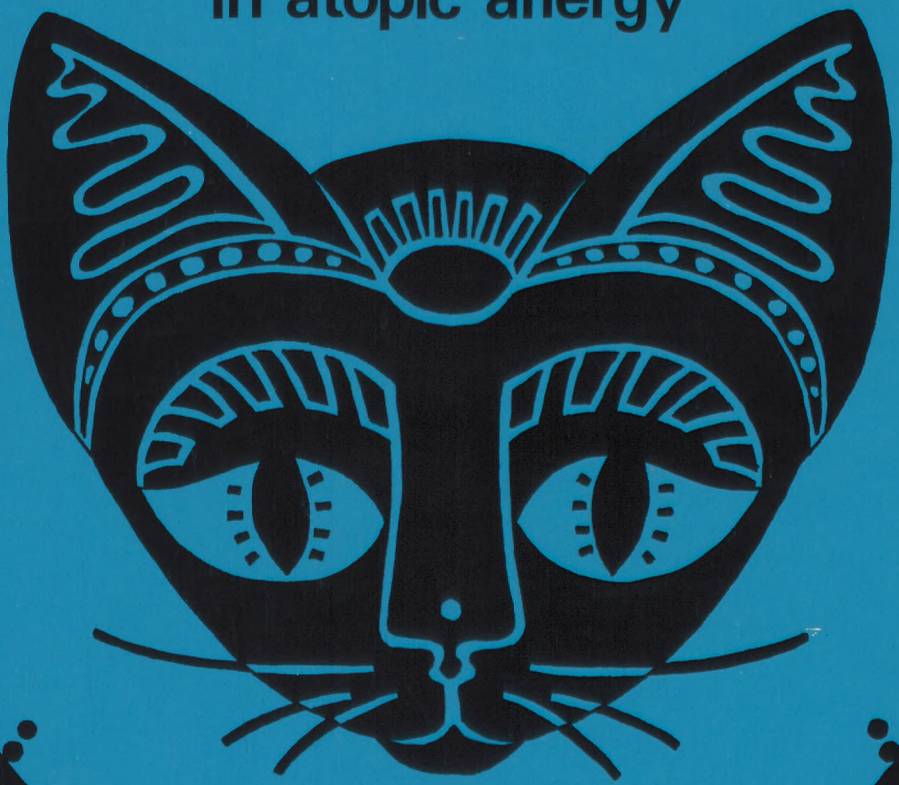
specificity and non-specificity in atopic allergy



W.J.KOERS

SPECIFICITY AND NON-SPECIFICITY IN ATOPIC ALLERGY

A STUDY PERFORMED IN PATIENTS ALLERGIC TO ANIMALS

Drukkerij S. Budde B.V. - Utrecht

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE UTRECHT, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. A. VERHOEFF, VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP DINSDAG 31 JANUARI 1978 DES NAMIDDAGS TE 4.15 UUR

DOOR

WILLEM JAN KOERS

GEBOREN OP 13 SEPTEMBER 1935 TE MIDDELBURG

PROMOTORES: DR. L. BERRENS
DR. E. YOUNG

To Marieke and Jan Willem, predisposed but asymptomatic, missing a 'pet' animal only.

To Heleen.

VOORWOORD

Dit proefschrift werd bewerkt in de afdeling klinische- en experimentele allergologie (Hoofden: Dr. E. Young, Lector in de dermatologische- en allergologische onderzoekingsmethoden en Dr. L. Berrens, Lector in de biochemie van de huid) van de Universiteitskliniek voor Huidziekten te Utrecht.

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CONTENTS

INTRODUCTION

In the Out-Patient Department of Dermatology and Allergy of Utrecht University Hospital, research in heterogeneous groups of patients suspected of allergy was conducted. As is customary, a classification into a number of groups was made according to the personal histories. However, after reviewing both the corresponding as well as the differing data from personal histories and skin tests, it became clear that in fact only two large groups of patients remained, with the exception of rare cases. Patients from the group with an allergy to house dust appeared to react quite differently as compared with patients from the pollinosis group. This difference was not only obvious in the pattern of clinical reaction, but was also evident in the pattern of skin reactions to tests with allergenic extracts.

Although patients with an allergy to animals should be considered as a separate group, animal dander allergy does not only occur in an isolated form, but is also much in evidence both in patients with pollinosis and in house dust-allergic patients, or in patients with a combination of these allergies. Nevertheless, it was remarkable to note that animal dander-allergic patients in their clinical reaction pattern closely resembled the pollinosis patients. As regards the skin reactions to tests with animal dander extracts in animal-allergic patients, a dual action appeared to be involved. Moreover animal dander allergy appeared to be an example of specific allergy. This clinical experience, taken together with the demonstrated *in vitro* differences between the allergenic action of house dust and of pollen (6) induced further research in this field. The choice of an investigation in patients with an allergy to animals was deliberate, because of the apparent trustworthiness of the personal histories and the reliability of the available purified allergens.

An attempt will be made to prove the correctness or incorrectness of the forwarded hypothesis, namely that positive skin reactions (and clinical reactions) caused by atopic allergens need not only be the result of an antigen-antibody reaction (Ag-Ab-reaction) but may also — or in some cases only — be the result of other reaction mechanisms, possibly non-immunological. The results of these studies are collectively presented in the form of a thesis because it was indeed possible to divide the *in vivo* skin reactions in atopic patients selected at random into relevant (R) and non-relevant (NR) reactions. It was also possible to relate these to specific (S) and non-specific (NS) immunological *in vitro* reactions. In consequence, the results of personal history-taking, skin tests and laboratory data must be interpreted somewhat differently than before, in order to draw correct conclusions and give advice for therapy, especially with regard to hyposensitization therapy.

CHAPTER I

A SHORT HISTORY OF ALLERGY

Although allergy is a phenomenon which has only drawn purposeful medical attention in the 20th century, in former times it must have occurred just as well, e.g. in pulmonary and cutaneous diseases. Little is known of allergy in ancient times, but because some of the asthmatic manifestations of today are due to a hypersensitivity reaction we may surmise that Hippokrates (460-357 b.C.) in his Aphorisms (16), when speaking of spasmodic asthma aimed at allergic influences without being aware of it. It was not untill the 19th century that mention was made of real allergic manifestations. The first description of symptoms due to hypersensitivity came from Bostock (7) in 1819 and dealt with the subject of hay fever. For a long time hay fever was called 'Bostock's Catharrh', after the publication of his treatise 'Case of a periodic affection of the eyes and chest'. In: 'On asthma: its pathology and treatment' (1860), Salter (39) gave the first accurate description of an asthma attack due to exposure to animal dandruff allergen.

Behring & Kitashima (1) in 1901, introduced the term hypersensitiveness. They used the term originally in another context than its present significance, viz. the existence of a condition of hypersensitiveness to a toxin (of tetanus). The sensitiveness to a primarily poisonous substance, to which an animal was naturally sensitive, was increased. Instead of developing immunity to administered proteins in experimental animals, such naturally sensitive animals apparently lost it and were without protection, or 'anaphylactic'. In 1902 Portier & Richet (35) introduced the term 'anaphylaxis' to designate this kind of hypersensitive state as one strictly opposed to prophylaxis and distinctly disadvantageous to the host.

The term 'allergy' was coined by Von Pirquet in 1907 (34). It concerned a changed reactivity to skin-testing with 'Alt Tuberkulin Koch'-toxoid in children without a clinical manifestation of tuberculosis. Later, it appeared that the term allergy had originally been given to an immune response to bacterial agents in healthy persons, thus indicating a developing immunity.

At that time, allergy was generally accepted to concern the opposite of immunity, because the patient demonstrated an adverse clinical reaction to a substance which was essentially harmless.

In 1906, Wolff-Eisner (45) suggested that hay fever was anaphylactic in nature. Meltzer (30) in 1910 suggested that bronchial asthma was anaphylactic as well, a suggestion which was largely based on the clinical analogy between the (experimental) reaction occurring in the guinea-pig and that in human beings during attacks.

The terms hypersensitiveness, anaphylaxis and allergy were used arbitrarily by various authors in different meanings. Some conformities were evident, however. The term allergy was used as a general designation for the phenomena of clinical

hypersensitiveness. Anaphylaxis was used in connection with experimental work, and in clinical allergy in connection with shock.

The need for a classification of the phenomena of hypersensitiveness was first recognized by Doerr (14). He subdivided the phenomena of hypersensitiveness into those exhibited to non-antigenic substances and those exhibited to antigenic substances. Coca (8) suggested not to use the term allergy in an immunological sense, but only the term hypersensitiveness: any form of specific peculiar reactivity in which the characteristic symptoms are different in the various animal species and are distinct from the normal physiological reaction to the agent in question.

Cooke (8) proposed a subdivision of the various forms of hypersensitiveness into a 'normal' group and an 'abnormal' group. To the group of normal hypersensitiveness belonged serum sickness and dermatitis venenata (41). The group of abnormal hypersensitiveness included anaphylaxis, hypersensitiveness of infection (bacterial allergy) and atopy, a term which was restricted to syndromes like hay fever and asthma.

The Greek word *atopia* suggested by Perry and introduced by Cooke in 1923 (8), meaning 'strange disease', included certain allergic manifestations in individuals with a hereditary background, who demonstrated skin reactions to tests with extracts of particular allergenic materials. Although in the course of years there were many differences of opinion about atopic allergy, Cooke's original opinion will be adhered to in this text.

Atopic hypersensitiveness

1. Is exhibited against non-precipitinogenic substances as well as against precipitinogenic substances.
2. Has not been shown to be passively transferable to normal individuals with the blood of sensitive individuals.
3. Can be greatly lessened but not completely removed by the suitable injection of the active substance (11).
4. Is inherited, subject to a dominant gen (12).
5. Is expressed in pathological changes the more important of which are different from those of the anaphylactic reaction in the guinea-pig, the rabbit or the dog.

The term atopic 'reagins' was applied to the active principle of allergic sera because it was doubted whether reagins could be regarded as true antibodies. Reagins were believed to be confined to the human species and to occur largely because of hereditary influences (9, 10, 27). The first evidence of antibodies in human allergy in point of fact was offered by Ramirez in 1919 when he reported the passive transfer of horse asthma by a blood-transfusion from a patient suffering from horse asthma (37). This phenomenon has been more intensively studied by Loveless (29). The nowadays more familiar test concerning passive transfer of serum was described in 1921 by Prausnitz & Küstner (PK-reaction). They demonstrated that the serum of an allergic patient injected intracutaneously into the skin of normal recipients could sensitize the site for subsequent challenge with allergen (36).

Intensive contacts with antigens like pollens and animal dandruff in some cases produced so many reagins — regardless of personal or family history — that they seemed to develop independently of the hereditary background.

Similar reaginic substances have been found in the serum of spontaneously sensitized dogs and cattle (44, 38), and in experimentally sensitized animals (33). In the latter, these were termed: homocytotropic antibodies.

Estimation of reagin titers in the sera of atopic individuals has for many years been possible only by way of the PK-technique. More sophisticated *in vivo* and *in vitro* methods were developed to estimate the reagin level, i.e. passive cutaneous anaphylaxis (PCA) in animals (28), histamine release tests with passively sensitized leucocytes, or chopped human or animal lung tissue (31, 15). However, these methods procured only rough estimations and, moreover, were unsuitable for routine purposes. Through the work of Ishizaka *et al.* (18, 17) and Johansson & Bennich (21) it was shown that the reagins in human atopic allergy belonged to a distinct class of immunoglobulins, IgE.

Elevated levels of IgE being firmly established in patients with atopic diseases like asthma (19, 46), hay fever (3) or atopic dermatitis (26, 24) supported the idea that IgE and reaginic antibodies are identical. This opinion was supported by the fact that IgE was shown to have the capacity of specifically inhibiting the PK-reaction (42). However, in healthy individuals the levels of total IgE in serum are not infrequently elevated as well (20), whereas in atopic patients it often happens that IgE-levels are within the normal range and vary considerably on different occasions (2, 22). Elevated IgE-levels in non-atopic diseases are found in parasitic infestations (25), in allergic aspergillosis (32), in the Wiskott-Aldrich syndrome (5) and in rare myelomas (23). So much overlap existed with total IgE values observed in the serum of non-atopic subjects, that a conclusive diagnosis of allergy in an individual atopic patient could not be made on this basis alone.

In 1967, Wide *et al.* (43) developed a method for the detection of specific antibodies of the IgE class. This method, known as the radioallergosorbent test (RAST), supplemented that of the proposed red-cell-linked antigen-antiglobulin reaction (RCLAAR) of Coombs *et al.* (13). In the early work, there appeared to exist a good correlation between the skin (and bronchial challenge) tests, and the specific IgE values (43, 4). In later years, however, failures occurred frequently (40) and questions arose as to the true identity of IgE and classical reaginic antibody; more particularly, the general acceptance of IgE as the one and only immunoglobulin involved began to waver. To this day, the puzzling question remains as to whether more than one mechanism underlies the allergic reactions in atopic individuals and whether perhaps non-immunological mechanisms are involved as well.

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CHAPTER II

ANIMAL ALLERGY IN MAN

A review

Handbooks on allergy generally make only cursory mention of allergy to animals or animal products (15, 21, 32, 50). Also, few publications are available on the subject. One reason for this paucity of information might be that attention was traditionally focused on more evident causes of allergy, such as to pollen or house dust. Another possibility is that animal allergy in the past was of less frequent occurrence, probably as a consequence of less intensive contacts with domestic animals than observed at the present time. The general assumption was that allergy to animals was not a frequent cause of asthma or rhinitis. Pronounced cases were in fact only observed in people with professional contacts. Today, however, we have the impression that the frequency of allergy to animals is increasing. Both the number and the species variety of domestic animals ('pets') and of laboratory animals appear to be increasing (43, 67), while contacts are more intensive as a result of smaller housing (apartments).

The earliest reports on animal allergy in man date back to the 16th century. These largely concern descriptions of asthma attacks induced by confrontation with cats. In a review on 'Diseases occurring in the 15th to 18th century caused by allergens', Schadewalt (48), like Unger and Coleman Harris (62), notes a few descriptions of symptoms that may have been caused by animal allergy. For example: in 1570, Mattioli suggested that the unrest, transpiration and paleness of one of his patients was traceable to the presence of a cat. In 1630 Santorio described a patient with attacks of asthma again due to the emanations of cats. At the time not only the hairs and odour of animals were suspect, but also the 'brains', which were thought to have an adverse influence on human sensibility. In 1684, Hannemann advised a patient — who underwent an allergic shock after coming into the presence of a cat — to use *'sal volatile'* as a prophylaxis in order to prevent the ascent of the presumed cat emanation by way of the olfactory system. This *'antipathia cum felibus'* was described earlier in 1681 by Schulz. According to this author, not only the presence of cats alive, but even the sight of pictures of cats could cause allergic symptoms. Descriptions of allergy to animals other than cats are not found in these early writings.

In 1851, Salter considered that it was materials originating in the animal's skin that caused the allergic symptoms. Apart from an allergy to pollen, Salter, who was an asthma patient himself, had developed an allergy to his own cat. In the chapter 'cat-asthma' of his wellknown treatise 'On asthma: its pathology and treatment' (46) he gives an accurate description of an asthma attack. Particularly worth noting is his consideration of the possible cause: 'The cause of this asthma is the proximity of a common domestic cat: the symptoms are very similar to those of hay fever, and, as in the case of hay fever, are occasioned by some

sudden influence inappreciable by the senses . . . I shall here notice the evidence of the more general influence of cats on my system — of the existence of what I am disposed to call cat-poison . . . The saliva of a cat is perfectly innocent, and a bite with the tooth in no way differs from ordinary wounds of the same character; in a word, I believe the influence is, in its source, exclusively cutaneous'. Salter also records that not only cat, but also rabbit might be a cause of allergy. An asthma attack caused by the latter animal was in point of fact described earlier, i.e. by Elliotsen. After Salter's work became known, the interest on animal dander allergy for the problem of asthma in man increased quite considerably. The scope of research in this field enlarged notably when skin tests with allergenic extracts came into general use after the pioneering work of Blackley (60).

The following descriptions mainly concern allergies to animals toward which people are frequently exposed. In this respect not only domestic animals as pets, but also animals contacted during professional exposure, are important sources of sensitization. As mentioned above, the attention of the medical profession was originally aimed at allergy to cats. This preoccupation shifted dramatically to horse allergy, when grave shock reactions were reported after the administration of therapeutic antisera.

Important work on the subject of 'horse-asthma' in man was done by de Besche (6). As far back as 1909 he drew attention to the fact that horse asthma in all probability is a form of anaphylaxis in man: quite similar to what was seen in guinea-pigs experimentally rendered anaphylactic to horse serum protein, an injection of horse serum in man was prone to provoke anaphylactic shock-like symptoms. De Besche described a patient with asthma due to the emanations of horses, who was submitted to a prophylactic injection of horse (anti-diphteria) serum. In about ten minutes this patient became acutely ill with all the symptoms of an anaphylactic shock. After his recovery, scratch tests were performed on his skin with horse serum. These proved the patient to be extremely sensitive to horse serum. For a period of 2-3 months after the shock reaction, the patient was free of symptoms, even in the presence of horses. This 'anti-anaphylactic' period did not last long, however. Ramirez (39) described a patient with a negative personal and family history of allergy who underwent a blood transfusion for primary anemia. Two weeks later, after a carriage ride, the patient developed an attack of asthma; subsequent skin tests with various allergens were negative, except with horse dandruff allergen. The blood donor was then found to have persistent asthma and to react strongly to horse dandruff on skin-testing.

De Besche (7), assuming that asthma due to horses was the most common form of animal-related asthma in man, advanced the theory that asthma in general must be regarded as a clinical manifestation of anaphylaxis in man. He also tried to differentiate between two forms of horse-related allergy. On the one hand he noted that in skin-testing most patients reacted to extracts of horse dandruff. On the other hand, particular patients reacted to horse serum exclusively. A number of people reacted to both. De Besche suggested that special attention be paid to these two forms of horse asthma, especially with reference to the risk involved in the injection of horse serum.

As a result of his investigations, the author propounded horse dandruff and horse serum to have an antigen factor in common (8). This might explain the observation that sufferers from horse asthma are prone to react pathologically to

the injection of horse serum. However, there are subjects who do not react to horse dandruff extracts, but do so with horse serum, even without prior sensitization by injections of horse serum. These people, as a rule, do not suffer from horse asthma and have 'latent allergy', according to Salén (45). In 1937, de Besche (9) reviewed several case reports on horse asthma. An important observation was that the majority of cases of horse asthma manifest themselves in childhood and early adolescence, in any event before the age of fourty. De Besche was the first to conduct experiments on the passive homologous transfer of antibodies against horse serum. Independent of these investigations, Prausnitz and Küstner (34) demonstrated this passive transfer phenomenon in the same year in a case of hypersensitivity in man to fish.

