

QUANTITATIVE STUDIES IN SKIN TESTING

III. THE ASSAY OF THE DIRECT SKIN REACTIVITY OF RAGWEED POLLEN EXTRACTS BY ENDERMAL TESTING UTILIZING AN "ALL OR NONE" RESPONSE*†

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THE first paper of this series¹ described a method for the assay of the direct skin reactivity of ragweed pollen extracts. It was an application of the log probit method of Bliss² using as the response the presence or absence of the wheal on testing the skin of specifically sensitive human subjects with the scratch technique. The second paper of this series³ described a shorter, simpler graphic calculation of the log probit technique which was an extension of the method of Miller and Tainter.⁴ It is the purpose of this paper, by using the endermal method of skin testing, to compare these two methods of calculation of the "all or none" assay of the direct skin reactivity of ragweed pollen extract.

The general plan of the experiment was almost identical to the previous papers.^{1, 3} A stock mixed giant and short ragweed extract was carefully diluted with a known amount of physiologic saline. The undiluted extract was termed for convenience the "Standard" (S); the diluted extract was called the "Unknown" (U). The dilution of S to make U, the true value, was known only to the technician who prepared the dilution and was made known to the experimenters only after the assay results had been calculated. The two dilutions were tested in the manner to be described. It was the object of the experiment to determine the validity of the assay procedure by seeing how closely the assayed ratio of the potencies, by the two methods stated, agreed with each other and with the "true value."

EXPERIMENTAL

The assay subjects were clinic patients receiving treatment for respiratory ragweed sensitivity. The subjects were divided into two groups of eight each. The first or "Monday" group consisted of male and female adults of relatively low skin sensitivity. The second or "Thursday" group was also made up of adults of both sexes who had relatively high skin sensitivity. In each group the members were selected who were as nearly equal in skin sensitivity as possible. Because some patients did not appear at each test period the groups were at times smaller than indicated.

Three dilutions of Standard each differing from the other by a factor of one-third or one-fourth and three of Unknown were prepared. Each dilution was injected endermally on the volar surface of both forearms so that each patient received six injections on both forearms. The skin test was read at the end of fifteen minutes as either positive or negative. Controls with the diluent were

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run simultaneously on both forearms. Both readings and injections were all made by one individual. The criteria for a positive reaction depended on the appearance of the control site. In the absence of any erythema at the end of fifteen minutes at the site of the control injection, a definite erythema encircling the injection site was considered positive. If the control site showed erythema, the reaction of a test site was considered positive if the area of erythema was definitely greater in diameter than that of the control site.

As in the preceding work on the scratch test and as previously demonstrated by Alexander,⁵ Bowman,⁶ and others, it was found that there was a progressive decrease in sensitivity down the forearm. There was also a clear difference in sensitivity between ulnar and radial sides of the forearm. These findings necessitated a scheme for randomizing the placing of the various dilutions on the forearm so that the overall response to any one dose would be an effect of the strength of that dose and not reflect the site on the forearm on which the dose happened to be injected. The method of randomization was the same as used in the preceding paper.¹ The position on the forearm where a given dilution was to be injected was indicated to the experimenter by the technician from the plan of randomization previously made. The experimenter did not know the concentrations which were being injected and when at the end of fifteen minutes he read the skin tests these were called positive or negative with no knowledge of the dilution to which the skin test corresponded.

At the conclusion of the experiment the assay results were independently calculated first by the method of Bliss,² to be called hereafter the "long method," and then by the shorter, graphical approximation described previously,³ to be called hereafter the "short method." The actual dilutions of the Standard and Unknown, the true value, were then revealed by the technician and the results of the long and short method were compared with the true value.

For a description of the methods of calculation the reader is referred to the excellent and simple description by Bliss² and to Becker.³

RESULTS

In all eight assays the regression lines for the Standard and Unknown were straight within experimental error as tested by χ^2 and the two curves (Standard and Unknown) were parallel as tested in each case by the χ^2 test as required for a valid assay.

The results of the last eight consecutive assays are given in Table I. The mean difference between the true value (column 5) and assay values calculated by the long method of Bliss (column 6) is $-8.98\% \pm 4.53\%$ (S.E.). This gives a *t* value of 1.98 with $P > 0.05$ (not significant).

The mean difference between the true value (column 5) and the assay calculated by means of the short method (column 12) is $-12.9\% \pm 6.23\%$ (S.E.). This gives a *t* value of 2.07 ($P > 0.05$) (not significant).

The mean difference between the assay results calculated by the long and short method is $-6.9\% \pm 1.72\%$ (S.E.), $t = 4.01$ with a *P* value $<< 0.01$. This demonstrates that the value obtained by the short method slightly but significantly overestimated the value obtained from the more reliable long method of calculation.

The two methods of calculations give not only an estimate of potency but also the error of this estimate. With both methods of calculation the estimated value was within ± 1 S.E. of the true value. However, it is clear from the data the S.E. calculated by the short method consistently and significantly overestimated the S.E. calculated by the more reliable long method.

DISCUSSION

The data of this paper confirm the findings of the preceding paper that the log probit method of Bliss is a valid method of assay for the direct skin reactivity of ragweed pollen extracts. There is, however, a large error entailed in this assay. The error has a number of sources, the greatest is undoubtedly the error in skin testing technique—the impossibility of injecting into the skin a constant volume at a constant depth. There is also a large variability within and between patients. Moreover, the end point is difficult to read consistently without practice. The slope of the dose-response curve (Table I, column 9) partly reflects these various sources of error and probably is partly inherent in the reaction of the allergen in the skin.

The short method when used for calculating the assay tends to overestimate the true value as far as the data of this paper go. The assay value calculated by the short method consistently and significantly overestimates the values obtained by the longer more reliable method of Bliss. This tends to confirm the findings of an earlier portion, I(B), of this series where while the difference between short method and long method was not significant it was suggested the data were such that further data might demonstrate that the graphically calculated ratio tends on the average to slightly overestimate the more accurate "maximum likelihood" solution. The S.E. of the assay calculated by the short method overestimates that of the long method. These findings seriously limit the use of the graphic approximation.

SUMMARY

1. The log probit method of Bliss and a shorter graphic procedure were applied to the assay of the direct skin reactivity of ragweed pollen extracts using the endermal method of skin testing.
2. The log probit method of Bliss was shown to be a valid method of assay.
3. The shorter graphic procedure tended to overestimate the assay values and standard error obtained by the more reliable method of Bliss. The error of the assay is relatively large.

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