Destruction of Hard Tissue by Biological Organisms

The four-session symposium on hard tissue destruction organized by the AAAS Section on Dentistry (Nd) for the 1962 Philadelphia meeting served as a logical sequence—the other side of the coin, as it were—to a previous section symposium which dealt with the constructive aspects of hard tissue biology [*Calcification in Biological Systems,* AAAS Publ. No. 64 (Washington, D.C., 1960)].

A variety of disciplines and tools have been applied to the study of a whole range of mineralized structures, such as corals, shells, antlers, bone, ivory, cementum, dentin, and enamel. The primary purpose of the symposium was to examine the conditions under which these solid substances are subject to destruction by various biological organisms—boring sponges, barnacles, mussels, snails, octopi, worms, algae, and fungi, as well as the giant cells of bone resorption and the bacteria of tooth decay. Beyond the morphological and cellular level of observation, the symposium finally served to delineate remaining areas of ignorance regarding the specific chemical agents which lead to the disruption and dissolution of the inorganic salts and organic matrices of mineralized structures (glandular secretions and various extracellular and intracellular metabolites, acids, chelators, enzymes, and combinations of chemical and physical factors). These problems are now being explored with the tools of molecular biology focused on unifying basic issues pertinent to decalcification of a variety of biological tissues.

The titles of the topics (23 presentations) and the names of the speakers (36 authors and coauthors) have been published previously [*Science* **138**, 1139 (1962)]. While no single conceptual scheme or master plan for biological decalcification mechanisms emerged, there came to light many examples of curious similarities, both in the approaches to and observation on the destructive processes in widely separate systems.

Rock and Shell Boring

Rock boring is widespread among marine organisms in search of housing and food. The tools for boring are possessed by a variety of filamentous green algae, some marine fungi, certain sponges, worms and sea urchins, a genus of barnacles, certain gastropods, and many bivalve mollusks. The substances bored into include not only relatively soft sedimentary rock but also densely mineralized calcareous products of animal secretion, notably coral skeletons and shells of mollusks.

The habit of boring confers a measure of protection and it may also provide nutrition. Fungi bore into bivalve shells and use the energy present in the organic (conchiolin) basis of the shell, the snail and octopus bore through oyster and abalone shells to reach the proteinaceous flesh below. The mode of boring includes the use of a chemical agent as well as mechanical means.

The bivalve mollusks are most ideally fitted for boring, and no less than seven superfamilies of bivalves have independently become borers. The anterior end of the body may penetrate deeply. Thus protected, the mollusks draw in water containing food and oxygen at the posterior end.

The true mussels (Mytilacea), which include the numerous species of date mussels (*Lithophaga*), are restricted to calcareous rock and are particularly common on coral reefs. Boring is assisted by a calcium-dissolving secretion by the animal, whose own shell is protected from decalcification by a thick periostracal, horny covering.

The rock borers are of supreme importance in the economy of coral reefs. They are among the major agents of erosion, countered by the exceptional powers of calcification possessed by reef-building corals.

The destruction of corals by boring sponges occurs primarily at the base of the coral reefs. At the same time, new growth occurs nearer the surface so that there is a constant remodeling of the corals through this interplay of destruction and reconstruction.

The boring snails which are a threat to oyster beds have the capacity to rasp off the periostracum of the oyster shell; then they soften the minerals by a glandular secretion, followed by further rasping by the radula. It was shown by electron microscopy and microradiography that there is a subsurface leaching out of minerals—an etching, that is—as part of the boring mechanism. Yet as far as could be determined the glandular secretion presumed responsible for this demineralization has a neutral *p*H. (Experiments in vitro showed that the snails cannot be made to bore into nonliving shells unless some oyster meat is placed under the shell, in which case the organic metabolites would invite the snail to take on the task of dissolving the shell minerals and bore its way into the underlying protein.)

It appears that the major groups of boring organisms have representatives which have evolved a decalcification mechanism to facilitate the search for food, protection, or a substratum.

