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Effect of Levamisole on Morphology, Bactericidal Activity, and Metabolism of Human Neutrophils *in Vitro* (39972)

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Introduction. The anthelmintic drug levamisole (L-2,3,5,6-tetrahydro-6-phenylimidazo-[2,1b]-thiazole-hydrochloride) has effects on phagocytes *in vitro*. We have demonstrated that monocytes incubated with levamisole have increased cytoplasmic spreading, chemotaxis, phagocytosis of bacteria, and activity of plasma membrane receptors for immunoglobulin (IgG) and complement (C3) (1). Levamisole was also shown to alter alveolar macrophage morphology and receptor activity (2). Recently levamisole has been found to increase random locomotion, stimulated random migration, and chemotaxis of neutrophils (3, 4). Levamisole is also the first agent to correct the defect in chemotaxis *in vitro* of neutrophils from patients with hyperimmunoglobulin E syndrome (4). Because of the increasing clinical importance of this agent (5), we decided to further investigate its influence on the morphology, bactericidal activity, oxygen consumption, glucose oxidation, nitroblue tetrazolium (NBT) reduction, and chemiluminescence of neutrophils from normal subjects and patients with chronic granulomatous disease (CGD).

Materials and methods. Investigation was approved by the Human Volunteers Committee of the University of Minnesota. Blood was obtained from normal subjects and two patients with well-documented CGD (6, 7). Techniques used to prepare neutrophils (8), bacteria (8), or serum (9) and to evaluate neutrophil morphology (1), bactericidal (8), and metabolic (6, 7, 10, 11) activity have all been reported in detail. Initially, the final concentration of levamisole ranged from 10 to 500 μg/ml. Concentrations greater than 200 μg/ml of levamisole in buffered Hanks' balanced salt solution produced an acid solution which was toxic to neutrophils. The majority of studies were done with 100 μg/ml. This is also the concentration which has produced optimal effects in prior investigations (3, 4).

Results. Morphology. Electron microscopy of control neutrophils and monocytes incubated with levamisole revealed large numbers of vacuoles (Fig. 1). Vacuoles were observed at final concentrations of levamisole which ranged from 10 to 100 μg/ml. Vacuoles also occurred in neutrophils and monocytes from patients with CGD.

Bactericidal activity. In contrast to findings for monocytes, levamisole-induced neutrophils showed no difference in phagocytosis of *S. aureus* when compared to controls. Bactericidal activity of control neutrophils preincubated in 10 to 100 μg/ml levamisole was not different from that of untreated neutrophils (Table I). Similar concentrations of levamisole did not improve the decreased bactericidal activity of neutrophils from patients with CGD.

Metabolism. Measurements of oxygen consumption or [1-^{14}C]glucose oxidation showed no difference between levamisole-treated neutrophils and controls (Tables II and III). Reduction of NBT on slides by endotoxin-stimulated neutrophils preincubated in levamisole (94 ± 5) was comparable to that by untreated neutrophils (96 ± 1.8; mean ± SE). Levamisole did not increase chemiluminescence of unstimulated or stimulated neutrophils (Fig. 2). Moreover, levamisole failed to potentiate the effects of phorbol myristate acetate (PMA) on neutrophil metabolism.

Discussion. Levamisole has been demonstrated to alter neutrophil chemotaxis presumably by maintaining intracellular cyclic GMP levels (3, 4). In the present study we found no significant effect of levamisole (10 to 100 μg/ml) preincubation on neutrophil bactericidal activity, oxygen consumption, glucose oxidation, NBT reduction, or chem-

