Experimental Studies on Pathogenesis of Angionecrosis Produced by Administration of Renal Extracts

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Received for publication, March 1, 1971


Necrotic change of the arterioles which is a hypertensive vascular change has been considered quite important as the cause of cerebral hemorrhage. However, the course of development and pathogenesis have not been clarified adequately. In this paper, the author attempted experimentally to prove that angionecrosis is caused essentially by the necrotizing factor which is present in the hypertensive injured kidney.

INTRODUCTION

There have been various studies on the correlation between hypertension and general vascular diseases. However, since Goldblatt noted the relation of the kidney with hypertension upon performing artificial renal artery stenosis, great importance has been placed on the role of the kidney in the development of hypertension and in the progress of vascular diseases following the hypertension. The experiments in pursuit of the relation between hypertensive vascular diseases and the kidney may be roughly classified into two categories: (1) to cause ischemic changes to experimental animals by surgical procedure, and (2) to inject renal extracts or other substances intravenously or abdominally to the experimental animals treated in various manners. For cateroty (1), various methods have been worked out such as renal artery ligation, direct aggression to the kidney, ureter ligation, and nephrectomy. In 1953, Haas-Goldblatt succeeded in the purification of renin and ever since then the group insisting that renin-angiotensin system is the cause of renal hypertension has become the majority. However, there are some papers...
insisting that the increased blood pressure cannot be considered as the main cause of vascular changes since there is no correlation between vascular disorder and blood pressure and that there must exist some vasculotoxic agent other than renin. In Japan in recent years, there have been made various studies in this field. Some investigators understood that the cause of vascular disorder in hypertension is vascular permeability factor deriving from the kidney, and some others reported that angionecrosis occurs frequently in rabbits administered with nonpressor fraction of the kidney.

The author prepared ultracentrifugal fractions of the kidneys of normal rats and of hypertensive injured rats, and studied primarily on (1) fraction with strong pressor effect, (2) fraction inductive of angionecrosis, (3) fraction with strong retention of effusions, and (4) fraction with strong vascular permeability. In addition, the same procedure was followed to investigate on the other organs in an attempt to compare them with the kidney.

METHODS OF EXPERIMENT

1) Preparation of Experimental Hypertension Kidney

Wistar rats, female, weighing 40—50 grams were used. Upon unilateral adrenalectomy and enucleation of the other adrenal, 1% salt solution was given as water to drink. One week thereafter, 0.5 ml of Nephrotoxin was injected in the caudal vein. The treated rats were kept in a constant room temperature of 20—25°C and underwent weekly measurement of the systolic pressure in the caudal artery by FRIEDMANN'S method. Those showing over 160 mmHg in the fourth experimental week were used for fractions of hypertensive kidney.

As shown in Fig. 1, the treated group indicated the systolic pressure of as high as 172 mmHg and marked proteinuria (300—1000 mg/dl) in the fourth experimental week. In the treated rats, the increase of body weight was delayed as compared with the control group and the
kidneys were somewhat enlarged with fine granular and anemic surface. Histological findings were necrotic changes of the glomerulus, edematous changes of the Bowman's capsule, infiltration of small round-cells in the interstitium and proliferation of the connective tissues. Remarkable were vascular changes particularly proliferative changes and necrotic changes in the interlobular arteries and the afferent arteries. These changes resembled closely the histological picture of malignant nephrosclerosis in human being.

Preparation of Nephrotoxin:

Normal Wistar rats weighing 100 g were thoroughly perfused by refrigerated physiological salt solution. The extirpated kidney except for the soft tissue of the pelvis was homogenized by ultrasonic generator (frequency 10 kc/s, 200 W, input 100 V 7 A). Then, together with Freund complete adjuvant, 1 ml was injected subcutaneously in the abdominal region of 2 kg rabbit and additional 0.5 ml was injected three weeks later. Upon confirming the production of antibody against kidney homogenate by OUCHTERLONY'S method in the fourth week, blood was drawn, inactivated by separation of serum and preserved below -20°C.

