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PATHOLOGICAL STUDIES ON THE RABBIT VIRAL HEMORRHAGIC DISEASE (RVHD) WITH SPECIAL REFERENCE TO THE USE OF VITAMINS A, E & C AS PROPHYLAXIS

(With 2 Tables and 9 Figures)

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دراسات باثولوجية على مرض النزف الدموي الفيروسي في الأرانب مع إشارة خاصه لاستخدام فيتامينات أ ، هـ ، ج كعامل وقائي

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أجريت هذه الدراسة لتوضيح تطور مرض النزف الدموي الفيروسي في الأرانب ومكان تكاثر الفيروس في الخلايا . ولمعرفة كفاءة استخدام فيتامينات أ ، هـ ، ج كعامل وقائي. أجريت الدراسة على حالات مرضيه إكلينيكية كذلك تم إحداث المرض تجريبيا . تم تسجيل الأعراض الإكلينيكية والهستوباثولوجية وكذلك التركيب الدقيق للتغيرات التي يحدثها المرض. وقد أظهرت النتائج أن تكاثر الفيروس يتم في حويصلات صغيرة محاطة بغشاء رقيق أو حول فجوات في داخل سيتوبلازم خلايا الكبد المصابة ثم يتم انتشار الفيروس في السيتوبلازم وينتقل إلى النواة. ووجد ان الفيروس له خصائص مورفولوجية وسيتوباثولوجية تشبه الفيروسات التابعة لمجموعة (كاليكيفيروس). وجد أن ارتفاع نسبة الوفيات في الأرانب المصابة إنما نتج من موت خلايا الكبد وظهور جلطات دموية كثيرة في الأوعية الصغيرة مما أدى إلى نزيف دموي حاد في معظم الأعضاء والأنسجة . وأوضحت الدراسة أن كثرة ظهور الجلطات في الأوعية الدموية نتج عن الموت الحاد لخلايا الكبد وكذلك الخلايا المبطنة للأوعية الدموية. في الأرانب التي أعطيت الفيتامينات (أ ، هـ ، ج) كوقاية وجد أن التغيرات الباثولوجية قد قلت حدتها مع انخفاض قدره الفيروس لتلازن الدم (HA) مع زيادة الاستجابة المانعة للأجسام المناعية المثبطة لتلازن الدم (H.I,Titer) مما يوضح دور هذه الفيتامينات على أضعاف قوة الفيروس المعدية كذلك قدرتها على حمايه وتنشيط الجهاز المناعي في الأرانب المصابة .

SUMMARY

This study was conducted to explore the pathogenesis and the site of replication of rabbit hemorrahagic disease (RHD) virus, as well as the efficacy of using of vitamins A,E and C as prophylaxis against infection .The study was performed on naturally and experimentally infected rabbits. The clinical observations, histopathological and ultrastuctural changes in different organs were recorded. Viral replication occurred in the membrane - bound cisternae and vesicles or scattered around the membrane - bound vacuoles of the cytoplasm of necrotic hepatocytes, then released and gain access to the nuclei. The viral particles have characters resembling that of caliciviruses in its morphology and cytopathology. The high mortality rate occurred due to severe hepatic necrosis, disseminated intravascular coagulation (DIC) and subsequent hemorrhages in different organs and tissues. The DIC phenomena occurred due to severe hepatocellular necrosis and necrobiosis of the endothelial cells. There were marked necrosis in the lymphoid tissue of the splenic follicles. In the vitamins supplemented rabbits , the histopathological changes were minimized, the mean HA titer was lowered and the mean HI titer was increased. This indicating the role of the vitamins in lowering viral antigenisity and protecting and enhancement of the immune system of the infected rabbits.

Key words: *Rabbit ,RVHD, Pathogenesis ,Ultrastructure, Vitamins.*

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is an acute viral disease characterized by necrotic hepatitis and disseminated intravascular coagulation (Park and Itakura, 1992). The disease is characterized by an acute onset with high morbidity and mortality rate. The pathological changes are severe generalized circulatory dysfunction (hyperemia, congestion & hemorrhage), marked degeneration of parenchymatous tissues, pronounced serohaemorrhagic pneumonia and extensive disruption of reticulolymphoid tissue (Xu and Chen, 1989). Since RHD was first reported in China (Liu et al., 1984), a few ultrastructural studies have been performed on tissues of rabbits with RHD.The site of replication is not clear, virus like particles were controversially reported

to be present in the nuclei or the cytoplasm of hepatocytes and other cells (Park et al., 1987; Gregg and Hense, 1989; Marcate et al., 1989; Park et al., 1992; Salem and El Ballal, 1992).

In Egypt the disease is responsible for severe economic losses among rabbits in different localities (Ghanem and Ismail, 1992; Salem and El-Ballal, 1992 and Abdel-Aziz et al., 1995).

Nutrition plays a significant role in the development and function of immune system. Essential nutrients, such as vitamins, may affect not only the humoral and cell mediated immune response, but also non specific humoral factors, such as lysozomes or hormones, which regulate the immune response (Weber, 1997). Vit. A (Chew, 1987), vit. E (Franchini et al., 1991) and vit. C (Lovell, 1989; Beisal, 1982 and Sayed and Abd-Elghaffar, 1999) have been used as immunostimulant, antibacterial and antiviral agents.

