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Donald George Skinner, B. A.
Wesleyan University, 1960

A Thesis
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Introduction

An association between septicemia and shock-like states has been known for many years. Laennac (1831) first described a condition characterized by severe infection with subsequent circulatory failure, falling blood pressure, anuria, cyanosis and shock. (38) Boise (8) included infection in a manuscript describing the differential diagnosis of shock. In spite of the advances that have taken place in medical and bacteriological research since the turn of the century, surprisingly little is known regarding the actual mechanisms of action of bacterial toxins on systemic homeostasis.

The investigations to be reported were made as an attempt to elicit information regarding the effect of staphylococcal toxin on the circulatory system with specific reference to renal function.

Review of Literature

In the past investigators have attempted in rather strict terms to differentiate between toxins produced by Gram negative bacteria and those produced by Gram positive organisms. The toxins were rigidly classified as exotoxins or endotoxins, depending on various physical, physiological or biochemical characteristics. As newer investigative techniques have evolved, however, and more

knowledge has been derived concerning bacterial toxins, these terms have become somewhat confusing and need clarification.

Endotoxins generally are defined as substances released from virulent strains of Gram negative organisms following autolysis. Historically Pfeiffer (1893) was first to demonstrate experimentally that specific bacteria were capable of producing a toxin causing lethal effects on mice. Following the intraperitoneal injection of living Cholera vibrio organisms, Pfeiffer observed that, although the host defense mechanism was capable of lysing the bacteria, the mice subsequently died. These experiments suggested that a toxin was contained within the organism and was liberated following lysis. Pfeiffer,therefore, formed the term endotoxin. (46)

It was not until 1959 that this substance was discovered to be a constituent of the bacterial cell wall. (11) Biochemically it is composed of a large protein-lipopolysaccharide-carbohydrate complex, (9) is relatively heat stable (74) and immunologically non-specific. (7) Physiologically, endotoxins are capable of producing irreversible shock secondary to peripheral vascular collapse, but the mechanism still remains largely unknown. (22) The most popular theory postulates an indirect action on the peripheral circulation via liberation of a histamine-like substance into the blood stream. (32, 54, 55, 64, 67)

Exotoxins generally are defined as substances produced and

secreted by virulent strains of Gram positive organisms. Biochemically exotoxins are composed of relatively simple proteins with specific immunological characteristics. (4) They are considered to be heat labile in contrast to endotoxins. Physiologically, exotoxins are also capable of producing irreversible shock secondary to peripheral circulatory collapse. The exact mechanism of this action also remains largely unknown although it has been suggested that they produce a more direct action on the peripheral circulation than endotoxins. (64, 71, 72)

As newer techniques are being devised in bacteriological investigation, it is becoming apparent that the former rigid distinction between exotoxins and endotoxins is less precise and somewhat questionable. It has recently been shown that substances, having the properties of exotoxins, can be obtained in high concentrations following the autolysis of certain bacteria, or by extracting bacterial bodies with various solvents. (4) Crude lysates of the Gram positive beta-hemolytic Streptococci are capable of producing many characteristic physiologic effects of endotoxins when injected into rabbits. (68) Altemeier and associates recently have obtained a toxin from the Gram positive Staphylococcus aureus which has some of the physical properties of an endotoxin. (2) To solve this confusing problem it has been suggested that toxins be classified

simply by their derivation; exotoxins being produced by Gram positive bacteria and endotoxins being derived from Gram negative organisms. The Gram positive toxins would thereby be soluble toxins found outside the parent cell, and the Gram negative toxins would be structural components of the bacterial cell wall even though they can be found in cell free autolysates. (4)

While most investigations regarding bacterial toxins were confined to the Gram negative group, experimentation was never-the-less taking place with crude toxins produced by Gram positive organisms. As early as 1894 it was observed that Staphylococcus aureus was capable of producing a toxin having direct effects on leukocytes. (76) Von Linglesheim in 1900 first described local skin necrosis following an intradermal injection of crude staphylococcal toxin, (79) but it was not until the studies of Lewis in 1927 that an attempt was made to explain the mechanism of action of the toxin. (39) His experiments, supported 10 years later by Feldberg, (15) suggested that staphylococcal toxin produced a direct cell injury that resulted in the subsequent liberation of a histamine-like substance. It was further postulated that the similarity between the effects of many injurious substances could be explained by this common mechanism.

Since this initial hypothesis a review of the published work

concerning the pathologic effects of staphylococcal toxin on the circulation indicates that very little is actually known. Indicative of this attitude are the many hypotheses which have been suggested but none has gained general acceptance. A few of the more pertinent observations will be discussed briefly in the following:

A. The direct action of staphylococcal toxin on the pulmonary circulation: Kellaway in 1930 proposed that schock following the injection of staphylococcal toxin was due to right-sided heart failure subsequent to increased vascular resistance within the pulmonary circulation. (37) Evidence for this was based on the observation of an engorged liver presumably secondary to right heart failure. Venous pressure within the inferior vena cava, however, was not measured and more recent work has shown that the engorged splanchnic and hepatic vascular beds noted by Kellaway are secondary to hepatic venule constriction, (73) not increased pulmonary resistance.

B. The direct action of staphylococcal toxin on cardiac tissue: The direct action of staphylococcal toxin on the myocardium was considered following electrocardiographic studies on the intact rabbit heart. (13) Large doses of toxin were employed with cardiovascular collapse occurring within a very short time after the injection. Electrocardiographic changes characteristic of myocardial

damage were observed preceeding the final phase of circulatory collapse. Direct blood pressure recordings, however, were made in only three experiments and the rapidity of circulatory collapse would make it doubtful that myocardial function could be assessed independent of the generalized circulatory response. (13)

C. The indirect action of bacterial toxins via the liberation of a histamine-like substance: As mentioned previously, Von Linglesheim and others have suggested that a histamine-like substance is released into the serum following the injection of bacterial toxins. (32, 54, 55, 64, 67) While this theory has become a popular hypothesis for the action of Gram negative endotoxins on the circulation, it presently appears that staphylococcal toxin exerts a more direct effect on the peripheral circulation. (64, 71, 72)

D. The direct effect of bacterial toxins on the peripheral circulation: Janeway (35) was the first to show, and more recently Warren, et al, (80) have agreed, that the collapse seen in the course of acute infections was not due to cardiac failure, but rather to failure of the peripheral circulation. Reports have also implicated the central nervous system as being the site of action of bacterial toxins with secondary autonomic reflex action causing peripheral vascular collapse. (74) Most of the observations supporting this concept, however, have been made following the injection of endotoxins.

Others have questioned the importance of vasomotor function and the central nervous system as mediators of toxin action. Holzbach (34) and later Windfield (81) observed the direct action of bacterial toxins on the capillary wall without implicating central vasomotor activity. Rigdon (48) and later Aub (6) described the pathologic effect of both staphylococcal toxin and clostridial toxin as being a direct action on capillary endothelial cells, causing stasis and the extravasation of blood into the surrounding tissues.

Although it is apparent that the exact mechanism of action of staphylococcal toxin is still uncertain, it does seem probable that some direct vascular paralysis causing irreversible peripheral circulatory collapse is evoked following their injection of lethal doses.

E. The effect of staphylococcal toxin on renal physiology: The ability of staphylococcal toxin to produce kidney lesions was brought to light during studies by Van de Velde (76) on the in vivo leukocytic action of the toxin. Autopsy studies showed extensive necrosis of the cortical portion of the kidneys. Neisser and Levaditi (45) were the first to investigate necrosis of the renal parenchyma in rabbits following the intravenous injection of crude staphylococcal toxin. They interpreted this necrosis as being secondary to infarcts as evidenced by thrombosed vessels. Others,

however, were unable to demonstrate thrombi within the renal vasculature and suggested that the toxin has a direct and simultaneous injurious effect on the epithelial and endothelial cells of the glomerular tufts and tubules. (49) Later observations by Von Glahn and Weld (78) indicated that the changes observed in the vascular elements of the kidneys were farther advanced than those noted in the epithelium of the tubules. They suggested, in addition, that the tubular damage was subsequent to interferance within the glomerular circulation. Studies were also reported on the effect of low doses of the crude toxin which suggested some degree of selectivity of the toxin to renal tissue since comparable lesions could not be demonstrated in other organs.

To add to this confusion, Glynn (23) used special dyes that accentuated mitochondrial structures and reported a direct effect of the crude toxin on tubular epithelium preceeding glomerular capillary damage. Glynn suggested that the tubular epithelium was affected directly as a consequence of excretion of the toxin through the glomeruli. He further observed that damage seemed to be limited to the cortex.

De Nauasquez (12) pointed out the similarity between the anatomical and histological appearances of the kidneys in rabbits

with staphylococcal toxemia and of the kidneys seen in women dying with symmetrical cortical necrosis during pregnancy. He suggested that the predominant injury produced by the toxin lies in the media of the smaller renal arteries. De Nauasquez observed the extreme dilatation of the afferent arterioles and glomerular capillaries which appeared stuffed with red blood cells without interviening plasma spaces. This observation let to the theory of a relative increase in hemoconcentration within the glomerular tuft subsequent to the rapid loss of plasma fluid through the injured arterioles and capillaries. By this explanation, the hemoconcentration caused obstructive stasis with subsequent ischemic necrosis of the renal parenchyma. The author did not suggest that staphylococcal toxin was the mediator of bilateral cortical necrosis in humans, but suggested that some agent with a similar vaso-selective action may initiate the human lesions.

Trueta et al. (75) have studied the activation of intrarenal medullary shunts following staphylococcal toxin injection with subsequent cortical ischemia and necrosis. These hypothetical shunts are believed to be located in the juxtamedullary glomeruli, whose efferent vessels and the vasa recta into which they empty are much larger in calibre than those of the cortical glomeruli. Evidence for the presence of these shunts stems from India ink studies and direct

visualization of the intact kidneys following the injection of staphylococcal toxin. (44, 75) Blanching of the cortex with bright red blood in the renal vein was observed. Trueta suggests that these intrarenal shunts are controlled by the autonomic nervous system. Neither total nor effective renal blood flow was measured in these experiments.

The most recent investigations regarding the effect of crude staphylococcal toxin on renal physiology have been performed by Thal and his associates. (69, 70, 71, 73) They have directly visualized the kidney following toxin injection in the intact dog, and also have studied the effect of the toxin on the isolated organ. Contrary to Trueta's observations, Thal has reported considerable spasm in the renal vein suggestive of marked diminution of total renal perfusion. India ink studies have demonstrated the engorgement of medullary vessels with blanching and ischemia in the cortical region of the kidney. From these experiments Thal has suggested that the toxin primarily causes a venule constriction resulting in stasis ischemia. In more recent work Thal has presented evidence that staphylococcal toxin exerts a selective effect on smooth muscle cells producing an initial prolonged contraction with subsequent paralysis. (71)

It, therefore, seems apparent from this brief review that staphylococcal toxin does exert a profound effect on kidney physiology.

The exact mechanism of this seems highly speculative at the present time since studies have not been performed in the attempt to measure either effective or total renal blood flow as an assessment of renal function. Nor have experiments been performed on the intact animal. It is well known that laporotomy and manipulation of the aorta or renal pedicle will markedly alter and depress renal function without the added insult of bacterial toxins. An isolated kidney is essentially a denervated kidney which also may show differences in renal function.

In summary, the methods thus far used in the assessment of renal function have not accurately measured renal perfusion, nor have they been correlated with other systemic blood pressure effects of the toxin.

Methods and Procedure

A. Experimental plan: The plan was to measure systemic blood pressure and estimate renal function following the intravenous injection of staphylococcal toxin under three experimental conditions: 1) when hypotension and shock were the result of cardiovascular alterations secondary to a large dose of toxin; 2) when hypotension and shock resulting from the toxin injection were temporarily reversed by infusion of the pressor drug, angiotensin; and 3) when

hypotension was not a factor in producing renal physiological alterations.

The toxin employed was originally produced by the Lederle Corporation (#42925-207; protein content 1.6 mg/ml; hemolytic titre 1:512). Preliminary studies at the University of Cincinnati have indicated that its classification lies somewhere between an exotoxin and endotoxin. It is an antigenic substance, thereby resembling an exotoxin; it is heat stable and thus resembles an endotoxin. It is recovered from the supernatant fluid and is diffusible, a property common to both exotoxins and endotoxins. (1)

B. Experimental procedure: A total of 23 adult mongrel female dogs weighing between 11.0 and 22.0 kg. were used in the three experimental studies. The dogs were anesthetized with Dial anesthetic (Ciba--0.5 ml/kg. initially with supplementary doses of 0.15 ml/kg. administered as needed) approximately 16-18 hours before experimentation.

In all experiments a one hour hydration period with the intravenous infusion of 0.2 ml/kg/min. of a 1:1 solution of Ringer's Lactate and 5% dextrose in water with PAH (1.0 - 1.3 g/L.) and creatinine (3.5 - 6.0 g/L.) added preceded a two hour control period. During the two hour control period continuous blood pressure

recordings were made and blood and urine collections were made at 30 minute intervals to estimate renal function. At the end of the two hour control period, staphylococcal toxin was injected intravenously followed by a two and one-half hour experiment. Blood pressure recordings were made from the carotid artery by means of a Sanborn 964 direct recording monitor.

The femoral vein was canulated for withdrawal of blood specimens, and the urinary bladder was catheterized with an indwelling No. 12 Foley catheter. In all experiments renal function was assessed by sequential determinations of urine and blood osmolality and concentrations of PAH, creatinine and sodium. Urine flow was measured via dependent catheter drainage. Blood urea nitrogen and hematocrit were determined during the control period, midway and at termination of the experimental period.

Group I experiments: 15 experiments were performed to study the blood pressure response to doses of toxin ranging from 0.10 ml/kg. to 0.25 ml/kg.

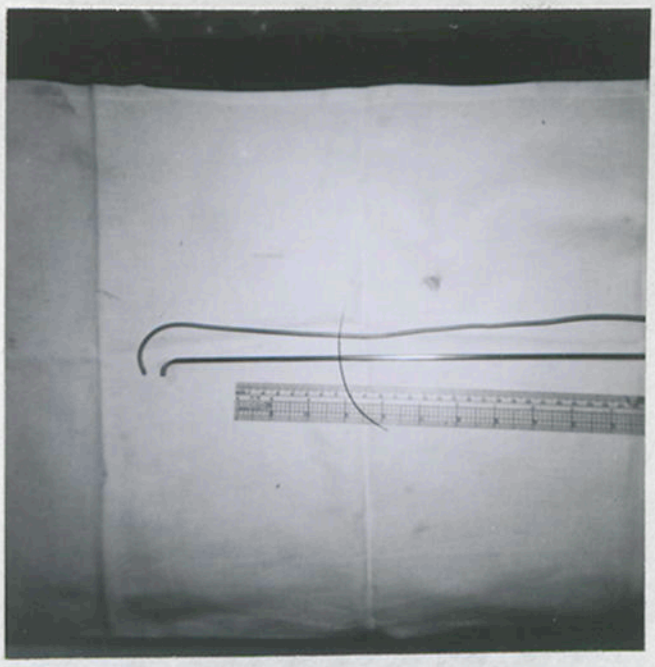
Group II experiments: Six experiments were performed in the attempt to reverse staphylococcal toxin shock with the pressor agent, angiotensin. Following the intravenous injection of a lethal dose of toxin (0.25 ml/kg) and a fall in the mean systemic blood pressure of 25 mm Hg., a continuous intravenous infusion of angiotensin was

started at the rate of 1 ug/kg/min.

Group III experiments: Five experiments were performed in the attempt to study the specific renal response in the intact dog to a dose of toxin insufficient to cause significant systemic blood pressure changes. Dosages of toxin ranged from 0.10 ml/kg. to 0.12 ml/kg.

Group IV experiments: Three experiments were performed in the attempt to study the direct effect of staphylococcal toxin on total renal blood flow in the intact dog with the aim of correlating with effective blood flow to determine the possible presence of intrarenal vascular shunts. A curved No. 15 radio-opaque Swedish catheter was inserted under fluoroscopy into the right renal vein via the left femoral vein. A specially designed aluminum catheter containing an equal length of No. 19 tubing and hollowed at the end so as to allow flow of blood into the renal artery was inserted into the right renal artery via the left femoral artery. (Plate 1) Through this catheter a known concentration of cardio-green dye could be injected into the renal artery with the simultaneous withdrawal of renal venous blood through a Curvette densitomer at a constant rate of 24.6 ml/min. (40) After approximately 20 cc. of blood had been withdrawn and the dilution curve had been recorded on a Sanborn direct recording monitor, the blood was reinfused into the dog via the renal vein catheter. Total renal blood flow less lymphatic drainage and urine flow can then be calculated

A.



B.

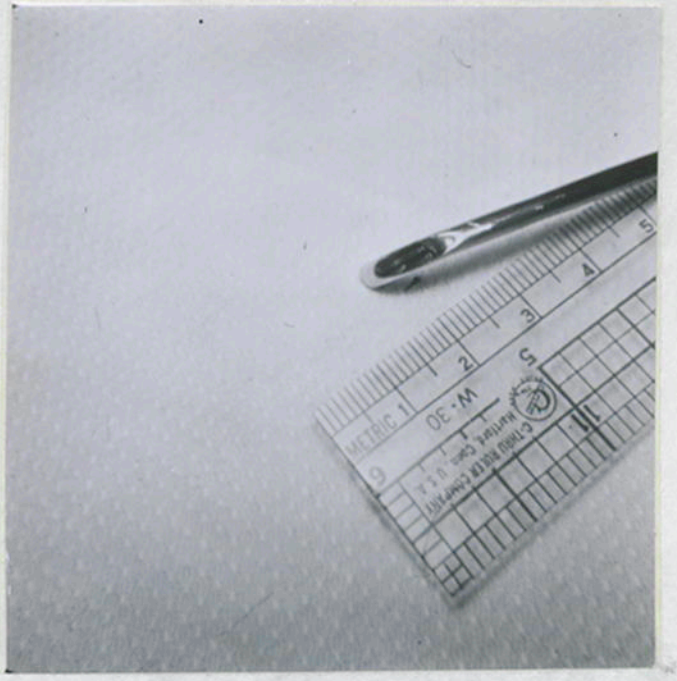


Plate 1. Plastic and metal catheters used to canulate renal vein and artery, respectively, shown in A. Closer view of curved end of metal arterial catheter shown in B. Note No. 19 tubing within hollowed end to facilitate dye injection without altering blood flow to renal artery.

by the standard dye dilution formula developed for cardiac output. (19, 20)

Group V experiments: Two experiments were performed in the attempt to reverse the renal failure resulting from low doses of staphylococcal toxin. The vasopressor drug, angiotensin, was used in one experiment and the vasodilator agent, hydralazine, was used in the other. A dose of staphylococcal toxin insufficient to cause significant blood pressure changes, 0.11 ml/kg., was injected followed 15 minutes later by the test drug. A continuous intravenous infusion of 0.1 ug/kg/min. of angiotensin was used in the vasopressor experiment. A single intravenous dose of 0.4 mg/kg. of hydralazine was used in the vasodilator experiment.

