

## ARTIGOS

### EFFECTS OF WATER DEPRIVATION ON RENAL HYDROELECTROLYTIC EXCRETION IN CHRONICALLY *TRYPANOSOMA CRUZI*-INFECTED RATS

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*The effect of an 8 hour-period of water deprivation on fluid and electrolyte renal excretion was investigated in male Wistar rats infected with the strain São Felipe (12SF) of Trypanosoma cruzi, in comparison with age and sex matched non-infected controls. The median percent reductions in the urinary flow (-40% v -63%) and excretion of sodium (-57% v -79%) were smaller in chagasic than in control rats, respectively. So, chagasic rats excreted more than controls. On the other hand, the median percent decrement in the clearance of creatinine was higher in chagasic (-51%) than in controls (-39%). Thus, chagasic rats showed some disturbed renal hydroelectrolytic responses to water deprivation, expressed by smaller conservation, or higher excretion of water and sodium in association with smaller glomerular filtration rate. This fact denoted an elevation in the fractional excretion of sodium and water.*

*Key-words: Water deprivation. Urinary flow. Urinary sodium and potassium. Glomerular filtration rate. T. cruzi-infected rat.*

Some studies have demonstrated that in patients with the indeterminate and cardiac chronic forms of Chagas' disease, there are disturbances in the renal control of the hydric and saline homeostasis, as well as changes in the osmolarity of the extracellular fluid, and in the balance of electrolytes, resulting in a higher natriuresis in comparison with healthy subjects<sup>1 10 18</sup>. The majority of these observations were based on protocols that used expansion of blood volume by intravenous infusion of saline solution to induce the effects studied. Large variations in the daily serum concentrations of potassium, greater than in normal individuals<sup>19</sup>, also observed in chagasic patients.

In chronic chagasic patients, in which acute changes in the levels of sodium in the kidneys were induced by intravenous injection of the loop diuretic furosemide, suggested that the alterations in the natriuresis were related to a mineralocorticoid effect<sup>16</sup>. This suggestion was based in a smaller sodium/potassium ratio observed in the saliva of chagasic patients when compared to that of control

subjects, assuming that this relation represent an indirect index of a mineralocorticoid excess<sup>2</sup>.

Presently it was investigated the effects of water deprivation on urinary flow, clearance of creatinine, and urinary excretion of sodium and potassium in a rat model of chronic chagasic infection. The objective of the study was to identify any possible disturbance in the hydroelectrolytic homeostasis in the rat infected with *T. cruzi*.

#### MATERIAL AND METHODS

Thirty male Wistar rats that survived the acute phase of *T. cruzi* infection, induced at 25-30 days of age, were used in the study. These rats, weighing 50 to 80g, were intraperitoneally inoculated with 2,000 to 6,000 trypomastigote blood forms per gram body weight of São Felipe (12SF) strain of *T. cruzi* obtained from blood of infected mice. After inoculation, rats were let to evolve for 8 months till reaching the chronic phase. Thirty non-infected age and sex matched rats formed the control group which were simultaneously observed.

Acute infection of all animals was proved by microscopic detection of circulating parasites in the peripheral blood during the first weeks following inoculation. Chronic disease in this rat model of chagasic infection, developed at the University of Brasilia Cardiovascular Laboratory, was identified

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by the presence of inflammatory and degenerative lesions of variable degree in the atrial and ventricular myocardial fibers, and by the intrinsic neuroganglionitis of the heart<sup>4 5 8</sup>.

### Experimental protocol

After 8th month of infection, chagasic rats were individually housed in metabolic cages, under a natural 12 hours light-dark cycle at the temperature of 21°C to 27°C and humidity of 60% to 80%. Control rats were treated as the chagasic group.