The objective of this work was directed to study:

- The pathomorphological changes in different affected organs in rabbits naturally and experimentally infected with RVHD.
- The ultrastructural changes induced by RVHD virus with special reference to the site of viral replication and its morphology.
- The efficacy of using vitamins (A, E and C) as prophylaxis.

MATERIALS and METHODS

Clinical cases:

Alive and freshly dead rabbits of different ages and breeds were obtained from outbreaks of RVHD in different localities at Assiut and Sohag Governorates. Cases were subjected to clinical and post mortem examination. Sample were taken from internal organs (liver, lung, kidney and spleen) and subjected to bacteriological and histo-pathological examination as well as for RHD virus identification. The percent of mortality as well as the age and sex of dead animals were recorded.

Experimental animals:

A number of 33 rabbits of native breed of two-months age old with no history of RVHD outbreaks or vaccination against the disease were obtained from private farms. Rabbits were kept under sanitary hygienic measurements at the Dept. of Pathology & Clinical Pathology, Fac. of Vet Med., Assiut Univ. Rabbits were fed on commercial pellets.

Organ suspension preparation:

10% bacterial free suspensions of liver from freshly dead rabbits in physiological normal saline was prepared by adding penicillin (10,000 IU/ml) and streptomycin (10 mg/ml) then the homogenate left at room temperature for 30 minutes in the antibiotic solution. The solution centrifuged at 3000 r.p.m for 15 min. The supernatant were collected and stored at -20°C until used for HA test and as inoculum for experimental animals.

Erythrocytes:

Citrated blood sample was obtained from human type 0.

1% Erythrocyte suspension was prepared in physiological saline to be used in HA and HI tests.

Preparation of RHDV hyperimmune serum:

RHDV hyperimmune serum used in HI test was prepared according to the method described by Ghanem and Ismail (1992).

Haemagglutination test (HA):

HA activity of liver suspension was detected using human type 0 RBCs according to the methods described by Chasey et al. (1995).

Haemagglutination inhibition test (HI test):

The HI test was made according to method described by Pu et al. (1985).

Experimental design:

The animals were divided into three groups:

Group A: (15 rabbits) were given normal drinking water for 15 days. Then infected with previously prepared antibiotic treated tissue suspension in a dose of 1 ml/rabbit orally.

Group B: (15 rabbits): Vitamin supplemented group were given vitamin A (10,000 IU/liter), vitamin E (500 IU/liter) and vitamin C (1000 IU/liter) in drinking water for 15 days. Then infected with previously prepared antibiotic treated tissue suspension in a dose of 1 ml / rabbit orally.

Third group: (3 rabbits) Served as control animals.

Three animals from the first and second group were sacrificed at 12, 24, 36, 45 and 72 hours post infection. The died animals during this period were deducted from the number. The control animals were sacrificed at the end of the experiment. Serum samples were collected from each rabbit and used for HI test.

Histopathological studies:

Specimens from liver, kidney, lung and spleen were taken from naturally and experimentally infected cases and fixed in 10% neutral buffer formalin, embedded in paraffin, sectioned at 5 micron and stained with hematoxylin and eosin stain (H & E stain). Then examined by light microscopy.

Preparation of semithin sections:

Specimens from the same organs (liver, kidney, spleen & lungs) were fixed in gluteraldehyde (5%), and approximately 1 X 1 X 1 mm blocks were perpared. These blocks were washed in cacodylate buffer (0.1 M, pH., 7.2) for 1 - 3 hours and then post fixed in 1% osmium. tetraoxide for 2 hours. After repeated washing in cacodylate buffer (4 X 30 min.) and dehydration in ascending grades of ethyl alcohol up to 100% (30 min. for every concentration.), the specimens were first placed in propylene oxide for 60 minutes, then in pure epon 812 and incubated in a special polymerization incubator (one day at 35°C, one day and 45°C and three days at 60°C). The blocks were trimmed with LKB ultratom. Semithin section were obtained and stained with toluidine blue for 2 minutes at 80°C and examined by light microscope.

Transmission electron microscope (TEM):

Representative fields of semithin section were selected. Ultrathin sections (70 nm) were cut with diamond knife using a reichert OMVs ultramicrotome. They were mounted in copper grids and stained with uranyle acetate lead citrate stain (Bancroft and Stevens, 1982). The ultrastructural investigation was carried out with TEM (Joel Cx II)

RESULTS

Clinical and post mortem finding:

The disease was observed in 2-20 months rabbits of different breeds. Both sexes were affected including pregnant females. The mortality rate was 30-60% in young age (2-4 months) and 70-100% among adult rabbits. Nothing was observed in those under two months of age.

The clinical examination of diseased rabbits showed incoordination, convulsion and epistaxis or vaginal bloody discharge. Sudden death without showing any clinical signs was usually observed specially after experimental infection. The majority of deaths occurred within 1 to 3 days from onset of infection. Post mortem examination

revealed severe congestion of subcutaneous blood vessels. Liver appeared congested at 12,24,36 hours post infection, became enlarged, friable and pale at 48, 72 hours post infection. Kidneys were congested with peticheal hemorrhages on their surfaces. Spleen was congested. Bleeding from nostrils, hemorrhagic trachitis, hemorrhagic and oedematous lungs were also observed. Large blood vessels contained clotted blood. Sanguineous fluids were seen in the thoracic and abdominal cavities.

