

Atropine Influence on Altered Hemodynamics of the Primate Kidney in Hemorrhagic Shock¹ (35927)

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One of the interesting hemodynamic alterations observed in the posttransfusion phase of hemorrhagic shock in the owl monkey is a reasonably well maintained renal blood flow but reduced GFR and reduced filtration fraction (FF) (1). Commonly associated with this phase in well-hydrated animals is a positive free-water clearance and reduced ratio of U/P osmolality, often below unity. In view of considerable evidence accumulating that there are cholinergic fibers supplying the medullary circulation, to vasa recta sphincters and juxtamedullary efferent arterioles (2-8), a possible mechanism resulting from this might be increased activity of cholinergic fibers, dilator to the juxtamedullary efferent arterioles, hence resulting in reduced filtration pressure. Such a mechanism might also be involved in maintaining medullary blood flow in hemorrhagic shock (9, 10), leading to washout of the osmolar gradient of the kidney as observed in the previous report, thus contributing to the reduced concentrating ability. Present evidence indicates that the cholinergic fibers are postganglionic (8), these ganglia lying either within the kidney or in the renal hilus, supplied by preganglionic nerve fibers of the renal plexus (6, 8, 11). Thus, one might speculate that during the overall stimulation of the autonomic nervous system during the stress of shock that these cholinergic fibers might also be activated, and alter the balance of adrenergic and cholinergic influence on critical segments of the vasculature, *e.g.*, afferent vs efferent arterioles, particularly of the juxtamedullary glomeruli.

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The investigation was carried out to attempt to see if alterations of the hemodynamic pattern observed in the posttransfusion phase of hemorrhagic shock might be prevented by the judicious infusion of atropine into the renal arteries during this phase of shock, thus possibly supplying evidence of cholinergic nerve activity. Support for the notion that cholinergic dilation of the efferent arterioles underlies the reduction in FF would be supplied if FF were restored to control values as the result of atropine infusion. Secondary changes in free-water clearance, if resulting from altered medullary circulation, might eventuate if cholinergic nerve activity were blocked by atropine, *e.g.*, increase in U/P of osmolality and improvement in the concentrating ability of the kidney.

Methods. Experiments were carried out on a total of 28 owl monkeys, mostly females, averaging ca. 800 g of body wt. They were anesthetized with 40 mg/kg of body wt of pentobarbital administered intraperitoneally, with additional minimal booster doses given when necessary during the course of the experiment. Surgical procedures included exposure of one or both kidneys by flank incision so that the ureter could be catheterized with fine polyethylene tubing for urine collection. Usually data were derived for only one kidney; when both were utilized (8 cases), clearance values were averaged for presentation of data. One femoral artery was catheterized for the registration of mean arterial pressure and for blood sampling, and the other artery was catheterized to allow bleeding into an overhanging reservoir. A femoral vein was catheterized for infusion of maintenance solutions.

The animals were allowed food and water *ad libitum* until the morning of the experi-

