

## Anaphylactic sensitivity of guinea-pigs drinking different preparations of cows' milk and infant formulae

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### SUMMARY

Guinea-pigs have been given various preparations of cows' milk or infant formulae to drink in an investigation of the capacity of these milk preparations to stimulate *per os* anaphylactic sensitivity.

The treatment, presumably heat, used to concentrate the 'Evaporated' whole cows' milks (three brands were tested) almost abolished their sensitizing capacity to  $\beta$  lactoglobulin. The brand presumed to be most heated as judged by the degree of caramelization had also lost most of its sensitizing capacity to casein. Injected parenterally, the 'Evaporated' milk adequately sensitized to anaphylaxis.

An infant formula, which in its spray dried form was only moderately sensitizing to both  $\beta$  lactoglobulin and casein, lost most of this sensitizing capacity when processed to a liquid concentrate state. The manufacturing process for the liquid concentrate involved greater and more prolonged heating than required for the spray-dried form.

The relevance of these findings in the guinea-pig to cows' milk protein intolerance in the infant and possibly also to cot death is discussed.

### INTRODUCTION

In 1976, Devey *et al.* reported that guinea-pigs (aged between 2 and 4 months) on a normal diet, but given pasteurized cows' milk to drink instead of water, very soon become anaphylactically sensitive to the proteins in cows' milk. After 14 days, all of the guinea-pigs could be fatally shocked following either intravenous injection or intra-tracheal inhalation of cows' milk.

In further studies (Coombs, Devey & Anderson, 1978) using 'Long Life' whole cows' milk (UHT), it was found that when the drinking of cows' milk was continued beyond 70 days or so, about 70% of the guinea-pigs became refractory to anaphylaxis on intra-tracheal challenge with cows' milk and about 30% on intravenous challenge.

The introduction of baby guinea-pigs to drinking cows' milk at 1–2 days of age resulted in what appeared to be immunological tolerance in 20–60% of the guinea-pigs, depending on the strain of guinea-pig used. Such 'tolerant' guinea-pigs did not enter the sensitive state. The anaphylactic sensitivity, particularly as measured by intravenous challenge, could be assessed by examining the sera for IgG1a antibodies to cows' milk by passive cutaneous anaphylaxis.

We now report experiments in guinea-pigs on the sensitizing capacity *per os* of different preparations of cows' milk or infant formulae. Considerable differences have been found, especially with regard to sensitivity to the individual proteins,  $\beta$  lactoglobulin and casein.

These model experiments are part of our investigations on the modified anaphylactic hypothesis for cot death. (Parish *et al.*, 1960), which maintains that a percentage of infants are sensitized by drinking cows'

milk and die with a modified anaphylactic reaction in their sleep, after inhaling a small amount of re-gurgitated stomach contents containing cows' milk protein. The model experiments are likely also to have relevance to cows' milk protein intolerance in infants.

## MATERIALS AND METHODS

*Guinea pigs.* Female Dunkin-Hartley guinea-pigs, aged 2-4 weeks on arrival from various commercial breeders, were used. The age of the guinea pigs at the start of each experiment is mentioned in the text.

*Diet.* RGP guinea-pig pellet diet (Rank, Hovis, McDougall). Although milk proteins are not listed as constituents of this diet, each bag had to be tested in Oucherlony tests for the presence of milk proteins. Contamination often follows in the pelleting process. Only bags shown to be free of milk proteins were fed.

*Milk fed.* Whole cows' milk 'Long Life' (UHT). Whole cows' milk raised to 140°C for 1-2 sec. This was the milk used in our previous experiments (Coombs *et al.*, 1978).

*Whole cows' milk (spray dried).* Self explanatory.

*Whole cows' milk ('Evaporated').* Whole cows' milk evaporated to one third of its volume by heat treatment. Nos. 1, 2 and 3 were from different manufacturers. No. 3 appeared to show a trace of caramelization suggesting a more prolonged heating process.

*Infant Formula 1 (spray dried).* A mixture of skimmed cows' milk, lactose and vegetable oils—spray dried—with supplementary minerals and vitamins.

*Infant Formula 2 (spray dried).* A mixture of electro dialysed heat-concentrated whey, skimmed cows' milk, lactose and vegetable fats, spray-dried—with supplementary minerals and vitamins.

