ible, aryl hydrocarbon hydroxylase may be induced in the lung, kidney, and skin. In addition, Burki *et al.* (*13*) studied induction of aryl hydrocarbon hydroxylase in explants of fetal mouse liver. Their data suggest that its induction is under multifactorial genetic control. Therefore, the exact inheritance of aryl hydrocarbon hydroxylase induction in mice remains controversial.

The genetic control of aryl hydrocarbon hydroxylase in human cells and tissues is also uncertain. Induction of aryl hydrocarbon hydroxylase by 3-methylcholanthrane has been studied in cultured human lymphocytes (*14*). In this study of 353 healthy individuals, both constitutive and 3-methylcholanthrane–induced levels of aryl hydrocarbon hydroxylase were measured. Inducibility varied from 1.3- to 4.5-fold among individuals, with three subpopulations consisting of individuals with low, intermediate, and high inducibility being evident. Analysis of the data suggested a single locus of genetic control with gene frequences of low and high alleles being 0.717 and 0.283, respectively. In a preliminary study (*15*), patients with lung cancer appeared to have a higher frequency of both intermediate and high inducibility than that of the patients serving as controls. These findings await confirmation.

The level of aryl hydrocarbon hydroxylase has also been measured in human placenta: a 70-fold variation of aryl hydrocarbon hydroxylase among women who smoked 15 to 20 cigarettes daily was observed (*16*). This variation is similar in magnitude to the variation in the level of BP bound to DNA in the study reported here. The possibility that factors such as drug intake, tobacco smoking, or occupation may have influenced our results is now being investigated.

The possibility exists that subpopulations of humans may be unusually susceptible to procarcinogens because of an enhanced genetically determined capability to activate these chemical carcinogens metabolically. This hypothesis requires further study (*1*).

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9. The distribution of bronchial specimens obtained at surgery was: (i) three patients (two black males and one black female) without lung cancer, and (ii) 29 patients (19 white males, nine black males, and one white female) with lung cancer. The bronchial specimens obtained at immediate autopsy were all from five patients (three white males and two black males) without cancer who died from head trauma. A retrospective analysis of hospital charts was done to determine the history of tobacco smoking for all of the patients; 28 of the lung cancer patients were smokers, one of the noncancer patients was a nonsmoker, and no information was listed for the remaining patients. Among the smokers, no correlation was found between the number of cigarettes smoked (number of packs per day times years smoked) and the amount of BP bound to DNA in cultured bronchial mucosa. Because of the incomplete listing of information concerning smoking, occupation, diet, and medication in hospital charts, this information is now being obtained for use in further studies.
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25-Hydroxyvitamin D_3: Autoradiographic Evidence of Sites of Action in Epiphyseal Cartilage and Bone

Abstract. *Tritiated 25-hydroxyvitamin D_3 was administered to growing rats to morphologically document its sites of action. Highly selective incorporation occurred in epiphyseal hypertrophic cells, epiphyseal matrix, osteoid, osteoblasts, and osteocytes of metaphyseal bone spicules. The labeled metabolite appeared in chondrocytic lacunar matrix coincident with hypertrophic cell death as evidenced by histological examination. The tritiated 25-hydroxyvitamin D_3 became localized only in areas of active mineralization.*

The major metabolites of vitamin D_3, 25-hydroxycholecalciferol [25-(OH)D_3] and 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$], have been shown to be active forms of the parent vitamin in the promotion of bone resorption (*1, 2,*), possibly by acting as permissive agents for the action of parathyroid hormone (*3*). The calcium-transporting function of such metabolites has usually been studied in the intestine (*4*). Recently, attention has turned to the binding of 25-(OH)D_3 and 1,25$(OH)_2D_3$ in mineralized tissues (*5*) where these metabolites participate in promoting cell-mediated bone calcium mobilization (*6*). As early as 1956 it was demonstrated that bones which included epiphyseal growth plates concentrated significant amounts of ^{14}C-labeled vitamin D (*7*). However, no direct histological evidence documents the effects of 25-(OH)D_3 on bone or cartilage, and the possible sites of action of 25-(OH)D_3 in bone, such as in the epiphyseal growth plate undergoing endochondral ossification (a site of active mineralization and mineral mobilization), have not been differentiated. Although it is known that 25-(OH)D_3 exerts a direct effect on epiphyseal cartilage metabolism (*8*), the cellular and extracellular sites of incorporation of this metabolite in calcifying connective tissues have not been demon-

Table 1. The incorporation of 25-(OH)[^{3}H]D_3 in growing rat bone and epiphyseal cartilage, as revealed by autoradiography. Each number represents the average of counts taken from eight separate areas of each respective region.