C. Analytical methods: Venous blood analyses included serum osmolality, sodium, creatinine and PAH concentrations. Urine volumes were measured to the nearest tenth of a milliliter. Urine analyses consisted of determinations of osmolality, sodium, creatinine and PAH concentrations. Hematocrit was measured by the microhematocrit technic using the Drummond microhematocrit apparatus.

Osmolalities of serum and urine were measured by freezing point depression using a Fiske osmometer. (62) Sodium concentrations in serum and urine were determined with an internal standard flame photometer. (62) Creatinine was determined from trichloracetic

acid filtrates of plasma by adsorption on Lloyd's reagent and color development with alkaline picrate, and in the urine by a modification of the Folin method with photometric measurement. (26) Serum and urine PAH concentrations were determined by the method of Smith and his associates. (62)

Two blood samples, one during the control and one late in the experimental period, in each experiment, were analysed for urea nitrogen, using the method of Van Slyke and Cullen. (77)

For determinations of total renal blood flow, blood was withdrawn from the renal vein catheter through a Cruvette densitometer at a constant rate of 24.6 ml/min. following the injection of 0.094 - 0.156 mg. of cardio-green dye into the renal artery. (40) The dilution curve was projected for calculation onto a Sanborn direct recording monitor.

D. Calculations: The rate of excretion of creatinine (mg/min.) divided by plasma creatinine concentration (mg/ml) was used to measure creatinine clearance (ml/min), an estimation of glomerular filtration rate. (62) The clearance of PAH (ml/min) was calculated by dividing the rate of excretion (mg/min) by the plasma PAH concentration (mg/ml), an estimation of the renal plasma flow. (62)

Free water clearance (C_{H_2O}) was calculated as the difference between urine flow (V) and osmolar clearance (C_{osm}). (28)

Because of hypertonic urine, osmotic clearance is greater than urine flow. Therefore, the difference between the two equals the amount of water which must have been reabsorbed to produce hypertonic urine. The active transfer of water is necessary to produce "free" water and when the kidney can no longer concentrate its urine a so-called "fixed" specific gravity of urine occurs. Therefore, values of free water clearance provide a good assessment of distal tubular function. (59)

Sodium clearance (C_{Na}) was calculated by dividing the rate of excretion (mg/min) by the plasma sodium concentration (mg/ml). (62) The percent of filtered sodium appearing in the urine was calculated by dividing the sodium clearance (C_{Na}) by the glomerular filtration rate (C_{Creat}). (28, 57) Since sodium is actively reabsorbed in the proximal tubules, the percentage of filtered sodium appearing in the urine provides a good assessment of proximal tubular function.

Total renal blood flow was calculated from the dye dilution formula developed for measurement of cardiac output. (20) Renal blood flow (ml/min) equals 60 (sec/min) multiplied by the quantity of dye (mg) injected into the renal artery divided by the total sum of the concentrations recovered in the renal venous blood. Renal blood flow (ml/min) is divided by the weight of the kidney and then multiplied by 100, giving flow in ml/min/100 grams of tissue.

Results

Group I experiments: A. Blood pressure changes: Nine experiments were performed employing an intravenous dose of 0.25 ml/kg of staphylococcal toxin. In six out of nine experiments there was an initial rise in blood pressure occurring within the first five minutes followed by a precipitous fall in pressure to shock levels from which recovery did not occur. (Figs. 1, 2) In the other three experiments there occurred an immediate fall in blood pressure to shock levels without recovery.

One experiment was performed using a dose of 0.15 ml/kg of toxin. This demonstrated an initial increase in mean blood pressure followed by a rapid fall to shock levels with a subsequent recovery of pressure to near control levels followed by a gradual decline in blood pressure over the two hour experimental period. (Fig. 3) This typical so called "biphasic" curve has been reproduced many times by Altemeier's group using identical doses. (1)

Five experiments were performed using doses of 0.10-0.12 ml/kg of toxin. These demonstrated relatively minor blood pressure changes. A slight gradual rise was noted in one experiment followed by a return to control levels. (Fig. 4) In the other experiments a very gradual decline in mean pressure was observed over the two and one-half hour experimental period, but not believed sufficient to

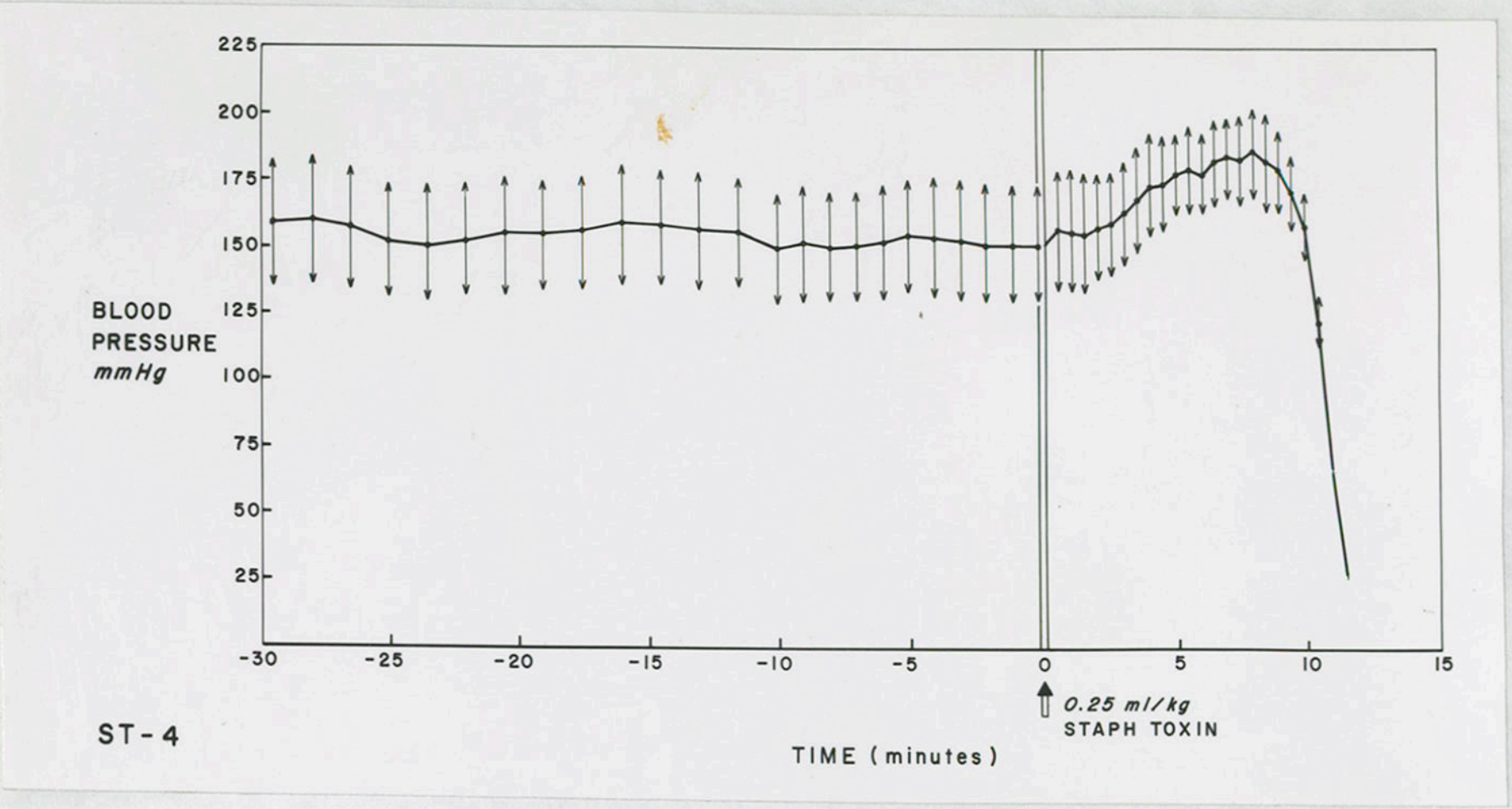


Fig. 1 The effect of 0.25 ml/kg. staphylococcal toxin on systemic blood pressure. Note initial rise in pressure followed by precipitous fall to shock levels.

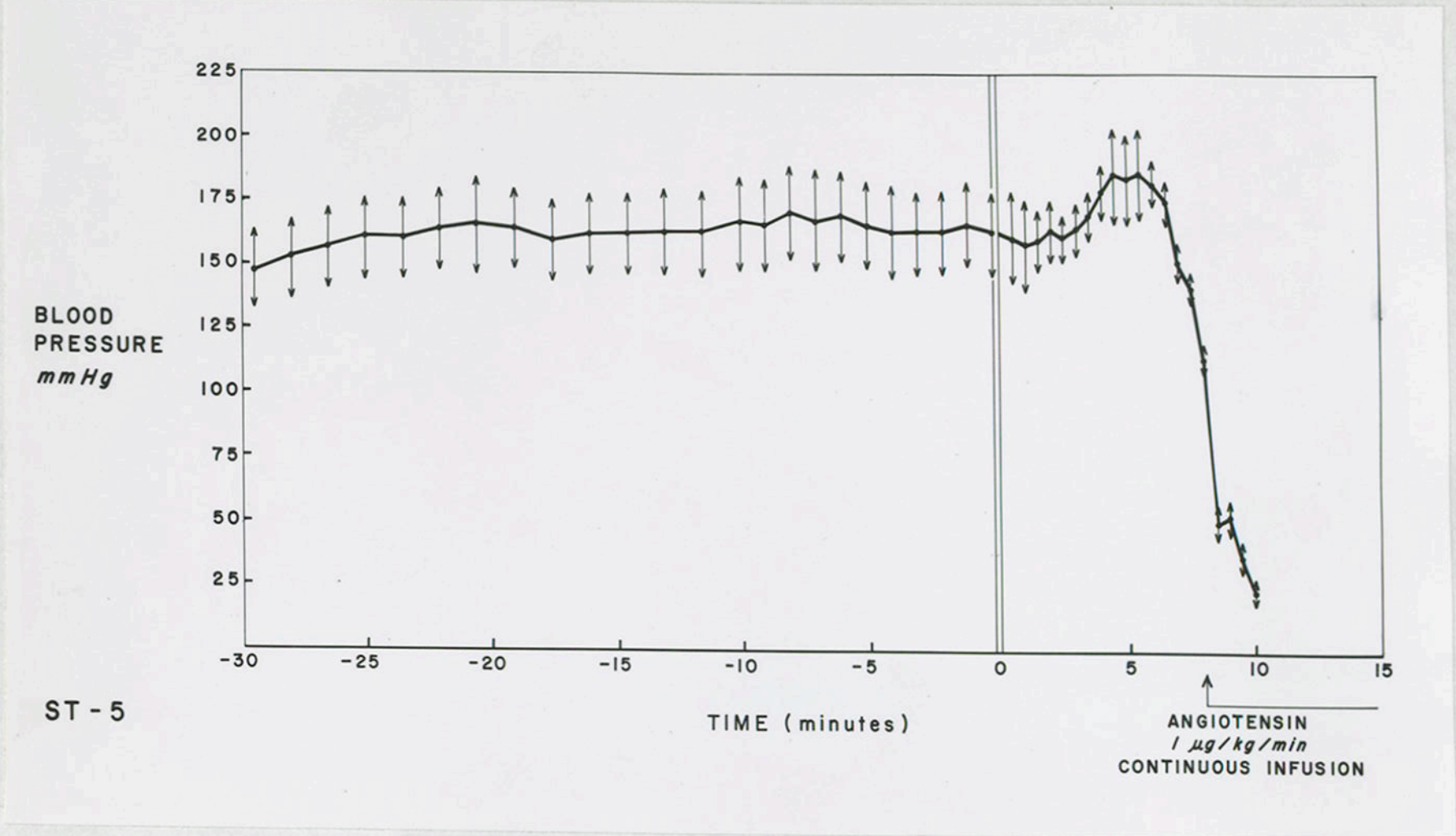


Fig. 2 The effect of 0.25 ml/kg. staphylococcal toxin (injected at Time 0) on systemic blood pressure. Note initial rise in pressure followed by precipitous fall to shock levels without recovery or response to intravenous angiotensin infusion.

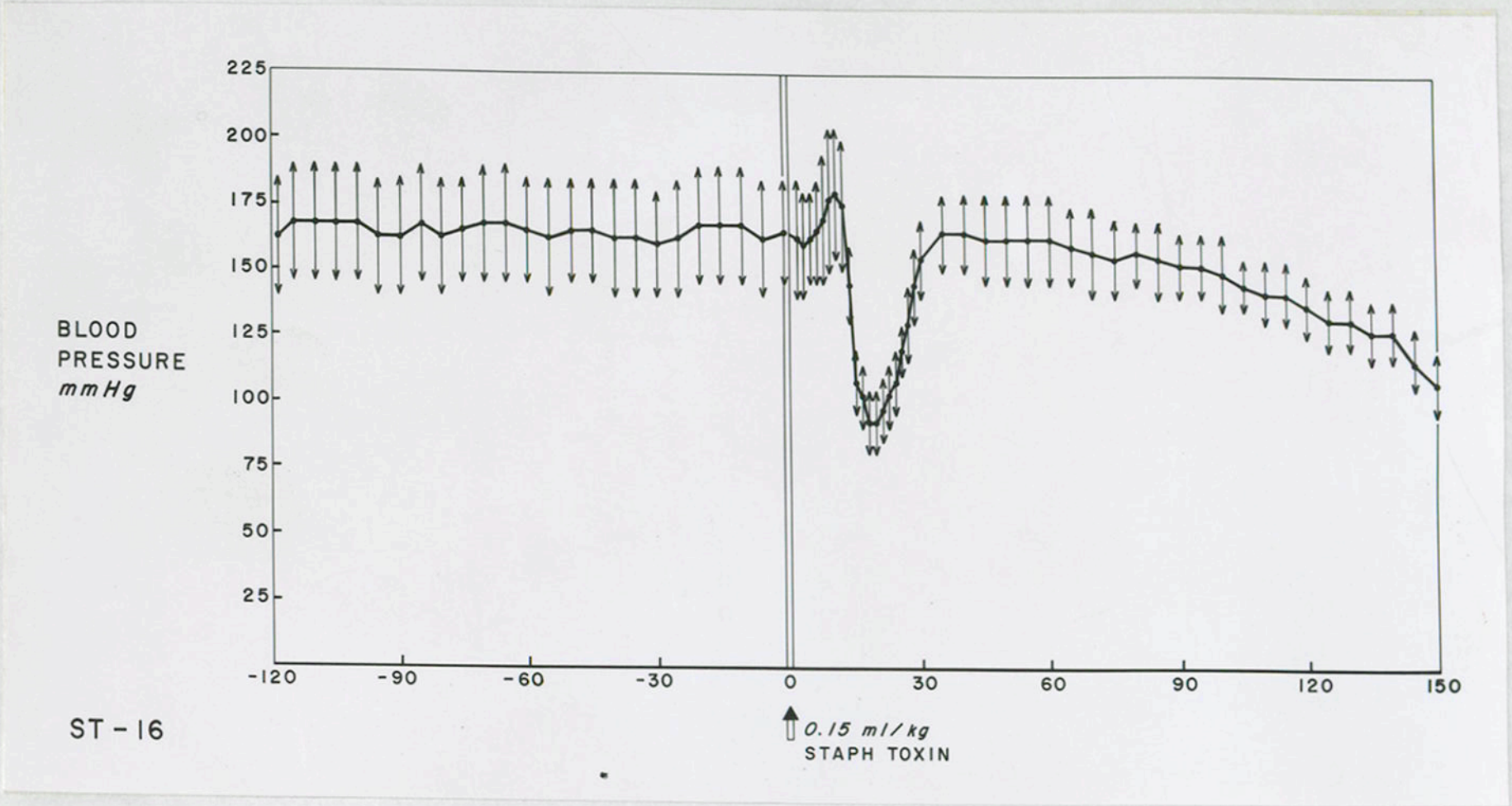


Fig. 3 The effect of 0.15 ml/kg. staphylococcal toxin on systemic blood pressure. Note initial rise in pressure followed by a rapid fall to shock levels with a subsequent recovery of pressure to near control levels followed by a gradual decline in blood pressure over the two and one half hour experimental period.

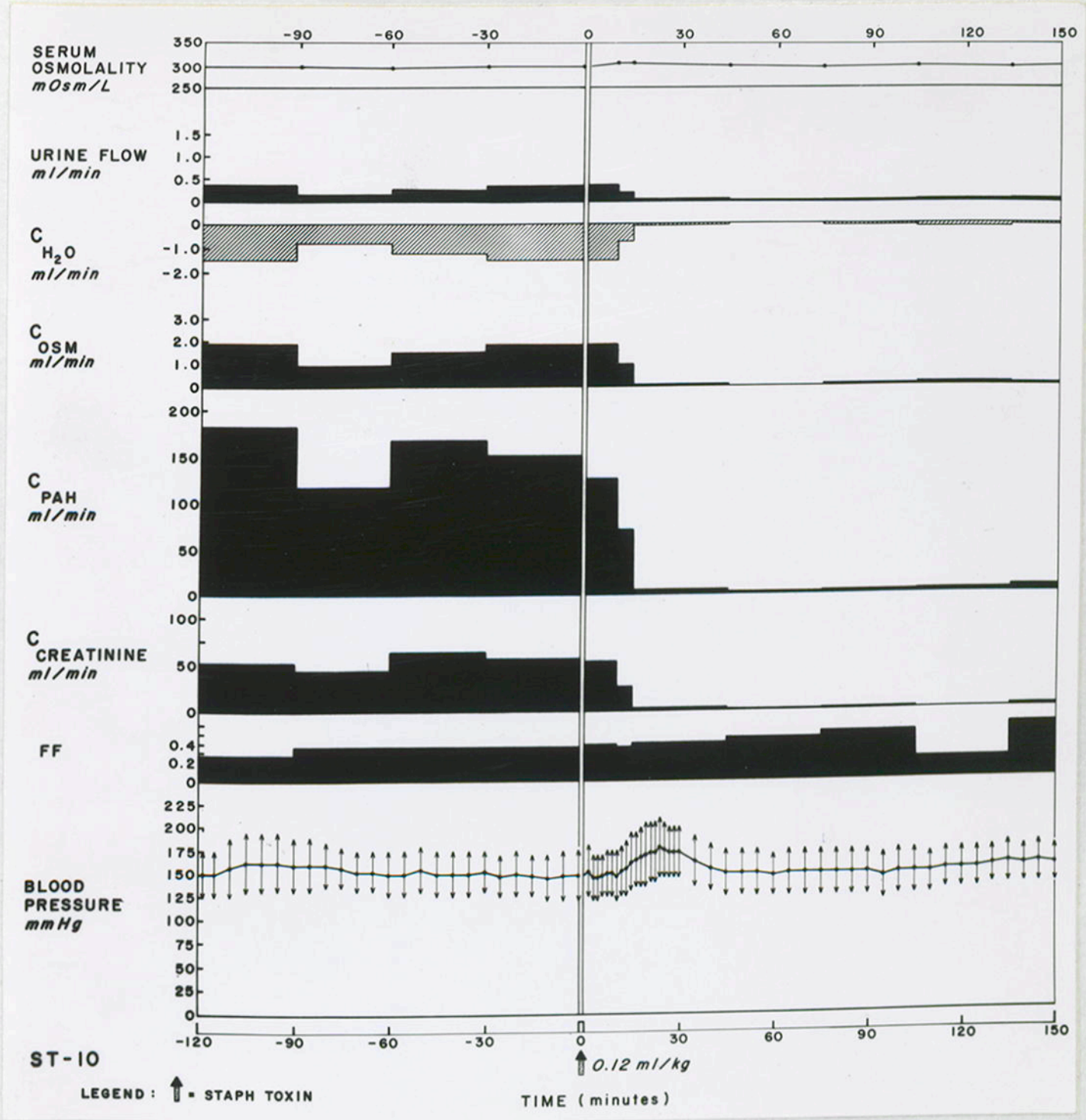


Fig. 4 The effect of 0.12 ml/kg. staphylococcal toxin on systemic blood pressure and renal function. Note relatively minor blood pressure changes but marked decrease in effective renal plasma flow (C_{PAH}) and glomerular filtration ($C_{creatinine}$). Osmolar and free water clearances are also markedly decreased.

cause alterations in tissue perfusion.

