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THE EVOLUTION OF BONE

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Abstract.—Vertebrates are practically unique among the Metazoa in their possession of a skeleton made from calcium phosphate rather than calcium carbonate. Interpretation of the origin of a phosphatic skeleton in early vertebrates has previously centered primarily on systemic requirements for phosphate and/or calcium storage or excretion. These interpretations afford no anatomical or physiological advantage(s) that would not have been equally valuable to many invertebrates.

We suggest the calcium phosphate skeleton is distinctly advantageous to vertebrates because of their relatively unusual and ancient pattern of activity metabolism: intense bursts of activity supported primarily by rapid intramuscular formation of lactic acid. Bursts of intense activity by vertebrates are followed by often protracted periods of marked systemic acidosis. This postactive acidosis apparently generates slight skeletal dissolution, associated with simultaneous vascular hypercalcemia. A variety of apparently unrelated histological features of the skeleton in a number of vertebrates may minimize this postactive hypercalcemia.

We present new data that suggest that postactive skeletal dissolution would be significantly exacerbated if bone were composed of calcium carbonate rather than calcium phosphate. The former is far less stable both in vivo and in vitro than is calcium hydroxyapatite, under both resting and postactive physiological conditions.

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The evolutionary history of the skeleton is the best understood of all vertebrate organ systems. The fossil record almost continuously reveals details of its biology virtually from the origin of vertebrates more than 510 million years ago (Romer, 1966). Nevertheless, biologists have long remained puzzled by an attribute of bone that sets the vertebrate skeleton apart from the calcareous skeletons of virtually all other metazoans: the hard inorganic component or "ground substance," of bone is primarily calcium phosphate, as crystalline calcium hydroxyapatite ($3Ca_3[PO_4]_2Ca[OH]_2$). In contrast, the mineral or hard fraction of almost all calcareous invertebrate skeletons since the Lower Tommotian (ca. 560 MY B.P.) of the Early Cambrian is primarily calcium carbonate, as crystalline calcite or aragonite ($CaCO_3$) (Lowenstam, 1981).

Why have vertebrates not used the skeletal support material that is almost universal among invertebrate groups? What, if any, special properties of calcium hydroxyapatite have led to its early incorporation into vertebrate structural material? A variety of explanations have been advanced (see below) but none of these is particularly compelling or deals with circumstances unique to vertebrates. After reviewing previous hypotheses, we present a new explanation for the evolution of the chemical composition of bone.

Previous Hypotheses

Phosphate Excretion.—The earliest vertebrates for which there is any fossil record were the agnatha of the early to middle Paleozoic Era, the ostracoderms. These were primarily small, jawless, perhaps microphagous feeders (Mallatt, 1985) (Northcutt and Gans [1983] believe they may have been at least moderately predaceous). Bone was limited to an extensive exoskeleton, which contributed nothing to internal support. Accordingly, Berrill (1955) suggested the phosphatic skeleton originated, at least in part, as a dermally deposited excretory product. He assumed that ostracoderms were originally freshwater and, therefore, were forced to deal with excess phosphate, which they would presumably have absorbed in rivers and streams. For unstated reasons, he believed the kidneys of early vertebrates were incapable of excreting excess phosphate. Thus, ostracoderms were forced to rid

themselves of excretory products as an exoskeleton, as is currently done by some arthropods.

Evidence for such a scenario is weak. Freshwater and marine phosphate concentrations vary little except in some river deltas where human-generated effluent may contain high phosphate concentrations (Halstead, 1968). Furthermore, it is now generally accepted that the earliest ostracoderms were marine, not freshwater, inhabitants (Halstead, 1973; Repetski, 1978). In any case, all extant vertebrates are capable of phosphate excretion (Eckert and Randall, 1983), and there is no reason to assume that early vertebrates were not similar in this respect.

Phosphate Storage.—Pautard (1961, 1962) suggested that a Paleozoic dearth of marine phosphates led early vertebrates to sequester phosphate as calcium phosphate. Accessible phosphate stores would have been of particular utility to any active animal, and bone-derived phosphate in early vertebrates could have been useful for manufacturing compounds essential for energy transfer during normal metabolism. Calcium is abundant in sea water and would have been a convenient agent for the precipitation of phosphate. Only later, with the advent of terrestrial vertebrates, is bone seen as playing a critical role in mechanical support.

There is little to indicate that present marine phosphate concentrations have changed much since the appearance of vertebrates (Walker et al., 1983; Holland, 1984), and present phosphate concentrations are, of course, sufficient to support an abundant and varied biomass even in invertebrate groups that do not possess such phosphatic storage material.