The relationship between horse dander and horse serum sensitivity has long remained the subject of much debate. Many animal experiments have been performed in attempts to define the relationship or difference between the antigenicity of horse dandruff extracts and of horse serum. In 1918 Rackemann (35) was unable to show cross-reactions between horse dandruff and horse serum. This was confirmed by Longcope, O'Brien and Perlzweig in 1925 and 1926 (26, 27). More successful in demonstrating a relationship between horse dandruff and horse serum was Forster (16). By cross-precipitations *in vitro* and by anaphylactic reactions *in vivo,* he demonstrated an as yet unidentified antigenic element common to horse dander and horse serum. In horse dander it occurred in relatively small proportion, however. Ratner and Gruehl (40) in 1929 substantiated an earlier observation of an antigenic relationship between horse dander and horse serum (41); the common factor occurring in only small amounts in horse dander most probably resides in the globulin fraction. Unfortunately, quantitative techniques were not employed in these studies, and no mention was made of the concentrations of the dandruff extracts used for sensitization and testing. This also applies to the work of Tuft (61), who tested sites passively sensitized with the serum of asthmatics in human volunteers. Tuft noted that prolonged contact with horse dander by predisposed individuals may result either in sensitiveness to the dander alone or in some instances to both the dander and serum.

Between 1934 and 1950 little work on allergy to horses was published. It was further taken up by Squire (58), who found the major antigen in horse dandruff extracts to have the properties of an albumin. Other horse serum proteins appeared to be allergenically inert except for those preparations contaminated with serum albumin. Squire's attempted identification of the major allergen in horse dandruff had been preceded by the early studies of Wodehouse (69, 70, 71). The latter was the first to show that the hair-keratins and -derivatives were allergenically inactive. This preliminary analytical work of Squire was extended by the thorough studies of Stanworth. This author (59) concluded from his experiments that the main allergen in horse dandruff is not serum albumin but a non-serum globulin with electrophoretic β-mobility. The work on the nature and chemistry of the allergens in human and animal dander extracts has been reviewed in detail elsewhere (4, 5).

Case histories of animal allergy.

In 1920 Walker (65) described ten patients with symptoms of rhinitis, con-

junctivitis, asthma or urticaria provoked by the emanations of horses. The contact with horses was either 'direct' by riding horseback, by taking care of horses or by just being around horses as was customary in those days in any busy street, or 'indirect', for example due to the 'dust' of the animals transferred by clothes of horse-men. Walker emphasized the importance of skin tests with epidermal protein as a diagnostic tool, and he advocated treatment by repeated inoculation in gradually increasing amounts of the epidermal protein (hyposensitization). In patients with perennial 'hay fever' caused by animals other than horses such as cats, dogs and laboratory animals (rabbits and guinea-pigs) discontinued contacts were advised.

In 1923, Rackemann (36) described an allergy to rabbits and guinea-pigs in a laboratory-assistant who developed a rhino-conjunctivitis after four years of handling the animals. The author emphasized the spontaneous occurrence of the allergy in this patient with an otherwise simple history of no atopy in himself or in the family. Scratch tests with a series of inhalant- and food-allergenic extracts produced very definite positive skin reactions to rabbit and guinea-pig dander only. To explain this special case of animal allergy, Rackemann assumed that the 'entire spontaneity' is only apparent and that at some time and in some way active sensitization must have occurred in an individual who possessed a natural 'tendency' to asthma or hay fever.

Lintz (25), in 1923, was the first to describe a patient with asthmatic attacks caused by the emanations of rats and mice. The attacks occurred mainly at night and appeared to be provoked by these rodents prowling under the floor of the bedroom. Skin tests performed with rat and mouse dander extract and with a routine series of inhalant-allergens provided no positive reactions. An inhalation-provocation challenge with the odorous emanations of a white mouse caused an acute asthmatic attack and also marked urticaria and dermatitis. The patient's symptoms disappeared after extermination of the rodents.

Chafee (13) reported two similar cases of bronchial asthma due to mouse (and rat) emanations. The first patient was a housewife, with a positive family history, who lived in a house infested with mice. By skin-testing, a 2+ reaction to mouse allergen was observed among other reactions, e.g. to house dust and feathers. She reported improvement after she had moved to another neighbourhood. The second case history does not differ much from the first. It concerns a factory worker, who's symptoms occurred almost immediately after starting work and disappeared on the way home. The factory was heavily infested with mice and rats. Skin tests showed a 3+ reaction to mouse and a 4+ reaction to rat dander extracts. PK-reactions corroborated the results of direct skin-testing. A hyposensitization therapy was instituted.

Gilday (19) reported sensitivity to rats in a veterinarian employed as a research worker. An attack of asthma occurred after entering the animal room where white rats were housed. No mention was made of the sensitization period, the personal or family history, but hyposensitization therapy was reported to be successful.

Other more detailed case histories on allergy to rats and mice have later been published by Romanoff (42), Arbesman *et al.* (2) and Sorrell and Gottesman (56).

Romanoff, in 1940, described a laboratory assistant who developed rhino-conjunctivitis after contacts with rabbits, mice and rats for one year. Whenever she handled rats she suffered from urticaria of her hands. Tests with extracts of

numerous materials including the allergens of the suspected animals provoked a positive reaction only to rat. The PK-reaction with the rat epithelium failed to show the presence of antibodies. In this patient with a clean personal history, but with a strong family history of atopy, the allergy to rats developed rather fast and could be proven by *in vitro* test methods.

Sorrell and Gottesman (56) described a young research worker who developed an allergy by handling mice for a period of two years. She complained of rhinitis only during her work with mice. Both the personal and family history failed to reveal an atopic constitution. The direct skin test with mouse dander extract as well as the PK-reaction was positive. A hyposensitization therapy was instituted.

A patient, described by Arbesman, Beede and Rose (2) in 1958, had been working with white mice for two years without trouble. Upon re-exposure three years later he developed symptoms of rhinitis and asthma when he entered the animal room. The patient had a past history of atopy. When he was a child, exposure to guinea-pigs, rats and dogs was known to produce allergic symptoms. By skin-testing a very strong positive reaction was observed not only to mouse dander extract, but also to mouse serum. Tests with other animal dander extracts, e.g. rat, guinea-pig, horse, rabbit and sheep, revealed positive skin reactions. In agreement with the findings of Simon (53) the patient reacted to a great variety of animal sera, including the sera of exotic animals. The observed ability of *in casu* mouse serum to cross-neutralize various animal sera reactions, led to the suggestion that 'false positive' skin reactions to sera of animals to which the patient was not exposed were due to cross-reactions with antigens occurring in mouse serum.

In a subsequent article Beede *et al.* (3), in 1958, gave a report of an immunological study of patient's serum. A substance different from reagin was found. This thermostable substance caused *in vitro* agglutination of red blood cells coated with all mammalian sera tested, except for human serum. The reactions appeared to be species-specific with the exception of the species with which the patient had no contacts. Serum of the mouse allergic patient of Sorrell and Gottesman (56) was investigated immunologically afterwards by Beede *et al.* The results were in conformity with the results of their own patient.

A case report of rhinitis and asthma in a technical assistant caused by guinea-pig was published by Braun (12). The symptoms developed four years after the start of her work with these animals. Like in foregoing case reports, the patient's personal and family histories were negative for atopy. Skin tests with extracts of several kinds of animal danders provided clearcut positive reactions only with guinea-pig allergens and a slight reaction with rat allergen.

An uncommon allergy to animal danders was reported by Munro-Ashman & Frankland (30). They described a sensitivity to deer scurf in a patient who had been hunting deer for ten years. Rather unexpectedly he began to notice an irritation of the eyes with an associated rhinitis and a local urticaria around small abrasions, whenever he skinned a deer. Within three years of the onset of his deer sensitivity he began to develop an associated asthma. Before this episode, he knew of no other allergic troubles, although in the past he had been in close contact with other animals, i.e. cows, horses and goats. The patient showed a marked positive reaction to prick tests with deer scurf extract. Though clinically there were no symptoms after contact with other animals, skin tests with extracts of horse, cattle, dog and rabbit dander also produced positive reactions; tests with

extracts of pollens, fungal spores and dust remained negative. The patient was treated by hyposensitization.

Other allergies to undomesticated animals were described by Blamoutier (10) and Spicer and McCormick (57). The first author reported the curious case of a patient already known to have an allergy to Siamese cats and to short haired dogs. After frequent visits to the Zoo, he started having attacks of asthma. He was soon able to tell that it was the tiger and more particularly the fox and wolf that provoked his symptoms. Later, this patient bought a monkey, only to have fits of asthma again a few weeks later. Skin tests with dander extracts provided proof for his allergy to the abovementioned animals. Another case report by Blamoutier concerned an allergy to lions. A gardener whose duties included feeding the lions, started to present asthmatic symptoms. Afterwards he became allergic to monkeys, i.e. after a change of animals in his care. Mention was also made of a dompteur in a circus who suffered from asthmatic attacks when he was in contact with lions. Skin tests performed with extracts of the relevant danders in these cases produced marked positive reactions. Finally, a case of a laboratory assistant working in the Pasteur Institute with mice, guinea-pigs, rats and hamsters, was described. After having worked for many years with these animals, this patient suddenly began to cough and had an attack of asthma. He also developed a very itching eczema of the hands. Skin tests with dander extracts of the animals concerned gave positive reactions. Control tests were negative, as were tests with other irrelevant allergenic extracts. The above observations by Blamoutier concerned allergies to animals which are rarely contacted, though in all the reported cases contacts were frequent and intensive.

In the field of atopy to exotic animals, the first and only recorded case of elephant allergy came from Spicer and McCormick (57). They described a Zoo keeper whose job it was to look after a baby elephant. He had been an elephant keeper four years earlier. Since starting the job with the baby elephant he had suffered from many minor attacks of bronchospasm in addition to some major episodes. There were no other symptoms of allergy, and there was no family history of atopy. The patient was examined for reactivity to mixed grass and tree pollens, goat and cow dander extracts, mixed moulds and *Alternaria* as well as to the elephant scurf extract. All tests were negative except for the elephant dander. He was advised to avoid further contacts, or to change his job.

Rajka (38) gave a report of ten research workers and assistants who developed an allergy to laboratory animals. All patients suffered from rhinitis, four patients had asthma. Urticaria and prurigo Besnier occurred in two patients. Three patients in addition had hay fever. By means of scratch tests nine patients appeared to have multiple allergy; only one had an isolated allergy to rat. Positive skin reactions were found to guinea-pig (5×), rabbit (5×), mouse and rat (3× each). Natural exposure tests with the suspected animals were positive in all cases. The author concluded that hypersensitivity to laboratory animals is not uncommon in research and laboratory workers in close contact with the animals. Guinea-pig and rabbit hair were the most frequent cause of hypersensitivity, but allergies to mouse hair were observed as well.

In 1971 Wilson (68) described four patients with asthma, due to hypersensitivity to hamsters. In these cases the hamsters were kept as domestic pets. The symptoms developed in one-half to two years. Until then none of the patients had ever

noticed allergic symptoms. In the author's view hamster hair contains a potent allergen, which is capable to provoke 'extrinsic' asthma in adults of low atopic status.

Investigations in groups of patients.

The first extensive studies by means of skin tests in groups of patients date back to 1916. The personal histories were not always conclusive about allergy to animals and skin tests with animal dander extracts were therefore performed to procure further evidence. Goodale (20) performed scratch tests with epidermal extracts in 49 subjects with rhinitis and/or asthma provoked by animal emanations. He observed that in all cases a definite reaction was elicited by the application of a 'keratin'-extract. While the majority of the patients were aware of their sensitization, yet for a number of individuals the first recognition of the cause of their trouble was 'obtained' through the test. It was noteworthy that 86% of the group of 49 patients presented a positive skin reaction to horse dander extract. This important observation by Goodale — as early as 1916 — has since been confirmed by many other investigators and has been reaffirmed as recently as 1977. Less frequent positive skin reactions were found to dog (17%), cat (12%), and other animals such as cow. Skin tests with the serum of the various animals were also performed. Goodale found a distinct difference in reactivity between the 'proteid' of the serum and that of the epidermal material. More than 25% of the horse dander-allergic patients did not react to the serum; an approximate similar proportion was found for dog and cat serum.

Walker (64), in 1917, performed scratch tests with horse dandruff, dog and cat 'hair' extracts in groups of asthmatic patients. About 22% gave a positive reaction to the horse extract and approximately 13% to dog and cat extract. Walker also performed skin tests with the serum of the animals; this produced positive reactions in 22%, 55% and 70% of the patients with a positive reaction to the corresponding dandruff extract, respectively.

Some case histories of patients with asthma, from a large group of hypersensitive persons, were reviewed by Rackemann (37). Emphasis was particularly laid on patients with horse asthma. Like others, this author observed a high incidence of positive reactions to horse dander extracts. But he commented: 'While a positive skin reaction to horse dander occurring alone among many negative tests, or more frequently as one of several other positive tests, has been observed repeatedly in scores of cases, a definite relationship between attacks of asthma and exposure to horse dander and, based on this, a diagnosis of horse asthma, has been confirmed in only 36 cases'. In a comparison with results obtained by skin-testing with horse serum and sera of other animals in a contemporary study, Mackenzie (29) remarks that it could not be proven that the observed non-specific hypersensitiveness was in fact acquired. He assumed that in certain individuals there must be a 'tendency' to develop this hypersensitivity.

In order to show that animal danders are not a rare cause of allergic disease, Rynes (44) performed skin tests in about 3000 definitely allergic patients. Positive reactions to cat dander were noted in 9% of the cases, 8% reacted to horse

dander, 5% to dog dander and 2% to rabbit dander. Rynes stated that much of the significance of positive reactions to dander allergens depends upon the frequency and nature of the contacts with the animal or animal products. In his study, 7% of the group of patients knew of such contacts with the animal under consideration, yet only 2,5% were able to associate this contact with actual symptoms. This made an overall incidence of only 24% of what may be termed 'symptom-provoking contacts'. This incidence varied greatly among the different animal contacts: 62% of the persons with horse contacts, 20% with dog and 18% with cat contacts could recall an aggravation of symptoms due to the presence of these animals. Concerning the remaining 76% positive skin responses, the patients were not aware of their allergy. According to the terminology of Salén (45), these can therefore be considered as subjects with 'latent' allergy.

In an extension of his 1934 studies (53), Simon (54), in 1941, performed skin tests with the extracts of the common inhalants, of foodstuffs and of miscellaneous substances, including horse serum and horse dander. Of 3630 patients, 22 were found to present large scratch test reactions to either horse serum, horse dander, or both. They were subsequently tested with 14 mammalian sera and two non-mammalian sera, viz. chicken and frog serum. Only seven patients were sensitive to horse serum but not to any of the other sera with which they were tested. Seven other patients were sensitive to some degree to all the mammalian sera. PK-reactions indicated that the skin sensitivities were often transferable. Simon emphasized that a patient sensitive to one mammalian serum is likely to be sensitive to other mammalian sera, including sera from animals to which the patients could not possibly have become sensitized, e.g. exotic animals like elephant or opossum.

Contrarywise, other investigators believe that there exists an extreme species-specificity. Blamoutier (10) remarks that the allergens of horse and donkey dander are antigenically distinct and this may also be the case in various different strains of dogs and cats. Voorhorst (63) noted differences in allergic reactions to common (European) cats and to Siamese cats. Nonetheless he proposed that there exists a common cat allergen, apart from strain-specific antigens.

To evaluate medical and social implications of rodent sensitivity in asthmatic children, Snyder and Kahan (55) undertook a study with a group of 210 patients and 60 non-asthmatics. The group of patients was subdivided on the basis of specific socio-economic situations. Scratch tests were performed, initially with both rat 'hair', and mouse 'hair' extract. It proved that people living in economically deprived areas were more likely to develop an allergy to mouse and rat hair than their fellow-men in the more affluent areas. In contrast to these data, other investigators reported the possibility of a positive relationship between educational achievement and allergic diseases (24).

Discussing the subject of animal allergy, Patterson (33) recognized that this can be a veritable problem in laboratory workers, which may on occasion require a change of occupation. About 10% of the general population has been estimated to display a genetic predisposition to allergy. An individual with one particular allergy is prone to develop allergic reactions to other allergens. In the case of animal dander allergy, antigens of species-related animals are particularly suspect. For example, the possibility of cross-sensitization between rat and mouse antigens was mentioned.

Disturbed by the fact that allergy to laboratory animals appears to be increasing,

Böhm and Braun (11) published a report of 19 research workers and assistants with allergic symptoms while working with animals. Rhinitis and conjunctivitis occurred most often; bronchial asthma was observed in six patients as well. Only five patients reported allergic symptoms such as hay fever or asthma before starting work with the animals. None of them had a positive family history of atopy. In three of the other 14 patients the family history was positive for atopy. Scratch tests revealed that all patients were allergic to rat, 42% reacted to mouse, and 26% to guinea-pig. Attention was given to the sensitization period, which varied from two weeks to three years. When there was a personal history of atopy, this interval was relatively short, as compared with patients with a negative history of atopy.

Lincoln, Bolton and Garrett (24), in 1974, examined 404 members of the ORNL Biology Division. These people were exposed to dander, hair, urine and saliva of intact animals and to tissues or sera during surgical procedures. The animals concerned were mice, rats, hamsters, rabbits and guinea-pigs: 58% of the workers had frequent exposure to animals. Of these 11% developed allergic symptoms. Most patients of the group (80%) complained of nasal congestion or rhinorrhea and itching of their eyes. About 50% experienced episodes of asthma. Only two of the patients (7%) developed angio-oedema after contact. Most of the patients had other allergic problems. For example, about 50% had typical seasonal hay fever. Prick tests were performed with a routine series of inhalant allergens and with extracts of relevant animal danders in the 27 patients; 77% reacted to pollens and 63% to house dust. A positive skin reaction to one or more of the animal danders was shown by 89%. A history and skin test survey was conducted among the members of the Biology Division and in a matched non-exposed population. There were few differences. Hay fever occurred in both groups with equal frequency, i.e. in 22%, as did asthma in about 6%. The obvious exception was a higher frequency of positive histories as well as positive skin reactions to animal danders in the exposed group (24% against 17%). There was a trend towards a positive relationship between educational achievement and allergic diseases, in contrast to the findings of Snyder and Kahan (55).