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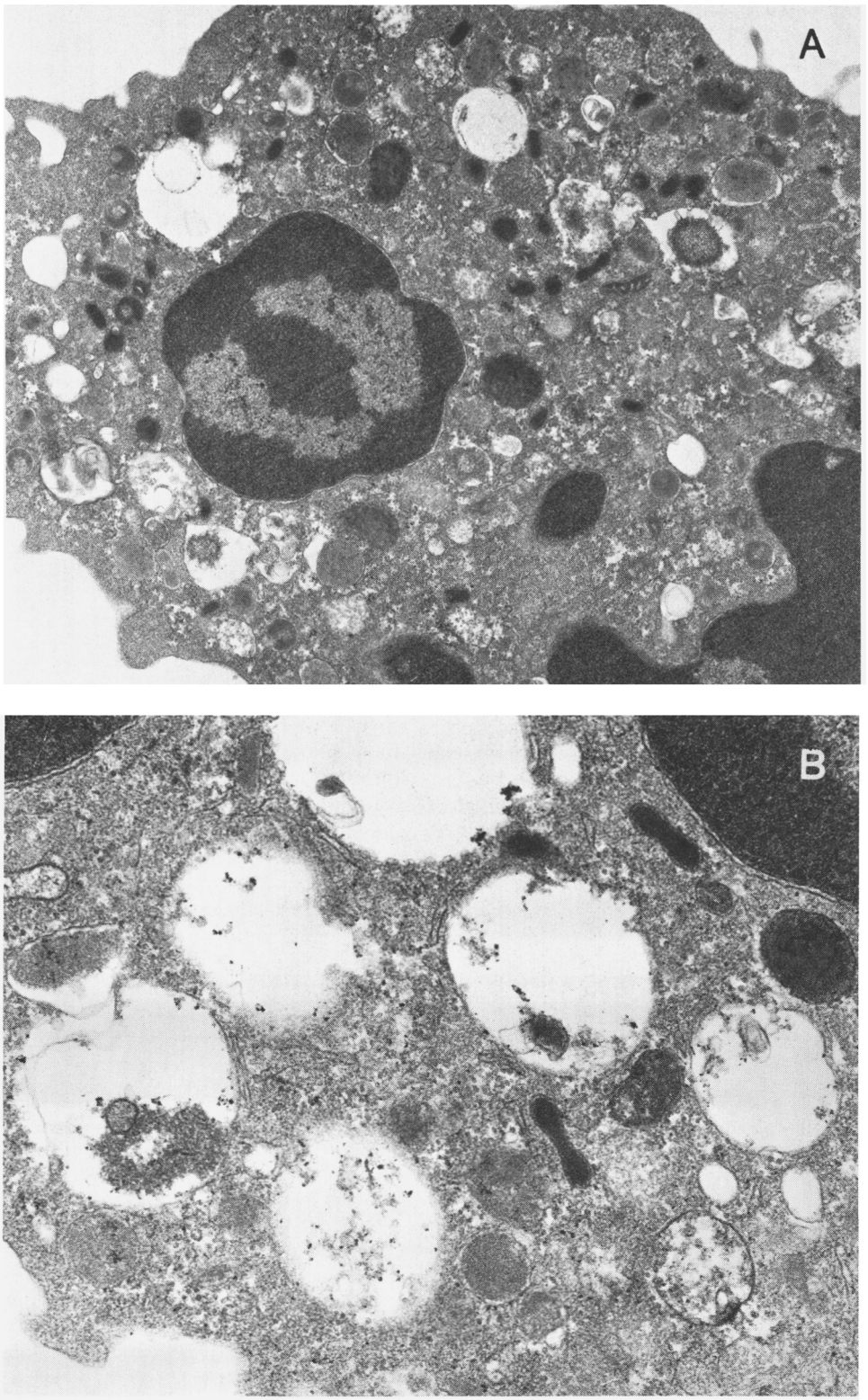

FIG. 1. (A) Neutrophil from normal donor incubated with levamisole (10 μg/ml) for 1 hr. Frequent vacuoles are present in the cytoplasm. × 22,500. (B) Higher magnification of neutrophil incubated with levamisole as in (A) showing cytoplasmic vacuoles. × 56,250.

TABLE I. BACTERICIDAL ACTIVITY OF PMN PREINCUBATED WITH LEVAMISOLE (100 μg/ml) FOR 1 hr.

Test conditions (bacteria to PMN ratios)	*S. aureus* killed in 60 min			
	Control PMN		CGD PMN	
	Without levamisole	With levamisole	Without levamisole	With levamisole
1.25:1	80 ± 8 (11)[a]	82 (3)	28 (2)	18 (2)
12.5:1	60 ± 14 (10)	67 (3)	7.3 (2)	5.3 (3)
50:1	37 ± 14 (6)	29 (3)	3.8 (2)	1.2 (2)

[a] Mean ± SD (*N*).

iluminescence. Neutrophils from CGD patients were also unaffected by levamisole. Morphologic studies did, however, demonstrate that cytoplasmic vacuolization occurs in normal or CGD neutrophils treated with levamisole. These findings are similar to those observed with human monocytes and rabbit alveolar macrophages (1, 2). The extent of vacuolization observed, however, was less than that which occurred with PMA (7). The present study affirms that the principal effects of levamisole on neutrophil function may be limited to its ability to improve random locomotion and chemotaxis (3, 4, 12, 13). However, more complete knowledge of the effects of this immunopotentiating agent is important, since levamisole is being utilized in clinical trials (4, 5).

Summary. In previous studies the anthelmintic agent, levamisole hydrochloride, has been shown to alter function of monocytes and macrophages as well as to increase random locomotion and chemotaxis of neutrophils. In the present investigation we have extended knowledge about this poten-

TABLE II. OXYGEN CONSUMPTION OF PMN PREINCUBATED WITH LEVAMISOLE (100 μg/ml) FOR 1 hr.

