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Vascular Lesions Produced by Transient Renal Ischemia

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It has been clarified by the studies of our laboratory that the renal cortical extract of normal rats and experimentally hypertensive rats contain vascular injurious factors in addition to pressor substances. For the purpose of demonstrating this evidence in vivo, observations were made mostly of mesenteric and pancreatic vessels upon performing unilateral nephrectomy and contralateral renal arterial and ureteral ligations and releasing the arterial ligation after 2 hours and the ureteral ligation after 24 hours. As the result of transient renal ischemia, abnormalities of electrolytes, serum blood urea nitrogen and creatinine were recognized but these were gradually recovered. For eliminating pressor factors, rats without significant increase of blood pressure were used for the study.

One day after the surgery, fibrinoid degeneration appeared in the media of arterioles and small arteries. This change developed with time into angiitis and panarteritis like lesions on the seventh day after the surgery. These vascular lesions were basically the same as those caused by the renal cortical extract. Accordingly, the vascular lesions resulting from transient renal ischemia were deemed to be caused by renal vascular injurious substances in vivo.

INTRODUCTION

After GOLDBLATT[4] reported that fibrinoid necrosis was induced in blood vessels by experimental hypertension by means of renal artery constriction of the dog, vascular lesions have been elucidated from the aspect of renal hypertension by giving various operations to the kidney. WINTERNITZ et al.[21] performed intravenous injection of extracts from normal kidneys or ischemic kidneys to bilaterally nephrectomized dogs and clarified that vascular lesions were induced by the renal extracts. Subsequently it has been elucidated that pressor factor, increase of vascular permeability and vascular injurious factor are present in renal extracts. Investigaters[3),5),10),11),18),19)] in our laboratory have studied the relation between

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renal extracts from normal and experimentally hypertensive rats and vascular lesions, and confirmed that localized degeneration or necrosis of medial smooth muscle cells accompanied with fibrin-like material induced in 24 hours by non-pressor renal vascular injurious factors develops into panarteritis with fibrinoid degeneration and proliferation of elastic fibers of the intima as well as cellulofibrous thickening of the media.

In the present study, the author studied the process of vascular lesions upon eliminating the hypertensive factor with the presumption that non-pressor renal vascular injurious substances are released from the kidney after transient renal ischemia.

MATERIAL and METHODS

Wistar female rats weighing 250-300 g were used for the experimental group, right nephrectomy and left renal arterial and ureteral ligations were performed under anesthesia with ether. The renal arterial ligation was released after 2 hours and the ureteral ligation after 24 hours. The control group was divided into 3 subgroups. For the control subgroup 1, right nephrectomy was performed, for the control subgroup 2, right nephrectomy was followed by left ureteral ligation which was released after 24 hours, and for the control subgroup 3, sham operation was made. Both the experimental group and the control group were further classified into groups of 1,2,3,5, and 7 postoperative days. The numbers of rats assigned to those classified groups were 10, 10, 10, 13, 13, respectively for the experimental group and 2 each for the control group. The rats were sacrificed after blood sampling from the abdominal aorta and microscopic sections mainly of the kidney and mesenteric and pancreatic vessels were prepared.

Materials for histological examination were fixed immediately in 10 percent formalin, embedded in paraffin and made into serial sections of 4 μ in thickness. The sections were stained with Hematoxylin-Eosin stain, Phosphotungstic acid Hematoxylin stain, elastic fiber stain by Weigert's Resorcin-Fuchsin method, Periodic acid Schiff reaction and Azan-Mallory stain. Selected sections were stained with calcium stain. Frozen sections were cut in some instances and stained with oil red O.

Blood pressure was measured daily with an automatic recording apparatus ISM 105-T type. Serum was separated from the sampled blood for determination of Na, K, BUN and creatinine.

Values are reported as the mean or mean difference $\pm$ the standard error of the mean (SEM). Statistical evaluation was made by use of the appropriate Student's t-test for paired or unpaired comparisons. P values (<0.05) were considered significant.

RESULTS

1. Blood Pressure

In the experimental group, the blood pressure showed no increase from the preoperative

one in most cases. For the purpose of eliminating the pressor factor, the rats without any increase of blood pressure and with an increase by less than 5 mmHg which was deemed transient were used in this series of experiments (75%). The rats with an increase by more than 5 mmHg and the blood pressure of under 150 mmHg (14.3%) and the rats with an increase by more than 5 mmHg and the blood pressure of over 150 mmHg (10.7%) were excluded from this series.

In the control group, a transient increase of blood pressure by more than 5 mmHg compared with the preoperative one was noted in 18% of the rats. However, there was no significant difference between the experimental group and the control group. The variations of blood pressure are shown in Fig. 1.