Parasitological and bacteriological examination of internal organs revealed negative results.

Histopathological findings:

I - In naturally infected cases:

The hepatocytes showed vacuolar degeneration with either diffuse or focal hepatocellular necrosis (Fig. 1a & b). Inflammatory cells were rarely seen. In the kidney, the glomeruli showed severe hemorrhages filled the Bowman's capsule (Fig. 1c). Other glomeruli showed microthrombi formation in the capillary tufts, in which the tufts were swollen and engorged with fibrin deposits (Fig. 1d). The renal tubules showed sever necrobiotic changes. Hemorrhages were also seen in the interstitial tissue either in the renal cortex or the renal medulla.

Alveolar hemorrhage was the prominent finding in the lung tissue associated with thrombosis in the pulmonary vessels and degeneration in the endothelial lining (Fig.1e). In spleen there were hemorrhages in the red pulps with evidence of necrosis in the lymphocytes in both white and red pulps (Fig.1f).

II- In experimentally infected cases:

The liver showed diffuse necrosis in the hepatic parenchyma (Fig.2 a), their were also vacuolar degeneration of the other hepatocytes with edema in the disse space (Fig. 2 b). Inclusion bodies were seen either in the cytoplasm (Fig. 2 b) or in the nuclei (Fig. 2 c) of the hepatic cells. Some hepatic sinusoids were engorged with fibrin thrombosis (Fig. 2 d). In animals died after 72 hours, there were inflammatory cell infiltration in the necrotic areas as well as in the portal areas (Fig. 2 e).

In ultrastructural examination:

It was found that, after 24 hours post infection (h.p.i) the hepatocytes showed hypertrophy of rough endoplasmic reticulum with increase in the free polyribosomes (Fig.3a). After 36 hours the hepatocytes showed multiple malformation in the mitochondria with marked increase in the free ribosomes and presnce of sidrosomes (Fig.

3b). After 72 hours there were swelling in the mitochondria with destruction of their cristae, as well as vesiculation of the rough endoplosmic reticulum and presence of many vacuoles in the cytoplasm of the hepatic cells (Fig. 3 c). The endothelial cells lining the sinusoids showed either cytoplasmic vacuolation with swelling and degeneration of their mitochondria (Fig. d) or lysis of their organells (Fig.3 e).

Viral inclusion:

Viral like particles were observed in the cytoplasm and nuclei of hepatocytes. In the cytoplasm the viral particles were present singly or accumulated irregularly in small number. Crystalloid (paracrystallin) arrays of the particles were observed in association with membrane - bound vacuoles (Fig. 4 a). Small membrane bound vesicles and cisternae containing the particles were sequestrated within a large membrane bound vesicle around and within which the particles were located (Fig. 4b). The particles were also seen within a large membrane bound vesicle (Fig. 4 c). The virus was unenveloped and more or less rounded. In the nuclei the viral particles usually present in groups in crystalloid arrays either in the center of the nuclei (Fig. 3 d) or paracentral (Fig. 3 e) or near the nuclear envelope (Fig. 3 f).

In the kidney, the main lesions include marked hemorrhages in the Bowman's capsule of the glomeruli (Fig. 5 a). The hemorrhage was also seen in the interstitium of the renal cortex and inside the renal tubules (Fig. 5b) associated with marked hyaline droplet degeneration of the renal tubular epithelial cells. Other cases showed in addition to hemorrhage and tubular necrosis, microthrombi formation in the glomerular tuft (Fig. 5c). In the renal medulla the hemorrhage was more severe and associated with marked necrobiosis of the collecting tubular epithelium (Fig. 5d).

In semithin section, multiple rounded deeply stained droplets, were infrequently seen in the renal tubular epithelium (Fig. 6a). With transmission electron microscope, these droplets appeared electron dense with variable shape and size (Fig.6b). The renal tubular epithelium showed vacuolar degeneration of cytoplasmic organells with swollen of mitochondria and destruction of their cristae. Lysis and disappearance of rough endoplasmic reticulum (Fig. 6c).

In the lungs the lesions, were more similar to that in the natural cases including alveolar hemorrhage (Fig. 7a), microthrombosis in the alveolar septal capillaries (Fig. 7b) and red thrombus formation in the

pulmonary vessels (Fig. 7c). Compensatory emphysema was infrequently seen in some areas.

In the spleen, hemorrhages in the red pulb as well as necrosis and exhaustion of the lymphocytes in both white and red pulb were the prominent findings (Fig. 5 a). Ultrastructurlly, their were pyknosis of the lymphocyte nuclei with destruction of their cytoplasmic organells (Fig. 8b). Few sidrosomes were infrequently seen in the cytoplasm of some macrophagal cells and endothelial cells.

In the vitamin supplemented group:

Marked improvement was seen in most of the examined organs except the liver. The liver showed multiple individual cell necrosis, in which the hepatic cells were shrunken and deeply eosinophilic stained (Fig. 9 a). The renal cortex appear more or less normal with slight degeneration in the tubular epithelium (Fig. 9b). The renal medulla showed only congestion of their interstitial blood vessels (Fig. 9c). Necrosis of some lymphocytes in the white pulb of the spleen was seen in some cases (Fig. 9d). However lymphocytic population in the splenic follicles were relatively bitter (Fig. 9e)

Haemagglutination activity:

As shown in Table (1), the HA activity could be detected as early as 12 hours post-infection in group A, while in group B it could be detected at 24 hours. The HA titers in group A were higher than in group B which supplemented with vitamins. The mean HA log2 titers were 10 and 8 at 72 hours post-infection in groups A and B respectively.

