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IDENTIFICATION OF THE PRESSOR SUBSTANCE PRODUCED IN EXPERIMENTAL NEPHRITIS AND PREVENTION OF THE HYPERTENSION BY A SURGICAL PROCEDURE

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ABSTRACT

In order to investigate the possible role of the liver in removing the humoral pressor substance from the renal venous blood of hypertensive dogs with experimental nephrotoxic nephritis, an anastomosis was made between right renal vein to portal main trunk or to superior mesenteric vein of dogs whose left kidneys were previously removed.

When adequate amounts of the nephrotoxic serum (serum of rabbit which was sensitized by dog kidney cortex) were given intravenously, elevation of the blood pressure, gross and microscopical hematuria, marked proteinuria, generalized edema and azotemia were observed.

The systolic and diastolic pressure began to rise in one or two days after the injection of the serum, and remained on moderately high level until surgical operation or autopsy was performed several days later.

When the reno-portal shunt was made before the injection, however, no increase in blood pressure could be seen, even though the condition of nephrotoxic nephritis had developed.

The mode of activities of vasoconstrictor and vasopressor effects of the plasma obtained from renal vein of nephritic dogs were compared with those of synthetic angiotensin and essential nature of the pressor substance was discussed.

In 1898, Tigerstedt and Bergmann[1] demonstrated the prolonged rise in blood pressure in animals which follows the intravenous injection of a crude kidney extract. This rise in blood pressure was attributed to the substance called renin that was found only in renal tissue. The production of persistent hypertension in dogs through the alteration of intra-renal hemodynamics by Goldblatt[2] in 1934, has opened a new field in experimental studies, since the condition so caused in the animals was similar to human hypertension.

Clinically, the first successful removal of a kidney for treatment of hypertension

in human being was reported by Butler[1] in 1937. Since then, many accounts in the literature have been described about vascular and parenchymal lesions of the kidneys associated with hypertension.

Experimental renal hypertension in early stage, as well as patients with acute severe hypertension, has been considered by many investigators to be the result of the release into the circulation of a pressor substance elaborated by the kidney. In 1940, a substance with theoretically required properties was described simultaneously by Page and Helmer[4] and Braun-Menendez and his coworkers[5]. In the course of experiments of former group, it was found that the potent kidney extract containing renin, which was known to cause a strong elevation in blood pressure when given intravenously, produced less constriction of the blood vessels that were isolated in saline. However, if small amount of plasma was added to the extract, an intense vasoconstriction was restored immediately. This indicated that renin could formed an active substance when it acted upon a plasma substrate.

The plasma substrate which reacted with renin, Kohlstaedt, Helmer and Page[6] called renin-activator. This substance could be found in the alpha-two globulin fraction of the plasma proteins produced by the liver. An active substance formed by the interaction of renin and renin-activator was called angiotonin by Page and Helmer[4]. Braun-Menendez also named this substance which was obtained by his experiments hypertensin. At a conference which was held in 1957 in commemoration of Goldblatt's investigation, Page and Braun-Menendez agreed on the term angiotensin for the pressor substance and angiotensinogen for the substrate upon which renin acts[7].

Purification and analysis of angiotensin were slowly established. Skeggs, Marsh, Kahn and Shumway[8][9] and Helmer[10] showed the presence of two forms of angiotensin. One form, angiotensin I which is inactive, was identified as a direct product of the action of renal enzyme renin upon its plasma substrate. Other form, angiotensin II, which was formed by the action of a converting enzyme from angiotensin I, was found to be the renal pressor substance. Angiotensin I, however, is equally active in vivo, because it is rapidly converted to angiotensin II by a chloride-activated enzyme in plasma when given intravenously.

The purification of a pressor peptide derived from ox serum was described by Peart[11] in 1956. In the same year Elliott and Peart[12][13] determined that ox angiotensin I was the sequence of ten amino acids. A ten amino acid structure of horse angiotensin I was also proved by Skeggs and associates,[14][15] who showed that angiotensin II was identical chain of the first eight amino acids of angiotensin I. According to Peart, however, valine is in the position of isoleucine which is fifth in order in horse or hog angiotentin by Skeggs.

Synthesis of deca- and octapeptides of the angiotensin series were accomplished

almost simultaneously by Page and coworkers[16] and by Schwyzer's group[17] independently. The intensive pharmacological studies conducted by those groups[18,19,20] have verified that synthetic angiotensin II is identical with the naturally occuring substance.

In view of these humoral renal pressor mechanism, Kahn, Skeggs, Shumway and Wisenbaugh[21,22] have reported the presence of increased amounts of angiotensin in arterial blood of patients with essential and malignant hypertension. Helmer and Judson[23,24,25] reported the presence of vasoconstrictor and vasopressor activity in the renal venous and peripheral blood of patients with malignant and renal hypertension. Thus, the existence of humoral pressor substance in blood have been generally accepted.

Levy and Blalock[26], Child and Glenn[27], Verney and Vogt[28] and Maluf[29] described that the complete portalization of the renal effluent blood could not prevent the renal hypertension produced by the constricting renal artery. On the other hand, the possible evidence to prevent or abolish the hypertension associated with renal ischemia by the reno-portal venous shunts on dogs was emphasized by Aoki[30].

The primary purpose of the present paper is to investigate whether there exist any significant depressor effect of the complete reno-portal shunt on hypertensive condition with the nephrotoxic nephritis in dogs. Secondary, properties of this pressor substance in renal venous blood were assayed for vasoconstricting activity by means of spirally cut strips of rabbit aorta or for vasopressor action by intravenous injection in 24 hours postnephrectomized dogs, and also these two activities were compared with those of the synthetic angiotensin.

MATERIALS and METHODS

1) Materials

Forty two healthy female dogs, each weighing, between 6.5 and 11 kg, were used to test the significant depressor effect of reno-portal shunt on the hypertensive dogs with nephrotoxic nephritis. In all dogs, the blood pressure was determined by use of dermo-carotid bridge method of Aoki[30]. On the normal condition without anesthesia, the previously left nephrectomized dogs showing higher blood pressure more than 90—140 mm/Hg before injection of nephrotoxic serum were discarded. Such selected animals were injected 3.5 to 4 ml. per kg. of nephrotoxic serum intravenously in a single dose.

2) Antigen

The antigen was prepared according to the direction of Shibata[31] with following modifications. Normal dog kidney was removed under aseptic condition, the renal artery was cannulated with polyethylene tube and the kidney was perfused with 0.9 %

sodium chloride solution under hydrostatic pressure of about 2 m. until the organ appeared free of visible blood. The kidney was then decapsulated and the cortex which was separated from its medulla was cut into small slices. It was homogenized in Waring Blender with equal weight of saline and then filtrated through 2 sheets of aseptic gauze. Then, approximately 20 % kidney cortex suspension was made in physiologic saline solution. Following this, phenol in proportion of 0.5 % and approximately 500 units of penicillin were added to per ml. of the kidney cortex suspension. Greenspon and Krakower[32)33)] demonstrated that a potent nephrotoxic serum can be obtained by using glomeruli only, as antigen. Therefore, such antibody was prepared only from kidney cortex.

