The Biochemistry of Food Allergens: What Is Essential for Future Research?

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In this presentation the topic of food allergens is approached from an angle different from that of comprehensive reviews. A number of such reviews are available (1–5). Together with a recent book on food allergy (6) they compile almost everything about food allergens that is worth knowing—and some information that is not so valuable. The present discussion is concerned only with IgE-mediated allergy and natural allergens found in food. It does not take into consideration possible antigenicity of additives used for preservation, flavoring, or food cosmetic purposes.

DEFINITION OF TERMS USED

Scientific work and clinical work in the study of allergy must both satisfy strict criteria with regard to specificity and precision. Thus it appears appropriate to be specific and precise with respect to the terms and definitions used.

Allergy is used in the context of hypersensitivity reactions caused by immune reactions that are harmful to the tissues or disruptive of the physiology of the host. The immune reaction triggers complex biochemical and/or inflammatory responses that result in clinical symptoms. These responses are dependent on the degree of reactivity of the involved tissue receptors and of the effector cells.

Allergen indicates the antigenic molecule that takes part in the immune reaction resulting in allergy. Food allergen indicates allergens found in food.

Allergenic source indicates the material (or food) that contains allergens.

Immunogen indicates the molecule (or part of it) that is able to initiate proliferation of immunocompetent lymphocytes or trigger the synthesis of specific antibodies.

ISOLATED ALLERGENS

In recent years many allergen sources have been studied. A wide variety of allergens have been isolated and characterized. The list of isolated allergens has be-
come too long to be given here. The time has come to bring order to a rather chaotic situation concerning allergen nomenclature. Those interested are referred to the nomenclature system advocated by the International Union of Immunological Societies (IUIS) presented in the Bulletin of the World Health Organization 1986 (7).

FOOD ALLERGENS ARE (MOSTLY) PROTEINS

All natural allergens that react with IgE antibodies have, so far, been shown to be proteins.

A protein is made up of a number of amino acids bound together in peptide linkages with or without additional carbohydrate residues in the primary structure. Each amino acid is characterized by its side chain. The side chains together represent chemically active sites and contribute to the final shape and the power field of the molecule. The chain or sequence of amino acids is twisted and is given its final shape through conformational changes resulting from the chemical forces between the side chains, which then fold the molecule into its tertiary structure. Chemical forces from outside also influence the final shape and the net chemical power of the molecule.

The amino acids can, in a way, be said to act as letters in a chemical alphabet containing 20 different letters. Different combinations of these chemical letters create a multitude of words (peptide fragments) and phrases (proteins) in the language of protein chemistry. Some of the words are made by the amino acid letters as found in the original sequence of the chain. These words are sequential denominators. Other words are made when amino acid "letters" that are remote in the primary sequence chain are brought close together by folding of the chain. These words are conformational denominators.

The complexity of proteins found in allergen sources and in extracts of allergenic foods is well demonstrated by a number of techniques: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); isoelectrofocusing (IEF); and PAGE or starch gel electrophoresis with immunoprinting and crossed radiimmunelectrophoresis (CRIE). A wide variety of modifications of these and similar methods have been used, and new methods are being invented. We can rightly speak about immunoacrobatics in this connection. Combined with each other and, for example, with immunosorbent techniques, as well as by means of selected patients' sera, monospecific antisera may be produced to almost any of the allergenic components in food. The monoclonal antibody technique adds further possibilities for research in the field (8). Progress in protein separation methods has been an important propagator. Many possibilities have been opened for those interested.

I am not saying that this is easy work! On the contrary, it is a demanding, tedious, and time-consuming process full of challenges, problems, and pitfalls. If we want to spend time and resources on it, we ought to consider our ultimate goals with this kind of work. An isolated and well-characterized allergen is not a goal
in itself but only a tool of value for further research. Then we have to ask ourselves: What is essential for future research with regard to food allergy? Answers to this question should influence (a) what food allergens we select for study and (b) the research protocol in allergen purification and characterization.

