asexual, extraintestinal cycle has been observed.

Toxoplasma appears to have originated as an intestinal coccidian in cats, with fecal-oral transmission. Of 13 hosts investigated, only cats excreted oocysts in feces. Unlike typical coccidia, *Toxoplasma* multiplies extensively in other tissues and hosts. The formation of a persisting cyst in brain and muscle has made it possible for carnivorism to become an additional means of transmission. Whether infected by oocysts or cysts, other mammals and birds can be regarded as facultative intermediate hosts.

With the newly identified *Toxoplasma* oocysts from cats, the transmission of *Toxoplasma* in the human ecosphere must be reassessed. Congenital transmission plays a small but medically important role. The ingestion of cysts or trophozoites through raw and undercooked meat, whether biologically determined, as in cats, or culturally determined, as in humans, appears important as a means of transmission (*9*). In cats, carnivorism serves not only as an efficient means of infection, but can result in contamination of the environment with large numbers of oocysts. *Toxoplasma* oocysts develop aerobically, and we have found them capable of inducing infection in mice and presumably in man after periods of at least 4 months in water or moist soil.

J. K. FRENKEL
J. P. DUBEY
N. L. MILLER

Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City 66103

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Dentin Matrix Transformation: Rapid Induction of Alkaline Phosphatase and Cartilage

Abstract. *An acid-insoluble fraction of rat dentin rapidly initiates a chain reaction in mesenchyma after allogeneic transplantation to the subcutaneous tissues. The tooth matrix induced alkaline phosphatase activity within 24 hours; cartilage appeared within 5 days; bone and bone marrow formed within 14 days. The induced cartilage disappeared within 5 weeks, but bone persisted at least 1 year.*

The stem cells of the connective tissue of animals in postnatal life possess a singular potential for alteration of their phenotype. The following experiments demonstrate the efficiency of dentin matrix as an inductor of differentiation of mammalian cells.

There are two different methods to convert fibroblasts of the mesenchyma into cells of other sorts such as cartilage or bone. In one, the surgical approximation of a transforming epithelium (*1*), for example, the bladder mucosa, and responding mesenchyma induces bone in autologous and allogeneic (*2*) transplants in dog and certain other animals, whereas cartilage is not observed. In the second method, both bone and cartilage are produced by amnion cells (*3*) with placenta and hard tissues acting as transformants of mesenchyma.

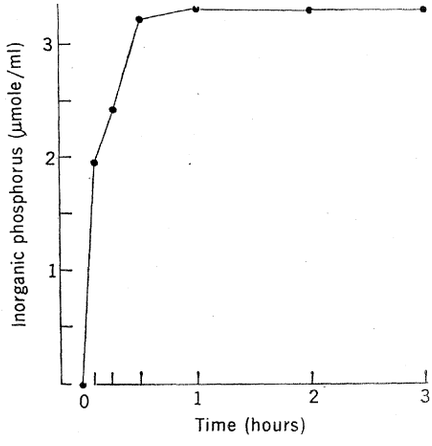

Fig. 1. Rate of extraction (μmole/ml) of inorganic phosphorus (*6*) from tooth in 0.5*N* HCl at 30°C.

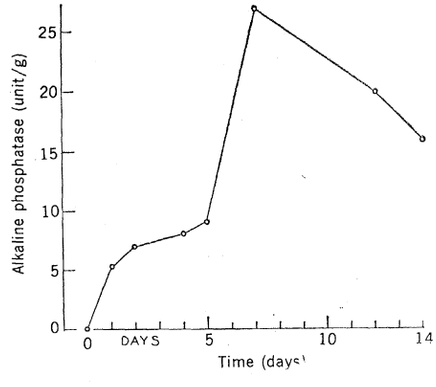

Fig. 2. Alkaline phosphatase (*7*) content (unit/g) of allografts of acid-decalcified rat tooth matrix in the subcutaneous tissues. Mean values of ten samples at each time interval are provided.

Autologous transplantation (*4*) of tooth to muscle leads to ossification that invades dentin and enamel and occurs in the pulp space. Urist *et al.* (*5*) discovered that the matrix of cell-free decalcified lyophilized bone, tooth, or cartilage can induce new cartilage and bone. The matrix was implanted as an allogeneic transplant in the muscle of rabbit and rat; deposits of new bone and cartilage were found 24 days after the operation. We now describe the early stages of the changes in responding cells induced by dentin matrix, which is highly cross-linked and exceptionally resistant to the deleterious action of strong acids.

