

Detection of reaginic and short-term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons

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Summary

Sera of 50% of persons extremely sensitive and of 30% of persons moderately sensitive to milk had heat-labile reaginic antibodies conferring passive cutaneous anaphylactic sensitivity on monkeys for 24 hr. More persons in each group had IgE antibodies that bound radio-isotope labelled β -lactoglobulin and passively sensitized monkey basophils for anaphylactic degranulation.

These tests, however, are not certain evidence of clinical allergy, because of the sera of nineteen atopic children, three mediated monkey PCA tests and five had γ E anti- β -lactoglobulin antibodies detected by antigen binding or basophil degranulation, though the serum donors were not clinically allergic to milk. Similarly a few normal, milk-tolerant adults also had γ E anti- β -lactoglobulin antibodies.

Sera of nine children with milk-induced gastroenteritis occurring 12–36 hr after exposure had no anti-milk reagins, and only one had γ E binding β -lactoglobulin. This allergy may be mediated by heat-stable IgG antibodies, because three sera conferred milk sensitivity on monkey skin for 4 hr and two others for 2 hr. The three sera had high titres of IgG agglutinins, one to β -lactoglobulin, one to α -lactalbumin and one to casein. All three fixed complement in the presence of milk, and one had precipitins. They did not passively sensitize human mast cells or monkey basophils for anaphylactic degranulation, but they prepared human lung for anaphylactic release of histamine, though in small amounts compared to that released from lung prepared with reagins.

The sera of two of three cases of cot death had IgG antibodies with similar properties.

Introduction

Allergy to milk is a hazard of childhood, though anaphylactic sensitization usually wanes or disappears later in life. Some of the disorders attributed to milk allergy are mediated by IgE reagins: others, e.g. gastroenteritis occurring 12 or more hours after exposure to milk, do not appear to be so.

Antibodies to milk are frequently present in the sera of normal as well as allergic

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children, and sometimes in adults, and as the antibodies in several disorders vary in their properties, different techniques are available for their detection. Precipitins are often present in children with recurrent pneumonia, bronchiectasis, some gastrointestinal disorders and failure to thrive (Heiner, Sears & Kniker, 1962; Peterson & Good, 1963; Handelsman & Nelson, 1964; Luz & Todd, 1964), but they are not usually found in children with anaphylactic milk allergy manifested by gastrointestinal disorders, eczema, urticaria or asthma (Saperstein *et al.*, 1963; Luz & Todd, 1964). Antibodies in these reaginic allergies can be detected by monkey passive cutaneous anaphylaxis (Parish, 1969), anaphylactic release of histamine from human lung passively sensitized *in vitro* (Parish, 1967), or the very sensitive binding of radio-isotope labelled antigens in gel precipitation tests (Freier *et al.*, 1969; Heiner & Rose, 1970; Kletter *et al.*, 1971). In sera of normal or allergic persons, or in cases of cot death, antibodies can be found by agglutination or antiglobulin agglutination tests (Gunter *et al.*, 1962; Parish *et al.*, 1964b; Steele & Coombs, 1964; Coombs *et al.*, 1965; Parish, 1969).

The results reported here extend the earlier investigation (Parish, 1969) by detecting γ E reaginic antibodies to milk by radio-isotope labelled antigen precipitation, and by anaphylactic degranulation of monkey basophils. They also show that gastrointestinal disorders starting 12–36 hr after exposure to milk are not mediated by reagins, but possibly by heat-stable IgG antibodies of the type conferring specific sensitivity on monkey skin for 4 hr (Parish, 1970) and which activate complement *in vivo* (Matthews & Soothill, 1970).

Materials and methods

Sera

Sera were those tested previously with many additions (Parish, 1969). They are divided into groups according to the clinical signs and history, as follows.