MAJOR, INTERMEDIATE, AND MINOR ALLERGENS

Only one or very few of the several proteins found in a given allergen source act as essential allergens in the majority of allergic patients. The most important ones are called major allergens. Less important allergens, statistically speaking, are called intermediate allergens and minor allergens. It should be kept in mind that a so-called "minor allergen" may play a major role in rare individual patients.

CRIE can be used to define these terms more precisely in order to promote meaningful communication, provided that a CRIE reference system is included (9). A major allergen is then defined as one that binds IgE antibodies in 50% or more of sera from all the patients allergic to the matter, with strong binding in at least 25% of the sera. A minor allergen binds IgE antibodies in not more than 10% of the sera from the same patient population. Allergens with binding capacities between these two are called intermediate allergens. Most, if not all, allergen sources seem to contain several distinct allergens of major, intermediate, or minor importance. The egg white in hen's egg, for example, is a complex mixture of at least 20 distinct proteins, but only four or five of them are allergenic (10,11).

Blands et al. (12) demonstrated 40 antigens in wheat flour. Eighteen of them were able to bind IgE, and three were considered to be major allergens. Theobald et al. (13), in a study of the sera from patients with "bakers's asthma," concluded that IgE and IgG antibodies seemed to react with the same components in wheat. A clear-cut distinction between antigens and allergens was not obtained in their study. Baldo et al. (14) found a high degree of allergenic cross-reactivity between several cereals, particularly between wheat, rye, barley, and oats. All the studies were concerned with inhalant allergies to flour, and the results are not necessarily applicable to cereals in food. Some individuals allergic to wheat in food tolerate moderate amounts of gluten-free cereals, whereas others do not. This suggests that some, but not all, major or intermediate allergens in wheat are removed or inactivated by the processing of gluten-free cereals.

Cow's milk contains more than 25 distinct proteins that may act as antigens in humans, and a few more antigenic characteristics may arise during the intestinal passage. Absorption of antigenic molecules leads to antibody production. Increased absorption results in more prominent immune response and higher serum concentration of antibodies to milk components. Most of the antibodies do no harm; they are only innocent waste products and can, in a sense, be compared with sewage from the immunocompetent cell populations in the host.

The antigenicity differs from protein to protein and seems to depend on host factors as well as a combination of genetic, environmental, and adjuvant factors.
This applies also to allergens. The most important allergens are found in beta-lactoglobulin (60–80% of cow’s milk allergic patients), casein (60%), lactalbumin (50%), and bovine serum albumin (50%) according to several investigators. Others claim that bovine serum albumin, casein, and bovine gammaglobulin rank the highest.

WHAT MAKES AN ALLERGEN AN ALLERGEN?

One may ask why some proteins act as strong allergens while others in the same food do not. Casein is by far the most prominent protein in cow’s milk but is not as important in allergy as beta-lactoglobulin, which represents approximately 10% of the total protein. In hen’s egg, one could get the idea that the proteins found in the highest concentrations may be the most important ones in allergy. Ovalbumin, which constitutes more than half of the total protein in the egg white, is the most important allergen. Lysozyme, however, is a very weak allergen even though it represents as much as 3.5% to 10% of the total protein.

It has not been possible to point out any physicochemical feature that is characteristic of major allergens reacting with IgE antibodies except for the fact that these allergens are proteins with a molecular weight usually between 10,000 and 100,000 daltons. Theoretically, there may be physicochemical traits that are important for the transport through living membranes, for passage of biochemical barriers, or for phagocyte handling without being directly related to antigenicity. Genetic host factors are probably as important as the molecular structure (4).

Those who study the classic scientific literature may easily become confused or be led astray. As evaluated today, some of the documentation has been confirmed, some has been shown to be only partially true, and some has been dismissed as being wrong. During one period (1960–1970) it was, for example, claimed that sugar moieties and N-glycosidic bonding elements were mandatory for allergenic activity. To study this, one has to work with completely pure allergen systems. The N-glycosidic bond theory was, however, a result of generalizations from studies with a restricted number of impure systems. These are pitfalls that many have fallen into. You may find many examples of this in the literature in question. Using a pure system we could show in 1971 that N-glycosidic bonds are not necessary for the allergic reaction as such.

