Influence of Geometry of Transplanted Tooth and Bone on Transformation of Fibroblasts[1] (37381)

A. H. Reddi and Charles B. Huggins

The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637

Certain fibroblasts (mesenchymal cells; stem cells of connective tissue) possess the attribute of transformability which can be elicited by bringing them in contact with epithelium (1) or with the matrix of bone (2) or tooth (3). By these procedures, the phenotypic expression of the competent responding fibroblasts is altered radically; enzymes (4) are induced followed by emergence of chondroblasts, osteoblasts and hemopoietic bone marrow. Bone and tooth are equipotent in their transforming ability (5).

Transplantation of acid-insoluble bone powder to the subcutaneous tissue causes the formation within 24 hr of discrete transformation plaques (6) in which cartilage, bone and hemopoietic bone marrow form in a sequence of scheduled events. The transformation products are confined to the central region of the plaque; the periphery consists of a translucent rim of fibroblasts devoid of cartilage and bone. The present experiments were undertaken to investigate the role of geometry in differentiation of fibroblasts using various sizes of powdered bone and various shapes of tooth as inductors.

The pulp chamber of incisor tooth of adult rat has a conoid shape, open at its base, with thick walls of highly cross-linked dentin. It seemed ideal for investigation of the influence of shape on differentiation since the interior of the incisors of adult rats is relatively deep (~8–11 mm) and repopulation can take place exclusively by entry of cells through its solitary aperture.

Material and Methods. Preparation of bone powders. Desiccated powdered acid-insoluble matrix (6) of rat bone was sieved into two distinct particle sizes: fine powder, 44–74 μm; coarse powder, 420–850 μm. Fine and coarse powders were transplanted in weighed amounts (15–20 mg) in symmetrical contralateral sites in 8 male rats, age 35 days. The day of transplantation is denoted Day 0. The transformation plaques were harvested on Days 7 to 35.

Radioactivity and enzyme assays. At harvest, the plaques were dissected out and weighed. Half of the plaque was subjected to histologic examination; the other half was used for assay of alkaline phosphatase (EC 3.1.3.1) activity (7) and ^{35}S and ^{32}P incorporation (6).

Preparation of teeth. Forty-eight whole incisors were dissected out from adult rats. Tooth pulp was removed with the aid of a needle and jets of water forced into the pulp chamber by means of a curved hypodermic needle and syringe. The teeth were washed in water 2 hr; absolute ethanol, 1 hr; diethyl ether, 0.5 hr; and dried overnight at 37°.

Teeth were demineralized as follows: 0.5 *N* hydrochloric acid (1 ml/mg) 5 hr at room temperature in a jar with magnetic stirring; repeated washing in copious amounts of water 3 + hr; absolute ethanol 1 hr; diethyl ether 0.5 hr; and dried overnight at 37°. Demineralized teeth were devoid of cellular elements. The transplants (6) of bone powder and whole tooth were allogeneic.

Results. Influence of transplant size. Fine and coarse powders transplanted in symmetrical contralateral sites elicited transplantation plaques of two classes which differed significantly according to the size of the transplant. On Day 7, $Na_2{}^{35}SO_4$ was injected iv and 18

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TABLE I. Incorporation of ^{35}S and ^{32}P in Transformation Plaques Elicited by Fine and Coarse Powders of Bone Matrix.[a]

Day	Size of powder	Alkaline phosphatase (units/g)	Incorporation (cpm/mg)	
			^{35}S	^{32}P
7	Fine	2.9 ± 0.3	287 ± 26	—
	Coarse	17.4 ± 3.4[b]	848 ± 204[c]	—
14	Fine	5.5 ± 0.7	—	1945 ± 145
	Coarse	74.7 ± 2.9[b]	—	5254 ± 502

[a] In each category, 18 plaques were harvested 4 hr after iv injection of $Na_2{}^{35}SO_4$ or $H_3{}^{32}PO_4$. ±, standard error of mean; $n = 9$ for all determinations.

[b] $p < 0.01$.

[c] $p < 0.02$.

plaques elicited, respectively, by coarse and fine powder were harvested 4 hr later. The "coarse powder" plaques were larger, alkaline phosphatase activity was higher and ^{35}S incorporation was greater (Table I) than in plaques elicited by fine powders. On Day 14, $H_3{}^{32}PO_4$ was injected and 18 plaques of each category were harvested at +4 hr; alkaline phosphatase activity and ^{32}P incorporation into acid-soluble activity were significantly higher ($p < 0.01$) in "coarse powder" plaques denoting greater *de novo* formation of bone mineral than in transformation plaques evoked by fine powder. Likewise, hemopoietic bone marrow, vivid red in the gross, was consistently more extensive in histologic sections of plaques created by coarse powder and harvested on Days 21 and 35.