- (1) Eight children, very allergic to milk, manifested by severe urticaria, sometimes with wheezing and asthma though the pulmonary signs are not known to be due to milk. All had strong skin responses to milk.
- (2) Twenty-three adults and eight children, moderately allergic resulting in urticaria and gastrointestinal disorders, mainly diarrhoea and abdominal discomfort.
- (3) Nineteen children, 2–12 years old, with atopic dermatitis. None were suspected of being allergic to milk and the condition of three (with monkey PCA antibodies) did not improve when milk was withheld.
- (4) Eight normal, milk-tolerant adults, 25–36 years old, reported to have been moderately allergic when young children.
- (5) Nine children, 10 months to 2 years 6 months old, with gastrointestinal disturbances, diarrhoea and vomiting starting 12–36 hr after taking milk. They gave a positive skin test to milk.

Antigens

Cow's milk soluble antigen was obtained by centrifuging pasteurized milk at 15,000 rpm on a Sorvall SS3 centrifuge for 1 hr, and removing the opalescent middle fraction by inserting a fine pasteur pipette through the edge of the top layer of fat and not disturbing the precipitated debris at the bottom of the tube. Beta-lactoglobulin was bought from British Drug Houses Chemicals Ltd, Poole, and bovine γ -globulin (BGG) and bovine plasma albumin (BPA) from Armour Pharmaceutical Co., Eastbourne. The

α -lactalbumin and casein were gifts from Dr Aschaffenburg (Shinfield). The bovine IgM (BGM) was prepared from bovine serum with high titres of anti-*Escherichia coli* antibodies. It was separated first on DEAE cellulose with a 0.1 M phosphate buffer, pH 6.5, and then once or twice by Sephadex G-200 gel filtration according to purity. The BGM preparation contained very small amounts of transferrin and α 2M globulin, but no BGG.

Monkey passive cutaneous anaphylaxis (PCA)

Baboons and Cynomolgous monkeys were fed diets free of milk and were tested as described previously (Parish, 1969, 1970). Sera for 24 hr periods of sensitization were tested unheated, and after heating at 56°C for 2 hr. Sera for 2 or 4 hr periods of sensitization were heated. In all tests, the 5% Evans blue dye was injected 10–15 min before intravenous antigen, to show that exudation of dye at 2 or 4 hr serum sites was not due to toxic substances in the sera.

Anaphylactic histamine release from human lung

Fresh human lung from one donor was prepared in slices 1–2 mm thick, treated with human sera and challenged with 50 μ g β -lactoglobulin, as described previously (Parish, 1967) with the exception that passive sensitization occurred at room temperature for 4 hr. Lung slices were subsequently fixed and examined for mast cell changes as described before.

Monkey basophil test

Venous blood from monkeys with spontaneous high numbers of basophils, or after injection with *Ascaris* extract, was taken into siliconed syringes and 2 ml of 5% EDTA per 18 ml blood. Initially the leucocytes were separated by treatment of 8 ml blood with 2 ml 4.5% dextran, mol wt 150,000 (Glaxo) as described by Goldsmith (1965). The washed leucocytes in Parker 199 medium and 5% monkey serum albumin were added to short columns of glass beads for 10 min at 37°C which were then washed through with Parker 199 medium. More monocytes and neutrophils adhered to the glass than basophils. The leucocyte suspension was then concentrated by centrifuging and resuspended in Parker 199 medium containing 0.5% monkey serum albumin. In later tests the basophils were separated with mainly lymphocytes, by the technique of Coulson & Chalmers (1967), by treating the blood with methyl cellulose 15 cps (R. W. Greeff & Co., London) and carbonyl iron powder S.F. (GAF Ltd, Calder Street, Manchester). The loss of basophils was small. The leucocytes including basophils were suspended in Parker 199 and 0.5% monkey serum albumin.

The leucocytes were sensitized with an equal 0.1 ml volume of the serum dilution in silicone treated tubes in a water bath at 37°C for 1 hr, which was longer than that shown by Osler, Lichtenstein & Levy (1968) to be necessary to sensitize human leucocytes for the anaphylactic release of histamine. They were gently centrifuged, the medium removed, and washed once in 1 ml medium containing 0.5 ml monkey albumin, and resuspended in that medium. Drops were tested on slides treated with neutral red dye (Shelley, 1962) or with toluidine blue and sealed with paraffin wax. Challenge was made with a drop of antigen at 5 μ g/ml. Control slides not tested with antigen, treated with normal serum and antigen, or antigen alone, were examined in every test. 100 or more basophils were examined on each slide or pair of slides.

