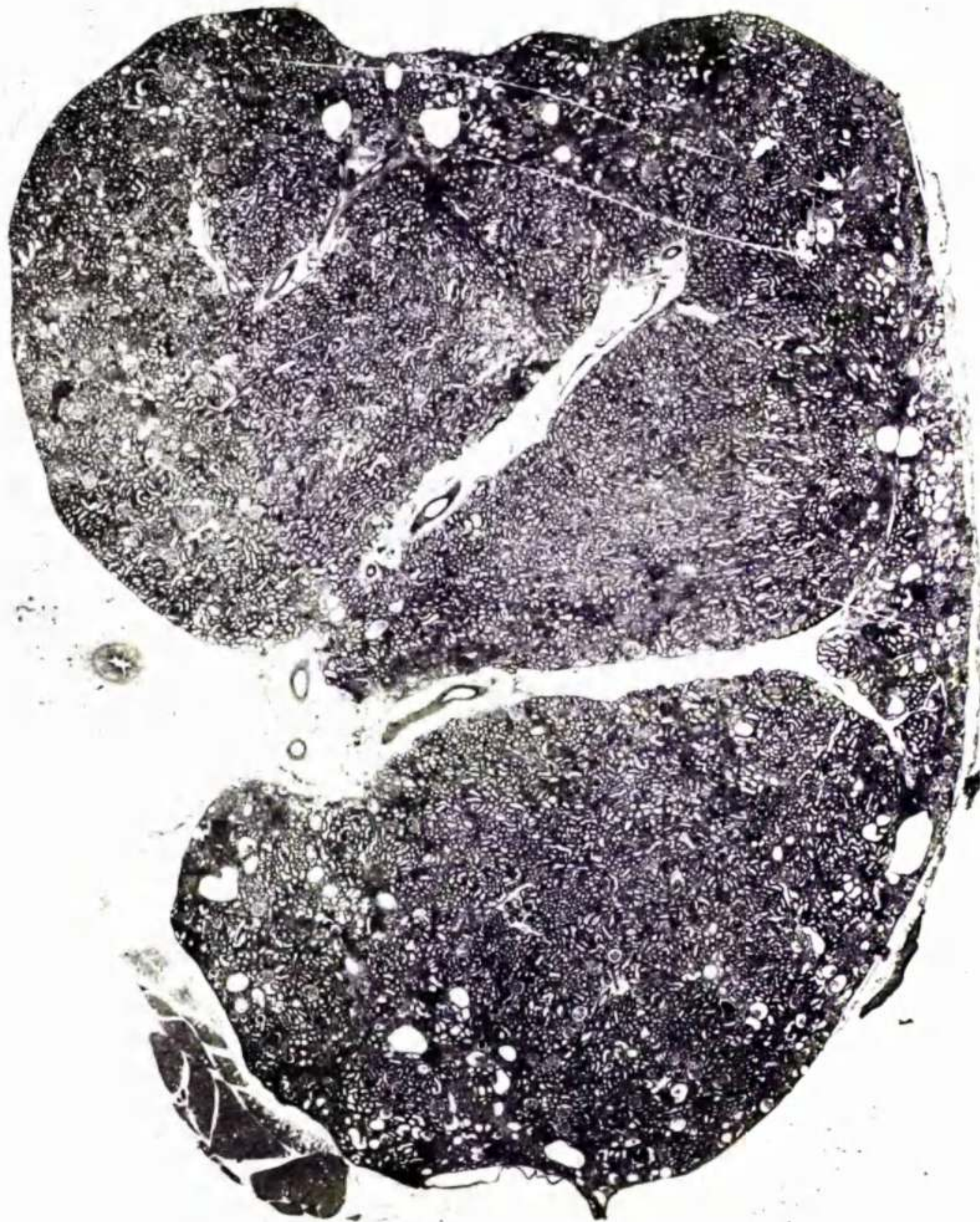


Figure 70: Gasil 200: 60 mg: 9 Months:

Low power photomicrograph of a whole kidney section showing narrowing of the cortex with multiple cyst formation.

H & E Mag. X 19.

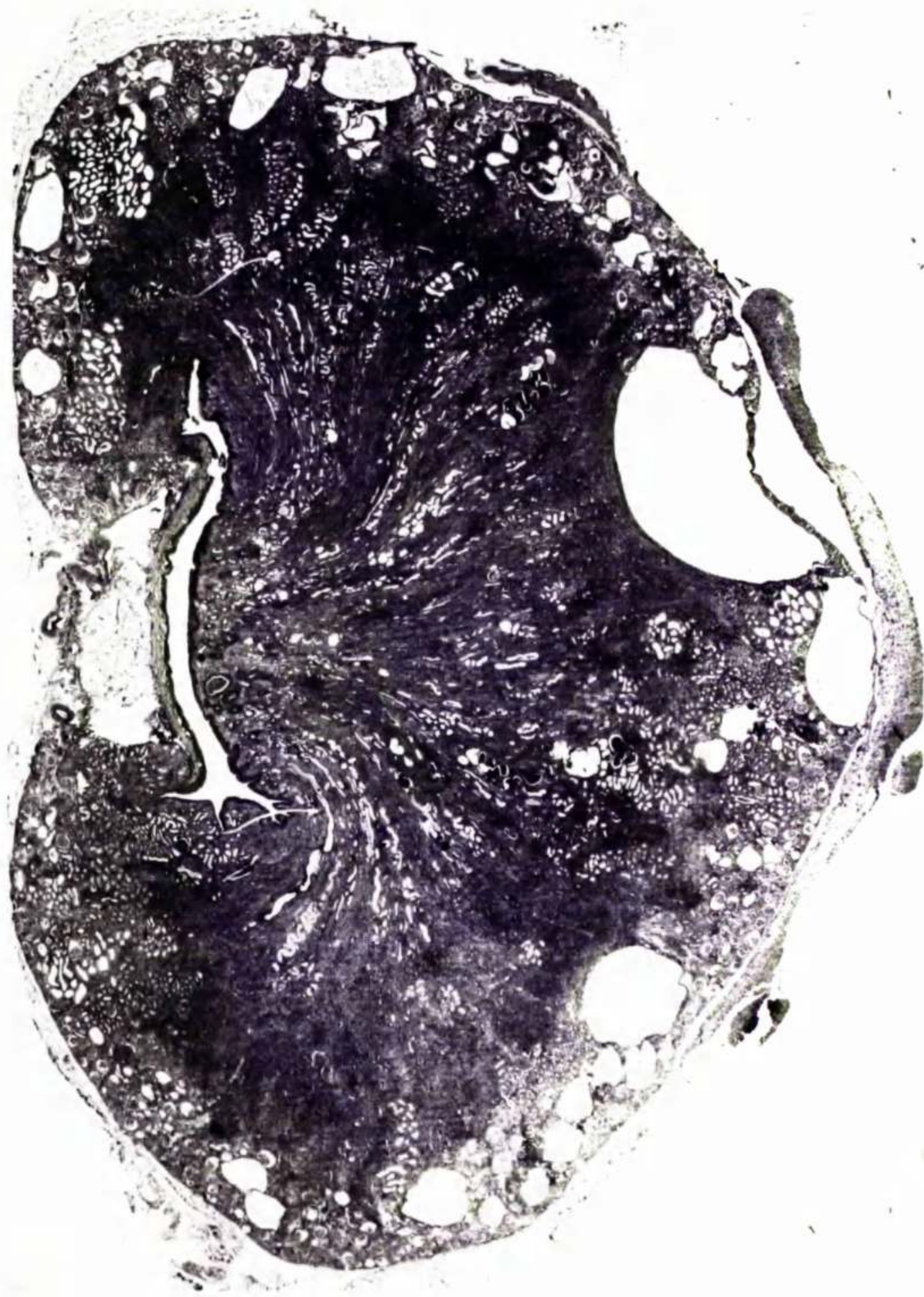


Figure 71: Gasil 200: 60 mg: 9 Months:

Medulla demonstrating extensive damage in the collecting ducts and thick loop of Henle. The epithelial lining shows obvious cytoplasmic swelling and vacuolation together with marked changes. There is gross thickening of the tubular basal lamina, peritubular fibrosis and interstitial inflammatory infiltrate.

H & E Mag. X 860

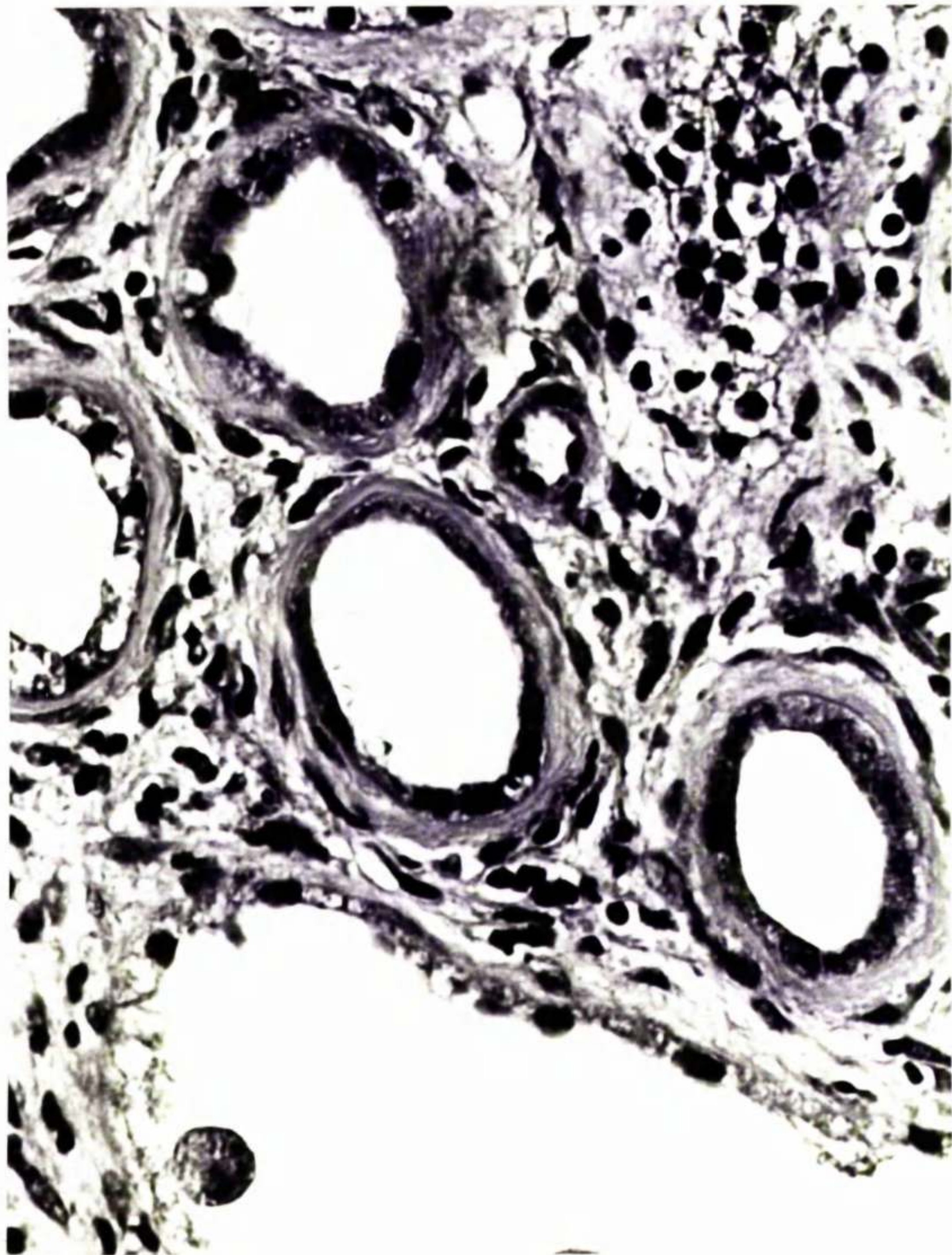


Figure 72: Gasil 200: 60 mg: 9 Months:

Corticomedullary region showing marked tubular loss, cyst formation and extensive interstitial inflammatory infiltrate. The glomeruli show marked dilatation of the urinary space.

H & E Mag. X 138.

Figure 73: Gasil 200: 60 mg: 9 Months:

Subcapsular region: The thin cortex shows glomeruli with marked widening of the Bowman's spaces and thickening of the capsules. The interstitium shows heavy infiltration by acute and chronic inflammatory cells,. Marked tubular loss is also in evidence.

H & E Mag. X 345

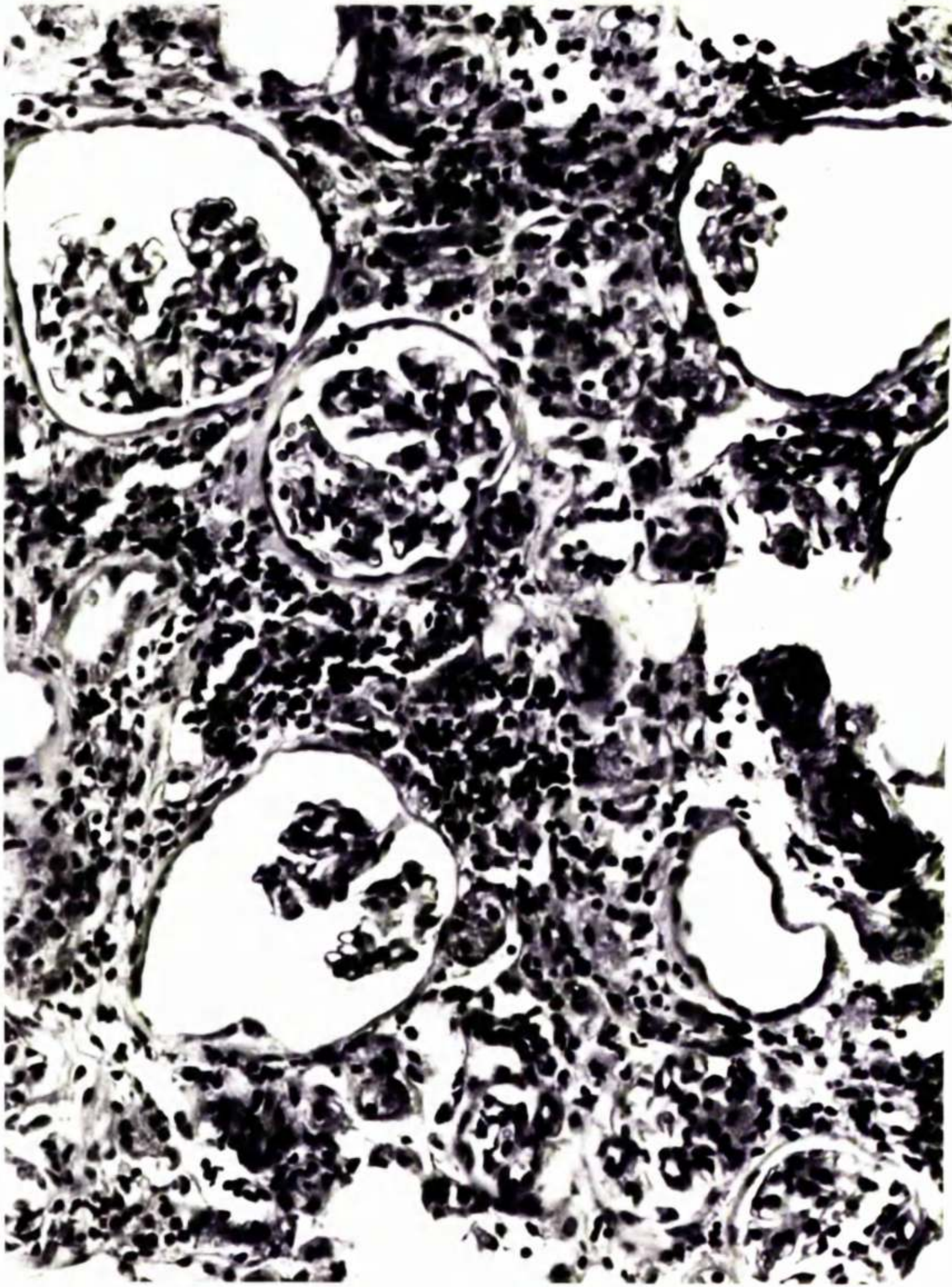


Figure 74: Control rat: Glomerulus:

Normal glomerular ultrastructural architecture.

X 12696.

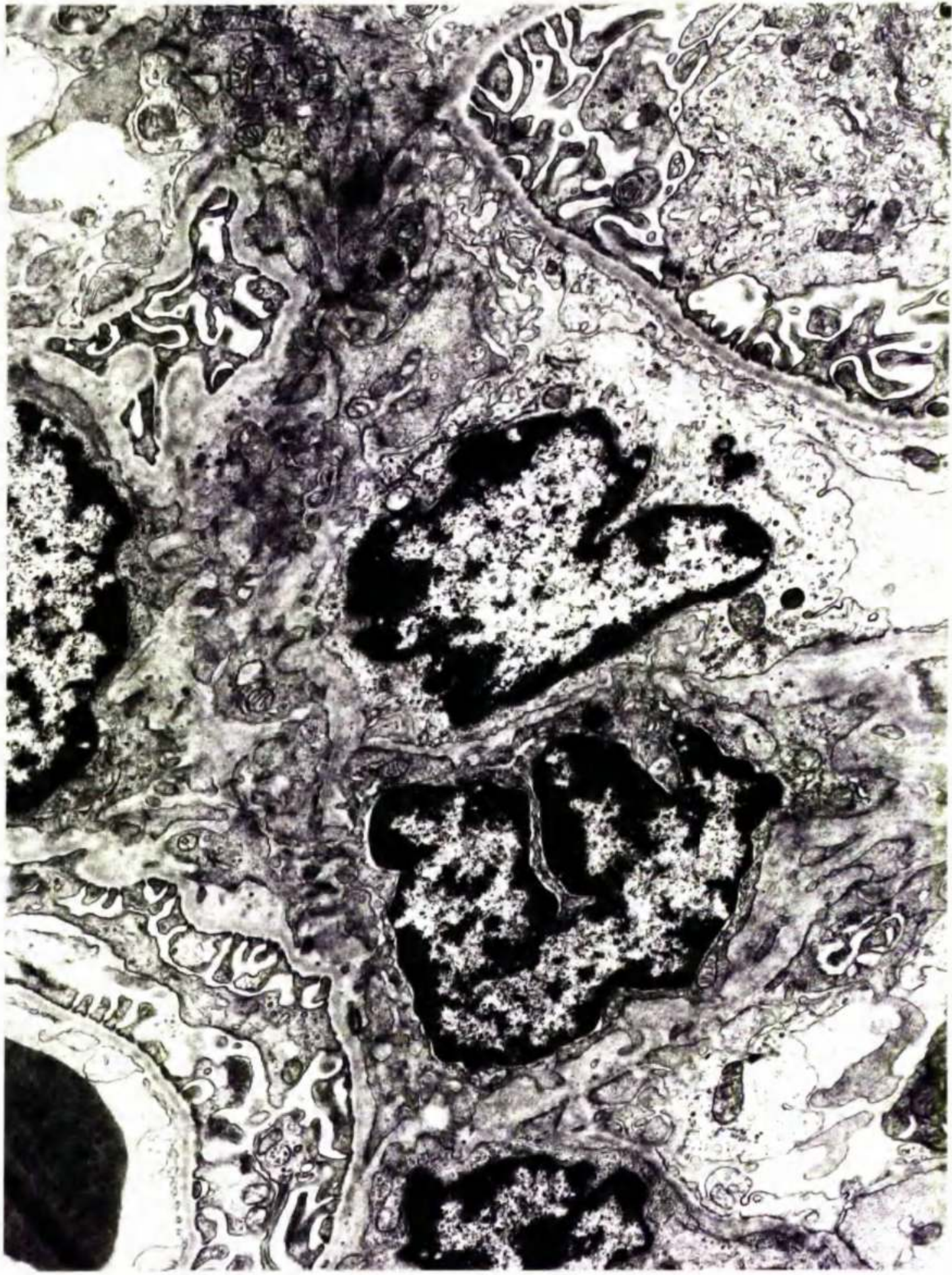


Figure 75: Gasil 200: 60 mg: 1 Month:

Glomerulus showing marked electron dense deposits in the paramesangial subendothelial region.

X 20930.

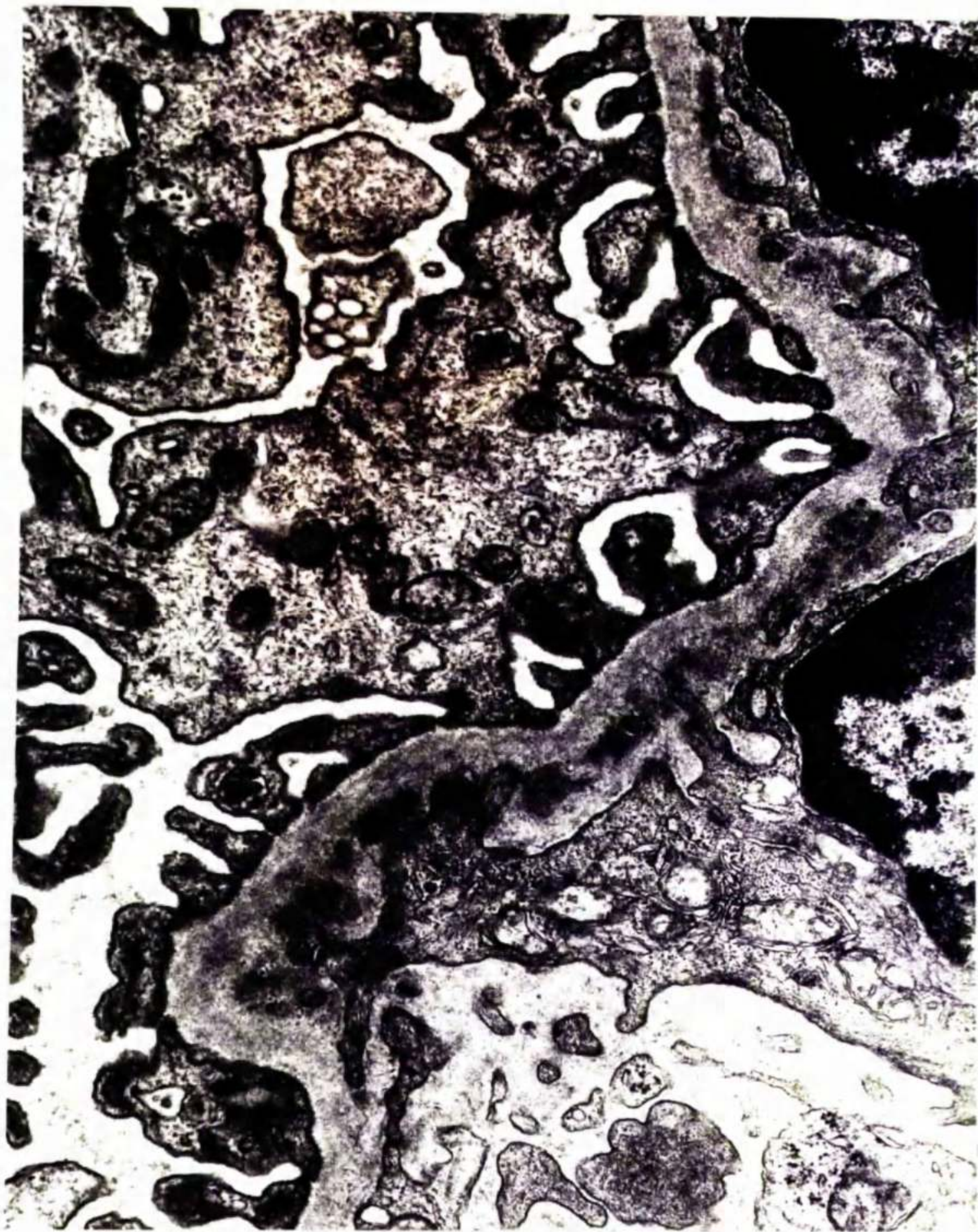


Figure 76: Gasil 200: 20 mg: 1 Month:

Glomerulus demonstrating small scattered electron dense mesangial deposits.

X 12696.

Figure 77: Gasil 200: 20 mg: 2 Months:

Glomerulus showing a visceral epithelial cell which contains a collection of cytoplasmic multilamellated bodies.

X 61870.