The acid-soluble components of teeth were extracted at room temperature (28° to 30°C). Female Long-Evans rats, 25 to 30 days old, were the source of incisor teeth. The teeth were weighed and placed in 0.5*N* HCl (1 ml/mg) in a jar with a magnetic stirrer where they were propelled continuously around the vortex created by vigorous stirring. The rate of extraction of inorganic phosphorus (*6*) from the teeth was determined on portions of the acid. After 2 hours the tooth pulp was discarded, and the acid was replaced with an equal volume of 2 percent sodium bicarbonate to neutralize and wash out the acid. After 2 hours of stirring the teeth were implanted in the wet state subcutaneously on the abdominal muscles of allogeneic littermates of the donors of the teeth; the day of operation was counted as day 0. The grafts were excised subsequently on day 1 to day 365. The content (*7*) and localization (*8*) of alkaline phosphatase (E.C. 3.1.3.1) in the grafts were determined.

Before acid extraction the lower incisor teeth of our rats, age 30 days, weighed 29.9 ± 1.3 mg. The alkaline phosphatase activity was, in units: dental pulp, 292 ± 40; tooth shell, 10.5 ±

4; abdominal muscle at the site of the graft, 1.2 ± 0.6. After acid extraction there was no alkaline phosphatase activity in these tissues. The concentration of alkaline phosphatase in tooth pulp greatly exceeded that of all other tissues of the rat which we have assayed. Extraction of tooth with HCl in our experiments removed 60 percent of acid-soluble phosphorus in 5 minutes (Fig. 1) and all of it before 1 hour.

The alkaline phosphatase activity of the grafts began to rise steeply within 24 hours (Fig. 2) and reached a maximum on day 7. Thereafter the concentration of alkaline phosphatase declined somewhat but the values still greatly exceeded those of the muscle at the site of the grafts.

The transformation (Fig. 3) of mesenchyma by grafts of acid-extracted dentin matrix was rapid. On day 1 the implant was encapsulated by fibroblasts that contained large quantities of cytoplasmic alkaline phosphatase; the cells were assembled in parallel rows, and the proliferating new cell population had blended with the implanted dentin matrix. On day 5 the innermost layer of cells next to the implanted dentin included many proliferating chondrocytes, which before day 7 (in 34 consecutive implants) became large aggregates of hyaline cartilage. The new cartilage was present in apposition to the dentin in the pulp space and on the surface of the tooth including its tip, and it extended considerably into the surrounding connective tissue as well. In the cartilage, alkaline phosphatase activity was both intracellular and in the pericapsular matrix. On day 10, osteoblasts were found in the grafts; on day 14, hemopoietic bone marrow was present. On day 29, we noted that large quantities of bone and bone marrow had differentiated in the implant whereas many of the cartilage cells had succumbed. On day 35 and at 1 year also bone was plentiful, but cartilage had vanished. It is remarkable that the life of the cartilage evoked by dentin matrix was brief whereas the induced bone persisted.

