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The Role of Chloride in Hypokalemic Alkalosis in the Rat *

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Cooke and co-workers (1) have suggested that in potassium depletion net movement of hydrogen ion occurs from the extracellular compartment into cells, thereby causing extracellular alkalosis and intracellular acidosis. Disappearance of plasma abnormalities following the acute administration of potassium chloride to nephrectomized potassium-deficient rats (2) seemed to support this hypothesis, as did the finding by some investigators (3–5) of a decrease of intracellular pH in potassium-deficient compared with normal skeletal muscle. Other workers, however, failed to find evidence of intracellular acidosis in potassium-deficient muscle (6, 7).

On indirect evidence Cooke and associates (8) suggested that hypochloremia in potassium deficiency was the result not of chloride loss but rather of expansion of extracellular fluid volume. Balance studies in potassium-deficient dogs (9) and in humans with primary hyperaldosteronism (10), however, showed that in potassium deficiency extracellular alkalosis is accompanied by chloride deficiency. Neither the potassium deficit nor the alkalosis could be fully corrected until chloride was administered.

To resolve these contradictory results experiments were designed to study in potassium-deficient rats the effects of acute and chronic administration of potassium chloride and potassium bicarbonate on the whole body inulin space and on the electrolytes of plasma, total carcass, muscle, and skin.

In all potassium-deficient animals a chloride deficiency was found. The inulin space was not increased. Complete correction of either extracellular alkalosis or of potassium deficiency did not occur until a chloride supplement was given. On acute potassium loading of nephrectomized potassium-deficient rats the penetration of potassium into muscle cells was independent of the plasma chloride concentration. During potassium repletion chloride was retained in excess of sodium and potassium, suggesting that during potassium deficiency no intracellular acidosis existed. The data suggest that chloride plays a critical role in the recovery and probably in the pathogenesis of extracellular alkalosis in the potassium-deficient rat.

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Methods

Studies were carried out on male, white rats derived from the Wistar strain, weighing 260 to 340 g at the start of the experiment. During the whole experimental period the rats were fed 50 g per kg body weight daily of a diet (for composition see Table I) with a potassium content of less than 0.4 mEq per kg.

In experiments I and III the diet contained 12 mmoles of sodium and 7 mmoles of chloride per kg body weight; in experiment II the chloride content was 0.5 mmole per kg body weight daily. A supplement of sodium chloride, 6 mmoles per kg body weight each day, was added to the diet during the depletion period in all experiments except in experiment II, where during the last 5 days of this period the equivalent amount of sodium bicarbonate was given instead. In experiments IA, II, and III all animals received an intraperitoneal injection of 2 mg desoxycorticosterone gluconate in water [1] (DOC) daily. Distilled water was given ad libitum. The rats were kept in individual cages. The general condition of all animals remained good throughout the experiment. Rats with diarrhea (in some cases caused by intraperitoneal injections of potassium solutions during the repletion period) were discarded. After a depletion period of 30 to 35 days, the administration of DOC and supplements was discontinued, and the following three potassium repletion experiments were carried out.

I. *Effects of administration of potassium chloride.* In experiment IA (27 rats) the animals were divided into four groups. Group 1 was sacrificed immediately (day 0) and served as the potassium-deficient control group. The dietary sodium chloride supplement of the remaining

[1] Percorten, water-soluble, Ciba, Basel, Switzerland.

TABLE I

Composition of diets

Components of basic diet		Vitamin mixture		Mineral mixture*	
	g/kg		*mg/kg diet*		*g/kg*
Sucrose	670	Thiamin	2.0	NaCl†	225
Casein	200	Riboflavin	2.5	NaH_2PO_4	310
Peanut oil	20	Pyridoxine	3.0	$Ca_3(PO_4)_2$	149
Cod-liver oil	20	Pantothenic acid	10	$CaCO_3$	210
Cellulose powder	50	Niacin	300	$MgSO_4$	90
Salt mixture	40	Choline	300	$FePO_4$	14.70
		Inositol	300	$MnSO_4$	0.20
		p-Aminobenzoic acid	10	$CuSO_4$	0.39
				$Al_2(SO_4)_3$	0.09
				NaI	0.05
				NaF	0.57

* Weights without water of crystallization.
† Changed to equivalent amount of $NaHCO_3$ in latter part of experiment II.

animals was changed to potassium chloride, 3 mmoles per kg body weight twice daily, administered as isotone solution by intraperitoneal injection. Groups 2, 3, and 4 were sacrificed 3, 7, and 10 days after starting potassium repletion. Experiment IB (26 rats) was similar to experiment IA in every detail except that no DOC had been given during the potassium-depletion period.

II. *Comparison of effects of potassium bicarbonate and chloride administration.* In this experiment (22 rats) the diet had been low both in potassium and chloride from the last 5 days of the depletion period onwards. The animals were divided into four groups. Group 1 was sacrificed immediately (day 0) and served as the potassium-deficient control group. Groups 2 and 3 were sacrificed after receiving a supplement of potassium bicarbonate, 3 mmoles per kg body weight twice daily for 3 and 11 days. Group 4 was sacrificed after receiving potassium bicarbonate for the first 6 days and then potassium chloride for 5 days.

III. *Comparison of effects of acute potassium bicarbonate and chloride administration after functional nephrectomy.* In this experiment 29 rats were divided into three groups. Four hours before sacrifice, immediately after bilateral functional nephrectomy by ligation of the renal pedicle, the animals were given as intraperitoneal injection per kilogram body weight: to group 1 (potassium-deficient group), 10 ml of a 5% solution of dextrose; to group 2, potassium chloride, 6 mmoles in 10 ml water; to group 3, the equivalent amount of potassium bicarbonate.

