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MINIREVIEW

Calcium Metabolism in Experimental Hypertension (42646)

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Chronic essential hypertension is characterized by increased vascular resistance with a normal cardiac output. The underlying processes responsible for the initiation and maintenance of increased vascular tone are unclear. Some important hypotheses that have been proposed to explain elevated blood pressure include centrally altered sympathetic nerve activity, vascular structural alterations, and excessive salt intake in genetically predisposed individuals. No single hypothesis, however, adequately explains the major portion of hypertension encountered in humans. Recently, a broad-based set of observations has emerged which suggests an association between hypertension and altered calcium metabolism. While the full significance of this association is presently unknown, it has opened a promising area in hypertension research at the epidemiologic, clinical, whole animal, and cellular levels.

Three major lines of evidence link calcium and hypertension. First, epidemiologic analyses of several large data bases suggest an inverse relationship between blood pressure and dietary calcium intake. Similarly, the prevalence of hypertension appears to be inversely correlated with calcium intake. Second, alterations in systemic and cellular calcium metabolism that may contribute to calcium deficiency have been described in experimental and human hypertension. Specifically, both calcium malabsorption and renal calcium wasting have been reported. Finally, supplemental oral calcium lowers blood pressure in hypertensive animals and humans, suggesting that correction of a calcium deficit favorably influences blood pressure. Thus, both low dietary calcium intake and abnormal systemic calcium handling are associated with hypertension. To integrate these findings, it has been proposed that some forms of hypertension are a manifestation of low calcium intake in an individual with a compromised ability to conserve calcium. Depending on the circumstances, one or both of these conditions may be sufficient to cause or maintain elevated blood pressure. The hypertension may arise from calcium deficiency per se or may be an incidental manifestation of the processes which cause or compensate for calcium deficiency. Altered calcium metabolism may play a role in essential or genetic hypertension as well as secondary forms of hypertension such as primary aldosteronism and renal vascular disease. In this review, we will focus on systemic and cellular calcium metabolism in experimental and human essential hypertension.

Epidemiology of Calcium and Blood Pressure. Population-based studies of calcium and hypertension were initially prompted by a report that untreated human hypertensives appeared to have a renal calcium leak despite elevated serum parathyroid hormone (1). It was suggested that individuals with essential hypertension were deficient in total body calcium compared with normotensive controls. Accordingly, a pilot population survey was undertaken looking at the relationship between dietary calcium intake and blood pressure (2). This survey and a subsequent larger analysis (3) of the Health and Nutrition Examination Survey data base (HANES I, National Center for Health Statistics) uncovered a significant, inverse relationship between dietary calcium intake and blood pressure. This relationship has been confirmed by other investigators using the HANES and other data bases covering many diverse populations and geographical locations (4–7, 116–127). While these epidemiologic surveys are consistent in showing a relationship between low calcium intake and hypertension, they do not establish causality. Rather, they have stimulated further investigation into systemic and cellular calcium metabolism. Subsequently a number of alter-

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ations in calcium metabolism have been described in human and experimental forms of hypertension.

Systemic Calcium Metabolism in Hypertension. Hypertensive animals and humans demonstrate several distinct alterations in calcium (Ca^{2+}) metabolism at the systemic level which may affect Ca^{2+} balance. The specific systemic manifestations of abnormal calcium metabolism in either experimental or human hypertension include low serum ionized Ca^{2+} concentrations, elevated serum parathyroid hormone (PTH) levels, hypercalciuria, and alterations of intestinal calcium absorption and vitamin D metabolism. Ultimately these alterations affect total body and bone Ca^{2+} balance. Taken as a whole, the systemic abnormalities of Ca^{2+} regulation in hypertension suggest whole animal calcium deficiency. Ca^{2+} metabolism has been best characterized in the spontaneously hypertensive rat (SHR) although significant information also exists for several other experimental models of hypertension and for human essential hypertension. As with other physiologic processes, blood pressure and calcium handling change as the hypertensive animal matures and is also affected by the gender of the animal. Therefore, in discussing findings in the SHR, we will emphasize age and sex of the animals studied with the aim of elucidating their behavior over time.

Normal calcium metabolism. Before discussing the disturbances of calcium metabolism in hypertension, it is worthwhile to consider normal Ca^{2+} metabolism. Intestinal calcium absorption is regulated by 1,25-$(OH)_2$ vitamin D_3 although a large portion of calcium absorption is vitamin D independent, passive or active transport. The major stimuli of 1,25-$(OH)_2$ D_3 production in the kidney are parathyroid hormone (PTH), phosphate depletion, and calcium depletion. Calcium deposition in bone is partly a mass action phenomenon while bone calcium resorption is controlled by PTH. Renal calcium excretion is a function of filtered load (determined by glomerular filtration rate and serum ultrafilterable calcium) and tubular reabsorption which is stimulated by PTH. The major stimulus for PTH secretion is a low serum ionized calcium concentration. Most cells are able to maintain an intracellular Ca^{2+} concentration of approximately 100 n*M* against a 10,000-fold gradient of external–internal calcium through an elaborate system of membrane-embedded channels and transport systems that regulate extrusion and sequestration mechanisms. Intracellular calcium constitutes a small percentage of total body calcium but nonetheless is finely regulated as determined by membrane permeability and active calcium exchange and transport systems.

When discussing differences in Ca^{2+} handling between hypertensive and normotensive animals, it must be realized that both intracellular and extracellular Ca^{2+} levels are tightly regulated by the processes described above. Small differences in markers of calcium homeostasis may reflect a significant metabolic defect, which may be minimized by compensatory regulatory processes. Until the basis for abnormal Ca^{2+} metabolism in hypertension is understood, it will be difficult to determine if the various hormonal and functional alterations are primary or compensatory. Similarly, basic information about the underlying defect is needed to understand any pathogenetic link between Ca^{2+} and hypertension.