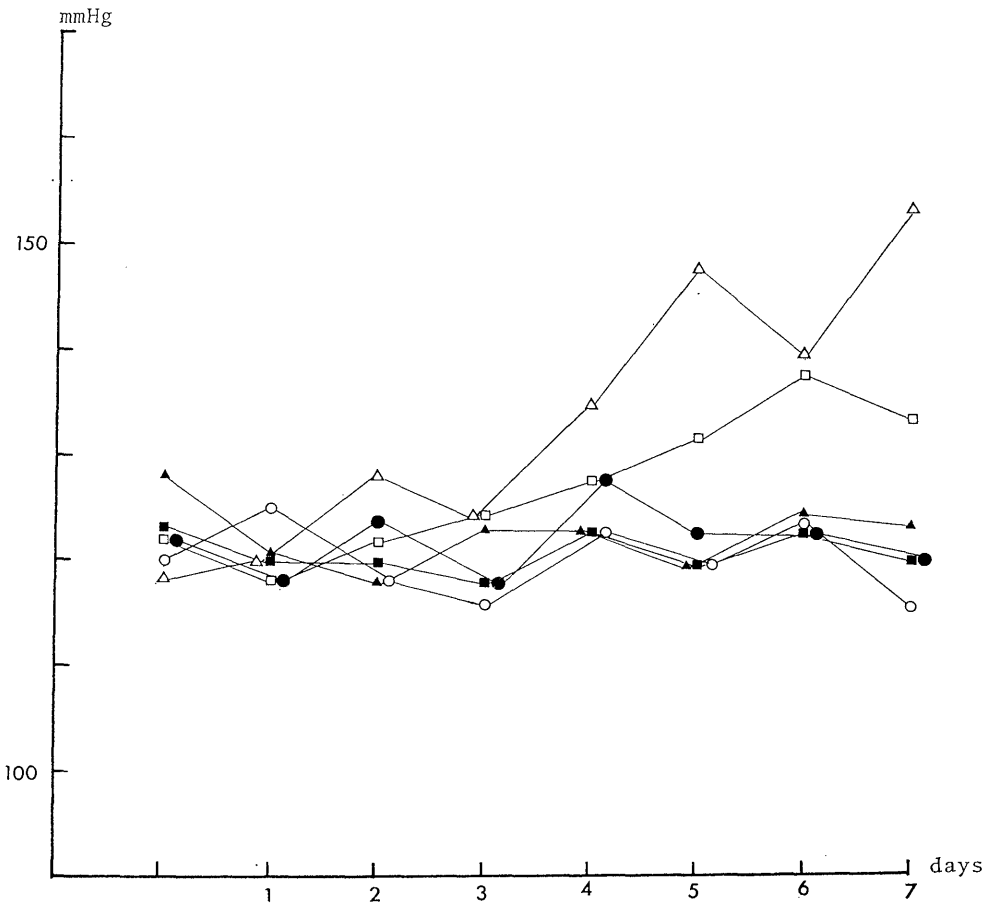

Fig. 1. Variation of blood pressure
■——■ : rats with an increase of blood pressure within 5mmHg
□——□ : rats with the blood pressure of under 150 mmHg
△——△ : rats with the blood pressure of over 150 mmHg
▲——▲ : control subgroup 1 (right nephrectomy)
●——● : control subgroup 2 (left ureteral ligation)
○——○ : control subgroup 3 (sham operation)

2. Serum Sodium and Potassium

The variations of serum sodium and potassium levels are shown in Figs. 2a and 2b. In the control subgroup 3, serum sodium level was 142.9 mEq/L in mean and serum potassium

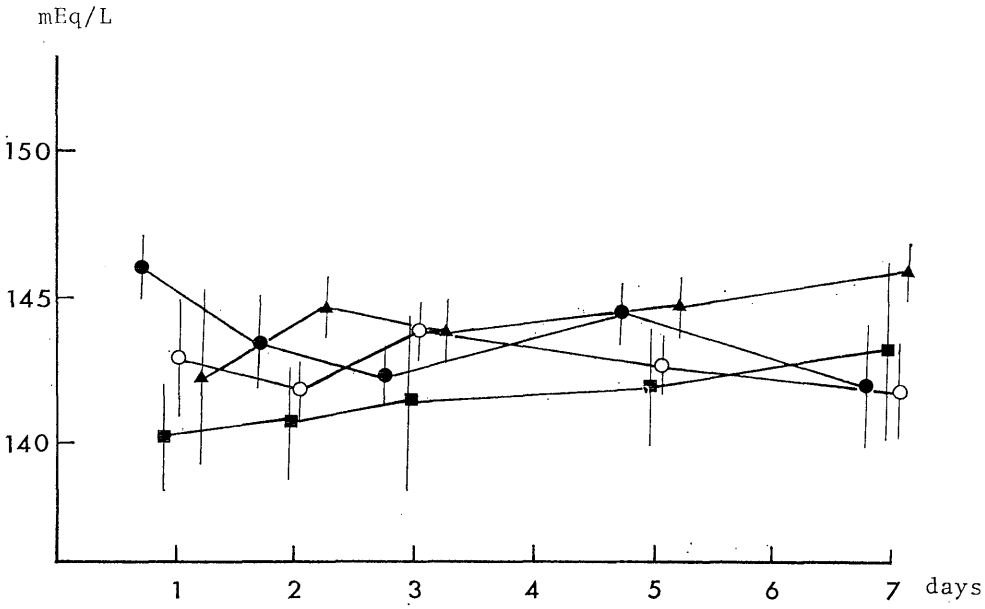

Fig. 2. Variation of electrolytes, serum BUN and creatinine
a : serum sodium (mEq/L)

