

10189

31957

Safety of casein hydrolysate formula in children with cow milk allergy

Hugh A. Sampson, MD, Jan Bernhisel-Broadbent, MD, Evelyn Yang, BS, MS, and Sheila M. Scanlon, BSN

From the Division of Pediatric Allergy/Immunology, Johns Hopkins University School of Medicine, Baltimore, Maryland

The purpose of this study was to determine whether a new casein hydrolysate infant formula, Alimentum, could be administered safely to children with cow milk hypersensitivity. The formula was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and with a sensitive enzyme-linked inhibition immunoassay, and it was judged to be safe for clinical trials in children allergic to cow milk. Twenty-five such children underwent double-blind, placebo-controlled oral food challenges with 10 gm of powdered cow milk and casein hydrolysate formula. All children were highly atopic and had positive skin prick reactions to cow milk. No patient reacted to placebo during a double-blind, placebo-controlled food challenge. Two patients lost their allergy to cow milk and did not react during the challenge; the remaining patients reacted with a variety of cutaneous, respiratory, and gastrointestinal symptoms within 15 to 90 minutes of challenge. All children tolerated the blinded challenge to the casein hydrolysate and were fed the hydrolysate openly without difficulty. We conclude that this casein hydrolysate is generally safe to feed to children with immediate hypersensitivity to cow milk. We recommend that all infant formulas promoted as "hypoallergenic" be tested in milk-allergic patients to assess their allergenic potential, in addition to standard nutritional evaluation and animal testing for antigenicity. (*J Pediatr* 1994;118:520-5)

The treatment of choice for cow milk hypersensitivity is total avoidance of cow milk proteins. In infants and young children, substitute milk sources are recommended: soy formulas, meat-based formulas (no longer commercially available in the United States), and hydrolyzed cow milk casein and whey formulas. Soy hypersensitivity occurs in 15% (authors' series) to 50%¹ of children sensitive to cow milk, and all unmodified proteins are capable of provoking sensitization; thus hydrolysate formulas are being recommended more frequently. The casein hydrolysates Nutramigen and Pregestimil (Mead Johnson/Bristol Myers, Evansville, Ind.), are generally regarded as safe ("hypo-

allergenic") for children with cow milk hypersensitivity. Although no formal study in human subjects documents this assumption, studies of rodents² and years of clinical experience suggest that these formulas are safe for the vast majority of infants allergic to cow milk.

The purpose of this study was to compare the potential allergenic properties of a new hydrolysate formula, Ali-

PBS	Phosphate-buffered saline solution
SDS	Sodium dodecyl sulfate

mentum (Ross Laboratories, Columbus, Ohio), with those of Nutramigen. In addition, preclinical testing of another new hydrolysate formula, Good Start HA, Carnation Co., Los Angeles, Calif., was also performed. Alimentum is an extensively hydrolyzed casein formula, and Good Start is a partially hydrolyzed whey formula. Physicochemical and immunochemical methods were used to examine the antigenic and allergenic properties of these formulas before oral challenges were done. On the basis of preclinical studies,

only Alimentum was tested in cow milk-allergic children to confirm its hypoallergenic properties.

METHODS

Physicochemical properties of the formulas were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with minor modifications of a previously described method.³ Briefly, each formula was diluted 4:5 with SDS sample buffer (0.125 mol of Tris hydrochloride [pH 6.8] buffer containing 2% SDS, 10% 2-mercaptoethanol, 10% glycerol bromphenol blue, and pyronin Y) and boiled for 10 minutes. Fifty microliters of each sample was loaded onto a discontinuous gel. The running gel was prepared from a stock of 33.5% acrylamide and 0.3% *N,N'*-bis-acrylamide to a final acrylamide concentration of 13.5%, and the stacking gel was prepared from a stock of 30% acrylamide and 0.44% *N,N'*-bis-acrylamide to a final acrylamide concentration of 3% in 0.5 mol/L Tris hydrochloride pH 6.8. Both gels contained 0.1% SDS and were polymerized with 3% ammonium persulfate and *N,N,N,N'*-tetramethylethylenediamine. After electrophoresis, gels were stained with silver nitrate.⁴

