A SURVEY OF SKIN TESTING, WITH OBSERVATIONS ON A SUGGESTED METHOD IN EPILEPTICS.

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Limitations of Skin Testing.

SKIN sensitivity had been mentioned and demonstrated in isolated instances over a long period of years before Schloss in 1912 extended and described its usage and established it as a definite means of detecting hypersensitiveness. Since then it has been universally recognized as a most useful adjunct to the study of the aetiological problems of allergy. At first it seemed that a miraculous means had been found of explaining both the cause of and the reason for the recurrence of certain common disorders of obscure aetiology in widely separated organs. It promised to show how totally different symptoms could be attributed to a common agent. The fact that the skin was sensitive but that the only clinical evidence of disease appeared, say, in the lungs gave the impression that all organs of the body must be in a like state of sensitivity. This has since been widely disputed and denied and on this subject Alexander (1936) states that such an assumption, at least from the clinical standpoint, is a fallacy. Man, he believes, may exhibit general hypersensitivity if a sufficiently large dose of protein be experimentally introduced.

If, then, hypersensitiveness is localized in certain tissues, it follows that the skin may or may not be sensitive in any given case and at any given time. In other words, because the ingestion of a specific food leads to colic or urticaria, it does not necessarily follow that the skin will give a positive reaction to an extract of that food in all cases or at all times. Indeed, a positive reaction may not occur at any time. This limitation of skin testing is now accepted ; but nevertheless, allowing for the fact that only 50–60 per cent. of allergics (and that is not an over-generous estimate) give positive results, it still remains a very valuable diagnostic aid.

Testing Materials.

The first and most important consideration in skin testing is that the testing materials should be potent and trustworthy. Many workers use the dry protein extracts exclusively ; others prefer standardized solutions of the extracts, and for this purpose many different extracting fluids may be used,

such as saline, dextrose, glycerin and alcohol. Should solutions be employed a careful watch must be kept for deterioration and frequent control tests performed on known allergic subjects. Some solutions are prone, in time, to develop histamine, the mere presence of which renders results useless by producing inevitable positives; on the other hand, negative findings may arise through a gradual loss of potency.

Extracts differ so much in their effectiveness and uniformity that it is of primary importance to find and maintain a reliable source of supply. This lack of uniformity is such a disturbing factor that several allergists now produce the required allergens in their own laboratories. The standardization of extracts is usually determined on the basis of total nitrogen or protein nitrogen. According to Simon (1936) two essential factors are involved: (1) What will the extract do when injected into the skin of a clinically sensitive patient? (2) What will it do in a normal and non-sensitive person? No interpretation of tests is possible unless an answer to each of these questions can be given, since the first must ensure its potency and the second provide a control.

Furthermore, certain vegetable and fruit extracts which contain non-specific irritating properties, and which will produce erythema and wheal formation in normal persons, must be so diluted that these non-specific reactions disappear before the solutions are suitable for testing.

The Different Methods employed in Skin Testing.

Several distinct methods exist of testing the skin for protein sensitivity, but the only two to be discussed at length will be the dermal or scratch method and the intradermal. Of other methods less commonly employed there is the patch test (1) largely used to detect sensitivity in dermatitis due to external irritants. In this method the suspected substance, in relatively large quantity, is applied on gauze to the back and three readings taken at 24-hour intervals.

The ophthalmic test (2) is usually reserved for determining the exciting or causative factor of pollen sensitiveness. It may be performed by dropping a quantity of pollen equal to the amount used for a scratch test into the conjunctival sac. A positive reaction is indicated by reddening of the inner canthus. According to Peshkin (1931) its main purpose is to determine, not the degree of sensitivity, but whether sensitization is present or absent when skin testing gives negative results.

