ma γ-GT produced by damage to nonrenal tissue would not lead to enzymuria because the molecular weight (84,000) is too high to allow filtration by the glomeruli (*28*). Furthermore, phenylmercury compounds are rapidly degraded to inorganic mercury in animals (*13*). In the infants virtually all the mercury in the urine was in the inorganic form (*29*), a potent renotoxic agent (*22*).

To our knowledge this is the only published study of concentration-effect relations for infants exposed to phenylmercury compounds or, for that matter, any other forms of mercury. Our results may be useful to pediatricians and to public health and regulatory agencies.

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Flurbiprofen: A Potent Inhibitor of Alveolar Bone Resorption in Beagles

Abstract. *The nonsteroidal anti-inflammatory drug, flurbiprofen, a potent cyclooxygenase inhibitor, significantly decreases the resorption of alveolar bone in naturally occurring chronic destructive periodontal disease in beagles. This observation indicates that arachidonic acid metabolites are important in the alveolar bone loss of periodontitis and suggests a use for flurbiprofen in the management of bone resorption disease.*

Arachidonic acid metabolites appear to be mediators of a wide range of pathological events. Certain metabolites such as the prostaglandins and prostacyclin have been associated with diseases marked by bone resorption. These include periodontal disease, dental cysts, the hypercalcemia of malignancy, and rheumatoid arthritis (*1*).

Nonsteroidal anti-inflammatory drugs (NSAID's) inhibit cyclooxygenase and lipoxygenases, products of the metabolic breakdown of arachidonic acid (*2*). Thus NSAID's may have an impact on disease processes that are mediated in part by metabolites of arachidonate. In current practice, NSAID's are used for their anti-inflammatory effect, primarily to decrease the symptoms of the disease rather than to halt the progression of the disease. Flurbiprofen (Ansaid), a phenylalkanoic acid, is a specific cyclooxygenase inhibitor. We now report that flurbiprofen, administered orally daily, is a potent inhibitor of alveolar bone resorption in naturally occurring periodontal disease in beagles. Our evidence suggests that this pharmacologic agent significantly arrests the progression of the injury to the alveolar bone without a clinically detectable anti-inflammatory effect on the gingival tissues covering the bone. This finding implicates cyclooxygenase metabolites in the pathogenesis of periodontal bone loss. If flurbiprofen has the same inhibiting effect on bone resorption in the human, this agent could provide an effective method for treating chronic destructive periodontal disease (periodontitis).

Twelve adult female beagle dogs with naturally occurring alveolar bone loss, classified as moderate to severe, were studied (*3*). The beagle was selected as a model because the pattern and progression of alveolar bone loss is similar to that found in humans (*4*), and the beagle has been used in numerous studies on periodontal disease (*5*). In both beagles and humans, periodontitis is characterized by alveolar bone loss, tooth mobility, calculus and plaque accumulation, and gingival inflammation and pocket formation.

The experimental design of our study was similar to one we used earlier and included a pretreatment and treatment period (*6*). In the first 6 months, designated the pretreatment period, the progression of alveolar bone loss around premolar teeth was measured by a standardized radiographic method. The pretreatment rate of bone loss was compared to a subsequent 12-month treat-

ment period in which five dogs were given flurbiprofen (0.02 mg/kg daily), while six other dogs were treated with placebo in the form of empty gelatin capsules. At the beginning of the treatment period, each dog had the upper and lower teeth on one side of the mouth treated locally with periodontal flap surgery, tooth cleaning weekly, and prophylaxis every 3 months. Thus the experimental design permitted comparison of the rate of alveolar bone loss in the pretreatment and treatment periods in four groups of periodontally diseased teeth: (i) nonsurgically treated in the placebo-treated group, (ii) surgically treated in the placebo-treated group, (iii) nonsurgically treated in the flurbiprofen-treated group, and (iv) surgically treated in the flurbiprofen-treated group. Radiographic progression of alveolar bone loss was measured from standardized sequential radiographs as described previously (*6*). The mean rate of bone loss per month in the pretreatment period was compared to the mean rate of bone loss at 3, 6, 9, and 12 months of the treatment period (*7*).

With the administration of flurbiprofen daily, the mean rate of alveolar bone loss was statistically decreased at 3, 6, 9, and 12 months of the treatment period in comparison with the baseline for the pretreatment period rate in both surgically and nonsurgically treated teeth (Fig. 1). In contrast, the rate of alveolar bone loss in the placebo-treated dogs did not decrease during the treatment period as compared with the baseline rate, except at 9 months for those teeth treated surgically. This decreased rate of bone loss was not sustained by 12 months (Fig. 1). Daily flurbiprofen administration did not significantly affect gingival inflammation (*8*).

