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Research Article

ELECTROLYTE AND ACID–BASE BALANCE DURING ACUTE LOADING WITH RUBIDIUM CHLORIDE *†

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Previous studies have shown that administration of rubidium to normal or potassium-depleted rats results in *a*) metabolic acidosis (1), and *b*) accumulation of rubidium in muscle tissue where it attains final concentrations considerably higher than that of potassium (2). Since it was found that rubidium was at least as effective as potassium in lowering the plasma CO_2 content of potassium-deficient nephrectomized animals (1), it was suggested that administered rubidium, like potassium, probably exchanges with intracellular hydrogen.[1]

In view of these facts it was deemed of interest to obtain information about the simultaneous renal and tissue exchanges of electrolytes during loading with rubidium. Short term balance studies were therefore carried out on rats given 40 mEq. per Kg. of RbCl over two days, and the results compared with those in control animals given an equivalent amount of KCl.

The data to be reported show that acute loading resulted in the rapid accumulation of very large amounts of rubidium in tissues with the simultaneous displacement of an almost equal amount of intracellular cation. Approximately half of the estimated cellular content of potassium was excreted in the urine together with a smaller quantity of sodium. A severe metabolic acidosis developed which was unaccompanied by compensatory increase in the urinary excretion of acid, thus demonstrating a renal defect which perpetuates the acidosis.

METHODS

Male albino rats of the Sprague-Dawley strain, weighing approximately 300 Gm., were used in all experiments. Four day balance studies were carried out, a two day control period on a standard liquid diet being followed by a two day period during which RbCl or KCl was added to the food. The composition of the diet fed during the control period is shown in Table I, its caloric value being approximately 2.5 calories per ml. The diet was administered by means of a stomach tube in four equal feedings per day; 87 ml. of diet per Kg. of weight was given, this being the largest quantity which could be administered without producing severe diarrhea. After 48 hours on the control diet the rats were divided into two groups. Group I, which consisted of 10 rats, was given a supplement of KCl, 20 mEq. per Kg. per day for the next two days, in addition to the standard control diet. Group II, consisting of 11 rats, received the control diet plus 20 mEq. per Kg. per day of RbCl to which tracer quantities of Rb^{86} had been added. Thus the total load of rubidium or potassium for the two days of loading was 40 mEq. per Kg., an amount approximating the total quantity of potassium in the body. Tap water *ad libitum* was provided for drinking purposes throughout the experiment.

To facilitate complete separation of urine and feces the animals were immobilized in light plaster casts. A modification of the cast described by Lathrop and Harper (4) was used, the chamber for the collection of expired gases being omitted from the design. The animals in

TABLE I

Composition of liquid diet

Glucose	344 Gm./L.
Mazola Oil®	105 Gm./L.
Casein	50 Gm./L.
Sodium	27 mEq./L.
Potassium	10 mEq./L.
Chloride	28 mEq./L.

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[1] As recently pointed out by Eckel, Norris and Pope (3), the administered potassium may restore extracellular CO_2 content to normal by the displacement of intracellular cationic amino acids which accumulate in potassium-depleted tissues.

TABLE II

*Plasma composition**

Group	Na	K	Rb	Rb + K	CO_2	Cl
	mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mMoles/L.*	*mEq./L.*
I (K)	144 ± 2.11	3.9 ± 0.58			23.9 ± 1.33	104 ± 3.46
II (Rb)	153 ± 6.41	2.6 ± 0.84	1.3 ± 0.24	3.9 ± 1.07	13.2 ± 4.83	114 ± 3.90

* Mean values ± S. D.

their casts were strapped onto a frame designed to permit the hind quarters of the rat to project over the edge. Urine was collected under mineral oil in a glass cylinder held in position beneath the penis, phenyl mercuric nitrite and toluene being used as a preservative. Stools were collected in a beaker suspended under the anus.

