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A Proposed Nonpathogenic Biological Indicator for Thermal Inactivation of *Listeria monocytogenes*†

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Listeria innocua **M1 was developed as a thermal processing indicator organism for** ***L. monocytogenes*** **by selection of a rifampin- and streptomycin-resistant mutant.** z_D **values were 5.6 and 5.8°C, and** $D_{(68°C)}$ **values were 3.8 and 4.9 s for** ***L. monocytogenes*** **and** ***L. innocua*****, respectively, in skim milk. The advantages of easy selection, similar heat resistance, and nonpathogenicity make** ***L. innocua*** **M1 appropriate for challenge studies designed to evaluate process lethality with respect to** ***L. monocytogenes*****.**

Foodborne illness outbreaks due to *Listeria monocytogenes* have been associated with coleslaw (23), whole milk (12), and cheese (3, 19); other foods have been implicated in sporadic listeriosis (16, 25, 31). Thus, control of *L. monocytogenes* has public health and economic importance. The control of *L. monocytogenes* in foods is challenging because of its ability to grow at refrigeration temperatures (2, 8, 13, 17, 22, 30) and its relative heat resistance. *L. monocytogenes* is as heat resistant as or more heat resistant than many other vegetative bacteria of concern in food. D'Aoust et al. (6, 7) and Farber (11) determined that *L. monocytogenes* was as heat resistant as *Salmonella seftenberg* 775W, a strain recognized for its extreme thermal resistance in raw milk. Schoeni et al. (24) reported that a five-strain composite of *L. monocytogenes* was about four times more resistant than salmonellae in ground beef roast.

The fate of *L. monocytogenes* in thermal processes has been determined by monitoring its survival in food under simulated process conditions. While it would be advantageous to monitor *L. monocytogenes* under actual process conditions, it is generally undesirable to risk working with pathogens under such conditions. Often, biological indicators (BI) are used in challenge studies to evaluate processes because they are nonpathogenic and typically more resistant than the pathogen, providing the processor with a margin of safety (21). For example, the canning industry uses *Clostridium sporogenes* PA 3679 as a BI of process adequacy with respect to *Clostridium botulinum*.

Biological indicators are used to verify the adequacy of a thermal process in the presence of many known and unknown factors in a food system. To be useful, the BI must have thermal resistance equal to or greater than that of the target organism and be carefully calibrated under controlled laboratory conditions in the product in which it is going to be used. Use of a BI to evaluate pasteurization-type processes has been limited or has not occurred because a specific target organism of concern is not identified and/or because the nonsterile nature of the food has made selection and enumeration of the BI impossible or impractical.

Availability of a BI to use in lieu of *L. monocytogenes* could help to enhance food safety. Foegeding and Stanley (14) identified *L. innocua* ATCC 33091 as an appropriate BI for evaluation of process lethality with respect to *L. monocytogenes* in milk. They transformed the proposed BI with pGK12 (18) to confer resistance to erythromycin and chloramphenicol and facilitate enumeration. The transformed BI was 1.5 to 3.0 times more heat resistant than *L. monocytogenes* between 56 and 66°C. Foegeding and Stanley (14) proposed that this strain be used as a BI of thermal process lethality with respect to *L. monocytogenes* but cautioned that it would not be appropriate to use a genetically engineered organism which depends upon plasmid-encoded traits in a commercial processing plant.

The objective of this research was to improve upon the concept of a BI for *L. monocytogenes* by avoiding use of a plasmid through selection of spontaneous antibiotic-resistant mutants. The effectiveness of the *L. innocua* mutants as a BI for *L. monocytogenes* was evaluated by comparing its heat resistance with that of *L. monocytogenes*.

(A preliminary report of this work has been presented [T. M. Fairchild and P. M. Foegeding, Abstr. Annu. Meet. Am. Soc. Microbiol. 1991, P18, p. 43].)

