CORRESPONDENCE

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Purification of *M. leprae* Isolated from Human Skin Biopsies

TO THE EDITOR:

For many years after its discovery in 1873 ([3]), biopsies of nodules from lepromatous leprosy patients constituted the only source of the non-cultivable bacillus, *Mycobacterium leprae*. Draper's method ([1]) is currently being used by the WHO-IMMLEP program for the isolation of large quantities of *M. leprae* from armadillo tissues. We used the same protocol to isolate *M. leprae* from human skin biopsies. The data presented in Tables 1 and 2 show that Protocol 1/79 can be adapted for human tissue, although its reproducibility was not totally satisfactory. The often low bacterial counts in skin tissues and the relatively massive amounts of collagenous tissues and fats greatly affect the recovery, especially at the density gradient centrifugation step. However, the isolated *M. leprae* cells from skin tissues are free from the brownish contaminants which are readily found in isolates from armadillo liver tissues. Moreover, other enzymes and protein contaminants were not detected by starch gel electrophoresis, nor were they seen by light and electron microscopy.

The type of disease in the patient from whom the starting material is obtained affects the percentage recovery of *M. leprae* (Table 2). The *M. leprae* cells in the old LL cases could have been in the disintegrated form and could have been lost during the purification process. Skin biopsies from new LL cases and BL patients gave percentages of recovery comparable to those from armadillo liver tissues. Skin biopsies from patients with LL with reactivation and LL with ENL gave very good yields of pure *M. leprae* cells.

Preliminary observations suggest that *M. leprae* isolated from human skin biopsies may be more specific than those isolated

TABLE 1. *Yield of bacilli and presence of tissue contaminants at different stages of the purification process.*

Purification step	No. of bacilli: $\times 10^5$ AFB ml^{-1} (yield in %)				Presence of tissues	
	Expt. 1[a]	Expt. 2[a]	Expt. 3[a]	Expt. 4[b]	LM[c]	EM[d]
Skin tissue homogenates	90 (100%)	650 (100%)	600 (100%)	66 (100%)	++++[e]	++++
End of homogenization	78 (86%)	480 (74%)	520 (87%)	47 (71%)	+++	++
End of enzymatic treatment	66 (73%)	420 (65%)	490 (82%)	39 (59%)	++	+
End of gradient centrifugation	51 (57%)	390 (60%)	420 (70%)	27 (41%)	+	−[f]
End of two-phase separation	48 (53%)	280 (43%)	310 (52%)	21 (32%)	±	−

[a] Expt. 1–3 = Human skin biopsies (pooled).
[b] Expt. 4 = Mouse foot-pad tissue (pooled).
[c] LM = Light microscopy.
[d] EM = Electron microscopy.
[e] + = Presence of tissue contaminants.
[f] − = Absence of tissue contaminants.

TABLE 2. *Recovery of* M. leprae *isolated from skin biopsies taken from patients with different types of disease.*

Disease type[a]	Biopsy pool: no. of specimens	Tissue homogenates ($\times 10^5$ AFB ml^{-1}) (a)	Two-phase separation ($\times 10^5$ AFB ml^{-1}): Lower layer (b)	Two-phase separation ($\times 10^5$ AFB ml^{-1}): Upper layer (c)	% Recovery $\left(\frac{c}{a} \times 100\%\right)$
LL old cases	5	8.9	0.0	0.0	—
LL new cases	8	281.0	0.0	131.1	47
LL with reactivation	4	131.4	3.1	87.6	67
LL with ENL	4	8.1	0.0	7.0	86
BL	7	71.0	0.0	31.4	44
TT	4	0.0	0.0	0.0	—
BT	3	0.0	0.0	0.0	—
Mouse foot pad	6	3.1	0.0	0.8	26

[a] LL = lepromatous leprosy; ENL = erythema nodosum leprosum; BL = borderline lepromatous; TT = tuberculoid leprosy; BT = borderline tuberculoid.

from armadillo liver tissues (supplied by IMMLEP) in that the former did not react with any of the tuberculosis patients' sera tested, while the latter reacted with 5%–9% of these sera ([2]).

—Sen-Chiew Gan, M.Sc., Dip. Ed., Grad.D.B.A., C.Biol., M.I.Biol., A.I.B.A., A.M.S.A.

Research Officer,
Tropical Diseases
Institute for Medical Research
50588 Kuala Lumpur
Malaysia

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