The experimental protocol included four periods:

1. adaptation, that lasted 5 days for the animals to habituate to the new environment;
2. basal or pre-hydropenia, that consisted in the collection of urine for 24 hours, and blood and urine samples for analysis;
3. hydropenia, initiated 48 hours following the basal period, consisting of total restriction of access to water (water deprivation) for 8 hours; during this period, urine was also collected, and at the end samples of blood and urine were further obtained;
4. recovery or post-hydropenia, consisted in the return to basal conditions, with further collection of urine following 24 hours after the period of hydropenia.

In all four experimental periods, rats were allowed to have free access to standard food and were weighed each day. Excluding hydropenia, in all the other periods animals were kept on water *ad libitum*.

The variables used for assessment of renal function during hydropenia were: urinary flow ( $V_u$ ); creatinine clearance ( $CL_{Cr}$ ), employing the method of Follin-Wu in a Micronal B-29511 spectrophotometer, for evaluation of glomerular filtration rate; and the urinary excretion of sodium ( $U_{NaV}$ ) and potassium ( $U_{KV}$ ) using a Micronal B-262 flame photometer.

### Statistical analysis

Since the data in the two groups of rats proved not to have a normal distribution the results were

analysed employing the Mann-Whitney test for unpaired comparisons between the groups, and the Wilcoxon signed rank test for paired comparisons within each group, using the STATGRAPHICS software (STSC'S Plus-Ware, 1985) in an IBM/PC microcomputer. A two-tailed p value < 0.05 was considered statistically significant. Results are reported as medians with the lower and upper quartiles.

### RESULTS

During the experimental periods the serial weight curves were similar for the two groups of rats, with the means  $\pm$  standard deviation (sd) varying between  $337 \pm 53$  and  $369 \pm 56$ g in the control, and  $347 \pm 56$  and  $377 \pm 38$ g in the chagasic group.

Table 1 shows the median with lower and upper quartiles of the functional variables in the basal and hydropenic periods, for control and chagasic groups of rats. No significant differences ( $p > 0.05$ ) were observed between the chagasic and control groups as to the basal values of urinary flow ( $4.17$  v  $4.17 \mu\text{l} \cdot \text{min}^{-1}$ , respectively), glomerular filtration rate expressed by the creatinine clearance ( $351$  v  $400 \mu\text{l} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ), and excretion of sodium ( $274$  v  $353 \mu\text{Eq} \cdot \text{min}^{-1} \cdot 10^{-3}$ ) and potassium ( $764$  v  $826 \mu\text{Eq} \cdot \text{min}^{-1} \cdot 10^{-3}$ ).

The percent changes induced in the variables after hydropenia is also shown in Table 1. Significant ( $p < 0.01 - 0.05$ ) and marked absolute reduction in all the variables was observed in both groups of animals. However, only the urinary excretion of potassium showed median percent decrement statistically similar ( $p > 0.05$ ) in the chagasic (-64%) and in the control group (-70%). The median reduction in the urinary excretion of sodium was relatively smaller ( $p < 0.05$ ) in the chagasic (-57%) than control (-79%) rats. The urinary flow was also reduced less ( $p < 0.05$ ) in the chagasic (-40%) than in control (-63%) rats. On the contrary, the median decrement in the clearance of creatinine was relatively higher ( $p < 0.05$ ) in the chagasic group (-51%) than in the control (-39%) group.

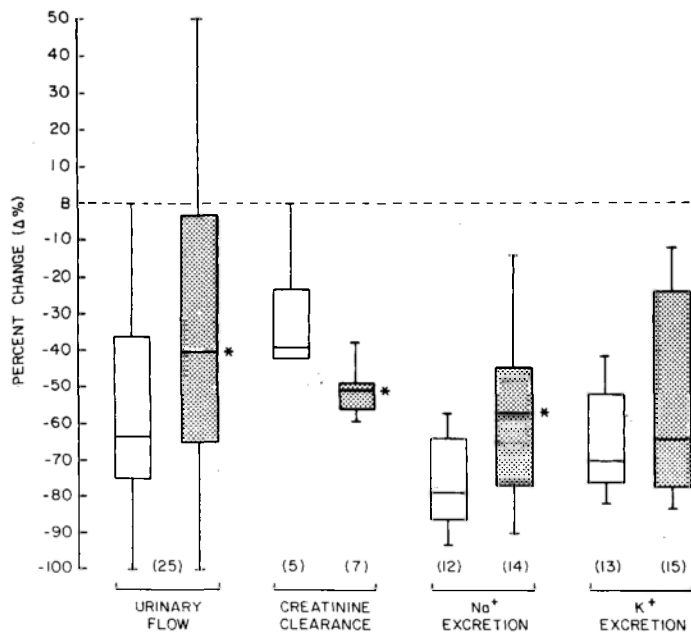
The statistical parameters of percent changes of the variables in each group of rats following water deprivation is depicted in Figure 1.