On the morning of the fifth day—about 16 hours after the last feeding—the animals were anesthetized with the intraperitoneal injection of a 2 per cent solution of Amytal Sodium® (approximately 0.75 ml. per 100 Gm. weight) and then exsanguinated by withdrawal of blood from the abdominal aorta into a heparinized syringe.

Total CO_2 content of the plasma and urine was estimated by standard manometric technique, and chloride in stool and diet was determined by a modified Volhard titration. Urine pH was determined at room temperature with a glass electrode, urine ammonia by the permutit method of Folin, and total titratable acid by titration to pH 7.4 with standard alkali. Sodium analyses were carried out with a flame photometer, as were analyses for potassium in those specimens in which no rubidium was present. For samples containing mixtures of rubidium and potassium a special analytical technique was devised based on the combined use of tracer Rb^{86} and the chloroplatinate titration. This is described elsewhere (2).

RESULTS

During the course of the experiment the potassium-loaded animals (Group I) lost, on the average, 43.6 ± 6.7 Gm. weight; the rubidium-loaded rats (Group II) had a mean weight loss of 50.8 ± 8.2 Gm., a value which is not significantly different.[2] The only obvious evidence of toxicity was the development of signs of increased irritability in most of the rubidium-treated animals.

The data on plasma composition at the end of the experiment are summarized in Table II. It will be seen that the rubidium animals had a striking metabolic acidosis, the mean value for the plasma CO_2 content in this group being 13.2 ± 4.83 mMoles per L. This was significantly lower than the mean CO_2 content of the rats loaded with potassium (23.9 ± 1.33 mMoles per L.). Plasma potassium in the potassium-loaded animals was 3.9 ± 0.58 mEq. per L. In the rubidium-loaded group the potassium concentration was 2.6 ± 0.84 mEq. per L., while the rubidium concentration was 1.3 ± 0.24 mEq. per L. Mean sodium and chloride concentrations in the rubidium-loaded group were slightly above normal and significantly higher than in the potassium group.

The external balance data for sodium, potas-

[2] Differences described as "significant" had a "p" value less than 0.01, unless stated otherwise.

TABLE III

*External balance data** †

Group	Na		K		Rb	Cl	
	Cumulative balance during control (days 1 and 2)	Cumulative balance during loading (days 3 and 4)	Cumulative balance during control (days 1 and 2)	Cumulative balance during loading (days 3 and 4)	Cumulative balance during loading (days 3 and 4)	Cumulative balance during control (days 1 and 2)	Cumulative balance during loading (days 3 and 4)
	mEq./Kg.		*mEq./Kg.*		*mEq./Kg.*	*mEq./Kg.*	
I (K)	+0.6 ± 1.21	−1.7 ± 0.37	−5.4 ± 0.77	− 0.7 ± 6.42		+1.8 ± 1.11	+5.6 ± 5.41
II (Rb)	+0.9 ± 3.19	−4.8 ± 0.93	−5.8 ± 1.72	−27.9 ± 4.75	+29.3 ± 2.29	+2.1 ± 1.88	+4.0 ± 5.39

* Do not include stool data (see text).
† Mean values ± S. D.

sium, rubidium and chloride are set out in Table III expressed, for purposes of comparison, as mEq. per Kg. of initial body weight.

It should be noted here that stool electrolytes were not included in the calculation of the balance data. However, complete electrolyte analyses were carried out on stools obtained from four randomly selected animals in Group I and from six in Group II, during control and loading periods. Electrolyte content was found to be negligible and stool analyses were therefore omitted in the other animals. For each electrolyte, where appropriate, two sets of mean data are presented. In the first column are shown the figures for the cumulative balance at the end of the second control day immediately prior to loading. The second column shows the cumulative balance for the two days of loading (Days 3 and 4). During the two day control period no significant differences between the groups were observed. Sodium and chloride balances were not significantly different from zero in either group, and in both groups there was a small negative balance of potassium (Group I: 5.4 ± 0.77 mEq. per Kg.; Group II: 5.8 ± 1.72 mEq. per Kg.).