Immunochemical properties of the formula were examined by means of sensitive enzyme-linked inhibition immunoassays to cow milk proteins. Antibodies to whole cow milk, casein, and whey were raised in rabbits and kindly provided by K. Mahan, PhD (Ross Laboratories). Casein and whey antisera were extensively adsorbed with whey and casein, respectively, to produce highly specific antibody. The enzyme-linked inhibition immunoassays were performed as follows: 96-well microtiter plates (Immulon plates, from Dynatech Laboratories, Inc., Chantilly, Va.) were coated with 0.1 ml of whole cow milk proteins, casein, or whey (5 µg/ml in carbonate buffer [pH 9.6]) and incubated overnight at 4° C. Before use, plates were washed with phosphate-buffered saline solution-Tween buffer and blocked for 1 hour with PBS-Tween. Milk protein standards were prepared in PBS-Tween: 0, 10, 30, 300, 1000, and 3000 ng/ml. One hundred microliters of anti-whole milk (1:75,000), anti-casein (1:150,000), or anti-whey (1:150,000) antisera were added to test tubes containing 100 µl of respective standards and formula samples and incubated at 37° C for 2 hours. One hundred microliters of the milk protein standards- or sample-antibody complexes was added to microtiter wells and incubated at 25° C for 1 hour. Plates were washed five times with PBS-Tween before 100 µl of peroxidase-labeled goat anti-rabbit IgG antisera (Sigma Chemical Co., St. Louis, Mo.) was added. After incubation at 25° C for 1 hour, plates were washed five times and then developed with H₂O₂-3,3',5,5'-tetramethylbenzidine and read on a kinetic microplate reader (Molecular Devices, Menlo Park, Calif.). As depicted in Table I, standard curves were generated for whole milk protein, casein,

Table I. Enzyme-linked inhibition assays intraassay coefficients of variation

Assay	Range (ng/ml)	High value (%)	Low value (%)
Casein	10-3000	8.0	6.2
Whey	10-3000	4.3	3.2
Milk	10-3000	7.6	6.2

The percentage of variation for values at the upper and lower portions of the standard curves are given for assays to casein, whey, and whole cow milk proteins.

and whey. Formula samples were compared with these standards to determine the quantity of immunologically recognizable cow milk protein that they contained.

Subjects allergic to cow milk. Twenty-five children with cow milk hypersensitivity previously documented by double-blind, placebo-controlled food challenges were enrolled in the study. The children ranged in age from 8 months to 9½ years of age [median 4.4 years]. All 25 subjects were highly atopic; 24 of them came from atopic families (one child had been adopted), total serum IgE levels ranged from 61 to 86,000 IU/ml (median 515 IU/ml), 17 had atopic dermatitis, 18 had allergic rhinitis, and 14 had asthma. All the children had evidence of milk-specific IgE antibodies as indicated by positive skin prick reactions. Skin tests to undiluted Alimentum, Good Start, Nutramigen, histamine control (1 mg/ml), and a saline control were applied to the backs of 10 children by the prick technique. The mean diameters of the wheal-and-flare lesions were recorded; a mean wheal 3 mm or greater than control was considered positive.⁵ All tests were applied by means of sterile bifurcated needles (Allergy Laboratories of Ohio, Columbus) and measured by the same nurse clinician throughout the study.

Previous blinded challenges to milk in 21 patients resulted in the following objective symptoms: skin rash or urticaria or both in 15 patients, vomiting or diarrhea or both in 13, and rhinitis or wheezing or both in 12. Four patients had previous isolated ingestions of milk that resulted in skin symptoms in two patients and in skin, respiratory, and gastrointestinal symptoms in two. Many of the 25 children in the study had documented allergy to other foods: six of them were allergic to one other food, six to two other foods, and six to more than two other foods (egg 18, peanut 7, soy 5, others 9).

Patients were admitted to the Johns Hopkins Pediatric Clinical Research Unit for double-blind, placebo-controlled food challenges to whole cow milk and Alimentum. As previously described,⁶ challenges consisted of administering up to 10 gm of dehydrated, defatted cow milk or 10 gm of powdered Alimentum in 100 ml of Nutramigen during a period of 60 to 90 minutes. Three different commercial lots of Alimentum were used in the study; two patients were chal-

Supported in part by grant A124439 from the National Institute of Allergy and Infectious Diseases, grant RR00052 from the General Clinical Research Centers Program, and a grant from Ross Laboratories, Columbus, Ohio.