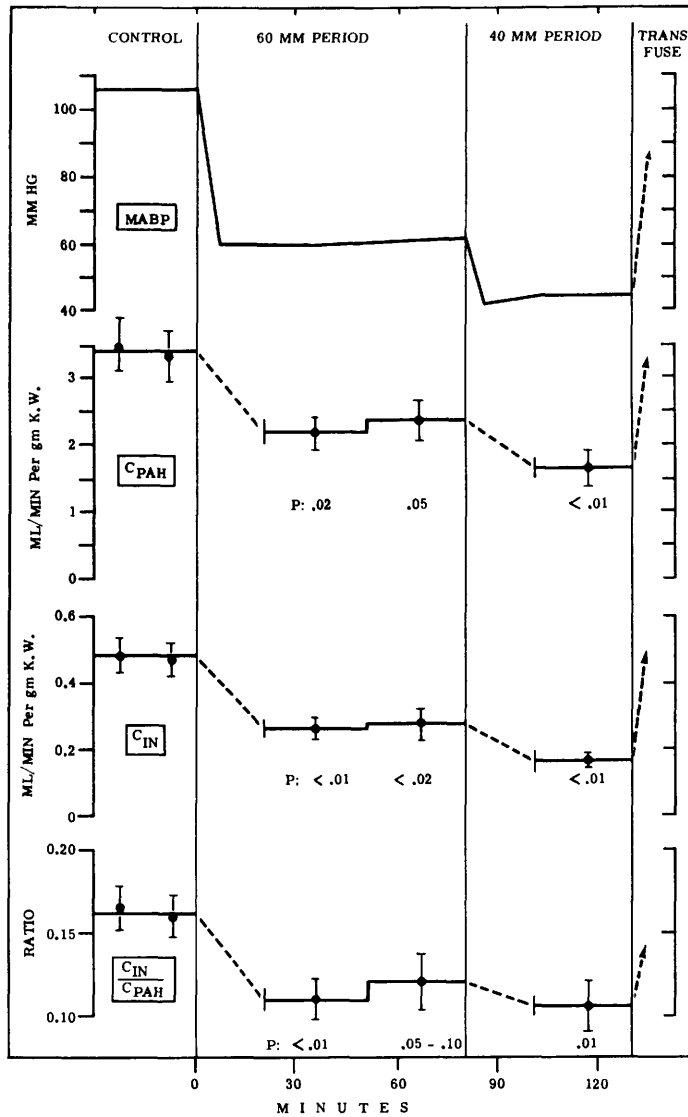


FIG. 1. Influence of prolonged hemorrhagic hypotension on MABP, C_{PAH}, C_{IN}, and the ratio C_{IN}/C_{PAH} (29 animals in series, showing mean trends, ± 1 SEM). Average bleeding volume was 28 ml/kg (± 1.5, SEM). Statistical significance calculated for periods during hypotension is compared to original control averages (Student's *t* test).

ment, and were assumed to be in normal fluid balance. The plan of the experiment was to maintain the arterial pressure at a constant value of 60 mm of mercury for 75 min following two control periods each of 15 min duration, by hemorrhage into the bleeding reservoir. Further hemorrhage then reduced the mean arterial blood pressure to 40 mm Hg where it was maintained for a second phase of 45 min duration, before transfusion

intra-arterially of the heparinized blood. At this time, 20 mg of protamine sulfate were administered to circumvent continued bleeding from surgical sites in the posttransfusion phase. After restoration of blood volume, the observations were continued for a total of 3 hr, at which time the animals were sacrificed.

PAH was infused at a rate adequate to give plasma concentrations suitable for calculation of effective plasma flow (EFP), and

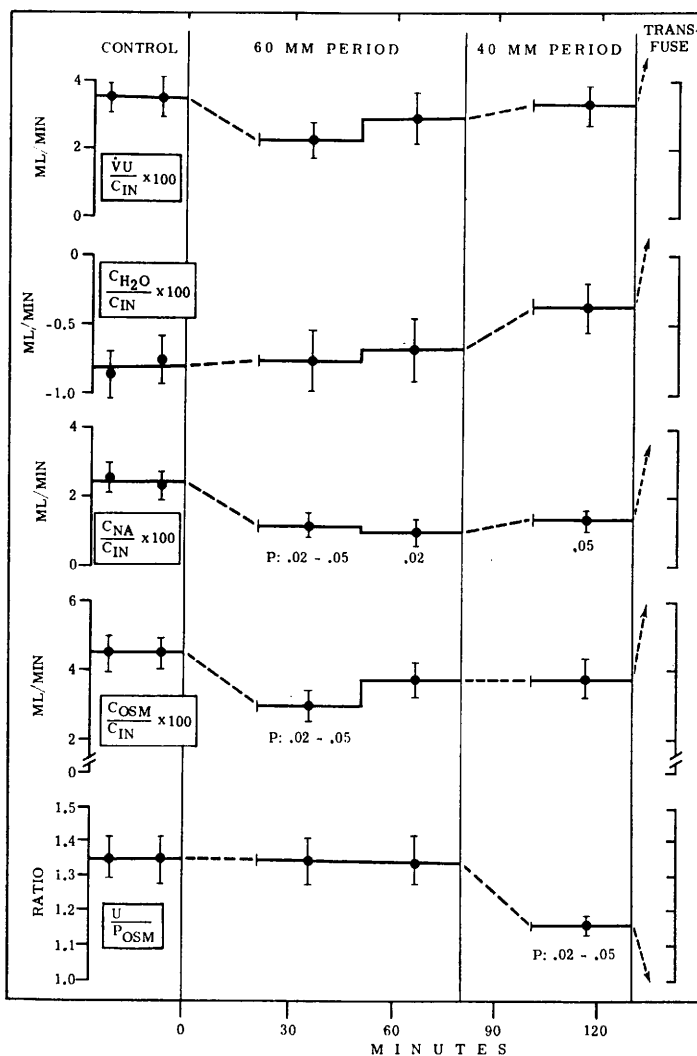


Fig. 2. Influence of hemodynamic alterations resulting from hemorrhage on urine volume (V_U), and the fractional clearance of free water, C_{In} , sodium, and osmolal constituents, and the concurrent influence on U/P osmolality. Same series as depicted in Fig. 1.

