

SOME CHARACTERISTICS OF THE ANTIBODIES INVOLVED IN ALLERGIC SKIN REACTIONS OF THE HORSE TO BITING INSECTS

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SUMMARY

Active skin tests were carried out on a horse with sweet itch using extracts of various biting insects and the resulting reactions compared with those produced when rabbit anti-human IgE was injected intradermally into a normal horse. The horse with sweet itch produced a large reaction to an extract of *Culicoides* which was similar to those produced by anti-human IgE. Absorption of serum from an allergic horse with anti-human IgE reduced its ability to sensitize skin of a normal horse to challenge with the extract of *Culicoides*. Passive transfer of allergic horse serum to guinea-pig skin gave significant reactions when challenged after a latent period of 4 h but not after 24 h.

INTRODUCTION

Allergic skin reactions in horses to biting insects have been reported from many countries. Sweet itch (a recurrent, seasonal dermatitis) in the British Isles and similar conditions found in other parts of the world have been associated with *Culicoides* species (Riek, 1954; Mellor & McCaig, 1974; Quinn, Baker & Morrow, 1983). Serum of affected horses is capable of sensitizing the skin of normal horses to challenge with insect extracts and that sensitization persists for at least 72 h (Quinn, Baker & Morrow, 1983). This suggests the presence of long-term homocytotropic antibody to antigens derived from *Culicoides* insects in the serum of affected horses. In man and some other species, IgE is the long-term homocytotropic antibody and its existence in horses was confirmed by Suter & Fey (1983). Although passive sensitization with heterologous serum has been reported in a number of closely related species, this has been of short duration only (Mota & Wong, 1969; Augustin, 1978). IgE antibodies in various species share antigenic determinants (Halliwell, Schwartzman & Rockey, 1972).

In this study the response of a horse with sweet itch to intradermal challenge with a *Culicoides* extract and extracts of other biting insects was compared with that produced by intradermal injection of anti-human IgE in a normal horse. Serum from an allergic

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horse, absorbed with anti-human IgE, was used in passive transfer tests. The ability of serum from affected horses to sensitize guinea-pig skin was also investigated.

MATERIALS AND METHODS

Active skin tests using insect extracts

Intradermal skin tests were carried out on a horse suffering from sweet itch using extracts of *Culicoides*, *Stomoxys*, *Tabanidae* and *Culex* and an isotonic saline control. Insect extracts were prepared in isotonic saline as previously described by Quinn, Baker & Morrow (1983). Skin tests were carried out by the intradermal inoculation of 0.1 ml of the insect extracts or isotonic saline at previously marked sites on the lateral aspect of the neck. Reactions were recorded by measuring the skinfold thickness at challenge sites prior to and at 20 min, 1, 4, 24 and 48 hours after challenge using vernier callipers.

Intradermal skin tests using anti-human IgE

Rabbit anti-human IgE was obtained from a commercial source*. It was reconstituted with the recommended volume of distilled water and dilutions of 1:10 and 1:30 (in isotonic saline) were prepared. Nine sites were marked on the previously prepared lateral side of a horse's neck. This horse did not have a history of allergic skin reactions. Three of the sites were challenged intradermally with 0.1 ml of either undiluted anti-human IgE, 1:10 or 1:30 dilutions and three other sites with similar dilutions of normal rabbit serum. Of the remaining sites one was challenged with a 1% extract of *Culicoides*, one with a 0.1% solution of sodium azide (which had been used as a preservative in the anti-human IgE) and one with isotonic saline. Skinfold thickness measurements were recorded prior to and at 20 min, 1, 4, 24 and 48 h after challenge.

Passive transfer (P-K) tests using allergic horse serum which had been absorbed with anti-human IgE

Absorption. Serum from an allergic horse was mixed with equal volumes of undiluted rabbit anti-human IgE or normal rabbit serum, incubated at 37°C for two hours and then placed in the fridge overnight at 4°C. Following centrifugation at 1450 g for 1 h in a refrigerated centrifuge at 4°C, the supernatant was removed and filtered through a sterile 0.22 µm filter. The sterility of the resulting serum samples was checked on blood agar incubated for 24 h at 37°C.