Seasonal Phosphate Storage.—More recently, Halstead (1974) has advanced a hypothesis that represents an amalgam of previous ideas on the subject. In this scenario, bone is postulated to have originated in response to the relative "overabundance" of marine calcium in concert with seasonal depletion of organophosphates from various strata of marine waters. Thus, early vertebrates seasonally sequestered phosphate for future use by complexing it with excess calcium, which was continually entering the animal by inward diffusion from the marine environment. A calcium phosphate precipitate was stored in the skin until seasonal phosphate diminution, when some of the bone-like exoskeleton was broken down and the phosphate utilized for metabolic purposes.

Halstead's ideas are vulnerable to criticisms similar to those that weaken earlier hypotheses. There is no reason to believe that the early vertebrate kidney would not have been able to excrete excess divalent calcium and retain or excrete phosphate ions, depending on need. In addition, most, if not all, marine invertebrates that do not store significant skeletal phosphate do not seem to have been adversely affected by such an omission.

All of these previous explanations for the unique chemistry of the vertebrate skeleton share a feature that makes none of them particularly compelling. They postulate no advantages to ancient vertebrates that would not have been equally useful to a variety of invertebrates. Thus, the original question remains: why was calcium phosphate selected as the building material for the vertebrate skeleton but not for the skeletons of most invertebrates?

A New Hypothesis

We offer a new explanation for the unusual chemical composition of bone: calcium hydroxyapatite has a greater chemical stability than calcium carbonate in the acidic conditions that prevail in most vertebrate systems, particularly after intense exercise. Specifically, the lower solubility product of the crystalline structure of calcium hydroxyapatite is better suited than that of calcium carbonate to the pH regime of the vertebrate internal milieu.

Following bouts of intense activity, osseous vertebrates customarily experience acute, marked depression of extracellular fluid (ECF) pH (Table 1). Among vertebrates with osseous skeletons, this acidosis generates slight skeletal dissolution and consequent vascular hypercalcemia (Ruben and Bennett, 1981). We suggest that the magnitude of this skeletal dissociation and hypercalcemia would be significantly greater were the skeleton calcitic rather than phosphatic, resulting in physiological diffi-

TABLE 1. Extracellular fluid pH at rest and after exhaustive activity in a variety of vertebrates and decapod crustaceans. Values in parentheses represent the percentage increment in extracellular fluid hydrogen-ion concentration following activity.

Species	Rest pH	Postactive pH	Reference
Lamprey	7.91	7.23 (378%)	Ruben and Bennett, 1981
Hagfish	8.03	7.31 (426%)	Ruben and Bennett, 1980
Shark	7.78	7.14 (336%)	Piiper et al., 1972
Rainbow trout	7.80	7.30 (217%)	Mulligan and Wood, 1986
Rattlesnake	7.38	6.79 (289%)	Ruben, 1979
Salt water crocodile	7.4	6.6–7.0 (157–545%)	Bennett et al., 1985
Human	7.41	7.15 (82%)	Cunningham et al., 1985
Six decapod crustaceans	7.83	7.53 (99%)	McMahon, 1981

culties for animals with a vertebrate pattern of metabolism and activity.

Of all the Metazoa, vertebrates rely on the most prodigious rates of production of lactic acid via glycolysis for ATP generation during periods of intense activity (Ruben and Bennett, 1980). This pathway of anaerobic energy metabolism enables vertebrates to attain levels of burst activity that would be impossible if they were dependent solely on aerobic metabolism. However, systemic release of this lactic acid is the causal factor in the disruption of postactive ECF pH (see Table 1). Blood acid levels, or hydrogen-ion concentration, in exercise-fatigued vertebrates are often at least 4–5 times normal, and blood pH levels may descend to 7.0 or lower. Activity-induced blood pH depression may persist for several hours or longer, following extended bouts of intense activity (Bennett, 1978). This pattern of activity physiology with its associated postexercise pH depression is as old as vertebrates themselves. Utilization of similar modes of activity physiology in extant gnathostomes, agnathans (lampreys and hagfish), and the nonvertebrate chordate amphioxus indicates the pattern's probable presence in the earliest common ancestor of these vertebrate groups, the early- to mid-Paleozoic ostracoderms (Ruben and Bennett, 1980).

Significantly, the extant nonvertebrate chordate amphioxus (Cephalochordata), which completely lacks ossification and is included in a taxon believed to be close to that of vertebrate ancestry (Northcutt and Gans, 1983), forms modest amounts of lactate during intense activity (Ruben and Bennett, 1980). Thus, development of an osseous skeleton in protovertebrates seems, most parsimoniously, to have followed earlier selection for the lactate-supported pattern of activity metabolism.