inulin was given to permit calculation of GFR. Sodium was measured in plasma and urine, as were osmolar constituents. All substances were infused in a small volume (0.12 ml/min) in order not to disturb the base-line urine volume and electrolyte excretion rates.

The animals were divided into three groups. Group A (13 animals): these were untreated after transfusion of the blood volume removed to establish the desired level and duration of hypotension, and served as a control group for the animals receiving atropine; Group B (8 animals): atropine infu-

sion was begun just prior to transfusion, and continued for 30 min after restoration of the bleeding volume; Group C (8 animals): blood volume was restored, and atropine infusion was started 30 min later, then continued for 30 min. Atropine was infused via a catheter advanced into a femoral artery so that the tip lay just above the junction of the renal arteries and the abdominal aorta, or by a fishhook needle of small caliber inserted into the junction of the aorta and renal artery. The average dosage of atropine sulfate given was 0.275 mg/kg/min. Urine col-

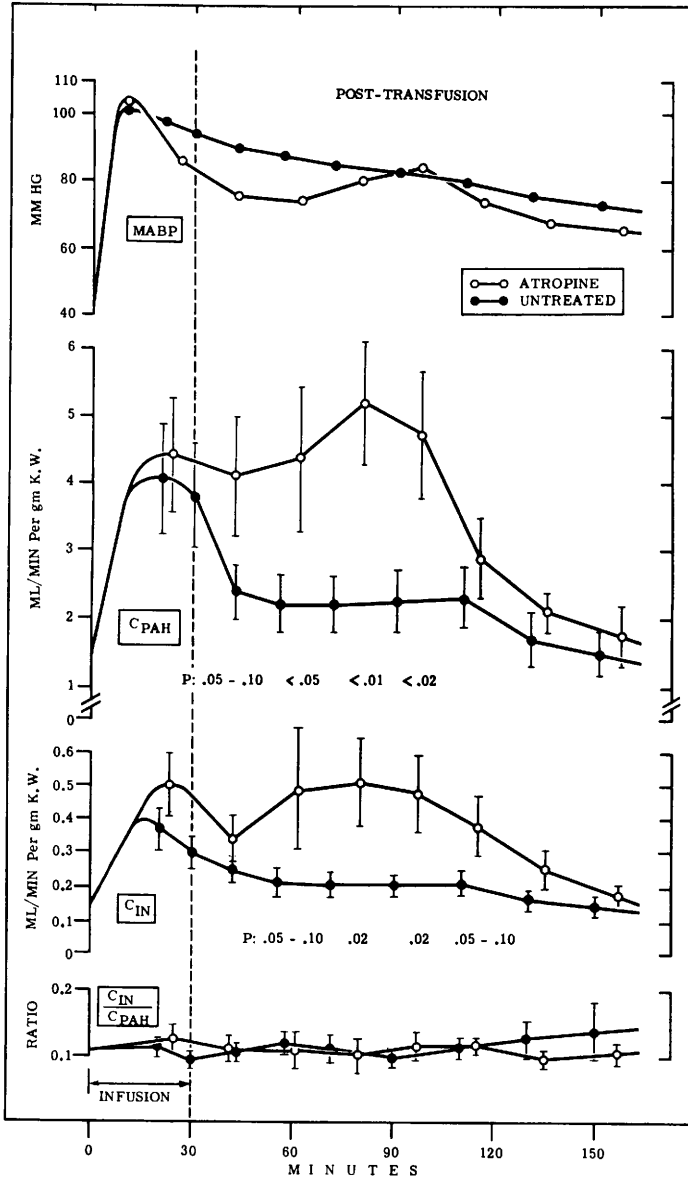


FIG. 3. Effect of intra-arterial atropine infusion (0.275 mg/kg/min) during the first 30 min post transfusion on MABP, C_{PAH} , C_{IN} , and C_{IN}/C_{PAH} ($N = 8$). Trends are compared with 13 animals not receiving atropine. The t and P values were calculated for samples with different numbers in each group:

$$t = (X_1 - X_2) \{ [n_1 n_2 (n_1 + n_2 - 2)] [(n_1 + n_2) \sum x^2] \}$$

(Snedecor, G. W., "Statistical Methods," p. 90. Iowa State Univ. Press, Ames, (1962)).

