Renal Response to Blood Volume Expansion in Conscious Rats with Acute, High Spinal Cord Transection[1] (35971)

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In anesthetized dogs, chronic high spinal transection (C-8) was shown to prevent a natriuretic and diuretic response to 25% intravascular volume expansion when artificial blood (washed canine red cells suspended in 6% bovine albumin in Ringer–Locke solution) was used. Pearce and Sonnenberg (1) advanced the hypothesis based on this work, and on that from intact dogs with denervated kidneys, that intact afferent spinal pathways were essential to the renal response to such expansion. Michaelis and Gilmore (2) later studied the problem in anesthetized, acute, high spinal (C-8) dogs after infusion of 6% dextran in saline equivalent to 33% of estimated blood volume. They reported an attenuated diuresis and natriuresis approximating 1/4 of their normal responses (3). It is to be noted, however, that their expanding solution also diluted blood constituents, which Nizet (4) and others (3, 5) have demonstrated to result in an increase in excretion without a change in intravascular volume. For this reason, and because of the lack of adequate control experiments, as well as the inconsistency of findings in the available reports, the importance of encephalorenal spinal pathways to the kidneys' response following blood volume expansion remained unclear.

The present experiments were therefore designed to determine whether such spinal pathways are essential to the development of volume natriuresis and diuresis. Two series of unanesthetized, acute, high spinal (C-8) rats were investigated. The first underwent an estimated 33% intravascular volume expansion with donor blood, and the second, an equivalent isovolemic exchange. The results indicate that spinal animals are able to respond to blood volume expansion with an increase in urine volume and sodium excretion within the range of those reported for intact, anesthetized rats which underwent a similar experimental protocol [(6), and see Table II].

Methods. Fifteen male Sprague-Dawley rats, weighing an average of 325 ± 19 g (mean ± standard error), were anesthetized with ether. After the injection of 1 ml of 1% procaine hydrochloride (The Vitarine Co Inc, NY) between the shoulder blades, a midline, high dorsal incision was made. The muscles above the spine were separated until the vertebrae could be directly palpated with the tips of a curved forceps. The tips of the forceps were then driven between two vertebrae using the T-2 spinal process as a guide. Destruction of the spinal cord was accomplished as quickly as possible by moving the forceps back and forth along the floor of the vertebral canal and along its sides. The bottom and sides of the canal were seen to be free of neural tissue, and this served as the first check that the cord was completely cut. This was further verified at autopsy. It has been determined by gross dissection that the procedure resulted in cord transection at a level where the C-8 nerve leaves the spinal cord. After the insertion of Gelfoam and cotton, the wound was closed. The animal was allowed to recover from the ether and continued to breathe spontaneously throughout the experiment.

A polyethylene catheter (PE 50) was inserted into a femoral artery to register the mean blood pressure (ABP) on a mercury manometer, and to obtain blood samples for

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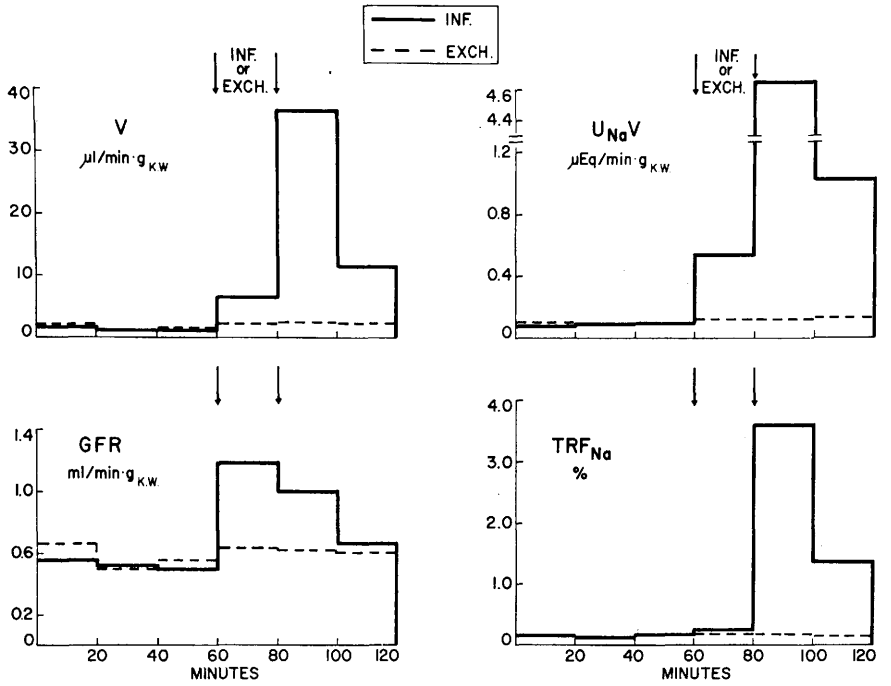

