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Refractoriness to anaphylactic shock after continuous feeding of cows' milk to guinea-pigs

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SUMMARY

Further studies have been undertaken on the anaphylactic sensitization to cows' milk in guinea-pigs given cows' milk to drink instead of water. With continuous feeding over a prolonged period of time (over 70 days), about 70% of the guinea-pigs became refractory to intratracheal challenge of cows' milk and about 30% to intravenous challenge. Some guinea-pigs given cows' milk from the second day of life appeared not to undergo an early sensitization at all and may have been displaying a tolerance to anaphylactic sensitization. The degree to which this was shown may be genetic. The anaphylactic sensitivity, particularly as regards intravenous challenge, could be assessed by examining the sera for IgG1a antibodies to cows' milk by passive cutaneous anaphylaxis.

INTRODUCTION

In a previous paper we have shown that guinea-pigs fed a normal pellet diet but given cows' milk to drink *ad libitium* become sensitive to anaphylaxis and may be shocked by either intravenous or intratracheal challenge with cows' milk (Devey *et al.*, 1976). These studies have been continued and the effects of long-term feeding of cows' milk and of feeding cows' milk from very early in life have been investigated. With continuous feeding over a prolonged period of time there is evidence that some guinea-pigs develop resistance to anaphylactic shock on intratracheal challenge and, to a lesser extent, on intravenous challenge. The object of this present report has been to define the time of onset of this refractoriness to anaphylaxis, its extent and characteristics, as the same phenomenon may well operate in human infants fed on cows' milk.

MATERIALS AND METHODS

Animals. Female guinea-pigs aged between 2 and 4 weeks were obtained from a random-bred Dunkin–Hartley strain. New-born guinea-pigs (Dunkin–Hartley, Frant, from a closed breeding colony, and inbred Heston–Birmingham) of both sexes were removed from their mothers 2 days after birth and immediately placed on the experimental diet.

Diets. Guinea-pigs were fed on a commercial pelleted diet that had been shown to be free of cows' milk (except in experiment 1) by immunological testing. Water or cows' milk was given to drink as indicated.

Cows' milk. Unigate 'Longlife' cow's milk (heated to 140°C for 1–2 sec) was used for feeding and for challenge. For the latter the milk was spun at 1400 rev/min and the middle layer used for injection.

Challenge for anaphylactic sensitivity. Intravenous (i.v.) injections of cows' milk (0·01 ml/g body weight up to a maximum of 0·5 ml) in conscious animals and intratracheal (i.t.) injections (0·2–0·3 ml) in animals lightly anaesthetized with Nembutal® were carried out as described by Devey *et al.* (1976).

Passive cutaneous anaphylaxis (PCA). Sera from milk-fed guinea-pigs were injected intradermally into sites on closely shaven guinea-pigs fed a cows' milk-free diet. The animals were challenged i.v. with 0·25 ml cows' milk and 0·25 ml 2% Evans blue dye after an interval of 4 hr for IgG1a, after 5 days for IgG1b and after 9 days for IgE (Parish, 1970). After 30

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min the animals were killed and the size and intensity of the skin reactions measured. Sera were also tested after heat treatment at 56°C for 1 hr and after treatment with 2-mercaptoethanol.

Schultz–Dale reactions. Milk-fed guinea-pigs were starved overnight and killed by a blow to the base of the skull. The terminal ileum was carefully removed and its lumen thoroughly washed with Tyrode's solution. Pieces of about 2·5 cm in length were suspended in a Schultz–Dale tissue bath containing oxygenated Tyrode's solution at 37°C. High and low doses of histamine were delivered to the bath using a modification of the automatic apparatus of Boura, Mongar & Schild (1954) and contractions were monitored using a pen recorder. Challenge was with various dilutions of cows' milk in Tyrode's solution.

Measurement of specific antibodies to cows' milk. Specific antibodies to cows' milk were measured by passive haemagglutination of tanned cells (mainly for IgM antibodies) and by the red cell-linked antigen–antiglobulin reaction, using a rabbit antiglobulin raised to a DEAE–IgG fraction of guinea-pig serum, but it was not monospecific for IgG (anti-7S globulins) as described previously (Devey *et al.*, 1976).

