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The Everted Rat Intestine Used as a Model System to Demonstrate Regulation of Sodium Absorption Following Hemorrhage (37740)

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(Introduced by E. Grim)

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In earlier work on canine ileal loops, Mailman and Ingraham (1) demonstrated an increase in the absorption of sodium following hemorrhage. Since these studies were done *in vivo* the results could not delineate whether the effects were from hemodynamic changes in intestinal circulation or if there were substances in the plasma that affected the sodium transport at a cellular level. This report shows evidence that following hemorrhage there appears to be a release of a circulating substance in the plasma that stimulates the absorption of sodium in the isolated everted rat intestine. Aldosterone, angiotensin and vasopressin were also investigated to a limited extent as to their effect on sodium absorption in the everted rat intestine.

Methods and Materials. Male retired breeder rats (Holtzman strain) were used as the assay animal in the experiments. They were anesthetized with ether and an abdominal incision was made. Following identification of the cecum, the distal 8 to 10 cm of ileum was removed, stripping the ileum away from the mesentery. The ileal contents were removed by flushing with isotonic saline containing glucose and the segment was everted with a 1 mm stainless steel rod. With the use of a pair of forceps the everted segment was mounted on a Lucite cannula designed to collect serial samples. A 5 cm section was tied off with cotton thread forming a sac. Within 30 to 50 sec the segment was placed into a 50 ml tube containing 40 ml of the Krebs solution (2) through which was bubbled a continuous oxygen supply. A constant temperature was maintained by use of a water bath at 36°.

In all experiments 10 m*M* of D-glucose was present in both the mucosal and serosal solutions. The Krebs bicarbonate solution was aerated with 95% oxygen and 5% carbon dioxide continuously for all studies and for 1 hr before use to maintain the solution at a pH of 7.4 and to adequately oxygenate the tissue.

The serosal solution was the same as the mucosal solution with the addition of 50 mg% of inulin, which is neither metabolized nor absorbed by the rat ileum, to measure the net transport of water. One milliliter of the serosal solution was pipetted through the cannula into the sac. The experimental periods were 1 hr in length with both serosal and mucosal solutions being changed at the beginning of each hour. Samples of 100 μl were taken by a Beckman self-measuring micropipette at the beginning and end of each hour for determinations of sodium, potassium and inulin.

The sodium and potassium samples were diluted with lithium (1:200) by use of a 20 ml automatic pipette. The readings were made with an Instrumentation laboratory flame photometer (Model 143). The inulin was measured by a colorimetric method developed by Kulka (3) that is insensitive to the presence of glucose. Readings were made with a Bausch and Lomb Spectronic 20 col-

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orimeter.

Four female mongrel dogs weighing 9 to 11 k were used as experimental animals. All dogs were anesthetized with Nembutal (30 mg/kg). The surgery was kept to a minimum, with only a femoral artery being isolated and cannulated for arterial sampling.

Control blood samples as well as those following hemorrhage were taken directly into heparinized centrifuge tubes and centrifuged at ½ maximum speed (approx 1500 rpm) in an International clinical centrifuge for 20 min. Plasma was added to the Krebs bicarbonate serosal solution and silicone antifoam agent was added to control the foaming. Blood samples were taken 15 min following a hemorrhage of 20% of blood volume (estimated at 8% of body wt). The hemorrhage took place over a period of 40 min with equal amounts of blood being removed every 10 min. The control plasma was added to the serosal solution of the everted rat segment at the beginning of the first hour. The experimental plasma was added at the beginning of the second hour. Each rat gut segment served as its own control.

In the formulae described below, 1 ml of the initial (zero time) serosal solution was used as a reference. The calculations were as follows:

$$\text{ion movement: } Q_{Na} = \frac{I_i}{I_f} \times Na_f - Na_i$$
$$= \mu\text{Eq/hr sac},$$

where

I_i Initial insulin concentration at zero time (mg/ml)

I_f Final inulin concentration at end of experimental hour (mg/ml)

Na_i^+ Initial sodium concentration at zero time (μEq/ml)

Na_f^+ Final sodium concentration at end of experimental period (μEq/ml)

The statistical analyses were done on a PDP-8 computer. Student's pairing test was used for statistical analysis and a P level of less than 0.05 was considered to be significant throughout this study.

Results. Plasma controls. A healthy mongrel male dog anesthetized with Nembutal was used for sequential control studies. A plasma sample from blood taken from the femoral artery was assayed on the rat gut during the control hour. After 45 min, during which the anesthesia level was maintained constant, another plasma sample was taken from the femoral artery and assayed for its effect on sodium transport during the experimental hour. The results of this control experiment are recorded on Table I.

TABLE I. Effect of Addition of Plasma from Anesthetized Dog (12% of Serosal Solution) upon the Movement of Sodium for Two Sequential Periods of Time.

Rat no.	Control $Q_{Na} = \mu$Eq/hr/sac	Experimental $Q_{Na} = \mu$Eq/hr/sac
1	34.1	69.3
2	100.7	91.0
3	55.0	40.6
4	152.1	65.8
5	113.0	137.3
Mean	91.0	80.8
SEM	16.8	27.2

Analyses revealed no significant difference in the movement of sodium between the control and experimental hour.

Contraction of extracellular fluid compartment. The data showing the effect of addition of plasma (12% of serosal solution) from dogs hemorrhaged approximately 20% of blood volume on the absorption of sodium in the rat ileum are presented in Table II.

In the control period the sac contents contained 12% plasma from the normovolemic animal. In the experimental hour the sac contained 12% plasma from the hemorrhaged animal. A significant increase in the absorption of sodium is demonstrated ($P < 0.05$). There appears to be the presence of a circulating substance in the plasma following hemorrhage that stimulates the absorption of sodium in the isolated everted rat intestine.

