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Irreversible Shock of Rats After Acute Renal Vein Thrombosis* ** ***

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Summary. After occlusion of the renal veins rats die quickly in progressive shock (within 4.5 h), but after ligating the renal hilum of both Kidneys they survive 27 h. To learn why renal vein occlusion is so rapidly lethal, and what substances are given off and by what method from the hemorrhagically infarcted kidneys, we studied eight groups of rats, each containing at least seven animals. The groups differed in the combination of hilar structures (renal veins, ureters, lymphatics) ligated. We compared: survival times, changes in blood pressure, blood volume, levels of plasma kinins, adenosine, and lactate, changes of blood pH, responses to Indomethacin, Trasylol®, and plasma expanders, tubular and capillary flow rates, histopathological changes in organs and cerebral blood flow and changes in the blood coagulation system. Our results suggest that the venous stasis, anoxia, and hemorrhagic necrosis caused by bilateral venous occlusion release into renal lymphatics toxic substances which reach the systemic circulation and induce irreversible shock. We have excluded prostaglandins and adenosine as the toxic substances inducing shock but could not rule out an action of the kallikrein-kinin-system. We postulate that the striking degenerative changes occurring in the arterioles of the brain after bilateral venous occlusion may mean these vessels are especially susceptible to high levels of lactic acid and that this may explain why these animals die so quickly. Our conclusions should help not only in understanding why high levels of lactate in shock portend a poor prognosis but also help in formulating appropriate therapy for circulatory failure of renal origin and for protracted hypotension after extensive tissue injury.

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** Presented in part: Jäckh and Steinhausen, 1976; Dallenbach et al., 1978; Zimmerhackl et al., 1979

*** We dedicate this paper to Wilhelm Doerr, Dr. med., Professor of Pathology, University of Heidelberg on the occasion of his 65th birthday (August 25th, 1979)

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Introduction

Following our studies of the microcirculation of the kidney (Steinhausen and Tanner, 1976) we observed that anesthetized rats with bilateral occlusion of the renal vein died in an irreversible lactacidotic shock within a few hours. In contrast to that observation, anesthetized control animals with bilateral ligation of the renal hilus lived much longer.

Although Buchwald and Litten (1876) first reported in Virchows Archiv that unilateral ligation of the renal vein in dogs and rabbits led to proteinuria and atrophy of the kidney, and numerous studies of renal vein thrombosis have appeared subsequently, none cite observations which resemble those we have reported (Brod, 1973; Dierixs et Moorloose, 1948; Friedberg, 1944; Harris et al. 1968; Heidbüchel et al. 1977; Kumar et al. 1975; Künzer and Breuer, 1970; Mandel et al. 1976; Mann, 1960; Rosenkranz und Esch, 1976; Schröder et al. 1976; Schürholz, 1977; Siderys and Kilman, 1966). The results which are most closely comparable to ours are those of Stahl et al. (1977) who found they could induce irreversible shock in rats by injecting microspheres into the animal's renal artery, and those of Darewics et al. (1976) who observed similar metabolic changes after temporary occlusion of one renal vein in dogs.

To discover why bilateral ligation of the renal vein leads to death by shock so rapidly, we studied our model of renal vein occlusion in various modifications. We soon learned that the renal lymphatics were of great importance, carrying from the hemorrhagically infarcted kidneys to the general circulation noxious substances which precipitated severe shock. In attempts to identify these noxious substances from the infarcted kidneys we analyzed the blood for changes in its pH, kinin-kallikrein content and the effects of kinin-kallikrein and prostaglandin inhibitors (Carvalho and Diniz, 1964; Torres et al. 1975). Because our histopathological studies disclosed fresh intravascular coagulation and unusual spasm with early necrosis of cerebral arterioles and capillaries we carried out extensive studies of changes in blood-coagulation and cerebral blood flow during the inception of severe shock.

Methods

For our studies we anesthetized with Inactin® (100 mg/kg body weight) male Wistar Furth rats that weighed between 190 to 250 g. To ensure that the pulmonary function of all animals remained comparable, we inserted tubes in their trachea and kept the airways clear. We cannulized the left carotid artery and monitored the blood pressure constantly (by Stathamtransducer and direct recording), and cannulized the left jugular vein for infusions. Through a mid-line celiotomy we freed the kidneys from their beds of fat and exposed the renal hilus, taking special care not to compromise the blood supply to either the kidneys or adrenal glands or to destroy renal lymphatic vessels. With the aid of the dissecting (stereo) microscope we separated the renal vessels and

placed loose ligatures about them. With an intravital microscope we examined the renal surface of some of the animals and studied the intratubular passage of lissamine green (Steinhausen and Tanner, 1976).

To find out why occlusion of the renal vein causes death so much sooner than ligation of the renal hilus we modified our shock-model in the following ways, labeling each as a special model:

Model A: Bilateral occlusion of the renal vein.

Model B: Unilateral occlusion of the renal vein with ligation of the *contralateral ureter.*

Model C: Unilateral occlusion of the renal vein with ligation of the *contralateral hilus.*

Model D: Bilateral occlusion of the renal vein after enclosing the kidneys in plastic capsules to decrease intrarenal hemorrhage. The plastic shells moulded from normal kidneys, were in two halves held firmly together by a lateral clamp, leaving the hilus free[1].

Modell E: Destruction of the renal lymphatics after enclosing the kidneys in plastic capsules and bilateral occlusion of renal vein. With the aid of the dissecting microscope we destroyed all hilar lymphatics with high frequency cautery.

Model F: Bilateral occlusion of the renal vein; one hour later bilateral ligation of the renal hilus.

Model G: Unilateral occlusion of the renal vein.

Model H: Bilateral ligation of the renal hilus.

We injected Indomethacin® (12 mg/kg body weight) on the evening before and again one hour before onset of the experiment. A phosphate buffer (pH 7.6) served as vehicle for suspension of the Indomethacin®.

Levels of plasma kallikrein were determined by measuring plasma esterase activity on the substrate benzoyl-L-arginineethylester. The by-product of that reaction, ethanol, served as the measure of kallikrein activity and was determined photometrically (Trauschold et al., 1974). The kallikrein preparation Padutin® of the Bayer firm was used as standard.

When infusions were given, we started them one hour before occluding the renal vein(s). Each experimental animal received a total of 10 ml Haemaccel® (iso-osmotic and iso-colloidal gelatine solution) or 10 ml Trasylol® (aprotinin 20,000 KIE/ml) infused at the rate of 0.375 ml/h. Levels of plasma lactate were measured with lactate dehydrogenase and NAD with the test combination of Boehringer, Mannheim.

The animals selected for histological study were autopsied immediately after death. We fixed the tissues and organs of interest in 5% buffered formalin and after appropriate dehydration and embedding in paraffin, prepared hematoxylin-eosin stained sections for study.

We measured blood volumes with FITC labelled dextran of 150,000 MW. As we previously reported (Steinhausen et al., 1977) dextran is not filtered by normal kidneys. One and a half minutes after injecting 0.1 ml of a 5% solution of the labelled dextran, its plasma concentration remained constant. Although glomerular permeability does change after occluding the renal vein, glomerular filtration decreases so greatly that loss of dextran may be regarded as negligible.

Following the method of Leichtweiss et al. (1969) we measured cerebral blood flow as hydrogen clearance with platinum microelectrodes (Betz, 1972). After craniotomy we inserted these with the aid of a micromanipulator into the brain at a depth of 1.5–2 mm below the cortical surface. The anterior and lateral coordinates were 5.1–5.6 mm and 2.7 mm respectively according to the stereotaxic atlas of König and Klippel (1963).