About 10% basophils were degranulated by the preparation and treatment with

antigen. Normal human serum sometimes reduced this to 5%. Occasional serum samples degranulated 15–20% basophils spontaneously without treatment with antigen. Therefore 25% was the lowest percentage of degranulated cells considered to be significant in the anaphylactic tests.

IgE binding of radio-isotope labelled β -lactoglobulin, and antiglobulin agglutination tests
Details of these tests are to be published separately.

The IgE binding of ^{131}I or ^{125}I labelled β -lactoglobulin was tested by the technique of Heiner & Rose (1970). Monospecific anti-IgE precipitated with the IgE of the test sera in gels, which were subsequently tested for their ability to bind the labelled antigen.

Antiglobulin agglutination tests were carried out by the red cell linked antigen-antiglobulin technique of Steele & Coombs (1964) as reported previously (Parish, 1969).

Results

Monkey passive cutaneous anaphylaxis

Half the serum samples from persons with milk allergy manifested by urticaria, and sometimes by asthma, had antibodies mediating PCA in baboons 24 hr after sensitization. Just less than one third of the samples from persons whose allergy was manifested by urticaria and gastrointestinal disturbances had similar anaphylactic antibodies (Table 1).

Table 1. Monkey PCA tests on the sera of milk-sensitive and normal persons

Clinical response to milk	Signs	Time of onset	No. of samples	No. of sera mediating PCA at:†			
				24 hr	Titre range	4 hr	Titre range
Allergic	Urticaria, asthma	Minutes to 1 hr	8	4	16–124	0	
Allergic	Urticaria, gastro-intest.	Minutes to 1 hr	31	10	2–124	1	4
Allergic	Gastro-intest.	12–36 hr	9	0		3	2–16
Normal	Cot death*	Within 3 hr	17	2	8	2 (of 3 tested)	
Normal	Atopic dermatitis	None	19	3	2–8	0	
Normal adult; allergic as child	Normal	None	8	0		0	
Normal	Normal	None	56	0		0	

* Parish (1969).

† Sera tested 24 hr after passive sensitization were unheated; those tested at 4 hr were heated at 56°C for 2 hr.

In contrast to the tests on sera from allergic persons, only two of seventeen post-mortem sera from cases of cot death mediated 24 hr PCA responses, both at a titre of 1/8 (Parish, 1969).

Three of nineteen sera from children with atopic dermatitis had low titres of PCA antibody to milk (Table 1). However, it was reported that milk did not appear to aggravate the disease, and excluding milk from the diet did not prevent it.

Reaginic antibodies were not found in the sera of nine children who had gastrointestinal disorders 12–36 hr after taking milk. In three, however, heat-stable antibodies at titres of 2, 8 and 16 mediated increased vascular permeability when challenged 4 hr after passive sensitization. Repeating the test with a 2 hr interval between passive sensitization and challenge showed that a further two sera mediated this response.

Two of the three cot-death sera tested 4 hr after passive sensitization, also mediated increased vascular permeability. Evans blue injected intravenously into the baboons before antigenic challenge did not appear at the serum sites, showing that the increased vascular permeability was not due to substances in the sera obtained after death.

Table 2. Sera giving monkey PCA reactions to whole milk, tested with separated milk proteins

Clinical group	PCA challenge after:	No. of sera mediating PCA after challenge with:						
		Milk	β -Lact.	α -Lact.	Casein	BPA	BGG	BGM
Asthma, urticaria	24 hr	4	4	1	0	0	0	0
Urticaria, gastrointest.	24 hr	10	7	3	0	0	0	1
Gastrointest.	4 hr	3	1	1	0	0	1	0
	2 hr	2	1	0	1	0	0	0
Cot-death	4 hr	2	1	0	1	0	0	0

When the reaginic sera mediating PCA to whole milk were tested against separated milk proteins, most reacted with β -lactoglobulin (Table 2). In the asthma-urticaria group, all four mediating PCA to whole milk reacted with β -lactoglobulin, though one also reacted with α -lactalbumin. In the ten sera of the urticaria-gastrointestinal group seven reacted with β -lactoglobulin, and one also with bovine IgM.

