

Leukotriene C₄ release in upper respiratory mucosa during natural exposure to ragweed in ragweed-sensitive children

Benjamin Volovitz,^{a,b,c} Scott L. Osur,^{b,d} Joel M. Bernstein,^{b,*} and Pearay L. Ogra^{b,f,e} Buffalo, N.Y.

*With high-pressure liquid chromatography and specific radioimmunoassay, the concentration of leukotriene C₄ (LTC₄) was measured in nasopharyngeal secretions (NPS) of groups of children with allergic rhinitis before, during, and after the ragweed-pollen season. Increase LTC₄ concentrations in NPS were noted in all the children during the pollen season; the concentration of LTC₄ increased from a preseasoned mean of 1.87 ± 0.43 ng/ml to 5.52 ± 0.7 ng/ml at the peak of the ragweed season ($p < 0.001$). The mean LTC₄ concentration 2 weeks after the end of the ragweed season declined only to about 4.45 ± 1.04 ng/ml. The seasonal variation of LTC₄ concentrations and symptom scores of the children paralleled the pattern of the ragweed-pollen count. All children studied were ragweed sensitive, and six children were additionally sensitive to *Alternaria* (mold). The mold-sensitive children demonstrated higher concentrations of LTC₄ in their NPS before and continued to have high LTC₄ levels after the peak ragweed season, coinciding with peaks of mold-spore counts. Data presented in this study suggest that mast cells and other target cells in the nasal mucosa of children with allergic rhinitis produce pharmacologically active mediators, such as LTC₄, during natural exposure to ragweed or *Alternaria*. (J ALLERGY CLIN IMMUNOL 1988;82:414-8.)*

Elevated concentrations of histamine have been demonstrated in the NPS of allergic subjects during experimental challenge with allergens, such as ragweed pollen and ragweed extracts.¹ Recently, increased concentrations of LTC₄ have been found in allergic adult volunteers after experimental inhalation of ragweed pollen and cold dry air.^{2,3} No data exist regarding leukotriene production during natural exposure to ragweed pollen. The objective of the present study was to evaluate the production of LTC₄ in the upper respiratory tract of ragweed-sensitive children during naturally acquired exposure to atmospheric

Abbreviations used

HPLC: High-pressure liquid chromatography
NPS: Nasopharyngeal secretions
LTC₄: Leukotriene C₄
PBS: Phosphate-buffered saline
RIA: Radioimmunoassay

ragweed pollen during the ragweed season in the community.

MATERIAL AND METHODS

Study population

Sixteen children, 11 male and five female subjects, ranging in age from 5 to 12 years, with a history of allergic rhinitis, were enrolled in the study. All subjects were ragweed sensitive, as demonstrated by positive skin tests. Of these subjects, six children were found to be sensitive to *Alternaria* mold. Additionally, a group of 12 normal healthy subjects, who had no history of allergy to ragweed or *Alternaria*, was included as control subjects. Informed, written consent was obtained from the parents of all patients. Symptoms such as rhinorrhea, nasal congestion, and sneezing were recorded on an arbitrary scale of 0 to 3 (0, none; 1, mild; 2, moderate; and 3, severe). The scores were compiled weekly from the daily diaries. Atmospheric pollen and mold-spore counts were recorded daily from August 1 to

From the Departments of ^aPediatrics, ^bMicrobiology, and ^cOtolaryngology, State University of New York at Buffalo, and Divisions of Infectious Diseases and ^dMicrobiology Laboratories, and ^eAllergy and Immunology, Children's Hospital, Buffalo.

Supported in part by National Institute of Allergy and Infectious Diseases Grant AI-15939 and National Institute of Child Health and Human Development Grant HD-19679.

Received for publication Aug. 14, 1987.

Accepted for publication March 2, 1987.

Reprint requests: Pearay L. Ogra, Division of Infectious Diseases, Children's Hospital, 219 Bryant St., Buffalo, NY 14222.

*Recipient of Fellowship from American Physician Fellowship; current address: Pediatric Department, Hasharon Hospital, Petach Tiqva, POB 121, Israel.

