Association of Alkali Metal and Alkaline Earth Cations with Subcellular Particles Prepared from Renal Cortex

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SUMMARY

A differential centrifugation procedure was used to separate homogenized renal cortex of normal dogs into four sediments and four supernatants, each of which was analyzed for alkali and alkaline earth cations and for dry weight. The amount of each of the cations associated with, or "bound" by, the particles sedimenting in each step was computed by a method which takes into account trapped supernatant. Trace cations were determined radiometrically, except for lithium, which was administered by priming injection and constant infusion for 2 hr prior to nephrectomy. The extent of association of each cation with each of the four types of particles was expressed as the percentage of total tissue cation sedimenting with those particles. For the largest ("nuclear") particles, the extent of association of five alkali cations averaged 5.6%, and of four alkaline earths (Mg, Ca, Sr, and Ba) averaged 24%; no selectivity was seen in either group. The second largest ("mitochondrial") particles showed pronounced selectivity, the extent of association increasing from 2% to 15% with increasing atomic weight in the first group, and from 19% to 70% in the second. The third ("heavy microsomal") fraction showed no selectivity; an average of 1.4% of the alkaline cations and 6.7% of the alkaline earths was associated with these particles. The fourth ("light microsomal") fraction showed the following selectivity in order of increasing atomic weight: $Li = Na > K < Rb = Cs$; $Mg > Ca > Sr \ll Ba$.

Thus the "mitochondrial" particles preferentially adsorb (or take up during homogenization) larger cations in both groups, while the "light microsomal" particles show least binding of cations of intermediate size. The sequence of tissue-to-plasma ratios of alkaline earths was similar to the sequence of their association with the "light microsomal" particles. For alkali cations, tissue-to-plasma ratios paralleled the extent of their association with the "mitochondrial" particles.

INTRODUCTION

Among the prevalent cations in body fluids, considerable differences are seen not only in the ratio of intracellular to extracellular concentrations, but also in relative transport rates across epithelial membranes. Both forms of selectivity may involve differences in affinity of binding sites on cell membranes or within cells, as well as differences in membrane permeability. In the kidney (1–3) as well as the gall bladder (4), relative transport rates of all the alkali cations and alkaline earths as a function of crystal ion radius can be described by a single curve with a maximum at about 1 A, the size of the sodium as well as the calcium ion. Intracellular-to-extracellular concentration ratios are known only for a

RESULTS

Whole Tissue Concentrations

Values for sodium, potassium, calcium, and magnesium per kilogram of wet cortex are shown in Table 1, together with their standard deviations. These are in accord with most previous estimates (11–17). In addition, the tissue-to-plasma ratio is shown for each of these four cations, for the four cations determined radiometrically, and for lithium in two additional experiments. No obvious pattern in relation to atomic size emerges. The alkali cation present in tissue at the highest ratio to plasma is rubidium and the alkaline earth is magnesium (beryllium and radium were not examined). In muscle, cesium is accumulated to a greater extent than rubidium (18). Sodium is evidently excluded from kidney cells even more completely than lithium, although a small portion of the difference between the two may be attributable to higher ratios of tubular-fluid-to-plasma for lithium. Strontium is evidently excluded from cells almost as effectively as sodium.

TABLE 1

Concentration of nine cations in plasma and in renal cortex of normal dogs (means ± SEM)

Parameter	Li	Na	K	^{86}Rb	^{137}Cs	Mg	Ca	^{85}Sr	^{140}Ba
Number of dogs	2[a]	9	9	3	6	9	8	6	3
Plasma concentration (mM)	2.80 3.06	143 ±2	3.9 ±0.2	—	—	0.75 ±0.02	2.52 ±0.06	—	—
Tissue concentration (mmoles/kg)	4.74 4.04	58.8 ±0.9	68.8 ±1.1	—	—	7.85 ±0.12	2.40 ±0.05	—	—
Tissue-to-plasma ratio	1.69 1.32	0.41 ±0.01	17.9 ±0.7	47.2 ±10.0	31.0 ±4.2	10.8 ±0.3	0.97 ±0.03	0.50 ±0.06	1.05 ±0.06

[a] These two dogs received LiCl by priming dose and constant infusion.

