Depletion of the Renal Medullary Osmotic Gradient
Following Hemorrhagic Hypotension in Hydropenic Rabbits

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Summary

The influence of blood pressure on the renal medullary osmotic pressure gradient was studied in hemorrhagic hypotension of hydropenic rabbits. Slice analysis was made for urea, sodium and potassium concentrations in the kidney slices. The tissue fluid osmolality was estimated as the sum of the urea concentration and $2 \times$ electrolyte concentrations. A drop of systemic blood pressure, induced by bleeding, resulted in decrements of urea and sodium concentrations with resultant reduced osmotic pressure gradient in the medulla, but did not significantly affect solute and osmotic concentrations in the cortex. A loss of urea from the medulla was more rapid and greater than that of sodium. The reduction of the medullary osmotic pressure gradient became greater as mean blood pressure decreased to 50–60 mm.Hg, but was reduced with progressively decreased blood pressure below 50 mm.Hg. Renal arterial occlusion for 1 hour gave rise to a slight decrease in the medullary osmotic pressure gradient, chiefly due to loss of urea from the medulla. The finding indicates that the depletion of the medullary osmotic pressure gradient in hemorrhagic hypotension is dependent upon the blood pressure level. The data were also discussed in relation to the renal hemodynamic alterations in hemorrhagic hypotension.

Additional Indexing Words:
Hemorrhagic hypotension Shock Renal tissue fluid osmolality Renal arterial occlusion

It is generally accepted that the vasa recta contributes to preservation of the hypertonicity of the mammalian renal medulla as one of the counterflow systems. If this is correct, intrarenal hemodynamic alterations caused by hemorrhagic hypotension, namely more severe curtailment of glomerular filtration than medullary blood flow, may favor depletion of the medullary osmotic pressure gradient. Selkurt et al. have described higher renal venous osmolality than arterial osmolality and a renal urine-concentrating defect after bleeding to 60 mm.Hg in dogs, suggesting the washout of osmolar

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constituents from the renal papilla by medullary blood flow. In support of Selkurt's speculation, some investigators\(^2\),\(^{12}\) have demonstrated depletion of the medullary osmotic pressure gradient after 1–3 hours at 60 mm.Hg mean blood pressure. If removal of solute is the primary cause for the reduced medullary osmotic pressure gradient in hemorrhagic shock, depletion of the medullary osmotic pressure gradient should be dependent upon the blood pressure level. Wright et al.\(^{12}\) demonstrated that the papilla is slightly hypertonic to the cortex at 40 mm.Hg mean blood pressure, while the papilla and cortex are isoosmotic at 60 mm.Hg.

The purpose of the present work was to investigate the possibility that the depletion of the medullary osmotic pressure gradient is dependent upon the blood pressure level in hemorrhagic hypotension of hydropenic rabbits.

**Methods**

Twenty-five rabbits, weighing 2.5–3.5 Kg., were caged in the laboratory for several days and maintained on a commercial rabbit chow. In order to obtain maximal hypertonicity of the renal medulla, the animals were deprived of drinking water for 36–40 hours prior to the experiments.

Rabbits, anesthetized with 25–35 mg./Kg. sodium pentobarbital given intravenously, were bled from the femoral artery or the carotid artery into a heparinized syringe until blood pressure stabilized at a constant level. In some experiments the left renal artery and the abdominal aorta just above the left renal artery were occluded by screw clamps. Systemic blood pressure was measured just below the origin of the left renal artery with a Statham pressure transducer (P23AA), connected to a catheter inserted into the abdominal aorta through the right femoral artery. After 30 min. or 1 hour both kidneys were exposed through a mid-abdominal incision and removed. The kidney was sectioned and a middle triangular block, containing the cortex and medulla, was frozen in a mixture of dry ice and acetone. While still frozen, the kidney block was cut with a razor blade into 6 slices of 30–120 mg. weight perpendicular to the longitudinal axis of the papilla: superficial and deep cortex, outer medulla, and outer layer, middle layer and papilla of inner medulla.

