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A Logarithmic Index of Bacilli in Biopsies

I. Method[1]

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The assessment of leprosy patients, both initially and when under treatment, requires a numerical indication of the numbers of bacilli present in the skin. Much reliance is customarily placed on the results of skin smears taken from several sites. Because the bacterial index of a skin smear was thought to reflect the density of bacilli in a granuloma, but not the size of the granuloma, it was felt that an index compounded of both these factors would serve a useful purpose ([2]). The assessment of both can be made from suitably stained histologic sections, and the resulting biopsy index ([3]) has been used in therapeutic trials, in the elucidation of classification, and for other research purposes.

The most accurate assessment of the bacillary content of the skin is made by counting in homogenates of weighed portions of biopsy material ([1]). This investigation necessitates taking a sample of skin in addition to that used for the usual histopathologic examination. It would clearly be of value if the latter could be carried out so as to provide a reasonably similar estimate of the bacillary content. The biopsy index falls short of this requirement largely because it is subject to the mathematic defect that it is a compound of an arithmetic with a logarithmic component. One of us (G. R. F. H.) has proposed that the best way to overcome this defect would be to adopt a logarithmic scale for both components. This has the disadvantage that the calculation of the index would be a little more complicated, and that the rate of fall of the index due to treatment of the patient would be much slower. Nevertheless, the greater accuracy of such an index as a measure of the numbers of bacilli in a lesion would outweigh these disadvantages for most purposes. The object of this paper is to describe a modified index that is mathematically sound.

THE LOGARITHMIC INDEX OF BIOPSIES (LIB)

The LIB represents the number of bacilli per unit area of a section. It is determined by using the relative area occupied by a granuloma to correct the figure for the density of bacilli in the granuloma. It is calculated as follows.

The density of bacilli in the granuloma, as seen in a well stained histologic section 5μ in thickness, is assessed on a 6+ logarithmic scale as in the original biopsy index (IB) or in the bacterial index (BI) of a smear ([3]). The fraction of the dermis occupied by the granuloma is estimated as accurately as possible by use of a low power (x4) objective. It is important that the biopsy specimen go through the whole thickness of the skin as far as the subcutaneous fat. The proportional area of the granuloma can be estimated visually to

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within about one-tenth; e.g., the granuloma may occupy three-tenths or four-tenths of the whole dermis in the section. For the assessment of very small granulomas (one-tenth or less) an eyepiece grid[3] is a help, although there is in any case a fairly wide random error with granulomas of this size. The logarithm to the base 10 of this fraction is taken, and added to the bacterial density figure. In arithmetic terms, this is multiplying the bacterial density by the granuloma fraction. The answer is the LIB. With a little practice it can be assessed easily from the examination of a section in two minutes.

The density of bacilli is defined in the same way as the BI:

6+ = 1,000 or more bacilli per field of the x100 objective

5+ = 100 or more bacilli per field of the x100 objective

4+ = 10 or more bacilli per field of the x100 objective

3+ = 1 or more bacilli per field of the x100 objective

2+ = 1 or more bacilli per 10 fields of the x100 objective

1+ = 1 or more bacilli per 100 fields of the x100 objective

In practice, 6+ is uncountable. The other values are easily assessed. Greater accuracy is achieved if, when the count is in the upper range of a "+ number," half units are introduced. For example, if bacilli are estimated to be in the region of 50-90 per field, the BI would be 4.5, or more accurately still, since this is a logarithmic scale, 4.7. Half units are also convenient when bacilli are distributed unevenly between parts of the granuloma.

The logarithms required for the area of a granuloma have negative values since they refer to fractions of 1. Thus log 0.6 is conventionally expressed as $\bar{1}.8$, which means $-1 + 0.8$, which is in effect -0.2. For simplicity the logarithms will be expressed in this latter form:

Log. 1.0 = 0	Log. 0.5 = −0.3
Log. 0.9 = −0.05	Log. 0.4 = −0.4
Log. 0.8 = −0.1	Log. 0.3 = −0.5
Log. 0.7 = −0.15	Log. 0.2 = −0.7
Log. 0.6 = −0.2	Log. 0.1 = −1.0

If the area of the granuloma is less than 0.1, the logarithms shown above are increased by −1.0; e.g., log. 0.05 = −1.3; log. 0.01 = −2.0.

