CHAPTER IV

RESULTS

Following single intramuscular injections, most animals were drowsy and developed hematuria. 1% of animals in group A (RVV 1 mg./kg.) and 50% of animals in group B (RVV 2 mg./kg.) died. All of deaths occured within 5 to 48 hours after envenomation. Some of the survivors in group B developed gangrenous limbs. In group C (control group), all animals survived until they were sacrificed. Although pathological studies in all non-survivors were not performed, the difference in renal pathology between non-survivors and survivors was not found.

By gross examination, most kidneys at 2 hour, 6 hour, and 1 day interval were enlarged and dark red in their color. Subsequent microscopic examinations indicated that it was a characteristic of renal vascular congestion. In some animals, their bladders contained black urine which would be hemoglobinuria. At 3 day, 10 day, and 30 day interval ,gross morphology of kidneys were usually normal.

Light Microscopic Study

In group A and B (RVV 1 and 2 mg./kg.), renal histological changes were demonstrated as early as 2 hours after Russell's viper envenomation. The renal histological changes in group A and B will be presented simultaneously because no significant difference in renal pathology between both groups was found. At the 2nd hour, tubular lesion was one of outstanding features. All kidneys in group A and B showed tubular

swelling, varying in severity from mild: swelling of luminal surface membrane to severe degree: vacuolar degeneration. These changes, accompanying with intratubular casts, were found in focal distribution, both in cortical and medullary area. Intratubular casts, eosin positive by H&E stain, would be hemoglobin casts since some of them still contained red blood cells.

Besides tubular lesion, renal vascular congestion was also noted both in glomerular and peritubular capillaries of all animals. Their capillaries were dilated, filled with many red blood cells. In severe degree of congestion, capillaries were marked engorged with numerous erythrocytes and leukocytes.

At the 6^{th} hour, renal pathology revealed similar changes as those of the 2^{nd} hour. Focal tubular swelling, intratubular casts and vascular congestion were found in all animals. Moreover, in 2 of 4 animals in group B, positive fibrin stain in glomeruli was also demonstrated.

At the 1^{th} day, characteristics of tubular necrosis were more clearly presented. Proximal tubular cells were flattened and losed their brush border, making proximal tubules looked like distal tubules. These pictures, all tubules resembled distal tubules, were called distalization. In more severe degree of changes, the smudgy appearance of tubular cells without nuclei was seen. Some tubular cells were shred from the tubular basement membranes. In one case of group A, tubulorhexis, characterized by tubular basement membrane disruption, was demonstrated. At this interval, vascular congestion also appeared.

Most glomeruli appeared normal; however, 3 animals in group A and 1 in group B, showed enlarged glomerular tufts with dilated capillary loops filled with clumps of erythrocytes and leukocytes. In these animals, some of their glomeruli contained eosin-positive substance, which was later

proved to be fibrin by PTAH staining and electron microscopic examination.

At the 3rd day, characteristics of tubular regeneration were seen. Many tubules appeared hypercellular with enlarged nuclei. Focal tubular swelling and degeneration were still seen in less severe degree than those of 1st day.

At the 10th and 30th day, renal histology was nearly normal; only mild tubular changes were found in some area. Vascular congestion was mild or absent and all glomeruli appeared normal.

In this study, uncommon pathology which has been reported in human such as extracapillary glomerulonephritis, interstitial nephritis and vasculitis was not detected.

In control group, only mild tubular change was seen. Renal vascular congestion was occasionally demonstrated as well; however, degrees of congestion were mild. Meanwhile, no glomerular fibrin thrombi was found.

By using the criteria reported by Mandal et. al. in 1982, tubular lesion and vascular congestion were scored as follow:

Renal tubular lesion score

0 : Normal.

1+ : Loss of luminal membrane or brush borders.

2+ : Tubular swelling and vacuolation.

3+ : Necrosis of the cell and separation of the cells from the basement membrane

4+ : Same as 3+ with basement membrane disruption and necrosis.

Vascular congestion score

0 : Normal.

1+ : A few red blood cells in peritubular capillaries and intrarenal veins.

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4+ : Same as 3+ with basement membrane disruption and necrosis.

Vascular congestion score

0 : Normal.

1+ : A few red blood cells in peritubular capillaries and intrarenal veins.

2+ : Moderate number of red blood cells in glomeruli, peritubular capillaries, and intrarenal veins.

3+ : Numerous red blood cells inside glomeruli, peritubular capillaries, and intrarenal veins and frequently engorgement of peritubular capillaries.

This criteria is reproducible and make the results more scientific and available for further analysis. The details of renal pathological scores are being shown in table 1, 2, and 3.

Electron microscopic study

Electron microscopic findings confirmed the results of light microscope. Intracellular edema and swollen organelles of tubular epithelial cells were demonstrated. In some cases, tubular cells showed disruption of the cell membrane with extrusion of cytoplasmic contents into tubular lumen. Under electron microscopic examination, intratubular casts and crystals were also noted. Moreover, some proximal tubular cells contained intracytoplasmic osmophilic crystal like substances.

The presence of fibrin, characterized by its fibrillar structure, was apparently demonstrated by electron microscopic examination. A significant amount of fibrin was found in only few animals, as same as the results of light microscopic study. In these animals, their glomerular tufts revealed swollen endothelium and the capillary loops were occluded by erythrocytes, leukocytes and platelets enmeshed in fibrin. This is the feature of intravascular thrombosis.

Vascular congestion was also presented under electron microscopic examination. Glomerular and peritubular capillaries were dilated, contained with numerous erythrocytes and leukocytes. Occasionally, small amount fibrin was found in peritubular capillaries.

Data Analysis

Three separated analyses were performed.

A. Correlation Between Lesions and Dosages of the Venom

By using One-Way Analysis of Variance (ANOVA), correlations of tubular lesion scores in 3 groups were calculated. Among these 3 groups, the mean tubular lesion scores at the 2^{nd} hour, 6^{th} hour, 1^{st} day, and 3^{rd}day were significantly different at a p-value < 0.05. However, at the 10^{th} and 30^{th} day ,the differences of mean tubular lesion scores among 3 groups were not found. These results proved that tubular lesions were the significant pathology in animals receiving RVV. Further analyses did not show positive correlations between tubular lesion scores and dosages of the venom, because animals in group B, which received higher dosages of the venom than those of group A, didn't have the higher scores of tubular lesions. In contrast, at the 2^{nd} and 6^{th} hour, the mean tubular lesion scores in group A were even higher. At other intervals, no difference in mean tubular lesion scores between both group was found.

Similarly, mean vascular congestion scores among 3 groups were significantly different (p-values < 0.05) at the 2^{rd} hour, 6^{th} hour, 1^{st} day and 3^{rd} day, but not different at the 10^{th} and 30^{th} day. Again, the difference in mean vascular congestion scores between group A and B was not found except at the 1^{st} and 3^{rd} day which mean vascular congestion scores in group A were higher.

Table 4 and 5 are showing the analytical results of renal tubular lesion scores and vascular congestion scores in 3 groups, respectively.

B. Correlation Between Tubular Lesions and Vascular Congestion

The Coefficient of Correlation between tubular lesion and vascular congestion scores was calculated. However, at the r-value = 0.53, our result doesn't show strongly positive correlation between both lesions.

C. Correlation Between Tubular Lesion Scores and Positive Fibrin Stain

The mean tubular lesion score of kidneys which fibrin was found was compared to the mean score of kidneys which fibrin was absent. Only the scores which showed statistically significant from kidneys of the 2^{nd} hour, 6^{th} hour, 1^{st} day, and 3^{rd} day were used. By using Unpaired-T test, the mean tubular lesion score of the group with positive fibrin stain was significantly higher at a p-value < 0.05. (figure 18)