Serum calcium (Table I). A low serum ionized calcium concentration ($[Ca^{2+}]_s$) in the SHR, relative to its normotensive control the Wistar–Kyoto rat (WKY), was found at 5 and 13 weeks of age in female rats (8) and at 8, 10, 16, 24, 33, 40, and 45 weeks in males (9–11). On average, $[Ca^{2+}]_s$ in WKY exceeded that in SHR by approximately 0.16 meq/liter. Whether the specimens were taken from fasted or fed animals was generally not specified. Wright also found decreased $[Ca^{2+}]_s$ in SHR as compared with normotensive Sprague–Dawley rats (12). By contrast, Lau *et al.* reported higher postabsorptive $[Ca^{2+}]_s$ in 25-week-old female SHR and no difference in fasting $[Ca^{2+}]_s$ between 26-week-old female SHR and WKY (13). However, these experiments were performed on parathyroidectomized rats. The same authors found that fasting plasma ultrafilterable Ca^{2+} was lower in parathyroid-intact, 23-week-old, female SHR. After parathyroidectomy, there was no difference in plasma ultrafilterable Ca between SHR and

TABLE I. SERUM IONIZED Ca^{2+} IN SHR

Author (Ref.)	Age (weeks)	Sex	Result
Lau (14)	3.5	F	—
	3.5	F	—
Wright (8)	5	F	↓
	13	F	↓
Bindels (9)	8	M	↓
Stern (10)	10	M	↓
McCarron (11)	16	M	↓
	24	M	↓
	33	M	↓
	40	M	↓
	45	M	↓
Lau (13)	23	F	↓[a]
	25	F	↑[b]
	26	F	—[b]

Note. Arrows indicate direction of difference compared with WKY; (—) indicates no difference found between SHR and WKY.
[a] Ultrafilterable Ca.
[b] Animals parathyroidectomized.

WKY. Plasma ultrafilterable Ca includes ionized calcium (~80%) as well as complexed, nonprotein-bound Ca (~20%). Thus a reasonable interpretation of these studies is that $[Ca^{2+}]_s$ is decreased in the parathyroid-intact SHR but the difference from WKY is obliterated by removal of the parathyroid glands. More recently Lau *et al.* reported no difference in $[Ca^{2+}]_s$ between 25-day-old parathyroid intact SHR and WKY, both male and female (14). At this age, blood pressure of SHR was not different from WKY. Thus, it appears that $[Ca^{2+}]_s$ is initially normal in SHR but subsequently falls as the arterial pressure rises. The uninephrectomized DOCA–salt hypertensive rat also has low $[Ca^{2+}]_s$ while the one-kidney, one-clip and two-kidney, one-clip rat models of renovascular hypertension do not (8).

Total serum calcium is not altered in SHR in any consistent direction. The combination of low $[Ca^{2+}]_s$ and normal total serum calcium implies an increase in serum protein-bound Ca^{2+} in SHR. Direct measurements in humans with essential hypertension confirm decreased serum ionized, complexed, and ultrafilterable Ca^{2+} and increased protein-bound Ca^{2+} (15). Other investigators have also reported low $[Ca^{2+}]_s$ in human essential hypertension (16, 17) and in some patients with primary aldosteronism (18).

Parathyroid hormone and cyclic AMP (Table II). Serum PTH has generally been found to be elevated in the SHR, presumably as a secondary response to the low $[Ca^{2+}]_s$. Stern *et al.* found that carboxy-terminal PTH was measurable in 43% of 6-week-old and 55% of 10-week-old male SHR, whereas all WKY of the same ages had undetectable C-terminal PTH (10). In this study, $[Ca^{2+}]_s$ was significantly lower in 10-week-old SHR than in WKY. Bindels *et al.* also found elevated immunoreactive PTH and decreased $[Ca^{2+}]$ in 8-week-old SHR although another assay for intact PTH showed no difference between SHR and WKY (9). McCarron *et al.* (16) found that amino-terminal PTH was significantly elevated in male SHR at 18, 24, and 29 weeks of age. A C-terminal assay also revealed higher serum PTH in the SHR at 29 weeks. Again $[Ca^{2+}]_s$ was significantly lower in the SHR at these ages. Since glomerular filtration rate is consistently normal in the SHR of this age range (13, 19), reduced clearance of immunoreactive PTH fragments is unlikely. A midmolecule PTH assay failed to find a difference between SHR and WKY (20). Recently, SHR were found to have hyperplasia of the parathyroid glands providing further evidence for chronic stimulation of the parathyroid cells (21). Elevated serum PTH concentration has also been reported in human essential hypertension (1, 22, 23) and with primary aldosteronism (18).

TABLE II. SERUM PTH IN SHR

Author (Ref.)	Age (weeks)	Sex	Result
Stern (10)	6	M	↑
	10	M	↑
Bindels (9)	8	M	↑[a]
	8	M	—[b]
Young (20)	13–15	M	—
McCarron (11)	18	M	↑
	24	M	↑
	29	M	↑

Note. Arrows indicate direction of difference compared with WKY; (—) indicates no difference found between SHR and WKY.
[a] Immunoreactive PTH.
[b] Intact PTH.

While serum PTH appears to be appropriately elevated in the SHR in response to decreased $[Ca^{2+}]_s$, end-organ responsiveness may be abnormal. PTH stimulates cyclic AMP synthesis in the kidney and urinary cyclic AMP reflects renal PTH activity. Basal nephrogenous cyclic AMP was lower in 11- to 17-week-old male SHR than in WKY (24). Total urinary cyclic AMP has been reported to be decreased (13, 24) or normal (19) in 13- to 16-week-old male SHR. Normal or decreased urinary cyclic AMP in the face of elevated serum PTH suggests relative renal unresponsiveness to the hormone. Further evidence for reduced renal response to PTH in the SHR includes hypercalciuria (11) and a subnormal increment in 1,25-$(OH)_2$ vitamin D production in response to PTH infusion (27). Furthermore, PTH infusion caused a smaller increment in $[Ca^{2+}]_s$ in 13-week-old male SHR than in WKY (29). *In vitro* studies also suggest a problem with cyclic AMP generation in that adenylate cyclase activity of isolated renal membranes (nephron sites not specified) from 5- and 16-week-old SHR was reduced in response to PGE_2 although the response to other stimuli and basal activity were normal (26). In sum, the SHRs ability to compensate for a low $[Ca^{2+}]_s$ by renal mechanisms is compromised. In contrast, in human essential hypertension, urinary cyclic AMP is appropriately elevated (22) in response to elevated serum PTH (1, 22). Other measures of PTH responsiveness in essential hypertension are lacking (i.e., calcemic or 1,25-$(OH)_2$D response to PTH).