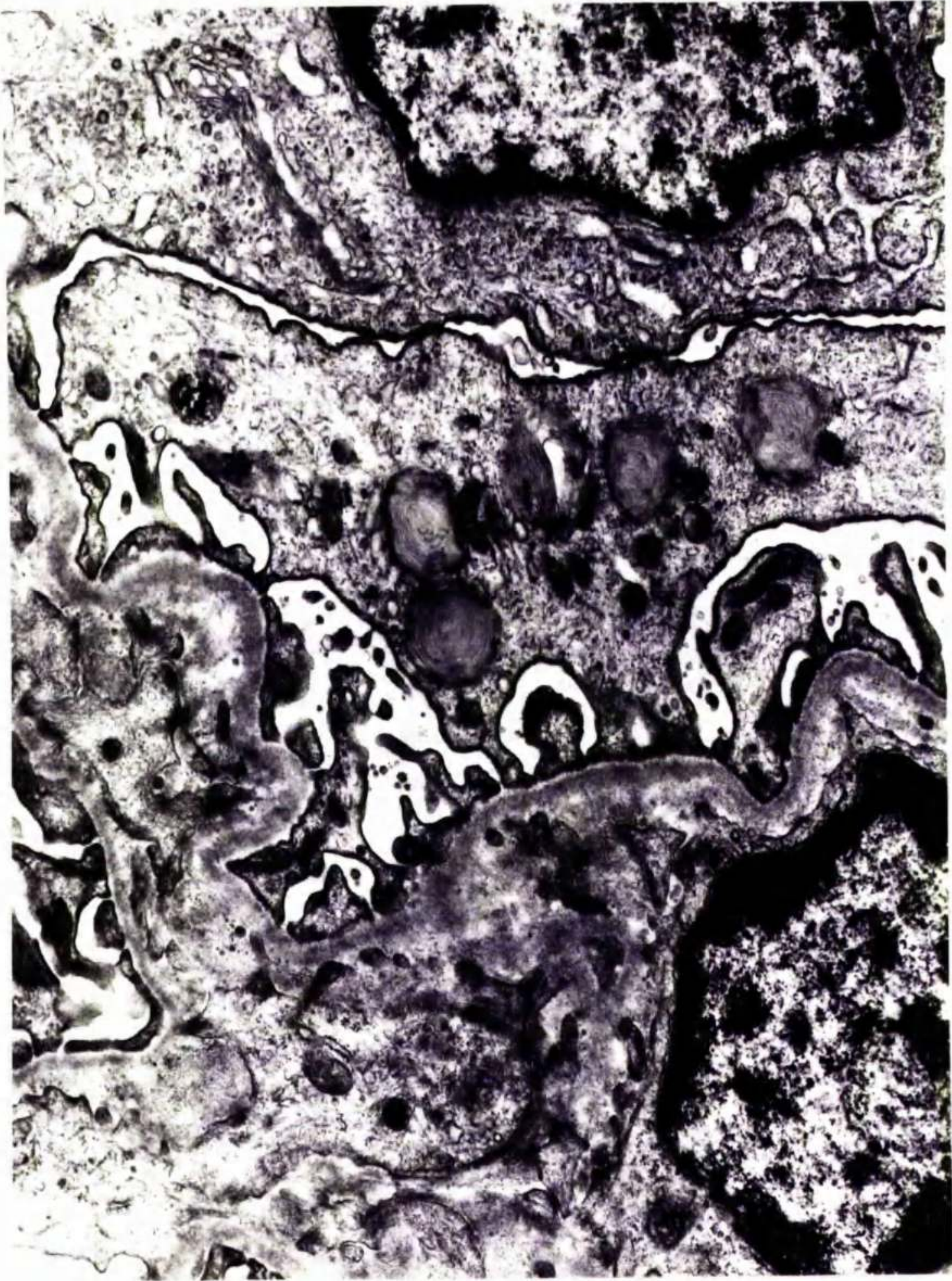


Figure 78: Gasil 200: 20 mg: 2 Months:

Thick loop of Henle showing a thickened convoluted basal lamina. There are multiple intracytoplasmic vacuoles and increased cytolysosomal structures. The intercellular space is widened.

X 12696.

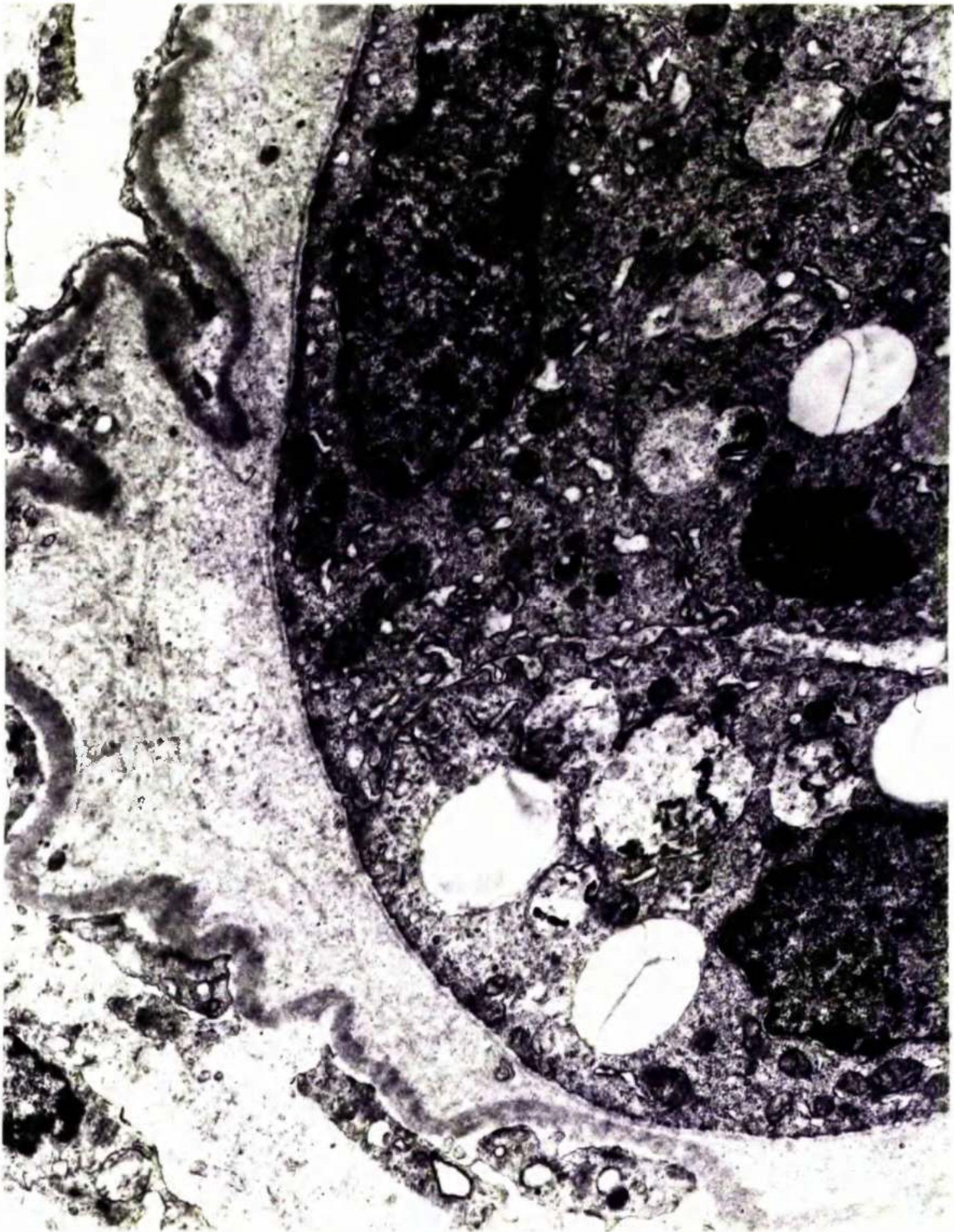


Figure 79: Gasil 200: 60 mg: 2 Months:

Segment of wall of a markedly dilated collecting duct showing the composition of its lumenal contents. On the ~~left~~ right there is a degenerate epithelial cell, on the ~~right~~ left a polymorphonuclear leucocyte. The ~~upper~~ lower cell which shows an irregular nucleus containing inclusions is possibly a macrophage. The epithelial lining of the collecting duct shows extensive vacuolation and prominent lysosomal structures.

X 3864.

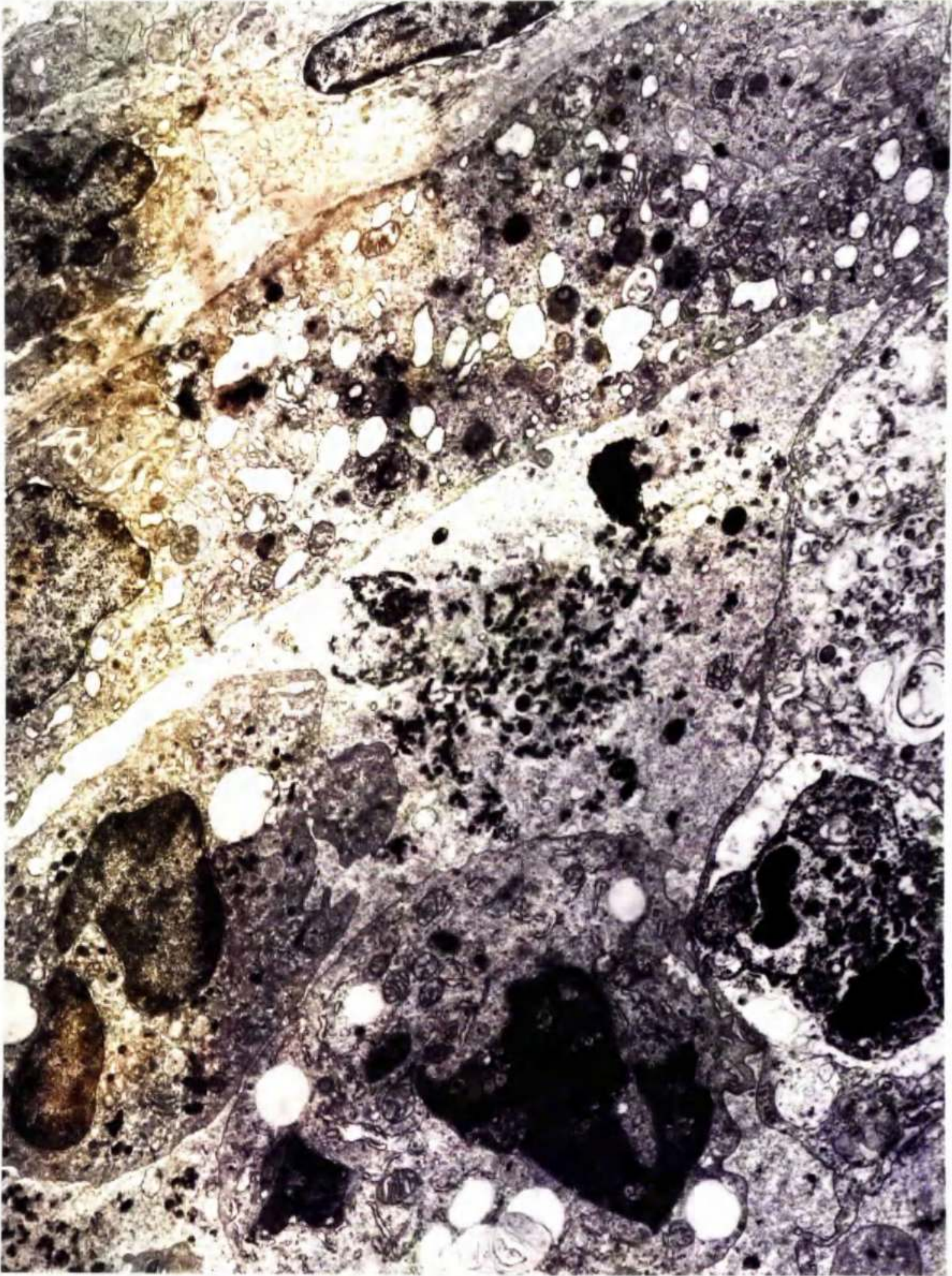


Figure 80: Gasil 200: 20 mg: 2 Months:

Interstitium showing infiltration with acute inflammatory cells. There is obvious fibroblastic activity with laying down of collagen fibres.

X 7728.

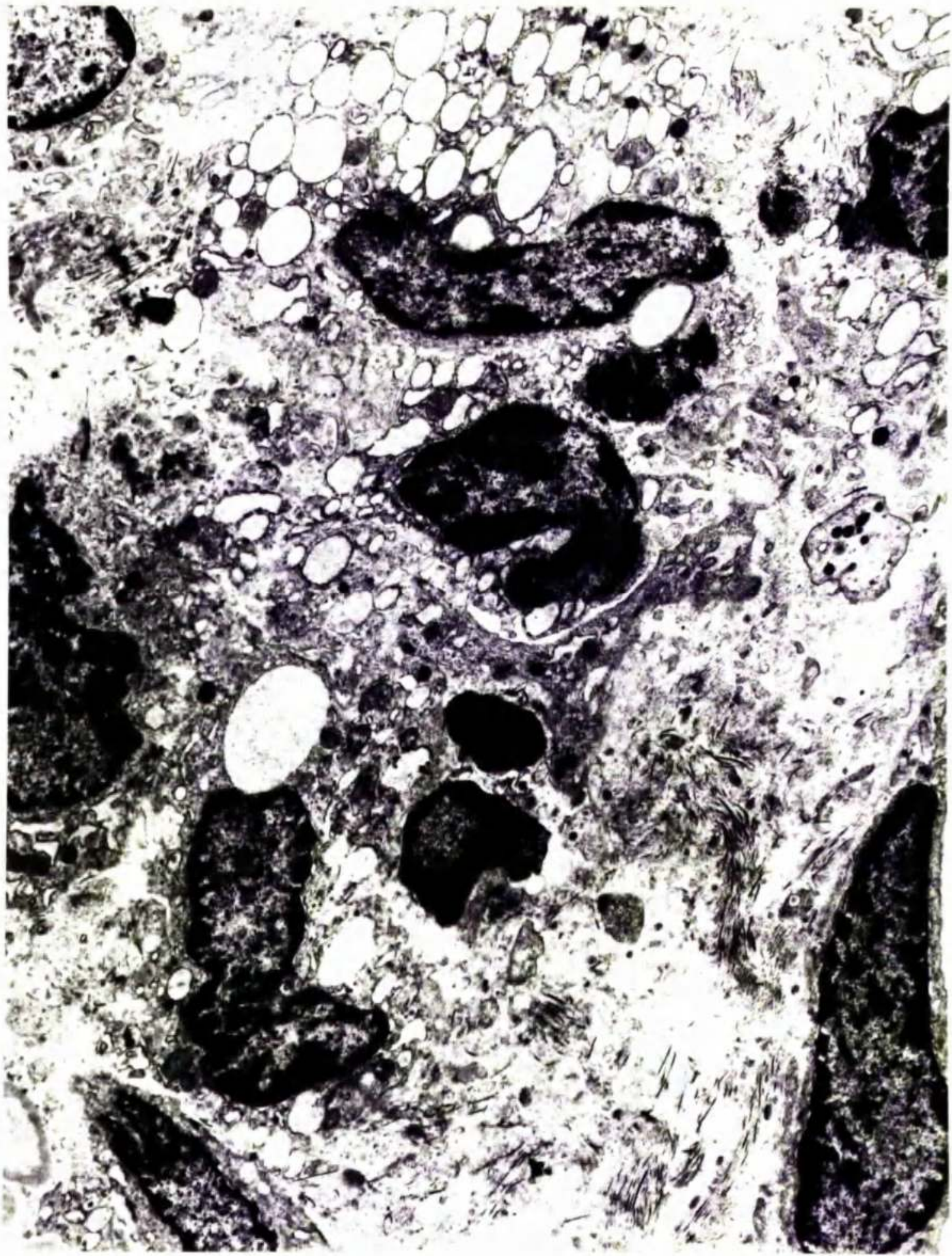


Figure 81: Gasil 200: 20 mg: 3 Months:

Glomerulus showing visceral epithelial cell damage consisting of excessive vacuolation and a large intracytoplasmic multilaminated body.

X 35880.

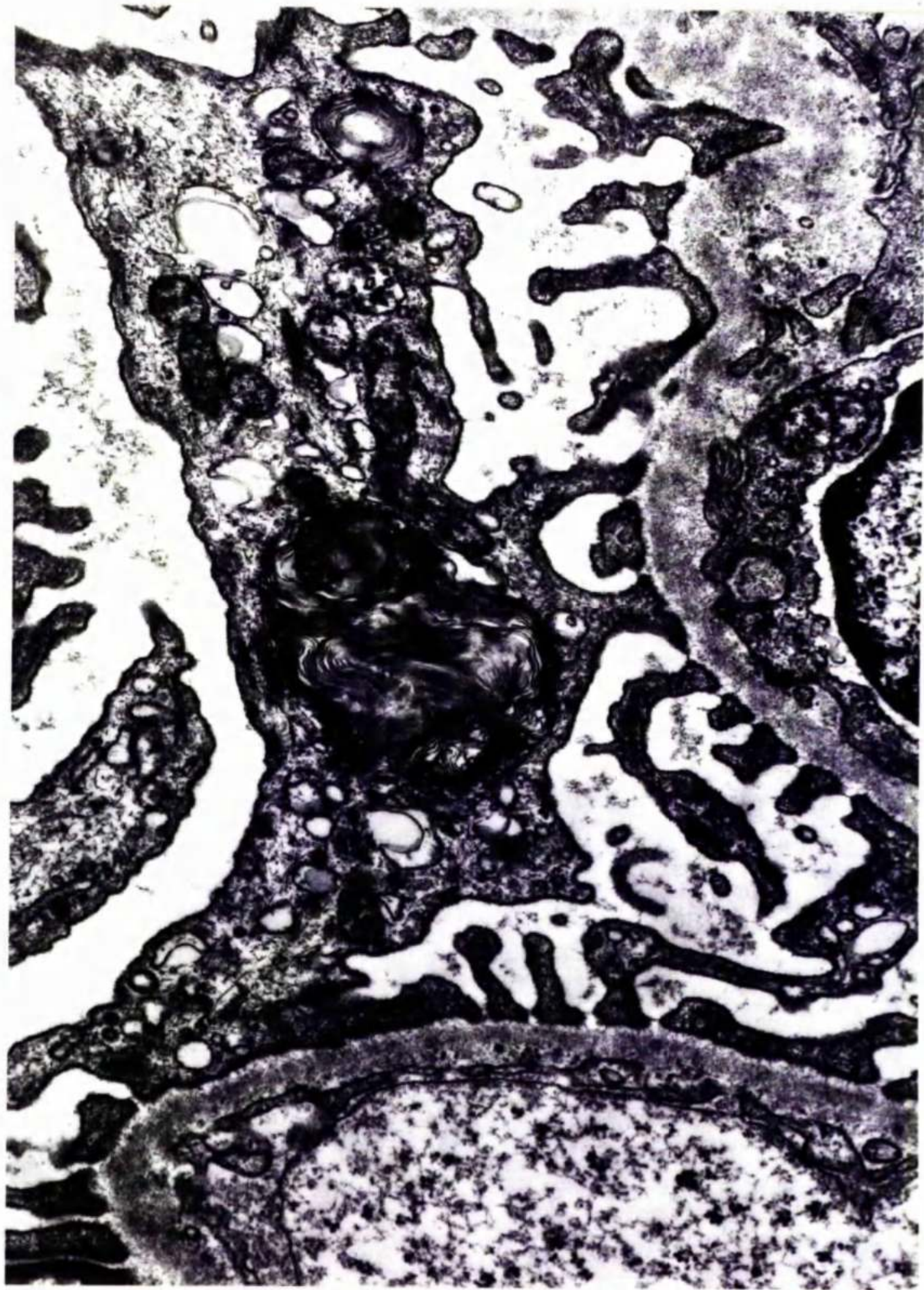


Figure 82: Gasil 200: 20 mg: 3 Months:

Glomerulus showing degenerate parietal epithelium in association with deposition of electron dense material on its inner aspect.

X 12690.

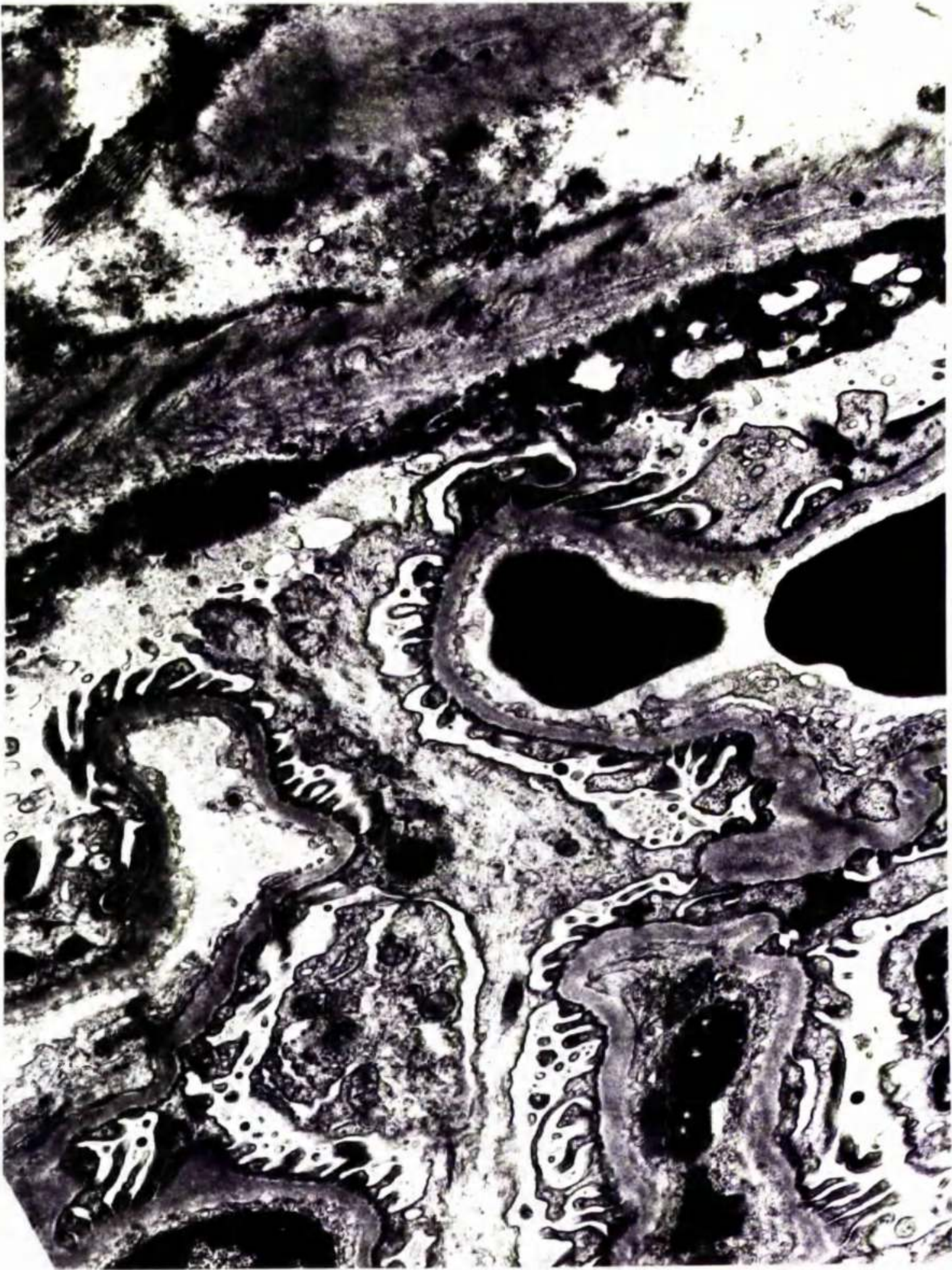


Figure 83: Gasil 200: 20 mg: 3 Months:

Proximal tubule showing extensive intra-cytoplasmic electron dense material (residual bodies).

X 12696.