The overall effect of flurbiprofen on preserving bone in the surgically and nonsurgically treated groups was quantified and the values were compared with those obtained for the placebo-treated dogs. At the end of the 12-month treatment period, a linear regression analysis was used to obtain an overall rate of bone loss for the treatment period. This overall rate was used to compute the observed alveolar bone loss in the treatment period for all groups. The pretreatment rate of bone loss was used to calculate the expected amount of bone loss during the 12-month treatment period (the pretreatment rate of bone loss multiplied by 12). This expected amount of bone loss was compared with the observed bone loss during the treatment period (Table 1). With flurbiprofen administration, the observed amount of bone lost in the treatment period was 66 percent less than the expected amount. When flurbiprofen administration was combined with local periodontal treatment, bone loss in the treatment period was 91 percent less than the expected amount ($P < 0.05$). When the flurbiprofen treatment was stopped at 12 months, the high pretreatment rate of bone loss returned during the next 6 months. In placebo-treated dogs, alveolar bone loss increased during the treatment period whether or not the teeth received surgical treatment (Table 1).

Goldhaber *et al.* reported earlier that the NSAID indomethacin blocks bone resorption in tissue culture (*9*). However, the effect of an NSAID on naturally occurring bone loss in vivo had not been previously demonstrated. We included local periodontal treatment for half the teeth in our experiment because we doubted that a chemotherapeutic agent other than an antibiotic could significantly decrease the rate of alveolar bone loss in this animal. We were also skeptical that the low dose of flurbiprofen administered to these dogs would exert a detectable effect on bone resorption. The dose of flurbiprofen we used was 1/200 of the human clinical dose used in the treatment of arthritis and ankylosing spondy-

Table 1. Efficacy of flurbiprofen administration on preserving alveolar bone in beagles.

Treatment	Bone loss			
	Rate before treatment (percent/month)	Amount after 12 months of treatment (percent)		Observed versus expected (percent change)
		Expected	Observed	
Flurbiprofen				
Without surgery	0.69	8.3	2.8	↓66
With surgery	0.68	8.2	0.7	↓91
Placebo				
Without surgery	0.78	9.4	10.9	↑16
With surgery	0.39	4.7	5.8	↑24

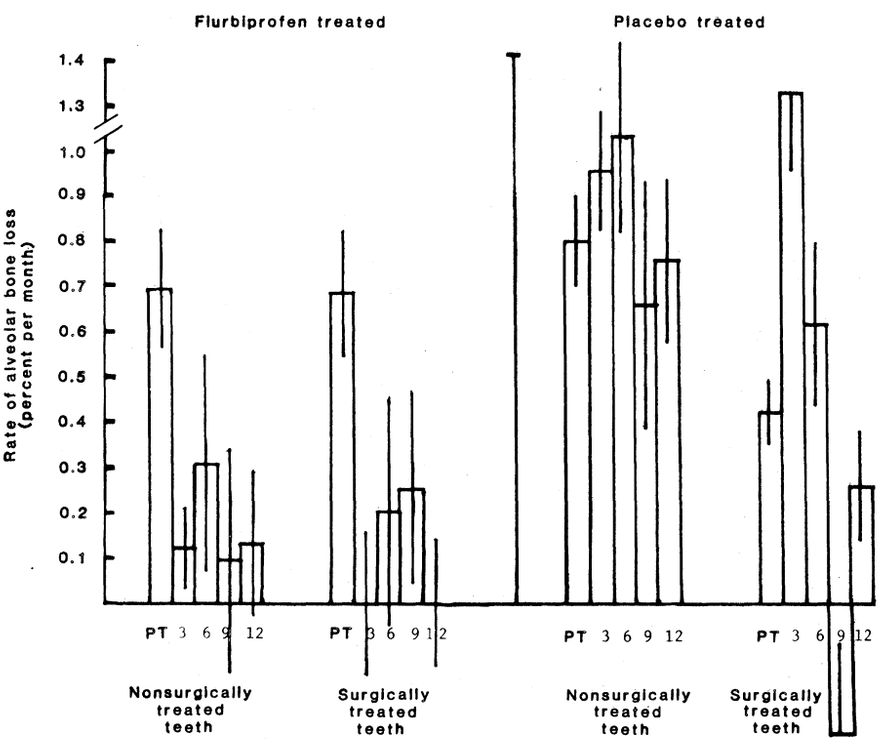

Fig. 1. Comparison of the rate of alveolar bone loss in dogs treated with flurbiprofen or placebo prior to treatment (PT) and at 3, 6, 9, and 12 months of treatment. Bars represent the mean, and vertical lines represent the standard error for each root surface studied in premolar teeth ($N = 458$). In the dogs given flurbiprofen, the values for the treatment period were significantly less than the pretreatment values. In the dogs given the placebo, only at 9 months was the rate of bone loss of surgically treated teeth significantly less than the pretreatment value.

litis (*10*). Thus, flurbiprofen, even at a dose that was too small to elicit an anti-inflammatory response (*11*), had a dramatic effect on alveolar bone loss without supplemental local treatment.