DENATURATION AND DIGESTION

Identification of allergens in a given food starts with a crude extract of the matter. This involves the risk that some allergenic components are not represented in the original form or not at all in the extract. Some of them may be insoluble and lost in the sediment if the latter is not examined. Others may be present in an altered form. Denaturation and inactivation with respect to IgE binding may occur
during the preparation of the extract. This occurs, for example, for some fruit allergens, as demonstrated by Bjørksten et al. (15).

Furthermore, the processes of fractionation and isolation of protein molecules imply great risks of inducing alterations in the conformation and charges of some of the molecules in question. The molecular folding and charge of proteins are influenced by forces exerted on them from the environmental electrolytes and other proteins. Dilution itself may induce marked changes. This may or may not affect the antigenicity of the molecule.

It is more likely than not that isolation and dilution will induce some changes in the allergenic molecules you try to purify. In fact, for a large number of allergens, you may find that a given fraction with all signs of immunologic homogeneity may be separated in an electrical field if you use the right kind of medium and buffer (or, in your opinion, the wrong kind). Such so-called isoallergens are antigenically identical molecules that migrate slightly differently in an electrical field. They may well represent molecules from the same origin which are slightly changed in conformation and charge during the separation manipulations, but the changes do not affect the antigenic sites.

Questions about the degree of resistance to denaturation and digestion are especially important for food allergens. Clinical observations indicate this. Thus many patients have fierce allergic reactions to fresh but not to cooked apples, carrots, and potatoes. A large number of bakers get allergic asthma or allergic rhinitis as occupational diseases from inhaling flour dust, but they tolerate the same cereals in the food.

Many of the allergens in question may be very susceptible to denaturation during preparation of the food. With regard to allergens in apples, for instance, inactivation may occur as soon as the apple is cut and crushed. The fruit contains phenolic compounds and enzymes that tend to denature the allergenic molecules quickly on manipulation. Apple allergens could, however, be extracted in active form with media containing agents which inhibit the phenol-protein reactions (15). Phenols combine with proteins by oxidation and hydrogen bonding. This results in denaturation with alterations of the antigenic properties of some molecules.

On the other hand, such foods as hen’s eggs, peanuts, nuts, peas, fish, and seafood elicit allergic reactions almost irrespective of what you do to the food in question. They provoke allergic reactions even when found as steam droplets from the food being cooked or fried. New antigenic forms may be created during cooking and/or digestion and may be important in very rare cases. Küstner, in 1921, who delivered his serum for the first scientific demonstration of the presence of circulating reaginic antibodies associated with allergy, claimed that he reacted to fish only after it had been cooked. This suggests that allergenic sites not present in the original fish proteins were formed or made accessible only by denaturation. Küstner must have been quite unique in this respect. I myself have seen hundreds of fish-allergenic patients and all of them react both to raw and cooked fish proteins.

In other words, the antigenic determinants in question in some food allergens
are affected and inactivated by denaturation and digestion. Others are not affected; they maintain the allergenic activity. Here we have arrived at something essential in the discussion of the biochemistry of food allergens.

**EPITOPES (ANTIGENIC/ALLERGENIC DETERMINANTS)**

The antibody (or immune receptor of an immunocompetent lymphocyte) binds specifically to a very limited part of the antigenic molecule. This binding site is called an epitope or antigenic determinant. In this review an allergenic determinant or epitope means an epitope in the IgE system. All proteins may be considered to be complex mosaics of epitopes—comparable, in a sense, to short or long words in the language of protein chemistry. Some feature may be decisive, others less so, as seen from the antibody viewpoint. Some components may act only as necessary spacing elements keeping the essential denominators at an optimal distance from each other, or they may take part through more or less essential binding forces. The factors that may determine the potency of the particular allergen, making it a major, intermediate, or minor allergen, are (a) the number of allergenic epitopes that are accessible for the specific antibodies and (b) the binding dynamics of these epitopes and antibodies.