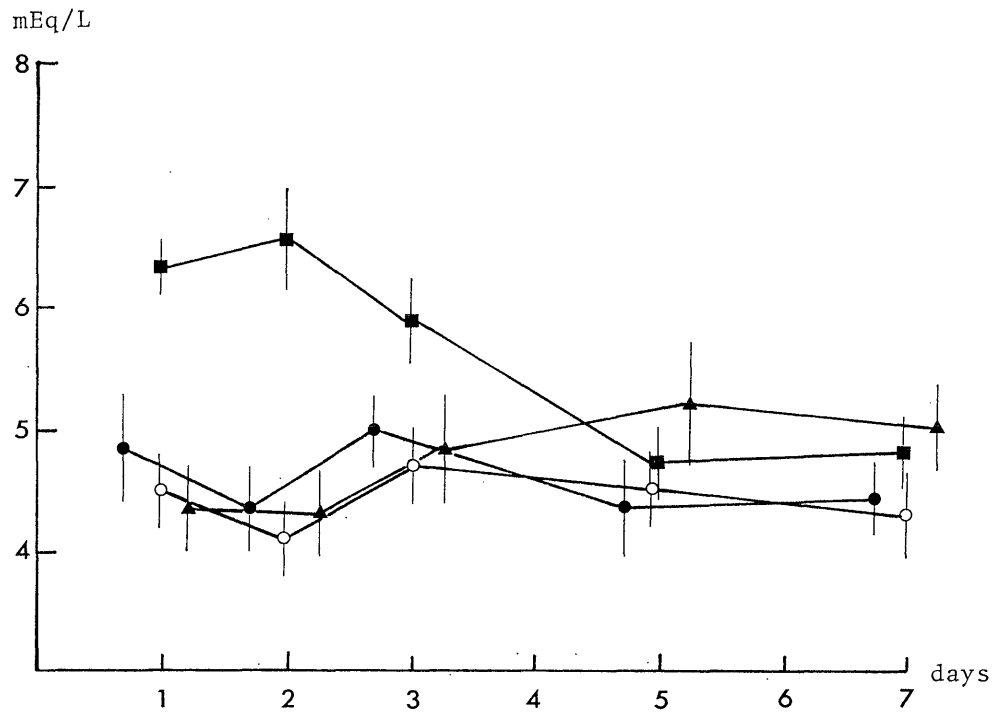

Fig. 2b. serum potassium (mEq/L)

level was 4.42 mEq/L. The serum sodium and potassium levels in the control subgroup 3 were of no significant difference from the levels in the control subgroups 1 and 2. In the experimental group, serum sodium level was 140.4±1.8mEq/L, 140.9±1.9mEq/L and 141.7 ±2.3mEq/L during the first three postoperative days showing a slight decrease. However, there was no significant difference from the control group. Serum potassium level was 6.30 ±0.23mEq/L, 6.49±0.42mEq/L and 5.95±0.35mEq/L during the first three postoperative days indicating a remarkable increase ($p<0.01$), but thereafter, there was no significant difference from the control group.