B. Renal function changes: All nine experiments using 0.25 ml/kg of toxin demonstrated a prompt and marked diminution of effective renal plasma flow as indicated by PAH clearances. Glomerular filtration was also markedly reduced as indicated by creatinine clearances. Oliguria and in some cases anuria were noted in all nine experiments. In four experiments marked diminution of effective renal blood flow was noted before hypotension occurred, as best illustrated in figures 5, 6, and 7. In the other five experiments hypotension occurred before renal function changes could be demonstrated.

Group II experiments: Six experiments were performed in the attempt to reverse staphylococcal toxin shock (0.25 ml/kg) with angiotensin. Following a drop in the mean blood pressure of approximately 25 mm Hg., continuous intravenous infusion of angiotensin, lug/kg/min., was started.

A. Blood pressure changes: An immediate rise in systemic blood pressure to hypertensive levels occurred in three out of six experiments. (Figs. 8, 9, 10) No increase was noted in one experiment as the pressure rapidly reached shock levels without recovery. (Fig. 2) Normotensive pressures could be maintained in only one of the six experiments following the angiotensin-induced initial

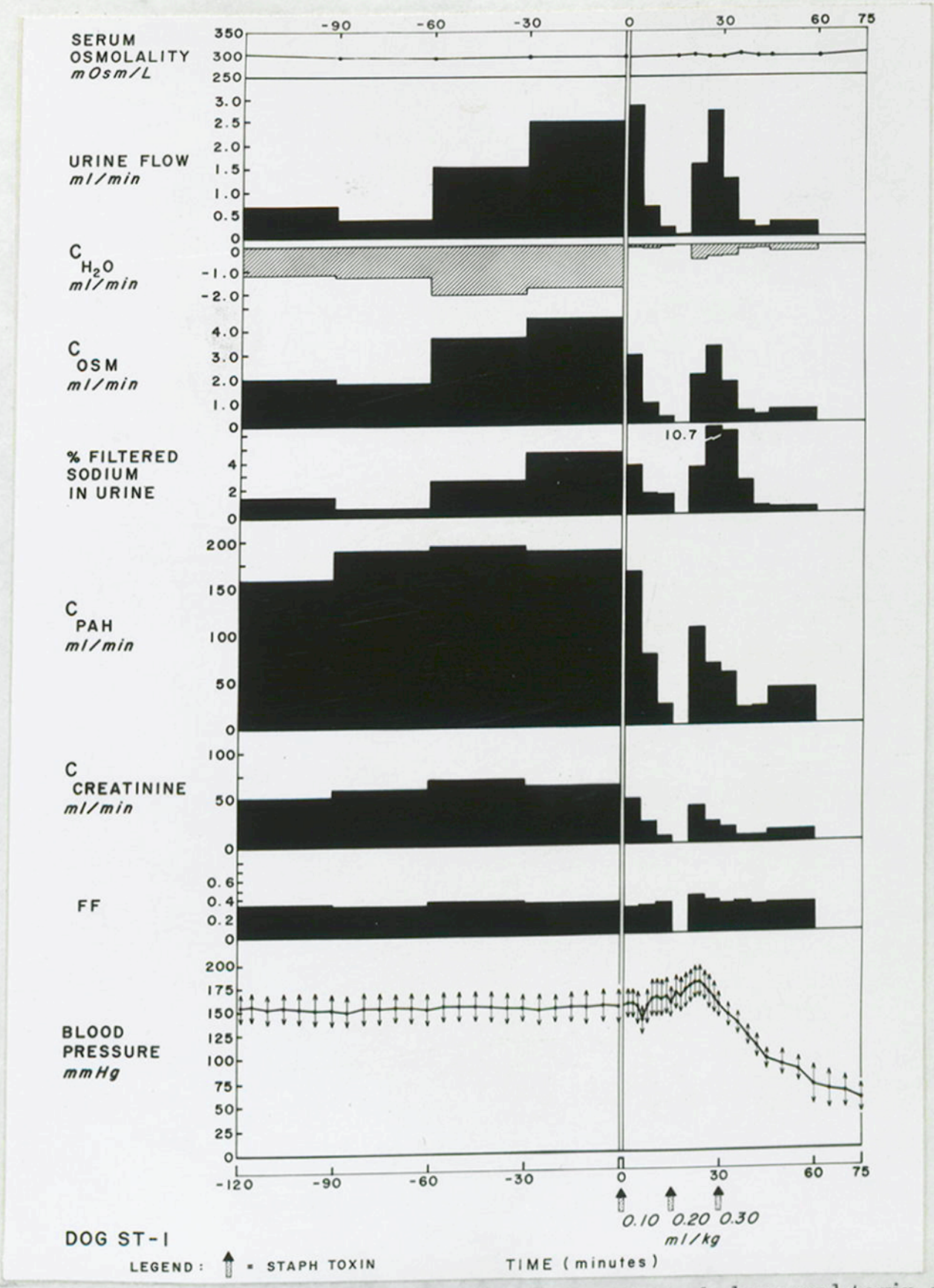


Fig. 5. The effect of increasing doses of staphylococcal toxin on systemic blood pressure and renal function. Note the marked decrease in effective renal plasma flow, glomerular filtration, and osmolar and free water clearances following the initial dose of 0.10 ml/kg. of toxin before significant changes in blood pressure occurred.

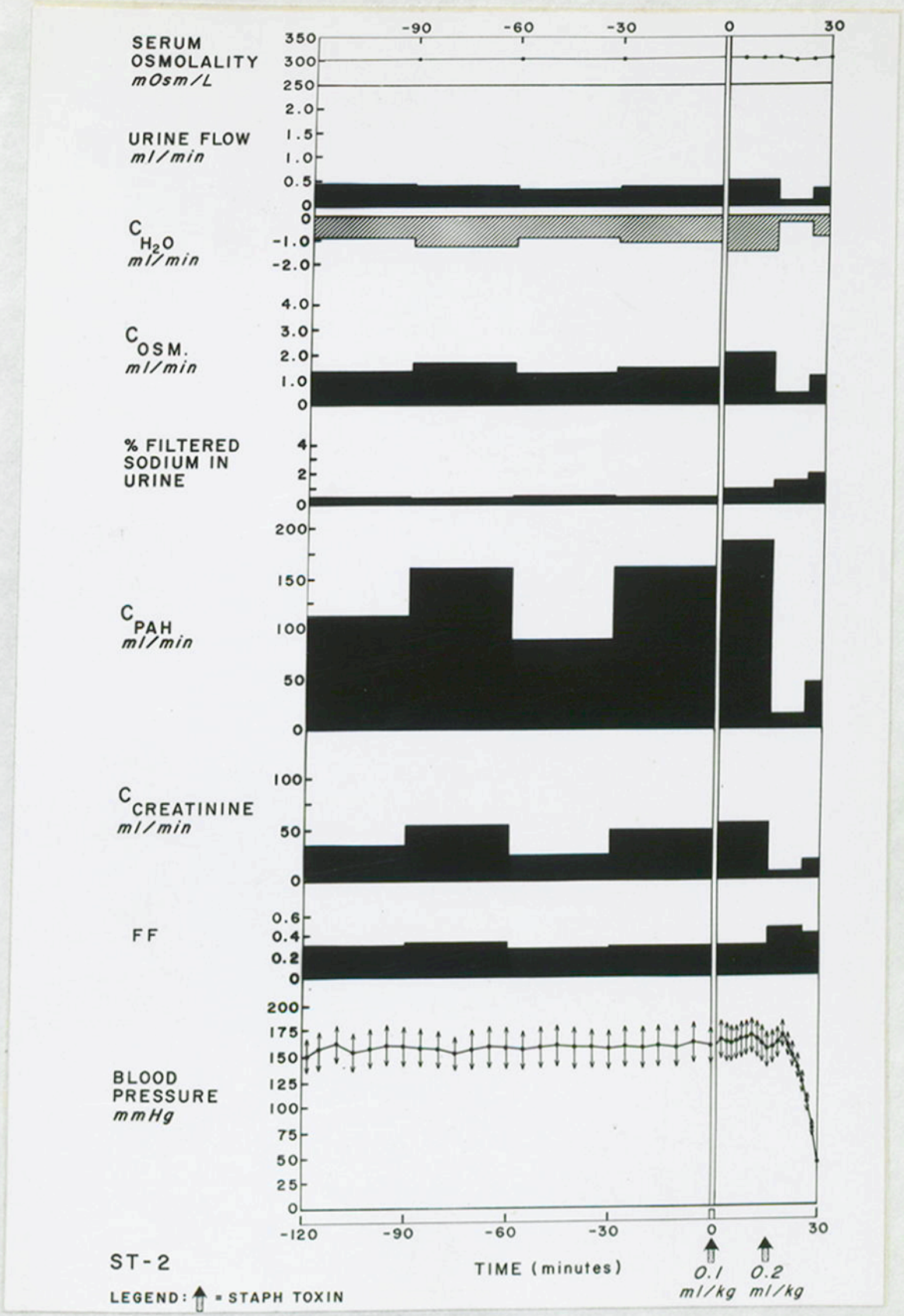


Fig. 6. The effect of increasing doses of staphylococcal toxin on systemic blood pressure and renal function. Note decrease in effective renal plasma flow and glomerular filtration occurring before significant hypotension. Also note the rise in percentage filtered sodium appearing in the urine.

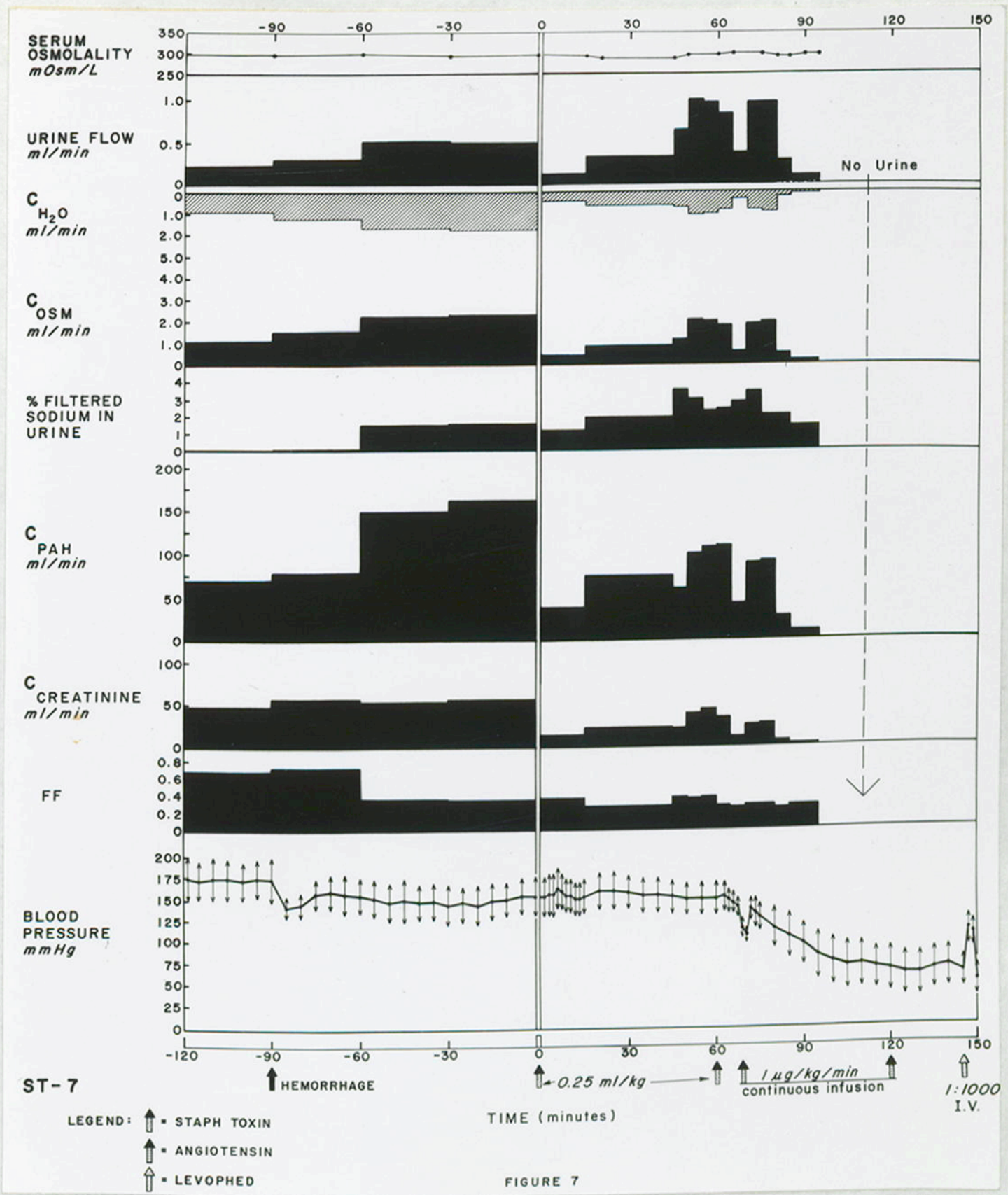


FIGURE 7

Fig. 7 The effect of multiple doses of 0.25 ml/kg. of staphylococcal toxin on renal function with attempted reversal of hypotension with antiotensin. Note decrease in renal function following initial injection despite normotensive blood pressure. Note initial blood pressure response to angiotensin but marked tachyphyloxis despite continuous infusion. Note pressor response to single injection norepinephrine at termination of experiment.

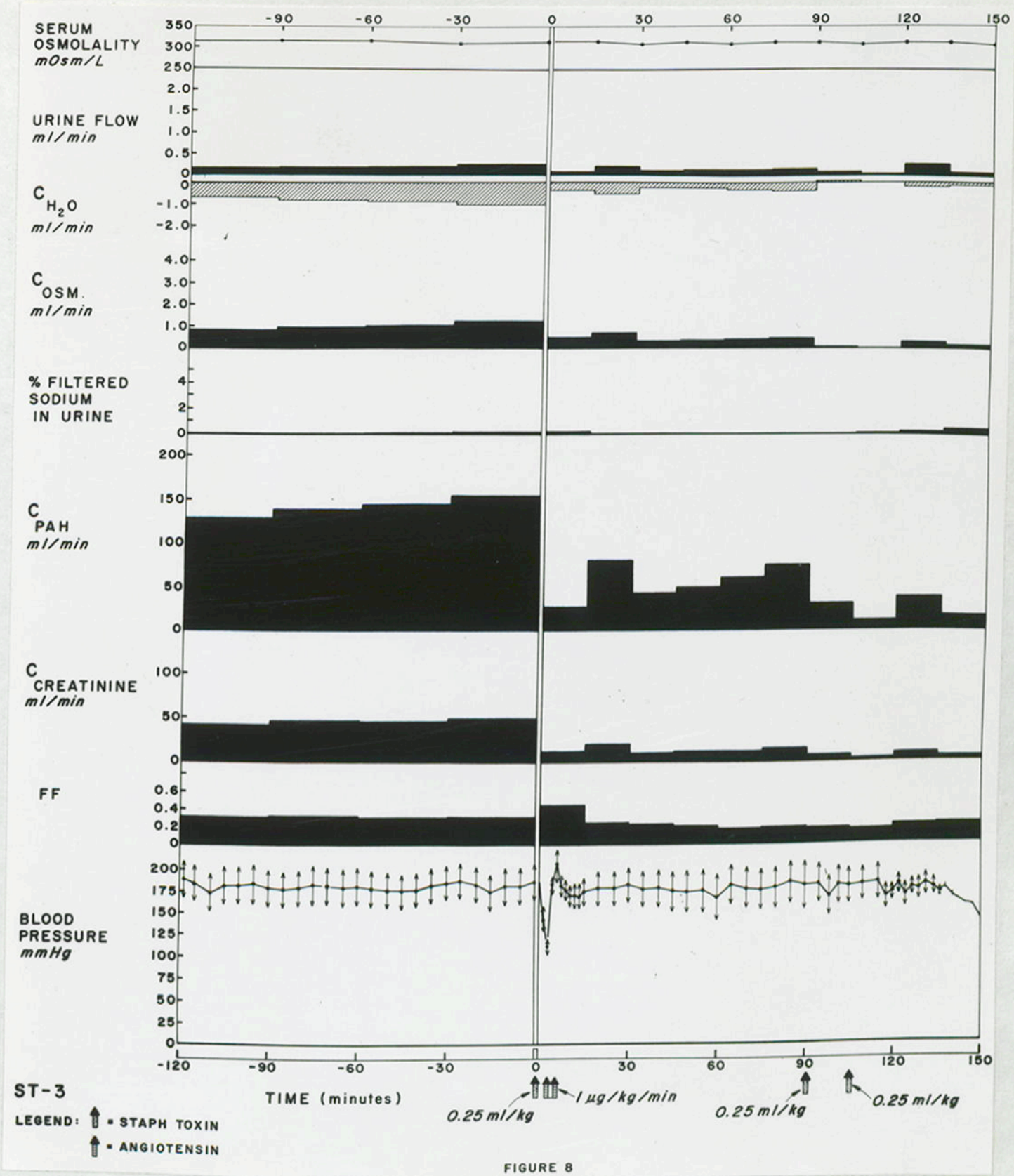


FIGURE 8

Fig. 8. The effect of antiotensin in reversing hypotension following the injection of 0.25 ml/kg. staphylococcal toxin. Note initial hypertensive response to the pressor drug with subsequent restoration of normotensive levels. Despite maintenance of normal blood pressure, however, renal function never returned to control levels.