Volumes of Sediments and of Particles

In four dogs, the calculated volumes of the sediments, expressed as fractions of the total homogenate, were compared with volumes measured directly in graduated cylinders. The results indicated a close correspondence for the nuclear sediment, but progressively larger discrepancies in the case of the subsequent sediments. This reflects the fact that losses occur during each transfer and these losses are accumulated. Furthermore, the volume of the last two sediments is only a small fraction of the volume of suspension during these steps. Consequently, determination of the sediment volume by difference is accompanied by a larger error. No significant difference was found between right and left kidneys.

TABLE 2

Calculated volumes and particle weights of four sediments obtained from renal cortex (means ± SEM for nine normal dogs)

Sediment	Conditions	Volume (% of homogenate)	Particle weight (mg/g of cortex)
I ("nuclei")	1,000 *g*, 20 min	28.0 ± 2.4	70 ± 10
II ("mitochondria")	10,170 *g*, 30 min	6.6 ± 1.1	26 ± 4
III ("heavy microsomes")	35,000 *g*, 30 min	3.2 ± 1.1	7 ± 2
IV ("light microsomes")	122,000 *g*, 120 min	2.6 ± 0.2	9 ± 2
		40.4	113

Therefore the values of sediment volumes and of particle weights in each dog were taken as the average between the right and left kidneys, giving a single value for each dog. The results are shown in Table 2. The "heavy microsomal" fraction was the most variable in volume; the "nuclear" sediment was the least. Particle weights (equated with particle volumes) varied from 4.5% of the sediments in the case of the "heavy microsomal" fraction to 8.1% of the sediment in the case of the "mitochondrial" fraction. Expressed in terms of whole tissue, the total particle weight represented 11% of the weight of wet tissue.

Cation Concentrations

Observed and derived data obtained from the analysis of one kidney are shown in Table 3. The supernatant concentrations of all six cations fall with each successive centrifugation. The concentrations in the sediments are in each case higher than in the corresponding supernatants. The most pronounced fall in supernatant concentration is seen with strontium and the smallest fall is seen with sodium. Also shown are the dry weights of each supernatant and sediment. A major portion of these dry weights is contributed by sucrose, which was present in the homogenate at a concentration of 0.2 M. This corresponds to a dry weight of approximately 68 mg/ml.

"Bound" Cations

Table 4 shows the average values for the percentages of total cortex cation associated with each of the four types of subcellular particles for each of these nine cations.

In the "nuclear" sediment as well as the "heavy microsomal" sediment there are no significant differences among the alkali cations or among the alkaline earths in the percentage associated with these particles. This is surprising, since most known ligands would exhibit greater selectivity among these metals, particularly when all are present simultaneously at concentrations varying by several orders of magnitude.

In the "mitochondrial" sediment, by contrast, there are pronounced differences among the cations in both groups. With increasing atomic size and atomic weight, individual cations are more extensively associated with the particles. Only barium is exceptional, being less extensively bound than strontium.

In the "light microsomal" sediment, considerable differences among cations are again seen, but here the least binding in both groups of ions is found near 1.2 A crystal radius.

Effect of Lithium Administration

In the two dogs who received lithium, all of the tissue concentrations and the percentages found were normal except for total magnesium values, which were both below the range of the normal dogs (6.9 and 7.1 mmole/kg, respectively, compared with a normal range of 7.3 to 8.5). The plasma concentration of lithium attained is within the range employed in the therapy of psychiatric disorders (19). It would be of interest to examine the effect of lithium administration on magnesium metabolism. Some preliminary studies have been reported (20).

Cation Binding by Particles in Relation to Their Weight

When the values in Table 4 are divided by the particle weights (Table 2) a ratio is obtained which expresses the percentage of each cation bound per milligram of particle weight per gram of tissue. This measure is presumably a better estimate of the relative affinity of these four macromolecular aggregates. The results of this calculation are as follows: The microsomal particles bind more lithium, sodium, magnesium, and barium per milligram than do the mitochondria or the nuclei; the mitochondria bind more of the other five cations than do microsomes or nuclei.

DISCUSSION

The method of calculating "bound" solutes used here is based upon the simplifying assumption that each sediment contains only sedimented particles of definite volume and a medium identical in composition to the supernatant. Fluid phases within

sedimented particles are not taken into account, and consequently the resulting estimates of "bound" solutes include amounts present within these fluid spaces, to the extent that interior solute concentrations exceed those in the supernatant. If interior solute concentrations were less than those in supernatant, the estimates of "bound" solute would be negative, unless the quantity adsorbed on the particle surface exceeded the deficit in the interior. The very fact that all of the estimates of bound solute are significantly greater than zero indicates that none of these cations is excluded both from the interior and from the surface of these particulate fractions.