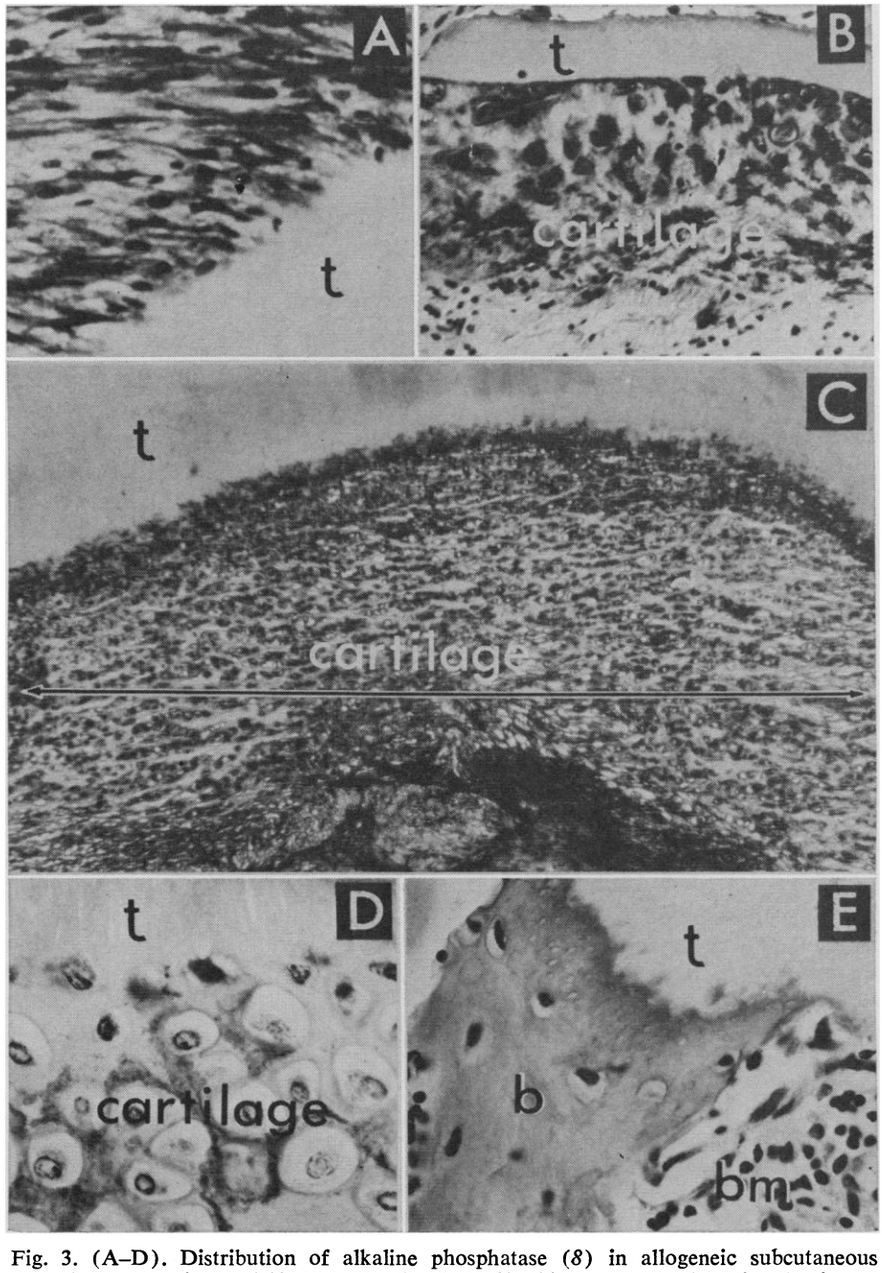

Fig. 3. (A–D). Distribution of alkaline phosphatase (*8*) in allogeneic subcutaneous transplants of acid-decalcified rat tooth: (A) fibroblasts surrounding the dentin on day 1 (× 150); (B) cartilage on day 6 (× 100); (C, D) calcified cartilage on day 7 (C, × 60; D, × 350); (E) hematoxylin-eosin stain: creeping osseous substitution with hemopoietic bone marrow on day 29 (× 200). *t*, Dentin; *b*, bone; *bm*, bone marrow.

An additional extraction of the dentin matrix with glacial acetic acid for 1.5 hours after decalcification in HCl did not diminish the transforming activity of the grafts. No inflammatory response was observed in 100 consecutive allografts of dentin matrix extracted with HCl or acetic acid. The preparation of tooth with the strong acids used in these experiments decalcified and sterilized dentin and eliminated its histoincompatibility.

Induction of alkaline phosphatase by adrenal cortical hormones has been observed in human neoplastic (*9*) and fibroblast (*10*) cells in culture. The experimental transformation of bacterial type was first accomplished in pneumococcus (*11*). Avery *et al.* (*12*) discovered that the pneumococcal transforming agent is DNA. The nature of the agent in tooth which creates a new morphogenetic pattern in the stem cells of the connective tissue has not been identified.

These transforming effects of dentin in postnatal life are comparable to embryonic induction. The extraordinary inductive productivity of acid-insoluble dentin can be assayed simply by measurements of alkaline phosphatase activity in the responding mesenchyma.

CHARLES B. HUGGINS
Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois 60637
MARSHALL R. URIST
Department of Surgery, University of California, Los Angeles

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5-Hydroxytryptamine: A Cytospecific Growth Stimulator of Cultured Fibroblasts

Abstract. *5-Hydroxytryptamine (serotonin) in micromolar amounts increased the growth of fibroblasts in culture while not affecting five other cell lines. Serotonin appeared to shorten the lag phase of cell growth. The effect was less when serotonin was added to the fibroblast culture after the initial 24-hour period. The two functional groups of the serotonin molecule were required for growth enhancement. Serotonin in millimolar concentrations was toxic to fibroblasts.*

In humans with the carcinoid syndrome, elevated concentrations of 5-hydroxytryptamine (serotonin) in the plasma are often causally related to excessive formation of connective tissue (*1*). Under experimental conditions, repeated injections of serotonin into joint spaces (*2*) or into the subcutaneous tissue (*3*) cause an overgrowth of connective tissue. Serotonin has been reported to increase the incorporation of radioactive thymidine into guinea pig skin slices in vitro (*4*). From these associations, the question was raised whether serotonin would directly stimulate the growth of fibroblasts in culture.

