The Temporal Course of Fluid Intake and Response to Fluid Loads in Perinephritic Hypertension in Rats

By D. M. Green, M.D., F. M. Sturtevant, Ph.D., and C. G. Van Arman, Ph.D.

The spontaneous intake of water and saline and their output under load were measured in the early and late stages of perinephritic hypertension in rats. The conclusion was drawn that sodium exchange is increased in the prehypertensive phase, but reverts toward or drops below normal with the development of chronic sustained hypertension.

RENAL hypertension caused by cellophane wrapping was reported by Oster and Martinez to be associated with polydipsia and polyuria.1 Braun-Menendez found that hypertensive rats with one normal and one perinephritic kidney showed a rise in water intake and output; in bilateral perinephritis these changes were exaggerated and were associated with decreased renal clearances and nitrogen retention.2 Saline loading of the unilaterally perinephritic animals increased the rates of sodium and water excretion relative to controls. On the other hand, bilaterally perinephritic hypertensive rats were found by Abrams and associates3 to consume less sodium chloride and bicarbonate and more water than controls, when given a free choice.

In these reports the temporal stage of the hypertension was not stated. In studies of desoxycorticosterone acetate (DCA) hypertension in rats, we observed that salt and water exchanges were increased early in the disease. Later, salt appetite as well as output under load regressed to normal or below.4 These findings prompted us to determine whether salt and water exchange in perinephritic hypertension might also be related to its temporal development.

Experimental Procedure

The experimental animals were 96 male Sprague-Dawley rats. They formed three test groups (T1, T2 and T3) and their respective control groups (C1, C2 and C3), each containing 16 animals (table I). The experiment itself consisted of two parts. In one, the outputs under load, voluntary intakes and blood pressures were measured from 2 weeks before until 17 weeks after encapsulation of one kidney (group T1) or both (group T2). In the other, responses during the three-week period immediately after bilateral encapsulation (group T2) were compared with responses seven months after bilateral encapsulation (group T3). Since the normal values of the measured factors vary with age,4-5 the responses in some instances were also expressed as the ratio (T/C) of the measurements obtained in the test group compared with its own control. The significance of changes shown by test groups from one time interval to the next, and of differences from the corresponding control values, was evaluated by “Student’s” t test or the F test. The details of the experimental procedure were as follows:

The animals in each group were housed four to a cage. Rockland rat diet, containing 0.53 per cent sodium and 0.56 per cent potassium, was fed ad libitum. Groups T1, C1, T2 and C2 were offered a choice of both distilled water and 0.56 per cent sodium chloride solution as drinking fluids throughout the experiment. At 5 weeks of age, under ether anesthesia, one kidney was removed from the animals in group T1. The other, after exposure and decapsulation, was enclosed in a latex envelope,6 loosely enough so that neither the kidney nor its pedicle was constricted. An identical operation was performed on the animals in group C1, except that the remaining kidney was not encapsulated. Bilateral latex encapsulation was performed on group T2, while a bilateral sham-operation was done on group C2.
FLUID EXCHANGE IN PERINEPHRITIC HYPERTENSION

TABLE 1.—Composition of the Six Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Treatment</th>
<th>Period of Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$</td>
<td>16</td>
<td>Unilateral nephrectomy. Contralateral renal encapsulation</td>
<td>From 2 weeks before to 17 weeks after day of operation, inclusive</td>
</tr>
<tr>
<td>$C_1$</td>
<td>16</td>
<td>Unilateral nephrectomy. Control for group $T_1$</td>
<td>Same</td>
</tr>
<tr>
<td>$T_2$</td>
<td>16</td>
<td>Bilateral renal encapsulation</td>
<td>Same</td>
</tr>
<tr>
<td>$C_2$</td>
<td>16</td>
<td>Control for group $T_2$</td>
<td>Same</td>
</tr>
<tr>
<td>$T_3$</td>
<td>16</td>
<td>Bilateral renal encapsulation</td>
<td>From 30 to 32 weeks after day of operation, inclusive</td>
</tr>
<tr>
<td>$C_3$</td>
<td>16</td>
<td>Control for group $T_3$</td>
<td>Same</td>
</tr>
</tbody>
</table>

Once a week all four groups were fasted for 18 hours. Half the members of each group were then given a water load of 50 cc. per kilogram of body weight by stomach tube. The other half received 50 cc. per kilogram of 0.80 per cent sodium chloride. The urine was then collected over five hours. Its sodium concentration was determined with a flame photometer and the sodium output calculated. For certain statistical analyses the loading responses were grouped for the two preoperative weeks and for postoperative weeks 1 through 5, 6 through 10, and 11 through 15.