Over the two day loading period the animals given KCl were virtually in potassium balance, the cumulative value being -0.7 ± 6.42 mEq. per Kg. However, if it is assumed that the average rate of potassium loss during the first two days (probably associated with the weight loss and tissue breakdown caused by immobilization in a cast) would have continued during Days 3 and 4 in the absence of loading, then the observed figure for the loading period may represent a slight retention of potassium, approximately 10 per cent of the load. There was a small negative balance of sodium during the period of potassium-loading (-1.7 ± 0.37 mEq. per Kg.), but a positive balance of chloride ($+5.6 \pm 5.41$ mEq. per Kg.).

The rubidium-loaded rats in Group II retained an average of 29.3 ± 2.29 mEq. per Kg. of rubidium during Days 3 and 4, this amount being approximately 70 per cent of the administered load. This retention of rubidium was accompanied by a markedly negative potassium balance (-27.9 ± 4.75 mEq. per Kg.). Correction of this figure for the negative potassium balance during the control period would yield a net change in potassium of approximately -22 mEq. per Kg. during the period of rubidium loading.

There was a moderate negative balance of sodium during the two days of rubidium loading (-4.8 ± 0.93 mEq. per Kg.), this figure being significantly different from both the control balance during Days 1 and 2 and the balance during loading with KCl. There was no accompanying loss of chloride. Chloride balance during Days 3 and 4 was slightly positive ($+4.0 \pm 5.39$) but not significantly different from the balance during the control period or from the balance during loading with KCl.

Table IV summarizes the data on urinary excretion of ammonium, titratable acid and total CO_2. In the control periods there was no difference between the two groups with respect to ammonium, titratable acid or total CO_2 in the urine. Urine pH was virtually identical in both groups (range, 5.8 to 6.8). Net excretion of acid (roughly estimated as ammonium plus titratable acid minus CO_2 content) averaged about 6 to 7 mEq. per Kg per day.

During loading with KCl there were no significant changes in ammonium, titratable acid or CO_2

TABLE IV

*Urinary excretion of acid and base**

Group	Ammonium		Titratable acid		CO_2	
	Control period (days 1 and 2)	Loading period (days 3 and 4)				
	mEq./Kg./day		*mEq./Kg./day*		*mMoles/Kg./day*	
I (K)	4.6 ± 0.93	4.7 ± 1.28	1.7 ± 0.48	1.2 ± 0.50	0.4 ± 0.32	0.7 ± 0.41
II (Rb)	5.0 ± 1.32	3.5 ± 1.31	1.8 ± 0.63	1.3 ± 0.35	0.3 ± 0.33	0.2 ± 0.24

* Mean values ± S. D.

excretion and no appreciable change in urine pH. In the Group II animals which were loaded with RbCl there were no significant changes in the excretion of CO_2 or titratable acid when compared to the first two days or to the KCl loading period. On the other hand, ammonium excretion showed a small but significant reduction during the period of rubidium loading, when compared with the excretion during the control. The "p" value for the difference between ammonium excretion during loading with KCl and that during loading with RbCl was between 0.02 and 0.05. Urine pH did not change significantly during the loading period.

DISCUSSION

These experiments clearly demonstrate the remarkable avidity of the tissues of normal animals for administered rubidium and the speed with which a large proportion of body potassium can be replaced by this ion. Slightly more than 70 per cent of the rubidium load was retained (29.3 mEq. per Kg.). Since the final extracellular rubidium concentration was quite low (1.3 mEq. per L.), it can be reasonably assumed that most of the retained rubidium entered cells. On the basis of the simultaneous negative potassium balance of approximately half of total body potassium (22.1 mEq. per Kg., corrected for control), it would appear that the accumulated rubidium largely exchanged for intracellular potassium. Muscle tissue analyses in three randomly selected animals in Group II confirmed these exchanges in revealing approximately equal quantities of intracellular rubidium and potassium.