3. Serum Blood Urea Nitrogen (BUN) and Creatinine

In the control subgroup 3, serum BUN and creatinine levels were 22.4 mg/dl and

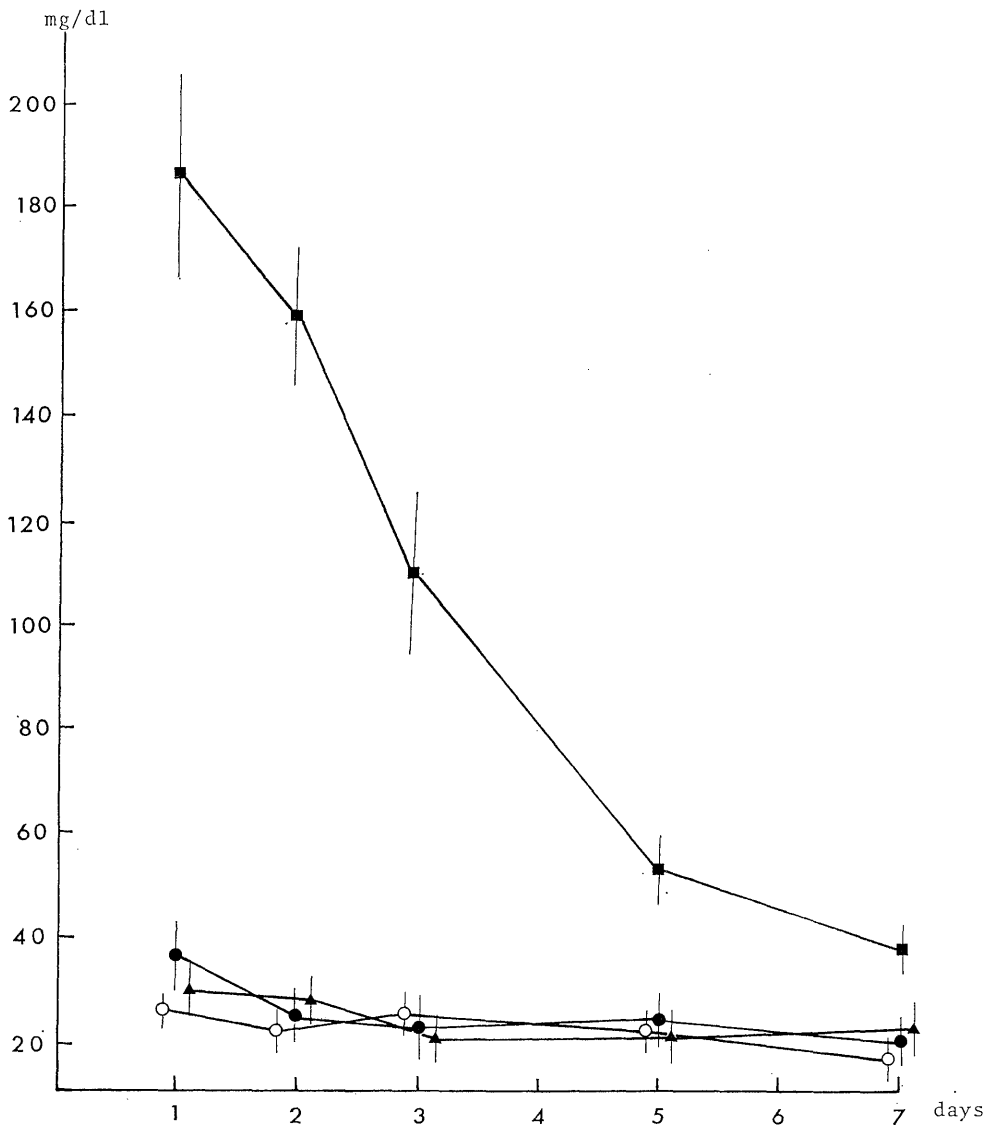

Fig. 2c. serum BUN (mg/dl)

0.64 mg/dl in mean respectively, without any significant difference from the levels in the other control subgroups. In the experimental group, serum BUN level was 186.1±19.2 mg/dl, 159.8±13.3 mg/dl, and 110.1±15.3 mg/dl during the first three postoperative days showing a remarkable increase ($P<0.01$) and was 54.9±6.7 mg/dl and 40.5±4.9 mg/dl on day 5 and day 7 respectively, being somewhat higher ($P<0.01$). Serum creatinine level

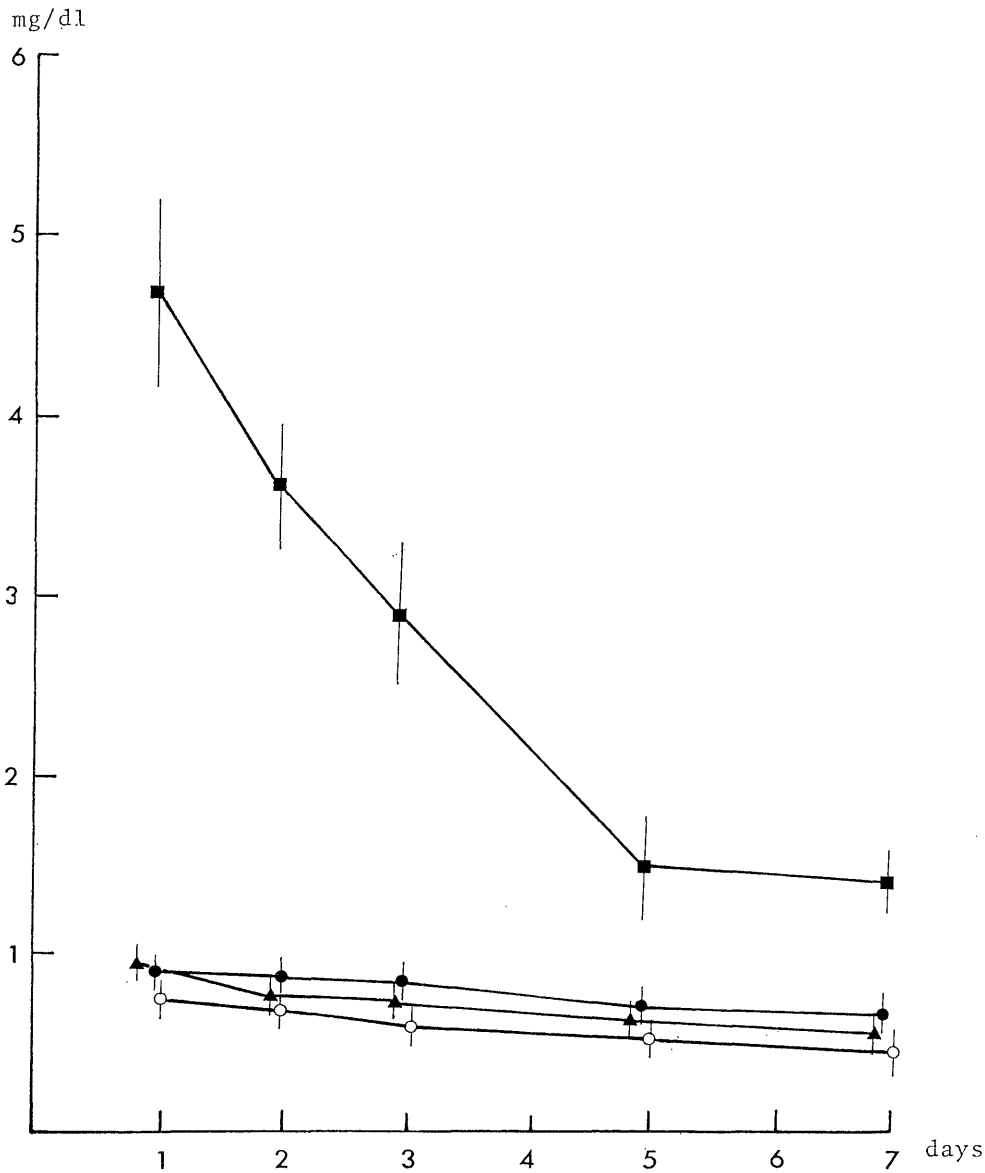

Fig. 2d. serum creatinine (mg/dl)
■——■ : experimental group
▲——▲ : control subgroup 1
●——● : control subgroup 2
○——○ : control subgroup 3
The vertical bars illustrate the standard error of the mean.

was 4.75±0.52 mg/dl, 3.65±0.35 mg/dl and 2.89±0.37 mg/dl during the first three postoperative days showing a remarkable increase ($P<0.01$) and was as low as 1.51±0.29 mg/dl and 1.40±0.11 mg/dl on days 5 and 7 respectively with a significant difference from the control group ($P<0.05$). The variations of serum BUN and creatinine levels are shown in Figs. 2c and 2d.