Apart from this ambiguity concerning the site of material estimated to be "bound," the most questionable assumption is that smaller, nonsedimenting particles are distributed in the same manner as the soluble portion of the supernatant. For example, the concentration of mitochondria in the first sediment, according to our assumptions, should be only slightly less than in whole homogenate. One can argue that in fact mitochondria should be more concentrated, owing to partial sedimentation, or less concentrated, owing to their exclusion from the fluid phases of the nuclei. Definite information is not available, and the magnitude of potential error owing to this cause is uncertain.

A more important question is whether the distribution of cations observed under these conditions bears any relationship to the distribution in the living cell. In all probability, the amount bound *in vivo* differ greatly, because of the radical changes in the environment of the subcellular structures which have been produced. Nevertheless, the results suggest that the relative extent of association of cations with these particles may be reflected accurately. As noted above, addition of lithium did not alter the percentage of each cation "bound" by the four particles. In further experiments, threefold augmentation of homogenate magnesium or potassium had little effect on the relative amounts of cations bound. These findings provide indirect support for the view that the smaller changes in ionic concentrations produced during homogenization have not altered relative affinities for these cations, as measured by this method.

A large number of reports have appeared in which the association of one or more cations with subcellular particles from various tissues have been examined, and no attempt will be made to review them here. These studies have included measurement of ion concentrations in sediments subjected to various degrees of washing (5, 21, 22) or resuspended in electrolyte media (6, 23, 24). Because of different techniques of separation as well as calculation, it is difficult to compare these results with ours. In particular, the extensive recent investigations of ion accumulation, as energy-linked processes, by mitochondria or microsomes are not strictly relevant to the present technique. While some ion transport and turnover of intermediates persists at 0° (25), it is clear that most of the differences found here in ion concentrations between various portions of the cell were either present *in vivo*, or were achieved passively during the fractionation procedures.

It is of interest to attempt to correlate the extent of association of individual cations with the intracellular-to-extracellular ratios and with the relative rates of transcellular transport. Tissue-to-plasma ratios, as shown in Table 1, are of course unreliable guides to intracellular concentrations because of the uncertain contribution of extracellular fluid and of tubular fluid of unknown composition. However, the gross differences seen among these cations must be in large measure attributable to a similar sequence of intracellular-to-extracellular ratios. Among the alkali cations, a progressive increase with atomic weight is seen, lithium being exceptional; the apparent decrease between rubidium and cesium is not statistically significant, and is contrary to previous findings in muscle (18). This tendency toward increasing cellular accumulation with atomic weight may therefore be in part a result of mitochondrial activity, since a similar increase is seen in cation binding by these particles. Among the alkaline earths the opposite is

TABLE 3

Observed and derived data from the analysis of one pair of kidneys in a normal dog given ^{86}Rb and ^{140}Ba by constant infusion[a]

Parameter	Homogenate	Step I		Step II		Step III		Step IV	
		Sup't	Sed.	Sup't	Sed.	Sup't	Sed.	Sup't	Sed.
1. Observed data	C_0	C_1	C_I	C_2	C_{II}	C_3	C_{III}	C_4	C_{IV}
Na, mM	10.82	10.19	12.37	10.08	12.40	9.65	14.43	9.53	13.70
K, mM	14.03	13.52	16.24	12.35	23.79	11.87	15.39	11.52	14.62
Ca, mM	0.488	0.346	0.812	0.168	2.058	0.116	0.760	0.094	0.852
Mg, mM	1.482	1.084	2.59	0.740	3.61	0.668	2.40	0.540	3.79
^{86}Rb, cpm	468	446	564	388	849	382	478	372	586
^{140}Ba, cpm	26.3	18.5	48.0	10.8	75.0	8.6	44.2	4.8	110.9
Dry weight, mg/ml	101	84	132	81	163	81	133	81	161
2. Derived data									
a. Sediment volumes, ml/ml of V_0		V_I	W_I	V_{II}	W_{II}	V_{III}	W_{III}	V_{IV}	W_{IV}
Estimated from Na		0.289	0.026	0.036	0.03	0.061	0.13	0.017	0.10
Estimated from K		0.185	0.023	0.084	0.42	0.100	0.05	0.070	0.04
Estimated from Ca		0.305	0.608	0.065	19.80	0.051	9.56	0.017	25.65
Estimated from Mg		0.231	0.733	0.092	4.62	0.028	3.10	0.026	14.32
Mean ($= \Sigma V_i W_i / \Sigma W_i$)		0.263	—	0.071	—	0.046	—	0.020	—
b. Particle wt., P, g/ml of V_0			0.0146		0.0063		0.0026		0.0017