Further research in the field of laboratory animal dander allergy (LADA) was carried out by Lutsky and Neuman (28). Data were collected and processed on about 1300 employees from laboratories where experiments with animals were conducted. About 15% of these subjects showed symptoms of hypersensitivity to the laboratory animals. This incidence falls well within the overall 10-20% incidence of atopic diseases reported for the U.S. of America. The allergic response was characterized by symptoms like rhino-conjunctivitis (100%), asthma (71%), contact urticaria (58%), cough (58%) and palatal itch (38%). In 93% of the group the symptoms became apparent within ten minutes after exposure to the animals. Of the group of employees with symptoms 30% were aware of allergy in the family. The time of exposure to the animals, prior to the onset of symptoms ranged from a few weeks to 22 years. In 86% of the cases symptoms appeared within four years of contacts. A duration of one to three years occurred most often. Frequent allergies were recorded to the danders of rat (56%), mouse and rabbit (37%), guinea-pig (24%), cat (13%) and dog (10%). Over 50% of the patients were allergic to more than one animal. No mention was made of skin tests in a control group or in a group of laboratory workers without clinical symptoms.

The diseases due to LADA were characterized as a bonafide occupational allergy. About 13% of the affected workers were advised to change their job; another 15% voluntarily resigned.

Next, Neuman & Lutsky (31) attempted to define and characterize the clinical and laboratory conditions for persons at risk of developing animal allergy. Detailed case histories were obtained from ten research workers with a history of allergic symptoms due to laboratory animals. Eight of them had a personal history of atopy, nine had a family history of atopy and all ten had positive skin reactions to environmental allergens besides the skin reactions to the animal dander extracts.

The time of onset of LADA symptoms varied widely, from immediate up to nine years after the first contacts. The authors assumed that the time of onset and the severity of the clinical symptoms depend on the patient's genetic background and on the intensity of the allergenic load. However, with no genetic background, it still proved possible for a specific allergy to develop.

Rudolph *et al.* (43) recently re-examined 79 patients belonging to a group of 214 individuals already known to have an animal dander allergy. Sensitization to guinea-pig was found in 49% of the patients, while 22% of them were allergic to cats, 14% to hamsters, 8% to rabbits and 2,5% to horses and dogs. In only 18% of the cases, an isolated sensitization was found to a particular animal; in the other cases co-reactions were observed, most frequently to the dander extracts of sheep, cattle and horses. In about 42% of the patients a history of allergy preceeded the registered animal sensitization. The family history of atopy was positive in 33% of the patients. The period of exposure to the animals before the appearance of symptoms varied from between some days up to many years. Rapid sensitizations occurred in cases where personal and family histories were positive for atopy. It is considered that individuals sensitized to one particular antigen, subsequently sensitize easier to other antigens, possibly as a result of cross-sensitization due to common antigenic structures. This may indeed be a plausible explanation in the case of related animals such as rodents. The high incidence of skin reactions to animal dander extracts of unrelated animals like horse, cow, sheep, goat and pig, however, was supposed to be due to an unknown mechanism.

Schultze-Werninghaus *et al.* (49) performed skin tests and bronchial provocation tests (BPTs) in a group of 355 asthmatic patients. Of these, 176 had actual and frequent contacts with animals, while 75 of this group noticed symptoms during, or after, only occasional contacts. All the patients were skin-tested with the dander extracts of dog, cat, horse, cow, goat, guinea-pig and hamster. In the group of 75 patients with symptoms due to animal contacts, an allergy could be proven in 88%. In the remaining 12% there was no response to the skin tests. Contrarywise, in patients with no animal contacts, 18% presented 'false positive' skin reactions. Obvious in this investigation was the large number of history-related sensitizations to guinea-pig; allergies to cat and to dog occurred less often, while sensitizations to horse and to cow were in the minority. Because of the negative skin reactions and the 'non-related' positive reactions, BPTs were performed. Negative results were not seen in patients with strong skin test reactions. Positive BPTs were recorded in some cases with a negative or weakly positive skin response, however.

Investigations with the aid of IgE measurement

The diagnosis of animal dander allergy in the above-described investigations had to rely on history-taking and on various methods of skin-testing and other provocation tests.

After the discovery of immunoglobulin E as carrier of reaginic antibody activity (23), a new diagnostic tool became available which measures specific IgE-antibodies in the serum by way of the radioallergosorbent test, RAST (66). Several studies indicated a good correlation between IgE-antibodies determined by RAST *in vitro* and 'reaginic' activity as evaluated by *in vivo* methods. As regards animal dander allergy, Fagerberg & Wide (14) were the first to apply the RAST technique in their study of dander allergens from different dog breeds. To answer the question whether there are breed-specific allergens and/or whether there are allergens in common among the various breeds, they performed skin tests, inhalation tests and RASTs with the dander extracts of several breeds of dogs. All 35 asthmatic patients involved in the investigation had been in contact with dogs and were suspected of allergy to dog epithelium. The test solutions consisted of two different mixed dog dander extracts — alsatian, boxer and mongrels — and nine different individual breed extracts, viz: alsatian, boxer, cocker-spaniel, poodle, drever, dachshund, dalmatian, swedish foxhound and collie. An overall agreement of 97% was found between RAST and clinical diagnosis when using the mixed dog extract. Comparisons between RAST results and inhalation challenge tests showed 95% agreement; between RAST and skin tests the agreement was 85%. RAST results with individual dog breed extracts agreed with inhalation provocation tests in only 59% and with skin tests in 65% of the cases.

These results indicate that there are differences between allergens from different breeds of dogs. Tests with breed-specific extracts showed less agreement than tests with mixed dog dander extracts. The authors concluded that mixed dog breed extracts are superior to the individual items for diagnostic purposes, which supports the idea of a common non-species-specific major determinant in dog allergens.

Foucard (17) investigated 81 asthmatic children with the aim of studying the development of reaginic allergy. The results of skin tests and RAST were related to the clinical cause of the disease. Tests were performed with extracts of pollens from birch, timothy and dandelion, of egg-white and fish, and with aqueous extracts of horse, dog and cat epithelium. In 24 of the 81 children 65 positive skin tests were found. There was a relationship between the size of the skin test reaction and the serum concentration of specific IgE antibodies. The correlation with the RAST results was best with the stronger skin reactions. As concerns IgE antibodies to animal danders, elevated RAST levels were present in eight children. A good correlation (86%) was again reported between the sizes of the skin reaction and IgE antibody concentration in the serum. Only a minority of the weak reactions was accompanied by positive RASTs. Furthermore, specific antibodies were not detected before the age of one year and a half. It was concluded that the significance of weak skin reactions must be interpreted with caution, but that strong skin reactions are highly indicative of reaginic allergy.

Provocation tests, skin tests and RASTs were performed in 156 asthmatic children, by Apold *et al.* (1) using 11 different allergens. These included pollens, moulds and dander allergens from horse, cow, dog and cat. Skin tests were per-

formed (by scratch, prick, and intradermal method) with appropriate allergen concentrations. Bronchial provocation tests (BPTs) were carried out with increasing allergen concentrations. The agreement between RAST and skin test was good, particularly for the pollen allergens. The overall agreement between BPTs and RAST was 84%; between positive BPTs and RAST the agreement was only 61%, being highest for the pollen, and poorest for the mould group. Within one particular group of allergens the agreement varied significantly. High RAST classes were conclusive for the presence of clinical allergy, but this was not so for the intermediate RAST classes. The sum-total of RAST and skin test score provided more conclusive correlation in a number of cases. However, the advantage gained from combining RAST and skin test was limited, particularly for animal danders. The results obtained with RAST by Apold *et al.* were less favourable than those recorded in preceding studies. The authors state that the reliability of RAST is dependent on the type and quality of the allergen, which is a probable cause of the observed differences.

Assuming that there is an agreement between the results of RAST and those of biological tests for specific atopic allergies, but considering that the reliability depends on the type of allergen extract used, Havnen *et al.* (22) compared the results of skin tests and of BPTs with RAST when allergen preparations from two different producers were used. Eleven allergic children were tested with dog allergens, and with birch and Timothy pollen allergens. A good overall agreement was obtained with the two extracts from different commercial sources. As concerns dog allergy, three patients had a highly suggestive history and in one it was uncertain. Skin tests, with the two different dog extracts showed one failure, and this was also the case with the BPT. When skin test and BPT results were compared, a complete agreement between these *in vivo* tests for one of the extracts existed. The other extract gave a 73% agreement (including the negative results). In those cases where skin tests gave only weak responses and BPTs were positive, RAST values were not detectable. In conclusion Havnen *et al.* state weak skin test reactions and low RAST values are not to be relied upon with respect to immunological specificity.

Finally, Sarsfield *et al.* (47) have recently investigated the possible clinical consequences of keeping pets. They employed the methods of history-taking, skin-testing and the measurement of specific IgE antibodies by RAST of an unselected group of 118 asthmatic children. Exposure to animals was assessed both at home and at school. Close and prolonged contact occurred more often at home than at school, though the latter should not be overlooked.

Inquiries revealed that in 86% of the schools, under investigation, animals were present. The most common pets were of the rodent family, especially gerbrils (61%) and rabbits (8%). A history of pet contact was obtained in 55% of 118 unselected asthmatic children. This group of 65 children was divided into two subgroups. First, a group of children in whom clinical sensitivity was not apparent and, second, a group of children with a history-related clinical animal allergy.

Positive skin tests in the first subgroup were obtained in 70% (latent allergy), and in the other subgroup in about 100% (related allergy). In the latent allergy group, specific IgE antibodies were detected in 8% of the children. No specific IgE was found in the skin test-negative children. In the group of children with relevant skin reactions, only 52% positive RAST results were obtained. This

correlation was lower than expected. The effect of avoidance of contacts or complete cessation of allergen exposure was discussed. The results indicated that the clinical history is a rather reliable guide of pet sensitivity, as judged by skin as well as by RAST results. Skin tests often provide an unrelated positive reaction (70%), whereas IgE results confirm a specific allergy in only 50% of the cases.

Discussion

The first accurate description of allergic symptoms caused by animal contacts was given in 1851 by Salter (46). Previous to this only historical reports are available in the literature (48, 62). Comparatively few case histories of animal allergy are recorded in the medical literature. Most case reports deal with descriptions of symptoms of patients having professional contacts with laboratory animals, such as guinea-pigs (10, 12, 36, 38), rats (10, 19, 25, 38, 42), mice (2, 10, 13, 25, 38, 56), hamsters (10) and rabbits (36, 38, 46).

In publications concerning groups of patients allergic to laboratory animals, records have been filed on guinea-pigs (11, 24, 28, 43, 49), mice (11, 24, 28), rats (11, 24, 28), hamsters (24, 43, 49), rabbits (24, 28, 43), cats (28, 43, 49) and dogs (28, 43, 49). In professional contacts outside the field of laboratory animals there is a hazard of becoming allergic to other animals as well. Unique case histories of a hunter (30), a dompteur (10), a zoo-gardener (10) and an elephant keeper (57) are on record. In the fur industry there is the possibility of becoming sensitized to animal dandruff. Rabbit epithelium is the most frequent offender (51, 52). Keeping animals as 'pets' must be a frequent cause of sensitization, but amazingly few case histories are available on this subject, e.g. cats (46, 37) and hamsters (68).

The case reports in this review are specimen of histories concerning 'relevant' allergies to animals. Most of the personal histories are so convincing for allergy to the corresponding animal that skin tests, in retrospect, would have been superfluous. In point of fact, these were performed solely to furnish clear proof of the suspected allergy. Because of the importance of history-taking, some corresponding data, obtained from the case reports and from the group investigations will be recorded here.

The time of onset of symptoms, after having acquired an animal, or after professional contacts with animals, on average is less than five years (2, 11, 12, 28, 36, 38, 42, 56, 68), with a range of some years to ten years (28, 30, 31, 38, 43, 68). The most frequently cited period is at three months (19%) to two years (27%) (28). Factors which influence this sensitization period are: a previous personal history of atopy, a positive family history, frequent and intensive contacts with animals, and the kind of animal concerned. When a positive personal and/or family history of atopy occurred the sensitization period appeared relatively short (11, 28, 31, 38, 42, 43, 56).

The term atopy originally included the suggestion that hypersensitivity runs in families. Although heredity undoubtedly is a predisposing and contributory factor in atopic allergy, this factor appeared to be of minor or no importance in the cases of isolated allergy to animals (11, 12, 36, 38, 43, 56, 57, 68). It might be said that these case histories in fact unveil the very first atopic manifestation of the family in

question. In only two cases there was a positive personal history of atopy before the acquired animal dander allergy became manifest (57, 68), and in one case there was a positive family history (42). Investigations in groups of patients revealed similar and even more convincing data with regard to the family history. Absence of atopy in the family was recorded in about 40-84% of the cases (11, 28, 38, 43).

Animal species which most frequently caused atopic symptoms have been the subject of research for several investigators. Few data can be extracted from the case histories, but the rodents are likely to be frequent offenders, e.g. guinea-pigs (10, 12, 36, 38), mice (10, 25, 38, 55, 56) and rats (10, 25, 38, 42, 55). Investigations in groups of patients produced more valid information, but definite conclusions can not be drawn because of many differences in the set-up of the research projects.

First, the period of investigation concerns 1916-1975 and this causes untractable variations, e.g. in the purification technique of the test extracts, the manner of skin-testing and the reading of the skin reactions. Second, the choice of animals differs among the various groups and, third, the composition of the groups of patients varied considerably. Despite these uncertainties a comparison has been made and some arbitrary conclusions are drawn.

In early times (1900-1940), horse-derived allergens were the most frequent cause of allergic symptoms, followed by dog and cat.

Investigations in later years (1972-1975) among laboratory workers with intensive animal contacts, revealed that rodents (43) — especially hamsters (11), guinea-pigs (11, 43, 28), rats (11, 28), mice (11, 28) and rabbits (28) — are potent allergy-provoking animals. When skin tests with extracts of these animals were positive, almost every patient had already been aware of the cause of his allergy symptoms. Furthermore, isolated allergies to rodents often occur, e.g. rat (11) in 37% and various other rodents (28). This contrasts with the positive skin reactions to extracts of sheep, cow (43) and particularly horse (24, 43, 49). In the latter publications mention is intentionally made of socalled 'false positive' skin reactions to horse dander extract. Prior to this, these non-history related reactions (NR-reactions) must have frequently been observed as well on skin-testing routine series of allergens in groups of patients, e.g. to the horse dander extract of Goodale's investigation (20), where 86% of the patients presented positive reactions. Rackemann (37) too observed a high incidence of reactions to horse dander extract, while a relationship between asthmatic attacks and exposure to horses could be confirmed in only a ten cases. Rynes (44) found in skin test-positive individuals an overall incidence of only 24% of what he called 'symptom-provoking contacts'. The remaining 76% of the patients, having animal contacts, but not being aware of their allergy, were considered as subjects with 'latent allergy'.

The first reported true NR-reactions were observed in a non-exposed population serving as control subjects in the investigation of Lincoln *et al.* (24). In 17%, NR-reactions were observed to skin tests with animal dander extracts. While only 18% of the patients investigated by Rudolph *et al.* (43) had an isolated allergy, co-reactions were noted in the remaining cases. NR-reactions occurred most frequently to the extracts of sheep, cattle and horse. It was considered that co-reacting to extracts of related animals, e.g. rodents, was possibly a result of cross-sensitization due to common antigenic structures, but as regards the unrelated animals an unknown mechanism was supposed to be involved. The patients from

the investigation of Schultze-Werninghaus *et al.* (49) presented in 18% false-positive skin reactions. Where the results of skin-testing and history-taking were not in conformity, BPTs were performed. These provided no better results, however.

After the discovery of IgE (23) and the development of RAST (66) a step forward was made to justify the term 'relevant' allergy or — because of the propounded specificity of the test method — 'specific' allergy. The RAST-parameter, however, also did not prove to be completely reliable. Although Fagerberg and Wide (14) obtained an overall agreement of 97% between RAST and clinical diagnosis (including negative data) for mixed dog extract, the results with individual dog-breed extracts agreed in only 60%. This supported the idea of common non-species specific determinants in dog allergen extracts. Foucard (17) obtained a score of 86% as best correlation with the strong skin reactions.

The same overall agreement was reached by Apold *et al.* (1). The advantage gained from combining RAST and skin test results, was limited, however, particularly for animal dander allergens. Only high RAST classes were conclusive for relevant allergy. Havnen *et al.* (22) arrived at the same conclusion. Finally, Sarsfield *et al.* (47) obtained 8% positive RASTs in children with a latent allergy (NR), while only 52% agreement was obtained in a group of children with relevant skin reactions.

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CHAPTER III

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THE SPECIFICITY OF ANIMAL DANDER ALLERGENS: A SEMI-BLIND CLINICAL AND EXPERIMENTAL STUDY

By

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Apart from the allergens in the skin flakes of man and horses, little is known of the chemical nature and immunological specificity of the skin-reactive allergens of animal epithelial tissue in general (3). However, in the practice of allergy, there is a need of accurate information on vital issues such as allergen content of various animal dander extracts, standardization, species-specificity, cross-reactivities, etc.

Though neither allergen has been isolated in pure form, it has been shown that highly purified human and horse dandruff allergens most probably represent 2.5-3.4 S α-glycoproteins with isoelectric points of pI 4.1-4.2 (2, 17). In the human material, trace contamination of (antigenically modified) serum albumin and IgG was noted, together with a considerable proportion of 2.5 S serum α_2-glycoproteins. The human allergen was demonstrated to occur in sweat collected from the skin surface (1).

In a recent series of experiments, Ceska and colleagues (10, 11, 18) have shown that the dander extracts of cat, dog, horse and cow, separated by gel isoelectrofocussing, contain common major components of pI 4.5-5.0. These bind IgE from the serum of sensitive individuals, as determined by radioallergosorbent test, using ^{125}I-labelled anti-(Fc) IgE (ND). However, binding antigens of different pI were also noted, which were believed to combine with distinct IgE counterparts. Though no *in vivo* tests were performed, the conclusion of these authors, namely that there may exist major common as well as minor specific determinants among dander allergens from different (dog) breeds, is corroborated by the results of Fagerberg & Wide (12).

We have approached this problem by performing skin tests with different animal dander extracts prepared under standard conditions and submitted to the clinical investigators under code; simultaneously, a newly developed *in vitro* assay based on human complement consumption was employed (8, 9). A preliminary account of this work has been given (7).

