Glomerular filtration barrier in experimental endotoxin shock: a histopathological and physiopathological study

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Summary. In the present work a morphopathological study is carried out of the glomerular filtration barrier in 20 Large-White pigs weighing 20 kg, subjected to experimental intravenous inoculations of endotoxin from *Salmonella enteriditis*.

The study is completed with the determination of protein plasmatic levels, through urine test with the determination of pH, density, proteins, glucose, ketone body levels and urinary sediment.

The histopathological and physiopathological results reveal alterations at the level of the filtration barrier with quantitative differences between the different experimental groups.

Key words: Endotoxin shock, Filtration barrier, Histology, Physiopathology, Pigs

Introduction

The interest in the morphopathological study of the shock process lies in the importance of the vascular and proliferative phenomenon which are established, as far as they can serve as a pathological model in order to understand the pathogenesis of particular present morbid processes, both in veterinary and human medicine.

In the experimental work we used the endotoxins as causal agents of a process of a shock, because of their direct action on the endothelial cells (Bradley, 1976), which produces alterations in the microcirculation and therefore release of the pheomenon of shock; a phenomenon which is more evident in organs with strong vascularization. This is the fact which led us to use the kidney as the organ for study.

We have used the pig as an experimental animal taking into account its susceptibility to endotoxins. A

susceptibiliby presented by the relatively light weight of the heart, high cardiac frequency, the slow phases of rest between beats and the general sensibility to heat through lack of sweat glands (Shultz and Drommer, 1985).

The aim of the present work was the approximation of physiopathological parameters (plasmatic proteins and urine test) to renal morphopathology in experimental endotoxin shock, and particularly to the structural architecture of the glomerular filtration barrier.

Materials and methods

In the present work we used twenty Large-White pigs, aged sixty days and with 20 kg body weight. They were exempt from any infectious or parasitic disease and to assure that they were not suffering from any morbid process, several blood and urine tests were practised.

The pigs were divided into four experimental groups with five animals in each one. One of them was the control group and the others experimental.

Each animal was intravenously inoculated with successive doses of endotoxins from *Salmonella enteriditis* (Table 1) (0.125 mg/kg of body weight).

The sacrifice of the animals was performed after being tranquilized and anaesthesetized with strecnil (2 c.c. I.V.) and thiobarbital (0.5 g. I.V.). Vascular perfusion was done with glutaraldehyde at 5%.

Samples were taken and fixed in 10% formalin and 5% glutaraldehyde. The samples fixed in formalin were processed according to the usual methods for light microscopy and embedded in paraffin. Cuts of two to three microns thick were made from them and they were stained with methenamine silver.

The fixation of the samples for the ultrastructural study was carried out with gluraraldehyde at 5% in sodium cacodylate solution pH 7.2 and they were refixed in 2% osmium tetroxide solution.

After, they were passed through washing solution and dehydrated in an ascending scale of acetones, adding 7% uranyl acetate and 0.5% phosphotungstic acid to the one

of 70%, in order to obtain a prior contrast.

Finally the samples were embedded in Durcupan ACM.

Cuts of 60 nanometres were made from the blocks obtained which were contrasted with uranyl acetate and then with lead citrate.

The study of the urine analysis was performed at the moment of sacrifice.

The following organoleptic measurements were made:
- turbidity.
- pH.
- density.
- protein concentration.
- glucose concentration.
- ketone bodies concentration
- urinary sediment

according to standard methodology of clinical analysis (Bush, 1982; Balcells, 1984).

In the blood analysis the levels of plasmatic proteins were determined. The analysis was performed at the time of sacrifice.

Results

Morphopathological study

The study of the structural and ultrastructural changes observed in the kidneys of pigs subjected to experimental inoculation of endotoxins from *Salmonella enteriditis* , was made of the components of the glomerular filtration barrier: endothelium (endothelial cells), basement membrane and visceral epithelium (podocytes).

The division in the experimental groups was decided by the number of doses administered which led to produce a chronic morbid state.

Group I

Ultrastructurally, the most significant changes observed in the podocytes were an intense development of the granular endoplasmic reticulum, along with disorganization of pedicels at the level of Bowman's space (Fig. 1). On the other hand the presence of polymorphous structures formed of a fine electrodense granulation were found, principally in the perinuclear region, although they have been detected more or less dispersed throughout the cytoplasm (Fig. 2).

Likewise, we observed focal alterations of the endothelial cell which were characterized by light electrondensity and disorganization of the intercellular unions.

