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IN VITRO CULTIVATION OF LEPROSY BACILLI *

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It has previously been reported (1, 2, 3) that a histochemically determined relationship exists between the presence of acid-muconolysaccharides and the presence of M. leprae and M. lepraemurium in the respective lesions they call forth. It was further reported that these bacilli have the necessary enzymes to utilize hyaluronic acid in their metabolism and that hyaluronic acid acts as a bacilli growth enhancing agent in mice inoculated with M. leprae It was further noted that pilot studies indicated that the use of hyaluronic acid significantly enhanced the probability of in vitro cultivation.

It is now noted that five strains of acid fast bacilli have been isolated, cultured and subcultured utilizing both liquid and agar plate forms of a hyaluronic acid based medium, designated LA-3. It has the following composition:

1.	0.006 M phosphate buffer (Na_2HPO_4-KH_2PO_4), pH 6.24	81 ml
2.	Glycerin	3 ml
3.	Hyaluronic acid (sodium salt, grade-III-S, from human umbilical cord, Sigma)	100 mg
4.	Bovine serum albumin (Cohn Fraction V, Sigma)	6 gm
5.	Fresh yeast extract (aseptic, Microbiological Associates, Inc., Bethesda, Maryland)	16 ml
6.	Potassium penicillin G (Eli Lilly and Co. Suspended in factory made sodium-citrate buffer 0.6 ml)	20,000 unit

Five cultures of acid-fast bacilli isolated from patients widely separate in time and Pacific/Asian geography, yield growths of the same characteristics within two to three weeks and are readily carried into subculture.

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* This work was not completed in time to meet the deadline for submission of manuscripts for this meeting. The chairman of the session was, however, kin enough to provide a strictly limited five minute period for its presentation at the conclusion of the first morning session.

The cultures are identified as M. leprae by specific immuno-fluorescence utilizing LL pooled serum from 18 patients different from the patients the bacilli are isolated from. The serum preparation follows on the work of Abe (4) in being fractionated to its IgG fraction, adsorbed against cardiolipin, and a battery of disparate mycobacteria, and coupled with FITC. Other mycobacteria do not fluoresce with this preparation but the isolates from the patients, and the primary and secondary cultures monitored with this technic, all yield positive reactions. The cultures are DOPA + but this is not presently acceptable as significant identification since several possibilities for false + reactions, not previously recognized, have been identified. Mouse foot pad inoculation is in process. The cultures do not grow on standard media for mycobacteria. Details are in press in the International Journal of Leprosy, Vol. 43, #3.

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