Two fibroblast lines were used, the mouse (3T6) and the human embryo lung. The origin and growth characteristics of these cells were described previously (*5*). The cells were maintained in Dulbecco's modification of Eagle's basal medium (*6*) with 10 percent bovine calf serum and antibiotics [streptomycin (0.1 mg) and penicillin (100 unit/ml)].

The HeLa (epithelioid carcinoma, cervix; human), KB (epidermoid carcinoma, oral; human), and BHK (kidney; golden hamster, *Mesocricetus auratus*) were obtained from the cell culture repository (American Type Culture Collection, Rockville, Maryland) and maintained in Eagle's minimum essential medium. The cell lines were adapted to the Dulbecco's modified Eagle's medium by two or three passages before being used.

Cell cultures were started by placing 5 ml of fresh medium containing 4×10^4 cell/ml into a 2-ounce glass bottle. The bottles were flushed with a mixture of oxygen and carbon dioxide (90 : 10), sealed with rubber stoppers, and then incubated at 34°C in a light-tight incubator. The cultures were grown for 1 to 7 days without a change in the culture medium. Under these experimental conditions, the fibroblasts were confluent at approximately 6 days.

The cells were harvested by first removing the medium; they were then washed with 5 ml of Hanks solution, and finally detached with 5 ml of a 0.05 percent trypsin and 2 percent ethylenediaminetetraacetate solution. In the trypsin step the cells were incubated with the trypsin solution at 34°C for 5 minutes, and the flask was mechanically agitated for 3 to 5 minutes. The cells were transferred to a test tube and centrifuged at 900*g*. The pellet was washed with Hanks solution, centrifuged, and resuspended in 0.9 percent sodium chloride solution. A cell count was made on a portion of the cell suspension with a Coulter counter. The counts were made in quadruplicate, and the variation around the mean was less than 3 percent. The bottles were checked by microscopic examination for cells that might have remained attached to the flasks.

Table 1. Effect of serotonin ($10^{-6}M$) given at different periods of cultured fibroblast growth. The cells were harvested at either 4 or 6 days. The values are the mean of six experiments.

Days	Percent increase
0	73
1	56
2	26
3	21
5	26

Serotonin bimaleate, when present in the culture medium throughout the period of growth, influenced fibroblast growth. At high concentrations (10^{-2} and $10^{-3}M$) serotonin was toxic; a concentration of $10^{-3}M$ significantly reduced cell numbers by more than 80 percent (Fig. 1). Serotonin concentrations of 10^{-6} or $10^{-7}M$ significantly increased fibroblast growth by nearly 100 percent. Our studies on fibroblast growth with serotonin were at a concentration of $10^{-6}M$.

Serotonin given at zero time increased the number of fibroblasts after 24 hours by 58 percent; control, $2.45 \pm 0.16 \times 10^5$ and treated, $3.86 \pm 0.16 \times 10^5$ cells ($n = 6$). Supplemented injections of serotonin at 3, 5, or 6 days caused a significant increase of cell

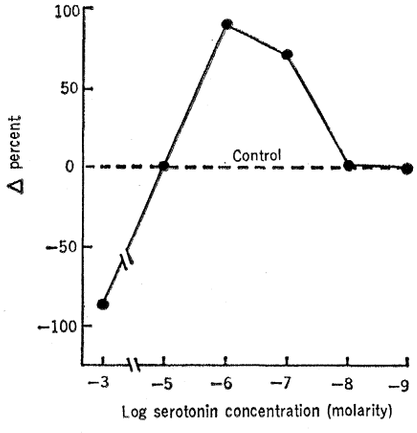

Fig. 1. Change in human fibroblast (96-hour cultures) numbers as a function of serotonin concentration. Control cultures were given concentrations of maleic acid equivalent to the amount in serotonin bimaleate. Maleic acid at this concentration had no effect upon cell growth. Compounds were included in the initial incubation medium, and supplements were added at 72 hours. The cells were harvested after 96 hours. Values are the mean of at least six experiments.