Submitted for publication Sept. 12, 1990; accepted Nov. 12, 1990. Reprint requests: Hugh A. Sampson, MD, CMSC-1103, Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21205.

9/20/26794

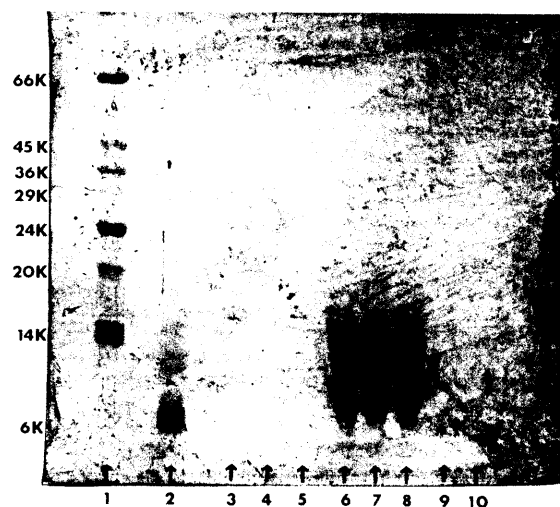


Figure. SDS-polyacrylamide gel electrophoresis and silver nitrate staining of hydrolysate formulas: lane 1, molecular weight markers; lane 2, low molecular weight markers; lanes 3 to 5, three different lots of Alimentum; lanes 6 to 8, three different lots of Good Start HA; and lanes 9 to 10, two different lots of Nutramigen.

lenged with two different lots of Alimentum. Ready-to-feed Nutramigen served as the test substance carrier and the placebo control. Patients received one test food and one placebo each day; randomization was performed by the research unit dietitian, who used a computerized program. Four children did not receive a blinded challenge to milk because two had recently been challenged and two had recently ingested milk accidentally and had had significant symptoms. Each challenge was initiated with 5 ml of formula, and the quantities administered were increased every 15 minutes until symptoms developed or the entire amount was ingested. All symptoms were recorded on a standardized symptom score sheet. If a patient had no reaction to the test material, he or she was fed 180 to 240 ml openly during observation in the research unit. Children tolerating the open feeding of Alimentum were given the formula to drink for the subsequent 6 months. Studies were approved by the Johns Hopkins University School of Medicine Joint Council on Clinical Investigation.

Statistics. To determine the expected safety of Alimentum on the basis of the sample size, we calculated binomial confidence intervals. For an experiment with a binomial (reaction/no reaction) outcome where p = probability of failure ($0 \leq p \leq 1$), n = number of independent observa-

tions, and X = number of observed failures, a $(1 - \alpha) \%$ 100% upper confidence interval can be generated for p given n observations and $X = 0$: $p \leq 1 - \exp(\log(\alpha)/n)$.⁷

RESULTS

SDS-polyacrylamide gel electrophoresis. As depicted in the Figure, there was no evidence of peptide fractions greater than 4000 daltons in the Alimentum and Nutramigen lanes, but clearly identifiable fractions were visible in the Good Start lanes. With this technique, previous experiments demonstrated that 10 ng/ml of protein loaded into the gel lane could be detected by silver stain. Not shown, electrophoretic transfer of protein fractions from the gel to nitrocellulose, followed by probing with patient antisera (i.e., Western blotting), also failed to demonstrate any detectable protein fractions in the Alimentum and Nutramigen lanes. Studies of rodent models have demonstrated that peptides less than 3400 daltons do not produce anaphylaxis.⁸ On the basis of information derived from rodent models and the physicochemical properties of these infant formulas, it appeared likely that milk-allergic infants would not react to Alimentum or Nutramigen but would react to Good Start.

Enzyme-linked inhibition immunoassay. Ten different lots of freshly opened Alimentum, and freshly prepared Good Start and Nutramigen, were analyzed by enzyme-linked inhibition assay to determine the quantity of immunologically recognizable cow milk proteins. As shown in Table II, Alimentum and Nutramigen had comparable amounts of cow milk proteins. However, Good Start had more than 700 times the amount of detectable whey proteins, more than 100 times the amount of casein proteins, and more than 700 times the detectable whole milk proteins than in the other two hydrolysate formulas. Data derived from immunochemical studies suggested that, like Nutramigen, Alimentum was very unlikely to provoke symptoms in children allergic to cow milk. Good Start, however, was likely to provoke symptoms, as was seen in one study patient who had a severe anaphylactic reaction after receiving this formula from his parent, and we thought that clinical studies were not warranted.