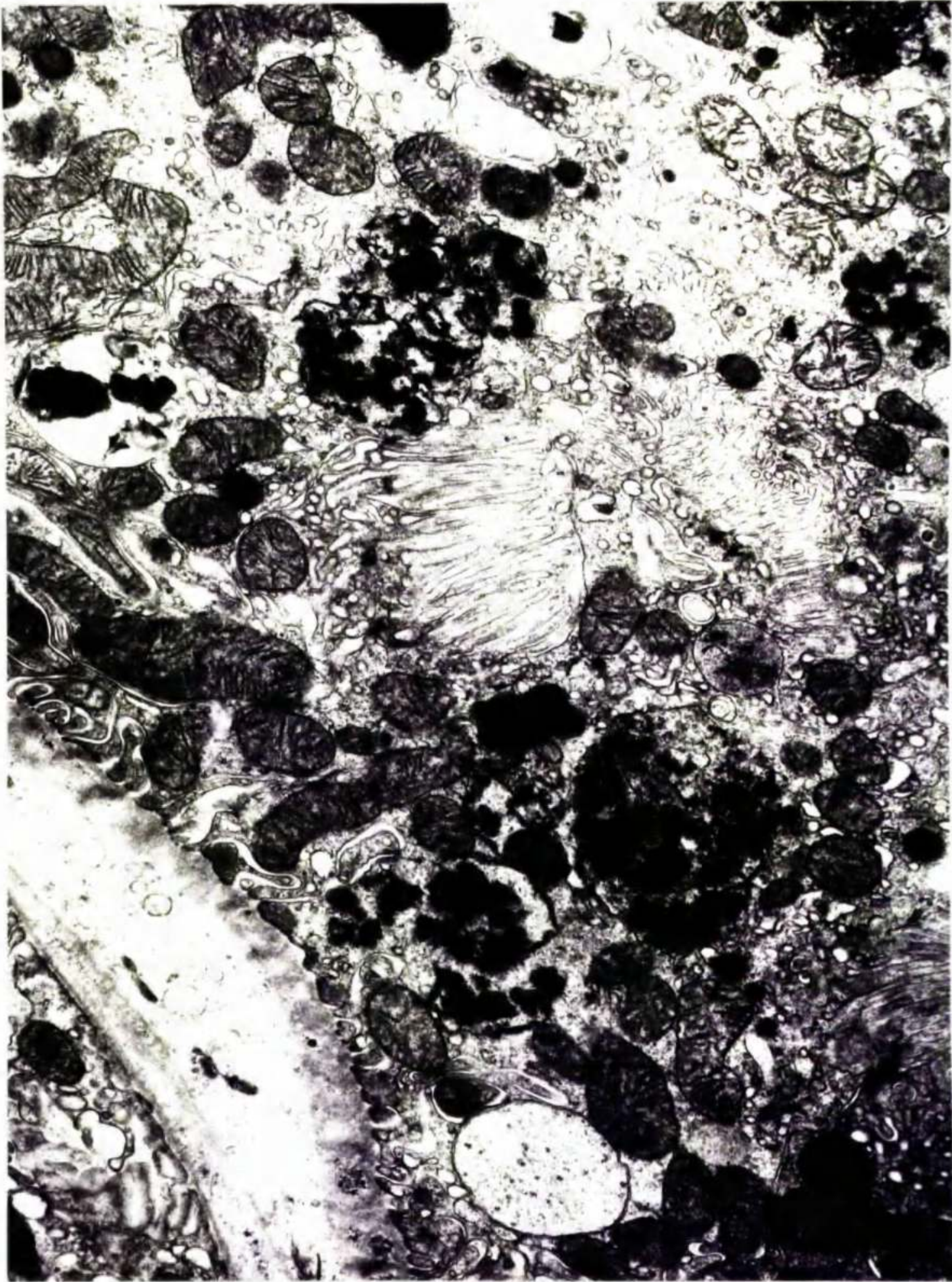


Figure 84: Gasil 200: 20 mg: 4 Months:

Podocyte trabeculum, packed with large multilamellated bodies, showing dilatation of cisternal systems.

X 61870.

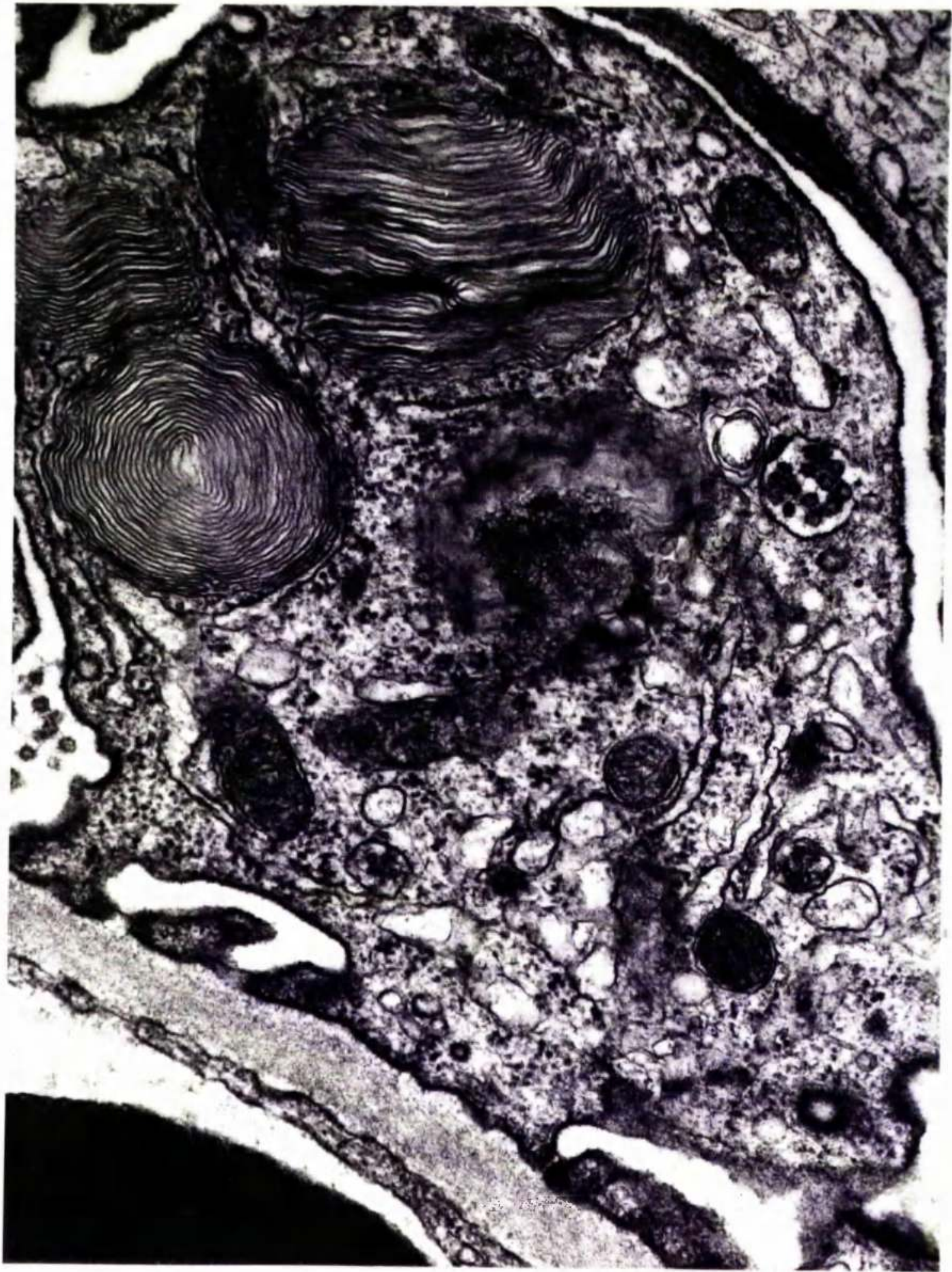


Figure 85: Gasil 200: 60 mg: 4 Months:

Proximal tubular cell with marked cytolysosomal activity.

X 61870.

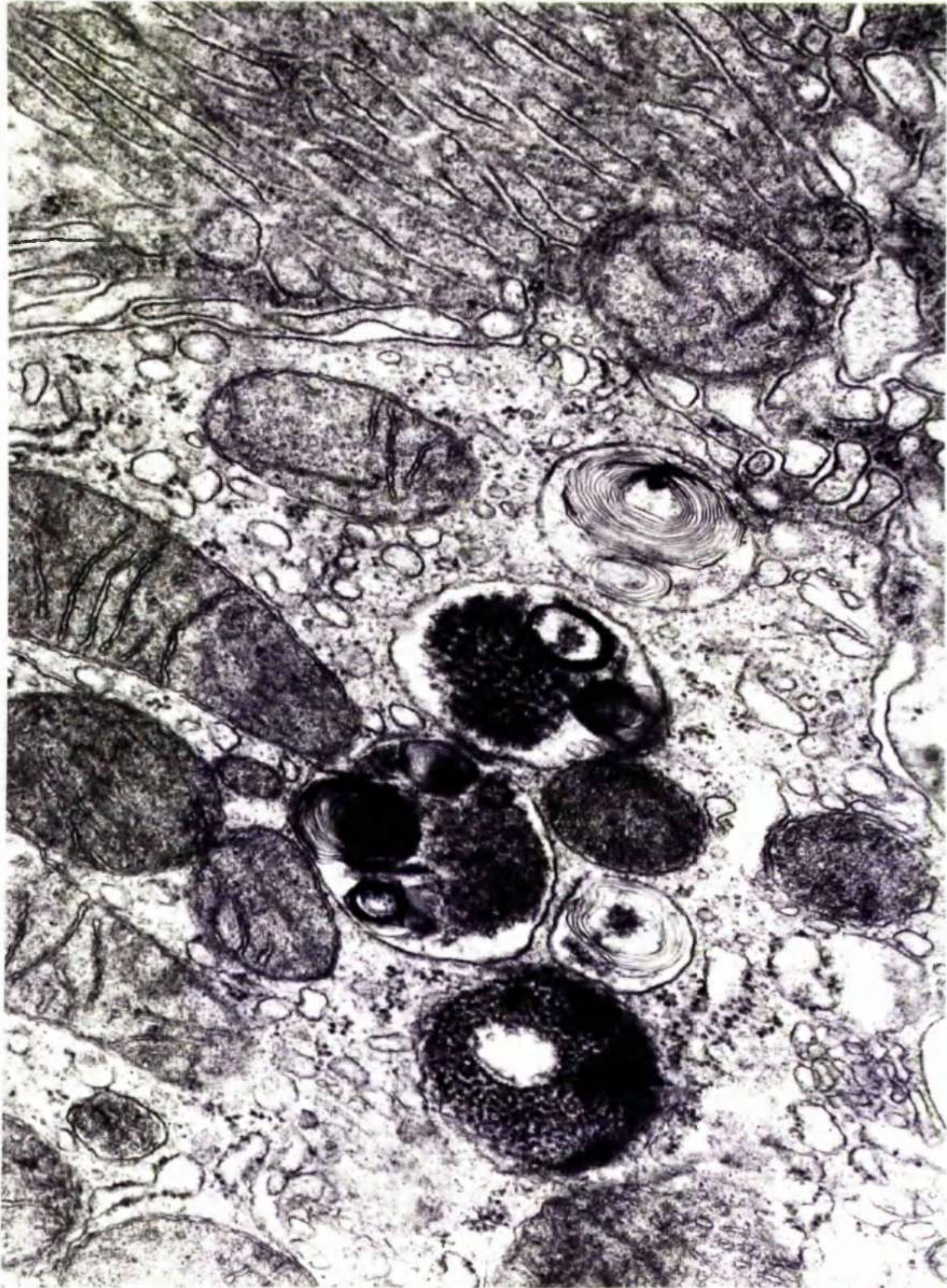


Figure 86: Gasil 200: 20 mg: 4 Months:

Basal region of proximal tubule showing large intracytoplasmic residual bodies.

X 20930.

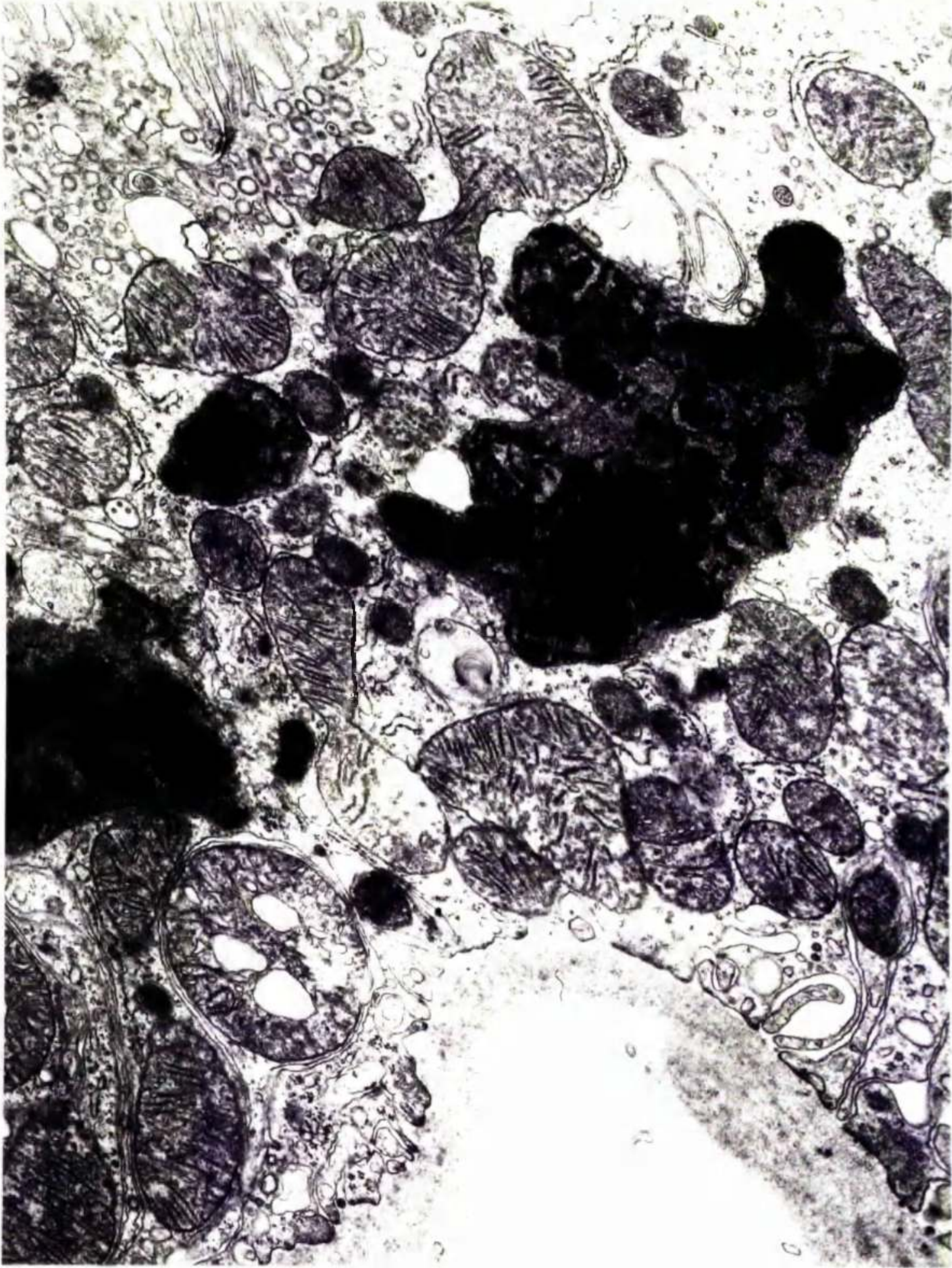


Figure 87: Gasil 200: 60 mg: 4 Months:

An electron micrograph showing severe tubular damage manifest as total organelle disruption and widespread formation of multilamellated and residual bodies. Due to the severity of the damage it is not possible to identify the part of the tubule so affected. A markedly thickened basal lamina is also noted.

X 12696.

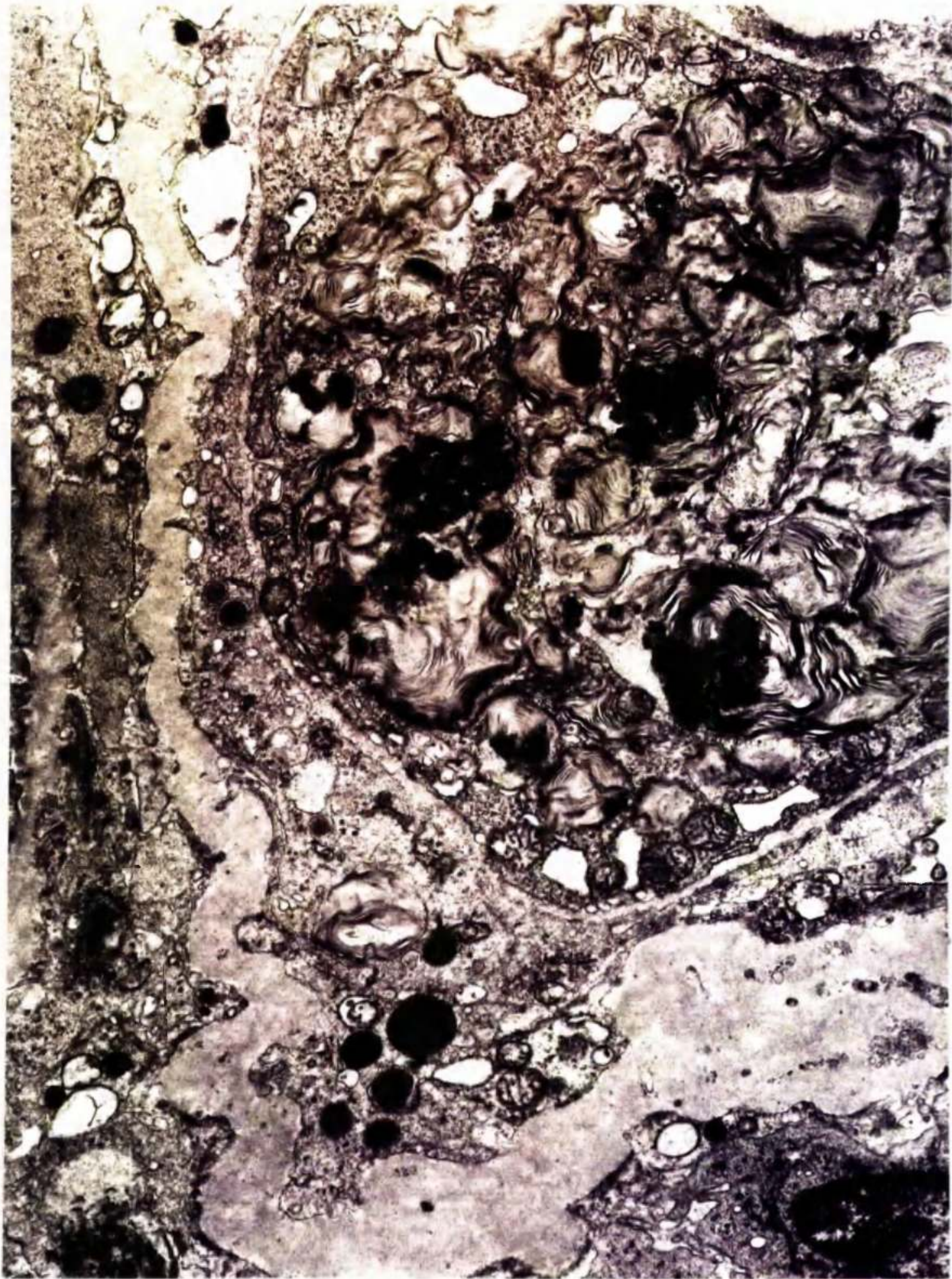


Figure 88: Gasil 200: 60 mg: 4 Months:

Thin loop of Henle demonstrating widespread cellular damage and thickened basal lamina.

X 61870.

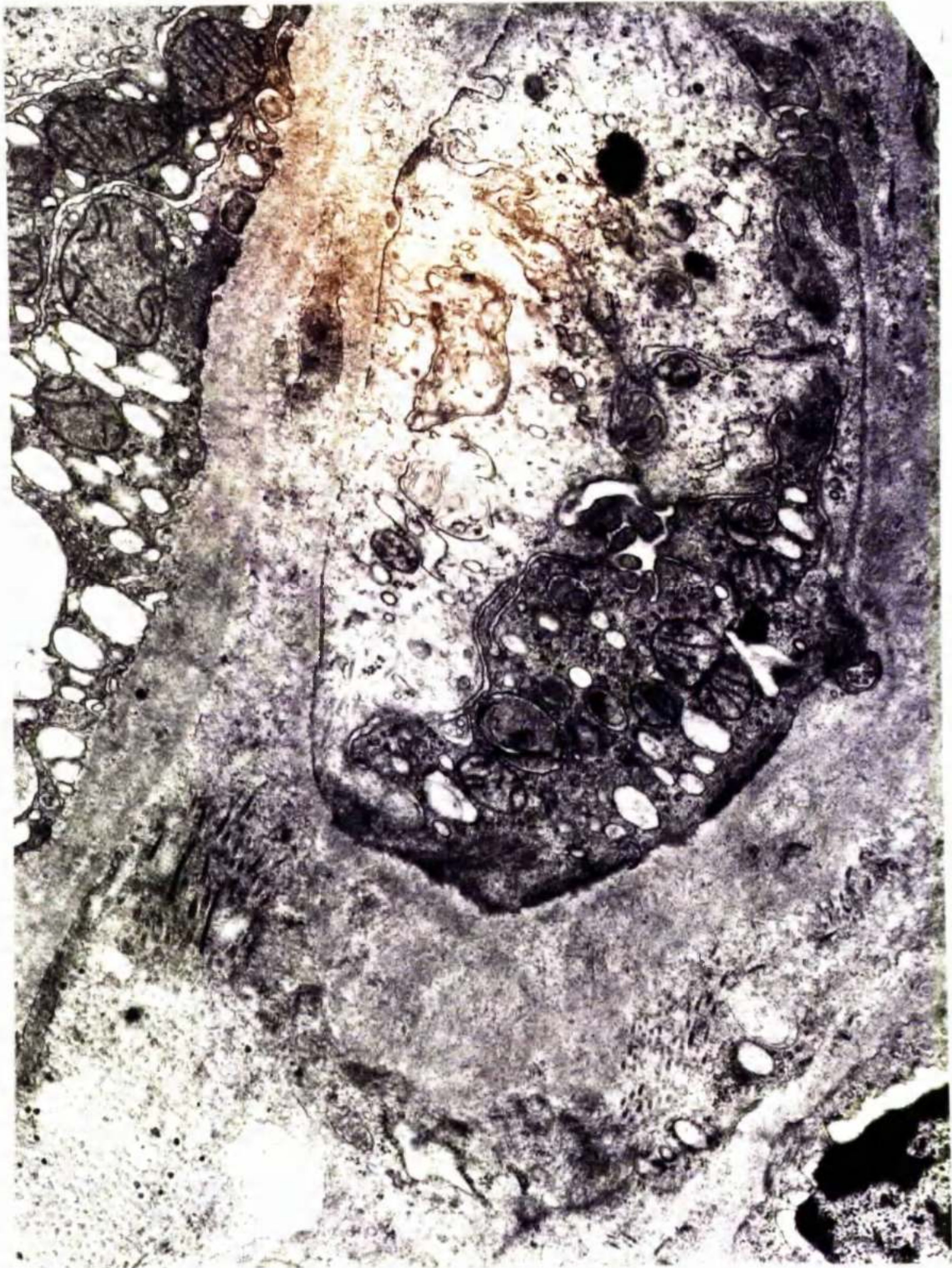


Figure 89: Gasil 200: 20 mg: 4 Months:

Interstitial infiltrate: The dark cell with condensed cytoplasm and swollen mitochondria is probably a damaged macrophage.

X 12696.