In experiments I and II water and electrolytes of plasma, total carcass, muscle, and skin of all animals were measured, and the inulin space was determined. In experiment III carcass analysis was not done. Comparison of the results with preliminary studies of normal and potassium-deficient rats showed that groups 4 of experiments I and II could be considered to be fully repleted, essentially normal animals (11).

The inulin space was determined as follows. Food and water were withheld 6 hours before sacrifice. Under light ether anesthesia blood vessels and ureters of both kidneys were ligated through small lumbotomies. Immediately afterwards inulin, 0.3 g per kg body weight in 3 ml water, was given by intravenous injection. Blood was drawn in heparinized syringes, 0.6 ml after 2 hours from the jugular vein, and 8 to 10 ml after 4 hours, at the time of sacrifice, from the abdominal aorta. Inulin was determined on both blood samples. As aorta blood was also used for other determinations, care was taken to obtain this blood anaerobically.

Specimens of thigh muscle and of skin were removed. Analysis of the total carcass, minus contents of the gastrointestinal tract, was then carried out. A modification of the method of Mickelsen and Anderson (12) was used, involving autoclaving of the carcass at 125° C for 15 minutes. As it was found that a sufficiently homogeneous tissue preparation could be obtained by prolonged use of a blender, a colloid mill was not used. Muscle and skin specimens were dried and pulverized before analysis.

Water content of tissues was determined by drying at 100° C for 24 hours. Fat content was determined by extraction with equal volumes of ether and petroleum ether. Inulin was determined by the method of Higashi and Peters (13). Sodium and potassium were determined by flame photometry; tissues were first prepared by digestion with sulfuric acid and perhydrol. Chloride was determined by electrometric titration (14); tissue was prepared by shaking the homogenized sample with 1 N nitric acid for 4 hours. Total CO_2 was determined manometrically. The pH was measured at 37° C with a pH meter with microelectrode.[2] Because of respiratory changes during anesthesia the total CO_2 is a better measurement of the degree of extracellular alkalosis than is pH. With the experimental technique used a certain decrease of total CO_2 occurs as a result of the elimination of renal function during the last 4 hours of life.[3]

[2] Radiometer, Copenhagen, Denmark.

[3] In a group of eight rats weighing 170 to 200 g, fed during 4 weeks the diet shown in Table I but containing potassium, 8 mmoles per kg body weight daily, total CO_2 4 hours after functional nephrectomy amounted to 18.1 ± 2.4 mEq per L (mean ± SD).

TABLE II

Effects of potassium chloride administration on plasma, carcass, muscle, and skin

Group	K repletion Duration	No. rats	Plasma					Carcass (per 100 g FFDS†)					
			Na	K	Cl	Total CO_2	pH	Na	K	Cl	H_2O	E_{Cl}‡	E_{In}‡
	days		*mEq/L*	*mEq/L*	*mEq/L*	*mEq/L*		*mEq*	*mEq*	*mEq*	*g*	*g*	*g*
1	0	6	146 ±1	2.1 ±0.2	82.9 ±1.7	29.0 ±2.5	7.50 +0.03 −0.03	25.3 ±1.4	21.4 ±0.8	10.8 ±0.8	268 ±3	118 ±9	82 ±5
2	3	6	147 ±2	3.5 ±0.7	96.7 ±4.1	23.3 ±4.5	7.33 +0.06 −0.05	21.5 ±1.3	27.9 ±2.1	11.9 ±0.8	280 ±12	112 ±10	83 ±6
3	7	6	149 ±5	4.3 ±0.3	99.4 ±0.7	21.5 ±1.9	7.40 +0.04 −0.04	19.8 ±2.5	28.2 ±0.3	13.8 ±1.0	272 ±12	126 ±9	81 ±8
4	10	9	149§ ±4	4.2‖ ±0.3	101.8‖ ±1.9	19.5‖ ±2.0	7.38‖ +0.05 −0.05	18.9‖ ±0.8	27.7‖ ±0.7	13.1‖ ±0.7	264 ±10	117 ±6	77 ±5

* All values are given as the mean ± standard deviation. DOC = desoxycorticosterone gluconate.
† FFDS = fat-free dry solids.
‡ E_{Cl} = chloride space; E_{In} = inulin space; Na_i (K_i) = intracellular Na (K).
§ $0.05 \geqslant p > 0.01$, when comparing groups 4 and 1.
‖ $0.01 \geqslant p$, when comparing groups 4 and 1.

Tissue data are expressed per 100 g FFDS (fat-free dry solids). Observed pH values were converted to hydrogen ion concentration for calculation of mean and standard deviation, and then reconverted to pH units. Chloride and inulin spaces were calculated as the volumes of distribution of chloride and inulin. The mean of the two plasma inulin determinations in each individual animal was used in the calculation. Intracellular sodium and potassium contents were calculated only for muscle, using the volume of distribution of chloride as extracellular space. The concentrations of electrolytes in extracellular water were calculated from their respective plasma concentrations with a combined factor of 1.1 assumed for chloride and 1.0 for sodium and potassium, for the Gibbs-Donnan effect and the water content of plasma. For inulin in plasma water a factor of 1.06 was used.