RESULTS

Evidence for refractories to anaphylaxis following continuous feeding of cows' milk to four-week-old guinea-pigs

Experiment 1. Eighty guinea-pigs (female Dunkin–Hartley) aged about 4 weeks were fed a pellet diet and given cows' milk to drink *ad libitium.* Twenty control guinea-pigs were given water to drink instead of cows' milk. At intervals the animals were challenged with cows' milk either i.v. or i.t. The results are shown in Table 1. As shown previously (Devey *et al.*, 1976), there was uniform sensitivity to fatal anaphylaxis following either i.v. or i.t. challenge with cows' milk after 14 days on the milk diet. However, on day 55 of drinking milk, although four animals challenged i.v. died, only one animal challenged i.t. died with two showing only mild reactions, such as short periods of apnoea, and three showed no reaction at all to challenge. Between day 71 and 86 of drinking milk the majority of animals showed refractoriness to fatal anaphylaxis after both i.v. and i.t. challenge. After 93 days, although most animals were still resistant to i.t. challenge, only one out of twelve animals survived the i.v. challenge. Control guinea-pigs, given water to drink, were challenged throughout the experiment and although most showed no reaction, occasionally very mild reactions such as peripheral irritation were noted. This suggested that the pellet diet was not entirely free of cows' milk and this was confirmed by immunological testing.

Unfortunately, at this stage of the experiment most of the guinea-pigs were found to be pregnant (due to four rogue males!) and so the results were complicated by the possible effects that pregnancy is thought

TABLE 1. Refractoriness to fatal anaphylaxis after long-term feeding of cows' milk to Dunkin–Hartley guinea-pigs (experiment 1)

Days fed cows' milk	Route of cows' milk challenge	Results of challenge			Percentage dead	Status
		Unaffected	Non-fatal reaction	Dead		
28*	i.v.	0	0	2	100 (100)†	Sensitive
55	i.v.	0	0	4		
71	i.v.	0	2	1	22 (78)†	Refractory
86	i.v.	2	3	1		
93	i.v.	1	0	11	92 (92)†	?
28*	i.t.	0	0	4	100 (100)†	Sensitive
55	i.t.	3	2	1		
71	i.t.	3	1	2		
86	i.t.	4	2	0	16 (40)†	Refractory
93	i.t.	4	0	1		
97	i.t.	1‡	1‡	0		

* Large numbers of guinea-pigs had shown fatal anaphylaxis at this stage previously (Devey *et al.*, 1976).

† Percentage either dead or manifesting definite anaphylaxis.

‡ Male guinea-pigs.

to have on anaphylaxis (Gluck & Gluck, 1976). Nevertheless, after 97 days of drinking cows' milk, the two surviving male guinea-pigs survived i.t. challenge, with one showing no reaction at all. This suggested that the refractoriness to anaphylaxis was indeed due to the length of time for which milk was fed. However, because of the complication of pregnancy, it was decided to repeat the experiment.

Experiment 2. This was essentially the same as experiment 1, except that all the young female guinea-pigs (Dunkin–Hartley) were very carefully screened for male animals and it was ensured that the pellet diet was entirely free of cows' milk proteins. Eighty female guinea-pigs aged about 4 weeks were given a pelleted diet and allowed to drink cows' milk *ad libitium*. Control guinea-pigs were given water to drink instead of milk. Animals were challenged either i.v. or i.t. as indicated in Table 2.