Streptomycin-resistant (Str^r) and rifampin-resistant (Rif^r) mutants of *L. innocua* ATCC 33091 (American Type Culture Collection, Rockville, Md.) were generated by selective enrichment using a modification of the gradient plate technique of Szybalski (29). *L. innocua* was sequentially plated on Tryptose phosphate agar (TPA; Difco) containing increasing concentrations of streptomycin (S-6501; Sigma, St. Louis, Mo.) to a maximum of 2,000 mg of streptomycin per liter. The process was repeated by transferring Str^r mutants to TPA with increasing concentrations of rifampin (R-3501; Sigma) to a maximum of 500 mg/liter. Five mutant strains (designated M1 through M5) were selected for evaluation. Stock cultures of *L. innocua* ATCC 33091 and mutants were maintained in brain heart infusion (BHI) broth (Difco) plus 20% glycerol and stored at −20°C. Cultures for all experiments were transferred twice in BHI and incubated for 18 to 24 h at 35°C. Initially, several antibiotic concentrations and rifampin-streptomycin ratios were tested for their effects on enumeration of the mutants (data not shown), and this resulted in the choice of TPA with 50 mg of rifampin per liter and 250 mg of streptomycin per liter (TPA+) as the selective medium.

Colony size, generation time, verification of phenotype and nonpathogenicity, and recovery on TPA+ were used to select mutants for further evaluation. Generation times were

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determined by using BHI cultures (18 h) diluted in BHI to 10^6 CFU/ml and incubated at 35°C with agitation. Generation times were determined from the straight-line portions of plots of log optical density (660 nm) versus time. Hydrolysis of esculin, catalase activity, nitrate reduction, methyl red and Voges-Proskauer reactions, and acid production from maltose, mannitol, rhamnose, and xylose were determined by methods outlined in the 6th edition of the *FDA Bacteriological Analytical Manual* (20). Nonpathogenicity was documented by the procedure of Stelma et al. (27), as applied by Conner et al. (5), by using intraperitoneal injection into immunocompromised female white mice. Recovery of *L. innocua* mutants on TPA and TPA+ incubated for 7 days at 35°C was compared. A two-tailed *t* test ($\alpha = 0.05$) was used as the criterion for significant difference.

The efficacies of the selective media for the recovery of heat-injured cells were compared with that of a nonselective medium. Thermal treatments were applied to capillary tubes filled with 50 μl of a 35°C overnight culture in sterile skim milk as described by Foegeding and Leasor (13). Tubes were heated for 35 s at 66.4°C and then spread plated in triplicate on TPA, Trypticase soy agar (TSA), TSA with 0.6% yeast extract (TSAYE; Difco), TSA with 50 mg of rifampin per liter and 250 mg of streptomycin per liter (TSA+), TSA+ with 0.6% yeast extract (TSAYE+), and TSA with 4.0% NaCl (TSAS). Colonies were enumerated after incubation for 7 days at 35°C.

A two-tailed *t* test of independent *L. innocua* M1 and M2 cultures enumerated in BHI and on TPA and TPA+ indicated no significant difference ($\alpha = 0.05$) in enumeration between the two media. These two mutants maintained colony sizes similar to that of the parent strain. The generation times at 35°C for *L. innocua* ATCC 33091, M1, and M2 were 36, 43, and 59 min, respectively. Accordingly, *L. innocua* M1 was selected as the potential biological indicator organism for *L. monocytogenes*.

L. innocua M1 was verified to be nonpathogenic. *L. innocua* ATCC 33091 and M1 hydrolyzed esculin, produced acid from rhamnose and maltose but not mannitol or xylose, and were catalase, methyl red, and Voges-Proskauer positive.