*Infant Formula 2 (liquid concentrate).* As above but the concentrated liquid mixture is not spray-dried but diluted with the appropriate amount of water so that it is twice the concentration recommended for feeding to infants. It is then sterilized by heating.

All milks were fed as recommended for infant feeding, at approximately comparable protein concentrations (20 mg/ml protein—Lowry estimation).

*Milk proteins for challenge.*  $\beta$  lactoglobulin and casein were obtained from the National Institute for Research in Dairying, Shinfield.

*Challenge for anaphylactic sensitivity.* The intravenous challenge consisted of 0.5 ml of protein at a concentration of 6.0 mg/ml.

*Passive cutaneous anaphylaxis reaction (PCA).* These were performed as described previously (Coombs *et al.*, 1978). The guinea-pigs were bled by cardiac puncture on days 22 and 35 of the experiment.

*Passive haemagglutination using coated tanned red cells and red cell linked antigen antiglobulin reaction.* As used in our previous report (Coombs *et al.*, 1978).

## RESULTS

### *Experiment 1: anaphylactic sensitivity (vis à vis $\beta$ lactoglobulin and casein) of guinea-pigs drinking different cows' milk preparations for 43 days*

Groups of fifteen guinea-pigs (aged 3-5 weeks) were given to drink the different milk formulae recorded in Table 1 for 43 days. They were bled for serum on days 24 and 38. On day 43 the guinea-pigs were challenged for anaphylactic sensitivity by intravenous injection of either  $\beta$  lactoglobulin (ten guinea-pigs) or casein (five guinea-pigs).

The results of the intravenous challenge are recorded in Table 1. The guinea-pigs drinking water were completely unaffected. Only four guinea-pigs were challenged as similar controls have been incorporated many times. The guinea-pigs drinking Infant Formula 1 (spray-dried) behaved more or less as anticipated from the previous experiments with 'Long Life' milk, although the reactions were not as strong or uniform as found previously. In previous experiments, however, the challenge had been with the whole milk product under test and not simply with the isolated component proteins. Although the numbers were small, Infant Formula 2 (spray-dried) appeared to be less sensitizing with regard to both  $\beta$  lactoglobulin and casein.

The most significant finding was that guinea-pigs drinking whole cows' milk ('Evaporated') No. 1 developed no anaphylactic sensitivity to  $\beta$  lactoglobulin, although they did to casein.

A further check on this pattern of sensitization was obtained by examination of the 24th and 38th day sera in PCA reactions. The results of these tests are given in Table 2. They corroborate the findings seen with direct intravenous challenge, although they are a less sensitive measure of sensitization. This is due

TABLE 1. Anaphylaxis after intravenous challenge of guinea-pigs drinking different preparations of cows' milk for 43 days

Milk drunk	Challenged with:	Results of challenge				
		Numbers			Percentage anaphylactic to:	
		Unaffected	Non-fatal anaphylaxis	Fatal anaphylaxis	$\beta$ lactoglobulin	Casein
Water only (controls)	$\beta$ lactoglobulin	2	0	0	0	
	Casein	2	0	0		0
Infant Formula 1 (spray-dried)	$\beta$ lactoglobulin	4	1	6	64	
	Casein	0	1	3		100
Infant Formula 2 (spray-dried)	$\beta$ lactoglobulin	4	4	2	60	
	Casein	3	0	2		40
Whole cows' milk ('Evaporated') No. 1	$\beta$ lactoglobulin	6	0	0	0	
	Casein	0	2	3		100
	$\beta$ lactoglobulin after casein	0	3	2		100

TABLE 2. Anaphylactic status (as measured by ability of sera\* to give PCA reactions) of guinea-pigs drinking different preparations of cows' milk

Milk drunk	Days on milk	Results of challenge					
		Numbers of sera giving positive PCA reactions to:			Percentage of sera with anaphylactic antibodies to:		
		$\beta$ lactoglobulin	Casein	Milk†	$\beta$ lactoglobulin	Casein	Milk†
Water only (controls)	24	0/5	0/5	0/5	0	0	0
	38	0/5	0/5	0/5	0	0	0
Infant formula (spray-dried) 1	24	7/14	7/12	9/15	50	58	60
	38	10/15	11/15	10/14	67	73	71
Infant formula (spray-dried) 2	24	2/15	3/12	4/15	13	25	27
	38	3/15	4/12	6/15	20	33	40
Whole cows' milk ('Evaporated') No. 1	24	0/11	4/9	5/11	0	44	45
	38	0/11	7/10	8/11	0	70	73

These results were obtained with the sera of guinea-pigs recorded in Table 1.