Table 1 - Urinary flow ( $V_U$ ), creatinine clearance ( $Cl_{CR}$ ), and urinary excretions of sodium ( $U_{Na}V$ ) and potassium ( $U_KV$ ) showed by control and chagasic rats in the basal state and after water deprivation.

		control rats			chagasic rats		
		basal	hydropenia	- Δ%	basal	hydropenia	- Δ%
$V_U$ ( $\mu$ l/min)	N	27	27	25	26	25	25
	Med	4.17	2.08	63%**	4.17"	2.08	40%** , +
	QI-QS	3.47-6.25	0.3-1.2	75%-36%	2.78-5.90	2.08-4.16	65%-3%
$Cl_{CR}$ ( $\mu$ l/min/100g)	N	19	5	5	16	7	7
	Med	351	217	39%*	400"	172	51%* , +
	QI-QS	255-418	199-255	42%-23%	351-449	165-224	56%-49%
$U_{Na}V$ ( $\mu$ Eq/min. $10^{-3}$ )	N	27	13	12	26	14	14
	Med	353	83	79%**	274"	109	57%** , +
	QI-QS	208-534	62-156	86%-64%	176-383	42-176	77%-45%
$U_KV$ ( $\mu$ Eq/min. $10^{-3}$ )	N	28	15	13	27	20	15
	Med	826	386	70%**	764"	332	64%**
	QI-QS	609-1123	260-490	76%-52%	601-962	218-482	77%-24%

Data are reported as medians (Med) with the inferior and superior quartiles (QI-QS) of N rats considered for analysis.

\*  $p < 0.05$ , \*\*  $p < 0.01$  (hydropenia x basal, Wilcoxon test); +  $p < 0.05$ , "  $p > 0.05$  (chagasic x control, Mann-Whitney test).



Medians and lower and upper quartiles are indicated as horizontal lines in the boxes, and the minimum and maximum values of distribution as vertical bars. In parenthesis the number of rats considered for analysis of the variables in each group.

\*  $p < 0.05$  compared to the control group (Mann-Whitney test).

Figure 1 - Percents changes in relation to the basal values, of urinary flow ( $V_U$ ,  $\mu$ l/min), creatinine clearance ( $Cl_{CR}$ ,  $\mu$ l/min/100g), and urinary excretion of sodium ( $U_{Na}V$ ,  $\mu$ Eq/min. $10^{-3}$ ) and potassium ( $U_KV$ ,  $\mu$ Eq/min. $10^{-3}$ ). Induced hydropenia in control (open boxes) and in chagasic (hatched boxes) groups of rats.

## DISCUSSION

The responses to water deprivation presented by the normal rats were, as expected, a marked reduction in the urinary excretion of sodium and potassium and in the urinary flow rate, associated with the decrement in glomerular filtration rate. Different mechanisms and hormonal actions promote these renal effects for the maintenance of the hydroelectrolytic homeostasis in hydropenia, the most important of which are the renin-angiotensin-aldosterone system, the arginine-vasopressin hormone, the atrial natriuretic factor, cardiorenal reflexes and the baroreceptor control of vasopressin<sup>3 11 13 14 17</sup>.