Calcium excretion (Table III). Urinary calcium excretion is increased in the mature SHR although there is disagreement as to whether the excessive calcium excretion reflects primary intestinal hyperabsorption or a primary renal leak. During early development, 24-hr urinary calcium has been variously reported as decreased (9, 14), not different (11, 13, 29, 30) or increased (13, 30, 31). As the SHR reaches maturity, its urinary calcium excretion begins to exceed that of the WKY. Longitudinal studies indicate that hypercalciuria develops in male SHR by 17 weeks of age and in females by 25–26 weeks. McCarron *et al.* (11) reported significantly increased calcium excretion in 17-, 22-, 28-, and 43-week-old male SHR with concurrently low $[Ca^{2+}]_s$ and elevated serum PTH. The hypercalciuria was interpreted to reflect a renal calcium leak. That is, the kidneys of maturing and mature SHR fail to normally reabsorb calcium despite a lower filtered calcium load and a higher level of serum PTH which should stimulate Ca^{2+} reabsorption. On a low Ca^{2+} diet, hypercalciuria was enhanced in 18- to 20-week-old male SHR (32) providing further evidence for a renal leak or

TABLE III. URINARY CALCIUM EXCRETION IN SHR

Authors (Ref.)	Age (weeks)	Sex	Result
Lau (14)	3.5		↓
Ayachi (30)	7	M	↑
	11	M	—
	15	M	↓
	19	M	—
Bindels (9)	6	M	—
	8	M	↓
Lau (13)	10	M	—
	16	M	↑
	13	F	↓
	14	F	↓
	15	F	↓
	16	F	↓
	18	F	↓
	20	F	—
	22	F	—
	24	F	—
	25	F	↑
	26	F	↑
	50	M	↑
	52	F	↑
McCarron (11)	12	M	—
	17	M	↑
	22	M	↑
	28	M	↑
	43	M	↑
Hsu (31)	5	F	↑
	14	F	—
Hsu (19)	8	M	↓
	9	M	—
	10	M	↓
	11	M	↓
	12	M	↓
	13	M	—
	14	M	↓
Kageyama (28)	7	M	—
	8	M	—
	9	M	—

Note. Arrows indicate direction of difference from WKY; (—) indicates no difference found between SHR and WKY.

abnormal humoral regulation of renal calcium excretion. Impaired renal tubular Ca^{2+} reabsorption may arise from diminished cyclic AMP generation as discussed above although alternate mechanisms are possible.

In female SHR, Lau *et al.* found higher 24-hr calcium excretion beginning at 25 weeks of age (13). Parathyroidectomy enhanced hypercalciuria in the 25-week-old female SHR. Urinary calcium excretion after fasting was decreased in 23-week-old females (13) and in 8- to 14-week-old males (19). However, the relevance of these findings is questionable because 24-hr urinary calcium excretion is only increased in older SHR as indicated above. Nonetheless, based on low fasting calcium excretion and normal urinary cyclic AMP (PTH was not measured), the 24-hr hypercalciuria was felt to result from increased intestinal calcium absorption (13) and not from diminished renal tubular calcium reabsorption.

The critical distinction between absorptive and renal hypercalciuria remains unsettled because of differences in sex and age of animals used in various studies and in actual measurements of calcium balance. A further complication arises because the SHR may have defective calcium metabolism involving several organs which prevents anticipated compensatory responses. Expected findings with a primary renal calcium leak include fasting hypercalciuria and ionized hypocalcemia; elevated serum PTH, urinary cyclic AMP, and serum $1,25\text{-}(OH)_2D_3$; parathyroid hyperplasia; and negative (or neutral) calcium balance relative to WKY (33). In short, renal hypercalciuria is consistent with whole animal calcium deficiency in SHR. Absorptive hypercalciuria predicts normal fasting urinary and serum ionized Ca; suppressed parathyroid function and gland size; and positive (or neutral) Ca balance relative to WKY (33). Pure absorptive hypercalciuria therefore indicates calcium surfeit rather than deficit. This matter will be readdressed after discussion of Ca absorption, vitamin D metabolism, and Ca balance.

Hypercalciuria is also found in the uninephrectomized, DOC-treated rat model in response to feeding sodium chloride but not sodium bicarbonate (34). In this model, hypercalciuria precedes the onset of hypertension. Similarly, urinary calcium excretion is increased in the Dahl salt-sensitive hypertensive rat (115) and in the Milan rat strain of spontaneous hypertension (36) compared to their respective normotensive controls. Hypercalciuria appeared to be on a renal basis in both the DOC and Milan hypertensive strains. Thus, hypercalciuria is common to several forms of experimental hypertension. A possible explanation for the hypercalciuria may be decreased renal adenylate cyclase response to PTH as described for Dahl salt-sensitive and DOC–NaCl hypertensive rats (37). The Milan hypertensive strains are said to have decreased renal Ca^{2+}-ATPase activity (38). Either of these abnormalities could contribute to a failure of normal renal calcium reabsorption (i.e., a renal leak).

Increased urinary calcium excretion has also been found in human essential hypertension (1, 39). Basal 24-hr urinary calcium excretion was higher in hypertensives than in normotensive control subjects despite comparable urinary sodium excretion (22). Calcium infusion resulted in higher urinary calcium excretion for a given filtered calcium load (22, 40).

Calcium absorption (Table IV). Intestinal calcium transport and net absorption have been studied by several techniques with seemingly conflicting results. The *in vitro,* everted duodenal sac preparation estimates net active and passive transfer of radiolabeled Ca^{2+} from the mucosal to the serosal surface. In the hands of several investigators, this technique has revealed no difference in duodenal Ca^{2+} absorption in 5- and 10-week-old male SHR (10, 41) and both increased absorption in 12-week-old male SHR (41) and decreased absorption in 5- and 12-week-old male SHR (42). Similarly, *in vivo* techniques for measuring duodenal radioactive Ca^{2+} transfer have indicated both increased absorption in 12-week-old male (41) and 50-week-old female SHR (13) and decreased absorption in 12-week-old male SHR (42). These discrepancies among different laboratories are not readily explained by differences in experimental technique or diet.

Another *in vitro* approach has been to employ the modified Ussing chamber technique which provides a direct measurement of ac-

TABLE IV. INTESTINAL CALCIUM TRANSPORT AND ABSORPTION IN SHR

	Author (Ref.)	Age (weeks)	Sex	Result
Everted duodenal sac (*in vitro*)	Toraason (41)	5		—
		12		↑
	Schedl (42)	5	M	↓
		12	M	↓
	Stern (10)	10	M	—
In situ duodenal uptake	Lau (13)	50	F	↑
In vivo duodenal perfusion	Toraason (41)	12	M	↑
	Schedl (42)	12	M	↓
Ussing chamber	McCarron (43)	12–14	M	↓
	Lucas (44)	12–14	M	↓
		20–24	M	—[a]
		20–24	M	↓[b]
	Lau (45)	35	M	—
Isolated duodenal enterocytes	Roullet (46)	12–14	M	↓
	Drüeke (47)	28–32	M	↓
Balance	Lau (14)	3.5	M	↑
			F	↑
	Bindels (9)	6	M	—
		8	M	—
	Stern (10)	10	M	—[c]
	Lau (13)	10	M	↓
	Lau (13)	25	F	↑
		50	F	↑
Ca load test	Hsu (19)	13–16	M	↑

Note. Arrows indicate direction of difference compared with WKY; (—) indicates no difference found between SHR and WKY.

