1. Ryan CA, Nickels MK, Hargrett-Bean NT, et al. Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. JAMA 1987;258:3269-74.
2. Gerding DN, Johnson S. Clostridium difficile. In: Blaser MJ, Smith PD, Ravdin JI, Greenburg HB, Guerrant RL, eds. Infections of the gastrointestinal tract. 2nd ed. Philadelphia: Lippincott Williams & Wilkins, 2002:763-84.
3. Neill MA, Opal SM, Heelan J, et al. Failure of ciprofloxacin to eradicate convalescent fecal excretion after acute salmonellosis: experience during an outbreak in health care workers. Ann Intern Med 1991;114:195-9.
4. Johnson S, Homann SR, Bettin KM, et al. Treatment of asymptomatic Clostridium difficile carriers (fecal excretors) with vancomycin or metronidazole: a randomized, placebo-controlled trial. Ann Intern Med 1992;117:297-302.
5. Gorbach SL. Probiotics in the third millennium. Dig Liver Dis 2002;34:Suppl 2:S2-S7.

Six Cities Revisited

TO THE EDITOR: The Six Cities Study by Dockery et al.[1] has captured renewed attention (Jan. 8 issue).[2,3] Claiming to identify an increased mortality rate in Steubenville, Ohio, as compared with the rate in Portage, Wisconsin, the authors posited that air pollution in Steubenville, an Ohio River Valley mining and steel-making city, was responsible for its increased death rate. That interpretation is not permissible, inasmuch as the socioeconomic and ethnic makeups of the populations of Portage and Steubenville are markedly different. Compounding that invidious comparison, the calculated difference in mortality rates is seen to be only a hair's breadth within the bounds of statistical significance. Indeed, as shown in Table 2 of the report,[1] there was no difference in mortality between women in Portage and women in Steubenville, who breathed the same air as men in that city.

Steinbrook[3] quotes David M. Michaels, formerly of the Clinton administration, who suggests that critics of clean-air studies are "hired guns working for dirty companies." Joel Schwartz, later associated with the clean-air group at Harvard, reportedly dismissed critics of clean-air studies as "industry thugs."[4] Is this the language of collegial scientific discourse?

John M. Bruner, M.D.

P.O. Box 617
Groton, MA 01450-0617
jmrb@post.harvard.edu

1. Dockery DW, Pope CA III, Xu X, et al. An association between air pollution and mortality in six U.S. cities. N Engl J Med 1993;329:1753-9.
2. Krewski D, Burnett RT, Goldberg MS, et al. Validation of the Harvard Six Cities Study of particulate air pollution and mortality. N Engl J Med 2004;350:198-9.
3. Steinbrook R. Peer review and federal regulations. N Engl J Med 2004;350:103-4.
4. Stix G. Where the bodies lie. Sci Am 1998;278:30-2.

Review of *Christian Science on Trial*

TO THE EDITOR: In our review of the book *Christian Science on Trial* (Dec. 25 issue),[1] we inadvertently misrepresented the views of physicians Larry Dossey and Andrew Weil. We made the following statement: "If the materialism of modern medicine tends to neglect the soul in order to cure the body, the spiritualized alternative medicine proposed by modern physicians such as Andrew Weil and Larry Dossey may, like the Scientists, neglect the body to cure the soul." We withdraw our comment and regret any inconvenience it may have caused Drs. Dossey and Weil.

Daniel E. Hall, M.D.
Harold G. Koenig, M.D.

Duke University Medical Center
Durham, NC 27710

1. Hall DE, Koenig HG. Review of: *Christian Science on Trial: Religious Healing in America*. N Engl J Med 2003;349:2574-5.

Boosting the Sensitivity of Real-Time Polymerase-Chain-Reaction Testing for SARS

TO THE EDITOR: In view of recent concern about the recurrence of severe acute respiratory syndrome (SARS) in Guangdong, China, we would like to highlight the tremendous importance of sensitivity in testing for the SARS-associated coronavirus (SARS-CoV). Because the initial symptoms of SARS

