Precautions for Injecting Uniform Doses of Lepromin [1, 2]

John H. Hanks[3]

This note has two purposes: (a) to show that in many currently available lepromins flocculating components, visible tissue particles, and large mycobacterial clumps, settle in syringes during the time required to make 8-10 intradermal injections, thus causing marked variations in the successive doses delivered from the same syringe; (b) to offer suggestions on minimizing these dosage irregularities during the use of existing stocks of lepromin.

A companion paper (1) describes production methods that eliminate the present difficulties.

METHODS

Twenty-two lepromins had been stored in vials containing two small glass beads. The vials were shaken to obtain maximal dispersion of each lepromin before withdrawal of samples into 1 ml. tuberculin syringes. Successive aliquots of 0.1 ml. were discharged from these syringes at intervals of 60 seconds. Meanwhile each syringe was laid horizontally on the bench. The total time for emptying each syringe was 10 minutes, the time usually required for 10 intradermal injections.

Inequities of injectable doses were judged by three criteria:

1. Observing whether sludge could be seen to accumulate ahead of the piston of the syringe during discharge of the 10 0.1 ml. aliquots;
2. Letting each aliquot fall into a separate depression in a Pyrex spot plate laid on black paper in order to compare the optical densities of the aliquots; and
3. Using the low power objective (100x magnification) of a microscope to compare the sizes of tissue particles and also the numbers of bacterial clumps containing 25 or more bacilli, i.e., "low power positive" clumps, in the successive aliquots.

The first criterion suffices to judge the sedimentation rates on a practical basis.

EXPERIMENTAL RESULTS

Among the 22 lepromins, two were sufficiently stable to assure the injection of 10 equivalent doses from one syringe during a period of 10 minutes. An additional three lepromins did not settle in horizontal syringes rapidly enough to cause serious inequality of dosage.

In the remaining 17 lepromins (85%), visible sludge accumulated ahead of the piston at variable rates. On average, the first two or three doses discharged into the spot plate appeared to be uniform. Doses Nos. 4 to 9 tended to become successively weaker, while dose No. 10 was extremely variable. If the sludge could be accommodated in the needle and the tip of the syringe, the No. 10 dose was "weaker" than the early doses. However, if the syringe had been rotated, or if it had deliberately been laid down so that the sludge ahead of the piston was against the "ceiling," the No. 10 dose could contain a great excess of sludge.

Microscopic studies demonstrated that injection of the sludge will deposit a great excess of tissue particles, including large masses and clumps of bacilli.

Therefore, even with standardized concentrations of bacilli, some 85 per cent of

[1]Received for publication 28 June 1967.
[2]This work was supported by the World Health Organization, Geneva, Switzerland, and by U. S. Public Health Service Grant AI-06944 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014.
[3]J. H. Hanks, Ph.D., Microbiologist, Johns Hopkins-Leonard Wood Memorial Leprosy Research Laboratory, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205.

the available lepromins will not yield 10 equivalent dosages from the same syringe.

DISCUSSION

In order to ensure the injection of uniform dosages from particle-rich or flocculating lepromins, either of two precautions seems feasible. One choice is to take smaller volumes into the syringe and to refill the syringe from the lepromin stock, which must be well shaken, more frequently. Another option is to drop a small glass bead into the barrel of the syringe prior to sterilizing the assembled barrel and piston, and then let the bead traverse the length of the barrel as a stirrer between injections.

Other maneuvers, tested with some lepromins, such as alternately laying the syringe "top side up" and "top side down," were less successful in preventing variations of dosage.

SUMMARY

Although standardization of bacterial concentrations contributes to the general reliability of measuring Mitsuda reactivity, it is also necessary for the individual worker to inject uniform dosages per skin site. Two suggestions have been made to achieve this second goal, viz., (1) refilling syringes with small volumes, more frequently, and (2) using a glass bead in the syringe, with which a uniform suspension between injections can be ensured.

RESUMEN

Aunque la uniformación de concentraciónes bacterianas contribuye a la confianza general de la medida de la reactividad en la prueba de Mitsuda, es también necesario para el trabajador inyectar dosis uniformes en cada sitio de la piel. Dos sugerencias se han hecho para lograr este segundo objetivo, viz., (1) llenar jeringas con volúmenes menores, mas frecuentes, y (2) usar una bola de vidrio en la jeringa, mediante la cual suspensiones uniformes entre inyecciones puedan ser asegurades.

RÉSUMÉ

Quoique la standardisation des concentrations bactériennes contribue à rendre plus fidèles les mesures de la réactivité de Mitsuda, il est également nécessaire que le travailleur individual injecte des doses uniformes dans la peau. Deux suggestions ont été émises afin d'atteindre ce dernier but: (1) recharger les seringues plus fréquemment avec de petits volumes; (2) utiliser une bille de verre dans la seringue, grâce à laquelle une suspension uniforme d'une injection à l'autre peut être assurée.

Acknowledgment. I am indebted to Dr. B. R. Chatterjee for his participation in the preliminary experiments.

REFERENCE

1. Hanks, J. H. Standardizable lepromins yielding uniform concentration of *M. leprae* per skin site. Internat. J. Leprosy **36** (1968) 66-75.