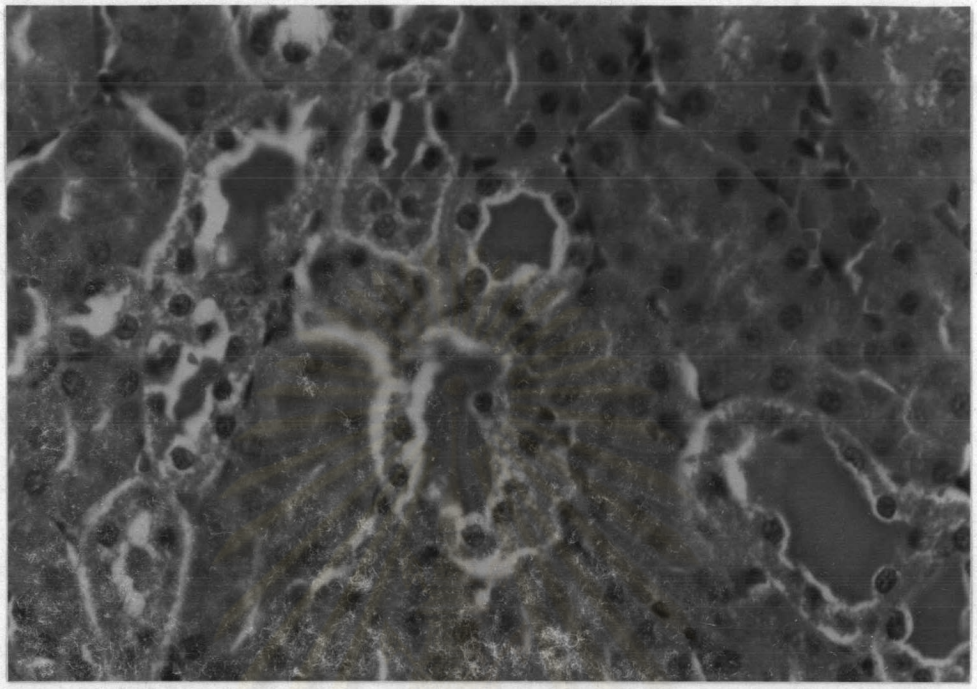


Figure 1 Renal pathology of a rat in group B at the 2nd hour

This picture reveals mild tubular swelling in focal distribution, together with intratubular casts, which are possibly heme casts. (H&E Stain, x 400)

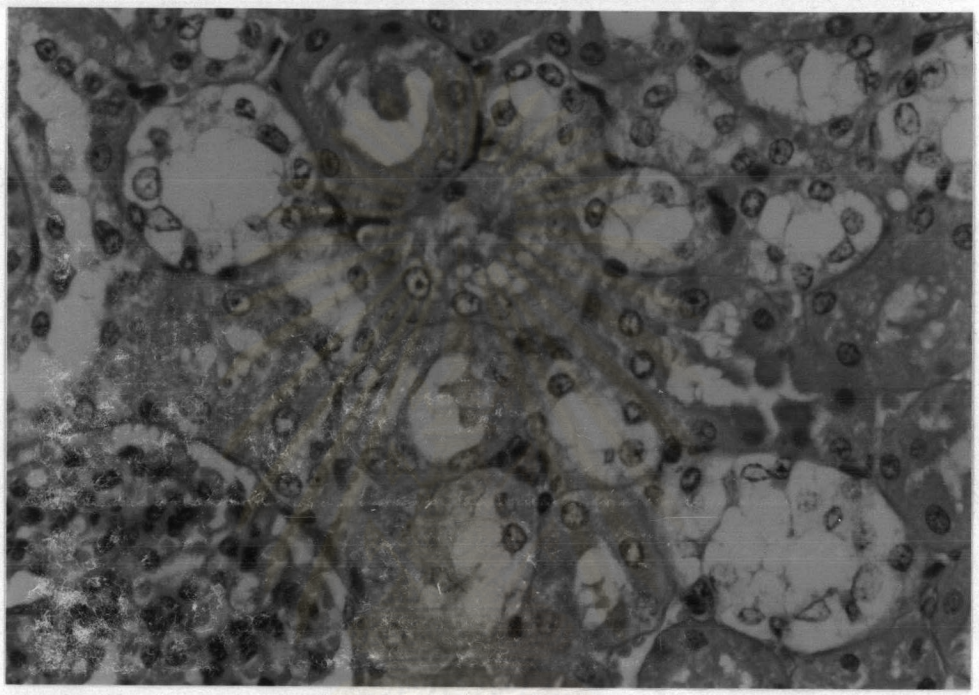


Figure 2 Renal pathology of a rat in group B at the 2nd hour

The renal pathology of another rat shows more severe degree of tubular swelling than that of figure 1; tubular cells are distended with large clear vacuole. The glomerulus, partially shown, appears normal. (PAS Stain, x 400)

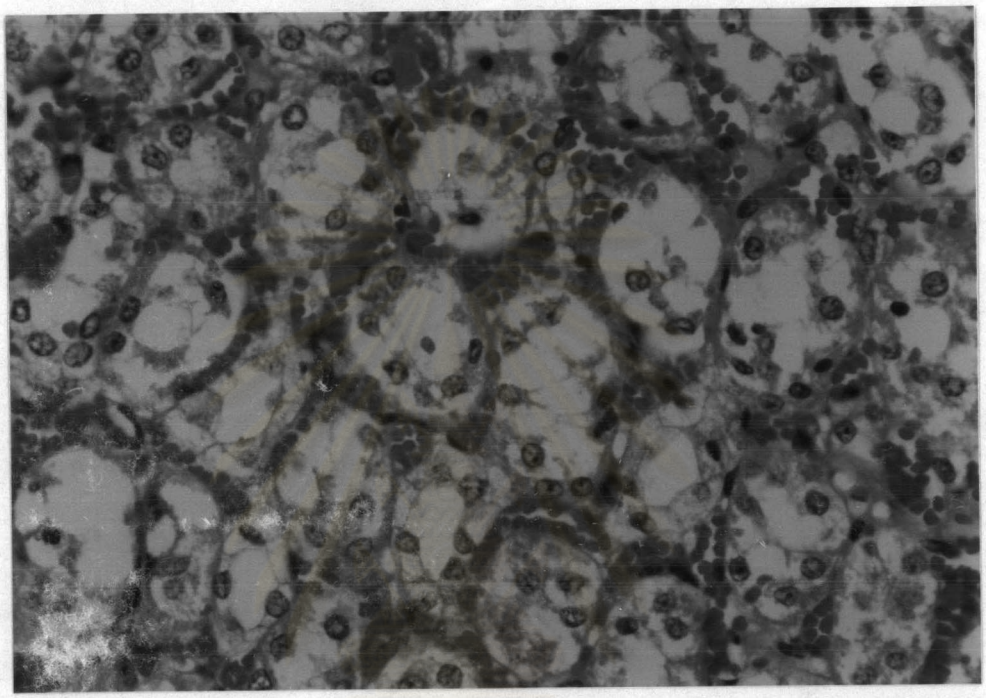


Figure 3 Renal pathology of a rat in group B at the 2nd hour

The characteristics of vacuolar degeneration and vascular congestion are both demonstrated. The peritubular capillaries are dilated, filled with densely packed erythrocytes. (H&E Stain, x 400)

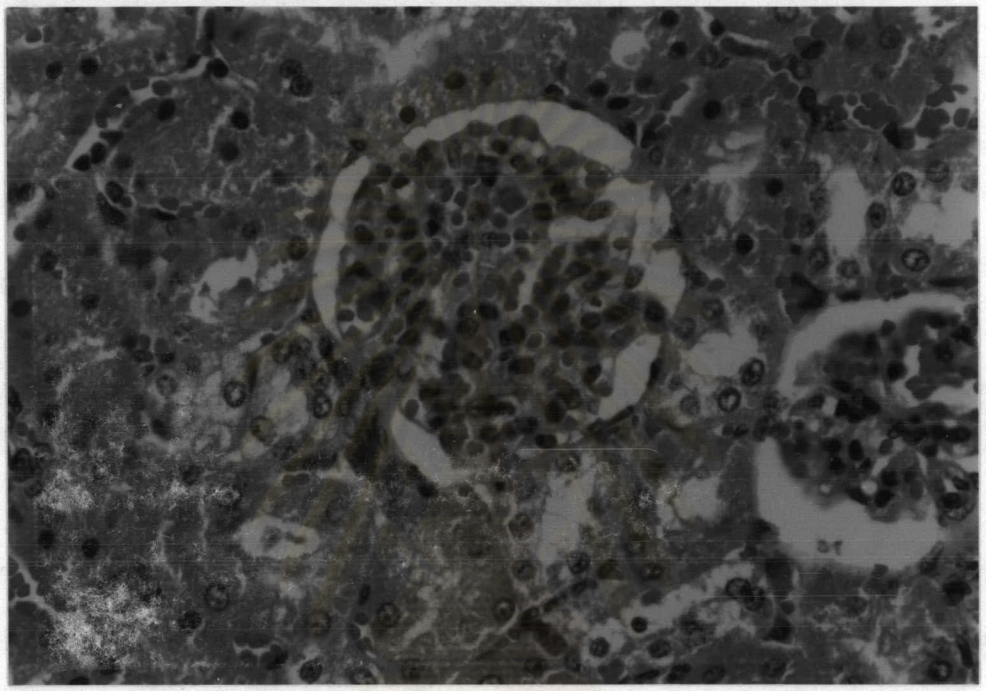


Figure 4 Renal pathology of a rat in group B at the 2nd hour

Taken from the same section as figure 3, this picture reveals glomerular congestion. A number of erythrocytes are in the capillary lumens. (H&E Stain, x 400)