The thermal resistance of *L. innocua* M1 was compared with that of *L. monocytogenes* F5069 (obtained from C. W. Donnelly, University of Vermont) in raw and sterilized skim milk by the method of Stern and Proctor (28) as modified by Fairchild (10). Thermal resistance studies were conducted in a split-plot design. The dependent variable, the *D* value, was determined for four *Listeria*-treatment combinations (*L. monocytogenes* in sterile milk, *L. innocua* M1 in sterile milk, *L. innocua* M1 in sterile milk recovered on selective medium, and *L. innocua* M1 in raw milk recovered on selective medium) at five temperatures (approximately 62, 64, 66, 68, and 70°C). Temperature was considered the whole plot, and *Listeria*-treatment combinations were considered the subplot. The experiment was replicated three times. Capillary tubes filled with 50 μl of an overnight culture in sterile skim milk or a 1:10 dilution of this culture in raw milk were exposed to the test temperature. A thermocouple sealed in a capillary tube served to record the time-temperature profile of each treatment. After exposure to the selected time-temperature treatment, capillary tubes were aseptically crushed and diluted in 0.1% peptone (Difco) water and spread plated. *D* values for each trial were determined as the negative slope^{-1} of the best-fit line of the survivor curve. The z_D values were determined as the negative slope^{-1} of the best-fit line of the decimal reduction-time curve. Log *D* values from the thermal resistance studies were subjected to analysis of variance in a general linear model program (SAS Institute, Cary, N.C.). Hypothesis tests were constructed to detect significant differences due to replication, temperature, and *Listeria*-treatment combination.

TABLE 1. Recovery of heat-injured *L. innocua* M1 on selective and nonselective media

Treatment, recovery media	Mean recovery (log CFU/ml)[a]
No injury, all[b]	8.19
Heat injury[c]	
TPA	5.59*
TSA	6.16†
TSAYE	6.54†
TSA+	5.73*
TSAYE+	5.58*
TSAS	2.89

[a] Treatments with identical symbols are not significantly different (P = 0.05).
[b] The noninjured population was not heated and was recovered equally on all of the media listed.
[c] Injury was inflicted by heating cells in skim milk for 30 s at 66°C.

Initial thermal resistance studies indicated that TPA+ caused a 1-order-of-magnitude reduction in detectable survivors at the longest heating times for each temperature compared with TPA (data not shown). This was attributed to the inability of TPA+ to recover heat-injured *L. innocua* M1 fully. Heat-injured *L. monocytogenes* cells do not recover on media containing 4 to 8% NaCl (1, 15, 26). Table 1 shows the relative recovery of heat-injured *L. innocua* M1 on TPA, TSA, TSAYE, TSA+, TSAYE+, and TSAS. The heat treatment resulted in approximately a 2-order-of-magnitude reduction in the population compared with unheated cells enumerated on TSA. Recovery on TSAS indicated that 99.95% of the cells were injured and recoverable on TSA. Recovery of 99.8% of the injured cells on TSA+ and TSAYE+ was not significantly different from that on TPA, suggesting that *D* values determined on TSA+, TSAYE+, and TPA would be similar.

TSA+ was tested for the ability to select against endogenous organisms in temperature-abused raw skim milk and found to select against approximately 10^3 to 10^4 CFU/ml (data not shown). Thus, TSA+ was considered an acceptable recovery medium.

Thermal resistance studies were conducted to (i) compare the resistance of *L. innocua* M1 with that of *L. monocytogenes* F5069, (ii) evaluate the effect of TSA+ on the *D* value, and (iii) evaluate the utility of the BI organism in raw milk. Average decimal reduction times for the different treatments are shown in Table 2. The thermal resistance of *L. innocua* M1 was compared with that of *L. monocytogenes* F5069 (Table 2, columns 2 and 3) because this strain of *L. monocytogenes* is recognized as one of the most heat-resistant strains in liquid whole egg (13), milk (8, 9), and phosphate buffer (14). Sterile skim milk was used as the heating menstruum to allow comparison on nonselective media. *L. innocua* M1 exhibited heat resistance equivalent to that of *L. monocytogenes* at each temperature; *D* values for *L. innocua* M1 and *L. monocytogenes* ranged from 38.5 s at 61.8°C to 1.4 s at 69.5°C. The z_D values of 5.5 and 5.6°C, for *L. monocytogenes* and *L. innocua* M1, respectively, were similar. Thermal resistance curves were subjected to a test of homogeneity of slopes by regressing the log *D* value on temperature in a general linear model program (SAS). There