Passive transfer tests. A normal horse was injected intradermally at previously marked sites with 0.2 ml of either the allergic horse serum which had been absorbed with rabbit anti-human IgE (absorbed serum), allergic horse serum which had been incubated with an equal volume of normal rabbit serum (diluted serum) or normal serum from the same horse (allergic serum). Each serum sample was injected at two sites. All six sites were challenged with 0.1 ml of a 1% extract of *Culicoides* following a sensitization period of 72 h. The horse was also challenged with 0.1 ml of a 1% extract of *Culicoides* and isotonic saline at normal skin sites.

Passive transfer of allergic horse serum to guinea-pig skin

The ventral and lateral aspects of the abdomen of eight mature guinea-pigs were closely clipped. Tests were carried out initially on two guinea-pigs, and later on the

*Miles Laboratories Ltd., P.O. Box 37, Stoke Poges, Slough, SL2 4LY.

remaining six. Ten sites were marked on each of the initial two guinea-pigs (GP1 and GP2). After disinfecting the skin with alcohol, 0.2 ml of allergic horse serum was injected into each of nine sites. Serum from the same horse was used in both cases. The tenth site, which had not been previously injected with horse serum, was challenged with *Culicoides* extract. Eight of the sites which had been inoculated with horse serum were challenged with 0.1 ml of a 1% *Culicoides* extract or the isotonic saline control after a latent period of sensitization of either 4, 6, 24 or 48 h. The remaining site was a serum control site at which skinfold thickness measurements were taken 20 min, 1, 4, 24 and 48 h after injection of the serum.

Skin sites on the six remaining guinea-pigs were inoculated with serum from different allergic horses. Of the six sites marked on each of these guinea-pigs, five were injected with 0.1 ml of serum and the sixth site was a control site for the *Culicoides* extract on its own. Four of the injected sites were challenged with either the *Culicoides* extract or isotonic saline: two after a latent period of 4 h and the remaining two after a latent period of 24 h. The fifth site was a serum control site. Reactions were recorded as the increase in skinfold thickness 20 min, 1, 4, 24 and 48 h after challenge with the *Culicoides* extract.

RESULTS

Active skin tests using insect extracts

The horse challenged intradermally with extracts of various biting insects produced a much larger reaction to the *Culicoides* extract than to any of the other insect extracts. This reaction reached its maximum recorded value at the 4 h reading (Fig. 1).

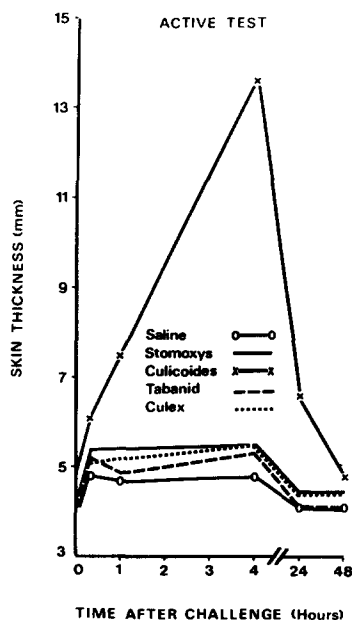


Fig. 1. Intradermal challenge of an allergic horse with insect extracts and isotonic saline. The reactions were recorded by measuring the skinfold thickness at injection sites before, and at 20 min, 1, 4, 24 and 48 h after challenge.

Intradermal skin tests using anti-human IgE

The reactions produced by all three concentrations of anti-human IgE followed a similar pattern (Fig. 2) and were concentration dependent. The maximum recorded values of these reactions also occurred at the 4 h reading.