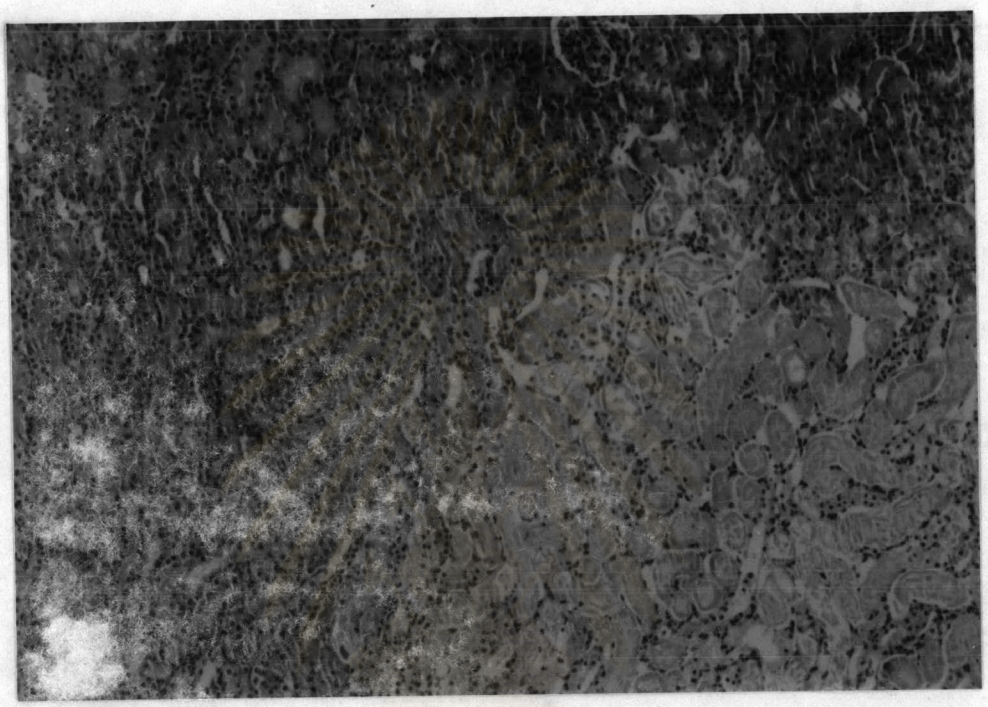


Figure 5 Renal pathology of a rat in group A at the 1st day

At 1 day interval, characteristics of tubular necrosis are more apparent. This picture shows tubular lesions which are focally distributed on the right side. (H&E Stain, x 250)

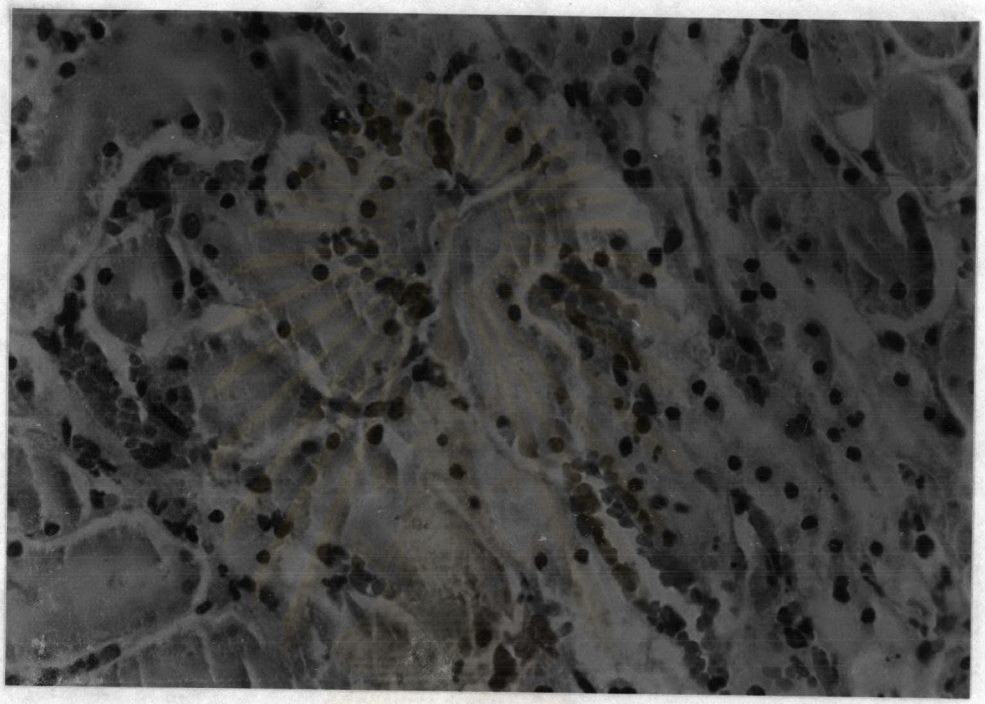


Figure 6 Renal pathology of a rat in group A at the 1st day

The higher magnification of figure 5 reveals necrotic tubules in smudgy appearance without nuclei. Tubular basement membrane is disrupted. Vascular congestion, packed erythrocytes in peritubular capillaries, is also seen. (H&E Stain, 400)

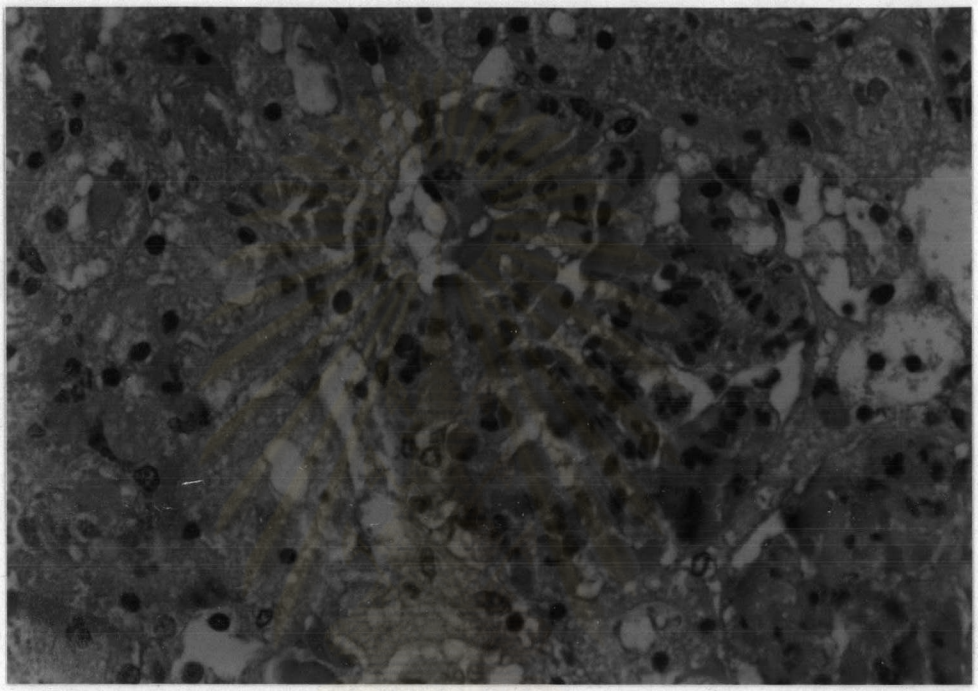


Figure 7 Renal pathology of a rat in group A at the 1st day

This glomerulus exhibits severe congestion; the capillary lumens are obliterated with clumps of erythrocytes and polymorphs. In this animal, fibrin was also detected by PTAH staining and transmission electron microscopic examination. (H&E Stain, x 400)