c. "Bound" cations, estimates	Susp	Sed	Susp	Sed	Susp	Sed	Susp	Sed
Na, mmoles/kg cortex	3.89	3.61	0.75	1.14	1.56	1.14	0.45	0.50
K, mmoles/kg cortex	3.50	4.57	4.71	4.43	1.76	1.16	0.96	0.41
Ca, mmoles/kg cortex	0.74	0.64	0.66	0.67	0.18	0.15	0.07	0.08
Mg, mmoles/kg cortex	1.82	2.07	1.29	1.04	0.25	0.40	0.40	0.33
^{86}Rb, cpm/kg cortex	142.	188.	226.	175.	25.0	27.8	35.5	25.1
^{140}Ba, cpm/kg cortex	40.4	40.1	28.6	23.0	7.6	8.3	11.8	10.6
d. Weighting factors	Susp	Sed	Susp	Sed	Susp	Sed	Susp	Sed
Na	0.082	0.918	0.006	0.994	0.004	0.996	0.001	0.999
K	0.083	0.917	0.015	0.985	0.003	0.997	0.000	1.000
Ca	0.528	0.472	0.946	0.054	0.800	0.200	0.704	0.296
Mg	0.631	0.369	0.237	0.763	0.159	0.841	0.312	0.688
Rb	0.604	0.396	0.794	0.206	0.633	0.367	0.652	0.348
Ba	0.604	0.396	0.794	0.206	0.633	0.367	0.652	0.348
e. Percentage "bound"								
Na	6.7		2.8		3.3		1.5	
K	6.4		8.5		2.0		0.9	
Ca	28.3		36.3		10.2		4.6	
Mg	26.7		20.5		7.8		7.7	
Rb	6.9		9.2		1.1		1.4	
Ba	30.7		20.9		6.0		8.7	

[a] Abbreviations: Sup't, supernatant; Sed, sediment; Susp, suspension; V, volume; W, weighting factor.

TABLE 4

Association of cations with subcellular particles from renal cortex in normal dogs (means ± SEM)

Particles	Li (2)	Na (9)	K (9)	Rb (3)	Cs (5)	Mg (9)	Ca (8)	Sr (5)	Ba (3)
	Per cent of tissue cation, (*n*)								
I ("nuclei")	5.3	5.1	5.7	6.6	5.1	24.2	22.6	17.6	28.3
	5.6	±0.4	±0.4	±0.3	±1.2	±0.3	±1.9	±3.6	±2.7
II ("mitochondria")	2.0	2.2	7.2	10.3	15.5	19.3	37.7	70.5	30.3
	2.4	±0.1	±0.4	±0.1	±0.3	±1.0	±1.6	±5.8	±2.3
III ("heavy	0.8	1.9	1.4	1.2	1.6	6.2	7.0	5.8	8.1
microsomes")	1.5	±0.3	±0.1	±0.2	±0.2	±0.6	±0.8	±0.2	±0.3
IV ("light microsomes")	1.6	1.5	1.1	2.3	1.9	9.0	5.4	2.4	12.9
	2.3	±0.2	±0.1	±0.3	±0.3	±0.6	±0.7	±0.5	±0.5
Total	9.7	10.6	15.2	22.0	24.1	58.7	72.7	96.3	79.7
	11.8	±0.6	±0.5	±0.5	±1.2	±1.4	±2.0	±0.8	±1.1
	Fraction of tissue cation per gram of particles per gram of tissue								
I ("nuclei")	0.74	0.73	0.82	1.04	0.81	3.51	3.14	2.70	3.65
	0.90	±0.04	±0.03	±.14	±0.15	±0.14	±0.19	±0.16	±.34
II ("mitochondria")	0.76	0.82	2.63	3.42	6.48	7.29	14.73	28.52	10.20
	0.84	±0.02	±0.08	±.34	±0.64	±0.32	±0.94	±2.57	±.67
III ("heavy	1.88	2.67	2.05	1.48	3.14	8.98	10.00	11.50	10.05
microsomes")	2.31	±0.18	±0.20	±.37	±0.67	±0.80	±1.58	±2.36	±2.81
IV ("light microsomes")	1.60	1.70	1.17	2.33	2.18	9.70	5.51	2.73	14.17
	1.48	±0.16	±0.08	±.07	±0.23	±0.63	±0.22	±0.54	±1.02

