THE EXCRETION OF INTRAVENOUSLY ADMINISTERED SALINE BY THE RAT

By

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Whilst administration of isotonic saline to man does not readily cause diuresis, it is known to have this effect in dogs (Chanutin, Smith & Mendel, 1924; Gross, 1948) and rats (Kellogg, Burack & Isselbacher, 1954). Furthermore, it is recognised that disturbances of sodium and water elimination may be of considerable clinical interest.

The present paper is one of a series of studies of the physiological mechanisms responsible for the renal excretion of saline loads. The total water and sodium excretion by rats after administration of a hypotonic load has been described (Cole, 1953; 1954 a, b). The time-course of renal excretion after giving intravenous saline has now been investigated in rats using 77 mM, 154 mM, 308 mM and 1740 mM saline. This was done in order to assess the relative importance of the amounts of solute and water, and of the load concentrations.

MATERIAL AND METHODS

Male albino rats of the »Glaxo« strain were used throughout; previous to the experiment they were given water and »Thorley« rat cubes ad libitum. Saline was given intravenously through a polythene cannula in the external jugular vein (Ginsburg & Heller, 1953). The rats were unanaesthetised during and after the infusion and were placed in wire cages over large polythene funnels which had been coated with a silicone water-repellent material (MS 1107; Hopkin & Williams Ltd., London). The cannulae were implanted 24 to 36 hours before the infusion was started, air-ether anaesthesia being used for the preliminary operation.

The infusions of saline lasted less than five minutes and, for the first ninety minutes

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after the infusion, urine was collected each twenty to thirty minutes, and thereafter at hourly intervals. Lifting the animals from their cages usually sufficed to make them urinate.

Blood was collected in oxalated capillary tubes before, and at intervals of about 30, 60 and 150 minutes after the infusion. The tubes were immediately sealed at one end and centrifuged. Plasma was diluted for the estimation of sodium and chloride. The experiment was terminated between six and seven hours after the infusion. The rats were killed by decapitation and blood collected in a beaker containing crystals of ammonium oxalate. This blood was centrifuged at once and all plasma and urine specimens were stored at −10° C. until the creatinine content had been determined.

In some experiments 'Pitressin' (Parke, Davis; London) was given. These fall into two groups:— those where a single infusion of 77 mM or 154 mM saline was given as described above, and those where diuresis was maintained by giving 77 mM as a continuous intravenous infusion. 'Pitressin' was given intravenously at different stages of the diuresis.

Other animals were also given a continuous infusion but received doses of up to 370 μg. deoxycorticosterone glycoside (DCG) (CIBA Aktiengesellschaft) intravenously, in place of 'Pitressin'.

Creatinine was estimated by a modification of the method of Muntwyler & Griffin (1953), using 0.5 ml. plasma or 0.05 ml. urine.

Sodium was determined with an internal (Li) standard flame photometer.

Chloride was determined electrometrically, using a silver/silver-chloride electrode (cf. Kolthoff & Kuroda, 1951).

**RESULTS**

Endogenous creatinine clearance was used as a measure of glomerular filtration rate (GFR) and it is recognized that errors may be present when small volumes of urine were collected. The concentration rate for creatinine, \( c' \) (equal to the urine creatinine concentrations ÷ the plasma creatinine concentration) was used as an index of renal tubular water reabsorption. The sodium concentration ratio, \( c \) (equal to the urine sodium concentration ÷ the plasma sodium concentration) was used to assess sodium reabsorption. That percentage of filtered water not reabsorbed in the renal tubules has been termed the »tubular rejection fraction« for water (TRF\(_w\)) Simmons, Harrev & Hoshiko, 1954), and is equal to:

\[
100/c'
\]

Similarly, the tubular rejection fraction for sodium (TRF\(_{Na}\)) is:

\[
TRF_{Na} = 100 \times c/c'
\]

As the rate of urine flow does not enter into these calculations they are less susceptible to collection errors than are the values for GFR rates of excretion.

