A Comparison of the Influence of Hormones, Vitamins, and Other Dietary Factors upon the Formation of Bone, Dentine, and Enamel

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I. Introduction

1. Chemistry and Physics

The chemistry of dentine and bone is fundamentally similar—each has an organic component consisting of collagen and ground substance and an inorganic phase of apatite crystals embedded in the organic fraction. There seems no doubt from the work of Solomons and Irving (1956) that, based on the reactivity of the ϵ and terminal amino groups, the collagen of dentine and bone is different from that of uncalcified connective tissue. The chemistry of ground substance is still incompletely understood; it contains very small amounts of mucopolysaccharide, probably chondroitin sulfate. The apatite fraction is almost certainly the same in bone and dentine.

Enamel has a high organic content when first formed, but mature enamel contains only 0.2–0.8% of organic matter. The protein is a eukeratin having an appreciable content of hydroxyproline. Mucopolysaccharides are present but in very small amounts. The inorganic phase consists of apatite crystals, but these are larger than those in bone or dentine.

The bone crystal, and presumably also that of the tooth, may be pictured as consisting of "a surface hydration shell containing nonspecific boundary anions in rapid equilibrium with the surrounding medium; an inner, crystal surface containing more or less specific cations and anions also in equilibrium with the solution (or the hydration layer); and interior ions with a slow but measurable equilibrium with the outer layers (recrystallization)" (Neuman and Neuman, 1953). It seems possible that recrystallization is relatively more important in the tooth in the incorporation of blood Ca into its structure than is new crystal formation or ion exchange (Stover *et al.*, 1957). While the theoretical molar Ca:P ratio of hydroxyapatite is 1.66, it has been shown both *in vivo* and

in vitro that by processes of adsorption and heteroionic exchange in the apatite lattice the Ca:P ratio can be quite considerably altered (Sobel, 1955).

2. Bone and Tooth Formation

The formation of these three tissues exhibits some marked differences, which account to a large extent for their characteristic responses to dietary and endocrine factors, since these chiefly operate during formation of the tissue.

In normal osteogenesis, whether endochondral or intramembranous, the formation of matrix and its calcification are virtually simultaneous, and only when bone formation is very rapid are osteoid margins found. It has been tacitly assumed that the osteoblasts make the matrix and also calcify it, although recent work (Irving, 1956a) has suggested that fibers analogous to Korff fibers in dentinogenesis may be formed by cells other than the osteoblasts.

In normal dentinogenesis, on the other hand, calcification lags behind matrix formation for a definite period, which varies in its length from species to species. In the rat incisor it is 24 hours. Thus dentine is always bordered on its pulp side by a layer of uncalcified predentine. Most workers now agree that the matrix is formed from Korff fibers which arise in the pulp and which are modified and calcified by the odontoblasts.

Amelogenesis is a process in which at first a lightly calcified matrix is laid down. With the passage of time, the matrix becomes more calcified (Deakins, 1942; Weinmann *et al.*, 1941), protein and water are withdrawn, and finally the tissue is completely matured. Evidence will be adduced later which seems to show that matrix formation and calcification are one inseparable process, and not two separate actions as in bone and dentine.

Bone has a resorptive mechanism which can be affected by dietary and hormonal influences and plays an important role in the mineral exchanges in the body. Teeth do not have such a mechanism under physiological conditions and thus do not enter into metabolic exchanges in the body as do bones.

The formative cells are under the control of several vitamins and hormones. In the case of bone-forming cells, considerable modulation can occur, probably centering round an undifferentiated spindle cell (Heller *et al.*, 1950) which can under certain conditions become an osteoblast and under others an osteoclast, the former almost certainly bone-forming, the latter at any rate associated with osteoclasis. These changes are under the control of vitamins A, C, and D and the hypophysis.

parathyroids, adrenals, and gonads. The odontoblasts, the dentine-forming cells, are not capable of as much modulation as are the bone cells, but are none the less controlled by many of the factors mentioned above. The ameloblasts, the active cells in the enamel organ, are much less affected by hormonal and dietary influences and require drastic changes in their environment before any striking effects are seen.

3. Comparative Studies of Bone and Teeth

Almost all studies have been made on growing animals, usually rats, since the effects of various factors are most marked on bone and teeth when they are forming. For studying endochondral ossification, the proximal epiphysis of the tibia is often used; it shows changes well and quickly, but has the disadvantage that it does not close in adulthood—in other words, the cartilage plate is sealed off with bone but not removed as is usual in other bones (Becks *et al.*, 1945). For intramembranous bone formation, the shafts of long bones have been studied. Bailie and Irving (1955) found the maxillary fundic bone of the rat very reactive and convenient. Bones can be studied over a long span of the animal's life, and even after bone growth has stopped the constant removal and renewal of bone can still be used as an index of bone metabolism.

In tooth studies on the other hand, it has been found that the tooth is reactive only during formation, and the metabolism of the fully erupted tooth, as judged by the exchange of radioisotopes, is at a very low level. Teeth in embryos are probably very sensitive to dietary and endocrine influences, but it is not easy to affect them via the mother, though this has been done with, for example, vitamin A (H. Mellanby, 1939). The 3rd molar of the rat erupts 35 days after birth and has been studied before eruption. But luckily for the worker in this field, the rodent incisor is a tooth of continuous eruption, replacing itself in the rat about every 45 days, and the processes of dentinogenesis and amelogenesis are the same as found in teeth of limited eruption. Thus it is possible to study in the same animal the effects of various factors upon bone and tooth formation. It is for this reason that most investigations in this field have been made with rats, mice, guinea pigs, and rabbits.

The inorganic metabolic turnover of the incisor tooth is lower than that of bone (Carlsson, 1951), as is its interstitial Ca metabolism (Tomlin *et al.*, 1955), though the tooth takes up initially more radioisotope than the skeleton (Chievitz and Hevesy, 1937). In general the incisor tooth is less sensitive to dietary and endocrine changes and seems able to protect itself against deficiencies when bone may be more severely affected. But once affected, the tooth, as might be expected, responds much sooner

to reparative agencies than does bone. In addition, the precise pattern of tooth formation makes it much easier to detect early reparative changes in them than in bone.

II. The Influence of Vitamins and Dietary Factors on Bone and Tooth Formation

1. Vitamin A

This vitamin has profound effects upon both bone and tooth formation, whether deficient in the diet or given in excess.

a. Avitaminosis A. Bone. The first worker to draw attention to the effect on bone was L. A. Moore (1939) who described constriction of the optic nerve in calves, caused by stenosis of the optic canal and preventable by β-carotene dosage. Since that time several workers have investigated the changes, which are in effect an overgrowth of bone of a very cellular type in both normal and abnormal situations (Irving, 1956a). The interpretation of the changes and the causes of them have differed. Wolbach (1946) held that intramembranous bone formation went on in the normal growth pattern, but that remodeling sequences ceased. E. Mellanby (1947), finding bone to be laid down in situations where this does not normally occur, considered that there was an orderly change in position or activity and number of osteoclasts and osteoblasts. Irving (1949b) agreed in general with Mellanby but felt that the prime influence of the vitamin was on the osteoblasts, which in the absence of the vitamin became uncontrolled and engaged in disorderly activity. The osteoclasts attempted vainly to reverse what the osteoblasts were doing. In a more recent study, Irving (1956a) found that the cells adjacent to the bone could modulate to osteoblasts with considerable mitotic activity during avitaminosis A.