Undemineralized incisor tooth transplants became encapsulated and their pulp chambers populated with fibroblasts; bone and cartilage were never observed. On Day 28, the pulp chamber of undemineralized transplanted incisor tooth was filled with a sparse population of slender fusiform fibrocytes surrounded with edema fluid. The blood vessels were dilated and hemorrhages always were found located near the apex of the conical pulp chamber. The findings were consistent with a hypoxic environment in the pulp chamber.

In *demineralized* incisor transplants, it was found on Day 7 that there had been an incursion of fibroblasts in the empty pulp chamber and cartilage was found just within its aperture. On Day 9 it was seen that capillaries were interspersed among the fibroblasts and that chondrolysis accompanied by osteogenesis had occurred; fibroblasts with new chondrogenesis continued centrifugally toward the apex of the conical pulp chamber. On Day 28 the incisor transplant was red. The space at the base of the pulp chamber was occupied by an ossicle (Fig. 1) containing hemopoietic marrow; the apex of the cone was populated by cartilage devoid of capillaries. At Day 100, there was persistence of cartilage at the apex of the conical pulp chamber with a red ossicle in its base.

The tip of a *demineralized tooth* was amputated truncating the conical pulp chamber to form a tooth tube with open ends. Thirty-six incisor tooth cylindroids of this sort were transplanted sc as in the last mentioned experiment and the results were utterly different. On Day 14, bone was present near both apertures of the cylindrical tube separated by an island of cartilage midway between. On Day 28 the transplanted tooth cylinder was filled with trabeculae of bone (Fig. 2) containing hemopoietic and fatty bone marrow; cartilage had vanished. The appearance of capillaries was detrimental to chondrocytes.

Discussion. Transformation of fibroblasts by bone and tooth is a regulated property. Tooth with minerals is ineffective in transformation; minerals must be removed for this property to become available.

These experiments demonstrate the profound quantitative influence of physical size of the inductor on differentiation of fibro-