At the level of the basement membrane a slight increase could be observed, along with the start of disorganization.

Group II

In the vascular endothelium, similar modifications to the previous group were present (Fig. 3). In the endothelial cells there was a greater number of ribosomes and

lysosomes, along with large development of the granular endoplasmic reticulum (Fig. 4).

In the cellular body of the podocytes we observed numerous microvilli (Fig. 4). Likewise, in the primary prolongations there existed very osmiophilic paracrysta-lline laminar structures, well isolated or embedded in granules of moderate electrondensity (Figs. 3, 4).

In the cytoplasm of the podocytes we observed large development of the endoplasmic reticulum, multivesicu-lar bodies and numerous microvesicles (Fig. 4).

We also detected, at the level of the glomerular filtration barrier, fusion of the foot processes which, in some cases, took up large areas and in Bowman's space could be seen as being disorganized and forming digital-like structures (Fig. 4). The development and the focal disorganization of the basement membrane were similar to those of the previous group.

Group III

In the vascular wall we observed vacuolation of endothelial cells, which compressed and deformed the nuclei (Fig. 5). In this experimental group endothelial alterations were frequent, such as convexity (Fig. 6), vacuolation and disorganization of the basement membrane which, moreover, presented electrondense poly-morphous structures and in some cases loss of their architecture (Figs. 6, 7).

In this experimental group homogeneous thickening of basement membrane was more evident. The basement membrane had on its surface an intense fusion of foot processes (Fig. 7).

The podocytes presented great development of cell organelles of the cytoplasmatic vacuolar system, princi-pally mitochondria and endoplasmic reticulum.

Likewise, we observed a certain increase in the number of multivesicular bodies and lysosomes. It is important to point out that in the pedicles there exists a greater hyaloplasmatic electrondensity, fundamentally in the area which relates to the basement membrane (Figs. 6, 8). In the same way we observed a great disor-ganization of foot processes, as they showed dislocated images in the areas which related to the endothelial cells and microvilli in the cellular body (Fig. 6).

In the glomerular capillary lumen (Fig. 6), platelets and mononuclear cells (Fig. 8), with «sticking effect» towards the wall, are evident.

Group IV

The increase of basement membrane reached its highest point in this experimental group (Fig. 9), presenting intense disorganization of its structural architecture in some areas (Figs. 10, 11).

From the ultrastructural point of view, at the level of the glomerular filtration barrier we observed similar changes to those of the previous group. The glomerular capillary presented endothelial alterations (Fig. 10) and in capillary lumen, mononuclear cellular elements (Fig. 11) and grouping of platelets. The alterations of the endothelium were represented by capillary protuberan-

Table 1. Development of the experiment.

Group	Experimental Animals	Control	Sock Time	Doses
I	4	I	10 days	2
II	4	I	20 days	4
III	4	I	30 days	6
IV	4	I	50 days	8

Table 2. Urine and blood test.

URINE TEST

	Control bright	Group I turbid +	Group II turbid +	Group III turbid + +	Group IV turbid + +
Transparency	bright	turbid +	turbid +	turbid + +	turbid + +
pH	6.2 ± 0.2	5.2 ± 0.3	5.3 ± 0.1	5.1 ± 0.3	4.9 ± 0.2
Density	1.015 ± 0.02	1.015 ± 0.1	1.020 ± 0.01	1.030 ± 0.02	1.035 ± 0.02
Proteins	–	–	5.4 ± 0.3 g/dl	6.3 ± 0.4 g/dl	7.9 ± 0.2 g/dl
Clucose	–	–	–	–	–
Ketone bodies	–	–	–	–	–
Sediment	–	–	–	–	–
Erythrocytes	–	–	–	–	–
Leucocytes	–	–	–	–	–
Epithelial Cells	–	+	+ +	+ + +	+ + +
Crystals	–	calcic oxalate+	calcic oxalate+	calcic oxalate+ +	calcic oxalate+ +
Cylinders	–				
–Hyaline		+ + +	+ + +	+	+
–Epithelial		+ + +	+ + +	+	+
–Cereous		+	+	+ + +	+ + +

BLOOD TEST

	Control	Grup I	Group II	Group III	Group IV
Total Proteins	8.5 ± 0.2 g/dl	7.2 ± 0.3 g/dl	6.3 ± 0.2 g/dl	4.5 ± 0.46 g/dl	4.3 ±0.3 g/dl

ces projecting into capillary lumen and focal densification of the cellular hyaloplasm (Fig. 10).