October 1. Ragweed pollen and *Alternaria* spores were collected with Rotorod model 62A (Ted Brown Associates, Los Altos Hills, Calif.) and a retracting collector rod head for intermittent sampling.

Collection of NPS

In Buffalo, N.Y., ragweed-pollen season began about Aug. 17, 1986, and ended by Sept. 17, 1986. NPS samples for LTC₄ concentration were collected on August 15, approximately 2 weeks before recording of the highest ragweed-pollen counts, on August 29, during the highest counts, and on September 26, 2 weeks after the end of the season. In 10 subjects, samples were taken before, during, and after the ragweed season. In five children, samples were collected only before and during the peak season, and in one child, only during and after the peak of the ragweed season.

NPS washes were collected by use of a suction catheter and a trap. A 3 ml volume of PBS was instilled gently into the nostrils of the children in six consecutive 0.5 ml volume instillations, followed each time by gentle suction. The catheter was then rinsed into the trap with 1 ml of PBS. Total recovery of the instilled PBS was $69.1 \pm 19.5\%$. Samples were kept on ice while they were being transported to the laboratory. The samples for LTC₄ determination were stored at -70°C until they were assayed.

Measurement of LTC₄ by HPLC

A 1 ml volume of NPS washes was incubated with 4 ml of HPLC-grade methanol (Aldrich Chemical Co., Milwaukee, Wis.) for 2 hours in ice and centrifuged at 1000 g at 4°C for 15 minutes. The supernatant was vacuum extracted to dryness, and the residue was dissolved in 5 ml of 20% methanol and applied to a Sep-Pak cartridge (Waters Associates, Milford, Mass.). The sample thus prepared was subjected to vacuum extraction to dryness, and the residue dissolved in 0.5 ml of 30% methanol. Five nanograms of purified preparation of prostaglandin B₂ (Sigma Chemical Co., St. Louis, Mo.) was added as an internal standard, and the entire sample was injected into a 4.6 by 25 cm C₁₈ reverse-phase column (Beckman Instruments, San Ramon, Calif.). Elution of LTC₄ was performed isocratically in an HPLC system (Pharmacia, Piscataway, N.J.) with a mixture of 80% methanol, 20% water, 0.05% trifluoroacetic acid, and 0.5% triethylamine, both from Pierce Chemical Co., Rockford, Ill. Recovery of LTC₄ was $84 \pm 9\%$. Purified preparations of LTC₄ were used as standards and tested in parallel with the samples.

RIA for LTC₄

The presence of LTC₄ in HPLC-separated fractions was verified by a specific RIA (New England Nuclear, Boston, Mass.). Peaks of LTC₄ collected after the separation with HPLC and other peaks as positive and negative controls were dried and then resuspended in the assay buffer. A tracer containing [³H]LTC₄ was added to the samples, and a small amount of standard assay antibodies was subsequently added. After incubation, absorption of the excessive an-

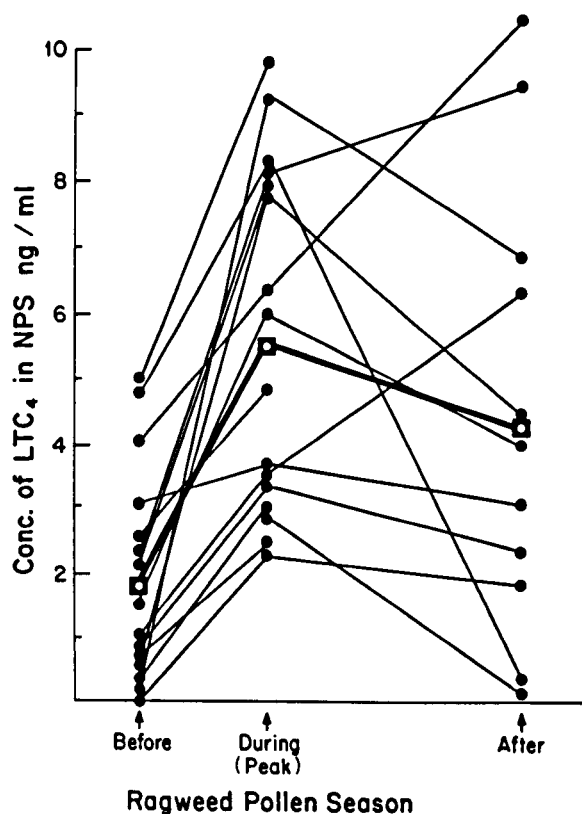


FIG. 1. Individual and mean LTC₄ concentrations in NPS of all children before, during, and after the peak ragweed season; individual concentration (●—●); mean concentration (□—□).