In contrast, few, if any, invertebrates experience such acute or severe activity-related acidosis. Some crustaceans form moderate levels of lactate during exercise (Full and Herreid, 1984), but increments in ECF $[H^+]$ are only about two times normal, with ECF pH levels seldom below 7.5–7.8 (McMahon, 1981). ATP production during periods of intense exercise in other groups is achieved by alternate, metabolic pathways that do not generate acid (i.e., high rates of aerobiosis in insects or anaerobically produced succinate and octapine in some molluscs) (Hochachka and Somero, 1984).

Bone in some groups is well vascularized and in contact with acid-laden postexercise blood and tissue fluid. This association is apparently sufficient to cause solubilization of a small fraction (probably generally less than 1%) of the calcareous skeleton. What are the possible deleterious results of this skeletal dissolution? First, there is a potential weakening of the skeletal support system. This is probably of minor concern with a phosphatic skeleton (but see below for calcitic skeletons).

Second, the skeletal dissolution produces varying degrees of acute vascular hypercalcemia in all osseous vertebrates, ranging up to a 70% increase in total blood calcium levels in some fish immediately following extended periods of intense exercise (Ruben and Bennett, 1981).

Change in extracellular calcium concentration substantially affects responses of cells to agonists that gate calcium (i.e., glucose-

stimulated insulin release from pancreatic islets [Curry et al., 1968]). Additionally, although modest hypercalcemia may supplement cardiac performance (e.g., Driedzic and Gesser, 1985) or aid in chelation of blood anions (Jackson and Ultsch, 1982), cardiac arrhythmia and a tendency toward systolic arrest is the typical response to more marked hypercalcemia in active animals (including myxines [Fange and Ostlund, 1955], petromyzontids [Augustinnson et al., 1956], bony fishes [Farrell and Milligan, 1986], frogs [Niedergerke, 1956], and rats [Nielsen and Gesser, 1983]). Moreover, hypercalcemia associated with acidosis, such as occurs after strenuous activity, may interfere with recovery following exercise (Gesser and Poupa, 1979). In mammals, the only vertebrates that have been examined for such effects, hypercalcemia also causes nausea, confusion, and disorientation (Parfitt and Kleerekoper, 1980).

Usually, however, vertebrate blood calcium levels are relatively tightly regulated, and various organs (including gut, kidneys, and skeleton) and glands (including parathyroid in mammals and Stannius corpuscles in fish) participate in calcium regulation (Taylor, 1985).

The dissolution of bone and its attendant hypercalcemia occur even with calcium phosphate as a structural material. It would undoubtedly be more severe were the vertebrate skeleton composed of calcium carbonate (see below for details). We hypothesize that a calcite-based skeleton would be chronically prone to dissolution under conditions of even submaximal activity and might be hazardously soluble after bouts of intense activity. Calcite might, therefore, be too unstable a mineral for use in animals with a vertebrate pattern of activity and metabolism.

The robustness of this hypothesis is enhanced by: 1) earlier observations that solubilization of calcium carbonate deposits in anuran endolymphatic sacs occurs at systemic pH values significantly higher than that at which bone apparently begins to dissolve (Simkiss, 1968); 2) dissolution of the calcitic pelycepod shell (in *Mercenaria*) at a relatively high tissue pH [>7.8], under conditions generating hypoxia accompanied by systemic acidosis (Gordon and Carriker, 1978; see also Crenshaw [1980] and Wilbur and Saleuddin [1983]); 3) persistent maintenance of the decapod crustacean carapace-fluid compartment at pH above 8.2 concomitant with a substantially lower range of ECF pH (Wood and Cameron, 1985); 4) the inorganic chemistry of hydroxyapatite and calcium carbonate and their dissolution limitations (described below); and 5) a series of in vitro and in vivo experiments (described below) in which calcite dissolution rates were consistently higher than were those of hydroxyapatite.

The Chemistry of Hydroxyapatite and Calcium Carbonate and Dissolution Limitations

The basis for variation in stability of hydroxyapatite and calcite lies in the different solubilization properties of these two compounds. Thus, the solubility products (K_{sp}) and/or Gibb's surface energies (G°) of these compounds in inorganic solution might, theoretically, be particularly useful in predicting the absolute stabilities of these compounds as bone minerals. K_{sp} for hydroxyapatite is $10^{-57.8}$, and for calcite (as aragonite) it is $10^{-8.2}$ at 25°C. G° is $-3{,}020$ Kcal mole^{-1} for hydroxyapatite and is -269.9 Kcal mole^{-1} for calcite (Krauskopf, 1979). Thus, the solubility of calcite is much higher and the surface energy is considerably lower than that of hydroxyapatite.