The etiological factors in chronic destructive periodontal disease are not well understood. It seems likely from our data that one of the major biochemical pathways of bone resorption in beagle periodontal disease may be mediated by cyclooxygenase. The use of flurbiprofen to further probe pathologic mechanisms and to treat bone resorption disease has promise.

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8. Gingival inflammation about each tooth was scored on a scale of 0 to 3 with the index of Rosenberg *et al.* [*J. Periodontol.* **37**, 208 (1966)]. Inflammation was not significantly reduced in the treatment period from the baseline pretreatment values ($P > 0.05$).
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Use of Restriction Fragment Length Polymorphisms to Determine the Clonal Origin of Human Tumors

Abstract. *A novel strategy to determine the clonal origin of human tumors has been devised. The strategy involves the use of a cloned polymorphic X-chromosomal gene and two restriction endonucleases. The first endonuclease distinguishes the paternal and maternal copies of the gene through a DNA polymorphism of restriction fragment length. The second endonuclease distinguishes active from inactive copies of this gene through changes in DNA methylation. As illustrations of this strategy, three human cancers were each shown to be monoclonal. The analysis described should have a wide variety of clinical and experimental applications.*

Knowledge of the clonal origin of a tumor has important conceptual and practical implications. A multiclonal tumor is logically inconsistent with the premise that a rare genetic event (for example, DNA mutation or chromosomal rearrangement) was responsible for formation of the tumor. Indeed, the assumption that tumors are monoclonal is a cornerstone of somatic mutation theories of carcinogenesis (*1*). Consistent with such theories, some human cancers, particularly leukemias and lymphomas, have been shown to be monoclonal (*2, 3*). However, several multiclonal human tumors have been reported (*4*). In animals, too, some experimentally induced cancers are polyclonal in origin (*5*). In humans, a major limiting factor in the clonal analysis of tumors is the availability of a technique that can be generally used to assess clonality. The analysis of glucose-6-phosphate dehydrogenase protein variants has yielded unambiguous and valuable information on clonality, but it is only applicable in 1 of 50 human tumors (*2*). We describe here a new strategy, based on the analysis of DNA polymorphisms of restriction fragment length, for determining the clonal origin of human tumors. To illustrate this strategy, we demonstrated that each of three human cancers was monoclonal.

This analysis of clonal origin is based on three underlying principles: (i) Only one X chromosome in each female cell is active (*6*). This activation occurs at an early step in embryogenesis and is stable throughout the lifetime of the cell, even if that cell is cultured in vitro or becomes neoplastic (*6, 7*). (ii) Activation of many genes, including those on the X chromosome, is accompanied by changes in methylation of cytosine (C) residues (*8*). Although these methylation changes do not affect all sites that potentially can be methylated (*8, 9*), they occur consistently at some sites and are easily monitored at those sites by using restriction endonucleases that have the capacity to recognize methylated C residues (*10*). (iii) The maternal and paternal copies of the X chromosome in female cells can be distinguished at the DNA level, using restriction fragment length polymorphisms (RFLP) (*11*). These polymorphisms are normal variations in DNA sequence that occur in the human population.

The strategy thus involves the use of a cloned polymorphic X-chromosomal gene and two restriction endonucleases. The first endonuclease distinguishes the maternal and paternal copies of the gene through an RFLP. The second endonuclease distinguishes active from inactive copies of this gene, through methylation changes. If the tumor developed from one cell—that is, if it was monoclonal—the paternal copy of the gene will be cleaved by the second enzyme in a much different fashion from the maternal copy, since the paternal copy will be either active in all the cells of the tumor or inactive in all the cells of the tumor. Conversely, in polyclonal tumors or normal tissue, approximately half the cells will have an active paternal gene and half will have an active maternal gene, so that the paternal and maternal copies of the gene will be affected identically by digestion with the second enzyme.

A gene that we have found useful for this technique is the hypoxanthine phosphoribosyltransferase (HPRT) gene. A map of the relevant segment of the gene is shown in Fig. 1A. The Bam HI endonuclease sites B_1 and B_3 are present in all X chromosomes, but 16 percent of X chromosomes contain an additional Bam HI site, B_2 (*12*); hence, 27 percent of females will be heterozygous at this locus. There are at least six Hha I sites within this region of the gene (Fig. 1A) (*13, 14*). Hha I cleaves at the sequence GCGC (G, guanine), but does not cleave this sequence when either C is methylated (*15*). Hha I site 1 is unmethylated in active chromosomes (*13, 14*); cleavage at