Test conditions	O_2 consumed/hr/4 × 10^6 PMN (μl) Control PMN	
	Without levamisole	With levamisole
Unstimulated	2.4 (3)[a]	2.9 (3)
Stimulated (bacteria to PMN ratios)		
1.25:1	6.3 (3)	6.2 (3)
12.5:1	7.5 (3)	8.2 (3)
50:1	21 (3)	19 (3)
PMA (0.1 μg/ml)	23 (3)	19 (3)

[a] Mean (*N*).

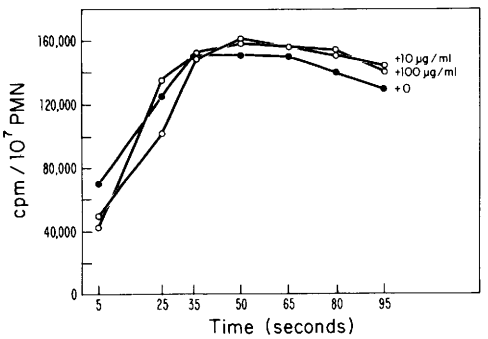

FIG. 2. Chemiluminescence of neutrophils preincubated with 10 and 100 μg/ml of levamisole (○) or HBSS (●). Neutrophils were tested in the presence of 4% serum and 50 heat-killed *S. aureus* per cell at 37°C.

TABLE III. GLUCOSE OXIDATION BY PMN PREINCUBATED WITH LEVAMISOLE (100 μg/ml) FOR 1 hr.

Test conditions	cpm [$^{14}CO_2$]/4 × 10^6 PMN/20-min incubation			
	Control PMN		CGD PMN	
	Without levamisole	with levamisole	Without levamisole	With levamisole
Unstimulated	240 ± 67 (8)[a]	74± 47 (8)	87 ± 21 (4)	123 ± 31 (4)
Stimulated (bacterial to PMN ratios)				
1.25:1	644 ± 229 (10)	535 ± 128 (8)	113 ± 20 (4)	140 ± 8 (4)
12.5:1	807 ± 46 (10)	804 ± 51 (8)	103 (2)	92 (2)
50:1	1911 ± 206 (10)	1433 ± 194 (10)	171 ± 48 (4)	225 ± 62 (4)
PMA	5095 ± 561 (10)	3840 ± 555 (8)	308 ± 37 (4)	199 ± 46 (4)

[a] Mean ± SE (*N*).

tially clinically useful agent. We found that in the optimal range of doses used in prior studies, levamisole produces vacuole formation but does not alter bactericidal activity, oxygen consumption, [1-^{14}C]glucose oxidation, nitroblue tetrazolium reduction, or chemiluminescence of neutrophils from normal subjects. We have also demonstrated that levamisole has no effect on neutrophils from patients with chronic granulomatous disease. The results suggest that the major effects of levamisole on neutrophil function relate to its ability to improve random locomotion and chemotaxis.

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1. Schmidt, M. E., and Douglas, S. D., Clin. Immunol. Immunopathol. **6,** 299 (1976).
2. Daughaday, C. C., Schmidt, M. E., and Douglas, S. D., Infect. Immun. **17,** 161 (1977).
3. Anderson, R., Glover, A., Koornhof, H. J., and Babson, A. R., J. Immunol. **117,** 428 (1976).
4. Wright, D. G., Kirkpatrick, C. H., and Gallin, J. I., J. Clin. Invest. **59,** 941 (1977).
5. Symeons, J., and Rosenthal, M., J. Res. **21,** 175 (1977).
6. Repine, J. E., White, J. G., Clawson, C. C., and Holmes, B. M., J. Clin. Invest. **54,** 83 (1974).
7. Repine, J. E., and Clawson, C. C., J. Lab. Clin. Med. **90,** 522 (1977).
8. Clawson, C. C., and Repine, J. E., J. Lab. Clin. Med., **88,** 316 (1976).
9. Repine, J. E., Clawson, C. C., and Friend, P. S. J. Clin. Invest. **59,** 802 (1977).
10. Beall, G. D., Repine, J. E., Hoidal, J. R., and Rasp, F. L., Infect. Immun. **17,** 117 (1977).
11. Ochs, H. D., and Igo, R. P., J. Pediatr. **83,** 77 (1973).
12. Versijp, G., vanZwet, T. L., and van Furth, R., Lancet **1,** 798 (1975).
13. DeCree, J., Cerhaegen, H., DeCork, W., Vanheule, R., Brugmans, J., and Schuermans, V., Lancet **2,** 294 (1974).

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