[a] Animals fed a normal calcium diet.

[b] Animals fed a low calcium diet.

[c] Trend for decreased Ca absorption in SHR.

tive Ca^{2+} flux across isolated intestinal segments. Ca^{2+} flux driven by the electrochemical gradient is eliminated by voltage clamping the preparation and by exposing the mucosal and serosal membrane surfaces to the same concentration of Ca^{2+}. Using this technique, the unidirectional mucosal-to-serosal Ca^{2+} flux was significantly reduced in 12- to 14-week-old male SHR when compared to the WKY (43, 44). Ca^{2+} secretion (serosal-to-mucosal flux) was not different between SHR and WKY with the result that net duodenal Ca^{2+} flux across the isolated duodenal segment was reduced in the adolescent SHR (43, 44). By contrast, Ussing studies on 24 (44)- and 35 (45)-week-old male animals revealed no difference between SHR and WKY in unidirectional and net Ca^{2+} flux across duodenum and colon on a normal Ca^{2+} diet although duodenal flux was reduced in 24-week-old SHR maintained on a low Ca^{2+} diet (44).

Isolated duodenal enterocytes provide another technique for assessment of Ca^{2+} metabolism in intestinal epithelium. Enterocytes can be isolated by mechanical vibration and remain viable in a cell suspension for approximately 90 min as judged by trypan blue dye exclusion. Influx of radiolabeled ^{45}Ca was decreased in enterocytes isolated from the proximal duodenum of 12- to 14- and 28- to 32-week-old male SHR as compared with age-matched WKY (46, 68). Also, ^{45}Ca efflux was decreased in enterocytes from 12- to 14-week-old SHR (47). While cellular flux data do not measure actual transmucosal Ca^{2+} flux, these data indicate that one step of intestinal Ca^{2+} transport is reduced in growing as well as in mature male SHR.

Because *in vitro* and segmental *in vivo* techniques may not fully account for differences in paracellular and segmental intestinal calcium absorption, measurements of

whole animal Ca^{2+} absorption have been attempted. Stern *et al.* (10) performed balance studies on male SHR and WKY from 6 to 10 weeks of age and reported no significant difference in cumulative Ca intake and mean daily urinary and fecal calcium excretion. Over the 4-week balance study, SHR retained 180 ± 15 mg Ca^{2+} as compared with 218 ± 24 mg Ca^{2+} in WKY. Cumulative Ca absorption calculated by us from these authors' measurements of Ca intake and fecal excretion was 89.2 mg for SHR and 169 mg for WKY over the last 2 weeks of the balance period suggesting a trend for reduced Ca^{2+} absorption and cumulative balance in growing male SHR. Similarly, Lau *et al.* found reduced fractional Ca^{2+} absorption and a trend for reduced absolute Ca^{2+} absorption and Ca^{2+} retention in 10-week-old male SHR over a 6-day balance study (13). By contrast, Bindels *et al.* found no difference in Ca^{2+} absorption in 6- and 8-week-old male SHR (9) and Hsu *et al.* reported increased Ca^{2+} absorption in 13- to 16-week-old male SHR on the basis of increased urinary Ca^{2+} excretion following an oral load of either cold or radiolabeled Ca^{2+} (19). Ca^{2+} hyperabsorption was also found in balance experiments performed on 3.5-week-old prehypertensive male and female SHR (14) and in mature, hypertensive 25-week and 50-week-old female SHR (13). In general, these data from several laboratories using different diets indicate that male SHR malabsorb Ca^{2+} as they mature and develop hypertension. Ca^{2+} malabsorption becomes less apparent as the SHR ages and, in very early life, the SHR may actually hyperabsorb Ca^{2+}. Female SHR hyperabsorb Ca at all ages studied (3.5, 25, and 50 weeks). There is currently no explanation for these differences between sexes, although the findings in the female remain to be confirmed in other laboratories. Unfortunately, the balance technique for measuring Ca absorption in small animals cannot accurately measure small differences in Ca^{2+} absorption and is subject to error, especially if urine or feces become contaminated with uneaten food. More accurate methods are desirable for definitive determination of Ca balance.

Bone (Table V). Since bone is the largest store of Ca^{2+} in the body, bone mineralization should provide an accurate index of whole animal Ca^{2+} balance. Bone mineralization reflects net Ca^{2+} retention as determined ultimately by intestinal absorption and urinary excretion of calcium and as mediated by PTH, $1,25\text{-}(OH)_2D_3$, and other Ca^{2+}-regulating hormones. In male SHR, bone Ca^{2+} content (mg Ca/gm fat-free dry weight) was not different from WKY at 6 and 18 weeks of age (9, 19) but was significantly decreased at 23 (44) and 54 (48) weeks of age. Similarly, ash weight/volume and ash weight/dry weight of the femoral bone were unchanged in 6-week-old male SHR (9) but were reduced in 26-week-old male SHR (49). Bone cortical thickness was also reduced in the 26-week-old animal (49). By contrast, a study of hydralazine-treated female SHR reported increased fractional bone mass and Ca^{2+} density at 1 year of age (13). Thus, in male SHR bone Ca^{2+} is normal in early life but falls below WKY as the animals reach late adolescence and early adulthood. Reduced bone Ca^{2+} and mineralization in SHR is consistent with reduced Ca^{2+} retention over time mediated by reduced Ca^{2+} absorption and/or increased excretion. The situation appears to be quite different for female SHR. To date, Ca^{2+} absorption, balance, and bone status have not been measured in other experimental models or in human hypertension.

TABLE V. BONE MINERALIZATION IN SHR

Author (Ref.)	Age (weeks)	Sex	Result
Bindels (9)	6	M	—
Hsu (25)	18	M	—
Lucas (44)	23	M	↓
Izawa (49)	26	M	↓
Metz (48)	54	M	↓
Lau (13)	52	F	↑

Note. Arrows indicate direction of difference compared with WKY; (—) indicates no differences found between SHR and WKY.

Vitamin D (Table VI). Vitamin D metabolism appears to be abnormal in the SHR and may partially explain the decreased intestinal Ca absorption. Altered vitamin D regulation may also be involved in the increased renal excretion of Ca^{2+} and altered cellular Ca^{2+} metabolism (see below) found in hyperten-

TABLE VI. SERUM 1,25-$(OH)_2D_3$ IN SHR

Author (Ref.)	Age (weeks)	Sex	Result
Lau (14)	3.5	M	↑
		F	↑
Kawashima (50)	4	M	—
	12	M	—
Schedl (51)	5	M	—[b]
	5	M	↓[c]
	12	M	↓[b]
Stern (10)	6	M	—
	10	M	—[a]
Bidels (9)	8	M	↑
Merke (21)	11	M	↓
	12	M	↓
Schedl (42)	12	M	—
Kurtz (52)	12	M	↓
Lucas (44)	12–14	M	↓
Young (27)	13	M	↓

Note. Arrows indicate direction of differences from WKY; (—) indicates no difference found between SHR and WKY.

