Calcium: Taste, Intake, and Appetite

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Tordoff, Michael G. Calcium: Taste, Intake, and Appetite. *Physiol Rev* 81: 1567–1597, 2001—This review summarizes research on sensory and behavioral aspects of calcium homeostasis. These are fragmented fields, with essentially independent lines of research involving gustatory electrophysiology in amphibians, ethological studies in wild birds, nutritional studies in poultry, and experimental behavioral studies focused primarily on characterizing the specificity of the appetite in rats. Recently, investigators have begun to examine potential physiological mechanisms underlying calcium intake and appetite. These include changes in the taste perception of calcium, signals related to blood calcium concentrations, and actions of the primary hormones of calcium homeostasis: parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D. Other influences on calcium intake include reproductive and adrenal hormones and learning. The possibility that a calcium appetite exists in humans is discussed. The broad range of observations documenting the existence of a behavioral limb of calcium homeostasis provides a strong foundation for future genetic and physiological analyses of this behavior.

I. INTRODUCTION

Calcium is essential for life. It contributes to the electrical potential of cell membranes and is involved in many fundamental processes, including DNA synthesis, enzyme activity, photo- and chemosensory transduction, neurotransmitter release, membrane permeability, and intercellular communication. In vertebrates, calcium is also the major component of bone. Given these and many other functions, it is not surprising that disturbances in calcium metabolism have been implicated in most of the major chronic diseases, including osteoporosis, kidney disease, obesity, heart disease, and hypertension (for reviews, see Refs. 8, 20, 115, 116, 186, 187, 195, 231, 325). This has led to concern among nutritionists that the majority of the United States population does not eat as

much calcium as they need (28, 191, 193, 196, 210, 212a, 310; see Refs. 118, 311 for contrasting views).

The pervasive contribution of calcium to physiology, coupled with recent advances in the ability to measure intracellular calcium levels, makes it a key area of study. For the past several years, there have been >1,000 peer-reviewed papers published each month that include "calcium" as a keyword (60a). There are several fine reviews of the homeostatic controls underlying the regulation of intracellular calcium, blood calcium concentrations, and bone (e.g., Refs. 159, 209). However, none considers the possibility that body calcium is regulated behaviorally as well as physiologically.

The purpose of this review is to readdress this imbalance. In fact, there is a moderately large body of work on calcium appetite, but it is widely scattered and badly splintered. In the avian literature, calcium appetite is a well-accepted phenomenon, although there is little connection made between studies on wild and domesticated birds. There have also been very few attempts to examine the physiological basis of the appetite and no studies of calcium gustation in birds. The mechanisms of calcium gustation have been studied most extensively in amphibians, but there are no accompanying behavioral or physiological studies for this class. Work on calcium appetite in mammals had a promising start in the 1930s (76, 242, 243) but foundered in the 1960s until recently. The lack of interest was due to work showing that rats could use arbitrary flavors to recognize sources of calcium (248, 263). From this, it was inferred that calcium appetite is solely a learned behavior, and thus uninteresting, or at least simply one of many learned appetites. This contrasted markedly with the appetite for sodium, which was considered to have innate properties and a clear anatomical and physiological substrate (248; see Refs. 67, 262 for review). The emphasis on sodium appetite as a unique behavior has overshadowed calcium appetite, and much of the information gleaned about calcium taste and intake comes from studies directed primarily at understanding sodium taste and intake.

Calcium appetite is the motivation to seek out or choose calcium-containing items. This implies that animals are capable of detecting calcium or some marker for it. The evidence for this is mustered in section II, which also deals with the spontaneous or "need-free" ingestion of calcium. Section III covers primarily behavioral observations that form the backbone of the phenomenology of calcium appetite. A major issue involves how calcium appetite is manifested. Does an animal deprived of calcium seek calcium ions, minerals, saltiness, bitterness, or something else? Section IV addresses this question of the appetite's specificity, or lack of it. Section V musters the evidence for and against various potential physiological mechanisms underlying calcium appetite. The final two sections raise the issue of whether or not humans have a

calcium appetite and provide a brief commentary on data needs and potential future directions of this field.

This review is limited to vertebrates. Although several invertebrates, including American cockroaches (95), aquatic beetles (123), and various moth caterpillars (94, 96) reject high concentrations of calcium, nothing is known about the mechanisms involved.

II. CALCIUM TASTE AND CALCIUM INTAKE BY CALCIUM-REPLETE ANIMALS

Sources of calcium could potentially be detected by sight, smell, or taste. Of these, taste appears to be the most important, at least for mammals. There is evidence that birds use visual cues to locate calcium (145; see sect. IV*A*). Moreover, chickens offered separate sources of $CaCO_3$ or $SrCO_3$ were unable to distinguish between them unless a red color cue was present (131). But visual cues are not required for calcium detection because chickens respond appropriately when given a choice between diet containing $CaCO_3$ and calcium-deficient diet with white flour added to obscure color cues (131).

Humans and other mammals can detect calcium salts by smell (78, 295). However, at least in humans, the thresholds for detection of calcium odor are many times higher than those for taste (295), and wearing a nose clip does not influence attribution ratings of $CaCl_2$ solutions (121). There is no evidence that calcium salts specifically activate trigeminal afferent fibers, which would imply thermal, textural, or irritant qualities for calcium. Thus the emphasis here is on calcium taste.

A. Sensitivity to Calcium

Amphibians can detect submillimolar concentrations of calcium, which is consistent with levels found in their habitat (141, 228). One comprehensive investigation found changes in activity of the glossopharyngeal nerve of the frog when as little as 10 μM $CaSO_4$ or $CaCl_2$ was applied to the tongue (150).[1] Other investigators report slightly higher threshold concentrations (4, 40, 141, 151, 324), and 1 mM $CaCl_2$ is routinely used as a taste stimulus. High concentrations of $CaCl_2$ (e.g., 250 mM) produce less response than do lower ones (e.g., Ref. 324).

In contrast to the work with amphibians, which was designed primarily to uncover mechanisms of taste trans-

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[1] All concentrations have been converted from the original units to System International units. Some error may have occurred when the form of salt was not specified. For example, many early studies used 2.4% calcium lactate. This is equivalent to 78 mM calcium lactate pentahydrate but 110 mM anhydrous calcium lactate. Here, it is assumed that the most common form of the salt was used, in this case pentahydrate.

duction (see sect. IIB), most of the early work on calcium sensitivity in mammals formed part of broad surveys of gustatory sensitivity to many compounds, with the focus being on NaCl (e.g., Refs. 17, 19, 87). The minimum concentration of orally applied $CaCl_2$ to evoke a response in the chorda tympani nerve of the rat is $\sim$30–100 μM (132, 133, 135, 204). This agrees reasonably well with rat behavioral studies, in which concentration-preference functions for calcium salts were determined. The "thresholds" at which taste solution intake was significantly greater than water intake were 10–400 μM calcium (135, 294). The wide range can probably be attributed to differences in the calcium salt tested and rats' age, diet, and previous experience with calcium solutions.

Human calcium detection thresholds are similar to or slightly higher than those of the rat. Early investigators report detection thresholds ranging between 2 and 30 mM (median $\sim$10 mM; Refs. 83, 96, 128, 309; see also Ref. 217). However, a recent study focused specifically on calcium found the mean detection threshold of 13 women for $CaCl_2$ was 0.16 mM (range 0.01–1 mM, Ref. 295). Detection thresholds for four other calcium salts (lactate, gluconate, hydroxide, phosphate) were similar to those for $CaCl_2$. The much lower values found in this compared with earlier reports are probably due to the use of more sensitive methods, the purity of the water used, the subject's recent calcium consumption, and the subject's sex. The calcium content of the water supply where this study was conducted (Philadelphia) averages $\sim$0.9 mM (range 0.35–1.40 mM) calcium, depending on the season and water's source (218). Thus calcium must contribute variably to the taste of tap water (see also Ref. 326).

Calcium detection thresholds are fairly close to salivary calcium concentrations. Depending on the test conditions, average total saliva calcium levels range between 0.9 and 2.1 mM and ionized calcium from 0.5 to 1.0 mM (see Ref. 247 for an authoritative review). It is possible that the lower limit on calcium detection is determined by the level of ionized calcium in saliva.

B. Calcium Transduction and Taste Quality

Although it is clear that animals can detect calcium in micromolar or low millimolar concentrations, it is less clear what they detect or how they detect it. One obvious possibility is that the mouth contains calcium receptors or ion channels involved in the transduction of calcium. On the other hand, the idea of calcium as a distinct taste quality is an anathema to many psychophysicists, who argue that there are very few basic taste qualities (sweet, sour, salty, bitter, and umami). To them, calcium taste is a complex of basic tastes, such as bitterness, sourness, and saltiness. The major question here, particularly in studies of mammals, has been whether the response patterns produced by oral calcium can be distinguished from the patterns produced by sodium and/or other cations.

1. Calcium transduction: electrophysiological evidence

Amphibian electrophysiology supports the idea of a specific calcium taste mechanism. Gustatory afferent fibers that are sensitive to $CaCl_2$ in the frog respond to calcium and strontium ions (150) but not undissociated $CaCl_2$, low concentrations of chloride, or water (141). The response to calcium is inhibited by NaCl, KCl, magnesium salts, and high ($\geq$0.5 mM) concentrations of chloride (40, 141, 150, 324). This inhibition, the finding that the frog is more sensitive to calcium than it is to sodium (4), and other evidence (see Ref. 4) indicates that the mechanism of calcium transduction differs from that of sodium and other monovalent ions (141, 160; see Ref. 255 for a review).

In the mudpuppy, calcium-dependent ion channels are found in both the apical (chemoreceptive) and basolateral (synaptic) membrane of taste cells, but not in surrounding nontaste epithelial cells (see Ref. 251). Calcium is involved in both the active and passive components of the taste receptor membrane potential (149). The receptor potentials evoked by calcium are not simply due to it entering the cell through calcium channels (see also Refs. 150, 151). The critical transduction process involves modulation of resting potassium conductance (24). Both this finding and work with the frog suggests that transduction of calcium salts involves adsorption of the ion onto the taste cell membrane (24, 144, 150, 151; reviewed in Ref. 256).

This notion is particularly intriguing given the recent discovery of extracellular calcium receptors in mammals, including humans (37, 38). Perhaps such receptors mediate the adsorption and transduction of calcium taste. We recently have found positive staining for antibody to the extracellular calcium receptor (CaR) in taste buds of rat and one of two human fetus tongues examined (N. E. Rawson and M. G. Tordoff, unpublished results). There is also evidence that metabotropic glutamate receptors are found on the tongue and may be responsible for umami taste (46). Metabotropic glutamate receptors in the central nervous system (CNS) are sensitive to extracellular calcium (156, 257), but it is unknown whether this is true of the lingual receptors. Another mechanism underlying calcium transduction may involve ecto-calcium-dependent ATPase, which is found in taste buds of amphibians and mammals (hamsters) and is related to oral calcium concentrations (9). A role for epithelial Na^+/Ca^{2+} exchange seems unlikely because blockade with 5-(N-4-chlorobenzyl)-2′,4′-dimethylbenzamil had no effect on human subjects' perception of $CaCl_2$ taste (259). Studies of the electrophysiological response to oral calcium in mammals are rudimentary. Early work com-

pared calcium and other minerals with sodium. The integrated response of the chorda tympani nerve to continuous stimulation of the tongue with 100 mM $CaCl_2$ declined more rapidly than it did during stimulation with 100 mM NaCl (17), calcium salts evoked a longer discharge after a water rinse than did sodium salts (17), and adaptation of the tongue to 100 mM $CaCl_2$ did not influence the response to 100 mM NaCl (18). Both whole nerve and single-unit recordings from the chorda tympani nerve of the rat showed the response to orally applied 100 mM $CaCl_2$ was ~55% of the magnitude of the response to 100 mM NaCl (17, 19, 87; see Ref. 19 for similar studies in hamster, guinea pig, cat, and dog). In contrast, a more recent study found that $CaCl_2$ applied to the rat tongue produced a whole nerve chorda tympani response that was larger than that to monovalent chlorides (204). This is consistent with work showing that changes in receptor potential produced by application of 100 mM $CaCl_2$ to the rat tongue were "sometimes" larger than those produced by NaCl (147). Such results argue that the perception of calcium and sodium involve different transduction mechanisms, but are otherwise uninformative.

Individual gustatory afferent nerve fibers can be classified by the taste solution that causes the most pronounced (or "best") increase in action potential frequency. Recordings from both the whole chorda tympani nerve (132) and individual fibers (133) indicated that HCl- and NaCl-best fibers are stimulated about equally by oral application of 3, 10, and 31 mM $CaCl_2$ solutions. However, there was considerable variability. All HCl-best fibers responded to $CaCl_2$, but only some NaCl-best fibers were sensitive to it. There was little or no response of sucrose-best fibers to $CaCl_2$. Recordings from the geniculate ganglion of the rat produced different results (31). Application of 50 mM $CaCl_2$ to the tongue caused approximately six times more spikes in acid- than NaCl-sensitive units. Most units in the petrosal ganglion showed only small responses to $CaCl_2$. These units were more sensitive to quinine or atropine, suggesting that $CaCl_2$ may activate a class of alkaloid-sensitive units (30).

Smith et al. (273) performed a comprehensive test of gustatory evoked electrophysiological activity in the nucleus tractus solitarius and parabrachial pons of the hamster. In the nucleus tractus solitarius, HCl-best and NaCl-best but not sucrose-best units responded to $CaCl_2$. In the parabrachial pons, HCl-best, NaCl-best, and many sucrose-best units responded to $CaCl_2$. This paper is noteworthy in that the authors attempted to compare the similarity of tastes based on electrophysiological and behavioral responses, the latter being determined by generalization of conditioned taste aversions (see sect. IIB2). Except for quinine, the correspondence between electrophysiology and behavior was very close.

An interesting study of the chorda tympani response of SIc:ICR mice found that, in contrast to most other species tested, integrated responses to 100 mM $MgCl_2$ and $CaCl_2$ were considerably greater than those to monovalent chlorides (207). Cluster analysis of the responses of individual chorda tympani fibers found that ~23% were narrowly tuned to calcium and/or magnesium. As far as I know, this is the only direct evidence for specific calcium-magnesium taste fibers in mammals.