4. Macroscopic Findings

Marked swelling and mild hydronephrosis were observed in the left kidney in which arterial ligation was released and ureteral ligation was maintained for 24 hours. On and after day 2, the marked swelling of the left kidney was still present but the mild hydronephrosis gradually disappeared. A small of ascites and mild edema of the pancreas and mesentery were seen throughout the course. Macroscopic hematuria was recognized in all rats within 1 hour after the release of recognized in all rats within 1 hour after the release of ureteral ligation, but not on and after day 2. In the control group with right nephrectomy, mild swelling of the left kidney was noted. In the control group with right nephrectomy and left ureteral ligation which was released 24 hours after, mild swelling of the left kidney and mild hydronephrosis were present 24 hours after the operation but the mild hydronephrosis disappeared on days 2-3.

5. Light Microscopic Findings

(1) Kidney

Ischemic changes of the kidney were intensive particularly in the tubular epithelium within the cortex mostly consisting of the proximal tubular epithelium. On postoperative day 1-3, cloudy swelling, hyaline droplets, fatty changes, calcification, necrobiosis and necrosis were observed in most of the proximal tubular epithelium within the cortex (Fig. 3). There was no increase in cellularity of the glomeruli. In a few of Bowman's space hyaline or protein like substances were present on day 1, but they gradually disappeared on days 2-3. The interstitial tissue showed dilatation of capillary vessels and congestion of blood but no such finding as fibrosis, inflammatory cell infiltration and hemorrhage.

Part of the tubular epithelium within the medulla showed degeneration and necrosis. Hyaline casts were seen numerously in the tubular lumen. Dilatation of capillary vessles was noted in the interstitial tissue.

On postoperative days 5-7, cloudy swelling and calcification were present in the tubular epithelium within the cortex mostly consisting of the proximal tubular epithelium which was also marked with flattening and dilatation (Fig. 4). There was no increase in cellularity of the glomeruli. The Bowman's space showed no hemorrhage, exudate, nor inflammatory cell infiltration. In the interstitial tissue, fibrosis and infiltration of inflammatory cells mostly consisting of lymphocytes were observed. The medulla was not remakable.

Fibrinoid necrosis was present scatteringly in the media of the arcuate arteries and interlobular arteries. There was no thrombus in the vessels of the kidney.

In the control group with ureteral ligation, a few of Bowman's space disclosed hyaline or protein like substances and the tubules were seen mild dilatation 24 hours after

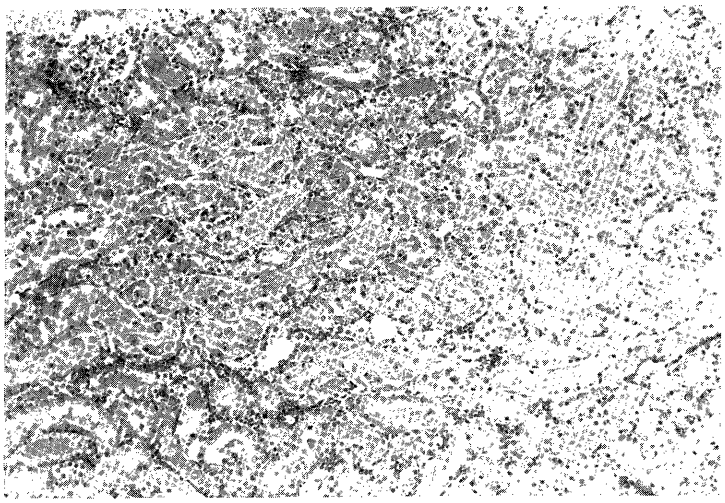

Fig. 3. Photomicrograph of the kidney, 1 day after operation.
The proximal tubular epithelia of the cortex are necrotic and the tubular lumina are filled with eosinophilic necrotic tubular epithelial cells. Hematoxylin and eosin stain.

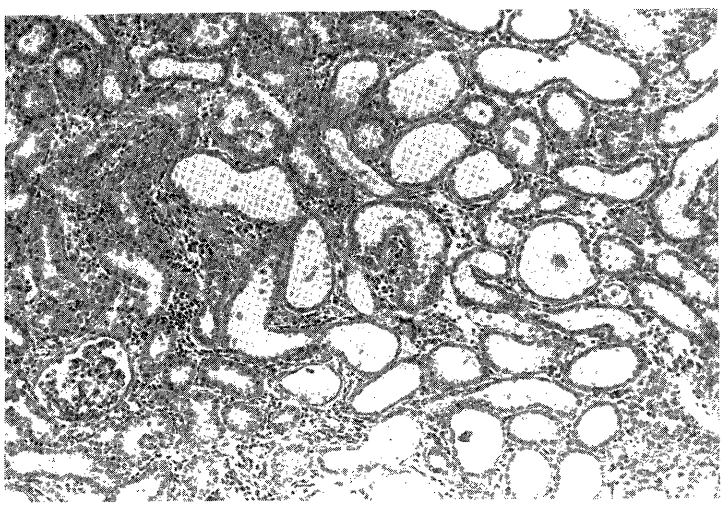

Fig. 4. Kidney, 7 days after operation.
The proximal tubular epithelia of the cortex are marked with flattening and dilatation. Hematoxylin and eosin stain.