Table 1: Mean HA Log2 titers in groups infected with RHDV.

Groups	Treatment	Mean HA Log2 titers				
		Hours post-infection				
		12	24	36	48	72
Group A	Virus	4	5	7	8	10
Group B	Virus + vitamins	< 2	3	5	7	8

Haemagglutination inhibition test:

As shown in Table (2), the HI antibodies against RHDV could be detected at 48 hours in group A. While in group B it appeared at 36 hours post-infection. The mean HI log2 titers were 6 and 7 at 72 hours in groups A and B respectively.

Table 2: Mean HI Log2 titers in groups infected with RHDV.

Groups	Treatment	Mean HA Log2 titers				
		Hours post-infection				
		12	24	36	48	72
Group A	Virus	-	-	-	4	6
Group B	virus + vitamins	-	-	4	5	7

DISCUSSION

RVHD is a worldwide, highly contagious disease of rabbits of different age and breeds. The disease is responsible for severe economic losses due to high mortality rate among the affected rabbits. The mortality rate was 30-60% in young aged 2-4 month and 70-100% in adult rabbits. These results are nearly similar to those reported by El-Zanaty (1994) and Abdel-Aziz et al. (1995).

The characteristic clinical picture of the disease, either in naturally or experimentally infected cases included sudden deaths with occasionally presence of hemorrhagic foamy discharge from the nostril and vagina. The majority of deaths occurred within 1-3 days from the onset of the infection. Similar symptoms were previously described by (Singer et al., 1989; Boucher, 1989; Salem and El-Ballal, 1992 and Amina Nawar et al., 1996).

The gross pathological finding were similar in both naturally and experimentally infected rabbits. These lesions were in the form of congestion and enlargement of the liver, congestion of the kidney with multiple peticheal hemorrhage on its surface, enlargement and congestion of spleen, hemorrhagic tracheitis, hemorrhage and oedema in the lung, presence of clotted blood in the large blood vessels and presence of sanguineous fluids in thoracic and abdominal cavity. These observations were similar to that described by Kolble et al. (1990); Nowotony et al. (1990) and collery et al. (1995).

In the liver the histopathological change include degeneration in hepatic cells to focal and diffuse hepatocellular necrosis, in addition to the presence of intranuclear and intracytoplasmic inclusions. Ultrastructerally, viral particles were demonstrated in the cytoplasm and nuclei of the hepatic cells. The increase number of ribosomes and polysomes in the early affected hepatocytes seemed to be related to the initial viral replication. The membrane - bound cisternae and vesicles

contained viral particles in the present cases were thought to have resulted from versatile variations (for example, S shape torsion) of endoplasmic reticulum, around and within which the viral particles were replicated. The presence of the viral particles in the nuclei in later time than that in the cytoplasm indicated that the virus was replicated early in the cytoplasm (in the vesicles or cisternae), then released from the vesicles and cisternae to the cytoplasmic matrix and gain access to the nucleus. These properties of RHD virus in addition to its presence in paracrytallin arrays and unenveloped manner resemble the character of caliciviruses of family picornaviridae (Godman, 1973; Studdert, 1978; Ghadially, 1988 and Park et al., 1992).

So the hepatocellular degeneration and necrosis seen in the disease could be related to viral replication in the cytoplasm and nuclei of the hepatic cells. Similar conclusion was recorded by Salem and El Ballal, 1992; Park et al., 1992) but they recorded viral replication only in the cytoplasm of the hepatic cells.

The other interesting findings were the degeneration and necrobiosis of the endothelial cells lining the hepatic sinusoids. Disseminated intravascular coagulation indicated by microthrombosis in the hepatic sinusoids, glomeruler capillary tufts ,in the alveolar septal capillaries and in the pulmonary vasculature; as well as hemorrhages in the lung alveoli, renal cortex, renal medulla and in the splenic red pulp. The cytopathological findings recorded in the hepatic cells and in the endothelial cells gave an explanation for disseminated intravascular coagulation and subsequently the extensive haemorrhoges in various organs and tissues. The pathogenesis of DIC in acute hepatic necrosis is multifactorial. Verstraete et al., 1974, have suggested that necrotic hepatocytes might activate blood coagulation factor in circulating blood plasma. However, Ueda et al., 1992, stated that severe hepatic necrosis in RHD, leads to flow out of tissue thromboplastin from hepatocytes, with reduction in clearance of activated coagulation proteins and synthesis of antithrombin III. In the present cases ,the thromboplastin could also released from injured endothelial cells as well as the roughness caused due to this injuries may act as cofactors causing DIC and subsequent hemorrhages in various organs and tissues.

Necrosis of the lymphocytes in the splenic follicles, indicated that the RHDV has an immunosuppressive effect on the affected rabbits.