\* Sera diluted  $\frac{1}{2}$  ( $\frac{1}{4}$  in a few cases).

† Whole cows' milk, 'Long Life' (UHT).

partly to the nature of the test and to the fact that the sera were used diluted  $\frac{1}{2}$  (and even  $\frac{1}{4}$  in some cases). It was a general finding that the numbers of animals sensitized and the intensity of the reactions increased from day 24 to day 38.

The results of the PCA reactions also showed that whole cows' milk ('Evaporated') No. 1 is non-sensitizing *per os* with regard to  $\beta$  lactoglobulin. Again, Infant Formula 2 (spray-dried) was less sensitizing than Infant Formula 1 (spray-dried); this was shown more clearly with the PCA tests than by direct challenge procedure.

As was found by direct challenge, whole cows' milk ('Evaporated') No. 1 did sensitize to casein and was apparently more sensitizing for this protein than was Infant Formula 2 (spray-dried).

*Experiment 2: a further experiment to confirm that whole cows' milk ('Evaporated') No. 1 is not sensitizing per os to  $\beta$  lactoglobulin*

The findings in Experiment 1 were so important that they had to be confirmed, or otherwise, in a further experiment. Also, as the degree of sensitization with the two spray-dried whole cows' milks tested in that first experiment did not seem to be as strong as might be expected from our previous investigations (Coombs *et al.*, 1978) which had utilized 'Long Life' whole cows' milks, a group of guinea-pigs drinking that milk was also included.

Five groups of ten guinea-pigs each (approximately 8–10 weeks old at the beginning of the experiment) were set up. One group acted as a control and had water to drink. Three groups respectively had the milks which were used in Experiment 1 and the fifth group had 'Long Life' whole cows' milk to drink.

As in Experiment 1, all the guinea-pigs were bled twice (on days 22 and 35) for subsequent PCA tests on the sera. On the 37th day all the guinea-pigs were challenged with  $\beta$  lactoglobulin.

The results of the anaphylactic challenge are recorded in Table 3. Of the guinea-pigs drinking 'Long Life' whole cows' milk, Infant Formula 1 and Infant Formula 2, 100% showed anaphylaxis, although the figures for fatal anaphylaxis were 90%, 80% and 30%, respectively. In confirmation of the findings in Experiment 1, only one out of ten guinea-pigs fed whole cows' milk ('Evaporated') 1 showed anaphylaxis on challenge with  $\beta$  lactoglobulin and this one reaction was not fatal.

TABLE 3. Anaphylaxis in guinea-pigs drinking different preparations of cows' milk for 37 days, on intravenous challenge with  $\beta$  lactoglobulin—a further experiment

Milk drink	Challenged with:	Results of challenge			
		Numbers:			
		Unaffected	Non-fatal anaphylaxis	Fatal anaphylaxis	Percentage anaphylaxis
Water only (controls)	$\beta$ lactoglobulin	5	0	0	0
Whole cows' milk 'Long Life' (UHT)	$\beta$ lactoglobulin	0	1	9	100
Infant Formula 1 (spray-dried)	$\beta$ lactoglobulin	0	1	8	100
Infant Formula 2 (spray-dried)	$\beta$ lactoglobulin	0	6	3	100
Whole cows' milk ('Evaporated') No. 1	$\beta$ lactoglobulin	9	1	0	10

Examination of the sera of these guinea-pigs by the PCA reaction (see Table 4) adequately substantiate the findings on direct challenge and those in Experiment 1. Whole cows' milk ('Evaporated') No. 1 was non-sensitizing to  $\beta$  lactoglobulin. 'Long Life' whole cows' milk (UHT) and Infant Formula 1 were more or less equally sensitizing to this protein, and Infant Formula 2 very much less.

On the other hand, all the milk preparations were sensitizing to casein, with possibly Infant Formula 2 slightly less so.