tibodies by charcoal suspension, and centrifugation at 1500 g for 15 minutes, the supernatant was added to Alomlight, a scintillation solution (DuPont Co., Boston, Mass.), and the radioactivity was measured in an LS 9000 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.). Recovery of LTC₄ concentrations from HPLC-separation fractions to RIA was $76 \pm 11\%$ ($n = 5$). The concentration of LTC₄ was extrapolated from a standard curve of purified LTC₄ provided in the assay kit and measured in the same time as the samples.

Statistical analysis

Two-tailed *t* test for dependent measures was used for analysis of LTC₄ concentrations and patient symptom scores. As the measurements of leukotrienes were done in duplicate, the standard error of the mean was used instead of standard deviation.

RESULTS

Changes in LTC₄ concentrations during ragweed season

The concentrations of LTC₄ in the NPS of non-allergic healthy subjects before the ragweed season ranged from 0 to 1.2 ng/ml (mean concentration, 0.39 ± 0.05). Eighty-three percent (10/12) of

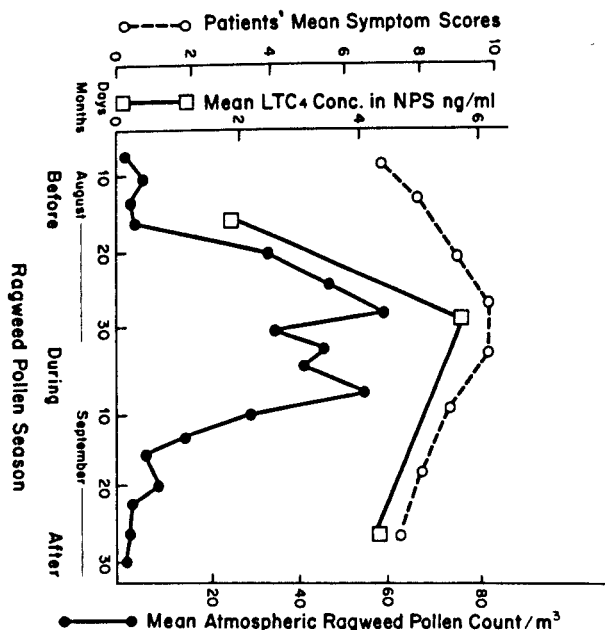


FIG. 2. Relationship of the mean atmospheric pollen counts per square meter (●—●) to the mean LTC₄ concentration (□—□) in NPS and to the mean symptom scores (○—○) of all the children before, during, and after the peak ragweed season.

the control subjects had LTC₄ concentrations of <1 ng/ml. The individual and mean LTC₄ concentrations did not exhibit significant changes during or after the ragweed season (data not presented). By contrast, the individual concentrations of LTC₄ in NPS of the ragweed-allergic subjects increased up to sixfold during the peak ragweed season compared to the pre-season baseline. The mean LTC₄ concentrations increased from 1.87 ± 0.43 ng/ml before to 5.52 ± 0.7 ng/ml during the peak ragweed season ($p < 0.001$). The activity declined after the season to about 4.45 ± 1.04 ng/ml (Fig. 1). However, this difference was not statistically significant.

Relationship of LTC₄ concentration to the mean symptom scores

The individual symptom scores in general were higher during the ragweed season, and the mean symptom scores of the study population as a whole increased from 6.8 ± 1.6 before to 10.61 ± 1.5 during the ragweed season ($p < 0.05$) and then decreased to about 7.8 ± 2.1 . The elevations of the mean LTC₄ concentrations often paralleled the increase of the ragweed-pollen counts and the mean symptom scores of the children. However, as illustrated in Fig. 2, both LTC₄ concentration and the symptom scores remained elevated well beyond the disappearance of most atmospheric ragweed pollen (Fig. 2).

