

The milk proteins responsible for milk allergy, an autoallergic disease of cattle

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It has been shown, using skin tests with various serum and milk derived proteins, that cows with a typical history of milk allergy, an autoallergic disease of cattle, are in fact sensitive to homologous milk and to the protein α -casein which is formed in the udder. The animals under test did not react to any of the other milk proteins used, to bovine serum, or to any of the serum proteins tested.

Milk allergy is a disease of cattle which has been recognized as a distinct clinical entity by farmers and veterinarians since the beginning of this century.¹⁻⁴ Its exact etiology has never been investigated, but it appears that certain cows, under conditions of undue engorgement of the udder, absorb their own milk and produce an allergic reaction to it. A similar condition may exist in the mare and the bitch, and in the field of human medicine there is one report⁵ of a woman with allergic eczema who was found to be allergic to her own breast milk. Campbell⁴ showed that these allergic cows produced a positive skin reaction of the immediate type when they were tested with autologous and homologous milk but not with the milk of heterologous species. He transferred the sensitivity to calves and suggested that he was dealing with an autoallergic disease mediated by antibodies and that the allergens responsible were likely to be those milk proteins which were actually formed in the udder. This work was undertaken to test the hypothesis that udder-derived proteins were, in fact, responsible for the hypersensitivity by determining the sensitivity of milk-allergic cows to individual milk and serum proteins. It was further hoped to identify the specific protein or proteins responsible for milk allergy in cattle.

MATERIALS AND METHODS

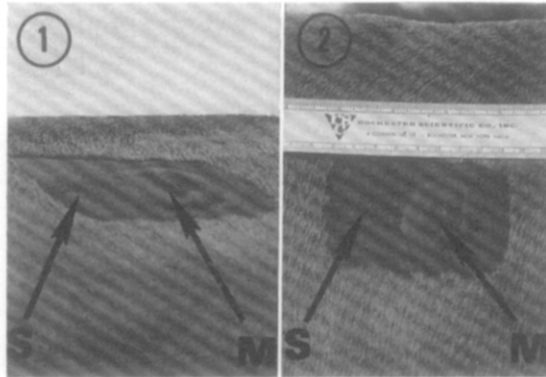
Cows

The 10 principal cows selected for test (Table I, Nos. 1 to 10) were animals which had a typical history of milk allergy⁴ and which had previously shown positive skin tests to cow's milk. In addition 10 control cattle were used; 5 of these (Table I, Nos. 11 to 15) were taken from the herds in which the allergic cows were found, and 5 (Table I, Nos. 16 to 20) were taken from the college herd. All 10 controls were lactating, and none had a history of urticaria, respiratory disease, or other manifestations of milk allergy.

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FIGS. 1 and 2. Typical positive skin tests (**M**) and saline controls (**S**) in a milk-allergic cow. Wheals photographed 30 minutes after intradermal injection of 0.1 ml. of saline (**S**) and cows' milk (**M**) at a dilution of 1:1,000.

Skin tests

The skin tests were carried out by clipping an area of skin over the scapular region of the cow and injecting 0.1 ml. of the allergen under test by the intracutaneous route. The diameter of the resultant wheal was measured with calipers after 30 minutes had elapsed. The statistical significance of the difference between the mean diameters of the skin reactions of principal and control cows was analyzed using the *t* test.

Milk and milk proteins

The milk used for the skin tests was a composite sample of milk from 5 Holstein cattle which had been centrifuged to remove the fat and Zeiss filtered. It was diluted with physiologic saline and was used at a dilution of 1:10 and 1:1,000. The following commercially available milk proteins were also used for skin testing: α -casein,* α -lactalbumin,* and α -lactoglobulin,* all at a dilution of 1:1,000 with saline. The individual milk and serum proteins used for skin testing were selected from a number which are available commercially after being screened for purity using a macroimmunodiffusion test in agar gel.

Bovine serum and serum proteins

Bovine serum was used for skin testing at a dilution of 1:10 and 1:1,000. The serum was obtained from a composite sample of the serum of 5 healthy bulls, because of the possibility of the presence of milk proteins in cow's serum.⁶ The following two serum proteins which are commercially available were also used for skin testing: bovine serum albumin† and bovine gamma globulin* (Cohn fraction II), both at a dilution of 1:1,000. Sodium chloride for injection‡ was used both as a diluent for all of the above proteins and as a saline control.