The method of passive transfer or the Prausnitz-Kustner reaction (3) is occasionally used. The rationale of this procedure lies in the fact that if the serum of a protein sensitive person be injected into the skin of one who is non-sensitive, the latter becomes sensitized at the site of inoculation to those allergens to which the former is normally positive. A few hours after injection it is possible to carry out intradermal tests on the prepared area, and this acquired sensitivity may persist for many weeks. Should the site become

desensitized to one allergen it will still react to others, provided the original serum was multiple sensitive.

According to Becker and Black (1931) passive transfer may or may not be obtainable with the serum of patients giving positive skin reactions together with marked clinical manifestations; also skin-sensitive individuals who never have had clinical manifestations may give a positive passive transfer. Passive transfer is not dependent on the degree of skin sensitivity, on clinical manifestations, on a temporary allergic state, or on previous desensitization treatment.

The above method has a limited field of application and is chiefly useful when it is difficult or impossible to perform skin tests in the usual way, as in infants, extensive skin disease, or dermographia. Subcutaneous (4) and nasal (5) tests are occasionally employed, but are of no great significance.

The Dermal or Scratch Method.

This method is more widely used than the intradermal, and has the initial advantage of being simpler to perform and thus requiring no particular experience. Once the site has been selected, a series of small scratches is made by a scarifier, a scalpel or something similar. The scratches should all be of equal length (about a quarter of an inch long), and made across the lines of the skin and not parallel to them. It is important that no blood be drawn. The extracts to be tested are applied to the prepared surface, directly if in solution, or with a drop of decinormal soda solution if dry. The substance is then gently rubbed into the scarified area with a clean glass rod and the result noted in ten to twenty minutes. Before reading each site should be lightly wiped with moistened cotton-wool.

The Intradermal Method.

This method is considerably more delicate and requires some preliminary experience to master its technique. To the uninitiated it may present serious pitfalls, as interpretation of results is often difficult, and the introduction of the protein extract may be sufficient to bring about a sudden exacerbation of an existing clinical condition, or even give rise to a general reaction. A tuberculin syringe is often used and should preferably be composed entirely of glass. The point of the fine intradermal needle is introduced between the layers of the skin with the bevelled surface upwards and a small quantity, about one fiftieth of a cubic centimetre, of the solution carefully injected. Great care must be taken to ensure that the total amount of solution introduced gets between the layers of the skin, and that none is given subcutaneously or escapes to the surface on withdrawal of the needle. Should such errors go uncorrected uniformity of results cannot be expected.

Interpretation of Results.

Reactions begin to appear in approximately five minutes, reach their maximum in 20 minutes, and then fade rapidly. The recorded reading is usually that taken at the end of 20 minutes, and it must be carefully compared with the control reaction which has been simultaneously produced by the injection of a like quantity of normal saline. Some writers believe that positive reactions may be delayed and only appear after several hours. Vaughan (1927), who found skin tests a reliable lead in 50 per cent. of his cases, reports this phenomenon in his treatise on migraine and suggests that this delayed reaction is characteristic of the condition. If this is so it may partly explain Foran's (1937) lack of success with skin tests in migraine. Rowe (1931) records similar findings to Vaughan in food hypersensitivities. Parlato (1933) takes an opposite view, believing that food reactions are important only when they are immediately positive. He says one should be very careful when speaking of delayed reactions.

Findings may be tabulated as true positive, pseudo-positive, traumatic-positive or negative. A true positive consists of a clearly defined wheal of appreciable size with a white pitted centre, or with irregular outgrowths (the so-called pseudopodia) reaching from its margin. Again there may be simply a pronounced area of erythema without wheal formation, or any combination of the above pattern types may occur. It should be noted that the size of the skin reaction bears no relationship to the degree of sensitivity.