[a] No statistical analysis provided. Absolute value lower in SHR but probably not significant.

[b] Animals fed a high Ca^{2+} diet.

[c] Animals fed a low Ca^{2+} diet.

sion. While serum levels of 1,25-$(OH)_2D$ are variously reported to be elevated (9, 14) or unchanged (10, 42, 50, 51) in young male SHR from 3.5 to approximately 13 weeks of age, several laboratories have detected low basal serum 1,25-$(OH)_2D$ concentration in SHR as early as 11 weeks of age (21, 27, 44, 51, 52). That is, low serum 1,25-$(OH)_2D$ levels are found just as the SHR are developing hypertension and Ca^{2+} malabsorption. Low or even normal serum 1,25-$(OH)_2D$ levels are clearly inappropriate since the SHR have low $[Ca^{2+}]_s$ (Table I), low serum phosphorus (9, 31), and elevated PTH (Table II). Serum 25-(OH)D tends to be elevated in the SHR indicating that vitamin D intake or substrate availability is not compromised and therefore cannot account for the inappropriately low 1,25-$(OH)_2D$ (25, 42, 51). Additional evidence for altered vitamin D metabolism in the SHR is indicated by subnormal increments in serum 1,25-$(OH)_2D_3$ in response to PTH (27, 50), cyclic AMP (27), and phosphate depletion (27, 52). The response of serum 1,25-$(OH)_2D$ to a low Ca^{2+} diet has been reported to be both decreased (44) and normal (51) in the SHR. The low basal and PTH-stimulated serum 1,25-$(OH)_2D$ in SHR is explained entirely by reduced production rate since metabolic clearance of 1,25-$(OH)_2D$ is not different in SHR and WKY (20). Still more evidence for abnormal vitamin D metabolism is found in the observation that SHR but not WKY develop hypocalcemia and low serum 1,25-$(OH)_2D$ when placed on a vitamin D deficient diet between the ages of 4 and 13 weeks (25). Serum 25-(OH)D falls to undetectable levels in both groups although only WKY are able to maintain 1,25-$(OH)_2D$ and Ca^{2+} homeostasis.

The mechanism of abnormal 1,25-$(OH)_2D$ production in SHR is unknown but intrinsic enzyme activity from isolated mitochondria appears to be normal or even enhanced (50). Cyclic AMP is involved in PTH stimulation of 1,25-$(OH)_2D$ synthesis (53) and urinary cyclic AMP generation is decreased in SHR. However, cyclic AMP infusion caused a smaller increment in serum 1,25-$(OH)_2D_3$ in SHR than in WKY suggesting an additional defect (27). Increased intracellular or mitochondrial concentration of Ca^{2+}, inorganic phosphate, or hydrogen ion could possibly suppress activity of the 1α-hydroxylase enzyme in renal proximal tubules since these are postulated regulators of the enzyme. There is some evidence for systemic acidosis (54) and phosphate retention (9, 55) in SHR although the intracellular ionic status has not been measured. Cytosolic free Ca^{2+} is elevated in some cells of the SHR (see below), but preliminary reports indicate unchanged or low levels in renal proximal tubules (56, 57).

Although some studies have suggested that SHR display intestinal resistance to the effects of 1,25-$(OH)_2D_3$ (41, 42), more recent work demonstrates normal (44) or increased (58) intestinal response to the hormone. Lucas *et al.* found that Ca^{2+} flux across isolated duodenal segments measured by Ussing apparatus is normalized in SHR when they are given supplemental 1,25-$(OH)_2D_3$ (44). Similarly, the calcemic response to systemic PTH infusion is blunted in SHR and is normalized when the animals are given 1,25-$(OH)_2D_3$ (29). Thus it appears that the

defective vitamin D metabolism found in SHR is of functional importance.

Vitamin D metabolism may also be altered in human hypertension. Resnick *et al.* reported high serum 1,25-$(OH)_2D$ in patients with low renin hypertension and low serum 1,25-$(OH)_2D$ in patients with high renin hypertension (59). For each renin group, serum 1,25-$(OH)_2D_3$ varied inversely with serum ionized Ca^{2+} although the appropriateness of the response was not assessed quantitatively. These investigators hypothesize that 1,25-$(OH)_2D_3$ is an important factor in the mediation of low renin hypertension. Further work on vitamin D metabolism in human hypertension and in other experimental models is needed.

Integrated View of Ca^{2+} Metabolism in SHR. In view of the above described alterations in Ca^{2+} metabolism in the SHR, it is worthwhile to attempt a synthesis of the various observations into a more unified view of calcium status. For now, too little information exists to attempt a synthesis for human hypertension although Ca^{2+} metabolism in the SHR and human essential hypertension appear to be quite similar. Figure 1 is a schematic, longitudinal, representation of systemic Ca^{2+} metabolism in the SHR relative to WKY based on data cited above in the text and tables. While the conditions of the various experiments vary markedly in terms of experimental design, diets, source of animals, and other important factors, the figure may be useful for identifying general trends. It appears likely that differences between SHR and WKY are more likely to be found when the animals are on a relatively low Ca^{2+} diet. Male SHR have been studied most extensively and are known to develop earlier and higher elevations of blood pressure than female SHR; therefore most comments will apply to the male. Figure 1 shows that the major discrepancies in the literature concern the issue of intestinal Ca^{2+} transport and absorption. Nonetheless, many investigators find evidence for reduced Ca^{2+} absorption in young SHR. As the animals age, the difference between SHR and WKY seems to decrease. Basal serum 1,25-$(OH)_2D$ may initially be elevated but ultimately falls. The time course data are insufficient to determine if Ca^{2+} malabsorption is solely the result of abnormal vitamin D metabolism. Nonetheless, the reduced serum ionized Ca^{2+} and elevated serum PTH levels at a very young age suggest that net calcium retention is reduced in the SHR. However, gross Ca^{2+} depletion is not manifest until adulthood when decreased bone Ca^{2+} concentration is found. Finally, hypercalciuria appears in late adolescence just prior to the time when a measurable reduction in bone mineralization occurs. While the cause of the increased urinary calcium has been disputed (e.g., 11, 13, 19), the predominant finding of reduced or normal calcium absorption and reduced bone calcium (Fig. 1) argues strongly against an intestinal source for the calcium. Furthermore, the findings of low serum ionized Ca^{2+} concentration, elevated PTH levels, and parathyroid hyperplasia (21) in SHR largely fulfills the criteria for renal hypercalciuria (cf. above). Urinary cyclic AMP and serum 1,25-$(OH)_2D$ are not elevated as predicted for renal hypercalciuria, perhaps because of a defect in the systems that control their production and release. The interpretation of