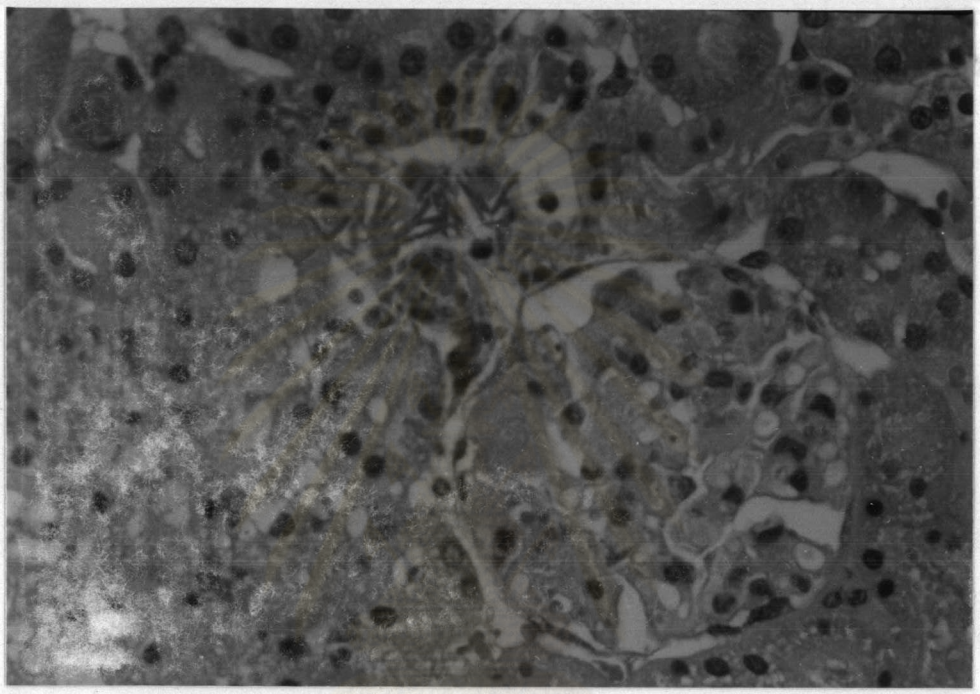


Figure 8 Renal pathology of a rat in group A at the 1st day

The renal pathology of another rat shows some eosin positive substance deposit in the glomerulus. Confirmed by PTAH stain, this feature is intraglomerular fibrin thrombi. The tubules contain intratubular crystals and casts, one of which on the upper left is red cell cast. (H&E Stain, x 400)

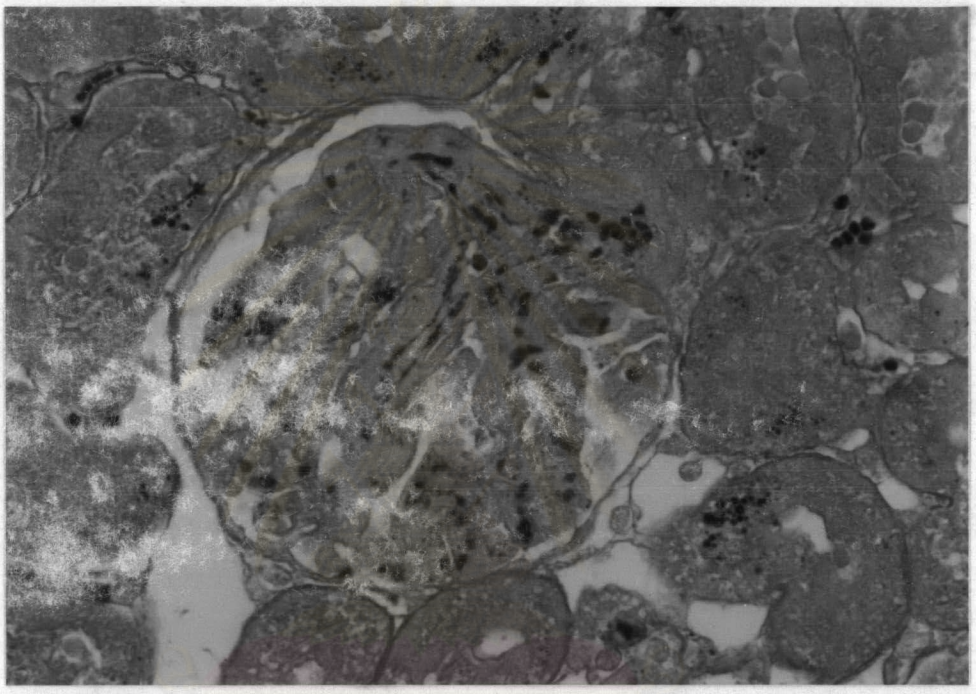


Figure 9 Renal pathology of a rat in group B at the 1st day

By PTAH Staining, fibrillar character of intraglomerular fibrin is being demonstrated. (PTAH Stain, x 400)

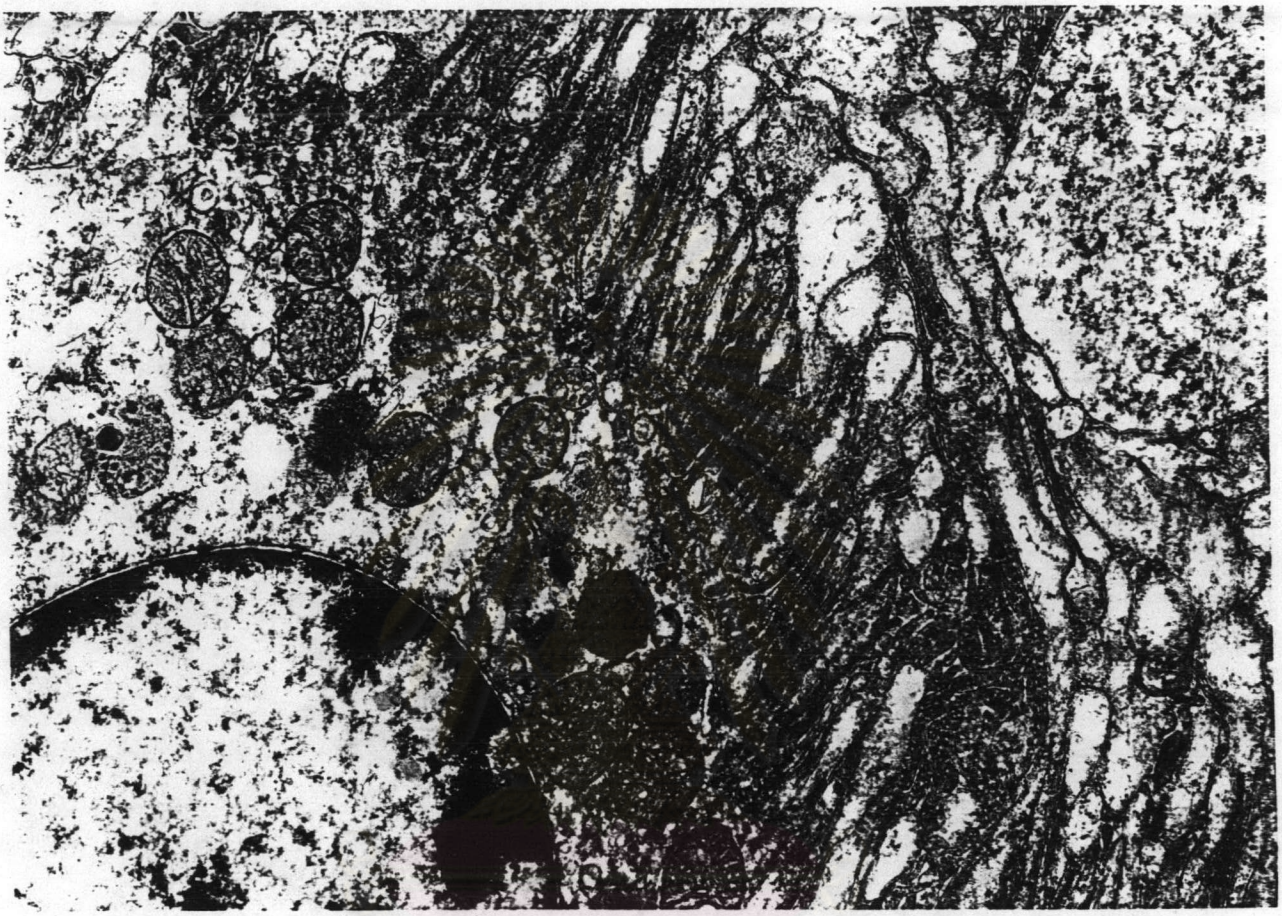


Figure 10 The electron micrograph of a rat in group B at the 2nd hour

This picture reveals some part of swollen proximal tubule; the brush borders appear in bleb-like projection. (x 25,000)