We performed the thromboplastin time, activated partial thromboplastin time, thrombin time and reptilase time according to standard techniques. We followed Koller's method (1951) to determine Factor V, with reagents from the Behringswerke (Marburg/Lahn) and from Boehringer (Mannheim). To measure concentrations of Factors IX, XI, and XII we used plasma supplied by Merz and Dade (München), and to measure Factor VIII according to Dahlmann (1967) plasma from Immuno GmbH (Heidelberg). We employed the Feissly and Lüdin method (1949) to determine the platelet count, and the Clauss method (1957) to measure levels of fibrinogen.

The values are given as mean and either standard deviation (SD) or standard error of the mean (SEM) significance was determined by Student's *t*-test.

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[1] We thank Mr. Walter Schmidt of the University of Heidelberg Dental School for kindly preparing the kidney capsules for us

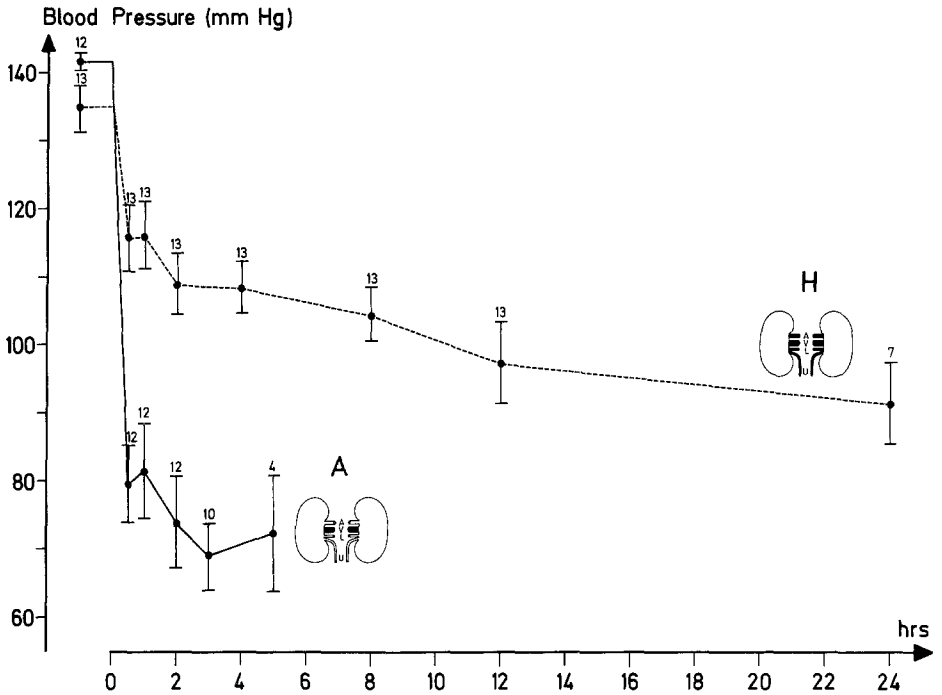

Fig. 1. Mean values and SEM of blood pressure after bilateral ligations of the renal hilum (Model H) and renal veins (Model A). The digits give the number of animals living at the time measurements were made

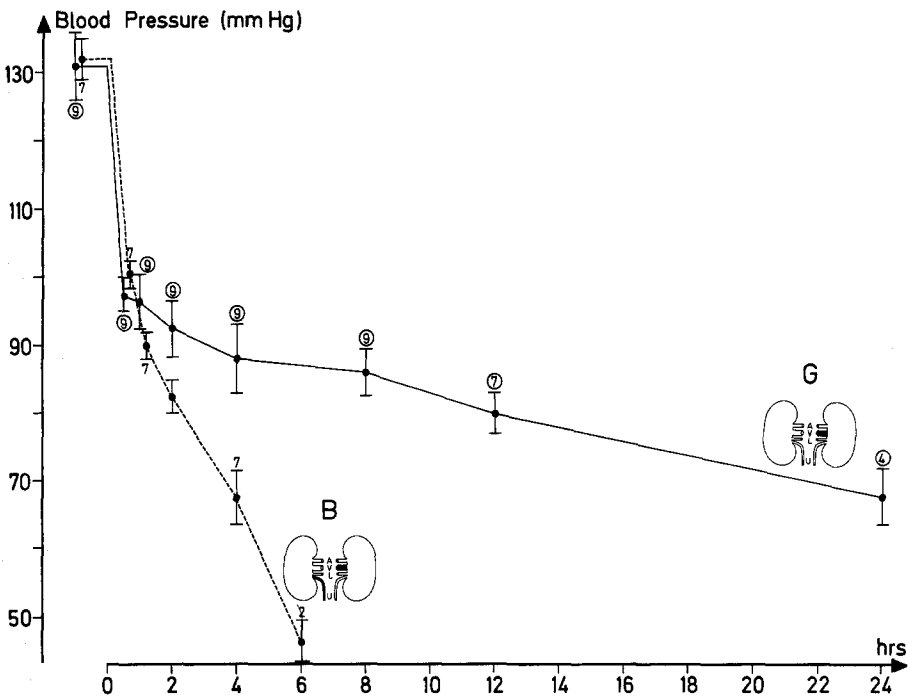

Fig. 2. Mean values and S.E.M. of blood pressure after unilateral occlusion of renal vein (Model G) and unilateral occlusion of renal vein and ligation of contralateral ureter (Model B). For numbers see Fig. 1

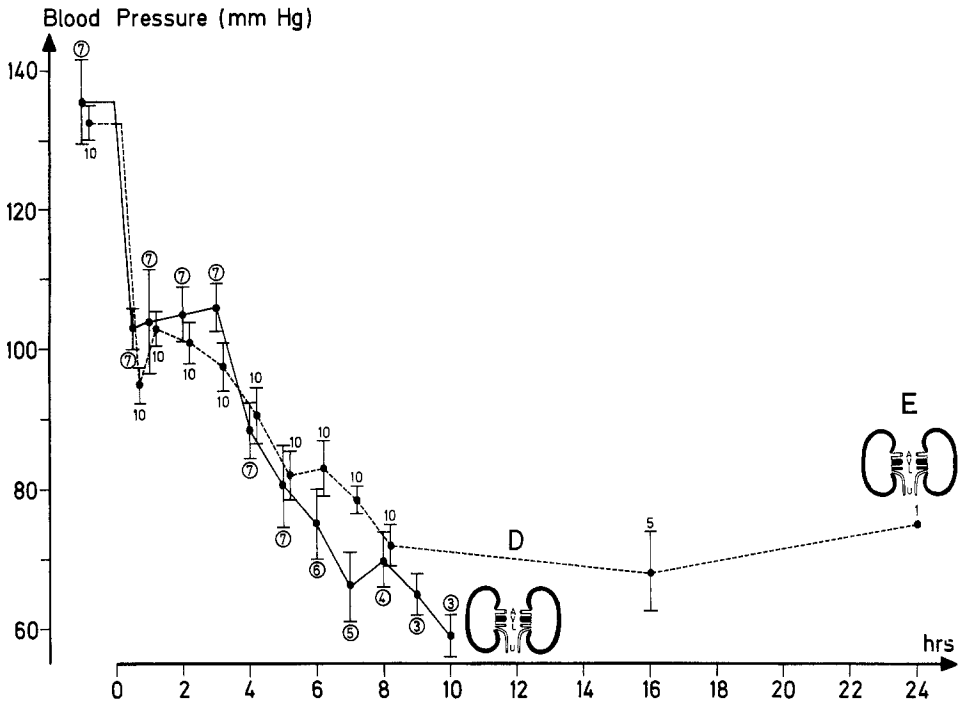

Fig. 3. Mean values and S.E.M. of blood pressure after bilateral occlusion of renal veins (model D) and coagulation of renal lymph vessels (Model E). Both kidneys are tightly enclosed in plastic capsules. For numbers see Fig. 1