The histopathological finding in the vitamin supplemented group represent a relative improvement in the examined liver, kidney, lung and

spleen. HA titers in group A was higher than in group B indicating that the vitamins have a role in lowering the multiplication of the virus in liver.Also the HI test revealed that RVHD antibodies appeared later in group A (at 48 hours) ,while in group B (vitamin supplemented group) it appeared earlier at 36 hours post-infection. It was also found that, HI titers in group B were higher than in group A. This indicating the role of vitamins in lowering the viral antigenisity as well as protecting and enhancement of the immune system of the infected rabbits. Many previous studies reported that, the use of vitamins as immune stimulant, antibacterial and antiviral agents were succeeded. (Chew, 1987; Franchini et al., 1991; Lovell, 1989; Beisal, 1982 and Sayed and Abd-Elghaffar, 1999).

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Fig. 3 e: Electron micrograph of endothelial cell lining hepatic sinusoid showing complete lysis of their cytoplasmic organells. X 20000.

Fig. 4 a: Electron micrograph of hepatocytes cytoplasm from an infected case (36 h.p.i). Viral particles are present along and within a membrane bound vacuole (arrows). X 1000.

Fig. 4 b: Electron micrograph of the cytoplasm of hepatocytes from an infected case (36 h.p.i). The cytoplasm has many membrane-bound, vesicles and cisternae. Viral particles are present within the membrane bound cisternae. Some viral particles show paracrystellin arrays (arrow). X 5000.

Fig. 4 c: Electron micrograph of the cytoplasm of hepatocytes from an infected case (36 h.p.i) showing presence of viral particles inside membrane bound vesicles. X 20000. *Inset*, semithin section of hepatocytes showing many vesicles contain inclusions. 10 X 40.

Fig. 4 d: Electron micrograph of the nucleus of hepatocytes from an infected case (48 h.p.i) showing a group of viral particles in the center of the nucleus. X 14000.

Fig. 4 e: Electron micrograph of the nucleus of hepatocytes showing paracrystellin arrays of viral particle in the paracentral zone of the nucleus. X 14000.

Fig. 4 f: Electron micrograph of the nucleus of hepatocytes showing crystalloid arrays of viral particles near the nuclear envelope X 20000. The particles were more or less rounded and unenveloped.

Fig. 5 a: Kidney from experimentally infected cases showing glomerular hemorrhages with necrobiosis of the renal tubular epithelium. H & E. 10 X 10.

Fig. 5 b: Kidney from infected case showing presence of erythrocytes in the lumen of the renal tubules in addition to the glomerular hemorrhages. H & E. 10 X 25.

Fig. 5 c: Kidney from infected case showing fibrin microthrombi in the glomerular tuft with necrobiosis of the renal tubular epithelium. H & E. 10 X 25

Fig. 5 d: Kidney from infected case showing medullary hemorrhages and necrosis in the collecting tubules. H & E. 10 X 10.

Fig. 6 a: Semithin section from kidney of infected case showing fibrin microthrombi in the glomerular tuft (F) and pyknosis of their nuclei. some of the renal tubular epithelium contain multiple deeply stained droplets. Toluidine blue stain. 10 X 100.

Fig. 6 b: Electron micrograph of renal tubular epithelial cell from infected case showing presence of multiple electron dense droplets of varying shape and size in their cytoplasm. X 4000.

Fig. 6 c: Electron micrograph of renal tubular epithelial cells from infected case showing swelling of mitochondria (M) with degeneration their cristae X 10000.

Fig. 7 a: Lung from infected case showing alveolar hemorrhage and emphysema.H & E. 10 X 40.

Fig. 7 b: Lung from infected case showing fibrin microthrombi in the septal capillaries. H & E. 10 X 10.

Fig. 7 c: Lung from infected case showing red thrombosis in small pulmonary blood vessels. H & E. 10 X 10.

Fig. 8 a: Spleen from infected case showing extensive necrosis in the lymphocytes in the white pulp and sever hemorrhage in the red pulp. H & E. 10 x 25.

Fig. 8 b: Electron micrograph of spleen from infected case showing multiple nuclear pyknosis of the lymphocytes (arrows). X 4000.

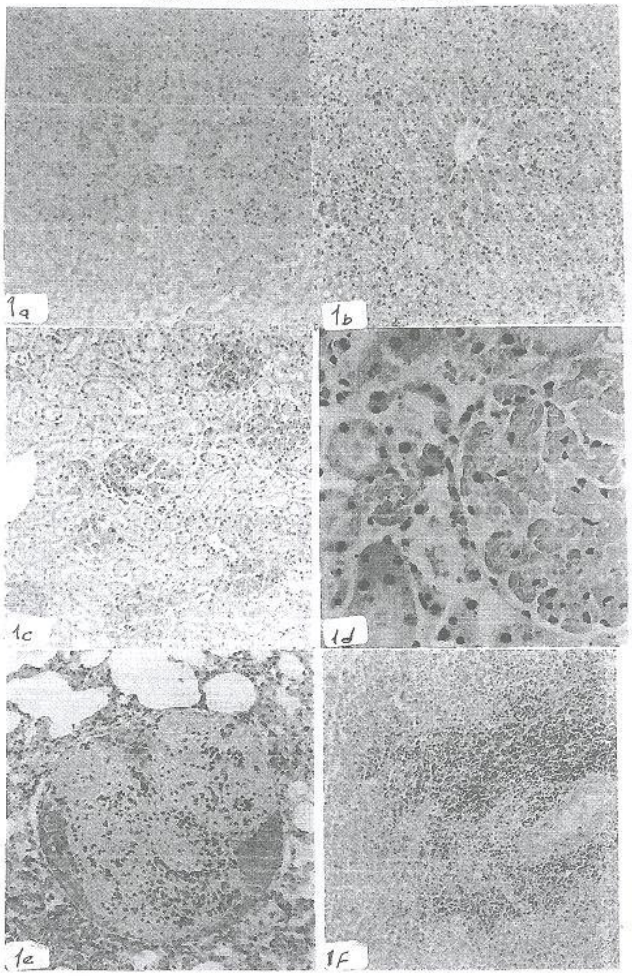
Fig. 9 a: Liver from vitamin treated case showing necrosis of some hepatocyte at the periphery of the hepatic lobules. H & E. 10X10.