lections during the control period were 15 min in duration. During hypotension, 30 min samples were collected. After transfusion, 10 min periods were taken early (before atro-

pine infusion), 15 min periods during infusion, and 20 min periods thereafter. Before each phase, an adequate discard period was observed (15 min). Blood samples were tak-

en at approximately midpoint of the urine collection periods, with delay time corrections made if plasma concentrations were changing. Usually, reasonable constancy of the plasma levels of the infused substances minimized clearance errors. Urine samples were collected under oil to minimize evaporative losses before analysis.

Chemical Procedures. Inulin determinations were made by the anthrone method of Davidson and Sackner (12) and PAH by the method of Smith *et al.* (13). Plasma concentrations were measured from TCA filtrates. Sodium was measured in a Beckman atomic absorption spectrophotometer, and osmolalities by an Advanced Instrument, Inc., osmometer.

Results. Hemorrhagic hypotension: hemodynamic alterations. Figure 1 shows the influence of hemorrhage to 60 mm Hg for 1.25 hour, then 40 mm Hg of mean arterial pressure for an additional 45 min, followed by rapid transfusion. All animals are lumped in this series ($n = 29$). The average maximal bleeding volume was 28 ml/kg, (± 1.5 , SEM). As shown, C_{PAH} , C_{In} , and the ratio of C_{In}/C_{PAH} fell markedly with the initial hemorrhage and even more so during the 40 mm Hg period (p values in the latter period were .01 or less from the original control averages).

Effects on urine volume, sodium clearance, and osmolar clearance. Figure 2 shows the influence of the period of hypotension on urine volume (V_U), free-water clearance (C_{H_2O}), sodium clearance (C_{Na}), osmolar clearance, (C_{Osm}) and the U/P osmolal ratio. All absolute values decreased with the progressive decrement in C_{In} , as observed in Fig. 1. In order to bring out any underlying influence on these functional parameters, the values are expressed as a fraction of the inulin clearance in Fig. 2. There was an initial tendency for $V_U/C_{In} \times 100$, $C_{Na}/C_{In} \times 100$, and $C_{Osm}/C_{In} \times 100$ to decrease early in the 60 mm period. $C_{H_2O}/C_{In} \times 100$ was negative and tended to become less so during this interval. U/P (Osm) was fairly stable but showed a later significant decrement during the 40 mm period. $V_U/C_{In} \times 100$ and $C_{Osm} \times 100$ seemed to rise again somewhat during the 40

mm period accompanying the drop in U/P (Osm). The negative free-water clearance value became even less so during this phase, and V_U , in the 40 mm period, was restored virtually to control value when considered as a fraction of the inulin clearance, reflecting the decrement in the negative water clearance. One notes, therefore, a progressive loss in the ability of the tubules to concentrate in urine as U/P (Osm) declines. A rising trend in the fractional sodium clearance underlies a similar trend in osmolar clearance.

Posttransfusion alterations in renal function: Hemodynamic alterations resulting from atropine infused immediately after transfusion. Figure 3 summarizes the alterations in mean arterial blood pressure, C_{PAH} , C_{In} , and the ratio C_{In}/C_{PAH} comparing the average of 13 untreated animals with 8 which receive atropine intra-arterially during the first 30 min of the posttransfusion period. Infusion of atropine caused a decrement in the systemic arterial blood pressure well below that anticipated in the untreated group, as atropine became distributed throughout the general circulation. This effect was restored to the control level about 60 min after cessation of infusion of atropine. Accompanying this, significant increases in C_{PAH} and C_{In} were observed. These functions also tended to be restored to control level about 1.5 hr after cessation of infusion of atropine. The lowest panel in Fig. 3 shows that the ratio C_{In}/C_{PAH} did not alter from the trend of the untreated series during the entire posttransfusion period, the ratio remaining slightly above 0.1, a value considerably below the pre-hemorrhagic control average.