TABLE 2. Decimal reduction times of *Listeria* spp. in sterile milk and raw milk recovered on TPA and TSA+

Heating temp (°C)[a]	Avg[b] (SE) decimal reduction time (s) of:			
	L. monocytogenes, sterile milk[c], TPA[d]	*L. innocua* M1, sterile milk, TPA	*L. innocua* M1, sterile milk, TSA+	*L. innocua* M1, raw milk, TSA+
61.8	38.5 (2.8)	38.4 (3.0)	38.1 (3.7)	49.5 (5.9)
63.7–63.8	15.3 (1.9)	20.7 (1.3)	18.1 (0.7)	18.3 (0.6)
65.6–65.8	7.6 (0.5)	8.4 (1.7)	9.4 (2.5)	7.6 (1.0)
67.3–67.7	3.5 (0.8)	5.1 (0.3)	4.6 (0.4)	5.4 (1.2)
69.3–69.7	1.4 (0.0)	1.4 (0.2)	1.3 (0.0)	1.3 (0.2)
z_D value (°C)	5.5	5.6	5.6	5.1

[a] Temperature range over triplicate trials.
[b] Averages of triplicate trials are reported.
[c] Heating menstruum.
[d] Recovery medium.

was no significant difference ($P = 0.05$) between the z_D values for *L. innocua* M1 and *L. monocytogenes* F5069.

D values for *L. innocua* M1 determined on TSA+ and TPA were essentially the same (Table 2, columns 3 and 4), ranging from 38.4 s at 61.8°C to 1.3 s at 69.5°C. The z_D value was 5.6°C in each case. Thus, the selective medium had no effect on the *D* value.

To evaluate the effectiveness of *L. innocua* M1 as a BI, it was tested in raw skim milk with an endogenous microbial population of 10^3 to 10^4 CFU/ml. Decimal reduction times for *L. innocua* M1 in sterile skim milk were compared with those in raw skim milk (Table 2, columns 4 and 5). *D* values at 61.8 to 69.7°C ranged from 38.1 to 1.3 s, respectively, for *L. innocua* M1 in sterile skim milk and from 49.5 to 1.3 s, respectively, in raw milk. The z_D values were 5.6 and 5.1°C for *L. innocua* M1 in sterile milk and raw milk, respectively. The test for homogeneity of slopes indicated no significant difference ($P = 0.05$) between the z_D values of *L. innocua* M1 heat treated in raw or skim milk. Populations of endogenous organisms recovered from uninoculated raw milk plated on TSA+ were <100 CFU/ml, indicating they did not interfere in determination of the *D* value of *L. innocua* M1.

The $D_{66°C}$ value for *L. monocytogenes* F5069 in skim milk was similar to that reported by Foegeding and Stanley (14); the z_D value of 5.5°C differed from their z_D value of 5.2°C (14). This may reflect the different temperature ranges studied. Shoulders were not observed on the survivor curves for *L. monocytogenes* as they were by Foegeding and Stanley (14). The $D_{68.9°C}$ values determined by Bradshaw et al. (4) for three strains of *L. monocytogenes* ranged from 2.8 to 4.0 s in raw milk and 6.2 to 9.1 s in sterile milk; our $D_{67.5°C}$ values for *L. monocytogenes* F5069 and *L. innocua* M1 in sterile milk were slightly lower (3.5 to 5.1 s). This may be attributed to strain and recovery medium differences. While Bradshaw et al. (4) reported larger *D* values in sterile milk than in raw milk, this study indicated there was no significant difference. However, Bradshaw et al. (4) estimated *D* values in sterile milk by a colony count procedure while *D* values in raw milk were estimated by a statistical procedure using a selective enrichment broth.