FIG. 1. Average effect over time of either infusion (—); or isovolemic-exchange (- -); using donor blood, in acute, spinal transected rats. The experimental maneuver is indicated by arrows.

analyses. A femoral vein was cannulated (PE 50), and, after a 1 ml priming dose (4 μC) of tritiated inulin (New England Nuclear), served for the continuous infusion of hydration fluid (0.02 ml/min, Ringer-Locke) incorporating inulin (4μC/ml) for glomerular filtration rate (GFR) determinations. The neck of the bladder was cannulated (PE 50) to allow urine collections. The majority of the bladder was tied off to reduce dead space.

Urine volume was measured by weighing tared polyethylene collection tubes. Hematocrits (HCT) were measured (International microhematocrit centrifuge and reader) from blood samples obtained at the midpoint of the urine collection period and are reported without correction for plasma trapping. Plasma and urine samples were used for determination of sodium concentration (P_{Na} and U_{Na}; IL internal standard flame photometer) and, after dilution in a toluene base scintillant containing Bio-Solv (7) for measuring radioactivity (Packard scintillation spectrometer). All renal function values were normalized to gram kidney weight (g_{kw}) and expressed on a per minute basis.

The plan of an experiment is as follows: At least 1 hr elapsed from the time of spinal transection and the initiation of 20 min urine collections. After a 60 min control period, one group of 10 rats (338 $\pm$ 26 g) received a 33% blood volume expansion with fresh, heparinized, donor blood obtained from ether anesthetized, decapitated rats. A second group of 5 spinal animals (301 $\pm$ 20 g) underwent an equivalent, isovolemic exchange with the donor blood, to provide the control protocol. The exchange procedure consisted of infusing donor blood into a femoral vein while simultaneously withdrawing an equal amount from a femoral artery. Renal function was studied during the above maneuvers and for 40 min thereafter.

For purposes of statistical comparison, a normalized value for the control period (60 min) was subtracted from that of the experimental segment (60 min), which resulted in a difference for each animal. These differences were averaged for each variable and significance between spinal infused and spinal exchanged animals was assessed by the use of Student's t test ($p<.05$).

Results. The average effect over time for the two series is shown in Figs. 1 and 2. Table I shows the average of the control and experimental periods. There were no differences between base lines for any of the measured variables. Expansion (see Fig. 1) resulted in a substantial increase in urine volume (V) and sodium excretion ($U_{Na}V$), as opposed to exchange. The GFR rose and peaked during the infusion period, then returned towards base line values, while that of the exchange animals remained essentially constant. The tubular rejection fraction for sodium ($TRF_{Na} = U_{Na}V/GFR \cdot P_{Na}$) increased to a peak of 3.60 ± 1.36% during the period following infusion and then returned towards control levels. Exchange resulted in little alteration of TRF_{Na}. The effect of the two procedures on mean ABP and hematocrit is displayed in Fig. 2. There was a large increase in ABP from hypotensive (84 ± 5 mm Hg) towards normotensive (126 ± 8 mm Hg) levels with infusion, and essentially no alteration with isovolemic exchange. A small increase in hematocrit was measured subsequent to infusion, with that of the exchange series remaining at base line values.