Teeth. The changes were first described by Wolbach and Howe (1933) and have since been confirmed and extended by a number of workers (e.g. Irving and Richards, 1939; Schour *et al.*, 1941). In part the dentinal change is the same as in bone. In the incisor tooth the labial odontoblasts produce excessive amounts of poorly formed dentine and may be included in the dentine so formed, reserve cells in the pulp taking over their functions. Osteoid tissue may be present in the pulp. The lingual odontoblasts, in contrast, atrophy and produce too little dentine, so that the pulp cavity is moved lingually. The enamel organ also atrophies, the change starting anteriorly and working backwards. The atrophy is unlike that seen in vitamin E deficiency (see heading II, 6, b). The papillae of the outer enamel organ proliferate and look like peninsulas of squamous epithelium, and the ameloblasts become flattened. Schour *et al.*

(1941) considered that the histodifferentiation, especially of the lingual odontogenic epithelium, was disturbed and incomplete, so that its normal organizing influence causing the pulpal cells to differentiate into odontoblasts was ineffective. The present writer considers this explanation inadequate, as it does not account for the activity of the labial odontoblasts. Nor is it an all-embracing enough theory since the bone changes, which are in many ways comparable, cannot be explained in such a way.

As a result of the changes in the enamel organ, the teeth lose their yellow pigment and turn white. This was shown by T. Moore (1943) not to be due to any action through vitamin E, in the absence of which the teeth also lose their yellow color. Using diets adequate in A but not in E, and vice versa, he obtained white teeth, which could be cured by restoring the vitamin missing from the diet.

Irving and Richards (1939) had found that the requirement of vitamin A necessary for tooth maintenance rose with age, and in a later paper (1956) carried out a study in which the reactions of various tissues to vitamin A deprivation were compared. They found in young recently weaned rats on a vitamin A-free diet that bone and teeth showed signs of vitamin A deficiency simultaneously and that these could be prevented by equal doses of the vitamin. But in older animals, the requirements of these tissues changed, that of the tooth being at least eight times that of bone. Of the other tissues studied, the central nervous system was found to be very sensitive to vitamin A lack in early life, but to require relatively little of the vitamin as age advanced. Since the fundamental action of vitamin A is still not understood, it is difficult to interpret such findings. Irving and Richards did not consider that the requirements of vitamin A were a function of body weight as has been claimed by some. The most one can say is that the requirement of each tissue is related to its metabolic activity.

b. Hypervitaminosis A. Bone. It has long been known that excessive doses cause spontaneous fractures of the bones of rats and a tendency to internal hemorrhage. Moore and Wang (1943) showed that this was a true vitamin A effect since they were able to reproduce it with a crystalline ester of the vitamin. Wolbach and his school have published several studies of the bone changes. They consider that the remodeling sequences are accelerated and a defectively calcified bone is laid down which fractures easily (Wolbach, 1946; Maddock *et al.*, 1949; Wolbach and Maddock, 1951). The effect was not mediated through the hypophysis (Wolbach and Maddock, 1952b) or parathyroids (Cohen *et al.*, 1955) but was accentuated by adrenalectomy (Wolbach *et al.*, 1955). Irving (1949b) studying the responses of the alveolar bones, found the rate of formation of bone to be greatly reduced and osteoblast action to be considerably

depressed. Some bones were slowly resorbed, and this removal was judged to be due to unopposed osteoclast activity. Fell and E. Mellanby (1952) cultivated embryonic-mouse limb bones *in vitro* and found, when excess vitamin A was added to the culture medium, that the bone was quickly resorbed but few osteoclasts were present. Qualitatively similar, but less drastic, results were obtained when plasma from a hypervitaminotic fowl was used instead. Barnicot (1950) implanted bone in contact with a particle of vitamin A acetate into the brain and found that where the bone was in contact with the vitamin, erosion with many osteoclasts occurred. Carotene had no such effect. It may be asked whether such a procedure is physiological enough for conclusions to be drawn. Nerunkar and Sahasrabudhe (1956) carried out balance experiments on rats during hypervitaminosis A and found negative Ca and P balances. Since the relative composition of the bones was unchanged, they considered the increased excretion must be due to thinning of the bones. Thus the balance of the evidence is against Wolbach's view—the remodeling sequences are not accelerated, but a variety of procedures has shown them to be retarded or stopped.

Teeth. Very little work has been done on teeth during hypervitaminosis A. Pohto (1938) stated that the condition had no effect upon incisor teeth. Wolbach (1946) considered that the "sequences of enamel and dentine formation are unaffected, perhaps they are somewhat accelerated."

Irving (1949b) made a series of measurements in the incisor teeth of the animals whose bones he had studied and found the predentine to be considerably narrowed and the rate of dentine apposition to be reduced; the odontoblasts were also reduced in height; no change was seen in amelogenesis. Irving considered the vitamin to have a direct effect upon the odontoblasts, damping down their action. In a recent paper, M. Mellanby and Holloway (1956) grew tooth germs *in vitro,* with and without excess vitamin A. The vitamin caused much slower growth compared to the controls, with relatively little differentiation especially of the enamel organ. They thought the vitamin exerted a direct action on the developing tooth.

c. Function of Vitamin A. E. Mellanby (1947) concluded that the general function of vitamin A as regards bone was to control its shape, and especially its fine molding, by influencing the position and activity of osteoclasts and osteoblasts. Bearing in mind the much more limited possibilities in teeth as regards apposition and resorption, it seems to the present writer that much the same may be said about the teeth, since the odontoblasts behave in a way similar in general to that of the osteoblasts in both avitaminosis A and hypervitaminosis A. While not wedded to his original view to the exclusion of others, the present writer

still feels that a direct action of the vitamin on osteoblasts and odontoblasts is the simplest explanation of the data so far published.

2. B Complex

Deficiency of these vitamins produces effects in bone like those seen in protein deficiency and inanition (Levy, 1950; Nelson *et al.*, 1947, 1950). Chase (1940) reported degeneration of the enamel organ and interference with enamel formation in rats on B_6 deficient diets. To the writer's knowledge, no reports of the effects of deficiencies of the B complex upon dentine formation exist. It is possible that, as with protein deficiency (see heading III, 3), the effects of such vitamin deficiencies upon dentine are very slight.

3. Ascorbic Acid and Scurvy

The only animals which are sensitive to ascorbic acid deficiency are guinea pigs, monkeys, and humans, and in all three the appearances of scurvy in bones and teeth are similar. Most experimental work has been done on guinea pigs, which have the advantage that their molars as well as incisors are teeth of continual eruption.

a. Histological Changes in Bone and Teeth. The histological changes in bone and teeth during scurvy are in general so well known that a detailed description is not necessary. One of the first and best accounts of the bone changes was given by Wolbach and Howe (1926), and Boyle *et al.* (1936) have given a good report of the tooth changes. In bone, apposition of new bone stops, the periosteum gradually thickens, and the cells composing it look like fibroblasts. Resorption continues and as a result the shaft, and especially that part of the metaphysis where remodeling normally occurs, becomes very thin and may fracture, with hemorrhage and damage to the cartilage trabeculae and cell columns, producing an area of debris and fibrin deposition known as the *trümmerfeld* zone. Capillary penetration into the epiphyseal cartilage is more upset than are the cartilage cell sequences. A mass of cells develops in the metaphysis on the diaphyseal side of the *trümmerfeld* zone and is known as the *gerüstmark*. In teeth, dentine formation ceases and the odontoblasts change to fibroblast-like cells. Enamel formation is apparently only secondarily affected by changes in dentinogenesis (Wassermann, 1944).

In both tissues, at the onset of absolute scurvy, the pre-existing matrix is sealed off from the tissues formed subsequently by a dense calcified band. The epiphyseal cartilage is so treated (Bourne, 1943) and so is the dentine (Fish and Harris, 1934). The cartilage lattice of bone becomes densely calcified (Park *et al.*, 1935). The formation of the *gerüst-*

mark in bone has been attributed to the trauma which occurs as the scorbutic process develops, since Follis (1943) found no *gerüstmark* if the limb was immobilized. However Irving and Boyle (1952) found a typical *gerüstmark* in the pulp of scorbutic guinea pigs' teeth. It thus appears possible that *gerüstmark* formation is a "natural" sequel of ascorbic acid deficiency. Most workers consider it to be a collection of inactive calcifying cells, and on the administration of ascorbic acid immediate calcification occurs among them in bone (Menkin *et al.*, 1934), and teeth (Fish and Harris, 1934). Irving and Boyle (1952) likewise considered the pulpal *gerüstmark* to be a collection of inactive odontoblasts.