**CROSS-REACTIVITIES**

A number of the proteins present in a given food may have some epitopes in common, as demonstrated for wheat flour by Theobald et al. (13) using monoclonal antibodies. This accounts for immunological cross-reactivities between different proteins within a given food as well as between different related food. Thus, patients allergic to the major allergen (Allergen M) in codfish therefore react also to haddock and carp white-muscle proteins. In fish allergy, some patients react to all fish species tested for, whereas others exhibit a marked species differentiation (16,17). Some patients who react to both codfish and salmon apparently have IgE antibodies reacting with identical allergenic epitopes on proteins found both in codfish and salmon. In other patients there are, however, quite distinct sensitizations to different allergens (epitopes) in the two species of fish.

Cross-reactivities are also found between eggs of different birds (11), between different seafoods (18), and between many other foods within the same order. Cross-reactivities are also found between certain pollen and vegetables/fruits (19).

**CONFORMATIONAL AND SEQUENTIAL EPITOPES**

Most epitopes are thought to be conformational. They result from the steric folding of the amino-acid–peptide chains. The folding of the molecule brings together important amino acids that are found quite remote in the amino acid se-
quence chain as such. Denaturation of the protein will usually alter the folding and break up this kind of epitope. Laboratory manipulation during protein fractionation may have similar effects, thus making it extremely difficult to identify essential epitopes. It is still more difficult to synthesize such epitopes.

A number of epitopes are sequential. They are organized from a number of amino acids (with or without sugar moieties) as found in the original linear sequence. They may be picked out directly from the amino acid sequence of the protein. This type of epitope often remains unchanged following denaturation of the protein and may be left untouched by enzymes not specific for amino acid bonds present within the epitope. Sequential epitopes lend themselves much more easily to identification and synthesis.

THE CODFISH ALLERGEN MODEL

The observation that the allergenic activity of hen’s egg white, peas, and fish is unaffected by cooking and digestion in human intestines suggests that these foods contain major allergens with sequential epitopes. To begin with, it is wise to select such material for purification and characterization purposes. This is what I myself thought when I wanted to pick a model for molecular research in the immunology of allergic reactions.

An infant with severe atopic symptoms was admitted to my department. He had been solely breast-fed but suffered from atopic eczema, bronchial asthma, and bouts of severe angioedema and diarrhea. We could demonstrate that he was excessively sensitive to fish and that he got worse every time his mother had eaten fish herself.

The allergenic molecules in question had resisted cooking (denaturation) followed by digestion (proteolysis) in the mother’s intestines, passage through several membranes (to the breast milk), and a second digestion in the infant’s intestines. They were still active when reaching reaginic antibodies in various tissues in the infant. From these observations, the allergenic molecules in question had to be associated with sequential units of amino acids and could probably be found in rather short fragments. I wanted a model for investigation of what makes an allergen an allergen, and here the case history of the infant told me about a suitable model.

I selected codfish for this purpose and started a tedious protein fractionation and purification process including quite a number of trials and failures before succeeding. Codfish contains one major allergen (Allergen M) found in the white-muscle tissues. All the codfish-allergic patients I have seen react to this allergen. Codfish white muscle also contains other intermediate and minor allergens and so does the blood serum of the fish. In the most sensitive patient systems the purified allergen was extremely potent. It provoked marked local whealing reactions in passive transfer or so-called Prausnitz-Küstner tests (PK test) in concentrations corresponding to less than 10,000 molecules injected into the sensitized sites. Double-
blind food challenges with microgram quantities given to the PK-test recipients provoked similar reactions. The latter experiments confirmed that the purified allergen was also absorbed in an active form through normal adult intestines. The major allergen is heat stable and quite resistant to proteolytic digestion and several denaturation procedures. This supported the idea that the allergenic activity is found in a linear sequence.

The purified allergen turned out to be a valuable tool for performing further work in basic study, as well as clinical study, within the immunology of allergy. Thus, it was shown that most of the IgG antibodies to codfish in patients' sera reacted with proteins other than the major allergen in question. Furthermore, some IgG antibodies (both human and rabbit) binding to the allergenic molecule reacted with the allergenic epitope as such, whereas others reacted with other epitopes on the same molecule. This is an important point in discussions about the role played by so-called blocking antibodies. It appears that only those blocking antibodies that react with the allergenic epitope as such are likely to affect the in vivo synthesis of the IgE antibodies in question. These antibodies could be referred to as 'allergenic epitope blocking antibodies'.