Tooth Destruction

Teeth and tusks, suspended in their bony alveolar sockets within the jaws, combine the full spectrum of vertebrate hard tissues—enamel, dentin, ivory, cementum, and bone—and are subject to mechanisms of eruption, shedding, resorption, attrition, erosion, abrasion, and caries (dental decay), as well as to postmortem destruction by subterranean saprophytes ("The ivory houses shall perish . . .", Amos 3 : 15).

Within certain limits, destruction of the dental hard tissues is a normal physiological process, accompanying masticatory function, growth, and aging. But in the absence of the lubricating action of the saliva, rapid wear of the teeth can take place even with regular function and food habits.

Extreme wear may occur as a result of the combination of mechanical and chemical influences. Thus, excessive wear reported in sheep's teeth has been attributed to decalcification by organic acids from herbage, combined with freshly expressed juices of grasses and clovers containing enzymes capable of acting on the organic portion of the teeth (proteolysis).

Experimental caries research in ro-

dents, utilizing germ-free systems in part, strongly indicates that dental caries is of bacterial origin, caused by a Gram-positive streptococcal infection and transmissible between animals. In the presence of such infection, only fluoride and reduced intake of carbohydrate have been found to give significant protection to the teeth. Examination of the initial carious lesion of enamel by means of polarized light and soft x-rays (microradiography) indicate that there is a differential demineralization extending deeply into the subsurface structure of enamel to a depth of about 1000 microns. Thus, there is already considerable subsurface demineralization of enamel before the proteolytic destruction of the organic matrix takes place. From a biochemical point of view, it is only at a late stage in the lesion before microorganisms actually invade the tooth substance. Electron microscopy of enamel caries, sectioned by means of a diamond knife, has shown that the substance between the minute enamel rods (prisms) is primarily involved. Yet the destruction is not entirely a one-way process. There is evidence of recrystallization of dissolved minerals, and the inorganic salts remaining within partially destroyed teeth have been found to contain a very high proportion of fluoride.

Incipient carious lesions of enamel, remarkably similar to those formed in vivo, have been produced by purely chemical systems containing a reactant (acid) capable of dissolving hydroxylapatite and an organic polymer capable of protecting the external enamel surface. These in vitro lesions have been explained by the dynamics of the heterogeneous system consisting of an acidic solution and a calcium phosphate solid involving chemical phase transformation, equilibria, and kinetics. The enamel dissolution appears to be a "first order" diffusion-controlled reaction which can be inhibited by protective deposits (salts of the reaction products) on the apatite surfaces. In equilibrium with acid solutions, there has been established the existence of dicalcium phosphate on the surface of enamel, bone, and synthetic hydroxylapatite, and the presence of calcium fluoride on the surface of fluorapatite.

In contrast to dental caries, the so-called idiopathic dental erosion is characterized by a much lesser subsurface demineralization and by the absence of bacterial invasion of the tooth substance. Consequently, one cannot detect the beginning of such erosion by any change in visual opacity which is so typical of the early "white spot" formation in dental caries. Histological examination suggests that dental erosion is not necessarily devoid of surface deposits of bacteria-laden mucus, though the lesion may look clean to the naked eye. Occasionally these superficial deposits may be separated from the tooth surface by a thin cuticular membrane, which stains orthocromatically with toluidine blue, presumably originates from saliva, and may possibly be of protective significance. Electron-microscopic examination of dentinal erosion has shown the presence of deposition of extremely large crystals which appear to block the dentinal tubules and thus may explain the failure of bacterial invasion of the tooth substance.

Even after death, the teeth are subject to destruction by biological organisms. Postmortem changes have been observed in human teeth of prehistoric, ancient, and recent times. The histological observations indicate that the commonest postmortem change consists in large irregular canals which penetrate the dentin and cementum, a condition not known to occur in teeth during life. The microscopic pattern of the postmortem destruction is suggestive of invasion by fungi capable of removing calcium phosphate and collagen virtually simultaneously, but incapable of attacking dental enamel. Postmortem changes are rarely found in the enamel and are limited to localized areas of decalcification or erosion of the enamel surface, possibly caused by algae.

