that inhibit DNA methylation, such as 5-azacytidine, result in the hypomethylation and expression of some genes but not others (*19*). If hypomethylation leads to expression of genes important in neoplastic growth, then cells exhibiting a defect in the control of methylation may obtain a selective advantage (*20*). Second, hypomethylation might inhibit chromosome condensation which, in turn, might lead to problems in chromosome pairing and disjunction. Indeed, experimentally induced hypomethylation leads to areas of chromosome decondensation with resultant mitotic chromosomal abnormalities (*21*). In support of such a mechanism for tumorigenesis, it has been shown that 5-azacytidine induces transformation of CHEF/18 cells at high frequency. In these cells, the transformation event is in every case associated with DNA hypomethylation and a specific chromosomal translocation (*22*).

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16. Repetitive DNA sequences were examined in 22 neoplasms with a probe for satellite DNA (a 0.3-kb Eco RI genomic DNA fragment derived from the plasmid pB(Eco RI-2°)6 [B. Shafit-Zagardo, J. J. Maio, F. L. Brown, *Nucleic Acids Res.* **10**, 3175 (1982)].
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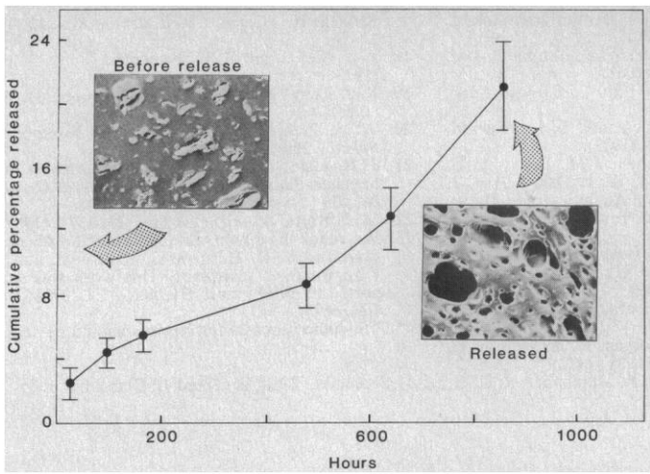
Inhibition of Calcification of Bioprosthetic Heart Valves by Local Controlled-Release Diphosphonate

Abstract. *Bioprostheses fabricated from porcine aortic valves are widely used to replace diseased heart valves. Calcification is the principal cause of the clinical failure of these devices. In the present study, inhibition of the calcification of bioprosthetic heart valve cusps implanted subcutaneously in rats was achieved through the adjacent implantation of controlled-release matrices containing the anticalcification agent ethanehydroxydiphosphonate dispersed in a copolymer of ethylene-vinyl acetate. Prevention of calcification was virtually complete, without the adverse effects of retarded bone and somatic growth that accompany systemic administration of ethanehydroxydiphosphonate.*

Bioprostheses fabricated from porcine aortic valves treated with glutaraldehyde are widely used to replace diseased cardiac valves (*1–3*). More than 300,000 of these bioprostheses have been used in clinical implants since 1971 (*4–7*). Cuspal calcification is the most frequent cause of the clinical failure of these devices (*4–6*) and necessitates removal of the prostheses after 5 years in more than 50 percent of the children (*5*) and 5 to 10 percent of the adults receiving the valve implants (*2, 6, 7*). The pathogenesis of cuspal calcification is not completely understood (*7*), and there is no effective therapy for its prevention (*7*). Its pathophysiology can be reproduced with orthotopic valve replacements in sheep (*8*) or calves (*9*), or with subcutaneous cuspal implants in rabbits (*10*), mice (*11*), or rats (*12*). Subcutaneous implants are used because the time course of the mineral-



