

EVALUATION OF ALLERGEN DIAGNOSIS

JAMES C. HEALY, M.D.

BOSTON, MASS.

The diagnosis of the specific substance or substances in any case of hypersensitivity is a relatively modern and not entirely accurate procedure. With the recognition of the allergic nature of several syndromes such as asthma, certain forms of rhinitis, eczema, urticaria, migraine, functional gastro-intestinal disturbances, and a variety of minor ailments, details of the cause-and-effect relationship are paramount. In a number of instances the affected individual is fortunate enough to have made his own observations and conducts himself accordingly. The investigating physician likewise is able to come to probable conclusions in many instances from the patients' history and his knowledge of types of reaction and the nature of reactions. In either event, however, observation and interpretation must be corroborated by some specific diagnostic test to justify a reasonable therapeutic plan.

According to our medical historians, the earliest experiment of this nature took place in 1835 when pollen was applied to the broken skin and the nasal mucosa of a physician who suffered from seasonal coryza. Since the specific cause was known to be ragweed, the test definitely established the cause-and-effect relationship. Later in 1873 this earlier informal procedure was applied in the testing of hay fever patients with various pollens. Great impetus to this form of diagnosis occurred in 1909 as a ramification of work being done with tuberculin skin sensitiveness. The skin scratch test with inhalant allergens was adopted for the detection of probable excitants of the allergic states by 1912. About the same time the intracutaneous method, employing solutions of various allergens, came into prominence.

Skin testing by the scarification technique has become a standard diagnostic procedure in all forms of allergic disease. Its efficiency, aside from inhalant allergens where it is highest, is not sufficient to justify complete confidence. Actually many investigators contend that skin testing with food allergens is no more than 50 per cent accurate at best. The intradermal method is more accurate but it

has the disadvantages of being somewhat painful, possibly giving rise to untoward systemic reactions, and being restricted in universal employment because the number of skin injections must be limited at any one sitting. The conjunctival test, although highly informative in cases of nasal and paranasal allergy, is naturally restricted because of anatomical limitations. Passive transfer testing is very valuable but here again practical difficulties are encountered in getting not only willing but also cooperative recipients. All these methods so far enumerated are based on the scientific duplication of at least one physiological change occurring in most allergic states: namely, capillary dilatation and increased capillary permeability with extravasation of blood elements into the tissues. The changes in the cellular elements of the blood, such as eosinophilia, have long been recognized as important in the diagnosis of the allergic state. The total leucocyte count and the lymphocyte-polynuclear relationship have been applied clinically as diagnostic criteria in the "leucopenic index" method of testing. In this method there is a great deal of laboratory work involving the prominent factors of errors in calculation and cell identity. Since well over a hundred allergens should be considered in practically every case the cooperation of the patient may be strained beyond his endurance. Hence the elaborate and interesting leucopenic index is relegated to a corroborative status.

While studying the effects of certain physical and mechanical respiratory impediments on the eosinophile content of circulating blood, it was found that the addition of histamine induced characteristic changes. Shed uncoagulated blood of experimental animals and of humans was smeared thickly on glass slides treated with supravital stains. A drop of 1:100,000 solution of histamine phosphate was added to the preparation and sealed in a hot box. There was a sharp rise of eosinophilic staining leucocytes in the preparation. This change was characterized not only by alteration of the staining affinity of the granulocytes but also by a marked increase in the size and coarseness of the nuclear particles. Changes occurred only in those polymorphonuclear neutrophils which had three or less segments in the nucleus, in other words the younger forms. It was further noticed that these changes occurred only in those blood specimens taken from experimental animals which had been previously traumatized or used in laboratory work involving some substances, such as drugs, foreign to the animal's normal environment. On checking the source of the human specimens it was found that in each instance the individual had an allergic state himself or gave a family history of the diathesis. Four of these persons were medical students who knew

the cause of their hay fever, ragweed. They became the subject of further study.

When a drop of a 1:100,000 dilution of ragweed pollen extract was added to the stained blood preparation, the eosinophile content rose from 2 to 9 per cent within a few minutes. These original studies were carried out during the month of January 1939. The following September three of these students had mild, moderate, and severe hay fever, respectively. None of the three had been hypo-sensitized. The differential leucocyte counts showed, in the order named, 4 per cent, 9 per cent, and 16 per cent eosinophiles. The addition of a drop of either the 1:100,000 ragweed dilution or the 1:100,000 histamine solution produced a rise to 8.5 per cent, 16 per cent, and 22 per cent, respectively, *in vitro*. The fourth student had received preseasonal and coseasonal graduated doses of ragweed extract. In this case the same experiment caused only an increase to 2 per cent over the basic 1 per cent eosinophiles. However, when the concentration of the pollen extract was increased to 1:10,000 or the histamine 1:100,000 a further rise of 6 per cent was obtained. In the three cases of active hay fever the blood cell changes did not occur after a sufficient amount of ephedrine or a similar pharmacological agent had been administered to practically obliterate symptoms.