Figure 3 summarizes the results of statisti-

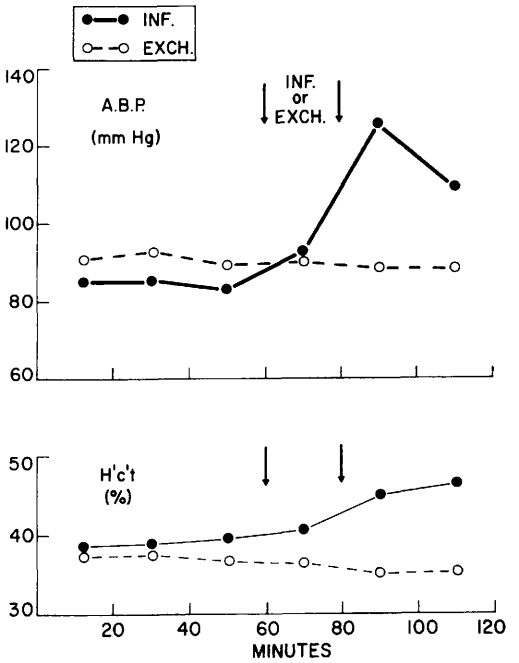

FIG. 2. Average effect, over time, of either infusion or isovolemic-exchange (notation as indicated previously) on mean arterial pressure and hematocrit. The experimental maneuver is indicated by arrows.

TABLE I. Comparison of Function for Exchanged (Exch.) and Infused (Inf.) Spinal Rats, Using Donor Blood, During Control and Experimental Periods.[a]

	Control (0–60 min)		Experimental (60–120 min)	
	Exch.	Inf.	Exch.	Inf.
V (μl/min·g_{kw})	1.70 ± 0.12 (5)	1.45 ± 0.16 (10)	2.38 ± 0.22 (5)	18.28 ± 3.52 (10)
$U_{Na}V$ (μEq/min·g_{kw})	0.10 ± 0.02 (5)	0.09 ± 0.01 (10)	0.14 ± 0.03 (5)	2.05 ± 0.53 (10)
GFR (ml/min·g_{kw})	0.58 ± 0.15 (5)	0.53 ± 0.08 (7)	0.62 ± 0.14 (5)	0.97 ± 0.10 (7)
TRF_{Na} (%)	0.15 ± 0.02 (5)	0.15 ± 0.04 (7)	0.18 ± 0.03 (5)	1.74 ± 0.62 (7)
ABP (mm Hg)	90 ± 3 (5)	84 ± 5 (10)	89 ± 5 (5)	108 ± 6 (10)
HCT (%)	36.6 ± 1.5 (5)	39.0 ± 1.2 (10)	35.6 ± 1.0 (5)	43.7 ± 1.1 (10)
U_KV (μEq/min·g_{kw})	0.45 ± 0.07 (5)	0.29 ± 0.06 (10)	0.62 ± 0.14 (5)	1.29 ± 0.12 (10)

[a] Values are means ± SE; numbers in parentheses are sample sizes.