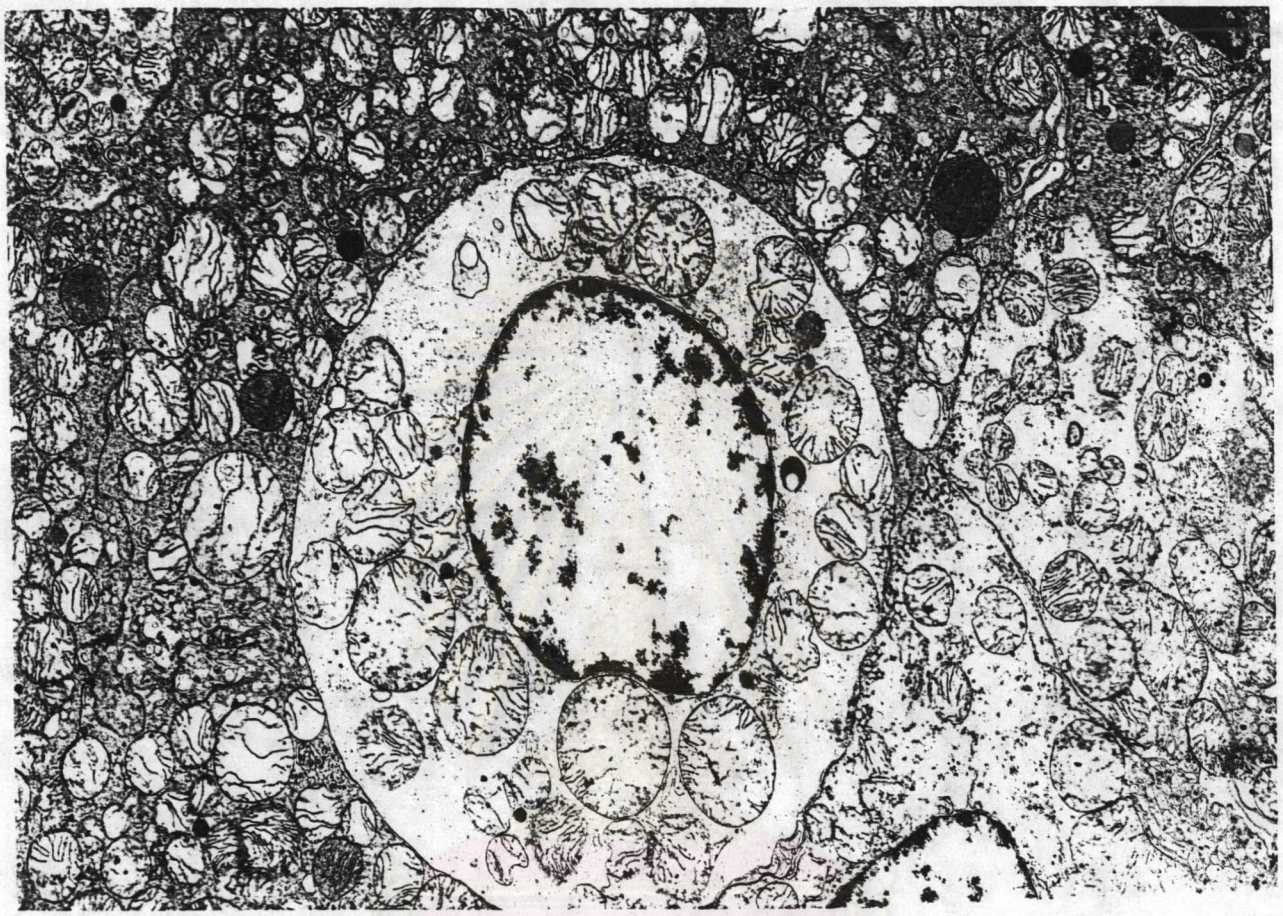


Figure 11 The electron micrograph of a rat in group B at the 2nd hour

Charateristic of tubular necrosis can be demonstrated as early as the 2nd hour by electron microscope. Intracytoplasmic organelles and nuclei are extruded into the tubular lumen. (x 10,000)

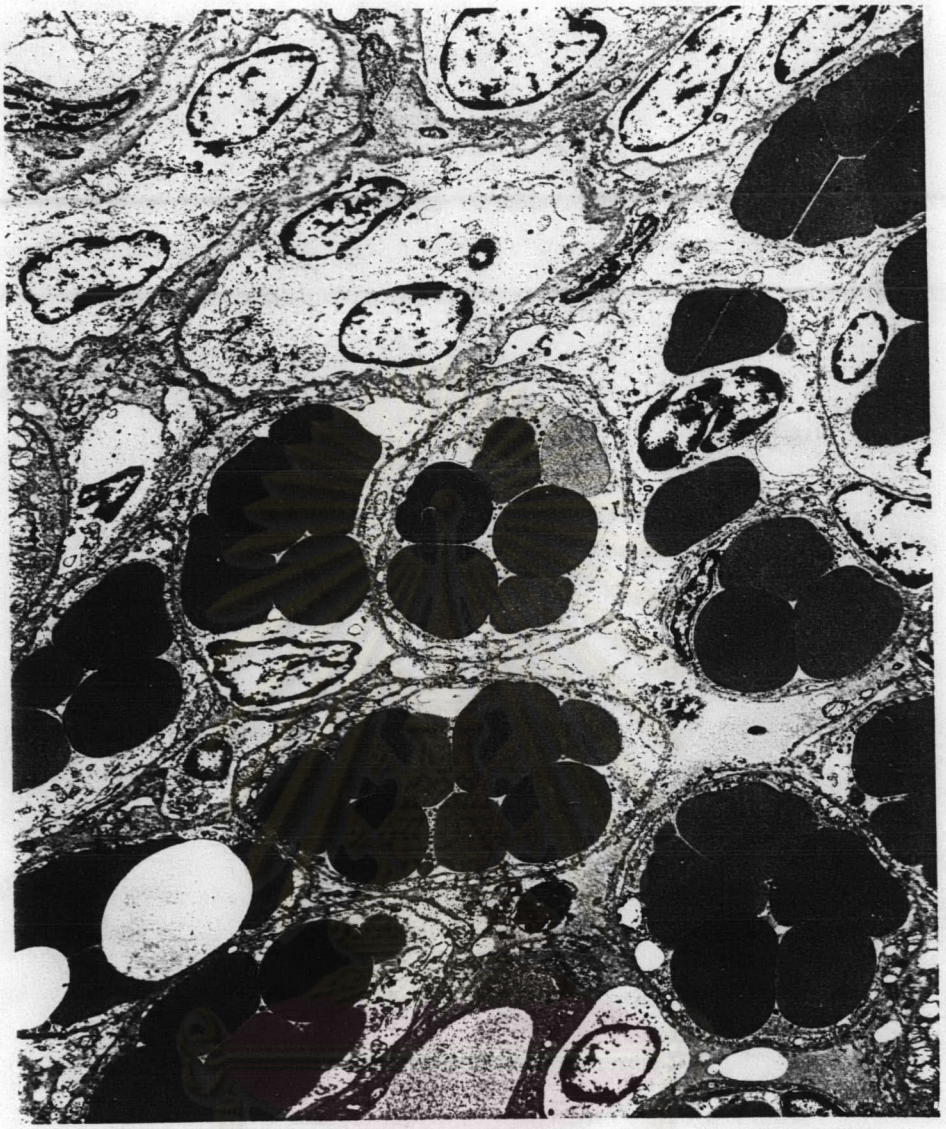


Figure 12 The electron micrograph of a rat in group B at the 2nd hour

This picture demonstrates a characteristic of congestion; the dilated vasculature is filled with densely packed erythrocytes. (x 3,750)