To determine the probability of a difference between

TABLE III

Effects of potassium chloride administration on plasma, carcass, muscle, and skin

Group	K repletion Duration	No. rats	Plasma					Carcass (per 100 g FFDS†)					
			Na	K	Cl	Total CO_2	pH	Na	K	Cl	H_2O	E_{Cl}‡	E_{In}‡
	days		*mEq/L*	*mEq/L*	*mEq/L*	*mEq/L*		*mEq*	*mEq*	*mEq*	*g*	*g*	*g*
1	0	6	148 ±2	1.9 ±0.2	89.1 ±3.3	30.7 ±2.3	7.43 +0.02 −0.02	24.7 ±1.2	21.0 ±1.1	19.6 ±0.4	264 ±23	108 ±6	81 ±7
2	3	6	155 ±3	4.2 ±0.6	100.5 ±5.4	22.6 ±1.2	7.39 +0.03 −0.03	20.0 ±1.7	26.9 ±1.4	12.3 ±0.3	259 ±10	111 ±5	72 ±3
3	7	6	155 ±3	4.0 ±0.3	102.7 ±2.7	22.6 ±2.7	7.35 +0.06 −0.05	19.6 ±0.9	27.2 ±1.5	12.8 ±0.2	260 ±13	114 ±4	75 ±2
4	10	8	157§ ±2	4.2§ ±0.2	105.8§ ±3.9	21.3§ ±3.9	7.37‖ +0.05 −0.04	19.4§ ±0.9	27.3§ ±1.3	14.0§ ±0.8	271 ±6	120‖ ±9	83 ±2

* All values are given as the mean ± standard deviation.
† FFDS = fat-free dry solids.
‡ E_{Cl} = chloride space; E_{In} = inulin space; Na_i (K_i) = intracellular Na (K).
§ $0.01 \geqslant p$, when comparing groups 4 and 1.
‖ $0.05 \geqslant p > 0.01$, when comparing groups 4 and 1.

TABLE II

*electrolytes in rats depleted by potassium-deficient diet and DOC (experiment IA)**

Muscle (per 100 g FFDS)							Skin (per 100 g FFDS)			
Na	K	Cl	H_2O	E_{Cl}	Na_i‡	K_i‡	Na	K	Cl	H_2O
mEq	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*mEq*	*mEq*	*mEq*	*g*
23.0 ±4.5	28.4 ±2.2	5.3 ±1.1	339 ±6	58 ±8	14.5 ±4.0	28.3 ±2.2	21.7 ±1.3	9.0 ±0.8	17.9 ±1.3	213 ±16
15.0 ±2.6	37.3 ±3.2	5.5 ±0.3	315 ±15	52 ±5	7.4 ±2.4	37.2 ±3.2	21.7 ±1.2	11.4 ±0.8	18.1 ±1.8	216 ±21
11.3 ±2.3	40.8 ±3.1	6.4 ±0.6	317 ±7	58 ±6	2.5 ±2.5	40.5 ±3.4	20.8 ±3.3	11.4 ±0.9	18.2 ±1.8	213 ±16
12.4‖ ±2.1	44.1‖ ±2.0	6.6§ ±0.9	323‖ ±8	59 ±9	3.6‖ ±1.6	43.8‖ ±2.0	23.1 ±1.8	12.3‖ ±0.5	21.4§ ±3.4	227 ±15

means arising by chance the following ranking tests were used: for two samples the method of Wilcoxon (15), for more than two samples the method of Kruskal and Wallis (16).

Results

Mean values and standard deviations are presented in Tables II to V. In experiments IA and IB (Tables II and III) the significance of differences between potassium-depleted and fully repleted animals (groups 1 and 4) is indicated. In experiments II and III (Tables IV and V) statistical comparison is between effects of either potassium bicarbonate or chloride administration (in experiment II, groups 3 and 4; in experiment III, groups 2 and 3).

TABLE III

*electrolytes in rats depleted by potassium-deficient diet (experiment IB)**

Muscle (per 100 g FFDS)							Skin (per 100 g FFDS)			
Na	K	Cl	H_2O	E_{Cl}	Na_i‡	K_i‡	Na	K	Cl	H_2O
mEq	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*mEq*	*mEq*	*mEq*	*g*
23.0 ±1.9	31.7 ±1.7	7.1 ±1.0	322 ±1	72 ±10	12.4 ±1.5	31.6 ±1.7	21.8 ±2.3	8.6 ±0.7	17.4 ±1.6	212 ±15
12.7 ±1.8	42.7 ±2.4	7.1 ±0.4	324 ±1	64 ±4	2.7 ±1.8	42.5 ±2.4	18.6 ±3.4	9.5 ±1.5	17.7 ±3.8	188 ±30
11.4 ±1.1	44.8 ±1.5	7.8 ±0.1	331 ±1	70 ±2	0.6 ±1.1	44.6 ±1.6	21.4 ±2.2	10.4 ±1.2	22.1 ±2.0	218 ±18
10.8§ ±1.3	44.0§ ±1.1	7.7 ±1.0	332 ±1	67 ±9	0.4§ ±1.2	43.6§ ±1.1	22.2 ±2.4	10.5§ ±0.9	23.5§ ±2.2	221 ±22

TABLE IV

Comparison of effects of potassium bicarbonate and chloride administration on plasma,

Group	K repletion Duration (and nature)	No. rats	Plasma Na	Plasma K	Plasma Cl	Plasma Total CO_2	Plasma pH	Carcass (per 100 g FFDS†) Na	Carcass K	Carcass Cl	Carcass H_2O	Carcass E_{Cl}‡	Carcass E_{In}‡
	days		*mEq/L*	*mEq/L*	*mEq/L*	*mEq/L*		*mEq*	*mEq*	*mEq*	*g*	*g*	*g*
1	0	6	149 ±3	1.9 ±0.2	75.4 ±3.7	38.9 ±4.7	7.54 +0.04 −0.04	23.5 ±1.4	21.8 ±0.6	10.0 ±0.5	277 ±14	121 ±8	87 ±8
2	3 ($KHCO_3$)	4	150 ±7	2.9 ±0.4	82.4 ±3.5	32.5 ±3.4	7.47 +0.07 −0.06	19.8 ±1.2	23.6 ±1.1	10.2 ±0.6	257 ±11	111 ±4	75 ±6
3	11 ($KHCO_3$)	6	150 ±3	3.0 ±0.3	90.6 ±1.4	27.4 ±1.9	7.46 +0.04 −0.03	20.2 ±1.2	23.7 ±0.8	11.4 ±0.3	260 ±12	114 ±3	83 ±11
4	6 ($KHCO_3$) + 5 (KCl)	6	146§ ±1	4.0‖ ±0.4	101.8‖ ±3.9	19.5‖ ±2.4	7.35‖ +0.04 −0.04	19.1 ±1.0	27.1‖ ±1.8	13.8‖ ±0.8	274 ±13	123§ ±6	94 ±9