RESULTS

The results of individual skin tests on milk allergic and control cows are recorded in Table I. Typical positive skin reactions in the cow are shown in Figs. 1 and 2. Wheal diameters of 20 mm. and larger have, in the past, been considered indicative of a positive test in cattle.⁴ Using this criterion, it will be

*Nutritional Biochemicals Corp., Cleveland, Ohio.

†Armour Pharmaceutical Company, Kankakee, Ill.

‡Abbott Laboratories, North Chicago, Ill.

TABLE I. Results of skin tests with milk and serum proteins on milk allergic (Nos. 1 to 10)

Milk and serum proteins	Dilution	Diameter of w									
		Allergic cows									
		1	2	3	4	5	6	7	8	9	
Milk	1:10	20	33	29	35	32	32	31	37	28	
Milk	1:1,000	18	35	21	25	29	29	28	30	25	
α -Casein	1:1,000	16	32	16	30	32	33	32	41	28	
α -Lactalbumin	1:1,000	8	7	13	9	19	22	13	18	16	
β -Lactoglobulin	1:1,000	11	10	11	17	12	0	14	10	11	
Saline		5	0	8	9	12	0	9	0	11	
Bovine Serum	1:10	10	0	9	12	15	5	9	12	3	
Bovine Serum	1:1,000	0	0	0	8	9	4	8	10	5	
Serum Albumin	1:1,000	11	0	8	9	6	2	11	13	11	
γ -Globulin	1:1,000	9	0	0	10	9	0	10	11	11	

noted that positive skin tests occur consistently in milk-allergic cattle when tested with milk at dilutions of 1:10 and 1:1,000 and α -casein at a dilution of 1:1,000. Only 2 cows were exceptions to this: No. 1 was negative to milk 1:1,000 and to α -casein 1:1,000, and No. 3 was negative only to α -casein.

On the other hand, both the milk-allergic cows and the controls produced negative tests to essentially all of the other allergens used. There were only two exceptions to this—cow No. 6, an allergic animal, had a positive skin reaction when tested with α -lactalbumin 1:1,000, and a control cow, No. 19, gave a positive reaction when skin tested with milk 1:10.

The mean wheal diameter for each allergen was calculated for both groups of cows. These are recorded in Table I. The largest mean wheal diameter (30.4 mm.) was produced by milk at a dilution of 1:10, the next largest (28.7 mm.) by α -casein 1:1,000, and the third largest (26.5 mm.) by milk at a dilution of 1:1,000. The mean wheal diameters of all other tests, both in allergic and control animals, were of a size considered to represent negative tests. This is illustrated graphically in Fig. 3.

Of the agents used for skin testing, 5 produced wheals of greater mean diameter in the allergic cows than in the controls. These were milk 1:10, milk 1:1,000, α -casein 1:1,000, α -lactalbumin 1:1,000, and β -lactoglobulin 1:1,000. A statistical analysis of these results (Table II) indicated that the difference in mean wheal diameter between allergic and control cows was highly significant in the case of milk 1:10, milk 1:1,000, and α -casein 1:1,000 in all instances ($p < 0.001$). In the case of α -lactalbumin 1:1,000 and β -lactoglobulin 1:1,000, there was no statistically significant difference between the mean wheal diameter of allergic and control animals ($p > 0.05$).

These results indicate that cows with a history of milk allergy produce a positive skin reaction to milk in a dilution of 1:10 and 1:1,000. In addition they give a positive skin test to α -casein at a dilution of 1:1,000. Upon statistical analysis, the diameters of the wheals produced by these allergens (milk 1:10 and 1:1,000, casein 1:1,000) were highly significantly greater than those produced by the other milk or serum proteins used in this study.

control (Nos. 11 to 20) cows

produced in 30 minutes
(mm.)

Mean	Control cows										Mean
	11	12	13	14	15	16	17	18	19	20	
30.4	8	9	11	0	7	11	17	18	25	11	11.7
26.5	9	10	11	0	8	10	13	14	13	9	9.7
28.7	11	11	11	0	8	9	12	13	12	9	9.6
12.5	9	9	11	0	12	11	13	12	10	10	9.7
9.6	9	10	12	0	10	11	0	13	12	10	8.7
5.4	10	12	12	0	9	0	11	15	14	9	9.2
7.5	11	12	13	0	10	12	11	18	14	10	11.1
4.4	10	10	10	0	8	9	9	15	12	11	9.4
7.1	12	9	9	0	8	11	12	15	13	0	8.9
6.0	10	9	8	0	5	9	10	13	11	9	8.4

