Physiological Bases for Different Effects of Extravascular Colloid Treatments on Water and NaCl Solution Drinking by Rats

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STRICKER, E. M. AND J. P. MACARTHUR. Physiological bases for different effects of extravascular colloid treatments on water and NaCl solution drinking by rats. PHYSIOL. BEHAV. 13(3) 389-394, 1974. - Extravascular injections of colloidal solutions gradually promote the sequestration of isosmotic protein-free plasma fluid which has been extruded from local capillaries. This procedure avoids many of the drawbacks of hemorrhage and seems ideal for studying appetitive behaviors elicited by hypovolemia. In the present study, rats given 30% polyethylene glycol (PG) solution subcutaneously increased their intake of water and, after a delay of 6-8 hr, also began to drink concentrated NaCl solution. Rats given the same colloidal solution intraperitoneally showed thirst but did not develop a sodium appetite. These behaviors can be related to the induced plasma volume deficits, which persist only so long as the colloid remains where it is injected. Thus, progressive decreases in plasma volume occur for 12-18 hr after subcutaneous injections, and PG does not appear in plasma during this time. In contrast, PG can be found in plasma within 6-12 hr after intraperitoneal injection, which rapidly vitiates the effects of this treatment and thereby removes the hypovolemic stimulus for NaCl intake.

Dehydration Hypovolemia Thirst Sodium appetite

IN EARLY RESEARCH on the behavioral contributions to the regulation of body fluids, controlled hemorrhage was used as a simple and direct means of reducing circulatory volume. However, reports from several laboratories indicate that blood loss does not consistently increase water intake in animal or human subjects (e.g. [8, 11, 15, 16]). These findings probably result from the facts that plasma volume is restored within minutes by fluid transfers from the interstitial space when losses are small, whereas with larger hemorrhages animals might become seriously anemic and/or hypotensive and therefore incapable of sustained behavioral activity. Because of these complications, alternative techniques for reducing circulatory volume have been sought. Extravascular injection of colloidal solutions is one such procedure. This treatment gradually promotes the sequestration of isosmotic protein-free plasma fluid which has been extruded from local capillaries, and thereby avoids anemia as well as compensatory repletion from interstitial fluid reservoirs (because that fluid is similarly leached into the extravascular depot).

Injections of colloidal solutions have been most effective in revealing the contributions of thirst and sodium appetite to the restoration of plasma volume [24] and, consequently, this general technique seems to be gaining widespread acceptance among investigators studying these appetitive behaviors in rats. Two slightly different methods of procedure are now being employed. In one, the colloidal solutions are injected intraperitoneally [8], whereas in the other they are administered subcutaneously, in the middle of the back [19]. Increases in water intake are proportional to the redistribution of extracellular fluid in either preparation [8,20]. However, whereas intake of NaCl solutions also increases markedly following subcutaneous administration of colloid [17, 25, 26], in preliminary work we did not obtain comparable effects when rats were treated intraperitoneally. We now provide a full report of our investigations confirming this difference and exploring its physiological bases.

METHOD

Animals and Pretreatment Maintenance

The animals used were adult male Sprague-Dawley albino rats, approximately 4-5 months old and weighing 360-440 g at the beginning of the experiment. They were housed individually in mesh-wire metabolism cages in a...
continuously illuminated temperature-controlled room (23–25°C). Purina laboratory chow pellets were available to all rats ad lib except during testing. Separate groups of rats were given either demineralized water (Groups A and A'), 0.15 M NaCl solution (Groups B and B'), or both water and 0.51 M NaCl solution to drink (Groups C and C'), in accordance with their later tests. The drinking fluids were continuously available in calibrated tubes attached to the front of the cages.

Procedures

Experiment 1. Four days prior to the experiment, 45 animals were lightly anesthetized with ether and sham-injected (i.e., a hypodermic needle was inserted but no fluid was delivered). They were then deprived of food for 24 hr, during which fluid intakes and urine outputs (+ 0.2 ml) were monitored every 1–4 hr. On the test day rats were again anesthetized with ether and given either subcutaneous (Groups A, B, and C; n = 6, 6, 7, respectively) or intraperitoneal (Groups A', B', and C; n = 9, 8, 9, respectively) injections of 5.0 ml of a 30% (W/W) solution of polyethylene glycol (Carbowax Compound 20-M; Union Carbide) in 0.15 M NaCl. Rats were returned to their home cages, where food had been removed, and intakes were recorded every hour for 8 hr and then every 1–5 hr for an additional 16 hr. Accumulated urine was collected and its volume was recorded at each time interval.