Resorption of Antlers, Bones, Teeth

As a normal consequence of resorptive processes, the shedding of antlers and teeth represents the only instance in which there is a natural loss en bloc of mineralized vertebrate tissues. The shedding of antlers is considered as one step in the normal growth process. Thus, the shedding is incidental to the growth of the new antler, that is, a transition between the destructive (bionegative) and the constructive (biopositive) part of the cycle. Antler shedding is under general systemic control. Unlike teeth, the antlers are shed at the same time. The process is associated with a fall in the level of testosterone in the blood at the conclusion of the reproductive period. Osteoclasts resorb the bone of the Haversian lamellae along the junction of the living bone (at the skull pedicle) and the dead bone of the antler base during a remarkably brief period of only 48 hours. In addition to the control by sex hormones, there is some evidence that some factor or factors of the pituitary gland tend to stimulate both shedding and regrowth of antlers.

Eruption and shedding of teeth is accompanied by extensive resorption and remodeling of the alveolar bone of the jaw. In a mutant strain of rats in which the incisors fail to erupt (strain *i.a.*) the eruption could be stimulated by administration of parathyroid hormone which induced resorption of the dense obstructing bone.

Pathological resorption of alveolar bone, which accompanies loosening and loss of teeth (pyorrhea), was shown to be associated with circulatory disturbances in the periodontal region, caused in part by infections and in part by functional stresses.

In the life cycle of the skeleton, the resorption and reconstruction of bone is part of the same system, continuously serving to remodel the skeleton and providing large surface areas for exchange reactions between body fluids and bone mineral.

Among the rarefying diseases of the human skeleton, the one most common disorder is osteoporosis, often superimposed upon physiological atrophy of bone in old age. Combined techniques of histology, autoradiography, and microradiography were utilized to study resorption in cortical bone. Among the findings reported with this analysis was the apparent indication that in osteoporosis the amount of resorptive surface relative to resting surface is increased. Human cases of osteoporosis, investigated by metabolic balance, radioisotope, and tetracycline techniques, suggest that the metabolic functions of the skeleton as a chemical "warehouse" are performed by the 1 percent of the bone that is reactive or exchangeable, and that this is not significantly abnormal in patients with osteoporosis. The deficiency is in the insufficient amount of the other 99 percent that comprises the so-called nonreactive, nonexchangeable, stable, or structural bone.

Recent work was interpreted to indicate that extreme dietary deficiencies of calcium and protein, various endocrinopathies, and many degenerative vascular system disorders produce premature or accelerated aging of bone, and that osteoporosis appears as a result.

With regard to the mechanism of resorption, the multinucleated giant cells

(osteoclasts) situated in typical Howships lacunae generally have been considered the sole culprits of destruction. Yet it was shown that the osteocytes of deeper areas of bone (well removed from bone surfaces) responded by resorbing the walls of their lacunae in a number of systemically accelerated resorptive states (brought about, for example, by the administration of parathyroid extract, cortisone, and the intravenous infusion of ethylenediaminetetracetic acid). This phenomenon was termed "osteolysis." If substantiated, these findings may represent the first step in delineating such a definite function for the osteocyte.

Two reports explored the degree to which resorbability is dependent upon the precise characteristics of the tissues being resorbed. In one study, the authors reported the results of experiments in which devitalized pieces of bone were implanted subcutaneously. The findings suggested that bone matrix need not be calcified in order to undergo resorption as long as it was calcified at some time in the past. Resorption of the implants was associated with the presence of an osteoclast-like cell, which appeared to be related to (or possibly identical with) foreign body giant cells. Histochemical studies demonstrated obvious similarities in enzyme reactions in these multinucleated cells, with the exception of acid phosphotase which appeared more highly concentrated in the bona fide osteoclasts.

