Restoration of intravascular fluid volume following acute hypovolemia in rats

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Stricker, Edward M., and John E. Jalowiec. Restoration of intravascular fluid volume following acute hypovolemia in rats. Am. J. Physiol. 218(1): 191–196. 1970.—Subcutaneous injection of hyperoncotic polyethylene glycol (PG) solution produced hypovolemia in rats by gradually withdrawing isosmotic plasma fluid into the local interstitium. Significant drinking was observed for 6–8 hr, although water ingestion subsequently diminished despite persistent hypovolemia. This inhibition of thirst in the presence of substantial plasma deficits has been attributed to osmotic dilution of body fluids. When 0.51 m NaCl solution was additionally present, rats manifested a sodium appetite 6–9 hr after treatment and water intake progressed without evident inhibition. Apparently, osmotic dilution potentiated a sodium appetite in the hypovolemic rats, which removed the inhibition of thirst by increasing body fluid osmolality. Because of the increased fluid intakes and low urinaria volumes and sodium concentrations, increasing amounts of near-isotonic fluid were retained and eventually plasma deficits were repaired. These results indicate that thirst and sodium appetite are interdependent behavioral mechanisms of volume regulation which complement well-known antidiuretic and antinatriuretic processes in providing for the restoration of intravascular fluid volume in hypovolemic rats.

Methods

Subjects and pretreatment maintenance. The animals used were adult male albino rats, weighing between 300 and 350 g, of the Wistar strain. They were housed individually in mesh wire metabolism cages in a continuously illuminated temperature-controlled room (73–77 F). Purina laboratory chow was available to all rats ad libitum except as noted. Some rats were given demineralized water to drink (Group A), others had isotonic (0.15 m) NaCl solution instead (Group B), while a third group had both demineralized water and 0.51 m NaCl solution (Group C). The drinking fluids were continuously available in calibrated tubes (±0.2 ml) attached to the front of the cages.

Procedures

Rats were made hypovolemic by subcutaneous injection of 5.0 ml of 30% polyethylene glycol (PG) solution (Carbowax, compound 20-M; Union Carbide) dissolved in 0.15 m NaCl. This procedure gradually reduces plasma volume by withdrawing increasing amounts of isosmotic protein-free plasma fluid into the local interstitium (6, 22, 23). Additional fluid from the general interstitium, drawn into the intravascular space by rising plasma oncotic pressure, is also drained into the injection area. Since ingested fluids do not remain exclusively in the circulation (because they raise hydrostatic pressure and lower plasma oncotic pressure), but replenish intravascular and interstitial fluid volumes concurrently, they are in considerable excess of actual plasma deficits.

Group A. Twenty-four rats were injected with 5 ml of 30% PG (n = 12) or the 0.15 m NaCl solution vehicle (n = 12). Food was removed and water only was available ad libitum for 24 hr. In 6 PG-treated and 12 control rats, water intakes were monitored every hour during the drinking test.

Group B. Fourteen rats were injected with 5 ml of 30% PG (n = 8) or the 0.15 m NaCl solution vehicle (n = 6). Food was removed and saline intakes were monitored every hour for 15 hr, and then after 24 hr. Previous studies had indicated that thirst in hypovolemic rats drinking 0.15 m NaCl was satiated within 14 hr after treatment (19).

Group C. Five rats were food deprived and their intakes of water and 0.51 m NaCl were monitored every hour for 24 hr. Five days later, each rat was injected with 5 ml of 30% PG solution, food was again removed from their cages, and fluid intakes were monitored for 24 hr as before.
During all drinking tests (except when food was present), urine voided was collected every hour and its volume measured in a graduated tube (±0.1 ml) attached to the base of each cage. Urine sodium concentrations were then determined by flame photometry. To obtain a cumulative index of sodium balance, urine sodium excretion (in mEq) was subtracted from ingested sodium (in mEq) each hour. It is recognized that this computation only estimates true sodium balance, since electrolyte excretion in the stools was not measured, although it should be noted that diarrhea was never produced by PG treatment.