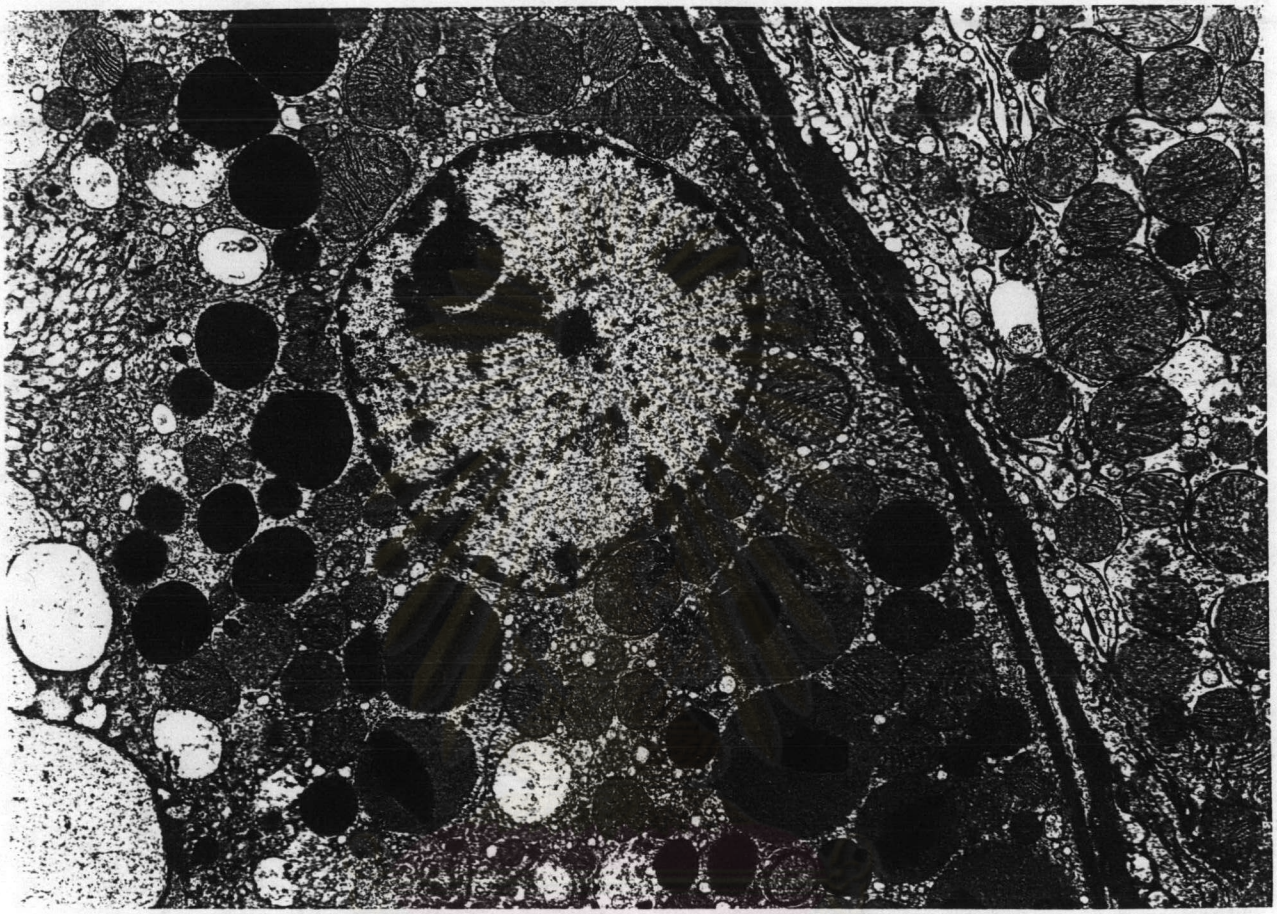


Figure 13 The electron micrograph of a rat in group B at the 6th hour

The proximal tubular cell reveals intracytoplasmic osmiophilic crystal-like substances. Suggested by some investigators, these dark bodies might be degenerated organelles or absorbed proteinaceous substances in tubular cells. (x 12,500)

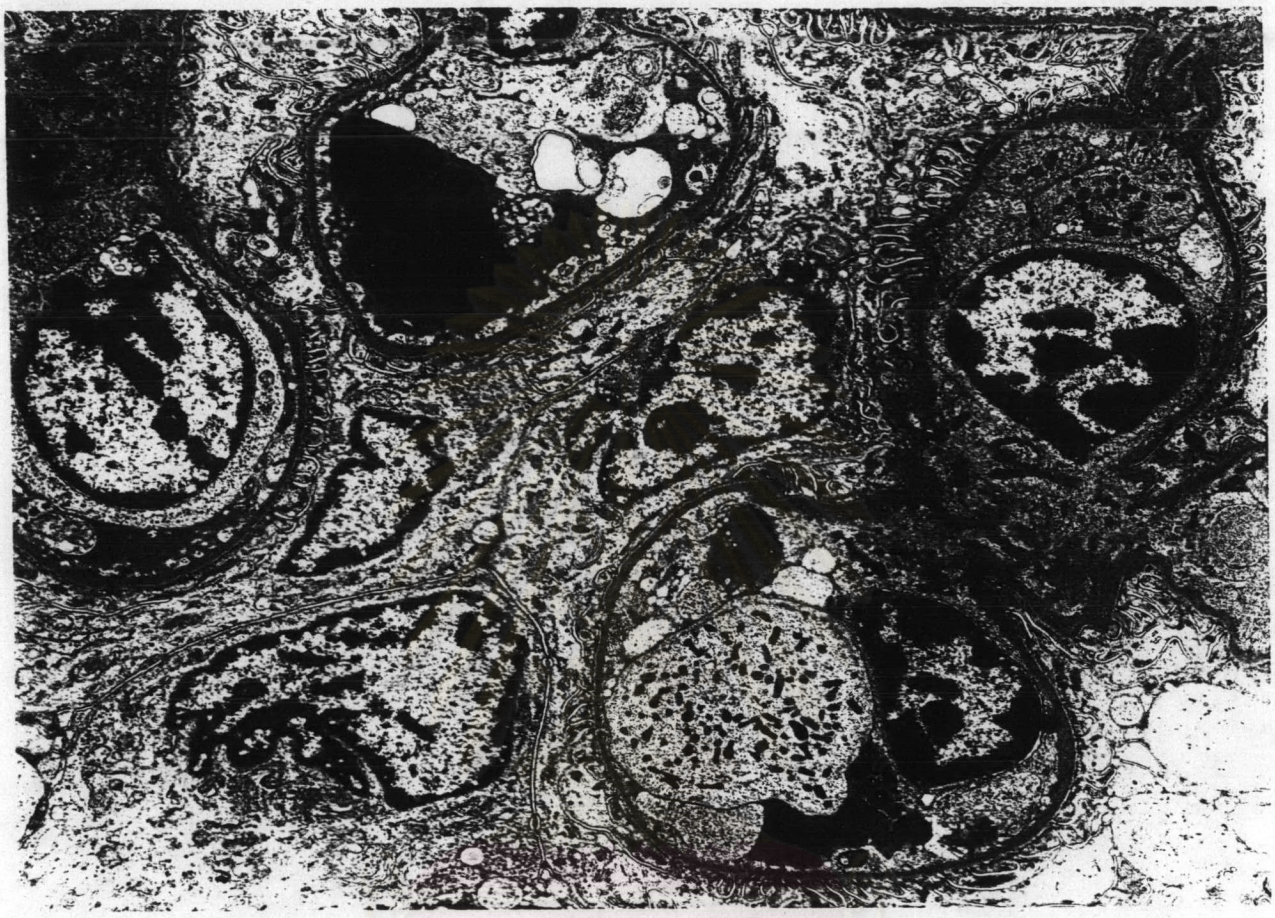


Figure 14 The electron micrograph of a rat in group B at the 6th hour

This picture shows some part of a glomerulus. The glomerular capillary loops are occluded with swollen endothelial cells, platelets, and fibrin. (x 6,250)