* All values are given as the mean ± standard deviation.
† FFDS = fat-free dry solids.
‡ E_{Cl} = chloride space; E_{In} = inulin space; Na_i (K_i) = intracellular Na (K).
§ $0.05 \geqslant p > 0.01$, when comparing groups 4 and 3.
‖ $0.01 \geqslant p$, when comparing groups 4 and 3.

Effects of potassium chloride administration (experiment I). In experiment IA depletion had been effected by potassium-deficient diet and DOC. Potassium-depleted animals (group 1) exhibited a marked hypokalemic, hypochloremic alkalosis (Table II). Administration of potassium chloride, 6 mmoles per kg body weight, resulted in correction of plasma electrolyte changes, noticeable after 3 and complete after 7 days. There was no significant difference [4] between results obtained from animals after 7 or after 10 days of potassium repletion. Comparison of group 1 with group 4 shows that the average reduction in carcass potassium (23% of normal content) was accompanied by a rise of equivalent magnitude in carcass sodium content. The carcass chloride deficit in the potassium-depleted animals was 2.3 mEq per 100 g FFDS. There was no significant difference between groups 1 and 4 either in carcass inulin or chloride space. Calculation of intracellular muscle data using the distribution of chloride as extracellular space showed an intracellular potassium deficit of 15.5 mEq per 100 g FFDS and an increase in sodium of 10.9 mEq per 100 g FFDS in group 1. Changes in muscle chloride content reflected changes in plasma chloride concentration. Potassium depletion had no effect on skin sodium content. Potassium content of skin in group 1 was lower than normal.

In experiment IB no DOC had been given during the depletion period. Comparison of experiment IB (Table III) with IA shows that the carcass potassium deficit was equal and approximated 23% of normal content in both experiments. In the potassium-deficient animals (group 1) of experiment IB plasma chloride was slightly higher and blood pH lower than in experiment IA. There was no difference in total CO_2 between the two groups. During potassium administration an unexplained elevation of plasma sodium occurred. In group 1 a carcass chloride deficit of 3.4 mEq per 100 g FFDS was found. Inulin spaces in groups 1 and 4 were of approximately equal magnitude as were the chloride spaces. Muscle analysis showed that the average reduction in intracellular potassium of 12.0 mEq per 100 g FFDS was accompanied by an equivalent rise in intracellular sodium.

Comparison of effects of potassium bicarbonate and chloride administration (experiment II). During the last 5 days of the depletion period the animals had been fed a diet low in both potassium and chloride. The carcass potassium deficit

[4] In the description of results a difference between two group means is said to be significant if the Wilcoxon test yields a value of p less than 0.01.

TABLE IV

*carcass, muscle, and skin electrolytes in potassium-depleted rats (experiment II)**

Muscle (per 100 g FFDS)							Skin (per 100 g FFDS)			
Na	K	Cl	H_2O	E_{Cl}	Na_i‡	K_i‡	Na	K	Cl	H_2O
mEq	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*mEq*	*mEq*	*mEq*	*g*
19.0 ±1.8	33.2 ±1.2	4.0 ±0.9	338 ±10	47 ±4	12.1 ±1.9	33.1 ±1.2	20.8 ±2.4	7.7 ±1.1	15.2 ±2.1	21.6 ±2.2
14.1 ±1.9	40.9 ±2.1	3.9 ±0.3	331 ±9	43 ±3	7.7 ±1.2	40.7 ±2.2	19.1 ±1.3	9.0 ±1.0	14.8 ±0.8	20.9 ±1.1
13.5 ±1.2	37.4 ±1.4	4.1 ±0.6	312 ±1	41 ±6	7.4 ±0.8	37.3 ±1.4	19.5 ±1.9	9.9 ±1.0	17.7 ±1.1	21.3 ±1.6
9.5‖ ±0.7	44.2‖ ±2.0	5.9‖ ±0.3	327‖ ±8	52§ ±4	2.0‖ ±1.1	44.0‖ ±2.0	20.4 ±1.6	9.2 ±0.7	20.4‖ ±1.1	21.4 ±1.3

was approximately 20% of normal. As shown in Table IV the potassium-deficient rats (group 1) were slightly more hypochloremic and alkalotic, but were otherwise not different from the same groups in experiment I. Likewise the potassium chloride-repleted rats (group 4) were similar to those of the same groups in experiment I and thus could be regarded as fully repleted animals. Administration of potassium bicarbonate, 6 mmoles per kg body weight daily for 3 days (group 2), resulted in only partial correction of plasma electrolyte changes and moderate retention of potassium. During administration of the same supplement for 11 days (group 3) a slight rise in plasma and tissue chloride and fall in plasma bicarbonate occurred. However, there was no further correction of the potassium deficit, which was still approximately 13% of normal content at the end of this period. Mean plasma potassium, chloride, and bicarbonate levels and blood pH of group 3 were intermediate between those of the potassium-depleted and the potassium chloride-repleted group. The same applies to the carcass potassium, sodium, and chloride, and also to the muscle potassium and sodium content. There was no significant difference between groups 3 and 4 either in carcass inulin or chloride space. The average difference in carcass chloride of 1.4 mEq per 100 g FFDS ($p < 0.01$) between groups 1 and 3 approximately equaled the total amount of chloride each animal of group 3 received with the diet during the potassium bicarbonate repletion period of 11 days. This indicates complete retention of dietary chloride during this period.