The finding of a low plasma concentration of potassium at the end of the period of rubidium loading (Table II) might suggest that the large kaluresis was the result of a primary increase in renal clearance of potassium with secondary tissue loss consequent to the hypokalemia. However, acute experiments in dogs given infusions of RbCl do not support this concept. These latter experiments revealed immediate rises in plasma potassium as rubidium entered cells (5). Increased renal excretion of potassium was shown to be the result of the hyperkalemia, there being no evidence for any direct effect of rubidium on renal tubular handling of potassium (6). Thus it would appear likely that in the present experiments transient elevations of plasma potassium were responsible for the kaluresis but had been dissipated in the 16 hours elapsing between the last load of rubidium and the time of blood sampling. The low plasma levels observed at this time presumably reflected the severe state of intracellular depletion which existed at the end of the study.

The large cellular uptake of rubidium was accompanied not only by potassium excretion but also by a diuresis of sodium which. although considerably smaller than that of potassium, was significantly larger than the sodium excretion of the Group I animals during the period of KCl loading. After correction for the sodium balance of the control period, there appeared to be a sodium loss of about 5.7 mEq. per Kg. during the rubidium-loading period. Unaccompanied as it was by any demonstrable loss of chloride, this diuresis of sodium probably represented additional intracellular cation displaced by the rubidium. As such, it could in part explain the observed discrepancy of approximately 7 mEq. per Kg. between rubidium uptake and corrected potassium loss. Additional support for this interpretation of the significance of the increased urinary sodium is derived from the finding of reduced intracellular sodium in the muscles of rubidium-loaded animals (2).

The powerful acidifying effect of rubidium was well demonstrated in the present experiments, the final plasma CO_2 content (13.2 ± 4.83 mMoles per L.) being significantly lower than that found in the potassium-loaded animals (23.9 ± 1.33 mMoles per L.). It has previously been shown in nephrectomized, potassium-deficient alkalotic rats that rubidium causes acidosis by the displacement of intracellular hydrogen (1).[3] Assuming constant endogenous acid production and a maximum value for extracellular volume of 300 ml. per Kg. body weight, transfer of only 3 mEq. per Kg. of hydrogen in the present experiments would have been required to produce the entire difference of 10 mEq. per L. in CO_2 content between the two groups. Together with the cell exchange of sodium discussed above, this relatively small displacement of hydrogen could approximately account for the entire discrepancy between the observed balance of rubidium and the corrected

[3] See footnote 1.

balance of potassium. However, the errors inherent in the balance measurements as well as in the correction of the potassium data are probably too large to permit accurate assessment of such small differences, and it is evident that these studies do not provide any definitive demonstration of cellular shifts of acid or their role in the pathogenesis of the acidosis.

On the other hand, these observations do permit certain conclusions about the role of the kidneys. It is clear from the unchanged urine CO_2 content in both groups during the loading period that there was no significant increase in bicarbonate excretion. Thus, renal loss of alkali played no role in the genesis of the acidosis in the rubidium-loaded animals. However, there appeared to be a slight reduction in excretion of acid due entirely to a small but consistent and significant reduction in ammonium excretion, as compared to the control period. If the endogenous production of acid were constant, this reduction in excretion of ammonium would represent the retention of a total of about 3 mEq. per Kg. of acid for the two day period of loading. To this extent, therefore, the kidneys might have contributed to the development of the acidosis.

Although the significance of the renal contribution to the pathogenesis of the extracellular acidosis in these experiments is somewhat uncertain, the data are unequivocal in demonstrating a failure of the kidneys to compensate for the acidosis. It is well known that rats are ordinarily capable of immediate and large increments in acid excretion (mainly ammonium) when challenged by an acidosis (7–9). It is apparent, therefore, that the kidneys of the Group II rats did not respond in the usual way to the extracellular acidosis which followed the rubidium load. Since renal excretion of acid constitutes the only mechanism by which animals normally regenerate their plasma bicarbonate after it has been lowered by acidosis, this failure of renal response, if maintained, would tend to perpetuate the acidosis.

