THE CONCENTRATION OF M. LEPRAE IN CURRENTLY AVAILABLE LEPROMINS

MICHEL F. LECHAT, M.D., M.P.H. and JOHN H. HANKS, Ph.D.

Johns Hopkins University Leonard Wood Memorial Leprosy Research Laboratory
The Johns Hopkins School of Hygiene
Baltimore, Maryland

This report deals with the concentrations of Mycobacterium leprae in 16 lepromins, which were kindly made available to us by friends and associates. Since lepromin is employed to assay Fernandez and Mitsuda reactivity to antigens from M. leprae, judgments concerning prognosis in individual patients and resistance in populations are drawn from the readings obtained.

If the results of the Mitsuda reaction are to be reproducible, and comparisons valid in various parts of the world, it is important that all lepromins should contain adequate and reproducible concentrations of bacillary bodies.

The purpose of this study has been three-fold:

1. To simplify methods for counting M. leprae in lepromin in order to make them feasible in other laboratories.

2. To learn whether the bacillary content of available lepromins is sufficiently uniform to justify confidence in existing data on Mitsuda reactivity.

3. To make suggestions regarding feasible concentrations of M. leprae, of lepromin. For this purpose, one must consider:

(a) the numbers of bacilli available in lepromins, and (b) existing data on the concentrations desired for the Mitsuda reaction.

METHODS

Lepromins: Requests for 5 cc. of each of two successive lots of lepromin were mailed to investigators thought to be making and using lepromin. The respondents were supplied with kits for mailing the samples and with questionnaires on which to record the methods employed for collecting lepromatous tissues and for preparation of the lepromins submitted. Each sample was transferred to a 20 cc. rubber-stoppered serum vial (containing two 3 mm. glass beads to facilitate resuspension of sediments) and stored by refrigeration. The lepromins were processed in group for uniformity of procedure, one previously counted lot being included with new lots as a control.

The method finally adopted for estimating the concentrations of M. leprae in lepromins will be described in an accompanying paper. It included three improvements of the original methods of Hanks (3). These were (a) addition of the diluent solution prior to dispersion of the deflocculated bacilli, (b) a simple, rapid method of transferring

1 This work was supported in part by a grant from the World Health Organization and by Grant E-3998, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service, Bethesda, Maryland. This material will be presented in part at the VIII International Congress of Leprology, Rio de Janeiro, Brazil, September 1963.
2 Leonard Wood Memorial-National Institutes of Health Fellow in Epidemiology.
replicate samples to glass slides, by means of pin heads, and (c) elimination of the usual calibrations of the areas of films on slides and of the optical fields of microscopes.

Satisfactory dispersions of bacilli and agreements of counts were observed, except in lepromins containing relatively large particles of tissue. Since this defect prevents the drawing of uniform samples into syringes and the injection of uniform aliquots into a series of persons, it is a problem which must be solved by the elimination of tissue particles during the preparation of lepromin. This problem is now under investigation.

RESULTS

Table I shows counts in the 16 samples of lepromin in which bacilli could be found. The highest count is 760 times higher than that of the lowest. In two instances two successive lots of lepromin from single sources differed by 100 and 200 times in bacterial counts.

A survey of reported methods of preparing lepromin indicates that the foremost causes of trouble are as follows: insufficient attention to established methods of selecting and pooling lepromas, excessive grinding of tissues and consequent disruption of bacilli, over-dilution, and failure to make adequate microscopic and clinical comparisons between successive lots.