To be a useful BI, *L. innocua* must be at least as heat resistant as *L. monocytogenes. D* values for *L. innocua* M1 were not significantly different from those of *L. monocytogenes* F5069 in skim milk between 62 and 70°C. *L. innocua* M1 was recovered from raw milk by direct enumeration on a medium containing rifampin and streptomycin without interference when the background populations were up to 10^4 times larger than the *L. innocua* M1 population. Furthermore, the selective recovery medium did not affect the *D* value compared with recovery on the nonselective medium, TPA. The antibiotic resistance of *L. innocua* M1 is the result of a chromosomal mutation and is stable in nutrient broth without the selective pressure of antibiotics. Plasmid-encoded antibiotic resistance probably would not be stable without selective pressure. Genetically engineered microorganisms require approval for use in commercial applications. *L. innocua* M1 was generated from a natural mutation; therefore, it is not a genetically engineered microorganism. Mutation did not impart pathogenicity. Thus, *L. innocua* M1 is proposed as a BI for evaluation of pasteurization process lethality with respect to *L. monocytogenes*. Application of this BI will permit evaluation of a process in situ rather than in a laboratory simulation.

L. innocua M1 would be useful for estimation of the fate of *L. monocytogenes* in foods processed over the temperature range of 61 to 70°C and probably outside this range (14). The ease with which *L. innocua* M1 can be cultured and recovered makes it applicable to challenge studies requiring large inocula. It currently is being used in raw skim milk to evaluate the effects of continuous-flow processing on thermal inactivation. Since the antibiotic resistance of *L. innocua* M1 would differentiate it from environmental *L. innocua* strains, it should not interfere with the use of naturally occurring *L. innocua* strains as indicators of environmental contaminants.

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REFERENCES

1. **Bailey, J. S., D. L. Fletcher, and N. A. Cox.** 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. J. Food Prot. **53:**473–477.
2. **Berrang, M. E., R. E. Brackett, and L. R. Beuchat.** 1989. Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. **52:**702–705.
3. **Bille, J.** 1990. Epidemiology of human listeriosis in Europe, with special reference to the Swiss outbreak, p. 71–74. *In* A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier, Amsterdam.
4. **Bradshaw, J. G., J. T. Peeler, and R. M. Twedt.** 1991. Thermal resistance of *Listeria* spp. in milk. J. Food Prot. **54:**12–14.
5. **Conner, D. E., V. N. Scott, S. S. Sumner, and D. T. Bernard.** 1989. Pathogenicity of foodborne, environmental and clinical

isolates of *Listeria monocytogenes* in mice. J. Food Sci. **54:** 1553–1556.

