Meetings

Biology of Hard Tissues

The biology of hard tissues was discussed at an interdisciplinary conference held in Princeton, New Jersey, 20–23 June 1965. Attendance was limited to 25 participants selected on the basis of their work in many disciplines, including chemistry, crystallography, physics, physical chemistry, biology, biochemistry, electron microscopy, histochemistry, pharmacology, physiology, and the medical sciences.

The conference was a departure from the currently popular style in which presentation of formal papers is followed by a limited time for discussion. A discussion leader opened each session with an introductory statement designed to orient the group to a specific topic, to emphasize unsolved technical and conceptual problems, and to stimulate lively discussion. The participants were encouraged to interrupt the discussion leader. The advantage of such an "off-the-cuff" approach is that the discussion leader does not make a speech but rather carries a theme consisting of a few major points for discussion by the group.

A unique approach was made on composition and structure of bone mineral. Wallace D. Armstrong (University of Minnesota) distributed a list of questions as a guide for discussion. The questions concerning the biological significance of the structure of apatites and of the ratio of calcium to phosphorus remain unanswered. There is some agreement that the most important aspect is the mechanism involved in the growth or maturation of bone crystals. There was some suggestion that small crystals have a low ratio of calcium to phosphorus; that octacalcium phosphate (OCP), apatite deficient in calcium, and mature crystals can exist together; that as bone mineral matures calcium is added and the ratio of calcium to phosphorus rises. It was further stated that bone mineral does not appear to be the same at different stages of development; it does not have the same stoichiometry as mineral apatite; and does not give the same response chemically. Bone mineral, however, does eventually achieve a stability which is concomitant with other growth factors.

Growth of hydroxyapatite (HA) crystals depends on a mechanism in which OCP is the initial precipitate. The current view is that bone mineral is first formed as OCP, and as hydrolysis occurs (aging changes) there is a conversion from OCP to HA. In young bone there tends to be more OCP in the microcrystals than in older bone where the reorganization of OCP to HA overtakes the precipitation process. It has not been established that the ratio of calcium to phosphorus in the initial bone salt is different from that in the salt finally formed.

D. R. Taves (University of Rochester) explored the adequacy of the existing model systems with the idea of unraveling the essential features of the mechanism whereby nucleation of a solid from a solution occurs in terms of physicochemical theories. The concept of diffusion was challenged, that is, the concept that the matrix is able to nucleate calcium phosphate salts from a solution at the local site and that the growth of crystals then can take place by diffusion alone since the extracellular fluids are supersaturated with respect to HA or bone mineral. If one depends only upon diffusion across a barrier, some other mechanism must concentrate the ions to form a critical size for precipitation. One such mechanism is the ion pump. The argument against the necessity for a pump, as is considered in a physiologically active transport system, was that the local factors involved in bone formation can produce conditions which will result in crystal formation. Diffusion will deliver the ions to the local site, and there the local factors determine the calcification. If there is an ion pump there would be no need for dependence of the calcium and phosphate concentration in body fluids.

It was agreed that the collagen model is inadequate. There is no evidence that pure collagen will initiate crystallization from concentrations of calcium and phosphate; there is no evidence that inorganic crystals are deposited. There was some suggestion that OCP may be a nucleator; chemical properties support this as compared with defect apatites in that OCP does hydrolyze apatite. The platey habit of these crystals is consistent with symmetry and normal morphology of OCP but is inconsistent with octacalcium apatite. The organic matrix itself may play a part in the crystal size. Current theory is that the actual control of collagen substrate physically limits the size in bone.

The model system of the rat aorta was presented. This system invokes the tightly bound phosphate as the local factor, except that a trace element is added. The criticism was that it was a good model for calcification but not for bone formation. Elastin was found to be the primary site of mineralization; there is no elastin in bone, and this system tells us nothing about nucleation of bone mineral.

A new model system which may prove to be adequate makes use of interactions of matrix chemical groups and nucleation of mineralization by preparing calcium phosphate derivatives of amino acids. Serine phosphate is prepared and calcified so that the serine phosphate dissolves or is precipitated with calcium phosphate to form the calcium derivative. In this system there is a fairly stable double complex. Calcification is inhibited if the matrix is first phosphorylated.

Additional information was presented on the serine found in hypertrophic cartilage and in primary spongiosa when they are subjected to lime salts. The concept is a phosphatizing, serine-binding calcium; as soon as calcification proceeds the lipid disappears. The lipid in bone is just present when calcification is initiated and disappears when calcification is underway. The area coincides with that of metachromasia.

Another point on the local factor in calcification, and one that has not been fully appreciated, is that zinc is deposited in the preosseous tissue in the same area where lipid deposition occurs. Deposition of zinc in this location indicates that the metal itself may activate a catalytic agent that probably has some role in calcification.