Comparison of effects of acute potassium bicarbonate and chloride administration after functional nephrectomy (experiment III). Potassium-deficient animals (group 1) exhibited a hypokalemic, hypochloremic alkalosis (Table V). Acute administration of potassium chloride, 6 mmoles per kg body weight, after functional nephrectomy 4 hours before sacrifice (group 2) resulted in partial correction of plasma and tissue electrolyte changes. The average increase in intracellular muscle potassium content of 6.5 mEq per 100 g FFDS approximately agrees with the rise expected if all of the administered potassium had entered the muscle cells. The mean reduction of intracellular muscle sodium was only 3.6 mEq per 100 g FFDS. The rise in plasma sodium of 8 mEq per L was sufficient to account for sodium expelled from muscle cells. The rise in plasma chloride of 16.2 mEq per L could be explained by assuming that all of the administered chloride remained in the extracellular space

TABLE V

*Comparison of effects of acute potassium bicarbonate and chloride administration to functionally nephrectomized potassium-deficient rats (experiment III)**

Group	Acute administration	No. rats	Plasma					Muscle (per 100 g FFDS†)							Skin (per 100 g FFDS)			
			Na	K	Cl	Total CO_2	pH	Na	K	Cl	H_2O	E_{Cl}‡	Na_i‡	K_i‡	Na	K	Cl	H_2O
	mmoles/kg		*mEq/L*	*mEq/L*	*mEq/L*	*mEq/L*		*mEq*	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*mEq*	*mEq*	*mEq*	*g*
1		9	147 ±3	2.0 ±0.3	84.5 ±3.9	27.7 ±2.2	7.51 +0.06 −0.06	20.3 ±2.5	30.7 ±2.5	4.6 ±0.3	322 ±8	49 ±4	13.0 ±2.8	30.6 ±2.5	19.0 ±1.0	10.0 ±0.8	16.9 ±1.3	213 ±8
2	KCl	10	155 ±2	2.8 ±0.4	100.7 ±3.3	22.4 ±1.8	7.41 +0.10 −0.08	17.4 ±2.0	37.2 ±2.0	5.7 ±0.4	323 ±6	51 ±4	9.4 ±2.1	37.1 ±2.1	20.3 ±1.0	9.4 ±0.7	19.8 ±1.8	219 ±9
3	$KHCO_3$	10	154 ±4	2.5§ ±0.2	83.8‖ ±2.5	33.8‖ ±1.9	7.55‖ +0.06 −0.05	18.3 ±1.7	36.7 ±2.5	4.8‖ ±0.3	328 ±6	52 ±4	10.2 ±1.5	36.6 ±2.5	19.4 ±1.8	9.3 ±1.0	16.2‖ ±1.6	209 ±16

* All values are given as the mean ± standard deviation.
† FFDS = fat-free dry solids.
‡ E_{Cl} = chloride space; Na_i (K_i) = intracellular Na (K).
§ $0.05 \geqslant p > 0.01$, when comparing groups 3 and 2.
‖ $0.01 \geqslant p$, when comparing groups 3 and 2.

and that this space did not increase.[5] There was an average fall of 5.3 mEq per L in total CO_2.

Administration of the equivalent amount of potassium bicarbonate to potassium-deficient rats resulted in a rise in plasma sodium (7 mEq per L), an increase in intracellular muscle potassium (6.0 mEq per 100 g FFDS), and a decrease in muscle sodium (2.8 mg per 100 g FFDS) not statistically different from those observed after potassium chloride administration.

There was no change in plasma chloride content. The rise in plasma total CO_2 (6.1 mEq per L) was much smaller than expected if it was assumed that all of the administered dose remained in the extracellular space.

Discussion

Potassium-deficient rats in the present experiments exhibited a marked hypochloremic alkalosis. The average carcass potassium deficit (experiments I and II) ranged from 20 to 23% of the normal content.

Changes in potassium and sodium contents of whole body during potassium repletion were compared with those of skeletal muscle, and changes in muscle-free carcass were calculated. On the basis of published data on rat composition (17) it was assumed that 50% of the fat-free whole body weight consisted of skeletal muscle.[6] Collective results are shown in Figures 1 and 2. The mean potassium and sodium contents of the muscle-free carcass were relatively constant in all groups. As can be seen in Tables II to IV, changes in skin electrolytes were not significant and closely followed changes in extracellular fluid concentration. The results suggest that changes in both potassium and sodium contents of the whole body accurately reflect those of muscle. With regard to the potassium data this agrees with observations made by Schwartz, Cohen, and Wallace (18) on young potassium-deficient rats.

[5] In another experiment in three groups of rats exactly similar to those described in experiment III the following values for the inulin space were obtained (g per 100 g FFDS, mean ± SD): group 1 (4 rats), 83 ± 4; group 2 (6 rats), 83 ± 5; group 3 (4 rats), 81 ± 4.

[6] Individual values for all animals were calculated as potassium and sodium contents of 1 kg fat-free wet carcass minus those of 0.5 kg fat-free wet muscle.