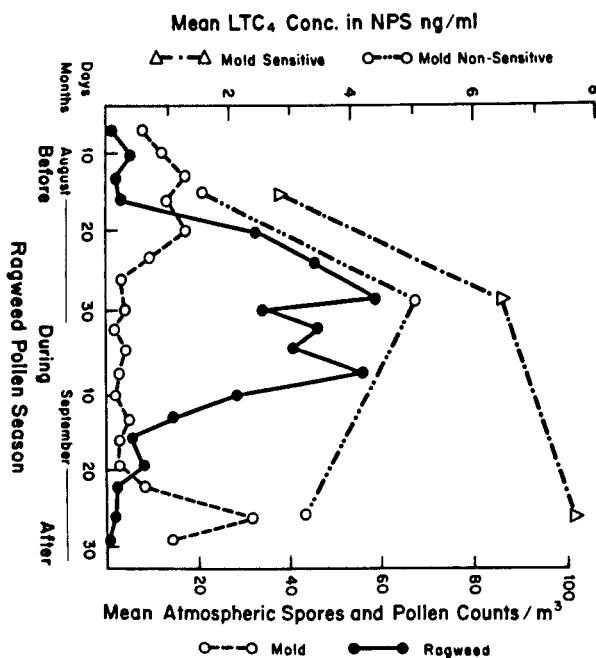


FIG. 3. Relationship of the mean atmospheric mold-spore counts per cubic meter (○—○) to the mean LTC₄ concentration in NPS of mold-sensitive (△—△) and mold-nonsensitive (○—○) children before, during, and after the peak ragweed season.

Relationship of LTC₄ concentration to mold-spore counts

An attempt was made to quantitate *Alternaria* mold-spore mass during the ragweed season and assess its impact on LTC₄ activity in the NPS in ragweed-sensitive children. The *Alternaria* spore counts demonstrated two peaks, one before and one after the ragweed season (Fig. 3). The mean spore counts were 12.3 ± 4.2 before, 4.3 ± 1.2 during, and 30.6 ± 6.7 spores per cubic meter after the ragweed season. The concentrations of LTC₄ in patients having mold sensitivity in addition to ragweed sensitivity were compared to the concentrations in mold-nonsensitive subjects. The children with both ragweed and mold sensitivity exhibited higher concentrations of LTC₄ during the entire study period. The possible effect of *Alternaria* on LTC₄ concentrations appeared to be greatest during prepeak and postpeak ragweed season, coinciding with the highest *Alternaria* counts, as illustrated in Fig. 3. It is of interest to note that the mean LTC₄ concentrations manifested further increases in the mold-sensitive group, even after the end of ragweed season.

DISCUSSION

Data obtained in this study provide evidence for the release of LTC₄ in the nasopharynx of ragweed-

sensitive children during natural exposure to atmospheric ragweed pollen. Both the individual as well as the mean LTC₄ concentrations increased significantly from a preseasonal low baseline level to high concentrations during the peak ragweed season.

LTC₄ has recently been found in a variety of disease processes, including cystic fibrosis, hypoxemia in neonates, adult respiratory distress syndrome, and during acute viral respiratory infections.⁴⁻⁷ Sulfido-peptide LTC₄ and LTD₄ are the most potent constrictors of bronchial smooth muscle and stimulate airway mucus production.^{8, 9} In addition, leukotrienes increase vascular permeability and possess important immunoregulatory functions.¹⁰⁻¹³ With the use of new separation techniques, release of sulfido-peptide LTC₄ and LTD₄ has been identified after stimulation of mast cells^{14, 15} and other inflammatory cells, such as eosinophils,¹⁶ monocytes, and macrophages.^{17, 18} Mast cells and the other inflammatory cells, such as basophils and eosinophils, are present in NPS of children during allergic reaction^{19, 20} and are most probably the major source for the LTC₄ found in NPS.²¹ To date, LTC₄ has been identified in the NPS of adult volunteers only under experimental conditions during challenge with ragweed antigen and exposure to cold dry air.^{2, 3} The study reported here clearly demonstrates that natural season exposure to atmospheric pollen is associated with the production of LTC₄ in the respiratory mucosa of ragweed-sensitive children in a manner similar to the observations reported with experimentally induced exposure.