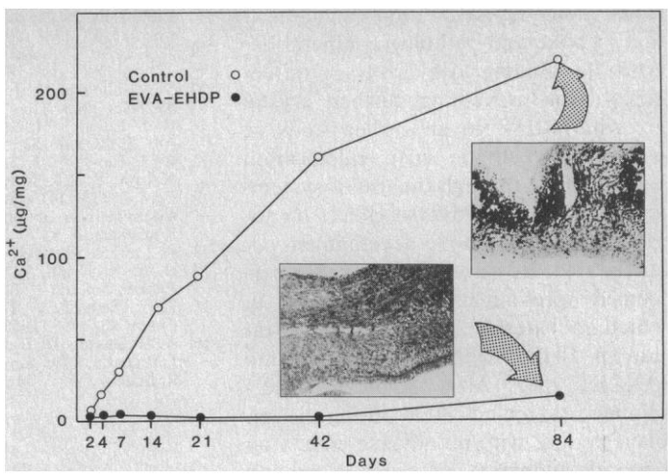


Fig. 1 (left). Cumulative percentage of EHDP released from an ethylene-vinyl acetate–EHDP matrix. Polymer matrices containing [^{14}C]EHDP were incubated at 37°C in 0.1*M* NaH_2PO_4, 7.40; released radioactivity was determined periodically. Inserts: scanning electron microscopy (ISI model DS-130) was performed on specimens before release and after 42 days of release in vitro. Fig. 2 (right). Ethylene-vinyl acetate (EVA)–EHDP inhibition of bioprosthetic valve cusp calcification. Calcification was assessed in cusps implanted subcutaneously next to 20 percent (by weight) ethylene-vinyl acetate–EHDP matrices or matrices containing only ethylene-vinyl acetate. Portions of retrieved cusps were fixed by the methods of Karnovsky (*22*) in cacodylate (0.2*M*)-buffered (*p*H 7.2) glutaraldehyde (2.5 percent) and formaldehyde (2.0 percent). Sections (3 μm) were stained with the von Kossa technique. Light microscopy (see inset) showed extensive intrinsic calcification at 84 days in a control specimen but no visible deposits in the implants treated with ethylene-vinyl acetate–EHDP.

ization is accelerated and the technique is simple (*12*).

In an effort to prevent this disease process, we administered the anticalcification agent ethanehydroxydiphosphonate (EHDP) directly into cusps implanted subcutaneously in rats. Local therapy was achieved with controlled-release matrices containing EHDP dispersed in a copolymer of ethylene-vinyl acetate.

Bioprosthetic heart valve cusps were prepared from porcine aortic valves that had been treated with glutaraldehyde (*12*) and stored in 0.2 percent glutaraldehyde (*p*H 7.4; 0.05*M* Hepes). Ethylene-vinyl acetate was obtained as a dried powder (ELVAX; 40 percent vinyl acetate; Du Pont), washed in alcohol and water, and dried (*13*). Ethanehydroxydiphosphonate was obtained as the disodium salt, both ^{14}C-labeled (9.2 mCi/mmol), and nonlabeled (Procter & Gamble). Rats used in these studies were 3-week-old male weanlings of the CD strain (Charles River).

Ethylene-vinyl acetate dissolved in methylene chloride (20 percent, weight to volume) was mixed with EHDP (20 percent by weight) and cast as hemispherical matrices (diameter, 1 cm) (*14*). This geometric structure provides nearly constant release rates for various test drugs (*14*). Before the release studies, the matrices were coated with ethylene-vinyl acetate in methylene chloride (15 percent, weight to volume) and air dried; a release aperture, 0.58 mm in diameter, was drilled into the flat face of the hemisphere.

Subcutaneous implants of ethylene-vinyl acetate–EHDP matrices were placed next to subcutaneous bioprosthetic cuspal (2 by 3 cm) implants in the rats (*12*). Control implants consisted of bioprosthetic cusps implanted next to ethylene-vinyl acetate hemispheres that did not contain EHDP. Animals were killed at intervals up to 84 days after implantation. Retrieved cusps were prepared for mineral analyses by hydrolysis with 6*N* HCl (*12*) and were analyzed for calcium by atomic absorption spectroscopy (*12*); the cusps were also analyzed for phosphorus (*15*). Blood specimens obtained by cardiac puncture at the end of the study were similarly analyzed for serum calcium. Potential adverse effects on bone development were assessed as follows. Representative femurs of the rats killed 84 days after receiving implants were fixed in neutral buffered formaldehyde solution, and the cortex was superficially decalcified to permit microtomy. The bones were embedded in glycomethacrylate, sectioned (3 μm) through the proximal femoral epiphyseal growth plate, and stained with hematoxylin and eosin to detect cellular abnormalities associated with diphosphonate toxicity (*16, 17*). The von Kossa technique was used to detect defective mineralization resulting from diphosphonate administration (*16, 17*).

Uniform, sustained release of EHDP [mean ± standard error of the mean (S.E.M.), 5.0 ± 0.4 μg/hour] was observed for more than 600 hours in vitro, with only 12 percent cumulative release by this time (Fig. 1). Scanning electron micrographs of unimplanted matrices of ethylene-vinyl acetate–EHDP showed a uniform distribution of EHDP particles ranging in size from 1 to 10 μm. After release of EHDP in vitro, scanning electron microscopy showed that canals had formed with dissolution of the EHDP (Fig. 1).

Minimal calcification was present in cusps removed from animals with hemisphere implants containing ethylene-vinyl acetate–EHDP. Control implants, in contrast, showed progressive extensive calcification (Fig. 2). Serum calcium levels were normal in both the group treated with EHDP for 84 days [Ca^{2+}, 9.5 ± 0.3 (S.E.M.) mg/dl; n = 4] and control groups (Ca^{2+}, 9.5 ± 0.3 mg/dl; n = 3). The body weight of the animals killed after 84 days of treatment with ethylene-vinyl acetate–EHDP [mean, 463 (± 48.3 standard deviation) g; n = 3] did not differ significantly from that of the controls [mean, 563 (± 23) g; n = 4], and histologic examination of representative von Kossa and hematoxylin-eosin sections of the femoral growth plates from the animals treated with ethylene-vinyl acetate–EHDP revealed no abnormalities in bone development.