Physiopathological Study

In relation to the organoleptic results we noted the more manifest turbidity of the last two experimental groups (Table 2).

In the urinary parameters we point out a fall in pH in the whole experiment, although it was more evident in group IV. On the other hand, the density of the urine was higher in the last two groups. Likewise, there was also a general increase in total protein levels (Table 2) (Figs. 12, 13, 15).

In the urinary sediment we detected the presence of calcium oxalate crystals in all the experimental groups, of hyaline cylinders and descamations of epithelial cells in groups I and II, and in addition, of cereous cylinders in groups III and IV (Table 2).

Study of the blood analysis. There exists a progressive reduction of plasmatic protein levels throughout the experiment down to levels of 4.5 ± 0.3 g/dl in the fourth experimental group (Table 2) (Fig. 14).

Discussion

The histopathological findings which are established in renal septic shock show alterations at 3 levels: glomeruli, renal tubules and interstitium (Mendez et al., 1987).

The morphopathological changes found in the glomerular filtration barrier were similar to those detected in acute renal failure in man (Rotter et al., 1962) and in neurotoxin shock in pigs (Drommer et al., 1982).

The presence of alterations in the vascular endothelium was significant in our experiment. These alterations were characterized by greater electrondensity of the hyaloplasm, disorganization of the cellular unions and cytoplasmic convexities (Kin et al., 1975; Bohle et al., 1979; Helchen and Thurau, 1980; Kaup et al., 1984), which provoked the possible passing of macromolecules to Bowman's space and thus, the presence of proteinuria, which has also been detected. Similar results have been described in studies in pigs of the glomerular alterations in different types of processes, such as shock (Shirota et al., 1986).

The glomerular basement membrane presented an increase in its development parallel to the number of doses injected and the time of shock; an increase which always coincided with the degree of structural disorganization (Redondo et al., 1987).

Numerous mononuclear cells mainly lymphocytes, lymphoblasts and some platelets are sequestered in the glomerular capillaries (Richman et al., 1980). We think that the before-mentioned cells are essential for the repairing of the endothelial alteration (Rosenbruck et al., 1984; Shultz and Drommer, 1985).

Likewise, we believe that the presence of these cells in the glomerular capillary would produce an increase in the glomerular area (Rosenbruck et al., 1984).

Fig. 1. Group I. Detail of glomerulus. Disorganization of pedicles (*) and cellular remains can be seen in Bowman's space. × 8,500

Fig. 2. Group I. Electrodense polymorphous granulation in the perinuclear region of the podocytes (arrow). × 20,000

Fig. 3. Group II. Glomerular capillary with aglutination of erythrocytes in the lumen. Endothelial alteration can be seen. Likewise electrondense structures in the pedicles can be seen (arrow). × 8,500

Fig. 4. Group II. Podocytes with large development of cytoplasmic organelles and electrondense laminar structure in the pedicles (arrow). Likewise, fusion of pedicles onto the basement membrane of the endothelial cells can be observed. × 21,000

Likewise, we have detected that the pedicles present a strong increase in their cytoplasmic organelles (Helchen and Thurau, 1980; Kin et al., 1975), correlated this with cellular hyperactivity. A hyperactivity which would provoke increase of the microvilli, observed throughout the experiment and which means a greater absorption ability.

Equally, both in the cellular body of the podocytes and in their prolongations, we found electrondense paracrystalline structures which can be interpreted either as a disturbance of the cellular metabolism, or as an increase of the synthesis of the glomerular capillary basement membrane.

The information extracted from the study of the plasmatic protein levels shows the existence of hypoproteinaemia, specially intense in groups III and IV, corresponding to the proteinuria detected in these same groups. This information correlates with the renal morphopathological modifications found.

The proteinuria detected from group II onwards is due to the conjunction of the glomerular alterations (Chinar et al., 1974; Lippman et al., 1974; Andrew, 1975; Lippman, 1981; Addis, 1984).

There is an increase of protein destruction which takes place in the last phases of shock, provoking hypoproteinaemia (Bush, 1982).

