sults fit the pattern outlined for tissue from *Picea mariana*, but it is not possible on this basis to draw conclusions concerning the degree to which our findings are representative of native woody tissue in general.

The most significant of our findings, we believe, is not so much that the orientation of the phenyl ring is most often preferentially in the plane of the cell wall but that the lignin is more highly organized at the molecular level than had heretofore been recognized.

A mapping of variations in molecular orientation throughout an individual cell would make possible detection of nodes in molecular organization and their relation to cell morphology. Such a mapping is not feasible at present, however, because the time needed to acquire spectra such as those in Figs. 1 and 2 is approximately 8 hours. It is anticipated that a new instrumental design incorporating multichannel detection will make it possible to acquire similar spectra in much shorter intervals. More comprehensive studies of the pattern of organization of lignin will then be possible.

RAJAI H. ATALLA
UMESH P. AGARWAL
Institute of Paper Chemistry, Appleton, Wisconsin 54912

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Early Biochemical Effects of an Organic Mercury Fungicide on Infants: "Dose Makes the Poison"

Abstract. *Phenylmercury absorbed through the skin from contaminated diapers affected urinary excretion in infants in Buenos Aires. The effects were reversible and quantitatively related to the concentration of urinary mercury. Excretion of γ-glutamyl transpeptidase, an enzyme in the brush borders of renal tubular cells, increased in a dose-dependent manner when mercury excretion exceeded a "threshold" value. Urine volume also increased but at a higher threshold with respect to mercury. The results support the threshold concept of the systemic toxicity of metals. γ-Glutamyl transpeptidase is a useful and sensitive marker for preclinical effects of toxic metals.*

Phenylmercury compounds belong to a broad class of aryl and alkoxyl aryl mercurials that have worldwide use as fungicides, contraceptive spermicides, and disinfectants. Despite the fact that human exposure has been extensive, quantitative information on dose-effect relations has not, to our knowledge, been reported for this important class of organic mercury compounds.

A massive exposure of infants to phenylmercury in Buenos Aires, discovered in spring 1980 (*1*), provided an opportunity to obtain quantitative information on the human toxicology of this class of mercurials. Three infants were diagnosed as having the childhood disease acrodynia, or pink disease. In the past this disease was associated with the use of mercury in teething powders (*2*). This practice ceased around 1950, and only one case of acrodynia was reported between 1950 and 1963 (*3*). In 1980 the clustering of a few cases of acrodynia at the same time and place alerted pediatricians to the possibility of mercury contamination. Analysis of infant urine samples revealed alarmingly high mercury concentrations. The source was discovered to be commercial diaper services, which had treated the cloth diapers with phenylmercury fungicide. About 7,000 to 10,000 babies had been exposed in this way. The prevalence of acrodynia, even in mercury-exposed babies, is very low—on the order of one per thousand (*4*)—and this proved to be the case in Argentina (*5*).

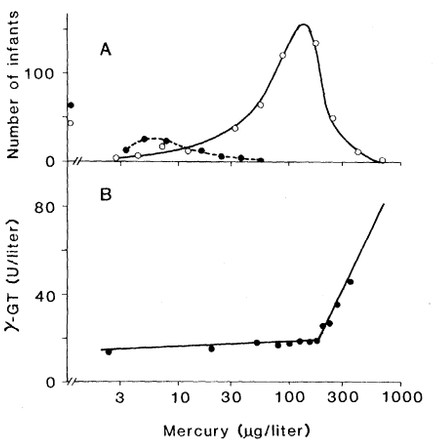

Fig. 1. (A) Distribution of mercury concentrations in the first samples of urine from exposed (○) and control (●) infants. (B) Relation between γ-GT and mercury concentrations in urine samples from infants exposed to phenylmercury. The lines were fitted by segmented linear regression analysis with nonlinear least-squares (*19*). Data points represent mean values for groups of infants (15 to 64 per group) and are present only to illustrate the goodness of fit.