2) Preparation of Fractions of Kidney and Liver

The normal rats and the hypertensive rats prepared by the aforementioned procedure were completely abstained from food and water from the day before sacrifice, thoroughly perfused with sterile refrigerated physiological salt solution from the abdominal aorta under the state of general anesthesia by ether, and nephrectomized. The other organs were also removed. The removed kidneys were fractionated according to the original method of HOGEBROON\(^7\)). Fractions were also prepared by the same procedure with the solvent of 0.9% NaCl. The fractions thus prepared may be divided into Fraction 1 with undestroyed cells, nuclei and connective tissue, Fraction 2 with mitochondria, Fraction 3 with microsome and Fraction 4 with soluble components. The same procedure was followed for the preparation of liver fractions and 0.25 M sucrose was used as solvent for normal rats.

3) Measurement of Vascular Permeability

Female Wistar rats weighing 100 g were anesthetized by ether, had the abdominal hair cut with scissors, and was injected from the caudal vein with 0.07 ml of Evans Blue (0.5 W/V %) per 100 g of body weight. Five minutes later, the fractions previously adjusted to be 0.05 ml 32 μg (protein volume) were injected subcutaneously at four locations which were changed according to Latin square. After the lapse of 30 minutes, the maximum diameter and rectangular direction were measured by slide calipers to obtain the arithmetic mean and the intensity of color tone was also measured (± ~ ++). The same measurement of permeability was then performed with the
group of one week pretreatment by means of gradually increased administration of Sinomenine hydrochloride and with the group treated with antihistamine (Diphenhydramine 6 mg intravenously injected). Measurement of permeability was also made with the fractions heated at 70°C for 20 minutes and at 90°C for 15 minutes.

4) Measurement of Retained Effusions After Administration of Fractions of Kidney and Other Organs, and Histological Study

Bilateral nephrectomy was performed on female Wistar rats weighing 80—90 grams anesthetized by ether. Nephrectomy was followed by complete fasting and an hour later by administration of 1 ml of each fraction (protein 6 mg) from the caudal vein. The rats were intravenously injected 0.1 ml of carbon (Pelikan cl1/1431a, Gunther

Rat Kidney

After being minced, the chilled Kidney was homogenized in 0.25 M Sucrose (1:10)

Homogenization was 2 min. in polytron (2000 r.p.m.)

Homogenate

Centrifuged for 30 min. at 14000 r.p.m.

International refrigerator

Model

Pellet

Suspended in 0.25 M Sucrose

Add 0.34 M Sucrose

Centrifuged for 10 min.

at 7000 (2000 r.p.m.)

Rotor No. 4

Fraction 1

Fra. 2

Fra. 4

Fra. 3

Supernatant

Centrifuged for 60 min. at 100000 G

Beckman Model L-2 Rotor type 50

sup. pellet sup. pellet

Dialysed against 0.9 NaCl overnight

Freezing and thawing 10 times

All operations were carried out at 0—5°C.

Determination of protein

Protein was determined by the method of Biuret and Folin

(560 Å)

Fig. 2. Fraction of rat Kidney
wagner, Co., Hanover, Germany) per 100 g of body weight one hour before sacrifice which took place 24 hours after nephrectomy. Upon measurement of pleural effusions and ascites, macroscopic and histological examinations were conducted. The cases died within 24 hours were autopsied at the time of death and subjected to the same examinations. The organs of injured hypertension rats were homogenized by Polytron homogenizer (200 rpm, 3 min.), 6 mg (protein) of each supernatant was intravenously injected and the same examinations were conducted. Histological examination was made for the brain, thymus, lung, liver, heart, pancreas, adrenal, small and large intestines, and mesentery upon fixation by 10% formalin and Zenker's solution and preparation of paraffin sections which were stained with H–E, PAS, Mallory-azan, Van-Gieson's, Weigert elastic, Fibrin, and PTAH.

5) Measurement of Pressor Activity of Fractions

Female Wistar rats weighing 100 g were nephrectomized bilaterally from the dorsal side and after 24 hours 0.5 ml of each fraction (protein 1.6 mg) was injected into the caudal vein. Then the pressure of the carotid artery was measured by automatic null balancing recorder.