Some considerable experience is necessary to interpret readings correctly; to distinguish strongly positive reactions from moderately positive and both from pseudo or traumatic. In some cases it may be impossible to differentiate a true positive from a pseudo, but if doubt exists repeated trials will show a lessening effect if the reaction is due to hypersensitiveness and an ever-increasing one if it is non-specific. It is suggested that these non-specific positive recordings are due to the liberation of histamine at the site of the inoculation. Some people have a skin unusually responsive to slight irritation and some have one that is frankly dermographic. In both these types the mere introduction of the needle or application of the scarifier is sufficient to set up a reaction, and the final result may be difficult if not impossible to assess owing to widespread erythema. Such incalculable results show the desirability of relating reciprocally the apparent causative agent with a history of recent contact or ingestion.

Various standards are employed in estimating skin tests. Results may be classified as one plus, two plus, three plus or four plus, and as negative. The area of erythema may be gauged roughly or measured in centimetres. Berkoff (1932) produced a scale with four eye-holes whose respective diameters grade the positive reactions as recorded above. More recently Abramson and Gorin

(1939) have described the construction and operation of a simple contour gauge, by means of which the rate of growth of the height and cross section of allergic wheals may be measured and recorded.

Choice of Application Site.

The site usually chosen for skin testing is either the forearm or the upper arm, but if the intention be to perform a large number of tests at one sitting then the back offers the more convenient area. The anterior surface of the thigh can also be successfully utilized. Different areas of skin have different sensitivities in the same individual, and according to Alexander and other observers, the subject may manifest varying degrees of positiveness in different sites or may be negative in one place and markedly positive in another. Alexander maintains that this variability reduces the worth of the test, as the investigator cannot know which finding is the true one.

According to Bowman (1936) the whealing process to pollen extracts is better in the upper part of the arm than the lower and better on the medial side than on the lateral. She adds that " Tests inserted in rows vertically on the arm influence each other more than those inserted in horizontal rows. This point should be taken into consideration in routine cutaneous testing for hypersensitivity."

Factors Modifying Results in Both the Scratch and Intradermal Methods.

Skin testing is far from being infallible, but once its limitations are appreciated it can prove, in the hands of a competent observer, most helpful and suggestive in diagnostic procedure. Certain facts, however, must be carefully borne in mind.

Because a person gives a positive reaction to a skin test it does not necessarily follow that he is presently allergic to that substance. It may simply be the expression of a former or a future sensitivity, or desensitization may have been achieved. Clinical symptoms may never have been present or the condition may have long disappeared, or there may as yet have been insufficient contact to produce it. The same applies to negative results. Here also an absence of reaction does not prove the person immune. It may indicate that sensitivity is not sufficiently established to respond to a locally administered allergen, although the shock organ will readily react to a small amount. Negatives may occur in cases with marked clinical signs of disease, or where there is other evidence to suggest the negative reacting allergen as the primary cause.

An accurate diagnosis can only be arrived at when a correlation of positive results with the history or with the present condition is possible. Variation in technique may account for the anomalous findings of different workers, as may also the potency of reagents. The location of the injection site has probably an influence. Age is a determining factor and children are infinitely

greater reactors than adults. Bray (1937) states that eight or nine out of every ten asthmatic children will give a positive result. Food sensitization rapidly declines towards the end of infancy and inhalant sensitization rapidly rises.

Multiple sensitiveness is the rule rather than the exception (Eyermann, 1938), but it must be remembered that because a person reacts to a number of allergens it does not follow that his clinical symptoms are due to their joint action. His condition may be due to only one of the suspected substances. Again sensitivities may be lost and gained from time to time or during treatment. Variations also occur with the time of performance of the tests, and results that are strongly positive just prior to or immediately following an allergic flare-up may be only mildly positive or even negative at other times.

Furthermore it must be remembered that although most people are potentially allergic to some substance or other at some period of their lives, only a few develop symptoms. In this connection Colmes, Guild and Rackemann (1935) write: "To become skin sensitive is a common property of mankind; while the capacity to express the sensitivity clinically is the property of those in whom the intrinsic factor, the activator, is operative."