There seems no doubt that some of the contradictory statements on tooth changes of the past were due to workers mixing up the signs of absolute scurvy and what may be called "chronic scurvy," when a small amount of ascorbic acid was still given. Thus Maclean *et al.* (1939) described the formation of osteodentine in the pulp in scurvy, which in the present writer's experience does not happen in absolute scurvy. Both Dalldorf and Zall (1930) and Fish and Harris (1934) recognized that this osteodentine formation was due to a condition of chronic or sub-scurvy. On the other hand, in bone, such a differentiation does not seem to have been made in the types of scurvy observed. Thus, as with other deficiencies, the teeth appear to be more sensitive to curative agents than does bone.

b. Chemical Changes. The biochemical changes that are governed by ascorbic acid have been recently reviewed by Meiklejohn (1953). Unfortunately hardly any of them seem to apply to the physiology of the hard tissues. During the development of scurvy, the collagen content of both bone and teeth fell (Robertson, 1950), that of the other tissues being unaffected. Robertson (1952) did not consider that this was due to a reduction in the existing amount of collagen but to the formation of new tissue with a defective collagen content. The histological results bear out this interpretation in both hard tissues. Ascorbic acid is essential and is probably a coenzyme for tyrosine oxidation (Sealock and Goodland, 1951), and since collagen does contain small amounts of tyrosine it is possible that a derangement in this system may play a part in the disturbances of collagen synthesis in scurvy. The alkaline phosphatase of bone fell during scurvy, but no very significant changes were observed in the incisor teeth (Gould and Schwachman, 1942).

The formation of chondroitin sulfate in cartilage, using the uptake of S^{35} as a criterion (which has been shown to be valid by Dziewiatowski, 1949), is considerably reduced in scurvy (Reddi and Nörstrom, 1954; Hill and Bourne, 1955), and since it is probable that this substance is also involved in dentine calcification (Bélanger, 1955, 1956) possibly the

same happens in teeth, though this has not yet been demonstrated. It would seem from these findings that in scurvy the bone- and tooth-forming cells secrete less and less collagen and a matrix defective in mucopolysaccharide, which many workers have shown is implicated in calcification. Wolbach and Howe (1926) originally thought that a fluid substance was produced in the pulp in scurvy which "gelled" when ascorbic acid was given. Only Mazoué (1937) has agreed with this theory. All other workers have considered that the pulp could not produce a collagenous matrix for the reception of lime salts (Fish and Harris, 1934), that the cells with specific functions did not differentiate or there was no stimulus to produce bone matrix (Meyer and McCormick, 1928; Maclean *et al.*, 1939), and that ascorbic acid was concerned with the growth and activity of cells in general and specialized cells in particular (Ham and Elliott, 1938). These theories apply equally to bone and tooth formation.

c. Endocrine Influences. Cortisone has been found to have no effect upon the sequence of events in the development and healing of scurvy in bone (Wolbach and Maddock, 1952a) or on the development of scorbutic changes in teeth (Pfander, 1952). The latter writer used the odontoblast height as an index of adequate dosage of ascorbic acid and found it much more sensitive than the weight changes in the adrenals (Pfander and Mitchell, 1952). He also found that hyperthyroidism caused an increased requirement of ascorbic acid as evidenced by lowered odontoblast height but that thiouracil did not affect the ascorbic acid requirement (Pfander, 1952). Crampton (1947) reported the odontoblast height to have a logarithmic relation to the dose of ascorbic acid.

d. Ascorbic Acid and Vitamin A. A relationship has been claimed between vitamin A and ascorbic acid, based on findings in both teeth and bone. Vedder and Rosenberg (1938) first drew attention to the fact that ascorbic acid protected rats against the toxic effects of jewfish-liver oil. They thought ascorbic acid acted to oppose the action either of excessive vitamin A or of a toxic factor in the oil; possibly the rat's own ascorbic acid was destroyed by this toxic factor since the symptoms produced were not unlike scurvy—failure to grow; hemorrhages from the eyes, nose, and mucous membranes; and an abnormal rarefaction and fragility of the bones. Jonnson *et al.* (1942, 1945) claimed that a *deficiency* of vitamin A induced scurvy-like alterations in the teeth. Rodahl (1949) stated that the changes caused by hypervitaminosis A (including those in the bones and teeth) in rats, mice, guinea pigs, rabbits, dogs, and chickens were like those of human scurvy. Gross doses of vitamin A were more toxic to guinea pigs on scorbutic diets than to those on normal diets. It may be stated that the tooth changes described by Rodahl during hypervitaminosis A were quite unlike those observed by the present writer

(Irving, 1949b). Morehouse *et al.* (1952) found in rats that hypervitaminosis A caused the liver ascorbic acid level to fall, but that giving ascorbic acid did not affect the symptoms of hypervitaminosis.

T. Moore and Wang (1945) were unable to repeat the results of Vedder and Rosenberg (1938), who had claimed that ascorbic acid protected against excessive doses of vitamin A, nor could they detect any abnormality in ascorbic acid metabolism in hypervitaminotic-A rats. They stated "it would be too much to expect that the combination of lesions in the rat should either resemble one particular form of scurvy as found in another animal, or should include all the lesions which are found in all forms of scurvy." It has certainly seemed to the present writer to be naive to expect that ascorbic acid deficiency symptoms in, say, rats, should resemble scurvy as seen in guinea pigs. But supposing such comparisons are indeed valid, it will be admitted by all working on the histological aspects of the matter that hypervitaminosis A produces entirely different appearances in both bones and teeth compared to those seen in scurvy.

4. Calcium, Phosphorus, and Vitamin D

The metabolic turnover of Ca and P in rat bone and teeth has been estimated by Armstrong and Barnum (1948), who found the ratio of relative specific activities of P:Ca to be as follows 5 days after administration of the isotopes: femur epiphysis, 0.85; incisor dentine, 0.94; incisor enamel, 1.0.

a. Rachitic Changes in Bone and Teeth. Histological changes. Almost all comparative work on bones and teeth in this field has been done on rats, which is in a sense unfortunate, since these animals differ from many others in their handling of Ca, P, and vitamin D. Provided the Ca:P ratio and Ca and P contents of the diet are satisfactory, the rat requires very little vitamin D (Irving, 1944a). The necessity for a vitamin requirement is seen only when the Ca:P ratio of the diet is grossly upset. With high Ca:P-ratio diets (as for example the well-known Steenbock-Black diet), rachitic changes occur in the epiphyses of the long bones which are indistinguishable from clinical rickets and osteoid margins appear in places where intramembranous bone formation occurs (Dodds and Cameron, 1934, 1939). This osteoid margin cannot be resorbed during remodeling processes and so accumulates. When the Ca:P ratio of the diet is lowered below approximately 0.5, a rachitic change also occurs, but the changes are not so extreme. Both conditions can be cured or prevented by an adequate intake of vitamin D. Thus it is impossible in the rat to get pure vitamin D-deficiency rickets.

The incisor tooth has almost invariably been used for the analysis of dental rachitic changes. These changes usually affect only dentine forma-

tion; unless special methods are used for causing extreme rachitic changes (which are described later), amelogenesis is not affected, apart from the occurrence of cysts of doubtful specificity in the enamel organ (Weinmann and Schour, 1945). In dentine the predentine widens, interglobular dentine appears, the incremental rate of dentine formation slows, and vascular inclusions are seen (Becks and Ryder, 1931; Weinmann and Schour, 1945). Using diets of high Ca:P ratio, Bailie and Irving (1955) found that the onset of rachitic changes was simultaneous (10 days under their experimental conditions) in the incisal dentine and in endochondral and intramembranous bone formation. As in bone, these dental changes could be averted or healed by vitamin D administration.

