The Membrane Control of Bone Potassium* (33618)

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Though the potassium content of bone has been occasionally studied over the past several decades, little interest was generated. There are several reasons for this. First, in relation to other elements, the potassium in bone is a minor constituent. Second, experimental values (except on scrupulously cleaned cortical bone) are frequently of uncertain validity. Since cells exhibit a potassium concentration 20–25 times that of the serum and extracellular fluids (1), a very small contamination of bone by cellular elements of marrow would result in aberrently high values. Finally, the levels of potassium in bone have not been associated with alterations of skeletal metabolism except in rare instances (2–4).

Recently, however, reinvestigation of the importance of potassium in bone was prompted by the discovery that the *apparent* concentration of potassium in carefully cleaned cortical bone is inexplicibly high (5, 6). In bone in which cellular water could account for less than 25% of the total water present, the average concentration of potassium as calculated for total bone water was 146 m*M*, considerably higher than normally found in cells (1).

One might invoke some physicochemical mechanism for concentrating potassium such as binding to the formed elements (principally collagen and crystals). However, crystals of hydroxyapatite have been synthesized in 0.16 *M* KCl and insignificant amounts of potassium was incorporated into the crystals (7). Nor was potassium concentrated to any degree in the crystals' hydration shells (8). Moreover, collagen's amino acid composition (principally glycine, proline, and hydroxyproline) does not render it an effective binder of cations. Even the mucopolysaccharides of connective tissue do not selectively concentrate potassium ions (9), and the concentration of mucopolysaccharides in cortical bone is very low. Thus, there appears to be no physicochemical basis for the high concentration of potassium in bone.

Confirming these deductions was the observation that the level of potassium in rat bone falls precipitously with age (10), a process in which the formed elements of bone (crystals and collagen) increase proportionately with the decrease in the water content. We are left, by exclusion, with the tentative conclusion that the potassium content of bone is maintained at high levels by the activity of living cells.

Equilibration of nonvital bone powder. If this conclusion were correct, it could be predicted that the potassium in *dead* bone should be easily leached out. Accordingly, compact, cortical parts of pelvic bone from a calf were frozen and ground to a 20-mesh powder at −15°. Twenty-g samples of this powder were equilibrated with varying volumes of buffer (20–100 ml) with stirring for 15 hr at room temperature. Thereafter, the bone powder was removed by filtration on Millipore membranes and the buffer was analyzed for sodium and potassium by flame photometry (Instrumentation Laboratories, Inc., model 143); for Mg by atomic absorption (Perkin Elmer spectrophotometer model 303); and for calcium by titration with Versene (11). The bone was analyzed for water content by drying at 105° for 24 hr, for hemoglobin by absorption at 400 mμ, and for DNA (12). The basic buffer had the following composition in mmoles/liter KH_2PO_4, 0.2;

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TABLE I. A Dilution Study of Ion-Binding by Bones.[a]

Ion studied	Buffer (vol/bone; ml/g)	Initial concn (mM)	Final concn (mM)	Apparent concn in bone water (mM)
K^+	1	4.0	13.5	90
	2	0.0	7.0	119
	5	8.5	11.5	132
	5	4.0	6.5	107
	5	6.5	9.0	110
Na^+	1	132	124	59
	2	0	22.5	385
	5	132	129	0
	5	129	124	—0.8
Mg^{2+}	1	1.8	1.7	0.9
	2	0	1.7	29.3
	2	0	1.1	18.9
	5	1.8	1.7	—2.2
	5	1.7	1.3	—14.9
Ca^{2+}	1	2.9	1.1	—13.4
	2	2.9	0.93	—30.4
	2	2.9	0.71	—34.9
	5	4.8	1.2	—152
	5	2.9	1.1	—71.0
	5	1.1	0.88	—9.3

[a] The original bone powder was 12.4% water, contained 0.12 mg of hemoglobin/g, 1.3 mg of DNA/g and its potassium content was 153 mM expressed in terms of total bone water. The "apparent concentration in bone water," C, was calculated by the formula:

$$C = C_f[(0.124a) + V] - C_i V / 0.124a,$$

where a = amount of bone powder in grams; V = volume of the solution in ml; C_i = initial concentration, mmoles/liter; C_f = final concentration, mmoles/liter.

$Na_2HPO_4 \cdot 7H_2O$, 0.9; $CaCl_2 \cdot 2H_2O$, 1.5; $MgCl_2 \cdot 6H_2O$, 0.9; Na_2SO_4, 0.35; $NaHCO_3$, 27.0; KCl, 3.8; NaCl, 103. Substitution of various salts were made, but the pH, ionic strength, and temperature were maintained at 7.4, 0.16, and 25°, respectively, and the slurry was continuously bubbled with N_2 containing 5% CO_2. The results of such equilibrations are summarized in Table I.