Finally it should be noted that negative results are especially common after specific treatment or the administration of adrenaline, or following severe allergic attacks or prolonged lack of contact with the offending protein. In respect to treatment Levin (1936) reported that energetic desensitization reduced the skin response and obliterated it in 14.9 per cent. of his cases. He believed the reduction in the skin test paralleled the clinical improvement.

Comparison of Scratch and Intradermal Methods.

For some time past controversy has existed over the relative merits of the scratch and intradermal methods of skin testing. According to Coca (1931), differences in results can be almost eliminated by the use of reliable extracts and a proper technique. He maintains that the increased number of non-specific reactions usual with intracutaneous testing results from the injection of too large a volume of the extract, and that they can almost always be avoided if the injected volume does not exceed 0·02 c.c. On the other hand, the scratch method often fails when the intradermal is successful, and he attributes this failure to the relative inactivity of the material used.

Whether this explanation be the correct one or not, it is an accepted fact that the intradermal method gives many more positives, and hence much valuable information would be lost were diagnosis to be based solely on the scratch. Simon states that results indicate the intracutaneous to be from one hundred to ten thousand times more sensitive than the scratch. Tuft (1937) maintains that the important advantage of the intracutaneous test is its greater sensitiveness and its ability to uncover positive reactions not detected by the scratch.

At the same time it is generally recognized that the intradermal is more difficult to perform and the results more difficult to interpret; that it is more dangerous and gives more false positives. Should a strong extract be given intradermally to a highly susceptible patient a general reaction may follow; while a weak extract or a mildly sensitive patient may give a false negative. Hence the absolute necessity of standardization. It is better to employ two extracts, one strong and one weak, than to test with a single of varying efficiency. Should two extracts be used the weak can be applied first and then, if results are negative, the other may be tried.

Rowe, writing in 1927, said that he favoured the scratch test and only used the alternative method when the former had completely failed, and then only in cases of pollen and dust sensitizations. But by 1934 his views had changed somewhat, and in the *Journal of Allergy* of that year he remarks that the intradermal test should certainly be performed with active extracts of important foods to which negative scratch reactions have occurred.

Allergy and Epilepsy.

It was evident on reviewing the literature that considerable diversity of opinion existed on skin testing procedure in general. Certain authorities favoured the intradermal method, others the scratch, while a few were sceptical of both. A similar lack of agreement distinguished the choice of site. Numerous investigators appeared content with any convenient skin area. Furthermore, they seldom confined themselves to one particular area, but employed different sites according to number and frequency of tests. Such lack of precision lent itself to considerable criticism since Alexander had stressed that, in the same individual, difference in reaction varied with difference in location.

Since allergy and the skin tests were so inseparably linked it seemed of primary importance to ensure standardization of method and technique, as otherwise results could not be assessed with any sufficient degree of accuracy. It was the writer's intention to conduct an investigation into the suggested allergic origin of idiopathic epilepsy, but he felt that before undertaking this problem he should apply himself to the more immediate task of elaborating a more accurate skin-testing technique. Before describing the inception and refinement of this method, however, one word is indicated on allergy and epilepsy.

The suggestion that certain types of epilepsy rest on a sensitization basis is no new one, as shown by the considerable research of the past twenty years. Many writers claim to have demonstrated, in certain of their cases, such an allergic aetiology, and especially where a personal or family history of other allergic phenomena existed. Although a good deal of work has been done on allergic epilepsy, no attempt has been made to standardize a method of skin-testing in epileptics. Each investigator has employed that method which

appeared to him best and most convenient. Some writers do not even specify the particular method employed.