Hemodynamic alterations resulting from atropine infusion 30 min after transfusion. Figure 4 shows that atropine caused MABP to decrease from 88 to 70 mm Hg, a 20% decrement during infusion, with a tendency to recover to the control trend at 52 min postinfusion. The untreated trend was from 94 to 87 mm Hg during the corresponding interval (30 to 60 min), only a 7.5% decline. C_{PAH} rose significantly as a result of the atropine infusion, and C_{In} increased proportionately, so that the ratio C_{In}/C_{PAH} (bottom panel) remained very similar to the untreated animals,

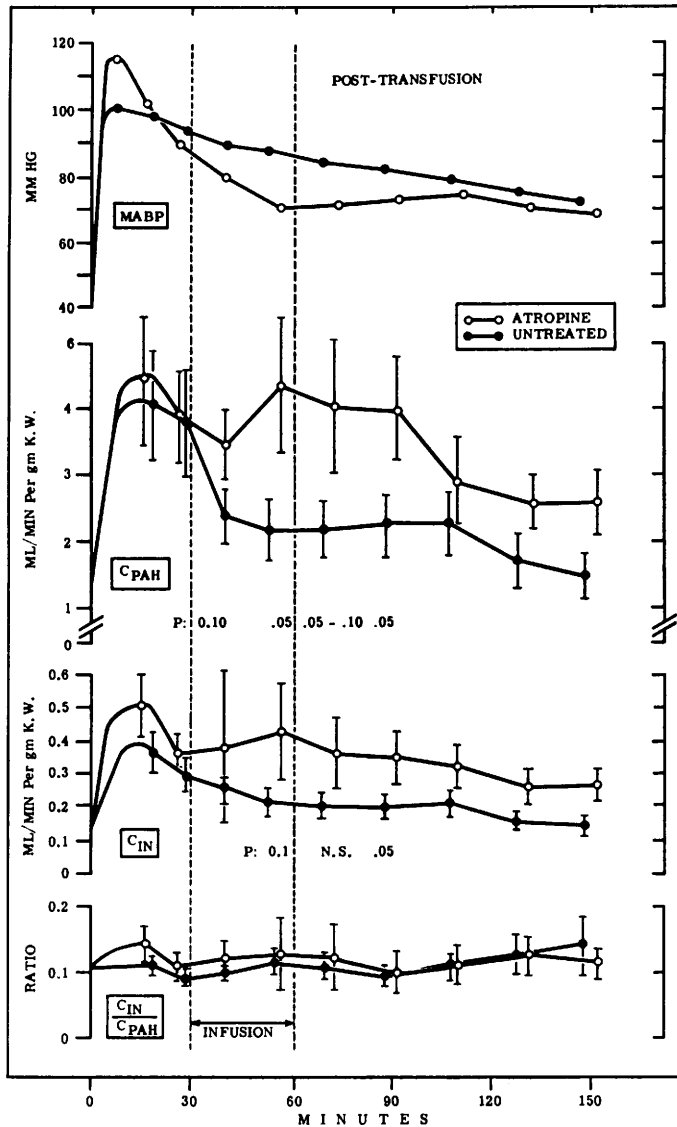


FIG. 4. Atropine infusion started 30 min after transfusion of shed blood, ($N = 8$), compared with trend of untreated group of animals.

again averaging slightly above 0.1. It was observed, when the infusion of atropine was delayed for 30 min, that the effect on C_{PAH} and C_{IN} , in terms of an increment above the expected control trend, was not as great as when the infusion was begun immediately before the restoration of blood volume.