Thus the fundamental change in both bone and dentine in rickets is the same, namely the loss of ability to calcify the matrix which is nevertheless still formed. The changes in dentinogenesis and intramembranous bone formation are quite analogous.

Chemical changes. Karshan and Rosebury (1933) studied the effects of altering the Ca:P ratio of the diet on bone and tooth composition. Gaunt and Irving (1940) extended these studies and reported that bone and teeth of rats differed in their reactions. With high Ca:P-ratio diets rachitic changes were more extreme in the bones than in the teeth, but low Ca:P-ratio diets affected the teeth to a greater degree. Normal teeth and bones were found only if the amount of Ca and of P in the diet was at least 0.3% of each, using dietary Ca:P ratios between 4.0 and 0.5. This was found in the absence of vitamin D, which is hardly needed at or above these levels of Ca and P intake.

These results are somewhat difficult to reconcile with those of Sobel and his colleagues. The PO_4:CO_3 ratio was higher in dentine than in enamel, while the Ca:PO_4 ratio was higher in enamel than in dentine. In both bone (1945) and teeth (1949a,b; Sobel and Hanok, 1948) they found the PO_4:CO_3 ratio to be related to the PO_4:CO_2 ratio of serum and the ratio in the serum to be related to the Ca:P ratio of the diet. In bone the CO_3:PO_4 ratio was higher if high Ca:P-ratio diets were fed. The ratio of PO_4:$2CO_3$, which is a measure of "n" in the apatite formula $[Ca_3(PO_4)_2]_n.[CaCO_3]_{1.0}$, varied from 2.08 to 7.72 in enamel and from 4.40 to 9.31 in dentine in various experimental groups—a far greater range than that found in bone under comparable conditions (1.86–3.33). Giving vitamin D caused denser bones but no change in the PO_4:CO_3 ratio; but the density of minerals in dentine and enamel were not influenced by vitamin D, though the CO_3:PO_4 ratio was increased (Sobel, 1955). Lund and Armstrong (1942) found conversely that low Ca diets inadequate in vitamin D had no effect upon tooth composition, but the Ca and P per unit volume of bone was significantly reduced. Hodge

(1955) considered that the changes in PO_4:CO_3 ratio observed in bone by Sobel *et al.* were at least in part due to an exchange of carbonate for surface phosphate. These observations go a long way toward reconciling the physical findings of an apatite structure in all three calcified tissues with the very varied chemical analyses reported.

The effects of lesser changes in the Ca and P of the diet upon teeth compared to bone have been demonstrated by Gaunt *et al.* (1942) and by Carlsson (1951). Gaunt *et al.* had rats on a diet low in Ca and P and found that, per unit of weight, the skeleton retained 50%, and the teeth 27%, more P^{32} than those of rats on a good diet when the P^{32} was administered at the end of the 28-day experimental period. The teeth, though affected by the deficiency, were less affected than the bones. Carlsson found that the ratio of femur:incisor Ca^{45} fell in growing rats on the Steenbock-Black diet to the same level as that in nongrowing animals—the bones grew but did not calcify, while the teeth were less affected. The ratio was increased by vitamin D dosage. On a Ca-deficient diet, on the other hand, no decrease was found in the ratio, and under this condition the formation of calcified tissue in bone and incisors was equally affected.

Amelogenesis during rickets. There appears to be a fundamental difference between amelogenesis and bone or dentine formation. Irving (1950) carried out the following procedure: rats were placed on a rachitogenic diet for 28 days and were then subjected to dietary restriction for a few days to cause healing of the rickets. They were then put back on to the rachitogenic diet, and the changes in the bones and teeth were observed. In bone and dentine a zone of calcification in the metaphysis and dentine corresponded to the period of dietary restriction and, on resuming the rachitogenic diet, this calcification stopped but matrix formation continued. Amelogenesis was differently affected. It had proceeded unchanged during the preliminary rachitic period and during the time of starvation, but on going back to the rachitogenic diet, amelogenesis stopped completely and the ameloblasts were considerably upset. After about 15 days, the ameloblasts recovered to a considerable extent, but they formed no more enamel and that which they had formed persisted in an acid-insoluble form. The subsequent ameloblasts were unaffected by these procedures. It was therefore concluded that calcification and matrix formation in amelogenesis must be inseparable processes and are unlike those in bone and dentine, where uncalcified matrix can be produced.

b. Action of Vitamin D. While in principle this vitamin acts in the same way on dentine and bone formation in that in the rat it will cause calcification to begin again in rachitic tissues, there are fundamental

differences which are inherent in the nature of the formative mechanisms. In endochondral bone formation, calcification begins again at the zone of provisional calcification of the epiphyseal cartilage, osteoid is calcified and removed, and the metaphysis is remodeled and disappears. In intramembranous bone-healing the osteoid is likewise calcified and removed. Uncalcified osteoid cannot be removed in remodeling, and it is of interest that it has been shown, in internal resorption of dentine, that predentine is likewise immune to resorption (Cabrini *et al.*, 1957). In the incisor tooth there is no reparative mechanism. Calcification of dentine starts again, but there is no change in the predentine formed before the vitamin was given, and it persists throughout the life of the tooth (Irving, 1944a). This lack of response of already formed dentine or predentine to subsequent influence has also been noted by Bevelander and Hoskins (1939) and Myers (1955). The new, calcified dentine is often laid down in incremental stripes which Irving (1944a) has shown each to correspond with 1 day's calcification, and thus it is possible to time the length of action of the vitamin. The incisor tooth was the most sensitive tissue, and healing could be detected 24 hours after one small dose (18 I.U.) (Irving, 1944a). Intramembranous calcification began 2 days after similar dosage (Bailie and Irving, 1955), but no calcification was seen in the epiphysis till at least 4 days after the dentinal reaction (Irving, 1944a). It is of interest that with small doses of vitamin D as many as 8 days could elapse before calcification of dentine began, indicating that the vitamin must act on other processes besides intestinal Ca absorption. It is not easy to say from present evidence exactly where this internal action of vitamin D is centered, but from work by Greenberg (1945), Underwood *et al.* (1951), as well as that by Irving cited above, the present writer thinks that there is a direct action on the calcifying mechanism in bone and almost certainly also in teeth.

The response of the teeth to various doses of the vitamin was, like that in bone, of an exponential type (Irving, 1944a).

c. Hypervitaminosis D. Hendricks *et al.* (1947) found that moderately large doses caused excessively mineralized bones in dogs and that the teeth were small and malformed. The effects of very large doses upon bones and teeth have also been investigated, but it is doubtful, at least in the present writer's view, if the results of such unphysiological procedures are of much value. In bone the reaction is so unexpected that it may well not be due to the antirachitic action of the vitamin but to some side effect detectable only with high dosage. Ham and Lewis (1934) found that the cortical bone already laid down was resorbed, but no osteoclastic reaction was seen, and an overgrowth of poorly mineralized bone occurred. In the incisor tooth, quite a different reaction is seen—at least

after one massive dose (Schour and Ham, 1934). This is the calciotraumatic response described by Irving (1943) and by Irving and Weinmann (1948) in which, after a hypercalcified line in the last-formed dentine, the existing predentine is not calcified and the next formed is temporarily hypercalcified. This reaction is caused by almost any agent interfering with dentine calcification, e.g. administration of F, parathyroid extracts, and Sr, and is not specific to large doses of vitamin D. Irving *et al.* (1949b) confirmed Schour and Ham's findings with vitamin D and also reported them still to occur after nephrectomy. Schour *et al.* (1937b) found the same response after vitamin D dosage in parathyroidectomized rats and concluded that the parathyroids were not necessary for the action of vitamin D. No one, to the writer's knowledge, has reported the histological effects of chronic vitamin D overdosage on the tooth. Amelogenesis is not affected by large doses of vitamin D.