3) Preparation of nephrotoxic serum

For sensitization with these dog kidney cortex suspension, rabbit weighing 2.5 and 3 kg. were used. The rabbits were given a single intraperitoneal injection of the material twice a week according to the following schedule : first 2 doses with 10 ml. of a 10 % suspension ; next 14 doses with 10 ml. of a 15 % suspension ; last 2 doses with 10 ml. of a 20 % suspension. One week after the last injection the rabbits were exsanguinated from the carotid artery. The blood was centrifuged under sterile conditions. The serum obtained was heated for 30 minutes at 56 °C, and phenol was added to the serum in proportion of 0.5 % after the determination of antibody titre of sera as shown below and then stored in frozen state until needed.

4) Antibody titration of the serum.

Antibody titration was made at one week after the last immunization. Antigen for the test was prepared by autolyzing a 20 % suspension of dog kidney cortex for 7 days at 4°C. The slightly opalescent supernatant fluid was diluted to 0.5 % protein concentration with saline : each 0.2 ml. of the serum to be tested were added to 0.2 ml. of antigen obtained by the serial double dilution method. The solutions were incubated for 30 minutes at 37°C, and then readings were made 2 hours later when reached to its maximum. The dog kidney precipitin titre of respective of eight rabbits had reached 1 : 128, except the one which showed 1 : 64.

5) Operative method of the complete anastomosis of the renal and portal veins. (Fig. 1. 2.)

The left kidney of each female dog had been previously extirpated. Under intravenous anesthesia with hexabarbital sodium, an median incision was made about 2 cm. below the ensiform process to ca. 7 cm. above the mons veneris. The right kidney was separated from the surrounding peritoneum except for the renal artery, vein and ureter, to prevent the subsequent formation of collateral circulation from pericapsular veins. The right renal artery was occluded temporarily by a silk ligation. The distal end of the right renal vein was anastomosed with the lateral aspect of

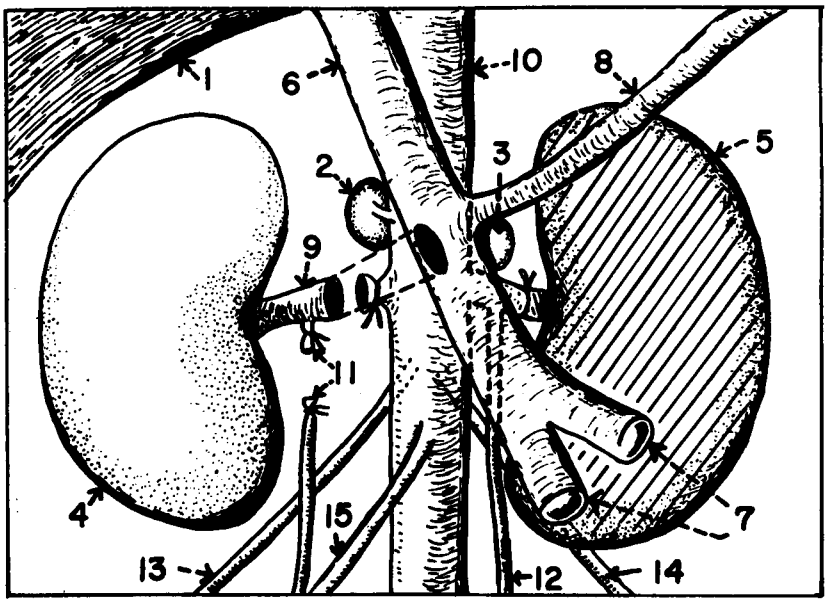

Fig. 1. Diagrammatic illustration showing complete anastomosis between right renal vein and portal system.
1, Liver; 2, right adrenal; 3, left adrenal;
4, right kidney; 5, left nephrectomy; 6, portal vein;
7, upper mesenteric vein; 8, splenic vein;
9, right renal vein; 10, inferior vena cava;
11, right ureteral vein; 12, left gonadal vein;
13, right lumbal vein; 14, left lumbal vein;
15, right gonadal vein.

portal trunk or superior mesenteric vein by hand, thus forcing entire circulation of renal effluent blood to return to the heart by way of the liver. The silk ligation was removed from the right renal artery and the incision was closed.

6) Other determinations

The urine was obtained by use of Nelaton's catheter. The determination of the urinary protein by 20 % sulfosalicylic acid and by the heat and acetic acid method, and of the content of the urinary sediments were done prior to the injection of serum and during the course of immunization. Determination of blood urea nitrogen (Kjeldahl-Nessler method), the total protein in serum (Hand Protein Refractometer, Hitachi), Na, K (Flamephotometer) and Cl in the blood (Schales and Schales method[34]) were carried out in control period and during the course of disease. The body weight was also measured periodically.

7) Pathological examinations

On 4 to 9 days after the injection of nephrotoxic serum, autopsy were performed,

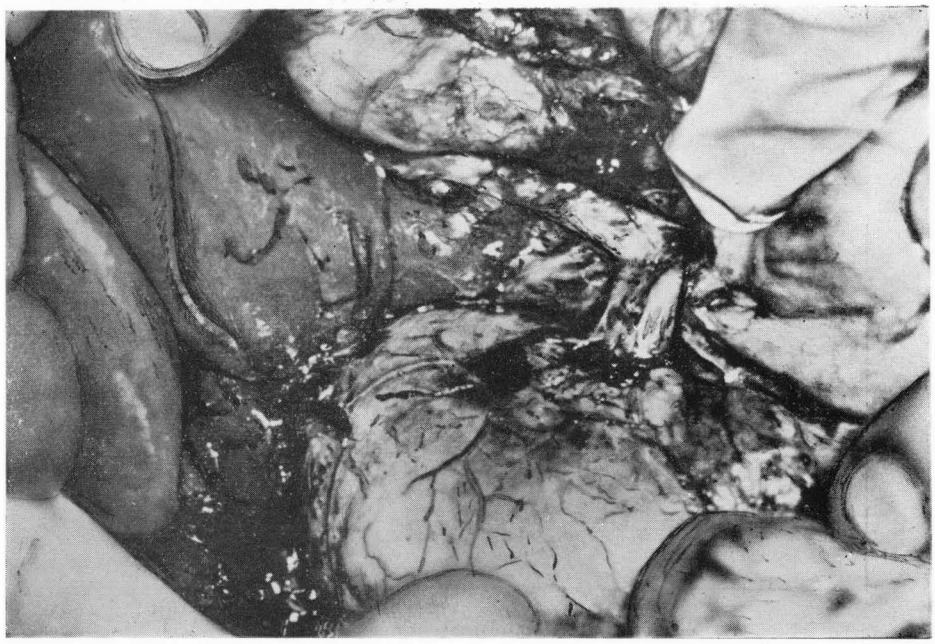

Fig. 2. Photograph showing patent anastomosis of right renal vein to portal system.

and made careful gross and microscopic examinations. The kidney and liver were fixed in cold 10 % formalin and 99 % alcohol, and stained with hematoxylin eosin and periodic acid Schiff reagent.