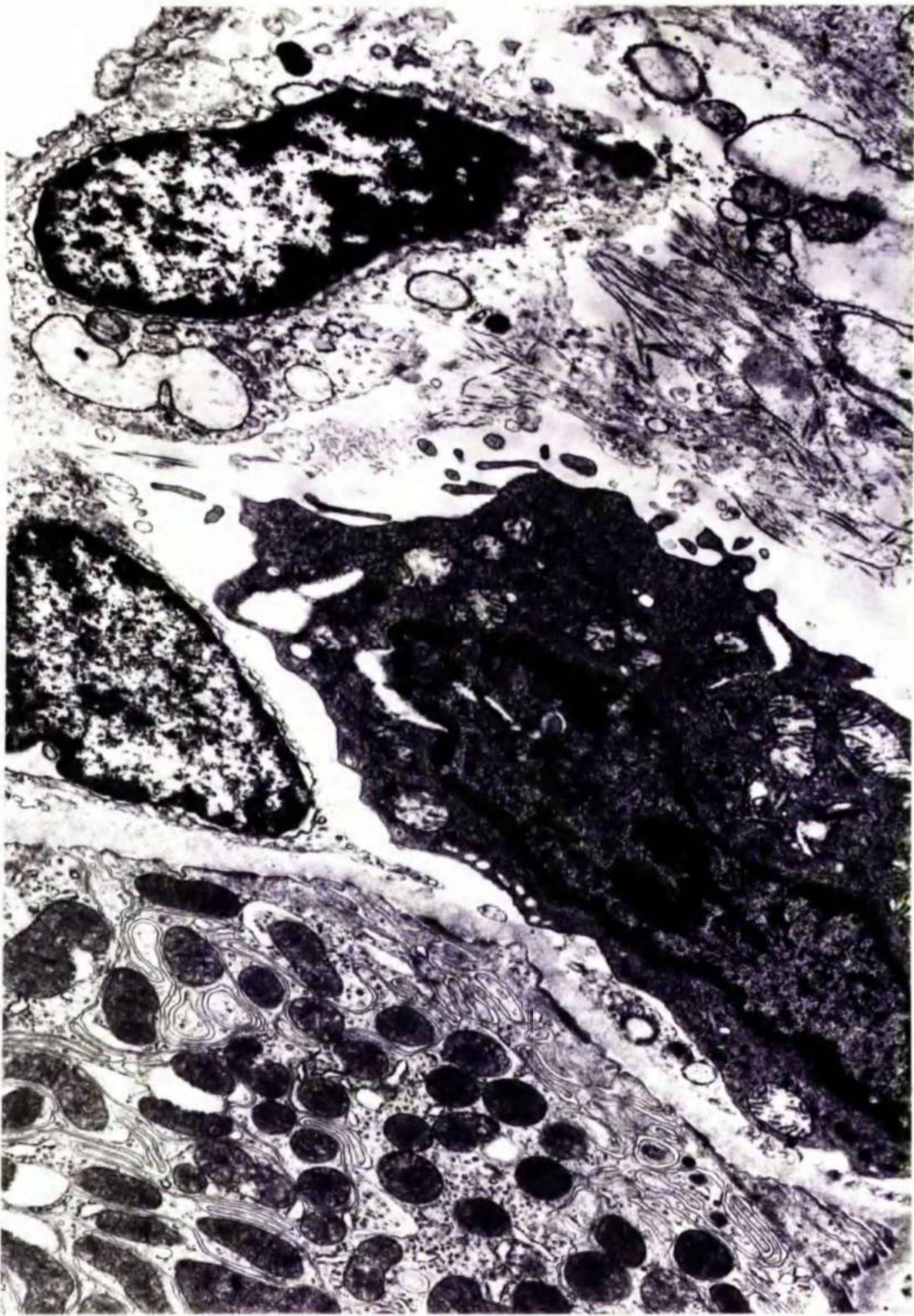


Figure 90: Gasil 200: 60 mg: 5 Months:

Glomerulus demonstrating widespread single membrane-bound structures containing electron-dense osmiophilic material. These varied widely in size. Other oeganelles are scanty.

X 7728.

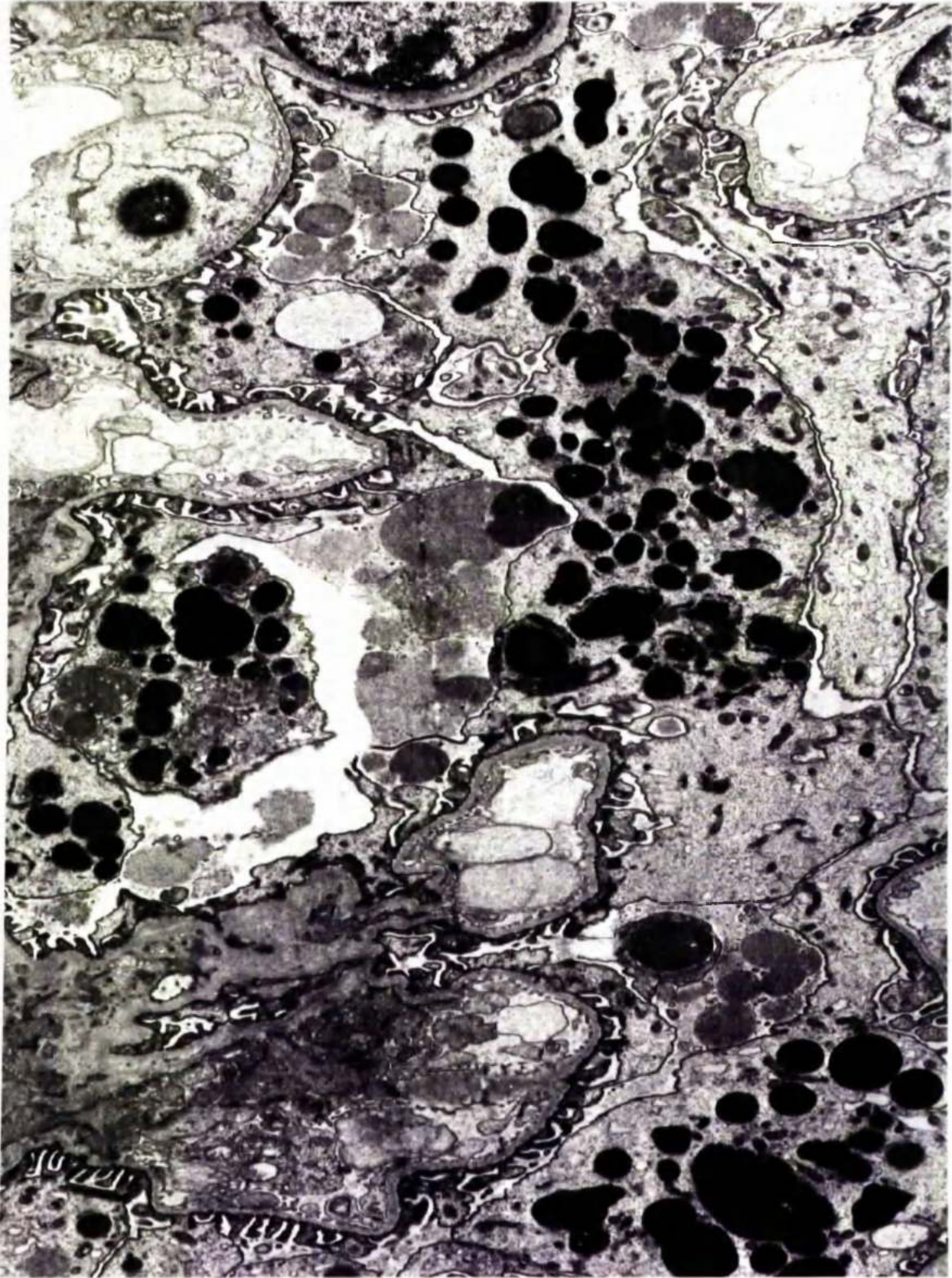


Figure 91: Gasil 200: 60 mg: 5 Months:

Glomerulus showing podocytes with multiple electron dense osmiophilic structures (high power).

X 20903.

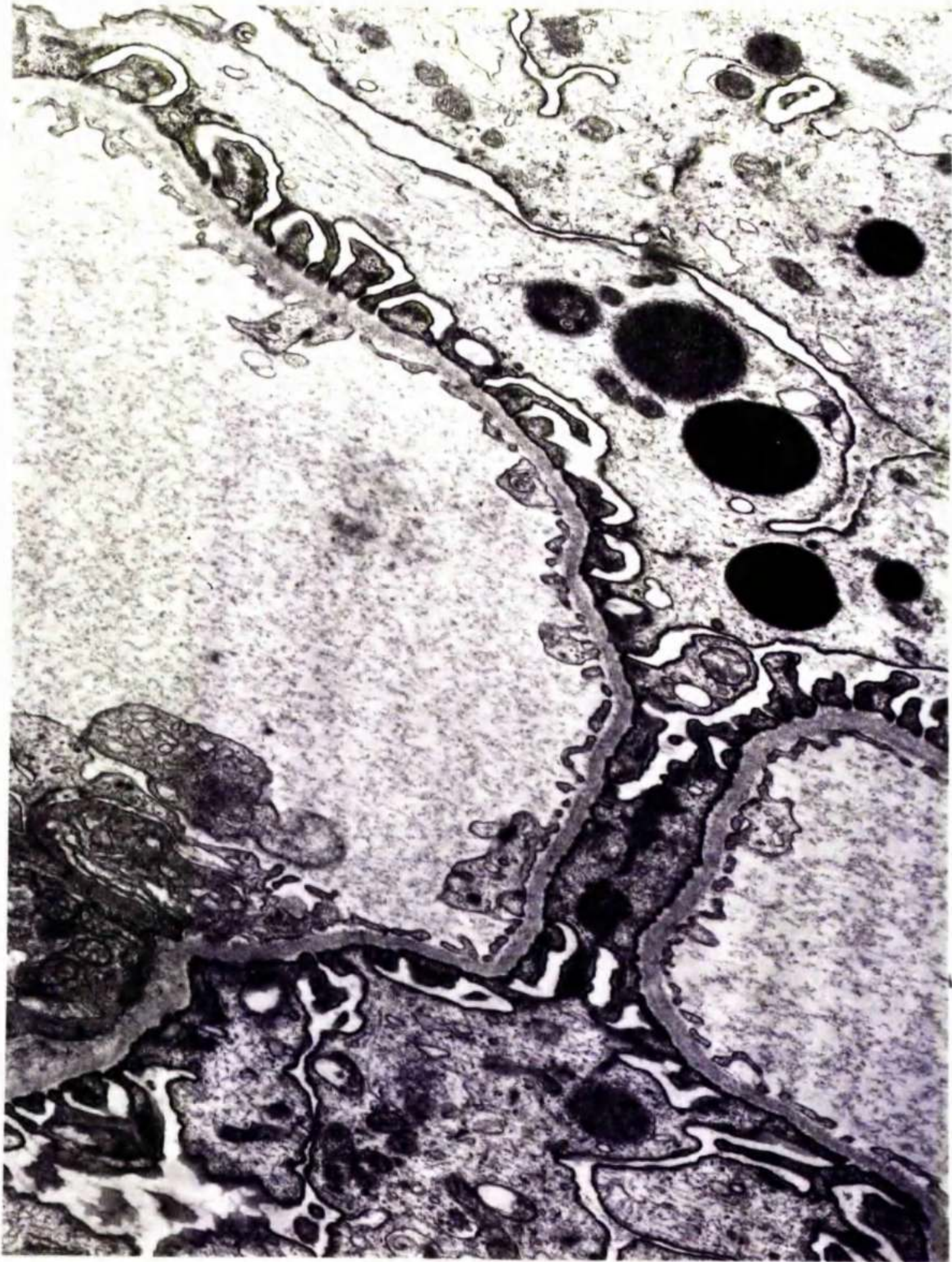


Figure 92: Gasil 200: 60 mg: 5 Months:

Collecting duct demonstrating dilatation of the cisternal system and opening of the intercellular clefts.

x 20930.

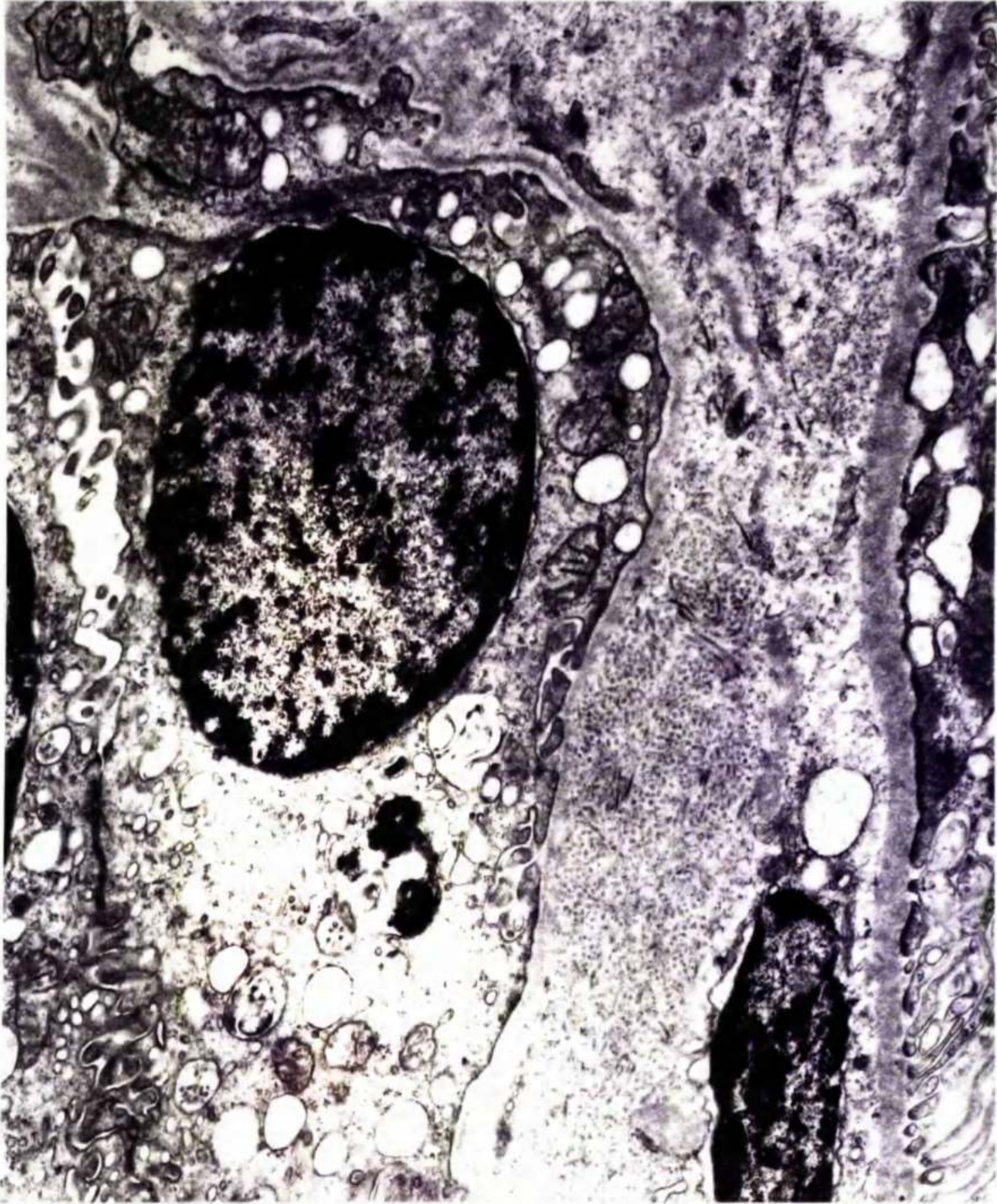


Figure 93: Gasil 200: 20 mg: 5 Months:

Proximal tubules showing large numbers of intracytoplasmic vesicles and vacuoles. There is increased cytolysosomal activity and dilatation of cisternal systems.

X 20930.

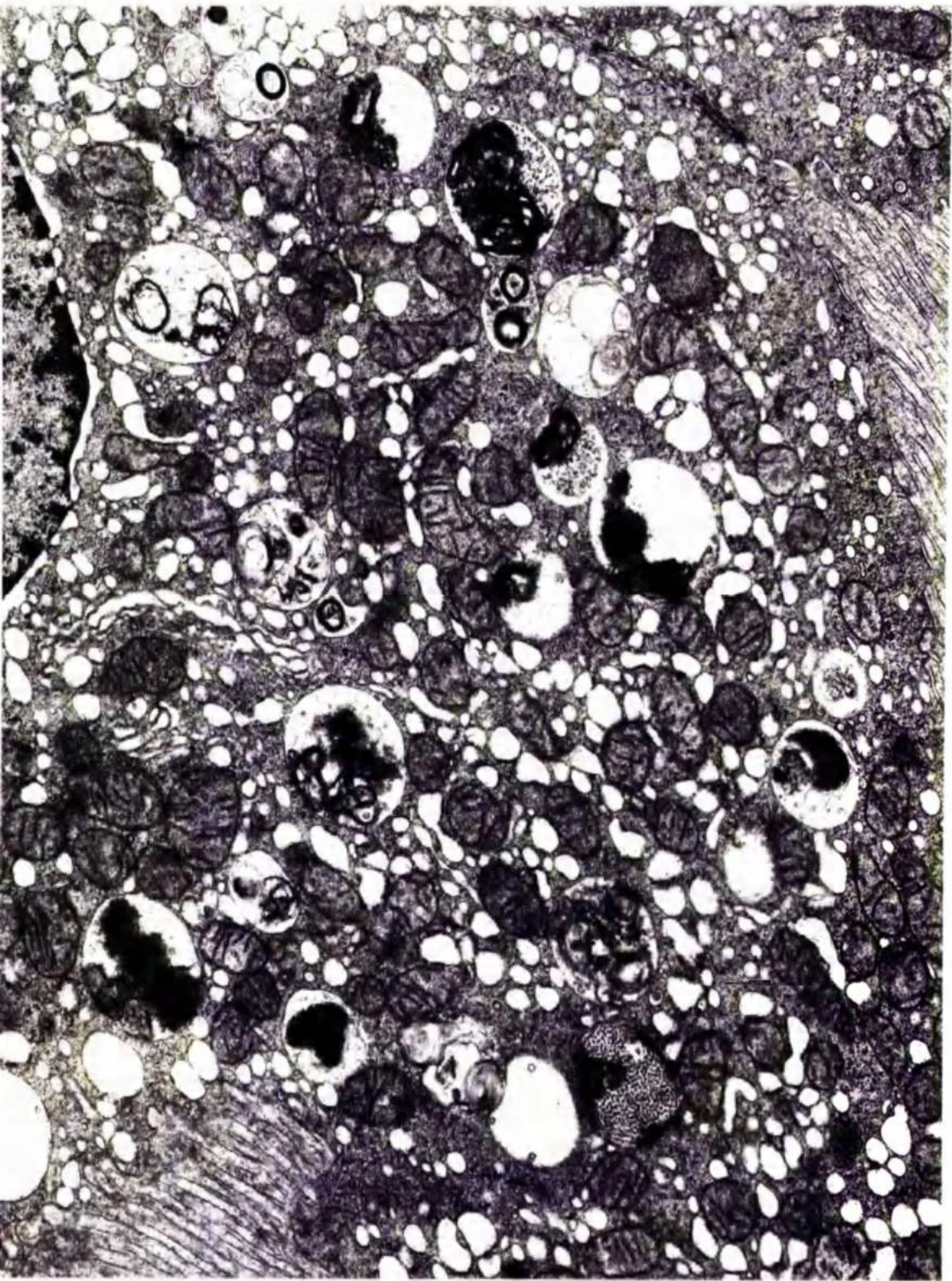


Figure 94: Gasil 200: 20 mg: 5 Months:

Distal tubule showing cellular oedema and nuclear damage consisting of chromatin margination and disruption of the nuclear envelopes.

X 20 930.

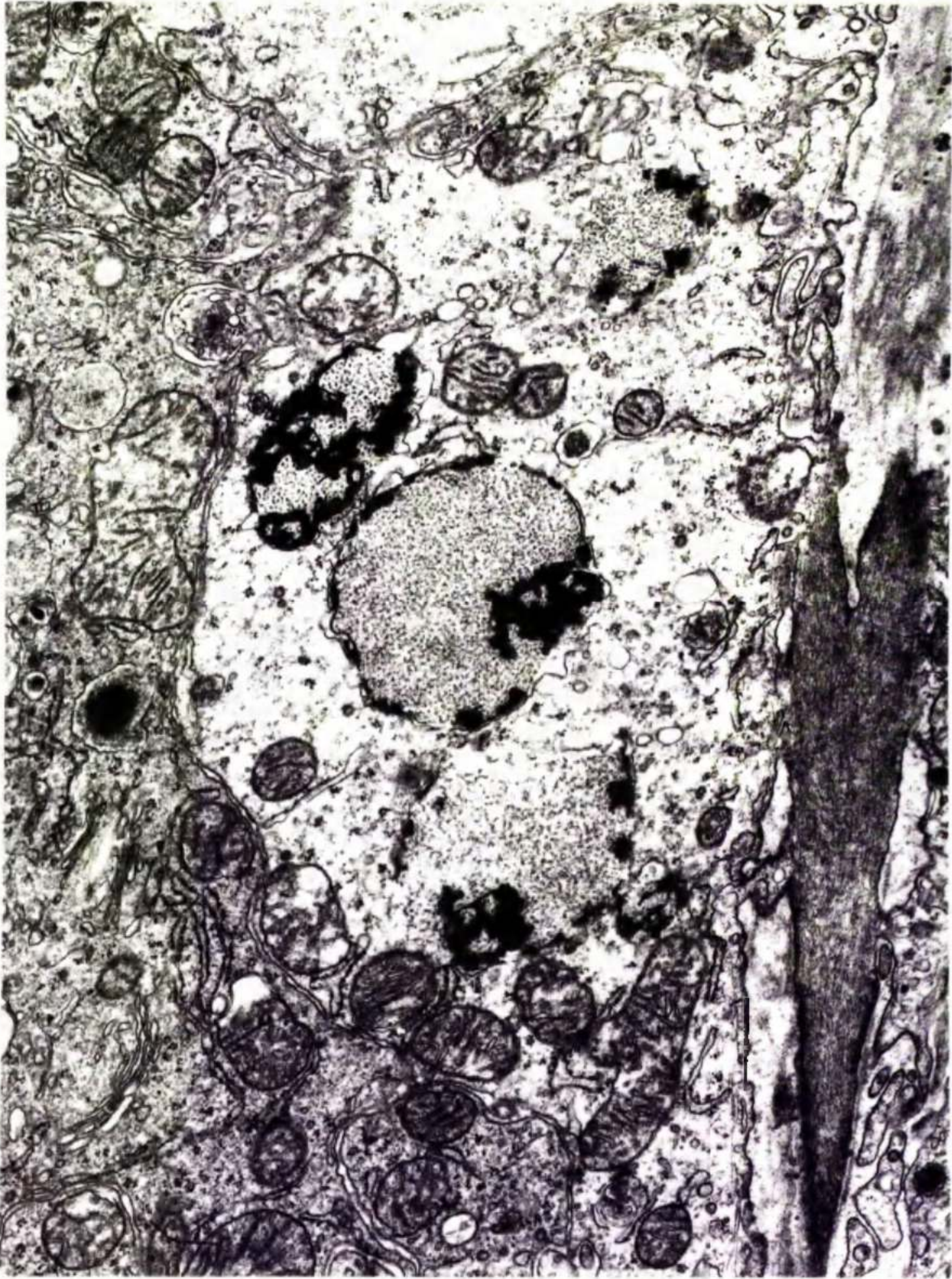


Figure 95: Gasil 200: 60 mg: 6 Months:

Glomerulus demonstrating foot process fusion.

X 20903.

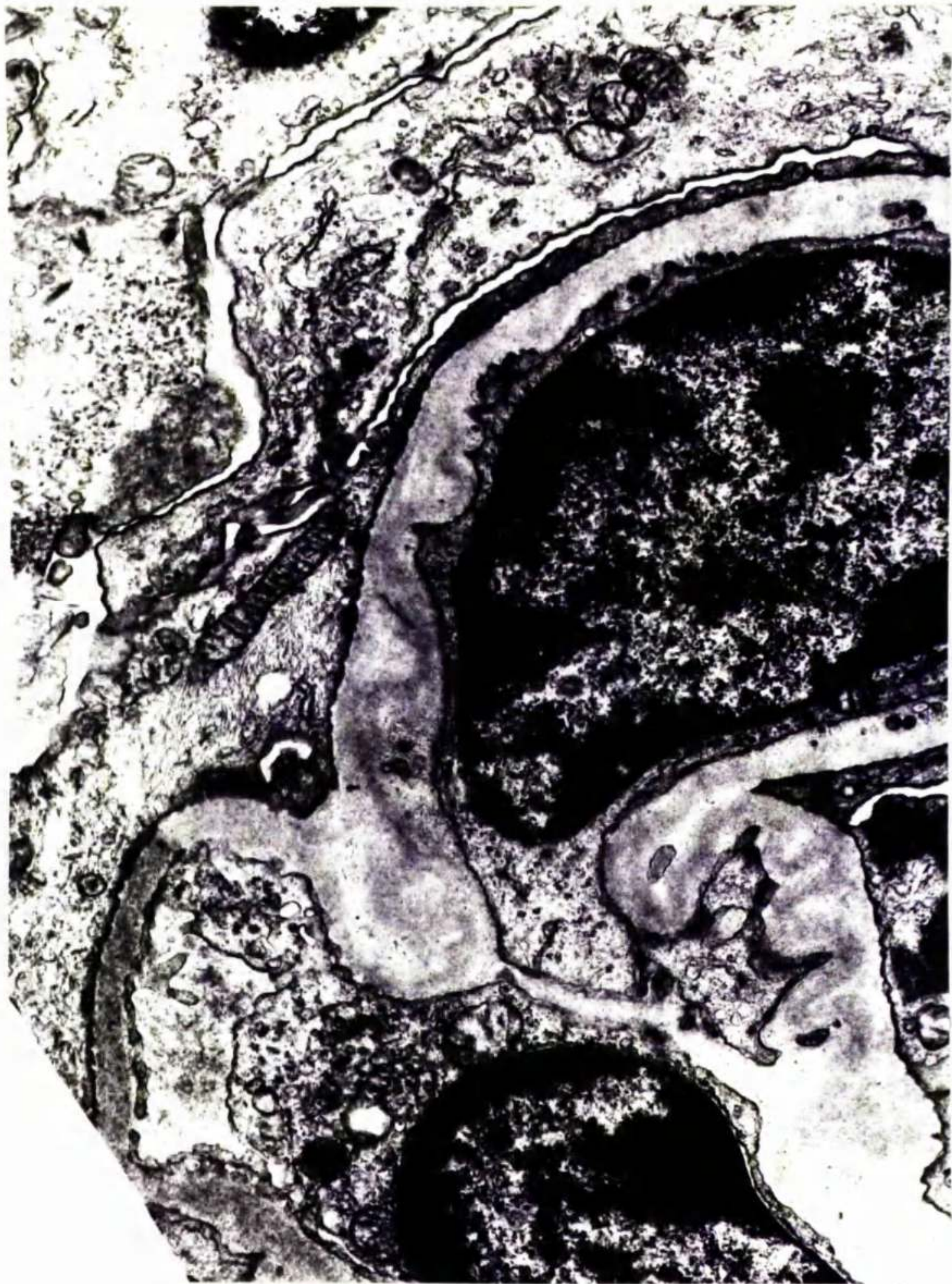


Figure 96: Gasil 200: 60 mg: 6 Months:

Interstitium demonstrating mononuclear cell infiltration and progression of the fibroblastic activity in the region around the glomerulus. Excessive collagen formation is also in evidence.