| Table 1. Rank of concentrations of M. leprae in 16 lepromins; average and median counts. |
|----------------------------------|-----------------|-----------------|-----------------|
| Number of bacilli/cm. (×10⁶)    | Apparent sub-groups | If the median count is regarded as 25 × 10⁶ (±10%) | ±15% |
| 228                             | (3)              |                |                |
| 218                             | (3)              |                |                |
| 218                             | (3)              |                |                |
| 98                              | (2)              |                |                |
| 62                              | (2)              |                |                |
| 37                              | (6)              | 22-28           | 21-29           |
| 33                              | (6)              |                |                |
| 25                              | (6)              |                |                |
| (34)                            | (6)              |                |                |
| 25                              | (6)              |                |                |
| 35                              | (6)              |                |                |
| 16                              | (6)              |                |                |
| 2                               | (5)              |                |                |
| 1                               | (5)              |                |                |
| 0.9                             | (5)              |                |                |
| 0.4                             | (5)              |                |                |
| 0.3                             | (5)              |                |                |

62 = Average.

1 The average and the median values (M) are low in part because of known over-dilution of some samples submitted. E.g., the count of 37 × 10⁶ = a 1.5 dilution of one lepromin. At these 4%, the counts of 0.4 and 0.5 would be 4 and 3 million, respectively. Incorporation of the indicated corrections, however, would not bring the average and median values into line with pre-existing data (see Table 2).
Table 2. Patterns of bacillary concentration in lepromas and lepromins.

<table>
<thead>
<tr>
<th>Lepromas or lepromins examined</th>
<th>Bacilli/cc (× 10^6)</th>
<th>Average number</th>
<th>Approximate median numbers assigned</th>
<th>Discrepancies between high and low counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual lepromas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual JH</td>
<td>210^1</td>
<td>23</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Individual W</td>
<td>38</td>
<td>28</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>185</td>
<td>555^2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled lepromins:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abe et al</td>
<td>220</td>
<td>185</td>
<td>185</td>
<td>1.5X</td>
</tr>
<tr>
<td>Wade, Mw</td>
<td>150</td>
<td>186</td>
<td>186</td>
<td>1.3X</td>
</tr>
<tr>
<td>Mabey #1</td>
<td>213</td>
<td></td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>Mabey #2</td>
<td>268</td>
<td></td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>Wade</td>
<td>164</td>
<td></td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>Mardin et al</td>
<td>158</td>
<td></td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Averages of above:</td>
<td>179</td>
<td>451</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sixteen samples in present study: 62 25 760X

1 Counts adjusted to tissue 3 per cent. These counts were made in the pre-saline run. They are thought to be low for two reasons: the bacilli were not declumped (clumps are lost from films more readily than single bacilli); fixation of films to slides was not as carefully controlled as at present.

2 Counts adjusted to tissue 3 per cent. The data in Table 2 are assembled to demonstrate three points.

1. The first two sections in the table show that the bacterial concentrations in first-quality, individual lepromas tend to differ by at least 10 times. The necessity of pooling at least five or six lepromas is inescapable.

2. The next two sections in the table show that when lepromas are carefully selected and pooled the bacterial counts fall in a fairly narrow range. In the studies illustrated, comparative skin tests demonstrated only small differences in Mitsuda reactivities to 220 and to 150 × 10^6 bacilli/cc. of lepromin.

3. At tissue 3-5 per cent, the bacterial concentrations in the first four sections of the table average 179 × 10^6.

Discussion

Extreme variations in the concentrations of M. leprae in currently available lepromas raise serious questions regarding the comparative value of existing data on Mitsuda reactions. If M. leprae is to be re-
garded as the major factor inducing Mitsuda reactions, it is urgent
that steps be taken to standardize bacterial concentrations.
As a result of careful investigations of bacterial concentrations in
relation to early and late skin reactions, the Japanese workers cited
in Table 2 (14) have adopted 160 million bacilli per cc. of lepromin.

Skilled laboratory workers are not always available where lepromas
may be obtained. Our laboratory, therefore, is prepared to cooperate
with all laboratories that will undertake to establish counting and pool-
ing procedures permitting re-distribution of a standard product on
an institutional or regional basis.