8) Bioassay of the dialyzed renal venous plasma

Angiotensin is a polypeptide which is thermostable and dialyzable, in contrast to its mother substance, renin, renin-substrate and converting enzyme which are protein and, therefore, thermolabile and non-dialyzable. For the assay of renin, about 50 ml. or more of renal venous blood was drawn in syringe with sufficient heparin as an anticoagulant, quickly immersed in ice and then centrifuged. The plasma was adjusted to pH 5.5 with HCL and dialyzed overnight in " Visking " casings against cold running tap water (7—9°C) to separate the protein from the dialyzable vasoconstrictor substance, such as catecholamines, serotonin, vasopressin, preformed angiotensin and other vaso-active materials. Avoiding a danger of denaturation of the plasma, no attempt was made to remove angiotensinase. After dialysis, plasma was made isotonic with addition of sodium chloride solution and centrifuged to remove insoluble protein. Just prior to be added to recipients for assay, the supernatant was adjusted to approximately pH 7.2 with NaOH. The remainder of the dialyzed plasma was stored in frozen state.

The dialyzed plasma was assayed for vasoconstrictor activity in spirally cut strips

of the rabbit aorta as described by Furchgott and Bhadrakom[15] and for vasopressor action in 24 hours postnephrectomized decerebrated dogs. The strips were mounted in a muscle chamber of 30 ml. capacity with the aid of stainless steel hooks, and bathed with Ringer's solution maintained at 37.2°C. Replacement of solution in muscle chamber was accomplished by over-flow principle. Through this solution in the chamber air was bubbled.

The isotonic lever was adjusted to give a ninefold amplification and counter-weight to exert 3 gm. tension on the strip, The contraction was recorded on a smoked drum. Synthetic angiotensin* was used to compared with the test substances and to standardized the mode of contraction of aortic strips. The substances added into the chamber were washed out within 4 minutes, because if working time was prolonged more than 4 minutes the lever was not restored sufficiently to its isotonic level.

For the determination of pressor activities, the test substances were assayed by intravenous injection in 24 hours postnephrectomized decerebrated dogs. The animal weighing between 6 and 9.5 kg. were decerebrated under anesthesia with hexabarbital sodium intravenously and the polyethylene catheter of 2 mm. internal diameter was inserted into the femoral artery and connected to a mercury manometer. The test substances were injected into the femoral vein of the opposite side in dose of 1 to 1.5 ml. per kg.

* Synthetic angiotensin was kindly presented by Ciba Pharmacological Institute.

RESULTS

1) The elevation of the blood pressure after the injection of nephrotoxic serum and the depressor effect of the portalization of renal venous circulation on the elevated blood pressure. (Group I).

It has been found that both systolic and diastolic blood pressures were noted to rise in one or two days following the serum administration. Although elevation of blood pressure, except for the Dog 30 in which systolic and diastolic pressures rose to 220 — 120 mmHg on the 4 th experimental day (Fig. 3), was less high in the other dogs, but elevation continued to remain moderately high up to the time either surgery or autopsy done several days later (Fig. 4. 5. 6).

Specimens of blood of the Dog 30 and 36 were collected from the right renal vein for bioassay and then they were sacrificed for autopsy, since they seemed too week to stand the further experiments. In Dog 10 the elevated blood pressure retured to the control level after the reno-portal venous anastomosis (Fig. 6). This dog died on the 3rd postoperative day, autopsy revealed no obstruction at the site of anastomosis. The

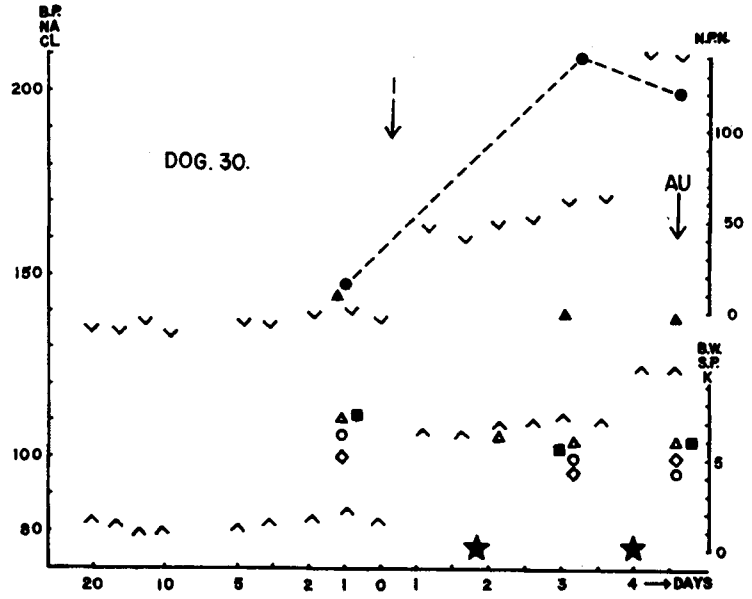

Fig. 3. Marked high blood pressure following intravenous injection of nephrotoxic serum and changes of components in blood and urine.

A : anastomosis of renal and portal vein
I : injection of anti-dog kidney rabbit serum
AU : autopsy
L ; left nephrectomy
D : death
∧ systolic blood pressure
∨ diastolic blood pressure — mmHg
● N.P.N. : non protein nitrogen — mg/dl
○ S.P. : serum protein — gr/dl
▲ N
□ K : in plasma — meq/l
■ Cl
△ B.W. : body weight — kg
★ gross hematuria
☆ : marked
☆ : moderate — micro. hematuria