TABLE II. Summary and analysis of results of skin tests with milk and serum proteins on milk allergic and control cows

Milk and serum proteins	Dilution	Mean diameter of wheals (mm.)		Difference	p
		Allergic cows	Controls		
Milk	1:10	30.4	11.7	+18.7	< 0.001
Milk	1:1,000	26.5	9.7	+16.8	< 0.001
α -Casein	1:1,000	28.7	9.6	+19.1	< 0.001
α -Lactalbumin	1:1,000	12.5	9.7	+2.8	> 0.2
β -Lactoglobulin	1:1,000	9.6	8.7	+0.9	> 0.5
Saline		5.4	9.2	-3.8	
Bovine serum	1:10	7.5	11.1	-3.6	
Bovine serum	1:1,000	4.4	9.4	-5	
Serum albumin	1:1,000	7.1	8.9	-1.8	
γ -Globulin	1:1,000	6.0	8.4	-2.4	

DISCUSSION

It was originally postulated by the author⁴ that cows which were sensitive to their own milk were likely to be sensitive to the udder-derived components of milk rather than those derived from serum, because intimate association with these proteins is restricted to the postpuberty period of life. The results presented above support this hypothesis and indicate that the milk-allergic cows used in this trial were sensitive to the udder-derived protein α -casein but not to the udder-derived proteins α -lactalbumin or β -lactoglobulin or to whole serum or the serum-derived proteins which were tested. It is appreciated that impurities may have been present in small quantities in the proteins tested. However, the dilution factor involved (1:1,000) reduces this risk, and it would still appear clear that the predominant protein which produces the naturally occurring autoallergic syndrome known as milk allergy in cattle is, in fact, α -casein.

It is interesting to note that in 1934 Lewis⁷ showed that under experimental conditions casein was isoantigenic in the goat. It is not surprising therefore that this protein which is produced by mammals relatively late in life is responsible for the autoallergic condition known as milk allergy in cattle, and one wonders

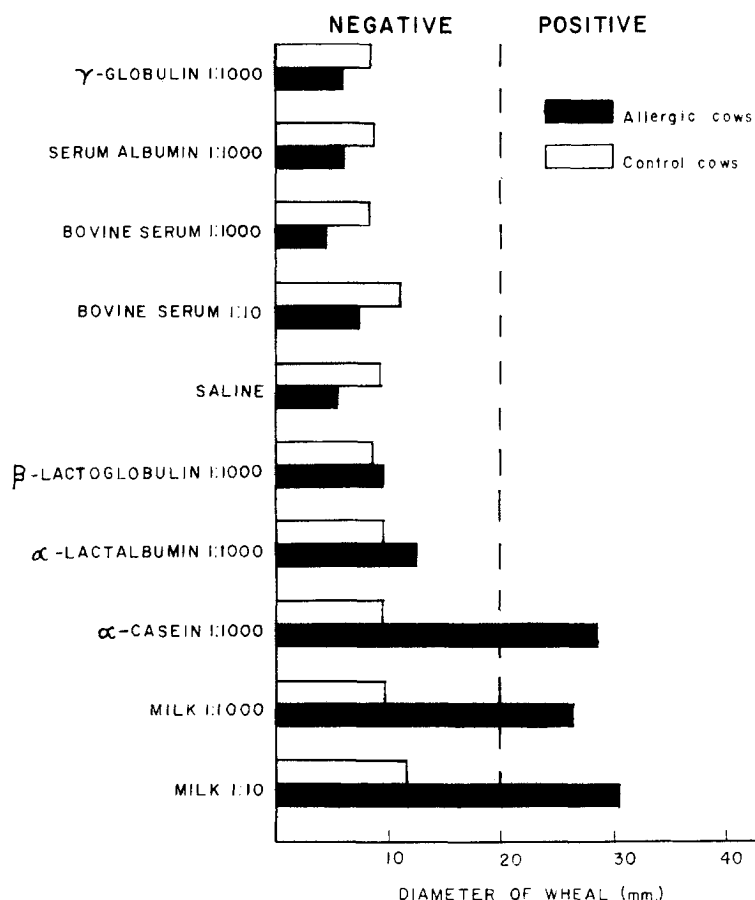


FIG. 3. Skin test reactions to milk and serum proteins in milk-allergic and control cows.

if this is the only condition or species in which casein acts as a consequential autoallergen.

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