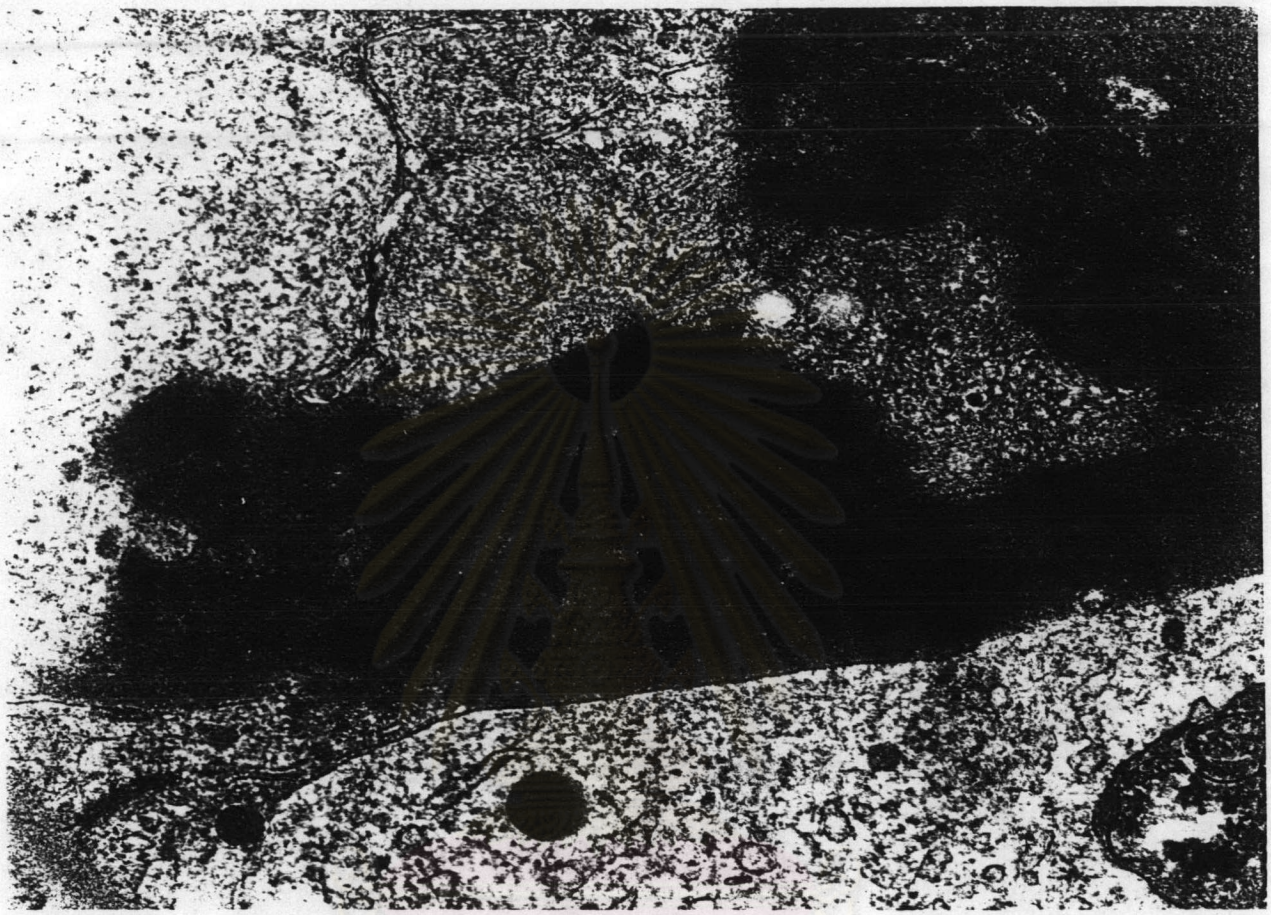


Figure 15 The electron micrograph of a rat in group B at the 6th hour

At a higher magnification, fibrillar character of fibrin is demonstrated. (x 50,000)

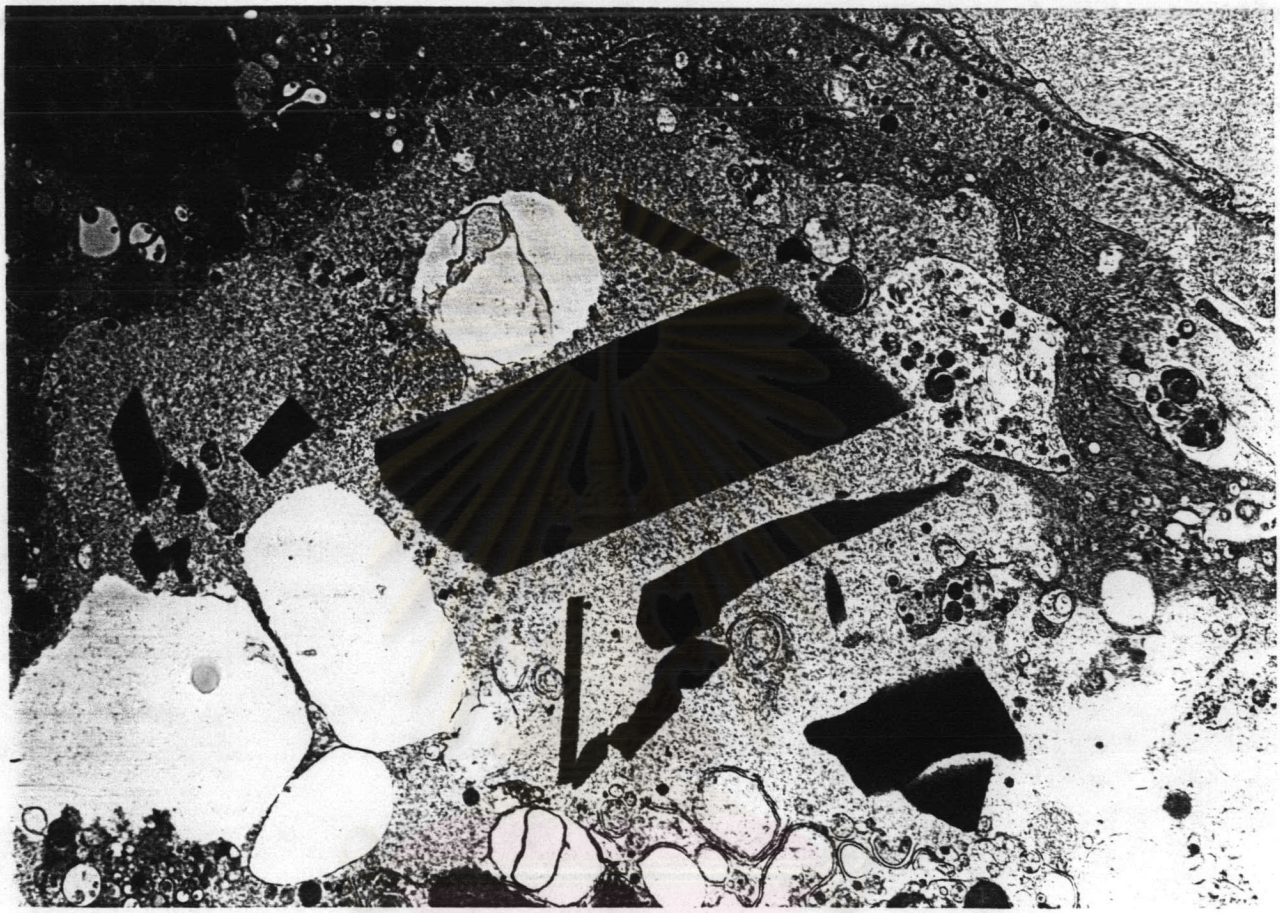


Figure 16 The electron micrograph of a rat in group A at the 1st day

This picture shows some crystal-like substances in the tubular lumen. (x 12,500)