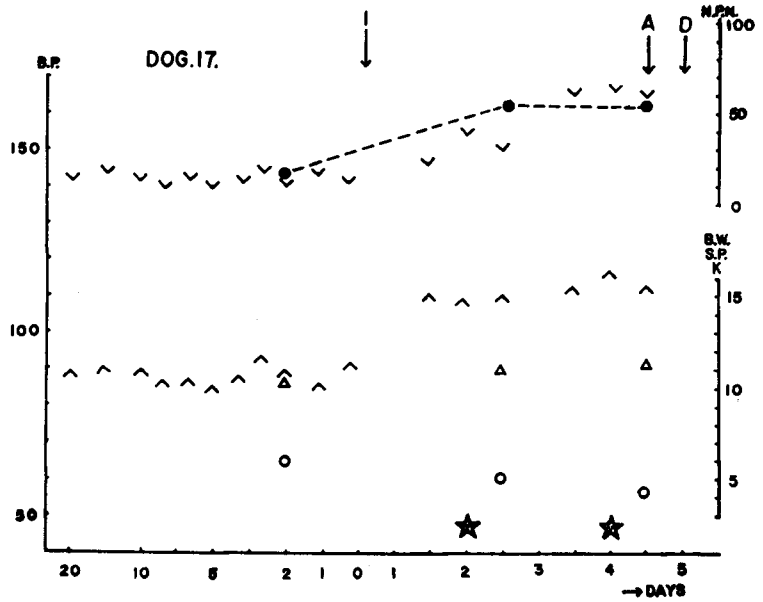

Fig. 4. Moderate rise in blood pressure following intravenous injection of nephrotoxic serum and changes of components in blood and urine.
(Refer to Page 8 for Abbreviations and Figures)

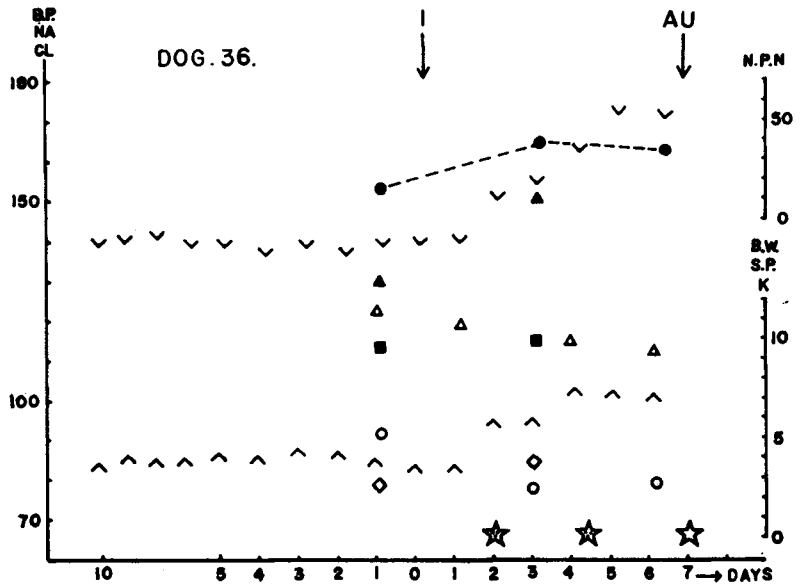

Fig. 5. Moderate rise in blood pressure following intravenous injection of nephrotoxic serum and changes of components in blood and urine.
(Refer to Page 8 for Abbreviations and Figures)

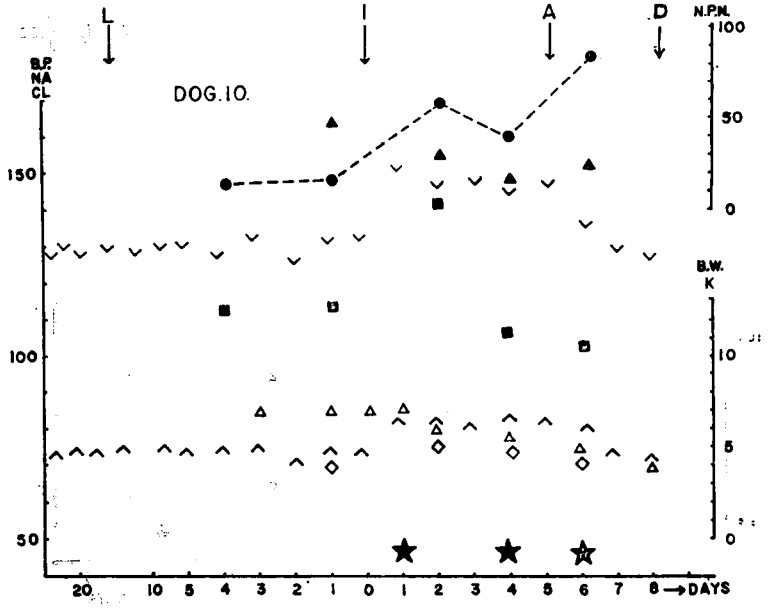

Fig. 6. Fall of the elevated blood pressure following successful portalization of renal vein and changes of components in blood and urine.
(Refer to Page 8 for Abbreviations and Figures)

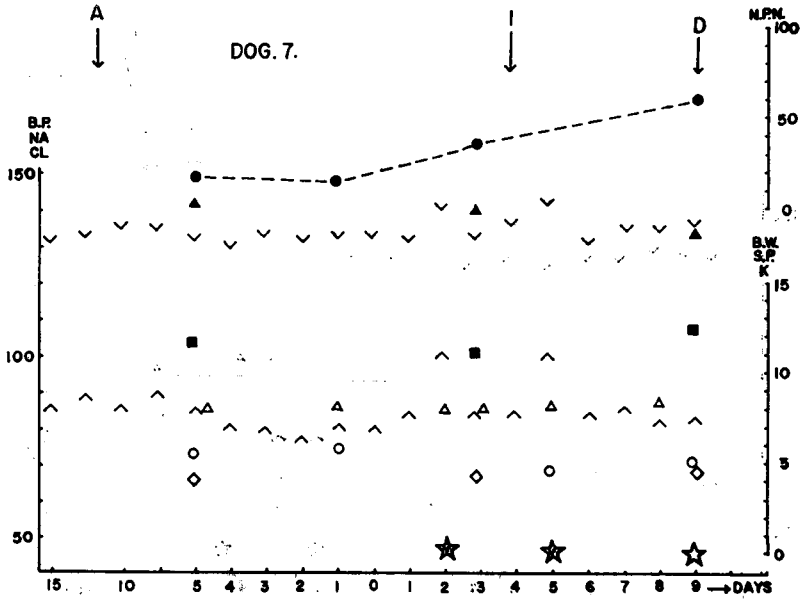

Fig. 7. Expected rise in blood pressure following injection of nephrotoxic serum was prevented by initial reno-portal shunt and changes of components in blood and urine. (Refer to Page 8 for Abbreviations and Figures)