The kidney slices were weighed and placed in 20 ml. Erlenmeyer flasks with 2 ml. of distilled water. The flasks with the slices were weighed and then heated to boiling for 5 min. in a water bath. After cooling, distilled water was added to the flasks to the same weight as before boiling. The flasks were closed and kept in a refrigerator of 6°C for 24 hours for diffusion to take place. The supernatant was analyzed for sodium, potassium and urea. Sodium and potassium concentrations were measured by means of a flame photometry. Urea concentration was measured by the Conway microdiffusion method.\(^4\) Concentration in renal tissue water was calculated from wet and dry weights of the slices. The osmotic concentration in the kidney tissue water was estimated as the sum of the urea concentration and 2× the sum of sodium and potassium concentrations.
RESULTS

In hydropenic rabbits sodium and urea concentrations were found to increase from the cortex to the papilla tip. Potassium concentration was

Fig. 1. Osmotic pressure (×), and urea (○), sodium (●) and potassium (▲) concentrations in renal tissue after 30 min. at 50 mm.Hg mean blood pressure (A) and 1 hour at 60 mm.Hg (B). Broken line illustrates values in control rabbits maintained at 100 mm.Hg. C: cortex. OM: outer medulla. IM: inner medulla.
slightly lower in the outer strip of the inner medulla than in the cortex and papilla tip, though the difference was not statistically significant. The average and S.D. of 10 cortex slices from 10 animals gave values for urea of $24.5 \pm 6.6$ mM./L. of tissue water, sodium of $78.2 \pm 11.7$ mEq./L. and potassium of $76.0 \pm 8.1$ mEq./L. The papilla-to-cortex ratio averaged 23.6 for urea, 6.4 for sodium and 1.3 for potassium concentration. The calculated osmotic concentration of the tissue water also increased progressively from the outer medulla through the papilla. The osmotic concentration in the cortex was $334.1 \pm 28.0$ mOsm/L. of tissue water. The osmolar ratio papilla/cortex averaged 5.3.

After 30 min. at 50 mm.Hg mean blood pressure urea concentration in the medulla decreased tremendously, while sodium concentration did not significantly change (Fig. 1, left). The solute concentrations in the cortex was not significantly affected. The medullary osmotic pressure gradient was reduced. After 1 hour at almost the equivalent blood pressure level, decrements of urea and sodium concentrations, and depletion of the medullary osmotic pressure gradient were more marked (Fig. 1, right), suggesting that depletion of the medullary osmotic pressure gradient in hemorrhagic hypotension involves time limits.2)

Fig. 2-left illustrates the tissue fluid osmolality in the cortex and papilla

![Fig. 2. Relationship of blood pressure level to tissue fluid osmolality (A) and solute content per unit dry weight (B) in cortex (O) and papilla tip (●).]
tip after maintenance of different blood pressure levels for 1 hour. Reductions of the fluid osmolality in the papilla tip was most conspicuous at 50–60 mm.Hg mean blood pressure. The osmotic concentration in the papilla tip at 50–60 mm.Hg was less than 30% of that at 100 mm.Hg. With a progressive blood pressure drop below 50 mm.Hg, the decrement of the tissue fluid osmolality in the papilla tip was reduced. The increase in cortical tissue fluid osmolality with decrease in blood pressure was not statistically significant. The same general relationship was found between blood pressure level and solute content per unit dry tissue weight of the papilla tip or the cortex (Fig. 2, right).

![Graph](image)

**Fig. 3.** Osmotic pressure (A), and urea, sodium and potassium concentrations (B) in renal tissue after 1 hour renal arterial occlusion in left kidney. Broken line illustrates values in control right kidney.

One-hour renal arterial occlusion gave rise to a slight reduction of the medullary osmotic pressure gradient in 4 kidneys, chiefly due to a decrease of urea concentration in the medulla (Fig. 3). Sodium concentration in the medulla did not decrease significantly. The tissue fluid osmolality values in the cortex and papilla tip after the renal arterial occlusion are also plotted at 0 mm.Hg blood pressure level in Fig. 2. The medullary osmotic pressure gradient after the renal arterial occlusion averaged 70% of that at 100 mm.Hg mean blood pressure.