Unfortunately, although the concept of solubility in simple salt solutions is relatively straightforward, such is not the case for complex biological media. Marked variation in intracellular and extracellular ionic composition, as well as ionic pairing, may result in a range of confounding solubility equilibria (Robertson, 1982). Nevertheless, the magnitude of variation in solubility between calcite and hydroxyapatite is such that it seems reasonable to hypothesize far higher solubility for calcite in vertebrate tissues.

Furthermore, the chemistry of these two compounds indicates that differences in their respective stabilities may well intensify over a range of hydrogen ion concentrations similar to those encountered in animal tissues. Accordingly, we estimate that the relative solubility of a calcite-based skeletal mineral, compared to the mineral of a phosphate-based skeleton, would increase dra-

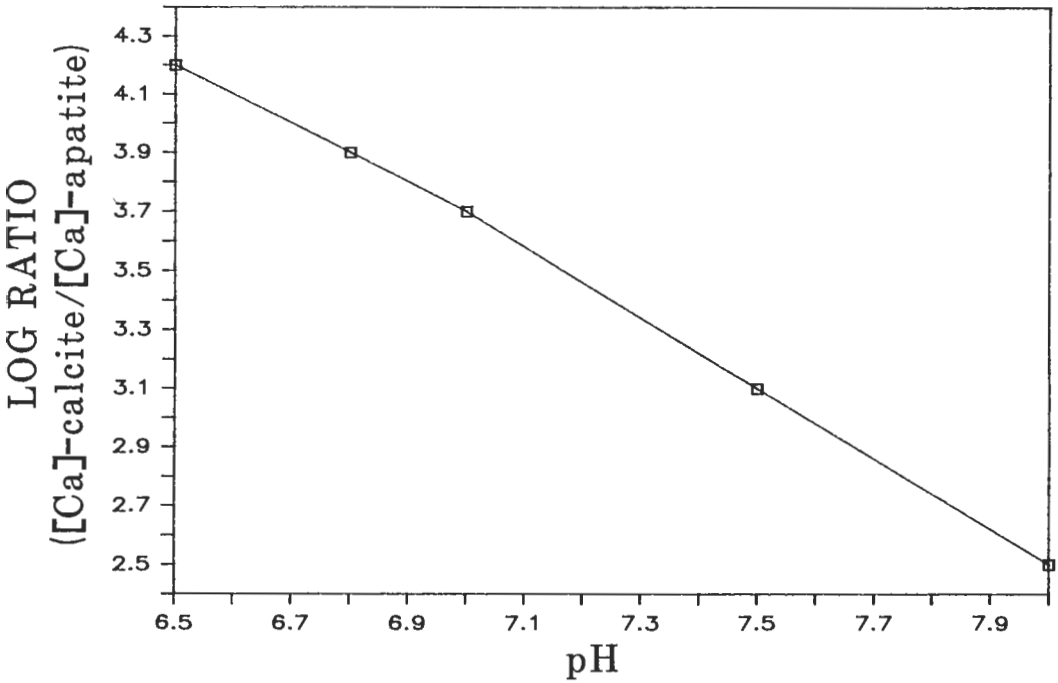

FIG. 1. Calculated ratio of $[Ca^{2+}]$ in a physiological solution saturated with calcite to $[Ca^{2+}]$ in a solution saturated with hydroxyapatite ($[Ca^{2+}]$-calcite/$[Ca^{2+}]$-hydroxyapatite). The pH regime is similar to that of vertebrate tissues. $[Ca^{2+}]$ for the calcite saturated solution was calculated for equilibrium among the species HCO_3^{1-} and CO_3^{2-} at pCO_2 = 2 mm Hg. $[Ca^{2+}]$ for the hydroxyapatite saturated solution was calculated for equilibrium among the species PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{1-}$, and H_3PO_4 at total phosphate concentration of 4 mM. Solubility products were $K_{sp} = [Ca^{2+}][CO_3^{2-}] = 10^{-8.01}$ for calcite (from Martell and Smith [1976]); $K_{sp} = [Ca^{2+}][PO_4^{3-}][OH^{1-}] = 10^{-58.33}$ for hydroxyapatite (from Nancollas et al. [1982]). Constants corrected to physiological strength.

matically in vertebrate tissues at lower pH values (Fig. 1). Activity-related hypercalcemia and physiological problems associated with it would have been exacerbated had vertebrates maintained a calcitic skeleton since their origin.