*Experiment 3: showing the anaphylactogenic property of whole cows' milk ('Evaporated') No. 1 following intraperitoneal injection*

The finding in Experiments 1 and 2 that whole cows' milk ('Evaporated') No. 1 did not sensitize to  $\beta$  lactoglobulin via the oral route was surprising as  $\beta$  lactoglobulin could be shown in this milk by Ouchterlony tests. It could also be shown by the more sensitive and semi-quantitative inhibition of passive haemagglutination (Boyden test), although the concentration (inhibition of four doses of anti- $\beta$  lactoglobulin with a 1/40 dilution of the milk) was 4–8 times less than that in two spray-dried whole cows' milk products.

To test the actual anaphylactogenic property of the  $\beta$  lactoglobulin in whole cows' milk ('Evaporated') No. 1, groups of ten guinea-pigs each were injected intraperitoneally with increasing doses of this

TABLE 4. Anaphylactic status (as measured by ability of sera\* to give PCA reactions) of guinea-pigs† drinking different preparations of cows' milk

Milk drunk	Days on milk	Results of challenge					
		Numbers of sera giving positive PCA reactions to:			Percentage of sera with anaphylactic antibodies to:		
		$\beta$ lactoglobulin	Casein	Milk‡	$\beta$ lactoglobulin	Casein	Milk‡
Water only (controls)	22	0/9	0/9	0/9	0	0	0
	35	0/9	0/5	0/9	0	0	0
Whole cows' milk 'Long Life' (UHT)	22	8/9	8/8	10/10	89	100	100
	35	9/10	9/9	10/10	90	100	100
Infant Formula 1 (spray-dried)	22	6/9	9/9	8/10	67	100	100
	35	9/9	9/9	9/9	100	100	100
Infant Formula 2 (spray-dried)	22	2/10	5/8	5/9	20	62	56
	35	2/10	6/6	7/7	20	100	100
Whole cows' milk ('Evaporated') No. 1	22	0/10	8/9	10/10	0	89	100
	35	0/9	7/7	8/9	0	100	89

\* Day 22 sera diluted 1/2; day 35 sera diluted 1/4.

† Guinea-pigs recorded in Table 3.

‡ Whole cows' milk, 'Long Life' (UHT).

'Evaporated' milk. A further group was injected intraperitoneally with 2.0 ml of 'Long Life' whole cows' milk as a positive control. As in the earlier experiments, the guinea-pigs were bled to obtain serum for PCA testing, this time on day 35. On day 37, the guinea-pigs were challenged with either  $\beta$  lactoglobulin or casein with the results recorded in Table 5, which also includes the results of the PCA testing.

It can be seen that whole cows' milk ('Evaporated') No. 1 proved to be an adequate sensitizer to  $\beta$  lactoglobulin via the intraperitoneal route. Sensitization to casein was also produced.

#### *Experiment 4: drinking experiments with two further examples of 'Evaporated' milks (Nos 2 and 3) and two other modified milks*

It was very important to see if this lack of, or extremely low, sensitizing capacity *per os* of whole cows' milk ('Evaporated') No. 1 to  $\beta$  lactoglobulin also held for other 'Evaporated' milk products. A similar experiment to Experiment 2 was therefore set up with two further 'Evaporated' milks, Nos 2 and 3, as well as the No. 1 tested in the previous experiments.

'Long Life' whole cows' milk and Infant Formula 1 (spray-dried) were re-tested as positive controls and two other milk products were also examined—a spray-dried whole cows' milk and a liquid concentrate of Infant Formula 2.

There were eight groups of guinea-pigs, seven drinking different milk preparations (see Table 6) and a negative control group drinking water. As before, all guinea-pigs were bled on days 22 and 35 and challenged intravenously with  $\beta$  lactoglobulin on day 37. Animals not dying on challenge with  $\beta$  lactoglobulin were re-challenged approximately 4 hr later with casein so that sensitivity to both proteins could be measured.

From the results of direct challenge (Table 6) and PCA tests (Table 7) it is clear that the three 'Evaporated' milks behaved similarly in not sensitizing to  $\beta$  lactoglobulin.

A further significant finding was that whole cows' milk ('Evaporated') No. 3 had a greatly reduced sensitizing capacity to casein. Evaporated milk No. 2 was again less sensitizing to casein than No. 1, (the product used in the earlier experiments). The liquid concentrated form of Infant Formula 2 behaved similarly to the 'Evaporated' milks in having very low sensitizing capacity for  $\beta$  lactoglobulin and casein.