6. **D'Aoust, J.-Y., D. B. Emmons, R. McKellar, G. E. Timbers, E. C. D. Todd, A. M. Sewell, and D. W. Warburton.** 1987. Thermal inactivation of *Salmonella* species in fluid milk. J. Food Prot. **50:**494–501.
7. **D'Aoust, J.-Y., C. E. Park, R. A. Szabo, E. C. D. Todd, D. B. Emmons, and R. McKellar.** 1988. Thermal inactivation of *Campylobacter* species, *Yersinia enterocolitica*, and hemorrhagic *Escherichia coli* O157:H7 in fluid milk. J. Dairy Sci. **71:**3230–3236.
8. **Donnelly, C. W., and E. H. Briggs.** 1986. Psychrotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. J. Food Prot. **49:**994–998.
9. **Donnelly, C. W., E. H. Briggs, and L. S. Donnelly.** 1987. Comparison of heat resistance of *Listeria monocytogenes* as determined by two methods. J. Food Prot. **50:**14–17.
10. **Fairchild, T. M.** 1992. A comparison of thermal death kinetics determined by continuous flow and batch systems using a biological indicator organism. M.S. thesis. North Carolina State University, Raleigh.
11. **Farber, J. M.** 1989. Thermal resistance of *Listeria monocytogenes* in foods. Int. J. Food Microbiol. **8:**285–291.
12. **Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312:**404–407.
13. **Foegeding, P. M., and S. B. Leasor.** 1990. Heat resistance and growth of *Listeria monocytogenes* in liquid whole egg. J. Food Prot. **53:**9–14.
14. **Foegeding, P. M., and N. W. Stanley.** 1991. *Listeria innocua* transformed with an antibiotic resistance plasmid as a thermal-resistance indicator for *Listeria monocytogenes*. J. Food Prot. **54:**519–523.
15. **Golden, D. A., L. R. Beuchat, and R. E. Brackett.** 1988. Inactivation and injury of *L. monocytogenes* as affected by heating and freezing. Food Microbiol. **5:**17–23.
16. **Ho, J. L., K. N. Shands, G. Friedland, P. Eckind, and D. W. Fraser.** 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. **146:**520–524.
17. **Ingham, S. C., J. M. Escude, and P. McCown.** 1990. Comparative growth rates of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked chicken loaf stored under air and two modified atmospheres. J. Food Prot. **53:**289–291.
18. **Kok, J., J. M. B. M. Van Der Vossen, and G. Venema.** 1984. Construction of plasmid cloning vectors for lactic streptococci which also replicate in *Bacillus subtilis* and *Escherichia coli*. Appl. Environ. Microbiol. **48:**726–731.
19. **Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome.** 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. **319:**823–828.
20. **Lovett, J.** 1987. *Listeria* isolation, p. 29.01–29.12. *In* FDA bacteriological analytical manual, 6th ed., suppl. Association of Official Analytical Chemists, Arlington, Va.
21. **Pflug, I. J.** 1990. Biological validation of preservation processes, p. 18.1–18.32. *In* I. J. Pflug (ed.), Microbiology and engineering of sterilization processes. Environmental Sterilization Laboratory, Minneapolis.
22. **Rosenow, E. M., and E. H. Marth.** 1987. Growth of *Listeria monocytogenes* in skim milk, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21, and 35°C. J. Food Prot. **50:**452–459.
23. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome.** 1983. Epidemic listeriosis: evidence for transmission by food. N. Engl. J. Med. **303:**203–206.
24. **Schoeni, J. L., K. Brunner, and M. P. Doyle.** 1991. Rates of thermal inactivation of *Listeria monocytogenes* in beef and fermented beaker sausage. J. Food Prot. **54:**334–337.
25. **Schwartz, B., C. V. Broome, G. R. Brown, A. W. Hightower, C. A. Ciesielskim, S. Gaventa, B. G. Gellin, L. Mascola, and the Listeriosis Study Group.** 1988. Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. Lancet **ii:**779–782.
26. **Smith, J. L., and D. L. Archer.** 1988. Heat induced injury in *Listeria monocytogenes*. J. Ind. Microbiol. **3:**105–110.
27. **Stelma, G. N., A. L. Reyes, J. T. Peeler, D. W. Francis, J. M. Hunt, P. L. Spauldin, C. H. Johnson, and J. Lovett.** 1987. Pathogenicity test for *Listeria monocytogenes* using immunocompromised mice. J. Clin. Microbiol. **25:**2085–2089.
28. **Stern, J. A., and B. E. Proctor.** 1954. A micro-method and apparatus for the multiple determination of rates of destruction of bacteria and bacterial spores subjected to heat. Food Technol. **8**(3)**:**139–143.
29. **Szybalski, W.** 1952. Microbial selection. I. Gradient plate technique for study of bacterial resistance. Science **116:**46–48.
30. **Walker, S. J., P. Archer, and J. G. Banks.** 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. **68:**157–162.
31. **Wenger, J. D., B. Swaminathan, P. S. Hayes, S. S. Green, M. Pratt, R. W. Pinner, A. Schuchat, and C. V. Broome.** 1990. *Listeria monocytogenes* contamination of turkey franks: evaluation of a production facility. J. Food Prot. **53:**1015–1019.