**DISCUSSION**

Sodium and urea concentrations in the renal cortex in hydropenic rab-
bits, measured with the same method as used by Schmidt-Nielsen et al.\textsuperscript{9)}, were slightly lower than those obtained with different methods in hydropenic dogs.\textsuperscript{2,11)} Also, the osmotic activity in the cortex was slightly lower in the rabbit than in the dog. The papilla-to-cortex ratio for sodium was higher in the hydropenic rabbit than in the hydropenic dog, while the ratio for urea was not different between the two animals.\textsuperscript{11)}

In contrast to the finding of Boylan et al.\textsuperscript{2)} that there was no depletion in the medullary osmotic pressure gradient after 30 min. at 60 mm.Hg mean blood pressure in the dog, reduction of the medullary osmolality was detected in the rabbit after the equivalent period at the same blood pressure. This reduction of the medullary tissue fluid osmolality was more marked with longer periods of hypotension, suggesting the possibility that the depletion of the medullary osmotic pressure gradient resulting from hemorrhagic hypotension is time dependent.\textsuperscript{2)} A loss of urea from the medulla was more rapid and greater than that of sodium.

The data support the finding of Selkurt et al.\textsuperscript{10)} that the renal venous-to-arterial ratios for osmolality and sodium concentration increase greater than unity in shock of the dog, suggesting the washout of solute from the papilla. Decrement of the medullary osmotic pressure gradient was enhanced with a progressive blood pressure drop to 50–60 mm.Hg. On the contrary, a drop of blood pressure below 50 mm.Hg resulted in less marked reduction of the medullary tissue fluid osmolality than at 50–60 mm.Hg. This finding implies that the depletion of the medullary osmotic pressure gradient is dependent on the blood pressure level. The same relationship was found between the blood pressure level and the solute content per unit dry weight of the papilla tip. Thus, the depletion of the medullary osmotic pressure gradient in hemorrhagic hypotension was chiefly due to a removal of solute from the medulla, and not due to a water shift.

In the other experiments, effects of hypotension on renal blood flow and urine flow were studied in left kidneys of anesthetized rabbits with the technique as previously described.\textsuperscript{13)} The average control values of mean arterial pressure, renal blood flow, urine flow and extraction ratio of para-aminohippurate were 88.2±11.2 mm.Hg, 27.2±8.0 ml./min., 0.1±0.06 ml./min. and 81.0±8.0\% respectively. With progressively decreased blood pressure induced by bleeding, renal blood flow and urine excretion decreased. It appeared that urine excretion ceases at 50–60 mm.Hg, while renal blood flow stops at 15–20 mm.Hg (Fig. 4). PAH extraction ratio decreased slightly as blood pressure dropped below 60 mm.Hg. The more severe reduction of urine flow than renal blood flow may be partly due to humoral-borne or neurogenic increase in the preglomerular vascular tone. Assuming that glomerular filtra-
tion ceases at the blood pressure level where urine is no longer formed during hemorrhagic hypotension, glomerular filtration will stop at mean blood pressure below 50 mm.Hg. On the other hand, decrement of medullary blood flow calculated from renal blood flow and PAH extraction ratio, according to the principle of Reubi, appeared to be less than that of renal blood flow or cortical blood flow during hemorrhagic hypotension. This is consistent with

Fig. 4. Effects of blood pressure on renal blood flow (RBF) and urine flow (UF).

Fig. 5. Schematic illustration of relationship of renal hemodynamic alteration to change of tissue fluid osmolality in papilla tip in hemorrhagic hypotension.
Thus, glomerular filtration might be severely reduced when compared with renal medullary blood flow. It is also possible that hypoxia will impair metabolically the hyperosmotic medullary operation at low blood pressure and blood flow rate. If the washout of solute is the primary cause for the reduced medullary osmotic pressure gradient in shock, a severe curtailment of glomerular filtration compared with renal medullary blood flow and a reduction of input to the countercurrent system will favor a more marked depletion of the medullary osmotic pressure gradient with progressively decreased blood pressure to 50 mm.Hg (Fig. 5). On the contrary, with a blood pressure drop below 50 mm.Hg, medullary blood flow may decrease gradually in the absence of glomerular filtration and thus, a slower removal of solute with resultant reduced depletion of the medullary osmotic pressure gradient may be produced. Below 15–20 mm.Hg mean blood pressure, both renal blood flow and glomerular filtration cease and the washout of solute by medullary blood flow does not occur. The slightly reduced medullary osmotic pressure gradient, seen after 1-hour renal arterial occlusion, might be partly due to physical diffusion.

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