TABLE 5. Anaphylactic status of guinea-pigs (injected intraperitoneally with whole cows' milk ('Evaporated') No. 1) as judged by either PCA tests on their sera (day 35) or direct intravenous challenge with antigen (day 37)

Milk injected intraperitoneally	Anaphylactic status assessed by:	Antigen of challenge	Results of challenge				
			Numbers of guinea-pigs:			Percentage of guinea-pigs anaphylactic to:	
			Unaffected	Non-fatal	Anaphylaxis Fatal	$\beta$ lactoglobulin	Casein
None (control)	PCA	$\beta$ lactoglobulin	10		0	0	
	Direct challenge		10	0	0	0	
	PCA	casein	10		0		0
	Direct challenge		2	0	0		0
Whole cows' milk ('Evaporated') No. 1 0.5 ml	PCA	$\beta$ lactoglobulin	3		7	70	
	Direct challenge		2	2	6	80	
	PCA	Casein	1		9		90
	Direct challenge		1	1	2		75
Whole cows' milk ('Evaporated') No. 1 1 ml	PCA	$\beta$ lactoglobulin	0		2	100	
	Direct challenge		0	2	1	100	
	PCA	Casein	0		1		
	Direct challenge		0	1	0		
Whole cows' milk ('Evaporated') No. 1 2.0 ml	PCA	$\beta$ lactoglobulin	0		10	100	
	Direct challenge		0	4	4	100	
	PCA	Casein	0		10		100
	Direct challenge		2	0	2		50
Whole cows' milk ('Evaporated') No. 1 4.0 ml	PCA	$\beta$ lactoglobulin	0		10	100	
	Direct challenge		0	4	6	100	
	PCA	Casein	0		10		100
	Direct challenge		1	0	2		67
Whole cows' milk 'Long Life' (UHT) 2.0 ml	PCA	$\beta$ lactoglobulin	0		10	100	
	Direct challenge		0	0	8	100	
	PCA	Casein	0		10		100
	Direct challenge		n.d.		n.d.		

n.d. = Not done.

As expected the spray-dried cows' milk was as sensitizing to both  $\beta$  lactoglobulin and casein as the 'Long Life' whole cows' milk.

## DISCUSSION

We had hoped to find differences in the sensitizing capacities of various preparations of cows' milk and infant formulae but had not anticipated the rather spectacular findings now recorded. That certain preparations do not sensitize to  $\beta$  lactoglobulin may be specially important should the modified anaphylaxis hypothesis hold for cot death, for we have some evidence that this protein could be the main operative allergen.

As infants in all probability become sensitized following absorption of sensitizing proteins via the gut, it is appropriate, and in fact essential, to test the sensitizing capacity of milk preparations in the guinea-pig via this route. Most previous investigations on the sensitizing capacity of different milk preparations have used the parenteral route for sensitization which neglects to allow for any differential digestion of modified proteins in the gut itself. In the present study whole cows' milk ('Evaporated') No. 1 which is relatively non-sensitizing to  $\beta$  lactoglobulin *per os* is nevertheless capable of sensitizing by intraperitoneal injection.

TABLE 6. Low sensitizing capacity *per os* of evaporated liquid milks as a category of milk preparations. Anaphylaxis in guinea-pigs (drinking different preparations of cows' milk for 37 days) after i.v. challenge with either  $\beta$  lactoglobulin or casein

Milk drunk	Challenged with:	Results of challenge				
		Unaffected	Numbers		Percentage anaphylactic to:	
			Non-fatal anaphylaxis	Fatal anaphylaxis	$\beta$ lactoglobulin	Casein
Water only (control)	$\beta$ lactoglobulin	5	0	0	0	
	Casein after $\beta$ lactoglobulin	5	0	0		0
Whole cows' milk 'Long Life' (UHT)	$\beta$ lactoglobulin	2	2	6	80	
	Casein after $\beta$ lactoglobulin	2	0	0		
Whole cows' milk (spray-dried)	$\beta$ lactoglobulin	0	2	8	100	
	Casein after $\beta$ lactoglobulin	1	1	0		50
Infant Formula 1 (spray-dried)	$\beta$ lactoglobulin	1	0	4	80	
	Casein after $\beta$ lactoglobulin	1	0	0		
Infant Formula 2 (Liquid concentrate)	$\beta$ lactoglobulin	9	1	0	10	
	Casein after $\beta$ lactoglobulin	5	0	0		0
Whole cows' milk ('Evaporated') No. 1	$\beta$ lactoglobulin	5*	0	0	0	
	Casein after $\beta$ lactoglobulin	2	1	2		60
('Evaporated') No. 2	$\beta$ lactoglobulin	10	0	0	0	
	Casein after $\beta$ lactoglobulin	3	2	0		40
('Evaporated') No. 3	$\beta$ lactoglobulin	10	0	0	0	
	Casein after $\beta$ lactoglobulin	4	1	0		10

\* Two of these five guinea-pigs gave a definite but extremely weak reaction.