The results for the bacterial density and the granuloma proportion are now brought together in a "biopsy formula," and the LIB is calculated from them, as illustrated in the examples below. The value of the biopsy formula is that it shows both the components of the LIB and at the same time provides a kind of "numeric picture" of the lesion; also in serial investigations on a single patient the individual values can be seen as they change with time. But it must be remembered that the shrinking granuloma is expressed as an increasing negative quantity.

Examples. Bacilli in granuloma 100 per field (5+); granuloma six-tenths of dermis; log. 0.6 = −0.2; biopsy formula 5—0.2; LIB 4.8. Bacilli in granuloma 4+; granuloma one-fiftieth of dermis; log. 0.02 = −1.7; biopsy formula 4—1.7; LIB 2.3. These would be typical values for a leproma before treatment and after three years of treatment. For borderline leprosy a typical formula would be 3—0.3, LIB 2.7. The last two examples show how the same or similar LIB values can be derived from very different bacterial density and granuloma values: the difference between LIB 2.3 and 2.7 indicates on the arithmetic scale a 2.5-fold difference in total bacillary content. In this instance it is the result of a 10-fold difference in bacterial density in one direction combined with a 25-fold difference in granuloma proportion in the opposite direction.

Whereas the IB and the BI can be no more than indices, the LIB forms the basis of a crude count. The antilog of the LIB would be the number of bacilli in 600 to 1,000 fields of the x100 objective, assuming that the granuloma was evenly distributed and that half units were used when required. With one microscope, using a wide-

[3]Supplied by Graticules Ltd., Holborn Viaduct, London, E.C. 1, England.

field x8 ocular, the diameter of the field of view of a x100 objective was 0.17 mm. and its area 0.025 sq. mm. The volume of tissue represented by 1,000 fields of a 5μ section is therefore 0.125 cmm. From this it can be calculated that an LIB of 1.0 indicates 80 to 250 bacilli per cmm. of dermis and an LIB of 6.0 indicates 8 million or more per cmm. Thus the bacillary content represented by these indices would usually be of the order of 10^5 per ml. and 10^{10} per ml. respectively, assuming a similar calibration of other microscopes. This range is consistent with the figures derived from counts on homogenates ([1]), if it is remembered that LIB 6.0 is a theoretic maximum seldom met in practice.

By a similar procedure the index could be modified to express the number of viable (solid-staining) bacilli present. If the percentage of solid organisms was 20, the proportion would be 0.2, the log of which is —0.7. If the LIB was 4.3, the LIB (viable) would be 4.3—0.7 or 3.6. Similarly the BI could be modified as an index of viable organisms.

DISCUSSION

Serial biopsies should be taken if possible from the same infiltrated areas. Nodules are not satisfactory, as there is too much random variation in their size and states of activity. If the LIB is to be followed up over a long period, the lesion must initially be of moderate size, i.e., the granuloma should occupy at least two-tenths of the dermis, and the initial LIB should be at least 1.0. It is desirable that two lesions at a time be biopsied, although if groups and not individual patients are the consideration this requirement is not essential. Two biopsies also help to exclude the possibility that one of them may not be representative of the patient's classification, although it is rare to find any significant difference in the histologic classification of two lesions in the same patient ([4]).

SUMMARY

A logarithmic index for bacilli in biopsies is described. It is probably the most accurate index of bacterial content yet devised, and numeric equivalents of index values are given. The index can be modified to express the quantity of viable bacilli in a lesion.

RESUMEN

Se describe un índice logarítmico para los bacilos en las biopsias. Este es probablemente el índice mas exacto de contenido bacteriano hasta ahora inventado, y se dan los equivalentes numéricos de los valores del index. El index puede ser modificado para expresar la cantidad de bacilos vivos en una lesión.

RÉSUMÉ

On a décrit ici un index logarithmique pour évaluer le nombre de bacilles dans des biopsies. C'est probablement l'index le plus fidèle encore mis au point pour estimer le contenu bactérien. On donne des équivalents numériques des valeurs de cet index. L'index peut être modifié pour exprimer la quantité de bacilles viables dans une lésion.

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