New Antigens in Lactose (35546)

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Bleumink and Young (1) reported over a 100-fold increase in skin reactivity on milk-sensitive persons of relatively inactive, crystalline β-lactoglobulin after prolonged heating with lactose at 50° at pH 7.0. They attributed the increased skin reactivity to a browning reaction condensation product of lactose with the ε-amino group of lysine in the β-lactoglobulin. They used 15 g of lactose/500 mg of β-lactoglobulin. After heating, the lactose was removed by dialysis and the undialyzed residue was recovered by lyophilization. The fact that such a large quantity of lactose was used and that no control test for nondialyzable antigens in the lactose was done prompted us to examine two reagent-grade, commercial samples of lactose for nondialyzable antigens which could account for the increased skin reactivity observed or for potential allergens *per se*. We found 4 unidentified antigens in the lactose as well as some β-lactoglobulin and α-lactalbumin. Less highly purified lactose would probably contain more of these antigens than were found in our two reagent-grade samples.

Materials and Methods. The α-lactose monohydrate had the following certificate of analysis (percentage basis) according to the supplier: dextrose 0.05; sucrose, 0.02; iron, 0.0001; insoluble matter, 0.001; residue on ignition, 0.016; dextrin, starch, 0.000; heavy metals (as Cu), 0.0003. Two properties of β-lactose by the supplier were: $(\alpha)_D$ +55° ± 1° (C = 6); mp, 224° dec. The dialyzer tubing retained materials with a molecular weight of 12,000 and higher. Total milk protein (TMP) was prepared from fresh skim milk by prolonged dialysis against many changes of 1 *M* sodium chloride. Sodium chloride was then removed by dialysis against distilled water until free from chloride ions. The proteins inside the membrane were recovered by lyophilization. The residue contained 13.7% nitrogen on an air-dried basis.

A 500-g sample of α-lactose monohydrate or anhydrous β-lactose was stirred up with 500 ml of water. The suspension, with toluene preservative, was dialyzed for about 8 days against running water or frequent changes of water until the dialysates gave negative or very faintly positive test for reducing sugar. The endo solution was filtered and lyophilized. The yield of brownish-colored residue, designated lactose-endo (LE) was 0.016 and 0.009% from α-lactose (α-LE) and β-lactose (β-LE), respectively. The nitrogen contents of various preparations ranged from 1.3 to 2.4%. Since α-LE and β-LE were both antigenic, a mixture containing 26% β-LE and 74% α-LE was used for this work. Unless otherwise indicated, LE refers to this mixture. The nitrogen content of LE was 1.8%.

Carbohydrate analysis of both unhydrolyzed and hydrolyzed LE was done using fractionation on a column of Type S Chromo-Beads (a resin by Technicon)[1] by an unpublished modification by Groves and Friedman of the method of Lee *et al.* (2). Hexosamine was determined on LE by the Elson–Morgan method (3) on the hydrochloric acid hydrolysate which was purified by Dowex-50(H^+) adsorption and elution. Losses were monitored by an internal radioactive control.

Antigenicity was determined by the Schultz-Dale technique (4) and by gel-diffusion analysis (5) using rabbit anti-LE. Vir-

[1] The use of a trade name, distributor or manufacturer is for identification only and implies no endorsement of the product or its manufacturer.

TABLE I. Summary of Results of Schultz-Dale Tests with α-LE, β-LE, LE, α-Lactalbumin, β-Lactoglobulin, Casein, and Bovine Serum Albumin.

Sensitization		Challenge		Results		
Antigen	Dose (μg of antigen N/guinea pig)	Antigen	Dose (μg of antigen N)	No. of guinea pigs tested	No. giving 4+ response[a]	No. with negative responses
α-LE	20	LE	10	6	4	2
		β-Lactoglobulin	10	6	1	5
		α-Lactalbumin	10	4	0	4
		Casein	10	1	0	1
		κ-Casein	10	1	0	1
		BSA	10	2	0	2
β-LE	10	LE	10	5	2	3
		β-Lactoglobulin	10	5	0	5
LE	200	LE	10	3	3	0
		β-Lactoglobulin	10	2	0	2
		α-Lactalbumin	10	3	1	2
		Total milk protein	100	1	0	1
None	—	LE	10	3	0	3

[a] 4+ reaction was a response 80 to 100% that of histamine.

gin female guinea pigs (about 225 g) were sensitized by subcutaneous injection (nuchal area) with two simultaneous 0.5-ml volumes of LE in physiological salt solution emulsified with Freund's complete adjuvant (1:1). Incubation was for at least 28 days. Uterine strips of the sensitized guinea pigs were used for testing. Rabbits were immunized by injection of 0.25 ml of LE emulsified with Freund's complete adjuvant in each of the footpads. The dosage was 2.5 mg of LE (45 μg of LE nitrogen). A booster dose of 3 mg of LE (54 μg of LE nitrogen) was administered intra-abdominally in 1 ml of solution 28 days later. Rabbits were bled out in 7 days after administration of the booster dose. Anti-LE was concentrated by filtration through a filter which retained material with a molecular weight of 20,000 or over.