In 1923 Wallis, Nicol and Craig scratch-tested 122 epileptics and 100 controls, and found that in the epileptic group 46 positive reactors occurred, while the control group gave only 4 slight positives. They did not record the site used, but found sensitivity varied according to the time tests were carried out. Ward and Patterson in 1927 skin-tested one thousand epileptics and found that 46·9 per cent. manifested sensitization, but they did not mention the site or method employed. Beauchemin (1936) tested a group of a thousand individuals comprising epileptics, psychotics and controls, utilizing the back as the testing site. He reported 80 per cent. of epileptics positive to one protein or another, and also the presence of many doubtful positives. Spangler (1927), Rowe (1931), Forman (1934) and many others have discussed the results of skin-testing various epileptic groups and individuals, but no mention is made of any special precautions observed, or of the adoption of any standardized technique.

A New Skin-Testing Technique in Epileptics.

(1) *Choice of Testing Material.*

The first step to be considered in the present investigation was the choice of testing material, and this material, as already pointed out, had to fulfil certain fundamental conditions. It had to be carefully standardized, of known potency and free from irritating substances, while supplies had to be readily accessible. It was not found convenient to use dry protein extracts, owing to the time and labour involved in standardizing solutions for intradermal purposes. After a survey of available sources it was decided to employ the preparations marketed by Messrs. C. L. Bencard, of London. In addition to individual allergens this firm produces a series of group reagents, and these were deemed admirable for the present purpose. A group reagent is a term signifying the testing extract resulting from the grouping together of approximately some half dozen distinct but biologically related allergens. Such a grouping is favoured by Vaughan (1929), although other competent authorities insist on individual testing.

The group reagents used were in solution and were put up in twelve groups as follows: (1) Mixed inhalants, standard; (2) other inhalants; (3) cereals; (4) eggs, milk, etc.; (5) vegetables; (6) meats; (7) fruits; (8) fish; (9) shellfish; (10) fabrics; (11) non-classified; (12) pollens.

(2) *Method of Investigation.*

Two groups of patients, each 20 in number and containing 10 epileptics and 10 psychotics, were selected. These groups were named A and B. Group A was equally divided into two sub-groups A1 and A2, each of which contained

5 epileptics and 5 psychotics. Group A1 was scratch-tested with the twelve group reagents, while group A2 was given the same reagents intradermally. Two days later this procedure was reversed, and group A1 was now tested intradermally and group A2 dermally. On each of the two following weeks the whole operation was again repeated. The same site was used throughout, namely, the anterior surface of the forearm. Each forearm was used alternately for scratch and intradermal testing. Separate standardized scratch and intradermal solutions were used.

Results are summarized thus :

Group.	Intradermal.		Multiple sensitivity.	Scratch.		Multiple sensitivity.
	Positive	Negative.		Positive.	Negative.	
Epileptic ..	7	3	5	4	6	3
Psychotic ..	2	8	1	1	9	0

The main inferences drawn were as follows :

1. The original number of positive findings obtained by (1) the scratch, (2) the intradermal method did not change on subsequent testings.

2. The specificity of the positive allergens did not vary.

3. The intradermal method gave almost double the number of positive reactors.

4 Multiple sensitivity was twice as common with the intradermal method.

5. In several members of the epileptic group the scarifying of the skin produced of its own accord some whealing and considerable erythema, which rendered interpretation of results unsatisfactory.

6. No general reactions of any kind were experienced.

Meanwhile location tests were instituted with group B. This group was likewise divided into two equal subdivisions, B1 and B2, each containing 5 epileptics and 5 psychotics. Group B1 was given a series of twelve injections, using the anterior aspect of the forearm and the patient's back, while in group B2 the surfaces employed were the upper arm and the anterior and medial aspect of the thigh. The method of testing favoured throughout was the intradermal. The injections were repeated after an interval of two days, and then the sites employed were reversed in the two divisions and another two series of injections given. This reversal of procedure was carried out until each sub-group had been tested eight times on each of four distinct sites.

Results were summarized thus :

	Forearm.		Upper arm.		Back.		Thigh.	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Epileptic group	6	4	6	4	4	6	5	5
Psychotic group	2	8	2	8	0	10	1	9

The main inferences drawn were as follows :

1. The largest number of positives was obtained on the forearm and upper arm.

2. The lowest number obtained was on the back.

3. No positives were present on any of the sites that were not also present on the forearm.

4. The positives present on each of the four sites neither changed their number or specificity on any of the eight occasions.