TABLE 2. Refractories to fatal anaphylaxis after long-term feeding of cows' milk to Dunkin–Hartley guinea-pigs (experiment 2)

Days fed cows' milk	Route of cows' milk challenge	Result of challenge			Percentage Dead	Status
		Unaffected	Non-fatal reaction	Dead		
12	i.v.	2	0	0	0 (0)*	Presensitive
22	i.v.	0	0	4	100 (100)*	Sensitive
41	i.v.	0	0	4		
83	i.v.	1	0	4		
92	i.v.	0	1	2	69 (85)*	Refractory
104–118	i.v.	1	1	3		
12	i.t.	3	0	0	0 (0)*	Pre-sensitive
22–30	i.t.	2	0	11		
40–44	i.t.	1	5	13	77 (87)*	Sensitive
62	i.t.	3	0	12		
71	i.t.	3	1	1		
83	i.t.	2	0	3	28 (50)*	Refractory
92–118	i.t.	4	3	1		

* Percentage either dead or manifesting definite anaphylaxis.

In the pre-sensitization period (before day 14), five out of five animals showed no reaction to either i.v. or i.t. challenge of cows' milk. Between days 22 and 41 of drinking milk, 100% of the animals died on i.v. challenge and between days 22 to 62 almost 80% died on i.t. challenge. Some of the animals that survived the i.t. challenge went into long periods of apnoea (up to 2 min), but then started breathing again spontaneously. If allowed to recover, these guinea-pigs appeared to be perfectly well, with very little sign that anything untoward had occurred. After 70 days of drinking milk there was a dramatic drop to 28% in the number of animals dying after i.t. challenge, and 50% showed absolutely no reaction at all on challenge. This refractoriness to anaphylaxis also occurred, but to a lesser extent, with i.v. challenge, with over 30% of the animals surviving. This experiment confirmed the findings in experiment 1 in that, after prolonged feeding of cows' milk, guinea-pigs may enter a refractory state in which they are resistant to fatal anaphylaxis, particularly after i.t. challenge of cows' milk.

PCA reactions. The PCA reactions were performed with sera from guinea-pigs fed cows' milk in experiment 2 to determine the properties and class of antibody responsible for anaphylaxis and to attempt to elucidate the mechanism of refractoriness to challenge. Sera from these animals sensitized normal guinea-pig skin optimally for PCA reactions at 4 hr, but not at 5 or 9 days. These skin-sensitizing antibodies were resistant to both heat and 2-mercaptoethanol treatment, suggesting that anaphylaxis was due to IgG1a antibodies, not IgG1b or IgE. The results are shown in Table 3. Between days 40 and 69 (the sensitive period for challenge), all the sera gave positive PCA reactions. From day 92 onwards (the refractory period), over 30% of the sera gave negative PCA reactions. By taking weekly blood samples from the same guinea-pigs, it was shown that sera from several animals giving positive PCA reactions in

TABLE 3. 4 hr PCA reactions (IgG1a) with sera of Dunkin–Hartley guinea-pigs fed cows' milk from 4 weeks of age (experiment 2)

Days fed cows' milk	Negative PCA	Positive PCA	Percentage positive	Status
9	4	0	0	Pre-sensitive
40–69	0	7	100	Sensitive
92–114	9	18	67	Refractory

the sensitive period subsequently became negative, suggesting that there was a 'turn-off' in IgG1a antibody production. By comparing the PCA reactions (Table 3) with the response to challenge (Table 2), it may be seen that there was a good correlation between the percentage of animals giving positive PCA reactions and the percentage dying after i.v. challenge. However, about half the animals whose sera gave positive PCA reactions survived the i.t. challenge, suggesting that there was another factor besides the 'turn-off' of anaphylactic antibody production that caused refractoriness to challenge in the lung.

The refractory state in guinea-pigs fed cows' milk from the second day of life

These experiments were carried out in an attempt to get closer to the situation that may exist in human infants who may be fed cows' milk from very early in life. We also wanted to see if earlier feeding of cows' milk affected the class of anaphylactic antibody produced, as it has been reported that injections of antigen, especially if alum-precipitated, in neonatal rabbits favour IgE antibody production (Pinckard, Halonen & Meng, 1972).