Blood analyses. Eighty-five other PG-treated rats were used to determine the effects of the different fluid intakes on plasma volume restoration. All rats were anesthetized with Nembutal (50 mg/kg, injected intraperitoneally) and blood samples were immediately removed from the abdominal aorta into heparinized vessels. Hematocrit (by microcapillary tubes), plasma protein (by refractometer), plasma sodium (by flame photometry), and plasma water (by drying to constant weight) values were obtained in duplicate from each sample. Changes in hematocrit and plasma protein values were used to estimate plasma deficits (23), whereas changes in plasma sodium concentration were used to estimate osmotic dilution (14).

Blood samples were taken at various times (1-24 hr) after PG treatment in Groups A and B (see Fig. 2). In Group C, the blood sample from each rat was obtained immediately following the urine excretion in which the sodium concentration rose above 40 mEq/liter (after 16-22 hr). This rise was abrupt and unambiguous, following many hours during which urine sodium concentrations were below 5 mEq/liter.

RESULTS

Group A. The effects of PG treatment on water intakes and urine volumes of food deprived rats are presented in Fig. 1. Control rats drank water gradually during the 24-hr drinking test, with much of the intake occurring in the last 8 hr, but they excreted all of it. Total intakes of 16.7 ml were significantly lower than the 32.0 ml these rats averaged each day when food was present (P < .001). In contrast, rats drank water rapidly during the first 6-8 hr following PG treatment (P < .001 within 2 hr) but then showed a pronounced inhibition of water intake that lasted 9-13 hr, during which time less than 3 ml of water was consumed and virtually all of the ingested water was retained. Water intakes gradually increased in the last 6-8 hr of the drinking test, as did urine excretions. After 24 hr, PG-treated rats had consumed an average of 30.1 ml of water but had excreted only 6.0 ml of it (n = 12; both P values < .001 in comparison with controls).

Water intake had no apparent effect on plasma volume during the first 8 hr following PG treatment and had only a slight restorative effect thereafter (as indicated by elevated hematocrits and plasma protein concentrations in Fig. 2). Moreover, because of the significant renal retention of the ingested water (Fig. 3), rats suffered pronounced osmotic dilution as well as hypovolemia. Note that plasma sodium concentrations decreased during initial stages of water consumption, then tended to rise during the period of low water intake (probably due to the losses of sodium-poor urine and insensible water), and finally decreased to their lowest values with the resumption of water ingestion (Fig. 2). Despite their marked hyponatremia, the rats were actually in only slight negative sodium balance (mean = −0.04 mEq; control: mean = −0.97 mEq; P < .001) since urine sodium concentrations were consistently below 5 mEq/liter after the first few hours of the test.
sodium balance was +7.91 mEq. The rate of fluid intake must have decreased while urine sodium increased in the final hours of the drinking test, since after 24 hr rats had consumed 86.3 ml of saline while sodium balance had stabilized at +7.30 mEq (Fig. 3).

Despite their large intakes, urine volumes in the PG-treated rats were comparable to control values. Urine sodium concentrations were always above 40 mEq/liter, and 75% of them were in the 200–300 mEq/liter range. Since considerably lower urine volumes and sodium concentrations are observed in hypovolemic rats (Group A), these results suggest that PG-treated rats drinking 0.15 m NaCl continuously avoided substantial plasma deficits. In fact, examination of their blood samples revealed no evidence of hypovolemia throughout the drinking test (Fig. 2).

As a result of the large fluid intakes and small urine volumes, substantial amounts of ingested fluid remained in the animals and could be observed readily as a huge subcutaneous edema surrounding the injection site. Renal retention of 7.15 mEq of sodium with sufficient water to maintain isotonicity (Fig. 2) represents approximately 45 ml of fluid added to the extracellular compartments (virtually the amount of fluid retained; Fig. 3). By comparison, it should be noted that a normal 300-g rat has only approximately 60 ml of extracellular fluid.