Sodium and potassium losses in urine excreted during the 24 hr test period was determined by flame photometry. Marked decreases in urine sodium concentration below the normal range of 50–250 mEq/l, and increases in urinary potassium/sodium ratios above the normal range of 0.5–2.0, were interpreted as indicating increased circulating mineralocorticoids during hypovolemia, while cessation of sodium retention was interpreted as indicating restoration of plasma volume [12, 23, 25].

All rats were anesthetized (Nembutal, 50 mg/kg, injected intraperitoneally) immediately following testing and blood was removed from the abdominal aorta into heparinized vessels. Plasma protein (by refractometry) and sodium concentrations (by flame photometry), and hematocrit values (with microcapillary tubes), were obtained in duplicate for each sample. Comparble measures in 10 untreated control rats also were obtained, for purposes of comparison.

Experiment 2. Fifty-four rats received 5.0 ml of 30% polyethylene glycol (PG) solution intraperitoneally and were given either water, 0.15 M NaCl solution, or nothing to drink. Subgroups of each (n = 3–7) were sacrificed at various times later (3 hr–18 hr). Ascitic fluid was collected from all animals, using a pipette, for determination of its volume and colloid concentration. In addition, aortic blood samples were collected and analyzed for hematocrit and plasma protein values. Plasma fluid was then deproteinated [18] and the presence of PG was measured by refractometry. This method could detect as little as 0.5–1.0% concentrations of PG in plasma, or approximately 0.06–0.12 g of PG in the 380–400 g rats that were used in this experiment.

Comparable blood measures also were obtained from eighteen rats receiving 5.0 ml of 30% PG subcutaneously and given nothing to drink, for purposes of comparison.

**RESULTS**

Experiment 1

Pretreatment control. Rats drank little water (range = 0–3.5 ml), 0.15 M NaCl solution (range = 0–6.0 ml), or 0.51 M NaCl solution (range = 0–0.3 ml) during the first 8 hr of food deprivation, and variable amounts during the remainder of the test period (24 hr intakes: ranges = 8–21 ml, 16–58 ml, and 0–2.7 ml, respectively). Fluid consumption was always followed by the excretion of comparable volumes of urine, and renal retention of water (i.e., fluid intake minus urine volume) and sodium balance never exceeded ± 5 ml and ± 0.5 mEq, respectively.

Subcutaneous colloid. The results obtained, summarized in Table 1 and 2, are similar to those reported previously.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
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<tbody>
<tr>
<td>EFFECTS OF PG INJECTIONS IN WATER AND SODIUM BALANCES</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Water Intake (ml)</th>
<th>Saline Intake (ml)</th>
<th>Urine (ml)</th>
<th>Na Balance (mEq)</th>
<th>Water Retention (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>32.6 ± 2.4*</td>
<td>–</td>
<td>10.8 ± 1.2</td>
<td>–0.1 ± 0.1</td>
<td>21.8 ± 2.1</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>85.7 ± 7.1</td>
<td>44.1 ± 4.6</td>
<td>+6.0 ± 1.1</td>
<td>41.6 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>52.0 ± 3.2</td>
<td>12.0 ± 2.3</td>
<td>17.5 ± 3.6</td>
<td>+5.8 ± 1.1</td>
<td>46.5 ± 3.4</td>
</tr>
<tr>
<td>A'</td>
<td>9</td>
<td>28.6 ± 2.9</td>
<td>–</td>
<td>18.9 ± 2.0</td>
<td>–0.2 ± 0.1</td>
<td>9.7 ± 1.5</td>
</tr>
<tr>
<td>B'</td>
<td>8</td>
<td>25.5 ± 3.3</td>
<td>9.6 ± 1.6</td>
<td>+2.7 ± 0.8</td>
<td>16.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>C'</td>
<td>9</td>
<td>26.4 ± 2.5</td>
<td>0.6 ± 0.2</td>
<td>16.6 ± 2.4</td>
<td>+0.2 ± 0.1</td>
<td>10.3 ± 1.9</td>
</tr>
</tbody>
</table>

*Mean ± standard error of the mean
using identical procedures [22, 23, 25]. Briefly, Group A rats increased intakes and consumed water steadily throughout the first 8–12 hr after PG treatment (Fig. 1), and retained most of the ingested fluid. Drinking rates decreased subsequently and seemed to parallel the slow excretion of urine. Urine sodium retention was marked (<10 mEq/l) throughout the 24 hr test period, and urinary potassium/sodium ratios were extremely high (>40).

Group B rats drank and retained large amounts of 0.15 M NaCl solution at very elevated rates for the first 12 hr (p<0.001 in comparison with Group A rats), after which further retention ceased and drinking rates became more moderate (Fig. 1). Urine sodium concentrations never dropped below 40 mEq/l, suggesting that plasma volumes were being replaced quite rapidly [25].