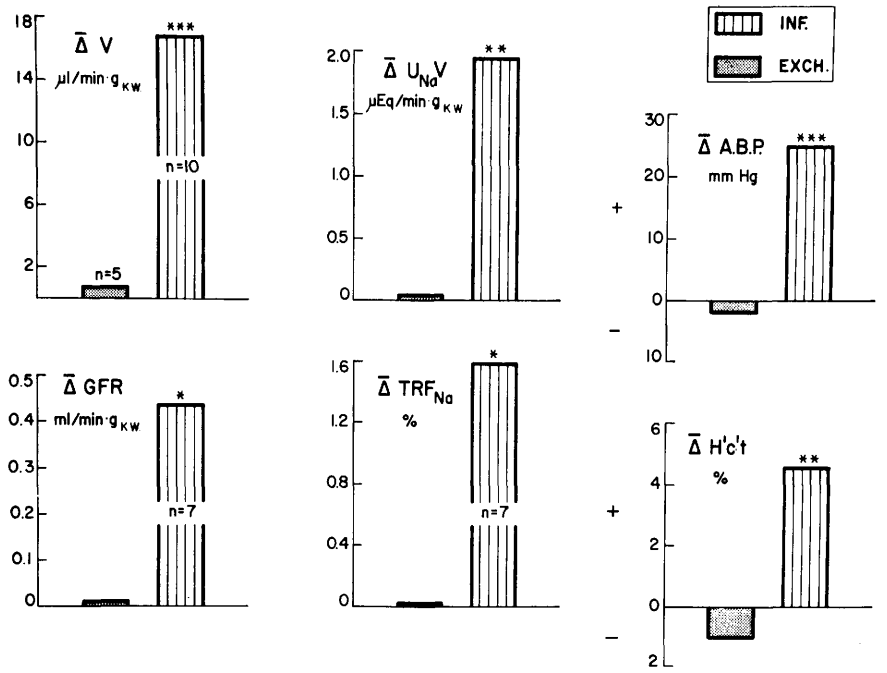

FIG. 3. Average difference between the experimental and control period for the indicated variables from spinal infused and exchanged rats; $*p<.05$; $**p<.01$, $***p<.001$; compared to exchange control series.

cal evaluation, as detailed earlier, for the experiments described. Expansion compared to isovolemic exchange resulted in significant increases for V ($p<.001$); $U_{Na}V$ ($p<.01$); ABP ($p<.001$); GFR ($p<.05$); TRF_{Na} ($p<.05$); and the hematocrit ($p<.001$).

Figure 4 shows the relationship between the rise in mean arterial pressure following infusion and the rise in sodium excretion ($r = 0.640$; $p<.05$) or urine volume ($r = 0.722$; $p<.02$) per minute gram kidney weight.

Discussion. The natriuresis and diuresis reported here for unanesthetized, spinal animals are within the range of those found in this laboratory for intact, anesthetized but untraumatized rats which underwent a similar experimental protocol [(6); Table II]. As shown by Tables I and II, conscious, spinal animals respond comparably to anesthetized, intact rats for sodium excretion, while the volume response for the spinal animals is somewhat blunted. This latter difference may be a consequence of altered antidiuretic hormone (ADH) levels attributable to the conscious state of the spinal rats, as well as the added surgical trauma and fluid loss accompanying spinal transection.

The rise in urinary excretion may be, in part, attributable to the measured increase in GFR. However, a tubular effect unassociated with the rise in GFR is suggested from the observation (see Fig. 1) that TRF_{Na} was still elevated, as was the sodium excretion, at a time when GFR had returned to base line values. It is unlikely that the observed responses can be attributed to a dilution of preexisting circulating levels of humoral substances such as ADH, aldosterone, or catecholamines, since no such responses are seen for the isovolemic, exchange control series. If the effects were humorally mediated, this must have been in response to the increased blood volume. Furthermore, although there was a significant increase in the hematocrit after infusion, such a change has been shown by Bengele, Houttuin, and Pearce (submitted for publication), to result rather in a decrease in urine volume and sodium excretion. Lastly, the responses may

TABLE II. Renal Function Values for Five Intact, Anesthetized, but Untraumatized, Rats During Control (C) and Experimental (E) periods.[a] [b]

	Control (0–60 min)	Experimental (60–120 min)	Δ (E — C)
V (μl/min·g_{kw})	1.48 ± 0.09	39.46 ± 1.70	+37.98 ± 1.70
$U_{Na}V$ (μEq/min·g_{kw})	0.10 ± 0.02	2.01 ± 0.15	+1.91 ± 0.16
GFR (ml/min·g_{kw})	0.96 ± 0.09	1.65 ± 0.04	+0.69 ± 0.10
TRF_{Na} (%)	0.07 ± 0.02	1.23 ± 0.10	+1.16 ± 0.11
ABP (mm Hg)	123 ± 1	124 ± 5	+1 ± 4
HCT (%)	45.2 ± 0.3	49.1 ± 0.5	+3.7 ± 0.5
U_KV (μEq/min·g_{kw})	0.42 ± 0.03	1.43 ± 0.08	+1.01 ± 0.07

[a] Values are means ± SE; experimental period refers to infusion (60–80 min; donor blood equivalent to 33% estimated blood volume) and postinfusion (80–120 min) segments.