Various forms of multidimensional scaling have been used to compare the response profiles of neurons in the rat nucleus tractus solitarius (81, 82, 264). Responses evoked by orally applied $CaCl_2$ clustered with those evoked by $MgCl_2$, KCl, and several bitter substances (e.g., strychnine, nicotine, quinine). There was also a close clustering of $CaCl_2$ with sour tastes (acids) in one analysis (based on activity profiles across neurons) but not in another (based on activity profiles across time, Ref. 264). One study compared the response of nucleus tractus solitarius neurons to a variety of taste solutions in sodium-deprived and control rats (136). The main finding was that response profiles elicited by sugars and by sodium salts were more similar in sodium-deprived than control rats. However, the most striking difference in response profile occurred with 300 mM $CaCl_2$. In the replete animals, the response to 300 mM $CaCl_2$ approximated those of HCl, KCl, and citric acid (replicating, Ref. 136), but in the sodium-deprived animals, the response to $CaCl_2$ was closest to quinine. Given that the response profiles relate well to taste qualities described by humans, this study suggests that the rat finds $CaCl_2$ to be predominantly sour when sodium replete but predominantly bitter when sodium deprived.

The method of cross-adaptation has been used to characterize the electrophysiological response to oral calcium (272). The principle of this depends on the observation that during continuous or repeated exposure to a taste solution, electrophysiological and behavioral responses decrease, or adapt. If the tongue is exposed to one taste solution and then another, the response to the second solution is reduced in relation to its similarity to the first. In a comprehensive study involving cross-adaptation of the rat chorda tympani nerve response to $CaCl_2$, $CaBr_2$, and 17 other compounds, Smith and Frank (272) found that the proportion of the response eliminated by adaptation (i.e., the similarity) was the following for each cation: Na^+ = 36%, Li^+ = 28%, K^+ = 76%, Mg^{2+} = 82%, and NH_4^+ = 59%. For bitter, sour, and sweet, the corresponding values were ~18, 46, and -13%, respectively. Cross-adaptation of $CaCl_2$ and $CaBr_2$ was 102%, that is, almost perfect.

In summary, electrophysiological evidence from amphibians supports the existence of a specific calcium-sensitive transduction mechanism, and there is histological evidence from mammals that is consistent with this. Electrophysiological studies of gustatory responses in mammals have produced a wide range of not always

consistent results. Calcium and sodium produce different patterns of integrated chorda tympani activity, and calcium stimulates fibers or neural units that are tuned to HCl, NaCl, sucrose, alkaloids, and calcium-magnesium. Multidimensional scaling and cross-adaptation methods indicate neural gustatory activity evoked by calcium is moderately similar to that evoked by potassium, magnesium, and ammonium, somewhat different from that evoked by acids, and very different from that evoked by sodium and sweeteners. The many differences between the results of various studies are probably due to the species being tested, the level of the nervous system where recordings are made, the other compounds used in comparison with calcium, and the methods used to determine which compounds produce the "best" firing.

It is noteworthy given the lack of specificity of calcium appetite (see sect. IV) that oral calcium probably modulates the transduction of other taste compounds. For example, K^+ and Cl^- channels in mudpuppy are dependent on extracellular calcium (182), so tastes modulated by these mechanisms may be influenced by calcium availability. Consistent with this, if the frog tongue was adapted to 1–100 mM $CaCl_2$ for 2 min, subsequent responses to bitter substances were prolonged. This effect was unaltered by calcium channel blockade and did not occur if the tongue was adapted to NaCl or $MgCl_2$ (146). Studies examining the chorda tympani response of mixtures of $CaCl_2$ with other salts and sweeteners in hamsters produced complex results, including several examples of calcium influencing the response to other taste solutions (132, 133).

2. Calcium taste quality: behavioral evidence

There has been very little attempt to determine how nonhuman animals perceive calcium. In a very clever study, Morrison (198) trained rats to press the appropriate bar after drinking quinine, HCl, or sucrose. He then presented them with 16 "test" salts including $CaCl_2$. When given 300 mM $CaCl_2$, the rats pressed levers associated with quinine and HCl about equally but did not press a lever associated with sucrose. The response pattern produced by $CaCl_2$ was "distinctly different" from all the other salts tested, except perhaps lead acetate. These findings were recently extended in a study involving tests of three concentrations of nine different taste compounds (100). Rats were trained to press one bar associated with NaCl and another associated with quinine, HCl, or NH_4Cl. After receiving 300 mM $CaCl_2$ the rats bar pressed in the ratio of 90% HCl-10% NaCl, 70% quinine-30% NaCl, and 80% NH_4Cl-20% NaCl. Thus they found 300 mM $CaCl_2$ to be about equally sour, bitter, and ammonium-like and not very salty. The rats responded more to NaCl and less to HCl and quinine after receiving 100 or 1,000 mM $CaCl_2$ than after 300 mM $CaCl_2$. Similar results were obtained in

a marmoset tested with 100 mM $CaCl_2$. The authors could not easily associate these results with their prior gustatory electrophysiological results (e.g., Ref. 265).

An alternative method to examine the similarity of tastes involves taste aversion learning. Animals poisoned after drinking a taste solution later avoid solutions with similar tastes. This method has been used with hamsters that were poisoned after drinking 50 mM $CaCl_2$ and tested with nine other compounds (or vice versa). The hamsters strongly avoided $MgSO_4$, NH_4Cl, HCl, and quinine to roughly the same extent and weakly avoided NaCl, $NaNO_3$, DL-alanine, sucrose, and tartaric acid (273).

For studies of taste quality, humans have the advantage of being able to give oral reports about what they taste. There have been several attempts to match the intensity or taste quality of $CaCl_2$ with a reference taste solution, usually NaCl. Thus it has been reported that $CaCl_2$ is 81% as salty as the same concentration of NaCl [(309) cited in (217)], that 26 mM $CaCl_2$ and 26 mM $CaBr_2$ are the same intensity as 100 mM NaCl (183), and that 25 or 50 mM $CaCl_2$ are the same intensity as 200 mM NaCl (258).

Although early investigators considered calcium salts to be salty and bitter (83, 95, 142, 194), humans are remarkably inconsistent in describing calcium taste. At least one investigator reported that "subjects did not agree at all on the names to apply to the tastes of such substances as calcium chloride" (96). More recently, Schiffman and Erickson (258) asked subjects to rate the similarity of $CaCl_2$ to 18 other taste solutions and to describe them using 33 semantic differential scales. Multidimensional scaling suggested that $CaCl_2$ was approximately equally similar to salty and bitter tastes and relatively dissimilar from sweet tastes (Fig. 1). The semantic differential scales suggested that $CaCl_2$ is "generally a simple, minerally taste which is moderately bitter, smooth, warming, and soft. Only one subject (of 4) considered it to have a salty component... Two subjects found it quite soapy, but not obnoxious. Two others found it obnoxious, but not soapy. For two subjects, $CaCl_2$ was rated slow developing, flat, and nauseous. For the other two, it was rated fast developing and neither flat nor nauseous. It was uniformly considered nonfoodlike, but was rated poisonous by only one subject. " (258). Either different subjects perceive $CaCl_2$ quite differently or they use different language to describe its taste.

The contribution of each of the four classic taste modalities to the taste of $CaCl_2$ and calcium lactate (CaLa) has been assessed (295). A 1 mM $CaCl_2$ solution was rated as 35% bitter, 32% sour, 29% sweet, and 4% salty. At higher concentrations, the sweet component diminished and the salty component increased so that 100 mM $CaCl_2$ was rated as 44% bitter, 20% sour, 1% sweet, and 35% salty. CaLa solutions were considered to be less bitter, less salty, and more sour than equimolar $CaCl_2$
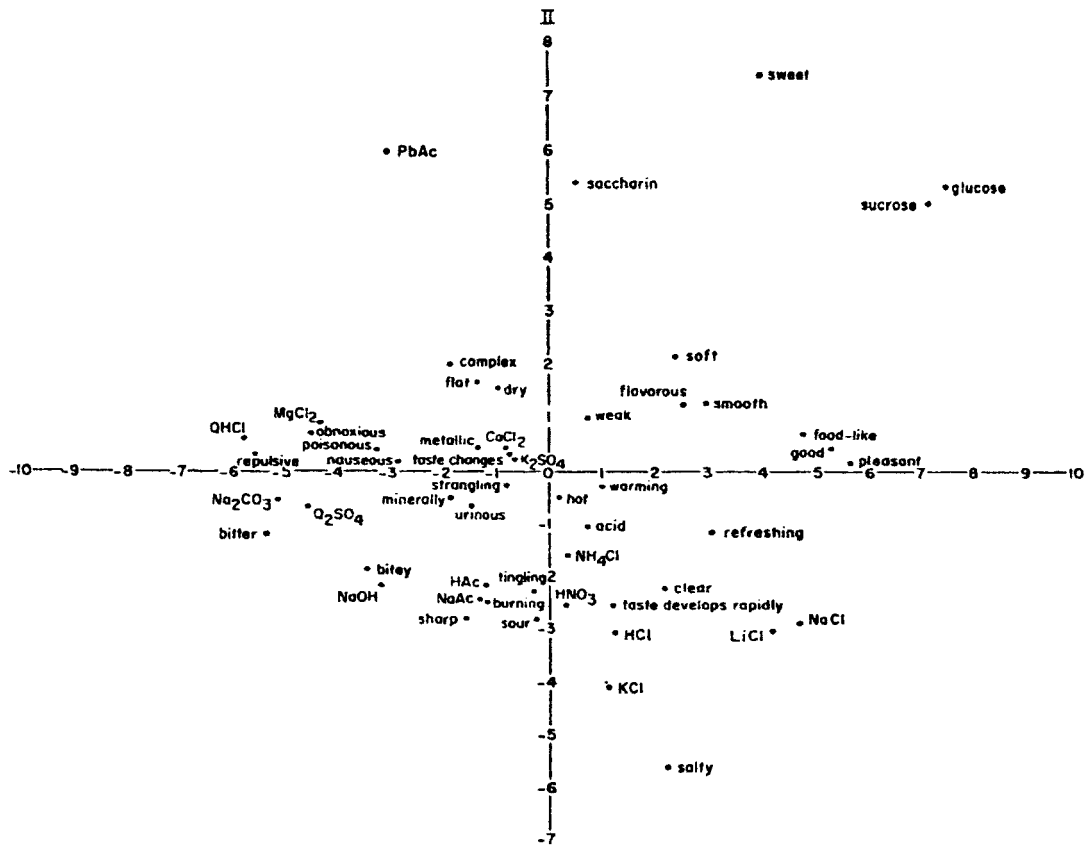
FIG. 1. Relationship of CaCl$_2$ to other taste solutions and descriptors. Results of multidimensional scaling are based on 33 semantic differential scales. Note that CaCl$_2$ (slightly left and above center) is not well described by either dimension and does not group with salty, bitter, or sour solutions. [From Schiffman and Erickson (258), with permission from Elsevier Science.]

solutions. In another study (121), subjects provided descriptors from a list of nine (sweet, sour, salty, bitter, soapy, sulfurous, metallic, other, none) that best described the taste of CaCl$_2$. The predominant rating at all three concentrations tested (10, 30, and 100 mM) was bitter; saltiness was not evident at 10 or 30 mM concentrations. The 10 mM CaCl$_2$ was also rated sweet/sour, soapy/metallic, other, and none; 30 mM CaCl$_2$ was rated salty, sweet/sour, sulfurous, and other; and 100 mM CaCl$_2$ was rated salty, sweet/sour, and soapy/metallic.

The method of cross-adaptation has been used to investigate the similarity of the tastes for calcium, sodium, potassium, and ammonium (183). Subjects were adapted to water, 100 mM NaCl, 110 mM KCl, 51 mM NH$_4$Cl, or 26 mM CaCl$_2$ by running the solution over the tongue. They then rated the intensity of the four chlorides and the equivalent bromides. Cross-adaptation between calcium and the other cations was minimal, suggesting different perceptual mechanisms were involved (see also Ref. 184).

In summary, the taste quality of calcium is not easily described, but it differs from the taste of sodium and other minerals. Calcium taste varies with both the form

and concentration of salt tested, but nearly always includes sour and bitter components. Only low concentrations are sweet, and only high concentrations of CaCl$_2$ are salty. Although there is some convergence of electrophysiological and behavioral results (273), there are also many differences (265). One difference is that behavioral studies generally find a much larger bitter component to calcium than do most electrophysiological studies, although this may well be due to the mammalian electrophysiologist's preference for recording from the chorda tympani nerve, which carries little bitter-related information (e.g., Ref. 278).

C. Calcium Preference and "Need-Free" Calcium Consumption

The previous sections have dealt with the detection and quality of calcium taste, but there is also a "hedonic" property of calcium that does not relate easily to its psychophysical qualities. It is clear that high concentrations of calcium are unpleasant to humans (295) and are avoided by animals (e.g., Refs. 135, 294). However, there

is also evidence that low concentrations are preferred over water and that animals frequently ingest more calcium than they need. Terminology in the literature is imprecise, but strictly speaking, this is a form of calcium intake that is unrelated to calcium appetite because it does not satisfy calcium homeostasis. The assumption is that under some circumstances calcium is consumed for no other reason than it tastes good.

The most common method to determine taste acceptability in nonhuman mammals is the two-bottle choice test. One bottle always contains water and the other contains a taste solution. Sprague-Dawley rats have been tested in this manner with a wide range of calcium solutions (0.2–300 mM, $CaCl_2$, phosphate, hydroxide, gluconate, and lactate; Ref. 294). Although there were slight differences in intake of the various salts, the general pattern of results was the same. Rats preferred calcium to water at calcium concentrations between ~0. 4 and 5 mM, showed indifference between 5 and 12 mM, and avoided higher concentrations. Slightly lower values were found in a follow-up study (294; Fig. 2). The inverted U-shaped preference curve is reminiscent of the curve shown by rats given NaCl solutions to drink (239).

This "spontaneous" or "need-free" intake is not confined to rodents. In a recent study (220), marmoset monkeys fed a calcium-replete diet drank significantly more 65 or 130 mM CaLa than water in two 7-h tests. Humans also follow the inverted U-shaped calcium concentration-liking function. Subjects reported 2 mM $CaSO_4$ to be preferred over water and other concentrations of $CaSO_4$. Similarly, 1.25 mM $Ca(HCO_3)_2$ tasted significantly better than 0.5 mM or 10 mM $Ca(HCO_3)_2$. There was no peak preference for $CaCl_2$, but the lowest concentration used (0.5 mM), which tasted about as good as water, may not have been low enough to reveal a preference (326). In other work (295), concentrations of $CaCl_2$ and CaLa of 3.16 mM or greater were disliked. High concentrations of $CaCl_2$ (31.6 and 100 mM) were rated as more intense and more disliked than equimolar CaLa solutions.