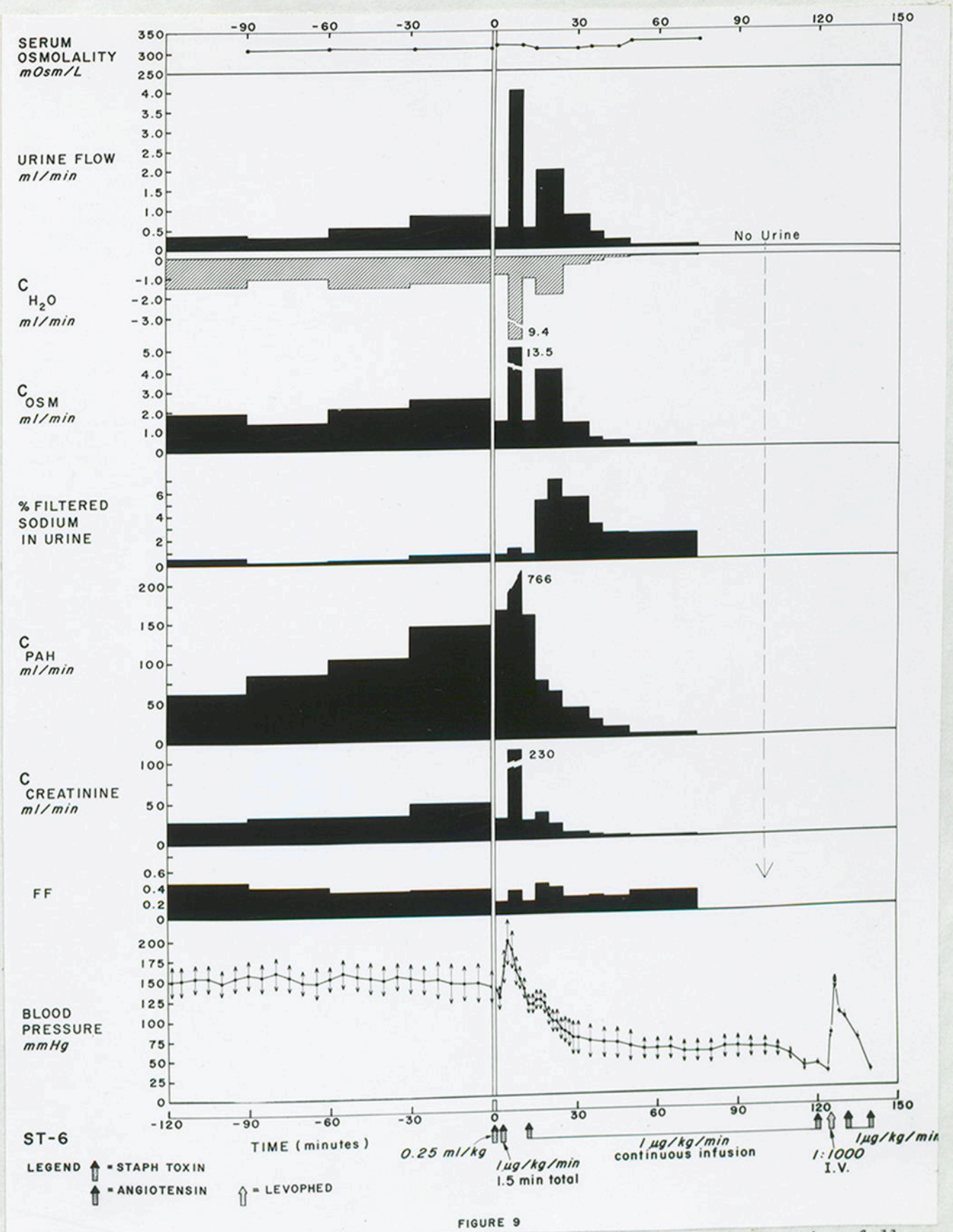


FIGURE 9

Fig. 9. The effect of angiotensin in reversing hypotension following injection of 0.25 ml/kg. staphylococcal toxin. Note initial hypertensive rise in blood pressure but subsequent gradual fall in pressure to shock levels despite continuous angiotensin infusion. Note transient increase in urine flow and osmolar clearance followed by marked decrease.

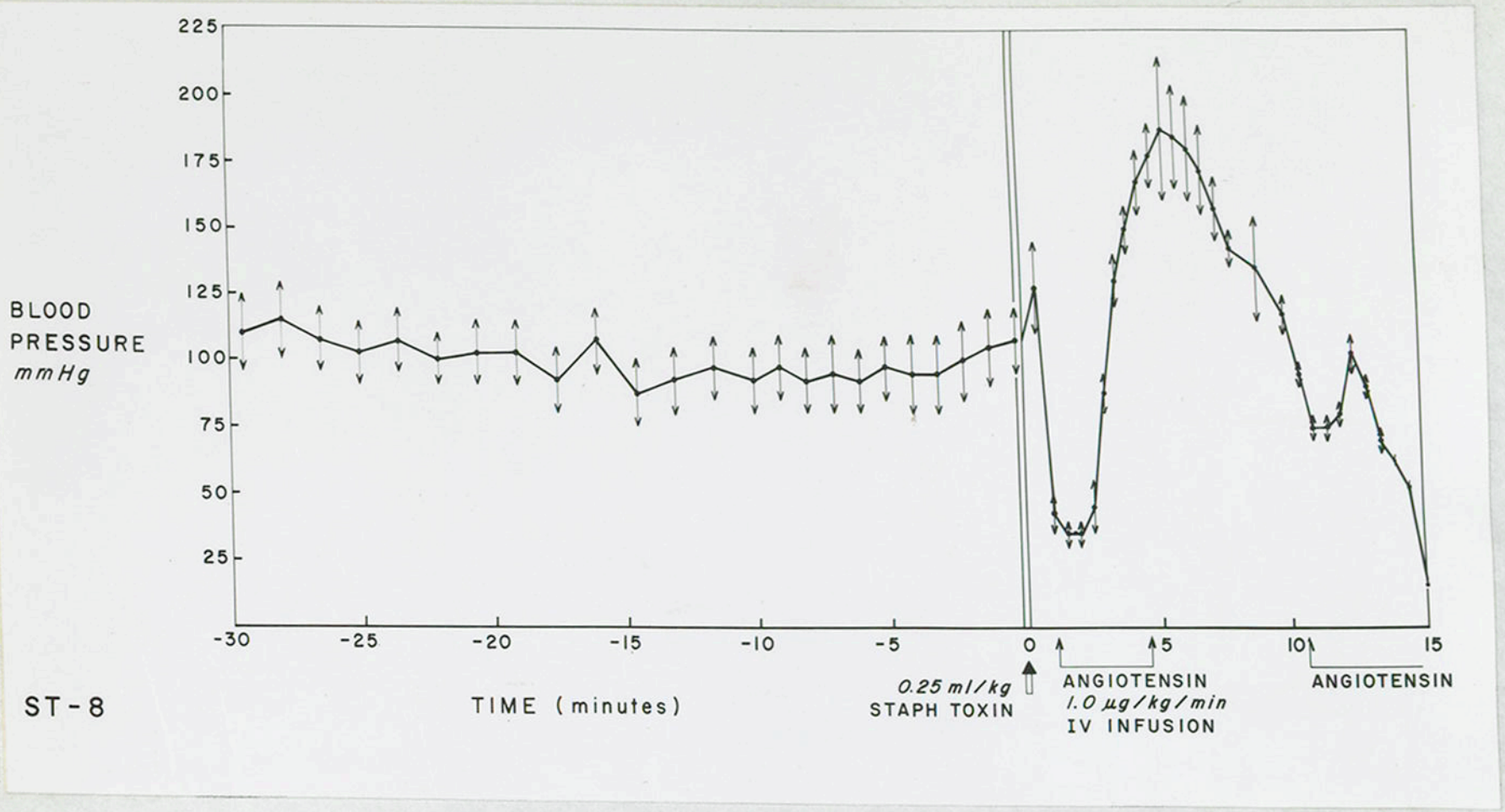


Fig. 10. The effect of angiotensin in reversing hypotension following the injection of 0.25 ml/kg. staphylococcal toxin. Note initial hypertensive response to the pressor drug followed by gradual fall in pressure with tachyphylaxis to subsequent angiotensin infusion.

hypertensive stage. (Fig. 8) In all other experiments the blood pressure steadily dropped to shock levels following the initial rise. Further administration of angiotensin was without sustained effect as the animals showed marked tachyphylaxis to the pressor drug. (Figs. 7, 9, 10, 11) At the termination of two experiments norepinephrine was injected intravenously with a marked blood pressure response, despite the continued tachyphylaxis to angiotensin. (Figs. 7, 9)

B. Renal function changes: In one out of four experiments transient improvement of urine flow and osmolar clearance was noted following the infusion of angiotensin. (Fig. 9) Control clearances, however, of PAH and creatinine could not be sustained despite continuous angiotensin infusion and showed marked diminution throughout the remainder of the experimental period. In the other three experiments angiotensin did not alter the typical renal response to the toxin.

Group III experiments: Since hypotension is known to alter renal perfusion, only those experiments in which hypotension was not a factor in altering renal function will be reported.

In three experiments using a dose of 0.25 ml/kg of toxin, hypotension was not immediately apparent. All three experiments, however, showed an immediate and marked diminution of urine flow and clearances of PAH and creatinine. (Seen best in Fig. 7)

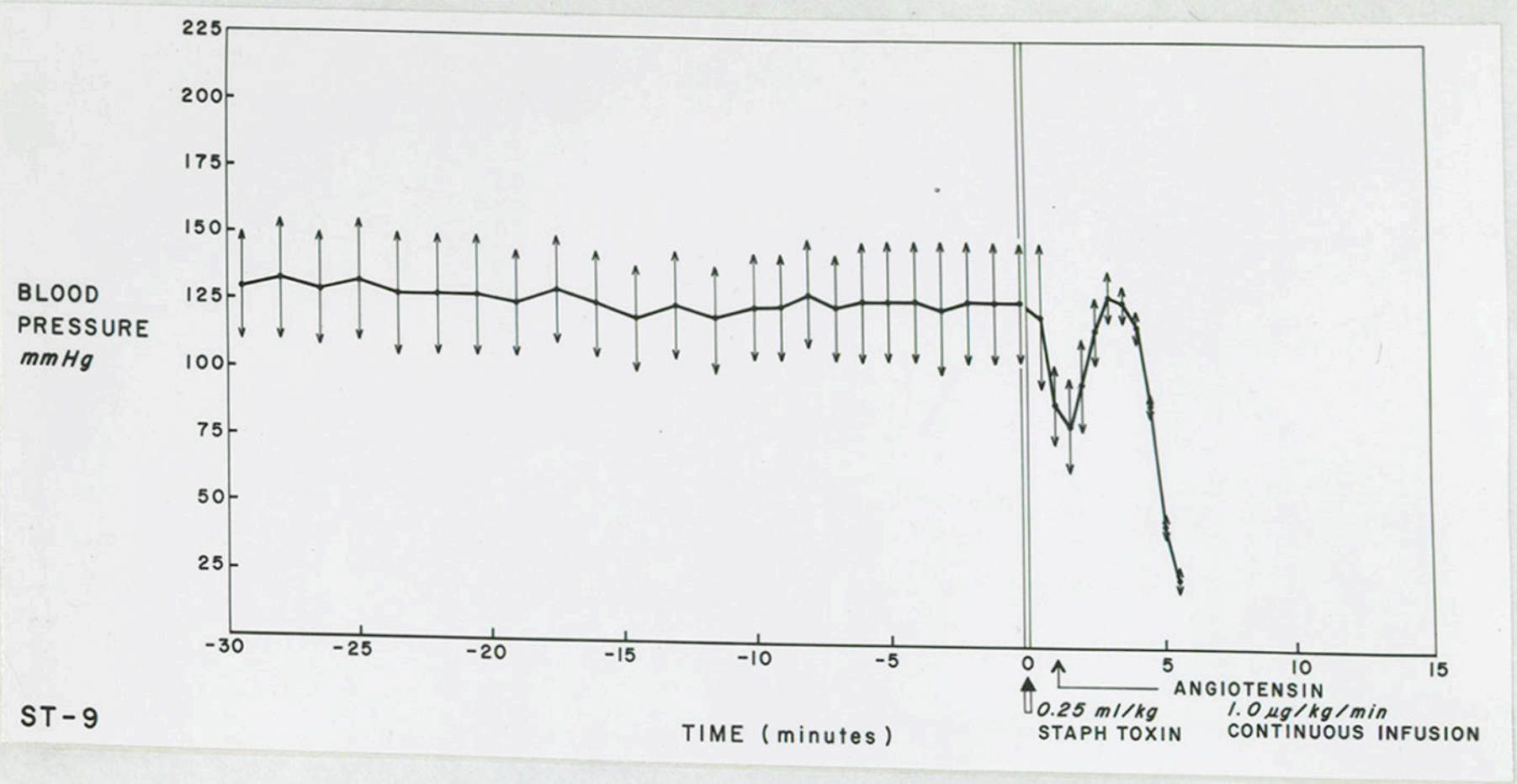


Fig. 11. The effect of angiotensin in reversing hypotension following the injection of 0.25 ml/kg. staphylococcal toxin. Note initial pressor response to the drug but subsequent tachyphylaxis despite continuous infusion.

Osmolar clearance and free water clearance were also markedly diminished prior to the terminal episode of hypotension. Although it cannot be stated with any certainty, it would appear that this renal response occurred independently and preceeding the hypotensive phase of toxin shock.

In contrast to experiments employing higher doses of staphylococcal toxin, hypotension was not a significant factor in animals receiving 0.10 - 0.12 ml/kg. Renal function and blood pressure curves are shown in figures 4, 12, 13, 14 and 15.

Oliguria and occasionally anuria were noted within 10 to 20 minutes following the toxin injection in all experiments. Subsequent but brief return of urine flow to near control levels did occur as a late event in three of five experiments. Even in these three experiments, however, the PAH, creatinine and osmolar clearances remained well below control levels throughout the experimental period indicating markedly diminished effective renal plasma flow, glomerular filtration and tubular function.

The free water clearance was markedly reduced throughout the experimental period in four out of five experiments. The percentage of filtered sodium appearing in the urine increased in all four experiments in which it was measured. These values are suggestive of tubular dysfunction. The filtration fraction tended to

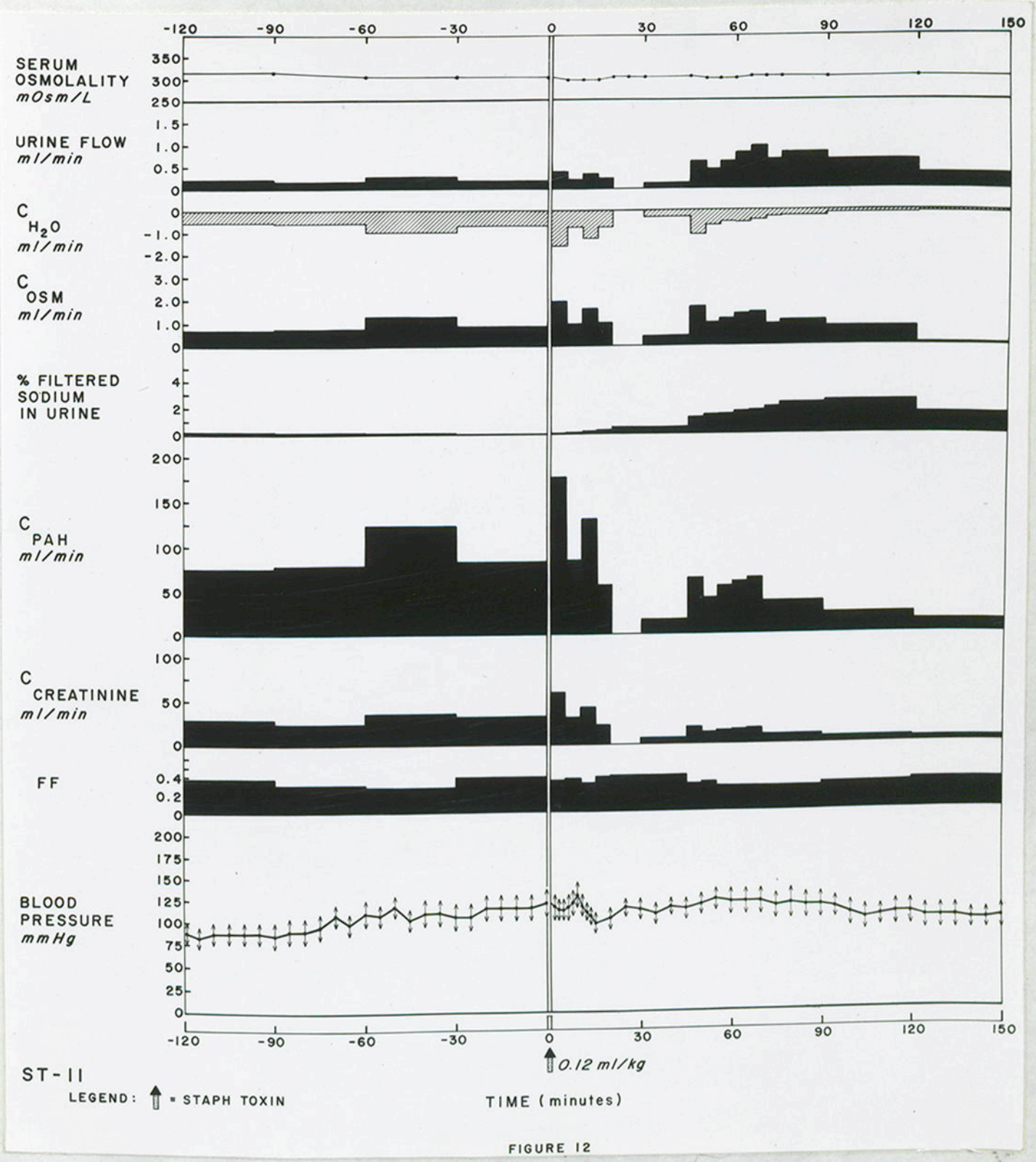


FIGURE 12

Fig. 12. The effect of 0.12 ml/kg. staphylococcal toxin on systemic blood pressure and renal function. Note marked decrease in renal function without significant blood pressure changes. Note rise in percentage filtered sodium appearing in the urine.