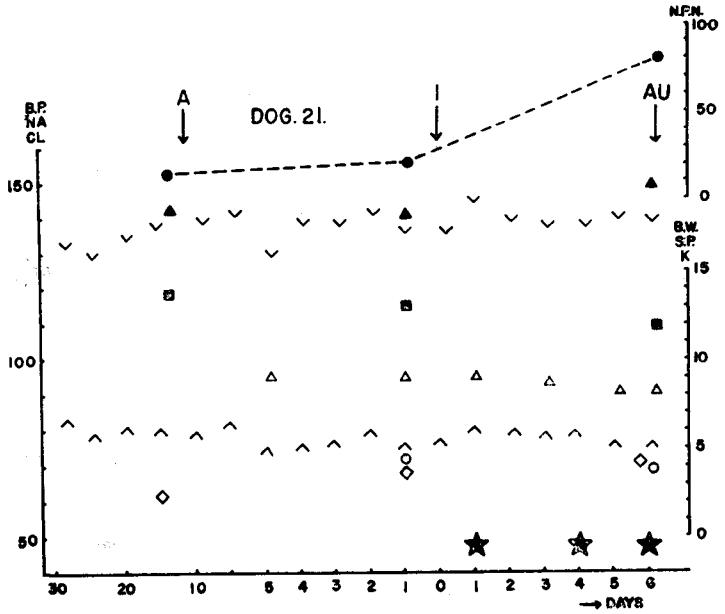

Fig. 8. Expected rise in blood pressure following injection of nephrotoxic serum was prevented by initial reno-portal shunt and changes of components in blood and urine. (Refer to Page 8 for Abbreviations and Figures)

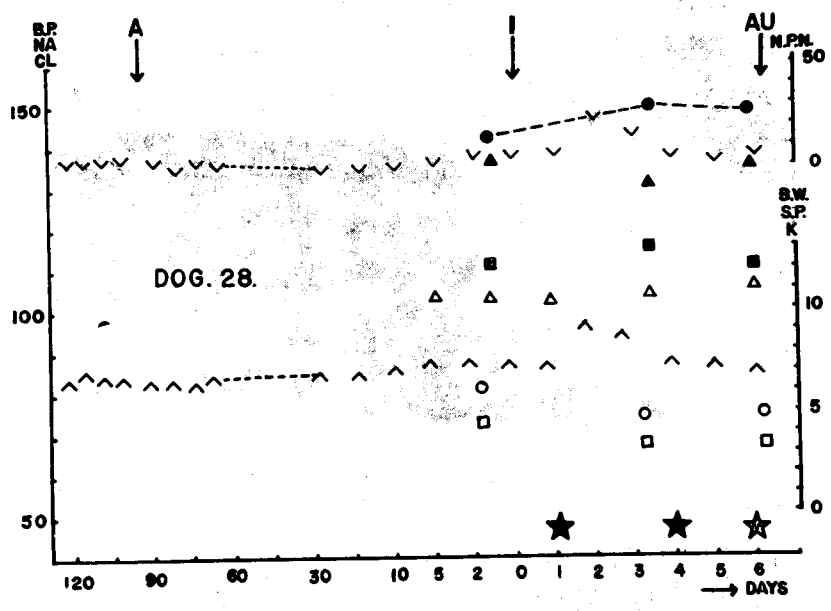

Fig. 9. Expected rise in blood pressure following injection of nephrotoxic serum was prevented by initial reno-portal shunt and changes of components in blood and urine. (Refer to Page 8 for Abbreviations and Figures)

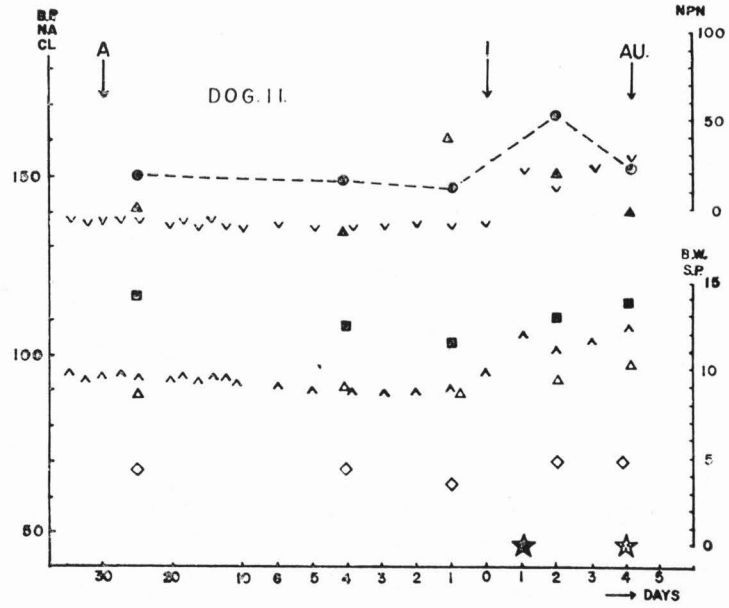

Fig. 10. In spite of the shunt, moderate rise in blood pressure occured following injection of nephrotoxic serum and changes of components in blood and urine. Occlusion of anastomosis was revealed by autopsy.
(Refer to Page 8 for Abbreviations and Figures)

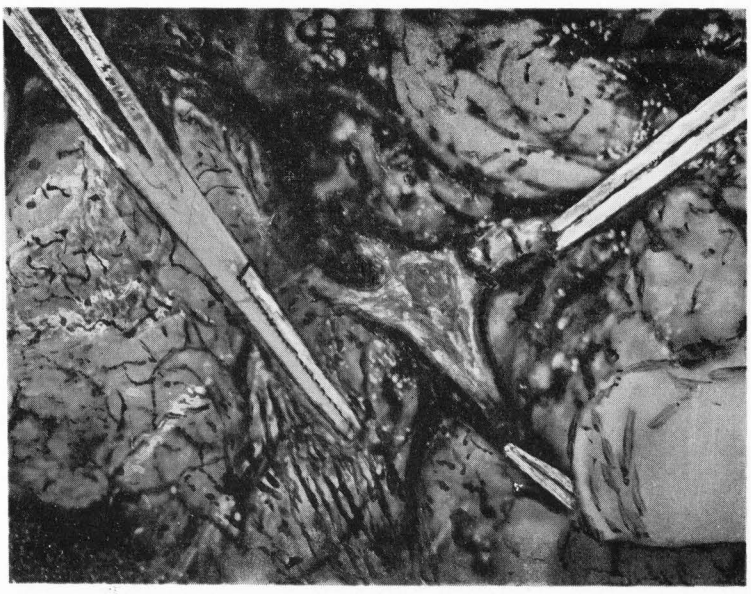

Fig. 11. Photograph, its centre showing the occlusion in the site of anastomosis by scar. The left hemostatic forceps showing the formation of marked collateral circuit from the renal surface and renal hilum to the inferior vena cava.

technique of portalization following the injection of the serum is extremely difficult procedure due to fragile wall of the edematous blood vessel.

2) The depressor effects on the blood pressure of connecting the renal vein to portal system prior to the injection of nephrotoxic serum. (Group. II).