The water and saline intakes were measured daily and averaged weekly. Since all watering bottles had tightly fitted stoppers and small tips of uniform diameter, leakage and evaporation losses were considered minor and randomly distributed. For certain statistical analyses the intakes were grouped for the two preoperative weeks and for the first and second seven-week periods after operation.

Each animal was weighed weekly. Blood pressure was measured 5, 8, 10 and 14 weeks postoperatively, without anesthesia, by a photoelectric tensometer. Four months after operation all surviving rats in these four groups were sacrificed. The kidneys, devoid of latex envelopes and fibrous perinephritic capsules, adrenals, testes, hearts and thymuses were weighed. Kidney sections were prepared for histologic examination.

Group $T_3$ represented 16 survivors of an initially larger number which had been subjected to bilateral silk encapsulation of the kidneys about 30 weeks earlier. Group $C_3$ acted as its control. No quantitative studies were made during the first seven months following encapsulation. From the thirty-first week inclusive, groups $T_3$ and $C_3$ were given a choice of both saline and water as drinking solutions, and alternately received saline and water loads. Outputs under load, voluntary intakes, blood pressures and weights were measured and compared with the values observed in groups $T_2$ and $C_2$ during the first three weeks following operation.

RESULTS

Since the results in groups $T_1$ and $T_2$ were essentially similar, they are presented together. Both groups showed increased water and sodium outputs after loading by the fifth day following operation (figs. 1, 2). These increases, which ranged as high as two and one-half times the control values, lasted for a variable time, which averaged about 10 weeks. Group $T_1$ showed a more marked response to water loading than to salt loading. The increases in output after both types of load were larger and more persistent in group $T_2$.

During the first postoperative week the water intakes of groups $T_1$ and $T_2$ rose from 25 to 50 per cent above control values (fig. 3). The saline intake of group $T_2$ was also significantly higher. These trends continued through the first seven-week period. During the second interval (weeks 8 through 14) the intakes of both groups regressed to normal. Although the blood pressures of these two groups increased from the fifth week onward, they did not reach significantly hypertensive levels relative to the controls until the eighth week (fig. 3). The mean pressures then rose progressively to a sustained level of approximately 180 mm. Hg between the tenth and fifteenth weeks. Four rats in each of these groups showed levels in excess of 200 mm. Hg.

No significant difference was observed between the body weights of groups $T_1$ and $C_1$. The mean body weight of group $T_2$ lagged behind that of group $C_2$ during the last preoperative week and the first two postoperative weeks but remained parallel thereafter. This difference averaged about 8 per cent over the four-month period.

Five of the animals in group $T_1$ and eight in group $T_2$ survived the experiment. The survivors among the controls numbered 16.
Fig. 1. Five-hour urine volume (cc./Kg.) and urinary sodium output (mM./Kg.) of encapsulated (—) and control (— — ) rats in response to an intragastric water load, 50 cc. per kilogram. Group T₁ (unilaterally encapsulated) and C₁ (its control) are on the left; group T₂ (bilaterally encapsulated) and C₂ (its control) are on the right. The S's over the intervals of 1-5, 6-10, and 11-15 weeks postoperative indicate significant differences between cumulative responses of experimental and controls (P < 0.05-0.01). (V = urine volume; Uₐₐᵥ = sodium output.)

Fig. 2. Five-hour urine volume (cc./Kg.) and urinary sodium output (mM./Kg.) of encapsulated (—) and control (— — ) rats in response to an intragastric load of 0.86 per cent sodium chloride solution, 50 cc. per kilogram. Other conditions as in figure 1.