Fig. 9 b: Kidney from vitamin treated case showing normal appearance of the glomeruli with minimum degeneration in the renal tubules. H & E. 10X10.

Fig. 9 c: Kidney from vitamin treated case showing only congestion in the interstitial blood vessels in the renal medulla. H & E. 10 X 10.

Fig. 9 d: Spleen from vitamin treated group showing necrosis of some lymphocytic elements in the white pulp. H & E. 10 X 25.

Fig. 9 e: Spleen from vitamin treated case showing normal lymphocytic population in the white pulb. H & E. 10 X 10.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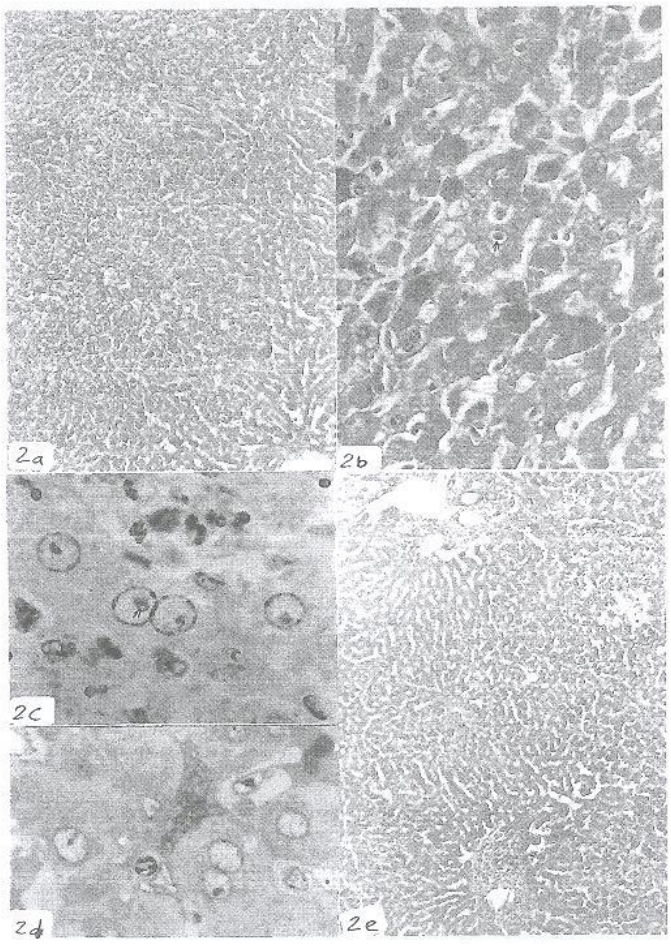
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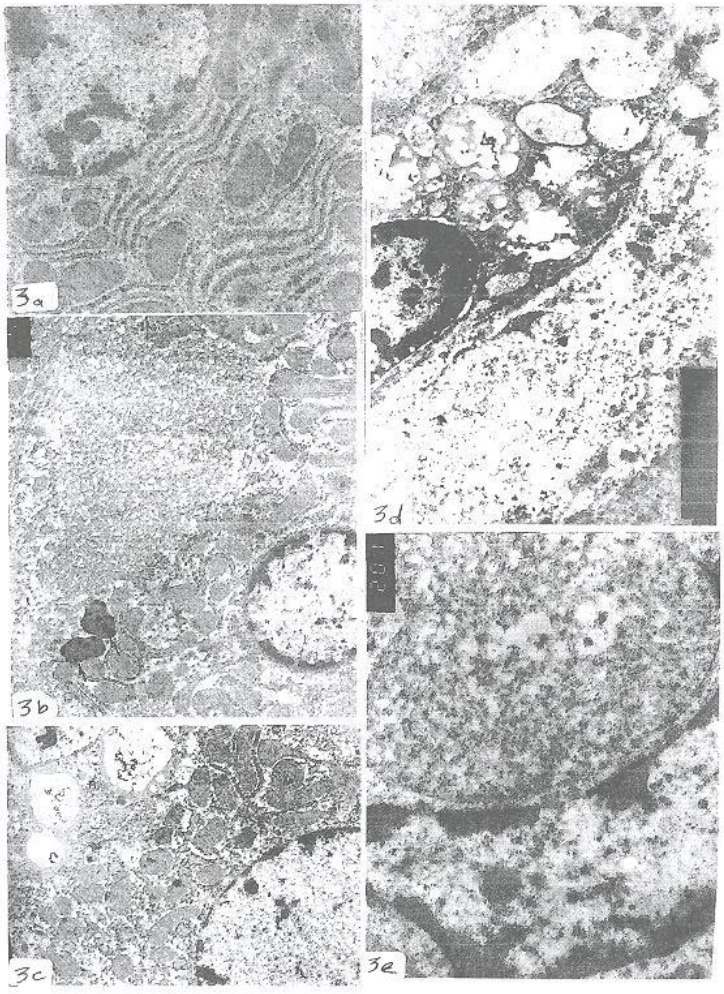
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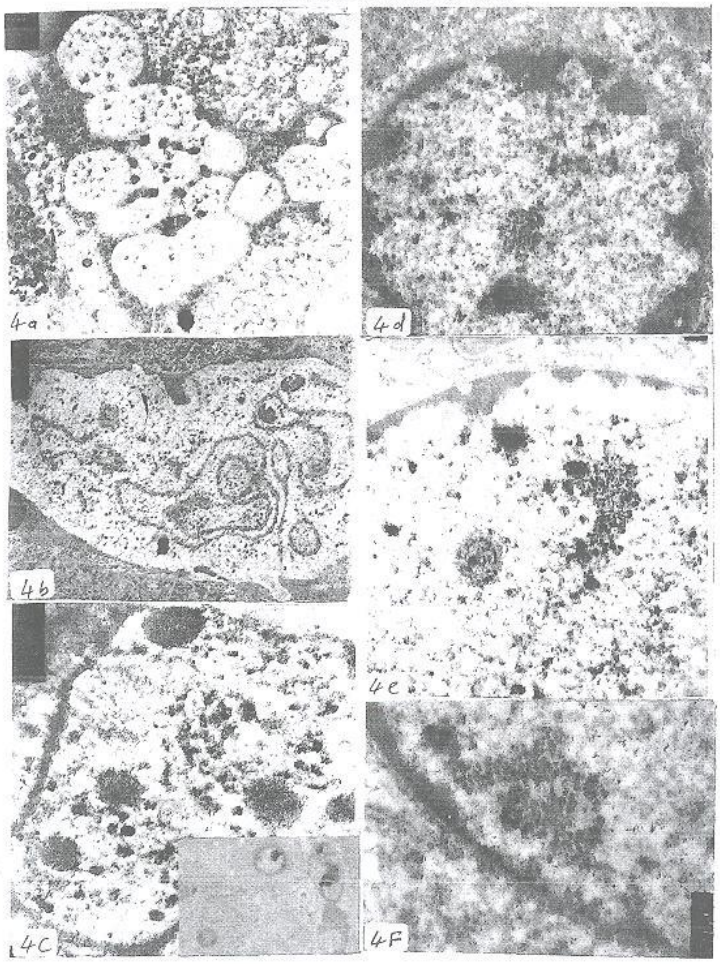
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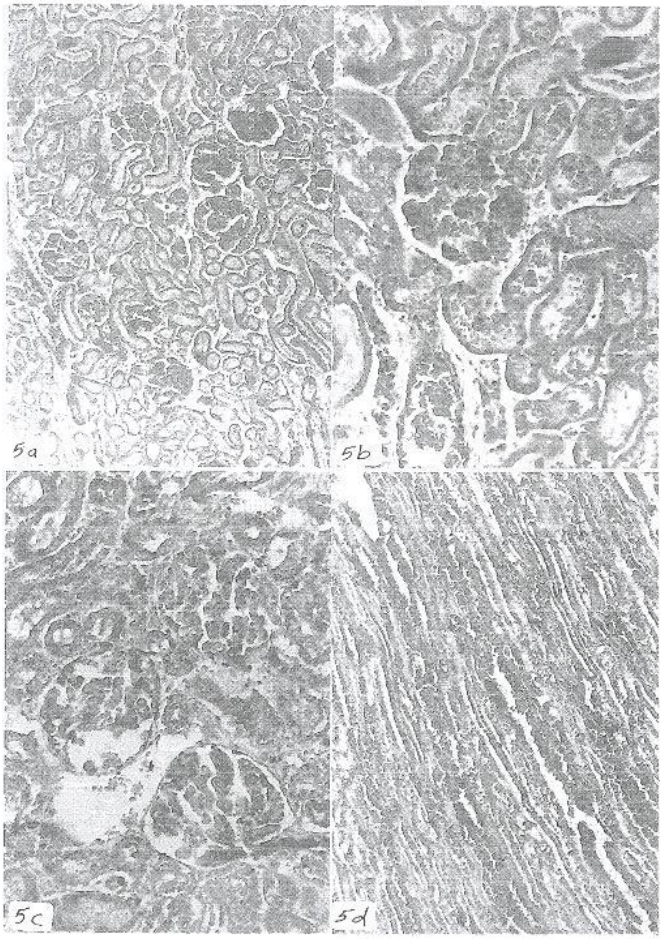
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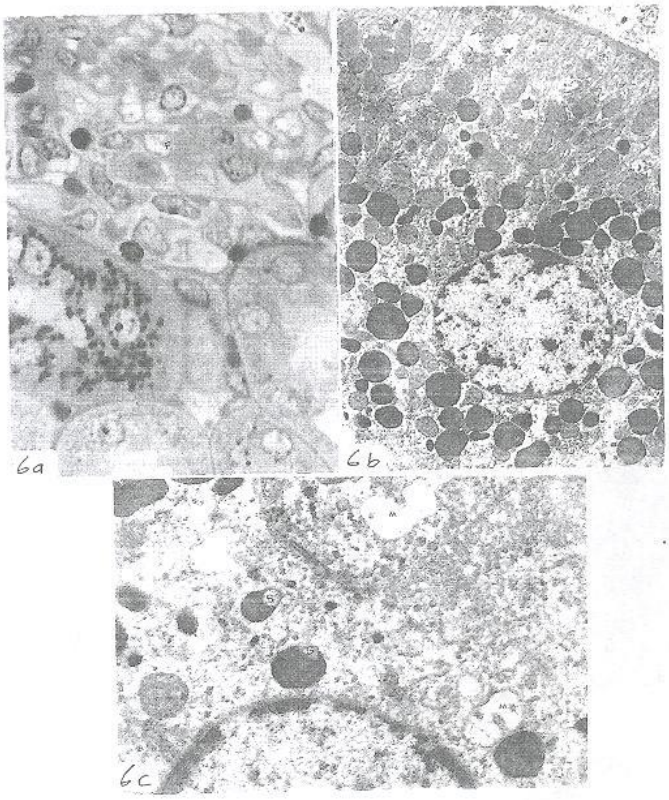