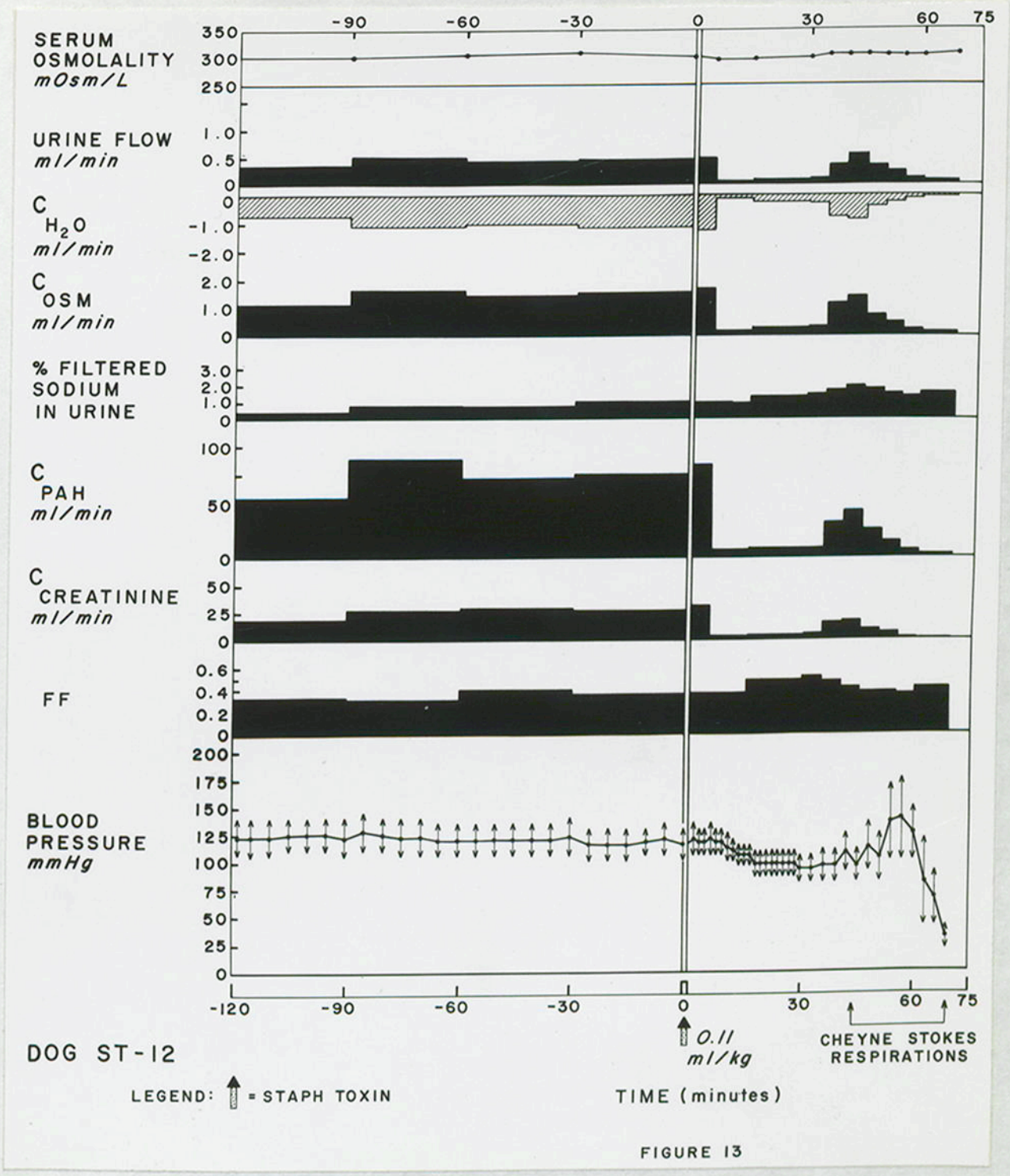


Fig. 13. The effect of 0.11 ml/kg. staphylococcal toxin on systemic blood pressure and renal function. Note marked decrease in renal function before hypotension and onset of Cheyne-Stokes respirations.

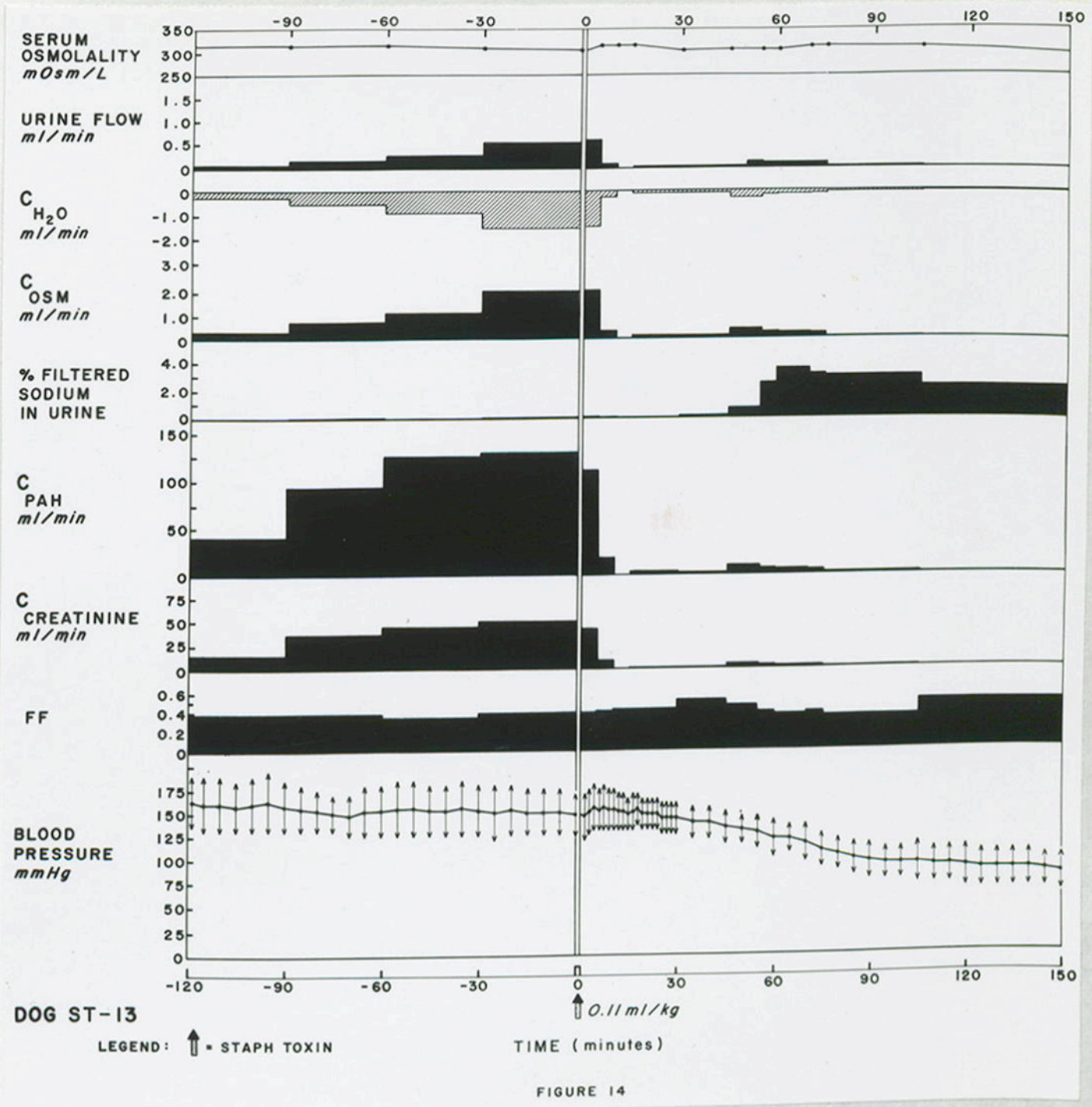


FIGURE 14

Fig. 14. The effect of 0.11 ml/kg. staphylococcal toxin on systemic blood pressure and renal function. Note decrease in renal function and rise in percentage of filtered sodium appearing in the urine.

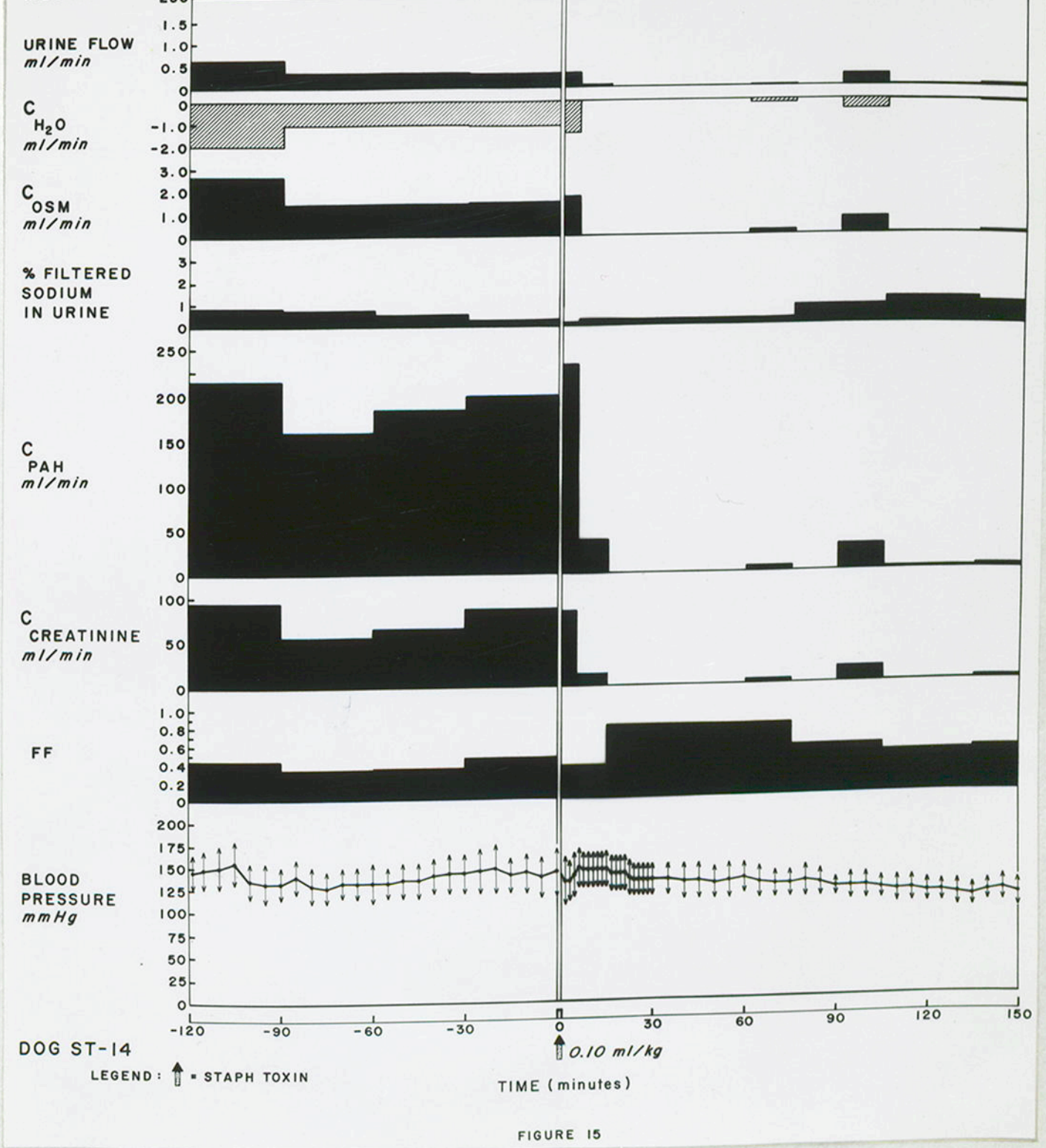


FIGURE 15

Fig. 15. The effect of 0.10 ml/kg. of staphylococcal toxin on systemic blood pressure and renal function. Note marked decrease in renal function without significant blood pressure changes.

increase in three of the five experiments.

Group IV experiments: In these experiments seven to nine determinations of total renal blood flow were calculated during the two hour control period. (Figs. 16, 17, 18) These averaged from 239 ml/min/100 grams kidney tissue in one experiment to 197 ml/min/100 grams kidney tissue in another. The third experiment, ST-23, averaged 204 ml/min/100 grams kidney tissue. Although these flows do not quite approximate the value of 300-400 ml/min/100 grams kidney tissue generally accepted for total renal blood flow, they are useful to show relative changes in flow.

Within five minutes following the injection of 0.11 ml/kg of toxin all three experiments demonstrated a marked flattening of the dye dilution curves to the point where they could not be accurately measured. In all experiments this occurred shortly before the clearances of PAH and creatinine became markedly reduced. (Figs. 16, 17. 18) This is due to the slight delay caused by the excretory dead space. In one experiment total blood flows once again were measureable at 25 and 30 minutes following the toxin injection, and accurately predicted a 10 minute diuresis. (Fig. 16) It, therefore, seems apparent that the total renal blood flow response to staphylococcal toxin parallels that of effective blood flow. Both become markedly reduced following the intravenous injection of low

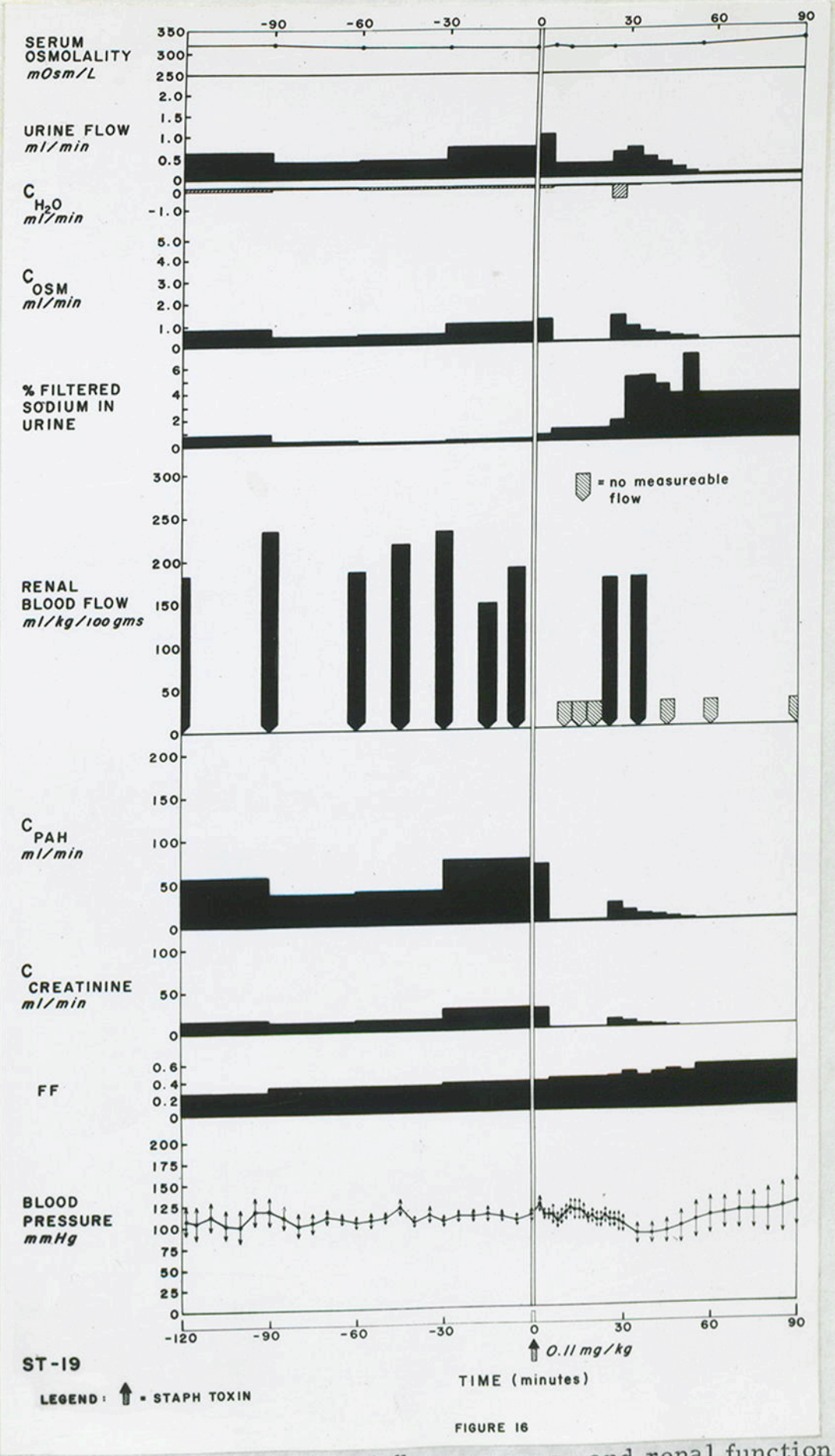


Fig. 16. Total renal blood flow response and renal function following injection 0.11 ml/kg. staphylococcal toxin. Note marked decrease in renal function following toxin injection without significant blood pressure changes.

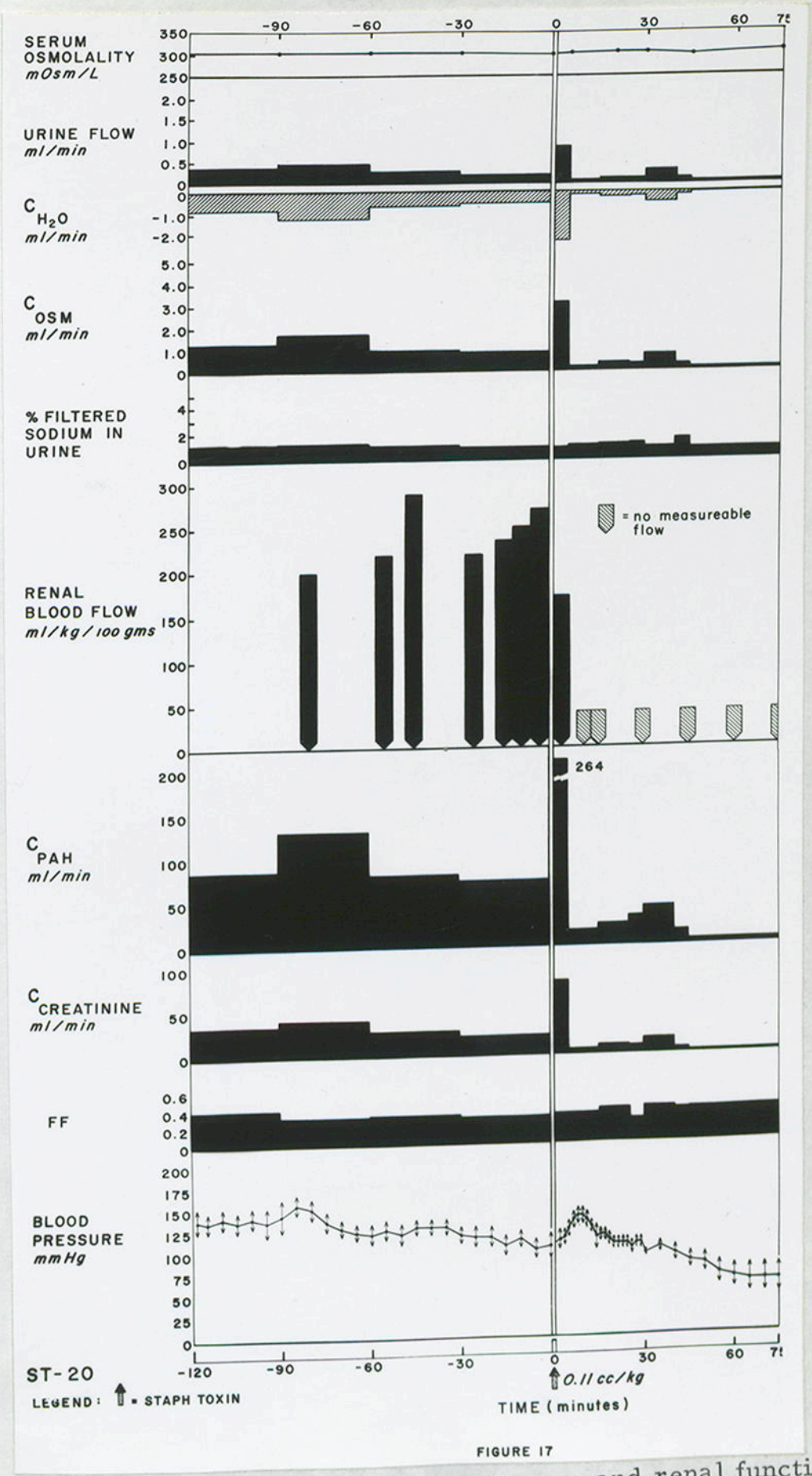


Fig. 17. Total renal blood flow response and renal function following injection 0.11 ml/kg. staphylococcal toxin.