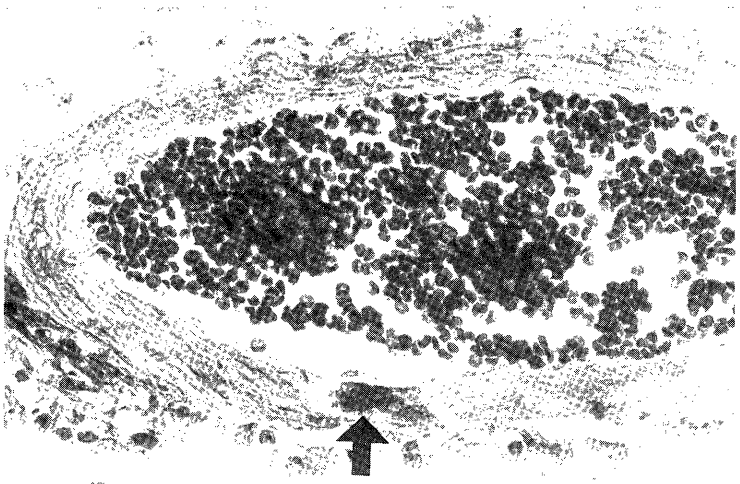

Fig. 5. Small mesenteric artery, 1 day after operation.
Partial fibrinoid necrosis (→) is seen in media of small artery.
Azan-Mallory stain.

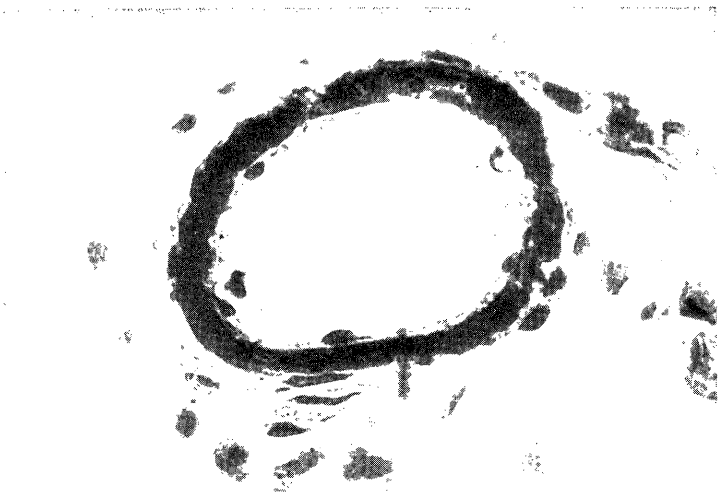

Fig. 6. Small mesenteric artery, 1 day after operation.
Fibrinoid necrosis extends the entire circumference of the artery.
Azan-Mallory stain.

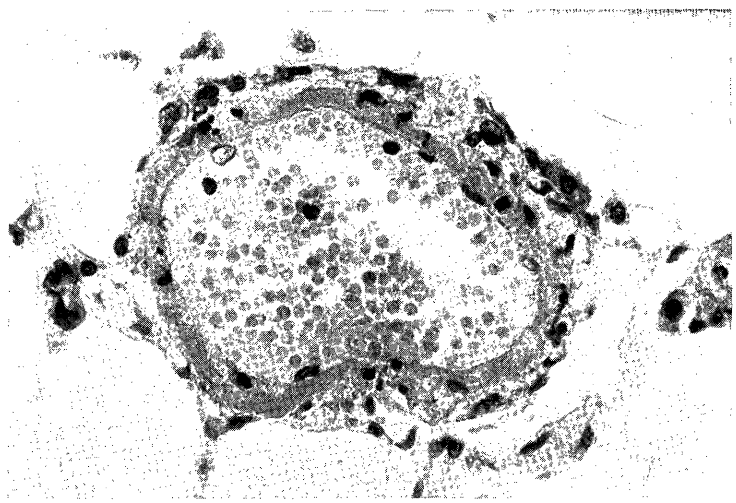

Fig. 7. Small mesenteric artery, 2 days after operation.
Fibrinoid necrosis is seen in the entire wall of the vessel.
Hematoxylin and eosin stain.

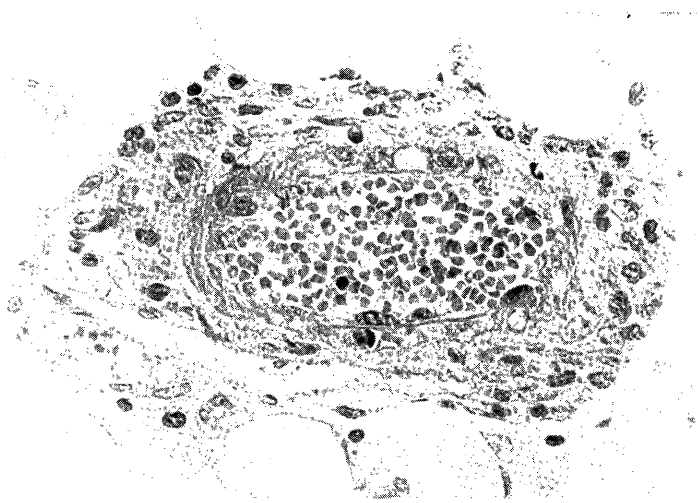

Fig. 8. Small pancreatic artery, 3 days after operation.
Proliferation of cells in the adventitia and fibrinoid degeneration in the media are seen. Hematoxylin and eosin stain.