In another study the author examined sites of preferential resorption in normal young rats and in comparable animals in which resorption had been stimulated by administration of parathyroid extract. In the latter group, the acceleration of resorption appeared to represent an exaggeration of this process in areas normally characterized by resorption. Combined histological, autoradiographic, and microradiographic analyses indicated that neither the age, degree of calcification (excepting uncalcified matrix, which was not studied), histological organization, nor chemical variations in the resorbable matrices was critical in determining sites of preferential resorption.

New information emerged regarding the ultrastructural characteristics of the osteoclasts. Electron-microscopic observations on osteoclasts in undecalcified preparations illustrated morphological differences in the cell membrane at the cell-bone interface, presumably related to functional differences in cellular activity. Among the most interesting findings was the observation that typically striated collagen fibers, evidently denuded of their inorganic crystallites, were present within the infoldings of the ruffled cell membrane juxtaposed to the resorptive bone surface. In terms of the mechanism of bone resorption, this may indicate that solubilization of the crystallites may precede lysis of the collagen component of the organic matrix.

Several in vitro studies dealt with biochemical aspects of bone destruction, including experimental resorption in tissue culture. When cranial vaults of immature mice were incubated in roller tubes, the addition of a number of substances to the culture medium, including parathyroid extract, vitamin D, and vitamin A, evoked an appreciable resorptive response, provided the oxygen tension of the medium was kept at a high critical level.

In tissue slices of resorbing bone, lactic acid rather than citric acid was found to be more concentrated in the supernatant end product of the system. While there is no question that bone cells can produce citrate, some question was raised as to whether significant concentrations of citrate accumulate and also whether parathyroid hormone influences this accumulation. It was suggested that the rate of operation of the Krebs cycle is controlled by the entry of metabolites through the pyruvic acid-oxidase complex to acetyl coenzyme A and through the condensing system or "citrogenase." There appears to be no real accumulation of metabolic citrate since the reactions of the Krebs cycle follow in sequence. If more citrate is produced in the cell, more is oxidized. The real consequence of increasing the flow through the bottleneck is a greater production of carbon dioxide.

As yet, there are not sufficient data to establish which of the two enzyme systems between pyruvate and citrate is the actual controlling factor. The fact that lactate, which accumulates in large amounts, is the major end product suggests that the crucial point in the sequence (the site of hormone action) is in the pyruvic acid-oxidase complex. Tentatively, it was proposed that an excess of parathyroid hormone induces an increased production of carbon dioxide from glucose or metabolites of glucose by cells in bone.

The appropriate research tools for making the next step toward understanding these destructive processes at the chemical level are now at hand and will undoubtedly be exploited with increased imagination and vigor by representatives of many fields of science.

Grateful acknowledgement is made to the National Institute of Dental Research for a grant which made it possible to invite a number of speakers from abroad to participate in this symposium.

For the next 4-year period (1963–66) Seymour Kreshover, scientific director of the National Institute of Dental Research, will serve as the new secretary of the Dentistry Section. Paul Boyle, dean, School of Dentistry, Western Reserve University, was elected vice president and chairman for the Cleveland meeting in December 1963.

REIDAR F. SOGNNAES
School of Dentistry,
University of California, Los Angeles

Evolution of Behavior

Evolution has a fascination for all biologists, for all scientists. Since it is the all-pervading concept of biology, every worker is able to categorize his findings, somehow, into an evolutionary framework. Thus a symposium entitled "The Evolution of Behavior" could become a *carte blanche* to its participants and might result in a disconcertingly diverse program. Such was not the case with the symposium organized by William Dilger (Cornell) and presented on 27 December at the Philadelphia meeting of the AAAS. Although the offerings were broad, the result was a coherent program.

Two general approaches were evident. The one most commonly used might be termed the functional orientation; the investigator decides upon an activity and seeks stages of its development within a systematic group. The objective is to reconstruct the evolution of the given activity by comparing existing forms; problems of convergence and direction of evolution are of little importance in such a first approximation.