Materials and methods

Allergens

Dander material was obtained from different groups of animals by carefully clipping the fur close to the skin with an electric razor. The samples were from Alaska female rabbits, female guinea-pigs, female Swiss random-bred mice, male hamsters, female Wistar random-bred rats, the fur of one cat and hair from one (poodle) dog. Hairs were weighed and washed twice in acetone. The acetone phase filtered through Whatman no. 1 filter paper. Powdered defatted dander remaining on the filters was air-dried, weighed, and extracted for one day at room temperature with 1 per cent NaCl-1 per cent phenol, then with 1 per cent NaCl, and finally with water. The combined extracts were dialysed against distilled water, and the non-dialysable material was lyophilized. Yields were recorded, ultraviolet absorption and fluorescence emission, nitrogen and carbohydrate were determined as before (4).

Complement consumption

Complement consumption tests with *human* complement in fluid phase were performed as follows (9). Samples of 15 µl of a patient's serum were incubated with varying amounts of dander allergen in a total volume of 0.5 ml Ca^{++} -Mg^{++}-veronal-saline buffer (VSB^{++}) made up according to Kabat & Mayer (13). Time of incubation: 30 min. at 37° C. Residual C^{hu} was then assayed by adding 2.5 ml of VSB^{++}, followed by 1.0 ml of sheep red blood cells (SRBC) optimally sensitized with rabbit anti (SRBC) haemolytic antibody (A), 2.5×10^8 cells/ml. After 60 min. at 37° C, residual C^{hu} was evaluated by observing haemoglobin in supernatants at 541 nm (13). Complement consumption was expressed in terms of decreasing Z-values (13, 16), where $Z = -\ln (1-y)$ and y represents percentage haemolysis.

Skin tests

Prick tests and intracutaneous tests were performed by routine techniques in atopic patients, employing the allergen concentrations mentioned in the Results section. Patients were selected on the basis of their clinical symptoms, a family history of atopy, a clinical history of animal dander contact and/or sensitivity, manifested by positive weal and flare skin responses to the injection of commercial dander extracts. The majority of these patients displayed positive skin reactions to purified house dust and human dandruff allergen.

Results

Recoveries and composition

It is common practice to adjust the strength of an allergenic extract on a weight (of dry substrate) per volume (of extracting fluid) basis. The allergen concentration per ml of extract then clearly depends on the allergen content of the dry dander, which may differ among danders of different sources. A first step towards better standardization might be based on the consideration that the common inhalant allergens possess molecular weights that do not allow them to pass through dialysis membranes (4). However, as shown in Fig. 1, the yield of total non-dialysable substance — which should embrace all the allergens — as well as the proportion of skin scales adhering to hair varies considerably among the different animal danders. Hence, conventional weight per volume extracts contain variable amounts of high-molecular weight material. The highest proportion of potential allergenic substance was obtained from the dander of dog, horse, cat and rabbits. In some cases, for example with the Swiss random-bred mice, the extremely low yields of soluble material hampered further analytical and isolation work. Even though it

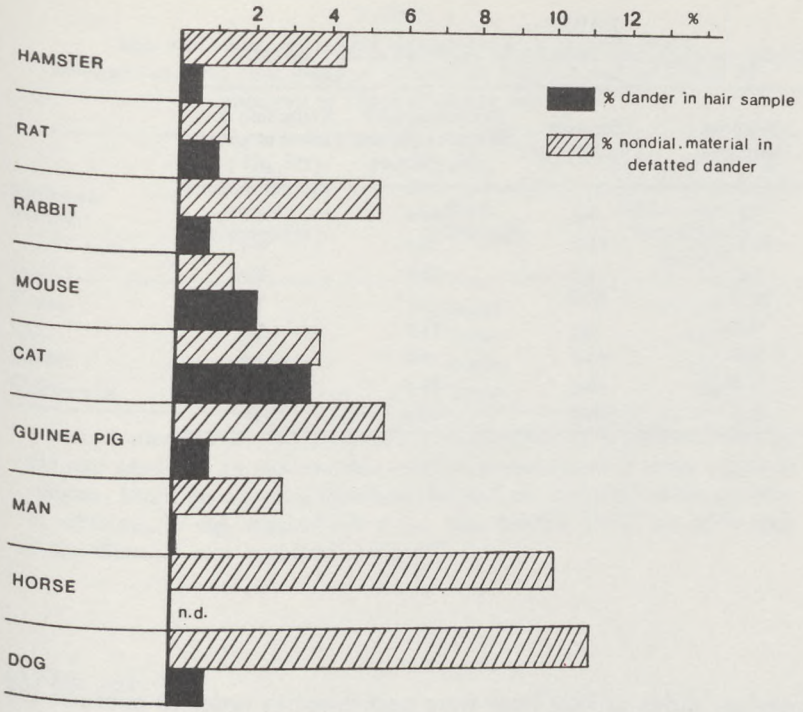

Fig. 1.
Diagram showing the percentage of dry dander obtained from the samples of hair used in this study, and the percentage of total extractable, nondialysable substance extractable from these danders.

was recognized that much of the total non-dialysable extract material must be non-allergenic components (e.g. mucopolysaccharides (4)), it was therefore decided to standardize test solutions for the present studies on the basis of equal concentrations of total non-dialysable soluble (lyophilized) substance per ml of aqueous solvent.

Physicochemically, the dander extracts all featured the UV-absorption spectra considered typical of allergenic extracts (4). Some numerical data have been listed in Table 1.

The dander extracts, at 3 per cent concentration, were examined by electrophoresis in agar gel at pH 8.6, using carbohydrate and protein stains for visualization (2). Except for the known protein composition of the horse (17), human (2) and cow dander extracts (14, 15), no polysaccharides or proteins that could be stained in a conventional manner were noted in the extracts of the other dander extracts. Only the horse dander extract produced a faint precipitation line during immunoelectrophoretic analysis against rabbit antiserum to purified human dandruff allergen, as described before (2).

TABLE 1

Analysis of total nondialysable sustance in different dander extracts.

Source of dander	Per cent Nitrogen	Per cent Total hexose	Extinction coefficient at 280 nm (1%, pH 7.0)	Extinction coefficient at 305 nm (1%, pH 7.0)
Human	6.1	6.4	10.0	5.2
Horse	10.2	13.9	10.4	4.5
Cow	7.5	5.7	18.0	9.0
Wistar rat	N.D.	N.D.	5.4	3.4
Rabbit	N.D.	4.4	13.2	8.0
Guinea pig	N.D.	15.2	19.0	11.9
Cat	N.D.	12.7	16.7	11.2
Dog	8.3	10.2	20.4	13.2

Skin tests

Three independent series of skin tests were performed in order to appraise the potency and the allergological specificity of the different extracts. For intradermal testing, stock solutions, of non-dialysable material were made up at 0.5 mg/ml, which were diluted 1:25 prior to testing (20 μg/ml); for prick testing, the 0.5 mg/ml solution was used undiluted. All extracts were coded by alphabetical numbering, which was different for the three separate series. The code was broken after evaluation of the completed sets of skin test results.

TABLE 2

Results of intradermal tests with purified house dust allergen (fraction E, 2 μg/ml) and different dander extracts (20 μg/ml), first series. Codes: C = horse dander; D = rabbit dander; E = guinea pig dander; F = cat dander.

	Number of patients	House dust positive; C, D, E, F positive	House dust negative; C, D, E, F positive	Negative controls
Total	26	18	4	4
Atopic	21	17	4	0
Non-atopic	5	1	0	4

TABLE 3

Skin-reactivities of soluble dander proteins, in units/mg, averaged over the total group of patients in Table 2, and prediction of potencies in classical (w/v) extracts.*

Source of dander	Nondial. soluble protein (mg/g dander)	Skin-reactivity found (units/mg)	Allergenic activity in units/ml predicted in 0.1 "percent" conventional extracts
Horse	100	113.400	11.340
Cat	38	13.100	498
Rabbit	53	10.250	543
Guinea pig	55	1.500	82

* 1 unit of allergenic activity is defined as the amount of lyophilized material (in mg) causing a 1+ positive skin reaction on average in a group of atopic subjects; hence, the activities filed here represent the reciprocal of the quantity of substance, in mg, required for a 1+ skin reaction (weal, no more than 5 mm diameter with associated erythema). (4)

a) *First series*

The number of patients involved, and the overall results of the tests have been collected in Table 2.

From an analysis of the skin test results and the history in individual patients, we were unable at the time (7) to decide whether or not the dander extracts had diagnostic value in detecting specific sensitization or whether the dander extracts were species-specific. Evaluation of the clinical data revealed several atopic patients who had not been suspected of animal dander sensitivities, yet reacted to the intracutaneous tests in an irregular fashion (most often to the horse dander allergen). For example, one patient — positive to house dust — reacted to horse and cat dander, despite the absence of animal contacts. Conversely, a patient was investigated who did not react to house dust, had a history of asthma and urticaria by horse dander, and presented impressive skin reactions to human, horse, rabbit, cat and guinea-pig dander.

Disregarding specificities, the mean allergenic potency of the dander extracts were calculated per mg of soluble substance on the basis of the overall positive tests, and expressed in units/mg as reported before (4). This permits an appraisal of the relative potencies of the dander extracts and — in combination with the recovery data of Fig. 1 — allows of a prediction of the test strength of conventionally prepared (w/v) extracts. The results are shown in Table 3.

b) *Second series*

For the investigation of a second series of patients, fresh solutions were prepared, and the codes were changed. Prick tests were performed in 11 patients, at

standard concentrations of 0.5 mg/ml. The results are shown collectively in Table 4.

TABLE 4

Results of prick-tests with house dust and different (coded) dander allergens in a second group of patients. Test concentrations: 0.5 mg/ml. A = cow; B = horse; D = dog; E = guinea pig; F = cat; G = rabbit dander; the + sign indicates a positive skin reaction, disregarding the gradation.

Patient	A	B	D	E	F	G	House dust	Remarks
1	o	o	o	o	+	o	+	
2	o	+	+	o	+	o	+	Clinically, sensitive to horse, dog and cat dander.
3	o	+	+	o	+	o	+	Previous i.c. tests positive to horse dander only.
4	o	o	+	o	+	o	+	Contact with horses, cats and dogs; previous i.c. tests positive to horse, guinea pig, cat and rabbit dander.
5	o	+	o	+	+	o	+	Contact with cow dander, previous i.c. tests positive to horse, guinea pig, cat and rabbit dander.
6	o	o	o	o	o	o	dubious	
7	o	o	o	o	+	o	+	Contacts: cat and dogs.
8	o	o	o	+	o	o	+	Contact: guinea pig (pet animal).
9	o	o	o	o	o	o	+	
10	o	o	o	o	o	o	+	
11	o	+	o	o	o	o	+	Contacts: cats and dogs.

Though, in the first group of patients, the correlation between the history of contacts and skin reactivity to the respective dander allergens was poor, the results of Table 4 show that the skin reaction can occasionally be highly specific indeed. This is especially evident in patients 2 and 8, where the test reactions to the coded allergens, in retrospect, correlated well with the clinical history. The negative reaction of patient 5 to the cow dander extract may be attributed to a very low allergen content of the non-dialysable material (see below). The test results in some subjects (no. 3, 4 and 5) who had also been included in the first series (a), dit not correspond entirely with the intradermal tests performed previously.

c) *Third series*

The above two groups of patients were investigated at the Paris Pasteur Institute. The subjects examined in the third series were patients attending the Out-Patient Department of Allergy at Utrecht. The dander allergens were again submitted under code number and were evaluated by intracutaneous assay at 20 μg/ml. The results collected in Table 5, demonstrate a tendency in most patients to react to

TABLE 5

Results of intradermal tests with different (coded) dander allergens (20 µg/ml) and purified house dust allergen (2 µg/ml) in a third series of patients. Key: + weal no more than 5 mm diameter with erythema; 2+ weal 5–8 mm and erythema; 3+ weal 8–12 mm, with erythema and pseudopods. P = cow dander allergen; Q = dog; R = cat; S = guinea pig; T = horse.

Patient	P	Q	R	S	T	House dust
1	—	2+	3+	3+	2+	2+
2	—	—	—	—	—	—
3	—	—	—	—	—	—
4	—	—	—	—	—	—
5	—	+	+	—	+	+
6	—	+	3+	+	2+	2+
7	—	+	2+	—	+	2+
8	—	—	—	—	—	—
9	—	+	3+	—	+	2+
10	—	+	3+	+	+	2+
11	—	—	—	—	+	2+
12	—	—	+	—	+	2+

each of the dander allergens, though in later investigations some striking cases of isolated allergies have been observed.

From the overall results of these pilot studies we gained the impression that two types of responsiveness occurred in our patients. The predominant situation was that positive skin reactions occurred to several different dander allergens, which did not correlate well with a history of animal contacts. Occasionally, however, isolated skin reactions were observed — not necessarily in typically atopic patients — to one special dander extract; in such cases, this 'specific' hypersensitivity usually fitted well with a history of sensitization to the particular animal species.

In vitro tests

As shown in two recent reports (8, 9), atopic allergens are capable of inactivating human, but not guinea-pig, complement in fluid phase. The inactivating capacity of individual allergens is related to the presence within the molecular framework, of 'allergenic' sites of lysine-sugar linkages. These structural sites have also been implicated as being responsible for eliciting non-allergen-specific skin reactions in man, by priming a universal mediator system of varying susceptibility among different people (5, 6, 8, 9).

The dander allergens employed in the present studies were examined for their capacity to induce *in vitro* consumption of human serum complement, by way of the simple technique described in the methods section. Fig. 2 collectively shows the experimental results obtained with a single serum sample (of patient 6, Table 5, who had not been desensitized to any of these dander allergens); similar results were obtained with other serum samples. It follows from this figure that dander allergens, like other atopic allergens (8), can induce consumption of human com-

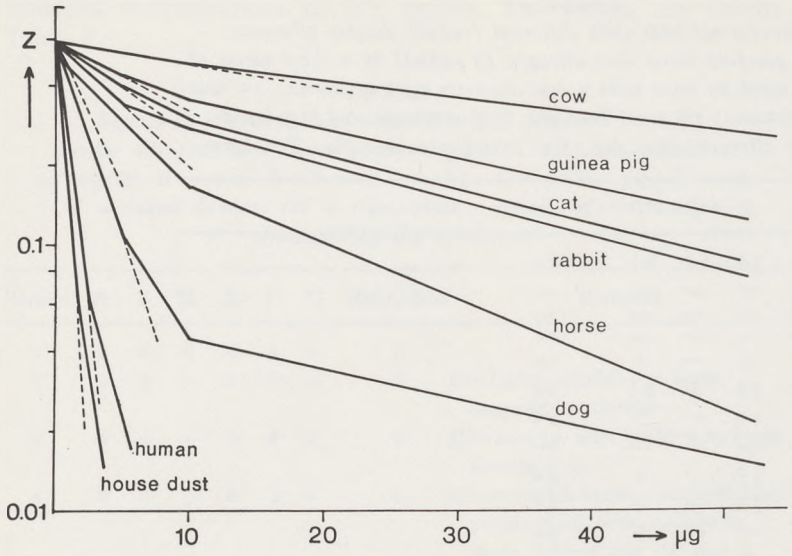

Fig. 2.

Graph showing the loss of human complement from the serum of patient 6, Table 5, by incubation under standard conditions (methods section) with house dust and dander allergens. Complement loss has been expressed in decreasing $Z = -\ln(1-y)$ values. (13, 16).

plement. Moreover, the different activities of the various dander allergens in this complement test system roughly coincide with their potencies in skin tests.

The C-inactivating power of individual allergenic extracts was compared by plotting complement-titers in the presence (Za) and absence (Zo) of allergen as Za/Zo ratios on a linear scale against allergen concentration on a logarithmic scale; by extrapolation to 100 per cent loss of haemolytic complement, this permitted comparing values for the *in vitro* activity of the different allergens.

The graph of Fig. 3 clearly demonstrates that there is a good correlation between the C-inactivating power of various dander allergens and the estimated mean skin reactivities in units/mg calculated over the whole population of the first series of patients (Tables 2 and 3). It is of note that the 'dose-response' relationship depicted in Fig. 3 is very steep. This has also been found with other allergens; the effect may be due to amplifying sequential reactions in the skin, which do not proceed in the *in vitro* assay.

Taken together with the observation that the human dandruff allergen invariably produced positive skin reactions in our patients, and noting that the poor *in vitro* activity of our cow dander sample appears to correlate with its relative impotency in skin tests, the results of complement inactivation measurements strengthen our conviction that the *in vitro* tests are a reflection of the non-allergen-specific action of the dander allergens.

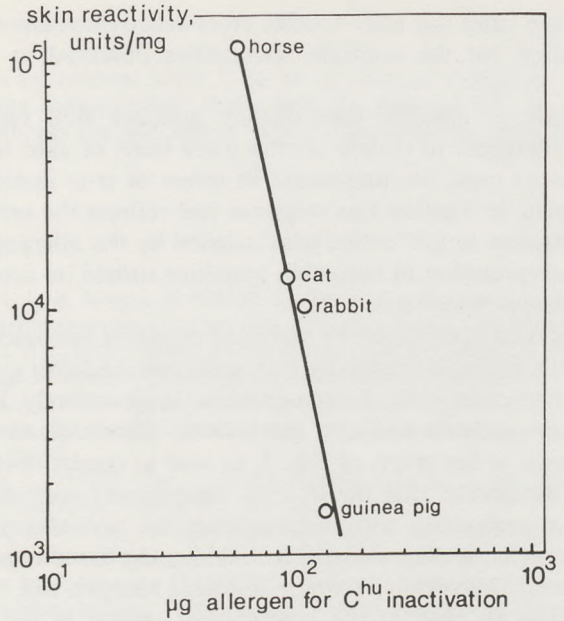

Fig. 3.

Relationship between *in vitro* capacity to inactivate complement in the serum of a single patient (expresed as µg of allergen required for 100 per cent complement inactivation under standard conditions), and the mean skin-reactivity (in units/mg) of the same allergens in a group of patients (series 1, Tables 2 and 3).

Discussion

The extremely low yields of total extractable and non-dialysable material from animal dander material has not permitted us to attempt purifying the allergens. Even though wide variations were noted in the percentage of total extractable material among the different danders (Fig. 1), this does not tell us anything about the true *allergen content* of the extracted material. This point is directly related to the problem of allergen standardization.

The results of the clinical studies tend to fit well with the concept developed along various experimental routes by one of us, namely that a distinction should be made between immunologically specific and non-specific mechanisms in human atopic disease (4, 5, 6, 8, 9).

In this approach to atopy, allergens are considered to exert a dual action. On the one hand, they may prime a universal mediator system, involving human serum complement *in vitro,* by virtue of a common 'allergenic determinant', or 'active site' of lysine-sugar bonding, which is not specific for the carrier macromolecule. Second, the specific *antigenic* determinants of an allergen molecule may induce the production of allergen-specific antibodies, probably of the IgE class. The clinical evidence presented in this paper indeed demonstrates that, whereas many patients react to several unrelated dander allergens, apparently in a non-specific fashion, isolated cases may occur of immunologically highly significant *specific* sensitiza-

tion. The latter observation rules out inter-species cross-reactivities among dander allergens as an explanation for the multiple sensitivities observed in the other patients.