TABLE 7. Low sensitizing capacity *per os* of evaporated liquid milks as a category of milk preparations. Anaphylactic status of guinea-pigs\* (drinking different preparations of cows' milk for 37 days) as measured by ability of sera† to give PCA reactions

Milk drunk	Days on milk	Results of challenge			
		Numbers of sera giving positive PCA reactions to:		Percentage of sera with anaphylactic antibodies to:	
		$\beta$ lactoglobulin	Casein	$\beta$ lactoglobulin	Casein
Water only (controls)	22	0/10	0/10	0	0
	35	0/10	0/10	0	0
Whole cows' milk 'Long Life' (UHT)	22	n.d.	n.d.		
	35	8/10	8/10	80	80
Whole cows' milk (spray-dried)	22	9/10	3/10	90	30
	35	10/10	5/10	100	50
Infant Formula 1 (spray-dried)	22	4/5	1/5	80	20
	35	4/5	3/5	80	60
Infant Formula 2 (Liquid concentrate)	22	1/10	1/10	10	10
	35	1/10	3/10	10	30
Whole cows' milk ('Evaporated') No. 1	22	1/5	4/5	20	80
	35	1/5‡	4/5	20	80
('Evaporated') No. 2	22	0/10	2/10	0	20
	35	0/10	4/10	0	40
('Evaporated') No. 3	22	0/10	0/10	0	0
	35	0/10	2/10	0	20

\* Guinea-pigs recorded in Table 6.

† Sera diluted 1/2.

‡ Besides this one reaction two other guinea-pigs gave extremely weak and faint reactions.

n.d. = Not done.

The outstanding finding in this investigation is this extremely low sensitizing capacity of the 'Evaporated' milks for  $\beta$  lactoglobulin and also, to varying degrees, for casein. Whole cows' milk ('Evaporated') No. 3—which incidentally from its colour appeared to be the most caramelized (suggesting more prolonged or heightened heat-treatment)—was totally free of sensitizing capacity to  $\beta$  lactoglobulin and practically free of sensitizing capacity to casein.

Infant Formula 2 (spray-dried) was less sensitizing to both  $\beta$  lactoglobulin and casein than Infant Formula 1 (spray-dried) (see Tables 1, 2, 3 and 4), and this same Formula 2 in liquid concentrate form had an extremely low sensitizing capacity to both proteins behaving in this respect very like cows' milk ('Evaporated') No. 3. Presumably the manufacturing process used to achieve the liquid concentrate form was responsible for the further reduction in the sensitizing capacity of this particular feeding formula.

We should stress that the sensitization being discussed in this paper refers specifically to tissue-sensitizing antibodies mediating Type 1 allergic reactivity, and to these antibodies only. This was also the case with regard to the 'tolerance' in very young guinea-pigs reported in our previous paper (Coombs *et al.*, 1978). Preliminary tests for IgM and IgG antibodies in guinea-pigs fed either whole dried milk (spray-dried) or whole dried milk ('Evaporated') No. 3 do not show the clear differences recorded with respect to anaphylactic antibody. Further, in the present experiments we have discussed the lack of sensitizing capacity of certain feeding formulae, yet we cannot exclude the possibility that some state of specific immunological tolerance may have been set up, as found (Coombs *et al.*, 1978) when fully sensitizing cows' milk was fed to baby guinea-pigs on their first or second day of life. This is presently under investigation.

It is now important, despite the difficulties, to investigate whether the pattern of sensitization being obtained in guinea-pigs is echoed in the human infant. Selection of the least sensitizing milk preparations for infant feeding must be important in the prevention and treatment of milk protein intolerance, and in the prevention of cot death, should the modified anaphylaxis hypothesis ultimately be shown to be the cause of these deaths.

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