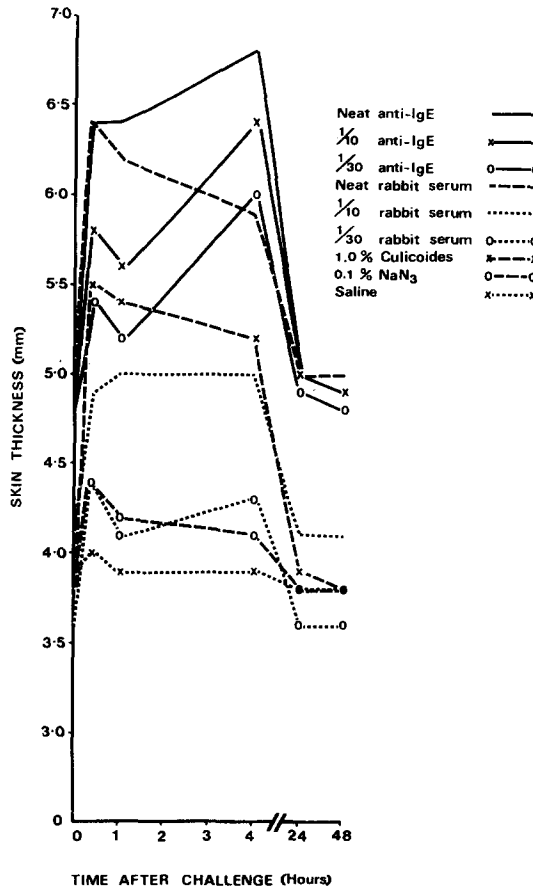


Fig. 2. Intradermal challenge of a normal horse with dilutions of rabbit anti-human IgE, normal rabbit serum, a 1% extract of *Culicoides*, a 0.1% solution of sodium azide and isotonic saline. The reactions were recorded by measuring the skinfold thickness at the injection sites.

Passive transfer tests using allergic horse serum which had been absorbed with anti-human IgE

The mean reactions (increase in skinfold thickness) produced following challenge with *Culicoides* at sites passively sensitized with allergic horse serum which had been absorbed with anti-human IgE, allergic horse serum which had been incubated with normal rabbit serum and non-absorbed allergic horse serum are shown in Fig. 3. The reaction produced by *Culicoides* at a normal control site is also shown. There was no measurable reaction at the skin site challenged with isotonic saline.

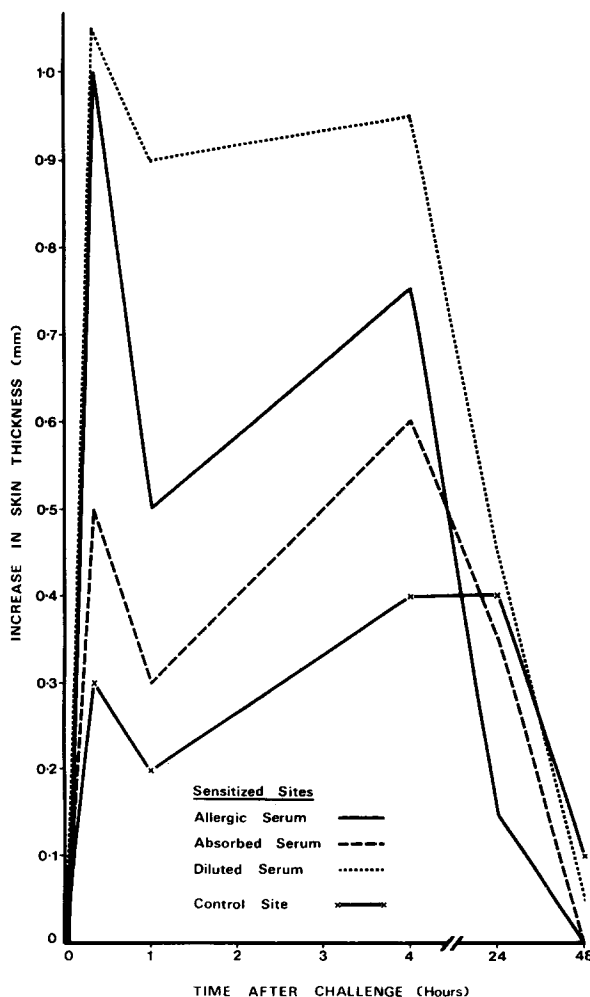


Fig. 3. Passive transfer experiment in which serum from an allergic horse (allergic serum), serum from an allergic horse absorbed with rabbit anti-human IgE (absorbed serum), and serum from an allergic horse diluted with an equal volume of normal rabbit serum (diluted serum) were transferred to two skin sites each on a normal horse and subsequently challenged with a 1% extract of *Culicoides* after a latent period of 72 h. In addition, a normal skin site (control site) was also challenged with a 1% extract of *Culicoides*. The reactions were recorded as the increase in skinfold thickness at injection sites 20 min, 1, 4, 24 and 48 h after challenge. The graph shows the mean increase in skinfold thickness at passive transfer sites.