The increase in the mean LTC₄ concentration during the peak ragweed season paralleled the increase in the pollen counts. However, the decrease of LTC₄ concentration after the peak season was only partial, and some children continued to exhibit high concentrations of LTC₄ after the decline in pollen counts from the atmosphere. It is of interest to note that the mean symptom scores of the children followed a similar pattern as the LTC₄ concentration, including parallel increase toward peak season and moderate decrease after the season. However, no direct correlation existed between individual levels of LTC₄ and the symptom score. The persistence of clinical symptoms and high concentrations of LTC₄ beyond the peak ragweed season may be due in part to the concomitant presence of other atmospheric allergens, such as *Alternaria* mold, in addition to the ragweed pollen. The persistence of symptoms beyond the peak pollen season, known as the "priming effect," is a well-described phenomenon in allergic subjects.²² Small amounts of ragweed-pollen antigen have been demonstrated to exist in the air long after the peak ragweed-pollen season.

Such environmental allergenic activity can lead to persistence of symptoms and mediator release in primed individuals.²² Furthermore, it has been suggested that natural exposure to ragweed can also prime an individual to subsequent exposure to other allergens.^{22, 23} This possibility is strongly supported by the observations that subjects sensitive to both ragweed and *Alternaria* exhibited highest LTC₄ concentrations during the entire ragweed season, and the concentrations even increased after the disappearance of ragweed. The potential role of plasma kallikrein and other late-phase mediators of inflammation in the persistence of symptoms has been studied by other investigators.^{24, 25} Their role, however, was not studied in the present population of subjects.

Based on the data presented in this study, it is suggested that LTC₄ is regularly released in the respiratory mucosa of children with allergic rhinitis. The LTC₄ concentration appears to increase significantly in ragweed-sensitive children during natural seasonal exposure to ragweed. It is suggested that mucosal mast cells and other effector cells in the nasal mucosa may produce mediators, such as LTC₄, during natural exposure to environmental allergens.

REFERENCES

1. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF Jr, Meyers DA, Norman PS, Lichtenstein LM. Mediator release after nasal challenge with allergen. *Am Rev Respir Dis* 1983;128:597.
2. Creticos PS, Peters SP, Adkinson NF Jr, Naclerio RM, Hayes EC, Norman PS, Lichtenstein LM. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984;310:1626.
3. Togias AG, Naclerio RM, Peters SP, Nimmagadda I, Proud D, Kagey-Sobotka A, Adkinson NF Jr, Norman PS, Lichtenstein LM. Local generation of sulfido-peptide leukotrienes upon nasal provocation with cold dry air. *Am Rev Respir Dis* 1986;133:1133.
4. Cromwell O, Walport MJ, Morris HR, Taylor GW, Hodson MF, Batten J, Kay AB. Identification of leukotrienes D and B in sputum from cystic fibrosis patient. *Lancet* 1981;2:164.
5. Stenmark KR, James SL, Volekel NK, Toews WH, Peeves JT, Murphy RC. Leukotrienes C₄ and D₄ in neonates with hypoxemia and pulmonary hypertension. *N Engl J Med* 1983;308:77.
6. Matthay MA, Eschenbacher WL, Goetzl EJ. Elevated concentration of leukotriene D₄ in pulmonary edema fluid of patients with the adult respiratory distress syndrome. *J Clin Immunol* 1984;4:479.
7. Volovitz B, Faden H, Ogra PL. Release of leukotrienes in human respiratory tract during viral infection: implication in virus-induced wheezing. *J Pediatr* 1988;112:218.
8. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C₄ and D₄, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* 1982;126:449.
9. Hanna CJ, Bach MK, Pare PD, Schellenberg RR. Slow-reacting substances (leukotrienes) contract human airway and