6) Ligation of Bilateral Renal Arteries and Liver Lobules

Of 15 female Wistar rats each weighing 100—200 grams, the renal arteries were adequately detached after approaching from the back and were ligated with silk thread approximately at the center between the abdominal aorta and the hilar region of the kidney. The ligation of the liver lobules was made upon upper abdominal median incision by means of ligating with silk thread the liver vein at the root of two lobules. Bilateral nephrectomy was also performed. All these rats were maintained in fasting condition at constant temperature. Animals were autopsied immediately after death for histological examination. Surviving cases were sacrificed 48 hours after nephrectomy.

RESULTS

1) Clinical Picture

There was no deceased case 24 hours after bilateral nephrectomy. In the group administered with kidney fraction, many cases died of apnea immediately after injection of Fr. 3. However, no deceased case was observed when the fraction was heat-treated. MELENA (tar stool) was demonstrated in approximately 60% of the rats administered with Fr. 3 but not in the groups of other fractions of the kidney and fractions of the liver.

2) Vascular Permeability

(1) Increased permeability of skin Vessels (Fig. 3)
Permeability of skin vessels was most strongly increased in the group of Fr. 3 of both normal kidney and injured kidney. The increase was least in the group of Fr. 4. In comparison between normal kidney and injured kidney, Fr. 3 with the strongest permeability showed no difference whereas Fr. 4 of the injured kidney with the

<table>
<thead>
<tr>
<th>0.25M Sucrose</th>
<th>Normal kidney</th>
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<tr>
<td>Fractions</td>
<td>1</td>
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<tr>
<td>Diameter</td>
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<tr>
<td>Intensity</td>
<td>+</td>
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<tr>
<td>Injured kidney</td>
<td>Diam. M.E.</td>
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<td>Int.</td>
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<th>0.9% MaCl</th>
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<tr>
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<td>+</td>
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<tr>
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<td>Diam. M.E.</td>
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<td>Int.</td>
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<td>Pretreated with SINOMENINE</td>
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<td>Diam. M.E.</td>
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Heat lability of vascular permeability

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<td>Diam. M.E.</td>
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Each group consisted of 6 animals.

Fig. 3 Measurement of Vascular Permeability

Pleural Effusion - Ascites
weakest permeability showed somewhat large difference (P < 0.05). The same trend was also observed when 0.9% NaCl was used as solvent. The administration of gradually increased dose of Sinomenine hydrochloride during the period of one week resulted in consumption of over 90% of histamine in the abdominal skin. In the rats thus pretreated, the change of vascular permeability was strongly restricted in all groups. The administration of Diphenhydramine 6 mg resulted in a slight restriction in all groups. As to heat resistivity, this factor was hardly affected by the treatment at 70°C for 20 minutes but somewhat affected by that at 90°C for 15 minutes.

<table>
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<th>Heart</th>
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<th>Intestine</th>
<th>Pancreas</th>
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AN: Angioneccrosis  H: Hemorrhage  E: Edema

Fig. 5 Histological changes caused by the administration of various organs.

Fig. 6 Histological changes caused by the administration of fractions of liver and various surgical procedures.
(2) Retention of effusions (Fig. 4)
The rats were administered with 1 ml (protein 6 mg) of respective fractions one hour after bilateral nephrectomy and were sacrificed after 24 hours. As shown in Fig. 4, the quantity of ascites and pleural effusions was the largest in the rats administered with Fr. 3 of the normal kidney, mean value of retention being 1.7 ml ± 0.3 ml. Fr. 3 of the normal kidney indicated a fairly stronger trend of retention as compared with Fr. 3 of the injured kidney (0.05 < P < 0.1). Among various fractions of the normal kidney, Fr. 3 showed much higher value of retention (P < 0.02). Among various organs administered in the same dose, the administration of the kidney homogenate indicated and apparently higher value of effusions than that resulting from the administration of the pancreas, liver or heart (P < 0.05). However, no significant difference was indicated from the value resulting from the administration of the spleen, lung or brain.

3) Macroscopic and Microscopic Findings of Changes Caused by the Administration of Fractions of the Kidney and Various Organs (Figs. 5, 6, 7)
The author studied on hemorrhage and edema, deposit of carbon

<table>
<thead>
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<th>Fraction</th>
<th>Rats</th>
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AN: Angionecrosis  H: Hemorrhage  E: Edema
■: Intensity of Angionecrosis
2/6: "AN" was found in two-sixths rats

Fig. 7. Histological changes caused by the administration of normal and injured kidney.
on the vascular wall and histopathological changes particularly angione-
crosis.