One of the characteristic morphopathological alterations of septic shock was the fusion of pedicels, an alteration which we have found throughout the experiment, and corresponds to the proteinuria also detected (Andrews, 1975; Zollinger, 1971).
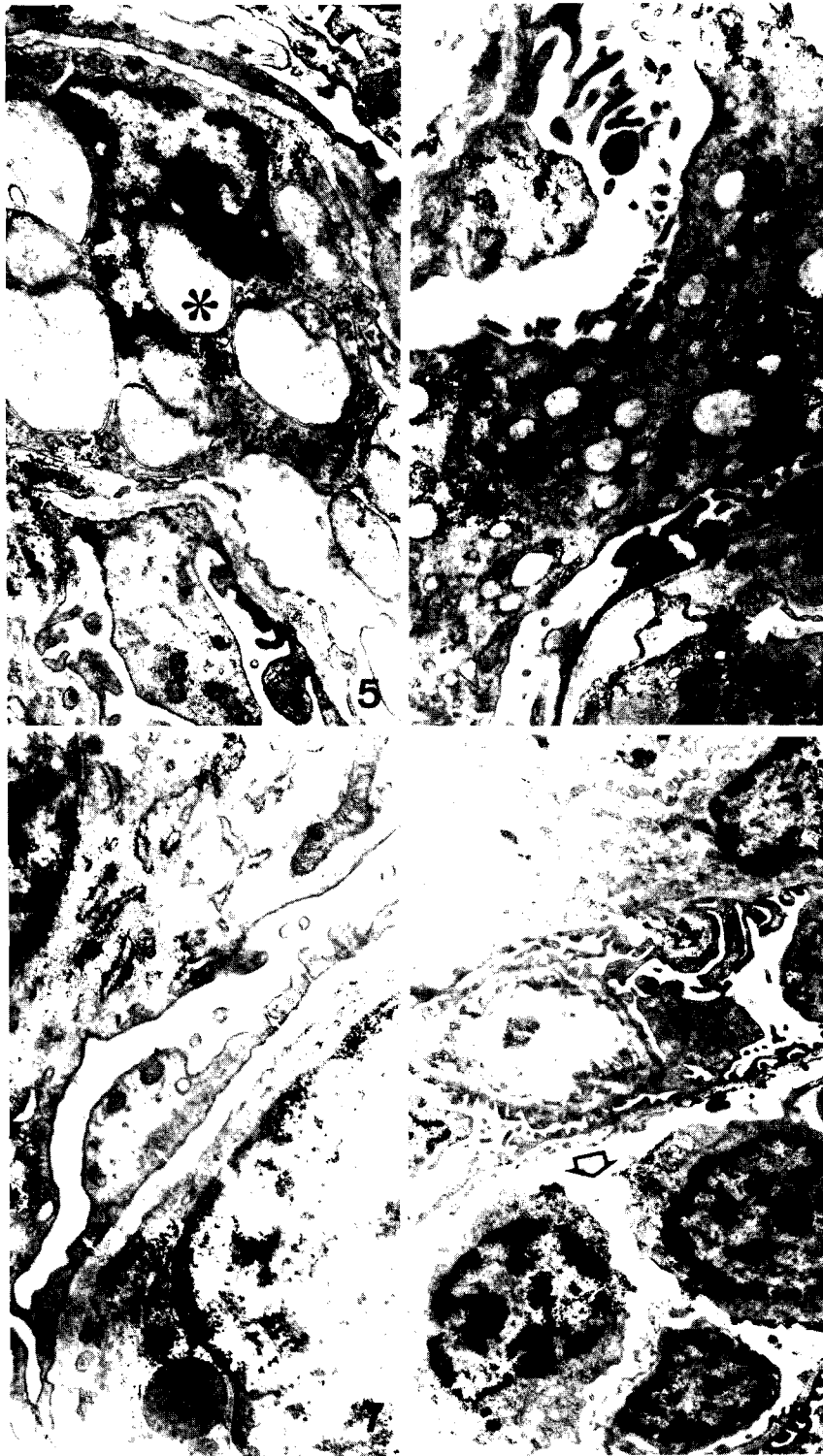
The effective and selective glomerular filtration barrier to diffusion is probably the basal lamina. The permeability of the basal lamina may be altered in shock

Fig. 5. Group III. Endothelial cells with cytoplasmic vacuolations (*). × 18,000

Fig. 6. Group III. Detail of glomerulus in which increase of basement membrane with electrodense polymorphous structures (arrow) can be observed. Evagination of endothelial cells and vacuolation of the cytoplasm of podocytes. × 18,500

Fig. 7. Group III. Detail of glomerular filtration barrier with fusion of pedicles (arrows). Likewise, electrondense vacuoles in the endothelial cells and intense development of the cytoplasmic organelles can be seen (arrow). × 19,000

Fig. 8. Group III. Presence of mononuclear cells in the glomerular capillary lumen (arrow). × 14,000

filtration (Bush, 1982). Decrease in pH levels of the urine, a characteristic of prolonged septic shock processes, is closely connected with the increase of protein catabolism and proteinuria (Bush, 1982).