Other aspects affecting the solubility of calcite and hydroxyapatite may provide further explanations for observed patterns of the evolution of the metazoan skeleton. During crystal dissolution, surface solids must be altered to the ionic phase and must pass outward toward solution immediately adjacent to the crystal surface. This is referred to as "surface process" (Christoffersen, 1981). To continue surface processes, dissolved substance must diffuse from the crystal-solution interface to the "bulk," or more general aqueous environment, in which the crystalline surface is suspended. This is "bulk diffusion process." If surface processes are sufficiently rapid compared to bulk diffusion processes, dissolving crystal surfaces will be surrounded by a narrow interface of nearly saturated solution, from which substance diffuses into the bulk. In such a case, crystal dissolution rates are limited by the bulk diffusion process. If surface processes are slower than bulk diffusion processes, crystals will be surrounded by solution of approximately the same composition as the bulk. In this case, dissolution rates are controlled by surface processes (Christoffersen, 1981). Dissolution rates for hydroxyapatite microcrystals in a wide variety of solutions are consistently about 10^{-4} times the predicted rate for bulk diffusion (Christoffersen, 1981). Thus, stability of hydroxyapatite in solution is probably mediated largely by surface processes. A number of observations indicate that this situation applies particularly at pH = 7.0. Calcite dissolution, however, seems more highly dependent on bulk diffusion pro-

cesses (Nancollas et al., 1982; R. Pytkowicz, pers. comm.).

These observations provide further evidence for the superiority of hydroxyapatite as the mineral for the vertebrate skeleton. Given the vertebrate pattern of activity physiology and accompanying pH fluctuation, "bulk" flow might result in excessive mineral solubilization if bulk processes were limiting factors to dissolution. However, utilization of hydroxyapatite, with its surface-limited processes of dissolution, may have allowed early vertebrates to vascularize the skeleton without losing control of rates of dissolution of skeletal mineral during periods of low ECF pH. In this context, it is noteworthy that no invertebrates possess skeletons of comparable vascularization, either superficial or internal, to that seen in vertebrates (Halstead, 1968). Perhaps bulk-process limiting factors restrict the degree to which invertebrates can safely vascularize their calcitic skeletons without risking excessive hazards of mineral dissolution and loss of skeletal structural integrity during periods of chronic hypoxia and acidosis.

A Test of the Hypothesis

We undertook a series of investigations designed to provide a more direct experimental test of our hypothesis. In a set of in vitro experiments, we measured total calcium concentration in serum from rainbow trout following incubation with powdered hydroxyapatite or calcite. In a separate set of in vivo experiments, we directly implanted hydroxyapatite and calcite crystals intramuscularly and intraperitoneally into rainbow trout that were maintained on varying exercise regimes. Subsequently, implants were removed and analyzed. Both sets of results furnish new evidence that, compared to calcite, the chemical stability of hydroxyapatite seems particularly suited to the distinctive vertebrate pH regime. These experiments are described below.

Materials and Methods

In Vitro Methods.—Pooled blood serum samples were obtained following exsanguination of 20 rainbow trout (Salmonidae: *Salmo gairdneri*), obtained from the Oregon Department of Fish and Game, Alsea River brood (mean mass = 310 g). Four 5-ml samples were prepared in 25-ml glass test tubes. Two samples were maintained at pH = 7.8 and two at pH = 7.1 by addition of Tris buffer. Five hundred mg of either powdered hydroxyapatite or calcite crystals were then added to serum samples at each pH. Hydroxyapatite crystals were obtained through the courtesy of the U.S. National Museum (Washington, DC), Division of Mineralogy (Museum Specimen No. R9498, Verde Antique quarry, Holly Springs, Cherokee Co., GA). Calcite crystals were obtained from the Department of Geology, Oregon State University. There was sufficient crystal to maintain a crystal/fluid interface throughout the experiments.

Serum-crystal mixtures were maintained in a "shaker" waterbath at 15°C (±0.5°C) for 24 hr. Following this period, 50-μl samples were removed from each mixture and analyzed for total calcium content by atomic absorption spectrophotometry.

In Vivo Methods.—Rainbow trout were obtained from the Oregon Department of Fish and Game, Alsea River brood (mean mass = 282 g, range = 242–310 g). These were maintained in 200-liter tanks, which contained flowing dechlorinated water containing small amounts of erythromycin antibiotic. Water temperature was 12°C (±0.05°C), an environmentally realistic temperature for these fish. Fish were fed Purina Trout Chow throughout the course of the investigations.

Experimental procedure involved direct implantation of crystals into the peritoneum or epaxial musculature. In the first series of experiments, preweighed (400–424 mg), granular-shaped crystals of both hydroxyapatite and calcite were directly implanted intraperitoneally into each of 20 fish (sedated with MS-222). The crystals were placed in opposite ends of the ventral region of the peritoneal cavity through two small (2–4 mm) incisions. Following implantation, each incision was closed with a single surgical stitch. Implanted fish were then marked for identification by fin-clipping. Erythromycin antibiotic powder was applied topically to each incision, and fish were allowed to recover from the effects of sedation before being returned to their 50-gal maintenance tanks.