We studied 509 exposed infants (*6*) and 166 matched controls. The infants received a clinical examination and urine samples were obtained for clinical chemistry tests, including mercury analysis. The urine samples were collected in containers that were free of mercury and that did not absorb mercury from urine. Portions of each sample were analyzed for total mercury (*7*), creatinine (*8*), γ-glutamyl transferase (γ-GT) activity (*9*), and total protein (*10*).

The form of mercury to which the infants were exposed was confirmed by chemical analysis of diapers treated by the commercial services (*11*). Samples of head hair from the infants also contained phenylmercury. A number of urine samples were analyzed for total and inorganic mercury by the Magos procedure (*12*). Inorganic mercury accounted for over 90 percent of the total mercury, as expected from previous studies of animals exposed to phenylmercury (*13*).

The first urine sample was collected usually within days and always within 1 month after the end of exposure. Figure 1A depicts the distribution of total mercury concentrations in the first urine samples from exposed and control infants. The median for the exposed group is approximately 20 times greater than that for the control group. The control values are comparable with reported values in the unexposed adult population (*14*).

Tests for proteinuria were all negative. It was decided, therefore, to measure γ-GT activity, since previous reports have

indicated this enzymuria to be a sensitive indicator of renal effects in experimental animals (*15*). Visual examination of a plot of γ-GT values against the logarithm of mercury concentrations clearly indicated the lack of a relation between γ-GT and mercury over a wide range of low mercury values. Elevated γ-GT levels were evident only at higher concentrations of mercury. Therefore, we chose a dose-effect model that included a threshold for toxicity (*16–18*). This was done by using a segmented linear regression model (*19*) consisting of two distinct lines meeting at a common point that, if the first line is nearly horizontal, we interpret as being a practical threshold for the effect of mercury (Fig. 1B). The two lines intersected at a urinary mercury concentration of approximately 190 μg/liter (95 percent confidence range, 178 to 203 μg/liter).

A relation between γ-GT and mercury values in these urine samples does not necessarily indicate a cause-effect relation. For example, high γ-GT and mercury might occur together in the most concentrated urine samples and thereby yield an apparent concentration-effect relation. Furthermore, age and body weight might affect urinary mercury and γ-GT in similar directions. To address these problems, two corrections were made. First, urinary concentrations of γ-GT and mercury were converted to 24-hour excretion rates by using the observed creatinine content of the urine samples and published tables relating age to 24-hour creatinine excretion (*20*). Second, the excretion rate was expressed per kilogram of body weight, again by using published tables relating weight to age (*21*). When the urinary excretion of γ-GT (units per kilogram per 24 hours) was plotted against that of mercury, essentially the same relation was seen as that shown in Fig. 1B. Segmented linear regression analysis gave a threshold value for mercury in urine of 6 μg/kg per 24 hours.

Twenty-four hour urine volume was also calculated from the creatinine content of the spot sample (*20*). A similar analysis of urine volume per kilogram versus urinary excretion of mercury also indicated a threshold relation with the threshold at 14 μg of mercury per kilogram per 24 hours. An increase in urine volume is not surprising in view of the diuretic properties of mercury (*22*).

To compare findings on γ-GT excretion and urine volume in the exposed and control groups, we carried out a dose-response analysis. Abnormal γ-GT levels and 24-hour urine volumes were defined as those values exceeding the upper 95 percent confidence limit of the values in the control infants. Logit (*19*) analysis (Fig. 2) indicates a sharp increase in the prevalence of abnormal values among exposed infants at 24-hour urinary mercury excretion rates of 4 μg of mercury per kilogram for γ-GT and 14 μg of mercury per kilogram for urine volume. These practical threshold values, estimated by comparison with control values (insets in Fig. 2, A and B), agree well with those estimated by segmented regression analysis of the exposed infants.