Epiphyseal zone or cell population	Grains per cell (No.)	Grains per unit area of matrix (No.)
Resting zone	0	0
Proliferating zone	2	1
Hypertrophic zone	18*	4
Provisional calcification	9†	38
Osteoblasts	13	36‡
Osteocytes	15	28
Osteoclasts	0	4

*Viable cells. †Nonviable cells. ‡Osteoid.

strated. This investigation documents histologically the specific sites of action of 25-(OH)D_3 in growing rat bone and cartilage.

Tritiated 25-(OH)D_3 (Amersham/Searle, specific activity 1.1 c/mmole, 2.7 mc/mg) was purified on a column (1.2 by 45 cm) of Sephadex LH-20 and developed with a mixture of chloroform and hexane (65 : 35 by volume). The fraction with the peak of activity was recovered, dried, and reconstituted in ethanol prior to being injected into rats (*9*). Weanling albino rats (Charles River, 75 to 100 g) were given a single intraperitoneal injection of 3.0 μc of the purified 25-(OH)[^{3}H]D_3. Forty-eight hours after the injection the animals were killed, and tissues including tibial epiphyses and costochondral junctions were removed, fixed in formalin, decalcified in ethylenediaminetetraacetate (EDTA), and processed for sectioning at 5 μm. Tissue sections were then coated with Kodak NTB-3 emulsion, exposed for 2 weeks, and developed in Kodak D-19 prior to being stained with hematoxylin and eosin. The amount of 25-(OH)[^{3}H]D_3 incorporated was determined by examining the autoradiographs with an optical micrometer. Counts of labeled cells and matrix were made from eight areas of each respective region and averaged.

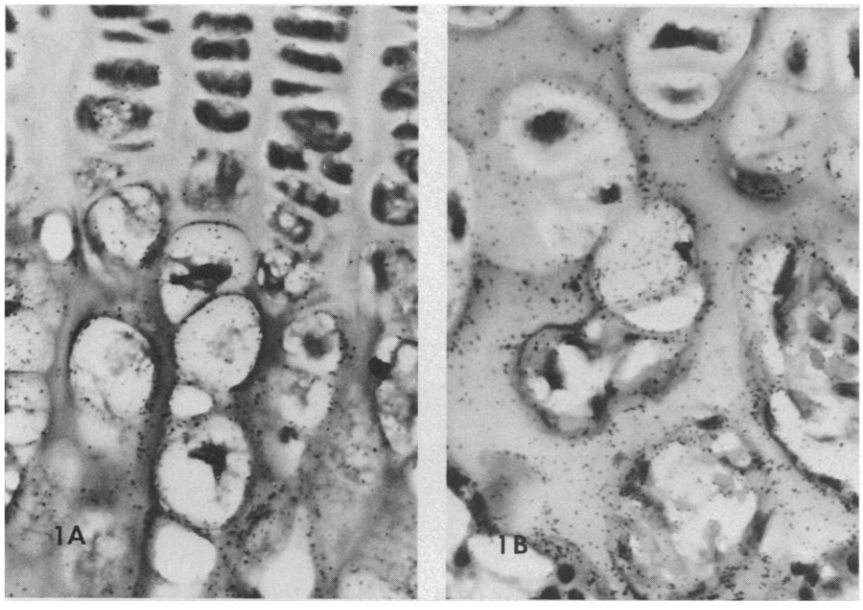

Fig. 1. (A) Photomicrograph of a tibial epiphyseal growth plate at the zones of cell hypertrophy and provisional calcification. The autoradiograph demonstrates the specific incorporation of 25-(OH)[^{3}H]D_3 into viable hypertrophic cells, and incorporation into the lacunar matrix of the intercellular septae. Note the paucity of metabolite in proliferating chondrocytes. Most of the 25-(OH)[^{3}H]D_3 is incorporated at the level of primary mineralization in the matrix regions (×200). (B) Photomicrograph showing the autoradiographic image of incorporation of 25-(OH)[^{3}H]D_3 into the hypertrophic lacunar regions of the matrix. Areas of vascular ingrowth into the emptied lacunae are bordered by labeled osteoblasts on the surfaces of matrix septae (×560).