Group C rats drank no 0.51 M NaCl solution but similar amounts of water as Group A rats during the first 6–8 hr after PG treatment. Thereafter, consumption of saline began and water intakes continued at the same steady rate throughout the 24 hr period. Renal sodium retention in all animals was pronounced until the last few hours of the test session.

Group A rats, given water but denied access to NaCl solutions, remained hypovolemic (as indicated by elevated hematocrit and plasma protein values) and became predictably hyponatremic; i.e., the 22 ml of water that was retained would be expected to reduce plasma sodium concen-

![Graph](image)

**FIG. 1.** Mean intakes of water (open symbols) or 0.15 M NaCl solution (closed symbols) after subcutaneous (Group A, n = 6; Group B, n = 6) or intraperitoneal (Group A', n = 9; Group B', n = 8) injections of 5.0 ml of 30% PG solution.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Hematocrit (g/100 ml)</th>
<th>Plasma Protein (mEq/l plasma water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>45.0 ± 0.8*</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>50.2 ± 0.9†</td>
<td>7.8 ± 0.1†</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>42.3 ± 0.5</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>41.8 ± 1.6</td>
<td>5.4 ± 0.2†</td>
</tr>
<tr>
<td>A'</td>
<td>9</td>
<td>41.4 ± 0.6†</td>
<td>6.0 ± 0.1†</td>
</tr>
<tr>
<td>B'</td>
<td>8</td>
<td>40.9 ± 0.6†</td>
<td>5.2 ± 0.1†</td>
</tr>
<tr>
<td>C'</td>
<td>9</td>
<td>40.5 ± 1.7†</td>
<td>6.2 ± 0.1†</td>
</tr>
</tbody>
</table>

*Mean ± standard error of the mean
†p<0.001 in comparison with control values
trations by approximately 13 mEq/liter in 400 g rats. In contrast, the plasma protein and sodium concentrations of Groups B and C were in the normal range, and their hematocrits were somewhat lower than normal. Thus, these PG-treated rats evidently had restored their plasma volume deficits during the 24 hr test period. As might be expected, the renal water retentions and sodium balances were similar in these two groups despite their different drinking tests, and indicated a net retention of approximately isotonic fluid.

Intraperitoneal colloid. Group A' rats, given only water to drink, had intakes that were similar to those of Group A (Fig. 1). However, Group B' rats, given only 0.15 M NaCl, drank much less than Group B rats did (p<0.001), and did not even consume more fluid than Group A' rats (Fig. 1). Group C' rats also drank similar amounts of water but, unlike Group C rats, drank virtually no saline at all (Table 1). Pronounced decreases in urinary sodium concentrations (< 10 mEq/l) and increases in potassium/sodium ratios (>40) were observed throughout the 24 hr period in Groups A' and C', but not Group B'.

Renal water retentions and sodium balances increased rapidly for the first 6–8 hr but then levelled off, and thus were smaller in rats given the colloid intraperitoneally than in the comparable groups injected subcutaneously (Table 1). Nevertheless, none of the rats given PG intraperitoneally showed evidence of marked hypovolemia 24 hr after the treatment (Table 2). In fact, hematocrits were lower than normal in all animals, as were plasma protein values in Group B'. Plasma sodium concentrations also were lower than would be expected from the net water retentions in Groups A' and C'.

**Experiment 2**

Fluid in the peritoneal cavity increased to 15–20 ml within the first 3 hr after injection of the hyperoncotic colloidal solution. Peak volumes of 20–25 ml were reached within 6–9 hr regardless of which fluid, if any, was available for drinking, and significant volumes of fluid (8–12 ml) still were present 24 hr after injection. Colloid concentrations in the ascitic fluid decreased to 5–6% within 3 hr in all animals and soon levelled off at slightly lower values (3–4%). These findings are consistent with previous studies of fluid accumulation following intraperitoneal injection of colloid [6,8].

In fluid-deprived rats given colloid intraperitoneally, plasma volume deficits (as suggested by elevated hematocrit values) increased rapidly during the first few hours after injection but did not increase further, and values had returned to the normal range by 12–18 hr after the treatment (Table 3). In contrast, plasma protein concentrations appeared to increase for the first 9 hr and still were elevated 18 hr after the colloid injection. However, it is important to note that these values were obtained by refractometric analysis, which could not differentiate protein from the appearance of other colloids in the plasma. In this regard, measurable PG was detected in the 12 hr and 18 hr plasma samples, at concentrations of 1–2%.