5. Other Elements Causing Rickets-like Changes

It is pertinent at this stage to mention the action of some other elements besides Ca and P, since many of these cause rickets or rickets-like changes. Unfortunately many of the results are not comparable; thus the effect of the given element in the diet may have been studied in bone, while the effect of injections had been studied in the tooth, and usually single or even several injections have no effect on bone. Excess Mn, Be, and Sr in the diet cause rachitic changes, and of these elements Sr is built into the apatite crystal. F, also built into the apatite crystal, has quite a different effect on bone and teeth. Mg deficiency only, to the writer's knowledge, has been studied, but this has afforded us additional knowledge of the comparative biochemistry of these two tissues.

a. Manganese. Excessive Mn ingestion causes rickets-like changes in the bones (Chornock *et al.*, 1942), but this was stated to be due to interference with Ca and P assimilation. It is not known if Mn has a direct effect upon bone formation. The same authors said that low Mn intakes had little or no effect on bone calcification when combined with normal or rachitogenic diets. Wessinger and Weinmann (1943) and Irving (1944b) found a calciotraumatic response in the dentine of the rat incisor after Mn administration, Irving stating that this also occurred if the animals were on a low Ca:P-ratio diet.

b. Beryllium. The action of Be upon calcified tissues was first described by Jacobson (1933) and by Guyatt *et al.* (1933), who found that the addition of Be salts to the diet caused severe rickets, which was supposed to be due to the precipitation of PO_4 in the gut. However the condition was not prevented by cod-liver oil, and Sobel *et al.* (1935) considered that there was a direct effect upon the "local factor" in bone,

and other workers (e.g. Gorlin, 1951) thought that the inhibiting action of Be on alkaline phosphatase should be considered. The present writer (1957) has stated elsewhere that he considers it unlikely on the balance of the evidence that phosphatase is implicated in the calcification process.

Gorlin has studied both bone and tooth changes in rats put on to Be-containing diets. The changes in dentine formation and endochondral and intramembranous bone formation were those of profound rickets, but amelogenesis was not affected.

c. Strontium. Bone. This element, when added to the diet, causes, as has been known for a long time, typical rachitic changes in bone. Shipley *et al.* (1922) stated it could not replace Ca in the bones. Follis (1955), however, considered that many of the effects produced by earlier workers had been due to the abnormal Ca:P ratios of the diets employed. Sr salts in diets of normal Ca:P content, or else injected intraperitoneally, caused a stimulation of intramembranous bone formation of an abnormal type but had little effect upon endochondral calcification. On the other hand, Macdonald *et al.* (1951) found Sr to inhibit calcification of bone even if the diet was adequate in Ca, P, and vitamins A and D.

Teeth. Sr differs from many of the other elements in its dental actions, since it affects both enamel and dentine formation. Klein *et al.* (1930) found that when Sr was added to the diet, poorly calcified dentine was produced, which they called dentinoid and which looks, from their photomicrographs, like predentine. Irving and Weinmann (1948) reported typical calciotraumatic responses in dentine after Sr injections, and Weinmann (1943) found that similar injections caused disruption of part of the enamel organ and cessation of amelogenesis, the ameloblasts laying down enamel at the time of the injection being affected.

It appears from this that the actions of Sr are unlike those of some of the other elements and more akin to those of Sobel *et al.* (1949c) considered that Sr competed with Ca for some constituent of the bone cell necessary for calcification. But it has also been stated that Sr cannot replace Ca in bone since it is not a competitor with Ca for a site of deposition (Lengemann, 1957). None the less it does enter the apatite crystal (Macdonald *et al.*, 1951). Hodge *et al.* (1946), studying Sr adsorption *in vitro* by bone and teeth, found bone to take up most, dentine next, and enamel least. The adsorption by bone *in vitro* was sufficient to account for the Sr taken up in the body *in vivo*. They concluded, on comparing their results with those found *in vivo* in the case of Ca, that the body did not handle Sr like Ca.

d. Magnesium. Bone. The changes that have been observed have been only those caused by Mg deficiency. Becks and Furuta (1943) found a dark-staining amorphous material to be deposited on the bone surface,

the bones becoming hard and brittle. Yamate and Singer (1953) stated that bone atrophy occurred. In the femur the parallel bone trabeculae in the metaphysis were lost and the zone of preliminary calcification just below the epiphysis was atrophic or absent. Osteoblastic activity was at a minimum.

Teeth. In the teeth most characteristic changes occur, which have been described by Klein *et al.* (1935), Irving (1940), Becks and Furuta (1942), and Yamate and Singer (1953), in rats and hamsters. Typical striations occur in the dentine, which appears to be imperfectly calcified. The odontoblasts atrophy and dentine calcification in the rat changes from a 1-day to a 2- or 3-day rhythm. Ectopic calcification occurs in the enamel organ, which later degenerates into a condition like that seen in vitamin A deficiency. Ectopic calcification is also seen in the pulp. When the Mg content of the diet is raised, normal dentine is immediately formed (Irving, 1940). The striations in the dentine were considered by Klein *et al.* (1935) to coincide with the convulsive seizures to which animals on low Mg diets are prone, but later workers still found striations in animals which had not had fits.

Chemical changes in bone and teeth. Comparisons of the chemical changes in tooth and bone have been carried out by Watchorn and McCance (1937) and also by Duckworth (Duckworth and Godden, 1941). Watchorn and McCance studied subacute Mg deficiency; their rats were on a diet containing 40 parts per million (p.p.m.) Mg, and the experimental period was 12 weeks. The Mg content of the incisor teeth fell to half and that in the bones to approximately two-thirds; the changes in the teeth were accompanied by the typical histological appearances. Even after 12 weeks the percentage Mg content of the teeth was higher than that of normal bones and a higher concentration of Mg seemed to be necessary for normal tooth structure than for normal bone. Duckworth studied acute Mg deficiency in conjunction with the experiments of Irving. His diet contained 6 p.p.m. Mg, the animals living for only a short time. When they were put on to the diet, the absolute amount of Mg in the incisor teeth remained constant, that in the teeth of the control animals rising over the experimental period. The Mg in the skeleton fell considerably. Since the incisor tooth was being continually worn away and replaced, Mg was not being mobilized from it but was actually being deposited, unlike what was happening in bone. When the Mg content of the diet was raised, the laying down of normal dentine and the increase in the Mg content of this tissue were simultaneous.

Mg is known to be a component of many enzyme systems and presumably the changes observed are due to interference with these systems, but why the changes take the form they do is not known. The body must

carry very low reserves of Mg since the changes in the teeth were observed by Irving only 4 days after the experimental period began.

e. Fluorine. Interest in this element has arisen from its effects on teeth in causing mottled enamel and also from its marked anticariogenic action. In chronic F intoxication in man, the enamel of the teeth becomes disfiguringly brown or black in areas and the teeth are very brittle. The nature of the pigment has not been determined, but it contains manganese (Ockerse and Wasserstein, 1955). Bone formation is severely disturbed and a crippling arthritis and spondylitis ensue, with the formation of many exostoses. Largent *et al.* (1943) examined the bone changes in rabbits after chronic F intoxication. The exostoses formed were composed of true bone and appeared like normal new bone, such as is seen in callus after a fracture.

Histological changes. Teeth. The histological effects on teeth have mostly been studied experimentally, using the rat incisor. Schour and Smith (1935) first analyzed the effect of continuous administration of F salts; this caused severe changes in the enamel organ, the papillae becoming fused and the ameloblasts laying down poorly stained globules of organic enamel. The odontoblasts were less affected, but the staining reaction of the dentine was altered by each injection, so that a series of stripes was seen. Irving (1943) studied the early action of single small doses on the incisor. In the enamel a pronounced stria of Retzius occurred at the time of the injection, while in dentine the calciotraumatic response was seen. This reaction in dentine was dependent on the blood Ca level, being more extreme when this was lowered (Irving, 1949a). Bélanger *et al.* (1954), using Ca^{45}, found the growth of dentine, cementum, and alveolar bone to be retarded in pigs fed fluoride.