X 7728.

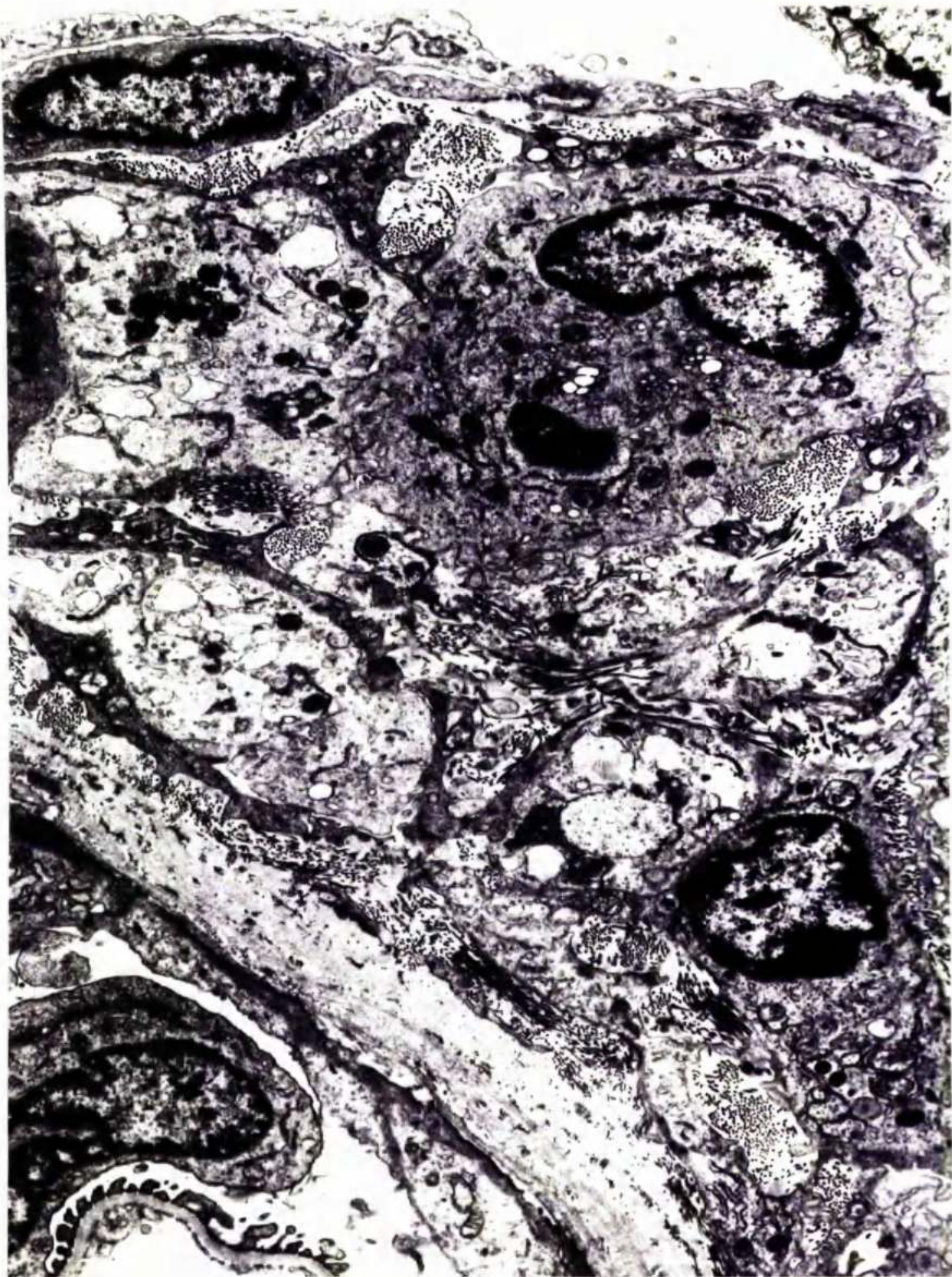


Figure 97: Gasil 200: 60 mg: 7 Months:

Lymphocytic infiltration of interstitium.

X 12696.

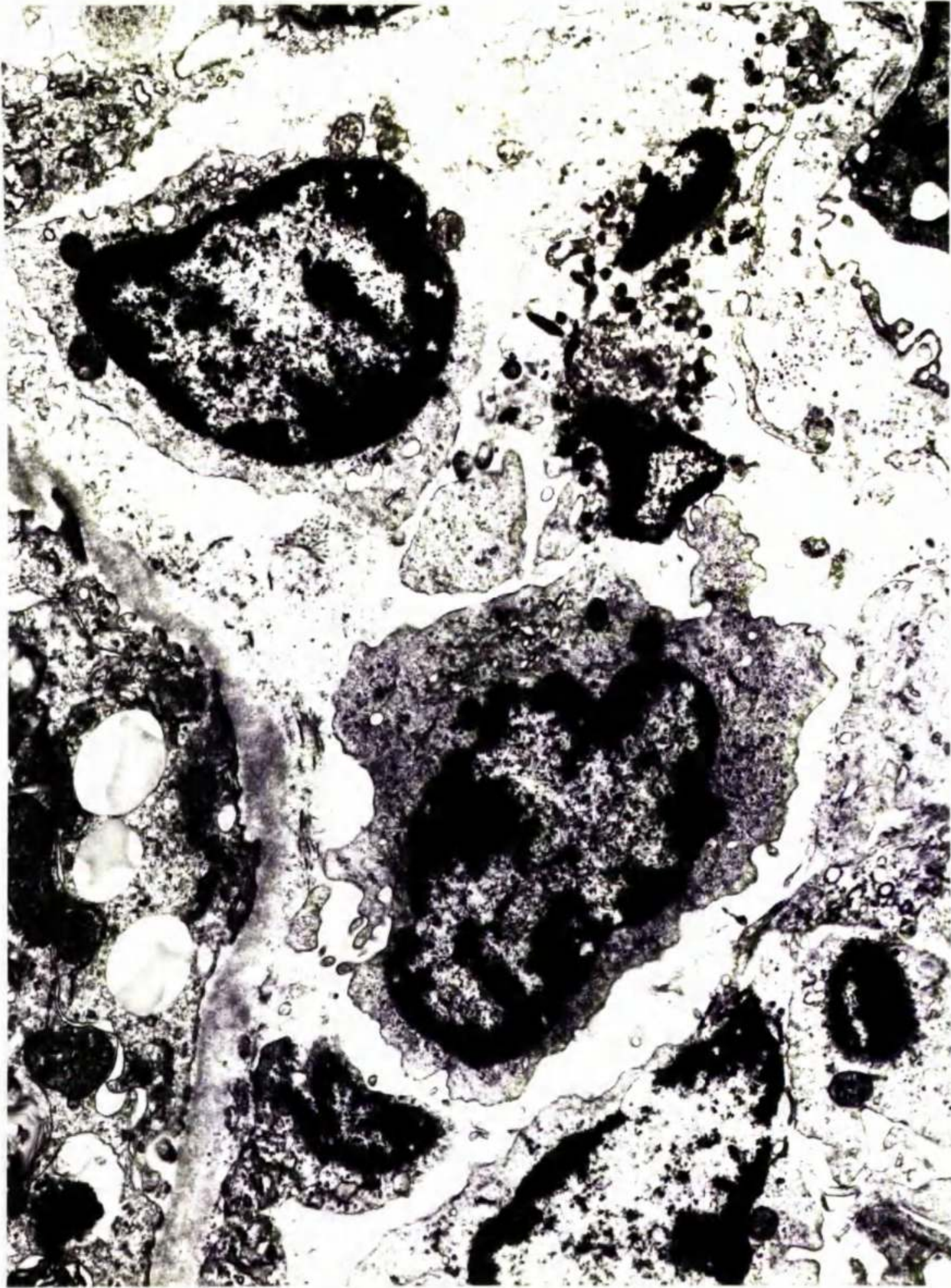


Figure 98: Gasil 200: 20 mg: 7 Months:

Interstitial infiltrate with plasma cells.

X 7728.

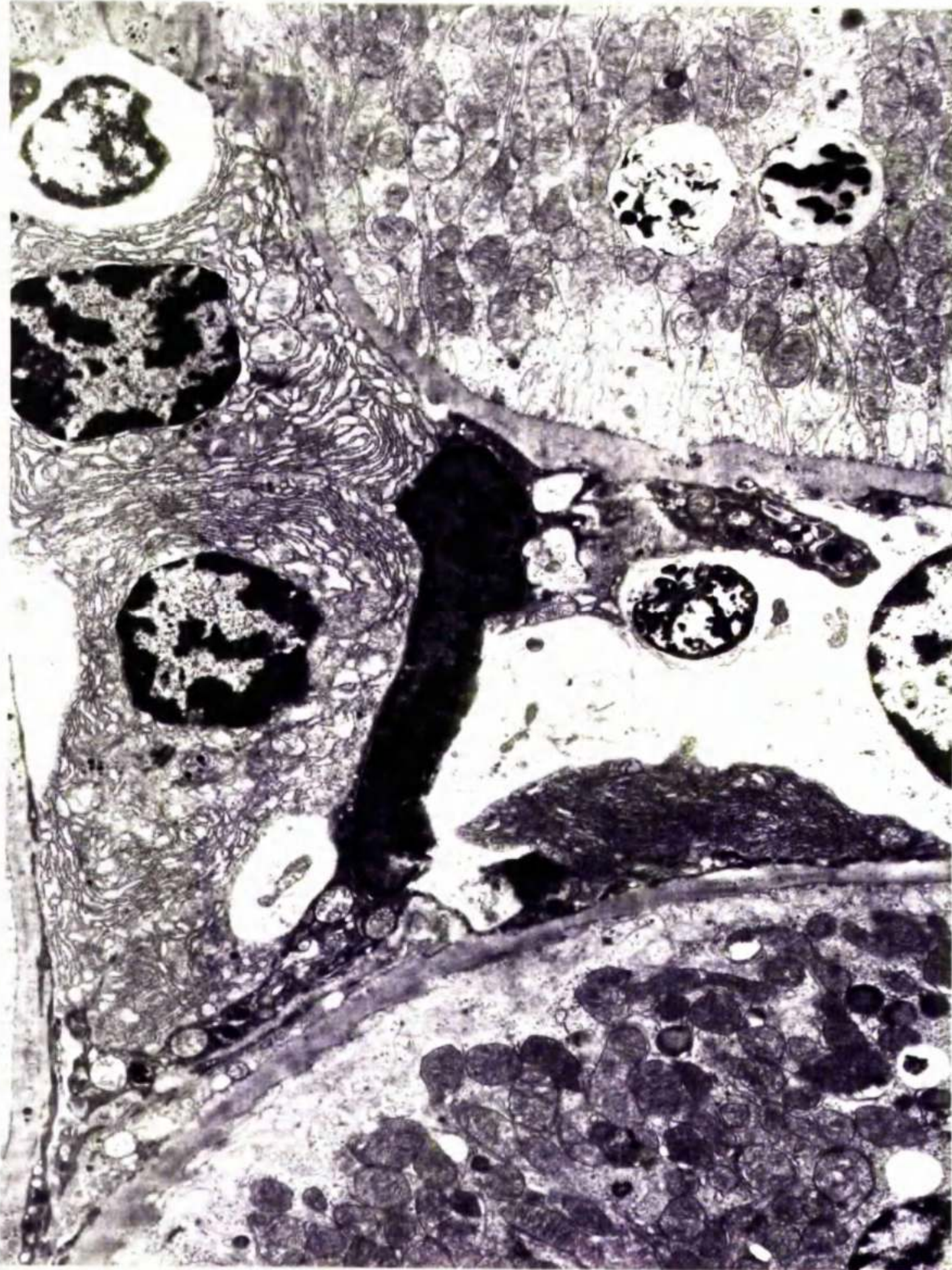


Figure 99: Gasil 200: 60 mg: 8 Months:

Glomerulus showing marked ultrastructural changes in the visceral epithelial cells consisting of irregular cystic swellings hugged by a narrow band of condensed cytoplasm In addition, multiple intracytoplasmic membrane bound structures, marked foot process fusion and condensation of electron dense material at the base of the podocytes and fused foot processes are encountered.

X 7728.

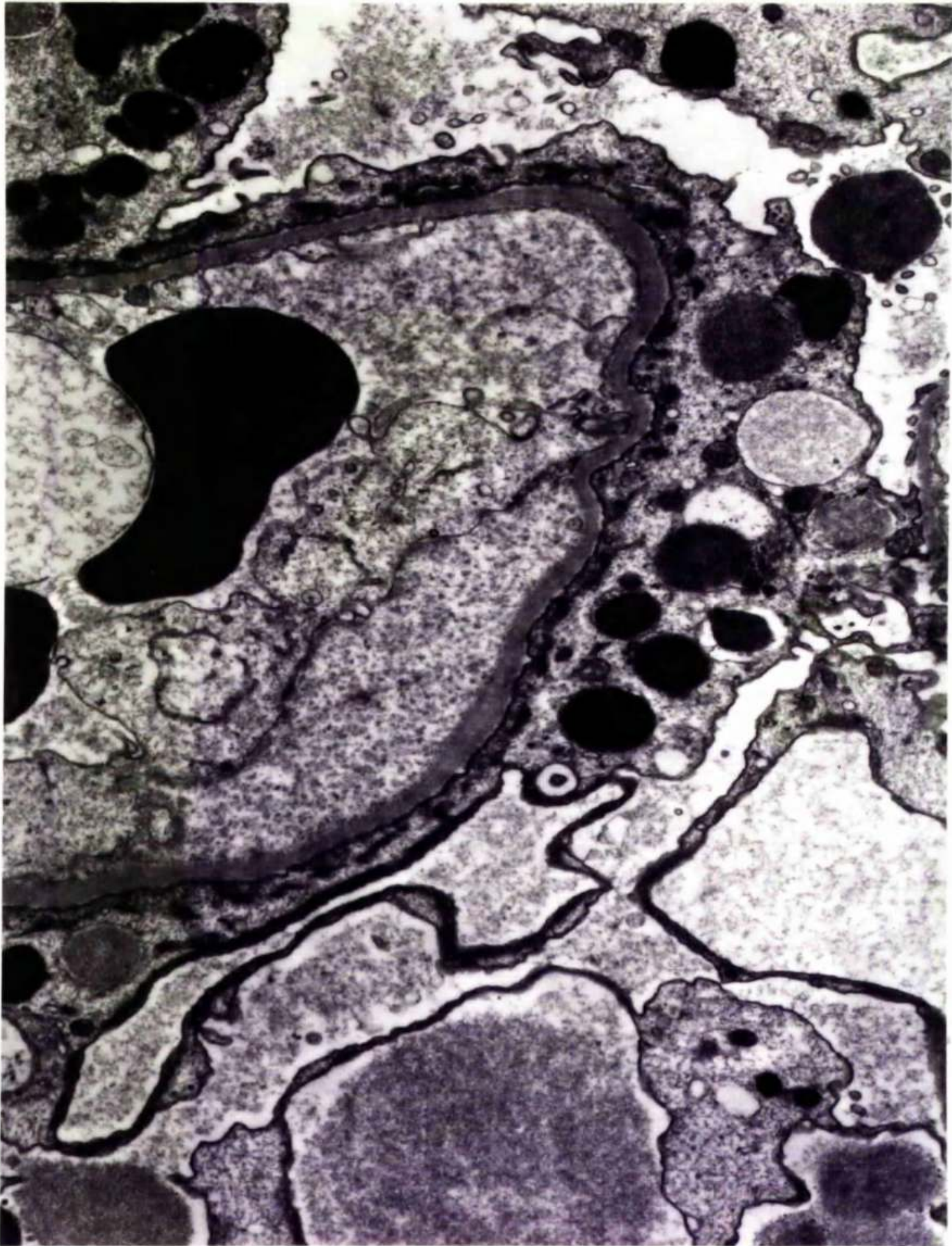


Figure 100: Gasil 200: 60 mg: 8 Months:

Glomerulus demonstrating in addition to the changes seen in the previous Figure, marked mesangial electron dense deposits and vesiculation of the mesangial cells.

X 7728.

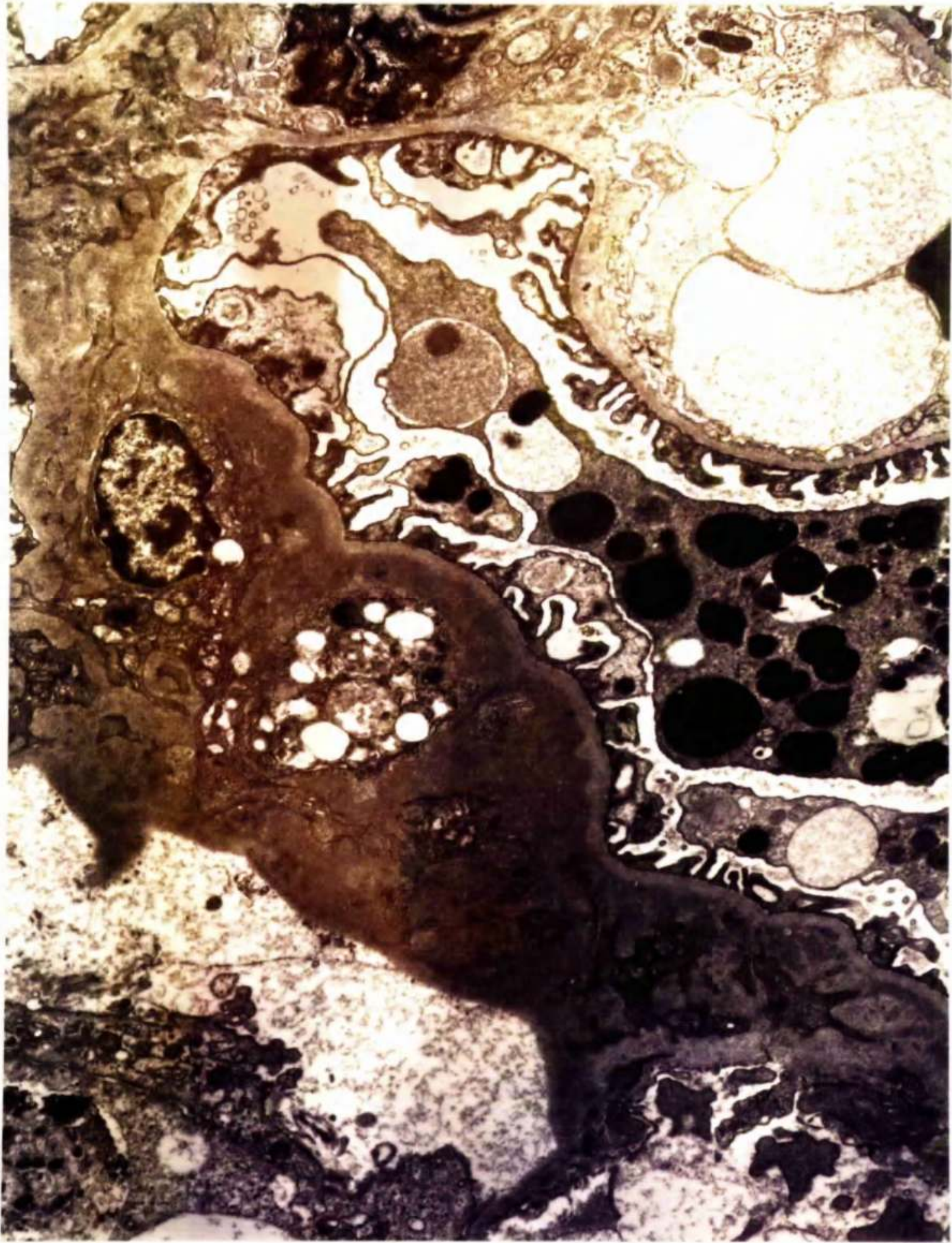
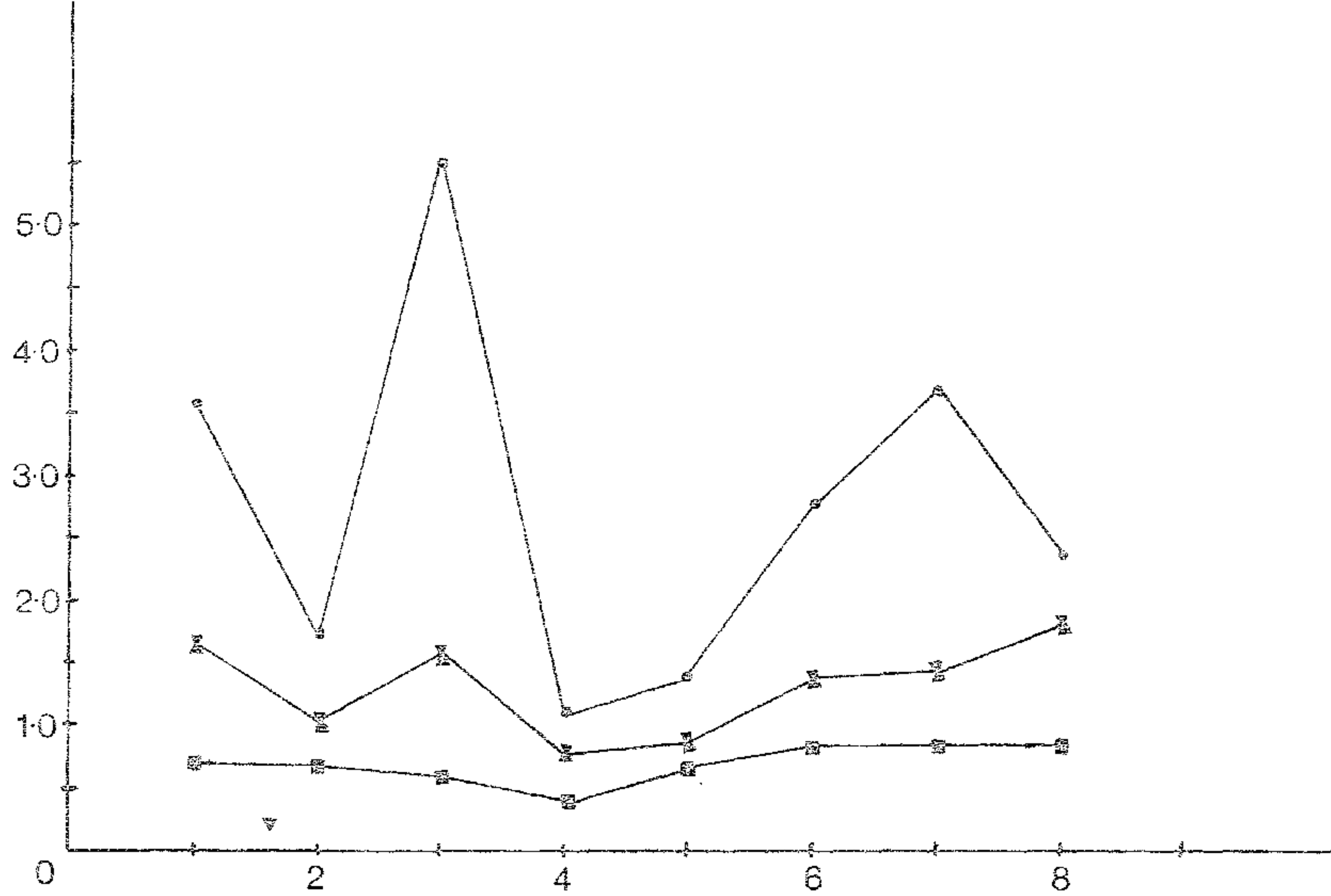


Figure 101: Showing monthly levels of serum silicon over an 8 month period in animals given weekly intraperitoneal injections of 20 mg and 60 mg gasil 200 together with values obtained from controls. There is considerable variation in the group receiving 60 mg, the peaks possibly resulting from repetitive effect of high silicon dosage. Both groups of tested animals however show silicon levels consistently higher than the control.