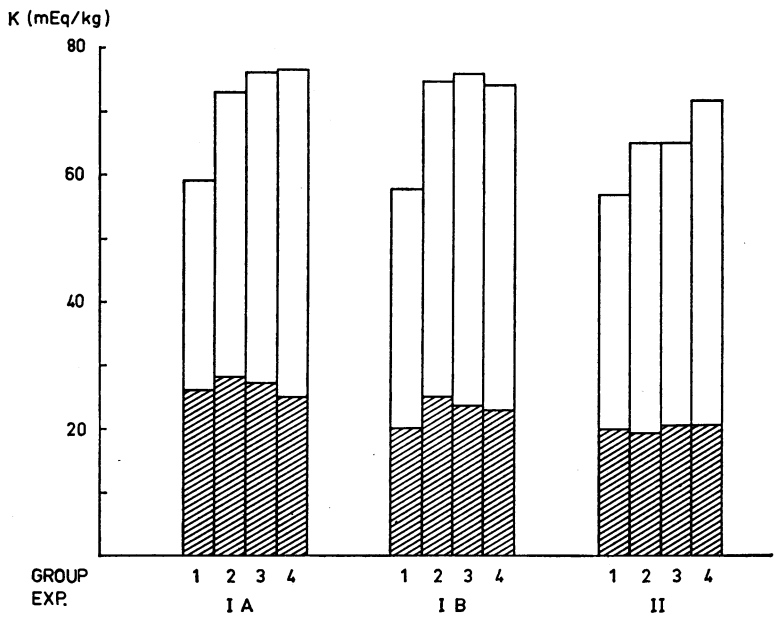

FIG. 1. POTASSIUM CONTENT OF WHOLE BODY COMPARED WITH THAT OF MUSCLE-FREE CARCASS DURING POTASSIUM REPLETION. Total columns = mean potassium content of total carcass; hatched columns = calculated mean potassium content of total carcass minus that of muscle (milliequivalents per kilogram fat-free wet tissue). In experiments IA and IB groups 1 are potassium-deficient rats and groups 2 to 4 animals during potassium chloride administration. In experiment II group 1 consists of potassium-deficient rats, groups 2 and 3 are animals during potassium bicarbonate administration, and group 4 consists of animals during potassium chloride administration. Differences in muscle-free carcass potassium content among groups are not significant. For all animals of experiment IA: $0.30 > p > 0.20$; experiment IB: $0.20 > p > 0.10$; experiment II: $0.50 > p > 0.30$ (test of Kruskal and Wallis).

Hypochloremia in potassium deficiency could be an expression of chloride deficiency, or the result of expansion of extracellular fluid volume (18), or a combination of these two (8). The value of the volume of distribution of chloride as a measure of extracellular fluid volume is questionable in a period when considerable changes in acid-base and in chloride balance take place. Chloride spaces were therefore compared with inulin spaces in experiments I and II. The inulin method is relatively insensitive. However, it was reasonable to expect that, if the observed changes in plasma chloride concentration of more than 15% resulted from changes in inulin space, these would be sufficiently large to be measured by this method. As shown in Tables II to IV there were only minor differences in chloride and in inulin spaces before and after potassium repletion, suggesting that during potassium deficiency extracellular space was not increased. This agrees with observations by Eckel, Botschner, and Wood (6) on the raffinose spaces of normal and potassium-deficient rats.

Data have been reported that show the whole body chloride content obtained by carcass analysis of potassium-deficient rats to be increased (18) or diminished (19). A high carcass chloride value can be found when potassium deficiency is associated with starvation, when the status of total body chloride does not correlate with the plasma chloride concentration (20). In the present experiments no weight loss occurred during the period of potassium depletion. The groups of potassium-deficient rats in experiments IA and IB showed a chloride deficit of approximately 2.8 mEq per 100 g FFDS (2.3 and 3.4 mEq per

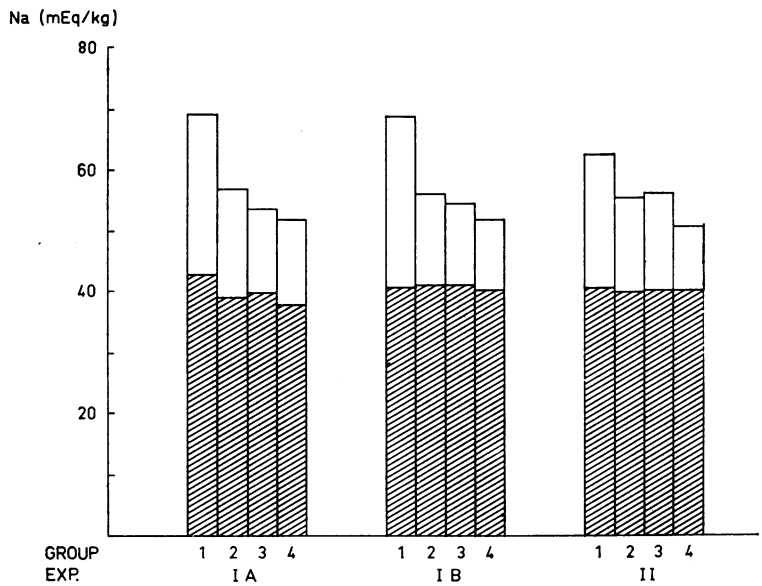

FIG. 2. SODIUM CONTENT OF WHOLE BODY COMPARED WITH THAT OF MUSCLE-FREE CARCASS DURING POTASSIUM REPLETION. Total columns = mean sodium content of total carcass; hatched columns = calculated mean sodium content of total carcass minus that of muscle (milliequivalents per kilogram fat-free wet tissue). In experiments IA and IB groups 1 are potassium-deficient rats and groups 2 to 4 animals during potassium chloride administration. In experiment II group 1 consists of potassium-deficient rats, groups 2 and 3 are animals during potassium bicarbonate administration, and group 4 consists of animals during potassium chloride administration. Differences in muscle-free carcass sodium content among groups are not significant. For all animals of experiment IA: $0.50 > p > 0.30$; experiment IB: $0.80 > p > 0.70$; experiment II: $0.70 > p > 0.50$ (test of Kruskal and Wallis).

