# Abnormal Sodium Regulation in Rats Following Chronic Intermittent Exposure to Carbon Tetrachloride[1] (35991)

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(Introduced by W. H. Hulet)

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Chronic intermittent exposure to carbon tetrachloride vapor causes the development of nodular cirrhosis of the liver in rats (1). The incidence of measurable ascites in this situation is variable, but has been reported to occur up to 50% (2). There is no information whether kidney function is normal or impaired in the sodium retaining states following $CCl_4$-induced liver disease.

The aims of this investigation were: (i) to examine the possibility that sodium retention prior to the development of the clinical signs of ascites and edema might be detectable with metabolic balance studies; and (ii) to examine inulin and PAH clearances and maximal concentrating ability ($U_{osm\ max}$) under these experimental conditions.

*Methods.* Twenty white, male Sprague-Dawley rats, with an initial average weight of 250 g, were allowed free access to Purina Rat Chow with an average sodium content of 20 mEq/100 g of diet. The experimental animals (14 rats) were individually placed into a loosely covered 6000 ml glass container and exposed to an oxygen–$CCl_4$ vapor mixture twice weekly for 20 min. Oxygen concentrations (measured in preliminary studies with a portable "Myra" oxygen analyzer) in the oxygen–$CCl_4$ mixture ranged from 10 to 24%. The amount of $CCl_4$ vaporized into the container ranged from 2.2 to 4.4 ml/20 min, depending on the tolerance of the animal. No further $CCl_4$ was added to the chamber after the animals were fully anesthetized. Vapor pressures of oxygen and $CCl_4$ were not measured. The remaining six rats served as controls.

The experimental animals were not exposed to $CCl_4$ vapor for at least 2 weeks prior to the metabolic balance studies (BS). These were performed 10 (BS-I), 16 (BS-II), and 40 (BS-III) weeks after initial exposure to $CCl_4$. Prior to the third balance study (BS-III), all animals received 0.9% NaCl solution as drinking fluid for 10 weeks.

A. For the balance studies, animals were acclimatized for 3 days in single metabolic cages. During the balance studies performed at 10 and 16 weeks, the rats were fed regular Purina Rat Chow and water. During the study performed at 40 weeks, animals received 0.9% saline and were fed their regular diet. During the following 5 days, weight, fluid and food intake, and urine volume were determined every 24 hr. The urine was collected under oil. The bladder was emptied by abdominal pressure at the end of each 24 hr collection period.

B. Maximal urinary concentrating ability was determined after 16 hr of water deprivation and after the administration of 250 mU of pitressin in oil.

C. Acute clearance studies, using techniques as described in detail elsewhere (3), were done after completion of BS-III. Methoxy-$^3$H-inulin and carboxy-$^{14}$C-hippuran were used and were given in a priming dose of 25 $\mu$Ci/ml of $^3$H and $^{14}$C and a sustaining dose of 2 $\mu$Ci/ml of $^3$H and $^{14}$C in Ringer's solution administered at a rate of 0.7 ml/100 g/hr. $^3$H-Inulin and $^{14}$C-

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hippuran in urine and plasma were counted in Beckman liquid scintillation counter. Sodium and potassium determinations were performed on an Instrumentation Laboratory flame spectrophotometer. Osmolality determinations were performed on a Fiske osmometer and on a Precision Systems Osmette A. Urinary creatinine was determined according to Chasson *et al.* (4) on a Technicon AutoAnalyzer. Plasma creatinine determinations were performed on microliter adaptations of the method described by Popper *et al.* (5) for creatinine.

Data of experimental and control populations were compared with Student's *t* test or the Mann-Whitney *U* test when the assumptions of the parametric *t* test were not applicable (6).

*Results. Pathology.* Four rats died during the first 3 weeks of exposure to carbon tetrachloride vapor. They had no obvious liver or kidney disease. Between the fourth and ninth week, three animals died revealing pathologic evidence of central necrosis and fatty metamorphosis of the liver. The remaining 7 animals developed nodular cirrhosis of the liver and one rat had ascites.

Kidneys of all experimental animals were normal in weight and gross appearance. On light microscopy there was evidence of thickening of the glomerular mesangium in two animals with nodular cirrhosis of the liver. Electron microscopic study of these two kidneys revealed thickening of the glomerular basement membrane (GBM) with electron dense deposits in the GBM and an increase of the mesangial stalk. Renal tubules were normal in the six experimental animals whose tissues were fixed for histology immediately postmortem and they revealed autolytic changes in the eight experimental animals whose autopsy was done from 10 to 24 hr after death occurred.

*Metabolic balance studies* (Table I). Six experimental and six control rats were studied at 10 weeks (BS-I). Three of the six experimental animals survived to be studied again at 16 weeks (BS-II) and 40 weeks (BS-III).

Experimental animals showed a significantly higher positive net sodium balance in all

TABLE I.[a]