Results

I. Changes in Blood Pressure and Survival Times

The changes that took place in the mean arterial blood pressure after the various procedures are recorded in Fig. 1–3. On bilateral occlusion of the renal veins (Model A) the arterial pressure fell in the first 30 min to an average value of 80 mm Hg. The animals died a few hours later after a further rapid decline in blood pressure (Fig. 1). In the experimental Model H (bilateral ligation of the renal hilus) the average arterial blood pressure fell to 110 mm Hg and remained at that level (Light Inactin® anaesthesia caused the blood pressure to rise to relatively high levels at the beginning of the experiments.).

After unilateral occlusion of a renal vein (Model G), the average arterial pressure decreased within 12 h to about 80 mm Hg. Most animals were able to tolerate that degree of hypotension several hours longer. On the other hand, when the opposite ureter was also ligated (Model B), then the blood pressure rapidly fell (Fig. 2).

When the kidneys were enclosed in the plastic capsules (Model D) thus reducing the swelling from hemorrhagic infarction, the shock-like state became protracted (Fig. 3). The average arterial pressure remained constant for 3 to

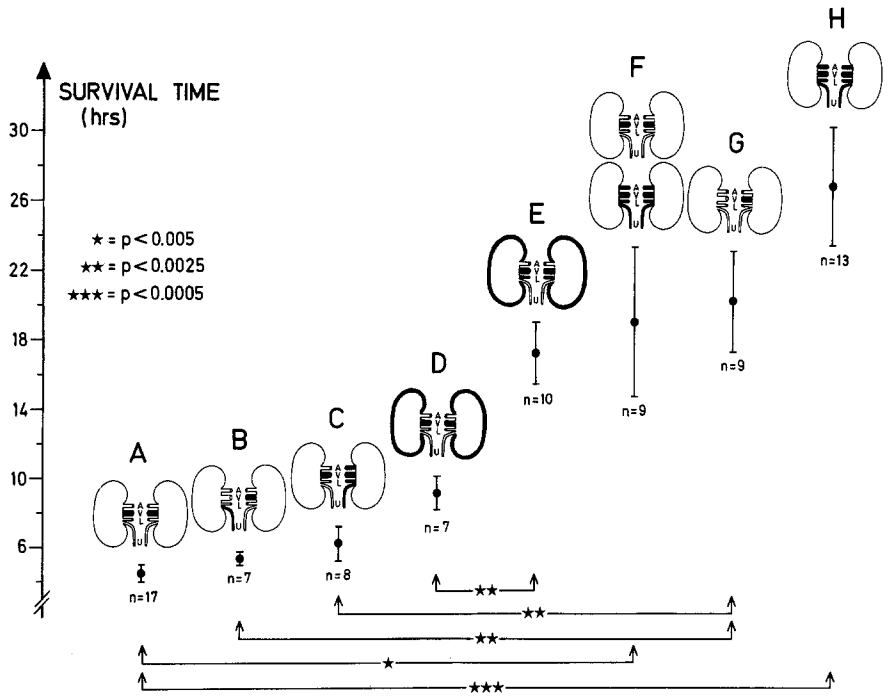

Fig. 4. Survival times of rats after occluding different structures of the renal hilus (mean values and S.E.M.). n=number of animals, *A* bilateral occlusion of renal veins, *B* unilateral occlusion of renal vein and ligation of contralateral ureter, *C* occlusion of one renal vein and ligation of the contraleral hilus, *D* bilateral vein occlusion after enclosing kidneys in plastic capsules, *E* bilateral vein occlusion after enclosing kidneys in plastic capsules, *F* bilateral vein occlusion followed by clamping hilum of both kidneys, *G* unilateral vein occlusion, *H* bilateral ligation of renal hilum

4 h at levels above 100 mm Hg, but sank in the fifth hour to levels below 80 mm Hg. If the hilar lymphatic vessels were then destroyed by coagulation (Model E), the blood pressure of only one of the animals dropped below 70 mm Hg in the first seven hours, whereas 5 animals were still alive 16 h after venous occlusion with blood pressure levels of 70 mm Hg.

If the renal hili were ligated one hour after bilateral venous occlusion when the blood pressure was less than 80 mm Hg (Model F), then the arterial blood pressure on the average slowly rose to levels above 80 mm Hg within 8 h.

The mean *survival time* of the animals of Model A (bilateral occlusion of the renal vein) was 4.5 h $\pm$ 0.4 (n=17) (Fig. 4). In Model B (unilateral occlusion of renal vein and ligation of contralateral ureter) the animals died in 5.4 h + 0.4 (n=7). In Model C (unilateral occlusion of the renal vein with ligation of the contralateral hilus) the mean survival time of the animals proved to be 6.3 h $\pm$ 1.5 (n=8). In Model D (when both kidneys were tightly enclosed in the plastic capsules before the renal veins were occluded bilaterally) their mean suvival time was extended to 9.2 h $\pm$ 1.0 (n=7). The animals of Model E (treated like those of Model D but with an additional destruction of the

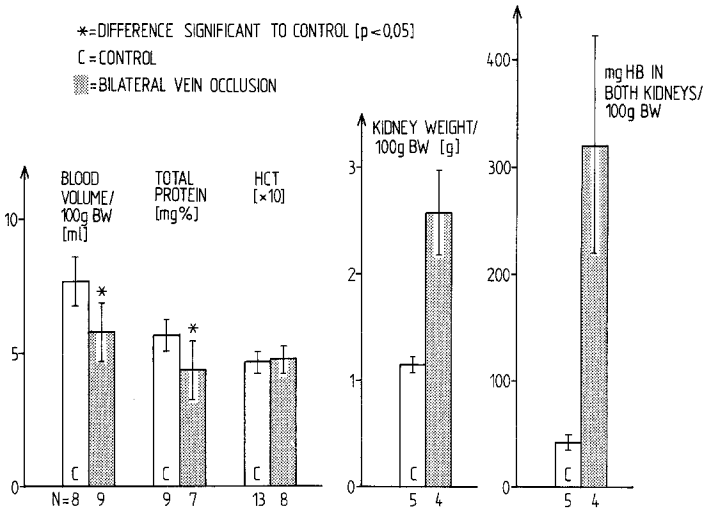

Fig. 5. Mean values and standard deviations (S.D.) of blood volumes, levels of plasma proteins, hematocrits, kidney weights, and hemoglobin contents of both control kidneys and kidneys 3.75 h after bilateral occlusion of renal veins (Model A)

renal lymphatics by cautery) survived a mean of 17.3 h ± 1.7 ($n=10$). In Model G (after unilateral occlusion of the renal vein) their survival time increased to 20.3 h ± 3.0 ($n=9$). In Model H (bilateral ligation of the renal hilus) the animals lived a mean of 27.0 h + 3.4 ($n=13$).

