

THE READING OF THE LEPROMIN TEST

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The increasing importance of the lepromin test in the study of leprosy makes it imperative that it should be better studied and known. It is not only the technique of preparation and use of the antigen that should be understood, but also—and principally—the manner of interpreting the reactions observed. This is necessary in order to obtain uniform results, of value in connection with the immunology and prognosis of the different forms of the disease.

Hayashi, observing on the 8th, 16th and 24th days the reaction lesions produced by the intradermal injection of the leproma antigen, established arbitrarily three degrees of positivity. These were based on the diameter of the nodule produced: +, 3 to 5 mm.; ++, 5-10 mm.; and +++, larger ones and those that suppurate.

These degrees of positivity, intended to indicate increasing degrees of resistance, have been considered by various authors in relation to the several forms of the disease, in order to characterize them immunologically. In this way an uncertain factor, the diameter of the reactive lesion, has been put in relation with another, equally uncertain one, the clinical form, and in consequence much confusion has resulted. Because of many doubts regarding our knowledge of them, neither the technique and reading of the lepromin test, nor the clinical form of the disease, can be taken as a "known" factor for the study of the "unknown" one.

Using the present system of reading the reaction, we have observed in about one thousand lepers of several forms, that the lesser degrees of the apparent positive reactions (+ and sometimes ++) do not always signify immunological defense, because such reactions were frequently seen in frank nodular cases. This fact would make it impossible to ascribe any prognostic value to reactions of such intensity. The large majority of unexpected results—positive readings in lepromatous leprosy or the negative ones in the neural form—are, in our opinion, due to the inadequate

definition of what constitutes a positive reaction, and perhaps also to the criteria of classification of cases, which vary with different workers.

The solution of the difficulty would lie in the discovery of a firm basis that would be the fixed factor around which the unknown factors would vary. This basis may be established as two forms of leprosy that are diametrically opposed from all points of view, clinical, bacteriological and histological. Ignoring questions of classification and nomenclature, these forms are the nodular and the fully characterized tuberculoid ones. The immunological distinction of these forms is also established, not only by the laws of general pathology (tuberculoid structure signifying allergy—Lewandowsky and Jadassohn) but also by all of the results as yet observed with the lepromin test; the nodular form is typically negative and the tuberculoid one positive.

It remains to be seen then, whether an exact definition of the positive reaction can be established that will assure that there will be correspondence of the results of the test and the immunological condition. This can be attempted by an investigator who has large numbers of well-characterized cases of both forms of the disease.

PERSONAL OBSERVATIONS

Of the total of 993 lepers tested, the study of which is detailed in a report presented to the Cairo conference, we may consider here 194 frankly nodular cases and 92 typically tuberculoid ones, confirmed histologically. The results of the lepromin test in these patients according to the present system of reading are shown in Table 1.

TABLE 1. *Results of lepromin tests in 194 nodular and 92 typical tuberculoid cases.*

Type of cases	Results of tests				Total
	—	+	++	+++	
Nodular.....	120	68	6	0	194
Tuberculoid.....	0	2	45	45	92

Observing the evolution of the reactive lesions in cases of both types of the disease, we noticed certain facts that merit consideration, and from which we have attempted to draw conclusions.

One of these conclusions is the uselessness of reading the reaction in the first week. It is true that within this length of time

differences may often be seen in cases of the two types (nodular reaction in tuberculoid cases and absence of any appreciable reaction in the nodular type); but on the other hand many tuberculoid cases do not react so soon. Such cases might be considered negative except that the delayed reaction is generally recognized and the practice of successive readings until the 4th or even the 6th week is an established one.

Of more concern is the opposite condition, namely, occurrence of reactive lesions in frankly nodular cases. Table 1 shows such reactions were noted in about one-third of the nodular cases, even though we ignored the readings of the first weeks. These reactions are due to unknown but probably nonspecific causes—foreign body irritation, sensitivity to the protein contents of the antigen, etc. Suitable control tests often give confirmation of nonspecificity; but sometimes the skin does not react to such controls, in which case the reaction must be attributed to a general group sensitization to acid-fast bacilli.

These reactive lesions may persist for a long time in involutive forms, visible and sufficiently palpable to be considered positive, even if only in slight degree. They are seen as little papular elevations, giving sometimes the impression of nodule formation, followed by cicatrization. In diameter they are seldom more than 5 mm. As is to be seen from Table 1, only 6 out of 194 nodular cases gave ++ reactions according to Hayashi's specifications, and actually they were only about 6-7 mm. in diameter.

It is evident that such reactions are not of any interest from the viewpoint of either immunology or prognosis, for they occur in nodular cases. From the viewpoints mentioned they must, in our opinion, be made equivalent to the totally negative test.

The clear distinction between the reactions to lepromin of the two types of cases is to be seen in the evolution of the reactive lesions. In the nodular cases the reaction, when there is any, reaches the peak before the 5th day, with a tendency to diminish thereafter, though it may persist for a longer time but without increase in size. In the tuberculoid cases, on the other hand, the reactions have a tendency to increase more and more, and three eventualities are generally observed:

1. The most frequent occurrence is that the reaction begins 2 to 5 days after the injection, with a small, erythematous papule that increases in size slowly, reaching its peak tardily, in most cases from the 2nd to the 6th week. At that time it consists of a

nodular lesion, more or less intensely red or bluish-red, usually measuring from 10 to 20 mm. in diameter but ranging from 5 to 40 mm. or more. Suppuration is frequent, especially in the larger lesions. These reaction lesions, even the lesser ones, have an objective aspect that to the experienced worker is distinctive from the false reactions of the nodular cases.