It is extremely difficult, in patients who display multiple skin reactivity to various distinct dander allergens, to decide on the mere basis of skin test results whether a positive response must be interpreted in terms of true immunological sensitization to the allergen, or whether this response just reflects the sensitivity of an underlying mediator system to the 'active sites' carried by the allergen. We feel that the future clinical interpretation of such skin reactions should be accompanied by the results of appropriate *in vitro* tests.

If the allergens exert a dual action, one by virtue of common biologically active sites detecting the patient's intrinsic sensitivity of a universal mediator system, and the other based on the interaction with allergen-specific (IgE) antibody, it becomes obvious that two *in vitro* methods and two methods of standardization will be required. The results shown in the graph of Fig. 3, as well as results obtained with other allergens (8, 9) demonstrate, that the *in vitro* complement consumption test is a reliable method for evaluating and standardizing the non-allergen-specific biological activity of allergens; it may also aid in establishing the allergen content of crude allergenic extracts. Conversely, a single standard allergen will be suitable to determine the sensitivity *in vitro* of the complement system in the serum of individual patients. It should be remarked here that current studies with the sera from house dust and grass pollen sensitive patients indicate no correlation between serum complement sensitivity and total or specific IgE titers established by RAST.

As regards the *specific immunological sensitization* to special dander 'antigens' in patients, recourse must be had to methods for appraising the level of specific antibody. The radioallergosorbent test discussed by Ceska and colleagues (10, 11, 18) may in such cases be a valuable tool. These different techniques may in the future offer two laboratory parameters, one for the 'atopic status' and one for the 'specific sensitivities', which might aid in establishing an accurate clinical diagnosis of animal dander sensitivity.

Summary

Several different animal dander materials have been extracted, and the yields and analyses of total non-dialysable substance have been compared. Various coded dander extracts were examined by prick tests and intracutaneous tests in atopic individuals; the allergens were also screened for their capacity to induce loss of haemolytic complement from human allergic serum. Wide variations were noted in the percentage of extractable material among the different danders. Many patients reacted to skin tests with several unrelated dander allergens, and these skin responses did not correlate with a history of animal contacts. However, isolated cases were observed of immunologically highly specific sensitizations. Calculated mean skin reactivities of the allergens correlated well with the capacity to inactivate human complement in fluid phase *in vitro*. It is suggested that the allergens may exert a dual action, both non-specific and/or immunologically specific.

Acknowledgements

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CHAPTER IV

Allergy '74: Proceedings of the 9th European Congress of Allergology and Clinical Immunology. Pitman, Kent, 357-363.

SPECIFICITY OF SKIN TESTS *

by

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Introduction

A discrepancy between personal histories and skin test results in atopic patients is a puzzling observation which presents a frequent enigma to practising allergists (7). Obviously not every positive skin reaction can be taken to mean that the patient has been specifically sensitized to the allergen under investigation. Mechanisms other than antigen-antibody reactions must be involved (5). After 1962 it became apparent that lysine-sugar configurations in allergen molecules represent biologically active, though immunologically non-specific groups, which are involved in skin reactivity and are shared by several of the common allergens (2, 3). Recently it has been demonstrated by *in vitro* methods that these allergenically active sites may participate in the initiation of the allergic reaction (4).

The experiments discussed in this paper are an extension of the preceding clinical pilot study where, in some of the twelve patients involved, non-history-related skin reactions (NR) could be demonstrated (5). As concerns preparation and purification of the extracts and methods of skin-testing, we will refer therefore to that previous paper. In the present work, another group of 146 individuals was tested intracutaneously with the dander extracts of cow, guinea-pig, dog, cat and horse, at a uniform concentration of 20 μg/ml.

The group was divided in three ways. First, a group of individuals with animal contacts and a group without contacts. Second, a division had to be made into

* Read at the 9th European Congress of Allergology and Clinical Immunology in London, September 1974; and at the 202nd Meeting of the Dutch Society of Dermatologists at Utrecht, November 1974. KOERS W. J. (1975): Specificiteit van intracutane huidtests bij 'atopische' patiënten. Ned. T. Geneesk. *119*, 1395-1396.

atopic patients and non-atopics. The third possibility was to subdivide the atopic group into grass-pollen-allergic and house dust-allergic patients.

Results

The lower part of the block diagram of Fig. 1 shows that after skin-testing of 99 individuals having animal contacts, many R-reactions occurred (white columns). Although a certain number of NR-reactions were expected, it was remarkable to note that their incidence was about the same as observed for the R-reactions (black columns). Another striking fact was that a relatively large number of R-reactions occurred to tests with dander extracts of guinea-pig and cat, whereas NR-reactions occurred twice as often as R-reactions to tests with cow and especially with horse dander extracts.

The averaged skin test score per animal, depicted in the upper part of Fig. 1, displayed no consistency with respect to R- or NR-reactions. For example, guinea-pig dander extract elicited strong R-reactions, but also strong NR-reactions. Weak NR-skin reactions occurred to tests with horse dander extract. Moreover, there was a high incidence of NR-reactions to horse dander allergen, while the opposite was demonstrated with guinea-pig allergen.

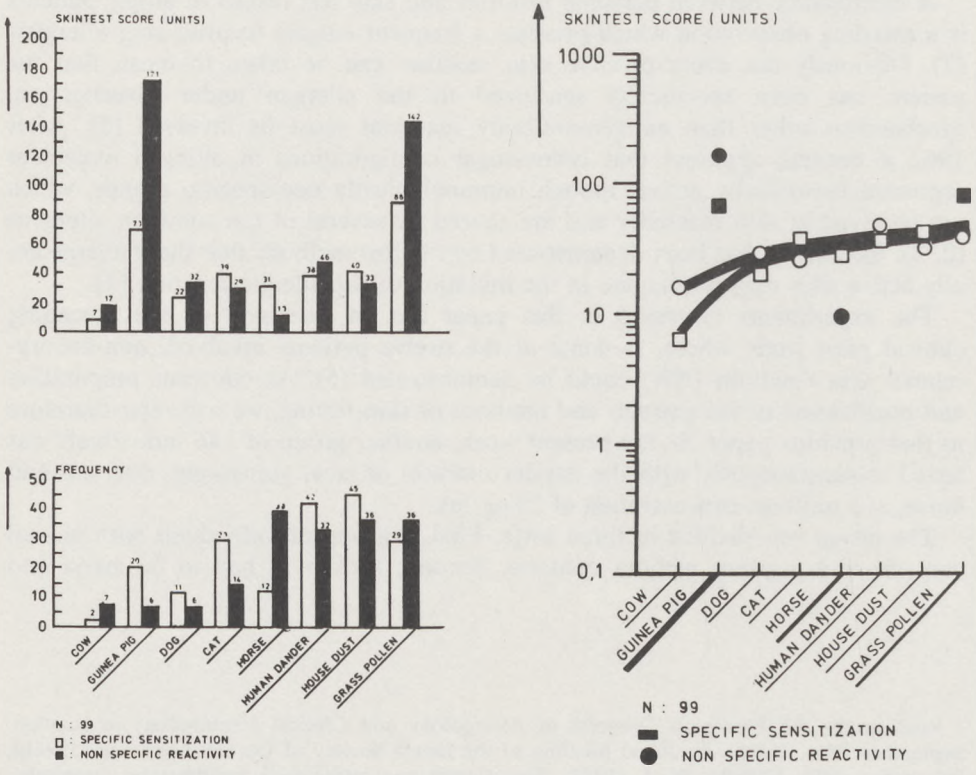

Fig. 1 and 2.

Semi-logarithmic plots of these skin test scores feature two lines running closely parallel (Fig. 2), which observation invites some comments. First, R-reactions as well as NR-reactions both occur frequently in atopic patients having animal contacts. Second, little difference in the strength of skin reactions exists between R-reactions and NR-reactions although some deviate above the basal lines (guinea-pig) or below them (horse).

Because grass pollen allergy is a prototype of a specific allergy and house dust allergy might represent a non-specific mechanism, we choose these parameters for further subdivision of the total group. On this basis eight groups were formed as shown in Tabel 1.

Table I

ANIMAL CONTACTS		NO-ANIMAL CONTACTS	
1a. House dust	+	1b. House dust	+
Grass pollen	+	Grass pollen	+
Animal contacts	+	No-animal contacts	—
2a. House dust	+	2b. House dust	+
Grass pollen	—	Grass pollen	—
Animal contacts	+	No-animal contacts	—
3a. House dust	—	3b. House dust	—
Grass pollen	+	Grass pollen	+
Animal contacts	+	No-animal contacts	—
4a. House dust	—	4b. House dust	—
Grass pollen	—	Grass pollen	—
Animal contacts	+	No-animal contacts	—

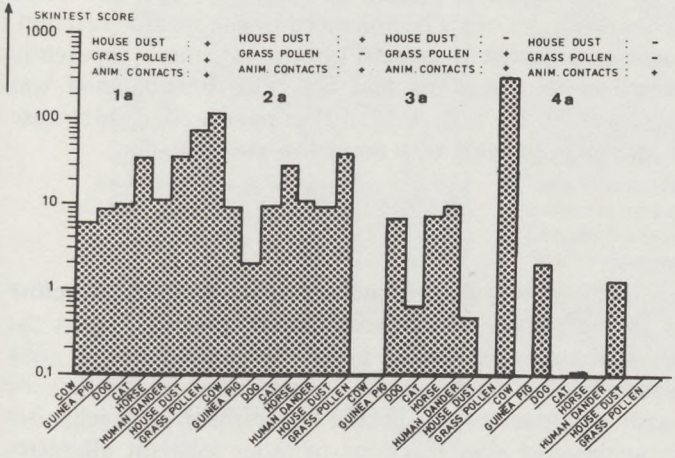

Fig. 3

The groups 4a and 4b represent non-atopic control subjects, with or without animal contacts. The groups 1b, 2b and 3b, in this study may be regarded as a 'novel' control group, comprised of atopic patients in whom tests with animal dander extracts should have been negative, because of the absence of the possibility of sensitization. The results of the skin test with the several animal dander extracts are summarized in the block diagrams of Fig. 4. In the top panel, the skin test results of the groups with animal contacts are shown (plotted on a semi-logarithmic scale) 'a' and at the bottom the 'novel' control groups 'b'.

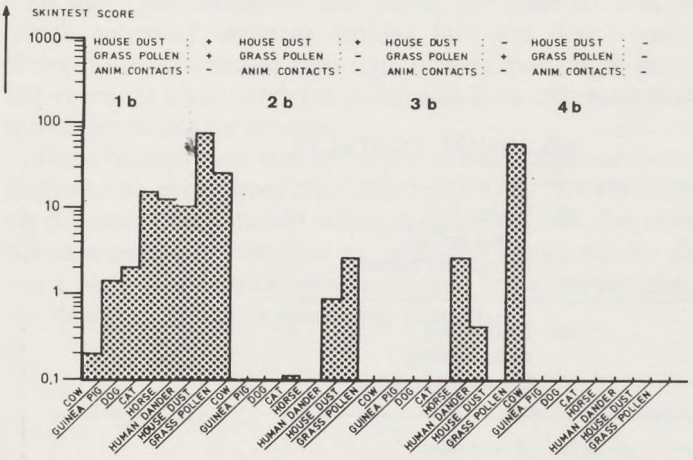

Fig. 4

In group 1b, many positive skin reactions were observed, at a relatively high level. These reactions must be ascribed to a non-specific mechanism as is the case in the other 'b' groups, with a lower grade of atopy than the first. In the 'a' groups with animal contacts, it is evident that the intensity of the skin reaction is higher than in the control groups 'b', apparently due to a specific reaction. This reaction may occur superimposed on a non-specific pattern of reactivity. The other way around, the non-specific basal reactivity might be enhanced by the specific reaction. When no evidence of atopic constitution is present (group 4a), there apparently remain purely specific reactions to guinea-pig and cat. This phenomenon was described earlier by some authors (9, 10, 1, 6, 8, 11, 12) in papers concerning case histories of animal dander allergy in patients with no or low atopic status.

Discussion

Apart from specific sensitizations we observed non-specific reactions as before (5), while cross-reactivity in these cases could be excluded. In this respect the importance of the intensity of the skin reaction to house dust extract and to grass pollen extract has been discussed. The results of our investigation indicate that the reaction to house dust mainly expresses a non-specific hypersensitivity mechanism (baseline) responsible for unexpected skin reactions to other inhalant allergens, such as animal danders. By contrast the reaction to grass pollen may have

significance as an indication of a predisposition to specific antigenic sensitization to such allergens. The baseline of skin reactivity seems to be dependent upon the degree of atopy; peaks on that baseline may have been induced by some acquired sensitizations to animals.

It can be observed that the larger the number of positive skin reactions, the more pronounced the non-specific reactivity of the patient. The interpretation so far was, that the number of positive reactions equalled the specific sensitization.

We can now take an opposite look at the usual interpretation of these skin reactions. The concept of atopy seems to be more closely related to a non-specific mechanism than to a specific immunological sensitization pattern. In the case of strong skin reactions to some test extracts in comparison with the basic level, one would therefore only have to consider the peak on the baseline as likely to be the specific portion of the skin reaction.

Since 1962 it has been demonstrated by *in vitro* methods that there must be an underlying non-specific mechanism in atopy. The reactivity of individual allergens and the frequency distribution of skin reactions within this non-specific mechanism can be predicted on the basis of the estimated number of lysine-sugar sites per mole of allergen, acquired by Maillard reaction. Several independent parameters can be chosen as estimates of the proportion of these sugar-blocked lysine residues per mole of allergen. The corrected ratio of extinction coefficients in the ultra-violet spectrum, E305/E280, has been most frequently employed to this end. The allergens may then be arranged into a rational sequence as illustrated in Figure 5.

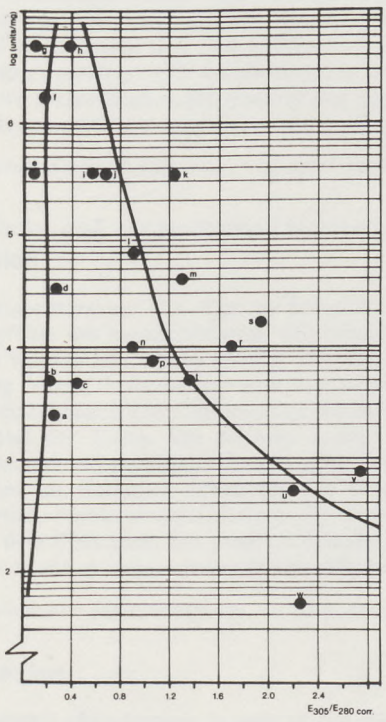

Figure 5: Semilogarithmic plot of skin reactivity (in direct tests) of purified atopic allergens in specifically sensitive atopic individuals versus the E_{305}/E_{280} ratio as a parameter for the mean number of sugar-blocked lysine side-chains per mole. Key: a cow's milk allergen VM 5; b total dialysed horse dandruff protein; c rye grass pollen allergen D (IEP); c 0–0.9 sat. ammonium sulphate precipitated fraction from dialysed aqueous rye pollen extract; d rye grass pollen allergen B; e egg-white allergen VE 9; f rye grass pollen allergen I-B; g rye pollen allergen II-B; h giant ragweed allergen Trifidin A; i ipecac allergen IPC-D; j caddis fly, pool 2; k house dust HE-E; l human dandruff HD-E; m cotton lint fraction CL-E; n alternaria tenuis, acetone-precipitated 2-month culture filtrate (Synthetic Czapek-Dox medium); p tomato allergen TO-G; r cottonseed allergen CS60C; s feathers FE-B; t purified trichophytin; u liquorice allergen SL-F; v kapok allergen KP-E; w hay extract HH-C; Ragweed pool C (on top of curve) and green coffee retentate (at $E_{305}/E_{280} = 4.5$) not indicated.

Fig. 5

The present *in vivo* investigations, by means of intradermal skin tests with allergenic extracts, may contribute to a better understanding of the NR-skin reactions, and serve as a guide to further clinical and laboratory investigations.

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CHAPTER V

Allergie u. Immunol. (1977) *23*, 261-267. J. A. Barth, Leipzig/DDR

SPECIFIC AND NON-SPECIFIC SKIN TEST REACTIONS WITH ANIMAL DANDER EXTRACTS

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Summary: Intradermal skin tests have been performed in atopic patients with allergenic extracts of different animal danders at uniform concentration. A high incidence of positive tests was recorded which could not be related to the clinical histories, especially with the allergens of horse, cow and dog dander (NR). Better correlation was observed with guinea-pig and cat allergens (R). The incidence of NR-reactions was highest in the house dust allergy group of patients; with the exception of horse dander allergen, the frequency of R-reactions was more pronounced in the grass pollen group. It is suggested that both allergen-specific and non-allergen-specific mechanisms may contribute to positive weal and flare reactions.

Key-words: Skin test reaction. — Animal danders. — Allergens.

Spezifische und unspezifische Hautreaktionen mit tierischen Hautschuppen-Extrakten

Zusammenfassung: Die Autoren haben bei atopischen Patienten intrakutane Hauttestungen durchgeführt mit standardisierten Allergenextrakten von verschiedenen tierischen Hautschuppen. Es wurden sehr häufig positive Hautreaktionen gefunden, die nicht mit der Anamnese in Einklang waren, insbesondere mit den Allergenen aus Hautschuppen vom Pferd, Rind und Hund. Eine bessere Übereinstimmung wurde beobachtet mit den Allergenen des Meerschweinchens und der Katze. Die höchste Anzahl nicht-klinisch relevanter Hautreaktionen wurde bei Patienten mit Hausstauballergie gefunden. Die Beziehung zwischen Anamnese und Hautreaktionen mit tierischen Allergenen war besser bei Patienten mit Graspollenallergie, mit Ausnahme vom Pferdeschuppenallergen. Es wird angenommen, dass möglicherweise zwei Mechanismen dem Phänomen der positiven Hautreaktion zugrunde liegen können, ein Allergenspezifischer und ein Allergen-unspezifischer Mechanismus.

Schlüsselwörter: Hautreaktionen. — Hautschuppen. — Allergen.