● 60 mg Animals

⧗ 20 mg Animals

■ Control Animals



5·0
4·0
3·0
2·0
1·0
0
2
4
6
8

Figure 102: Showing the monthly levels of serum urea over a 9 month period in animals given weekly intraperitoneal injections of 20 mg or 60 mg gasil 200 together with values obtained from the control groups. There is a considerable variation in level of serum urea in tested groups, possibly due to the obligatory paucity of sampling (values obtained from sacrificed animals). There is little difference between the urea levels in the control and the group receiving 20 mg gasil 200.

● 60 mg Animals

⧗ 20 mg Animals

■ Control Animals

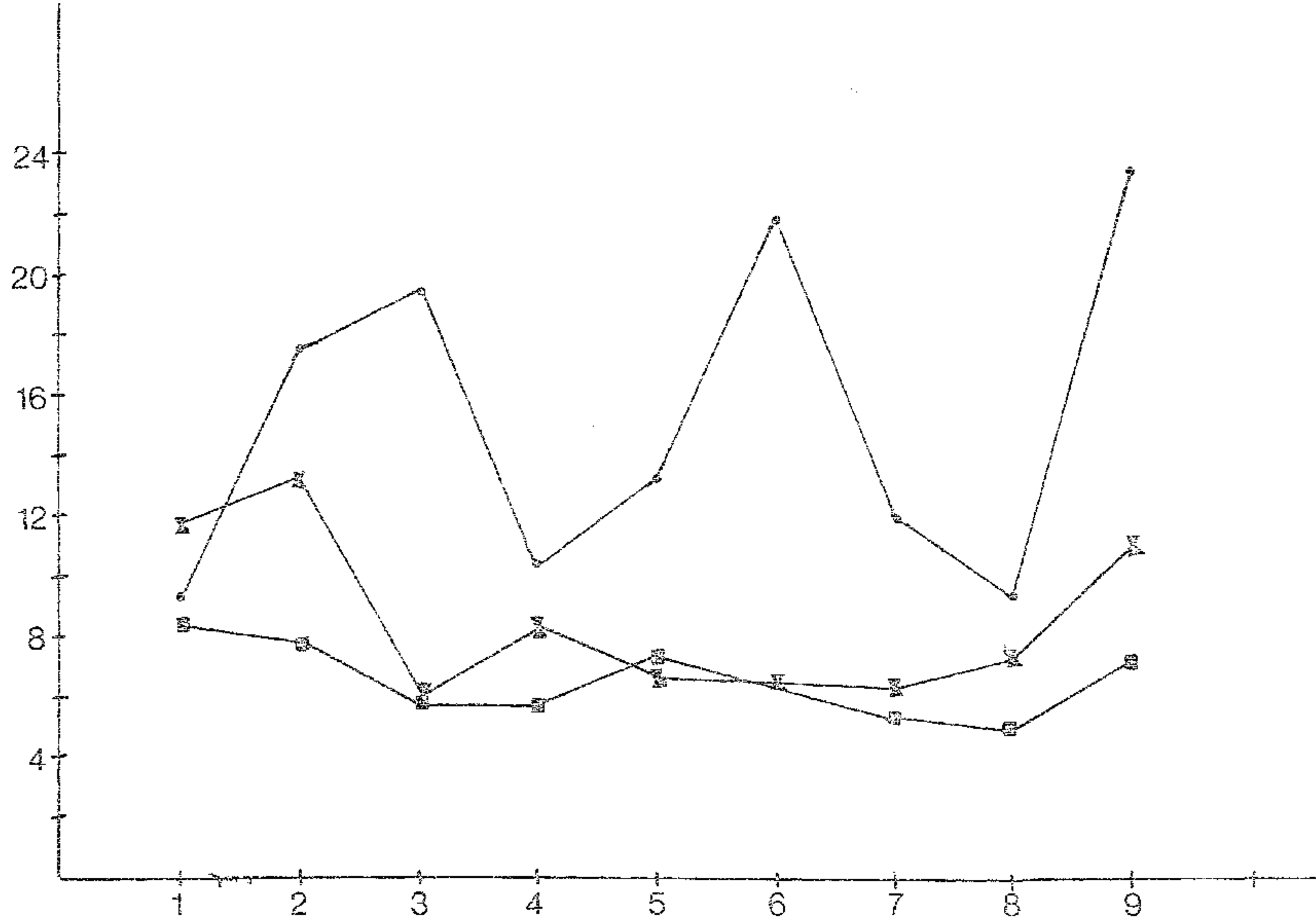
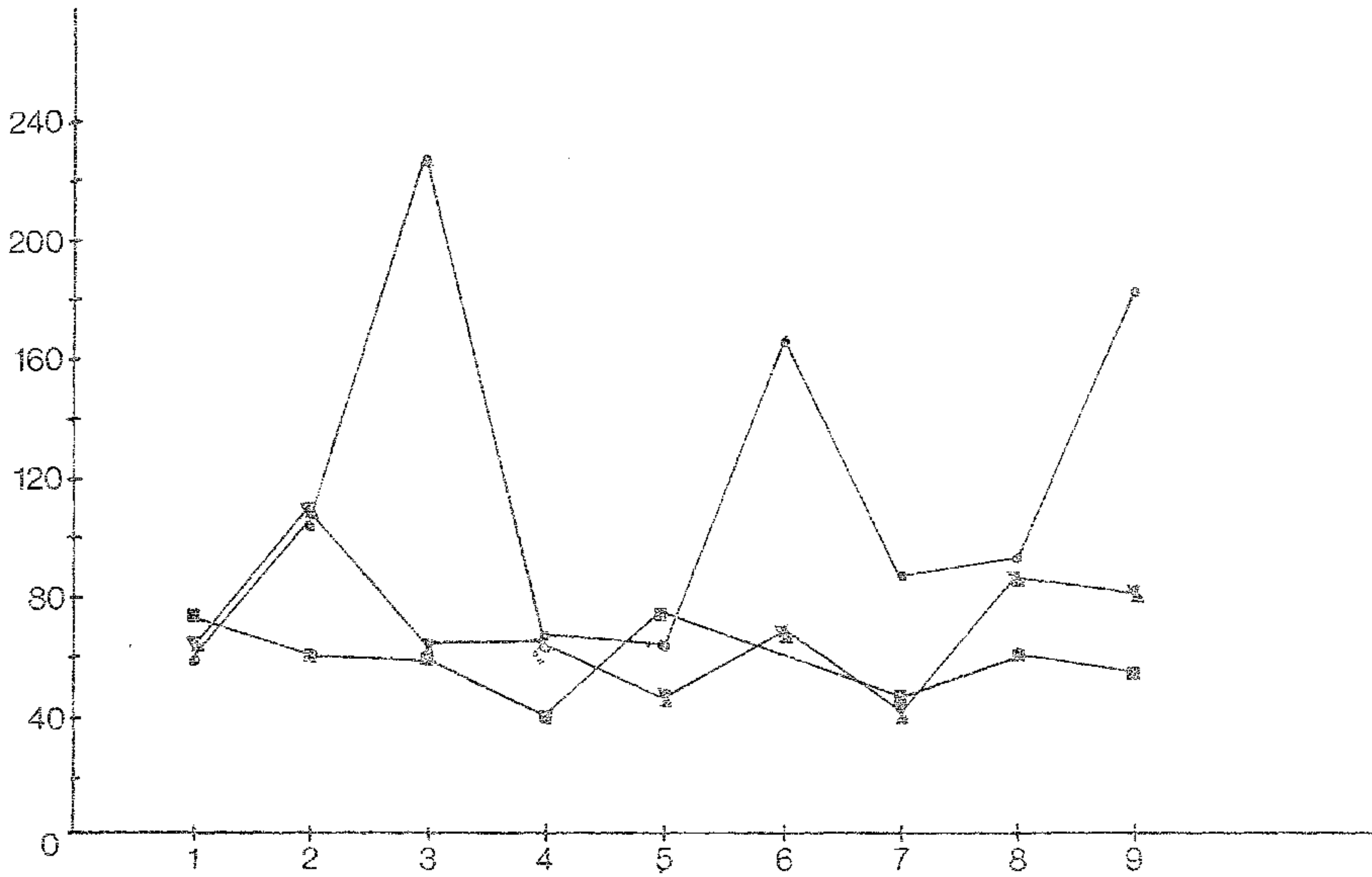

24
20
16
12
8
4
1
2
3
4
5
6
7
8
9

Figure 103: Showing the monthly levels of serum creatinin over a 9 month period in animals given weekly intraperitoneal injections of 20 mg or 60 mg gasil 200 together with values obtained from the control groups. There is a considerable variation in levels of serum creatinine in the tested groups, possibly due to the obligatory paucity of sampling (values obtained from sacrificed animals). There is little difference between the creatinine levels in the controls and the group receivin 20 mg.

● 60 mg Animals

⧗ 20 mg Animals

■ Control Animals


240
200
160
120
80
40
0
1
2
3
4
5
6
7
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9

Figure 104: Gasil 200: 60 mg for 7 Consecutive Days

Medulla: Showing the extent of dilatation, damage and inflammation of collecting ducts and interstitium.

H & E Mag. X 13

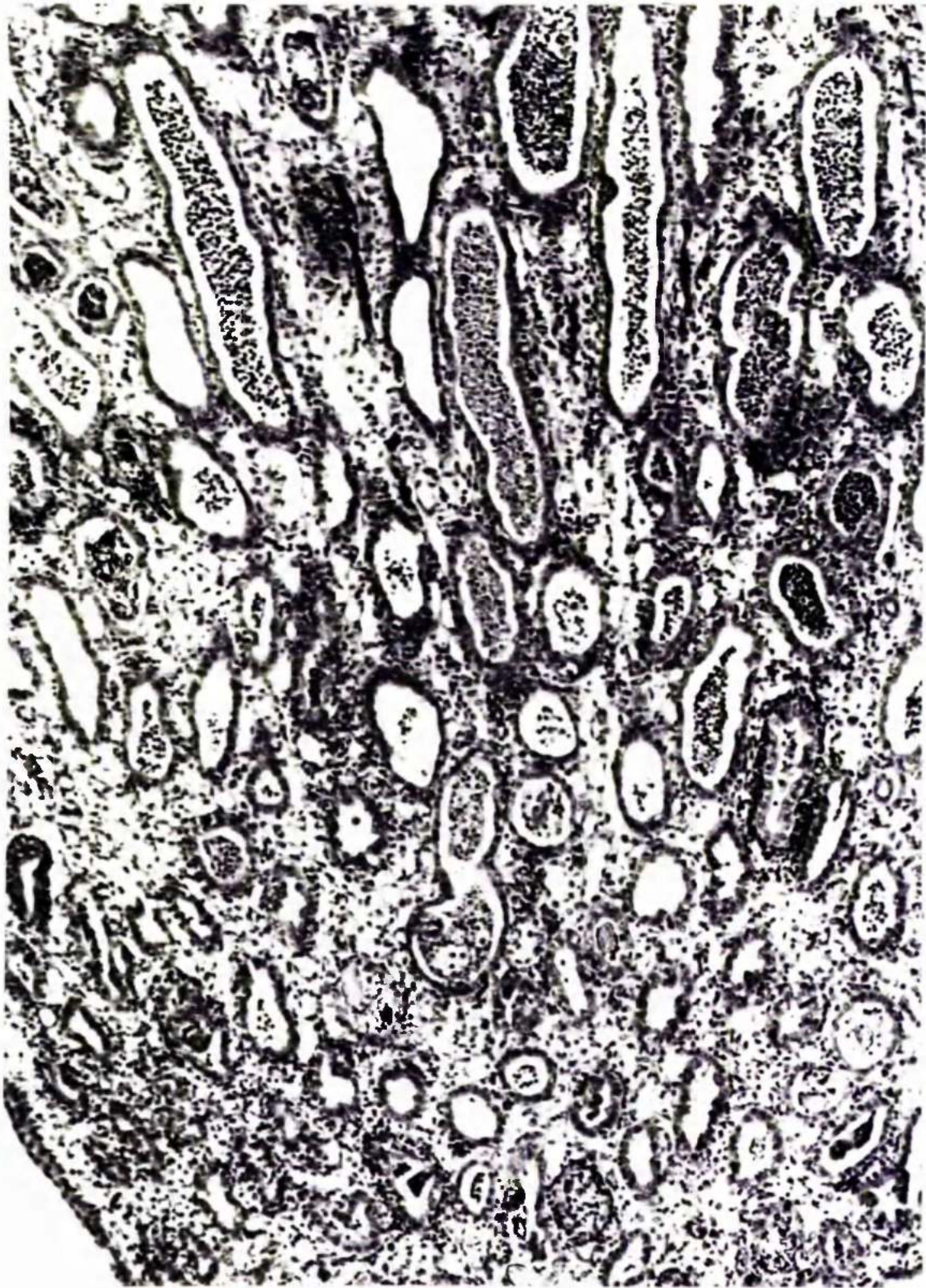


Figure 105: Gasil 200: 60 mg for 7 Consecutive Days

Medulla: Showing widespread tubular dilatation. The epithelial lining exhibiting a grading of degenerative changes. The lumena contain large numbers of desquamated cells, polymorphonuclear leucocytes and granular casts. Peritubular cuffing and extensive acute inflammatory infiltrate are encountered.

H & E Mag. X345

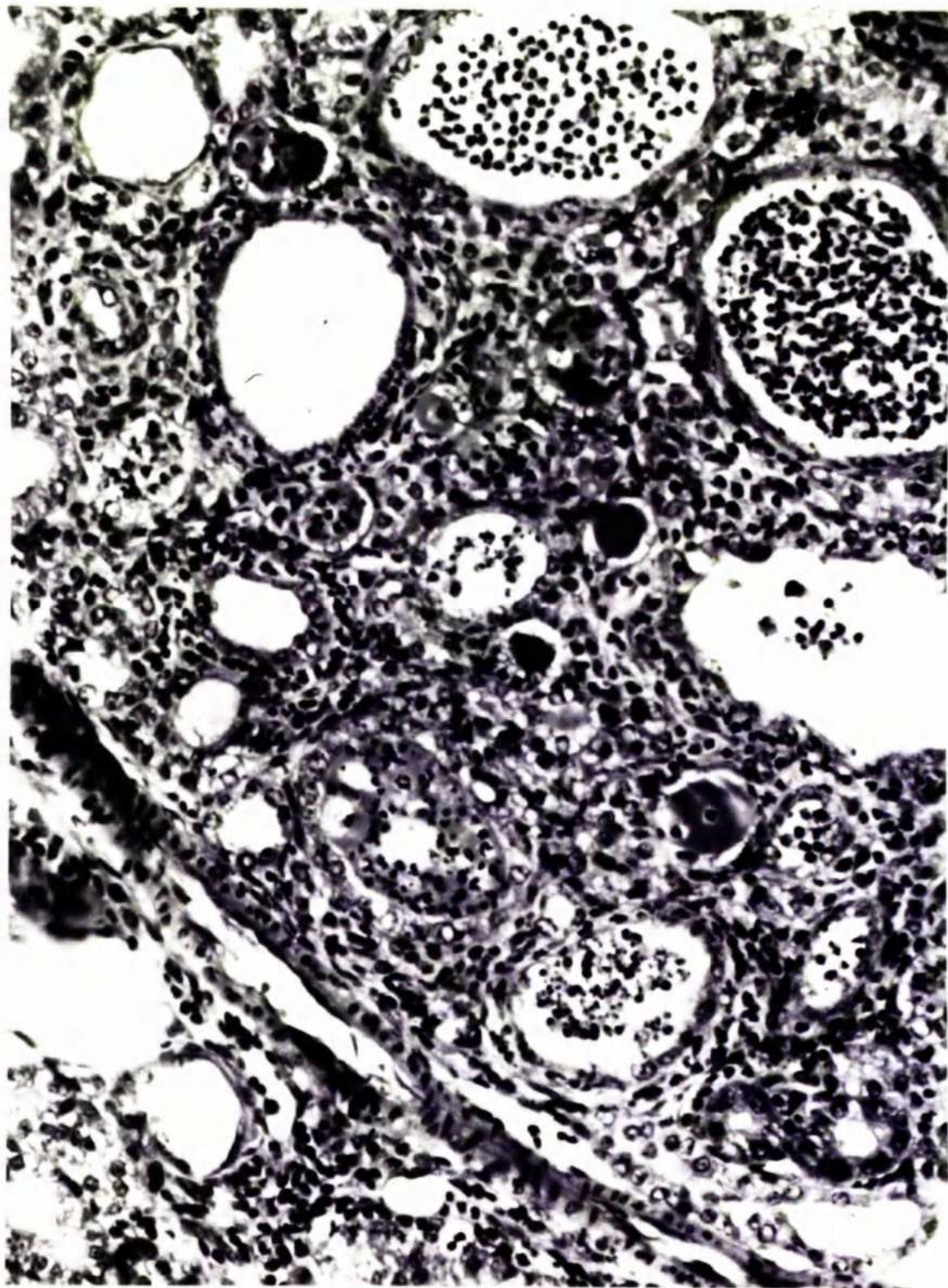


Figure 106: Gasil 200: 60 mg for 7 Consecutive Days

Normal glomerular architecture, the capillaries, however, contain mono and polymorphonuclear leucocytes.

X 7728.

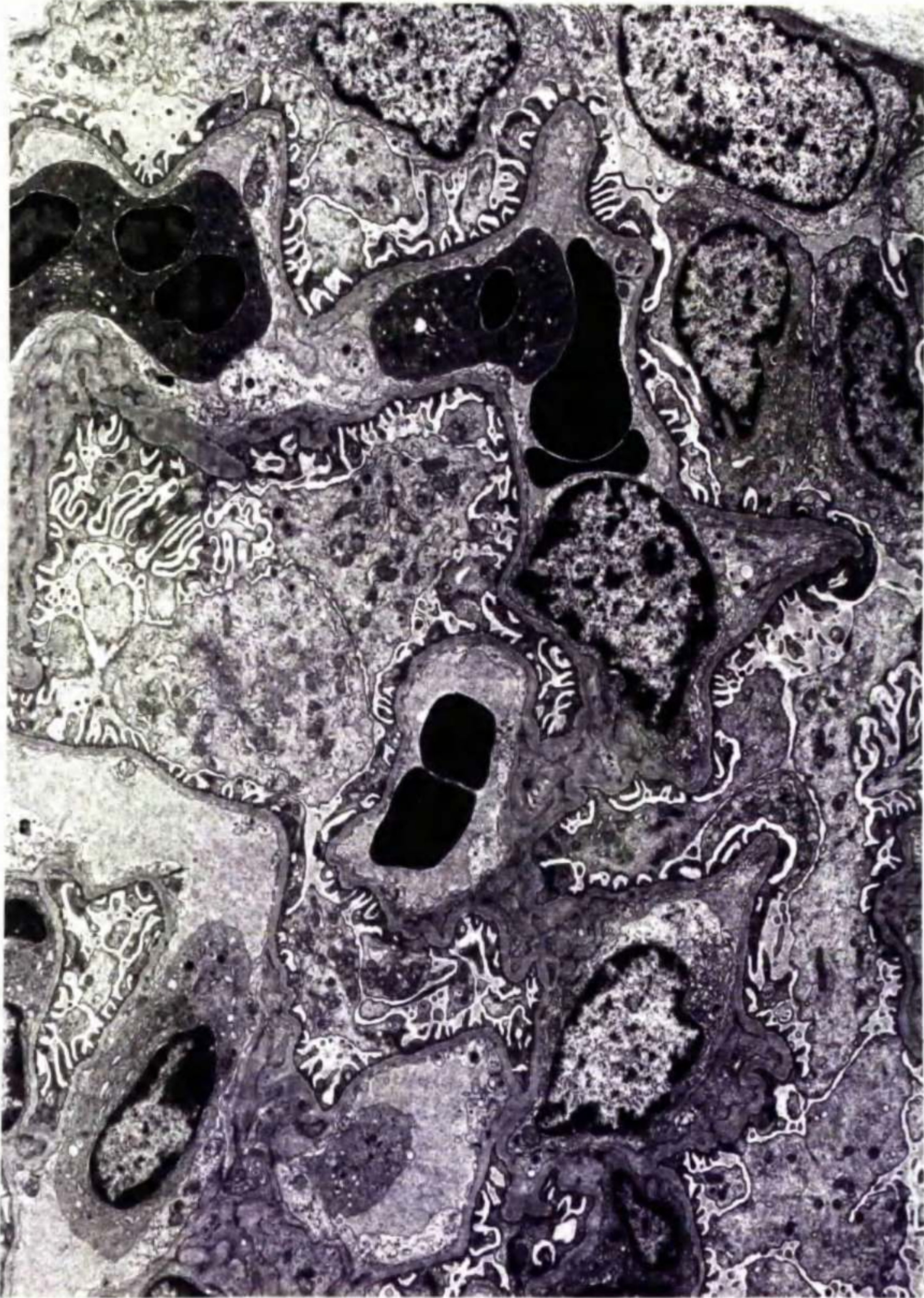


Figure 107: Gasil 200: 60 mg for 7 Consecutive Days

An example of severe tubular damage, shown here in the thin loop of Henle, where there are extensive changes in the epithelial cytoplasm and thickening of the basal lamina A prominent interstitial inflammatory infiltrate with polymorphonuclear and mononuclear cells is observed.

X 5520.

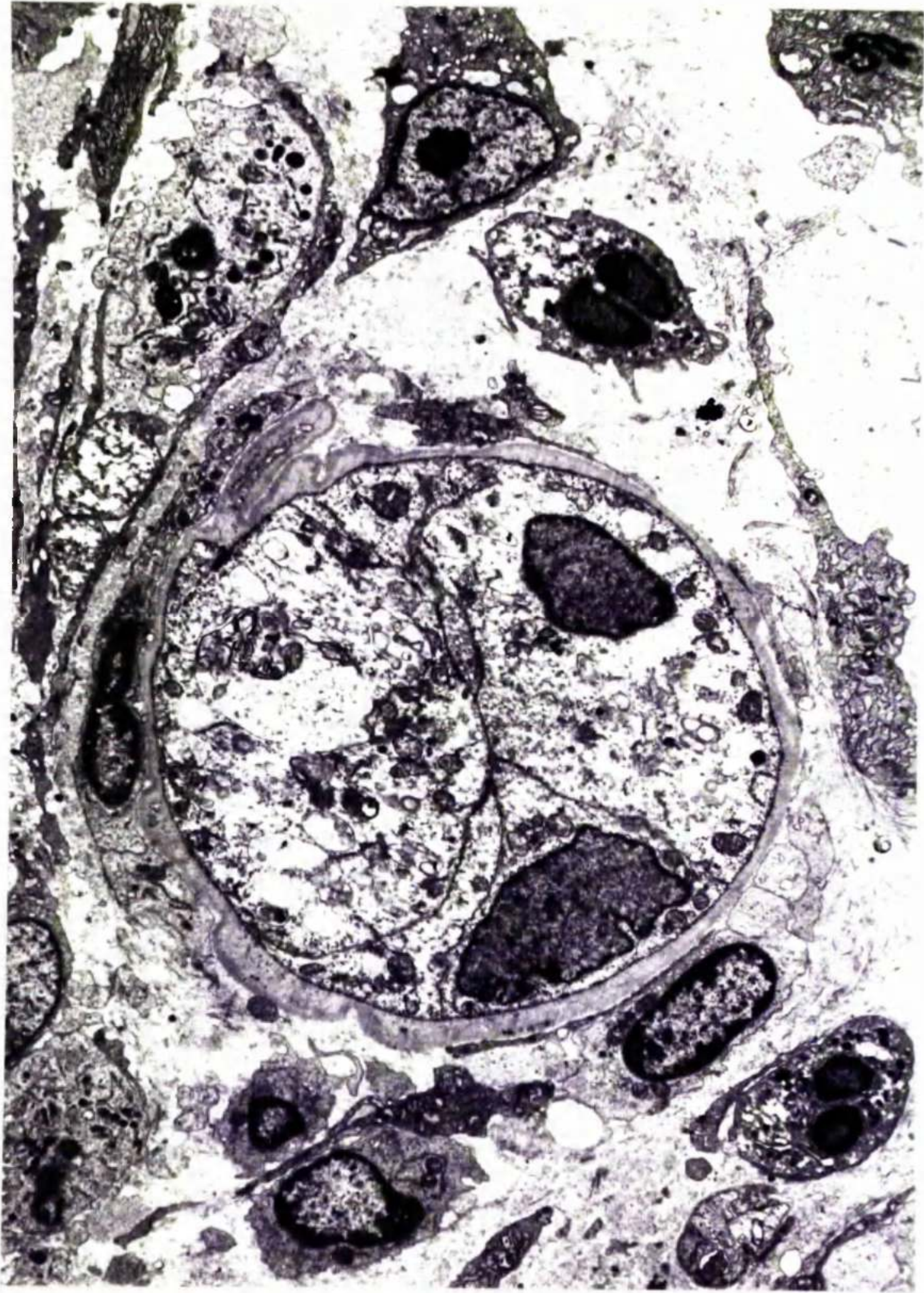


Figure 108: Gasil 200: 60 mg for 7 Consecutive Days

Electron micrograph showing a segment of the wall of a collecting duct, some of its lumen content and surrounding interstitium. The lumen is packed with markedly degranulated and degenerated polymorphonuclear leucocytes The epithelial lining of the duct demonstrat cytoplasmic and nuclear damage.