(group C₁) and 15 (Group C₂). Two preoperative deaths (group T₂) were due to introduction of fluid into the trachea during the loading procedure. Nearly all the remaining fatalities took place in two rather sharply defined intervals. Seven (six in group T₁; one in group C₂) occurred during the first four days after operation, before the first postoperative loading test. Nine of the remaining 11 deaths in test animals came during the fifth to ninth weeks, the period of most rapidly rising blood pressure. During this period death occurred in 6 out of 8 animals with pressures greater than 200 mm. Hg, as compared with 3 out of 14 with pressures under this level. Virtually all of these deaths were acute, without obvious signs of chronic illness, such as progressive weight loss or refusal to eat and drink. About a third of the animals showed premortal edema. No deaths occurred from the tenth week onward. The rats in groups T₁ and T₂ uniformly exhibited gross perinephritis of the originally encapsulated kidneys, together with varying degrees of cardiac and renal enlargement (table...
FLUID EXCHANGE IX PERINEPHRITIC HYPERTENSION

**Figure 3.** Blood pressure (lower graphs), self-selected intakes of 0.86 per cent saline solution (middle graphs) and of water (upper graphs) in unilaterally encapsulated (—) and control (—) rats. Group T (unilaterally encapsulated) and C (its control) are on the left; group T (bilaterally encapsulated) and C (its control) are on the right. The S's above the intake curves indicate significant differences in the cumulative intakes over the first seven postoperative weeks (P < 0.05-0.01). The S's above the blood pressure curves indicate significant differences (P < 0.01); vertical bars signify standard errors of means.

**Table 2.**—Average Organ Weights in Hypertensive Rats Sacrificed Approximately Four Months after Induction of Unilateral (Group T) or Bilateral (Group T2) Perinephritis and in Their Respective Controls (Groups C1 and C2).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Heart</th>
<th>Kidneys</th>
<th>Adrenals</th>
<th>Testes</th>
<th>Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>5</td>
<td>0.347</td>
<td>0.630</td>
<td>0.0154</td>
<td>1.051</td>
<td>0.0649</td>
</tr>
<tr>
<td>C1</td>
<td>16</td>
<td>0.332</td>
<td>0.534</td>
<td>0.0144</td>
<td>0.967</td>
<td>0.0748</td>
</tr>
<tr>
<td>T2</td>
<td>8</td>
<td>0.389</td>
<td>0.848</td>
<td>0.0126</td>
<td>1.006</td>
<td>0.0630</td>
</tr>
<tr>
<td>C2</td>
<td>15</td>
<td>0.332</td>
<td>0.779</td>
<td>0.0111</td>
<td>0.961</td>
<td>0.0667</td>
</tr>
</tbody>
</table>

**Table 3.**—Self-Selected Fluid Intakes and Five-Hour Loading Responses in Rats during the Early, Prehypertensive Phase (Weeks One through Three) and the Late, Chronically Hypertensive Phase (Weeks 30 through 32) of Bilateral Perinephritis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks</th>
<th>Intakes</th>
<th>Outputs after I.G. loads, 50 cc./Kg.</th>
<th>(Load: NaCl, 0.86%)</th>
<th>(Load: H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NaCl, 0.86%  cc./Kg./day</td>
<td>H2O cc./Kg./day</td>
<td>mEq/Kg./6 hrs.</td>
<td>cc./Kg./6 hrs.</td>
</tr>
<tr>
<td>T1</td>
<td>1-3</td>
<td>120</td>
<td>140</td>
<td>8.74</td>
<td>61.0</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>190</td>
<td>110</td>
<td>5.50</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>1-3</td>
<td>142</td>
<td>127</td>
<td>1.50</td>
<td>6.16</td>
</tr>
<tr>
<td>T2</td>
<td>30-32</td>
<td>63</td>
<td>99</td>
<td>2.82</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>30-32</td>
<td>89</td>
<td>77</td>
<td>4.38</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>30-32</td>
<td>0.71</td>
<td>1.28</td>
<td>0.64</td>
<td>0.75</td>
</tr>
</tbody>
</table>

In both groups the majority of the latex envelopes were found in the abdominal cavity, filled and secured with connective tissue. The degree of perinephritis suggested that the envelopes had been displaced as a result of fibrous tissue overgrowth, which all such kidneys showed. Only one kidney manifested any gross evidence of constriction. None of the rats had periarteritis nodosa of the intestinal mesentery, or gastroduodenal ulcers. Five rats in group T1 and five in group T2 exhibited glomerulonephritic changes with hyaline casts, intercellular leukocytic infiltration and tubular hypertrophy or atrophy; two rats had severely necrotic kidneys, fibrous invasion of the cortex and arteriolar occlusion; one rat remained without serious renal involvement. There were no relevant pathologic changes in the kidneys of the control groups.