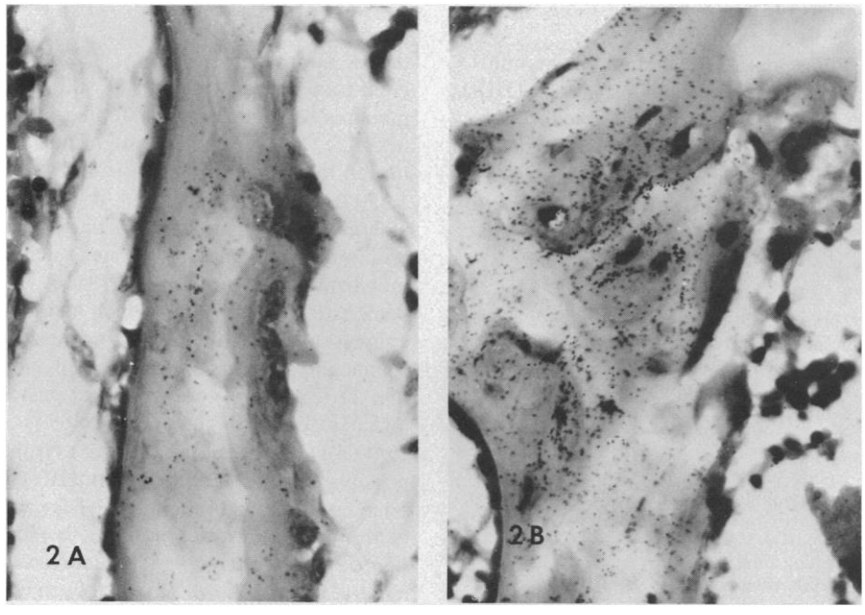

Fig. 2. (A) Photomicrograph of an autoradiograph demonstrating 25-(OH)[^{3}H]D_3 incorporation into bone spicule osteoblasts and into the calcifying osteoid regions. Cartilagenous cores of such metaphyseal bone spicules also demonstrate the persistence of labeled metabolite which was previously incorporated into the matrix at the zone of cell hypertrophy (×560). (B) Autoradiographic image of a region of a heavily labeled bone spicule undergoing active mineralization. Labeled osteocytes and osteoblasts are surrounded by calcifying cartilage which is heavily labeled with 25-(OH)[^{3}H]D_3 (×560).

Inspection of the autoradiographs revealed a high concentration of 25-(OH)[^{3}H]D_3 in bone and epiphyseal cartilage. Other tissues (liver, kidney, muscle) showed minimal incorporation of the labeled metabolite. The epiphyseal growth plate revealed zone-specific incorporation of 25(OH)[^{3}H]D_3 (Table 1). Of the four major zones of the epiphyseal growth plate (zone of resting cells, zone of proliferation and cell columns, zone of cell maturation and hypertrophy, zone of provisional calcification) the labeled metabolite was selectively incorporated into the viable, more mature cells of the hypertrophic cell zone. Scant amounts of label appeared in the proliferating zone cells and none appeared in the zone of resting chondrocytes. Of special interest was the incorporation of 25-(OH)[^{3}H]D_3 into the calcifiable matrix of the epiphyseal growth plate at the site of initial mineralization (Fig. 1). Where hypertrophic chondrocytes exhibited morphological evidence of cell death, 25(OH)[^{3}H]D_3 appeared to be released to the matrix immediately surrounding the chondrocyte. This site would be consistent with the location of the matrix vesicles which serve as extracellular, membrane-bound loci of the mineralization process (*10*). Similarly, all regions of active mineralization on bone spicule surfaces gave evidence of large amounts of 25-(OH)[^{3}H]D_3 incorporation. Labeled metabolite was observed in large amounts within the calcifying osteoid bordering the metaphyseal bone spicules as well as within surface osteoblasts, entrapped chondrocytes, and osteocytes of more calcified bone spicules (Fig. 2). No 25-(OH)[^{3}H]D_3 was observed in osteoclasts. Labeled metabolite was present in persistent cartilage

cores of bone spicules, apparently as a result of its earlier incorporation into matrix at higher regions of the growth plate.

The movement of 25-(OH)[^{3}H]D_3 to its target tissue reflects the demand by growing bone and cartilage for the metabolite. Quantitatively, 25-(OH)D_3 is the major metabolite found in bone (*11*) although other metabolites possess greater resorptive activity. In growing animals the demand for calcium is great because of the rapid growth of the skeleton. Although it is widely held that 25-(OH)D_3 promotes bone resorption, the histological evidence presented in this study suggests that it is involved in mineral accretion and calcium movements in epiphyseal matrix and osteoid undergoing mineralization. The long half-life of 25-(OH)D_3 combined with the availability of the metabolite to the target tissues for the 48-hour duration of the experiment did not result in its incorporation into zones of the growth plate or spicular bone where mineralization was not occurring. Growth plate chondrocytes and other bone cell populations associated with the process of mineralization of matrix were the only cells capable of incorporating the metabolite. The incorporation of 25-(OH)[^{3}H]D_3 into matrix regions suggests its ultimate association with structures or substances present at sites of initial mineralization (*12*).