5. A difference in degree of positiveness was noted between the forearm reactions and those on other sites, the former being definite and distinct, the latter, especially on the back, being usually ill defined and of less intensity.

6. In group B1 one epileptic complained of headache and slight dizziness after each series of injections. In group B2 one epileptic experienced flushing and tachycardia on three occasions and this was followed, in one instance, by a seizure.

General Deductions.

1. To ensure the greatest possible number of positive reactions the method of choice must be the intradermal and the site the forearm.

2. Provided the site remained the same uniformity of results could be expected.

3. The intradermal method tended to produce symptoms of a general nature in susceptible individuals.

(3) *The Selected Testing Method.*

From a study of the findings there appeared some justification for adopting that method which afforded the higher number of positive reactions. Such a method, admittedly, involved a greater danger from false positives and necessitated extra labour in tracking down ultimate offending proteins. Nevertheless, it seemed preferable to the constant uncertainty that must accompany any other method. Many sensitivities would remain unsuspected if reliance were placed exclusively on the scratch test, or again were the back utilized in preference to the forearm. On the other hand, it was recognized, from reference to the literature, that serious consequences had not infrequently followed the indiscriminate use of the intradermal test. Furthermore, the present investigation had not been entirely free from minor symptoms of a general nature and in one instance a seizure had occurred. Whether or not this seizure resulted from the introduction of the protein extracts could not, of course, be stated positively, but the prospect it opened up was not a pleasant one.

It was decided, after due deliberation, to adopt a combination of scratch and intradermal testing and the method of procedure was as follows : Each patient

was, in the first instance, scratch-tested and then, provided no alarming sensitivities had been encountered, was again tested one week later intradermally. This interval of one week was considered advisable on account of some skin discoloration persisting at the site of the tests and tending to confuse readings, and furthermore, because of the possibility, however remote, of local desensitization having been temporarily produced. Separate standardized scratch and intradermal solutions were used as before. If an exceptionally marked positive reaction occurred with any group reagent on scratch testing, then the corresponding intradermal solution was diluted with carbol-saline to one-half or one-quarter its strength before injection. The site of maximum efficiency, the forearm, was used throughout. By this means the safety of the scratch method was allied to the greater sensitivity of the intradermal, at the same time eliminating the disadvantages attendant on the lesser sensitivity of the former and the inherent dangers of the latter.

The above method was employed throughout the writer's further work on allergy and epilepsy, the results of which are presently being prepared for publication. During this later investigation a total of 72 persons were skin tested, a third of this number being epileptics. Each individual was injected with a selected series of protein extracts, either in the form of group reagents or as separate allergens. These latter were naturally more potent to specific reactors than the group reagents, on account of their greater concentration of specific protein Nevertheless, no general symptoms of any kind were encountered and no seizures were induced. Furthermore, by adhering rigidly to the prescribed procedure all possible assurance was given of the greatest number of positive skin recordings being demonstrated and of no possible clues to hidden sensitivity being overlooked. Finally standardization of technique made for a more strictly accurate assessment and comparison of results.

I am indebted to Dr. Grant, Medical Superintendent of Renfrew District Asylum for permission to carry out this investigation

Summary.

1. Reference is made to the role of the skin test in allergy and its uses and limitations, as an aid to diagnosis, are shortly summarized.

2. The accepted but less commonly employed methods of skin testing are listed and briefly discussed.

3. The scratch and intradermal tests are compared and contrasted, with special reference to technique and fallibility.

4. Attention is drawn to the association of allergy and epilepsy.

5. A method of skin-testing epileptics is described, embodying the use of the scratch and intradermal tests and favouring the forearm as the site of election.

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