Typical data for GFR, TRF\(_w\) and TRF\(_{Na}\) are shown in Figs. 1 a and 1 b. The values for the means and the ranges of the times between commencing the infusion and attainment of the maximum values for TRF\(_w\) and TRF\(_{Na}\) are given in Table 1.
Table 1

<table>
<thead>
<tr>
<th>Infusion Volume (ml.)</th>
<th>Concentration (mM)</th>
<th>Time to reach maximum TRF&lt;sub&gt;w&lt;/sub&gt; (min.)</th>
<th>Time to reach maximum TRF&lt;sub&gt;Na&lt;/sub&gt; (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>77</td>
<td>Mean: –</td>
<td>57 (35-90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: –</td>
<td>95 (45-170)</td>
</tr>
<tr>
<td>9</td>
<td>154</td>
<td>Mean: –</td>
<td>47 (30-83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: –</td>
<td>47 (25-90)</td>
</tr>
<tr>
<td>5–9</td>
<td>308</td>
<td>Mean: –</td>
<td>27 (20-70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: –</td>
<td>27 (15-70)</td>
</tr>
<tr>
<td>0.9</td>
<td>1740</td>
<td>Mean: –</td>
<td>85 (60-110)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range: –</td>
<td>105 (90-137)</td>
</tr>
</tbody>
</table>

Fig. 1a. Glomerular filtration rate (GFR) and tubular rejection fraction for water (TRF<sub>w</sub>) after intravenous infusion of 77 mM, 154 mM, 308 mM and 1740 mM saline. ● = GFR (ml./min.). ○ = TRF<sub>w</sub>.

Fig. 1b. Tubular rejection fraction for sodium (TRF<sub>Na</sub>) after intravenous infusion of 77 mM, 154 mM, 308 mM and 1740 mM saline. Data for TRF<sub>Na</sub> obtained from those animals used for calculation of GFR and TRF<sub>w</sub> in Fig. 1a. × = TRF<sub>Na</sub>.
In most experiments there was an increase of GFR during the first 30 minutes, followed by a slight fall. Except in the 1740 mM group there was no correlation between GFR and TRFW. The correlation coefficient between GFR and TRFW in the 1740 mM group was $+0.516 \ (0.02 > P > 0.01)$, and the regression coefficient $+0.84$. Correlation between GFR and TRFNa was not significant in this group. The relationship between GFR and TRFW was probably due to less efficient urine collection at lower rates of urine flow.

There was an increase of both TRFW and TRFNa some 30-90 minutes after the infusion in all except the 1740 mM group. In the 77 mM group the maximum value for TRFW was reached before the maximum for TRFNa. Changes of TRFW in the 1740 mM group were small and not consistent.

The lines in Fig. 2 were obtained by plotting the initial sodium load minus the amount excreted for each collection period. The residual loads at 30, 60, 90, 120, 150, 180, 240, 360, and 420 minutes were obtained by interpolation. This estimation was performed for each animal and the group-mean values for the above time intervals calculated. The enclosed areas in Fig. 2 represent the group mean values $\pm 2 \sigma$. The lines in Fig. 3 for the residual water load were calculated in the same way. In no case were less than nine values used to estimate the group mean. In both figures steepest curves, i. e. the most rapid

Fig. 2.
Residual sodium load (initial sodium load minus total excreted sodium) after administration of 77 mM, 154 mM, 308 mM and 1740 mM saline. The enclosed areas represent the mean $\pm 2 \sigma$. 400
Residual water load (initial water load minus total excreted water), after administration of 77 mM, 154 mM, 308 mM and 1740 mM saline. The enclosed areas represent the mean ± 2 σ.

rates of excretion, correspond to the animals given the greatest loads. Values for GFR, TRFₜₚ and TRFₙₐ obtained from rats which had been given ‘Pitressin’ or DCG are shown in Figs. 4 and 5. Values for plasma sodium from rats given 77, 154, 308 and 1740 mM NaCl, are shown in Fig. 6, the enclosed areas representing the mean ± 2 σ.

DISCUSSION

The findings in the present experiments cannot be explained solely in terms of an osmo-receptor and an anti-diuretic hormone (ADH) system. Animals which received 77 mM saline responded with increases of TRFₙₐ and TRFₜₚ in a manner similar to those given 154 mM or 308 mM saline. It is reasonably certain that the osmo-receptor stimuli must have differed in these cases. Unless some other factors had intervened, ADH formation in the three groups would also have differed.

In these experiments after saline administration, ‘Pitressin’ increased neither the TRFₙₐ nor the rate of sodium excretion. Shannon (1942), Corey & Britton (1941) and Pasqualini (1951) have suggested that pituitary ADH reduced renal
TRF$_{Na}$, TRF$_w$ and GFR after administration of saline and Pitressin intravenously. Amounts of Pitressin are given in $\mu$U and shown as solid columns.

$\bigcirc \ldots \bigcirc = \text{GFR}, \quad \bigtimes \ldots \bigtimes = \text{TRF}_{Na}, \quad \bullet \ldots \bullet = \text{TRF}_w$.