II. Changes in the Circulating Blood Volume

With bilateral occlusion of the renal vein (Model A) the kidneys increased in weight from 1.2 g to 2.6 g within 3.75 h (see Fig. 5). The increase was due primarily to intrarenal hemorrhage. Since the hematocrit of the animals failed to change after occlusion of the renal veins despite a fall in blood volume, the loss of plasma into the kidneys paralleled that of the blood cells. No ascites or subcutaneous oedema developed.

III. Acid-Base Balance Studies and Hyperpotassemia

Analysis of the acid-base balance after occlusion of the renal veins disclosed a severe acidosis. Figure 6 shows the base excess in three experiments after either bilateral or unilateral occlusion of the renal vein. Whereas the animals with unilateral occlusion of the renal vein often survived up to three days and the rise in their base excess above – 6 mmol first developed preterminally, that critical level was reached within a few hours in animals with both renal veins occluded. The acidosis was associated with a sharp rise in concentration of lactate in the arterial blood (Fig. 7). As the concentration of lactate rose,

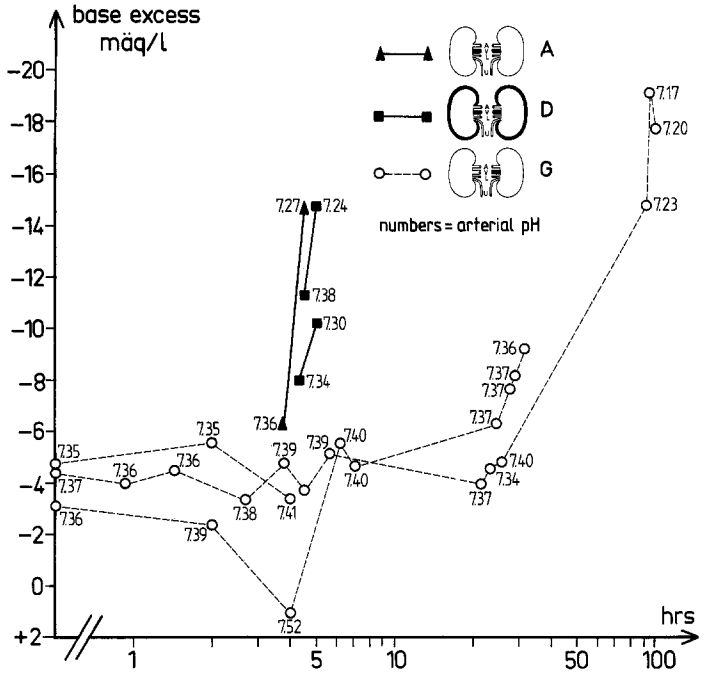

Fig. 6. Level of base excess of arterial blood: —— heavy unbroken line gives the values of three rats after bilateral occlusion of renal vein (Model A), two with capsules (Model D). ------- thin, broken line shows the value of three rats after unilateral occlusion of renal vein (Model G)

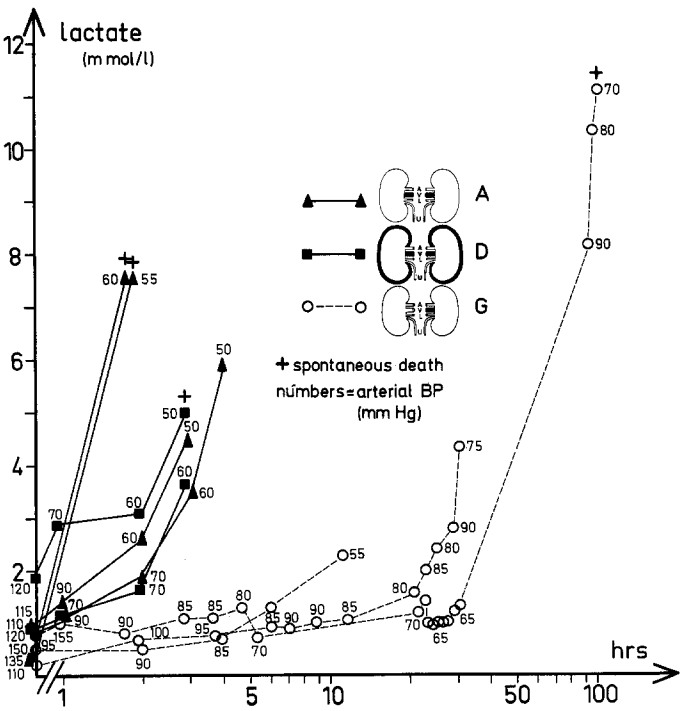

Fig. 7. Lactate levels of arterial blood and mean arterial blood pressure: —— heavy unbroken line represents values from six rats after bilateral occlusion of renal veins (Model D), four of which had no capsules applied (ModelA). -------- thin, broken line gives the values from three rats after unilateral occlusion of renal vein (Model G)

the blood pressure fell. With unilateral occlusion of the renal vein (Model B) the contralateral (functioning) kidney prevented a rise in lactate. Analyses here showed that the amount of lactate excreted in the urine regularly increased by a factor of 4–10 over normal values. Only terminally did the plasma concentration of lactic acid rise in the animals. Four hours after bilateral occlusion of the renal vein (Model A) the levels of serum potassium had reached 6–7 m val/l but rose before death to 9–11 m val/l.

IV. Intravital Microscopy of the Renal Surface

With unilateral occlusion of the renal vein, blood flow through the vessels of the renal surface ceased completely only after about 3 h. One hour after unilateral occlusion of the renal vein the velocity of blood flow at some welling points of the renal surface measured about 0.1 mm/s (normal values are 4.1 mm/s; see Steinhausen et al. 1973). Our studies indicate that after venous occlusion, filtration in the congested kidney persisted up to 3 h since at that time with the aid of intravital microscopy we could see the tubular loops fill with FITC-dextran. It should be noted, that under physiologic conditions FITC-dextran (150,000 molecular weight) is not visible in the tubular filtrate (Steinhausen et al., 1977). After occlusion of the renal vein, however, it becomes increasingly more conspicuous in the tubules up to 3 h. That finding implies that venous occlusion produces changes in glomerular permeability, as others have suggested (Ryan et al., 1976; Allen, 1962). Measurements of intratubular pressures with the servo-nulling device gave the following values: 39.0 ± 8.2 mm Hg (M $\pm$ S.D. $n=5$) 30 minutes after unilateral venous occlusion and 32.1 ± 4.7 mm Hg ($n=7$) 3 h after occlusion. In two animals we *incompletely* occluded the renal vein bilaterally with silver clips. The space between the clip-halves varied between 100–150 µ. Observations with the intravital microscope of the passage of lissamine green into the renal surface revealed that at 1 h and 6 h after occluding the veins, lissamine green appeared in the peritubular vessels in about 30 s. (Under normal conditions, lissamine green becomes visible in 2–3 s). Although tubular loops filled up with dye within three to five minutes, it was impossible to measure tubular-passage times. One of the animals of this model survived six hours, the other twenty-one hours. Because it proved difficult to ensure that the venous occlusion with these clips was always alike in every animal of this model, we did not persist with it, although these animals with bilateral incomplete occlusion did die sooner than the control animals.

V. Levels of Plasma Kinins

The kallikrein-like activity of plasma taken from animals four hours after occlusion of their renal vein (Model A) was not increased over that of control animals. The values of activity obtained for controls were 480 ± 40 mU/ml plasma ($n=4$), and for the rats with occluded veins 360 ± 40 mU/ml plasma ($n=4$). The somewhat lower values were perhaps due to the decreased renal excretion of inhibitors (Dietz and Gross, 1977).

