

When the results of specific IgE determination using the RAST technique were followed, an increase was noted in the percentage of positive tests with food antigens in December 1977 and in the percentage of positive RAST results for house dust mite in September 1978.

DISCUSSION

Our results indicate that by using a systematic quality control of skin tests and specific IgE determinations the following can be achieved: Changes in the skin testing by the prick technique and in the quality of the antigens used can be detected by these monthly controls (Table 1).

When the percentages of positive skin tests and RAST determinations for birch pollen antigens are compared, it can be seen that the results from the two analysed years 1977 and 1978 are comparable, indicating that the methods used are quite stable, and that no change occurred in the percentage of positive tests when the skin testing method was changed from the intracutaneous to the prick technique on July 1, 1977. One interesting point is the seasonal variation: there is a lower percentage of positive reactions 2–3 months before the birch pollen season and then a sudden increase in the percentage of positive reactions immediately after the birch pollen season in June and July.

This is evidence of the variations of specific IgE which occur in seasonal atopy caused by pollens from plants with intense but time-limited flowering.

As to the determination of specific IgE using the RAST technique, it appears that the percentage of the positive RAST reactions to food antigens has increased since December 1977 and the number of positive reactions to house dust mite has also increased since September 1978. We were later on informed by the producer that there had been a change in manufacturing discs for food RAST in November 1977. We assume that the same kind of change was made in the manufacturing of the discs for determination of specific IgE to house dust mite.

Finally, it can be mentioned that a quality control system of the type described above, which includes monthly discussions with the staff, provides a good opportunity to discuss and check the indications for the tests used.

Address:

Alf Bohman, M.D.
Hospital for Allergic Diseases
University Central Hospital
Meilahdentie 2
SF-00250 Helsinki 25
Finland

Standardization of Allergen Preparations

A. W. FRANKLAND

St. Mary's Hospital, London, England

Allergenic extracts used by physicians are usually obtained from commercial sources. We find that different commercial firms produce extracts which can be grossly non-comparable and unfortunately the same firm will sell extracts which from batch to batch have very different strengths. The physician cannot usually afford radio-allergosorbent tests (RAST), but he can very easily and cheaply carry out skin prick tests (SPT).

I would agree with the suggestions of Aas and colleagues (1) when considering appropriate methods of standardization of allergenic extracts to combine the use of skin prick tests (SPT) with RAST. But for the physician who cannot afford RAST, he can very easily and cheaply obtain much information from standardized skin prick tests.

It also seems very reasonable to use a histamine equivalent (HEP) by using a similar prick test of histamine hydrochloride (HCl) 1 mg/ml. (The com-

mercially available histamine preparation in the U.K. is histamine acid phosphate. I do not know the advantages of one histamine salt compared with another.) The concentration of the extract producing an identical reaction is stated to have 1000 biological units (BU)/ml. I would like to make a plea to use an agreed strength of histamine in a prick test, as the positive control, and I would particularly stress that prick testing is preferable to intracutaneous testing which is favoured by Voorhorst (9).

REPRODUCIBILITY OF HISTAMINE SKIN TESTING

It has been found that 20 prick tests with one concentration of a purified timothy pollen extract in two different individuals deviated less than 10% from the mean (6). It must be remembered that in any one patient there is a

day to day variability of skin size response to the histamine SPT. When the skin test is performed on the arm, the flexures are much more reactive than elsewhere on the arm. A test substance can be standardized to correspond to 1HEP, or 1000 BU/ml, but clinically instead of using a standard solution, it is informative to carry out SPT with four or five 10-fold dilutions. The end point from positive to negative can be very variable using the same allergen in two clinically similarly affected patients.

It has also been shown (10) when using a bio-assay to study standardization of extracts that the length and width of the flare is linearly correlated with the logarithm of the sum of the length and width of the wheal. On this basis a "skin reaction index" was proposed. Furthermore, the log-dose response curve of the skin reaction in the medium sized reactions proved to be a straight line, although the most probable shape of the log-dose response curve is an S-shaped line. It is well to remember that over 25 years ago, Swain & Becker (8) showed that an increase of the testing dose by a factor of 1000 causes an increase in the diameter of the wheal only by a factor of two. It has also been found (unpublished) that when using the skin of the back, if a Latin square (6×6) was used, it was easy to see that the SPT of histamine, by the time the 36th test was done, was much smaller than the earlier histamine tests. This was entirely due to the fact that the subject's skin had cooled during the half hour that the investigation took to carry out.

PURITY OF ALLERGENIC EXTRACTS

Many extracts are grossly contaminated. These contaminants may themselves be allergens. When collecting grass pollen at a "Pollenarium" (5) it was impossible to be sure that one grass pollen was not contaminated with any other grass pollen that would be flowering at the same time. Techniques were copied from those used in hospital operating theatres, but microscopically we could never be sure that one grass pollen was not contaminated with other grass pollens. The contamination occurred in the fields from other pollinating grasses. It was hoped that it was not greater than 1%. However, plantain pollen is easy to characterize under the microscope, and this quite often contaminated our "pure grass pollen" sample. Contamination of up to about 3% could easily occur with plantain pollen. Other pollens such as nettle (urtica), and many mould spores particularly of *cladosporium* and *alternaria*, were usually present.

Much small amorphous debris seemed likely to be due to insect, spider and mite particles, because when the pollen had been sieved twice and spread out thinly on to drying trays, many very small creatures would walk away during the next 2 or 3 days. Lime (tilia) pollen is very difficult to collect and is always grossly contaminated with other pollens. Cross antigenicity between unrelated

pollens has been shown by immunological tests, but may only be evidence of the impurity (contamination) of the pollens studied. There can be a standard "pure" preparation of ragweed pollen, ragweed E, but not all allergic patients with ragweed hay fever react to this pure standard preparation (7). So there is a danger that we may be asked to use pure standard preparations which clinically might not be useful diagnostically or therapeutically.