**Group B.** The effects of PG treatment on 0.15 m NaCl intakes, urine volumes, and sodium balances of rats during the first 15 hr of the drinking test are presented in Fig. 4. Control rats drank 0.15 m NaCl slowly during this time but excreted all the sodium and remained in near-zero balance. In contrast, all PG-treated rats drank the saline rapidly, retained most of the sodium ingested, and maintained a net positive sodium balance. Note that rats drank in considerably greater volume and without evidence of inhibition when isotonic saline was presented instead of water (in comparison with Group A, P < .001 within 3 hr and thereafter). After 15 hr, rats had consumed 65.0 ml, and

![Graph showing cumulative mean sodium balance and water retention](image-url)

**Fig. 3.** (Upper) Cumulative mean sodium balance (intake minus urine sodium), and (lower) cumulative mean water retention (total intake minus urine volume), of 30% PG-treated rats given water, 0.15 m NaCl, or water and 0.51 m NaCl to drink (n = 6, 8, 5, respectively).

**Fig. 4.** Cumulative mean volumes of 0.15 m NaCl ingested and urine excreted by rats injected with 5 ml of 30% PG (n = 8) or 0.15 m NaCl solution vehicle (control) (n = 6). Dotted lines represent intervals during which data were not collected.
The effects of PG treatment on body-fluid distribution have been discussed in detail elsewhere (22, 23). Briefly, PG raises the oncotic pressure of the local interstitial fluid, increasing the withdrawal of protein-free plasma fluid into these tissues. Consequently, circulating plasma protein concentrations increase, causing a movement of fluid from the general interstitium into the intravascular compartment, whereupon it is rapidly drawn into a growing edema localized at the injection site. This gradual sequestration of extracellular fluid can become considerable. For example, plasma protein changes indicate plasma volume deficits of approximately 45% 16 hr after 30% PG treatment (Fig. 2), which must have been paralleled by substantial losses in general interstitial fluid volumes as well. Further increase in plasma protein concentration was not evident during the remainder of the test period. This leveling off is probably due to increased tissue turgor local to the injection site, to a counterbalancing of increased oncotic effects of plasma proteins with decreased oncoticity of the injected material (as the fluid volumes in the two compartments changed), and to absorption of PG from the injection site. With regard to the latter, we have observed that hemococoncentration in fluid-deprived rats injected with 10% PG solutions virtually disappears 24 hr after treatment (unpublished data).

The effects of PG treatment on water and sodium excretion became apparent almost immediately. Significant decreases in urine volume, observed within the first 1–2 hr, were initially associated with plasma deficits of 5–10%, which are known to elicit ADH secretion and to reduce renal plasma flow and glomerular filtration rate (3, 10). Although altered renal hemodynamics can produce significant changes in urine sodium excretion (e.g., 29), the pro-
nounced antinatriuresis (urine sodium concentration <5 mEq/liter) observed after 4-6 hr is probably more related to the presence of aldosterone, since a comparable hypovolemia does not reduce urine sodium concentration below 20-50 mEq/liter in untreated adrenalectomized rats (unpublished data). These considerations are consistent with other findings that increased ADH secretion and water retention always precede increased aldosterone secretion and sodium retention during hypovolemia (8, 9).

The effects of PG treatment on water and sodium ingestion paralleled these changes in fluid retention. Increases in water intake were observed in the first 1-2 hr after injection, whereas increases in the intake of 0.51 M NaCl were not seen until 6-9 hr later. Although rats also drank 0.15 M NaCl almost immediately (Group B), ingestion of this highly palatable solution can be attributed to thirst rather than to sodium appetite (19), which may be operationally distinguished from thirst by the rat's acceptance of concentrated NaCl solutions that are normally avoided. Thirst preceding sodium appetite in onset has also been observed following acute sodium deficiency (13).

Differences in fluid ingestion determined the course of plasma volume restoration and, consequently, of renal excretion. When water was the only drinking fluid available (Group A), water ingestion slowed considerably after the first 6-8 hr and animals remained hypovolemic. Because of continued plasma deficits, water and sodium retention were observed throughout the test period. With a concentrated NaCl solution additionally present (Group C), fluid intake and retention gradually increased without evident inhibition until plasma volumes were restored. Thereafter, retention ceased and all ingested fluids were rapidly excreted. Finally, when isotonic NaCl was the only drinking fluid available (Group B), rats drank saline continuously and fluid accumulation proceeded most rapidly (Fig. 3). These rats apparently repaired their intravascular deficits as rapidly as they were formed (suggesting an extremely low threshold for thirst) and, in the absence of hypovolemia, their urine volumes and sodium excretions remained normal. Although fluid retention ultimately doubled total interstitial fluid volume, significant water and sodium excretion appeared only when the local edema could no longer accommodate ingested fluid.