Introduction

Although skin-testing is one of the methods most commonly used for the detection of specific allergies in atopic patients, the results cannot always be interpreted

in direct relation to the patient's clinical history. The incidence of positive skin reactions quoted for various allergens in the literature varies widely, moreover. Major reasons for the observed discrepancies must undoubtedly be sought in differences in the selection of patients, the preparation and standardization of the allergenic extracts and in the recording of the results. However, even under rigid control of these variables it remains a fact that positive skin reactions to certain allergenic extracts do not always appear clinically relevant. Positive intracutaneous reactions have been reported in many non-allergic individuals (7, 8, 9, 10, 13). The incidence of such reactions was higher in asymptomatic individuals with positive family histories of allergy (i.e. about 30 percent) than in people with no family history of atopy (about 4 percent) (7,12). Positive skin reactions in these subjects have commonly been classified as 'false-positive' or have been taken as presumptive evidence of 'latent allergy'.

As regards animal dander sensitivity, Rynes (14) examined a group of 2,829 patients for skin responses to test with one or more dander extracts of the common domestic animals. Positive skin reactions were observed in 13 percent of the group; yet, only 25 percent of these cases had been aware of their animal dander sensitivity. Simon (15), performing skin tests with several mammalian sera in a guinea-pig sensitive patient, found evidence for the existence of a non-species specific skin-reactive factor. In an extension of this work, he selected 22 highly sensitive subjects out of 3,630 clinically allergic patients (16). A 50 percent incidence of positive reactions to horse dander extracts was observed; furthermore, 50 percent of his patients who presented positive reactions to horse serum also reacted to several mammalian sera apparently unrelated to their clinical history. Arbesman, Beede and Rose (1) presented the case report of a patient with a strong skin reactivity to mouse, rat, guinea-pig, rabbit and horse dander; the patient's contacts with the former could be established, but actual contacts with rabbit or horse were not mentioned. Skin tests with the sera of these and other domestic animals were positive; likewise, the sera of exotic undomesticated animals (lion, tiger, elephant, coyote) produced positive skin reactions.

Multiple skin reactivity in highly sensitive patients is most often explained as either multiple specific sensitization or as being due to cross-allergenicity among the antigens in dander extracts. Alternatively, the possibility has been raised that reactions apparently unrelated to the patient's proven antigenic contacts might reflect the non-specific hyperreactivity of a common physiological mediator system. Studies on the chemistry of atopic allergens and their interaction with human haemolytic complement have led to the concept of two mechanisms underlying skin reactivity (2, 3). It was proposed that atopic allergens exhibit the common property of activating physiological enzyme systems, including complement, in a non-specific fashion. Second, the allergens might become recognized as distinct antigens by specific (IgE) antibody. There is some evidence that complement factors are involved in skin reactions (17). If both allergen-specific and non-specific mechanisms contribute to skin reactions, positive skin tests apparently unrelated to the patient's antigen exposure may indeed be expected. It was the purpose of this investigation to verify this point in cases of animal dander sensitivity. This paper describes the relevancy of skin reactions on purely clinical grounds.

Materials and methods

Allergens

Animal dander material was obtained from various groups of animals by clipping the fur close to the skin with an electric razor. The samples were from female guinea-pigs, mixed cow hair, the fur of one cat and hair from one (poodle) dog. The horse dander protein was a gift (in 1962) of Dr. D. R. Stanworth; the material had fully retained its activity over the years, kept in the lyophilized state in a desiccator. Powdered defatted dander was obtained and extracted as described (4). The total non-dialysable solubles were dried by lyophilization; the horse and dog material was further purified by collecting the protein substance precipitating with ammonium sulphate in the 0.3—0.8 saturation range at 4°C.

Skin tests were performed by the intradermal route, using 0.05 ml for injection. All allergen solutions were standardized at 20 μg of lyophilized material per ml, so that a standard dose of 1 μg was administered throughout. The skin reactions were assessed by visual appraisal. The atopic subjects were selected from among the patients attending the Out-Patient Department of Allergy on the guide of their clinical history (animal contacts), a family history of atopy, and positive skin reactions to commercial allergenic extracts of grass pollen and/or house dust. Patients were questioned carefully and repeatedly in order to obtain certainty concerning their contacts with the animals belonging to the test series.

Haemolytic complement consumption. The capacity of the dander allergenic preparations to inactivate human haemolytic complement was evaluated as before (4). The complement-inactivating power was expressed in μg required for 50 percent reduction of haemolytic complement in a dilute human serum sample under standardized conditions.

Results

Skin tests with routine series of the common allergens were performed in a group of 99 atopic and non-atopic patients, whose personal history featured definite contacts with one or more of the animals in the test series. After an initial screening of skin reactivity, towards the common allergens and histamine, 27 subjects were excluded because of hypo- or hyperreactivities, i.e. weak reactions to histamine or positive reactions to the control solution. Of the remaining 72 subjects, 50 were atopics and 22 were not. A group of 37 atopic subjects whose history reliably revealed no contacts — past or present — served as control. All patients were examined by intradermal tests with the specially prepared animal dander extracts of horse, cat, dog, guinea-pig and cow, at the uniform concentration of 20 μg/ml. The results have been collected in table 1.

Table 1 Distribution of positive skin reactions in a group of 50 atopic patients with known animal contacts, and regrouping on the basis of history-related (R) and non-related (NR) reactions; percentage positive reactions in the population is given in parentheses

Allergen	Skin reactions		
	Whole group	R	NR
Dog	19 (38)	6 (12)	13 (26)
Horse	38 (76)	9 (18)	29 (58)
Cow	8 (16)	3 (6)	5 (10)
Cat	30 (60)	21 (42)	9 (18)
Guinea-pig	15 (30)	8 (16)	7 (14)

As shown in the table, the number of positive skin reactions to, for example, horse dander allergen appeared unusually high in relation to the known or suspected actual antigenic contact. Patients were therefore carefully questioned again about possible exposure to animal material in their environment; they were also asked whether they were at all aware of clinical hypersensitivity symptoms due to any of the animals in the test series. On this basis, the skin test results were divided into two series. In the whole group, 47 skin reactions appeared to be related to the personal history, i.e. exposure and symptoms (R); similarly, 63 positive skin reactions were apparently unrelated (NR). Subdivision of the skin responses into apparently related and non-related reactions produced strikingly different results for the individual animal dander allergens. Table 1 shows that the highest percentage of NR-reactions occurred with the horse and dog allergens, whereas high R-scores were observed with the cat allergens.

A total number of 27 positive intradermal tests was observed in the control group of 37 atopic subjects without any animal contacts. Table 2 shows that the incidence of these 'false-positive' cutaneous reactions to individual dander allergens was remarkably similar to the sequence of NR-responses observed in the group of patients with known animal dander contacts. Since the questioning provided no evidence for immunological sensitization in the control group, an explanation for the positive test results as due to cross-allergenicity among animal danders appeared unlikely. The identical frequency distribution of NR-reactions in the patient's group indicates that the skin test responses in both groups may be due in part to the reactivity of a non-immunological system.

Table 2 Positive intradermal reactions in a control group of 37 atopic subjects without known exposure to animal dander allergens, as compared to skin reactions not related to the clinical history (NR) in the group of 50 atopic patients with known animal contacts. Percentages of the total number of positive skin tests are given in parentheses

Allergen	Not exposed (n = 37)	Exposed (n = 50)
Dog	4 (15)	13 (21)
Horse	13 (48)	29 (46)
Cow	2 (7)	5 (8)
Cat	5 (19)	9 (14)
Guinea-pig	3 (11)	7 (11)
Total	27 (100)	63 (100)

Positive skin reactions related to the clinical history appeared to be associated more frequently with cat and guinea-pig sensitivity than with horse, cow or dog sensitivity. This frequency distribution coincided with the intensity of the skin test response. The activity of allergenic preparations has in the past been expressed in units per mg of lyophilized substance, based on the logarithmic relationship between the intensity of the skin reaction and the allergen dose (6). The activities of the cat and guinea-pig allergens calculated in this fashion over the whole group of patients were about 5 fold higher than found with the other allergens. Since identical dosages were administered in the present studies, the results listed in table 3 indicate that the size of the skin reaction was more pronounced with those allergens that correlated best with the patients' histories.

Table 3 Complement-inactivating power of dander allergenic preparations, in μg for 50 percent reduction of haemolytic complement in 25 μl of human serum (huC50), and mean skin reactivity, in units/mg of lyophilized extract, compared to the incidence, in percent, of non-history-related intradermal reactions in 50 atopic patients with animal contacts.

Allergen	huC50, μg	Spec. activity, units/mg	%NR-reactions
Dog	2.0	4.3×10^3	26
Horse	5.1	7.9×10^3	58
Cow	47.2	1.3×10^3	10
Cat	10.3	1.5×10^4	18
Guinea-pig	17.6	2.4×10^4	14

Atopic allergens have been shown to inactivate human haemolytic complement. Evidence has been forwarded to support the idea that this property represents a common non-immunological property of allergens (3, 5). The complement-inactivating power of each of the animal dander extracts was determined and has been included in Table 3. As shown, the C-inactivating capacities of the individual dander extracts roughly corresponded to the incidence of non-related skin reactions. The results strengthen the hypothesis of two possible mechanisms involved in skin test responses. The first reflects the non-immunological reactivity of a system activated by allergens in a qualitatively similar, but quantitatively distinct fashion. The second mechanism might represent an immunologically specific system of antigen recognition. To explore this further, the group of 50 atopic patients in this investigation was divided into subgroups on the arbitrary basis of their atopic status as expressed by house dust — or grass pollen reactivity, respectively. This approach was taken because (perennial) house dust allergy, in a clinical sense, might represent a relatively non-allergen specific phenomenon of hypersensitivity (NS), whereas (seasonal) grass pollen allergy may be considered a prototype of an allergen-specific disease (S).

Table 4 Distribution of history-related (R) and non-related (NR) positive intradermal reactions in a group of 72 patients classified according to major symptoms of pollen hay fever (S) or perennial house dust allergy (NS). Subgroups:

I. Grass pollen positive, house dust negative (S)
II. Grass pollen negative, house dust positive (NS)
III. Grass pollen positive, house dust positive (mixed group)
IV: Grass pollen negative, house dust negative (non-atopic group)

Results given in percent of the total number of positive reactions within the subgroup; n = number of patients

Allergen	Group I (S) n = 16		Group II (NS) n = 15		Group III n = 19		Group IV n = 22	
	R	NR	R	NR	R	NR	R	NR
Dog	0	6	13	27	21	42	0	0
Horse	6	62	20	47	26	63	0	0
Cow	0	0	13	7	5	21	0	0
Cat	25	6	33	20	63	26	5	0
Guinea-pig	12	0	13	7	21	32	18	0

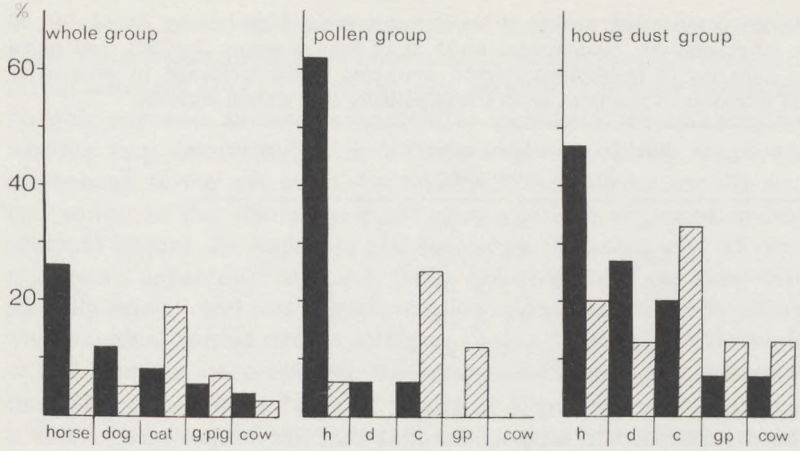

Figure 1. Graphical representation of the distribution of positive intradermal reactions to animal dander extracts in a group of 50 atopic patients; skin reactions were divided into history-related (R, hatched columns) and non-history-related responses (NR, black columns). The pollen group and the house dust group represent Groups I and II of Table 4, respectively. Results are given in percent of the total number of positive reactions within the group or subgroup.

Reconsideration of the skin test results on these criteria of specific and non-specific allergy produces an even stronger segregation of history-related and -unrelated skin reactions R and NR. As shown in Table 4, a very high percentage of the reactions to horse and dog allergens in the group S with grass pollen allergy was not related to the personal histories, whereas the reverse was true with guinea-pig and cat allergens. The latter were the only allergens, moreover, which provoked markedly specific reactions in the non-atopic group of 22 subjects, indicating truly specific sensitizations. High percentages of NR-reactions to horse dander occurred throughout all subgroups: horse dander allergen, moreover, produced more non-related reactions than any of the other allergens. This has been summarized graphically, for the major groups of patients, in figure 1.

Discussion

In this investigation, many positive intradermal reactions with animal dander extracts were observed which could not be related to the patient's complaints or known antigenic contacts. In the (control) group of allergic patients with no former or actual animal contacts, such reactions would usually be regarded as 'false-positive'. An explanation for positive reactions to animal allergens other than those actually available in the patient's environment would normally be based on the proposed cross-allergenicity of different dander extracts. However, both the incidence of non-related reactions and the sequence of these for the individual allergens was identical for both groups of patients (Table 2). Because no such NR-skin reactions were observed in the non-atopic control group, the responses can obviously not be attributed to primary irritants in the extracts. An explanation suggests itself by referring to the proposal of a dual action of atopic allergens, viz. the non-allergen-specific, non-immunological activation of a common mediator system ('atopen' activity), and an allergen-specific immunoglobulin-mediated reactivity

('antigen' recognition). In this view, the sequence of individual allergens inducing non-immunological skin reactions is dictated by the mean number of common allergenic (lysine-sugar) groups in each allergen (3). This figure, i.e. the non-specific potency of individual allergens, can be estimated by observing their capacity to inactivate a standard dose of human fluid phase complement *in vitro* (5). Comparison of the relevant data of Tables 2 and 3 demonstrates that the array of complement-inactivating potentials of the dander allergens *in vitro* indeed largely coincides with the observed sequence and frequency distribution of non-related skin reactions *in vivo*. This correspondence, of course, provides no direct evidence for the participation of complement in the skin reaction.

If it must be assumed that both allergen-specific and non-allergen-specific mechanisms may contribute to an observed positive weal and flare reaction, the question arises of the relative contribution of each of these mechanisms. This problem was approached by noting the incidence of NR- and R-reactions in patients with grass pollen allergy, which can be considered as a prototype of an allergen-specific hypersensitivity disease. House dust allergy was chosen as an example of a possibly less allergen-specific condition. Analysis of the skin test data indeed showed a reduced rate of NR-scores to cat, guinea-pig, dog and cow dander allergen, but not to horse dander allergen, in the group of hay fever patients as compared to the house dust allergy group.

The dander allergens with high R-scores in all groups were those of cat and guinea-pig; significant NR-scores were observed with horse dander allergen. The only specific allergies recorded in non-atopic controls were cat and guinea-pig sensitivities. Tentatively, this may be interpreted to mean that immunologically specific allergies may occur isolated and that the reactivity of non-allergen-specific systems may in some way be related to the atopic condition.

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CHAPTER VI

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ANIMAL DANDER ALLERGY

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Summary

In a group of twenty-three atopic patients skin tests were performed with the dander allergens of horse, cat and guinea-pig, and with house dust and Timothy pollen allergens. A good agreement was observed between positive skin reactions and the results of RAST with these various allergens. In a number of cases, positive skin reactions were not related to the clinical histories. Likewise, positive RAST scores in several instances proved clinically meaningless. In patients with history-related skin reactions better correlation with RAST was observed. In such patients, skin reactions with photo-inactivated allergens remained strongly positive.

Introduction

Previous investigations based on skin tests in patients with suspected animal dander allergy have led to the conclusion that weal and flare reactions to epithelium extracts of different species are often unrelated to the patient's clinical history of known antigenic contacts (Berrens, Hénocq & Koers, 1974). To explain these results, it was proposed that two mechanisms underlie skin reactivity, viz. an allergen-specific antibody-mediated reaction and a non-allergen-specific activation of a common mediator system. The present paper describes the results of experiments designed to verify this point.

As a working hypothesis, it was assumed that the specific mechanism might be equated with the well-described allergen-induced reaction mediated by IgE antibody. With regard to the possible contribution of a non-specific mechanism, both native and photo-inactivated allergens were considered for intradermal testing. The latter have been shown to be deprived of their skin-reactive potency in a large percentage of atopic subjects and to have lost their property of inactivating human haemolytic complement *in vitro* (Berrens, Hénocq & Radermecker, 1973).

Several authors have sought correlations between clinical parameters for reaginic allergy and serum IgE concentrations (RIST values) and/or the level of allergen-specific IgE antibodies (RAST values). On the subject of animal dander sensitivity,

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Fagerberg & Wide (1970) reported a 97% positive correlation between the clinical diagnosis and RAST with extracts of mixed dog epithelium. Intracutaneous tests and RAST agreed in 85% of the cases with mixed dog dander extracts and in 65% with dander allergens of individual breeds. Foucard (1973) examined animal dander allergy developing in children with asthmatoid bronchitis and found 86% agreement between strongly positive skin tests and high RAST values. However, no more than 20% and 42% correlation was found between high RAST scores and weak to moderate skin test responses, respectively. Studies by Apold *et al.* (1974) and Havnen *et al.* (1974) have shown less correlation between the results of RAST, provocation tests and skin tests. They emphasized that the reliability of skin tests and RAST is allergen-dependent. Besides possible variation in the nature and quality of the different allergenic extracts (Aas & Lundkvist, 1973), discrepancies between RAST, skin tests or other provocation tests may also be understood by assuming that there is more than one mechanism contributing to skin reactions. The relative preponderance of these may be allergen-dependent.

The non-specific action of atopic allergens has been attributed to common structural sites of lysine–sugar bonding among distinct allergens (Berrens, 1971). The selective destruction of these sites by u.v. irradiation leaves the carrier antigen sufficiently intact to be recognized by specific antibody in the skin or bronchi of particular patients (Berrens *et al.*, 1973). Photo-inactivated allergens of this kind were employed in the present work to investigate further the hypothesis of two mechanisms in atopic allergy.

Materials and methods

The epithelial allergenic extracts of horse, guinea-pig and cat were prepared as described previously (Berrens *et al.*, 1974). A highly purified house dust allergen preparation, 74E, was isolated from pooled vacuum-cleaner dust according to published procedures (Berrens & Young, 1961). The grass pollen preparation was an unfractionated dialysed extract of ether-defatted pollen grains of Timothy grass (*Phleum pratense*, harvest 1971, donated by Bencard Laboratories). Photo-inactivated allergens were prepared from the active parent by u.v. irradiation. The inactivation process was monitored by measuring the fall of human complement-inactivating power (Berrens *et al.*, 1973). The irradiated products were recovered by lyophilization.