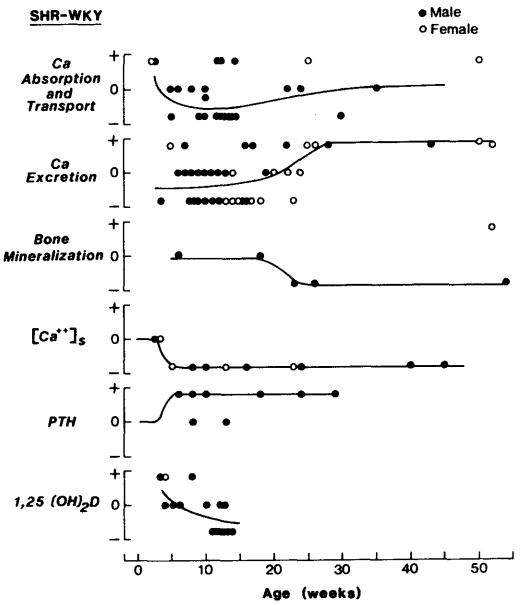

FIG. 1. Schematic representation of data from many reports showing calcium status over the life of the SHR. Ordinate indicates qualitative differences (+, 0, −) between SHR and WKY. Lines are meant to indicate a stylized fit of data as discussed in the text.

renal hypercalciuria indicates that the male SHR is indeed Ca^{2+} deficient. In female SHR, $[Ca^{2+}]_s$ falls early but hypercalciuria occurs later than in males for unclear reasons. Ca^{2+} hyperabsorption and increased bone mass are found in the female although the data are limited. Clearly, more complete longitudinal studies of systemic Ca^{2+} metabolism in males and females would be valuable.

Effect of calcium loading. Although provocative, the systemic alterations in Ca^{2+} handling are of uncertain relevance to hypertension. Since Ca^{2+} is the critical intracellular mediator of vascular smooth muscle contraction, an attractive explanation for abnormal systemic Ca^{2+} metabolism invokes an intrinsic abnormality of pan-cellular Ca^{2+} metabolism that results in elevated vascular tone and altered intestinal and renal Ca^{2+} handling. An important link between abnormal systemic Ca^{2+} metabolism and hypertension is the observation that dietary Ca^{2+} supplementation lowers blood pressure in SHR (11, 28, 30, 43, 60), in other experimental models of hypertension (35, 61, 62), and in human essential hypertension (39, 63). The mechanism of the antihypertensive effect of dietary Ca^{2+} is not known. Calcium may decrease sympathetic nerve activity as judged by circulating catecholamines or the systemic response to stress (64). Some investigators have found that dietary Ca^{2+} promotes natriuresis (30), although others have not (28, 60) and supplemental dietary Na appears to augment the hypotensive effect of Ca (43). The effect of dietary Ca^{2+} has also been ascribed to phosphate depletion (60). However, oral phosphate loading causes a decrease in blood pressure, not an increase as would be predicted by the phosphate depletion hypothesis (65, 66). Ca^{2+} may also lower blood pressure by virtue of its effect on PTH, vitamin D metabolites, or other hormones such as calcitonin gene related peptide (67), all of which may have acute and chronic vascular actions. Finally, dietary calcium may favorably modulate a defect in cellular Ca^{2+} handling leading to normalization of systemic Ca^{2+} handling and blood pressure. For example, a high calcium diet caused increased Ca^{2+} influx in enterocytes isolated from SHR and reduced Ca^{2+} influx in enterocytes isolated from WKY; the net result was that Ca^{2+} influx became the same in SHR and WKY as compared with a normal Ca^{2+} diet (68). Whatever the mechanism by which Ca^{2+} lowers blood pressure, an understanding of the cellular processes behind abnormal systemic Ca^{2+} metabolism is essential.

Cellular calcium metabolism. The questions of whether organ level disturbances in Ca^{2+} metabolism in humans with essential hypertension and the SHR are the result of alterations in cellular calcium metabolism and whether these alterations play a causal role in chronically elevated blood pressure are pertinent. One would predict that Ca^{2+} metabolism of vascular smooth muscle is abnormal in the hypertensive state if indeed this cell type is involved in a primary fashion in the pathogenesis of the disease as opposed to playing a passive role. Direct investigation in the human situation has been limited mostly as a result of difficulties encountered in obtaining appropriate tissue samples. As a result, the majority of information has been drawn from studies on formed elements of the blood derived from humans with essential hypertension or using vascular tissue isolated from animal models of hypertension. The latter case will be addressed first since it is most relevant.

Vascular smooth muscle. One primary hypothesis accounting for hypertension of vascular origin is that there is a defect in the ability of the vascular smooth muscle cell to maintain normal levels of intracellular Ca^{2+}, principally through a depressed control system. Cellular Ca^{2+} metabolism has been studied using a variety of techniques including measurement of flux of ^{45}Ca into intact tissues and isolated membrane preparations, enzymatic analysis of pumps or carriers believed to play a role in the regulation of intracellular levels of Ca^{2+}, and more recently, the use of intracellular indicators to assess free levels of intracellular Ca^{2+}.

Studies of vascular smooth muscle isolated from the SHR, which have examined ^{45}Ca influx into whole tissue, have shown an increase (69), no change (70), or a decrease in influx (71). These results, of course, are dependent upon the age of the animal, the time course over which influx was determined,

and the method of defining intracellular vs extracellular ^{45}Ca. Generally, only limited conclusions about specific pools of Ca^{2+} or binding sites can be drawn from this type of investigation. While early studies focused on conduit arteries, Cauvin *et al.* have recently examined unidirectional influx of ^{45}Ca into mesenteric resistance vessels of the SHR (72, 73) and observed enhanced influx of Ca^{2+} under both basal and norepinephrine-stimulated conditions. These studies derive support from the finding of Mulvany and Nyborg (74) that resistance arteries of the mesenteric bed have an increased sensitivity to extracellular Ca^{2+}. These results, in addition to the observations that Ca^{2+} channel blockers have a greater blood pressure-lowering effect and vasodilator action in essential hypertension than in control subjects, have been interpreted (76–78) to indicate that the cell membrane of vascular smooth muscle is more permeable to Ca^{2+} in the hypertensive state.