In addition, kallikrein activity by kinin formation was measured in a bioassay test using rat uterus[2]. The animals in Model A gave values of 3.65 ng/300 µl ± 0.9 ($n=4$); four hours after celiotomy the values for control animals were 8.2 ng/390 µl ± 0.46 ($n=2$). The plasma kininogen levels (Diniz and Carvalho, 1963) of animals 4 h after occlusion of the renal vein showed no fall as compared with the levels of control animals (for animals of Model A 19.9 ng bradykinin/mg ± 0.58 ($n=4$), for control animals 20.0 ng bradykinin/mg protein ± 0.5 ($n=2$)). These results provide no evidence of increased kallikrien and kinin activities.

The plasma adenosine level was not significantly increased in Models A and D four hours after occluding the renal veins.

VI. Effects of Indomethacin, Haemaccel® and Trasylol®

When indomethacin was given, neither the survival times of the animals in experimental Model A and Model D were prolonged nor was the fall in blood pressure prevented. The survival times observed, in fact, were somewhat shorter: 1.9 h ± 0.4 ($n=9$) for experimental Model A, and 4.2 h ± 0.4 ($n=6$) for Model D. Finally, we could show that the animals infused with Haemaccel® and Trasylol® (experimental Model D) survived longer. These solutions, however, showed no significant differences in their effect when used separately in Model D: 21.0 h ± 5.6 ($n=4$) for infusions with Trasylol®, and 22.8 h ± 2.4 ($n=4$) for infusion with Haemaccel®. The infusions of Haemaccel® in the animals of Model A did not affect their survival times ($t=4.8$ h ± 2.05; $n=6$). In contrast, the infusion of Trasylol® prolonged the average survival time to 13.7 h ± 3.04; $n=8$.

VII. Pathological Changes After Bilateral Occlusion of Renal Veins

1. Kidneys

Gross. After formalin fixation the kidneys appeared remarkably dark red-purple, swollen and thicker but softer than normal. When transected, the glistening, dark red-purple of the fresh surface obscured the usually prominent landmarks distinguishing the cortex and medulla.

Microscopic. Although fresh uncoagulated blood seemed to fill all interstital spaces, widening and distending them so as to compress parenchymal structures, the degree of extravasation of blood did vary somewhat from one part of a kidney to another and from one rat to another. The glomeruli were conspicuous because their intact Bowman's capsules separated them sharply from the sea of engulfing blood. In general, the glomerular capillaries were clogged with blood. Where bloodless, however, they were pushed aside by a proteinaceous fluid that distended Bowman's space. Admixed with the fluid were blood cells,

[2] We thank Dr. R. Geiger of the Institute for Clinical Chemistry and Clinical Biochemistry, München, for performing the bioassay of the kininogen and kinins

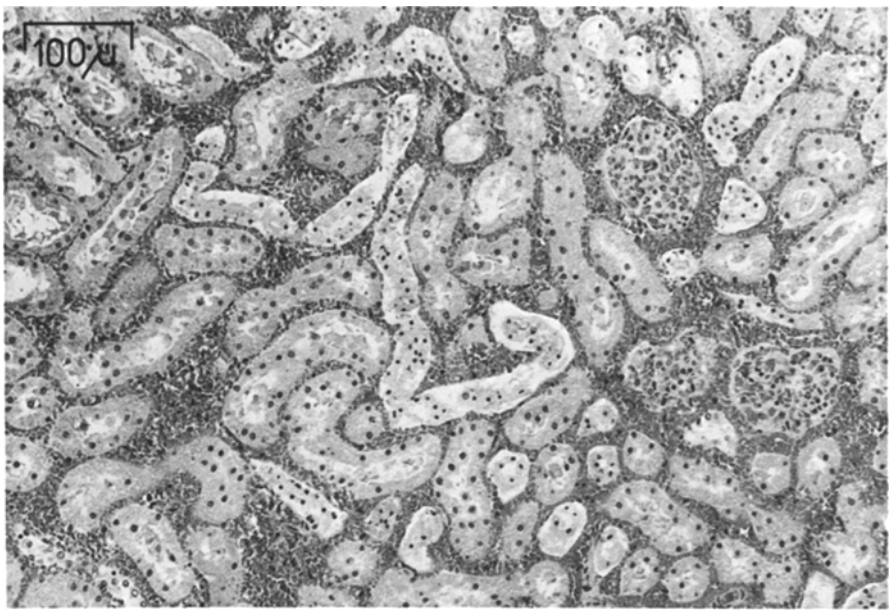

Fig. 8. Hemorrhagic infarction $3^5/_6$ h after bilateral occlusion of renal vein, with kidneys enclosed in capsules. Interstitium filled with blood. Glomerular capillaries engorged. Tubular epithelium degenerating, their nuclei pyknotic. Hematoxylin-eosin stain

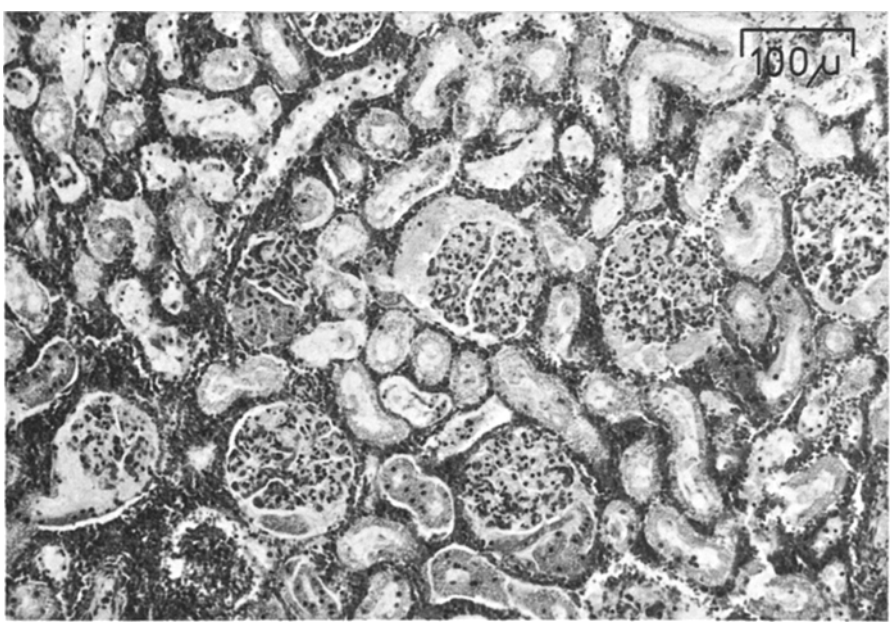

Fig. 9. Hemorrhagic infarction 11 h after bilateral occlusion of renal vein, with kidneys enclosed in capsules. Interstitium filled with blood. Proteinaceous filtrate and herniated tubular cells in Bowman's space. Severe tubular necrosis. Hematoxylin-eosin stain

fibrin threads, and degenerating cells of proximal tubulus that had herniated into Bowman's space (Fig. 8).

The epithelial cells of the proximal convoluted tubules revealed various but usually advanced degrees of coagulation necrosis. In most instances they formed irregular rings of amorphous cytoplasmic debris about an ill-defined floccular protein-rich filtrate that filled what should have been the tubular lumen. Remnants of nuclei appeared as dark, condensed clumps of chromatin or as dust-like deposits (Fig. 9).