The reaction produced by *Culicoides* at the control site was smaller than those produced at passively sensitized sites. The mean reaction produced at the two sites sensitized with absorbed horse serum was smaller than those produced at sites sensitized with either non-absorbed allergic horse serum or allergic horse serum incubated with normal rabbit serum as shown by the 20 min, 1 and 4 h readings.

These results suggest that serum from the affected horse was capable of transferring skin reactivity to a normal horse and that the ability of the serum to sensitize homologous skin could be decreased by absorption with anti-human IgE.

Passive transfer of allergic horse serum to guinea-pig skin

The reactions produced in eight guinea-pigs following challenge with a *Culicoides* extract at sites injected intradermally with allergic horse serum 4 h previously and at normal skin sites are shown in Figs. 4 and 5. The reactions produced following challenge

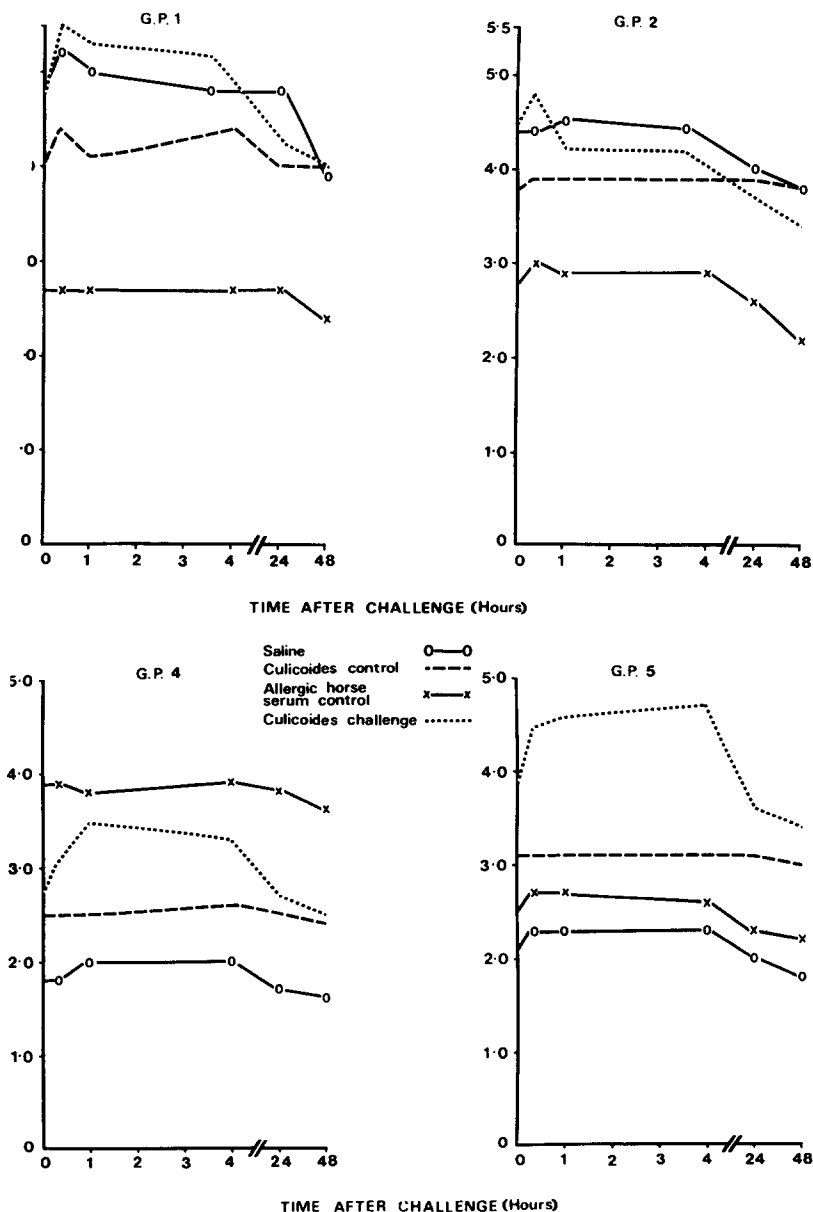


Fig. 4. Passive transfer of serum from allergic horses to skin sites on four guinea-pigs. Subsequently, after a latent period of 4 h these sites were challenged with an extract of *Culicoides* or isotonic saline. Two normal sites on each guinea-pig were challenged with an extract of *Culicoides* or allergic horse serum. Guinea-pigs 1 and 2 received serum from the same allergic horse; guinea-pigs 4 and 5 each received serum from a different allergic horse.

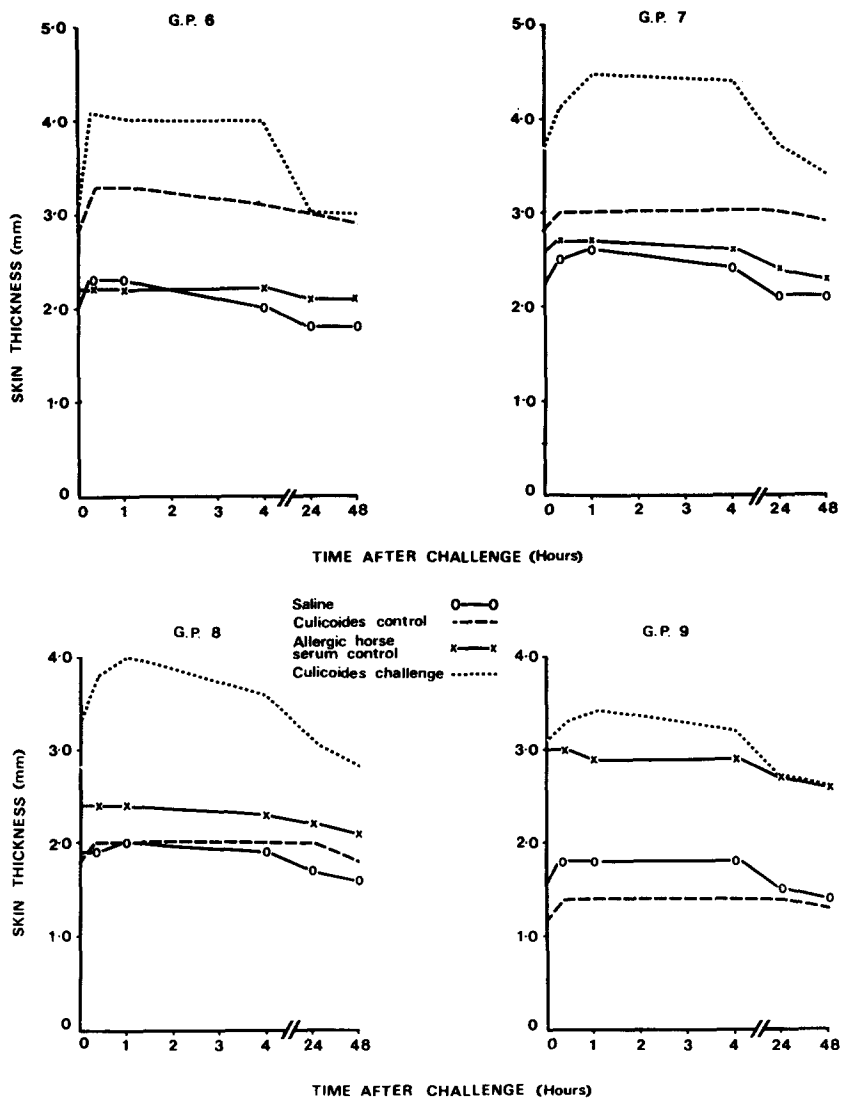


Fig. 5. Passive transfer of serum from allergic horses to skin sites on four guinea-pigs. Subsequently, after a latent period of 4 h sites were challenged with an extract of *Culicoides* or isotonic saline. Two normal sites on each guinea-pig were challenged with an extract of *Culicoides* or allergic horse serum. Each guinea-pig received serum from a different allergic horse.