SUMMARY AND CONCLUSIONS

1. This survey of concentrations of M. leprae in lepromins avail-
able to us has been combined with a re-study of methods for estimating
the numbers of bacilli and the collection of information on the prepara-
tion of lepromin.
2. Enumerations in 16 valid samples of lepromin from 10 sources
revealed that: (a) the richest lepromins contained 760 times more
bacilli than the poorest; (b) two lots from individual investigators
may differ by as much as 100 or 200 times; (c) the average in this
series was only 35 per cent of those in other base-line studies.
3. A survey of reported methods indicates that foremost problems
in the preparation of lepromins are: (a) insufficient attention to estab-
lished methods of selecting and pooling lepromas, (b) excessive grind-
ing of tissues and consequent disruption of bacilli, (c) over-dilution,
and (d) failures to make adequate microscopic and clinical compar-
sions between successive lots. This is taken to mean that the prepara-
tion of lepromin has been left too long for persons who are occupied
with other duties, and that more assistance is needed from national
and regional laboratories.
4. Assembled data indicate that an international standard con-
taining 3-5 per cent tissue and 160 \times 10^6 bacilli per cc. should be at-
tainable by proper care in pooling of lepromas. An offer has been made
to cooperate with laboratories interested in achieving this standard.

RESUMEN

1. Esta investigación de concentraciones de M. leprae en leprominas disponibles ha sido combinada con un re-estudio de los métodos para la estimación del número de bacilos y la recopilación de información en la preparación de leprominas.
2. Enumeraciones en 16 ejemplares válidos de lepromina de 10 fuentes, revelaron que: (a) las leprominas más ricas contenían 760 veces más bacilos que las más pobres; (b) dos lotes de investigadores individuales pueden diferir tanto como 100 a 200 veces; (c) el término medio en estas series fue solamente el 35% de aquellos en otra línea histórica de estudio.
3. Una investigación de métodos comunes indicó que los principales problemas en la preparación de leprominas son: (a) insuficiente atención a métodos establecidos de selección y pool de lepromas; (b) excesiva pulverización de tejidos y consiguiente disrupción de bacilos; (c) demasia dilución, y (d) fallas en las comparaciones
La preparación de lepromina ha sido deuda prolongada tiempo a personas que están ocupadas con otras tareas, y que mayores dificultades es necesaria desde los laboratorios nacionales y regionales.

4. Los datos juntados indican que un estándar internacional conteniendo 3-5% de tejido, y 100 × 10^6 bacilos por cc, debe ser obtenible con cuidado apropiado al pool de los lepromas. Una oferta ha sido hecha para cooperar con los laboratorios interesados en lograr esos estándares.

RESUMÉ

1. Los autores han profite de esta análisis sistemática de la concentración de M. leprae. y en los leprominas, que dan a su disposición para proceder a un nuevo examen de las células utilizadas para estimar el número de bacilos. Han también analizado la sensibilidad de las informaciones se reportando de la preparación de la lepromina que han sido realizadas.

2. L'enumeración de los bacilos en 16 échantillons de lepromina provenientes de 10 origines diferentes y los siguientes hechos: (a) los leprominas los más ricos contenían 760 miles de bacilos que los más pobres; (b) dos lotes de misma origen pueden diferir en una proporción de 100 a 200; (c) las leprominas en este estudio de leprominas no ha sido que de 35% inferior a celdas obtenidas en otras estudios de referencia.

3. Una reseña de las palabras de preparación y su uso están son indicativos: (a) no se puede usar a los métodos estables para la selección y el rasenamiento de los bacilos; (b) el lavado de los tejidos está excesivo, extraería una estructura de los bacilos; (c) la dilución está muy grande; (d) sin embargo, de proceder a un comparaciones microscópicas y clínicas de un lot au suivant.

4. Las observaciones dan razón a pensar que la preparación de la lepromina podrá ser muy largos, bien que las leprominas de diversas origen. Los autores proponen de colaborar con los laboratorios interesados a preparar las leprominas correspondiendo a este estándar.

REFERENCIAS