Unlike mammals, birds do not appear to find calcium solutions palatable, even after a period of dietary calcium deprivation (131, 145, 322). Nevertheless, hens fed diets containing sufficient calcium to meet requirements consume supplementary calcium grit when this is available (254; cited in Ref. 285). Under conditions of free access, calcium intakes can be highly variable and depend on the source of calcium (114).

D. Diet Choice by Calcium-Replete Animals

Animals sometimes show "nutritional wisdom"; that is, they can regulate their diet choices to satisfy their physiological needs. One method to examine this with respect to calcium is to present calcium-replete animals with a continuous choice between a food without calcium and a separate source of calcium. This can be a "pure" powder or grit, a solution, or a second food incorporating calcium. This method differs from "need-free" calcium consumption (see sect. II*C*) because in the need-free approach, the animal has its physiological requirement for calcium met by its maintenance diet but in the "diet choice" approach, it must consume the calcium source to avoid calcium deficiency. In fact, animals can recognize the nutritive value of calcium even while calcium replete (50). They most likely make appropriate diet choices before obvious signs of deficiency occur so the acquisition of calcium ingestion under these circumstances is probably not a response to deficiency.

Some of the earliest evidence for the existence of nutritional wisdom with respect to calcium comes from studies in which rats received a cafeteria choice of as many as 10 separate sources of nutrients. The rats consumed enough of each nutrient, including calcium, to match or exceed rates of growth produced by standard composite diets (e.g., Refs. 76, 78, 240–244). With the use of a much simpler method, rats given a choice between calcium-deficient diet and the same diet containing a calcium salt (CaLa, citrate, $CaCO_3$, $CaHPO_4$, or $CaSO_4$) chose between 20 and 34% of their calories from the calcium source, depending on the form and concentration of the calcium (263, 315). All the rats grew normally. Calcium intakes were higher in the rats given the high-calcium diets than those given the low-calcium diets, even though the high-calcium diets were preferred less.

Similar studies have been conducted with poultry. For example, hens given a choice between diets of high- or low-calcium content ate sufficient calcium to maintain normal egg production, although some groups were mildly hypocalcemic and had less dense eggs relative to birds given calcium-replete diets (124). In another study, hens given a choice between a high-energy, low-calcium
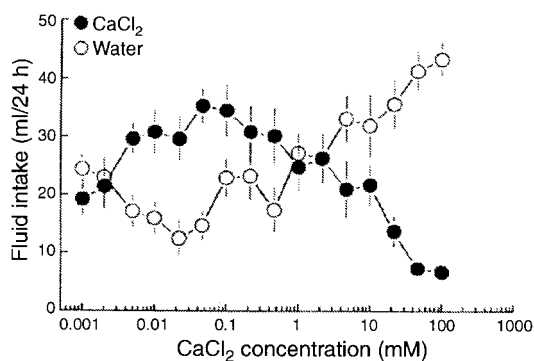

FIG. 2. Inverted U-shaped concentration intake function for $CaCl_2$ seen in Sprague-Dawley rats fed a calcium-replete (AIN-76A) diet. Humans also prefer low concentrations of calcium to water (326). [Adapted from Tordoff (294).]

diet and a low-energy, high-calcium diet had lower energy intakes and higher calcium intakes than did controls fed a composite diet. In this case, they produced eggs of superior shell quality (165).

A notable failure to find evidence for regulation of calcium intake involved cows fed diets containing various levels of calcium, and which also had access to solid $CaHPO_4$ for 1 h daily (58). In one experiment, $CaHPO_4$ intake increased during the first 10 wk of maintenance on low-calcium diet, but ~35% of the animals never approached the calcium source, and of those that did, there was great variability in intakes (0–1 kg $CaHPO_4$/day). In another experiment, heifers were fed diets containing 350, 3,100, or 4,150 mmol/day calcium and given continuous access to a choice of $CaHPO_4$ or defluorinated phosphate. Preference for the calcium source when fed the low-, medium-, and high-calcium diets was 77, 80, and 90%, respectively, which is the opposite result of what would be expected if calcium intake was regulated (see also sect. IIID).

The "diet choice" method has proven uniformly poor to determine calcium requirements, but this is not necessarily because the animal fails to show nutritional wisdom. It must balance the adverse, progressively developing effects of calcium deficiency against consumption of an unpleasant tasting calcium source. Sources of calcium differ substantially in acceptability, so intakes can be quite varied despite similar requirements (see discussion in Ref. 292).

III. DEMONSTRATIONS OF CALCIUM APPETITE

Calcium concentration in the sea is ~10 mM, although primieval levels may have been lower. Fresh water contains between 0.02 and 1.75 mM calcium (228), and calcium on land can be scarce. Thus the move from a marine environment to fresh water and land required the development of physiological mechanisms to help conserve calcium. It seems reasonable to assume that behavioral mechanisms to help locate calcium developed at the same time.

In some terrains, calcium is readily available as sedimentary rock (e.g., chalk and limestone). However, in many other areas, the ground does not provide sufficient calcium to support vertebrate life. Plants are generally an insufficient source of calcium (see Refs. 102, 307), so herbivores have a particularly difficult task. Many animals seek out and ingest salt licks if these are available. Although the emphasis has been on the behavior of animals obtaining sodium from salt licks, in many cases calcium is consumed as well (review in Ref. 67). However, the use of saltiness as a cue for calcium can be misleading because calcium and sodium do not always co-occur in rock outcrops (see Ref. 293).

An important source of calcium for many animals is the bones of their prey. Old bones are also chewed by herbivores, although this is most frequently associated with phosphorus deficiency (e.g., Refs. 26, 27, 67, 105, 112, 284). Calcium also comes from teeth, birds' eggs, and anthropogenic sources (e.g., mortar, putty), and in mammals, mother's milk.

There are three long-lived but disconnected literatures concerning the effects of calcium deficiency on subsequent calcium intake, which are summarized separately below.

A. Wild Birds

"March 26, 1981 was a sunny day with light frost in the morning; in some places, the snow had completely melted. At ~6:00 A.M. on the wall of the house where I had spent the night, there were 50 red crossbills and the whole time they were eating mortar. Small flocks would fly to nearby spruce trees and then return to the wall. During the following two days, the number of crossbills increased, so that on the third day there were between 100 and 150" (283).

The above extract is typical of many published reports of calcium intake by wild birds. Most birds eat insects and/or plants that are relatively low in calcium, so these cannot fulfill calcium requirements (111; review in Ref. 108). For some species, restricted calcium availability limits geographical distribution (61, 101, 185, 318 and citations therein). However, many others supplement their diet by eating calcium-rich material such as bones, owl pellets, mortar, grit, and the shells of snails, crabs, marine mollusks, and other birds (33, 106, 109, 110, 129, 139, 155, 176, 252). Boreal chickadees consume ash rich in calcium (86); sandpipers eat grit, lemming bones, and teeth (176); sandwich terns eat shell fragments; and crossbills in coniferous forests eat bones, putty, and cement (206, 214, 283).

Graveland (106) provides a table of literature citations covering 28 species of birds that consume calcium-rich material. Observations of two species exemplify the rich repertoire of calcium-obtaining behaviors. First, banded-tail pigeons drink from mineral springs with high calcium content and pick up salts that have dried on pebbles on the seashore (178). In the summer in Hope, British Columbia, $CaCl_2$ was used to reduce dust on gravel roads. Immediately after $CaCl_2$-coated pebbles were scattered on the road, large flocks of pigeons were seen picking them up. The pigeons did not frequent this area at any other time. Second, the red-cockaded woodpecker caches pieces of bone by wedging them into the bark of trees (230). The purpose does not seem to be to store the bone for times of need. Instead, caching may afford the birds some advantage by reducing the time they

spend obtaining calcium from raptor pellets on the ground, which is particularly hazardous for a woodpecker (230).

The demand for calcium is especially high during egg laying (see sect. v*E*). The importance of calcium appetite to reproductive success has been underscored by recent work aimed at understanding the decrease in bird populations in regions afflicted by acid rain. Acidity reduces the abundance of many invertebrates, such as snails, crayfish, and insects (see Ref. 279 for citations). Moreover, poor soil quality due to acid rain reduces the calcium content of leaves and needles (73). This, in turn, reduces the calcium content of caterpillars, which are an important food source for many species of tree birds (73). Reduced calcium availability leads to egg shell abnormalities and the consequent decline in bird populations (e.g., Refs. 73, 279; see sect. v*E2*).

B. Poultry

For obvious commercial reasons, there has been considerable interest in dietary calcium levels and calcium appetite in chickens. Intuitively, the laying hen seems to be a good model for studying calcium appetite because of its substantial calcium requirement for egg production. However, egg production quickly ceases if a laying hen is fed a low-calcium ration (103). Moreover, bone stores of adult hens are substantial, and calcium is conserved very efficiently, making a long period of deprivation necessary (see Ref. 131). Thus most investigators have studied growing chickens or the combination of growing and egg-laying chickens.

Calcium requirements are ~100 mmol/day for the growing hen and 2.5–5.0 mmol/day for the adult (citations in Ref. 102). Typical diets contain 750–1,000 mmol Ca^{2+}/kg, with the calcium provided as ground limestone. Gilbert (102) has written an outstanding review of the factors affecting calcium balance during egg production of hens.

The earliest reported experiment on calcium appetite (119), by Hellwald in 1931, involved chickens that were deprived of dietary calcium. Four hours before receiving free access to calcium, some of the birds were fed pulverized egg shell (calcium) inside macaroni, and the others were fed macaroni alone. The macaroni prevented the birds from tasting the calcium. When egg shells were made freely available, the group prefed calcium-containing macaroni ate 27 g, whereas the group prefed macaroni alone ate 91 g. This demonstrated not only that calcium-deprived chickens develop an appetite for calcium, but also that it can be assuaged without oral stimulation (see sect. v*B*).

Calcium-deprived chickens that received a choice between two diets initially preferred the alternative containing calcium (131, 322). Similarly, chickens given free access to $CaCO_3$ granules consumed the supplement in inverse relationship to the calcium content of the diet (285). This may have practical significance, because birds given low-calcium diets but free access to $CaCO_3$ granules consumed more calcium and laid more eggs than did controls fed a calcium-adequate diet but not given a supplement. Similar results were found with hens given cockleshell grit as a calcium supplement (286).

Whereas it has been relatively easy to see an effect of diet calcium availability on the intake of a solid calcium supplement, there have been two failures to find a preference of calcium-deprived chickens for calcium solutions. In one (322), calcium-deprived and control chickens drank similar amounts of 92 mM CaLa. This was attributed to the high sensitivity of birds to unpalatable fluids. In the other (131), calcium-deprived chickens preferred to drink water rather than 4% calcium borogluconate. The same birds tested with a choice between solutions of 4% calcium borogluconate and 1.4 μM quinine drank mostly quinine, but this was probably due to learning (see sect. v*A*). On the other hand, calcium-deprived birds preferred to drink considerably more of a 100 mM $CaCO_3$ suspension during a 10-day test than did birds that had never been calcium deprived (131).

C. Rats

Estimates of the amount of calcium required by rats for maximal growth and bone accretion range from ~0.55 to 1.2 mmol Ca^{2+}/day (22, 44). The National Research Council recommends a minimum daily calcium intake of 1.5 mmol Ca^{2+}/day (205), and most rodent diets provide considerably more calcium than is required [e.g., AIN diets contain ~125 mmol Ca^{2+}/kg (1, 227), grain-based laboratory diets contain ~200 mmol Ca^{2+}/kg].

Given that extracellular fluid (ECF) is ~20% of the rat's body weight, and calcium concentrations are ~2 mM, a 250-g rat has ~0.1 mmol calcium in its ECF. This is equivalent to a few bites (0.5 g) of Purina Chow or only 1 ml of 78 mM CaLa! It is a gross oversimplification to think that all ingested calcium contributes to ECF, but this calculation points out that intake of very small amounts of food or calcium solution have the potential to produce dramatic changes in the rats' extracellular environment.

The skeleton contributes 10.9% of the weight of the rat and is 47.4% water (271). We typically find calcium content of dry femur ash to be ~8 mmol/g, which implies that total skeletal calcium content of a 250-g rat is ~115 mmol. However, not all this calcium is accessible. After near-total calcium deprivation of growing rats for 3 wk, femur calcium concentration drops slightly (5–15%), and femur weight is considerably decreased (~50% relative to controls of the same age; unpublished results). These figures suggest that rats have available ~50–60 mmol of calcium in bone.

The experimental study of calcium appetite in rats began with a series of four papers by Richter and students (241–243, 245). In parallel with his work on adrenalectomy and NaCl appetite, Richter took advantage of the fact that rats without parathyroid glands do not maintain calcium balance. He found that rats with parathyroidectomy (PTX) maintained on a low-calcium diet increased intake of 65 or 78 mM CaLa solution (241, 243; see also Ref. 316; Fig. 3). Rats with PTX also drank more of other calcium salts (acetate, gluconate, or nitrate) and were able to select an adequate amount of calcium when given a choice of four different salts to drink (78 mM CaLa, 268 mM KCl, 357 mM NaLa, 180 mM $CaCl_2$, and 290 mM NaH_2PO_4; Ref. 242).

Several early studies found evidence for a calcium appetite based on calcium-deprived rats' choice between a calcium-free and calcium-containing diet. For example, deprived rats ate ~67% of their food as diet containing 375 mM CaLa, whereas replete controls ate only 33% (315). Similar findings have been made using several concentrations of $CaCO_3$ and other calcium salts (citrate, phosphate, and sulfate; Refs. 248, 263, 315). More detailed discussion of these and related studies is deferred until section V.