Studies of Ca^{2+} transport by isolated membrane fractions of vascular smooth muscle show a consistent trend that suggests a depressed ability of the smooth muscle cell membrane to actively transport Ca^{2+}. When ATP-supported Ca^{2+} uptake was measured in microsomal fractions of aorta isolated from SHRs (69, 75, 79) or from cell membrane-enriched fractions of mesenteric arteries of the SHR (80, 81), uptake was attenuated compared with fractions isolated from normotensive controls. It is possible that this decrease in uptake is a reflection of an abnormal cell membrane Ca^{2+}-ATPase moiety, although potential effects of factors such as differential protein content of the membranes, which is used as a normalization factor, have not been eliminated.

Perhaps a more rigorous approach would combine Ca^{2+} uptake studies using isolated membrane vesicles with kinetic analyses of the purified Ca^{2+}-ATPase. While this enzyme has been purified from both aortic muscle of the cow (82) and smooth muscle of the pig antrum (83, 84), it has thus far been resistant to attempts at purification from vascular smooth muscle of the rat primarily as a result of limited availability of tissue. Therefore, the enzyme has not been well characterized in experimental hypertension. On the other hand, Ca^{2+}-ATPase isolated from other smooth muscle systems has been shown to be activated by calmodulin and has a requirement for Mg^{2+} as well as Ca^{2+} and ATP and is thus similar to membrane-associated Ca^{2+}-ATPase from other organ systems, i.e., skeletal and cardiac muscle (85). It may therefore be inferred that the enzyme is similar in the various smooth muscle types but knowledge of potential differences in either its activity or activation characteristics during hypertension does not exist.

In addition to the Ca^{2+}-ATPase transport system, the Na^+–Ca^{2+} exchange carrier may also play a role in the regulation of intracellular Ca^{2+}. At least two groups have detected the presence of this carrier in cell membrane-enriched fractions of vascular smooth muscle (86, 87). In the former study, a comparison of Na^+–Ca^{2+} exchange activity of cell membrane-enriched fractions of mesenteric arteries of the SHR and WKY was carried out and no differences were detected. Thus, while it is likely that this carrier plays a role in modulating cell Ca^{2+} in vascular smooth muscle, there is currently no direct biochemical evidence to suggest that its activity is altered during experimental hypertension of genetic origin.

Besides these transport systems which are directly involved in Ca^{2+} transport, the Na^+,K^+-ATPase/sodium pump system is believed to play a role in the regulation of intracellular Ca^{2+} levels by its electrogenic contribution to the membrane potential and thus an indirect effect on potential-operated Ca^{2+} channels (88). The sodium pump also regulates levels of intracellular sodium and thus may help set the level of intracellular Ca^{2+} via the Na^+–Ca^{2+} exchange mechanism which is discussed above. To date, several models of experimental hypertension show depressed sodium pump function when assessed indirectly via either ^{86}Rb uptake (89) or potassium-induced relaxation of isolated vessels (90). However, when the number of pump sites in aorta of SHR and WKY was determined using [^{3}H]ouabain binding, no differences were detected (91), indicating that the pumps may be different physiologically but perhaps not enzymatically or biochemically. In addition to the sodium pump, a second transport system that may be linked

to Ca^{2+} regulation via alterations in intracellular Na^+ is the Na^+–H^+ exchanger. This carrier has been identified in membrane preparations of vascular smooth muscle (92) and is activated by the diacyl glycerol arm of the phosphotidylinositol pathway (93). While it has been suggested to provide a major Na^+ influx pathway (94), it has not been studied to date in vascular smooth muscle of the SHR or its normotensive control.

Given this discussion of the various systems involved in the regulation of intracellular Ca^{2+} levels, the primary question remains as to whether intracellular Ca^{2+} concentration is altered under basal or stimulated conditions. Studies using ^{45}Ca allow some estimation of influx rates and total exchangeable Ca^{2+} binding sites, but do not allow delineation between bound and free Ca^{2+}. A recent advancement in this area has been the application of the intracellular Ca^{2+} dyes Quin-2, Fura-2, and Indo-1 to both smooth muscle and formed elements. At least two reports exist which have examined intracellular Ca^{2+} levels in vascular smooth muscle during hypertension. Nabika *et al.* (128) examined monolayer of cells grown on coverstrips and found no differences in $[Ca^{2+}]_i$ between the SHR and WKY. On the other hand, Sugiyama *et al.* (129) examined both primary and passaged smooth muscle cells and found that $[Ca^{2+}]_i$ was higher in vascular smooth muscle cells of SHRs from 12-week-old rats. While reasons for these differences are not apparent, careful assessment is needed of potential differences in loading efficiencies and the effects of cell dispersion techniques or culture conditions on subsequently observed differences or similarities of the response between cells from hypertensive and normotensive animals.

Platelets. In addition to vascular smooth muscle of hypertensive animal models, Ca^{2+} metabolism has also been studied in formed elements of the blood of humans with essential hypertension and from the SHR, with the expectation that differences observed in these cell types are reflections of differences also present in vascular smooth muscle cells or that the same (humoral or pharmacological) factors that induce changes in formed elements also impinge on vascular smooth muscle cells and resistance vessels. To date, platelets have been most extensively studied, primarily because of the relative ease and quantity in which they are obtained as well as their similarity to vascular smooth muscle in terms of second messenger responses (including cAMP and inositol triphosphate pathways) and the presence of the myosin light-chain kinase–myosin light-chain system which plays a functional role in agonist-mediated shape changes (97).

Several groups have identified an increase in the intracellular levels of Ca^{2+} in platelets derived from people with essential hypertension using Quin-2 (98–102) and more recently Fura-2 (103) as an intracellular indicator. Of those groups, Erne *et al.* (98) and Le Quan Sang *et al.* (101) observed a positive relationship between blood pressure and intracellular Ca^{2+} concentration. Furthermore, Erne *et al.* (98) have observed a fall in intracellular Ca^{2+} upon lowering of blood pressure with antihypertensive therapy. The latter result may be interpreted to indicate either that the humoral profile of the plasma compartment was altered to provide for a lowering of intracellular Ca^{2+} in the platelet or that the platelets themselves were affected in a manner parallel to that of the vascular smooth muscle. While these suggestions are possible, it also needs to be considered that shear stress experienced by the platelets during their course through resistance vessels and capillaries may vary with mean arterial pressure and thus be a direct cause of elevation of intracellular Ca^{2+} as has been reported for red blood cells (104). If this were to be the case, then the applicability of elevated intracellular Ca^{2+} in platelets as a marker for changes in vascular smooth muscle is limited.

In addition to intracellular Ca^{2+} concentration, platelet Ca^{2+}-ATPase has been examined in essential hypertension and it was observed that overall Ca^{2+}-ATPase activity was greater in platelets of hypertensives while calmodulin stimulated the ATPase to a lesser extent than in control (105). While these results do not readily explain the elevated levels of intracellular Ca^{2+} reported in platelets, these investigators suggest that the elevated Ca^{2+}-ATPase capacity reflects a negative feedback control mechanism by which the cell protects itself from a Ca^{2+} overload.