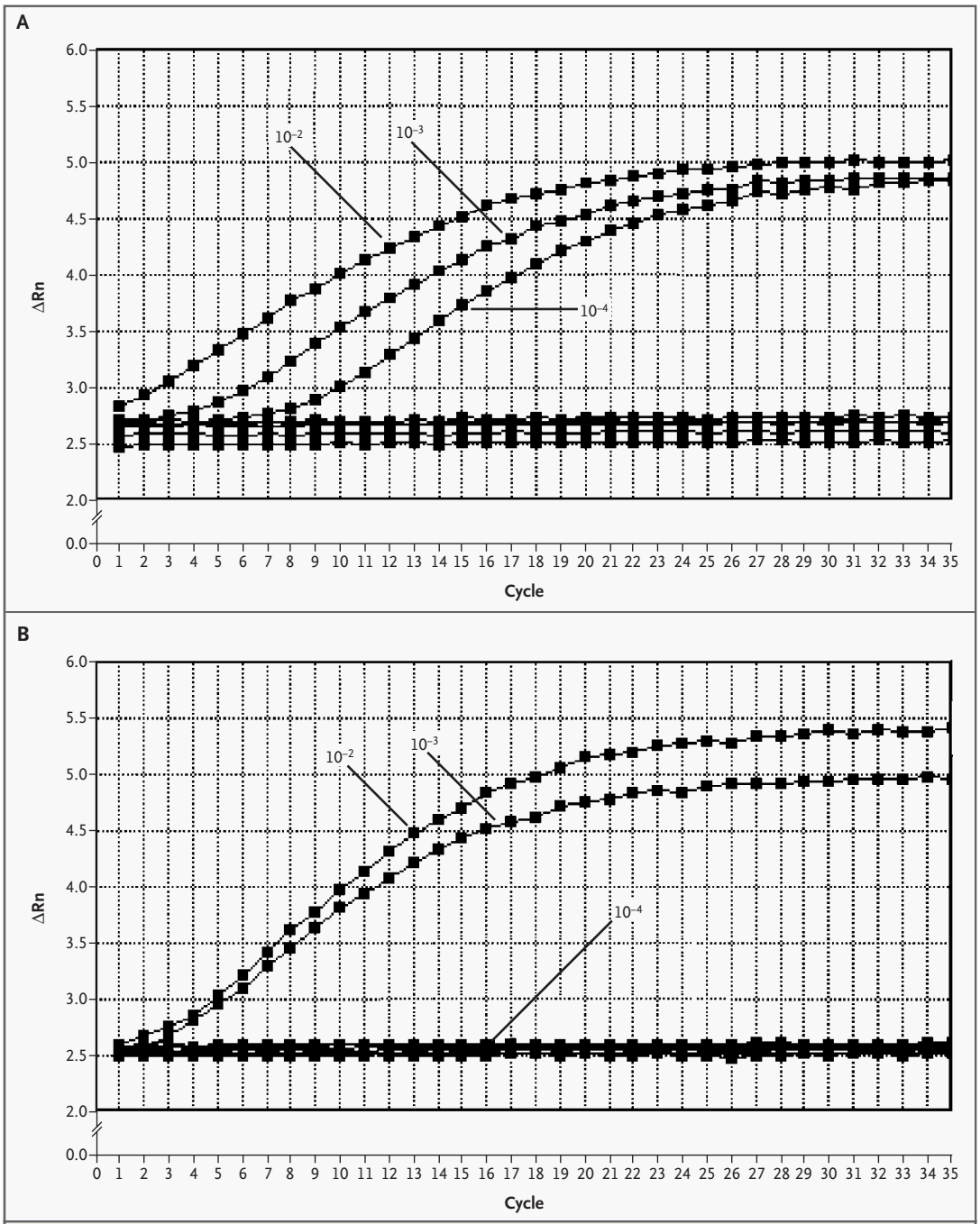

Figure 1. Results of Modified Enhanced Real-Time (ERT) Polymerase Chain Reaction (PCR) with One-Step Reverse-Transcriptase PCR (RT-PCR).

The sensitivity of the ERT technique is 10 times that of regular real-time PCR. Serial dilutions (10^{-2} to 10^{-4}) of a known amount of SARS-CoV nucleic acid (as previously described[1]) were prepared and used to compare the sensitivity of the ERT technique with one-step RT-PCR (Panel A) or separate RT and PCR steps (Panel B), followed by real-time PCR (TaqMan) under identical conditions. The change of normalized reporter signals (ΔRn) is calculated by normalizing the reporter signals with the fluorescent signals given by the passive reference. The region of the membrane-protein gene of the SARS-CoV was detected in this case. Positive signals are clearly displayed as prominent amplification curves on both real-time PCR plots, whereas negative signals remain unambiguously flat along the abscissas. The results clearly demonstrate that one-step RT-PCR boosts the sensitivity of the ERT technique by a factor of 10 as compared with the regular ERT technique.

are similar to those of other common respiratory diseases, making a specific diagnosis of SARS poses difficulties to medical professionals. Our enhanced real-time (ERT) polymerase-chain-reaction (PCR) method (first presented in June 2003 at a symposium on SARS[1]) has been designed for the detection of SARS-CoV with high sensitivity and easy-to-interpret results.[2] The power of the ERT technique has now been extensively explored with the development of ERT-based diagnostic tests for various infectious diseases, including avian influenza and foot-and-mouth disease.