with the insect extracts after a latent period of 24 h, and in the case of two guinea-pigs after latent periods of 24, 48 and 72 h were in all cases less than those where a latent period of 4 h was used. A comparison of the 20 min increase in skinfold thickness at injection sites produced by the *Culicoides* extracts with those produced by isotonic saline at sites which had been inoculated with allergic horse serum four hours previously (using the paired Student's *t*-test) showed that the *Culicoides* extracts produced reactions which were significantly greater than those produced by normal saline ($p < 0.0025$). The

reactions produced by the *Culicoides* extract at sites which had been injected with horse serum four hours previously were also significantly greater than those produced by *Culicoides* at normal control sites ($p < 0.0025$). However, the reactions produced by the *Culicoides* extract at sites which had been sensitized 24 h previously were not significantly greater than those at sites challenged with isotonic saline ($0.2 > p > 0.1$).

DISCUSSION

The study reported here using insect extracts on an affected horse, together with previous reports on sweet itch (Baker & Quinn, 1978) indicate an immediate type hypersensitivity in such horses to extracts of *Culicoides*. This is consistent with the results of passive transfer (P-K) tests reported by Quinn *et al.* (1983) which suggest the presence of a long-term skin sensitizing antibody to *Culicoides* allergen in the serum of allergic horses. The histopathology of skin lesions and intradermal challenge sites with *Culicoides* showing sub-epidermal oedema and eosinophilia reported by Baker & Quinn (1978) lend further confirmation.

The reactions produced by *Culicoides* extracts and anti-human IgE, when used in active tests, tended to follow a similar pattern reaching their maximum recorded values at the 4 h reading. However, the slope of this graph also changed at the 20 min reading revealing a tendency to form a minor peak at the 20 min reading. A similar feature was observed in other horses skin tested with extracts of *Culicoides* (Quinn *et al.*, 1983). Wasserman & Center (1979) reported that the pathological process following IgE-mast cell dependent immediate hypersensitivity reactions have both immediate and late phases. The former phase in the skin is characterized by a weal and flare reaction. The late phase occurs between 6 and 12 h after antigen exposure and is characterized in the skin by an indurated erythematous painful lesion (Solley, Gleich, Jordan & Schroeter, 1976). Experimental confirmation of this concept has been provided by studies employing purified antigen-specific IgE in P-K reactions and by the injection of antibody to IgE into the skin (Dolovich & Little, 1972; Dolovich, Hargreave, Chalmers, Skien, Gauldie & Bienenstock, 1973; Solley *et al.*, 1976). These studies also demonstrated that the inflammatory process in the late phase is characterized by a profound leucocyte infiltration involving neutrophils, eosinophils, basophils and mononuclear leucocytes, and that vasculitis may be a consequence of such a reaction. The reactions occur in the absence of IgG immune complexes or of complement activation or deposition. The development of the late stage is antigen-dose dependent, which may explain why it is not universally observed (Wasserman & Center, 1979).

Riek (1954) reported that in animals susceptible to Queensland itch, evidence was obtained for the presence of a thermolabile skin sensitizing antibody which was capable of passive transfer to guinea-pig skin as well as to homologous skin. That passive transfer of allergic horse serum to guinea-pig skin produced reactions when challenged with *Culicoides* extracts after 4 but not after 24 h suggests the presence of a short-term heterocytotropic antibody. These results are similar to the findings of Riek (1954) where passive transfer of sensitivity by serum from allergic horses could be demonstrated when challenged after a sensitization period of between 6 and 12 h but not after 24 h. Cross-sensitization occurs between closely related species such as the mouse and rat (Mota & Wong, 1969; Konig, Okudaira & Ishizaka, 1974; Prouvost-Danon, Wyczolkowska,

Binaghi & Abadie, 1975) and between monkey and man (Augustin, 1978). It has also been claimed that not only can human reagins sensitize rat and mouse mast cells (Korotzer, Haddad & Lopapa, 1971; Gillman, Korotzer & Haddad, 1972; Vijayanagar, Attalah, David & Sehon, 1972; Frick & Ashton, 1973) but that rat IgE is antigenically related to human IgE (Kanyerezi, Jatón & Bloch, 1971). It is also reported that human and canine IgE are immunologically related (Halliwell, *et al.*, 1972). However, Wyczolkowska & Prouvost-Danon (1976) reported that human IgE is unable to bind firmly to mouse peritoneal mast cells *in vitro*.