Furthermore, the density of the urine is higher in the last groups, which correlates with the tubular disintegration detected and the presence of calcium oxalate crystals (Iovine and Selva, 1981; Redondo et al., 1987). Likewise, this increase of density level is characteristic of prolonged shock processes.

From the study of the urinary sediment we note the presence of epithelial cells related to the tubular destruction, being more manifest in the last experimental groups, where the tubular degeneration is more intense (Balcells, 1984).

Equally significant is the presence of hyaline and epithelial cylinders in groups I and II, indicative of acute renal failure (Bush, 1982).

In the last two experimental groups cerous cylinders are more frequent which is related to the chronic renal failure present in the final phases of septic shock (Redondo et al., 1987).

There are alterations of the glomerular filtration barrier whose morphological expression is qualitatively similar in the different experimental groups, only showing quantitative differences; alterations which correlate with the analytical determinations of blood and urine.

process in which high molecular compounds (immune complexes) are deposited on or within the barrier. Proteinuria may result from a decreased capacity of tubular resorption or tubular damage, but most proteinuria is a consequence of altered glomerular

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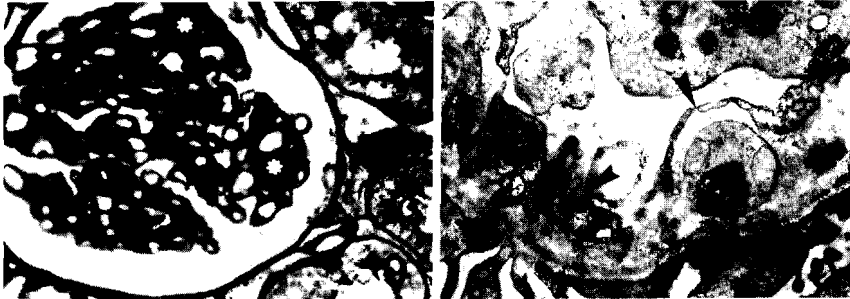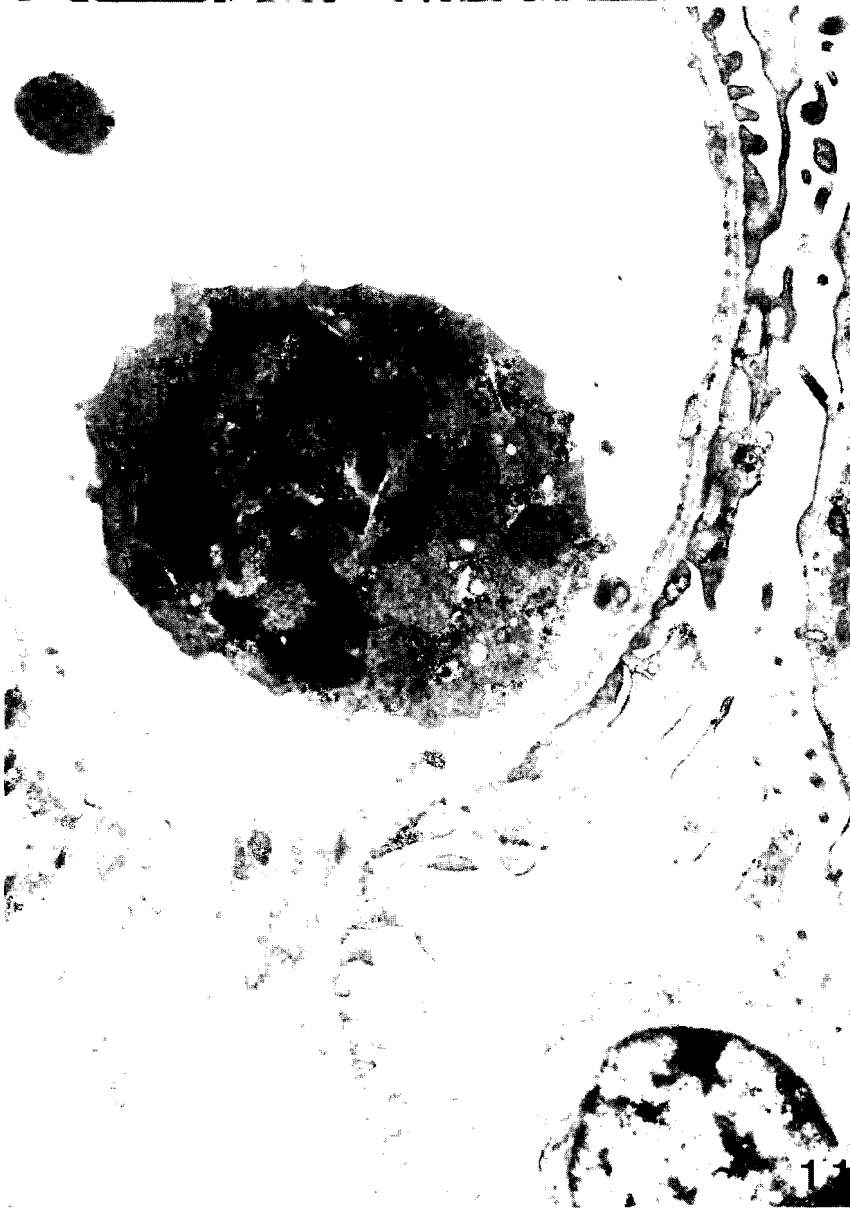Andrews P.M. (1975). Scanning electron microscopic of the