100 g FFDS, respectively), corresponding to 7.6 mEq per kg fat-free wet weight. As the present diet contained 13 mEq of chloride per kg body weight daily and diarrhea was absent, chloride deficiency must have been the result of urinary loss of chloride. It is of interest that a chloride deficit of similar magnitude occurred both when potassium deficiency was caused by dietary restriction of potassium and the administration of DOC (experiment IA), and when only a potassium-deficient diet was given (experiment IB).

Schwartz and co-workers (9, 21) have shown in dogs that neither plasma electrolyte changes due to potassium deficiency nor the potassium deficiency itself can be fully corrected by the administration of a potassium salt other than chloride. The daily intraperitoneal administration to potassium-deficient rats of potassium bicarbonate, 6 mmoles per kg body weight, during periods ranging from 3 to 11 days (Table IV), failed to fully correct either the potassium deficit or the hypochloremic alkalosis. Slow correction of alkalosis occurred, but this was associated with a significant increase in mean carcass chloride content of 1.4 mEq per 100 g FFDS, corresponding to 4.9 mEq per kg fat-free wet weight. The increase closely corresponds to the total amount of chloride provided in the diet during the entire 11-day period of potassium bicarbonate administration (5 mEq per kg body weight). In all experiments chloride deficiency had arisen during the period of potassium depletion in spite of a chloride administration of 13 mmoles per kg body weight daily. The rise in carcass chloride content in a period when a low chloride diet and a potassium bicarbonate supplement were given confirms the suggestion by Cooke and co-workers

(8) that administration of potassium in itself increases chloride reabsorption.

The results show that in potassium-deficient rats complete correction of electrolyte changes and potassium deficiency does not occur so long as no chloride is provided, as suggested by Cheek and West (19). In this respect rats resemble dogs (9) and patients with primary hyperaldosteronism (10). The results reported here are not in agreement with those of Cooke and co-workers (8), who demonstrated complete correction of both abnormal plasma composition and potassium deficiency in rats after the administration of a daily supplement of potassium bicarbonate, 6 mmoles per kg body weight for 3 days. In the absence of analytical data it might tentatively be suggested that the intrinsic chloride content of the diet used in those experiments exceeded that in the present studies. The period of 4 days on low salt diet that preceded the repletion period in the experiments of Cooke and associates might further explain some of the differences in results between the two studies. It should further be mentioned that the administration of sodium bicarbonate to potassium-deficient rats was shown to result in chloride depletion (8). In the present studies in experiment II during the last 5 days of the depletion period the daily sodium chloride supplement of 6 mmoles per kg body weight was changed into sodium bicarbonate. In spite of this the carcass chloride content of the potassium-deficient group in this experiment was not markedly different from that in the other experiments, probably because chloride was merely replaced with bicarbonate while sodium intake was kept constant.

Darrow, Schwartz, Iannucci, and Coville (22) demonstrated that the sum of intracellular potassium and sodium contents of potassium-deficient skeletal muscle was less than that of normal muscle. Cooke and associates (1) suggested that the resulting cation deficit was compensated by net movement of hydrogen ion from the extracellular compartment to cells, causing extracellular alkalosis and intracellular acidosis. Disappearance of plasma abnormalities on the acute administration of potassium chloride to nephrectomized potassium-deficient rats (2) and the finding of intracellular acidosis in earlier investigations (3–5) seemed to support this hypothesis. Recent studies, however, failed to demonstrate intracellular acidosis in potassium-deficient muscle (7) and showed that anions and cations other than potassium and sodium play a role in cellular pH regulation (6). In the present studies the sum of intracellular potassium and sodium of potassium-deficient muscle was smaller than that of normal muscle only in experiment IA. In experiments IB and II no cation deficit during potassium deficiency was found. The hydrogen ion balance using whole body "balance" data of fixed cations and anions was calculated. After potassium repletion the excess of chloride retained over the sum of potassium and sodium retained was 2.4, 2.4, 2.8, and 2.9 mEq per 100 g FFDS in experiments IA, IB, II ($KHCO_3$), and II (KCl), respectively. If it is assumed that this excess is an indication of hydrogen ions retained, these results would suggest that a positive hydrogen ion balance contributed to the fall in plasma CO_2 content during potassium repletion. A similar observation was made during potassium repletion of patients with primary hyperaldosteronism (10).

In experiment III the acute administration of potassium chloride after functional nephrectomy resulted in decrease of plasma total CO_2, as demonstrated earlier by Orloff, Kennedy, and Berliner (2). On the administration of potassium bicarbonate the rise in plasma total CO_2 was much smaller than expected, if it was assumed that all of the administered dose remained in the extracellular space. Both these observations could be the result of titration of extracellular bicarbonate by hydrogen ions originating from the intracellular compartment, or conversely by a shift of bicarbonate or hydroxyl ions into muscle cells. Our data do not allow a definite conclusion in this respect. It has been demonstrated (23) that the administration of potassium chloride or rubidium chloride to nephrectomized normal rats gives rise to intracellular alkalosis and extracellular acidosis. It is obvious that decrease of plasma total CO_2 as a result of acute potassium repletion therefore does not necessarily constitute evidence of intracellular acidosis before the administration of the potassium salt.