Influence of atropine on urine volume, sodium clearance, and osmolar clearance. Accompanying the increase in arterial pressure and GFR resulting from the transfusion of

blood, urine volume, C_{Na} , and C_{Osm} increased markedly early in the posttransfusion period. When expressed as a ratio to the inulin clearance, these functions, on the whole, approximately doubled compared to the prehemorrhage control values. The striking finding was that the free-water clearance, which had been negative throughout the hypotensive phase, now became positive, and the U/P osmolal ratio decreased to average values somewhat below unity (Fig. 5). However,

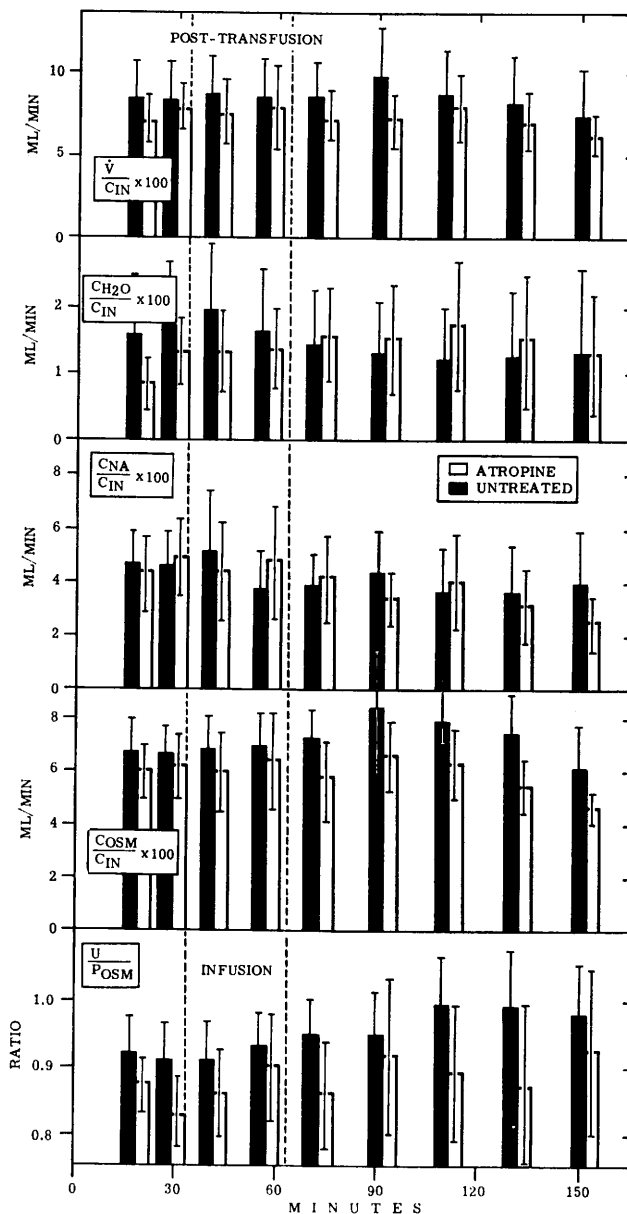


FIG. 5. Influence of atropine on V_U , C_{H_2O} , C_{Na} , C_{Osm} , expressed as fractions of C_{In} , and on U/P (Osm). Same group as depicted in Fig. 4.

when all of these parameters of the animals receiving atropine were compared with the untreated series as a fraction of the inulin clearance, no significant influence on V_U , C_{H_2O} , C_{Na} , C_{Osm} , and the U/P ratio of osmolality was detected.

Nor was there any influence on the comparative values for V_U , C_{Na} , and C_{Osm} when the atropine infusion was begun immediately

before transfusion. Accordingly, these values need not be presented in detail. In summary, when the influence of alterations in GFR resulting from atropine treatment were taken into account by expressing the clearances as a fraction of the inulin clearance, no significant alterations in urine volume, sodium handling, and concentrating ability of the kidney were observed, either in the series in which

atropine infusion was begun immediately or in the group in which atropine infusion was delayed until 0.5 hr posttransfusion.

Discussion. The most notable effect resulting from the atropine infusion was evidence of the increase in renal plasma flow indicated by significant increase in C_{PAH} over the trend in the untreated series. GFR as measured by C_{In} also was maintained well above the trend of the untreated series. The ratio of C_{In}/C_{PAH} remained constant, suggesting that filtration fraction had not altered significantly. Evidence of reduced renal vascular resistance was suggested by the relatively enhanced C_{PAH} accompanying the tendency for the arterial blood pressure to decrease more than the trend observed in the control series. Thus, using the ratio, mean arterial pressure/ C_{PAH} , as a rough index of renal vascular resistance, it can be assessed that this ratio fell significantly during the peak of the atropine action in both series. The fact that the atropine infusion immediately prior to transfusion gave the most significant effect can probably be explained by the fact that when the drug infusion was delayed until 30 min after the restoration of the blood volume, renal blood flow would be decreasing, as manifested by the rapidly falling trend of C_{PAH} in the untreated series. Hence, delivery of atropine into the renal vasculature would not be as effective as when administered early in the posttransfusion period at a time when C_{PAH} was maximal. It is also of interest that, in both series, the maximal effect on these functions was delayed somewhat until about 30 min after the atropine treatment was initiated.