ANIMAL EXTRACTS

Traditionally it has always been considered that a subject who has become allergic to an animal with inhalant allergic symptoms, is allergic to airborne scurf (dander) from the animal. But we know that clinically this may not be so. A salivary allergen may be the only part of a dog that first sensitizes a small child, later on perhaps all or any part of the family kept dog may cause allergic problems. But it is frequently observed that other breeds of dog cause no problems. When we carry out skin, nasal or bronchial provocation tests in a known dog allergic subject, using a "mixed dog" extract, and all tests are negative, quite obviously we have not used the correct dog extract however well it has been standardized. It is to be hoped that animal extracts in future will specify exactly how many kinds of dog have been included and also what part of the dog was extracted.

We sometimes see patients who have allergic asthma produced only by Siamese or Burmese cats, but a routine skin test with a (standard) cat extract may give a negative response. It is not going to be easy when standardizing animal extracts to legislate for these problems. Rat asthma in laboratory workers is due to allergy to a urinary protein, and the ordinary commercial "rat extract" usually has no urinary allergic rat moiety present in it (4). Commercial firms should perhaps specify that a rat extract is from the hair and skin of the rat and the clinician should know that this extract is probably not worth using diagnostically. Some patients are allergic to rat serum albumin and not the glycoprotein in the urine, but this is unusual.

It should soon be possible to standardize a dust mite extract of *D. pteromyssinus* now that the main allergic component has been characterized (2). An extract of mites that have been fed on horse scurf will cross react with those patients who give a positive skin test to horse scurf. In passing we must remember that if collected in the summer this horse scurf is also itself contaminated with pollen. We occasionally find patients allergic to horse serum albumin and not horse scurf.

This means that whenever an extract is being standardized, there are many variables in the patient's specific pattern of response to allergenic components. The usual pattern of allergenic response may be absent in some allergic patients and a standard extract may be quite inactive in such patients.

What I wrote in 1962 (3) still applies: "Advances in the understanding of the pathogenesis of the atopic diseases will only come when standardized methods using "pure" antigens and antibodies are available". Progress seems slow but progress is taking place, particularly in the Scandinavian countries.

REFERENCES

1. Aas, K., Bachman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. *Allergy* 33, 130-137, 1978.
2. Chapman, M. D. & Platts-Mills, T. A. E.: Measurement of IgG, IgA and IgE antibodies to the house dust mite *Dermatophagoides pteronyssinus* using a partially purified allergen (F₁P₁). *Clin. Exp. Immunol.* 34, 126-136, 1978.
3. Frankland, A. W.: Pathogenesis of allergic diseases. In Gell, P. G. H. & Coombes, R. R. A. (eds.): *Clinical aspects of immunology*, 2nd ed., p. 656. Blackwell, Oxford, 1968.
4. Frankland, A. W.: Rat asthma in laboratory workers. *Allergy*, p. 123, *Excerpta Medica*, Amsterdam, 1974.
5. Freeman, J.: Hay fever — a key to the allergic disorders. Heinemann, London, 1950.
6. Frostad, A. B., Bolle, R., Grimmer, O. & Aas, K.: A new well-characterized, purified allergen preparation from timothy pollen. II. Allergenic in vivo and in vitro properties. *Int. Arch. Allergy* 55, 35-40, 1977.
7. King, T. P.: Ragweed pollen allergens. *Allergology*, pp. 394-399, *Excerpta Medica*, Amsterdam, 1974.
8. Swain, H. H. & Becker, E. L.: Quantitative studies in skin testing. V. The whealing reactions of histamine and ragweed pollen extract. *J. Allergy* 23, 441, 1952.
9. Voorhorst, R.: Standardization of allergens by an improved skin testing technique. *Develop. Biol. Stand.* 29, 352, 1975.
10. Voorhorst, R. & Krieken, H.: Atopic skin test re-evaluated. III. The wheal flare ratio, the log-dose response curve and the bio-assay of allergen extracts. *Ann. Allergy* 34, 77, 1975.

Address:

A. W. Frankland
St. Mary's Hospital
Praed Street
London W2 7NA
England

Analysis and Standardization of Allergen Extracts Intended for Therapeutic Use

E. H. RELYVELD¹ AND E. HENOCQ²

¹Institut Pasteur, Unité des Vaccins
Bactériens, Garches, and

²Hôpital de l'Institut Pasteur, Consultation
d'Allergologie, Paris, France

Over the past decade, many papers and international meetings have been devoted to establishing methods and requirements for analysis and standardization of allergen extracts. In fact, manufacturers and control authorities are in demand to produce procedures enabling precise and viable quantitation of allergenic activity as well as analysis of extracts. Such an approach would allow physicians to prescribe uniformly labelled extracts of known quality and guarantee therapeutic procedures.

Minimum requirements have also to be established for drafting monographs for Pharmacopoeia.

It has been stated (3) that potencies determined by skin testing or by RAST inhibition of commercial extracts of short ragweed pollen showed about a 1000-fold difference as to their real activity and one can conclude

that allergenic extracts, actually on the market, often do not satisfy the general requirements of drugs. At present, it is even not advisable to substitute an extract of one manufacturer with that of another without again carrying out skin testing to ensure safety and therapeutic activity, because none of the quantitation systems actually used indicates a precise expression of the biological activity in standardized units, a condition leading to much confusion.

The concentrations of extracts are also for the time being expressed in different units such as the Noon unit, the total nitrogen unit, the protein nitrogen unit or weight by volume (5).

The flaw in these methods of standardization of allergenic extracts is that correlation of biological activity

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.