The three sera from the gastrointestinal group and the two cot-death sera that contained heat-stable, short-term sensitizing antibodies tended to have other specificities, in that two reacted with casein and one with bovine γ -globulin, which did not occur in the reaginic sera.

Agglutinins and complement-fixing antibodies

In the three sera from the gastroenteritis group which had antibodies mediating increased vascular permeability on challenge 4 hr after cutaneous injection, the first had precipitins to β -lactoglobulin and an IgG antiglobulin titre of >4000, the second had no precipitins to α -lactalbumin but an IgG antibody titre of 2000, the third, also without precipitins, had an IgG titre to BGG of 2000. All three sera fixed complement when tested with milk antigen at titres of 1/64, 1/16 and 1/16 respectively.

The two cot-death sera had no precipitins. One had an IgG antiglobulin titre to β -lactoglobulin of 2000, and the other to casein of >4000. Both sera when tested with milk antigen fixed complement at 1/32.

Anaphylactic histamine release from human lung

Sera from normal persons did not prepare thin slices of lung for the release of histamine on challenge with β -lactoglobulin, or mediate changes in mast cells, whereas sera from two persons with reagin-mediated allergy to milk, passively sensitized the lung for release of histamine and for mast cell changes on challenge (Table 3). Fewer mast cells could be identified in the anaphylactic than in the non-anaphylactic lung slices, and of those remaining many were vacuolated or had shed intact granules; some released homogeneous metachromatic substances.

One serum sample, from a milk-tolerant adult who had been allergic when a child, which in other tests was found to have γ E anti- β -lactoglobulin antibodies, and passively sensitized monkey basophils for antigenic degranulation (Table 4), neither released histamine nor prepared lung mast cells for antigen-induced changes.

Table 3. Anaphylactic release of histamine from human lung passively sensitized *in vitro*, and post-challenge mast cell changes; histamine expressed as μ g base per gram wet weight lung

Test serum	Control serum	Control* β -lact.	Serum + β -lact.	Test, less sum controls	Total in lung	Anaphylactic release (%)	Changed mast cells/total
Normal	0.24	0.79	0.68	—	26	—	9/200
Normal	0.41		1.48	0.28		1.1	16/200
Normal (allergic child)†	0.25		0.71	—		—	15/200
Milk allergy a.u.	0.3		6.9	5.81		22.3	26/80
Milk allergy a.u.	1.6		8.6	6.21		23.9	43/76
Milk allergy g.i.	0.32		3.4	2.29		8.8	12/200
Milk allergy g.i.	0.27		2.8	1.74		6.7	11/200

a.u. = asthma and urticaria. Sera contain reagins mediating PCA at 1/64 and 1/256; g.i. = gastrointestinal. Sera contain heat stable 4 hr 'PCA' antibody at 1/8 and 2 hr 'PCA' at 1/4.

* Mean of three samples 0.77, 0.79, 0.81.

† Normal adult, but allergic when young. Serum contains γ E and mediates basophil disruption (Table 4).

Two sera with heat-stable antibodies to β -lactoglobulin that sensitized monkey skin, one for 4 hr, the other for only 2 hr, prepared human lung for the antigenic release of histamine in significant amounts (8.8 and 6.7%) when compared with that released after treatment with normal sera, but much less than that released from lung prepared with reagin. Moreover, the number and morphology of the mast cells in lung treated with the heat-stable antibodies did not differ from the cells in lung pre-treated with normal sera.

Detection of γ E antibodies to β -lactoglobulin by precipitation with radio-isotope labelled antigen and by monkey basophil degranulation

Sera examined previously by monkey PCA were tested for γ E anti- β -lactoglobulin antibodies by the binding of radio-isotope labelled antigen to precipitates containing IgE, and by passive sensitization of monkey basophils for antigen-induced degranulation (Table 4). Some sera reported not to contain γ E anti- β -lactoglobulin by the precipitation test had IgE which did not bind the antigen; others had no IgE or too little

to be detected by this method. Details of these findings are to be described separately.