This group of experiments was performed to test whether there exist any possible role of the liver in inactivating the humoral vasoactive substance from the renal effluent blood of the hypertensive dogs with the experimental nephritis. No significant elevation of the blood pressure was observed in 3 of 4 dogs for 4 to 9 days after the immunization (Fig. 7. 8. 9). Even if the rise in blood pressure to be observed, it was of only mild and transient nature. It is interpreted that the expected rise in pressure to hypertensive levels could be prevented by means of complete portalization. The moderate elevation of blood pressure was found in Dog 11 (Fig. 10). Of this dog, occlusion in the site of anastomosis and production of collateral circulation from renal hilum and pericapsular membrane to inferior vena cava have been confirmed at autopsy on the 4th day after the injection (Fig. 11). This evidence shows a fact that the renal effluent blood containing hypothetical pressor substance had passed into the inferior vena cava through the collateral circulation directly to the heart without passing through the liver. The mean elevation of the systolic and diastolic pressure of the Group I was 26.5 and 23.2 mmHg above the control level as compared to 3.3 and 3.2 mmHg in Group II with the exception of Dog 11.

3) Other findings

The findings of the signs during the course of experimental nephritis were characterized by complete loss of appetite with occasional vomiting, elevation of blood pressure, edema, hematuria, marked proteinuria, ascites, decreased serum protein and occasional pleural effusion. Gross hematuria was observed on 1 to 2 days after the injection in 2 (Dog 10 and 30) of 4 dogs (Group I) and in 3 of 4 except for Dog 7 (Group II). In 2 (Dog 17 and 36) of the former group and in 1 (Dog 7) of the latter group, hematuria was only microscopic. Blood urea nitrogen was elevated over 50 mg/dl in 3 of 4 dogs in Group I and II respectively. One of the these dogs (Dog 30) showed marked high blood urea nitrogen (123—146 mg/dl). Remaining one of each group which failed to show elevated urea nitrogn, their values were 27.2 to 25.6 mg/dl and 28.2 to 25 mg/dl respectively (Dog 36. 28). The significant change of electrolytes in blood was not found.

4) Pathological examinations (Fig. 12. 13)

In general, these animals showed noticeable emaciation with the exception of 1 dog (Dog 11) with visible edema. Ascites over 30 ml, was found in 5 of 8 dogs. Especially, Dog 11 was found having approximately 600 ml. of ascites, 220 ml. of the fluid in the pleural cavity bilaterally, a small amount of pericardial fluid, edema of

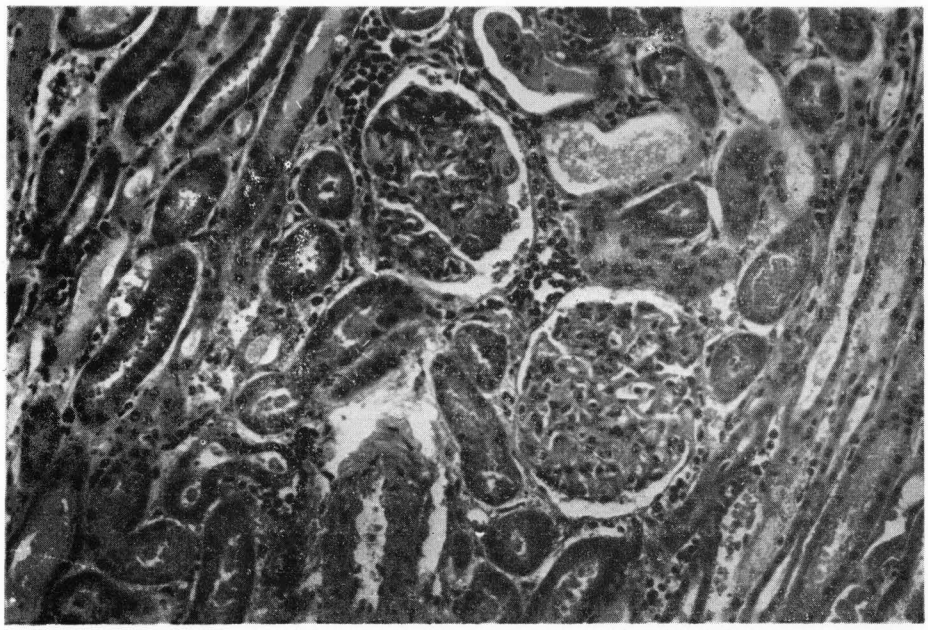

Fig. 12. Photomicrograph of microscopic section prepared from the kidney removed from Dog 21. Note enlarged glomeruli, adhesions of tuft to capsules, albuminous fluids and red cells in capsular spaces and renal tubules.
Hematoxylin-eosin, X 100.

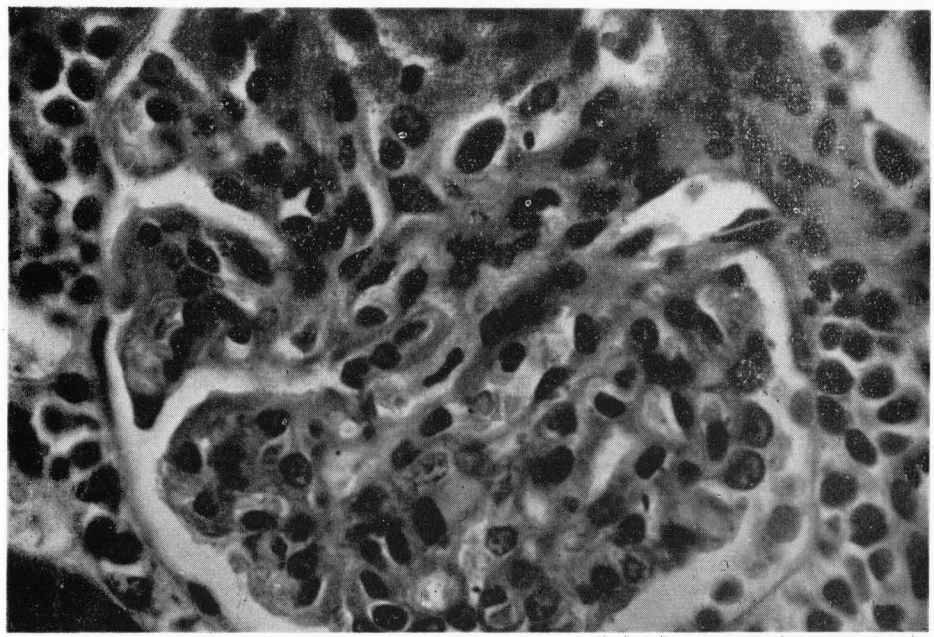

Fig. 13. Photomicrograph of microscopic section prepared from the kidney removed from Dog 30. Note ischemia in enlarged glomeruli, swelling of vascular endothelial cells, especially of mesangial cells and red cells in capsular space.
Hematoxylin-eosin, X 400.

mucous membrane of whole digestive tract and of entire peritoneum.

The kidney was enlarged and swollen. Its outer surface was smooth and reddish and showed tiny hemorrhages. All of the experimental animals showed the following microscopic changes of the kidney ; enlarged glomeruli, adhesions of tuft to capsule, a varying amounts of exudate, such as serum, fibrin, red cells and a small amount of leucocytes in the capsular spaces.