If, as we have suggested, potassium does not interact with the constituents of solid phase, its final concentration should be determined only by the total amount of potassium and the total water present. Such was found to be the case, Assuming a simple dilution of the bone fluid, the potassium content was calculated to be 112 ± 15 mM, a constant within experimental error. Clearly, in the absence of cellular activity, the potassium in bone does leach out and shows no appreciable interaction with any component of the solid phase.

On the other hand, sodium, magnesium, and calcium, calculated in the same way, showed strong interactions with the solid phase. In many instances the solid removed a considerable amount of the cation from the buffer. In such cases, the apparent concentration in bone water calculated on a simple dilution basis, was less than zero.

The loss of bone potassium on incubation in vitro. If the potassium in bone is indeed concentrated there by active cellular processes the study of the phenomneon would be greatly facilitated by a system in which the effects of metabolic inhibitors and other agents could be observed under culture conditions. Accordingly a variety of bone tissue samples were studied under a variety of incubation conditions.

Among those tissues examined were rat embryonic long bones (radius, ulna, tibia and fibula from 19-day embryos), calvaria from newborn rat pups, and calvaria from chick embryos (20 day). Among the media tried were Ringer's buffer, rat serum, BGJ (13), and 1% serum albumin in Ringer's and in BGJ.

It soon became apparent that the potassium of bone was very labile. For example, if a calvarium taken from a newborn rat pup is scraped with a scalpel and merely dipped in saline, a very significant fraction of the total potassium is lost from the tissue.

Accordingly, the following procedure was adopted. Calvaria from rats 1–2 days old were very crudely dissected and placed immediately in medium gassed with 5% CO_2 in air. Following various periods of incubation at 37° (2–6 calvaria in 3 ml) the calvaria were removed, carefully trimmed and wiped with a cotton swab. (Cotton may contain surprising amounts of potassium; some caution is required at this point.) The cleaned specimens were then weighed and dried for 16 hr at 105°, weighed again, then extracted for 16 hr with a mixture of nitric (0.1 *M*) and acetic acid (10%). The acid solutions after centrifugation were then analyzed.

There was a considerable variation in the potassium content of the calvaria as taken. We have reason to attribute this to variation (0–2 days) in the ages of the rat pups. This variation (from 250 to 450 μg K/100 mg of dry bone) necessitated taking random control specimens from the same litter without incubation and expressing the results on the incubated specimens as percentage of its own unincubated control.

Control specimens exhibited the following characteristics (mean of six experiments): water content 19.7 ± 0.4%, organic matter 33 ± 1.2%, ash 46.8 ± 1.0%, oxygen uptake 32 ± 3 μmoles/hr/g of dry bone. The extracellular space measured by inulin-^{14}COOH (14) was 85 ± 4% of the total water content. The modified Krebs–Ringer buffer had the following composition (parts by volume): 100 of 0.9% NaCl, 21 of 1.3% $NaHCO_3$, 4 of 1.15% KCl, 3 of 1.22% $CaCl_2$, and 1 each of 3.82% $MgSO_4$ and 2.11% KH_2PO_4. Glucose, 3.06 g/liter was added.

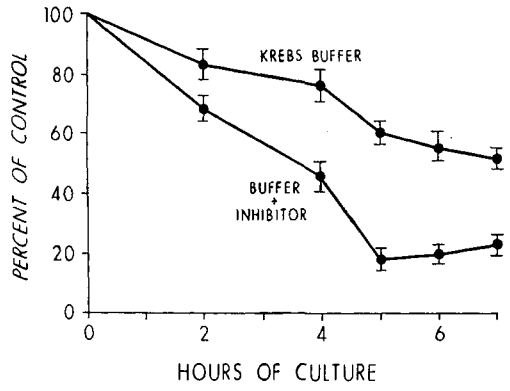

FIG. 1. The loss of bone potassium on incubation *in vitro*. From 4 to 11 experiments are averaged per point. The vertical bars indicate the standard deviation. The inhibitor was iodoacetate, 10^{-3} *M*.

There was a loss of potassium during the incubation as shown in Fig. 1. Also shown are the data obtained when iodoacetate ($10^{-3}M$) was present in the medium. In this case the loss of potassium was even greater and more rapid.

Consideration of these data give the impression that, even in the absence of the inhibitor, the culture conditions are such that the cellular integrity of the membranes responsible for maintaining potassium gradients gradually deteriorates. This impression was strengthened by the finding that the water content of the bone increased in a 6-hr incubation from 19.7 to 32.7%. Most of this could be attributed to an expansion of the extracellular water volume as measured by insulin-^{14}COOH, 88%.

These observations prompted an attempt to improve the culture medium by employing either freshly prepared rat serum or commercially available, frozen bovine serum (Grand Island Biological). These data are assembled in Table II.

The presence of serum prevented the loss of significant quantities of bone potassium during a 6-hr incubation. Moreover, the serum very significantly protected the calvaria from the effects of iodoacetate (10^{-3} *M*).

TABLE II. The Protective Effect of Serum on Bone Potassium.