Double-blind, placebo-controlled food challenges. Before undergoing blinded food challenges, 10 patients underwent skin tests for cow milk allergy and for allergies to full-strength Alimentum, Nutramigen, and Good Start by the prick technique. All 10 children had positive skin test reactions to cow milk, nine had positive responses to Good Start, six to Nutramigen, and five to Alimentum.

Patients received one test food and one placebo (Nutramigen) on each of 2 days during observation in the clinical research unit. No patient responded to placebo challenges. Two children did not react to the milk challenge; 23 of 25 patients reacted to the cow milk challenge. Symptoms provoked by the milk challenges included cutaneous symptoms in 16 of the 23 children, gastrointestinal symptoms in 11 of them, and respiratory symptoms in 15. No significant symptoms occurred during the Alimentum challenge. One child had a small hive on his cheek after ingesting 75% of the Alimentum test material (the hive was not believed to be related to the test material) but completed the challenge, ingested 240 ml of Alimentum under observation, and consumed the formula for 4 months at home without any evidence of reactivity. All children tolerated the open feeding of Alimentum under observation without difficulty. Using confidence intervals generated for a binomial (reaction/no reaction) outcome, this study provides 90% confidence that at least 90% of milk-allergic children ingesting Alimentum will have no reactions.

Follow-up. Attempts were made to feed the patients Alimentum for 4 to 6 months to confirm the efficacy of this formula. However, most of the older patients refused to drink the formula after a few days because of its objectionable taste. Overall, only seven patients ingested Alimentum for 2 days to 14 months (median 1 week). No child experienced any adverse reaction while ingesting the formula.

Table II. Immunologically identifiable cow milk proteins in hydrolysate formulas

Formula	Whey ($\mu\text{g/ml}$)	Casein ($\mu\text{g/ml}$)	Whole milk ($\mu\text{g/ml}$)
Alimentum	1.27 ± 0.3	0.19 ± 0.06	0.03 ± 0.01
(Range)*	(0.9-1.7)	(0.63-0.71)	(0.02-0.05)
Nutramigen	0.40 ± 0.1	0.07 ± 0.03	0.09 ± 0.03
(Range)*	(0.2-0.7)	(0.03-0.12)	(0.05-0.12)
Good Start	751.9 ± 124	24.2 ± 7.0	65.3 ± 28.3
(Range)*	(606-963)	(15.5-38.2)	(37.7-1145)

*Range of values seen in the 10 lots examined.

DISCUSSION

The only effective treatment for cow milk hypersensitivity is complete avoidance of cow milk protein. Alternate sources of protein such as soy formulas, meat-based formulas, and goat milk⁹ have been utilized, but these unaltered proteins are potentially allergenic and reactions to them have been documented. Extensively hydrolyzed infant formulas potentially provide an alternative source of nutrition that is unlikely to provoke immune responses. Nutramigen and Pregestimil, extensively hydrolyzed casein formulas, have been widely used in milk-sensitive children and have been assumed to be "hypoallergenic." However, as noted in the American Academy of Pediatrics Committee on Nutrition's statement on hypoallergenic formulas,¹⁰ "No published, well-controlled, double-blind studies exist to support the use of either casein or whey hydrolysates for prophylaxis or treatment of infants with milk hypersensitivity."

The U.S. Food and Drug Administration has recently requested the recommendations of the American Academy of Pediatrics Subcommittee on Nutrition and Allergic Disease regarding requirements for a formula to be designated as "hypoallergenic." The subcommittee recommended that a formula could be designated hypoallergenic when the base protein has been modified to reduce antigenicity so that 90% of subjects allergic to the base protein can tolerate the formula without symptoms.¹¹ In addition, several physicochemical and immunochemical tests were recommended to evaluate hydrolysate formulas. These criteria are consistent with most physicians' perception of the term "hypoallergenic," that is, a formula that is safe for ingestion by persons allergic to milk.