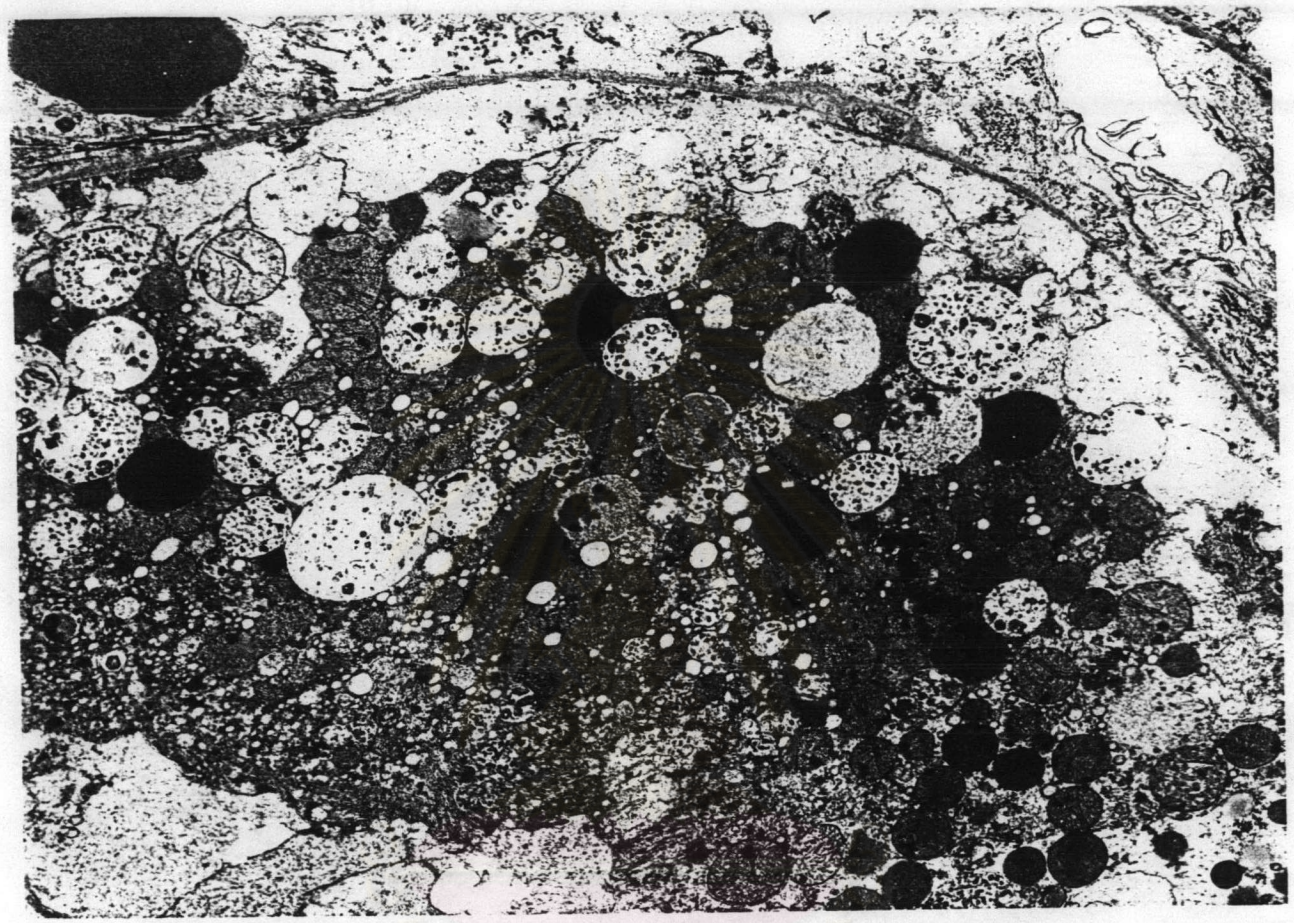


Figure 17 The electron micrograph of a rat in group A at the 1st day

The characteristic of tubular necrosis is shown. The tubular organelles are disintegrated and nuclei are absent. (x 7,500)

Table 1 The Renal Pathological Scores in Group A

PATHOLOGY	TUBULAR LESION	CONGESTION	FIBRIN
A 02.1	2+	2+	N
A 02.2	2+	2+	N
A 02.3	2+	1+	N
A 02.4	2+	1+	N
A 06.1	2+	2+	N
A 06.2	2+	2+	N
A 06.3	2+	2+	N
A 06.4	2+	3+	N
A 1.1	3+	3+	P
A 1.2	3+	3+	P
A 1.3	4+	2+	P
A 1.4	3+	3+	N
A 3.1	1+	1+	N
A 3.2	1+	2+	N
A 3.3	1+	2+	P
A 3.4	1+	1+	N

P = positive stain, N = negative stain

Table 1 (cont.) The Renal Pathological Scores in Group A

PATHOLOGY	TUBULAR LESION	CONGESTION	FIBRIN
A 10.1	0	2+	N
A 10.2	0	1+	N
A 10.3	1+	2+	N
A 10.4	1+	2+	N
A 30.1	0	0	N
A 30.2	0	0	N
A 30.3	0	0	N
A 30.4	0	0	N

P = positive stain, N = negative stain

Table 2 The Renal Pathological Scores in Group B

PATHOLOGY	TUBULAR LESION	CONGESTION	FIBRIN
B 02.1	2+	3+	P
B 02.2	1+	2+	N
B 02.3	1+	3+	N
B 02.4	1+	1+	N
B 06.1	1+	1+	P
B 06.2	2+	2+	N
B 06.3	1+	3+	P
B 06.4	1+	2+	N
B 1.1	1+	1+	N
B 1.2	1+	0	N
B 1.3	3+	1+	P
B 1.4	3+	1+	N
B 3.1	1+	0	N
B 3.2	1+	1+	N
B 3.3	1+	0	N
B 3.4	1+	0	N

P = positive stain, N = negative stain

Table 2 (cont.) The Renal Pathological Scores in Group B

PATHOLOGY	TUBULAR LESION	CONGESTION	FIBRIN
B 10.1	1+	2+	N
B 10.2	1+	1+	N
B 10.3	1+	2+	N
B 10.4	0	0	N
B 30.1	0	1+	N
B 30.2	0	1+	N
B 30.3	0	0	N
B 30.4	1+	0	N

P = positive stain, N = negative stain

Table 3 The Renal Pathological Scores in Group C

PATHOLOGY	TUBULAR LESION	CONGESTION	FIBRIN
C 02.1	0	0	N
C 02.2	0	0	N
C 06.1	1+	1+	N
C 06.2	1+	0	N
C 1.1	0	0	N
C 1.2	1+	1+	N
C 3.1	0	0	N
C 3.2	0	0	N
C 10.1	0	1+	N
C 10.2	1+	1+	N
C 30.1	0	0	N
C 30.2	0	0	N

N = negative stain

Table 4 Comparison of Mean Tubular Lesion Scores among 3 Groups

INTERVAL	GROUP A N = 4	GROUP B N = 4	GROUP C N = 2	F-value
HOUR 2	2.0 ± 0	1.25 ± 0.5	0	24.97*
HOUR 6	2.0 ± 0	1.5 ± 0.58	1.0 ± 0	8.17*
DAY 1	3.25 ± 0.5	2.0 ± 1.15	0.5 ± 0.71	6.90*
DAY 3	0.75 ± 0.75	1.0 ± 0	0	6.30*
DAY 10	0.5 ± 0.58	0.75 ± 0.5	0.5 ± 0.71	0.23
DAY 30	0	0.25 ± 0.5	0	0.70

Group A : RVV 1 mg./kg., Group B : RVV 2 mg./kg/, Group C : control group

N = The number of animals in each interval

* = Significant statistical difference at a p-value < 0.05

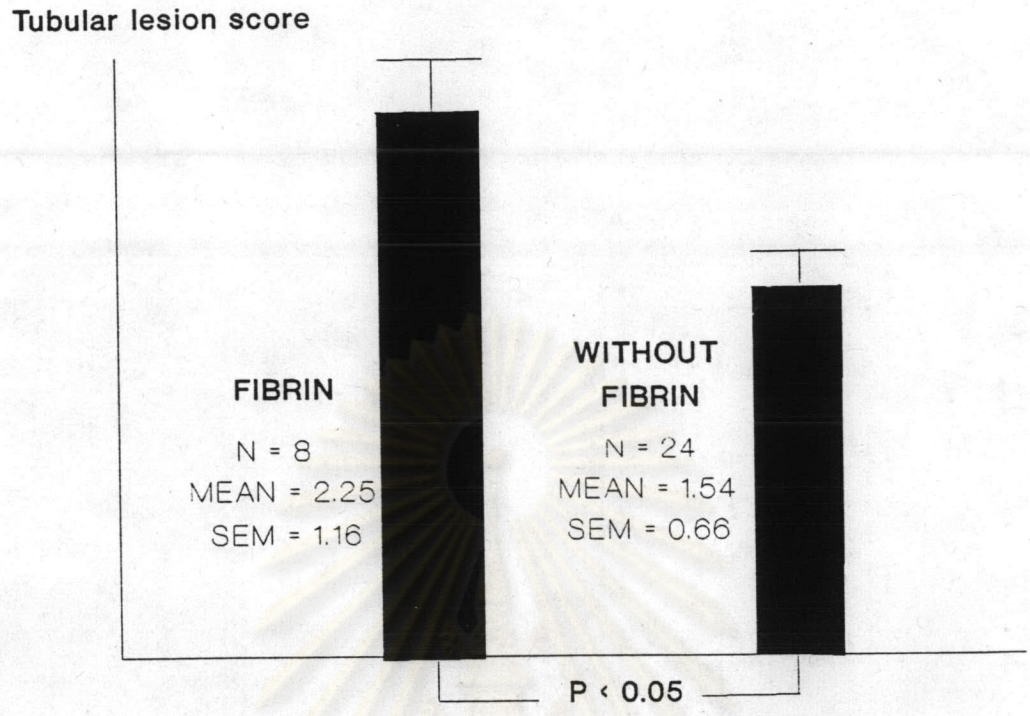
Table 5 Comparison of Mean Vascular Scores among 3 Groups

INTERVAL	GROUP A N = 4	GROUP B N = 4	GROUP C N = 2	F-value
HOUR 2	1.5 ± 0.58	2.25 ± 0.96	0	6.30*
HOUR 6	2.25 ± 0.5	2.0 ± 0.82	0.5 ± 0.71	4.68*
DAY 1	2.75 ± 0.5	0.75 ± 0.5	0.5 ± 0.71	18.38*
DAY 3	1.5 ± 0.58	0.25 ± 0.5	0	8.70*
DAY 10	1.75 ± 0.5	1.25 ± 0.96	1.0 ± 0	0.9
DAY 30	0	0.5 ± 0.58	0	2.1

Group A : RVV 1 mg./kg., Group B : RVV 2 mg./kg., Group C : control group

N = The number of animals in each interval

* = Significant statistical difference at a p-value < 0.05



N = The Number of Animals in Each Group

Figure 18 Mean Tubular Lesion Score of the Group with Positive Fibrin Stain Comparing to the Score of the Group with Negative stain