Antigenic degranulation of passively sensitized monkey basophils is believed to be mediated by γ E reagins, because the ability of the serum to sensitize basophils passively is destroyed by heating the sera at 56°C for 2 or 3 hr, and because basophils treated with unheated sera were not damaged when tested with rabbit or sheep anti-human IgG, IgA and IgM (adsorbed with monkey erythrocytes and liver powder). They were degranulated when tested with anti-IgE in dilutions up to 1/10,000. However, monkey basophils, not treated with human sera, were similarly degranulated when tested with anti-IgE diluted to 1/1000, indicating that these basophils normally have a monkey globulin on their surface that has antigenic determinants similar to human IgE.

All eight sera from persons extremely sensitive to milk contained γ E anti- β -lactoglobulin detected by radio-isotope antigen binding, and six of them anaphylactically

Table 4. Number of sera, previously tested for monkey PCA (Tables 1 and 2), containing γ E antibodies to β -lactoglobulin detected by radio-immunodiffusion precipitation, and by passive sensitization (P.S.) of monkey basophils for antigenic disruption

Clinical response to milk	Signs	No. of sera			P.S. basophil titre		
		Samples	With 24 hr PCA β -lact. (Table 2)	With γ E anti- β -lact.	P.S. basophils for disrupt.	Mean	Range
Allergic	Asthma, urticaria	8	4	8	6	256	16-1000
Allergic	Urticaria, gastrointest.	31	7	11	8	64	16-256
Allergic	Gastrointest.	9	0	1	0		
Normal	Atopic dermatitis	19		5	3	16	8-32
Normal adult; allergic as child	Normal	8		3	2	16	16 and 16
Normal	Normal	16		0	0	0	

sensitized monkey basophils (Table 4), though in the previous tests only five mediated monkey PCA. Similarly in the group of persons with gastrointestinal disorders believed to be reagin-mediated, more sera had IgE antibodies detected by precipitation and basophil degranulation than by monkey PCA.

These tests confirmed the absence of IgE antibodies in the sera of persons with gastroenteritis occurring 12-36 hr after exposure to milk, with the exception of one weak reaction in the precipitation test.

Sera from some children not known to be allergic to milk, or from normal, milk-tolerant adults, had IgE antibodies to β -lactoglobulin. The three children with atopic dermatitis whose sera mediated monkey PCA also had IgE antibodies detected by binding of isotope-labelled antigen and by antigenic degranulation of passively sensitized basophils. Two other sera not conferring anaphylactic sensitivity on monkey skin or basophils, had γ E antibodies binding the labelled antigen. Similarly three of eight milk-tolerant adults had γ E antibodies binding β -lactoglobulin, two of which also anaphylactically sensitized basophils. The one serum tested failed to sensitize human lung for anaphylactic release of histamine (Table 3).

Discussion

The results confirm that IgE reagins to milk are found in a third to a half of the sera from milk-allergic persons by monkey PCA (Parish, 1969). The results also show that γ E antibodies to milk are found by *in vitro* tests in some sera of persons who are milk-tolerant. In contrast, no γ E reagins were found in the sera of children with gastroenteritis starting 12–36 hr after exposure to milk, but they tend to have heat-stable, short-term anaphylactic sensitizing antibodies. Two of three cot death sera had similar antibodies. The significance of these findings are discussed in turn.

IgE reaginic milk allergy

Half the sera, or a third of the sera, according to the state of milk sensitivity of the donors, which confer anaphylactic sensitivity on monkey skin, are high proportions of those tested when it is considered that not all monkeys are suitable for testing human reaginic anti-milk antibodies (Parish, 1969) and that some sera are likely to be obtained from persons with a physiological intolerance to milk masquerading as allergy (Frankland, 1971). Monkey PCA, however, is not a very discriminating technique for detecting human reagins. The sensitivity of monkey skin for aggregated γ E is a tenth of that of human skin (Ishizaka *et al.*, 1970), as is monkey PCA compared to human PK tests (Parish, 1969), and histamine release from monkey compared to human lung (Assem & Schild, 1968).