Experiment 3. New-born Dunkin–Hartley guinea-pigs (male and female) were removed from their mothers 2 days after birth. They were immediately transferred, without difficulty, to a solid pellet diet with cows' milk to drink *ad libitium*. Cows' milk challenge was as before, either i.v. in conscious animals or i.t. under light anaesthesia. The results are shown in Table 4. Between days 25 and 29, when there was 100% fatal anaphylaxis on i.v. challenge in guinea-pigs given milk from 4 weeks of age, only 75% of these animals died on i.v. challenge. There was also 38% survival after i.t. challenge during this period. After a longer period (see Table 4), there was evidence for loss of tolerance and a later development still of refractoriness.

PCA reactions were performed as before, and again it appeared that IgG1a antibodies (as defined by PCA reactivity) were responsible for anaphylaxis. IgE antibodies to cows' milk were not detected. The

TABLE 4. Cows' milk challenge in Dunkin–Hartley guinea-pigs fed cows' milk from the second day of life (experiment 3)

Days fed cows' milk	Route of cows' milk challenge	Result of challenge: Unaffected	Non-fatal reaction	Dead	Percentage dead	Status
8–10	i.v.	3	0	1*	25 (25)†	Pre-sensitive
25–29	i.v.	2	0	6	75 (75)†	25% 'tolerant'
50–58	i.v.	0	1	7	87 (100)†	? Loss of 'tolerance'
81–87	i.v.	2	1	4	57 (71)†	? Some refractoriness
25–29	i.t.	3	0	5	62 (62)†	38% 'tolerant'
43–58	i.t.	0	0	7	100 (100)†	? Loss of 'tolerance'
85–97	i.t.	6	1	0	0 (14)†	? Refractory

* Death appeared to be anaphylactic at post-mortem.

† Percentage either dead or manifesting definite anaphylaxis.

results in Table 5 show that 27% of the guinea-pigs fed cows' milk from day 2 of life did not appear to produce skin-sensitizing antibodies to milk between days 16 and 37 (falling to 10% between days 41 and 46). Therefore refractoriness to both i.v. and i.t. challenge was probably mainly due to the lack of anaphylactic antibody, rather than some factor protecting against the effect of anaphylactic antibody after lung challenge, as in experiment 2.

Guinea-pigs continued on the diet beyond 80 days again showed development of refractoriness—43% after i.v. injection and 100% after i.t. injection.

In further experiments, guinea-pigs of a different strain had to be used because of problems of supply.

TABLE 5. 4 hr PCA reactions (IgG1a) with sera of Dunkin–Hartley guinea-pigs fed cows' milk from the second day of life (experiment 3)

Days fed cows' milk	Negative PCA	Positive PCA	Percentage positive	Status
7–13	12	2*	14	Pre-sensitive
16–37	16	43	73	27% 'tolerant'
41–46	1	10	90	Loss of 'tolerance'

* One of these guinea-pigs was challenged on day 10 and died.

TABLE 6. Refractoriness to fatal anaphylaxis after feeding cows' milk to Frant guinea-pigs from the second day of life (experiment 4)

Days fed cows' milk	Route of cows' milk challenge	Results of challenge: Unaffected	Non-fatal reaction	Dead	Percentage dead	Status
13	i.v.	1	0	0	0 (0)*	Pre-sensitive
16–39	i.v.	11	3	5	26 (42)*	60% 'tolerant'
46–57	i.v.	2	0	5	45 (64)*	? Breakdown of tolerance
66	i.v.	2	2	0		
83–91	i.v.	3	2	10	60 (80)*	? Further breakdown of tolerance ? Some refractoriness
13	i.t.	1	0	0	0 (0)*	Pre-sensitive
16–39	i.t.	11	2	0	0 (15)*	85% 'tolerant'
46–57	i.t.	4	1	3	27 (36)*	? Breakdown of 'tolerance'
66	i.t.	3	0	0		
85–93	i.t.	9	0	0	0 (0)*	Refractory

* Percentage either dead or manifesting definite anaphylaxis.