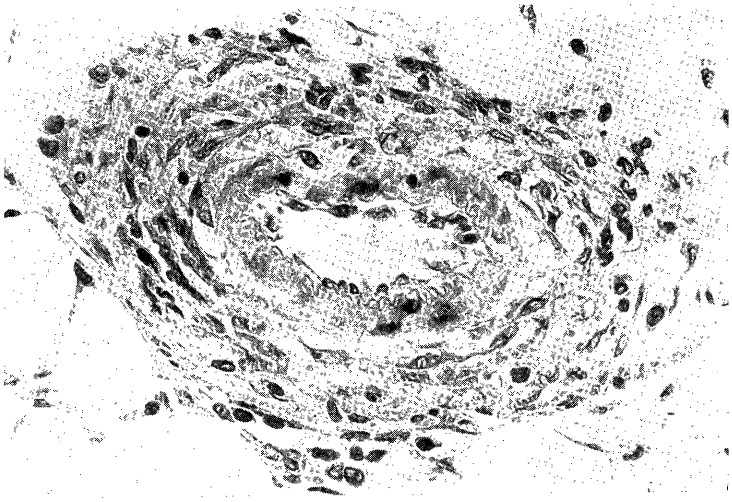

Fig. 9. Small pancreatic artery, 5 days after operation.
Fibrinoid necrosis is seen in the media. Proliferation of cells in the adventitia extends more than that of the 3rd day.
Hematoxylin and eosin stain.

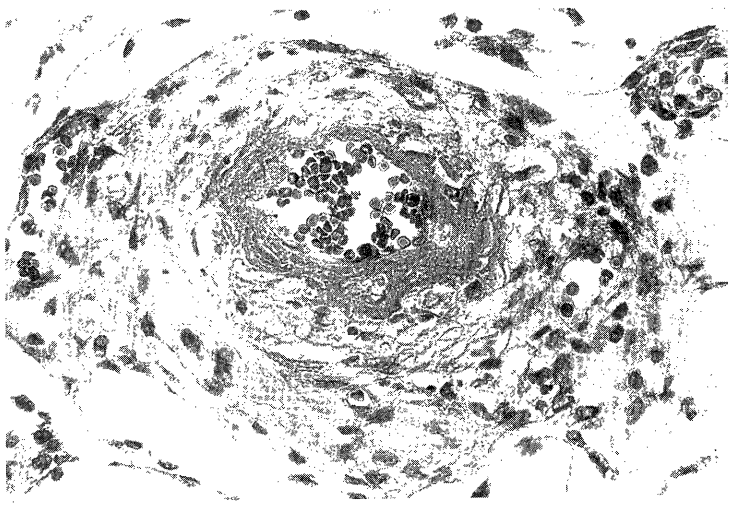

Fig. 10. Small pancreatic artery, 7 days after operation.
Cell proliferation with fibrinoid degeneration is seen. This change can be called panarteritis. Hematoxylin and eosin stain.

the surgery, but these changes gradually disappeared on and after day 2. There was no remarkable change in the other control group.

(2) Pancreatic and mesenteric vessels

On Postoperative day 1, arterioles and small arteries showed degeneration and necrosis of medial smooth muscle cells also involving fibrinoid degeneration partially (Fig. 5) or circumferentially (Fig. 6). On postoperative day 2, fibrinoid necrosis covered the entire wall of the vessel. The number of vessels with degenerated and necrotized cells decreased. There was no infiltration of inflammatory cells (Fig. 7). On days 3 and 5, fibrinoid necrosis was observed in the media, and infiltration of inflammatory cells consisting mostly of fibroblasts, lymphocytes, plasma cells and neutrophils was seen in the adventitia (Figs. 8 and 9). On day 7, panarteritis with marked cell proliferation in the intima, media and adventitia was recognized. These changes well resembled the lesions of vessels in periarteritis nodosa (inflammatory stage). Panarteritis was accompanied by fibrinoid degeneration in the intima and media in some vessels (Fig. 10) or not accompanied in some others. Elongation and fragmentation were caused in the internal elastic lamina, and the vascular lumen was narrowed where cell proliferation of the intima was enhanced. At this time, the number of vessels with changes was reduced as compared with that of day 1. There was no change in the control group throughout the course.

DISCUSSION

There have been many reports on renal injuries caused by interruption of renal blood circulation. After 2 hours' ligation of the rat renal artery and release thereof, REIMER et al.[15] studied the changes after 24 hours and reported that ischemic change was initially seen in the proximal tubules of the renal cortex. The author's experiments also disclosed severe degeration and necrosis of the renal cortex mostly consisting of the proximal tubular epithelium.

On postoperative days 1-3, a marked increase of serum BUN and creatinine was noted and it tended to be improved subsequently. In view of the behavior of serum BUN and creatinine and of the histological findings of the kidney, acute renal degeneration was considered. Consequently, transient renal ischemia resulted in marked degeneration of the renal parenchyma, particularly of the cortex, and in exsudation of cytoplasmic substances into the blood, and the exsudates should enter the circulating blood as soon as the vascular ligation was released. Moreover, since the 24-hour ureteral ligation disturbed the excretion of those substances, the concentration in the blood might be increased. As the result, the vascular lesions were caused by the effects of those substances. Although hypertensive rats were excluded from this series, pressor substances might have been released in these rats.