Almost identical results were obtained when drinking water was available to rats given colloid intraperitoneally, with the exception that apparent plasma protein values were much lower at 18 hr (6.2 ± 0.3 g/100 ml; p<0.01). However, when 0.15 M NaCl solution was available hematocrits did not show significant increases and PG appeared in the plasma (at concentrations of 1–2%) by 6 hr. Comparable hematocrit and plasma protein values were observed 3 hr after subcutaneous administration of colloid, but plasma volume deficits continued to increase progressively throughout the 12–18 hr period of observation (Table 3). PG was not detected in the plasma of any of these animals.

### TABLE 3

**EFFECTS OF PG INJECTIONS ON HEMATOCRIT AND PLASMA PROTEIN CONCENTRATIONS**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>n</th>
<th>Subcutaneous Hematocrit (g/100 ml)</th>
<th>Plasma Protein* (g/100 ml)</th>
<th>Intrapertitoneal Hematocrit</th>
<th>Plasma Protein* (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>10</td>
<td>45.0 ± 0.85</td>
<td>5.8 ± 0.1</td>
<td>45.0 ± 0.8</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>50.4 ± 0.9</td>
<td>6.9 ± 0.2</td>
<td>50.1 ± 1.0</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>53.9 ± 1.4</td>
<td>7.9 ± 0.2</td>
<td>50.7 ± 0.7</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>57.3 ± 0.4</td>
<td>8.8 ± 0.1</td>
<td>51.2 ± 0.3</td>
<td>8.1 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>66.4 ± 1.1</td>
<td>9.8 ± 0.4</td>
<td>46.8 ± 0.8</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>18</td>
<td>6</td>
<td>62.3 ± 1.1</td>
<td>10.1 ± 0.2</td>
<td>44.3 ± 1.3</td>
<td>7.1 ± 0.2</td>
</tr>
</tbody>
</table>

*That is, protein plus PG
†From Table 2
‡Mean ± standard error of the mean
Hypovolemic rats require water and sodium to repair their plasma volume deficits. Rats given colloid subcutaneously increase their water intakes in proportion to growing plasma volume losses, but most of this fluid is distributed extravascularly [20]. Nevertheless, drinking is not sustained despite continued hypovolemia (Group A), apparently due to some consequence of the osmotic dilution that results from the renal retention of ingested water [21, 22]. If osmotic dilution can be avoided, as when 0.15 M NaCl solution is presented instead of water (Group B), rats drink continuously until plasma volume deficits are restored; similarly, when concentrated NaCl solution is available in addition to water (Group C), rats concoct the isotonic NaCl solution that is required for volume restoration [21, 22, 23, 25]. These interactions between the stimuli for thirst and sodium appetite and relevant inhibitory and satiety mechanisms have been observed and discussed in detail previously [24].

Group A' rats, given colloid intraperitoneally, consumed and retained volumes of water during the first 6–8 hr that were comparable to those of rats after subcutaneous treatment, and thus diminution of further intake by these animals also may be attributed to inhibition caused by osmotic dilution. However, in contrast to the effects of subcutaneous colloid, fluid intakes after intraperitoneal treatments were not greater when 0.15 M NaCl solution was available instead of water (Group B'). Furthermore, there was no sodium appetite (Group C'). Evidently, a colloid solution does not have the same effect on drinking behavior regardless of its route of administration.

The results of Experiment 2 reveal prominent differences in the magnitude and duration of the effects of the two treatments, which begin to appear several hours after the injections. Fluid-deprived rats given colloid subcutaneously show progressive increases in both hematocrit and plasma protein concentration for 12–18 hr (see also [25]), whereas rats given the colloid intraperitoneally do not become nearly as hypovolemic or remain so for as long. Apparently, the relatively early appearance of PG in the blood following intraperitoneal injections rapidly vitiates the effects of that treatment, both by decreasing colloid concentration in the extravascular space and by increasing oncotic pressure in the intravascular fluid.