- pulmonary vascular smooth muscle in vitro. *Nature* 1981; 290:343.
10. Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, Corey EJ. Comparative airway and vascular activities of leukotrienes C₄ and D in vivo and in vitro. *Proc Natl Acad Sci USA* 1980;77:4354.
 11. Casey L, Clark S, Fletcher J, Ramwell P. Cardiovascular, respiratory and hematologic effects of leukotriene D₄ in primates. In: Samuelsson B, Paulelli R, eds. *Leukotrienes and other lipoxygenase products*. New York: Raven Press, 1982: 201.
 12. Dahlen SE, Bjork JS, Hedquist P, Arfors KE, Hammerstrom S, Lindgren JA, Samuelsson B. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules in vivo effects of relevance of acute inflammatory response. *Proc Natl Acad Sci USA* 1981;78:3887.
 13. Atluru D, Goudwin JS. Control of polyclonal immunoglobulin production from human lymphocytes of leukotrienes: leukotriene B₄ induces an OKT-8 (+), radiosensitive suppressor cell resting, human OKT-8 (-) T-cells. *J Clin Invest* 1984;74: 1444.
 14. MacGlashan DW, Schleimer RP, Peters SP, Schulman ES, Adams GK, Newball HH, Lichtenstein LM. Generation of leukotrienes by purified lung mast cells. *J Clin Invest* 1982; 70:747.
 15. Peters SP, MacGlashan DW Jr, Schulman ES, Schleimer RP, Hayes EC, Rokach J, Adkinson NF Jr, Lichtenstein LM. Arachidonic acid metabolism in purified lung mast cells. *J Immunol* 1984;132:1972.
 16. Weller PR, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RB. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C₄. *Proc Natl Acad Sci USA* 1983;80:7626.
 17. Ferreri NR, Howland WC, Spiegelberg HL. Release of leukotrienes C₄ and B₄ and prostaglandin E₂ from human monocytes stimulated with aggregated IgG, IgA, and IgE. *J Immunol* 1986;136:4188.
 18. Scott WA, Rouzer CA, Cohn ZA. Leukotriene C release by macrophages. *Fed Proc* 1983;42:129.
 19. Okuda M, Sakaguchi Y, Suzuki F, Ohtsuka H, Kawabori S. Ultrastructural heterogeneity of the basophilic cells in allergic nasal mucosa. *Ann Allergy* 1985;54:152.
 20. Gomez E, Corradi OJ, Baldwin DL, Swanston AR, Davies RJ. Direct in vivo evidence for mast cell degranulation during allergen-induced reactions in man. *J ALLERGY CLIN IMMUNOL* 1986;78:637.
 21. Naclerio RM, Proud D, Peters SP, Silber G, Kagey-Sobota A, Adkinson NF Jr, Lichtenstein LM. Inflammatory mediators in nasal secretion during induced rhinitis. *Clin Allergy* 1986;16: 101.
 22. Connell TJ. Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. *J ALLERGY* 1969;43:33.
 23. Ricketti AJ. Allergic rhinitis. In: Patterson R, ed. *Allergic diseases*. Philadelphia: JB Lippincott, 1983:207.
 24. Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65.
 25. Baumgarten CR, Nichols RC, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Plasma kallikrein during experimentally induced allergic rhinitis: role in kinin formation and contribution to TAME-esterase activity in nasal secretions. *J Immunol* 1986;137:977.

AVAILABLE NOW! The FIVE-YEAR (1981-1985) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$42.00. This comprehensive, 156-page reference guide is a current presentation of all topics included in the JOURNAL from January 1981 to December 1985 (volumes 67-76)—the past 10 volumes. It incorporates complete references to over 672 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1,525 Subject Headings, under which there are 5,316 references. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 4,780 Author Entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: The C.V. Mosby Company, 11830 Westline Industrial Dr., St. Louis, Missouri 63146-3318, or telephone FREE 1-800-325-4177, Journal Fulfillment, ext. 531 (in Missouri call collect at 314-872-8370, Journal Fulfillment, ext. 531). Please reference book code number 2605-6 when placing your order. PREPAYMENT REQUIRED. Make checks payable to The C.V. Mosby Company. (All payments must be in US funds drawn on a US bank.) Price: \$42.00 in the US, \$44.75 international (price includes mailing charges).