In addition to the changes in the ATPase activity, increased responses of platelets from people with essential hypertension to PGE_1, in terms of cAMP levels, and to serotonin and epinephrine in terms of phosphorylation of proteins and shape changes have also been observed (106). The latter results suggest that platelets are altered in the hypertensive state but it remains to be determined whether these changes are linked to Ca^{2+} metabolism in a direct fashion.

Erythrocytes. In addition to platelets, erythrocytes have also been studied in human and experimental hypertension, primarily because of the major role that the cell membrane Ca^{2+}-ATPase plays in regulating red cell Ca^{2+} concentration and the ease of obtaining samples. Postnov *et al.* (107) examined Na^+ permeability and Ca^{2+} binding capacity (defined as the pool removable by incubation with EDTA) and found that Na^+ flux was elevated while extracellular Ca^{2+} binding capacity was depressed during hypertension. Furthermore, intracellular Ca^{2+} was observed to have an inhibitory action on Na^+–K^+-ATPase function in red cells of essential hypertensives and this has been interpreted to reflect a depressed inner cell membrane binding capacity. The authors provide no mechanistic link between binding of Ca^{2+} to the inner side of the plasma membrane and Na^+–K^+-ATPase activity. In a later study, Postnov *et al.* (108) reaffirmed the findings of depressed binding of Ca^{2+} to outer membranes of red cell ghosts and additionally have carried out a study of Ca^{2+}-ATPase activity of erythrocytes obtained from humans with essential hypertension and found that upon depletion of the preparation of calmodulin, no differences in Ca^{2+}-ATPase activity were observed between hypertensives and normotensive controls. However, upon readdition of calmodulin, the erythrocyte Ca^{2+}-ATPase from hypertensive subjects was stimulated to a lesser degree, while there was no difference in the distribution of calmodulin in red cells of the two groups. These data, and an additional report of a biochemical characterization of calmodulin isolated from the brains of SHRs and WKY controls (109) which showed no differences in the affinity of the activation protein for Ca^{2+} or in its ability to stimulate phosphodiesterase activity, have been interpreted to indicate that a defect in the enzyme that is specific to the enzyme's capacity to be stimulated by calmodulin may be present in hypertension. In addition membrane binding of Ca^{2+} by the red cell is altered.

Confirmation of the findings of Postnov's group by other laboratories will permit greater emphasis to be placed on these findings. To this end, Vincenzi *et al.* (110) examined Ca^{2+}-ATPase activity of red cells of hypertensive and normotensive humans. The results showed that basal Ca^{2+}-ATPase activity, defined as ATPase activity resistant to inhibition by the calmodulin inhibitor, trifluoroperazine, was depressed in those with high blood pressure, while maximal stimulated activity was not different. While these data do not agree with those of Postnov *et al.* (109), a study by Olorunsogo *et al.* (111) reported that maximum red cell Ca^{2+}-ATPase activity was depressed with hypertension, but activation by calmodulin was normal. It is clear that additional work is needed for total clarification of these issues.

Other formed elements. In addition to platelets and red cells, intracellular Ca^{2+} levels have also been examined in neutrophils by Lew *et al.* (112) and no differences were observed between normotensive and hypertensive individuals. These authors concluded that not all cell types demonstrate elevated levels of intracellular Ca^{2+} during hypertension. Brushi *et al.* (113) also examined lymphocytes from the SHR and observed a higher concentration of free intracellular Ca^{2+} compared with cells derived from control, but these levels were not responsive either to alterations in extracellular Ca^{2+} or to the addition of ouabain or catecholamines. The efficacy of the loading procedure used for Quin-2 is questionable when no transients are observed since unhydrolyzed Quin-2 ester produces a fluorescence signal that is unresponsive in terms of transients.

Summary of cellular Ca^{2+} metabolism. It is concluded from this review of cellular Ca^{2+} metabolism in hypertension that vascular smooth muscle in experimental hypertension may exhibit changes in basic mechanisms of Ca^{2+} regulation, but more work is required before the picture will be complete.

Additionally, studies on formed elements have demonstrated elevated Ca^{2+} in platelets of humans and in Ca^{2+}-ATPase of red cells from experimental models. While of interest in their own right, the results obtained with formed elements allow only limited insight into the state of Ca^{2+} metabolism in vascular smooth muscle during hypertension. It may well be that changes in Ca^{2+} metabolism of the formed elements reflects altered physiology of the organism in terms of responses to humoral factors, but the degree to which they reflect similar changes in vascular muscle remains to be answered.

Conclusion. Clearly, Ca^{2+} metabolism is altered in hypertension at both the whole animal and cellular level. Furthermore, dietary Ca^{2+} deficiency is associated with human hypertension at the epidemiologic level and oral Ca^{2+} supplementation lowers blood pressure in human and experimental hypertension. In this review, we have presented the integrated view that a constellation of systemic abnormalities of Ca^{2+} metabolism in hypertension results in inefficient Ca^{2+} conservation and relative Ca^{2+} deficiency. Furthermore, the cellular findings in hypertension suggest increased cell membrane Ca^{2+} permeability and a compromised ability of the cell to remove or sequester intracellular Ca^{2+}. Paradoxically this leads to increased intracellular free Ca^{2+} stores in the face of reduced extracellular Ca^{2+}. Unfortunately most of the whole animal and cellular work has been carried out using different tissues. Nonetheless, it seems reasonable to hypothesize that abnormal Ca^{2+} handling at the cellular level is ultimately reflected at the organ and whole animal level so that alterations in vascular smooth muscle, intestine, kidney, erythrocytes, and so on are due to the same underlying defect manifested in many cell lines. The underlying defect may involve the cell membrane Ca^{2+}-ATPase (68), an intrinsic membrane binding protein (114), a cell membrane Ca^{2+} channel, or perhaps some other process.

If an underlying cellular defect in Ca^{2+} metabolism exists in some forms of hypertension, then what is the link with elevated blood pressure? While altered Ca^{2+} metabolism in hypertension may be an epiphenomenon of some other metabolic process that is primarily responsible for the elevated blood pressure, a more central role for altered Ca^{2+} metabolism in hypertension is suggested by the myriad of defects that have been reported. However, the issue will remain unsettled until the underlying bases for disrupted calcium and vascular homeostasis in hypertension are found. Understanding of calcium metabolism and blood pressure regulation in hypertension, whether as shared or separate mechanisms, should prove to be a challenging but productive area of future research.

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