The question may well be asked as to the validity of the C_{PAH} as an estimate of EPF in the posttransfusion phases following over 2 hr of hypotension. The extraction ratio of PAH (E_{PAH}) was measured in 8 of the animals of the control (untreated series). The control average for E_{PAH} was 0.8 (± 0.017 , SEM). After transfusion, E_{PAH} averaged 0.7 (range, 0.73 ± 0.06 , 15 min posttransfusion to 0.66 ± 0.09 , 165 min posttransfusion). This would appear to indicate about a 10–15% reduction in the ability of the tubules to extract PAH as a result of

the hypotensive regimen pursued in the present series. This would suggest that the estimates of PF would be low by this approximate percentage value. The other possible source of error would be an overshooting of clearance values immediately after transfusion resulting from the flushing of stagnant urine out of the nephrons. This source of error was minimized by discarding the first 15 min of urine collection after transfusion, at a time when urine flow was highest. In any event, the influence of atropine on C_{PAH} and C_{In} is so striking that even gross quantitative differences are very apparent when compared to the untreated group, particularly in the group which received atropine immediately with transfusion (Fig. 3).

The unexpected finding of proportionately enhanced EPF and GFR could not be explained in terms of abolition of cholinergic fiber influence on the juxtamedullary and inner medullary circulation (efferent arterioles and sphincters of the vasa recta), which would favor reduction in total renal blood flow. A possible explanation of the present results might be that atropine blocked the preganglionic fibers of the sympathetic nervous system supplying the kidney, to account for the apparent enhancement of blood flow. Although atropine action at the ganglionic level is not ordinarily accepted, this seems possible at the dosage given by the intra-arterial route (15). The probable sites would be the aorticorenal ganglia, which supply postganglionic adrenergic constrictor fibers to the kidney vasculature via the renal plexus (16). The transient fall in blood pressure during atropine infusion indicated that this was probably a rather widespread systemic effect, affecting other sympathetic, preganglionic fibers, innervating arterioles of the systemic vasculature with adrenergic postganglionic vasoconstrictor fibers. An added possibility is that atropine, in the dosage given, depressed the function of the neurons of the vasoconstrictor centers of the medulla (17). McGiff *et al.* (18) studied the influence of the frequency of renal nerve stimulation on the relative vasoconstrictor response of canine kidneys and observed a diminution of the renal vascular constrictor response after

atropine (1 to 2 mg/kg, intra-arterially), particularly at frequencies of nerve stimulation between 2 and 7/sec. They favored the notion, in a qualified manner, that there exists a cholinergic participation in the response of canine renal blood vessels to sympathetic nerve stimulation, along the lines of the Burn-Rand hypothesis (20).

The possible role of cholinergic fibers which supply the outer medulla and the mechanism of their action is receiving increasing attention (6, 7, 14, 19). Weitsen and Norvell (8) studied the degeneration of acetylcholinesterase-positive nerve fibers after degeneration of the renal nerves, leading them to suspect their origin in viable ganglion cells in intimate relationship with the kidney. Special staining techniques were used to demonstrate the presence of such ganglia in the hila of several of their preparations. Shvalev (11) also observed the consistent localization of ganglia subcapsularly in the canine kidney. The preganglionic fibers, which course through the renal plexus, are probably thoracolumbar in origin (16). The possibility of a craniosacral contribution was considered by Shvalev, who also allowed for the possibility of free-lying intramural ganglion cells.