Since the first report of ERT results for SARS,[2] the ERT technique has been modified to increase its sensitivity for the detection of SARS-CoV by at least 10 times (Fig. 1). This improved sensitivity has been achieved by combining the reverse-transcriptase (RT) and PCR steps into a single step (described in Supplementary Appendix 1, available with the full text of this letter at www.nejm.org). In addition, the procedural change makes the diagnostic procedure more convenient. These salient features of one-step RT-PCR have thus far been overlooked by other researchers in this field. Because the single RT-PCR step and the subsequent real-time PCR step require only 35 cycles, the detection of SARS-CoV by the modified ERT technique yields results quickly and with higher sensitivity than regular real-time PCR assays reported to date.

As noted by the World Health Organization with respect to the shortcomings commonly seen in available diagnostic tests for SARS,[3] it is important to unify a molecular test for SARS that can provide sensitive, reliable, and accurate results. Currently, many research groups claim that their methods are accurate, but the way in which they evaluate accuracy is not clearly described.[4] The usefulness of an accurate test that lacks sensitivity has yet to be determined. Unless a unified molecular test for SARS with high sensitivity and reliability is available, we may face the risk of false negative test results, which would allow infected patients to slip into the community and avoid control measures set up to isolate carriers.

Over a year after the start of the 2003 SARS outbreak, many people are still struggling to recover from the physiological and psychological scars inflicted at that time. Identifying potential SARS-CoV carriers by a method with high sensitivity and reliability and as early as possible is crucial to avoid a repetition of the 2003 outbreak.

Albert Cheung-Hoi Yu, Ph.D.

Peking University
Beijing 100083, China
achy@dnachip.com.hk

Lok-Ting Lau, Ph.D.
Yin-Wan Wendy Fung, Ph.D.

Hong Kong DNA Chips
Hong Kong, China

1. Lau LT, Wang CG, Yu CH. Enhanced detection of the coronavirus associated with severe acute respiratory syndrome (SARS). Presented at the ASEAN, China, Japan and ROK (10+3) High Level Symposium on SARS, Beijing, China, June 3–4, 2003. abstract.
2. Lau LT, Fung YW, Wong FP, et al. A real-time PCR for SARS-coronavirus incorporating target gene pre-amplification. Biochem Biophys Res Commun 2003;312:1290-6.
3. Update 3: Announcement of suspected SARS case in southern China: investigation of source of infection for confirmed case begins tomorrow: 8 January 2004. Geneva: World Health Organization, 2004. (Accessed March 19, 2004, at http://www.who.int/csr/don/2004_01_08/en/print.html.)
4. Grant PR, Garson JA, Tedder RS, Chan PKS, Tam JS, Sung JJY. Detection of SARS coronavirus in plasma by real-time RT-PCR. N Engl J Med 2003;349:2468-9.

Central Nervous System and Limb Anomalies in Case Reports of First-Trimester Statin Exposure

TO THE EDITOR: The cholesterol-lowering statin drugs are contraindicated in pregnancy[1]; therefore, few data exist regarding their safety in human gestation. We reviewed 178 cases of first-trimester statin exposure reported to the Food and Drug Administration (FDA) from 1987 through 2001 for patterns suggesting possible drug-related effects on embryogenesis. After the exclusion of cases involving first-trimester elective or spontaneous abortions (46 and 42 cases, respectively), pregnancy loss due to maternal illness (15), fetal genetic disorders (3), transient neonatal disorders (5), or loss to follow-up (15), 52 cases were considered evaluable (Table 1).

Among these cases, there were 20 reports of malformation, including 5 severe defects of the central nervous system (2 of which were holoprosencephaly) and 5 unilateral limb deficiencies; one patient had both of these malformations. The two simvastatin-exposed cases of limb deficiency were complex lower-limb anomalies including both long-