AN ALLERGENIC EPITOPE

The amino acid sequence of the purified allergen was analyzed. This provided enough material to propose the hypothetical model for a sequential allergenic determinant (IgE-binding epitope) presented during the 1975 Nobel symposium in Stockholm (3). This hypothetical model has subsequently been substantiated and confirmed. It was based on the assumption that the epitope may be formed by a few critical amino acid side chains found in a sequence while one or several amino acids, acting only as rather indifferent spacers, keep the critical amino acids at the optimal distance from each other.

Furthermore, at that time it had become evident that the biological reaction in IgE-mediated allergy was brought about when the allergen bound two IgE antibody molecules on the surface of mast cells or basophils. To be allergenically active the allergen had to have at least two accessible allergenic determinants, probably of identical composition. Assuming this, the molecule in question could have six (or even more) allergenic determinants (IgE-binding epitopes) composed of two closely connected carboxylic side chains (ASP + GLU) kept at a critical distance from the basic residue (LYS). According to this the sequences (-ASP-GLU-LEU-LYS-), (-ASP-GLU-ASP-LYS-), and possibly (-ASP-ASP-LYS-) stood out as particularly interesting candidates. The same constellations could possibly also occur as conformational units (Fig. 1). Short enzymatic cleavage products from the native Allergen M resulted in peptides containing ASP-GLU-LEU-LYS and ASP-GLU-ASP-LYS, which were allergenically active.

Preliminary efforts to synthesize such a tetrapeptide rendered a molecule that was only partially able to block PK-test reactions to the major allergen. At this
point I had to abandon molecular and immunological research in order to devote most of my time to other tasks. The molecular research work was, however, carried on by my former collaborators.

The region composed of residues 41 through 64 of Allergen M encompassed three of the tetrapeptides described, kept apart by two segments of six variable amino acid residues. Elsayed and co-workers (20,21) produced synthetic peptides corresponding to the peptides in question. A peptide composed of 16 amino acids corresponding to residues 49 through 64 of Allergen M was produced by solid-phase synthesis (SPS). This hexadecapeptide bound IgE antibodies in the sera from cod-allergic patients. The peptide interfered also with rabbit antiserum IgC antibodies to Allergen M (20,21). The nature of the interspacing amino acids appeared to be without significance.
IMPLICATIONS FOR FUTURE RESEARCH

At least two of the epitopes in question are necessary for the binding to IgE antibodies in biological tests of allergy to codfish. Immunogenicity for IgE antibody synthesis in predisposed individuals probably demands only one epitope. Theoretically, a carrier substance may be provided by the host itself (i.e., as serum albumin or heparin). In any case, extremely small fragments containing one epitope would suffice for sensitization of a disposed individual. Then it is not likely that sensitization depends on the degree of permeability of the gut but rather on a combination of genetic and adjuvant factors in the host.

Peptides of the kind described could serve as valuable tools for molecular and cellular studies in this field. The availability of synthetic peptides that represent the allergenic determinants of natural allergens may open the field for studies involving variable fragments, the effects of amino acid substitution, the effects of conjugation to various carrier substances, and so on. This kind of information may serve as a useful key to some of the hidden mysteries of immune reactions in allergy. It may also prove valuable in efforts to unveil mechanisms of induction, as well as of suppression, of the immune responses in question.

There is, however, no room for generalization. The codfish allergen model functions only for certain limited aspects of our many scientific problems. Other food allergens may behave in quite different ways at different levels of immune responsiveness and responses.

Conformational allergenic determinants are probably more common than sequential ones. They are much more demanding with respect to characterization and synthesization. To me it is surprising that the codfish allergen model is—after 10 years—still the only one for which important epitopes have been defined. There are many other food allergens that most probably contain sequential epitopes that could be defined as well.