(1) Hemorrhage and edema

Hemorrhage caused by the administration of fractions of the
kidney was noted in the brain, lung, intestinal wall, pancreas, liver
and mesentery; frequency was high in the lung and mesentery particu-
larly of the cases administered with Fr. 3. One case administered
with Fr. 3 of the normal kidney and another with Fr. 3 of the injured
kidney showed extensive subdural hemorrhage centered about the right
lateral head and cerebellum. In the cases administered with Fr. 3
and Fr. 4 of the injured kidney, intensive hemorrhage was observed
in the brain parenchyma centered about the cerebellum. The hemorr-
hage in the mesentery showed the trend of frequent occurrence near
the area attached to the intestine. In the cases administered with Fr.
3 of the injured kidney, hemorrhages due to the rupture of the necro-
tic vessel were observed. This finding is interesting so as to know
the causal relationship between vascular necrosis and hemorrhage,
which is discussed below.

Edema was apt to be found histologically in the lung and abdomi-
nal organs and the occurrence was in parallel with the quantity of
pleural effusinos and ascites. The hemorrhage in the lung and
mesentery was widely noted in the areas of venous hemorrhage in
addition to the areas of vascular necrosis. Hence, the cause of
hemorrhage cannot be considered uniform.

(2) Deposit of carbon

It is well known that granulopexy of carbon in the intima is used
as histological expression of vascular permeability. In the rats
with only bilateral nephrectomy, carbon did not deposit on the vascu-
lar system but was phagocyted by the reticulo-endothelial cells of
various organs. In the hemorrhagic region of the mesentery, carbon
was noted remarkably in the capillary portions of arterioles and the
carbon deposit on the media corresponded to the necrotic portions of
arterioles. The deposit was always found in the vicinity of necrosis
or in the necrotic tissue. The carbon deposit in the mesentery was
not always found in the region of angionecrosis and quantitative
evaluation was difficult, but it was considered as an evidence of
vascular permeability.

(3) Angionecrosis

"Characteristic" histological change 24 hours after bilateral neph-
rectomy and administration of kidney fractions was angionecrosis.
The necrotic tissue is stained strongly reddish with H.E. stain, posi-
tive to PAS stain, stained red with Mallory stain, stained dark
yellow with Van-Gieson stain, and positive to PTAH stain. The
necrotic vascular wall was accompanied with pyknosis and karyolysis
of degenerative nuclei around the cells. Angionecrosis was noted in the mesentery, intestinal wall, spleen, liver, pancreas and heart but it was frequent particularly in the mesentery. As shown in Fig. 7, these lesions were noted mostly in the arterioles of 80—100 μ
(diameter) and frequently in Fr. 3. However, these lesions were in common with the cases with fractions of normal and injured kidney. The same experiments were conducted with various organs of hypertensive rats and, as the result, angionecrosis was frequently noted in the mesentery and interintestinal wall of the group administered with kidney fractions as shown in Fig. 5. In other groups, angionecrosis was found only in one case administered with liver fraction.

As shown in Fig. 6, the changes in the rats administered with Fr. 3 and Fr. 4 of the liver were less in intensity and frequency as compared with those in the rats administered with respective fractions of the kidney.

The histological examination conducted after heat treatment of Fr. 3 of the kidney at 70°C for 20 minutes, revealed no angionecrosis.

4) Pressor Activity of Fractions of the kidney

The pressor activity was determined by measuring the carotid pressure upon administration of each fraction 24 hours after bilateral nephrectomy. As shown in Fig. 8, the blood pressure increased approximately by 50 mmHg in the cases administered with the normal kidney homogenate and decreased to 2/3 of normal value in the cases administered with the injured kidney homogenate. The pressor activity was observed exclusively in the groups administered with Fr. 4 of both the normal kidney and the injured kidney. However, it was particularly weak in the groups with Fr. 3 of the normal kidney as well as of the injured kidney which showed the strongest occurrence of angionecrosis, and was hardly noted in the group with administration of the injured kidney.