In addition to affecting fluid intake, the presence of 1–2% PG in plasma provides an explanation for three other discordant observations. First of all, the PG confounds determinations of plasma protein by refractometry, as indicated previously. Thus, plasma protein values for rats given colloid intraperitoneally (in Tables 2 and 3) are undoubtedly inflated and misrepresent plasma volume changes. For this reason, changes in hematocrit and plasma protein values for rats given colloid intraperitoneally (in Experiment 1) despite their increased plasma volumes. The presence of major lymphatic capillaries underlying the diaphragm, which drain the intestinal area, undoubtedly provide for the clearance of PG from the peritoneal cavity. For example, it has been reported that 50 ml/kg of whole plasma fluid (approximately 6% protein) can be absorbed within 5 hr after intraperitoneal injection, whereas little is absorbed if the thoracic and right lymph ducts are obstructed [6, 7]. Such high rates of absorption would not be expected with the hyperoncotic colloidal solutions used in the present experiments, both because fluid is initially drawn into the peritoneal cavity and because lymph flow should be retarded by the accompanying hypovolemia [6]. Nevertheless, proceeding from the observation that the colloid concentrations are lower than would be expected from the simple entrance of 15–20 ml of fluid into the peritoneal cavity, it can be estimated that 30–35% of the administered PG has been absorbed by 6 hr after treatment. Some transudation of absorbed PG out of the intravascular fluid compartment must occur [9], since the colloid cannot be detected in the circulation until 12–18 hr after intraperitoneal injection.

Comparable hematocrit and plasma protein values observed 3 hr after the two treatments (Table 3) suggest that the respective losses of plasma volume are quite similar at first. Identical plasma renin activities (which are closely related to plasma volume deficits; [14]) 2 hr after either treatment further suggest similar early effects (E. M. Stricker and F. H. Leenen, unpublished observations). Thus, the 10–15 ml of fluid which entered the peritoneal cavity following intraperitoneal PG treatments was probably comparable to the volume of fluid entering the local interstitium following subcutaneous injection of the same colloidal solution. Moreover, since plasma volume deficits increase at a steady rate for at least 12 hr after subcutaneous PG injection (Table 3), the same rate of fluid accumulation in the extravascular compartment may be projected. That is, approximately 40–60 ml of isotonic fluid may have left the circulation and general interstitium during the first 12 hr after injection, to be sequestered in a large localized edema. It is noteworthy that these rates of fluid loss are similar to the rate at which 0.15 m NaCl solution is ingested during the first 12 hr after subcutaneous PG injection (Fig. 1), and are consistent with previous observations that plasma volumes are repaired almost as rapidly as they develop in these animals [25].

The hypovolemia elicited by intraperitoneal colloid disappears more rapidly when saline is ingested instead of water, not only because saline is more effective in repairing plasma volume deficits but also because of the extraordinary ability of saline to stimulate lymph flow [13] and, hence, clearance of PG from the peritoneal cavity. Nevertheless, it is striking that rats receiving subcutaneous injections of PG solution continue drinking after plasma volumes are repaired and consume much more fluid than rats injected intraperitoneally, which drink little saline after the first 6–8 hr of the test period (Fig. 1). In this regard, it should be noted that fluid ingested by rats injected subcutaneously is distributed largely to the interstitial space until plasma volume is restored, after which the excess is excreted in urine [25], whereas with rats injected intraperitoneally much more of it should remain in the intravascular compartment due to the relatively large amounts of PG there. It is tempting to speculate that rats given the
colloid intraperitoneally are inhibiting saline intake because of the expansion of circulatory volume and consequent hypertension that it would cause. As such, these results are consistent with previous reports that hypertensive rats tend to avoid NaCl solution [1].

To summarize, the present results demonstrate that the behavioral effects of extravascular colloid treatments depend on the induced plasma volume deficits, which persist only so long as the colloid remains where it is injected. Of the two routes used most frequently in studies of appetitive behaviors, subcutaneous administration in the middle of the back seems to be much more effective than intraperitoneal injections, apparently due to considerable differences in the rates of lymphatic drainage of the two areas. Thus, hypovolemia produced by subcutaneous injections of 30% PG develops without complication for at least 12–18 hr, long enough for most studies of thirst and sodium appetite, whereas the preparation begins to deteriorate noticeably within 6 hr after administration of the same solution intraperitoneally and, consequently, it appears to be adequate only for brief tests of thirst and not at all for studies of sodium appetite.

Because detailed physiological effects of intraperitoneal colloid treatments have not been reported heretofore, previous studies using this procedure may have to be re-evaluated. For example, Blass and Hanson [4] observed that rats with bilateral septal lesions drank more water than control rats did during a 2 hr drinking test that was begun 6 hr after intraperitoneal PG injections, and proposed that the septal area mediated the mechanisms associated with osmotic dilution that inhibited hypovolemic thirst. However, later work [2,10] demonstrated that rats with septal lesions did not drink to greater levels of dilution during this test, because urine volumes paralleled their increased intakes, nor did they drink more water than controls when PG was administered subcutaneously. Collectively, these observations now suggest that rats with septal lesions are hyperreactive to thirst stimuli during hypovolemia (cf. [3]), but that this leads to increased water drinking only during conditions of modest hypovolemia in which excessive osmotic dilution is avoided because rats can excrete the extra water that is consumed [14,24].

REFERENCES