All animals thus implanted were maintained for 35 days under either of two routines. Ten individuals remained undisturbed for the duration of the experiment. The second ten were forced to exercise each day for a period of 90 seconds. During this period fish were agitated to burst activity by prodding with a wooden stick. Fish almost invariably reacted initially to the noxious stimulus by violent evasive behavior that was followed, about 45–60 sec later, by increased signs of fatigue. In a separate set of experiments, such forced activity was found to have been associated with generation of significant whole-body levels of lactic acid, accompanied by marked blood acidosis (resting: lactate = 0.21 mg/g tissue [±0.02 SE], blood pH = 7.83 [±0.07 SE]; postexercise: lactate = 1.4 mg/g tissue [±0.08 SE], blood pH = 7.22 [±0.09 SE]; t test, $P < 0.01$; lactate analysis as in Bennett and Licht [1972]; blood pH analysis as in Ruben and Bennett [1980]).

All but one individual survived the duration of the experiment, although most of the exercised group were noticeably less active in response to stimulation during the last 7–10 days. Following this period, all subjects were humanely killed, and crystals were then retrieved from the peritoneal cavity, dried thoroughly with a vacuum-flask and reweighed.

An additional twenty fish were implanted with artificial "bones" (Fig. 2). These consisted of hollow, 1 mm × 35 mm Silastic® semipermeable tubes filled with finely ground crystals of either calcite or hydroxyapatite. The tubes were sealed at each end with silicon glue and weighed. With the aid of sewing needles and thread, these were then stitched into the epaxial muscle masses of sedated fish in the manner illustrated in Figure 2. Each subject received one implant of calcite and one implant of hydroxyapatite in the left and right epaxial muscle masses, respectively. Following implantation, these fish were divided into two groups of ten each and maintained in a manner and duration identical to that described for the first experiment. Experimental treatment and behavior of these individuals were similar throughout to that of the intraperitoneally implanted group. All fish survived. Following humane destruction of experimental subjects, "bones" were removed, dried in a vacuum-flask for seven days, and reweighed.

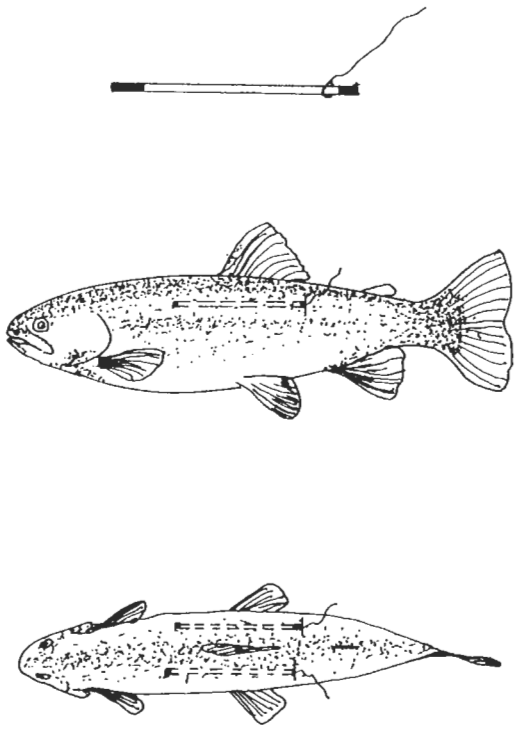

FIG. 2. Diagrammatic representation of mode of placement of artificial, Silastic® "bones" (above) into the epaxial muscle mass of experimental rainbow trout. Each fish received one implant filled with powdered, crystalline calcite and one filled with powdered, crystalline hydroxyapatite. Small (approx. 400 mg), intact calcite and hydroxyapatite crystals (not shown) were also implanted into the peritoneal cavity of other individuals.

RESULTS

In Vitro Experiments.—Except for the hydroxyapatite mixture at pH = 7.8, serum calcium concentrations in all other serum-crystal mixtures were greater following the 24-hr incubation period (Fig. 3). Additionally, all postincubation calcium concentrations of serum-calcite mixtures were greater than equivalent concentrations in serum-hydroxyapatite incubations (Fig. 3). Elevation of calcium concentration in serum-calcite incubations was most pronounced at pH = 7.1, where serum calcium concentration increased by 48% over that at pH = 7.8. Equivalent increment in serum-hy-