Furthermore, the characteristic lesions of these glomeruli were marked ischemia, swelling of vascular endothelial cells, especially of interstitial cells so called mesangial cells. The hyalinization of afferent arterioles of glomeruli were also seen. Tubular lumina were filled with albuminous fluids and red cells. These changes on glomeruli were diffuse, although not all glomeruli appeared affected to an equal degree. In both Group I and II, no difference was shown in the microscopical findings which were indicative of acute glomerulonephritis. The liver also failed to show any significant gross and microscopic changes.

5) Characteristics of vasoconstrictor and vasopressor activity of the dialyzed renal venous plasma. (Fig. 14. 15).

In 4 animals with glomerulonephritis (Dog 21. 28. 30 and 36) the dialyzed plasma from the renal vein has been assayed for vasoactive properties. The reactivity of the

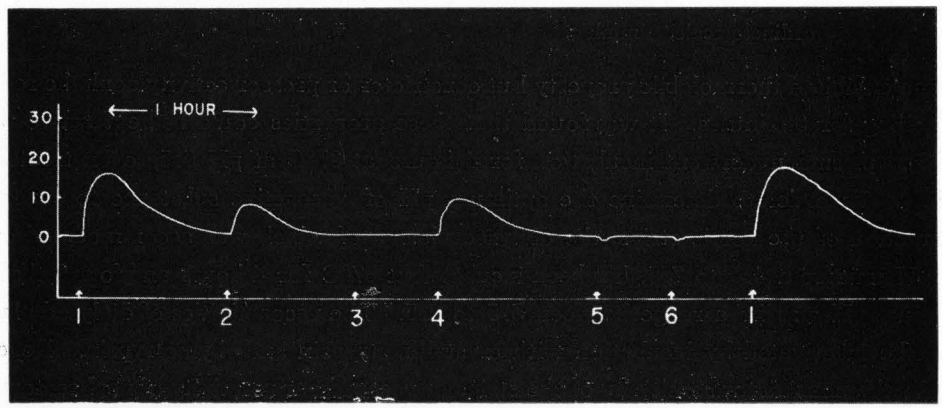

Fig. 14. Comparison of the constrictor effects of the dialyzed renal vein plasma treated by various procedures with synthetic angiotensin.

1. synthetic angiotensin 0.007mcg.
2. 2ml. of boiled plasma after incubation.
3. 2ml. of boiled plasma without incubation.
4. 2ml. of incubated plasma.
5. 2ml. of incubated plasma with chymotrypsin.
6. 2ml. of incubated plasma with trypsin.

ordinate scale. mm. rise.

aortic strips and of the nephrectomized dogs were not constant against the equal amount of test substances and of synthetic angiotensin. The freshly dialyzed non-incubated

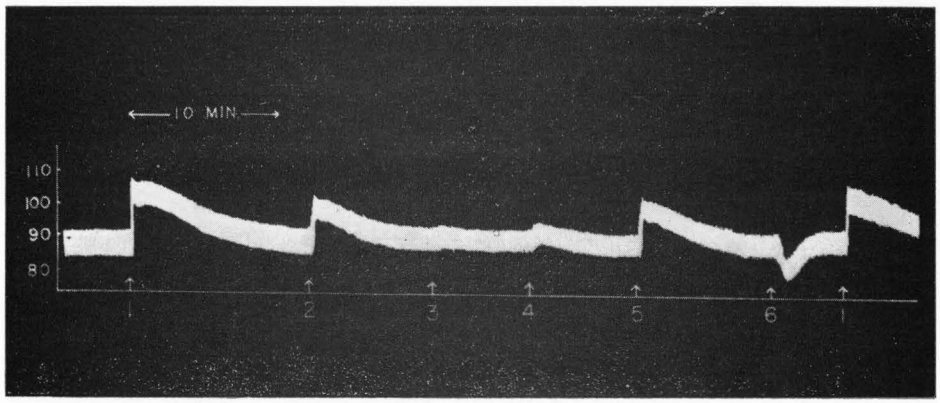

Fig. 15. Comparison of the pressor responses of the dialyzed plasma obtained from the renal vein with the synthetic angiotensin in a decerebrated dog, nephrectomized 24 hours before.

Substances injected intravenously.

1. synthetic angiotensin 0.025mcg.
2. 6ml. of incubated plasma.
3. 6ml. of incubated plasma with chymotrypsin.
4. 6ml. of boiled plasma without incubation.
5. 6ml. of boiled plasma after incubation.
6. 6ml. of incubated plasma with trypsin.

ordinate scale, mmHg.

plasma exhibited thermolabile property but constrictor or pressor activity could be reduced by boiling for 5 minutes. It was found that these properties could be kept active when dialyzed plasma underwent incubation for 4 hours at 37°C at pH 5.5, even treated by boiling. In order to determine the optimum pH of vasoactive substance to cause the contraction of the aortic strips, the dialyzed plasma of which was accommodated every 1.0 pH unit from 4.5 to 7.5, has been incubated at 37°C for 4 hours and found that the optimum pH was in a range between 5.5 to 6.5. The vasoactivities of dialyzed plasma could be inactivated by incubation with crystalline trypsin and chymotrypsin. Depending on the constrictor sensitivity of the strips or vasopressor reactivity of nephrectomized dog, the contour of constrictor or pressor curve of the dialyzed incubated plasma obtained from nephritic dogs is quite similar to the one which was obtained by synthetic angiotensin. These findings suggest that the kidney may elaborate a vasoactive substances in dogs with experimental glomerulonephritis and this elaborated substance might be identical with the synthetic angiotensin. (Fig. 14 & 15 showing the results of vasoactivities of renal vein sample in Dog 36.)

DISCUSSION

Recently, Aoki,[30] one of our colleagues, has reported his success proving possible

ability of the liver to inactivate the pressor substance released from the kidney in the hypertensive dogs by means of connecting the renal vein to the portal system and suggested possible clinical application of this procedure for the treatment of renal hypertension. In his experiments, the blood pressure was elevated by partial constriction of the renal artery. For the hypertension due to constriction of the renal artery, revascularization is the choice of treatment more so than a method of portalization. Prior to the clinical application of this procedure, the effect of portalization of renal effluent blood upon the renal hypertension, especially, upon the parenchymal lesions should be further investigated.

In this study, we attempted to confirm that the hypersentive conditions of the nephrotoxic nephritis could be due to renin in active form from the involved kidney and that these hypertentive conditions could be prevented or abolished by complete portalization of the corresponding renal venous blood.

The presence of increased amounts of renin or angiotensin have been clinically detected in the blood of patients with fluminating acute glomerulonephritis[25)36)] as well as that of essential and malignant hypertensive patients.[21)22)23)24)25)] Peart and his co-workers[37)38)] showed that no increase in activity of renal vein samples of both hypertensive rabbits caused by renal artery constriction and of the renal and malignant hypertension in human beings.