Bone. The histological changes in bone are less extreme and more general. Irving (unpublished results) never found any detectable change in rats after a single injected dose of NaF (10 mg. F per kilogram body weight). Comar *et al.* (1953) reported in pigs that continuous feeding of F caused a reduction in bone growth and an increase in bone resorption in the primary and secondary spongiosa. Paff and Boyd (1952), using tissue cultures, found inhibition of cartilage calcification, but not of growth; they thought this was possibly due to competition of F with phosphate for Ca or to inhibition of enolase. Retardation of calcification of bone has also been reported by Fleming and Greenfield (1954).

F Metabolism. The uptake of F by bones and teeth, when F is administered, appears to be similar in the two tissues. Younger rats took up more F in both bones and teeth than did older animals (Wallace-Durbin, 1954). This worker found that per gram of tissue, 1.6% of the dose of F went into bone and 3% into the incisor. Zipkin and McClure (1952) re-

ported essentially the same. Rats stored F in their bones and teeth when on an F-containing diet, but stored more during the period of rapid growth and then accumulated little or no more. If F was given at different ages, the same was found, less and less being taken up by the hard tissues as the animals got older. With a stabilized F intake, the F content of storage was fairly consistent at maturity. Savchuck and Armstrong (1951) fed rats water with a high F content for a definite period. When F was withdrawn from the diet, 10–15% of the F in the skeleton was lost over a period of 40 days, but the rest of the F was so bound that no more was detectably excreted over a period as long as 110 days. By 150 days after terminating the high F diet, the F content of the incisors was appreciably higher in the experimental animals compared with the controls, indicating an intraskeletal turnover of small amounts of F, though this turnover could not be detected chemically. It should be remembered that the incisor teeth replaced themselves several times during this 150-day period.

This uptake of F by the hard tissues is presumably adsorption followed by heteroionic exchange in the apatite structure. Leach (1956), however, found in adsorption studies with powdered enamel and dentine that the quantity of phosphate liberated at the same time as the adsorption of F was less than the stoichiometric amount of F adsorbed, and Neuman *et al.* (1950), using bone, reported that the F ion did not replace surface phosphate by exchange adsorption, but replaced either hydroxyl or bicarbonate in the surface of the mineral phase. As the animal gets older, the available tissue for these processes becomes reduced, presumably owing to the lessening of the extent of the hydration shell of the apatite crystal. The same reduction of availability of these tissues has been found using Ca^{45} and P^{32}. But some process other than this must also operate, at least in bone, since Miller and Phillips (1956) have reported that in older bone, sites originally available to F become closed to subsequent refluoridation.

Peckham *et al.* (1956) found that the F in human fluorosed teeth was associated with the inorganic fraction only, but the crystalline nature of the compound formed when F enters bone or teeth seems to be a matter of some dispute. Lindemann (1956), using an X-ray diffraction method that would have detected 0.5% of CaF_2, and working with bones and teeth of severely fluorosed rats, stated that no CaF_2 was found. On the other hand, Fischer *et al.* (1954, 1956) found, also using X-ray diffraction methods, that CaF_2 occurred in appreciable amounts in enamel treated with fluorine reagents *in vitro*. They determined the diffraction patterns of synthetic hydroxyapatite and fluoroapatite and stated that that of hydroxyapatite persisted after F treatment of enamel and that fluoroapatite was not the dominant product formed.

Very few endocrines have been studied with regard to the action of F on the hard tissues. Muhler and Shafer (1954) found in rats that thiouracil increased the uptake of F by the skeleton, but that thyroid extract had no effect on F uptake. Presumably a similar action would be found in the case of teeth.

There seems no doubt that F can enter the apatite structure equally in bone and teeth, but in common with many other ions which do this, can only be taken up to any large extent by apatite of young animals. Apart from that, F has undoubtedly some metabolic effect, which is probably a general one on all cells at some place in the carbohydrate glycolytic cycle. This effect would be most noticeable on the most active cells, and thus those involved in tooth formation, which is a process of much greater intensity than osteogenesis, are more affected than the osteoblasts or osteocytes.

Irving (1944b) studied the effects of a number of elements on enamel and dentine formation and found of those he tested only F and Sr to affect both processes. These two are known to enter the structure of the apatite crystal. It was possible to modify the reactions of dentine and enamel by altering the Ca:P ratio of the diet. After the animal had been on a high Ca:P-ratio diet, F affected only amelogenesis; by lowering the Ca:P ratio of the diet, the actions of many elements on dentine formation was abolished.

6. Vitamin E

a. Bone. While this vitamin is undoubtedly essential for certain aspects of tooth formation, it is still uncertain if it is necessary for the formation of bone. Barrie (1937) had found that the offspring of vitamin E-depleted rats had very soft bones and ossification appeared to be noticeably less than in normal animals. Weissberger and Harris (1943) quoted unpublished data of Harris and Joffe, who stated that rarefaction of the long bones occurred when large doses of vitamin E were given. Irving and Budtz-Olsen (1955) did not detect any difference in endochondral ossification in rats on diets with and without the vitamin. The present writer has searched carefully through his abundant material on vitamin E-deficient rats and has never detected any bone changes attributable to this deficiency.

b. Teeth. The effects of vitamin E deficiency on the teeth have been found only in the incisors of rodents and probably have something to do with the deposition of the brown pigment, the teeth usually turning a white color when the animals are on vitamin E-deficient diets, though this is not invariable. Irving (1942) first described the characteristic degeneration, the whole enamel organ atrophying in its anterior part and

being replaced by fibrous tissue. In these experiments the teeth retained their brown color. Granados and Dam (1945, and many other papers) found that a diet containing fats with a large content of highly unsaturated fatty acids, and deficient in vitamin E, produced white teeth and a characteristic degeneration of the enamel organ like that described by Irving. In addition, the enamel organ was invaded with macrophages laden with pigment (Granados *et al.*, 1946). Enamel hypoplasia did not occur. All these changes were prevented by including vitamin E in the diet.

It can be concluded that vitamin E probably has nothing to do with calcification mechanisms. However its action is tied up in some way with that of protein, and not only in the teeth but also in other organs such as the liver. In the teeth, protein can replace vitamin E to a large extent in protection of the enamel organ and enamel pigmentation when the animals are on a vitamin E-deficient diet (Hove, 1946; Hove and Harris, 1947; Granados, 1949; Granados *et al.*, 1949; T. Moore, 1949). Irving and Budtz-Olsen (1955) found protein to protect the enamel organ but not to prevent depigmentation when the animals had 20% of cod-liver oil in the diet. In recent experiments (Irving, unpublished results) in which the animals were on a vitamin E-deficient diet containing 2.5% of cod-liver oil and were then given supplementary protein, both the pigment and enamel organ were restored. Hove and Harris (1947), judging from effects on the efficiency of protein utilization, considered α-tocopherol and protein to act by separate and independent mechanisms, but Lindan and Himsworth (1950) considered from their protective action on the liver that vitamin E and protein "focus on a common point in metabolism." The experimental methods used by different workers in tooth studies are so diverse that it is difficult to compare the results obtained. But it is of interest that bone requires adequate protein for proper formation irrespective of the vitamin E content of the diet, while the incisor teeth only require adequate protein for enamel-organ protection if the E content of the diet is inadequate.

III. The Influence of the Endocrine Glands on Bone and Tooth Formation

1. Thyroid

a. Bone. The thyroid glands are well known to have a profound effect on bone. Becks *et al.* (1950) found that after thyroidectomy growth and differentiation of the epiphysis of bones of young rats were retarded. The bone of a 72-day-old thyroidectomized rat had the same appearance as that of a normal animal 15–20 days old (Becks *et al.*, 1948). Growth

hormone stimulated growth but not differentiation, while thyroxine stimulated both.

b. Teeth. Thyroidectomy or thiouracil invariably decreased the eruption rate of rat incisors and retarded dentine and root development (Ziskin *et al.*, 1940; Glickman and Pruzansky, 1947). The same was found in monkeys, in which almost pure predentine was formed in place of dentine after the operation (Ziskin and Applebaum, 1940); the operation of thyroidectomy caused a calciotraumatic response (Ziskin and Applebaum, 1941).