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Rod Outer Segment Disk Shedding in Rat Retina: Relationship to Cyclic Lighting

Abstract. *When albino rats are reared in cyclic light, a burst of rod outer segment disk shedding occurs in the retina soon after the onset of light. The number of large packets of outer segment disks (phagosomes) in the pigment epithelium at this time is 2.5 to 5 times greater than at any other time of day or night. The subsequent degradation of large phagosomes to smaller structures within pigment epithelial cells proceeds rapidly. The burst of disk shedding follows a circadian rhythm for at least 3 days, since it occurs in continuous darkness at the same time without the onset of light.*

Vertebrate rod photoreceptor cells continually renew their photoreceptive outer segments. The renewal process involves (i) synthesis of new membrane to form rhodopsin-containing disks at the base of the outer segment, (ii) displacement of these disks outward toward the tip of the outer segment by newly formed disks, and (iii) disposal of disks by detachment at the tip of the outer segment, followed by phagocytosis of the disks by the adjacent pigment epithelial cells to form phagosomes and degradation of the phagosomes within the pigment epithelial cell cytoplasm (*1–6*). Disks detach intermittently in groups, a process called disk shedding (*7*). Since rod outer segments maintain a relatively uniform length throughout life, the rate of disk disposal must equal the rate of disk synthesis. The rate of disposal is high, for in monkeys, rats, and mice the entire complement of rod outer segment disks is replaced every 9 to 13 days (*1, 4, 8*). It has been estimated that in the rat each pigment epithelial cell must phagocytize and degrade 25,000 to 30,000 rod outer segment disks each day (*9*). Given this high number, it is surprising to find relatively few phagosomes within pigment epithelial cells in most published studies (*3, 5, 8, 10, 11*). Either (i) degradation of ingested disks within pigment epithelial cells is rapid, (ii) phagocytized disks pass rapidly through the pigment epithelial cells and are degraded elsewhere, (iii) disks are degraded without passage into the pigment epithelial cells, (iv) most disks are phagocytized at a time when investigators usually have not taken the eyes for cytological examination (for example, at night), or (v) some combination of these factors occurs.

To explore this problem, I examined rod outer segment disk shedding in the rat retina in relation to the lighting cycle. I found that (i) a burst of disk shedding occurs soon after the onset of light in the morning and far less occurs at any other time of day or night (*12*), (ii) degradation of large phagosomes within pigment epithelial cells is rapid, and (iii) the burst of disk shedding follows a circadian rhythm since it occurs at the same time without the onset of light.

Fischer inbred albino rats were maintained in an environment with 12 hours of light and 12 hours of darkness (lights on in the morning at 0700 hours, off at 1900 hours) at a room illumination of approximately 20 to 35 footcandles (215 to 375 lu/m^2) from overhead fluorescent lamps. The illuminance levels within the cages varied from front to back, but did not exceed 15 footcandles (160 lu/m^2). The rooms were temperature-controlled at 23° ± 1°C, and the animals were fed Purina Formulab freely. At various times of the lighting cycle (*13*), the rats were killed, and their eyes were embedded in plastic, sectioned at 1.5 μm, and stained with toluidine blue (*14*). For quantification, all inclusion bodies in the pigment epithelial cell somas and the intensely staining structures among their processes (*15*) (Fig. 1a) that were greater in any dimension than 0.75 μm (half the diameter of the rod outer segments) were defined as large phagosomes and were counted as a measure of disk shedding (Fig. 2a, legend).

Some large phagosomes were present at all times of the lighting cycle. However, a sudden burst of outer segment disk shedding occurred in the morning soon after the lights were turned on (Figs. 1a and 2a). Between 0.5 and 2.25 hours after the lights came on, the number of phagosomes was 2.5 to 5 times the number found throughout the rest of the day or night (Figs. 1b and 2a). This burst of disk shedding occurred in both male and female rats ranging from 2.5 to 8 months of age.

At the peak period of disk shedding, almost every cell contained large phagosomes, and the number per 180-μm field was fairly uniform around the eye. For example, in 44 consecutive fields around a single section of an eye taken 2 hours after the onset of light, the number of phagosomes per 180-μm field was 26.3 ± 0.9 (mean ± standard error of the mean) with a range of 13 to 40. Considerably more variation occurred among animals than within an individual