D. Other Mammals

Drummond (74) reported that pigs fed a ration deficient in lime when let into a small brick-floored courtyard "spent a large part of their time attempting to lick whitewash from the walls or to root out a fragment of cement or mortar from the brickwork." This was in contrast to pigs deprived of vitamin A that "search(ed) for the smallest blade of grass or a chance weed which might be growing in the cracks of the floor," or a nutritionally
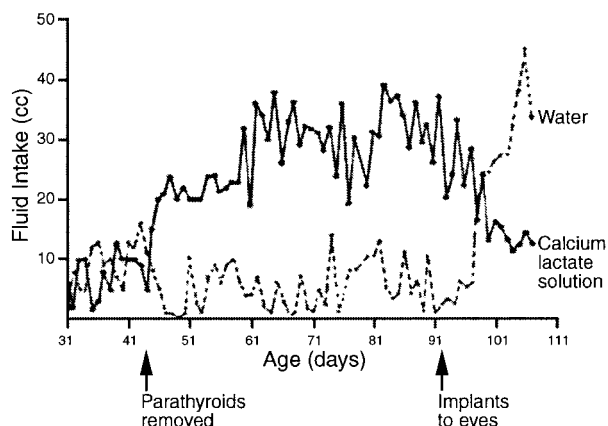

FIG. 3. One of the first demonstrations that parathyroidectomy of rats increases calcium lactate intake and that this can be reversed by reimplantation of the parathyroid glands. [From Richter and Eckert (242).]

complete control group, which apparently did none of these behaviors.

There are many observations that cattle eat old bones (e.g., Refs. 26, 27, 67, 105, 112, 284), but this appears to be due to phosphorus deficiency. Phosphorus-deficient cows do not consume calcium salts in cafeteria experiments (67, 112). Lactating cows fed a low-calcium diet for 9 wk and then give 1 h/day access to calcium phosphate ate ~2.5 times more supplement after the low-calcium period than before it (57). Despite this increase, the authors concluded that the cows did not have a calcium appetite because they did not consume the calcium requirements computed by the National Research Council. However, during the low-calcium diet period, body weight and plasma calcium levels increased. Milk production decreased, suggesting the cows were very efficient at conserving calcium. Also, the use of calcium phosphate as the test compound was unfortunate because calcium-deficient rats avoid phosphorus (see Refs. 242, 245, 262).

IV. SPECIFICITY OF CALCIUM APPETITE

The literature described above shows that calcium-deficient birds and rats preferentially ingest calcium when given the opportunity. However, several studies suggest that they also ingest other substances, which raises the question of how specific is the appetite for calcium? The evidence is summarized below.

A. Pica and Grit Intake

There are scattered allusions that calcium deficiency causes pica (intake of nonfood objects). However, these appear to be based on incidental observations in the wild (e.g., Refs. 212b, 257a) without empirical evidence that the animals were calcium deficient or that this was the only deficiency they suffered. An engaging review of the history of pica in humans mentions only infrequently the consumption of chalk or other calcium-containing items as a diagnostic symptom of pica, and the popular theory was that intake of such substances combatted gastric acidity (213). Moreover, pica appeared to be most often recognized in adolescent girls rather than subpopulations with high calcium demand (i.e., young children, pregnant and lactating women). A recent finding that calcium supplementation reduced premenstrual cravings for sweets and salts (288; see also Ref. 287) is consistent with a connection between calcium and indiscriminate ingestion, although the reduction in cravings may have been secondary to changes in appetite, pain, or other premenstrual symptoms. Perhaps more relevantly, a study of pregnant urban African American women, who consumed <60% of the recommended daily allowance for calcium, found ~8% reported pica, but this was mainly pagophagia

(ice chewing) or amylophagia (ingestion of laundry starch). None ingested chalk or antacid tablets (77). Thus, if calcium-directed pica occurs in humans, it must be very rare.

Evidence for pica related to calcium deficiency in birds is much stronger. Early investigators reported that chickens given a low-calcium diet in a lime-free room became greatly excited when fed, pecking at the feeder's buttons and fingernails (119), and that calcium-deprived chickens were "much more active" than controls and five times more likely to peck at novel objects (buttons, chalk, cake balls, acorns; Ref. 322). Egg-laying female great tits maintained without calcium spent considerable time searching on the ground and frequently dug in the soil. The birds showed particular interest in white-colored objects (109). This was not simply a general activation of behavior because the birds increased their search efforts on the ground but not in trees or elsewhere in the aviary (109). Laying red-cockaded woodpeckers repeatedly investigated white pieces of paper on the ground (230). These behaviors could be considered cases of calcium deficiency-induced pica, but at least from the birds' viewpoint they may be appropriate because calcium-rich material is often found on the ground and is frequently light-colored (i.e., snails, bones, grit).

Some birds eat grit when calcium deprived. An early study tested growing pheasants given access to a grain-based (low-calcium) diet and various forms of grit (185). Intake of quartz grit increased progressively from basal levels of ~ 1 to ~ 7 g/day. When bone meal or $CaCO_3$ was added to the pheasants' diet, quartz grit intake dropped back to ~ 1 g/day. In another experiment, pheasants ate <2 g/day grit obtained from glacial gravel mix, which looks like quartz grit but contains calcium. They were then switched to quartz grit, and intakes rose progressively to >6 g/day. When returned to the glacial gravel mix, intakes returned to baseline within 2–3 days. These results with growing birds were replicated with adults, although, consistent with their lower demand for calcium, the effects were muted (185; see also Refs. 185, 252).

These studies provide solid evidence that grit intake depends on calcium status, although grit is also consumed to help grind food in the crop. It seems likely that the intake of quartz by calcium-deficient pheasants is a mistaken attempt to gain calcium. The finding that quartz intake increases even though this does not alleviate calcium deficiency is consistent with the possibility that some species of birds innately recognize calcium as small lightly colored particles.

B. Intake of Salts

The degree of specificity of calcium appetite has been exhaustively studied in rats and to some extent also in poultry. Attempts have been made to look for common properties between compounds calcium-deficient animals ingest preferentially. Among the properties examined were *1*) class IIa elements, *2*) divalent minerals, *3*) polyvalent minerals, *4*) chlorides, *5*) compounds stimulating type "A" gustatory afferent fibers, or *6*) bad-tasting compounds. All of these hypotheses either have received mixed support or were refuted.

It should be noted that the ingestion of non-calcium compounds by calcium-deficient animals is often considered "aberrant," but the behavior is generally forced by the experimenter because calcium is unavailable. Calcium-deficient animals given a choice between a non-calcium compound and calcium will always consume the calcium, unless the calcium source is extremely unpalatable (e.g., Refs. 167, 172).

1. Strontium

Strontium (Sr) and calcium are in the same periodic group, and it thus might be expected that animals generalize from the taste of one compound to the other, in the same manner that sodium-deprived animals ingest LiCl and KCl (84, 203). Moreover, strontium can sometimes act as a physiological surrogate for calcium (see Refs. 166, 282).

Voluntary intake of 0.75 mM $SrCl_2$ or 0.56 mM strontium lactate solution by rats was increased by PTX (242) and could be reversed by reimplanting parathyroid glands. Dietary calcium deficiency increased the intake of 1 and 10 mM $SrCl_2$ solutions by rats in 24-h acceptance tests (48), but not 100 mM $SrCl_2$ in a 30-min test after water deprivation (49). Chickens could discriminate between diets containing 135 mmol/kg $SrCO_3$ and 200 mmol/kg $CaCO_3$ but not after flour had been added to hide the color (131). This strongly suggests that chickens cannot easily discriminate between the taste of strontium and calcium (see also sect. v*A*).

2. Magnesium

Like strontium and calcium, magnesium is a group IIa element, and thus it might be expected that animals confuse the three. However, magnesium has an intensely bitter taste. Calcium-replete rats showed a slight preference for very low concentrations of $MgCl_2$ (0.005–10 mM) over water in 24- or 48-h two bottle choice tests, and strongly rejected concentrations above ~ 50 mM (48, 294). Nevertheless, calcium-deprived rats drank more 10, 100, and 316 mM $MgCl_2$ than did replete controls (48), and rats with PTX increased intake of 61 mM $MgCl_2$ in one experiment (167) and 25 mM $MgCl_2$ (but not 0.69 mM magnesium lactate) in another (242). The increased intake of 25 mM $MgCl_2$ was reversed by reimplanting parathyroid glands (242; see also Ref. 168 and sect. v*F2*).

3. Phosphorus

Phosphorus counteracts some of the physiological actions of calcium and consequently exacerbates the effects of calcium deficiency. It thus makes sense for calcium-deficient animals to avoid it. Consistent with this, rats fed a low-calcium diet showed lower intakes than did controls of several concentrations of sodium phosphate and potassium phosphate (48). Similarly, PTX reduced rats' intake of 37 mM dibasic sodium phosphate, and this could be reversed by administration of parathyroid extract or dihydrotachysterol (78, 242, 245, 316). The effects on phosphate intake were more immediate and greater than those seen in parallel experiments on calcium intake (75, 242, 243, 245, 316).

In contrast to the results with deprivation and PTX, during pregnancy and lactation phosphorus intake increases (36, 240). Perhaps this is the result of different physiological mechanisms at play in the different models of calcium intake. However, a simpler possibility is that the decrease in phosphate intake occurs only when calcium is scarce, which was not the case in the studies of ingestion during reproduction.

4. Lead

Snowdon (274) has argued that calcium deficiency is a cause of lead poisoning in children. This is based on findings that calcium-deprived rats (274, 275) and monkeys (137) ingested more lead acetate solution than did controls. Deprivation of other minerals (magnesium and zinc, but not iron) also increased lead acetate intake, but to a lesser extent (274).

Lead acetate is toxic so the question arises of why do calcium-deprived rats drink a solution with deleterious effects? Lead is easily incorporated into tissues of calcium-deficient animals (164), suggesting that in the absence of sufficient calcium, the animal may be able to use lead as a surrogate. Also, lead can elevate concentrations of 1,25-dihydroxyvitamin D [$1,25(OH)_2D$; Ref. 98], which increases calcium absorption and thus reduces the severity of calcium deficiency (see sect. vD). Consistent with this, calcium-deprived rats given access to lead acetate solutions gain more weight than do rats maintained on the same diet without lead to drink (274). Moreover, a flavored drink that was followed by an injection of lead acetate was avoided by calcium-replete but not calcium-deprived rats (274). Either the toxic effects of lead ingestion by calcium-deprived animals do not support a conditioned aversion, or the benefits of drinking lead to the calcium-deprived animal outweigh its deleterious effects.

Naive calcium-deprived rats licked more frequently than did replete controls for 20 mM lead acetate solution in a brief-exposure test (49). Such a rapid effect on intake argues that the response to lead acetate is driven by taste factors. Lead acetate has been called "lead sugar" and labeled as sweet by humans (see discussion in Ref. 274). However, calcium-replete rats avoid it in long-term tests (180) and do not generalize between lead acetate and sucrose (198). Even if lead acetate tasted sweet, this would be an unlikely cause for the increase in intake because calcium deprivation reduces intake of sweet solutions (see below). One explanation for the high intake of lead acetate is that it may taste like calcium. Morrison (198) trained rats to respond to "test" salts by pressing a lever associated with bitterness, sourness, or sweetness. $CaCl_2$ and lead acetate showed similar response profiles, which differed from the other 16 salts tested (198).

5. Sodium

The relationship between calcium intake and sodium intake has been investigated in detail, in part because of the known link between calcium and sodium excretion (e.g., Refs. 34, 99, 148, 215), and in part because of the involvement of both calcium and sodium in hypertension (see reviews in Refs. 115, 186, 231, 276). NaCl intake is inversely related to both the amount of calcium in the diet and the duration of calcium deprivation (292, 305). Feeding a low-calcium diet to growing male rats progressively increased voluntary intake of 300 mM NaCl from control levels of ~10 to >60 ml/day after ~14 days. More severe calcium deprivation (>28 days) increased 300 mM NaCl intake to impressive levels (>125 ml/day; Ref. 305). Dietary calcium deprivation increased intake of a range of concentrations of NaCl (50–500 mM) (305) and several sodium salts including sodium acetate, bicarbonate, and glutamate (48, 290). Most observations were made with Sprague-Dawley rats, but the phenomenon has been demonstrated in other strains, including Fisher 344 rats, which are generally reluctant to drink NaCl (291), spontaneously hypertensive rats (SHR), and Wistar-Kyoto rats (WKY). The SHR appeared to be particularly susceptible to the effects of dietary calcium. Moreover, supplementing the calcium content of the SHR's diet decreased their already high NaCl intakes almost to levels of WKY controls (292).

The effects of PTX on NaCl intake are not as clear as are those of dietary calcium deprivation. Richter and Eckert (242) reported that with rats given PTX, "numerous single and multiple choice experiments made with sodium and potassium solutions. . . did not reveal cravings for these minerals." Similarly, rats with PTX did not increase NaCl intake when given a choice between 500 mM NaCl and 61 mM $CaCl_2$ solution (167). On the other hand, there are two reports, including one from Richter's laboratory, that rats with PTX increase NaCl intake in cafeteria choice experiments (76, 78), and a demonstration that rats with PTX pressed a bar to obtain NaCl in preference to $CaCl_2$ (172). One reason for the discrepancies is that animals with PTX given a choice between calcium and sodium solutions may ingest enough of the

calcium solution to negate their calcium deficiency, and thus do not always need calcium.

Several studies have examined potential physiological mechanisms underlying calcium deficiency-induced NaCl intake. The renin-angiotensin-aldosterone system (RAAS) is the primary mediator of NaCl intake (67, 79, 88, 92), but indicators of RAAS activity, including blood pressure, urinary sodium excretion, and ECF volume, appear unperturbed in calcium-deficient rats (297, 303, 305). Moreover, treatment with an aldosterone antagonist or angiotensin II receptor inhibitor had no effect on the NaCl intake of calcium-deprived rats (303). Adrenalectomy, which generally increases NaCl intake, decreased the already high NaCl intakes of calcium-deprived rats, even during aldosterone replacement therapy (296). Thus calcium deficiency is arguably the clearest example of high NaCl intakes where a role for the RAAS has been effectively ruled out (see review in Ref. 297).

It seems more likely that the high intakes of NaCl produced by calcium deprivation are due to increased NaCl palatability (49, 189) and learning. Immediately after calcium-deprived rats drank NaCl they had increased plasma ionized calcium concentrations and reduced concentrations of PTH and $1,25(OH)_2D$ (298). In vitro studies indicate that physiological levels of sodium modulate the binding of calcium to plasma proteins. Small increases in plasma sodium concentrations release bound calcium into the ionized pool, which has the effect of temporarily reducing the severity of calcium deficiency, and thus reinforcing NaCl drinking behavior. Of course, the effect of drinking NaCl on plasma ionized calcium is only temporary, and the excess NaCl must be excreted, carrying calcium with it. Thus the rat is in a vicious cycle in which it exacerbates its deficiency because it attempts to benefit in the short term (see Refs. 297, 298).