Data on the potential antigenic and allergenic properties of infant formulas are limited and are based on sensitized rodent models. For example, among 10 guinea pigs fed cow milk for 37 days, anaphylaxis ensued in all 10 animals challenged intravenously with cow milk, in seven challenged with goat milk, and in none challenged with casein hydrolysate (Nutramigen).¹² However, results of animal studies cannot always be extrapolated to human beings. An ul-

trafiltered whey hydrolysate that provoked no immune response in guinea pigs^{13,14} led to anaphylactic reactions in five cow milk-allergic infants challenged with the formula.¹⁵ Similarly, studies of heat-treated cow milk whey in guinea pigs suggested a loss of allergenicity.¹⁶ However, when five cow milk-allergic children were challenged with an ultrafiltered, heat-treated whey preparation, four developed immediate hypersensitivity reactions.¹⁷

The purpose of this study was to assess the "hypoallergenicity" of two hydrolysate formulas recently introduced in the United States. Physicochemical and immunochemical studies indicated that Alimentum, an extensively hydrolyzed casein formula, was similar to Nutramigen and was unlikely to provoke hypersensitivity reactions in cow milk-allergic patients. Good Start, a partially hydrolyzed whey formula, contained significant peptide fractions greater than 4000 daltons, as determined by SDS-polyacrylamide gel electrophoresis, and significantly more immunologically identifiable cow milk proteins by enzyme-linked immunosorbent assay. On the basis of these preclinical studies, it seemed highly likely that cow milk-allergic infants would react to this latter formula (a finding supported by several reports of allergic reactions to Good Start in cow milk-allergic children, including two of our patients); blinded challenges in cow milk-sensitive subjects therefore did not seem warranted.

Prick skin testing with undiluted hydrolysate formulas resulted in small immediate wheals in 5 of 10 patients tested with the extensively hydrolyzed Alimentum and in 6 of them with Nutramigen. This indicates that the minimal residual peptide fractions of cow milk proteins are sufficient to provoke cutaneous mast cell activation. However, as seen with all forms of IgE-mediated food hypersensitivity, positive skin test results frequently do not indicate symptomatic reactivity; only 30% to 40% of positive prick skin test reactions correlate with positive results on blinded challenges.¹⁸⁻²⁰ Therefore skin prick test results cannot be considered indicative of hypersensitivity to extensively hydrolyzed formulas. The utility of performing skin prick tests with Alimentum or Nutramigen in infants is questionable for several reasons: (1) The vast majority of positive tests will not correlate with symptomatic reactivity; (2) several forms of milk hypersensitivity in infants are not IgE mediated; and (3) a negative test result in children less than 2 years of age must be interpreted with caution because IgE-mediated activation of cutaneous mast cells is often depressed compared with that in older persons.²¹

Given the physicochemical and immunochemical similarity to Nutramigen, it is likely that virtually all milk allergic-children will tolerate Alimentum and that Alimentum can be considered hypoallergenic (i.e., safe for ingestion by milk-sensitive subjects). However, the designation

"hypoallergenic" does not mean "nonallergenic." Two appropriately confirmed cases of reactions to Nutramigen have been published,^{22,23} indicating that sufficient protein fractions (e.g., small peptides containing the relevant allergenic epitope) are present to cause reactivity in the rare patient. In milk-allergic children who have had a severe life-threatening reaction after the ingestion of milk, it may be prudent to administer the first dose of Alimentum in a physician's office, where resuscitation can be initiated in the event that allergic symptoms develop.

Although the taste of Alimentum, like that of other extensively hydrolyzed formulas, is unacceptable to most older children, it should still prove useful in young infants, who have the majority of allergic reactions to milk. We did not test Alimentum in subjects with other forms of milk hypersensitivity, but it is unlikely to provoke symptoms in them because IgE-mediated hypersensitivity reactions represent one of the most sensitive indicators of antigenicity and allergenicity. In addition, this study demonstrates methods for the preclinical and clinical testing of potential "hypoallergenic" formulas, and methods that can be used to compare various formulas and to monitor their batch-to-batch variability. Physicochemical analyses, immunochemical analyses, and animal studies are insufficient to ensure lack of allergenicity in human beings. At present, oral challenges in allergic patients are the only means of confirming hypoallergenicity.