It seems likely that these altogether inconsistent results obtained are rather due to the difference of analytical methods used than species or individual differences, although these might play some role in this matter. It is necessary that the analytical method should be sensitive enough to detect the minute quantities of renin or angiotensin. Moreover, a direct method to isolate angiotensin from the blood is also required.

In this study, the constrictor and pressor activity of the vasoactive substance released from the involved kidney was demonstrated by use of the spirally cut strips of rabbit aorta or the postnephrectomized decerebrated dogs. The degree of response of the aortic strips and of the nephrectomized dogs was not always constant to the equal dose of test substances. Dialyzed plasma of renal venous blood and synthetic angiotensin showed a similar reactivity on the strips and dogs. Some aortic strip was not active both to the synthetic angiotensin and the dialyzed plasma, even if it was quite reactive to catecholamines. This aortic strip method was useful in testing vasoconstrictor substance.

Because of our presenting evidence of depressor or regulating effect of blood pressure by the reno-portal shunt, it is reasonable to assume that the vasoactive substance released from the kidney was inactivated by only one passage through the liver and that this function of the liver certainly should have some limited capacity.

Since, slight or temporary rise in blood pressure was noted on some dogs with reno-portal shunt after the injection of serum (Dog 7 and 28). The hypertension had developed in Dog 11 in spite of the reno-portal shunt. In this case, it was found at the autopsy that the shunt was obstructed, thus allowing the renal effluent blood pass into the inferior vena cava through the collateral circuit directly to the heart without passing through the liver. This hypertensive condition is caused by the vasoactive substance in renal venous blood acting upon the peripheral vessels without inactivated by the liver.

This view is also confirmed by following evidences. Tabata[39] in our laboratory, by using same analytical method, demonstrated that the left nephrectomized dogs with the complete portalization of renal venous blood before and after the partial constriction of right renal artery resulted in gradual reduction of vasoactivity in dialyzed plasma of the renal, portal and hepatic veins from marked actve, to moderate and to minimum or no changes respectively. Furthermore, it is interesting to note that the injection of synthetic angiotensin through the tributary of upper mesenteric vein caused no significant elevation of blood pressure on the postnephrectomized dog, whereas when given through the femoral vein it showed a significant rise in blood pressure (Tabata).

The portalization would depress or prevent the experimental renal hypertension and would maintain normal blood pressure. However, other control mechanism such as nervous or humoral origins,[40] on the other hand, should play some role to prevent the further drop of blood pressur by the portalization.

Portalization of renal venous blood was first reported by Dragstedt[11] to detect its effect on blood sugar. However, no mention was made on its effect upon the blood pressure. The portalizations described by Levy and Blalock,[26] Child and Glenn[27], Verney and Vogt[28], and Maluf[29] were impossible to prevent the elevation of blood pressure following the production of renal ischemia. The methods of portalization, such as end to end anostomosis of reno-splenic vein, and side to side anastomosis of inferior vena cava and portal vein with ligation of cephalad of the shunt with or without ligation of caudal of the shunt, were entirly complicated. So far as the operative methods are concerned, following three factors must be considered to exist. First, the occlusion is frequently recognized at the site of anastomosis between renal and splenic vein during our experiments. Secondary, portal blood which has the renal effluent blood, also contains the suprarenal effluent blood. Third, the disturbance of venous return from the lower limbs might be produced by the ligature cephalad and caudal of the shunt. The operative method used in this experiments is, therefore, more completely portalized than these methods.

Against the above-mentioned evidence concerning the depressor effect by the complete portalization of renal effluent blood which contain renin in active form, the

following conflicting views may exist. If the possible impairment of liver function had occurred by the portalized renal venous blood, it is considered by the experiments of Page and his co-workers[42] and Munoz, Braun-Menendez Fasciolo and Leloir[18] that renin-activator, which is produced by the liver as the mother substance of angiotensin, may have been produced in lower quantity in the blood. Participation of so-called hepatorenal vasotropic factors is also considered. Especially, VDM which has been identified as ferritin[44] may give rise to its high content in blood through the possible liver damage by the portalization. Shorr[45][46] also showed that the blood pressure was reduced by the high amount of VDM in blood which resulted from the experimental liver cirrhosis and from hypertensive liver. The microscopic sections of the liver in our experiments have no significant changes and exclude these possible factors. Moreover, Tabata[19] showed that the complete portalization imparted no notable effect upon the liver function in the hypertensive dogs by partial constriction of renal artery. Davis, Tanturi and Tarkington[47] suggested that the formation of the vasodepressor factors which is considered to play a role in the experimental renal hypertension might have occurred by the reduction of the blood flow to the liver following the partial occlusion of the hepatic arteries and portal veins. In this experiment, however, autopsy finding was negative for such occlusion.

Because of the depressor effect of portalization, it is of interest to speculate whether VEM and antipressor agent,[48][49] which is contained on the medullorenal extract and acts upon small arteries or arterioles than directly related to the renin-angiotensin system, might be activated by the liver. Although the present report did not treat such VEM and antipressor agent as responsible factors, it is reasonable to pressume that the depressor effect of the portalization is due to the inactivation of renin by the liver.

At present, the surgical treament[50] available for the management of hypertension includes, thoracolumbar or transthoratic sympathectomy and splanchnicectomy, unilateralnephrectomy or direct surgery on renal artery and subtotal and total adrenalectomy. Recently, Schmidt[51] summarized the possible vicious circle involving such components as the renin-aldosterone-vascular reactivity. In order to intercept the formation of renin in the circle, the complete portalization of renal vein, which was first devised by Ito in our laboratory, should contribute for the treatment of the renal and essential hypertension in human beings, if this method is to be adopted in an appropriate stage of hypertensive patients.

SUMMARY

1. Experimental acute glomerulonephritis was produced in dogs by the injection of anti-dog kidney cortex rabbit sera, and hypertension, edema, increased blood urea and

gross and microscopic hematuria were observed.

2. In the present experiments, it was demonstrated that the vasoactive substance obtained from the renal venous blood of the nephritic dogs appeared to be identical with the synthetic angiotensin in modes of constrictive reaction to spirally cut strips of rabbit's aorta and of pressor effect to dogs nephrectomized 24 hours previously.

3. Hypertension of dogs with nephrotoxic nephritis could be prevented or abolished by means of the complete portalization of renal effluent blood, as the result of possible inactivation of the pressor substance by the liver.

4. There was no significant difference in microscopic findings of nephritis of dogs with or without reno-portal anastomosis. Complete portalization of the renal blood, therefore, showed an anti-hypertensive effect without affecting essential origin of the hypertension.

5. The possible clinical application of this procedure on the hypertensive patients of renal parenchymal origin is suggested.

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