Medium[a]	No. of tests	Mean (% of control)	SD
Rat serum	7	97	3.5
Bovine serum	11	98	5.7
Modified Ringer's buffer	22	64	11
Rat serum + IA	5	68	7.6
Bovine serum + IA	5	65	6.2
Modified Ringer's + IA	7	38	7.9

[a] See text for further details; IA contained iodoacetate, 10^{-3} *M*. Incubations were for 6 hr at 37°.

Discussion. Data in the literature and the results of the present studies strongly suggest that, with respect to potassium, the extracellular compartment of bone appears to be separated from the extracellular fluids generally by some functional cellular membrane or barrier. As a concept this is not entirely new. It was proposed over a decade ago (15), numerous allusions to the idea have been made, and very recent evidence (16) obtained from perfusion studies are in support of the idea. However, as a working hypothesis the bone-membrane has never been widely accepted.

Some 10 years ago, a review of the then current literature (17) stressed the important conclusion that the bone mineral could not possibly be in equilibrium with the circulating fluids. At that time, there was insufficient data to choose between two possible mechanisms: (a) a pH gradient maintained by the secretion of metabolic acids by bone cells (17, 18), or (b) a functional membrane separating the bone compartment. The hypothetical pH gradient is totally inadequate to explain the behavior of potassium in bone and we have some unpublished evidence that no pH gradient exists. We are left, then, by exclusion, with the conclusion that regulation of the flow of ions to and from the skeleton is under the control of a cellular membrane.

Implicit in this argument is the assumption that, if the bone fluid differs from serum in its potassium content, it most probably differs with respect to other electrolytes (19). The formidable task now confronting investigators is that of determining with reasonable precision the composition of normal bone "fluid" and its variations in disease- and deficiency-conditions.

Summary. An attempt was made to define, at least in an approximate way, the mechanism by which potassium is selectively concentrated in the fluid compartment of cortical bone. Equilibration of nonvital powdered bone revealed that potassium does not interact significantly with any of the solid phase constituents of formed bone. Therefore, the concentrating mechanism is attributable to cellular activity not to some physicochemical property of bone. Incubation of living bone preparations *in vitro* revealed that the integrity of the vital concentrating processes was very sensitive to manipulation of the specimens and to culture conditions. Cultured in serum (bovine or rat), calvaria of newborn rat pups retained their potassium content without significant loss for periods up to 6 hr.

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Creatine Phosphokinase in Detection of Visceral Muscle Injury* (33619)

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Reactions of the Embden-Meyerhof pathway and transamination occur in the majority of mammalian tissues. As a consequence, aldolase, lactic dehydrogenase (LDH) and glutamic-oxalacetic transaminase (GOT) have a ubiquitious distribution. Activity of these enzymes is high in striated muscle, liver, and other tissues limiting their value in specific detection of muscle cellular injury.

Significant creatine phosphokinase (CPK) activity is found only in muscle, brain, thyroid, and kidney (1). Very low CPK activity is found in serum, red cells, and liver. Therefore CPK activity determination is a better indicator of injury to the cellular integrity of those organs in which it is concentrated. Discussion of the advantages and limitations of the enzyme in the diagnosis of skeletal muscle disease employing CPK as an indicator of cellular injury have been published (2). Serum CPK alterations were useful in the diagnosis of myocardial infarction but to the authors' knowledge have not been employed in the detection of injury to visceral muscle.

Urinary bladder muscle was used as a model for visceral muscle in these studies. Since bladder muscle bulk is small and serum dilution effects were anticipated, an *in vivo* method of monitoring arteriovenous CPK differences was employed. The method is unique and permits inferences concerning specific organ injury. Muscle biopsy for CPK estimation during physiologic studies prevents reproducible electrophysiologic experimentation and is limited in patient application. Development of techniques of visceral muscle electronic reflex stimulation and diagnosis of visceral muscle disease demand more sophisticated means of evaluating detrusor muscle cellular changes and energy mechanisms. Accompanying studies of bladder muscle glycogen concentration were employed to assess changes in substrate reserves for energy metabolism. Vesical vein lactate concentration was assessed to determine the extent of anaerobic metabolism during the course of these experiments.

Materials and Methods. Adult female dogs were anesthetized by intravenous pentobarbital (25 mg/kg). The bladder was exposed by suprapubic incision. Ureteral catheters were employed for bladder filling and monitoring of intravesical pressure. Bipolar electrodes were affixed to the bladder by means of sutures (3). Blood was withdrawn from a vesical vein. Stimulation of the bladder muscle every 10 min over a period of 1 hr with 20 V, 1/msec pulses in a pulse train of 40–60-sec duration induced massive bladder contractions to the point of rigor. Vesical vein blood was collected again after the 1-hr period elapsed. The internal iliac arteries were then ligated. This procedure required about 20 min. Collateral circulation was such that ischemia rather than infarction occurred. After 60 min, blood was once again drawn from the vesical vein. The bladder fundus was

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