Fig. 9. Group IV. Detail of glomerulus with intense development of the basement membrane (*). Methenamine silver. × 320

Fig. 10. Group IV. Endothelial cells with electrondense and irregular protrusions. (arrows). × 17,500

Fig. 11. Group IV. «Sticking effect» in the glomerular capillary lumen. × 14,000

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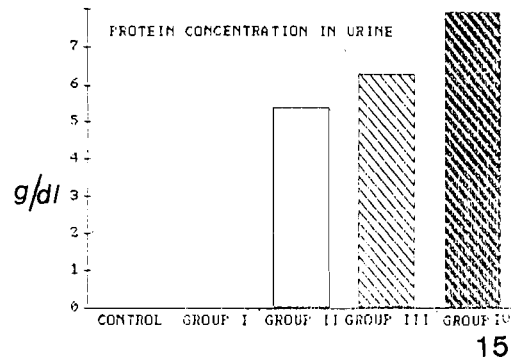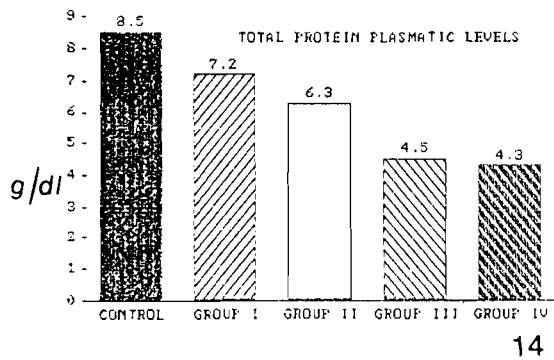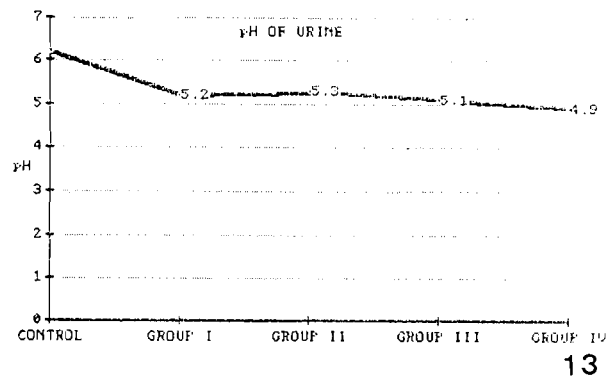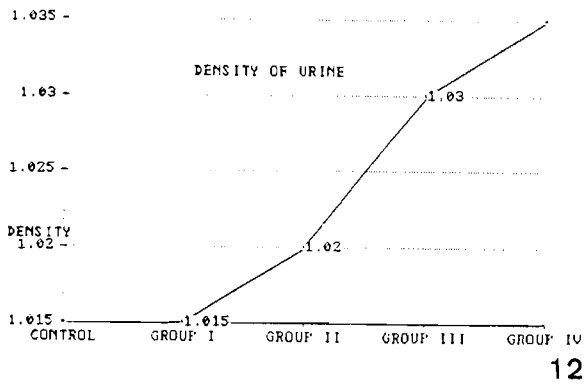
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