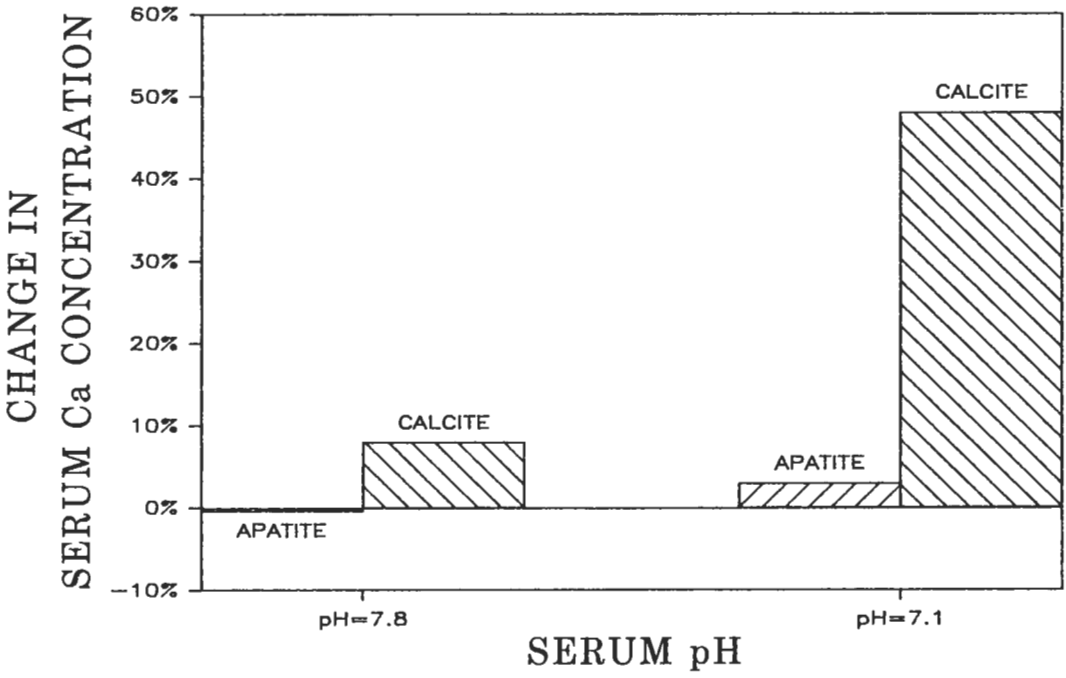

FIG. 3. Percentage increment in *Salmo* serum calcium concentration following 24 hr incubation at pH = 7.8 and pH = 7.1 with excess calcite or hydroxyapatite crystals.

droxyapatite calcium concentration was only about 3%.

In Vivo Experiments.—Dissolution rates of calcite implants, as expressed by reduction of implant dry-weight, were consistently greater than those of hydroxyapatite implants. In both nonexercised and exercised groups, intraperitoneal and epaxial calcite implants had higher rates of dissolution than were observed for equivalent hydroxyapatite implants (t test, $P < 0.05$; Fig. 4). Effects of chronic exercise on dissolution rates of implants were also marked. Both calcite and hydroxyapatite implants in individuals subjected to daily forced exercise showed significantly higher rates of dissolution than were observed in equivalent implants in nonexercised fish (t test, $P < 0.05$; Fig. 4).

DISCUSSION

The results from these experiments add corroborative evidence that, other factors being equal, hydroxyapatite can be reasonably predicted to constitute a relatively more stable vertebrate skeletal material than might be expected with a calcitic mineral. This is especially true at lower pH ranges associated with intense exercise. Solubilization of calcium from all experimental calcite preparations was higher than for all equivalent hydroxyapatite preparations (Figs. 3, 4). Differences were more marked when crystals were exposed to particularly low pH (i.e., exercised individuals in the in vivo experiments or at prescribed low pH in the in vitro experiments).

Dissolution rates of serum-crystal mixtures or artificial implants are, of course, not precise predictors of the absolute stability of either hydroxyapatite or calcite in vertebrate skeletons. Many parameters of real bone (including, for example, vascularization and cellular barriers to diffusion, etc.) were impossible to reproduce in the incubations or implants. These might reasonably be expected to affect rates of dissolution of any skeletal mineral, whether calcitic or phosphatic (see Cameron, 1985). Nevertheless, at the ultrastructural level, the models are useful to the degree that they help predict relative activity at the bone-fluid interface where mineral solubility product and fluid solute concentration will