5) Ligation of bilateral renal arteries and of liver lobules

The kidneys following the ligation of the bilateral renal arteries showed ischemic necrosis extending over 90% of the area resulting in severe renal infarction. The rats were sacrificed 24 or 48 hours after the operation and relatively slight angiosclerosis was noted in the mesentery, intestinal tract and pancreas as shown in Fig. 6. In the cases of ligation of the liver lobules, the retention of voluminous ascites was noted but, as to the vascular lesion, slight mesenteric angionecrosis was noted only in two cases.

DISCUSSION

1) It had long been well known that angionecrosis is present in the cases of hypertension especially malignant hypertension, and there have been conducted many experiments to study the pathogenesis. The first experiment was made by WINTERNITZ who produced angionecrosis by administering ischemic kidney homogenate prepared by renal artery ligation in nephrectomized dogs. ASSCHER et al. also
conducted similar experiments and assumed that the cause is attributable to the kidney. However, Pickering et al.\textsuperscript{24) attempted to seek the cause in the increased blood pressure. After the success in the purification of renin, there are many theories seeking the cause in renin-angiotensin system. Giese et al. also produced angioneurosis after administering rats with kidney homogenate prepared by bilateral renal ligation but insisted that the cause is the emigration of renin from the ischemic kidneys\textsuperscript{5,6}. Other investigators emphasized uremia or unbalance of electrolytes. Thus, opinions on the cause have been various among investigators.

The author, dividing the normal kidney and injured kidney into four fractions, reported that pressor effect is strong in Fr. 4 and angionecrosis in Fr. 3. Accordingly, it is suggested that pressor substances such as renin are not the main cause of angionecrosis. Fr. 3 consists mostly of microsome and the main cause of angionecrosis should be sought among the constituents of this fraction. In this series of experiments, the author presumed the presence of a factor or factors of vascular disorder deriving from the kidney. From the facts that angionecrosis was caused strongly by the administration of kidney homogenate among other organs, that angionecrosis was the most strong in the cases administered with Fr. 3 of the kidney and that angionecrosis was stronger in the cases administered with fractions of the kidney than in the cases with liver microsome fraction and liver soluble fraction, the presence of a factor or factors peculiar to the kidney was estimated. Recently, Nakao et al.\textsuperscript{19} reported that angionecrosis was frequently observed in the brain after the administration of nonpressor fraction.

2) Bilateral nephrectomy added with ischemic change of liver lobules and bilateral renal artery ligation

Histological examination 24 hours after bilateral nephrectomy revealed little change as previously reported by many investigators\textsuperscript{5,14,18). In the cases of bilateral renal artery ligation, angionecrosis was noted in the arterioles of the mesentery, intestinal tract and liver. Some investigators have been of the opinion that bilateral renal artery ligation causes stronger changes than bilateral nephrectomy\textsuperscript{26) and the author's experiments showed the same trend. The presence of ischemic kidneys, i.e., emigration of the factors of angionecrosis which is intensive in Fr. 3, is possibly presumed. The author holds the view that the appearance of the factors of vascular disorder in the living body is participated by the change of cells due to ischemia.

3) Factors of vascular permeability and factors of angionecrosis:

There have been various theories concerning the pathogenesis of angioneurotic changes and they may be classified into three major groups: (1) "Intrinsic theory that the changes of vascular wall
precede", (2) "Extrinsic theory that plasmic components invade into the vascular wall", and (3) a combination of the intrinsic theory and extrinsic theory

In the present series of experiments, the author observed effusions of Evans Blue out of the blood vessel, bleeding, edema, and carbon deposit as the expressions of vascular permeability increased by the administration of various fractions. NAIRN also observed the retention of effusions after the administration of the normal kidney and renin. The author noted that the retention of effusions was stronger in Fr. 3 of the kidney than in Fr. 4 of the kidney which showed strong pressor effect. However, there was noted no trend that kidney fractions were particularly remarkable among the fractions of all organs which were administered. Therefore, it is not evident whether or not a peculiar factor to cause the retention of effusions may possibly exist.