Because aldosterone levels were not measured, it is not clear whether the sudden decrease in sodium retention by Group C rats was due to the cessation of aldosterone secretion associated with plasma volume restoration or to an "escape" from the retention effects of elevated mineralocorticoid levels (12, 16). With regard to the latter possibility, we have observed in other 30 % PG-treated rats drinking water and 0.51 M NaCl solution that the reduction in renal sodium retention is associated with reciprocal changes in potassium excretion (unpublished data). Since elevated potassium excretion may be expected to continue following the escape from urine sodium retention (12, 16), it seems likely that aldosterone secretion was reduced with the restoration of plasma deficits. Actually, rats were always slightly hypervolemic when sodium retention ceased (as indicated by low hematocrits and plasma protein concentrations in Fig. 2) and thus plasma volumes probably had been restored somewhat before blood samples were taken. This is consistent with observations that aldosterone can be rapidly removed from the circulation (half-life = 30-35 min in humans and dogs) (2, 5).

Since PG-treated rats remained hypovolemic despite the ingestion of water and the renal retention of both water and sodium, plasma volume restoration was clearly dependent upon the intake of sodium. The physiological mechanisms which control sodium appetite have not yet been specified. Three stimuli frequently considered are hypovolemia, hyponatremia, and increased mineralocorticoid levels (28, 32), and all three were probably active at the onset of sodium appetite in the present 30 % PG-treated rats. Similar findings have also been obtained in 20 % PG-treated rats, with an immediate thirst followed by the appearance of sodium appetite 7-10 hr after treatment (unpublished data). In contrast, no sodium appetite was observed in isonatremic 20 % PG-treated rats denied access to water and 0.33 M NaCl until a 1 hr drinking test 8 hr after injection (25). Apparently, hyponatremia potentiates the sodium appetite of hypovolemic rats.

These considerations of thirst and sodium appetite permit some clarification of the behavioral mechanisms that direct the restoration of plasma volume in rats following acute hypovolemia. They are represented schematically in Fig. 6. Proceeding from right to left, intravascular dehydration activates a hypothalamic "thirst center" that results in increased drinking (27). This progressively lowers body fluid osmolality below normal and eventually activates a thirst "satiety system," inhibiting further water intake (24). Hypovolemia continues, since both water and sodium are necessary to repair plasma volume deficits. However, osmotic dilution (hyponatremia?) and hypovolemia stimulate sodium appetite which, if ingestion of concentrated NaCl solutions is permitted, removes the inhibition of thirst by increasing body fluid osmolality and thus promotes renewed water intake and ultimate restoration of plasma volume.

This schema draws attention to three major hypotheses: that hypovolemic thirst and sodium appetite are interdependent in restoring plasma deficits (Fig. 2); that osmotic dilution could inhibit hypovolemic thirst but potentiate sodium appetite (24, 25); and that central nervous struct-
tures are involved in mediating these appetitive drives (e.g., 17, 21, 27, 31). Various experimental evidences support each of these hypotheses, with the exception that the neuroanatomical substrates controlling the inhibition of hypovolemic thirst due to osmotic dilution (if such a system exists) are still unknown.

Finally, it should be recognized that this scheme focuses only on the body fluid parameters which elicit hypovolemic thirst and sodium appetite. Other factors may also be important. In particular, it is possible that the renin-angiotensin-aldosterone system stimulated by hypovolemia (e.g., 11, 70) is involved in the behavioral regulation of intravascular fluid volume in addition to its well-known effects on renal sodium excretion. In this regard, both renin and angiotensin have been shown to increase thirst in rats (7), whereas both renin and aldosterone elicit sodium appetite (1, 30). However, it must be noted that neither renin, angiotensin, nor aldosterone is essential in mediating these appetitive behaviors, since hypovolemia elicits thirst in nephrectomized rats (6) and sodium appetite in adrenalectomized rats (32), and thus the nature of their contribution remains to be determined.

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REFERENCES