Experiment 4. New-born Frant and a small number of inbred Heston–Birmingham guinea-pigs of both sexes were removed from their mothers and given a solid diet with cows' milk to drink from day 2 of life. The cows' milk challenge was as before. The results of challenge in the Frant guinea-pigs are shown in Table 6. Between days 16 and 39 (when there was 100% fatal anaphylaxis on i.v. challenge in experiments 1 and 2), approximately 70% of these guinea-pigs survived i.v. challenge and over 50% showed no reaction at all. During this period of time no guinea-pig died after i.t. challenge and only 15% showed any sign of anaphylaxis. After day 40, there was an increase to 45% in the number of guinea-pigs dying after i.v. challenge and to 27% after i.t. challenge, and also in the number of guinea-pigs

showing nonfatal reactions. Table 7 shows the result of challenge in Heston–Birmingham guinea-pigs. From the small numbers of animals challenged it appeared that a high percentage, as in the case of the Frant guinea-pigs, were refractory to anaphylaxis between days 28 and 68 of drinking milk. However, after day 80 refractoriness to anaphylaxis (i.v. challenge) appeared to increase rather than decrease, unlike the situation in Frant guinea-pigs. PCA reactions were performed with sera from these guinea-pigs and again all the skin-sensitizing activity appeared to consist of IgG1a antibodies. Tables 8 and 9 show that IgG1a antibodies to cows' milk were only detected in 26% Frant and 33% Heston–Birmingham guinea-pigs between days 16 and 37 of drinking cows' milk. Again the presence of skin-sensitizing antibodies correlated quite well with the number of animals dying after i.v. challenge. The PCA results also confirmed that the number of Frant guinea-pigs producing IgG1a antibodies to cows' milk increased with the length of time on milk, whereas the number of Heston–Birmingham guinea-pigs producing IgG1a antibodies decreased. But larger numbers of animals will be required before any generalization can be made.

TABLE 7. Refractoriness to fatal anaphylaxis after feeding cows' milk to Heston–Birmingham guinea-pigs from the second day of life (experiment 4)

Days fed cows' milk	Route of cows' milk challenge	Result of challenge: Unaffected	Non-fatal reaction	Dead	Percentage dead	Status
28–33	i.v.	1	0	1	43 (57)*	43% 'tolerant'
42–68	i.v.	2	1	2		
71–91	i.v.	9	1	0	0 (10)*	90% 'tolerant' or refractory
42–53	i.t.	1	1	0	25 (50)*	50% 'tolerant'
64–68	i.t.	1	0	1		

* Percentage either dead or manifesting definite anaphylaxis.

TABLE 8. 4 hr PCA reactions (IgG1a) with sera of Frant guinea-pigs fed cows' milk from the second day of life (experiment 4)

Days fed cows' milk	Negative PCA	Positive PCA	Percentage positive	Status
12–14	13	2	13	Pre-sensitive
15–38	52	18	26	74% 'tolerant'
45–82	30	18	38	? Breakdown of tolerance

TABLE 9. 4 hr PCA reactions (IgG1a) with sera of Heston–Birmingham guinea-pigs fed cows' milk from the second day of life (experiment 4)

Days fed cows' milk	Negative PCA	Positive PCA	Percentage positive	Status
18–36	8	4	33	67% 'tolerant'
45–74	16	3	16	84% 'tolerant' or refractory

In vitro challenge of guinea-pig ileum with cows' milk

The ileum was removed from Dunkin–Hartley guinea-pigs in experiment 2 during the sensitive period (days 20–60) and during the refractory period (days 90–118) and from Frant and Heston–Birmingham guinea-pigs in experiment 4 whose sera gave either positive or negative PCA reactions between days 30 and 40 of drinking milk. When strong contractions were obtained with standard doses of histamine in a Schultz–Dale tissue bath, a piece of ileum was challenged with 1 : 4–1 : 20,000 dilutions of cows' milk. On challenge, the ilea from guinea-pigs whose sera gave positive PCA reactions gave an immediate contraction (after a few seconds lag), similar to that caused by histamine, followed by a second, longer-lasting contraction, suggestive of Slow Reading Substance A (SRS-A). Ilea from some animals responded to as little as a 1 : 20,000 dilution of cows' milk. After a high dose of cows' milk a second challenge had little effect, indicating that all the mast cells bearing antibody specific for cows' milk had been exhausted. Ilea taken from refractory guinea-pigs with negative PCA reactions showed no reaction on addition of cows' milk, showing that no tissue-sensitizing antibodies to cows' milk were present in the ilea of these guinea-pigs.