X 7728

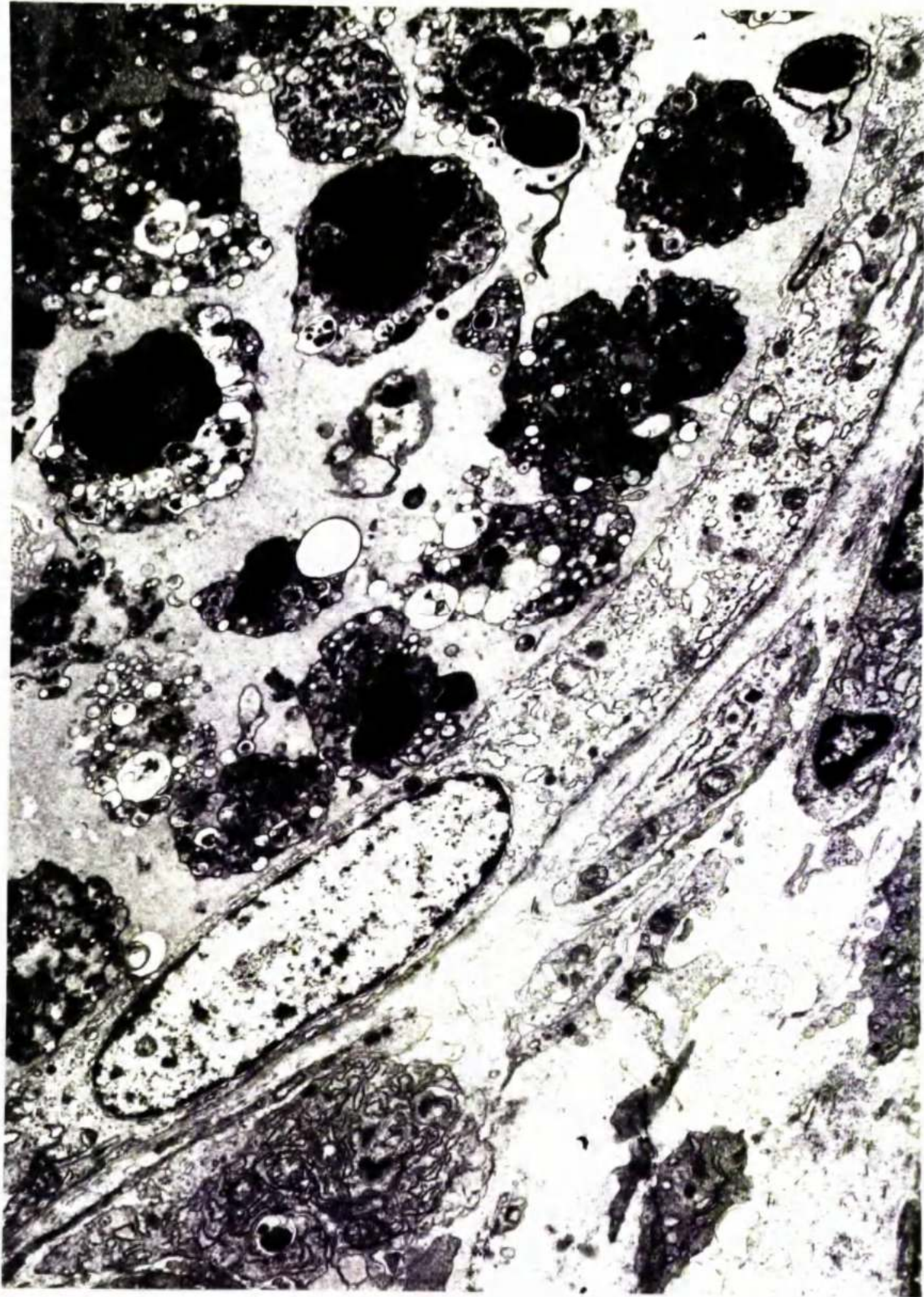


Figure 109: Gasil 200: 60 mg for 7 Consecutive Days

Interstitial infiltrate showing the nature of the inflammatory cells. The central cell is a plasma cell, others include lymphocytes, macrophage and polymorphonuclea cells.

X 7728.

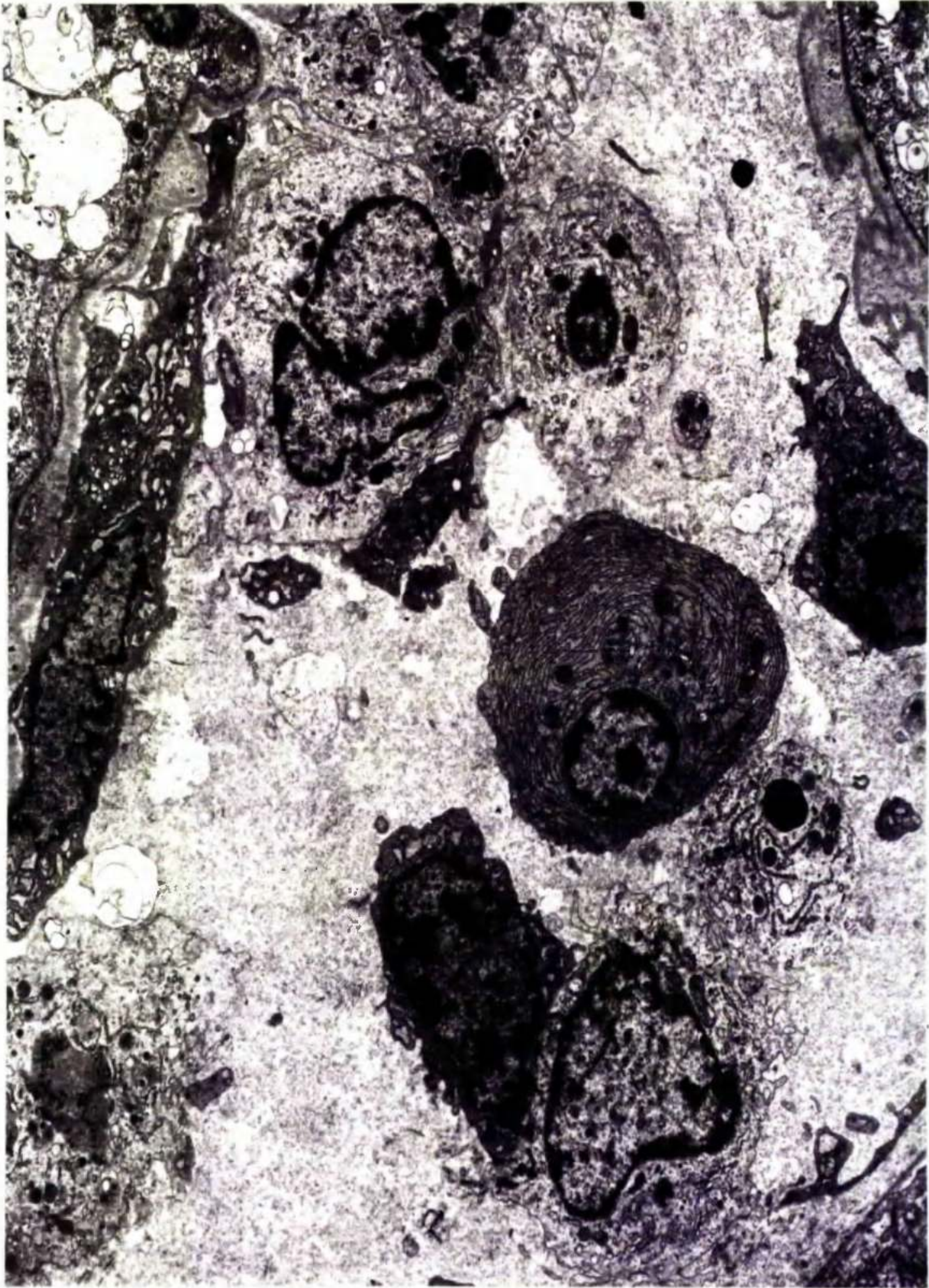


Figure 110: Gasil 200: 60 mg for 7 Consecutive Days

Unlike the cells in the ductal lumen, the interstitial inflammatory infiltrate, as demonstrated in this electron micrograph, shows much better preservation of cytologica integrity and absence of degranulation in the polymorphonuclear leucocytes.

X 3864.

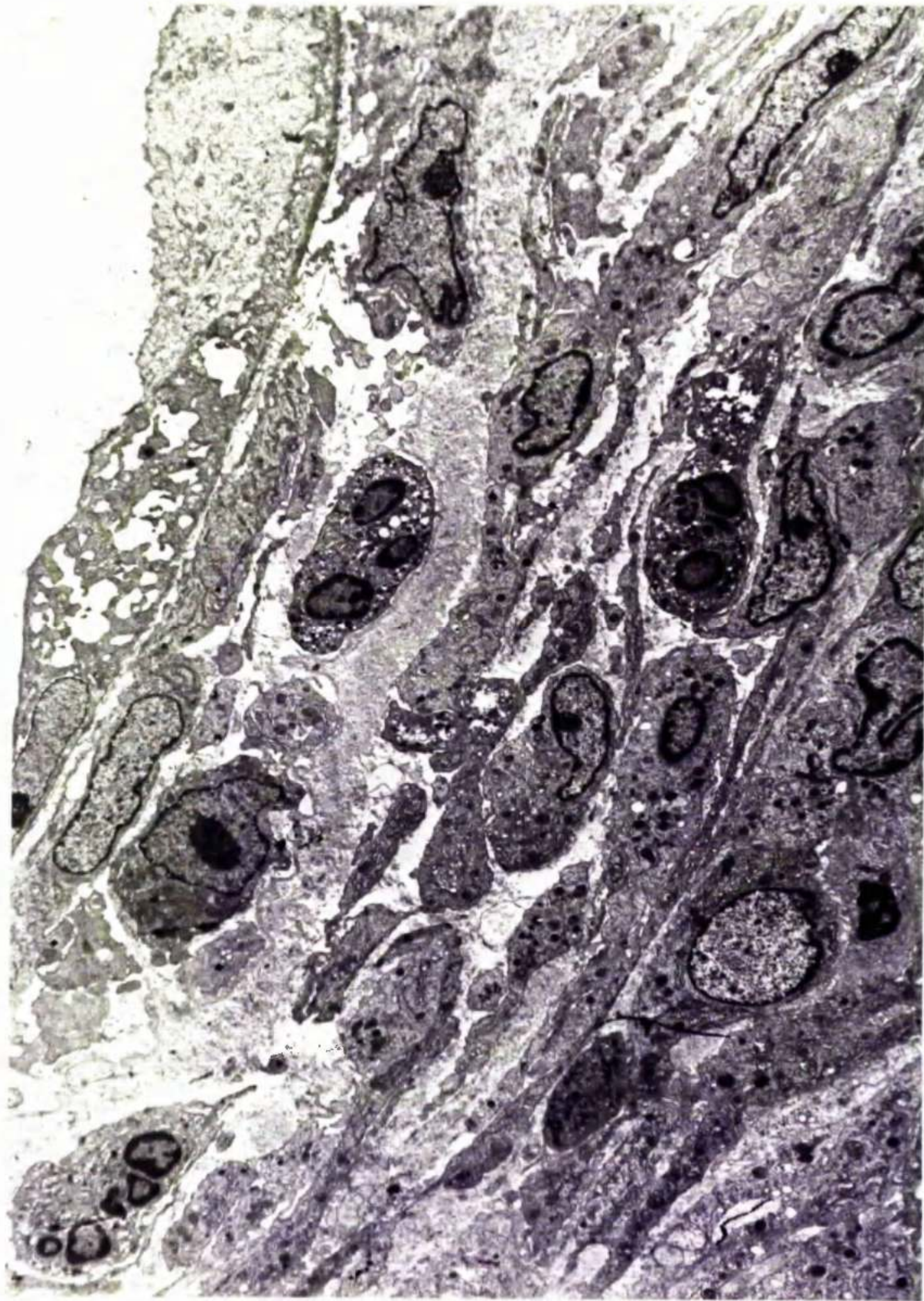


Figure 111: Immunofluorescence: anti-rat total immunoglobulin: Showing positivity in mesangium and capillary loop in the glomerulus of a control rat.

Mag. X 1450.

Figure 112: Immunofluorescence: anti-rat C_3 showing positive staining in mesangium and capillary loops in the glomerulus of a tested rat.

Mag. X 1

Figure 113: Immunofluorescence, anti-rat total immunoglobulin staining of cells in dilated collecting ducts. Although the interstitium was shown to be infiltrated by large numbers of acute and chronic inflammatory cells, non in this site took up the stain.

Mag. X 600

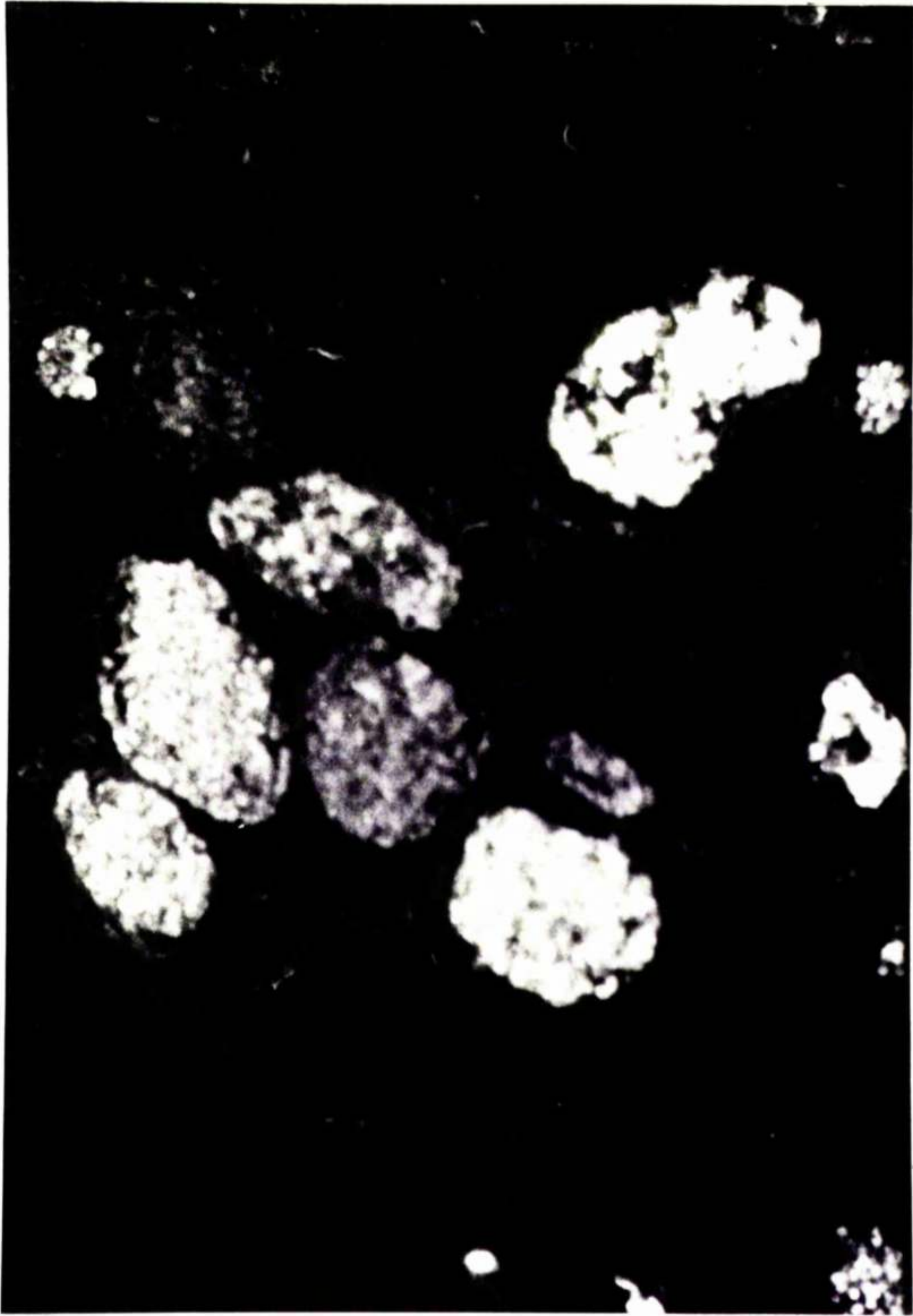


Figure 114: Immunofluorescence: anti-rat total immunoglobulin showing details of staining of lumenal cells in collecting duct.

Mag. X 1450

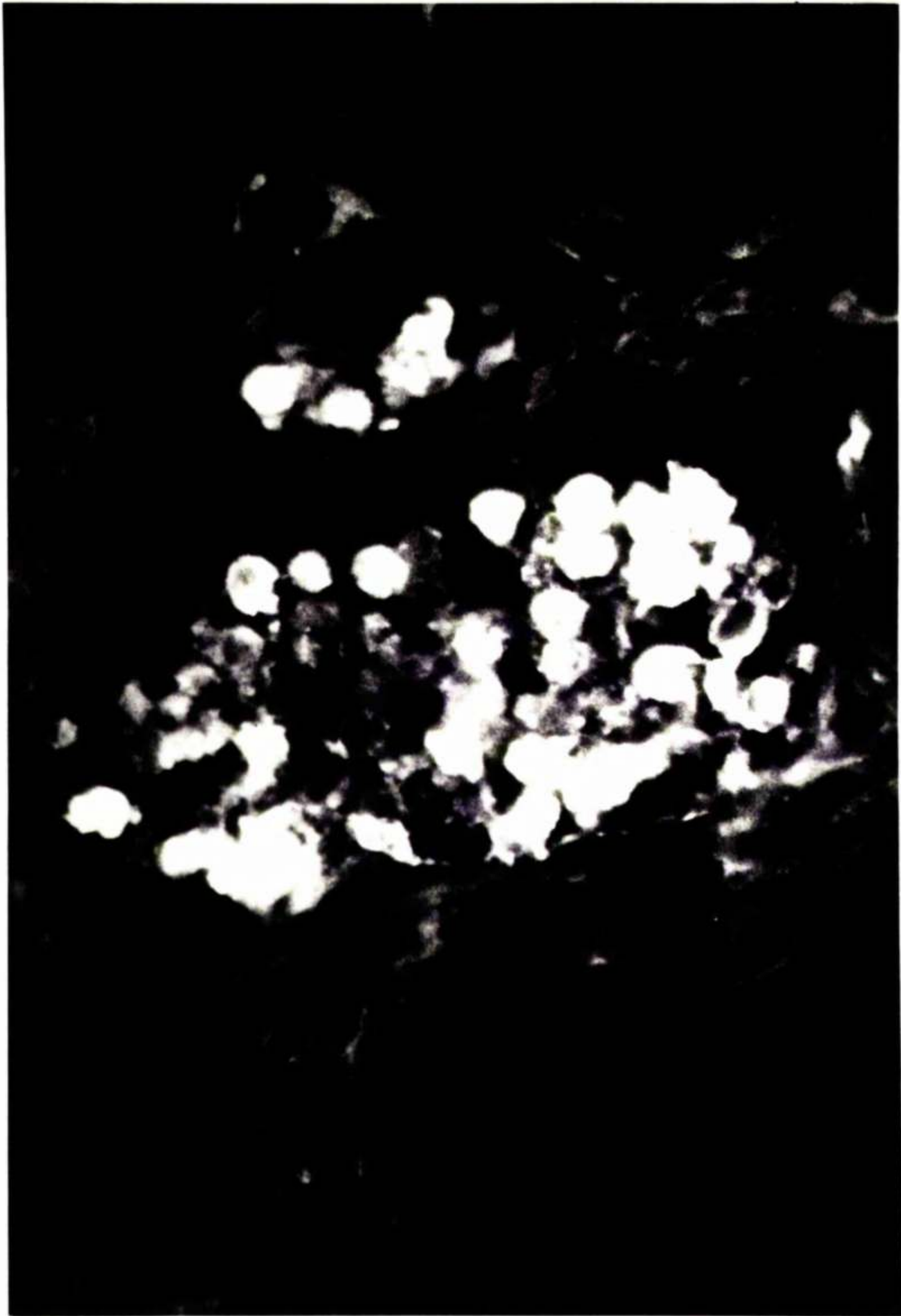


Figure 115: Para-aminophenol Hcl and Sodium Salicylate: 2 Months

Corticomedullary region showing tubular dilatation. The dilated tubules are lined by flattened epithelium which shows cytoplasmic and nuclear changes.

H & E Mag. X 86

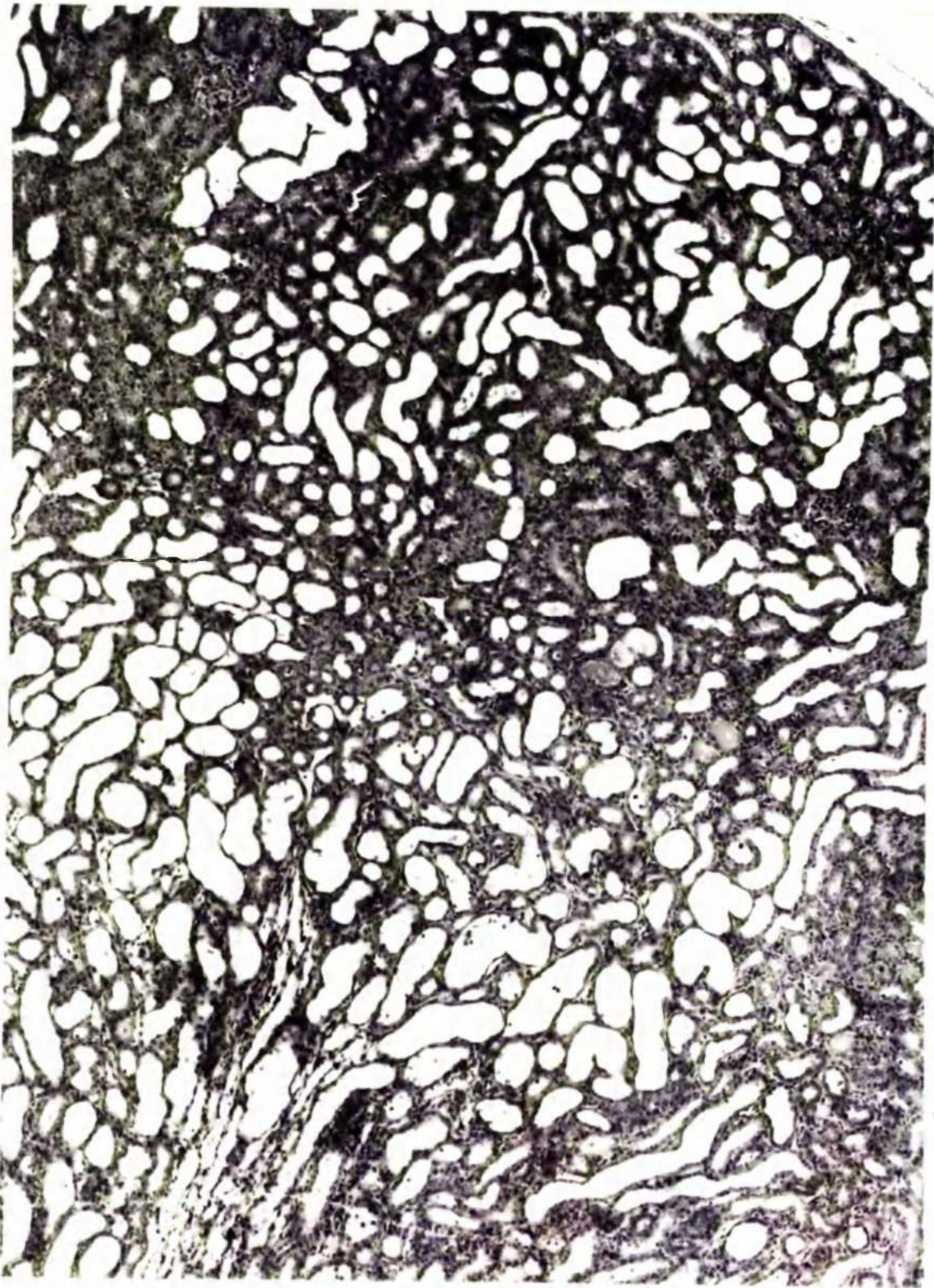


Figure 116: Para-aminophenol Hcl., Sodium Salicylate and Silicon: 2 Months

Corticomedullary region demonstrating tubular dilatation, flattening of the epithelial lining and chronic interstitial inflammatory infiltrate.