the case, tissue-to-plasma ratios varying with atomic size as follows: Mg > Ca > Sr < Ba. This is the same as the order of binding exhibited by the light microsomes. In both cases the sequential differences are statistically significant. Thus these particles may be involved in the transport of alkaline earth cations into or out of the cell.

When the relative rates of tubular reabsorption of these cations are calculated neglecting the unknown contribution of tubular secretion to normal action excretion, it is seen that cations larger or smaller than calcium and sodium are transported less readily (1–3). No such sequence is seen in any of the parameters measured here. Therefore the source of selectivity in the transepithelial movement of these cations may involve differences in permeability rather than differences in affinity of subcellular structures concerned with transport.

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APPENDIX

Computation of statistical weighting factors. The following approximation formulas (26) were employed (Eqs. 1–5):

$$\sigma^2(y \pm x) = \sigma^2(y) + \sigma^2(x) + 2\sigma(yx) \quad (1)$$

$$\sigma^2(y \cdot x) = x^2\sigma^2(y) + y^2\sigma^2(x) + 2yx\sigma(yx) \quad (2)$$

and

$$\sigma^2(y/x) = \sigma_2(y)/x^2 + y^2\sigma^2(x)/x^4 - 2y\sigma(yx)/x^3 \quad (3)$$

where $\sigma(yx)$ is the covariance of y and x.

If $y = l_1z_1 + l_2z_2$,

$x = k_1z_1 + k_2z_2$,

$$\sigma(yx) = l_1k_1\sigma^2(z_1) + (l_1k_2 + l_2k_1)\sigma(z_1z_2) + l_2k_2\sigma^2(z_2) \quad (4)$$

The weighted least squares combination of two correlated estimates is

$$\frac{[\sigma^2(x) - \sigma(yx)]y + [\sigma^2(y) - \sigma(yx)]x}{[\sigma^2(x) - 2\sigma(yx) + \sigma^2(y)]} \quad (5)$$

In the estimation of sediment volumes, the weighting factor W_i, for the ith ion, derived from the above formulas, is given in the first sediment by:

$$W_i = 1/[(C_0^2 + C_1^2)/(C_I - C_1)^2 + (C_0 - C_1)^2(C_1^2 + C_1^2)/(C_I - C_1)^4 - 2(C_0 - C_1)C_1^2/(C_I - C_1)^3]$$

and by corresponding equations in the other sediments.

If y is the suspension estimate and x is the sediment estimate of bound cation, then in the first step

$$\sigma(yx) = (W_i/\Sigma W_i)(C_0^2 + C_1^2)$$

$$\sigma^2(y) = C_0^2 + C_1^2$$

$$\sigma^2(x) = \bar{V}_I^2(C_I^2 + C_1^2) + 2\bar{V}_I(W_i/\Sigma W_i)[(C_I^2 + C_1^2) - (C_0 - C_1)C_1^2/(C_I - C_1)] + (C_I - C_1)^2/\Sigma W_i$$

where $\overline{V}_I$ is the weighted mean sediment volume, expressed as a fraction of the total homogenate. Errors in P_I are neglected, since they are much smaller than errors in $\overline{V}_I$.

These formulas all depend upon the assumption that analytical error for all four ions measured spectrophotometrically is a constant fraction, k, of the measured concentration. k can be estimated from the reproducibility of the individual estimates of sediment volumes as $k^2 = \Sigma[W_i(V_i - \overline{V}_i)^2]/(n - 1)$. The average value of k in the fourteen experiments was 0.03.

In estimating bound quantities of ions determined isotopically, the weighted mean is simply $[y\sigma^2(C_I) + x\sigma^2(C_0)]/[\sigma^2(C_I) + \sigma^2(C_0)]$, the quantities $\sigma^2(C_I)$ and $\sigma^2(C_0)$ being obtained directly from the counts and minutes of samples and backgrounds.

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