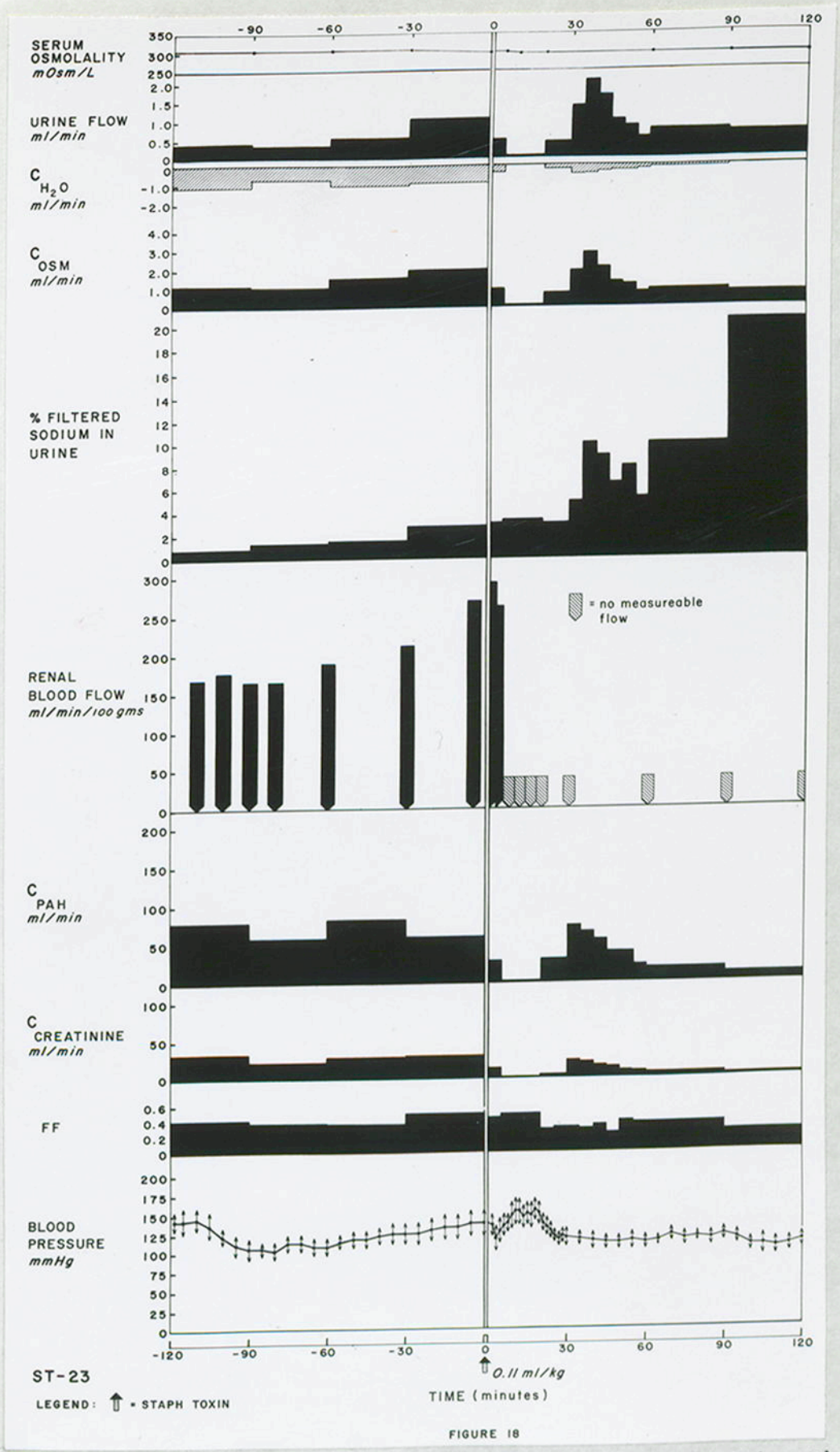


Fig. 18. Total renal blood flow response and renal function following injection 0.11 ml/kg. staphylococcal toxin. Note marked increase in percentage filtered sodium appearing in the urine.

doses of toxin. Figures 16 and 18 both demonstrate the characteristic increase in the percentage of filtered sodium appearing in the urine, indicative of tubular dysfunction.

Group V experiments: Fifteen minutes following the injection of 0.11 ml/kg of toxin, angiotensin was infused intravenously at the constant rate of 0.1 ug/kg/min. As seen in figure 19, there was an immediate pressor response with the mean systemic pressure maintained at slightly greater than control levels. Despite continued angiotensin infusion the osmolar, PAH and creatinine clearances remained markedly diminished. Shortly after the infusion was initiated, the urine became grossly hemorrhagic and continued that way throughout the experiment. The filtration fraction and percentage of filtered sodium appearing in the urine increased markedly throughout the experiment.

It would, therefore, seem apparent that angiotensin is of no benefit in the treatment of renal failure resulting from staphylococcal toxin. It actually appears to intensify the vascular response produced by the toxin itself resulting in greater pathologic damage to the renal parenchyma.

In the second experiment a single intravenous injection of the vasodilator drug, hydralazine, 0.4 mg/kg, was administered fifteen minutes following the injection of 0.11 ml/kg of toxin. The

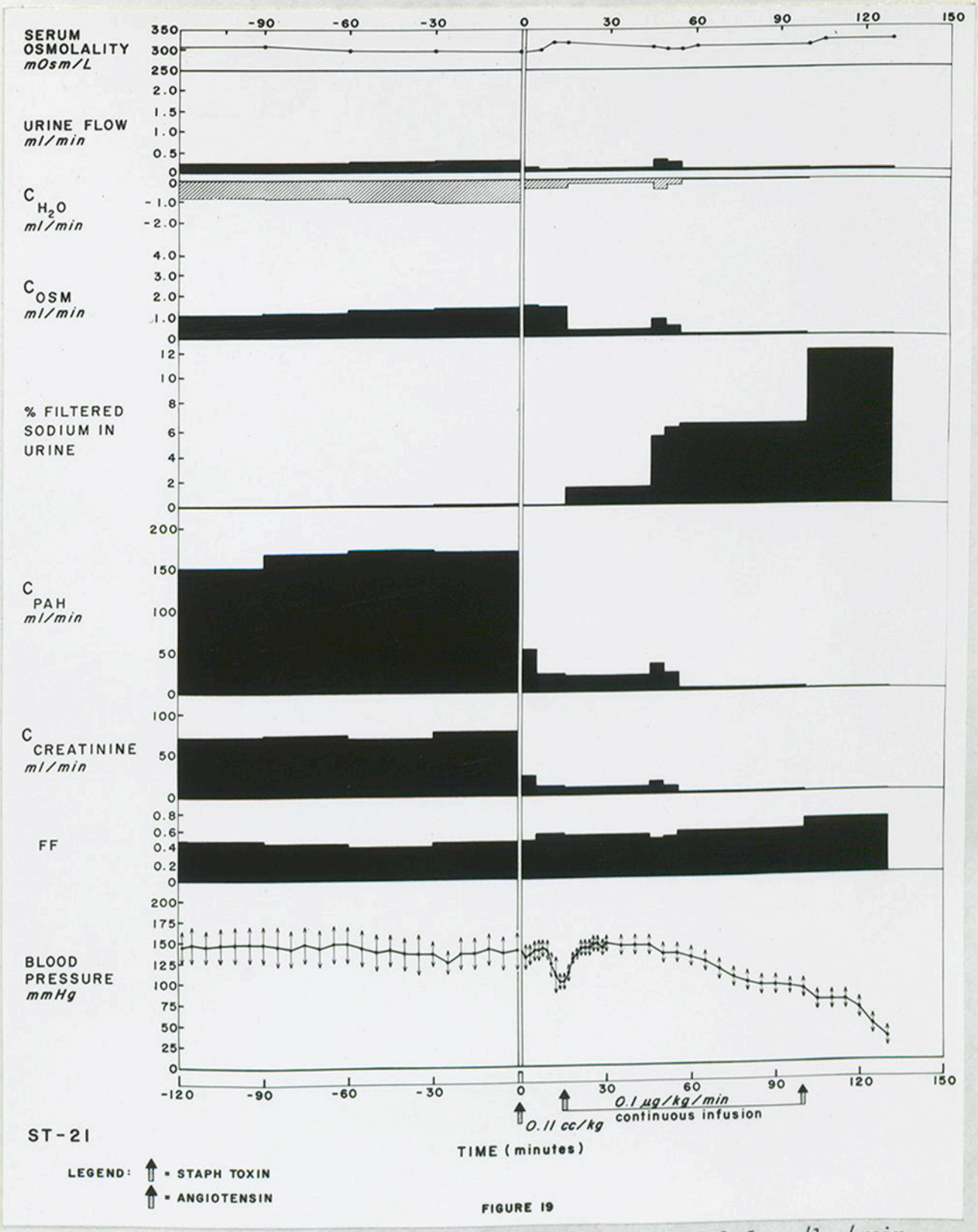


Fig. 19. The effect of continuous infusion of 0.1 ug/kg/min. angiotensin on renal function following injection of 0.11 ml/kg. staphylococcal toxin. Note decrease in function and rise in percentage filtered sodium appearing in the urine.

mean arterial pressure dropped from the control average of 142 mm Hg to approximately 105 mm Hg where it was maintained throughout most of the experimental period. As demonstrated in figure 20, the osmolar, PAH and creatinine clearances remained markedly reduced and were not improved by the drug's administration. Since hydralazine exerts a moderate adrenergic blockade via sympathomimetic inhibition it would appear that the toxin is acting directly on the renal vascular tree and does not involve a neurogenic vasopressor response.

Pathology

Post mortem examinations were performed on 21 dogs. Primarily gross alterations were limited to the kidneys in all experiments. Examination of the bisected kidneys demonstrated marked blanching of the cortex with focal congestion of the medullary region and particularly the cortico-medullary junction. (Plate 2) This is in contrast to the control kidney seen in plate 4-A. In two experiments India ink was injected into the right kidney near the termination of the experiment via the arterial catheter. Post mortem examination demonstrated ink distributed to both the cortex and medulla, but in a random and focal manner. (Plate 3)

Microsections of specific organs were made on 21 animals immediately following termination of the respective experiments.

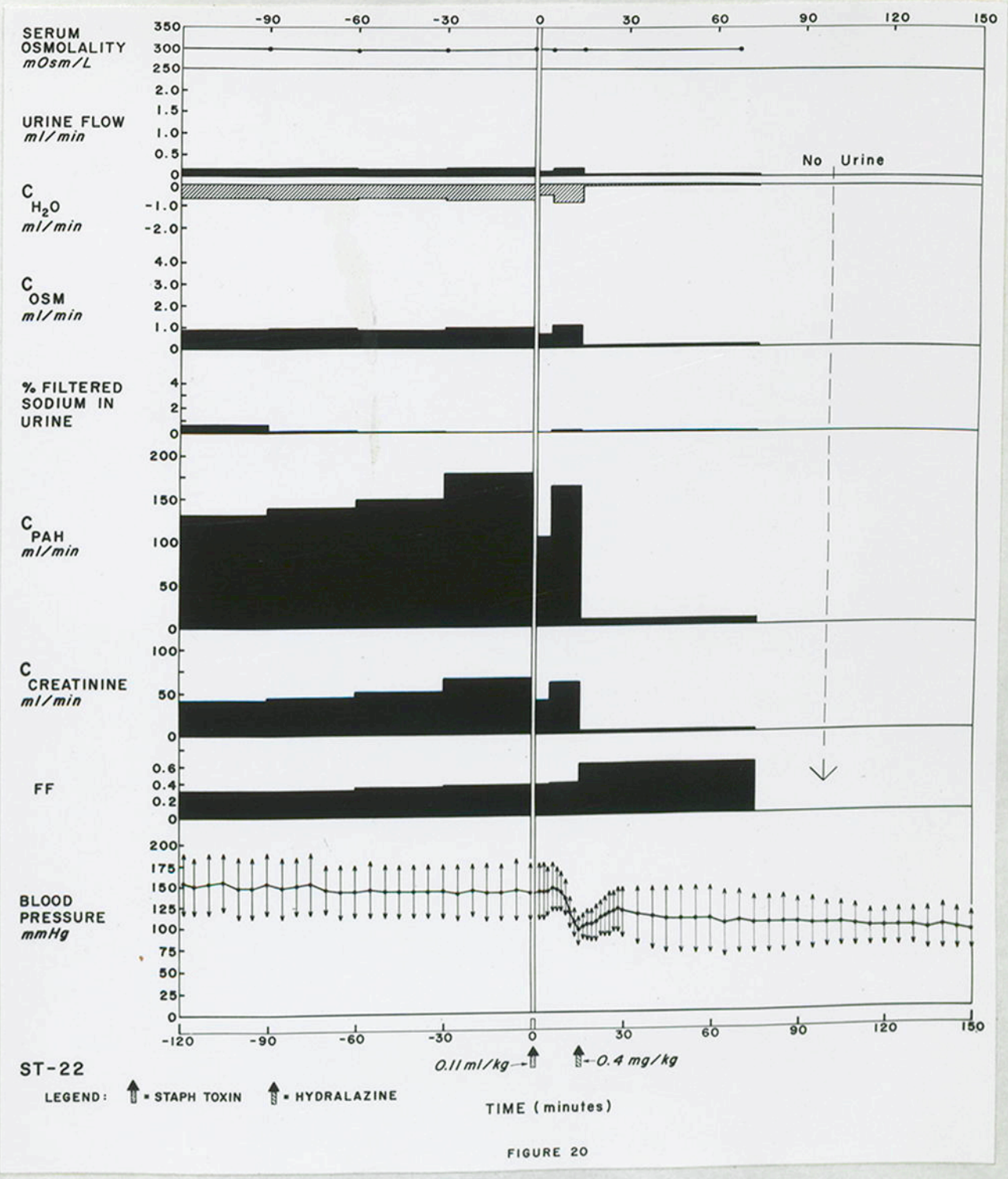
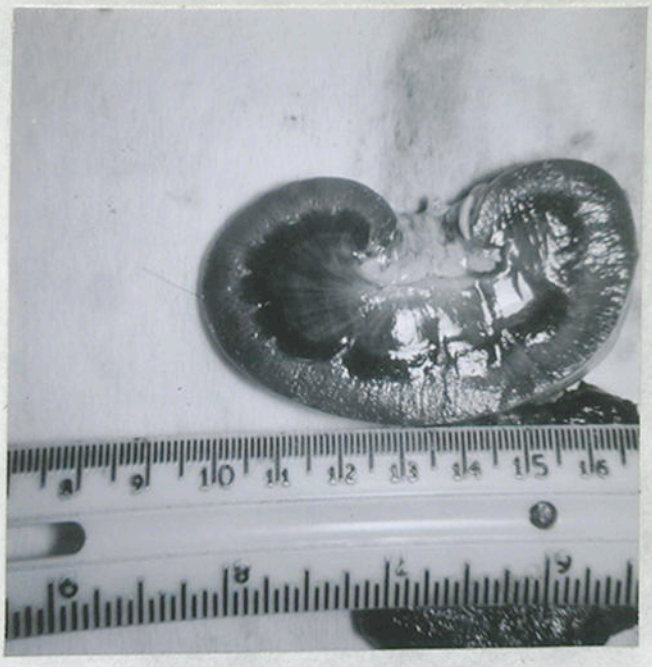


Fig. 20. The effect of hydralazine on renal function following the injection of 0.11 ml/kg. staphylococcal toxin. Note decreased renal function despite maintenance of good systemic blood pressure.

A.



B.

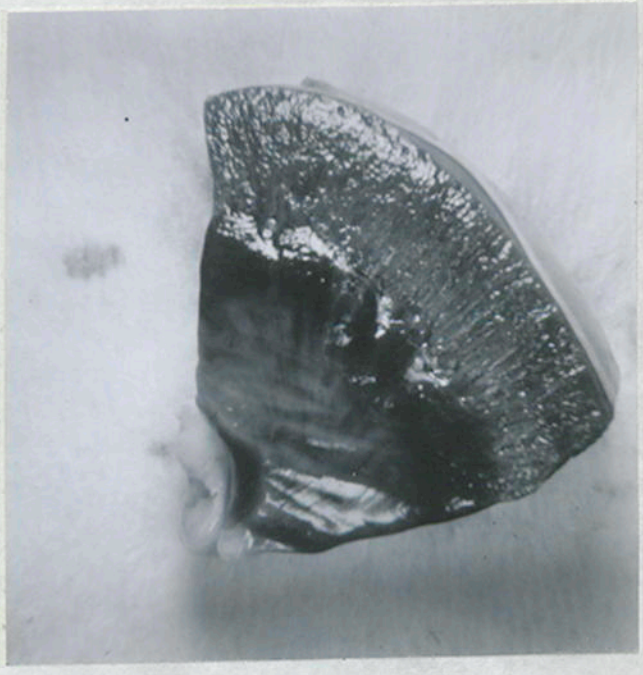


Plate 2. Photograph of a bisected kidney taken at post mortem examination. (A) Higher magnification shown in B. Note marked blanching of cortex with conjestion of the medullary region, particularly at the cortico-medullary junction.

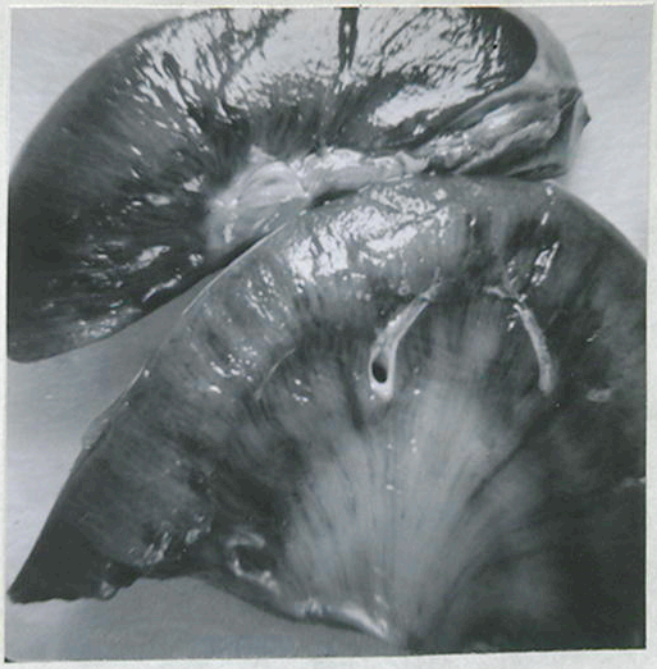
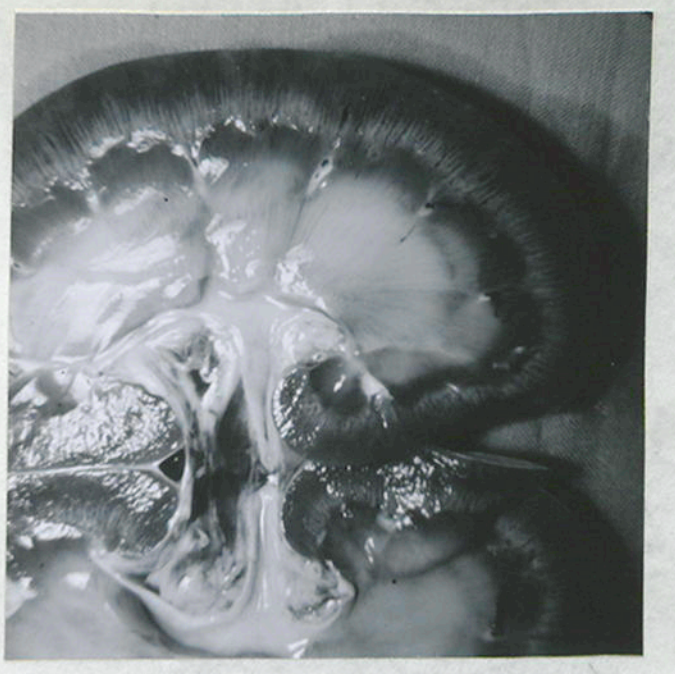


Plate 3. Photographs of bisected kidneys following India ink injection. Note distribution of the ink and its focal concentration within the medulla and cortex with adjacent areas of relative ischemia.