Chagasic rats also showed significative retention of electrolytes and water, and reduced glomerular filtration rate following hydropenia. However, the urinary flow rate, the clearance of creatinine and the excretion of sodium were reduced in these rats in significantly different proportions in relation to the normal rats; urinary flow rate and the excretion of sodium showed smaller decreases, and the creatinine clearance exhibited a greater decrement in the chagasic rats, expressing diverse functional responses to hydropenia. Therefore, water deprivation did not prevent a higher excretion of sodium in these animals. Unexpected high natriuresis was described previously<sup>1 18</sup> in chagasic patients submitted to the expansion of blood volume and to changes in plasma osmolarity.

The decrease in the excretion of potassium was similar in both groups of rats.

To explain the smaller capacity for the maintenance of sodium and water balance during hydropenia in the present experimental model of chagasic infection, some possibilities can be raised. One of these is that the hormonal control of the hydroelectrolytic homeostasis would be altered, resulting in higher natriuretic and diuretic effects. Isolated or associated deficient secretion of arginine-vasopressin, higher plasma levels of the atrial natriuretic factor, or inhibition of the renin-angiotensin-aldosterone system are the mechanisms probably implicated.

Another possibility is an altered renal tubular response to hormonal action in chagasic rats, i.e., smaller sensitivity to aldosterone and vasopressin, or higher sensitivity to atrial natriuretic factor.

Tubular-interstitial immunological lesions cannot be excluded as an alternative<sup>12</sup>.

It should be pointed out that in chagasic patients alterations in the osmotic threshold for vasopressin secretion was observed in association with induced increase in the plasma osmolarity<sup>18</sup>. Increased plasma concentration of renin<sup>6</sup> and higher levels of aldosterone<sup>15</sup> were also described in these patients. Regarding the latter observation, patients with primary aldosteronism showed increased plasma atrial natriuretic factor, resulting in higher natriuresis following acute sodium load<sup>9</sup>.

Another important conjecture concerns the interplay of cardiovascular and renal influences on the hormonal responses, considering the widespread impairment of the autonomic nervous system in Chagas' disease. Particularly, reflex cardiac autonomic dysfunction is a striking disturbance in chronic chagasic patients<sup>7</sup>. In the rat model of chagasic infection presently studied, reduced baroreflex bradycardia due to impaired efferent parasympathetic activity of the heart caused by intrinsic neuroganglionitis was verified<sup>8</sup>. Inflammatory and degenerative lesions of variable degree were also observed in atrial and ventricular myocardial fibers in the majority of infected rats<sup>4 5</sup>.

In conclusion, disturbed homeostatic response to water deprivation expressed by higher elimination of sodium and water in association with smaller glomerular filtration rate was observed in chronically *T. cruzi*-infected rats. Possible explanations include disturbances in the hormonal and/or neural systems concerned to hydroelectrolytic homeostasis; cardiac and renal autonomic mechanisms, and renal tubular processes can also be implicated.

## RESUMO

*O efeito de 8 horas do jejum hídrico sobre a excreção renal de sódio e água foi investigado em ratos Wistar machos infectados com a cepa São Felipe (12SF) do T. cruzi e comparados com ratos controles, de mesma idade e sexo. Durante a hidropenia, a mediana da redução no fluxo urinário (-40% v -63%) e a excreção urinária de sódio (-57% v -79%) foram menores nos chagásicos do que nos controles, respectivamente. Assim os ratos chagásicos excretaram mais do que os controles. Por outro lado, a mediana do decréscimo percentual na depuração de creatinina foi maior nos chagásicos (-51%) do que nos controles' (-39%). Portanto, os ratos*

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chagásicos mostraram alteração na resposta excretória hidroeletrólítica renal expressa por menor conservação de água e sódio em associação à menor taxa de filtração.

*Palavras-chaves:* Hidropenia. Fluxo urinário. Sódio e potássio urinários. Taxa de filtração glomerular. Infecção murina por T. cruzi.

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