Thus, local controlled release of EHDP from ethylene-vinyl acetate copolymer matrices directly into bioprosthetic cuspal implants inhibits calcification of the subcutaneous implants for up to 84 days without detectable untoward effects associated with diphosphonate therapy. The amount of calcification in the control cusps by 84 days was comparable to amounts occurring after several years of clinical implantation (*7*). Diphospho-

nates, including EHDP, inhibit calcification in bone and pathologic mineral deposits by binding to hydroxyapatite deposits and preventing further crystal growth (*16–18*). In an earlier study in which bioprosthetic cusp calcification was inhibited through the use of systemic EHDP, severe adverse effects on somatic growth and bone development occurred (*19*). However, we have now performed preliminary experiments in which short-term (2 weeks) administration of EHDP with an osmotic pump (ALZET 2001, Alza, Stanford, Calif.) inhibited the calcification during the implant period, with no adverse effects on bone development or somatic growth (data not shown). Technical constraints would prohibit the use of this type of osmotic device for long-term local therapy. The long-term (84 days) controlled-release system we have developed to inhibit cuspal calcification delivered EHDP at an approximate total body dosage of 6 μg/kg. Ethylene-vinyl acetate–EHDP matrices could potentially be incorporated into clinical valve prostheses during fabrication. In addition, ethylene-vinyl acetate–EHDP may be useful in controlling calcification in other types of prosthetic implants (*20, 21*).

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Ocelli: A Celestial Compass in the Desert Ant *Cataglyphis*

Abstract. *In addition to multifaceted lateral compound eyes, most insects possess three frontal eyes called ocelli. Each ocellus has a single lens, as does the vertebrate eye. The ocelli of some flying insects, locusts and dragonflies, have been shown to function as horizon detectors involved in the visual stabilization of course. In a walking insect, the desert ant* Cataglyphis, *it is now shown that the ocelli can read compass information from the blue sky. When the ant's compound eyes are occluded and both sun and landmarks are obscured, the ocelli, using the pattern of polarized light in the sky as a compass cue, help in guiding the ant back home.*

Insect physiologists have been reflecting for a remarkably long time on what use insects make of their ocelli (*1*). Although a lot is now known about the anatomical and physiological organization of the ocellar visual system (*2*), it is still a matter of dispute what function or functions insect ocelli serve (*3*). Recently, the old hypothesis (*4*) that flying insects (*5*) use their ocelli as horizon detectors, and thus as some means of visually stabilizing their body positions against movements about the roll and pitch axes, has been revived (*6*). We report that in a fast-running insect, the Saharan desert ant *Cataglyphis bicolor*, the ocelli can be used as a celestial compass and thus serve a function that has usually been attributed exclusively to the compound eyes (*7*).

The ants were trained to an artificial food source located 15 m from the nest. Upon arrival they were divided into three groups: COMP animals (compound eyes left open and ocelli occluded), OC animals (ocelli left open and compound eyes occluded), and blind animals (both types of eye occluded). Thereafter all ants were individually placed in unfamiliar territory, and their homing paths were recorded with the use of a grid of white lines painted on the hard desert ground. A specially designed trolley was moved along with the homing ant to restrict the ant's field of view to small parts of blue sky and to control the skylight factors displayed to the ant (for example, radiance, spectral composition, and orientation of the E vector). The trolley prevented the ants from seeing either landmarks or sun and from detecting wind direction (*8*).

In a first set of experiments the ants could view an annulus-shaped celestial window centered about the zenith (width 30°, mean elevation 40°, elevation of sun > 65°). The COMP animals were oriented in the homeward direction as precisely as controls, in which compound eyes and ocelli had been left open (Figs. 1A and 2A). We observed that even the OC animals were able to determine homeward courses, but their behavior differed strikingly from the behavior of both COMP animals and controls. First, the OC animals moved extremely slowly along tortuous paths, continuously swinging their body axes to the left and right and thus giving the impression of scanning the sky (Fig. 1B). Second, the scatter in the ant's bearings was significantly larger in the OC animals (Fig. 2B) than in ants that could use their compound eyes (Fig. 2A) (for example, 6-m distance, F-test, two-sided, d.f. = 17 and 23, $P < 0.002$). Any orientation was completely abolished when compound eyes as well as ocelli were painted out (blind animals, Figs. 1D and 2D).

This first set of experiments showed that ants that could use only their ocelli did not behave as though blind but were able to derive compass information from the sky even when the sun was not visible. However, because the ants could see a rather large part of the natural blue