The results in group T2, secured during the later phase of chronic perinephritic hypertension, are perhaps best presented in conjunction with the observations made on group T2 during its prehypertensive phase (table 3). Group T2 showed virtually normal responses to water loading. In response to saline loading, this group excreted only 75 per cent as much water and 64 per cent as much sodium as group C2. (These results contrasted with the very high...
rates of excretion displayed by group T1.) The water intake of group T3 was somewhat elevated, and averaged 28 per cent above its control. The saline intake was relatively subnormal and amounted to 29 per cent less than the control value. (This result contrasted with the 42 per cent increase seen in group T2 during its prehypertensive phase.) The mean blood pressure of group T3 was stable, and averaged 194 mm. Hg, approximately one and one-half times that of group C3, its control. The weights of groups T3 and C3 were comparable throughout. Three of the animals in group T3 died during the observation period, at intervals approximately a week apart. All of group C3 survived.

**DISCUSSION**

The results indicate that in the early weeks following unilateral or bilateral encapsulation of the kidneys (prehypertensive phase) the urinary output after water or saline loads was elevated and the appetite for water and salt was increased. These disturbances preceded the development of significant blood pressure elevation and its associated mortality. Water and sodium exchanges returned toward or to normal during the period of progressively increasing hypertension. At a later stage of chronic, sustained hypertension, the intake and output of sodium were subnormal.

The possibility was considered that the disappearance of the initial disturbance in salt exchange was due to the deaths of the animals in each test group with the greatest salt appetites and highest outputs under load. This possibility was explored by calculating the effect of deaths of individual rats on the mean outputs or intakes for their cages. In general, the immediate effect of a rat death was a transitory increase in intake for that cage. A similar increase was found in loading responses; in only one instance was it possible to explain a secondary fall in output on the basis of the death of a high excretor. Nor did it seem possible to explain the disappearance of the initial disturbances as a result of the unstable state of the test animals during the period of their highest mortality (weeks five through nine). Normal levels of water and salt exchange persisted during the succeeding eight weeks, in which no animals died, and some subnormal values were found in chronically hypertensive animals 30 to 32 weeks after encapsulation.

The possibility was also considered that the alterations in salt and water exchange were the result of reciprocal changes in food intake. In other studies in this laboratory we found the intake of this strain of rats to be highly correlated with weight (rxy = 0.91) and to average 77.9 ± 2.0 Gm. (S.E.) per kilogram per day, during a three-month period of active growth. Since differences in growth rate between the treated and control groups in the present experiments were never large, and in all cases were absent from the third postoperative week onward, the changes in fluid intake and loading responses do not seem explicable as the result of disturbances in food intake.

We have concluded that the early elevations in intakes and loading responses and their later return to normal or below were real changes associated directly with the induction of perinephritis and the development of hypertension, and were not artefacts produced by the coincidental morbidity or mortality.

Our findings support the observations of Oster and Martinez1 on the polydipsia and polyuria of perinephritic rats. They confirm those of Braun-Menendez2 as to the increased water and sodium output of perinephritic rats after saline loads but indicate that these responses are temporary and limited to the early stage of perinephritis, becoming less evident as permanent and fixed levels of hypertension develop. Our results are also consistent with the decreased appetite for salt reported by Abrams and co-workers3 but indicate that this phenomenon is characteristic of a later stage of perinephritis, and follows a definite but temporary stage preceding the development of sustained hypertension in which salt appetite is increased above that of comparably treated controls.

**SUMMARY AND CONCLUSIONS**

The rates of water and sodium excretion after fluid load and the self-selected intakes of water and saline were studied in rats following unilateral and bilateral renal encapsulation. Loading responses and intakes increased soon after the operative procedure and tended to
remain elevated during the prehypertensive stage, after which they returned toward or dropped below control levels. The disappearance of these disturbances coincided with the development of sustained hypertension.

The results are consistent with the hypothesis previously suggested\(^4\) that hypertension may be a compensatory reaction which serves to overcome a more fundamental disturbance in fluid and electrolyte metabolism.

REFERENCES

The Temporal Course of Fluid Intake and Response to Fluid Loads in Perinephritic Hypertension in Rats

D. M. GREEN, F. M. STURTEVANT and C. G. VAN ARMAN

Circ Res. 1954;2:73-78
doi: 10.1161/01.RES.2.1.73

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1954 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/2/1/73

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/