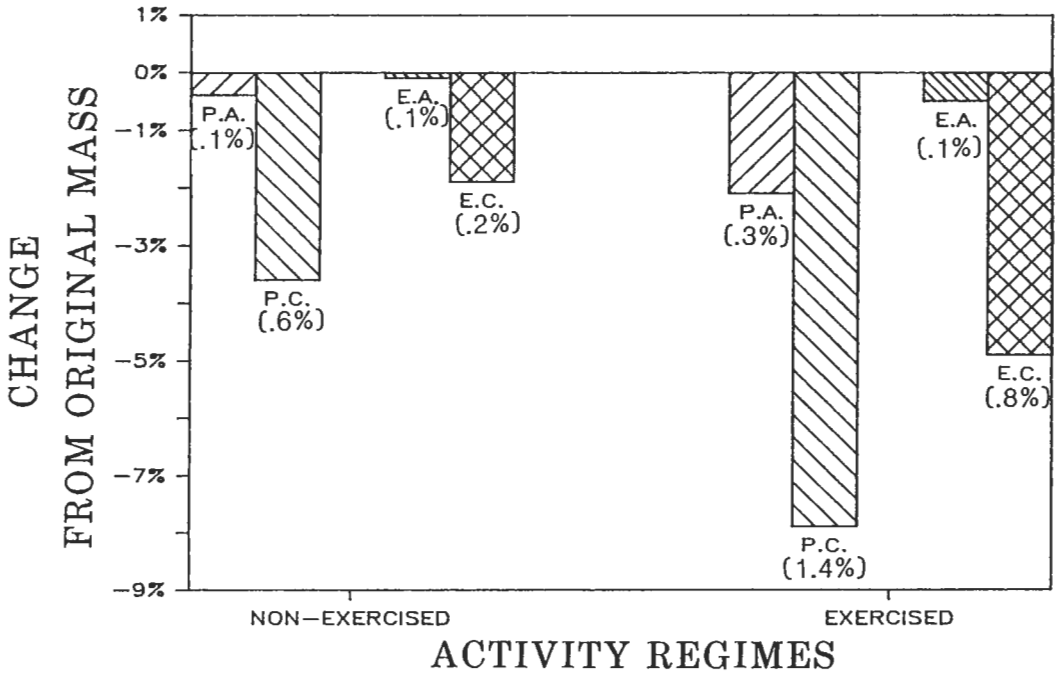

FIG. 4. Percentage change in mass of crystals and artificial "bones" 35 days after implantation into experimental subjects. Abbreviations: P.A. = peritoneal apatite crystals; P.C. = peritoneal calcite crystals; E.A. = epaxial apatite (in Silastic® "bones"); E.C. = epaxial calcite (in Silastic® "bones"). Standard errors are given in parentheses.

be primary factors effecting mineral dissolution (see Cameron and Wood, 1985; Cameron, 1985).

Exogenous implants used in our experiments also provide at least a facsimile of the overall structure and composition of the bone of the earliest known vertebrates, the Heterostraci (Repetski, 1978). These were small ostracoderms of early- to mid-Paleozoic times whose skeletons were composed of aspidin, which, like the implants used in this study, was a noncellular substance composed primarily of hydroxyapatite (Hancox, 1972). Moreover, it has been suggested that aspidin was the "ancestor" of true, cellular bone, which evolved sometime after the origin of vertebrates (Tarlo, 1963, 1964; Halstead, 1969*a*, 1969*b*).

Paleobiological Implications

Interpretation of physiological processes in extinct taxa is necessarily speculative; however, data described here may bear upon a number of interesting problems in vertebrate evolution.

Current theories on vertebrate origins focus on selection in early Paleozoic protochordates for an increasingly active lifestyle facilitated by enhanced capacity for anaerobic generation of ATP during periods of burst activity (Ruben and Bennett, 1980; Northcutt and Gans, 1983). These modifications seem to have been accompanied by refinements in sensory physiology to assist in perception of the ambient environment. Indeed, bone itself may have evolved initially in intimate association with superficial electroreceptors critical for perception of the external environment (Gans and Northcutt, 1983). If this scenario is correct, formation of a stable, phosphatic skeletal mineral compatible with emerging patterns of activity physiology may have been one of a number of components essential for the origin of vertebrates.

Given the antiquity of the vertebrate pattern of activity metabolism, it is reasonable to hypothesize continuous selection for reduction of its deleterious side-effects. Thus, the likely necessity to minimize activity-re-

lated skeletal solubilization and hypercalcemia may provide at least a partial explanation for frequent evolutionary trends toward separation of osseous structures from surrounding soft tissues and the circulatory system. This selection may, at least in part, account for the evolution of several seemingly unrelated skeletal features in different groups of extant vertebrates. These include cartilaginous skeletons in elasmobranchs, cyclostomes, chimerans, and primitive actinopterygians and acellular bone in teleost fishes. Both cartilage and acellular bone lack vascularization. Consequently, the relative surface areas of these skeletons exposed to postactive acidified ECF are probably orders of magnitude less than would be the case for skeletons of cellular bone, which is highly vascularized. Accordingly, taxa with nonvascularized skeletons experience relatively reduced postactivity hypercalcemia (Ruben and Bennett, 1981).

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