A.



B.

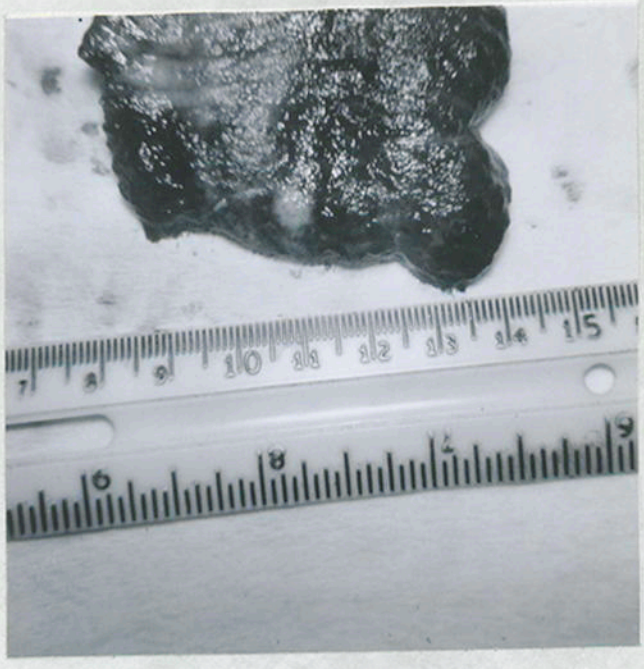


Plate 4. A. Bisection of normal post mortem kidney. B. Portion of duodenum demonstrating hyperemia and gross hemorrhage into the mucosa. Animal had received angiotensin in addition to 0.11 ml/kg. staphylococcal toxin.

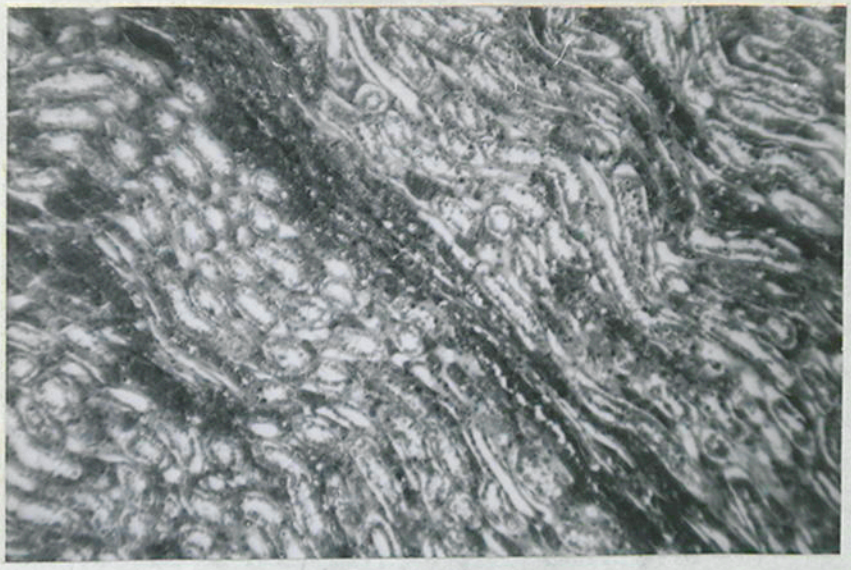
All specimens for microscopic examination were fixed in formalin; standard hemotoxylin and eosin preparations were made on the following organs: kidney, adrenal, liver, spleen, colon, small intestine, lung and the apex of the left ventrical.

Examinations of sections of heart, lung, adrenal, spleen and colon were essentially normal. Sections of the small intestine were unremarkable except for the one experiment in which angiotensin was administered in attempt to reverse renal failure. (Fig. 19) A section of the duodenum showed marked hyperemia of the mucosa with congestion of the villae. This provided microscopic evidence for the gross hemorrhage and hyperemia noted on gross examination at autopsy. (Plate 4-B)

Those experiments in which 0.25 ml/kg of toxin was injected showed mild congestion of the liver sinusoids. Necrosis was not observed and the liver was unremarkable in all other experiments.

Nearly all the pathological lesions were limited to the kidneys. Microscopic examination in 12 out of 17 experiments demonstrated massive focal congestion of the medulla, moststriking near the cortico-medullary junction. (Plate 5)

Focal congestion of many glomeruli within the cortex was also noted in the majority of experiments, most striking near the cortico-medullary junction. (Plate 6) Marked venous congestion



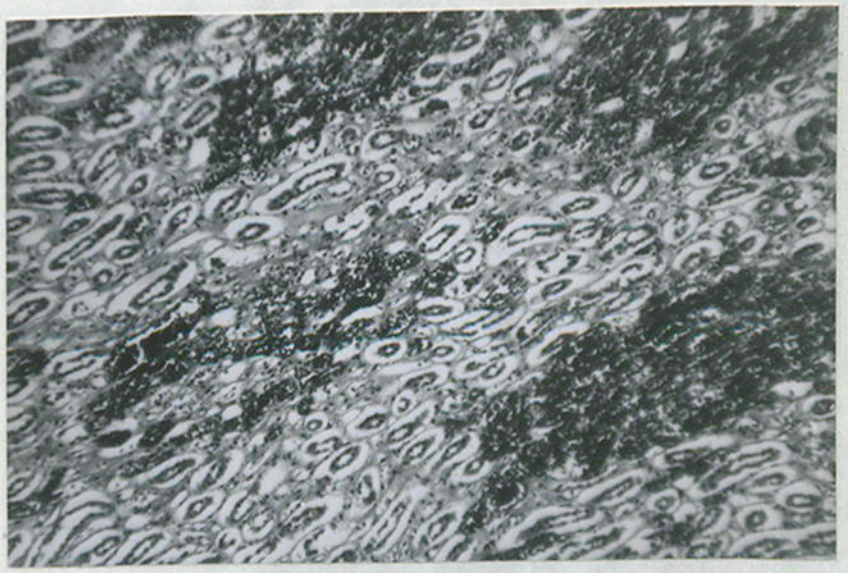


Plate 5. Photomicrographs of kidney sections demonstrating massive focal congestion of the medulla.

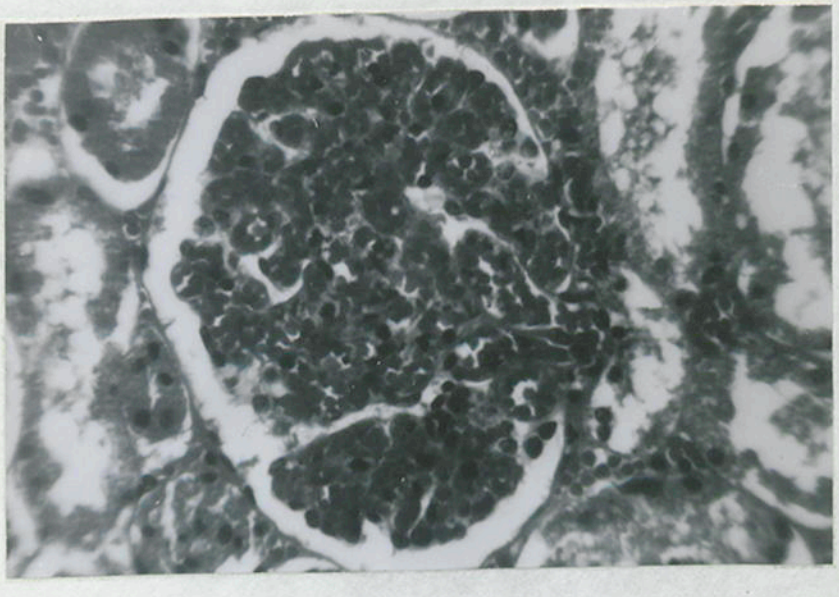


Plate 6. Photomicrographs of kidney sections showing congestion of the glomeruli located near the cortico-medullary region.

within the cortex was observed in one experiment. (Plate 7)

Amorphous proteinaceous material could be demonstrated in Bowman's spaces of many glomeruli. (Plates 8, 9) Tubules also contained much of this material, (Plate 10) and red blood cells could be demonstrated in several tubules and within Bowman's space. (Plates 11, 12-A) Edema of the kidneys was marked in all experiments characterized by an increased interstitial space, dilatation of the tubules and a discrepancy between the size of Bowman's capsule and the glomerular tuft. (Plate 12-B)

Narrowing of the arcuate arteries was observed in two experiments but was not striking in the other animals. In one experiment glomeruli at the cortico-medullary junction gave evidence of early necrosis. Besides marked congestion and edema of the glomerular tuft many pyknotic nuclei can be seen. (Plate 13) A large zone of mid-cortical glomerular and tubular necrosis was observed in one other experiment.

In general, the kidneys gave microscopic evidence of massive, focal medullary and glomerular congestion. Amorphous proteinaceous material was prominent within many tubules and in Bowman's space. Edema of the parenchyma was striking but structural changes were not remarkable except for two experiments. The appearance of many glomeruli and tubules examined in the cortico-medullary junction

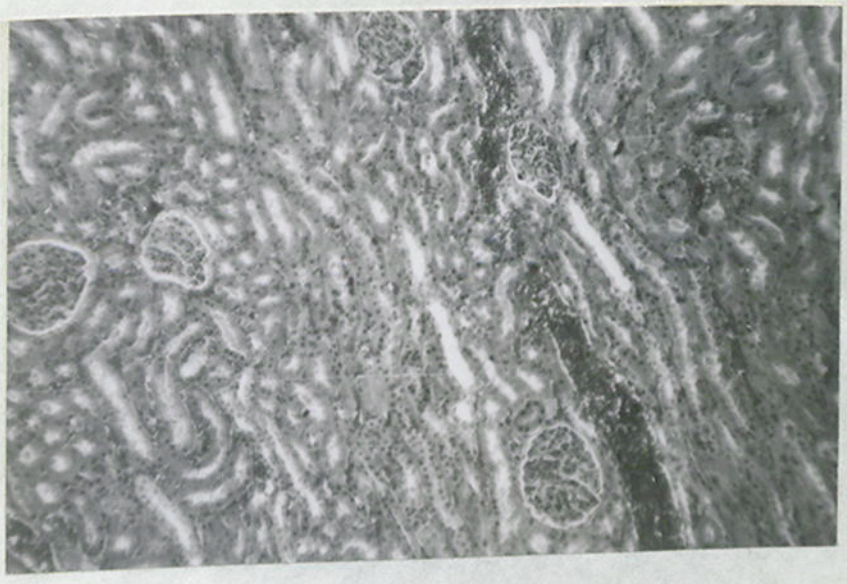
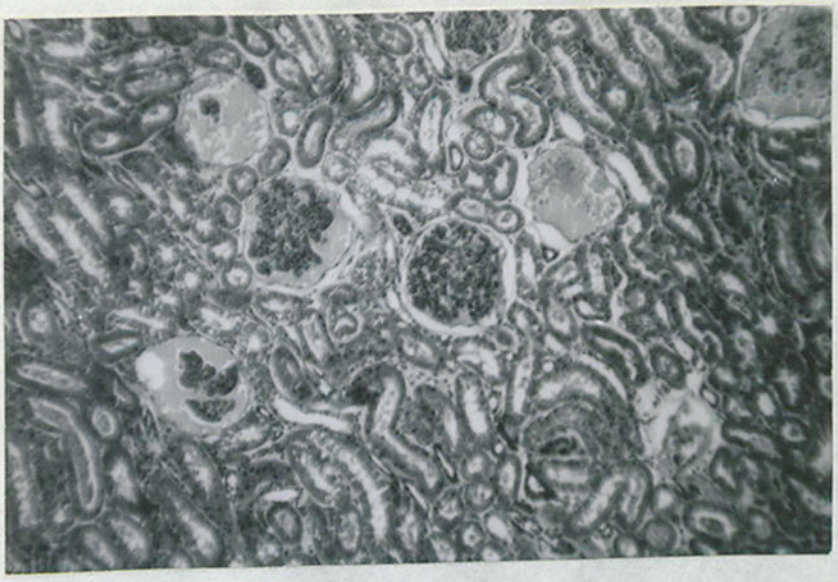


Plate 7. Photomicrograph of kidney section demonstrating venous congestion within the cortex.



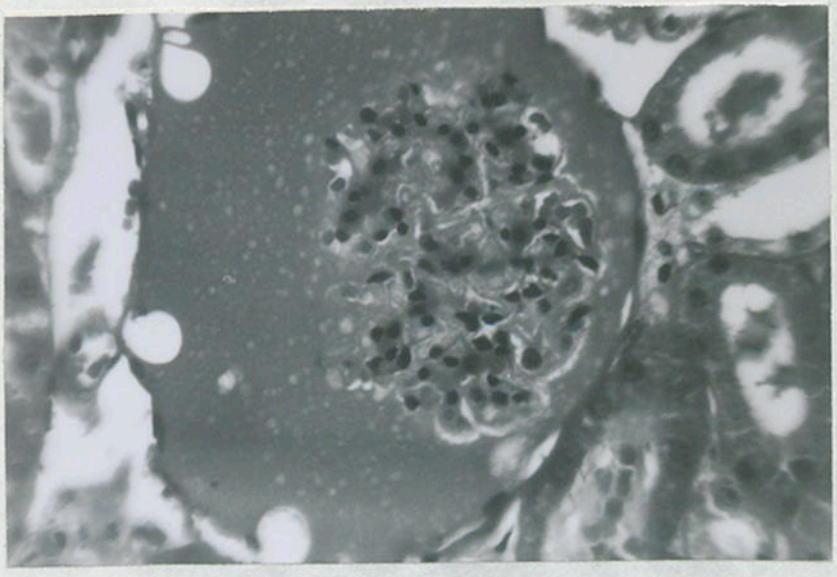
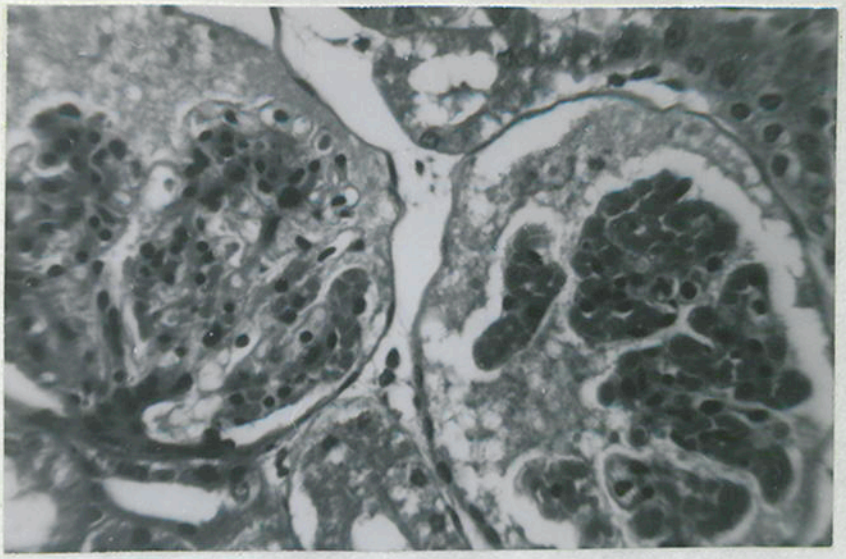


Plate 8. Photomicrographs of kidney sections demonstrating amorphous, proteinaceous material within Bowman's space.



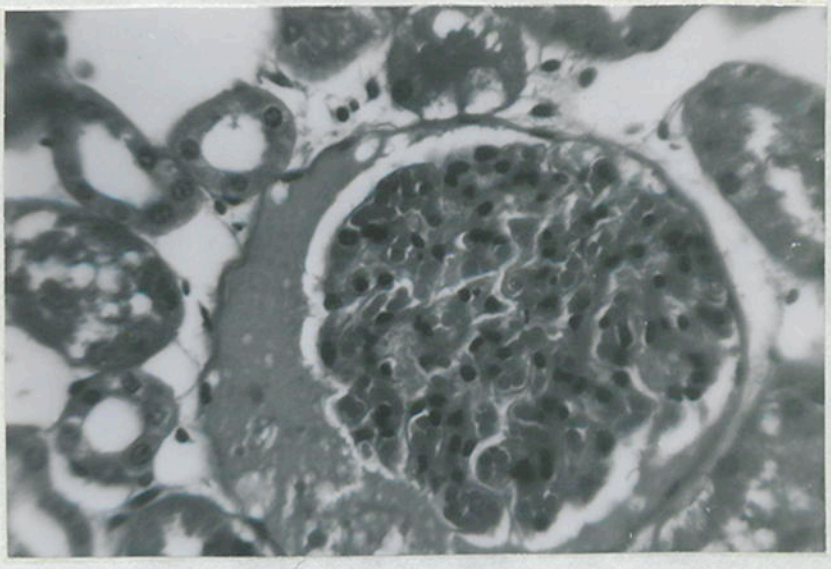
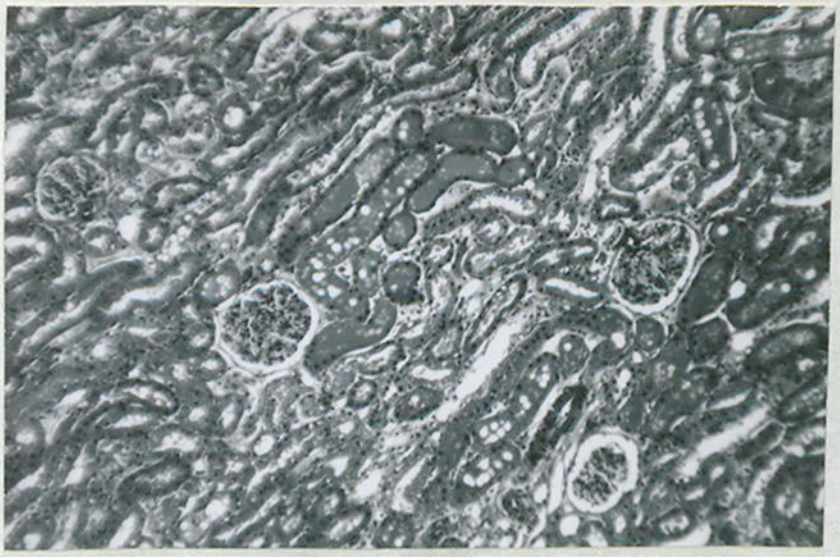


Plate 9. Photomicrographs of kidney sections demonstrating amorphous, proteinaceous material within Bowman's space.