A second urine sample was collected from 15 infants about 1 month after the first sample. Comparison of samples from the same infant showed that, with only one exception, the γ-GT excretion rate changed in the same direction as mercury excretion. The reversibility of the mercury effect was confirmed when 23 of the infants who had high γ-GT excretion were checked 2 years later and found to have normal values. Effects on urine volume were also reversible.

The concept of a threshold for toxicity has been challenged for genotoxic agents that may produce a biological effect (mutations, tumors) with a single exposure. However, there is mounting evidence that the threshold concept is useful in evaluating the systemic toxicity of several metals, such as the renal effects of cadmium (*23, 24*) and of inhaled mercury vapor (*24*), the neurological effects of methylmercury in adults (*18*) and in prenatally exposed infants (*25*), and the hematological effects of lead in children (*17, 18*). Indeed, several national and international groups of experts have used the threshold concept in evaluating dose-effect relations (*21, 26*). Our data lend further support to the concept in the sense that a sharp increase in urinary excretion of γ-GT is seen after mercury excretion has risen to a critical level.

Our findings are consistent with the results of animal studies (*27*) showing that the kidney is a target organ of prolonged exposure to phenylmercury compounds. Indeed, increased urinary excretion of γ-GT should not be possible without kidney damage. Elevated plas-

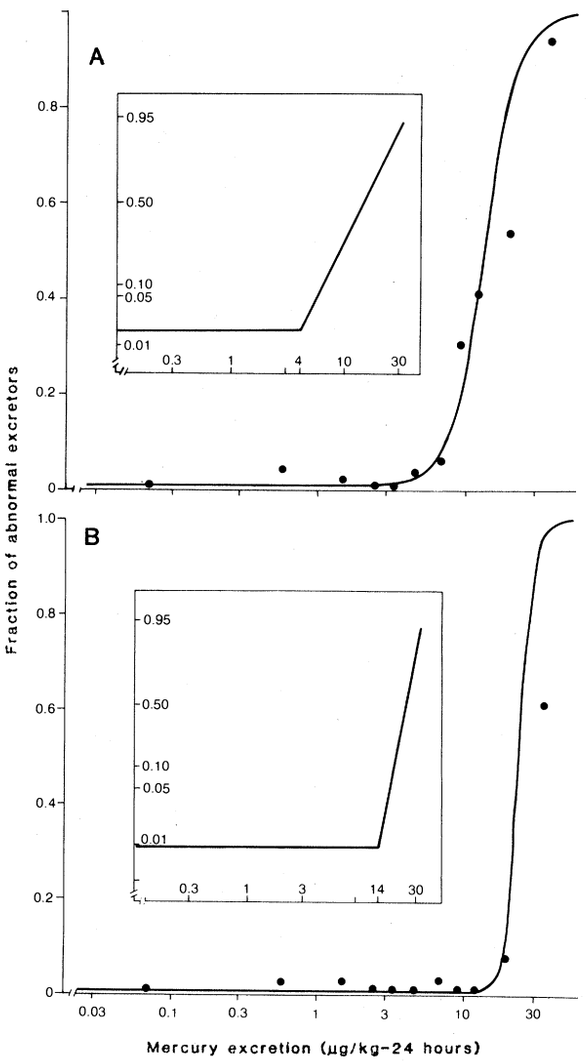

Fig. 2. Logit analysis of the prevalence of (A) abnormal γ-GT values and (B) abnormal urine volume versus urinary excretion of mercury. The insets show the linearized response function with a logit scale.

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.

CARLOS A. GOTELLI
EMILIO ASTOLFI
Department of Toxicology, Faculty of Medicine, University of Buenos Aires, Buenos Aires 1121, Argentina
CHRISTOPHER COX
Division of Biostatistics, University of Rochester School of Medicine, Rochester, New York 14642
ELSA CERNICHIARI
THOMAS W. CLARKSON
Division of Toxicology, University of Rochester School of Medicine

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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-