6. Nonsodium salts

Relative to replete controls, calcium-deprived rats have greater intakes of several chlorides (aluminum, ammonium, ferric, ferrous, potassium, zinc) but not nonsodium nonchlorides (ferrous sulfate, magnesium sulfate, potassium gluconate; Ref. 48).

C. Intake of Sweet Solutions

Relative to replete controls, calcium-deprived rats have reduced intakes of several sweet solutions, including sucrose, saccharin, D-phenylalanine, aspartame, and cola beverage (48, 49, 190, 290, 293, 305; see also Ref. 39). They also avoid a sweet, solid carbohydrate when allowed to choose between separate sources of carbohydrate, fat, and protein (290). The reduced appetite for sweetness does not appear to be due to a general loss of appetite for carbohydrates or calories. Intake of Polycose, alcohol,

and several fats was either increased or unaffected by calcium deprivation (290). The effects are reversible. The reduction in daily sucrose intake produced by feeding a calcium- or mineral-deficient diet could be eliminated by feeding bone meal or calcium gluconate (190).

The mechanism for these changes in sweet solution intake is unknown. One possibility is that they are mediated by changes in taste sensitivity. The sweeteners tested have diverse postingestive effects, and calcium-deprived rats ingest less saccharin or saccharin plus glucose mixture than do controls in brief-duration tests, which minimize postingestive effects (49, 298). Sweeteners increase intracellular calcium content of gerbil taste cells, and this depends on the presence of extracellular calcium (306). A complex relationship between extracellular calcium and sweetness perception has been observed in other species (157). It is thus conceivable that extracellular calcium modulates sweet taste transduction. It is also possible that changes in the concentrations of circulating hormones or other factors induced by calcium deficiency, rather than low levels of calcium per se, could be responsible for the changes in preference.

The possibility that a more general mechanism is involved is underscored by findings that deficiencies of sodium and zinc also influence sweet solution acceptance (14, 41, 42, 223, 237). This is not a function of general malnutrition because no changes in saccharin intake were observed in rats deprived of iron, magnesium, or phosphorus (293). One possibility is that deficiencies in calcium and other minerals interfere with CNS opioid activity, which governs intake of sweet compounds (see sect. v*C1* and Ref. 290).

D. Intake of Bitter and Sour Solutions

Calcium-deprived rats and controls had similar intakes of 0.368 mM sucrose octaacetate (bitter) and 2.5 mM citric acid (sour) in two experiments (293, 305). Calcium deprivation increased intake of 0.026 μM quinine sulfate but not higher concentrations (0.13–0.54 μM; Ref. 274, see also Ref. 48). Perhaps this extremely dilute quinine produces bitterness similar to the bitter component in the taste of calcium. Calcium deficiency had no effect on the intake of a number of other compounds, including citric, hydrochloric, and sulfuric acids (48).

V. MECHANISMS UNDERLYING CALCIUM INTAKE

A. Learning

There is strong evidence that the appetite for calcium involves an innate component. Most critically, calcium-

deprived rats recognize and ingest novel calcium solutions within seconds of receiving them (49). They also sham-ingest $CaCl_2$ solutions even though this provides little or no postingestive benefit (189) and show latent learning for calcium solutions; that is, they associate an arbitrary taste with calcium consumption even though they are not calcium deficient and thus have no motivation to learn this association (50).

Nevertheless, it is clear that calcium-deprived animals can learn to prefer calcium-containing foods and drinks. In a series of studies using different calcium salts ($CaCO_3$, $CaHPO_4$ or $CaSO_4$), Scott et al. (263) fed rats diets with or without calcium and with or without anise flavor, according to a 2×2 design. All groups then received a choice between flavored diet and the unflavored diet of the opposite calcium content for 5 days, followed by 5 days with the initial diets. The results are difficult to extract from the paper, but it appears that the calcium-deprived but not replete rats' initial preferences were eliminated when the diet choice was first switched, and these were reinstated when the flavors were switched back again.

A simpler study was conducted by Rodgers (248), who tested rats maintained for 3 wk on one of two calcium-deficient diets. The rats then received a choice between one diet with 133 mmol/kg $CaCl_2$ added and the other without calcium. All the rats preferred the novel diet. This was surprising because one group ate novel calcium-deficient diet in preference to "familiar" maintenance diet with added calcium. This paper also included a demonstration that, in contrast to the results with calcium, sodium-deprived rats consumed a sodium-containing food, irrespective of its novelty. This disparity was the main impetus behind the conclusion that sodium appetite is purely innate, whereas calcium appetite is purely learned.

Given the repressive effect of this study on subsequent research into calcium appetite, it is worthwhile noting some of its shortcomings. First, no independent measures that the rats were calcium deficient were collected, and given that they consumed at least 1.325 mmol calcium in the first day of the 4-day test, it is unlikely that intakes during the test reflected calcium deficiency. Second, the design did not involve counterbalancing the complex diets used. It is impossible to know whether the low intake of calcium-containing diet is due to its bad taste or the rats' desire for novelty. Given that calcium-containing diets are unattractive to replete rats, it may be that the rats consumed as much calcium as they required over the first few hours of the 96-h test and then avoided the calcium source. Third, even if the experiment was methodologically sound, the conclusions do not follow from the results. A demonstration that learning can occur does not preclude innate mechanisms from also being involved.

The role of learning in determining calcium intake has been examined much more thoroughly in poultry. Wood-Gush and Kare (experiment 2 in Ref. 322) noticed that calcium-deprived chickens had more difficulty selecting a high-calcium diet on the day after food cup position was rotated than on the second day of each 2-day test. This led Hughes and Wood-Gush (131) to examine the contribution of learning to calcium appetite. Their most convincing demonstration involved calcium-replete and deprived chickens given a choice between diets containing 135 mmol/kg $SrCO_3$ and 200 mmol/kg $CaCO_3$. In one experiment, flour was added to the $CaCO_3$ source to hide its color. In another, a pink dye was added to the $CaCO_3$ source to make discrimination easier. With no color cues available, most birds showed positional preferences but not diet preferences. On the other hand, with a color cue present, 8 of 11 deprived birds but only 2 of 12 replete birds preferred the $CaCO_3$- to $SrCO_3$-containing diet. When, after a 10-day test period, the color was switched from the $CaCO_3$ diet to the $SrCO_3$ diet, the replete birds continued to eat ~50% from each cup, whereas the "calcium-deprived" group initially switched preference to the $SrCO_3$ diet (i.e., followed the color), but this preference dissipated to indifference within 3–4 days. The simplest explanation for this is that when initially given the choice, the calcium-deprived birds were able to *1*) determine that the $CaCO_3$-containing diet was beneficial and *2*) associate the diet's appearance with its beneficial effects.

These results were confirmed using slightly different methods (130). An extensive series of studies were conducted to characterize potential mechanisms that could account for the learned preference for calcium. Four hypotheses concerning the benefit that chickens could potentially derive from consuming calcium were tested. These included a general reduction in anxiety or arousal, the reversal of bone calcification, and thus reduced bone pain, a nonspecific general sense of well-being or a learned aversion to the deficient (maintenance) diet. There was no unequivocal evidence found for any of these mechanisms. Thus, although it is clear that chickens can associate the chemosensory aspects of calcium with its postingestive effects, the nature of the unconditioned stimulus remains obscure.

Learning is involved in two other areas of calcium appetite research. First, there is strong evidence that cues about calcium can be transmitted by social learning (see sect. v*F4*). Second, learning has been used to determine the strength of the rat's drive for calcium. Frumkin (97) gave intact rats and rats with PTX a series of daily 1-h tests with access to 81 mM CaLa, 2.4 mM saccharin, or both solutions simultaneously. The rats were poisoned with LiCl each time they drank one or other of the solutions. Intact rats poisoned after drinking CaLa stopped drinking this solution but rats with PTX continued to drink it. This was not because the calcium-deficient rats

failed to learn to avoid a solution associated with illness because all animals poisoned after drinking saccharin stopped drinking it. Thus it appeared that the motivation to drink calcium of rats with PTX was greater than their learned aversion to the solution (see also Ref. 274). It is noteworthy that the toxic effects of lithium can be ameliorated by calcium supplementation (152).

B. Taste

Taste plays a dual role in the control of calcium intake. First, the taste of calcium and its vehicle (i.e., the rest of the diet) provides cues that can direct intake and be associated with the postingestive consequences of consuming calcium (see sects. II and VA). A good example of this is work showing that most rats with PTX given a cafeteria selection of nutrients could not select adequate amounts of calcium gluconate solution following lesions of ventrobasal thalamic gustatory regions (78). Second, the oral acceptability of calcium is mediated by calcium status. Whereas replete animals largely ignore concentrated calcium solutions, calcium-deprived ones ingest them avidly within seconds of receiving them (49), and sham ingest large volumes even though this provides little or no postingestive benefit (189).

Electrophysiological recordings from gustatory afferent nerves suggest that physiological calcium status influences calcium perception in the periphery (135, 158). In the frog, sensitivity to oral calcium depends on the concentration of calcium inside the tongue. The glossopharyngeal nerve response to water and a 1 mM $CaCl_2$ taste stimulus was positively related to the concentration of $CaCl_2$ infused through the lingual artery (158). The responses to quinine, HCl, and ethanol were either unaffected or decreased by lingual artery $CaCl_2$ infusion. In the rat, sensitivity to oral calcium is influenced by diet. The threshold concentration of oral calcium that elicited an electrophysiological response in the chorda tympani nerve was an order of magnitude lower in calcium-deprived than replete rats. The calcium-deprived animals also displayed greater integrated activity in response to low concentrations (<3 mM) of oral $CaCl_2$ and CaLa. In contrast, calcium-deprived rats were less sensitive than were replete controls to high (>32 mM) $CaCl_2$ concentrations. These results are consistent with the idea that the increased chorda tympani response at low calcium concentrations enhances the animal's ability to detect calcium, and the reduced response to high $CaCl_2$ concentrations lowers the perceived intensity of calcium and thus increases the acceptance of these normally unpalatable solutions (135). This link between calcium status and palatability is reinforced by recent data showing that calcium deprivation specifically increases the activity evoked by oral calcium of sucrose-best units in the nucleus tractus solitarius (188).

The mechanism by which calcium status influences gustatory sensitivity is unexplored. Richter and Eckert (242) believed that the craving for calcium depended on "chemical changes in the taste mechanisms in the oral cavity, making the calcium more desirable after parathyroidectomy than before," but this was apparently based solely on conclusions from his concurrent studies of sodium intake in rats with adrenalectomy (cf. Refs. 236, 238, 239). One obvious possibility is an effect of calcium on saliva composition. Diet calcium supplementation increases salivary calcium concentrations of children (23), although generally there is little or no relationship between calcium levels in plasma and saliva (72, 253). Alternatives include the direct modulation of transduction by extracellular calcium (see sect. IIB), or an effect of calciotropic hormones on taste mechanisms. There are parallels here to the literature on the modulation of sodium taste by sodium status (e.g., Refs. 52–54).

It is clear that the mouth does not act as an oral meter of calcium consumption. This was demonstrated in the earliest published experiment on calcium appetite (119), in which calcium-deprived chickens that were fed calcium-filled macaroni ate very little of a subsequent test "meal" of pulverized egg shells. More recently, Hughes and Wood-Gush (130) found that subcutaneous injections of calcium borogluconate reduced the calcium preference of calcium-deprived chickens given a choice between a calcium-deficient and calcium-replete diet. Finally, latent learning experiments indicate that calcium-deprived rats are motivated to consume arbitrary flavors that have been associated with calcium (50). Thus, although the acceptance of calcium is under oral control, the appetite for calcium can be assuaged without oral stimulation.

C. Hypocalcemia

Richter and Birmingham (241) proposed that "changes in blood calcium affect. . . the taste buds of the tongue. The presence of a high or low amount of calcium in the blood must thus affect the taste of calcium." The possibility that blood calcium concentrations provide a signal-regulating calcium intake is intuitively congruent and consistent with findings showing that calcium appetite is aroused by treatments that reduce blood calcium [e.g., dietary calcium deficiency, PTX, dietary vitamin D deficiency, and acute administration of calcitonin (289)], and decreased by treatments that increase blood calcium [e.g., high-calcium diet, chronic administration of $1,25(OH)_2D$ (289)].

Lobaugh et al. (173) were the first to provide direct evidence for the contribution of blood calcium levels to calcium appetite. In addition to the study just described, in which calcium intake of chickens was suppressed by subcutaneous injections of calcium gluconate, they at-

tempted a technically elegant study in which elevations in calcium levels were localized to the upper neck and head. During a daily 2-h access period to calcite, chickens were simultaneously infused into the carotid artery with $CaCl_2$ and into an intrabrachial (wing) vein with the chelator EGTA. Relative to control infusions, these combined intracarotid-calcium, intrabrachial-EGTA infusions reduced calcite intake by ~33% without affecting food intake. Unfortunately, the results are difficult to interpret because each infusion was not given separately, and there was no verification that brain calcium levels were raised. Nevertheless, they point to the possibility that CNS calcium receptors are responsible for the control of calcium intake.

Lobaugh et al. (173) suggested that calcium appetite is controlled by plasma ionized calcium levels stimulating the brain. The emphasis on ionized rather than total blood calcium resolves the problem that chickens given estrogen injections have markedly elevated plasma calcium levels despite no change, or a slight increase, in calcium intake. This is because estrogen facilitates the synthesis of the calcium-binding protein vitellogenin, increasing the total amount of calcium carried in the blood without altering levels of ionized calcium (see Ref. 173).

Work with rats also supports the possibility that blood ionized calcium concentrations are related to calcium appetite. In one study in which metabolic measures were collected after various durations of calcium deprivation, plasma ionized calcium levels fell within the first 3 days, but plasma total calcium levels were unaffected until 17 days of calcium deprivation (289). Under identical test conditions, a demonstrable calcium appetite developed after ~4 days of deprivation. Thus the calcium appetite emerged before changes in plasma total calcium occurred. In another study, rats given subcutaneous injections of polyethylene glycol showed a marked drop in plasma ionized calcium levels and a strong calcium appetite despite unchanged, or slightly elevated, plasma total calcium levels (299).