The shaded area in the lowest figure represents the rate of saline infusion.

tubular reabsorption of sodium or chloride. This, however, has not been confirmed universally: Hare, Hare & Phillips (1943), Sinclair-Smith, Sisson, Kattus, Genecin, Monge, McKeever & Newman (1950) and Chalmers, Lewis & Pawan (1951). TRF$_w$ and water excretion were not readily inhibited by 'Pitressin' (cf. Brunn, 1920); neither was TRF$_{Na}$ increased. There seems little ground for supposing that the renal tubular responses were due only to changes of pituitary ADH.

The experimental procedures described above must have caused a temporary increase of extracellular fluid volume. This may have stimulated increased fluid excretion, although the mechanism is obscure. Changes of ADH pro-
TRF\textsubscript{Na}, TRF\textsubscript{w} and GFR after intravenous infusion of 154 mM saline and DCG. Amounts of DCG are given in \( \mu g \) and shown as solid columns. The rate of infusion is shown as a solid area at the base of the figure.

\( \bullet \bullet \bullet = GFR, \quad \bigcirc \bigcirc \bigcirc = TRF\textsubscript{w}, \quad \times \times \times = TRF\textsubscript{Na} \)

production, whether due to osmo-receptor stimuli or volume changes (\textit{Welt \& Orloff}, 1951) do not completely explain the results.

Any explanation of renal water excretion in terms of tubular activity and of ADH alone offers problems similar to those for sodium. It will be seen that TRF\textsubscript{w} was increased in rats given 308 mM NaCl and where plasma sodium was raised. Under these conditions therefore, the renal tubular response, shown by alterations of TRF, appeared to depend on sodium and water load rather than on concentration.

The changes of TRF\textsubscript{Na} and TRF\textsubscript{w} are not necessarily interdependent; TRF\textsubscript{Na} increased when 0.9 ml. 1740 mM NaCl was given but TRF\textsubscript{w} was much less than in the 154 mM NaCl series. This is in general agreement with \textit{O’Connor}’s findings (1950) that in dogs, the total chloride excretion depended on the amount as well as the concentration of the load. A degree of independence between sodium and water excretion in rats was indicated by \textit{Dicker} (1948). Likewise, \textit{Gross}’s work with dogs (1948) shows some independence of sodium and water excretion.

DCG in considerable doses, tends slightly to reduce sodium excretion, but not to normal values. This argues against an interpretation of saline excretion
Plasma concentrations of sodium, in mEq/L, after administration of 77 mM, 154 mM, 308 mM and 1740 mM saline during the period 0—5 minutes after the start of the experiment. Enclosed areas represent means ± 2 σ.

Fig. 6.

in terms of decreased production of DCG-like material or of mineralo-corticoid — ADH antagonism.

Selkurt & Post (1950) and Selkurt (1954) stress the importance of GFR in regulating sodium excretion. We did not find that alterations of GFR were related consistently to TRFNa and TRFW so as satisfactorily to account for the changes observed. No information about the intra-renal distribution of blood is available.

It is possible to postulate three partially independent systems in order fully to explain the renal excretion of a saline load:—

(i) the osmoreceptor-pituitary ADH system, described by Verney (1947, 1954).

(ii) a system causing decreased tubular water reabsorption after fluid has been given.

(iii) a system causing decreased tubular sodium reabsorption after saline has been given.

In theory, any two of these systems acting together would suffice to account for the responses observed. It is tempting to suppose that osmotic and fluid-volume changes may act synergistically although the physiology of such a system is so far uninvestigated.
SUMMARY

A study has been made of the excretion of sodium and water by unanaesthetised rats to which hypo-, iso-, and hyper-tonic saline had been given by means of an external jugular vein cannula. Renal tubular reabsorption of sodium and water decreased in each case after 77 mM, 154 mM or 308 mM saline were given. 1740 mM saline decreased the reabsorption of sodium but not that of water.

These changes bore no consistent relation to any increase of glomerular filtration rate. »Pitressin« was not found to increase renal sodium excretion or reduce renal tubular reabsorption. Deoxycorticosterone glycoside, given after or during the saline infusion reduced renal sodium excretion, but not to the normal levels. Tubular sodium reabsorption was not restored to normal values.

The renal responses described are not explicable in terms of osmoreceptor-posterior pituitary activity alone, nor, it seems, in terms of antidiuretic hormone-adrenal antagonism or decreased output of adrenal steroids.

While it is possible that these renal responses are ultimately initiated by changes of body fluid or sodium content, as well as by concentration, no physiological pathway can be proposed for this response at present.

REFERENCES

Dicker, S. E.: J. Physiol. 107, 8, 1948.