More sera were shown to have IgE antibodies to β -lactoglobulin by binding of radio-isotope labelled antigen to precipitates *in vitro* and by passive sensitization of monkey basophils for anaphylactic degranulation, than by monkey PCA (Table 4). The *in vitro* tests were so discriminating as to show IgE antibodies in the sera of children with atopic dermatitis with no history of milk allergy, or of milk-tolerant adults. The one serum of a milk-tolerant adult with γ E anti- β -lactoglobulin antibodies which was tested on human lung *in vitro*, failed to prepare it for anaphylactic release of histamine, though this technique can detect anti-milk reagins (Parish, 1967, and Table 3).

It is possible that *in vitro* techniques are so discriminating that they detect amounts of IgE below the serum concentrations necessary to confer anaphylactic sensitivity. Other possibilities are that not all human IgE is capable of anaphylactic passive sensitization, or that changes occur, particularly in adults who were allergic as children, that alter the receptor sites of cells for the antibody, or the susceptibility of the tissues to the pharmacological mediators of anaphylaxis.

There is little doubt that the monkey PCA and basophil degranulation tests used in this study detected γ E reaginic (tissue passive-sensitizing) antibodies, though the radio-isotope antigen binding technique may, conceivably, have detected some non-anaphylactically sensitizing γ E.

It is well established that human IgE reagins sensitize monkey tissues anaphylactically, detected by PCA, and by histamine release from lung passively sensitized *in vitro* (Goodfriend, Kovacs & Rose, 1966; Ishizaka *et al.*, 1969). Monkey skin also responds immediately to injections of aggregated human γ E (Ishizaka *et al.*, 1970).

It is also well established that human reagins sensitize human leucocytes for the anaphylactic release of histamine (Osler *et al.*, 1968), and that basophils from sensitized persons, or from normal persons and treated with human reagins *in vitro*, degranulate on antigenic challenge (Shelley & Juhlin, 1961; Shelley, 1962) and human basophils degranulate and release histamine when treated with anti-human γ E antiglobulin (Ishizaka, Tomioka & Ishizaka, 1971). In a very elegant technique, Wilson, Marchand

& Coombs (1971) showed the presence of IgE antibodies on the surface of basophils by 'rosette' agglutination with allergen-coupled red cells. It is noteworthy that they occasionally detected basophils treated with normal sera that reacted with their allergen-coupled cells. These possibly correspond to the sera from milk-tolerant persons that occasionally prepared monkey basophils for anaphylactic degranulation.

Basophil degranulation as used in the experiment reported here, does not discriminate between sera with and without reagin as clearly as the rosette test of Wilson *et al.* (1971). There are always some cells that degranulate during the treatment and washing. Moreover a few sera degranulate basophils without antigen treatment, though it was not ascertained whether this was due to cytotoxic antibodies or substances, or due to preformed reagin-allergen complexes in the serum. In its favour, however, it detects very small amounts of biologically active γ E; it is sometimes a hundred-fold more sensitive than monkey PCA. It also detects reagin more frequently than degranulation of human mast cells (Tables 3 and 4). This is probably due to the membrane of blood basophils being entirely exposed to the reaginic sera, whereas mast cells in tissue slices are protected from the antibodies, or there is insufficient reagin to sensitize the mast cells after some has been adsorbed by other cells in the lung.

Evidence obtained in this study, confirmed that the antigen-induced degranulation of monkey basophils pretreated with human reaginic sera is mediated by IgE. The basophils treated with human sera were unharmed by monospecific anti-human IgG, A or M: they were all disrupted by anti-IgE. However, anti-human IgE antiglobulin also damaged monkey basophils not treated with human sera, indicating that a globulin on the surface of monkey basophils shares determinants with human IgE. Monkey neutrophils and eosinophils were unharmed.

Heat-stable short-term sensitizing IgG antibodies

The sera of the nine children whose gastroenteritis started 12–36 hr after exposure to milk had no heat-labile antibodies mediating monkey PCA (Table 1) or degranulation of monkey basophils *in vitro* when challenged with antigen (Table 4). Only one serum contained γ E antibodies that bound, weakly, radio-isotope labelled β -lactoglobulin in gel plate tests, though other milk antigens are still to be tested. It is evident that this is not a γ E reagin mediated disease.