1. Brain mechanisms

The brain is an obvious candidate for detecting changes in blood calcium concentrations. Oral administration of calcium increases brain calcium content within 30 min (117). Extracellular calcium stabilizes neural membranes and reduces excitability. Consistent with this, spontaneous activity of neurons in the paraventricular nucleus of the hypothalamus is inversely related to calcium content of the perfusate over the range of free calcium levels in the cerebrospinal fluid (0.75–1.26 mM; Ref. 219). Thus it is feasible that fluctuations in ECF calcium can influence behavior through the direct, physicochemical effects of calcium on neural membranes.

Calcium may also influence receptor activity. At least three classes of receptors have been identified in the CNS that respond to modest changes in extracellular calcium concentrations, including CaRs (250), metabotropic glutamate receptors (156, 257), and $GABA_B$ receptors (319). CaRs are located in high concentrations in the subfornical organ and other circumventricular organs that are crucial for the regulation of sodium appetite (250). This is circumstantial evidence that these receptors are involved in calcium appetite, but evidence of their function has not been forthcoming.

We have conducted several studies to characterize the effects of intracerebroventricular administration of calcium on the calcium appetite of rats. Intracerebroventricular calcium produced dose-related reductions in 50 mM $CaCl_2$ solution intake of both intact rats and rats with PTX. However, we found similar results after intracerebroventricular administration of EGTA and have been unable to demonstrate that the reduced intake is not simply due to malaise or general debilitation (unpublished results). Other investigators report that intracerebroventricular or hypothalamic administration of calcium increases food intake (6, 200–202, 266–268), but also causes hypothermia and catatonia (201, 308). It is doubtful whether any of these effects have physiological significance. For example, in the sheep, the minimum effective dose to induce feeding was 1.5 μmol $CaCl_2$ icv (267). With the assumption that the injectate is dispersed in 5 ml CSF, this would raise calcium concentrations by >0.3 mM, which is over twice the total calcium content of CSF (247).

Another approach has been to use calcium antagonists to influence calcium intake. There are many reports that calcium channel blockers reduce intake of sweet solutions and alcohol (e.g., Refs. 39, 64, 65, 221, 232), and although they also can produce conditioned taste aversions (222), at least one, [(−)-nimodipine], produces a significant place preference (63). In unpublished pilot work, we found that intraperitoneal injections of nifedipine or verapamil increased 50 mM $CaCl_2$ intake of rats in short-term tests. However, it was unclear whether this was a specific effect on calcium appetite or simply a response to the rapid drop in blood pressure produced by these compounds. Graded doses of nifedipine administered in the diet (100–1,600 mg/kg diet) had no effect on intake of 50 mM $CaCl_2$ solution, despite doubling daily water intakes.

Thus it remains to be demonstrated whether CNS calcium levels have a physiological role in the control of calcium intake. This may be similar to the failure of CNS sodium to influence sodium appetite in the rat (see Ref. 67). Circulating calcium levels may also be detected at other sites involved in the control of ingestion, such as the gastrointestinal tract or liver. Perfusion of the hepatic-portal vein with EGTA, calcium, or a calcium ionophore (A-23187) activated neural activity in the hypothalamo-

neurohypophysial tract (281), suggesting that receptor elements in the portal vein are sensitive to calcium. However, the relevance of this for calcium appetite has not been examined.

Calcium antagonizes the binding of morphine and/or enkephalins to CNS receptor sites (175, 280). Moreover, opiate-mediated antinociception is modulated by calcium, calcium antagonists, and vitamin D deficiency (13, 21, 51, 179, 280, 312). An extensive series of studies by Way (312) led to the hypothesis that neuronal calcium content is inversely related to analgesia. Given the role of the opiate system in the control of palatable food and fluid intake (e.g., Refs. 55, 56, 104), it is possible that circulating calcium interferes with opioid neurotransmission and thus affects intake of calcium and other compounds.

D. Primary Hormones of Calcium Homeostasis

The primary mechanism underlying sodium appetite involves the synergistic action of the hormones angiotensin II and aldosterone (for reviews, see Refs. 67, 79, 92, 262). Given the parallels between sodium and calcium appetite, it is reasonable to consider whether the hormones underlying physiological calcium homeostasis also underlie calcium appetite.

1. Parathyroid hormone

Richter and co-workers (241, 243) showed that rats with PTX maintained on a low-calcium diet increased intake of 65 or 78 mM CaLa. This could be reversed by several factors known to influence calcium metabolism, including feeding a high-calcium diet (241), reimplanting the parathyroids (243; Fig. 3), or injecting parathyroid extract, dihydrotachysterol, ergosterol, cholesterol, vitamin D_2, or vitamin D_3 (241). Rats with PTX also drank more of other calcium salts (acetate, gluconate, or nitrate) and were able to select an adequate amount of calcium when given a choice of five different salts (78 mM CaLa, 268 mM KCl, 357 mM sodium lactate, 136 mM $CaCl_2$, and 150 mM NaH_2PO_4; Ref. 242). Other studies involving rats with PTX are cited in section vE. In addition, Leshem et al. (167, 168) recently conducted studies in which intakes of $CaCl_2$ and $MgCl_2$ of rats with PTX were compared. They found that relative to intact controls, PTX increased intake of both solutions. Intake of $CaCl_2$ was generally greater than that of $MgCl_2$, suggesting some specificity of the appetite (see sect. $vF2$).

Administration of PTH generally reduces calcium intake. Lobaugh et al. (173) found that bolus intravenous injections of bovine parathyroid hormone (PTH) into chickens produced hypercalcemia and reduced intake of calcite ($CaCO_3$) grit. Chronic infusions of PTH into intact birds produced inconsistent effects on plasma calcium levels and only transient reductions in calcite intake. This

was probably due to a compensatory increase in calcitonin production (134, 211). Clearer results have been obtained using rats with thyroparathyroidectomy and thyroxine replacement, which lack both PTH and calcitonin. Chronic infusion of various doses of PTH into these animals produced marked and persistent reductions in intake of 50 mM $CaCl_2$ solution (Fig. 4). Some doses of PTH reduced calcium intake without affecting food or water intake (289).

2. Calcitonin

The role of calcitonin in the control of calcium appetite has not been investigated in any detail. This is surprising given work showing that CNS injections of calcitonin inhibit intake of food and alcohol (91, 161, 171, 216), and there is speculation that, at least in rodents, calcitonin acts to prevent the hypercalcemia following a meal. Calcitonin also influences water balance. Intracerebroventricular administration induces pronounced acute diuresis (91), subcutaneous injection causes water-satiated rats to begin drinking water (260), and daily subcutaneous doses increase water intake (as well as alcohol intake, Ref. 161).
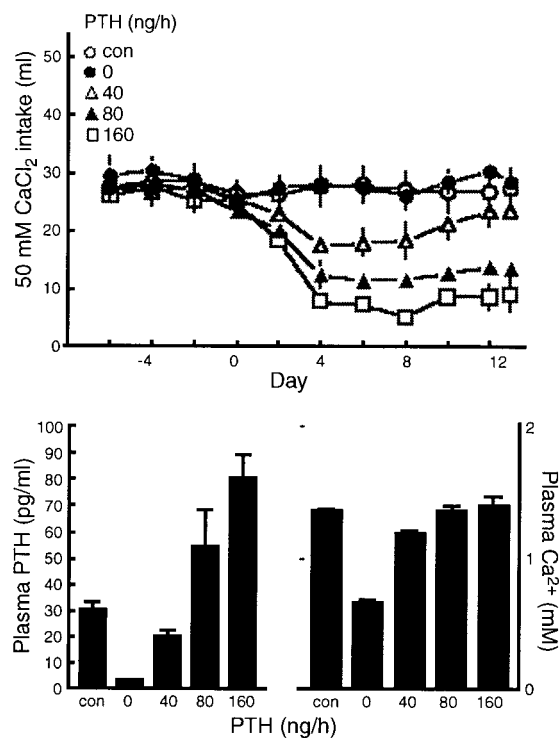
FIG. 4. Effect of various doses of parathyroid hormone (PTH) on 50 mM $CaCl_2$ intake by rats with thyroparathyroidectomy (TPTX). *Bottom*: plasma PTH and total calcium concentrations at the end of the infusion period (*day 11*). CON, surgically intact control group. It would be expected that $CaCl_2$ intakes would be higher in the untreated TPTX group than CON group. The lack of a difference is probably because the CON group was much bigger rats (281 vs. 151 g). [Data redrawn from Tordoff et al. (289).]

In vitro evidence suggests that calcitonin acts in the subfornical organ, and this is apparently independent of angiotensin because calcitonin-induced excitation could not be blocked by angiotensin receptor blockade (260). Of course, calcitonin has several other actions, including locomotor retardation, decreased gastrointestinal motility, cognitive abnormalities, decreased pain sensitivity, increased core temperature, and inhibition of sex hormones (reviewed in Ref. 62), which could be responsible for its effects on ingestion.

Removal of endogenous calcitonin by thyroidectomy (with thyroxine replacement treatment) had no effect on rats' intake of 50 mM $CaCl_2$ solution (289, 301). However, it is unclear whether this is an appropriate test of calcitonin's contribution to calcium appetite. Although calcitonin levels drop in response to acute calcium deprivation, they recover and are either unchanged or elevated during chronic deprivation of the rat (32, 163, 225, 289). Thus manipulations that increase calcitonin concentrations may be more informative than those that decrease it.

Chronic intravenous infusions of calcitonin into intact chickens did not affect intake of calcite (173). However, transient increases in 50 mM $CaCl_2$ solution intake were produced by subcutaneous infusions of various doses of calcitonin into rats with thyroidectomy or thyroparathyroidectomy. The organ ablations were required to remove endogenous calcitonin and endogenous PTH, which counters the effects of exogenous calcitonin (211). Doses that increased $CaCl_2$ intake also produced transient hypocalcemia, suggesting that the effects of calcitonin on intake may be secondary to its effects on blood calcium concentrations.

3. Vitamin D and its metabolites

In vitamin D deficiency, symptoms of calcium deficiency occur even with adequate calcium in the diet (reviewed in Ref. 90). Not surprisingly then, vitamin D deficiency can induce an appetite for calcium. Female rats deprived of vitamin D but given a choice among diets of different calcium and phosphorus contents ate more high-calcium diet than did replete controls. Vitamin D-deprived lactating rats given the same choices had even higher calcium intakes (36). Male rats maintained from weaning on a vitamin D-deficient diet for 9 wk also showed a strong calcium appetite relative to controls. This was due to the induced calcium deficiency and not the lack of vitamin D per se because rats showed no increase in calcium intake if fed the same vitamin D-deficient diet with sufficient extra lactose, calcium, and phosphorus to maintain normocalcemia (301).

Vitamin D improves gastrointestinal absorption of calcium, so eating food containing vitamin D helps to maintain calcium homeostasis. Along these lines, it has been shown that the high calcium intake of rats with PTX could be attenuated by vitamin D_2 or D_3 injections (241). A good source of vitamin D is cod liver oil, and there is evidence for a cod liver oil appetite. Richter (238) found that cod liver oil was accepted almost universally by 5-yr olds, but this acceptance decreased with age. This is consistent with the progressively reduced requirement for calcium as children age. Richter reported that "some children. . . had an almost insatiable appetite for cod liver oil. When allowed to satisfy their craving they took as much as 16 tablespoonfuls in one day, and continued to take high amounts for 5–10 days. After that they took only small amounts, and finally stated that they no longer liked it. Rats kept on diets deficient in vitamins A or D responded in much the same way when offered cod liver oil" (238; see Ref. 241 for studies with rats). Perhaps the children were mildly calcium deficient, and the high intake of cod liver oil provided vitamin D, which would counteract the deficiency by maximizing calcium retention.

The active component of vitamin D is $1,25(OH)_2D$. Marmoset monkeys with serum $1,25(OH)_2D$ concentrations in a range considered to indicate a marginal deficiency (<50 ng/ml) tended to drink more 78 or 156 mM CaLa solutions than did marmosets with adequate $1,25(OH)_2D$ levels (220). In contrast, two iguanas with low 25-hydroxyvitamin D levels, produced by restricting exposure to ultraviolet light, ingested less calcium (ground oyster shell) than did six iguanas with high 25-hydroxyvitamin D levels (212). A convincing explanation for this finding was not apparent.

Chronic infusions of $1,25(OH)_2D$ into rats produced complex effects on calcium intake. Intake of 50 mM $CaCl_2$ solution was persistently increased by moderate doses and transiently increased by high doses of $1,25(OH)_2D$. The high doses produced sustained reductions beginning 2–4 days after the start of infusions. The increases in $CaCl_2$ intake occurred despite simultaneous increases in total plasma calcium concentrations (289). Given the emphasis on blood ionized calcium (see sect. vC), it is unfortunate that total but not ionized calcium was measured. Nevertheless, this experiment appears to show a rare dissociation of hypocalcemia from increased calcium intake.

E. Reproductive and Adrenal Steroids

Female rats consume more CaLa solution than do male rats (261, 275). Gonadectomy of adults had no effect, but gonadectomy of neonates increased CaLa intake of adult males and decreased CaLa intake of adult females (229). This result argues that during a neonatal critical period, reproductive steroids have organizational effects on the tongue, gustatory nerves, and/or brain to influence calcium acceptance.

Administration of reproductive steroids to adults generally increases calcium intake. Chickens that were gonadectomized when 21 days old and tested 21 days later increased intake of a calcite supplement during 12 daily treatments with testosterone, estradiol, or the combination (173). Similarly, female rabbits given four minerals to drink in a cafeteria choice increased intake of 250 mM $CaCl_2$ when injected with 17β-estradiol (59) or a mixture of estradiol and progesterone (70). These effects appear to be restricted to need-free calcium intake. Although there was a sex difference in calcium intake of rats fed a nutritionally complete diet, there was no difference between the sexes in the initial intake of CaLa after 3-wk calcium deprivation (261). It is also unclear whether the sex differences are specific for calcium appetite or a general increase in acceptability of all taste solutions by female rats.

There was no difference in intake of 65 or 130 mM CaLa solutions by male and nulliparous female marmoset monkeys (220) given two 7-h tests. It is unclear whether this failure to see a sex difference is due to a difference between species, the use of an insufficiently long test, or some other methodological issue.

