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Renal Function in the Choline Deficient Rat[1] (39103)

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(Introduced by M. Epstein)

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Choline deficiency is known to induce both renal and hepatic changes in the growing rat (1–5). These changes are particularly striking when weanling rats are fed a choline-deficient diet. Indeed, administration of the deficient diet to weanling rats for only 1–4 weeks is sufficient to cause definite arterial hypertension, which increases in severity as the rat grows older (3, 6). It has been assumed that the hypertension is somehow related to the morphological changes seen in the kidneys of choline-deficient rats (3, 6); however, other observers prefer to postulate a functional rather than a structural abnormality (7). Recently, Kratzing and coworkers have examined renal function in choline-deficient rats and have noted a progressive decline in the proportion of an oral normal saline load excreted over a 4-hr period as choline deficiency progressed (8). Since glomerular filtration rate (GFR) was not determined in their study, it was not possible to decide whether the decline in sodium excretion was caused by a decrease in GFR or by increased tubular reabsorption. We therefore decided to study effective renal plasma flow (RPF), GFR, and the excretion of sodium following the infusion of 5% saline in hydropenic choline-deficient rats and their age-matched controls. Since many rats die in acute renal failure when choline deficiency is induced in weanling rats (3), the choline-deficient diet in this study was begun when the rats had attained a weight of 178 g. Only a few animals died during the course of the dietary regimen.

Methods. Male Sprague–Dawley rats were started on a hypolipotropic diet when they had attained an average weight of 178 g. The special diet for the experimental (Ch-D) and control (C) animals is described in Table I. Since acute renal failure develops frequently in choline-deficient rats, approximately half of the Ch-D and C animals were given normal saline to drink *ad libitum*, which has been described as lessening the severity of acute renal failure (9). The other half of the Ch-D and C animals consumed tap water. This corresponds to a sodium intake of approximately 7–10 mEq/24hr/kg in the saline-drinking and of 2–4 mEq 24hr/kg in water-drinking rats. Studies of maximal renal concentrating ability and renal free water reabsorption (T^cH_2O) were performed 10 months after the start of the dietary regimen. Maximal renal concentrating ability was tested after 44 hr of fluid deprivation. Rats were housed in metabolic cages, had free access to food only, and received 250 mU vasopressin intramuscularly after 24 hr of fluid deprivation. Four hours after this injection, their bladders were expressed by gentle abdominal pressure and a urine collection under oil was started and continued for 16 hr. This allowed the accumulation of large enough a urine sample (> 2 cc) for examination of osmolality, urea, sodium, and potassium. At the end of the urine collection, blood was sampled from free flowing tail blood.

T^cH_2O studies. Animals were prepared for renal clearance studies as described previously (10). Animals had been deprived of fluid for 24 hr prior to commencement of the clearance studies, and they had received 250 mU of vasopressin in oil intramuscularly 15 hr before the study. Following a loading dose of 25 μCi each of ^{3}H-inulin and ^{14}C-*p*-amino hippurate (PAH), and of 5 mU vasopressin in 1 ml, a sustaining infusion of Ringer's solution, containing 2 μCi each of ^{3}H-inulin and ^{14}C-PAH, and 8.3 mU of vasopressin per ml was given at a rate of 0.6 ml/hr/100 g BW. Two baseline collection periods, consisting of two 30-min urine col-

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TABLE I. COMPOSITION OF DIETS[a]

Choline-deficient diet		Control diet	
Casein	9 (g/100g)	Casein	18 (g/100g)
Soy protein	9	—	
Sucrose	60	Dextrose	59.7
Corn oil	10	Corn oil	10
Cholesterol	2	—	
Alphacel	4	Alphacel	6
Salt mixture[a] (Hegsted)	4	Salt mixture (Hegsted)	4
Vitamin mixture[b]	2	Vitamin mixture	2
Choline chloride	—	Choline chloride	0.3

[a] As supplied from Nutritional Biochemical Corporation, Cleveland, Ohio.

[b] Composition of vitamin mixture, see (5).

lections and three blood samples were completed before an infusion of 5% saline was begun. Hypertonic saline was infused at a rate of 0.62 ml/hr/100 g for 15 min and thereafter increased to 1.24 ml/hr/100 g. Forty-five minutes after starting this infusion, urine collection was resumed and five to seven 5–10-min collection periods were completed. A similar number of tail blood samples were obtained, and animals were killed by exsanguination at the end of the clearance study.

Pathological studies. Liver, heart, and kidneys were dissected free from adjacent tissue, weighed and put into 10% formalin-normal saline for fixation. Tissue was prepared for light microscopy by thin (2–4 μm) sectioning and staining with Hematoxylin-Eosin and Masson's trichrome stain.