Some of these children formed antibodies conferring short-term anaphylactic sensitivity on monkey skin for about 4 hr (Table 1) as previously found in persons who were skin sensitive in the absence of γ E antibodies for the test antigen (Parish, 1970). These antibodies appear related to, or identical with, those described by Matthews & Soothill (1970) in children with vomiting and diarrhoea occurring later after exposure to milk than in children sensitized by reagents. Matthews & Soothill (1970) found that exposure to milk was accompanied by *in vivo* complement activation, and they suggested that the disorder resulted from IgG complement-fixing antibodies causing an Arthus reaction in the gut.

The heat-stable antibody conferring short-term antigenic sensitivity on monkey skin is found in the IgG fraction of sera; its activity appears to be complement dependent, and the increased vascular permeability is mediated mainly by histamine. It differs from the classical Arthus reaction in being independent of neutrophils (Parish, 1970).

It is apparent from the results reported here that the antibody mediating a PCA-like increased vascular permeability in monkey skin when challenged 4 hr after passive

sensitization, induces tissue changes in a different manner from those induced by reagins. The heat-stable, short-term sensitizing antibody does not prepare monkey basophils (Table 4) or human mast cells (Table 3) for degranulation on antigenic challenge, and prepares human lung for the release of smaller amounts of histamine than released after passive sensitization with reaginic IgE (Table 3). Moreover the IgG and the IgE antibodies do not compete for the same cell receptors, because preparations of each antibody do not significantly reduce or inhibit the tissue sensitivity mediated by the other (Parish, to be published).

Until more is known about the receptor cells for the IgG antibody, it is not possible to describe its activity as anaphylactic, or anaphylactoid. Nevertheless, it confers cutaneous sensitivity on monkey skin which is antigen-specific, and the response resembles that resulting from challenge of reaginically sensitized skin, though the manner of the histamine release differs.

Short-term sensitizing IgG antibodies in cot death

It is particularly interesting that sera from two of three cases of cot death had heat-stable 'anaphylactic' antibody. Cot death, or sudden unexplained death in normal infants, is thought to follow an allergic reaction after inhalation of regurgitated milk by a sensitized, sleeping infant (Parish *et al.*, 1964b). The sera of these cases have higher titres of agglutinins to milk proteins than are found in normal infants of the same age (Parish *et al.*, 1964b; Coombs *et al.*, 1965; Hunter, Feinstein & Coombs, 1968). Reagins mediating monkey PCA were not found in six cot-death sera by Hunter *et al.* (1968) and in only two of seventeen sera by Parish (1969), and cot-death sera did not prepare human lung for anaphylactic release of histamine though the sera, without antigen, released histamine, either because they contained complexes or toxic post mortem products (Parish, 1967).

If cot death is due to allergy, it is almost certainly not mediated by reaginic IgE. It is tempting to suggest that death may sometimes be anaphylactic or anaphylactoid, mediated by heat-stable IgG-type antibodies whose presence is reflected by the high agglutinin titres. The particular cells releasing the histamine are still to be identified. Mast cells in cot-death lungs do not show anaphylactic changes (Parish *et al.*, 1964b) and these changes do not occur in human lung treated with short-term sensitizing antibody and antigen (Table 4). Though histamine can be released from intact mast cells, it is more likely that the increased vascular permeability mediated in monkey skin by human heat-stable IgG-type antibodies is analogous to the vascular changes induced in rabbits by antibody and antigen releasing histamine from platelets in the presence of complement (Henson & Cochrane, 1969a, b). In a previous experimental model of cot death, guinea-pigs were tested for their response to inhaled antigen (Parish, Barrett & Coombs, 1960), but death in these animals follows bronchoconstriction not observed in cot-death lungs (Parish, Akester & Gregg, 1964a). It is probable that rabbits which more readily form precipitins, and less readily form anaphylactic homocytotropic antibodies than guinea-pigs, would provide a more suitable experimental model.

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