H & E Mag. X 345.

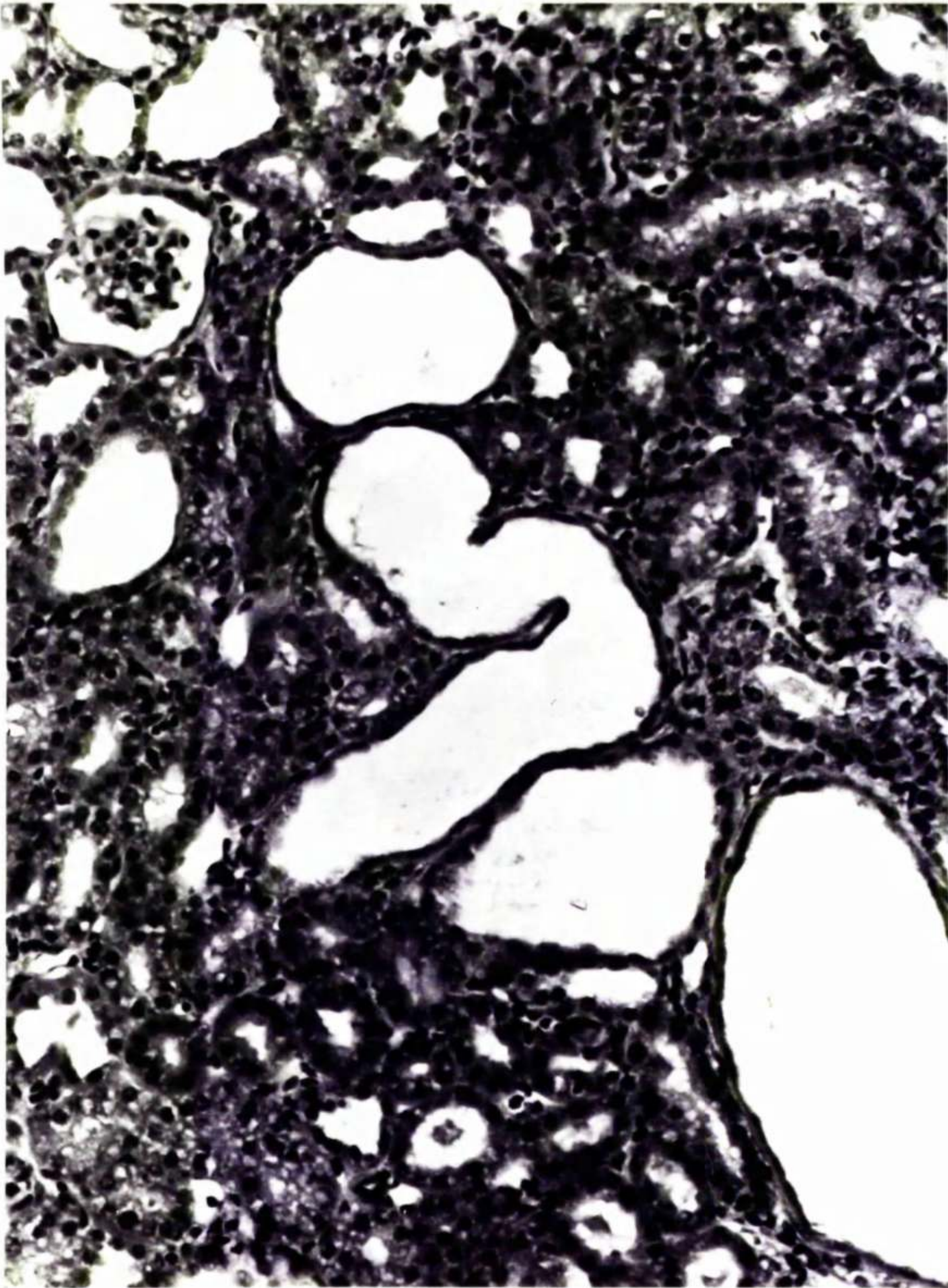


Figure 117: Para-aminophenol Hcl., Sodium Salicylate and Silicon: 3 Months

This photomicrograph showing marked degenerative changes in the epithelial lining of a variety of tubules mainly from the distal part of the nephron together with an interstitial inflammatory infiltrate

H & E Mag. X 345

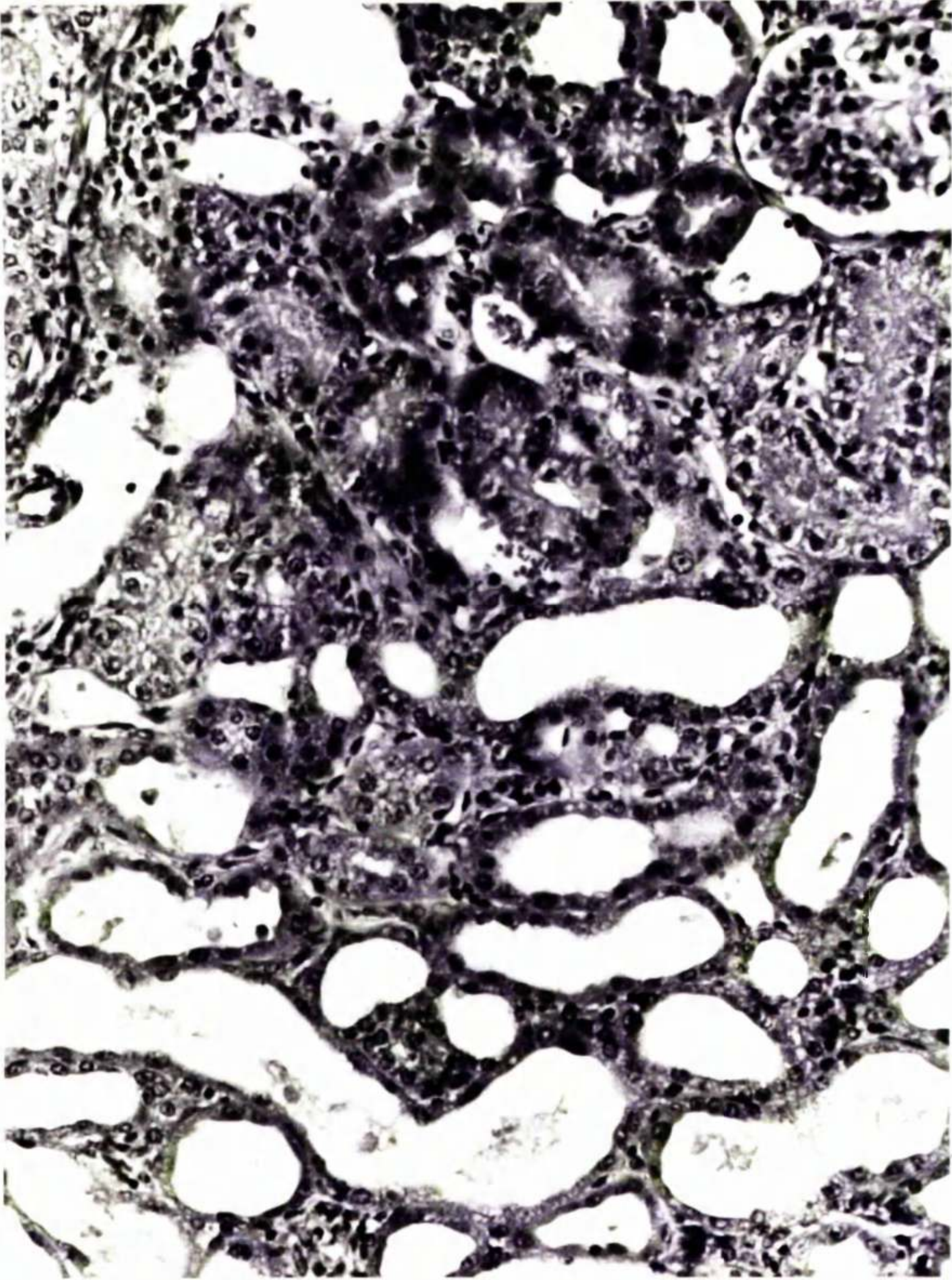


Figure 118: Para-aminophenol Hcl., Sodium Salicylate and Silicon: 3 Months

Medulla: Showing extensive distal tubular dilatation. The epithelial cells in non-dilated tubules demonstrate cytoplasmic vacuolation. There is also a chronic interstitial inflammatory infiltrate.

H & E Mag. X 860.

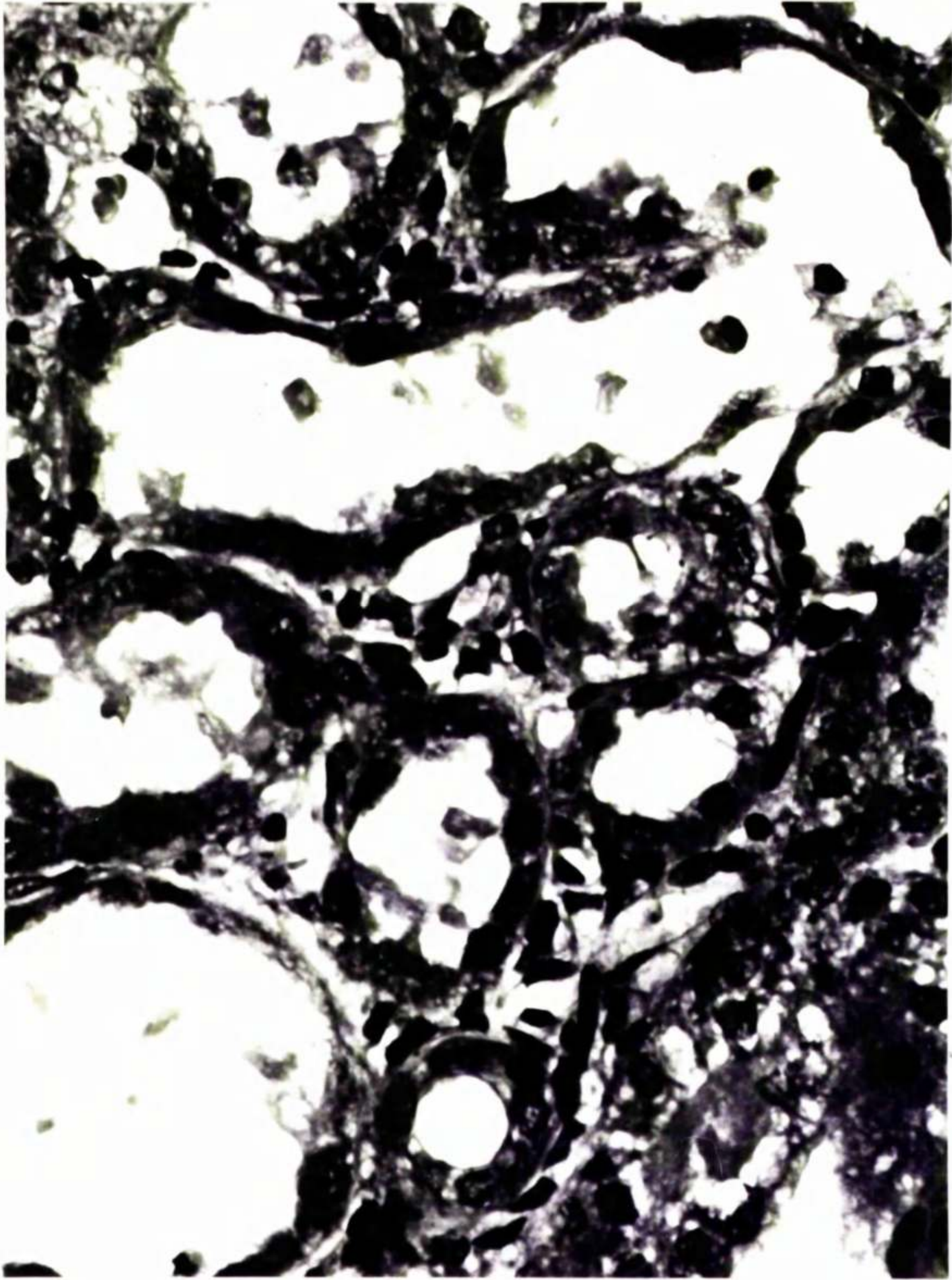


Figure 119: Intravenous pyelogram (2 minutes) of control animal showing kidneys of normal size and shape. The contrast medium is present in the bladder.

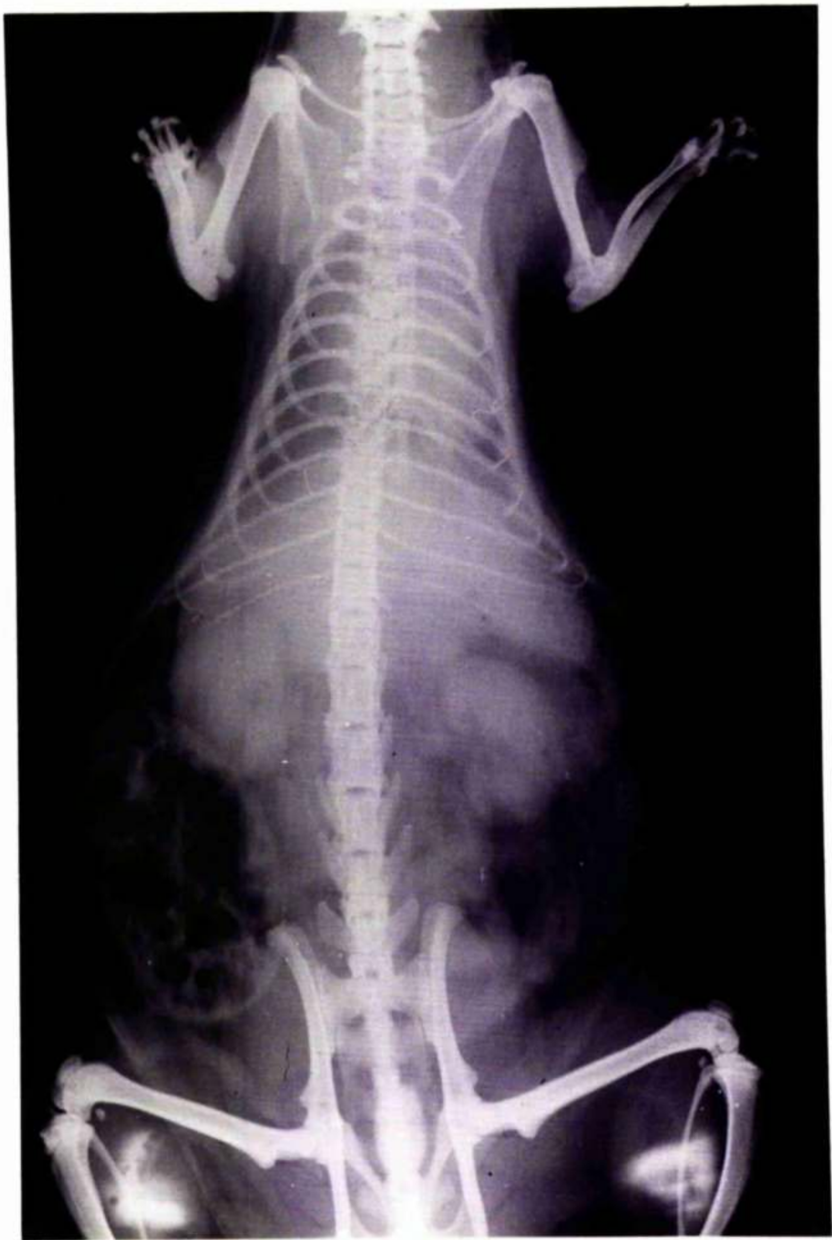


Figure 120: Intravenous pyelogram (2 minutes) of an animal subjected to acute silicon nephrotoxicity and demonstrating swollen, enlarged kidneys.

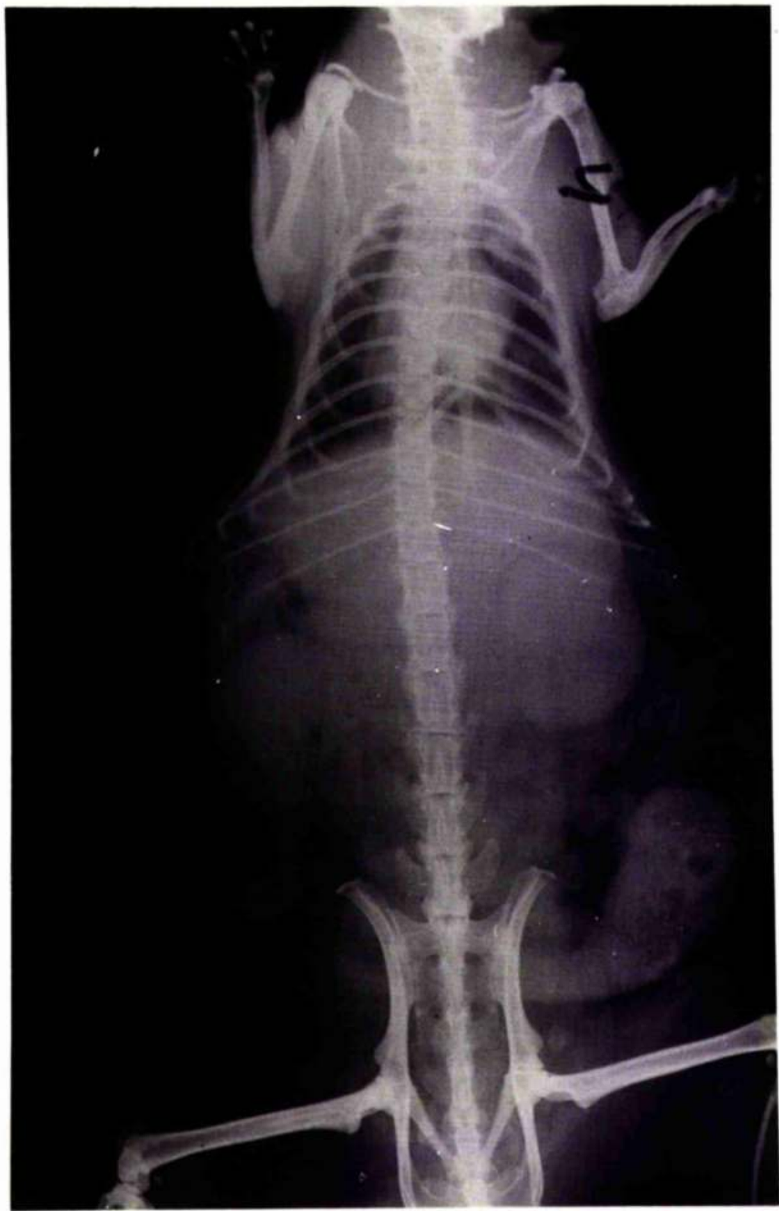


Figure 121: Intravenous pyelogram (2 minutes) of an animal subjected to repeated I/P injections of gasil 200 resulting in chronic silicon nephrotoxicity. The kidneys are small and irregular in outline.

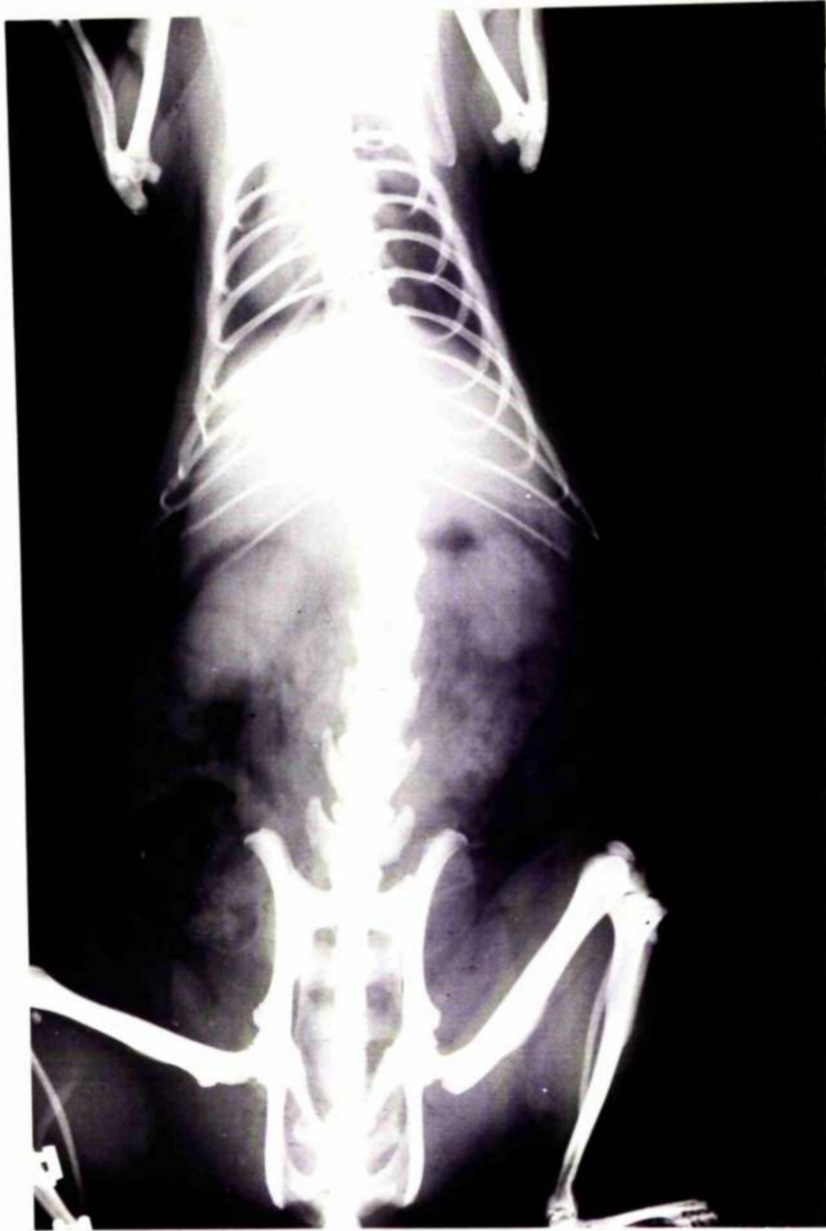
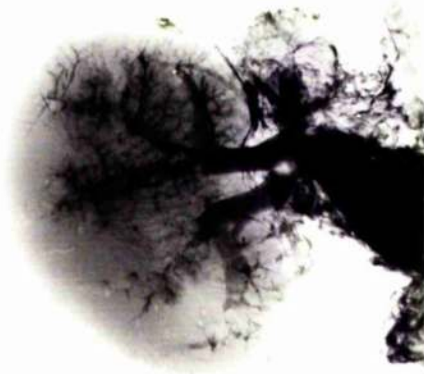
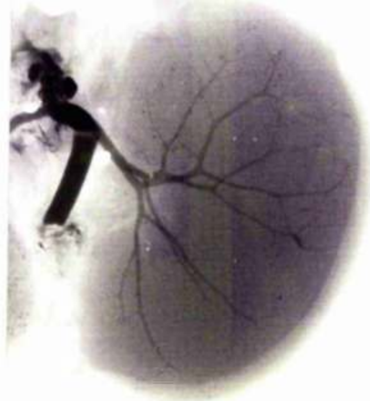


Figure 122: Arteriogram (A) and venogram (V) of normal control rat kidney. No significant abnormalities were encountered in the renal vessels of the animals with either acute or chronic silicon nephrotoxicity.

Mag. X 2.3

V





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