The session on the biochemistry and

physiology of mitochondria included discussion of the structure of mitochondria, enzymatic machinery, the calcium-transport system, the nature of deposited mineral, the calcium release system, and phosphate transport dependent on parathyroid hormone.

In the calcium and phosphate loading of mitochondria the ratio of calcium to phosphorus is 1.7. When the membrane of loaded mitochondria was removed it was found that the mineral was amorphous (noncrystalline). When calcium accumulates, the mitochondrion is no longer capable of doing work and respiration decreases. Although in mitochondria the flow of electrons in the respiratory chain is compulsively linked with the synthesis of adenosine triphosphate and with the energy of oxidative phosphorylation, a similar linkage among electron transport, generation of energy, and resorption of bone has not been shown. Mitochondria of osteoclasts accumulate this combination of calcium and inorganic phosphate; this accumulation is not specific for osteoclasts. As yet mitochondria cannot be directly implicated in calcification or resorption.

W. H. Boyce (Bowman Gray School of Medicine), reviewed mineralization from the standpoint of kidney-stone disease. Uromucoid, or substance A, was isolated from the urine of kidney-disease patients who do not form stones; the substance is not present in normal kidneys. John Eager Howard (Johns Hopkins University and Hospital) reviewed the work on inhibition of calcification. A proteinaceous matrix is present in kidney stones of all types; there are some similarities to bone and cartilage, as shown by histochemical studies of these matrices. Hypertrophic cartilage from rachitic rats will mineralize in vitro when provided with adequate concentrations of calcium and phosphate. Substances from serums of uremic patients, known to contain an inhibitor, will prevent mineralization of cartilage from rachitic rats. Urine from subjects with renal calculi will mineralize cartilage matrix from rachitic rats; the addition of metaphosphate prevents mineralization.

The last day of the conference was a recapitulation and a summing up of concepts. Participants were invited to present a wide variety of material, all bearing on the general theme of the conference. Discussion included: limitations of the applicability of physicochemical theories to biological situations; the application of the electrolyte theory to biological solutions; studies on the relationship between ion transport and the physicochemical state of ions in body cells and the extent of binding of ions by subcellular sites of structure; metabolic and structural bone; and material on cell populations, with a plea for an agreement on terminology.

Participants were encouraged to submit five questions or statements relating to unsolved problems covering topics of each major discussion. In retrospect, the conference, as an experiment of "conversation *en group*" was commendable in that no feature or topic by itself was abruptly concluded or dismissed; many of the problems and questions raised in earlier sessions were brought into the discussion during later periods. These unanswered questions and unproved theories may well be resolved in the future conferences of this series.

The conference, held under the auspices of the Interdisciplinary Communications Program of the New York Academy of Sciences, was supported by the Office of Naval Research and the National Aeronautics and Space Administration. A transcript will be published before the second conference, to be held in 1966.

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Population Growth

The World Conference on Population, organized under the auspices of the United Nations and held in Belgrade, Yugoslavia, 30 August–10 September 1965, had as its objectives improvement of understanding of population problems, especially as they relate to problems of economic and social development; stimulation of interest in scientific research and in securing of data pertinent to these problems; and enhancement of the effectiveness of work in these fields.

Underlying all the discussions were the facts of the recent rapid growth of the world's population, especially in the developing countries. It was pointed out that there are at present no developed countries with gross reproduction rates above 2.0 and no developing countries with rates below 2.0. With declines in mortality and a maintenance of traditionally high levels of fertility, rates of population growth in the developing countries have recently increased and generally show no signs of abatement. Much attention was given to the implications of current rates of population growth for the process of economic and social development and to the processes by which birth rates, and thus rates of growth, might be reduced.

There were wide differences of opinion on the role of governments in reducing rates of growth. One view was that governments should take positive action to bring knowledge of methods of fertility limitation to the people, and should make available the means by which such control could be accomplished. Another view was that such action was not a proper function of governments and that the most effective course would be to promote economic and social development which would, in turn, lead to a reduction of fertility, as it had in the developed countries. Some participants expressed the view that there was no problem of excessive rates of growth in underdeveloped areas and that, therefore, no public or private action was needed.

Although food supplies have been increasing about as rapidly as population, there was concern over the fact that increases in food production had not kept pace with population growth in most of the developing countries. In general these countries were exporters of food supplies before the war, but have been importing an increasing amount of food in recent years. Even with these imports, diets in many parts of the world fail to provide adequate nutrition. Very large increases in food production, especially in the developing countries, are needed if the growing population is to be supplied with necessary food. It was felt that the resources for such increases are available and that the problem of securing needed increases is a problem of the ways in which the available resources are used and of the extent to which capital and modern scientific and technological developments are applied. In an opening address, the Director General of the United Nations Food and Agriculture Organization urged that side by side with a concerted effort to increase productivity of agriculture in the developing countries, population stabilization must be undertaken without further delay as a social policy of urgent priority.

With regard to natural resources generally, it was pointed out, on the one hand, that although the earth is