In the expermental group without blood pressor elevation, fibrinoid necrosis was

recognized in the media at the initial stage, which in the course of time developed into panarteritis. The process of these vascular lesions was compared with FUKAZAWA'S experimental results using non-pressor renal cortical extracts. FUKAZAWA[3] examined mainly mesenteric and pancreatic vessels upon intravenous injection of non-pressor renal extracts from normal rats to rats with unilateral nephrectomy and contralateral ureteral ligation followed by release of ureteral ligation after 24 hours. Fibrinoid necrosis was seen in the media at postoperative 24 hours, and panarteritis with marked cell proliferation was noted in the intima, media and adventitia during postoperative days 5 to 7. Fibrinoid necrosis in the intima was either present or absent. Elongation and fragmentation were present in the internal elastic lamina.

These vascular lesions caused by non-pressor renal extracts were basically identical with those observed in the author's experimental series. In view of this, it is considered that transient renal ischemia results in the release of non-pressor vascular injurious factors from the kidney. Among the organs to have vascular injurious substances, the kidney has been paid greatest attention. There have been many reports by various methods of experiment and preparation concerning the vascular injurious substances that are considered to be present in the kidney. ASSCHER[2], MASSON[7] and ONOYAMA et al.[13),14)] assumed renin or renin like substances as the vascular injurious substances, while MURAKAMI[8], NAKAO[9], and YAMAGUCHI[24] observed the presence of bascular injurious factors in non-pressor fractions and reported that these factors are other than renin. Our laboratory has reported that the vascular injurious factors in the kidney are included in non-pressor fractions of the renal cortical extract[3),5),10),11),18),19)]. The vascular injurious substances released from the ischemic kidney are considered to be basically identical with the non-pressor renal cortical extract reported from our laboratory in view of the absence of increased blood pressure and the similarity of vascular lesions.

The vascular lesions resulting from the author's series of experiments are morphologically the same as periarteritis nodosa in man. Methods of producing experimental panarteritis similar to periarteritis nodosa in man are numerous such as the renovascular hypertension method by LOOMIS et al.[6] and ZEEK et al.[26], hypertension following administration of DOCA by SELYE[17], and adrenal regeneration hypertension by SKELTON[20]. In these methods, durative hypertension is produced and vascular lesions are taken as its consequent phenomena. There have also been reports that panarteritis occurs spontaneously in various animals[1),22),25)] and is observed in spontaneously hypertensive rats (SHR)[12),21)]. A problem in panarteritis as well as in hypertension is allergic mechanism. RICH et al.[16] induced hypersensitivity arteritis in rabbits by injecting a quantity of horse serum and attempted an immunological approach in the study of vascular lesions. However, FUKAZAWA[3] has reported that there was no production of antibody against non-pressor renal cortical extract in rats with panarteritis caused by non-pressor renal cortical extract. Immunological mechanism is not possibly considered for the vascular lesions resulting from transient renal ischemia. For the pathogenesis of panarteritis observed in the above experiments, various possibilities have been considered but none has been thoroughly elucidated. As one of

the causes of panarteritis, it may be worth while to consider the presence of non-pressor vascular injurious substances that are deemed to be released from transient renal ischemia.

In view of the fact that almost all vessels 1 day after the operation show some lesions but the frequency decreases during the course of long term observation, it seems that early vascular lessions are reversible and some of them may be repaired completely without leaving any trace.

Transient hyperkalemia was recognized during postoperative days 1-3. However, there has been no report that vascular lesions are caused by transient hyperkalemia. Hence, trasient hyperkalemia is considered to have resulted from acute renal degeneration without participation in the pathogenesis of vascular lesions.

In this series, only the rats without increase of blood pressure in the experimental group were examined for vascular lesions. The rats with increased blood pressure were excluded from the study. This is because increased blood pressure was considered not to be the cause itself of vascular lesions although it may be a factor to enhance vascular lesions.

CONCLUSION

For the purpose of verifying the presence of non-pressor vascular injurious factors in rats in vivo, observations were made of mesenteric and pancreatic vessels upon performing right nephrectomy and left renal and ureteral ligations and releasing the arterial ligation after 2 hours and the ureteral ligation after 24 hours. The rats without significant increase of blood pressure were used so as to eliminate pressor factor. Conclusion obtained was as follows.

Fibrinoid necrosis was observed in the media of mesenteric and pancreatic vessels 24 hours after transient renal ischemia and infiltration of inflammatory cells was seen in the adventitia during days 3-5. This change developed into panarteritis like lesions with fibrinoid necrosis on day 7. Since the number of vessels showing these changes decreased with the lapse of time, it is considered that some of these early vascular changes is reversible.

These vascular lesions consisting mostly of medial degeneration are similar to the vascular lesions induced by intravenous injection of non-pressor vascular injurious substances contained in the renal cortex as has been studied in our laboratory, and hence the pathogenesis seems to be the same. Although various factors may be involved in the pathogenesis of vascular lesions, the possibility that the vascular injurious substances contained in the renal cortex acted in vivo is considered to be strong. Arteritis changing with time or panarteritis involving fibrinoid degeneration should be regarded as a histological feature of the in vivo reaction, and the participation of immunological mechanism can hardly be considered.

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