The proportion of postganglionic fibers which are adrenergic compared to which are cholinergic has not been quantitatively assessed, but the evidence seems to indicate that the adrenergic type of fiber would predominate. Thus, atropine would tend to abolish the predominant adrenergic vasoconstrictor influence of enhanced sympathetic tone of the renal plexus by blocking the preganglionic transmission, in our view, while possibly also blocking the influence of the postganglionic cholinergic fibers (such as have been observed to enter into the medullary circulation). The logical explanation of the apparent enhancement in blood flow resulting from atropine infusion would thus be the result of the predominant abolition of vasoconstrictor activity by blockade at the preganglionic level as atropine entered the general circulation. If the medullary cholinergic fibers were blocked by the atropine, as one might expect, the effects were covered up by

the more profound influence of the apparent dilation of the afferent arterioles of all glomeruli. Changes in filtration fraction were not large enough to give positive evidence of change in resistance of efferent arterioles of the juxtamedullary circuits, nor could inferential conclusions be drawn about possible influence on the vasa recta circulation in the present investigation. Further studies will require instrumentation of a type that will accurately measure medullary blood flow of both the outer and inner zones to ascertain with certainty the discrete operation of the cholinergic nerve fibers that penetrate into the renal substance, particularly the outer medulla.

When the parameters were analyzed as a fraction of the concurrent GFR, no dramatic influence on urine volume, free-water clearance, osmolar clearance, and sodium clearance was observed which could not be explained by alterations in GFR, rather than by specific action of atropine on these functional aspects of the kidney. This is in accord with the finding of other workers who have studied the influence of atropine. Vander (21) studied the effect of acetylcholine in the EFP, GFR, and excretion of sodium, potassium, and water in dogs. When these functions were increased by infusion of acetylcholine into the renal artery, atropine abolished them by blocking the acetylcholine effect. In the absence of pretreatment with acetylcholine, there was no influence of atropine on these renal functions. Similarly, Lavendar *et al.* (22) found that, in dogs anesthetized with pentobarbital, atropine blocked the response to acetylcholine which, like the finding of Vander, was manifested by an increase in ERPF and GFR with concomitantly increased excretion of calcium, phosphate, sodium, potassium, chloride, and water, but that the drug exerted no renal actions in the absence of exogenous acetylcholine.

Although the ability of atropine to oppose the development of progressive renal vasoconstriction in the posttransfusion period was clearly demonstrated in the present study, no significant additional light was shed on the cause and nature of several interesting

renal aberrations in hemorrhagic shock, *viz.*, the development of positive free-water clearance and the concomitant reduction of U/P (Osm) below unity, and the possible relationship to the persistent reduction in FF. The evidence seemed to indicate that the adrenergic vasoconstrictor action is predominantly on the afferent arterioles, since reduction of such an influence by the atropine blockade did not alter the ratio C_{In}/C_{PAH} . On the other hand, since C_{PAH} reflects predominantly cortical blood flow, it is possible that subtle changes in medullary blood flow might have escaped analysis. With blockade of the intrarenal cholinergic fibers, atropine would be expected to reduce medullary blood flow, and, together with improvement of GFR, would work in the direction of restoration of the medullary gradient of osmolality, normally washed out under these circumstances (1). Indeed, the slight upward trend of U/P (Osm) in Fig. 5 supports this possibility [U/P (Osm) rose from 0.83 to 0.90 during atropine infusion]. However, it should be interjected at this point that positive C_{H_2O} and U/P (Osm) below unity reflect more subtle impairment of the urinary concentrating ability of the collecting duct in response to ADH than simple washout of the gradient (1). This could not produce urine below isotonicity. Thus, restoration of the gradient represents only one important phase of the restoration of the concentrating capability of the kidney. The mechanism of the failure of response of the epithelium to ADH under present experimental conditions is not understood.

Summary. Atropine sulfate was infused intra-aortically (0.275 mg/kg/min) to attempt blockade of medullary cholinergic fibers which might function during hemorrhagic shock to promote washout of the medullary gradient of osmolality by maintaining MBF. It was found that infusion of atropine in the dosage used and by the route described, resulted in relative increase in C_{PAH} and C_{In} over the trend observed in a group of untreated animals, similarly subjected to hemorrhagic hypotension, with no change in FF. Results could be explained by blockade of preganglionic (aorticorenal) cholinergic fibers, resulting in reduction of

postganglionic adrenergic vasoconstrictor influences on afferent arterioles. No influence of atropine could be detected on urine volume sodium clearance, and osmolar clearance when expressed as a fraction of C_{In} , nor on U/P osmolality. If intrarenal cholinergic fibers were blocked, the expected results of this (increase in FF and reduced total RBF) were covered up by the more marked influence resulting from diminution of enhanced vasoconstrictor activity.

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