ASSCHER asserted that the retention of effusions is fairly different by individual and its quantitative evaluation is difficult. In the literature, there have been many reports which observed the vascular permeability factor in the skin and the blood vessel. Some reported that the factor exists in the injured kidney but not in the normal kidney; that the factor exists in the lysosome of the normal kidney; that the factor is often noted in the lymph nodes; and that RNA is the substance of the factor. The author holds the view that a peculiar factor may possibly exist, since this factor is present most intensively in Fr. 3 and not entirely dependent on histamine.

Moreover, the author conducted carbon injection and observed carbon deposit in the region of angionecrosis. The invasion of plasmic components during the progress of angionecrosis was also confirmed. This may suggest the possibility of intervention of the above-mentioned vascular permeability factor. However, it is still unknown if the necrotizing factor which was remarkably observed in Fr. 3 is identical with this vascular permeability factor, and how this factor participates in the development of angionecrosis.

CONCLUSION

(1) No remarkable change was noted light microscopically 24 hours after bilateral nephrectomy. The administration of Fr. 3 of the kidney resulted in quite intensive angionecrosis. In the groups administered with Fr. 3 and Fr. 4 of the liver, angionecrosis was slight. Among various organs which were administered, only the administration of kidney fractions resulted in angionecrosis. It is indicated therefore that the necrotizing factor is present in Fr. 3 of the kidney.

Angionecrosis was remarkable in the arterioles of the mesentery.
and intestinal tract, showing initial change in the media in light microscopy.

Severe stenosis by means of bilateral renal artery ligation resulted in angionecrosis in the intestinal wall and mesentery. It is suggested that the ischemic kidney participates in the emigration of necrotizing factor.

(2) In respect to Evans Blue permeability and retention of pleural effusions and ascites, vascular permeability was the strongest in the group administered with Fr. 3 of the injured kidney.

(3) Among the kidney fractions, Fr. 4 showed the strongest pressor effect while Fr. 3 showed very weak effect. Accordingly, the pressor factor was attributed to one fraction while the necrotizing factor and the vascular permeability factor were attributed to another fraction.

(4) Diagram on the morphogenesis of angionecrosis

ACKNOWLEDGEMENT

The author is grateful to Professor I. NISHIMORI for his advice and encouragement. During this study valuable assistance was given by Professor M. KOIKE, Dr. M. HAMADA and Dr. M. KINOSHITA.

REFERENCE


Fig. 9. Hypertensive injured kidney showing extensive necrotizing vasculitis of the interlobular artery and devastation of the glomerulus. (Mallory stain)

Fig. 10. Same case as Fig. 9. (Mallory stain)

Fig. 11. Same case as Fig. 9 showing panarteritis of the interlobular artery. (Mallory stain)

Fig. 12. Mesentery of a case administered with Fr.3 of the injured kidney. Necrotic change of the media and hemorrhage due to rupture. (H.E. stain)
Fig. 13. Liver arteriole of a case administered with Fr. 3 of the injured kidney. The nuclei of muscle cells in the media are mostly karyolytic and the remaining nuclei are pyknotic. The necrotic region of the media appears homogenous. However, the cells of the elastic lamina and of the intima in the vicinity of the lesion are maintained almost normal. (H.E. stain)

Fig. 14. Mesentery of a case administered with Fr. 3 of the normal kidney. Angionecrosis is noted relatively towards the adventitia showing a trend of forming aneurysms. (H.E. stain)

Fig. 15. Pancreatic artery of a case administered with Fr. 3 of the normal kidney. Strongly positive to Mallory-Azan stain.

Fig. 16. Mesentery of a case administered with Fr. 3 of the normal kidney. Positive to PAS stain.
Fig. 17. Mesentery of a case of bilateral renal artery ligation. Mural hemorrhage is noted adjacent to the necrotic region. Carbon deposit is seen in a part of red cell mass (arrow). (H.E. stain)

Fig. 18. Mesentery of a case of bilateral renal artery ligation, showing heavy carbon deposit in the necrotic region (arrow). (H.E. stain)

Fig. 19. Mesentery of a case administered with Fr. 3 of the normal kidney. The elastic lamina is maintained almost normal. Deposit of necrotic substances is seen in the media somewhat towards the adventitia. The muscle cells in the vicinity are enlarged and vacuolated. (Mallory stain)