Allergens in hen’s egg white, for example, are tempting targets for such studies. In fact, more than 75 years have elapsed since Schloss indicated this through his elegant investigations of the biochemistry of egg-white allergens (22). Availability of synthetic epitopes reacting with human IgE antibodies may provide tools for important research at the very basis of the immune reactions in question. Indeed, we need much more knowledge of this kind for all types of allergen, particularly in the confusing field of intolerance and allergy to food.

REFERENCES


**DISCUSSION**

**Prof. Reinhardt:** In the codfish model, the allergic molecule must not only fit into the IgE molecule but it must also create a cross-bridge between two IgE molecules. What is the cross-bridging in the cod? Is it due to two tetrapart peptides within the codfish structure, or what?

**Dr. Aas:** In most cases there will be two identical epitopes on the same molecule, but not necessarily always. They could be similar but not identical, binding to somewhat different IgE molecules on the mast cell. The distance between the epitopes may not be very important, because the electrophysical power field exerted by the two types of molecules (i.e., the antibody molecule on the mast cell and the epitope on the allergenic molecule) will make the IgE molecules move a little in the membrane of the mast cell, and possibly also induce slight changes in the conformation of the allergenic molecule, so that the molecules are drawn together, after which they are electrophysically “sucked” into place. It is
interesting to study this point in the traditional Prausnitz-Küstner reaction. If you sensitize the skin with fish-allergic serum and then inject only about 50,000 molecules of the purified system into the other arm, you will get a strong reaction after 20 to 40 min, which shows how these molecules are rapidly distributed in body fluids and how, when a molecule reaches the sensitized site, it is stored there in a kind of "depot" until there is enough to make a visible reaction.

Dr. de Week: What do you consider to be the minimum size of an antigenic epitope? In your studies and in previous classical studies it appears to be about three amino acids. However, there are indications from studies of allergy to chemicals that an antigenic or allergenic epitope may be much smaller—for example, the quaternary ammonium compounds or the $\equiv N-O-CH_3$ side chains in cephalosporins, which are very small groupings but which can cause unexpected cross-reactions. Do you feel that sensitization to smaller epitopes might be a possible cause of unexpected cross-reactions in food allergy as well?

Dr. Aas: There is a considerable literature on the size of epitopes, and it is known that they may be quite large—for example, 8 to 12 amino acids in IgG antibody studies. I have no material to discuss specifically with regard to whether three amino acids is the minimum size, but when I read the literature I wonder what role is played by carrier substances in the material that has been used for the studies. With such a small number of amino acids in the epitope there is a good chance of cross-reactivities. For example, some of my patients who are allergic to codfish and salmon may also cross-react with a minor allergen in the salmon, while others have quite distinct IgE antibodies both to cod and salmon.

Dr. Guesry: I'd like to probe this point further. In the field of allergy to cow's milk it is said that in order not to be allergenic a molecule should be less than 5,000 daltons in size. You showed that a cod peptide of 16 amino acids—that is, about 2,000 to 2,500 daltons—is powerfully allergenic. Do you know the minimum size of the epitope of allergens such as casein and beta-lactoglobulin?

Dr. Aas: I think that some allergens of very small size present in cow's milk may be substances such as penicillin, and perhaps Professor de Weck may wish to comment on this. As far as native milk proteins go, there are always hydrolysates of milk of large enough size to carry two epitopes, which may be allergenic for the subgroup of patients who are allergic to the original molecule. However, I do not know their exact size, though from fish experiments allergenic hydrolysate peptides may be as small as 2,500 to 3,000 daltons in size, or even smaller.

Dr. de Week: I cannot answer specifically about milk antigens, but experiments have shown that peptide molecules less than about 2,000 daltons in size are not usually very antigenic. However, there are exceptions, and a number of small molecules are known to be immunogenic, probably through covalent binding or very strong hydrophobic binding which enables them to bind to a cell membrane and thus be presented to T-cells. Such a molecule may elicit an immune response but may be too small to elicit an allergic reaction.