Specific antibodies to cows' milk

Table 10 shows the serum titres of specific antibodies to cows' milk proteins in Dunkin–Hartley guinea-pigs fed cows' milk from 4 weeks of age (experiment 2). Two of these animals (8743 and 8747), after 99 days of feeding milk, gave positive PCA reactions and were sensitive to i.v. challenge; two animals (8744 and 8749) were in the refractory state and gave negative PCA reactions, and were resistent to challenge, while one animal (8748) gave a positive PCA reaction, but resisted i.t. challenge. There was very little difference between the titres of directly agglutinating antibodies (IgM) to the cows' milk proteins, alpha-lactalbumin (αLA), beta lactoglobulin (βLG) and casein, nor between the antibodies to casein measured by the guinea-pig anti-7S globulins. However, the two animals that gave negative PCA reactions appeared to have lower titres of 7S antibodies to βLG.

TABLE 10. Specific antibodies to cows' milk in Dunkin–Hartley guinea-pigs fed cows' milk from 4 weeks of age (experiment 2)

				Specific antibodies					
				αLA		βLG		Casein	
Serum	Days fed cows' milk	PCA result	Challenge result	IgM	7S	IgM	7S	IgM	7S
8743	99	+	Dead (i.v.)	8	n.t.*	2	>1024	8	64
8747	99	+	Dead (i.v.)	4	n.t.	4	512	64	16
8748	99	+	Alive (i.t.)	16	n.t.	16	128	32	256
8744	99	−	Alive (i.t.)	64	n.t.	8	8	64	256
8741	99	−	Alive (i.t.)	8	n.t.	4	32	32	128

* Not tested.

The titres of specific antibodies to cows' milk proteins in Frant or Heston–Birmingham guinea-pigs fed milk from 2 days of age (experiment 4) are shown in Table 11. Again, there was little difference between the titres of IgM antibodies to αLA, βLG and casein, nor between 7S antibodies to casein in animals with positive and negative PCA reactions. After 27 days of drinking cows' milk, two Frant guinea-pigs with negative PCA reactions (8553 and 8719) gave extremely high titres of 7S antibodies to βLG, but titres of these antibodies appeared to fall to quite low levels in animals drinking milk for longer periods. As the 7S antiglobulin sera was not monospecific, and specific antiglobulin sera for guinea-pig IgG and IgA were not available, we were not able to characterize the class of these antibodies further. Therefore, it was possible that the high titres of 7S antibody in some cases, for example to βLG in animals fed milk for 27 days, reflected the IgA antibody production. All the guinea-pigs fed

TABLE 11. Specific antibodies to cows' milk in Frant or Heston–Birmingham guinea-pigs fed cows' milk from day 2 of life (experiment 4)

Serum	Days fed cows' milk	PCA result	Challenge result	Specific antibodies					
				αLA		βLG		Casein	
				IgM	7S	IgM	7S	IgM	7S
8638	51	+	Dead (i.t.)	2	n.t.†	8	32	16	16
8631*	52	+	Dead (i.t.)	2	n.t.	2	64	32	128
8548‡	68	+	Dead (i.t.)	8	n.t.	4	0	8	32
8553*	27	–	Alive (i.t.)	2	n.t.	2	>1024	8	2
8719*	27	–	Alive (i.v.)	2	n.t.	4	>1024	32	64
8551*	32	–	Alive (i.v.)	8	n.t.	4	64	32	64
8654*	45	–	Alive (i.t.)	0	n.t.	0	4	4	4
8634*	51	–	Alive (i.t.)	2	n.t.	4	32	4	8
8549‡	68	–	Alive (i.v.)	8	n.t.	4	4	4	32

* Frant guinea-pig.
† Not tested.
‡ Heston–Birmingham guinea-pig.

cows' milk from day 2 of life produced some antibodies to cows' milk, although these were not necessarily skin- or tissue-sensitizing antibodies.