Hertzberg and Schour (1941) found that when thyroxine was given to rats, the eruption rate of the teeth was increased, but not the dentine apposition rate. In monkeys, however, Ziskin and Applebaum (1941) found thyrotropic hormone to increase the rate of apposition of dentine. Baume *et al.* (1954a) confirmed that thyroidectomy reduced the eruption rate, which was increased in thyroidectomized animals by giving thyroxine but was not affected by growth hormone.

The implications of these effects will be discussed in the section on the hypophysis.

2. Hypophysis

a. Bone. It is well known from clinical findings that this gland has a considerable control of bone growth.

After hypophysectomy all endochondral bone sequences ceased, the changes being like those in aging normal animals (Walker *et al.*, 1952). If growth hormone was given, a virtually normal picture was seen, but the endochondral sequences persisted and no closure of epiphyses occurred. If thyroxine was given to hypophysectomized rats, then normal closure of the epiphyses occurred (Asling *et al.*, 1949). Bone repair was inhibited in hypophysectomized rats, but when growth hormone was given, normal repair occurred (Simpson *et al.*, 1953). Becks *et al.* (1950) considered that growth hormone affected only growth in bone but that the thyroid gland controlled both growth and differentiation.

b. Teeth. Schour and van Dyke (1932) showed many years ago that hypophysectomy slowed and finally stopped the eruption of the rat's incisor. The enamel organ degenerated and enamel could be completely missing at the formative end. Dentine, normal in structure, overgrew and sometimes filled the pulp space entirely. Multiple foldings of the tooth, chiefly at the formative end, could occur. The cementum was likewise much thicker than normal. These results have been confirmed by Becks *et al.* (1946). Baume *et al.* (1954c) have suggested that the folding is due to the effect of masticatory stresses upon the little-consolidated apical structures. Sicher (1942) suggested that pulpal growth, the chief factor

in eruption, stopped but that epithelial growth and the induction of dentine growth were much less affected. In the monkey, apparently a somewhat different picture is seen since dentine apposition is retarded in developing permanent teeth, the dentine and enamel being of normal calcification (Ziskin *et al.*, 1949).

Baume *et al.* (1954b), working with hypophysectomized rats, found growth hormone to increase the size of the teeth but not the eruption rate. Thyroxine increased both, but not to normal levels. Both hormones given together had an optimal effect upon the eruption rate and restored completely the atrophied enamel organ. Baume and co-workers considered that growth hormone stimulated the basic process of growth and that the thyroid hormone controlled differentiation or maturation.

It will be seen that the effects of the hypophysis upon bone and tooth formation are comparable, and the same applies to the thyroid; also that after either hypophysectomy or thyroidectomy the thyroid hormone is essential for recovery of the tissue and cannot be replaced by growth hormone. The operation of hypophysectomy is most unsatisfactory in these studies, since it must drastically affect the thyroid and adrenal glands as well as the processes of growth in general.

It would appear that, in the rat, bone growth, tooth eruption, and amelogenesis are comparable processes and that dentine apposition, at least in this context, is somewhat different, since it can continue to a large extent unaffected by hypophysectomy; but in the monkey, dentine apposition is reduced after hypophysectomy. There is no doubt that the results on both tissues obtained with the purified growth hormone are far more clear-cut than those caused by hypophysectomy.

3. Protein and Amino Acid Deficiency

A comparative study of bone and tooth changes in protein deficiency has enabled us to draw conclusions about the relative actions of the hypophysis and protein on calcifying tissues.

a. Bone. Low-protein diets, or those deficient in protein, cause marked changes in endochondral bone formation. Irving and Budtz-Olsen (1955), working with rats, found about 10% protein (albumin) in the diet to be the limiting concentration. On low-protein diets the animals do not grow and the bone changes that occur are: narrowing of the epiphyseal cartilage, loss of the proper cell alignment, increase in ground substance, slowing and finally stoppage of cartilage erosion, cessation of formation of bone trabeculae, and ultimately sealing off of the cartilage with bone. This occurs on low-protein diets (Hunter, 1950; Follis, 1950; Frandsen *et al.*, 1954) and during phenylalanine (Schwartz *et al.*, 1951), lysine (Bavetta and Bernick, 1955), and tryptophan deficiencies (Bavetta *et al.*,

1954; Scott, 1955; Bavetta and Bernick, 1956). The same changes occur after hypophysectomy and during inanition. Frandsen *et al.* (1954) did not think that diminished action of the hypophysis was responsible for the changes during protein deficiency, and Follis (1950) stated that the question had not "been satisfactorily answered." However Schwartz *et al.* (1951) did attribute the changes they saw in phenylalanine deficiency to a reduction in the action of the hypophysis. It is not considered that the changes are due to inanition, since pair-fed controls, on an adequate protein diet, did not show comparable bone changes (Frandsen *et al.*, 1954; Scott, 1955). Estremera and Armstrong (1948) found the composition of the bone ash unchanged by low-protein diets.

b. Teeth. The teeth seem much less sensitive to protein or amino acid deficiency than is bone (Hunter, 1950; Irving, 1956b), since diets which can cause these marked bone changes may not affect the teeth at all. The teeth are able to draw on nitrogen from the general metabolic pool at times when, for example during fasting, the protein from other tissues, including bone, is being withdrawn (Fritz and Burnett, 1953). However, if the deficiency is stringent enough, changes are seen. Thus Bavetta and Bernick (1955) and Bavetta *et al.* (1954) found, after lysine or tryptophan deficiency, hypocalcified appearances in the dentine, with interglobular dentine and irregular predentine. Thus the tooth reaction is quite different, and, while that in bone is presumably due to diminished formation of the organic phase, the changes in teeth seem to be due to the formation of a poorly calcified, or possible poorly calcifiable, matrix. Bernick and Bavetta (1957) studied the ground substance of dentine during tryptophan deficiency and found it to be in a depolymerized state, which they considered could prevent normal calcification from occurring. One thing however seems certain: Hypophysectomy causes the most profound changes in the incisor teeth (see heading III, 2, b), entirely different from those (if any) caused by protein or amino acid deficiency. It thus seems impossible that the changes seen in bone in these deficiencies can be due to underaction of the hypophysis; and the fundamental actions of the hypophysis and of protein on the hard tissues must be completely unconnected (Irving, 1956b).

4. Parathyroids

The action of these glands has recently been reviewed by Bartter (1954), Greep and Kenny (1955), and by Irving (1957) and need not be discussed further here except in certain aspects.

The chief arguments at present are whether the glands act directly on the hard tissues, governing apposition and resorption, or primarily on the renal tubule by affecting P reabsorption; another school of thought

considers that two hormones, phosphaturic and calcemic, exist in parathyroid extracts, the former possibly being an artifact. A study and comparison of the bone and dental actions of the hormone may help to settle some of these arguments.

a. Bone. The changes in bone in clinical and experimental hyperparathyroidism are so well known that little need be said of them here. They are similar in both conditions and are in essence an excessive osteoclastic resorption. Heller *et al.* (1950) described the modulations that can happen among the cells concerned with bone formation and destruction, which occur round a central spindle cell. There seems no doubt that, whether the parathyroids have a renal action or not, they do act directly on bone as well. Thus both Ingalls *et al.* (1943) and Stoerck (1943) found bone changes in nephrectomized rats after treatment with parathyroid extract and Barnicot (1948) and Chang (1951) have demonstrated a local action of the gland on bone.

b. Teeth. Since the teeth have no resorptive mechanism, it might be expected that their responses to parathyroid treatment would be different from those in bone. Thus the cellular changes described by Heller *et al.* (1950) are not seen in the pulp or enamel organ. Almost all observations have been made on the rat incisor. Erdheim (1906) was the first to describe the effects of parathyroidectomy in the rat. The changes were virtually those of rickets and the doubt has been expressed as to whether the diet was adequate in all respects. However, more recent workers have in general confirmed his findings. Bevelander and Hoskins (1939) found after parathyroidectomy that in animals on a normal diet dentine calcification was much disturbed with incremental lines and interglobular dentine, while if the diet was Ca-free only predentine was laid down in the teeth. In general the effects of parathyroidectomy in rats on a normal diet were similar to those in normal rats on the Steenbock-Black diet. Similar results are obtained in normal rats with diets of low Ca:P ratio, which lower the blood Ca (Irving, 1944a). Schour *et al.* (1937a) did not find such profound effects as Erdheim had, but in rats which survived for longer periods of time, the incisal dentine showed zones of poor calcification and enamel hypoplasia could occur. The molars were unaffected, so that there was no histological evidence of Ca withdrawal from the teeth.