Dr. Walker: A clinical problem in the food allergy field is that the RAST test for the allergens is notoriously unreliable in making the diagnosis, especially in the case of milk proteins. Do you think that this is likely to be because the test involves the native protein rather than its digested product and that the process of digestion may expose certain epitopes or allergenic determinants which could, in turn, create an allergic reaction?

Dr. Aas: Küstner became world-famous because he was allergic to fish, and he was happy to have the collaboration of Prausnitz, who did the experiments! Küstner stated that he reacted only to cooked fish and never to raw fish, which would indicate that digestive
denaturation had created an epitope to which he was reactive. I have never seen such a patient, and I believe that in Küstner's case there was probably a species difference, in that he ate some species raw and some cooked. I have no indication in the fish group that epitopes arise during digestion which represent something not found in raw fish. The difficulty in using RAST for diagnosis of fish allergy is that the commercial RAST uses a codfish allergen, so fish-allergic patients who are not allergic to codfish and do not have a true immunologic cross-reaction with cod-fish will have negative RAST. Another problem is that, even in the presence of persistent clinical fish allergy, the RAST results vary from highly positive to zero. A skin-prick test using the right sort of fish antigen in the correct concentration is more reliable.

Dr. Guesry: When you defined major and minor antigens you took the number of people reacting to these antigens as your guide, but if you look through the world literature you find huge discrepancies. For example, citrus fruit seems to be the most important allergen in Scandinavia, cow's milk in southern Europe, soya bean in the USA, and so on. Don't you think that exposure to an antigen in early life may be as important as its biochemical properties?

Dr. Aas: When I spoke about major allergens I was referring to the role of the different proteins within a certain group of allergic people. Thus if you take the fish-allergic population of Norway you find that cod is the major allergen, while in central Europe it is carp. However, they are completely identical with respect to the major allergens: They have the same epitopes. That means that when you use the system in the carp-allergic population you will find exactly the same basis for definitions of major, intermediate, and minor allergens as you do in the cod-allergic population. My own feeling is that it is not so much a question of what is in the food as of what is in the air: If you eat carp in your home I can find and define the major allergen of carp in your house dust.

Dr. Frick: Some earlier work by Berrens from Holland showed that sugar linkage onto proteins was an important factor in allergenicity, through the browning reaction. Nothing has been said about this. Could you comment?

Dr. Aas: I think the statement by Berrens about the importance of N-glycosidic bonding was probably an unjustified generalization from one finding, especially since that finding was based on an impure system. The codfish allergen has one sugar molecule, and we have been able to split it into two peptides, one with and one without sugar. The one without sugar is the most active. So I should warn against generalizations. There may be cases when N-glycosidic bonding is important, but it is not essential for allergenicity.

Dr. Wahn: I would like to comment briefly on the question of allergenic epitopes. There is a good deal of experimental evidence now that not only are there two different epitopes on one molecule but also that the two epitopes responsible for one single bridging of IgE antibodies may be different. For example, it has been shown that bivalent haptens do not have to be identical. It was a tantalizing idea that we might be able to find epitopes which could block anaphylactic reactions, as has been done by Dr. de Week with penicillin, but since we now know that on one single molecule there may be 6, 8, or even 10 nonidentical determinants responsible for cross-linking in different combinations, I fear that we shall not be able to find blocking haptens.

Dr. Aas: I agree with your skeptical view about the results of epitope studies. I am equally skeptical about the use of the words "blocking antibody" in discussions concerning immunotherapy. This often-used term creates confusion in light of today's knowledge of allergenic molecules. You have to follow it with the question: What kind of epitope does
it block? We have shown that IgG antibodies may react with the same epitope as IgE, but they may also react with quite different epitopes on the same molecule, yet they are all called "blocking antibodies." There are other ways of studying this, however, even if we are skeptical about the final aim of being able to block all types of allergens with single epitope substances. I think that some allergies may perhaps be blocked by a couple of epitopes—the major allergens of codfish, for instance—but epitopes could also be used to study the mechanisms of the reaction between receptors on antibodies or cells and the epitope—for example, by the use of cytotoxic agents to differentiate the cell stages during the process. Allergen research should now be allergen epitope research.