Blood samples were obtained by venous puncture; after clotting in glass, the blood sera were separated and stored in 0·5 ml aliquots in polypropylene tubes at −70°C until use.

Total serum IgE was assessed by radioimmunoassay (RIST), using the reagents provided with the Phadebas® test kit. Allergen-specific IgE was determined by the RAST technique, using the allergenic preparations described above coupled to Sepharose® beads according to the procedure of Aalberse (1974). Results were expressed as percentage radioactivity bound from a standard dose of ^{125}I-labelled anti-IgE per tube. Values at over 2% binding were considered positive.

Skin tests were performed by the intradermal route, using 0·05 ml per injection. All dander extracts were tested at 20 μg/ml; the test concentrations for house dust 74E and for Timothy pollen extracts were 2 μg/ml and 1 μg/ml, respectively. The skin reactions were scored under a visual evaluation system as before (Berrens & Young, 1961; Maat-Bleeker, 1971).

Results

In a group of twenty-three atopic patients positive by skin test to animal dander allergens, total serum IgE levels were determined together with RAST values for specific IgE antibody against the allergens of horse, guinea-pig and cat dander, house dust and Timothy pollen. The results are shown collectively in Table 1. Breakdown of the analyses in relation to the skin tests and the clinical histories provided the following results.

Table 1. Total and specific IgE in the serum of twenty-three atopic patients with positive intradermal reactions to unmodified animal dander extracts. Skin reactions, graded according to intensity from 0 to 4, are shown in parentheses

Patient No.	Total IgE (iu/ml)	RAST (% binding)				
		Horse	Guinea-pig	Cat	Dust	Pollen
1	n.d.	21 (3)	3 (0)	42 (4)	38 (3)	52 (3)
2	960	6 (1)	2 (1)	22 (3)	28 (3)	0 (0)
3	2000	39 (3)	4 (0)	11 (1)	34 (3)	0 (0)
4	n.d.	0 (0)	3 (0)	10 (1)	15 (1)	4 (0)
5	115	1 (0)	1 (0)	46 (2)	32 (2)	1 (2)
6	1020	20 (2)	0 (0)	1 (0)	17 (2)	54 (4)
7	1800	39 (3)	10 (2)	56 (3)	52 (2)	0 (0)
8	3800	12 (2)	2 (0)	25 (2)	35 (2)	35 (2)
9	660	1 (2)	3 (0)	2 (3)	12 (3)	0 (0)
10	1180	24 (2)	57 (3)	19 (3)	44 (2)	9 (3)
11	820	12 (2)	45 (3)	5 (3)	35 (1)	6 (3)
12	440	12 (2)	0 (0)	0 (1)	12 (0)	54 (3)
13	970	1 (2)	0 (0)	2 (2)	15 (2)	16 (2)
14	147	4 (2)	23 (3)	1 (0)	19 (1)	0 (0)
15	430	20 (2)	0 (0)	1 (0)	20 (2)	52 (3)
16	285	0 (0)	0 (1)	0 (0)	2 (2)	0 (0)
17	1150	7 (1)	0 (0)	0 (0)	18 (0)	55 (4)
18	430	1 (2)	4 (2)	4 (0)	29 (2)	5 (0)
19	255	11 (1)	0 (0)	1 (0)	16 (0)	48 (3)
20	850	1 (2)	43 (4)	2 (2)	39 (0)	0 (0)
21	n.d.	0 (0)	0 (2)	0 (0)	1 (0)	0 (0)
22	n.d.	4 (0)	1 (0)	11 (1)	17 (3)	3 (1)
23	235	3 (1)	0 (0)	2 (2)	7 (0)	33 (3)

n.d. = Not done.

Total IgE in relation to the diagnosis

Total serum IgE levels were determined in nineteen patients reacting by skin test to animal epithelial extracts (Table 1). The mean level (923 iu/ml) was raised as compared to the mean for non-atopic subjects (100–200 iu/ml). There was a wide range in the individual IgE concentrations, extending downwards into the normal region (Table 1). In confirmation of earlier reports (Juhlin *et al.*, 1969; Johansson, 1967) it was observed that the diagnosis of atopic dermatitis and extrinsic asthma was often associated with high RIST values, while rhinitis occurred in even distribution in both the high-level and low-level groups.

On the basis of the personal histories, the group of nineteen patients was divided

Table 2. Mean RIST and RAST values in a group of nineteen patients arranged according to skin reactions related (R) or not-related (NR) to the clinical history. Mean ± s.d. indicated, except in series of less than five observations

Allergen	Related mean IgE (iu/ml)	Mean RAST (%binding)	Non-related mean IgE (iu/ml)	Mean RAST (%binding)
Timothy pollen	604 ± 388	37 ± 22	2490	16
Guinea-pig	685 ± 401	29 ± 23	1015	4
Cat	1338 ± 1194	21 ± 19	823	7
Horse	1607	30	1026 ± 985	11 ± 8
House dust	1383 ± 1063	27 ± 15	434 ± 310	24 ± 9

into those with history-related (R) and non-history-related skin reactions (NR). Table 2 shows that the highest RIST values in the R-group were observed in the sera of patients sensitive to house dust and horse dander, whereas in the NR-group, the highest total IgE values occurred in patients with skin reactions to grass pollen or guinea-pig dander, although natural exposure to these allergens apparently caused no complaints.

RIST in relation to RAST

Specific IgE antibody as evaluated by RAST was found twice as often in patients with RIST values >800 iu/ml than in patients with total IgE below this value (twenty-five out of fifty against eleven out of forty-five positive RASTs, see Tables 1 and 2). A diagnosis of house dust allergy combined with other allergies was prominent in the high RIST–RAST group. Five patients with serum IgE values of ⩾1000 iu/ml had three or more concomitant allergies. By contrast, specific animal dander and grass pollen allergy occurred singly even in the group with low RIST values. These results confirm those of Wide, Bennich & Johansson (1967), who reported that multiple allergies and the number of corresponding positive RASTs are frequently associated with high RIST values. Elevated RIST values need not necessarily accompany pronounced monospecific allergies, e.g. to grass pollen.

RIST, RAST and skin reactions in relation to the history

Table 2 shows the mean RAST values in groups of patients chosen on the basis of the history-related or non-related skin reactions. Depending on the allergen, the trend was an association of only moderately elevated RIST values with significant RAST scores in the R-group, especially with the pollen, guinea-pig and horse dander allergens. Conversely, in the NR-group, markedly increased RIST values were associated with low-grade RAST scores, in particular with horse dander and house dust allergens.

As shown by the overall results in Table 1, there appeared to be a good agreement between the results of RAST scores and intradermal testing. A statistically significant positive correlation was observed with the Timothy pollen allergens (Spearman rank correlation coefficient 0·84, $P<0·005$). The collected results in Fig. 1a and b demonstrate that the intensity of the skin test reactions correlated significantly with the RAST score with animal dander allergens in the R-group of patients, but not in the NR-group.

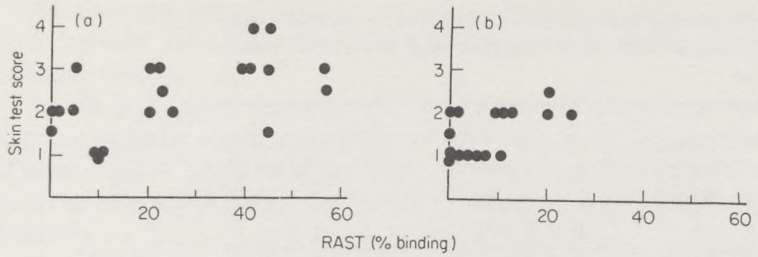

Fig. 1. Scatter diagrams of relationship between skin test score, intensity grade 0 to 4, and RAST to animal dander allergens in: (a) twenty-one cases of positive skin reactions related to the personal history (Spearman rank correlation coefficient $r = 0{\cdot}53$, $P < 0{\cdot}05$); and (b) sixteen observed positive skin reactions not related to the personal history ($r = 0{\cdot}46$, $0{\cdot}05 < P < 0{\cdot}1$).

However, these results and those of others indicate that many skin reactions have no clinical importance. In many cases there is no evidence of actual antigen exposure apparently having caused sensitization. Moreover, actual antigenic contact quite often causes no clinical complaints (Berrens *et al.*, 1974; Rudolph *et al.*, 1975; Schultze-Werninghaus *et al.*, 1976). Table 3 demonstrates that the skin reactions to horse

Table 3. Statistical relationship between personal history of allergy and skin reactions to animal dander allergens. *P*-values calculated by Fischer test

	Horse $P > 0{\cdot}20$ Skin reaction			Cat $P < 0{\cdot}05$ Skin reaction			Guinea-pig $P < 0{\cdot}05$ Skin reaction		
History	−	+		−	+		−	+	
−	4	14	18	5	7	12	12	3	15
+	1	4	5	0	11	11	2	6	8
	5	18		5	18		14	9	

dander did not correlate significantly with the personal histories in the present group of patients. Because of the agreement between RAST and skin reactions previously mentioned the authors therefore examined whether positive RASTs might be clinically meaningless.

As demonstrated by the statistical results collected in Table 4, there was no relationship between the results of RASTs and the patients' personal histories as regards allergy to horse and guinea-pig dander. Correlation was better with the allergens of cat and pollen, though in one RAST-positive case (Table 1, no. 8) there was no evidence of past or present complaints of pollen hay fever. With the dander allergens, 'false-positive' RAST results unrelated to the clinical history were often observed, especially with the horse dander allergens. False-positive RASTs to house dust allergen were also found, especially in the group of patients with skin reactions unrelated to their history. These results, and the distribution in R- and NR-groups of patients, are listed in Table 5.

From the results so far it appears that the RAST technique *in vitro* provides results comparing well with the skin test *in vivo*. As shown in Table 5, this implies that positive RASTs, not related to the patient's clinical history, are to be expected in

Table 4. Statistical relationship between personal history to animal dander allergens and RAST results with these allergens and the patient's sera. *P* values by Fisher test

	Horse $P>0{\cdot}20$ RAST			Cat $P<0{\cdot}05$ RAST			Guinea-pig $P>0{\cdot}20$ RAST		
History	−	+		−	+		−	+	
−	7	11	18	9	3	12	8	7	15
+	1	4	5	2	9	11	3	5	8
	8	15		11	12		11	12	

Table 5. Distribution of positive skin reactions with native and photo-inactivated (u.v.) allergens together with RAST results in patients with skin tests related to their clinical history (R) and in a non-related group (NR)

						R-group
Allergen	Contacts	Symptoms	Pos. skin reaction		RAST	
			Native	u.v.	⩾2% <20⩾20% binding	
Grass pollen	23	11	10	n.d.	4	7
Guinea-pig	14	8	6	4	1	4
Cat	17	11	11	4	6	5
Horse	8	5	4	4	1	4
House dust	23	11	11	8	4	7

						NR-group
	No contacts	No symptoms	Native	u.v.	⩾2% <20⩾20% binding	
Grass pollen	0	12	3	n.d.	2	1
Guinea-pig	9	9	3	0	7	0
Cat	6	6	4	0	4	0
Horse	15	15	14	1	8	2
House dust	0	12	6	0	7	4

approximately the same proportion as false-positive skin reactions. The RAST data for horse dander allergen in this respect (Table 5) correspond with our skin test data.

On considering a possible cause of non-related skin reactions, the suggestion has been forwarded that a second—unspecific—mechanism might underlie skin reactions. This mechanism was thought to be primed by common sites of lysine–sugar conjugation among different allergens. Removal of these sites by controlled u.v. irradiation consequently provides allergen-derivatives which might be expected to cause fewer non-related skin reactions (Berrens *et al.*, 1973).

Skin tests performed with u.v.-modified allergens in the present group of twenty-three patients did reduce the number of false-positive reactions in the NR-group to only one (horse).

In sharp contrast, however, the reactions to photo-inactivated allergens in the R-group of patients remained positive or even displayed increased intensity in 36–80% of these cases of highly specific allergy, depending on the kind of allergen. The results with the native and photo-inactivated specimen of each allergen have been listed in Table 5.

As carried out by other investigators (Apold *et al.*, 1974; Havnen *et al.*, 1974) the results of skin test reactions and the RAST score were compared. Arbitrarily, the RAST values were divided into a medium score group of 2–20% binding, and a high score group over 20% binding of added radioactivity. As demonstrated by the numerical data listed in Table 5, a good agreement was thus obtained with the high RAST scores and the results of intradermal tests with photo-inactivated allergens in the R-group of patients. It must be pointed out that in this particular group of patients the personal history was already highly convincing for a specific allergy to one of the animal dander allergens (e.g. cat, or guinea-pig). However, in the NR-group of patients, RAST scores over 20 were still found six times (to house dust or horse dander), although the skin reactions to photo-inactivated horse dander remained positive in only one case.

Intradermal skin tests with photo-inactivated allergens in our experience proved more valuable for diagnosing highly specific history-related allergies than skin tests with the native allergens *in vivo*, or RAST tests *in vitro*. More recent evidence in this respect indicates that highly specific allergies, especially to cat and guinea-pig dander, can easily be detected with the photo-inactivated allergens, even in those cases where the corresponding RASTs with anti-IgE are negative. Presumably, these may be immediate-type allergies mediated by an antibody class other than IgE (Bryant, Burns & Lazarus, 1975; Parish, 1970).

Discussion

In confirmation of earlier results, skin test reactions with animal dander extracts, and also with house dust and occasionally grass pollen allergens, are often unrelated to the patient's personal history of antigenic contacts or complaints on exposure. With IgE as an additional parameter, the present study shows that high total IgE values and low RAST scores are often observed in the NR-group of patients, particularly with house dust and horse dander allergens causing the positive skin reactions. In the R-group of patients, high RAST scores to the skin test positive allergens (i.e. grass pollen, guinea-pig and horse dander) were often associated with low or only moderately elevated total IgE values.

As was to be expected from the recorded overall agreement between RAST and skin tests, confirmed here, positive RAST results not related to the patient's history did occur. In this connection it is of note that other investigators have also observed that the mere possession of allergen-specific IgE need not imply clinical allergy to that allergen (Hoffman, 1975).

It would appear that such results are compatible with a dual action of allergens and the existence of at least two mechanisms underlying skin reactions and clinical allergy (Berrens, 1971, 1976). The present results with horse dander allergen are particularly telling in this respect. This atopic allergen has the characteristic properties of an atopen, i.e. the non-specific activation of mediating systems in NR-patients. It

also exhibits the properties of an allergen or antigen, i.e. the induction of, and combination with, specific IgE antibody in R-patients (Berrens, 1976). Discordance between clinical allergy and the results of skin tests or RASTs to dog, cow and horse dander allergen has also been noted by other investigators (Öhman & Johansson, 1974; Rudolph *et al.*, 1975; Schultze-Werninghaus *et al.*, 1976). In the present study it remains unexplained why positive RASTs to horse dander allergen not only occurred in the R-group of patients, but were also frequently observed in the NR-group.

A relationship was noted between parameters for horse dander and grass pollen allergy, both *in vivo* and *in vitro*. When separated into R- and NR-groups, the R-reactions to pollen (ten times) were accompanied by seven NR-skin reactions to horse dander. Conversely, the six counted R-reactions to horse dander allergen were in no instance related to NR-pollen reactions, excluding the possibility of cross-contamination of the extracts (Frankland, 1975). Further studies are intended to clarify the phenomenon of non-specific co-reactivity to horse dander allergen in hay fever patients in contrast to the reverse situation.

For detecting highly specific allergies, RAST proved no better than direct intradermal testing. In the cases of highly convincing personal histories, where impressive isolated skin reactivity was associated with high RAST scores, the authors have come to prefer skin tests with photo-inactivated allergens. As indicated in the Results section, the use of these derivatives drastically reduces the proportion of NR-reactions and convincingly aids in the detection of R-reactions. Furthermore, photo-inactivated allergens for skin testing may detect specific allergies in those cases where the anti-IgE RAST is negative, i.e. where the immediate type hypersensitivity reaction is apparently mediated by a different class of immunoglobulin.

The diagnosis of allergy and its causes may require a combination of quite different *in vivo* and *in vitro* techniques to define the precise mechanism underlying the disease in individual cases. Apart from skin and provocation tests with both native and photo-inactivated allergens (Hénocq, Garcelon & Berrens, 1973), RAST tests with both anti-IgE and possibly anti-IgG (Bryant *et al.*, 1975; Devey & Panzani, 1975), other parameters may be helpful in screening and guiding the individual patients. In patients with unspecific hyper-reactivity, complement-sensitivity *in vitro* may be an important factor (Berrens, 1975). In patients with highly specific allergies, parameters for evaluation may include simple data on HLA-type (Marsh *et al.*, 1975) or blood groups. A subsequent paper will describe the prevalence of A-type blood groups in specific grass pollen allergy.

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CHAPTER VII

SUMMARY

In chapter I a survey is given of discoveries and opinions concerning allergy in general. A consensus of opinion appears to exist as regards 'the different way of reacting' of the atopic patient. Repeated contacts with an essentially harmless agent evoke an 'anaphylactic' reaction instead of immunity. Prophylaxis is supposed to be secured when the allergen enters the organism by another route than the original 'porte d'entrée'. It is generally accepted that allergens only act as specific antigens. Traditionally therefore, the examination of atopic subjects focussed exclusively on specific causes. When 'false-positive' test results occurred, these were likewise interpreted in terms of immunological specificity, i.e. cross-sensitivity.

The original definition of atopy could not be maintained. In non-predisposed individuals, allergic symptoms occurred like in the atopic. In animals, both anaphylactic and constitutional atopic reactions were demonstrated. The number of symptoms belonging to the atopic syndrome so far remains uncertain. Skin reactions to tests with allergenic extracts could be provoked in asymptomatic individuals. Parameters such as inhalation challenge tests, the number of eosinofilic leucocytes and the levels of total and specific IgE antibodies supported the diagnosis of manifest clinical atopy. However, there are many non-atopic conditions in which the normal levels of these parameters are exceeded.

The available literature concerning allergy to animals is reviewed in chapter II. Unfortunately, few papers appeared on this subject as compared with, for example, allergy to pollens. A division was made on a chronological basis, according to case histories, to reports on group investigations and as regards examinations with the aid of assessment of antibodies of the IgE class (RAST).

The case histories relate allergies to several kinds of animals, varying from laboratory animals, small and big domestic animals, to exotic ones. Usually, the cause was evident from the personal history; skin tests were performed with the sole aim of proving the suspected allergy. The kind of animal most frequently provoking an allergy was subject of research in investigations in groups of patients. In the years 1910-1930, horse was an important offender, while laboratory animals became a more prevalent cause in the years after 1950.