[b] Data to be published by A. Veress and J. W. Pearce.

also have been caused, in part, by the large rise in ABP, either by way of a pressure diuresis (8) or the action of intrarenal physical forces at the peritubular capillary level (9). With respect to the latter, Martino and Earley (10, 11) demonstrated that a reduction in renal vascular resistance alone can lead to an increase in urinary excretion. Further, they proposed that under such conditions a rise in renal perfusion pressure would be more effective in altering peritubular capillary hydrostatic pressure and in turn affecting tubular reabsorption. Although renal resistance was not measured in the present experiments, it has been shown by Takacs *et al.* (12) that acute, spinal transection in the rat is associated with a reduction in total vascular resistance which included the kidney vessels. It is apparent from the present data displayed in Fig. 4, that as the expanded animal's blood pressure rises a greater urinary excretion is indicated, perhaps implicating intrarenal physical forces as a mechanism for the alteration in excretory pattern with vascular expansion of spinal transected rats.

The results demonstrate that unanesthetized, acute, high spinal (C-8) rats are able to respond to blood volume expansion. Although the mechanisms may not be the same as in the intact anesthetized animal, it can be concluded from this study that

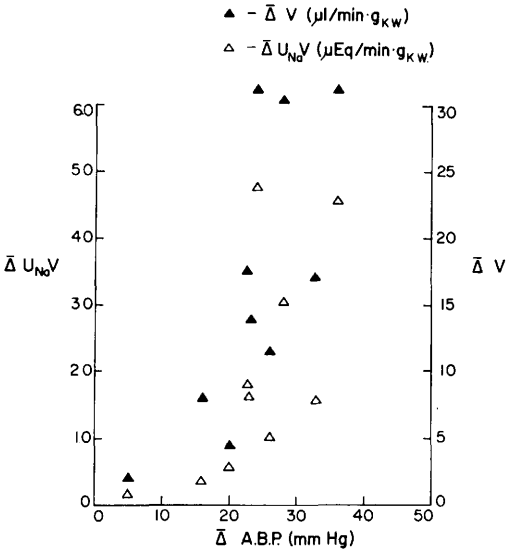

FIG. 4. Relationship between the rise in urine volume (ΔV) or sodium excretion ($\Delta U_{Na}V$) and the rise in mean arterial pressure (ΔABP) with infusion in 10 spinal rats. Correlation coefficient (r) was 0.722 ($p<.02$) and 0.640 ($p<.05$), respectively.

encephalorenal, spinal pathways, afferent or efferent, are not essential for the usual renal responses to such expansion.

Summary. In the present study, the importance of the encephalorenal, spinal pathways in the kidney response to acute blood volume expansion (2.3% body wt) was examined. Studies were performed in conscious rats at least 2 hr after spinal transection at the C-8 level. The results were compared with those obtained from a series of similarly prepared rats which underwent an isovolemic, exchange transfusion to provide the control protocol. The increased urine flow, sodium excretion, glomerular filtration rate, tubular reflection fraction for sodium and mean arterial pressure were significantly different from those of the exchange-control series. The natriuresis was comparable to that for anesthetized, but untraumatized, normal rats which underwent a similar experimental protocol. The diuresis, however, was somewhat blunted perhaps attributable to a disparity in ADH levels as a result of the differences in anesthetic level and degree of surgical trauma.

It can be concluded that encephalorenal, spinal pathways are not essential for the renal reaction to an expanded intravascular volume. A correlation between the rise in urinary excretion and the rise in mean arterial pressure with infusion implicates intrarenal physical forces as one probable mechanism for the described responses.

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