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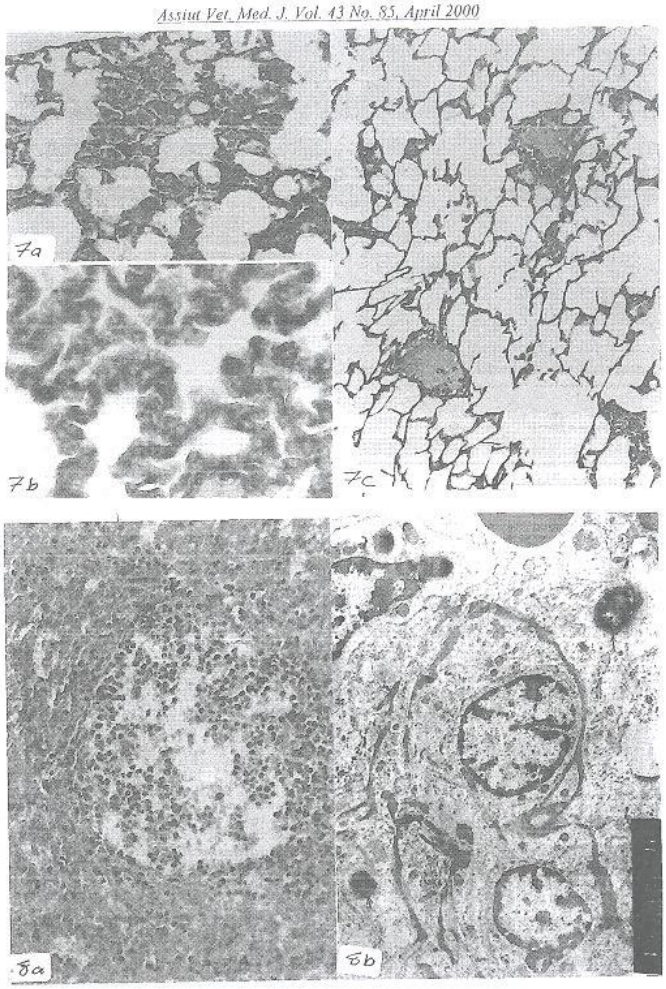
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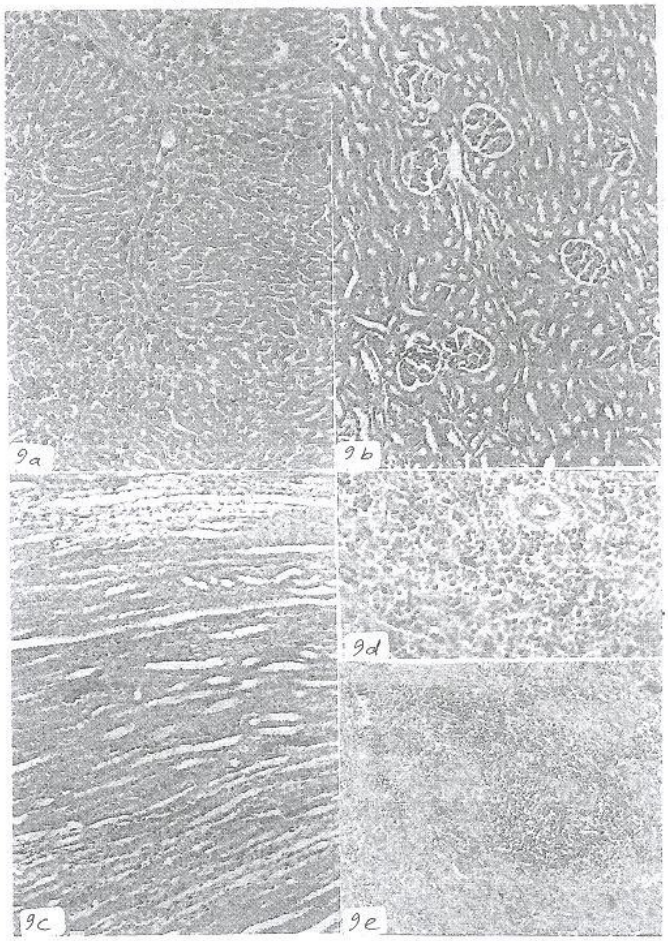
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