Domestic animals like dog and cat used to occupy an intermediate position as a cause of allergy. Little attention was paid to socalled 'false-positive reactions', because investigators were preoccupied by allergen-specificity, with emphasis on investigations performed with the aid of the RAST-method.

In chapter III attention is being paid to the standardization of allergenic extracts of animal dandruff. The proportion of crude allergenic material obtained from the hair samples of different animal species varies considerably. Moreover, the yield of total non-dialysable substance varies among the different animal danders. The conventional percentage notation of the strength of test extracts was abandoned

and replaced by standardization on the basis of equal concentrations of total non-dialysable substance per ml of aqueous solvent, expressed in μg/ml.

In the clinical section of the investigation, skin tests were performed with coded animal dander extracts of cow, dog, cat, guinea-pig and horse at a uniform concentration of 20 μg/ml. After the code was broken, and after evaluating the test results, many non history-related (NR) reactions were recorded besides related (R) reactions. On the basis of this pilot study, a tentative conclusion could already be drawn. The observation of strong isolated R-skin reactions rules out cross-reactivity among dander allergens as an explanation for the multiple sensitiveness observed in other patients. The newly developed *in vitro* assay based on human complement consumption yielded useful data concerning non-specific factors. Calculated mean skin-reactivities of the allergens correlated well with the capacity to inactivate human complement in fluid phase *in vitro*.

In chapter IV a purely clinical investigation is described, which substantiates the results of the pilot study of chapter III. A large group of patients was skin-tested with newly coded animal dander extracts. After the code was broken, about the same proportion of NR-reactions and R-reactions was recorded. Moreover, little difference was observed in the averaged strength of the R- and NR-reactions. R-reactions were most often observed to guinea-pig, while NR-reactions frequently occurred to the test extract of horse.

In order to obtain further information concerning the nature of R- and NR-reactions, the total group of patients was divided into eight subgroups. The criteria were: a) the presence or absence of an atopic condition, b) animal contacts positive or negative, c) positive skin reactions to grass pollen or house dust extracts. It became evident that the R-reactions were likely to occur more frequently in the group of grass pollen-allergic patients than in the group of house dust-allergic patients. The same, or the reverse, does not apply to the NR-reactions. The hypothesis that R-reactions are indicative of specific antigen-antibody reactions, whereas NR-reactions reflect a non-immunological mechanism, appeared to be confirmed by the results of this investigation.

In chapter V the ability of animal dander allergens to inactivate human haemolytic complement was investigated more closely. It was again established that skin test reactions occurred related (R) or non-related (NR) to the patient's history. The *in vivo* data and *in vitro* data demonstrated a relationship between NR-reactions and complement consumption. Skin tests performed with animal dander extracts in a group of atopic patients without any animal contacts revealed that both the incidence and the sequence of the 'false-positive' reactions were remarkably similar to those of the NR-skin responses observed in the group of atopic patients with known animal contacts. An explanation for these positive test results as due to cross-allergenicity among different animal danders therefore appeared unlikely. Rather, NR-skin test results are indicative of a common non-specific factor. The occurrence of isolated R-reactions in non-atopic patients indicates that the atopic condition is not a *conditio sine qua non* for specific sensitization. On the contrary, the fact that 'false-positive' reactions do not, or hardly ever occur in non-atopic individuals supports the idea that the atopic condition is a prerequisite for NR-reactions.

In chapter VI a description is given of the observed agreement between R-skin reactions and specific IgE levels on the one hand, and between the reduction of

NR-reactions due to UV-irradiation on the other. The investigation was performed in a group of atopic patients with actual animal contacts. In agreement with the results of the preceeding investigations many NR-reactions were observed. A high total IgE level (RIST) and a normal mean binding of specific IgE (RAST) was recorded in many cases of NR-reactions. However, positive RAST values were often observed in connection with NR-reactions.

The use of photo-inactivated allergenic extracts in skin tests provided two advantages over native allergens. First, the recognition and the appraisement of the R-reactions were simplified by, a) the absence of false-positive reactions and, b) the absence of non-relevant contributions to the R-reactions. Second, R-skin reactions were obvious already in those cases where IgE measurements (RAST) did not yet indicate specific sensitization.

SAMENVATTING

In hoofdstuk I wordt een overzicht gegeven van ontdekkingen en meningen betreffende de allergie in het algemeen. Er blijkt een bijna unanieme opinie te bestaan over het 'anders reageren' van de atopische patiënt. Op herhaalde contacten met een van origine niet schadelijk agens ontstaat een 'anaphylactische' reactie in plaats van immuniteit. 'Prophylaxe' wordt verondersteld gegeven te worden indien het allergeen via een andere dan de natuurlijke 'porte d'entrée' in het lichaam gebracht wordt. Algemeen wordt aangenomen dat allergenen slechts werkzaam zijn als specifieke antigenen. Het allergologisch onderzoek is er dan ook historisch op gericht de specifieke oorzaken op te sporen. Ook bij 'fout-positieve' testresultaten wordt de oorzaak gezocht in het kader van de specificiteit, bijvoorbeeld een kruisallergie.

De oorspronkelijke definitie van atopie kon niet gehandhaafd worden. Allergische symptomen manifesteerden zich ook bij niet-atopisch gepredisponeerde personen. Niet alleen anaphylactische reacties, maar ook atopisch-constitutionele reacties konden in dieren worden opgewekt. Het aantal tot de atopie behorende symptomen staat tot op heden niet vast omschreven. Bij asymptomatische personen zijn huidreacties door tests met allergeenextracten op te wekken. Gegevens verkregen uit inhalatie-provocatie tests, het bepalen van eosinofilie en van het niveau van totaal IgE en specifieke IgE antistoffen gaven steun aan de diagnose klinische manifeste atopie. Er bestaan echter vele niet-atopische condities waarbij de normaalwaarden van deze onderzoekmethoden worden overschreden.

In hoofdstuk II wordt nader ingegaan op de beschikbare literatuur over allergie voor dieren. In verhouding tot bijvoorbeeld allergie voor stuifmeel, zijn er helaas weinig publikaties over dit onderwerp verschenen. Behalve in chronologische zin werd een indeling gemaakt volgens casuïstische mededelingen, onderzoekingen in groepen patiënten en onderzoek waarbij gebruik gemaakt werd van de bepaling van antistoffen behorend tot IgE klasse.

De 'case histories' vermelden allergie voor diverse dieren, variërend van laboratoriumdieren, kleine en grote huisdieren tot exotische dieren. Anamnestisch was de oorzaak hier meestal al duidelijk; de huidtest diende als bewijs voor de verdachte allergie. Het bepalen van de diersoort die het meest frequent allergische reacties veroorzaakt is een onderwerp bij groepsonderzoekingen geweest. In de

jaren 1910-1930 was het paard een belangrijke oorzaak, terwijl sedert 1950 laboratoriumdieren meer op de voorgrond staan. Huisdieren als kat en hond hebben steeds een tussenpositie ingenomen. Geringe aandacht werd aan 'foutpositieve' reacties besteed; de belangstelling was meestal gericht op allergeenspecificiteit, culminerend in publikaties waarin onderzoek met behulp van IgE testmethoden werd beschreven.

In hoofdstuk III wordt aandacht besteed aan de standaardisatie van het allergeen extract van dierlijke huidschilfers. Tussen de hoeveelheden te extraheren ruw allergeen-houdend materiaal (huidschilfers) van de diverse diersoorten bestaat een groot verschil. De daaruit verkregen niet-dialyseerbare substantie bleek opnieuw van dier tot dier te verschillen. De conventionele procentuele concentratieaanduiding voor testextracten werd verlaten en vervangen door gelijke hoeveelheden niet-dialyseerbare oplosbare stof per ml waterig oplosmiddel uitgedrukt in μg/ml.

In het klinische gedeelte van het onderzoek werden huidtests verricht met gecodeerde huidschilfer extracten van koe, hond, kat, cavia en paard in een uniforme concentratie van 20 μg/ml. Na decodering en evaluering van de resultaten bleek dat er behalve relevante (R) reacties, relatief veel niet-relevante (NR) reacties waren waargenomen. Op grond van deze 'pilot study' kon reeds een voorlopige conclusie getrokken worden. Het voorkomen van geïsoleerde, sterke R-huidreacties, pleit tegen een 'kruisallergie' als verklaring voor NR-reacties waargenomen bij patiënten met een multipele allergie. Daarentegen leverde de nieuw ontwikkelde *in vitro* methode voor het meten van complement consumptie bruikbare gegevens op, aangaande niet-specifieke factoren.

In hoofdstuk IV wordt een zuiver klinisch onderzoek beschreven dat een vervolg is op de pilot study uit hoofdstuk III. Een grote groep patiënten werd getest met opnieuw gecodeerde dierextracten. Na decodering bleek dat ongeveer evenveel R-reacties als NR-reacties genoteerd waren. Ook in de sterkte van de R- en NR-reacties werd niet veel verschil waargenomen. De R-reacties werden het meest frequent waargenomen voor cavia, de NR-reacties voor paard. In grafiek gebracht, werd het bovengeschetste beeld duidelijker. Ten einde meer informatie te verkrijgen over de aard van de R- en NR-reacties werd de onderzochte groep onderverdeeld in acht subgroepen, op grond van de volgende criteria: a. atopie of geen atopie; b. diercontact of geen diercontact; c. huidtests positief voor graspollen of voor huisstof. Nu bleek duidelijk dat de R-reacties meer frequent voorkwamen in de graspollen-allergische groep, dan in de huisstof-allergische groep.

Voor de NR-reacties gold niet hetzelfde noch het omgekeerde. De gedachte dat de R-huidreacties een indicatie vormen voor een specifieke antigeen-antilichaam reactie, en de NR-huidreacties voor een niet-immunologisch mechanisme, leek door dit onderzoek bevestigd te worden.

In hoofdstuk V wordt een nader onderzoek naar het vermogen van de dierlijke huidschilferallergenen om humaan haemolytisch complement te inactiveren beschreven. Het relevant (R) zijn, en niet-relevant (NR) zijn van huidreacties werd opnieuw vastgesteld, waarnaast gepoogd werd een relatie te vinden tussen deze *in vivo* gegevens en de *in vitro* gegevens. Deze relatie bestond tussen complement verbruik en het optreden van NR-huidreacties. Uit de huidreacties werden voorts aanvullende gegevens verkregen. Bij tests verricht met extracten van dierlijke huidschilfers in een groep atopische patiënten zonder diercontacten, werd een frequentie en volgorde van voorkomen van positieve huidreacties gevonden in onge-

veer eenzelfde verhouding als in de groep atopici met diercontacten. Een gemeenschappelijke antigene factor (kruisreacties) als oorzaak van NR-reacties bleek hierdoor opnieuw onaannemelijk geworden. Veeleer suggereerde dit resultaat een gemeenschappelijke niet-specifieke factor. Het opnieuw en relatief frequent voorkomen van geïsoleerde R-reacties bij niet-atopische patiënten met diercontacten, duidt er op dat een atopische constitutie geen absolute voorwaarde is voor een specifieke sensibilisatie. Het zelden of niet voorkomen van NR-reacties in een niet-atopische controlegroep betekent dat de aanwezigheid van irritantia in testextracten uit te sluiten is, doch toont vooral de noodzaak van een atopische conditie aan voor het optreden van NR-reacties.

In hoofdstuk VI wordt enerzijds het aantonen van een relatie tussen R-huidreacties en hoge specifieke IgE (RAST) waarden beschreven, anderzijds de relatie tussen de reductie van NR-huidreacties en de inactivering van het testextract met behulp van UV-bestraling. Het onderzoek werd verricht bij een groep geselecteerde atopici met diercontacten. In overeenstemming met de resultaten uit vorige onderzoekingen werden vele NR-reacties waargenomen. Hierbij werd vaak een hoge totaal IgE spiegel (RIST) doch een normale specifiek IgE binding gemeten. Ten aanzien van specifiek IgE werd bij de R-huidreacties het omgekeerde waargenomen, nl. een hoge RAST waarde met een normale of matig verhoogde RIST waarde. Niet zelden werden bij NR-huidreacties toch specifieke IgE waarden gevonden. Het gebruik van UV-geïnactiveerde allergeenextracten leverde twee voordelen op ten opzichte van huidtests met natuurlijke allergenen. Ten eerste werd het herkennen en de waardebepaling van R-reacties vereenvoudigd door: a) afwezigheid van fout-positieve reacties, en b) afwezigheid van het NR-gedeelte van R-reacties. Ten tweede bleken reeds R-huidreacties te verschijnen wanneer IgE-bepaling (RAST) nog geen aanwijzing voor specifieke sensibilisatie gaf.

CONCLUSIONS

It has been demonstrated that the allergen content of the danders of different animals differs quite considerably. Therefore, the strength of allergenic extracts should not be expressed on a weight per volume (percentage) basis. A high percentage of non-dialysable material in the dandruff, moreover, is not indicative of the true allergen content. A distinction should be made between the immunologically specific part (quality) and the non-specific part (quantity) of the skin-reactive substance of an allergen. Skin tests with series of relevant and non-relevant allergenic extracts revealed many history-related as well as non history-related reactions.

Cross-reactivity among dander allergens as an explanation for the multiple sensitiveness observed in particular patients is ruled out by the observation of strong isolated R-skin reactions in other patients. Also, because of the observed similarity of both the incidence and the sequence of NR-reactions in groups of atopic patients with or without animal contacts, cross-reactivity appears unlikely.

Rather, such NR-skin reactions are indicative of a common non-specific factor. The human complement consumption assay yielded useful data concerning non-specific factors. The use of photo-inactivated allergenic extracts reduced both the number of NR-skin reactions and the complement consuming capacity. This relationship supports the idea of common structural sites of lysine-sugar bonding among distinct allergens responsible for the non-specific action of atopic allergens. It is assumed that both allergen-specific and non-allergen-specific mechanisms may contribute to skin reactions and to atopic symptoms as well.

Scheme of types of allergy.

personal history →	negative		positive	
skin test result ↓		eos. BPTs		eos. BPTs
negative :	I no all.	+ or —	II A: no all.	—
			B: devel. all.	+
positive :	III A: NR-all.	—	IV A: R-all.	—
	B: lat. all.	+	B: man. R-all.	+
HS pos. only :	C: atop. const.	+ or —	C: atop. all.	—
			D: man. atop. all.	+

type of allergy	RAST + (UV+)	UV —
I : no allergy	NS-immun. react.	no allergy
II B : devel. all.	devel. S-all.	no allergy
III A : NR-all.	NR-S-react.	no all., atop. const.
III B : lat. all.	S-lat. all.	no all., atop. const.
III C : atop. const.	S-all.	NS-all., atop. const.
IV A : R-all.	S-all.	NS-all., atop. const.
IV B : man. R-all.	man. S-all.	NS-all., atop. const.
IV C : atop. all.	S-all.	NS-all., atop. const.
IV D : man. atop. all.	man. S-all.	NS-all., atop. const.

Abbreviations

pos., neg. : positive, negative.
all. : allergy.
R., NR. : related, non-related.
S., NS. : specific, non-specific.
BPTs. : bronchial provocation tests.
HS. : house dust group.
eos. : eosinophillia.
man. : manifest.
lat. : latent.
devel. : developing.
const. : constitution.
atop. : atopic.
H.S. : house dust.

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STELLINGEN

I De pijlers van het oorspronkelijke begrip atopie zijn alle aangetast.

II Inhalatie-allergenen kunnen bij daarvoor ontvankelijke individuen een dubbele rol spelen: in kwalitatieve zin als antigeen, in kwantitatieve zin als atopeen.

III Een specifieke sensibilisatie voor emanaties van de dierehuid is niet afhankelijk van een belaste persoonlijke of familie-anamnese; de sensibilisatieperiode kan dit wel zijn.

IV Het bij routineonderzoek verrichten van intracutane huidtests met oplossingen van wel en niet door UV-licht geïnactiveerde allergenen biedt voordelen boven het laten bepalen van IgE-waarden.

V Tot op heden is hyposensibilisatie therapie een tijdrovende behandelingsmethode gebleken waarvan, althans met betrekking tot een allergie voor dieren, geen nuttig effect werd gezien.

VI Fout-positieve reacties op intracutane huidtests met allergeenoplossingen worden vaak ten onrechte verklaard op grond van kruisreacties.

VII Het bepalen van het totaal IgE met behulp van de PRIST-methode biedt geen reële voordelen boven een bepaling door middel van de RIST.
Kjellman, N. I. M. et al. (1976) Clin. Allergy, *6*, 51-59.

VIII Het door Makino et al. aangevoerde bewijs voor de aanwezigheid van een abnormale B_2-adrenerge respons bij asthmatici berust op een foutieve proefopzet.
Makino, S. et al. (1977) J. Allergy & Clin. Imm. *59*, 348-352.

IX Lineaire deposities van IgA in het basale membraangebied van de huid, door middel van immunofluorescentie aangetoond bij patienten met bulleuze dermatosen, pleiten in alle gevallen voor parapemphigus.
Meer, J. B. v. d. et al. (1977) Arch. Dermatol. *113*, 1462.

X In principe kan elke pathogene bacterie resistentie verwerven ten aanzien van elk antibacterieel geneesmiddel.

XI De holten in de long, ontstaan tijdens een primaire of secundaire staphylococcenpneumonie, zijn pneumatoceles, die ten onrechte longabcessen worden genoemd.
Hendren, W. H. & Haggerty, R. J. (1958) J. Amer. Med. Ass. *168*, 6-16.

XII In de allergologie en de immunologie geeft de term CARA eerder aanleiding tot verwarring dan dat zij verhelderend werkt.
Orie, N. G. M. et al. (1961) Ned. T. Geneesk. *105*, 2136-2139.

XIII Verschil in virulentie van Haemophilus Ducreyi stammen zou de oorzaak kunnen zijn van het voorkomen van "formes frustes" van ulcus molle; het verklaart echter niet dat ondanks een toenemende frequentie de ziekte bij vrouwen vrijwel niet meer wordt gezien.
Richter, R. (1948) Zschr. Hautkrankheiten *5*, 373-380.

XIV Het predicaat "goedgekeurd" na een lichte sportkeuring, kan een vermeend gevoel van zekerheid geven hetgeen risicoverhogend werkt.

XV De autonomie waarmee vele gemeenten zich zo gaarne sieren ontaardt vaak in onbegrepen willekeur, met name door de wijze waarop zij omspringen met de toeristenbelasting.

XVI Voor de patient met een Eczematoid van ROST is RUST belangrijker dan RIST en RAST.

Stellingen behorende bij het proefschrift van W. J. Koers, "Specificity and non-specificity in atopic allergy".
Utrecht, 31 januari 1978.