1. Pregnancy and lactation

Producing offspring imposes a high calcium requirement and causes changes in calcium metabolism that are reflected by increased calcium intakes in rats (7, 192, 240, 323), rabbits (69, 70), marmoset monkeys (220), and probably humans (3, 113, 143, 153, 154, 162, 174, 277). With the possible exception of Marmoset monkeys (220), increases in calcium intake are generally small during pregnancy. Rats increased intake of 78 mM CaLa by only ~1 ml/day (240, 323) and rabbits increased intake of 500 mM $CaCl_2$, but because intake of food (and thus calcium in food) decreased, total calcium intake was unaltered (69).

Much more substantial increases in calcium intake occur during lactation. These have been demonstrated in experiments involving calcium presented as a single source (192, 220, 323), in a cafeteria choice of solutions (69, 240), or a cafeteria choice of foods (36). In the rat, intakes of 78 mM CaLa increased by ~9 ml/day (240). Rat mothers with large litters drank more CaLa than did those with small litters (192).

Even with ad libitum access to calcium, the lactating mother loses bone calcium. Consistent with this, rats and some rabbits continued to ingest large amounts of calcium for several weeks after their pups had been weaned (69, 240). However, the increased appetite in response to both pregnancy and lactation is probably hormonally mediated rather than driven by the loss of calcium to offspring. Pseudopregnancy increased calcium intake of rabbits (59, 70). Moreover, rat dams with galactophores cut (preventing milk production) still increase 78 mM CaLa

solution intake (192). The physiological mechanism for this is unclear but probably involves a reflex response to suckling (see Ref. 192) because $CaCl_2$ intake of rabbits with litters removed at birth rapidly returned to normal (70).

Denton and Nelson (70) have performed several studies on the changes in mineral intake produced by daily injections of reproductive hormones, using as subjects rabbits given simultaneous access to four mineral solutions ($CaCl_2$, NaCl, KCl, and $MgCl_2$). The increased calcium intake seen during lactation could be mimicked to some extent by administration of hormones that are normally elevated during lactation. Male, normal female, and/or pseudopregnant female rabbits increased 250 mM $CaCl_2$ intake when administered an ACTH-prolactin-oxytocin mixture (70), an ACTH-oxytocin mixture (70), or prolactin alone (269). A prolactin-oxytocin mixture (70), oxytocin alone (269), or growth hormone alone (269) were ineffective. ACTH alone maintained the high calcium intakes of rabbits previously administered the ACTH-prolactin-oxytocin mixture (70), but ACTH without pretreatments was ineffective (25). ACTH also had no effect on 250 mM $CaCl_2$ intake by rats (314) or sheep (313) given the same cafeteria choice as the rabbits.

2. Shell formation

Like female mammals, female birds consume more calcium than do males. For example, the time spent consuming calcium-containing snail shells is greater in female than male great tits (109). During egg laying, calcium requirements increase dramatically (e.g., Refs. 109, 129). Female birds need 10–15 times as much calcium to produce eggs as do similarly sized mammals to produce embryos (270). A hen can produce an egg containing 60 mmol calcium daily for 50 wk. Up to 30 times the hen's own total calcium is produced as shell in a normal laying year (102). For some species, the calcium content of a single clutch of eggs can exceed that of the female's skeleton (see Ref. 224). Some calcium is obtained by liberation of calcium from medullary bone, which is a tissue found only in birds, but this is insufficient to meet all needs (29, 155, 270). Even birds such as terns, which eat a diet of small fish, cannot gain sufficient calcium from the bones they swallow to satisfy the requirements for egg production (33, 208).

Graveland and colleagues (106–111) have conducted a series of elegant observational and experimental studies involving great tits, *Parus major*, in the Netherlands. The work was initiated by observations that tits nesting in areas with poor soil and a scarcity of snails produced eggs with thin and porous shells, and clutches were often deserted. Young tits had high incidences of bone malformation and frequently died (108).

Providing snail or chicken egg shell increased the tits'

egg shell thickness and egg viability. Some females spent over 30% of their evening prior to egg laying handling and consuming the shells. Males showed little interest but were occasionally seen to feed shell fragments to females (109). These behaviors led to a dramatic increase in calcium intake of females from baseline levels of ~5 to ~70 mg/day during egg laying (in comparison, one egg contains ~30 mg calcium, of which ~60% is deposited in <8 h; Fig. 5).

Experimental studies confirmed the field observations. Great tits maintained in an aviary with poor quality soil and without access to snail shells produced less eggs (4.2 vs. 8.5), with ~33% being noticeably defective. The females spent 42% of their time searching on the ground, frequently dug in the soil, and may also have eaten their own eggs. When snail shells were introduced, most females started eating them immediately (within 5 s) and consumed 132 mg calcium [(109), see similar results with the cowbird (125), and failures to observe an effect of calcium supplementation on egg quality in blue tits (224) or house wrens (138)].

The relationship between calcium intake and egg formation is probably hormonally mediated. The greatest calcium intake is seen before the period that egg calcification occurs, suggesting the intake of calcium anticipates, rather than follows, calcium need (see Ref. 129 and comments to Ref. 286). Moreover, calcium intake still increased when shell formation was prevented by ligating the oviduct or causing shell-less eggs to be produced (129). This may parallel the increased calcium intake that occurs in mammals that are unable to nurse their pups (70, 192). There has been no attempt to associate the increase in appetite under these conditions with changes in particular hormones.

3. Adrenal steroids

ACTH, which activates the synthesis and release of adrenal steroids, had no effect on 250 mM $CaCl_2$ intake of
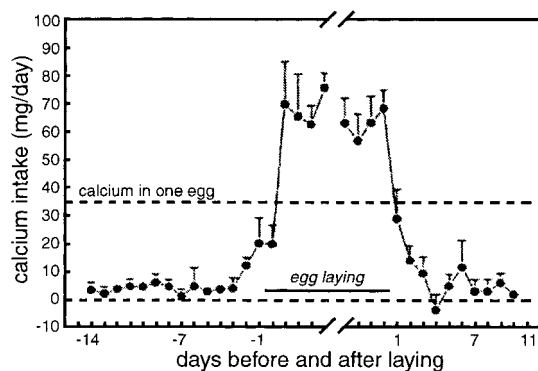

FIG. 5. Intake of snail shells by egg-laying Great Tit females in captivity. Values are means ± SE for 11 females. [From Graveland (108).]

rats (314), sheep (313), or rabbits (70) (see also sect. vE1). On the other hand, chronic administration of cortisol acetate or cortisol combined with corticosterone doubled 500 mM $CaCl_2$ intake of rabbits (25). The increased calcium intake persisted throughout the 10-day posttreatment period. Corticosterone alone had no effect on calcium intake, and the effect of cortisol was not seen in four rabbits with adrenalectomy (25).

Administration of the mineralocorticoid deoxycorticosterone acetate to rabbits increased intake of 500 mM $CaCl_2$ solution in a choice with 500 mM $MgCl_2$, NaCl, and KCl, but not 150 mM $CaCl_2$ solution in a choice with 150 mM $MgCl_2$, NaCl, and KCl. Low doses of aldosterone had no effect on intake of any of the minerals offered to the rabbits (68).

F. Other Factors Influencing Calcium Intake

1. Genes

There are several observations of strain differences in calcium intake. Wild female rabbits caught in the Snowy Mountain regions of Australia drank more 500 mM $CaCl_2$ solution than did rabbits bred in Canberra (69). Rats of the SHR strain drank two to four times more $CaCl_2$ solution than did WKY controls (85, 292). They also ate more of a calcium-containing food than did WKY when allowed to choose between a calcium-deficient and replete diet (292). In unpublished work, we have also found large differences in calcium intake between various strains of mice. For example, during 48-h two-bottle tests with a choice between water and 32 mM $CaCl_2$, 129X1/SvJ and AKR/J mice drank more $CaCl_2$ than water, BUB/BNG and C3H/HeJ mice showed indifference, and CE/J and KK/H1J strains strongly avoided the $CaCl_2$ solution. The same strain differences were observed with other calcium salts and related compounds (CaLa, $SrCl_2$, $MgCl_2$) but not with NaCl solutions, suggesting that the differences are not due merely to a general increase in avidity for salt or novel tastes. There has been no attempt to isolate the genes involved.

2. Age and growth

Calcium requirements vary considerably depending on the animal's growth rate. In mammals, calcium is provided in mother's milk during the immediate postnatal period. However, the demand continues after weaning and into adolescence. Consistent with the negatively accelerating growth rates, voluntary intake of CaLa and $CaCl_2$ is greatest in young male rats and declines with age (234, 292). Similarly, CaLa intake is reduced during a period of restricted growth produced by vitamin B deficiency and increased during the period of growth rebound when the vitamin is restored (233, 235). Whereas it re-

quires only 1 or 2 days of dietary calcium deprivation to induce a measurable increase in $CaCl_2$ solution intake of weanling rats, it can take several weeks in adults (unpublished results; see also Ref. 323).

Leshem et al. (168) conducted several studies to assess the developmental onset of calcium appetite in the rat. In one study, they examined the acceptance of orally infused 1 M $CaCl_2$ and 1 M $MgCl_2$ to pups of different ages. They found that in intact pups, there was no difference in intake of $CaCl_2$ or $MgCl_2$ at any age tested (2, 6, 8, 10, 15, or 20 days). The lack of a preference for calcium before *day 6* was supported by findings that intact 3-day-old rats apparently show no aversion to 1 M $CaCl_2$ (168) and that sodium-deprived pups mildly preferred $CaCl_2$ to NaCl (169). This may reflect the immaturity of the orosensory apparatus at this age (cf. Ref. 122). At older ages, it is clear that the rat is capable of differentiating between $CaCl_2$ and $MgCl_2$ because pups with PTX drank more $CaCl_2$ than $MgCl_2$ (they drank more of both salts).

Birds often feed their nestlings items rich in calcium. For example, vultures feed their young leg bones and teeth of large mammals and bivalve shells (199). Nestlings of the song thrush and blackbird receive sufficient calcium only because their main food, earthworms, contain soil rich in calcium (reviewed in Ref. 107). Young hens regulate calcium intake better than do old ones (124).

3. Time of day

Hughes (129) established the importance of circadian factors in determining calcium intake of chickens. Intake of cockleshell grit was highest during the afternoon and did not mirror food intake, which peaked during the morning. In contrast, Holcombe et al. (124) found that hens given continuous access to both low- and high-calcium diets ate most calcium in the early morning and early evening. It is unclear what accounts for the difference in results between the two studies.

The circadian pattern of calcium intake has not been directly studied in mammals, although our own observations of rats suggest they consume large volumes of calcium solutions during the dark period (unpublished results). Calcium-deprived rats with free access to 300 mM NaCl solution drink most solution during the second half of the dark period (302).

4. Social factors

Griminger and Lutz (114) compared the effect of housing conditions on calcium intakes of hens given free access to either oyster shells or calcite grit. The hens consumed more of these sources of calcium if communally housed than if individually housed in floor pens with litter. However, hens housed individually in suspended cages had the highest calcium intakes. The authors spec-

ulate that the high intake of the hens maintained in individual cages was due to boredom.

Joshua and Mueller (140) deprived individually housed chickens of calcium for 28 days and gave them a choice between sand and sand mixed with calcite. They found that only one replete and two calcium-deficient birds ate an appreciable amount of calcium. In contrast, a group of communally housed birds fed calcium-deficient diet ate approximately six times more calcite than did communally housed replete controls. In a second study, the calcium intake of birds reared together but tested in isolation was measured. When communally housed, the birds fed calcium-deficient diet ate approximately five times more calcite than did replete controls. When individually housed, they continued to eat more calcium, and this difference persisted even when the calcium-deficient birds were switched back to control diet. Although there are several methodological issues that cloud interpretation, one explanation of the results is that the birds learned to eat calcium when communally housed, and this behavior was maintained even when individually housed, and even when there was no longer a need for calcium.

VI. DO HUMANS HAVE A CALCIUM APPETITE?

The possible existence of a calcium appetite in humans has not received serious consideration. There are occasional allusions to a human calcium appetite in the anthropological literature. Almost 50 years ago, De Castro (66) used the term *calcium hunger* to refer to the "hidden hunger" state produced by calcium deficiency. He provided as an example the consumption of animal bones by Eskimos (66). Drummond (74) mentioned that "in East Africa the search for edible earths rich in calcium was frequently a cause of tribal raids and the evidence points to these products being instinctively consumed to make good the lack of lime in the customary diet." Unfortunately, this statement is not substantiated by the author, and I cannot find other references to it. Baker and Mazess (5) report that Quechua Indians from the high Peruvian Andes supplement their diet by eating a powdered rock porridge (*cal*) and chewing ashed stalks (*llipta)* along with coca. This may help alleviate calcium deficiency, but it is unlikely to be the primary purpose. The Quechua also consume a clay substance (*cha'qo*) that contains no calcium. Moreover, their daily calcium intake is relatively high (10.75 mmol/day) relative to other native groups, which presumably do not ingest these compounds. It may be that lime is chewed because it increases the availability of active alkaloids from coca.

There is evidence that Western Europeans prefer water containing millimolar quantities of calcium to pure water (326). The clinical literature contains some intriguing anecdotes. In addition to his work on cod liver oil

acceptance by children (see sect. vD), Richter (238) wrote that "children with parathyroid deficiency have been reported to show a craving for chalk, plaster, and other substances with a high calcium content" but unfortunately he did not provide details. There is one study showing that patients with pseudohypoparathyroidism have reduced sensitivity to selected odors and sour and bitter tastes (120). Perhaps these changes are due to an underlying altered sensitivity to calcium, but this was not tested directly, and there were equivocal effects on sensitivity to sweetness and saltiness, which makes interpretation difficult. Recently, Leshem and Rudoy (170) have reported that patients undergoing dialysis, which causes several metabolic effects including hypocalcemia, preferred higher concentrations of calcium mixed in cheese after than before treatment. There has been no work on the taste perception or calcium intake of patients with other diseases that perturb calcium metabolism (see review in Ref. 177).

Several studies show that calcium intakes are higher in lactating than nonlactating women and that calcium intakes increase as the children become older (and suckle more milk; e.g., Refs. 3, 143, 153, 154, 162, 174, 277). Intriguingly, women nursing twins consume significantly more calcium than do women with only one child to nurse but have higher serum calcium concentrations (113). Moreover, the ratio of calcium intake to calorie intake is higher in mothers of twins than singletons (113), suggesting there may be preferential intake of calcium during lactation. Of course, it is unclear whether this is due to changes in diet selection to favor calcium-containing products, incidental to increased energy intakes, or the result of nutritional counseling.