REFERENCES

- Burks AW, Casteel HB, Fiedorek SC, Williams LW, Pumphrey C. A prospective food challenge study of two different types of soy protein isolates in patients with possible milk or soy protein intolerance. Presented at Southern Society for Pediatric Research, New Orleans, La., Jan 1991.
- Knights RJ, Manes JD. Composition, molecular weight, and antigenicity of casein hydrolysates used in a formula for food allergic and malabsorptive infants. In Chandra RK, ed. Food allergy. St. Johns, Canada: Nutrition Research Education Foundation, 1987:273-85.
- Bernhisel-Broadbent J, Taylor S, Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates. *J Allergy Clin Immunol* 1989;84:701-9.
- Gooderham K. High-sensitivity silver staining of proteins following polyacrylamide gel electrophoresis. In: Walker JM, ed. Methods in molecular biology. I. Proteins. Clifton, N.J.: Humana Press, 1984:113-8.
- Sampson HA. Comparative study of commercial food antigen extracts for the diagnosis of food hypersensitivity. *J Allergy Clin Immunol* 1988;82:718-26.
- Sampson HA. Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1983;71:473-80.
- Rao CR. Linear statistical inference and its application. 2nd ed. New York: John Wiley & Sons, 1973:470-3.
- Poulsen OM, Hau J. Murine passive cutaneous anaphylaxis test for the "all or none" determination of allergenicity of bovine whey proteins and peptides. *Clin Allergy* 1987;17:75-83.
- Fries JH. Milk allergy: diagnostic aspects and the role of milk substitutes. *JAMA* 1957;165:1542-5.
- Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 1989;83:1068-9.
- Kleinman RE, Powell GF, Bahna S, et al. (American Academy of Pediatrics Subcommittee on Nutrition and Allergic Disease). Infant formulas and allergic disease. *Pediatrics* (in press).
- McLaughlan P, Anderson KJ, Widdowson EM, Coombs RRA. Effect of heat on the anaphylactic-sensitizing capacity of cow's milk, goat's milk, milk, and various infant formulae fed to guinea pigs. *Arch Dis Child* 1981;56:165-71.
- Granti B, Marioni L, Rubaltelli FF. Evaluation in guinea pigs of the allergenic capacity of two infant formulae based on hydrolyzed milk proteins. *Biol Neonate* 1985;48:122-4.
- Pahud J, Monti JC, Jost R. Allergenicity of whey protein: its modification by tryptic in vitro hydrolysis of the protein. *J Pediatr Gastroenterol Nutr* 1985;4:408-13.
- Businco L, Cantani A, Longhi MA, Giampietro PG. Anaphylactic reactions to a cow's milk whey protein hydrolysate (Alfa-Re, Nestlé) in infants with cow's milk allergy. *Ann Allergy* 1989;62:333-5.
- Kilshaw PJ, Heppell MJ, Ford JE. Effects of heat treatment of cow's milk and whey on the nutritional quality and antigenic properties. *Arch Dis Child* 1982;57:842-7.
- Ebbeling W, Buckley RH. Double-blind placebo-controlled challenges with heated cow milk whey protein in milk-sensitive patients [Abstract]. *J Allergy Clin Immunol* 1989;83:238A.
- Bock SA, Lee Y, Remigio LK, May CD. Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978;62:327-34.
- Sampson HA, Albergo R. Comparison of results of skin tests, RAST and double-blind placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984;74:26-33.
- Sampson HA, MacCaskill CM. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J PEDIATR* 1985; 107:669-75.
- Zeiger RS. Atopy in infancy and early childhood: natural history and role of skin testing. *J Allergy Clin Immunol* 1985; 75:633-9.
- Lifschitz CH, Hawkins HK, Guerra C, Byrd N. Anaphylactic shock due to cow's milk protein hypersensitivity in a breast-fed infant. *J Pediatr Gastroenterol Nutr* 1988;7:141-4.
- Bock SA. Probable allergic reaction to casein hydrolysate formula [Letter]. *J Allergy Clin Immunol* 1990;84:272.

FELLOWSHIPS

Available fellowships in pediatric subspecialties and those for general academic pediatric training are listed once a year, in May, in THE JOURNAL OF PEDIATRICS. Each October, forms for listing such fellowships are sent to the Chairman of the Department of Pediatrics at most major hospitals in the United States and Canada. Should you desire to list fellowships, a separate application must be made each year for each position. All applications must be returned to Mosby-Year Book, Inc., by February 15 of the listing year to ensure publication. Additional forms will be supplied on request from the Journal Editing Department, Mosby-Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318/314-453-4317.