To explain the absence of any renal response in the acidotic rubidium-loaded rats, it might at first seem reasonable to implicate the very rapid excretion of potassium which resulted from the rubidium load. There is good evidence in man and dog that loading with potassium inhibits secretion of acid (10), but the absence of any change in acid excretion in the potassium-loaded animals of this study, as well as other data in the literature (7, 9), suggest that this phenomenon is not readily demonstrable in the rat. In any event, recent studies have shown that possible hydrogen-potassium competition cannot explain the behavior of rubidium-substituted rats, because there is no increased acid excretion even when urinary potassium and rubidium are very low (11). Further work will be required to clarify this question.

SUMMARY AND CONCLUSIONS

Electrolyte and acid-base balance studies were carried out on normal rats fed 40 mEq. per Kg. of RbCl over a period of two days and the results compared with those resulting from an equivalent load of KCl.

Loading with KCl resulted in little or no change in electrolyte balance or plasma CO_2 content. By contrast, loading with RbCl resulted in cellular accumulation of approximately 29 mEq. per Kg. of rubidium and simultaneous renal loss of approximately 28 mEq. per Kg. of potassium and 5 mEq. per Kg. of sodium. When corrected for the negative balance during the control period, net potassium loss during rubidium loading amounted to approximately 22 mEq. per Kg. These renal losses seemed largely to represent the displacement of intracellular cation by rubidium.

The administration of rubidium also produced a severe extracellular metabolic acidosis which was not accompanied by increased urinary excretion of acid. There was, instead, a slight reduction in ammonium excretion. Thus it would appear that defective renal compensation plays an important role in the maintenance of acidosis in rubidium-substituted rats.

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REFERENCES

1. Relman, A. S., Roy, A. M., and Schwartz, W. B. The acidifying effect of rubidium in normal and potassium-deficient alkalotic rats. J. clin. Invest. 1955, **34**, 538.

2. Relman, A. S., Lambie, A. T., Burrows, B. A., and Roy, A. M. Cation accumulation by muscle tissue: The displacement of potassium by rubidium and cesium in the living animal. J. clin. Invest. 1957, **36**, 1249.
3. Eckel, R. E., Norris, J. E. C., and Pope, C. E., II. Basic amino acids as intracellular cations in K deficiency. Amer. J. Physiol. 1958, **193**, 644.
4. Lathrop, K. A. and Harper, P. V., Jr. Long-term recovery of metabolic products from rats. Science 1954, **119**, 447.
5. Kunin, A. S., Dearborn, E. H., and Relman, A. S. Effect of infusion of rubidium chloride on plasma electrolytes and the electrocardiogram of the dog. Amer. J. Physiol. In press.
6. Kunin, A. S., Roy, A. M., Dearborn, E. H., Burrows, B. A., and Relman, A. S. A comparison of the renal transport of rubidium and potassium. Amer. J. Physiol. In press.
7. Leonard, E., and Orloff, J. Regulation of ammonia excretion in the rat. Amer. J. Physiol. 1955, **182**, 131.
8. Rector, F. C., Jr., Seldin, D. W., and Copenhaver, J. H. The mechanism of ammonia excretion during ammonium chloride acidosis. J. clin. Invest. 1955, **34**, 20.
9. Muntwyler, E., Iacobellis, M., and Griffin, G. E. Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. Amer. J. Physiol. 1956, **184**, 83.
10. Berliner, R. W., Kennedy, T. J., Jr., and Orloff, J. Relationship between acidification of the urine and potassium metabolism. Effect of carbonic anhydrase inhibition and potassium excretion. Amer. J. Med. 1951, **11**, 274.
11. Hall, P. W., III, and Relman, A. S. Acid excretion in rubidium- and cesium-substituted rats (abstract). Clin. Res. 1959, **7**, 30.