Taken together, these results make an enticing case for the existence of a human calcium appetite, but it is far from compelling. There are at least three reasons why this may be so. First, nobody has systematically looked for a human calcium appetite. Second, it is possible that humans have innate behavioral mechanisms to respond to calcium deficiency but never use them. This is because the "low" human calcium intakes abhorred by nutritionists are rarely as low as those used in animal studies. The United States recommended daily allowance and other calcium intake recommendations are based on protection from diseases that generally strike after the reproductive years, and so failure to meet these intakes exerts little evolutionary selective pressure. It may be that calcium deficiency in humans is never sufficiently severe to activate behavioral mechanisms.

The third issue pertains to how a calcium appetite would be observed. Even under the most propitious circumstances, there is poor correspondence between physiological need and food selection in humans. For example, there is a general belief that patients with adrenal insufficiency consume large amounts of NaCl. Although this certainly occurs sometimes (317), only about one adult patient in six has noticeably high NaCl intakes (see Ref. 15). Patients with adrenal deficiencies who ate foods with a high salt content such as ham and sauerkraut "did not in any way associate the craving for these foods with their high salt content. All they knew was that the food had an unusually pleasant taste to them" (238).

Relative to the case for sodium, where there is a well-defined taste quality, the circumstances for detection of calcium are far from ideal. Social and cultural restrictions inhibit the consumption of "pure" sources of calcium such as bones, shells, and calcareous grit. Dairy products, which form the most appreciable source of calcium in the United States diet, have complex flavor profiles. Thus, if there is a distinctive taste to calcium, it may well be masked. The effects of calcium deficiency on food choice are made even more complicated by the acquisition of flavor preferences due to the association of particular flavors with their beneficial effects. For example, data from the rat showing that sodium liberates calcium from plasma proteins led to the proposal that one response to calcium deficiency is to consume sodium because this temporarily alleviates calcium deficiency (298). Consuming sodium would reduce the appetite for calcium until the additional sodium was excreted. Under these circumstances, salty foods would become preferred during calcium deficiency even though saltiness does not signal calcium.

There are indirect but persuasive data to support this hypothesis. Populations with a high demand for calcium have elevated NaCl preferences [e.g., pregnant and lactating women (249), children (16, 60, 71)]. Moreover, subjects with low calcium intakes prefer significantly higher concentrations of NaCl in tomato juice and other salted foods than do subjects with high calcium intakes (43, 297). More generally, a large, well-controlled study found that calcium supplementation reduced cravings of women suffering from premenstrual symptoms (288). The point here is that calcium appetite would be difficult to identify because it would not necessarily produce a change in liking of calcium-containing foods only.

VII. COMMENTARY

The main function of this review is to alert readers to the wealth of evidence for the existence of a calcium appetite. The notion held by several early investigators that calcium appetite was not interesting because it was learned is incorrect. Although there is no doubt that animals can associate chemosensory cues with the postingestive effects of calcium ingestion (e.g., Refs. 50, 130, 131, 248, 263, 322), there is also strong evidence for innate controls of the appetite (e.g., Refs. 49, 50, 189). Thus, like most other behaviors, calcium appetite has both innate and learned components.

There are many unresolved issues about calcium appetite. Perhaps the most important one is the nature of the physiological event or events that initiate and terminate it. Almost all the data are consistent with a simple model in which the appetite is inversely related to blood ionized calcium concentrations (e.g., Refs. 130, 131, 289; see Fig. 6). Such a mechanism is refreshingly simple but stands in marked contrast to those underlying other appetites, where multiple hormonal and neural controls are involved, and different mechanisms participate in initiation and satiation (e.g., Refs. 67, 79, 89, 92, 93). I suspect that the straightforward explanation of the underlying mechanism of calcium appetite reflects the paucity of pertinent data rather than the simplicity of the mechanism. Nevertheless, it is important to discover how and where changes in blood calcium concentration are detected by the CNS and how these signals are processed to influence behavior.

It is tempting to compare the appetite for calcium with the much more thoroughly studied appetite for sodium. However, the parallels are not very strong. For sodium, the primary initiator of the appetite is the synergistic action of angiotensin and aldosterone in the brain (67, 79, 88, 92). The mechanism of satiation is less clear, but at least in the rat, it probably involves gastrointestinal and hepatic sodium and osmoreceptors rather than a direct action of sodium in the brain (67, 80, 300, 304). For calcium appetite, the evidence is against a synergetic combination of the three primary hormones (289), and unlike the situation with angiotensin and salt appetite, there is no evidence that parathyroid hormone or calcitonin can influence calcium intake independently of their actions on blood calcium concentrations. The parallel between aldosterone and $1,25(OH)_2D$ is intriguing because both are steroids that enhance intestinal ion absorption and renal reabsorption, and both can influence appetite. However, large doses of aldosterone increase NaCl intake (320), but large doses of $1,25(OH)_2D$ decrease calcium intake (289). Thus an understanding of sodium appetite has not proven particularly useful in understanding calcium appetite. Indeed, calcium appetite might more profitably be compared with the appetite for glucose or
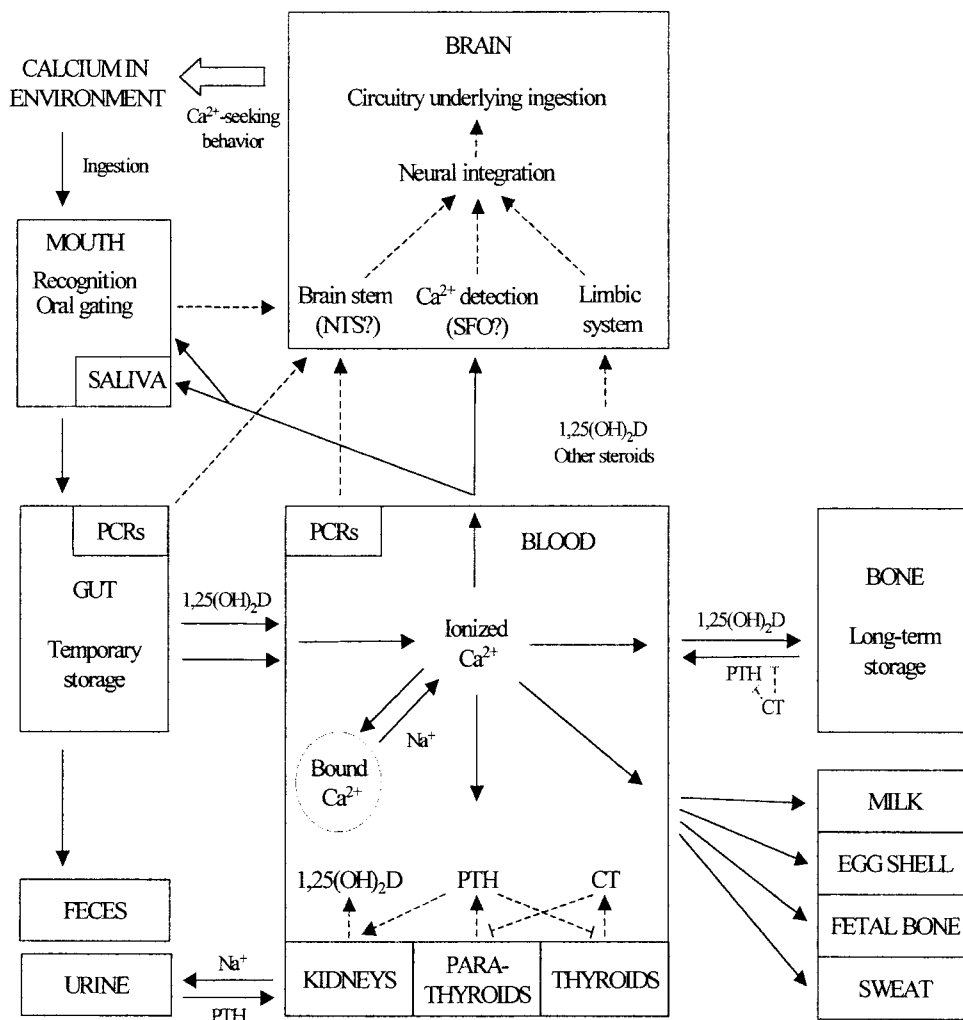

FIG. 6. A simplified model of the relationship between calcium homeostasis and calcium appetite. According to this model, the primary signal for calcium appetite is derived from blood ionized calcium levels, which are detected by calcium receptors, perhaps in the subfornical organ (SFO). Signals concerning calcium status also arise from the oral cavity and peripheral pre- and postabsorptive calcium receptors (PCRs), and these may be integrated in the nucleus tractus solitarius (NTS) or other early brain stem nuclei. The influence of parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D [$1,25-(OH)_2D$] are mostly secondary to their actions on blood ionized calcium. However, there may also be direct effects of $1,25(OH)_2D$ and other hormones on the brain to influence calcium appetite (289). Note that blood calcium levels can modulate calcium taste by influencing salivary and extracellular calcium in the mouth.

energy, because unlike sodium homeostasis, these involve a reservoir (bone for calcium, glycogen and/or fat for glucose and energy; see Ref. 321 for a reservoir theory of sodium appetite).

The contribution of bone to the control of calcium appetite is unexplored. Some role seems likely because it is generally more difficult to produce a calcium appetite in large animals (with big bones) than small ones (with little bones; see sect. III). It is unknown whether calcium-deprived animals given access to a source of calcium ingest sufficient to recoup all the bone calcium they have lost. Rats and chickens can ingest enough calcium to replete extracellular calcium levels within a few minutes. If blood calcium levels are related to calcium intake, one might expect that deficient animals would ingest calcium in discrete bouts as extracellular calcium stores are repeatedly filled and then drained to replenish bone stores. It is also conceivable that calcium appetite could respond to a feedback signal from bone. This would be analogous in action to leptin, the "fat-feedback" hormone, involved in the control of energy appetite.

The low blood calcium-high calcium intake hypothesis does not easily accommodate findings that administration of reproductive and adrenal steroids increase calcium intake (e.g., Refs. 68–70, 269). One of the biggest issues with this literature is the specificity of the increases in calcium appetite. Most of the studies involved rabbits given a choice between four mineral chlorides (calcium, potassium, magnesium, and sodium). In nearly every case where calcium intake increased, there was also an increase in NaCl intake. Moreover, energy intake is increased during pregnancy and lactation, and by administration of estradiol or testosterone (see Refs. 47, 67 and references therein). On the other hand, hens given a separate source of calcium during egg laying ate the supplement and did not show the usual increase in food intake (see Ref. 286), suggesting that, at least in this case, energy intake is incidental to the need for calcium. It remains to be determined to what extent the three appetites are independent or respond to a single physiological control.

The specificity of calcium appetite has been an enduring concern. Rats deprived of dietary calcium increase ingestion of diverse salt solutions, including most monovalent and divalent chlorides, some acids, and lead acetate (48, 49, 274, 275, 290). Some of these solutions may be ingested because they taste similar to calcium. Others, such as NaCl and lead acetate, may at least temporarily ameliorate calcium deficiency (298). Nevertheless, this lack of specificity is inconsistent with a specific appetite. Recently, it has been suggested that the appetite for calcium produced by PTX is more specific than that produced by dietary deprivation (167, 168), perhaps because of the rapid onset of deficiency after PTX. However, the studies using rats with PTX involve a choice test ($CaCl_2$ vs. NaCl or $MgCl_2$), whereas the dietary deprivation stud-

ies involve tests of single solutions. I suspect that this difference in procedure accounts for the difference in results and thus I do not think it necessary to distinguish between the appetites induced by PTX and dietary deprivation.

Another issue is the nature of calcium taste. Does calcium have a specific gustatory receptor or is it recognized simply as the sum of other taste modalities? Humans find the taste of calcium difficult to describe. Most people have tasted the $CaCO_3$ found in blackboard chalk or masonry lime and describe it as "chalky," but they would be hard pressed to reduce this to sweet, sour, salty, and bitter components. Subjects in controlled psychophysical studies tasting calcium solutions also give inconsistent descriptions (e.g., Ref. 258). One reason for this is that the taste of calcium is influenced by its concentration (295). It is noteworthy in this respect that most psychophysical and electrophysiological studies involve oral stimulation with high concentrations of pure calcium salts, yet these are never experienced outside the laboratory. Many psychophysicists consider calcium to have a salty component, but it is doubtful that saltiness is a property of calcium in the diet because this is present only at high concentrations (i.e., 31.6 or 100 mM $CaCl_2$; Ref. 295). Skim milk, which is probably the closest food in concentration and consistency to these "pure" solutions contains <32 mM calcium, of which ~92% of this is bound to casein or phosphate (see Ref. 247 for a review), and so is probably not tasted.

The recent genetic characterization of many bitter taste receptors (e.g., Refs. 2, 45, 127, 181, 226) reinforces older psychophysical data (e.g., Refs. 10, 35), suggesting that there are probably several types of bitterness. Perhaps one of them is mediated by a calcium receptor. It is interesting in this regard that there is moderate homology between at least one bitter receptor (T1R1) and the extracellular calcium receptor CaR (127). On the other hand, a dedicated gustatory pathway is not a prerequisite for the existence of a specific appetite (cf. protein appetite) so "calcium taste," if it exists at all, may be due to stimulation of a combination of gustatory receptors coding other taste qualities in a particular pattern. Arguing against a specific gustatory receptor is the lack of data from mammalian electrophysiological recordings of afferent fibers that can be activated by calcium alone (see Ref. 207 for an exception). However, the great majority of mammalian recordings have been made from the chorda tympani nerve. Given that fibers in the frog glossopharyngeal nerve are highly and specifically sensitive to calcium (e.g., Refs. 4, 141, 150, 151), it would be interesting to survey the mammalian glossopharyngeal nerve for calcium-sensitive fibers. Other major issues with respect to calcium taste include the contribution of extracellular calcium to the perception of other tastes (e.g., Refs. 132, 133, 157, 182, 197) and the mechanism by which body calcium

status influences gustatory calcium sensitivity (135, 158). In particular, there is very little work on the role of saliva in determining sensitivity to calcium.

Despite the rich behavioral data available, and the importance of calcium intake to human health, our understanding of the physiological basis of calcium appetite lags behind that of other appetites, particularly sodium appetite. There is a need for concerted examinations of calcium appetite's ontogeny, neuroanatomy, neurochemistry, and genetic basis. There is also a need for a direct test to determine whether calcium appetite exists in humans. I hope this review acts as an impetus to encourage concerted research into these neglected but important questions.

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