Previous work had suggested that aldosterone may be the substance involved in increased absorption in the intestine following hemorrhage (4). Since it is difficult to get aldosterone into solution except by dissolving it in alcohol, and this would be toxic to the gut, the decision was made to use angio-

TABLE II. Effect of Addition of Plasma (12% of Serosal Solution) from Dogs Hemorrhaged Approximately 20% of Blood Volume on Absorption of Sodium in the Rat Ileum.

Rat no.	Control $Q_{Na} = \mu Eq/hr/sac$	Experimental $Q_{Na} = \mu Eq/hr/sac$
1	39.2	40.9
2	61.3	85.6
3	61.1	66.3
4	85.6	93.3
5	211.0	275.0
6	76.0	108.0
7	89.0	91.0
8	96.0	82.0
9	89.0	115.0
10	86.0	109.0
11	16.5	137.9
12	39.2	93.5
13	39.6	60.8
14	28.4	176.0
15	44.1	105.1
Mean	70.8	109.3
SEM	11.9	14.4

tensin II to stimulate the release of aldosterone in the donor dog and assay the resulting plasma for its effect on sodium transport in the gut. Mulrow and Ganong (5) found that in dogs which had been hypophysectomized, nephrectomized on the left side and having the right lumboadrenal vein cannulated, infusion of 1 μg/min of angiotensin II increased the secretion of aldosterone in the adrenal vein without stimulating glucocorticoid secretion. The effects of the addition of plasma obtained from a dog receiving an infusion of angiotensin on sodium movement in the everted rat intestine are summarized in Table III.

TABLE III. Effect of Addition of Plasma (12% of Serosal Solution from Dogs Infused with Angiotensin 1.0 μg/min for 60 min on the Absorption of Sodium in the Rat Ileum.

Rat no.	Control $Q_{Na} = \mu Eq/hr/seg$	Experimental $Q_{Na} = \mu Eq/hr/seg$
1	48.1	77.9
2	62.0	81.7
3	77.0	74.0
4	16.0	136.0
5	98.0	120.0
Mean	60.2	97.9
SEM	12,2	12.6

After 60 min of continuous infusion of angiotensin II into a dog the plasma obtained significantly increased sodium absorption during the experimental hour ($P < 0.05$). This type of evidence suggested that angiotensin infusion stimulated the release of some substance, possibly aldosterone, that increased the absorption of sodium in the rat intestinal segment.

Since there is the possibility that angiotensin itself influences the absorption of sodium in the everted rat segment, the next experiment involved the addition of angiotensin 10^{-12} g/ml to the serosal side at the beginning of the experimental hour. There was no consistent or significant effect on the transport of sodium.

It is generally accepted that, following hemorrhage, there is a release of vasopressin. Since there was an increase in the transport of sodium in the gut in these studies, the possibility that this resulted from vasopressin needs to be considered. From work done by Baratz and Ingraham (6) with a bioassay using the specific gravity of rat urine as an indicator of concentration, the average level of vasopressin found during a 20% hemorrhage in dogs was approximately 3000 μU/ml of solution. The control values from the same animals before hemorrhage averaged 100 μU/ml of solution (6). Since in the previous experiments 12% of the serosal solution was plasma, for this experiment 12% of the serosal solution contained vasopressin at the concentration of 3000 μU/ml in order to approximate the circulating level of vasopressin.

There was no significant or consistent effect on sodium transport with the addition of vasopressin at the level of a 20% hemorrhage during the experimental hour.

Discussion. The experimental results presented here show that plasma obtained from dogs who had been hemorrhaged 20% of blood volume increased sodium absorption in the everted ileum segment of the rat. From work reported by Farrell, Rosnagle and Rauschkolb (4) it is accepted that following hemorrhage there is an increase in the concentration of circulating aldosterone. In some of these experiments an attempt was made

to increase the level of aldosterone by stimulating its release by infusion of angiotensin II, a potent stimulator of aldosterone release. The plasma obtained from normovolemic dogs after angiotensin II was infused for 60 min caused a significant increase in sodium transport. Whether the responsible substance was actually aldosterone was not determined in these experiments and it can only be concluded that following an infusion of angiotensin II some factor appeared in the plasma that increased sodium absorption in the everted rat intestine. To preclude the possibility that angiotensin II itself might affect sodium transport in the everted rat segment, experiments were carried out in which the angiotensin was added to the serosal solution in the intestinal segment during the experimental hour. There was no consistent effect on sodium transport.

Vasopressin is known to increase membrane permeability for water (7) and there is some evidence that vasopressin stimulates the transport of sodium (8). Vasopressin was added to the serosal solution during the experimental hour to give a concentration equivalent to that found *in vivo* following a 20% hemorrhage (6). The results from this experiment revealed no consistent or significant change in the movement of sodium.

Summary and Conclusion. Plasma obtained from dogs during the posthemorrhage period increased the absorption of sodium in the everted rat segment over control values.

The plasma obtained after dogs were infused for 60 min with angiotensin (1 μg/min) also resulted in a significant increase in sodium absorption in the everted gut preparation over the control values.

Angiotensin added directly to the serosal solution in the everted rat gut did not increase sodium absorption.

Vasopressin at a concentration equivalent to that found following a 20% hemorrhage had no effect on sodium absorption.

In conclusion, the experimental evidence supports the hypothesis that following contraction of the blood volume there is a change in the composition of the plasma that does significantly affect the absorption of sodium in an everted rat segment. This mechanism present in the intestine, then, should complement the role of the kidney in the homeostatic regulation of total body sodium.

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