DISCUSSION

As we have previously described (Devey *et al.*, 1976), feeding of cows' milk to Dunkin–Hartley guinea-pigs from 2 to 4 weeks of age causes them to become sensitive to fatal anaphylaxis after either i.v. or i.t. challenge with cows' milk. The antibody responsible for anaphylaxis was shown to be IgG1a, as defined by PCA testing.

With continuous feeding of cows' milk over a prolonged period of time (after about 70 days), 30–70% of the guinea-pigs became resistant to anaphylaxis, either showing only mild signs of anaphylaxis, followed by complete recovery, or in some cases showing no reaction at all. It was shown by PCA testing that this refractoriness was due to a 'turn-off' in the production of IgG1a antibodies to cows' milk. In addition to this, there was also greater refractoriness to i.t. challenge, as compared to i.v. challenge, in guinea-pigs that still had anaphylactic antibodies to cows' milk. This may have been due to greater protection at the lung surface, perhaps due to blocking antibody (possibly IgA).

When Dunkin–Hartley guinea-pigs were allowed to drink cows' milk from 2 days of age, about 25% appeared not to undergo the early sensitization and these may have been displaying a tolerance to anaphylactic sensitization. In other strains of guinea-pig (Frant and Heston–Birmingham) also fed cows' milk from 2 days of age, this 'tolerance' appeared to be even more marked, with 50–80% of the guinea-pigs not entering a sensitive stage. However, this appeared to be only a failure to make anaphylactic antibodies to cows' milk, as antibody production in other classes remained unimpaired. In those guinea-pigs that became anaphylactically sensitized to cows' milk there was, as before, always greater refractoriness to i.t. challenge. In Frant and Dunkin–Hartley guinea pigs there appeared to be a 'breakdown' in 'tolerance' after about 40 days of drinking cows' milk, and an increased number underwent anaphylactic reactions after both i.v. and i.t. challenge. In contrast, in the small group of Heston–Birmingham guinea-pigs studied, the number undergoing anaphylaxis decreased even further after 70 days of drinking cows' milk. PCA tests suggested that this was again due to a 'turn-off' of IgG1a antibodies to cows' milk.

So-called tolerance induced by ingestion of antigen (the Sulzberger–Chase phenomenon) was first

described by Chase in 1946 and has since been described by a number of workers who have usually studied the influence of ingested antigens on the effects of subsequent parenteral sensitization (Pomeranz, 1970; Thomas & Parrott, 1974). It has been suggested by Andre *et al.* (1975) that this hyporesponsiveness, which can be transferred to normal animals by serum, is due to IgA antibody–antigen complexes which paralyse B cells.

In our experiments, in which guinea-pigs were fed cows' milk for long periods of time, three phenomena appeared to occur. Firstly, 'tolerance', in that there was a failure to make anaphylactic antibodies (but not those of other classes) to cows' milk, secondly, a 'turn-off' of anaphylactic antibody production after the animals had passed a stage during which they were sensitive to anaphylaxis; and thirdly, additional protection against the effects of i.t. challenge in animals that were in the anaphylactically sensitive stage. The occurrence of these phenomena appeared to depend on the age at which the guinea-pigs were first allowed to drink cows' milk, the length of time for which milk was fed and on the strain of guinea-pig.

These observations have considerable significance if the modified anaphylaxis hypothesis is found to be the explanation for cot death in infants. The relatively low incidence of death may, in some measure, be due to frequent tolerance to anaphylactic sensitization or to refractoriness once the anaphylactic state has occurred. The experiments in the guinea-pig show quite clearly that the tolerance may be confined to the tissue-sensitizing (IgG1a) antibodies to cows' milk. Feeding regimens should be carefully and thoroughly examined with regard to these phenomena in both guinea-pigs and human infants.

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