The facts that changes in blood can be provoked independent of the controlling body influences within a few minutes and that the leucocyte granules changed not only in their chemical affinity but also morphologically, convinced us that further attempts along chemical lines might facilitate allergic diagnosis. This method of inquiry was employed in 24 cases of known inhalant allergy with perfect results. A small number of atopic eczemas in children (6 cases) which were known to be caused by milk or egg in the diets were studied in the same way with uniformly accurate results. The allergen reagent used was a 1:5000 dilution of milk protein in the milk sensitive patients and a 1:10,000 dilution of egg in the egg sensitive patients. Here again, as in the other methods of testing, the procedure is impractical for routine use. For example, it would take constant observation of blood preparations under the microscope to determine even a few antigens without the investigator's vigilance being overtaxed to avoid possible errors in technique and calculations. Finally, because of previous work done on blood titres of precipitins and agglutinins and the contradictory evaluations of these determinations in blood sera and plasma of allergic individuals, a modification of this principle was investigated. Using first serum and

later plasma it was found that the addition of either pollen extract or histamine solution caused a turbidity which could be seen macroscopically in many instances and in higher dilution by means of the nephelometer in all. For purposes of extensive testing with a large number of allergens high dilutions of sera or plasmas are necessary and to carry out such a program modifications were developed which form the basis of this clinical report.

During the past six years a group of 2164 patients who had one of the major allergic disturbances was studied. Each patient was observed for from two to five years to be certain of accuracy. They were all of the white race, between 8 and 34 years of age, of both sexes, of all ordinary occupations, and all residents of New England, mostly of greater Boston. In each instance the procedure and the ultimate goal were the same: namely, to limit the cause of each particular allergic syndrome to one outstanding etiologic factor. Depending upon the clinical substantiation of the laboratory diagnosis in each case the individual study was considered a success or a failure.

Technique. Five cc. of blood is aspirated from the aseptically prepared antecubital vein with a sterile needle and syringe. This is immediately hemolyzed with an equal volume of distilled water in a test tube, which is tightly stoppered and refrigerated at 5° C. or as close to it as possible. Because of the possibility of confusion in reading the tests, it is our policy to have patients abstain from any medication for one or two days before a blood specimen is taken. Since it is probably a colloid phenomenon any extraneous clinical influences are not only undesirable in general but also specifically, as noted elsewhere with regard to ephedrine and similar compounds. This precaution also applies to the sulfonamides since they lead to methemoglobin formation and interfere with accurate dilution of the hemolyzed blood.

The hemolyzed blood is centrifuged to separate out cell fragments and cellular elements. The clear supernatant liquid is decanted and enough distilled water added so that compared with a hemoglobin standard it is colorimetrically the equivalent of 1.3 grams per 100 cc. In this way one need not be concerned with the corrections otherwise necessary if there is an anemia. Furthermore, a uniform dilution of non-cellular elements is available in sufficient quantity for about 200 tests. No preservative is required since the preparation is refrigerated and used within a reasonable time.

The allergen medium is made up in bulk and kept refrigerated. For convenience the prepared medium is placed in small glass vials

2.5 cm. long, 0.5 cm. in diameter, with a capacity of 0.5 cc. A number of tubes for each particular allergen are prepared at the same time and can be stored for several weeks before use without deterioration. Various substances have been used as medium bases, gelatine, agar, glycerinated gelatine, and glycerite of starch. Each of these has its limitations and at the present time the best base appears to be a vegetable albumose. This is taken in the dry state and a sufficient amount of a 1:50,000 allergen solution added to bring it into a liquid state. Two cc. of this preparation is then placed in each glass vial to be stored for immediate or later use and refrigerated. It rapidly solidifies on standing a half hour. The method of Coca has been employed for the preparation of most allergen solutions. Special methods are used for the pollens, dusts, animal epidermals and bacterial protein extracts. To prevent drying of the media if they are not to be used within a few days, the refrigerator must be carefully humidified and the tubes covered with moistened filter paper or a blotter.