The amount of filtrate and cellular debris filling the tubular lumina decreased as the loop of Henle was approached. Here, as along the ascending loops, the intermediate segments and the distal convoluted tubules, the shrunken lining cells, having shed from their delicate basement membranes, lay free and partially disintegrated in the tubular lumen. Their cytoplasm was shredded and their condensed nuclei extruded. The occasional short portions of distal tubules and collecting tubules lined by intact epithalial cells with visible boundaries and more normal chromatin patterns attested to the patchy nature of the degenerating processes.

Widespread extravasations of blood and deposits of abundant coagulated protein extended around larger veins, throughout sinus fat tissues, beneath the epithelium of the renal pelvis, along the ureter, and into the renal capsule and perirenal fat tissues.

Diagnosis. Hemorrhagic infarction of the kidney.

2. Liver

Gross. In all instances the livers were swollen, heavy, and covered by glistening capsules. Fresh sections through the lobes revealed a pale, moist parenchyma with prominent vascular markings.

Microscopic. In general, all livers showed the same changes, which, however, varied slightly in severity from one animal to another. The central veins were often clogged with finely granular thrombi of aggregated platelets, coarser globules of proteinaceous substance, cellular debris, and intact red blood corpuscles. These thrombotic masses often adhered to the endothelium and extended into and along contiguous dilated sinusoids. Individual liver cells nearby showed various stages of degeneration, in general having shrunken and retracted from their neighbors. Their cytoplasm was condensed, stained darkred, and their nuclei distorted, either swollen and pale, or dark, pyknotic and fragmented (Fig. 10).

Diagnosis. Early thrombosi of central veins with focal necrosis of pericentral liver cells.

3. The Brain

The *arteries* and *arterioles* supplying the gray and white matter revealed pathological changes of variable severity. Most of these vessels appeared

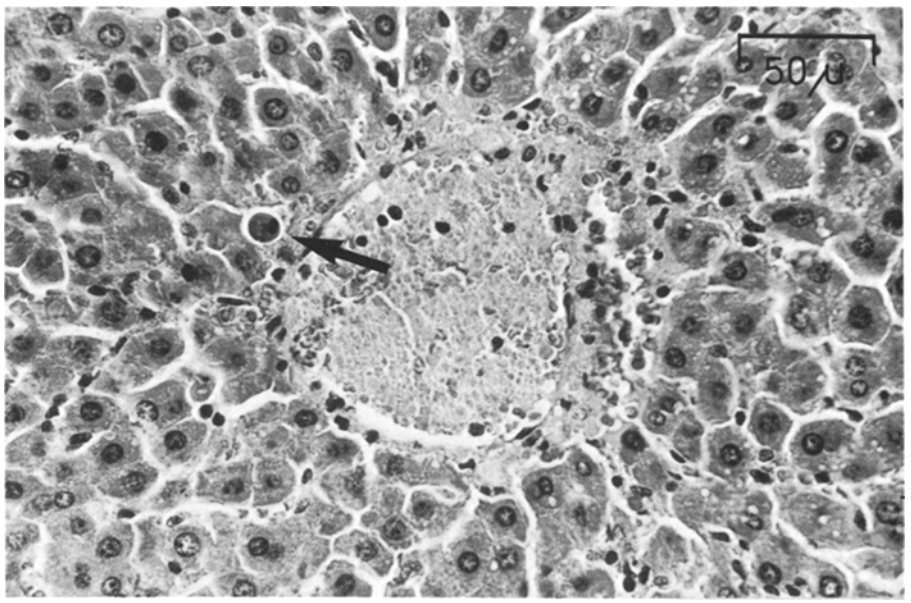

Fig. 10. Fresh hyaline thrombus of hepatic central vein of rat five hours after bilateral occlusion of renal vein. Degenerating liver cells at →. Hematoxylin-eosin stain

constricted, and their slender, often twisted lumina were either empty or contained elongated, degenerating or fragmented red blood corpuscles, remnants of swollen endothelial cells, cellular debris, and/or thrombi of clumped platelets and coagulated plasma. The smooth muscle cells of their walls exhibited virtually every stage and type of degenerative change possible, from intracytoplasmic vacuoles and hyaline droplets to shrunken condensed cytoplasm, coagulation necrosis and lysis, whereby adjacent cells seemed to melt together, occluding the vessel lumen (Fig. 11). The nuclei of these degenerating muscle cells also varied; some were oedematous and pale-staining, others merely clumps of pyknotic chromation often fragmented. The space about the vessel was usually dilated, spanned by elongated fine filaments that stretched between the collapsed vessel and the surrounding spongy neuropil and swollen neuroglial cells, which like occasional neighboring neurons, also revealed various stages of degeneration or necrosis. Many of the *capillaries* were filled with plump endothelial cells, hyaline thrombi or agglutinated, sometimes degenerating red blood corpuscles. Other capillaries, however, were greatly distended with packed blood cells and were surrounded by small hemorrhages at breaks in their walls. Some capillaries resembled strands of coagulated proteinaceous material still connected to the oedematous neuropil by thin filaments. The neighboring neurons or glial cells were shrunken and darkly stainded, manifesting various stages of degeneration (Fig. 12). Although the majority of the *veins* were overdistended with stacked rolls of intact red blood corpuscles, their endothelial cells and walls were intact. No veins were found containing thrombotic material or desquamated cellular debris. Their perivascular spaces in general were normal.

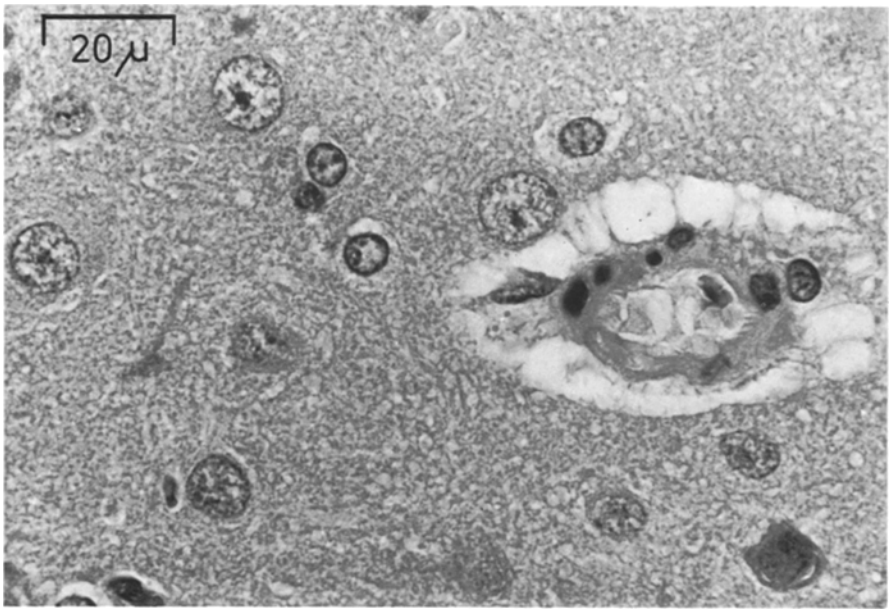

Fig. 11. Degenerating cerebral arteriole of rat five hours after bilateral occlusion of renal vein. Nuclei of smooth muscle cells pyknotic, cytoplasm condensed. Lumen contains thrombotic material, perivascular space edematous. Hematoxylin-eosin stain