Analysis of the blood and urine samples for sodium, potassium, ^{3}H-inulin, and ^{14}C-PAH was done as previously reported (10). Osmolality in the plasma was determined with an Advanced Nanoliter Osmometer; urine osmolality was measured on a Precision Systems Osmette A. Urea concentration in the urine was analyzed on a Technicon Autoanalyzer, utilizing a slight modification of the method of Marsh *et al.* (11). Renal clearances and solute free water reabsorption (T^cH_2O) were calculated according to standard formulae. Student's unpaired *t* test was used for the comparison of group means of the C and Ch-D animals. Within-group comparisons were done with a paired *t* test (12). Linear regression lines were calculated by the method of least-squares and were compared by analysis of covariance (12). Since no significant differences in the clearances of inulin, PAH, sodium, and solutes, or in urine flow and T^cH_2O were observed between the animals receiving saline and those drinking tap water, the data are reported after combining them in one group. Data are reported as means ± SEM. Differences in the groups attaining a two-tailed P value of < 0.05 were considered statistically significant.

Results. Renal function studies. (Table II and III). Maximal renal concentrating ability was impaired in Ch-D: Uosm (max) 2113 ± 66 mOsm/kg H_2O (Ch-D) as compared to C rats; 2525 ± 127; $P < 0.005$. This was entirely accounted for by a decrease in nonurea solutes (Uosm − Uurea): Ch-D, 604 ± 21 mOsm/kg H_2O; C, 1016 ± 98; $P < 0.001$. Urea concentration was the same in the two groups, while sodium concentration and excretion was less in Ch-D rats. Studies of fractional free water reabsorption under the stimulus of 5% saline infusion showed a 26% decrease in Ch-D at comparably high fractional solute clearances (COsm 10–20 ml/min/100 ml GFR) (Table III). Analysis of the regression of fractional T^cH_2O on fractional osmolar clearance showed a significantly smaller increase in T^cH_2O formation per increase in solute clearance in the Ch-D rats: $T^cH_2O = 0.34\ COsm + 0.67$, $r = 0.91$ (Ch-D); $T^cH_2O = 0.44\ COsm + 0.34$, $r = 0.98$ (C); F (slopes) = 20.1879, $P < 0.005$. Solute clearance was smaller in Ch-D secondary to a decrease in sodium clearance in these animals ($P < 0.02$). This was presumably due to a 26% decrease in inulin clearance, resulting in a decrease in the filtered load of sodium in Ch-D. Infusion of hypertonic saline resulted in a slightly but significantly higher serum sodium concentration in Ch-D ($P < 0.05$). Mean arterial blood pressure before the start of the saline infusion was 92 ± 2 mm Hg in C and 107 ± 5 mm Hg in Ch-D ($P < 0.02$). Effective renal plasma flow was 26% lower in Ch-D, and the filtration fraction was similar in the two groups [0.28 ± 0.02 (C); 0.29 ± 0.02 (Ch-D); $P > 0.6$].

TABLE II. MAXIMAL URINARY CONCENTRATION AFTER 28 HOURS OF FLUID DEPRIVATION AND VASOPRESSIN[a]

	Control (9)	P	Choline deficiency (23)
Uosm (mOsm/kg H_2O)	2525 ± 127	< 0.005	2113 ± 66
Volume (μl/min/kg BW)	4.48 ± 0.30	> 0.10	5.75 ± 0.58
Uurea (mM/liter)	1509 ± 90	> 0.995	1509 ± 55
U_{Na} (mEq/liter)	301 ± 32	< 0.001	159 ± 10
$U_{Na}V$ (μEq/min/kg BW)	1.37 ± 0.17	< 0.025	0.92 ± 0.10
Posm (mOsm/kg H_2O)	289 ± 4	> 0.3	294 ± 2

[a] 16-hr collection period after 28 hr of dehydration.

TABLE III. FREE WATER REABSORPTION IN RESPONSE TO A 5% SODIUM CHLORIDE INFUSION[a]

	Control (7)	P	Choline deficiency (10)
C_{in} (ml/min/kg BW)	6.4 ± 0.5	< 0.02	4.7 ± 0.4
C_{PAH} (ml/min/kg BW)	22.9 ± 1.7	< 0.05	17.2 ± 1.9
Volume (μl/min/kg BW)	578 ± 45	> 0.05	472 ± 37
Uosm (mOsm/kg H_2O)	581 ± 19	< 0.05	529 ± 15
UosmV (mOsm/min/kg BW)	0.34 ± 0.02	< 0.01	0.25 ± 0.02
$U_{Na}V$ (μEq/min/kg BW)	153 ± 12	< 0.02	109 ± 10
Cosm (μl/min/kg BW)	1107 ± 87	< 0.005	770 ± 59
Cosm/C_{in} (%)	17.5 ± 1.5	> 0.30	16.3 ± 0.5
T^cH_2O (μl/min/kg BW)	521 ± 41	< 0.001	297 ± 33
T^cH_2O/C_{in} (%)	8.3 ± 0.7	< 0.02	6.2 ± 0.4

[a] Values are the average of the two periods with the highest solute clearance.