2. Another occurrence is that reaction reaches the peak more rapidly, in the first week, but involution does not occur at once; a nodular lesion, generally more than 5 mm. in diameter, persists for a long time. Suppuration frequently occurs.

3. In the third eventuality there is no sign of reaction in the first week, and the case would seem negative, but sometimes in the 2nd week, often not until the 3rd or 4th, a lesion appears and follows the same course of evolutions as the less delayed reactions.

In these three kinds of reactions are comprehended, we think, the great majority of those that occur in tuberculoid leprosy. This description reveals the fact that at about the end of the 4th week, the reactive lesion is at its peak or even still developing, and consists of nodule or an ulceration of characteristic aspect, rarely less than 6 mm. in diameter. In the third, delayed type of reaction described, that size may be reached only in the 7th or the 8th week, but its positiveness is indicated precisely by this extreme tardiness of appearance. By this time none of the reactions that may have been observed in nodular cases is in evidence; they have involuted, leaving either no traces or only an involutive papular lesion, seldom more than 5 mm. in diameter.

As a practical conclusion we would take as the criterion for positivity, significant of immunity defense, the dimensions of the reaction lesion at the end of the 4th week, considering positive only those that are more than 5 mm. in diameter at that time. However, everyone who works with skin tests knows how difficult it is to make a qualification based exclusively on the size of the reaction lesion, and how important are its objective aspects. For this reason the study of specific skin reactivity in leprosy should be undertaken only by men trained in the objective and evolutive characteristics of the reactions, both in nodular and tuberculoid cases.

Even to an expert, however, a large number of reaction lesions will be difficult to qualify, due to some uncertain feature of appearance or evolution as to borderline dimensions (4-6 mm.). We think that it is preferable to put such reactions in a "doubtful" class, considering it better to let them go unclassified than to adopt

an imperfect qualification that is liable to lead to surprises of prognosis.

Thus considered, our positive reactions include the classes ++, +++, and ++++ generally used, but based on more rigorous criteria. First, the persistence of size (diameters more than 5-6 mm.) until the fourth week is demanded, and second, the evaluation of the objective and evolutive signs by a trained expert is required. Our proposal is only to remove the false positive tests, and we will not discuss a possible and desirable subdivision of the real positive tests according to degrees of strength.

We think that a consideration of the features here discussed will reduce the frequency of paradoxical results often reported (the "positive" tests in nodular cases, and easy change from positivity to negativity) that may lead to the discrediting of this very important test.

STANDARDIZATION OF LEPROMIN

Lepromin is an antigen prepared by triturating lepromata in normal saline, and sterilizing and carbolizing the resulting suspension, according to various techniques. From filtration experiments it is concluded that the activity of the antigen is related to its bacillary content. Standardization of such an antigen would not be an easy task, because of the difficulty of making bacillary counts, and also because of the possible inequality of antigenic value of bacilli from various origins. To assay each lot of antigen by performing the test in an individual with known reactivity would be difficult of practice and of limited usefulness.

Such a standardization becomes of less interest than it may seem to have if we desist from attempting an excessive, and as yet impossible, precision in evaluating reactions. Wide variations of concentration of the antigen do not give correspondingly different results. A nodular case, anergic to the standard lepromin of Hayashi (1 gm. of leproma to 20 cc. fluid), does not react to lepromins of two or even three times the concentration, though there may be increase of the nonspecific reaction, which is quite different in appearance and evolution from the true positive reaction. On the other hand, an allergic tuberculoid case reacts, though with less intensity, even to 1:300 lepromin, the maximum dilution employed by us. In some instances the reaction with this dilution was still so strong that it was evidently far from the limit.

If these considerable differences in number of bacilli do not essentially change the result of the test, it is logical to conclude

that the relatively small variations of bacillary content of antigens prepared by different workers will have very little importance. It is sufficient to establish a uniform technique and concentration of antigen, and to discard as uncertain the reactions that are not characteristic, thus giving protection against imperfect determinations and resolving at least provisionally the question of the inequalities of the antigenic power of different lots of lepromin.

SUMMARY AND CONCLUSIONS

The present system of reading the lepromin test was established arbitrarily, and the findings have been related to the different forms of leprosy, the immunological characteristics of which are still uncertain. From this combination of unknown factors much confusion may arise. For example, of 194 nodular cases 74 gave "positive" reactions that could evidently have no immunological or prognostic significance.

Studying the evolution of the reaction in two very distinct groups of cases, nodular and tuberculoid, with undoubtedly opposite conditions of immunity, the author points out the essential distinctive features of the reactions typical of these forms of the disease.

In the nodular form the test is negative: there is no reaction, or only a small papular one, due to nonspecific factors, which reaches its maximum before the fourth or fifth day, seldom is larger than 5 mm., and may persist until the fourth week.

In the tuberculoid form the true positive reaction occurs: a nodular lesion, often suppurating, usually belated in appearing, of progressive evolution, generally reaching its maximum from the second to the sixth week, seldom less than 5 mm. in diameter in the fourth week.

The dimensional criterion of positivity is not sufficient, and the test must be performed by trained observers familiar with the clinical signs. Even so, it is necessary to admit a large group of doubtful reactions, of indefinite aspect and borderline dimensions (4-6 mm. diameter). Recognition of doubtful groups permits not only avoiding error of reporting reactions, but also avoids possible faults due to different antigenic values of different lots of lepromin, the standardization of which still presents a problem.