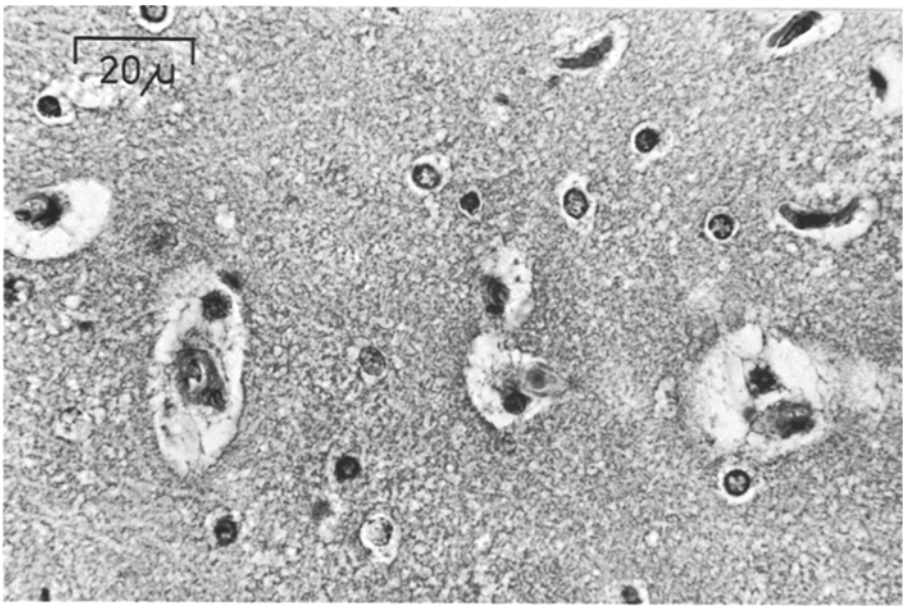

Fig. 12. Thrombosis and severe degeneration of cerebral capillaries and contiguous glial cells, perivascular edema, five hours after bilateral occlusion of renal vein. Hematoxylin-eosin stain

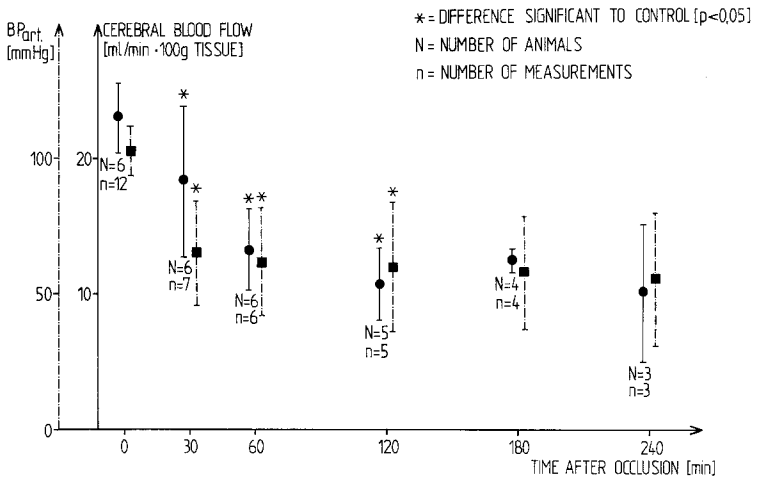

Fig. 13. Cerebral blood flow (circles) and mean arterial pressure (squares) of rats after bilateral occlusion of renal veins (Model A). N = number of animals, n = number of measurements ($M \pm SD$)

Diagnosis. Degenerative changes and focal thrombosis of small arteries, arterioles and capillaries of the brain. Oedema of the brain.

Changes in Other Organs. Except for generalized endothelial edema and rare newly-formed hyaline thrombi of small pulmonary and epicardial arterioles, no other pathologic changes were found in the adrenals, spleen or thymus.

VIII. Cerebral Bloodflow After Occlusion of the Renal Veins

The pathological changes found in the brains of rats dying in severe shock after bilateral occlusion of the renal vein may well have been caused by a reduced cerebral blood flow. To determine what effects shock has on cerebral blood flow, we measured with Pt microelectrodes the hydrogen clearance of the white matter of six rats after occluding their renal veins (Model A) (see Fig. 13). Although the mean arterial pressure in some of the animals fell within the first half hour after occluding the renal veins, their cerebral blood flow decreased only slightly. That suggested an adaptive response by autoregulatory mechanisms. Sixty minutes after occlusion of the renal vein, however, cerebral blood flow had decreased to half of the control values.

IX. Changes in Blood Coagulation After Bilateral Occlusion of the Renal Veins

The intravascular microthrombi found in histological sections of organs, primarily of the liver and lung, stimulated us to investigate changes occurring in the blood-coagulation system. As Figure 14 shows, $3^3/_4$ h after occluding the renal veins, the fibrinogen levels in the plasma decreased by 16%. If we produced shock in rats by withdrawing blood (2 ml/100 g body weight), then the fibrinogen levels were similarly decreased. Only in shock following occlusion of the renal veins were the values for the Quick Test, factors VIII and IX significantly low, but those for the thrombin time, reptilase time and A PTT were significantly

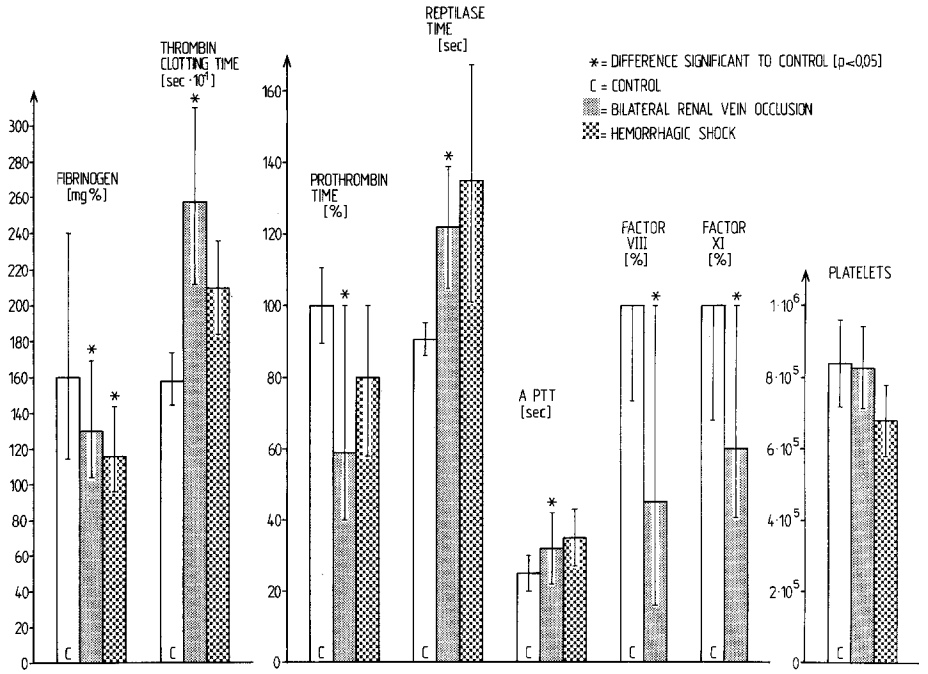

Fig. 14. Levels of fibrinogen, thrombin clotting times, prothrombin times, reptilase times, activated PTT, factors VIII and IX, and platlet counts of control rats, and of rats $3^3/_4$ h after occluding their renal veins (Model A), and of rats bled 2 ml blood/100 body weight ($M \pm SD$)

increased. The results of all tests pointed to the onset of a disturbance of blood coagulation but the changes in the coagulation system were too moderate to be regarded as the cause of death.