Human reagins do not produce passive cutaneous anaphylaxis (PCA) reactions in guinea-pigs (Fisher, Middleton & Menzel, 1956) although they are produced by non-reaginic human IgG antibodies. Human IgG does not give PCA reactions in monkeys while reagin fractions do (Augustin, Connolly and Lloyd, 1964). However, if challenged at 2.5 to 3 h after intradermal inoculation of antibodies, it has been found that human IgG antibodies have some, though shortlived, affinity for monkey skin (Augustin, O'Sullivan & Connolly (1966) and Augustin (1978). Cordal & Margni (1974) reported that of the non-precipitating antibodies localized in the IgGa, IgGb and IgGc fractions of serum of horses inoculated with dinitrophenylated human gamma-globulin only IgGa and IgGb non-precipitating antibodies mediate PCA in the guinea-pig and mouse. These reactions were produced following a 3 h passive sensitization period. However, McGuire, Crawford & Henson (1972) found variable results following the use of equine serum to produce PCA in guinea-pigs, mice and rats. They claimed that the lack of ability of the equine serum to induce PCA in these species compared to the same amount of rabbit antibody correlated with the complement-fixing activity of the equine serum. It has been shown that complement-fixing activity enhances the PCA reaction (Osler, Hawrisiak, Ovary, Siqueira & Bier, 1957). It is possible that the short-term sensitization produced in guinea-pigs by allergic horse serum was the result of an IgG antibody rather than IgE which would suggest the production of IgG as well as IgE to insect allergens in allergic horses.

Homocytotropic skin sensitizing antibodies have been demonstrated in the serum of horses sensitized with bovine serum, appearing between 7 and 10 days after the initial injection of bovine serum (Eyre, 1972). Suter & Fey (1981) separated the homocytotropic antibodies from equine serum and found that they were analogous to human IgE. Intracutaneous injection of anti-IgE antibody results in the induction of a reverse type allergic reaction (Ishizaka & Ishizaka, 1968). Using antisera specific to five different classes of human immunoglobulin they found that only anti-IgE antiserum induced erythema-wheal reactions in monkeys and man. The results obtained following the intradermal challenge of a horse with different concentrations of rabbit anti-human IgE suggest the presence of an antibody (probably horse IgE) on mast cells in the skin of the horse which shares certain antigenic determinants with human IgE. Absorption of allergic horse serum with anti-human IgE appeared to be successful in reducing its ability to transfer skin reactivity to a non-allergic horse. It has been suggested that IgE of rats, monkeys and rabbits also share antigenic determinants with human IgE (Ishizaka, Ishizaka & Tada, 1969; Zvaifler & Robinson, 1969; Kanyerezi *et al.*, 1971). Nielsen (1977) using radial immunodiffusion found that sheep anti-human IgE heavy chain produced reactions with horse serum which were so weak that he doubted if cross-reactions occurred, but anti-bovine antiserum produced considerable cross-reactivity with horse serum.

Sweet itch is a manifestation of an immediate type hypersensitivity resulting from the interaction of *Culicoides* allergen with mast cell bound IgE. Equine IgE is immunologically related to human IgE and the intradermal inoculation of anti-human IgE results in a reverse type allergic reaction similar to that produced by insect allergen. Passive transfer of serum from allergic horses failed to sensitize guinea-pig skin when challenged after 24 h although reactions were produced when challenged 4 h after sensitization. This finding suggests that the antibody in horse serum responsible for transfer of allergic activity to normal horses is homocytotropic in nature and that other antibodies which can sensitize guinea-pig skin for a short interval are of the IgG type.

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