Pathological studies. The livers of all Ch-D rats showed a significant increase in weight: 30.3 ± 2.3 g (Ch-D); 15.4 ± 0.8 g (C); $P < 0.001$. The percent of total body weight accounted for by the liver was higher in Ch-D (3.6 ± 0.3%) than in C (2.4 ± 0.2%), $P < 0.005$. Most of the increase in liver weight could be attributed to a marked increase in liver fat. In the five choline-deficient rats whose livers were examined by light microscopy, there was one animal with nodular portal cirrhosis and one animal with marked increase in fibrous tissue in the liver. The livers were normal in the two C rats examined. Three of five Ch-D rats had calcium deposits in the cortico-medullary zone of the kidney. All five animals had increased interstitial mononuclear cellularity, and there were hyaline casts in many of the tubules. One animal had thickening of the glomerular basement membrane and increase of the mesangial stalk. This same animal also showed thickening of the tubular basement membrane and thickened arteriolar walls. One of the two control animals studied had many tubular casts and increased interstitial cellularity. Neither one had any evidence of glomerular changes or nephrocalcinosis. Heartweights were somewhat higher in Ch-D (1.84 ± 0.06 g) than in C (1.60 ± 0.08), $P < 0.02$; however, heartweight factored by bodyweight was not different in the two groups.

Discussion. The present studies have defined some abnormalities of renal function in choline-deficient rats. These functional changes consist of a decrease in effective renal plasma flow and glomerular filtration rate. Sodium excretion during saline infusion is diminished mainly as a consequence of a decreased filtered load of sodium due to the decrease in GFR. There is a decrease in maximal concentrating ability that is characterized by a decrease in the urine concentration and excretion of nonurea solutes, and a normal urinary urea concentration. Solute free water reabsorption is also diminished, presumably as a consequence of decreased sodium chloride delivery to the loop of Henle. However, other causes for a

decrease in T^cH_2O, i.e., a defect in sodium chloride reabsorption in the loop of Henle or changes in medullary blood flow cannot be excluded by the present studies.

An explanation for the underlying mechanisms of these functional changes is not forthcoming from these studies. However, studies by Nagler *et al.* (13) of the mesoappendiceal circulatory bed of choline-deficient rats have demonstrated a marked hypersensitivity of the precapillary sphincters and precapillary arterioles to doses of epinephrine less than 10% of those required to elicit a maximal response in control animals. These authors have postulated that the substance antagonistic to epinephrine in this circulatory bed (presumably acetylcholine) is present in less than normal amounts or activity (13). If a similarly increased reactivity exists in the preglomerular arterioles of choline-deficient rats, this could explain the observed decrease in effective renal plasma flow noted in the present studies. Whether it could account for a depression of filtration rate of comparable magnitude cannot be decided from the available studies in the literature. Infusion of l-epinephrine in man causes about a 20% decrease in renal plasma flow with a much smaller decline in GFR and an increase in the filtration fraction (14, 15), which was not observed in the choline-deficient animals.

Correlation of the morphological findings with the observed functional changes cannot be made, particularly since these morphological abnormalities appeared to be rather modest. However, the dystrophic cortical calcifications observed might indicate a loss of functioning nephrons.

Choline deficiency in the diet of weanling rats causes hypertension (3, 6). Any maneuver to slow the growth rate in animals on a hypolipotropic diet will result in much less injury to the kidneys (16) and less severe hypertension (8). Blood pressure was higher in our choline-deficient rats than in the controls, despite the start of the deficient diet at an older age. Only one animal, however, was clearly hypertensive (BP 140 mm Hg). Therefore, the question arises whether the hypertension per se could account for some of the renal functional abnormalities seen in the choline-deficient rats. Buckalew *et al.* (17) have reported an impaired free water reabsorption in hypertensive patients. It is well known that saline infusion results in an exaggerated natriuresis in hypertensive patients (18), even those in whom GFR is decreased by up to 30% (19). While having an impaired T^cH_2O formation, choline-deficient rats had a decreased sodium excretion during 5% saline infusion. We therefore feel that the mild increase in blood pressure was not the cause of the renal functional changes observed.

Lastly, consideration should be given to the possibility that the liver disease in choline deficiency might be responsible for the renal abnormalities. The most consistent renal abnormality in cirrhotic patients is an increased tubular reabsorption of sodium (20). Fractional sodium reabsorption at maximal solute clearance, however, was not lower in the choline-deficient rats. We therefore conclude that the main reason for the decreased sodium excretion in the choline-deficient rats was a decreased filtered load of sodium due to the diminution of GFR and not increased tubular reabsorption. One other finding, that of a normal urea excretion during the concentration studies, allows us to differentiate the mechanism of a decreased solute free water reabsorption in the choline-deficient rats from that observed in patients with cirrhosis, in whom a lower urea concentration and excretion in the urine is found (21). Considering the renal morphological abnormalities and the above-mentioned differences in renal function, we conclude that the choline-deficient rat is unsuitable as a model for the elucidation of the renal functional changes observed in cirrhosis.

Summary. Abnormalities in renal concentrating ability and free water reabsorption, and a diminished sodium excretion, glomerular filtration rate, and effective renal plasma flow were observed in adolescent rats which ingested a lipotrope deficient diet for 10 months.

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