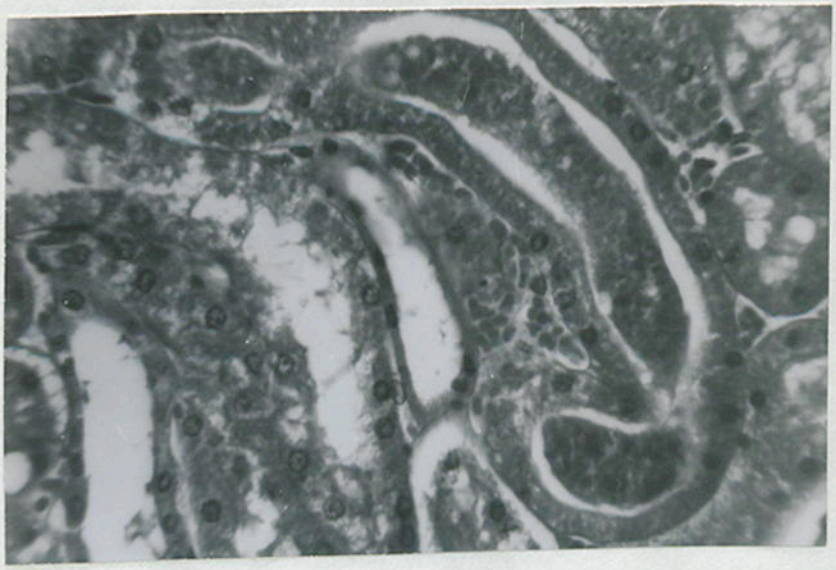


Plate 10. Photomicrographs of kidney sections demonstrating amorphous, proteinaceous material within many tubules.

A.

B.

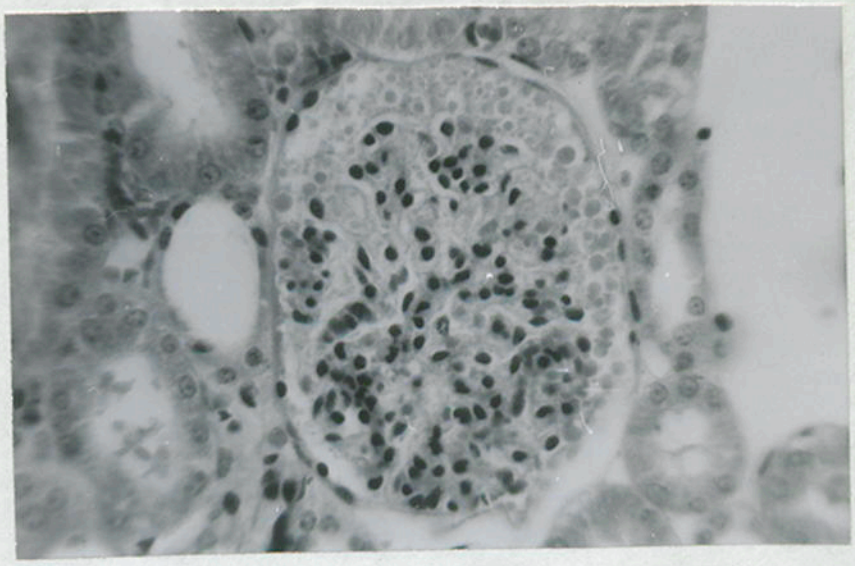
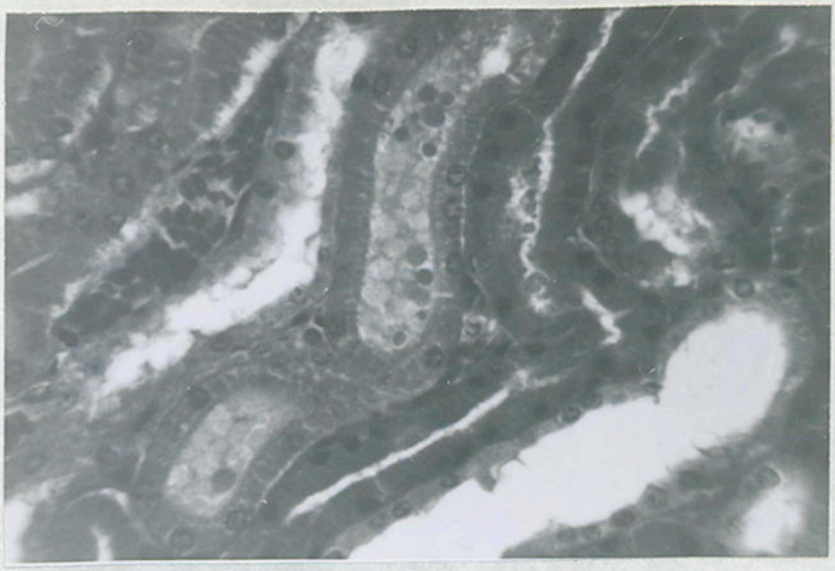


Plate II. Photomicrographs of kidney sections demonstrating red blood cells within many tubules (A) and Bowman's space. (B)

A.



B.

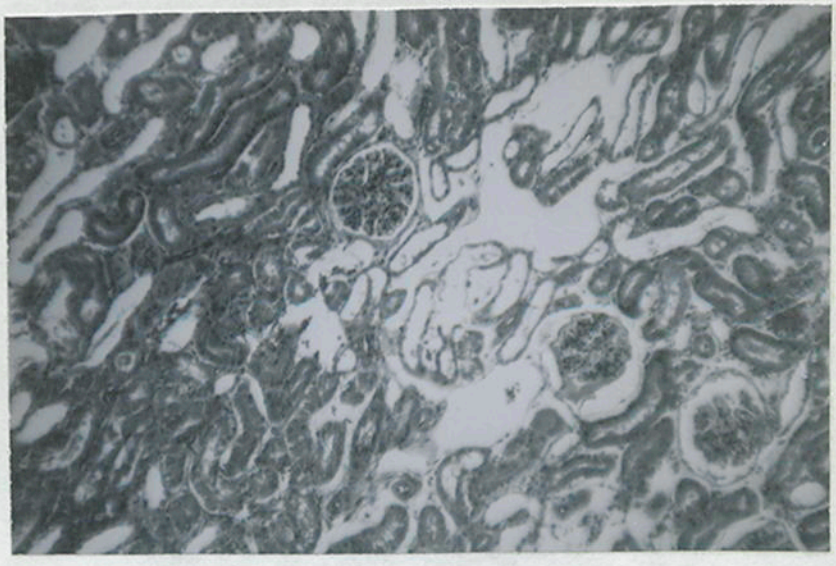
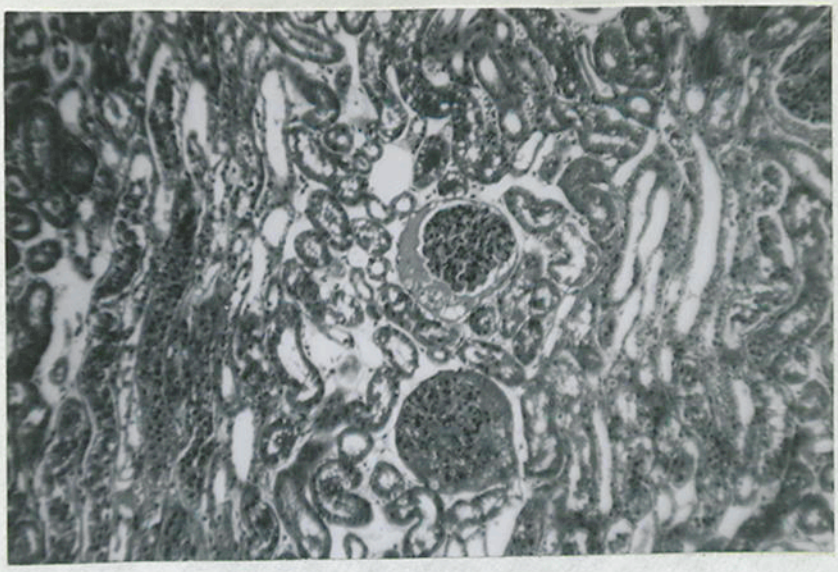


Plate 12. A. Photomicrograph demonstrating red blood cells within individual tubules. B. Photomicrograph of cortical region demonstrating increased interstitial space characteristic of edema.

A.



B.

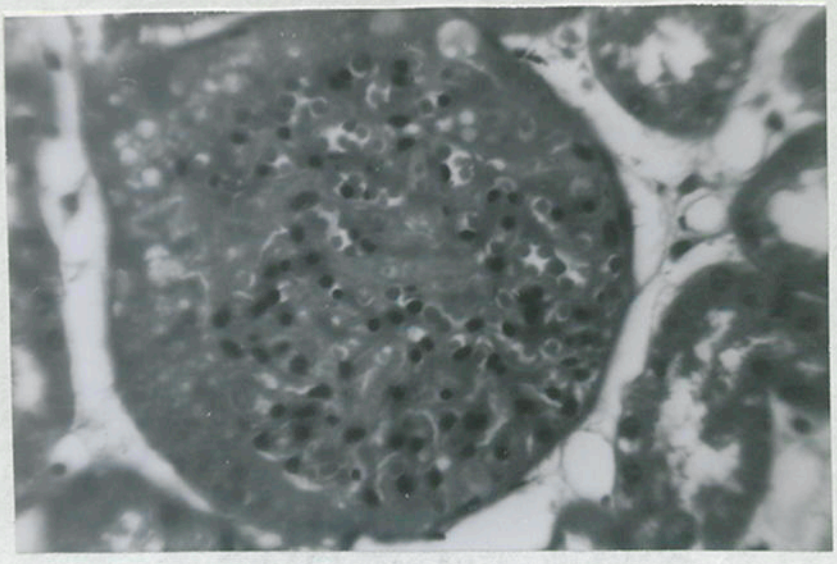


Plate 13. Photomicorgraphs of kidney sections demonstrating interstitial edema (A) and glomeruli with evidence of early necrosis. Note pyknotic nuclei within the glomerular tuft. (B)

suggested early changes characteristic of acute tubular necrosis. Clinically these pathological lesions represent the findings seen in acute renal failure.

It would, therefore, appear from microscopic examination that the renal response to staphylococcal toxin was primarily a vascular one resulting in marked focal congestion with possible secondary stasis ischemia of the renal parenchyma.

Analysis of Data

Results have demonstrated that staphylococcal toxin can produce irreversible shock in the dog. Of even greater interest is the demonstration that staphylococcal toxin in low doses acts selectively on the kidney. In all experiments total as well as effective renal blood flows were markedly decreased with doses apparently insufficient to cause significant pathological alterations in other organ systems. Larger doses of toxin were capable of producing more generalized changes but many of these experiments showed renal function alterations preceeding systemic changes.

Gross post mortem examinations of the bisected kidneys revealed blanching of the cortex with marked engorgement of the medullary region. Microscopic sections demonstrated massive focal medullary and glomerular congestion with marked edema of

the renal parenchyma. Although structural changes were not apparent, the appearance of glomeruli at the cortico-medullary region was suggestive of impending acute renal failure. This all lends evidence for the hypothesis that staphylococcal toxin exerts a selective and direct action on the renal vascular system producing congestion with secondary stasis ischemia to the peripheral cortical regions.

Diminution of total renal blood flow at the same time as diminution of effective renal blood flow would indicate a generalized decrease in renal perfusion without implicating the existence of an intra renal shunt of blood.

In many experiments the free water clearance diminished and the percentage of filtered sodium appearing in the urine increased significantly to indicate both distal and proximal tubular dysfunction. Since both the transfer of sodium and free water against an osmolar gradient requires the activity of a complex enzyme system in the tubular epithelial cells, ischemia secondary to the toxin-induced hyperemic stasis could easily explain this dysfunction.

Since the vasopressor drug angiotensin appeared to intensify the congestion and the sympathomimetic inhibiter, hydralazine, was without benefit, it would appear that the toxin exerts a direct action on the renal vascular system characterized by congestion and stasis

ischemia. The exact mechanism of this response remains unknown, but does not appear to be mediated by the nervous system.

Discussion

The blood pressure response to lethal doses of bacterial toxin has been demonstrated on many occasions. Hypotension and irreversible shock secondary to peripheral vascular collapse have been emphasized in most reports. There have been some studies on the effect of relatively small doses of various toxins on specific organ physiology, but investigation has been mostly limited to the toxins produced by Gram negative bacteria. (30, 31, 33) Surprisingly little is known in regard to the effect of small doses of staphylococcal toxin on systemic homeostasis.

At the same time, however, a review of the clinical literature reveals a significant percentage of cases with staphylococcal septicemia associated with clinical shock and renal failure. Of 37 cases of bacterial shock caused by a single organism at the St. Louis City and St. Louis University Hospitals between 1950 and 1956, 14 were due to Gram positive cocci and 23 due to Gram negative bacilli. (14) Oliguria was noted in 16 of the 37 cases and the authors stress the inability to differentiate clinically between shock produced by Gram negative and Gram positive organisms. (14) Of 35 cases of

bacteremic shock at the Minneapolis Veterans Administration Hospital reported between 1948 and 1955, 34% were due to Gram positive cocci and 66% were due to Gram negative bacilli. (24)

Altemeier in a recent review emphasizes the frequency with which staphylococcal bacteremia is associated with clinical shock. (4) At the University of Cincinnati Hospital 64 of 93 cases of clinical shock secondary to serious infections demonstrated marked oliguria. (3)

In reviewing the cases of bacteremic shock at the Mayo Clinic, renal insufficiency and hypotension were noted in a high percentage of cases. (41) In a ten year period at the University of Minnesota Hospital 150 cases of shock secondary to sepsis were observed. A majority of the cases were associated with Gram negative septicemia, but in a surprisingly large number antibiotic resistant staphylococci were cultured from the patients blood. Once again the typical clinical pattern was characterized by hypotension and severe renal failure. (17, 64, 65, 66) The above cases of clinical shock and renal failure associated with infections are reported only to point out the possible significance bacterial toxins might have on renal function.

There have also been many clinical reports in the literature describing prolonged periods of diminished renal function following brief episodes of hemorrhagic or hypovolemic shock. (5, 27) This

diminished renal function is characterized by oliguria and a marked diminution of creatinine and PAH clearances. Even those reports of diuresis following brief periods of hypotension and shock often show a fixed specific gravity and poor clearances of PAH and creatinine. (27)

At the same time, however, experimental cross-clamping of the thoracic aorta for up to three hours produces only minor residual variations in renal function. (43) There are other experimental reports indicating that total renal ischemia for up to two hours is without residual effect on renal function. (21, 50, 56, 59) Therefore, it appears that in the intact animal hypotension, or shock, has a far greater effect on renal function that pure ischemia.

In support, various hypotheses have been established giving evidence that severe and often irreversible shock results from the release of a diffusible substance into the serum. Although the identity of this substance is largely unknown and quite controversial, there is general agreement that it mediates a peripheral vascular collapse. The two most prominent schools of thought as to the identity of this substance are led by Shorr and associates on the one hand and Fine's group on the other. Shorr suggests that there is a vasodepressor substance (VDM) that is released by the anoxic liver producing peripheral vascular collapse and irreversible shock. (60, 61) This VDM substance, however, has not been isolated or satisfactorily

identified, and still remains largely hypothetical. (18)

Fine believes that the irreversible factor in shock is due to the action of bacterial toxins on the circulation also mediating peripheral vascular collapse. (16, 17, 18, 58) Under Fine's hypothesis there is a normal and passive diffusion of bacterial toxins, primarily from the coliform group, through the intestinal wall into the portal circulation. (47) Radio-active labelling of these toxins in the dog indicates that they are normally detoxified by the reticuloendothelial system of the liver. (10, 29, 36, 52, 53) Anoxia secondary to hypotension and shock serves to depress the detoxifying ability of the reticuloendothelial system thereby allowing the toxins to circulate in the systemic circulation. (58) Under this scheme differences in mortality rate, survival time and alterations in organ physiology can be explained by the amount of bacterial toxin reaching the systemic circulation. Since its forumlation, this hypothesis has been controversial as being too superficial to explain the complex physiological alterations occurring in irreversible shock. Other investigators have been unable to reproduce certain results reported by Fine, (25) and recent work with so-called germ free animals indicates that irreversible shock occurs with the same incidence following hemorrhage as it does in the normal animal populations. (82)

Regardless of whether bacterial toxins are the cause of irreversible shock it does seem apparent that they are capable of

producing profound effects on systemic homeostasis.

The renal response seen both in experimental animals and patients suffering from septicemic shock is quite similar to that observed in hemorrhagic and hypovolemic shock states. This response is characterized by oliguria and marked diminution of effective renal blood flow. The mechanism of this response is largely unknown, but it does seem apparent that the renal dysfunction cannot be explained by hypotension alone. The subsequent morbidity and mortality in such patients has been high in spite of many various approaches to therapy. Renal insufficiency is often the final cause of death long after hypotension has been corrected.

Experiments herein reported have demonstrated that staphylococcal toxin can produce irreversible shock in the dog. But what may be of greater significance is the evidence that staphylococcal toxin in low concentrations acts selectively on the kidney without significant blood pressure changes. This renal response is characterized by a marked diminution in renal perfusion with oliguria a natural consequence. Because of the similarity between the renal response noted in these experiments and those observed in clinical cases of septicemic shock it seems quite essential that further investigation be undertaken in attempt to understand the exact mechanism by which various bacterial toxins exert their influence on renal function.

Summary

A study has been made in 23 dogs of the effect of varying intravenous doses of a staphylococcal toxin on renal function. Results indicate that the toxin in low doses acts selectively on the kidney. Total as well as effective renal blood flows, and glomerular filtration were decreased by doses of toxin insufficient to cause significant blood pressure changes, or pathological alterations in other organ systems.

In many experiments there was a significant decrease in free water clearance and a corresponding increase in the percentage of glomerular filtered sodium appearing in the urine to indicate severe tubular dysfunction.

The pressor drug, angiotensin, was ineffective in improving renal function and actually intensified renal vasocongestion, producing gross hemorrhage into the tubules. The sympathomimetic inhibitor, hydralazine, was also ineffective in improving renal function.

Gross post-mortem examinations of the kidneys revealed blanching of the cortex with marked engorgement of the medullary region. India ink injection demonstrated a distribution to all areas of the kidney, but in a random and focal manner. Microscopic examination revealed massive, focal medullary and glomerular

congestion with edema of the renal parenchma. Although actual necrosis had not yet become apparent, the appearance of many glomeruli and tubules examined in the cortico-medullary junction suggested early changes of tubular damage, "acute renal failure."

The conclusions are that a potent staphylococcal toxin in small doses exerts a selective and direct action on the renal vascular system.

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