The injection of parathyroid extract into rats causes the calciotraumatic response in the teeth already described (see heading II, 4, c) (Irving *et al.*, 1949c). The same was found after nephrectomy, but nephrectomy alone also caused a calciotraumatic response (Irving *et al.*, 1949a). The question is whether the various agents causing this response act directly on dentine formation or indirectly by changing the composition of the blood. Tweedy and McNamara (1936) had studied the blood

chemistry of the animals whose teeth were examined by Irving *et al.* (1949a,c). Forty-eight hours after nephrectomy the blood Ca was at a normal figure, but these authors stated that the blood inorganic P rose rapidly. This change has been commented on by Greep and Kenny (1955) and makes difficult the assessment of the results of such experiments where it was not prevented (e.g. by peritoneal lavage, Grollman, 1954). Injections of parathyroid extract, in the experiments of Irving *et al.* (1949c) were reported by Tweedy and McNamara to cause a considerable rise in blood Ca, but after nephrectomy such injections did not alter the blood Ca concentration, probably owing to various abnormal states such as the high blood P. However both injections caused a calciotraumatic response. Irving and Nienaber (1946) studied the serum Ca and P after the injection of NaF and found with normal Ca:P-ratio diets that no changes occurred in these figures although good histological responses were found in the teeth. It is possible, indeed probable, that changes in the blood chemistry, too slight to be detected chemically, might influence dentine formation, and in fact Bevelander and Hoskins (1939) have stated that the histological picture in the teeth was a more sensitive index of changes in Ca metabolism than was the serum Ca. The possibility that blood Ca levels do play a part in influencing calciotraumatic responses is suggested by the fact that the action of F on the teeth was dependent on the blood Ca level, being much more intense if the blood Ca was low (Irving, 1949a).

Furthermore, such a variety of otherwise unrelated agents produce the calciotraumatic response that it seems impossible that they can all act specifically on the teeth, and rather that they must operate through some common medium—the composition of the blood. The fact that nephrectomy causes such a response seems to be conclusive, since as far as is known, the kidney does not exert any endocrine influence on tooth calcification.

Thus the calciotraumatic response caused by parathyroid extracts is almost certainly the result of a change in the chemical environment of the tooth and nothing more. The tooth has no resorptive mechanism and so the calciotraumatic response is prominent. It is possible that such a change also occurs in bone but is overshadowed by osteoclasis. The changes in the teeth after parathyroidectomy also support this concept since they seem to be identical with those caused by diets which lower the Ca content of the blood. As far as the questions asked at the beginning of this section are concerned, it is to be regretted that they are not answered —the changes that are seen in the teeth after administration of parathyroid extract are due to chemical changes in the environment which may equally be due to action on the hard tissues or to changes induced in the kidney.

5. *Adrenals*

The action of ACTH or cortisone is similar on bone and teeth and "antianabolic," as might be expected from their actions on other tissues. Thus Becks *et al.* (1944) found ACTH to retard chondrogenesis and osteogenesis in the normal rat, and Sissons and Hadfield (1955) reported cortisone, when administered to rabbits, to cause changes in the epiphysis like those seen after hypophysectomy or during protein deficiency. Larow *et al.* (1956) found that the healing of rachitic changes in dentine by the administration of Ca and vitamin D was retarded by cortisone.

6. *Gonads*

While gonadectomy affects dentine calcification, estrogen has no effect at all on tooth formation in mice or rats, in which animals its effects on bone formation are well known. Little space need therefore be devoted to this aspect.

a. Bone. The formation of medullary bone in chickens during the egg-laying cycle was described many years ago and can be reproduced by the administration of estrogen (Gardner and Pfeiffer, 1939). Of all the other animals tested, only mice show the same response (Urist *et al.*, 1948); in young rats a large primary spongiosa develops, owing to lack of resorption of cartilage and spongy bone (Budy *et al.*, 1952).

b. Teeth. Ziskin and Applebaum (1941) found that castration in monkeys reduced the rate of growth of dentine, and Schour (1936) reported upsets in dentine formation of the squirrel after gonadectomy. The administration of estrogen has however no effect at all on the teeth of mice or rats, though causing osteosclerosis of the alveolar bone of mice (Stahl *et al.*, 1950). It is a curious anomaly that mouse bone should be affected by estrogen at all, since the mouse does not develop medullary bone during pregnancy. It is somewhat unfortunate in this regard that chickens have no teeth.

IV. Conclusions

It would appear that the reactions of bone and dentine are similar under nutritional and endocrine influences as regards changes affecting apposition of tissue. This is seen in avitaminosis A and hypervitaminosis A and during scurvy and rickets. Thyroidectomy and hypophysectomy also affect these tissues in a comparable manner as do also ACTH and cortisone. Both these tissues consist of a calcified collagen, and thus it is not unexpected that these resemblances occur. When changes affecting

resorption are considered, however, no resemblance at all is seen, since the teeth have no resorptive mechanism. Thus the action of vitamin D after rickets, that of hypervitaminosis D and that of parathyroid extract are quite different on these two tissues. It is possible that changes like those in dentine do also occur in bone but are overshadowed by the resorptive changes. The only way to investigate this would be to use the well-known *ia* rat, in which bone resorption does not occur in early life, or the manatee, but these animals are not normally available to research workers. In normal rats, birth usually causes a calciotraumatic response in the dentine, due to the metabolic changes occurring at this time. Dr. Bhaskar has shown the present writer similar reactions in the long bones of *ia* rats. The effect of other factors causing a calciotraumatic response in these animals would be most interesting.

Amelogenesis is a totally different type of process and is comparable neither to bone nor dentine formation—the calcification sequences are different and the matrix has a different composition. The only reaction seen in the enamel organ, caused by any procedure which affects it, is some kind of atrophy, and the only "productional" change is a reduction in enamel formation, never an excess. This occurs in avitaminosis A, avitaminosis E, and after hypophysectomy. Changes in rickets are seen only if the rachitic change is extreme, and one can say in general that the enamel organ is not very sensitive to nutritional effects. Some conditions, such as hypervitaminosis A do not affect amelogenesis at all. Vitamin E, which is essential for the maintenance of the rodent enamel organ, is apparently not needed for bone or dentine formation. Amelogenesis seems to be more comparable to nail or hair formation, and some workers have investigated it from this point of view.

Some of the instances in which dentine and bone formation differ quite radically in their reactions to endocrine effects have enabled us to draw conclusions about the fundamental action of these agencies. Thus it seems quite clear that protein deficiency does not affect the hard tissues through any influence on the hypophysis. The claims that vitamins A and C are metabolically interrelated appears to be very unlikely from a comparative study of their actions on bone and dentine.

As stated at the beginning, the teeth are able to protect themselves better against nutritional and other deficiencies than is the skeleton. Arguing teleologically, this would seem to be a valuable property. It would therefore be expected that teeth would be very sensitive to curative agencies. By studying the response of the teeth to these curative agencies it is possible to detect their action early and time it accurately, and quite apart from purely dental interests, the information thus obtained has proved of great general value.

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