Two cc. of the diluted hemolyzed blood is superimposed on the prepared medium in the tubes and the set of each individual's tests placed in an incubator at the same time. They are kept at 37° C. for several hours, usually overnight. Readings are then made by use of the nephelometer. A positive reaction has been interpreted by us to be a zone of density at the junction of the blood dilution and the medium. A control, containing untreated medium and a portion of the untested blood dilution, is used for comparison. A second control consists of untreated medium and distilled water.

Obviously this method entails considerable work in the preparation of tubes and in care of the glass. At the present time experiments are under way to reverse the process; that is, using the untested blood in the medium solidified in petri dishes. These may be spotted with allergen dilutions so that 32 to 64 tests may be done on each plate.

Since the histamine reaction in tissues so closely resembles the allergic and since changes occur in the cellular and non-cellular elements of the blood as described above, all blood specimens were routinely tested with histamine-containing media. Of 2164 patients a positive reaction to histamine *only* was obtained in 139 cases, or 6.4 per cent, of the whole group. Fifty-four additional histamine reactors, or 2.5 per cent, had also a positive test for some extrinsic factor as a complication of our laboratory diagnosis. One hundred sixteen of the first sub-group of histamine reactors, or 89 per cent,

obtained from marked to complete relief by hyposensitization with histamine solutions. In the second sub-group (54 cases) identical treatment together with avoidance of the indicated extrinsic factor proved valuable in 42 patients or 77 per cent of the group. A single course of histamine hyposensitization is not a permanent cure and must be repeated. In our experience these intervals varied from a month to a year and in several instances continuous histamine therapy was necessary for control of the patients' symptoms. Here a plan of treatment has been established similar to the perennial program of pollen hyposensitization.

In the large group where extrinsic factors *only* were established by our laboratory findings, the symptoms of 1732 patients of the 1963, or of 88 per cent, were effectively controlled as long as they cooperated by following rigid regulations for the avoidance of the offending substance. Out of this group only cases of bacterial or inhalant hyperergy were treated "actively" by specific parenteral hyposensitization. Institution of the "passive" regime of allergic control necessitates in many instances careful education of the individual concerning not only the composition of his diet but also the ultimate nature of its ingredients. Compliance or failure in this respect decides the issue, clinically and statistically. This is particularly true of primary foodstuffs such as wheat, egg and milk, which are universally the ingredients of so many dishes. Here the science of the dietitian, the art of the epicure and the mental toil of the allergic patient must be sublimated.

	CASES	PER CENT
Total number cases	2156	
Total number histamine reactors (only)	139	6.4%
Histamine desensitization successful	116	89%
Histamine desensitization unsuccessful	23	11%
Histamine factor plus extrinsic factor	54	2.5%
Symptoms successfully controlled	42	77%
Symptoms not successfully controlled	12	23%
Total number reacting to extrinsic factors (only)	1963	91.1%
Symptoms successfully controlled	1732	88%
Symptoms not successfully controlled	231	12%
TOTALS		
Successful Cases	1890	87.7%
Unsuccessful Cases	266	12.3%

In tabulating the list of extrinsic factors it has been impossible to present any orderly grouping of the offending substances. No common denominator of chemistry, biology, physics, nutritional needs, or frequency of use was adequate as an index. According to the incidence of reactions they have been listed in the arbitrary fashion: foods 69.4 per cent, inhalants, 12.8 per cent, bacterial proteins 11.7 per cent, beverages 1.9 per cent, clothing materials 1.4 per cent, and a miscellaneous group of 2.8 per cent which includes tobacco and the condiments. In the 231 failures (12%) of the extrinsic group the breakdown of figures is roughly the same as that for the successful except for the higher incidence of laboratory findings of bacterial hypersensitiveness.

The food group is interesting in that the vegetable-fruit, the cereal, and the fish sensitivities were most frequent, the meats least and dairy products and poultry intermediate. Comparison of these figures with those published reports of many investigators on skin tests are not entirely satisfactory but close enough to confirm the general accuracy of all methods as far as they go.

CONCLUSIONS

1. A method for routine study of allergic problems is reviewed. This method necessitates a minimum of discomfort for the patient, is entirely objective, and does not interfere with symptomatic relief pending etiologic diagnosis.
2. This review suggests further simplification and application of the method.
3. It offers through finer diagnosis an easier existence for those who have been overwhelmed heretofore by staggering lists of positive tests by other methods.

520 COMMONWEALTH AVENUE.