| | | No. of animals | Wt (g) | Fluid intake (ml/24 hr) | Urine vol (ml/24 hr) | Positive net-sodium balance (mEq/24 hr) | Positive net potassium balance (mEq/24 hr) | Endogenous creatinine clearance (ml/min) |
|---|---|---|---|---|---|---|---|---|
| BS I | C | 6 | 517 ± 10 | 36.6 ± 2.6 | 19.1 ± 1.8 | 0.65 ± 0.07 | 0.63 ± 0.09 | 1.1 ± 0.1 |
| | E | 6 | 441 ± 28 | 60.4 ± 7.2 | 31.1 ± 5.6 | 1.40 ± 0.25 | 1.65 ± 0.42 | 1.8 ± 0.2 |
| | *p* | | <.05 | <.02 | NS | <.02 | <.05 | <.005 |
| BS II | C | 6 | 569 ± 11 | 39.6 ± 1.9 | 17.9 ± 2.5 | 1.31 ± 0.19 | 1.56 ± 0.09 | 1.7 ± 0.2 |
| | E | 3 | 537 ± 40 | 65.4 ± 3.0 | 36.6 ± 1.2 | 2.13 ± 0.12 | 1.71 ± 0.23 | 1.5 ± 0.1 |
| | *p* | | NS | <.001 | <.005 | <.025 | NS | NS |
| BS III | C | 4 | 612 ± 26 | 66.2 ± 4.7 | 44.6 ± 5.2 | 0.87 ± 0.12 | 0.25 ± 0.20 | 1.6 ± 0.1 |
| | E | 3 | 619 ± 36 | 98.6 ± 5.9 | 75.4 ± 3.6 | 1.64 ± 0.15 | 0.61 ± 0.30 | 1.8 ± 0.3 |
| | *p* | | NS | <.01 | <.01 | <.01 | NS | NS |

[a] BS I, balance study at 10 weeks; BS II, balance study at 16 weeks; BS III, balance study at 40 weeks under chronic saline load; C, control animals; E, rats exposed to $CCl_4$. Results are averages of last 3 days of BS and reported as mean ± SEM; *p* values are given only when difference is significant.

TABLE II.

| | No. of animals | No. of observations | $C_{IN}$ (ml/min/kg of body wt/kidney) | $C_{PAH}$ (ml/min/kg of body wt/kidney) | FF (%) |
|---|---|---|---|---|---|
| C[a] | 4 | 16 | 4.37 ± 0.39 | 16.24 ± 1.38 | 29 ± 2 |
| E | 3 | 12 | 4.40 ± 0.34 | 20.60 ± 1.71 | 23 ± 2 |
| $p$ | | | NS | $.05 < p < .1$ | $.05 < p < .1$ |

[a] For explanations, see Table I.

three studies (see Table I). The difference in positive net sodium balance between experimental and control animals was 0.75, 0.85, and 0.77 mEq/24 hr in BS-I, BS-II, and BS-III, respectively.

Endogenous creatinine clearances were significantly higher in the experimental group during BS-I and there was no significant difference in clearance values between experimental and control animals in BS-II and BS-III. This is best explained by the death of the experimental animal with the highest creatinine clearance prior to BS-II. Plasma creatinine concentrations were equal in the two groups during BS-II and BS-III and there was no difference in creatinine excretion in all three studies.

Positive net balance of potassium was significantly higher in the experimental group in the first study, but was not different in BS-II and BS-III.

*Maximal urinary concentrating* ability was not significantly different between the two groups: 2156 ± (SEM) 70 mOsm/kg in the experimental animals vs 2400 ± 149 mOsm/kg in the controls ($.1 < p < .2$).

Results of the *acute clearance studies* are listed in Table II. The $C_{IN}$, $C_{PAH}$, $C_{IN}/C_{PAH}$ and fractional excretion of sodium and water were not statistically different between the two groups.

*Discussion.* Greater positive net sodium balance in the experimental animals was observed during 2 out of the last 3 days of each of three balance studies, indicating that intermittent sodium retention occurs in the animals exposed to $CCl_4$ vapor, which have developed cirrhosis of the liver. The magnitude of sodium retention was higher in the animal which developed ascites, being 1.9 mEq/24 hr higher than in the controls and more than twice the amount retained by the experimental animals as a group. Since no visible edema or ascites developed in the remaining experimental animals, they either must have had periods of increased natriuresis, or the sodium was retained in excess of water retention (7). Since total body sodium was not determined, we cannot comment on that possibility. GFR was the same in both groups, as measured with inulin clearances after BS-III and it was higher in the experimental group during BS-I as judged from endogenous creatine clearances. Higher aldosterone activity in the experimental animals cannot be excluded. However, we believe that any physiological stimuli for increased aldosterone secretion should have been minimal during chronic saline loading in BS III. At that time, the experimental animals were retaining sodium to the same degree as in the previous two studies. The $P_{Na}/P_K$ ratios, which were similar in the two groups during BS-I and BS-II, were lower in the experimental animals during BS-III (25.2 ± 0.8 vs 30.0 ± 1.2) ($p < .05$) while $U_{Na}/U_K$ ratios were slightly, but not significantly higher, in the experimental animals, making an increased aldosterone effect unlikely.

While there was no difference in inulin clearances between the experimental and control animals, the PAH clearances in two experimental animals were the highest of all rats examined (24.0 and 22.5 ml/min/kg of body wt/kidney, respectively). Similarly high PAH clearances have been described in some patients with alcoholic cirrhosis of the liver (8) and suggest that the $CCl_4$-intoxicated rat model might be a suitable one to examine aspects of renal function in liver cirrhosis, which are not amenable to experimental study in humans.

Although there was no significant difference in $U_{osm\ max}$, there seemed to be a trend for it to be lower in the experimental animals. A decrease in $U_{osm\ max}$ and solute-free water reabsorption has been described in humans with liver cirrhosis and no detectable renal disease (9). Whether it was due to late effects of $CCl_4$ or to other factors related to the liver disease in these animals cannot be decided with the present data.

*Summary.* Rats with nodular cirrhosis due to $CCl_4$ intoxication showed a higher net positive sodium balance than their age-matched controls during three metabolic balance studies, done 10, 16, and 40 weeks after initial exposure to $CCl_4$. Renal hemodynamics and $U_{osm\ max}$ were not different between both groups. The data suggest that intermittent periods of sodium retention precede the development of ascites in rats with $CCl_4$-induced cirrhosis of the liver and that these changes occur in the presence of intact glomerular and tubular renal function.

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