Discussion

Our results indicate that when we occlude the renal vein of the rat, toxic substances are released from the infarcted parenchyma and carried by way of lymphatics into the systemic circulation, where they induce hypotension.

These results differ from those observed by Friedberg (1944), who reported that when he partially constricted the renal veins in dogs they developed a mild hypertension. On complete constriction, however, the dogs died in uraemia in from one to five days. Friedberg gave no explantion of their death. In contrast, Dierixs and de Moorloose (1948) found that after partial occlusion of renal veins of rabbits their blood pressure remained normal. Whether these differences in response to renal vein occlusion are specific for animals species (possibly related to inelasticity of the renal capsule) or more related to the experimental techniques employed we are unable to say.

The evidence that thermic coagulation of the hilar lymphatics prolonged the survival of our rats and slowed the development of the hypotension proves how important the lymphatic channels are. The absorbed toxic substances are apparently eliminated from the body by a functioning kidney, since with uni-

lateral occlusion of the renal vein the toxic and hypotension-inducing effects are potentiated by ligating the opposite ureter.

The results of the experiments with Indomethacin help to rule out prostaglandins as agents causing the fall in blood pressure in the experimental models we used, indeed since Indomethasin actually intensified toxic effects and shortened survival of the animals. Had prostaglandins been responsible for the hypotension we would have expected the Indomethacin to exert an ameliorative effect. None of the other tests we performed pointed to free proteases or kinins in the plasma as the toxic substances.

The favorable effect of Trasylol® (as compared with Haemaccel®) in prolonging survival of animals of Model A may be explained by its inhibitory effect on kallikrein. Trasylol®, however, also inhibits other proteases, and through various effects on the microcirculation modifies the development of shock. This nonspecific effect of Trasylol®, as compared with Haemaccel®, was not observed in the animals of Model D, perhaps because the survival times obtained almost equaled those of the control animals (Model H). Analysis for adenosine excluded it as one of the toxic substances. In no animals were the plasma levels of adenosine significantly elevated. The increase in blood pressure characteristic of excessive adenosine never developed. Furthermore, when we injected 1.0 g of adenosine intraperitoneally, the rats survived for long periods, even those bilaterally nephrectomized.

Although four hours after occluding both renal veins the plasma levels of potassium of the rats had risen to 6.7 mval/l, we found it difficult to believe these animals died because of the hyperkalaemia. To test that possibility, however, and to learn how hyperkalaemia might affect our animals in total renal failure, we used four nephrectomized rats with a solution of 300 mval/l KCl. Sudden cardiac standstill eventually developed after four hours of infusion when the plasma level of potassium had reached 14 mval/l. Yet none of the animals developed the gradual, persistent fall in blood pressure that typically developed after bilateral occlusion of the renal vein. Of the causes of the circulatory failure, more important, in our opinion, was the lactic acidosis resulting from the renal anoxia and subsequent massive parenchymal necrosis. Whether the lactic acid merely served as indicator of the other more potent and active substances or alone induced circulatory failure are questions we cannot answer completely. In order to learn more about the effects of high levels of plasma lactic acid or lactate, we infused these substances into normal rats. The lethal hypotension that ensued resembled that seen after occlusion of the renal vein. Although the lactic acid can be neutralized with bicarbonate, permitting the plasma lactate to reach especially high levels before circulatory failure sets in, the animals nonetheless develop an irreversible lactic acidosis.

Although the histological studies failed to provide a clearcut explanation of the death of the animals after bilateral occlusion of the renal vein, the extensive degenerative changes in arterioles, capillaries and perivascular parenchymal cells of the brain and liver clearly indicate the metabolism in these organs was seriously disturbed. The intravascular thrombi also found served as additional morphological evidence that biochemical equilibria in the blood had become upset. Hardaway (1968) reported that an acidosis greatly accelerated the clotting of blood.

The unusual predilection of injury for the vessels of the brain and the type of degenerative change seen suggest that more than protracted hypotension and subsequent hypoxemia had been responsible, especially since vessels of other organs failed to reveal similar injury. Whether the striking degenerative changes of the smooth muscle cells of the arterioles of the brain means that they are especially susceptible to high levels of lactic acid we do not know. Such changes as we found are unlike those described after uncomplicated hypoxia (Brown and Brierley, 1971; Hirano, 1976). Recent electronmicroscopic studies by Dahl (1976) and by Roggendorf et al. (1976) report that the cerebral arterioles are structurally different from other arterioles of the body. Although these authors avoided trying to explain what the differences might mean physiologically, it does seem plausible from our studies to suggest the cerebral arterioles, because of their unique intercellular contacts, may be unusually sensitive to high levels of lactic acid. Oldendorf (1976) explains how an excess of lactate in brain tissue would "overburden that tissue's buffering capacity with a lowering of the brain pH". Betz (1976) has stated that a decrease in H^+ or a sharp increase in K^+ in the perivascular space of pial arteries causes vasoconstriction. Whether a hyperphosphatemia, which O'Connor et al. (1977) observed in lactic acidosis, contributes to a imbalance of ionic autoregulation of cerebral blood-flow is not known. Wahl et al. (1973) found that the pial arteries are also sensitive to changes in perivascular osmolarity: hypo-osmolarity brings about vascular constriction whereas hyperosmolarity causes the vessels to dilate. These authors (Wahl and Kuschinsky, 1977) also state that in studying the effects of various ions (or metabolic factors) on the smooth muscle cells of the cerebral arteries, one must consider the complicated interactions of these ions or factors with one another. They suggest the differences in responses noted between cerebral and coronary arteries may mean these vessels either react differently to combined stimuli, or the experimental procedures used to investigate them were responsible. As Herrschaft (1976) points out, even the several theories of autoregulatory mechanisms, contrived to explain how the cerebral blood-flow is controlled under normal conditions, contradict one another. What ionic shifts might occur during lactic acidosis and whether they might induce persistent contraction of cerebral arterioles causing them to degenerate is unknown and can only be resolved by further studies.

If, as all results here imply, high levels of lactic acid ('representing under-utilization as well as over-production' – Alberti and Nattrass, 1977), evoke vasodilatation in arterioles outside of the brain, leading to protracted hypotension and hypoxemia, then these in turn would be expected to aggravate tissue hypoxia and the lactic acidosis, setting into motion a vicious circle that would end fatally.

Lactic acidosis, so exceedingly difficult to remedy (Cohen and Simpson, 1975) has assumed special interest and relevance in the discussion of the use of Biguanides (Alberti and Natrass, 1977). A prerequisite for therapy with these antidiabetic agents is a healthy kidney, which is able to eliminate lactate.

With the aid of incident-light ciné-photomicroscopy we were able to detect distinct alterations of glomerular permeability in haemorrhagic infarction of the kidney. Such alterations explain the proteinuria so often described with thrombosis of the renal vein (Mann, 1960; Omae et al. 1958; Brod, 1973; Romen, 1971).

The results we present here should prove of help not only in devising therapy for circulatory failure of renal origin, particularly renal vein thrombosis of small children, but also aid in understanding why lactic acidosis and hypotension after severe injury should be treated immediately and adequately. If high blood levels of lactate early in shock portend a poor prognosis, as Peretz et al. (1964) and Cohen and Woods (1976) maintain, that may well reflect injury to sensitive cerebral arterioles, capillaries and brain cells, a complication requiring early and vigorous measures if it is to be prevented.

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