The impossibility of fully correcting the potassium deficit without the administration of chloride could be due to a direct effect of a low plasma

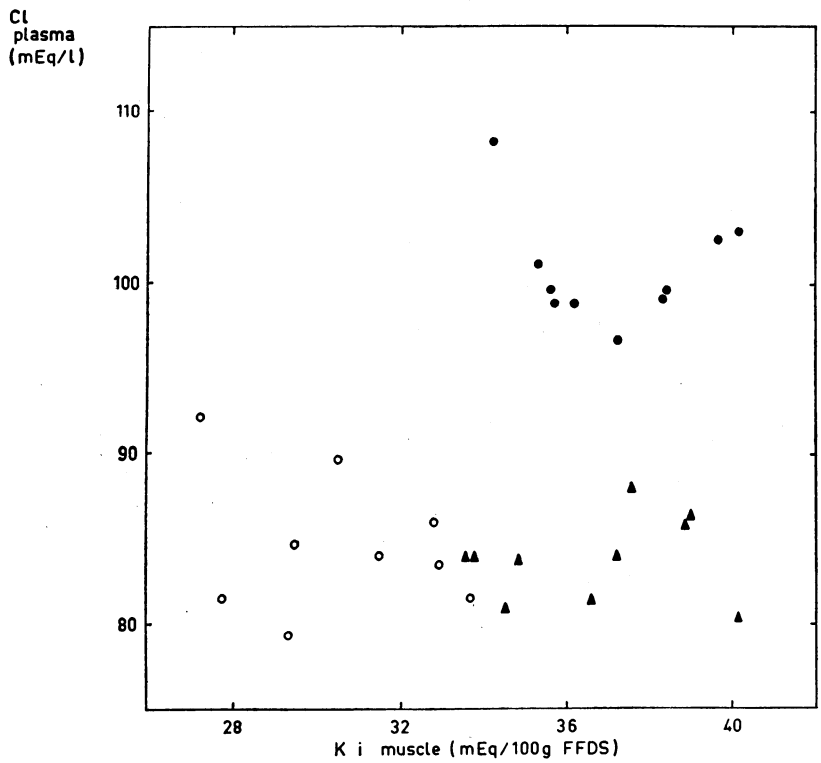

FIG. 3. ACUTE LOADING OF POTASSIUM CHLORIDE OR POTASSIUM BICARBONATE TO FUNCTIONALLY NEPHRECTOMIZED POTASSIUM-DEFICIENT RATS. Relation between intracellular potassium content of muscle (mEq per 100 g FFDS) and plasma chloride concentration (mEq per L) in experiment III. The increase of intracellular potassium content is independent of plasma chloride concentration. ● = KCl group; ▲ = $KHCO_3$ group; ○ = potassium-deficient control group.

chloride concentration on the influx of potassium ions into muscle cells. On acute loading of either potassium chloride or of potassium bicarbonate after functional nephrectomy, all of the administered potassium entered muscle cells within 4 hours (Table V). Approximately 50% of this potassium was exchanged for intracellular sodium. There was no significant difference between the potassium chloride and the potassium bicarbonate group either in cellular potassium gain (Figure 3) or in sodium loss. This would indicate that on acute administration of potassium to nephrectomized animals the influx of potassium into muscle cells is not influenced by the plasma chloride concentration.

The data presented show that potassium deficiency in rats is accompanied by chloride deficiency and that adequate chloride is necessary for the correction of extracellular alkalosis and for the optimal renal retention of administered potassium. The demonstration that on acute loading of potassium to functionally nephrectomized rats the penetration of potassium into muscle cells is not influenced by the plasma chloride concentration suggests that the effect of chloride deficiency is via altered renal function rather than through a direct effect on the muscle cell. The observations are consistent with the suggestion of Schwartz and co-workers that in chloride deficiency the disproportion between sodium reabsorbed and the availability of penetrating anion causes a rise in transtubular potential, increasing the fraction reabsorbed through exchange with potassium and hydrogen ions.

The cause of chloride deficiency in potassium deficiency is not known. Decrease of renal tubular reabsorption of chloride in potassium deficiency has been demonstrated (24, 25). Three possible mechanisms can be suggested. Decreased reabsorption of chloride could be secondary to a rise of bicarbonate threshold (26). A decreased permeability of the mucosal membrane to chloride

ions might result from the low internal potassium content of renal tubular cells. A remote possibility would be a decrease in active transport of chloride. The present data allow no conclusion whether decrease in chloride reabsorption or whether increase in bicarbonate threshold is the primary factor.

Summary

Rats were made potassium deficient and alkalotic by dietary restriction of potassium and, in part of the experiments, by the intraperitoneal administration of desoxycorticosterone gluconate. The changes in whole body inulin space and in electrolytes of plasma, whole body, muscle, and skin were studied during chronic or during acute administration of potassium chloride or potassium bicarbonate.

Potassium-deficient animals exhibited a potassium deficit of approximately 20% of normal carcass content. Changes in potassium and sodium as a result of potassium deficiency were nearly wholly confined to muscle. Changes in skin were of minor importance.

In the potassium-deficient animals a chloride deficit of approximately 2.8 mEq per 100 g fat-free dry solids was found, corresponding to 7.6 mEq per kg fat-free wet weight. There was no significant difference either in inulin spaces or in chloride spaces between potassium-deficient and completely repleted animals. It was not possible to fully correct either hypochloremic alkalosis or potassium deficiency without the administration of chloride. Administration of potassium as chloride or as bicarbonate increased renal chloride reabsorption.

On acute administration of a potassium salt to functionally nephrectomized rats the penetration of potassium into muscle cells was not influenced by the plasma chloride concentration.

The results demonstrate the critical role of chloride in the recovery and probably in the pathogenesis of extracellular alkalosis in the potassium-deficient rat.

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