Results and Discussion. Antigenicity of LE, α-LE, and β-LE and their antigenic relationships with some known milk proteins as determined by Schultz-Dale technique are shown in Table I and Fig. I. The β-LE (10 μg of nitrogen, each) sensitized 2 of 5 guinea pigs to LE and none of these 5 were sensitive to β-lactoglobulin. α-LE (20 μg of nitrogen, each) sensitized 4 of 6 guinea pigs to LE, 1 of 5 of these was sensitive to β-lactoglobulin, none of the 4 tested was sensitive to α-lactalbumin, none of the 2 was sensitive to BSA and none of the 1 each tested was sensitive to soluble casein or to κ-casein. LE (200 μg of nitrogen each) sensitized 3 of 3 guinea pigs to LE, none of 2 of these was sensitive to

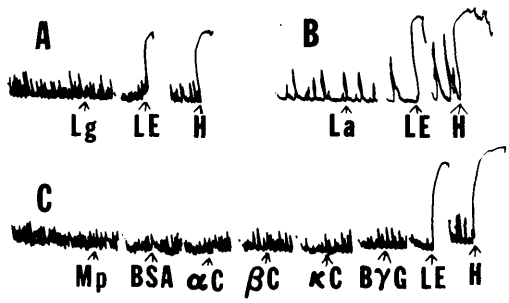

FIG. 1. Demonstration of antigenicity of LE and nonidentity of LE with several milk proteins by the Schultz-Dale technique using 3 segments of uterine horns from a guinea pig sensitized with 200 μg of LE nitrogen: challenge dose (μg of N) of respective substance (H = histamine for maximum response): (A) Lg (β-lactoglobulin), 10; LE, 10. (B) La (α-lactalbumin), 10; LE, 10. (C) Mp (mucoprotein), 1; BSA (bovine serum albumin), 1; C (α-casein), 1; βC (β-casein), 1; κC (κ-casein), 1; BγG (bovine gamma globulin), 1; LE, 1.

β-lactoglobulin, and 1 of 3 tested was sensitive to α-lactalbumin. LE gave no nonspecific reactions with nonsensitized guinea pig uteri. The nonidentity of LE antigen with several milk proteins is apparent from the Schultz-Dale test shown in Fig. 1 using 3 segments of uterine horns from a guinea pig sensitized with LE (200 μg of nitrogen). Figure 1A and B show that 10 μg of LE nitrogen produced 4+ responses (80 to 100% the reaction to histamine) following negative responses with 10 μg of β-lactoglobulin and α-lactalbumin nitrogen, respectively. Fig. 1C shows that 1 μg of LE nitrogen produced a 4+ response following negative responses to successive 1 μg nitrogen of each mucoprotein (6, 7), BSA, α-casein, β-casein, κ-casein, and bovine γ-globulin. In another experiment 10 μg of LE nitrogen produced a 4+ response following a negative response with 100 μg of TMP nitrogen in the uterine strip of a guinea pig sensitized with 200 μg of LE nitrogen. This result shows that the TMP preparation either contains none or an undetectably small amount of LE antigens. The proportions of β-lactoglobulin and α-lactalbumin present in LE were estimated by the Schultz-Dale test. Titration with β-lactoglobulin and LE of uterine strips of guinea pig sensitized with β-lactoglobulin showed that LE contained less than 10% β-lactoglobulin. Likewise, titration with α-lactalbumin and LE of uterine strips of guinea pigs sensitized with α-lactalbumin showed that LE contained less than 1% α-lactalbumin.

Four antigens which were not identifiable with any of the milk proteins tested were demonstrated by gel diffusion analysis using 5-fold concentrated rabbit anti-LE as shown in Fig. 2. A qualitatively similar precipitate pattern was obtained using unconcentrated rabbit anti-LE and no precipitate was obtained in this test using the following antigens, β-lactoglobulin, α-lactalbumin, soluble casein, and BSA. LE gave no precipitate with normal rabbit serum.

LE contained 33% lactose, whereas the acid hydrolyzate of LE contained 24 and 21% glucose and galactose, respectively, along with traces of mannose and of two unidentified substances. Although 100% composition of LE has not been determined, the fact that

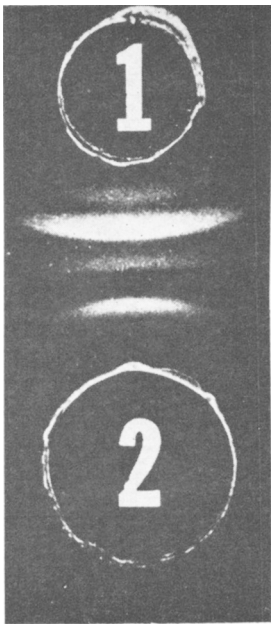

FIG. 2. Demonstration of 4 unidentified antigens in LE by gel double diffusion analysis: Well 1, 0.1 ml of LE, 0.2 mg of LE nitrogen/ml; Well 2, 0.2 ml of rabbit anti-LE, concentrated 5-fold. Photographed 5 days after test started.

the sum of glucose and galactose significantly exceeds that of the unhydrolyzed lactose, indicates that some lactose may be combined with antigenic nitrogenous components. This could explain the retention by the dialysis membrane of lactose-containing antigens. LE contained 0.68% hexosamine.

Every antigen is a potential allergen. Consequently, a possibly significant allergenic role of these unidentified antigens must be considered not only in the experiment of Bleumink and Young (1), but also in all cases of adverse response to ingestion of lactose until clinical evaluation of these antigens can be made. Whether the four unidentified antigens are chemically combined with lactose or present as a contaminant is not known. In either case, their association with lactose has provided a means for their first detection and only further studies will show whether or not these antigens can be more readily obtained in greater yields directly from milk. Further work on the nature of these antigens is contemplated.

Summary. Four unidentified antigens,

which are distinct from known milk proteins, are contained in commercial, reagent-grade α-lactose and β-lactose. These unidentified antigens, rather than the condensation product of lactose with the ε-amino group of lysine of β-lactoglobulin, may cause, or partially cause, the reported increase in allergenicity of crystallized β-lactoglobulin on heating with lactose. The unidentified antigens present in lactose have possible, hitherto unrecognized, allergenic significance.

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