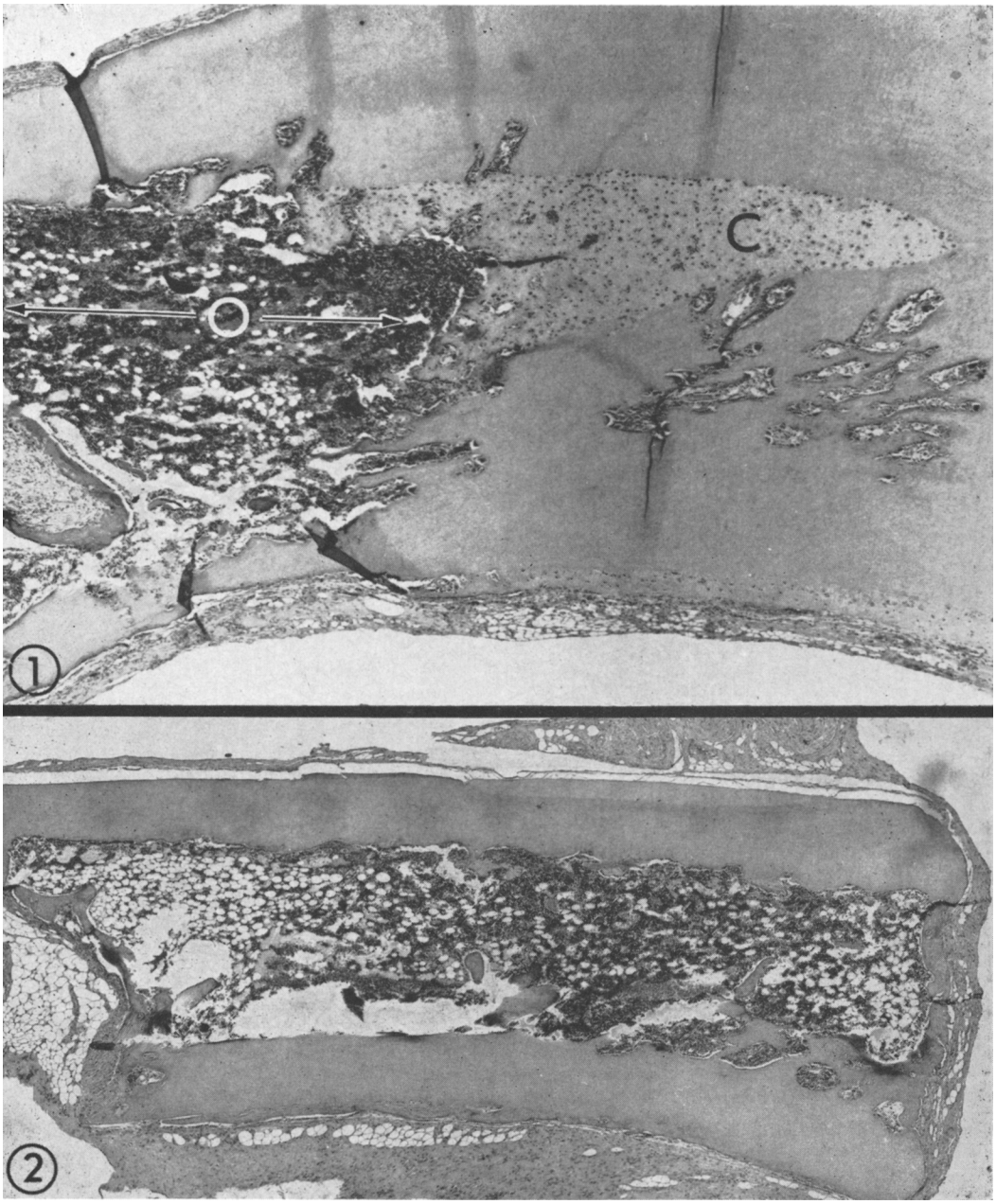

FIG. 1. Transplant of whole demineralized incisor tooth on Day 28. In the pulp chamber an ossicle (O) containing bone marrow is situated near the aperture whereas the apex is populated with cartilage (C) devoid of capillaries. ×30.

FIG. 2. Transplant of demineralized incisor tooth tube on Day 28. The transplant is filled with bone which contains bone marrow; cartilage is absent. ×20.

blasts. It was advantageous to transplant coarse demineralized bone powder to create large yields of cartilage and bone which formed *de novo*. In order to obtain large amounts of newly formed red bone marrow it was useful to transplant coarse powder, har-

vesting the transformation plaques 21 days later.

The origin of the responding fibroblasts in the transformation of the sort described herein is obscure at present. Insofar as the chondroblasts and osteoblasts are concerned it is probable that they are derived from neighboring fibroblasts. However, with respect to bone marrow cells, one should keep in mind that these cells may originate from vascular endothelium or as newcomer cells from blood-borne erythroblasts.

It is noteworthy that the temporal sequence of fibroblast–chondroblast–osteoblast transformation can be greatly altered by the shape of the inductor. Cartilage persisted for months when whole tooth was transplanted but chondrolysis resulted within 3 wk when (a) powdered bone, or (b) tooth tubes open at both ends were transplanted. The current experiments comparing tooth tube and whole tooth demonstrate an important role for the arrival of oxygen-carrying, invading capillaries initiating chondrolysis. Furthermore, in the present study cartilage was always seen first in the deeper regions of the transplant or in the pulp chamber, where presumably the oxygen tension is low.

The studies of Bassett (8) have shown that low oxygen tension favors chondrogenesis. Shaw and Bassett (9), using organ culture techniques, investigated 11 day chick embryonic tibiae and observed that extensive osteogenesis resulted in gas mixtures containing 35% O_2 whereas the explants failed to calcify when grown in 5% O_2. Direct measurements of O_2 tension (10) with an oxygen microelectrode of rat costochondral junctions *in vitro* showed low O_2 tensions in cartilage cells. Sledge and Dingle (11) observed an oxygen-induced resorption of cartilage in organ culture of limb bone of chick embryos. Selye, Lemire and Bajusz (12) induced bone formation by implanting glass tubes in the subcutaneous tissue of rat.

Summary. Allogeneic transplants of demineralized powdered bone or whole teeth in different shapes transformed fibroblasts to cartilage and bone. Coarse powders of bone elicited significantly higher yields of transformation products than fine powder did; criteria of transformation included histologic findings, alkaline phosphatase activity, ^{35}S and ^{32}P incorporation. The temporal sequence of fibroblast–chondroblast–osteoblast transformation was profoundly influenced by the geometry of the transformant. The incursion of capillaries among the transformed cells resulted in chondrolysis.

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