Macrophage Microbicidal Mechanism

TO THE EDITOR:

Jolly and Mahadevan have attempted to study the role of reactive oxygen intermediates in the intracellular killing of *Mycobacterium leprae* in human macrophages ([3]). The authors have reported 89 nmol of H_2O_2 and 0.3 nmol of superoxide (O_2^-) in the macrophages of lepromatous leprosy patients.

During phagocytosis, macrophages produce substantial quantities of O_2^- and H_2O_2 as shown by the following reactions: Superoxide is formed by the one electron reduction of oxygen: $2O_2 + NADPH \rightarrow O_2^- + NADP^+ + H^+$. Superoxide is converted to H_2O_2 by the reaction $2O_2 + 2H^+ \rightarrow O_2^- + H_2O_2$. This reaction is catalyzed by the enzyme superoxide dismutase.

Considering the fact that all of the oxygen taken up during the respiratory burst is converted to O_2^-, and that 80% of this O_2^- is converted to H_2O_2 by dismutation ([1]), it is difficult to understand from the paper ([3]) how 89 nmol H_2O_2 could be accounted for when only 0.3 nmol O_2^- was produced (Table 1).

However, there is a report which claims a direct conversion of molecular oxygen to H_2O_2 ([2]), as shown by the reaction: $NADH + O_2 + H^+ \rightarrow H_2O_2 + NAD^+$. This has been reported in guinea-pig neutrophils *in vitro*. It has also been argued that the rather high Km (0.4 mM) for NADH observed *in vitro* for this enzyme militates against significant activity during phagocytosis ([2]).

Under these circumstances, more confirmation is needed regarding the role of superoxide and H_2O_2 in the killing of *M. leprae* by human macrophages.

—Raji Swamy, M.D., Ph.D.

Department of Immunology
Tuberculosis Research Centre
Spur Tank Road
Chetput, Madras 600031, India

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A Comparison of the Ziehl-Neelsen and Kinyoun Methods in Staining Smears from Leprosy Patients

TO THE EDITOR:

The cold staining method of Kinyoun ([3]) for acid-fast bacilli is widely used in tuberculosis bacteriology, and is recommended by the U.S.A. Centers for Disease Control (CDC) ([2]) and the Institut Pasteur, France ([1]). To our knowledge, this method was not evaluated in leprosy diagnosis. Because the Kinyoun method was applied in our mycobacteriology laboratory, we decided to evaluate it for leprosy diagnosis since this disease is highly endemic in the state of Amazonas, Brazil (estimated incidence and prevalence were 69/100,000 and 11/1000 inhabitants in 1987).

Smears from leprosy patients were prepared in the outpatient leprosy clinic of the Centro de Dermatologia Tropical e Venereologia "Alfredo da Matta," Manaus, Brazil. One smear from each site was stained in the outpatient clinic laboratory using the Ziehl-Neelsen method as recommended by Leiker and McDougall ([4]). The method of Kinyoun was applied as recommended by the CDC and Institut Pasteur ([1, 2]), except that the destaining solution was 1% instead of 3% hydrochloric acid in ethanol. The smears stained by the Ziehl-Neelsen method were read and classified by an experienced microscopist at the outpatient clinic.

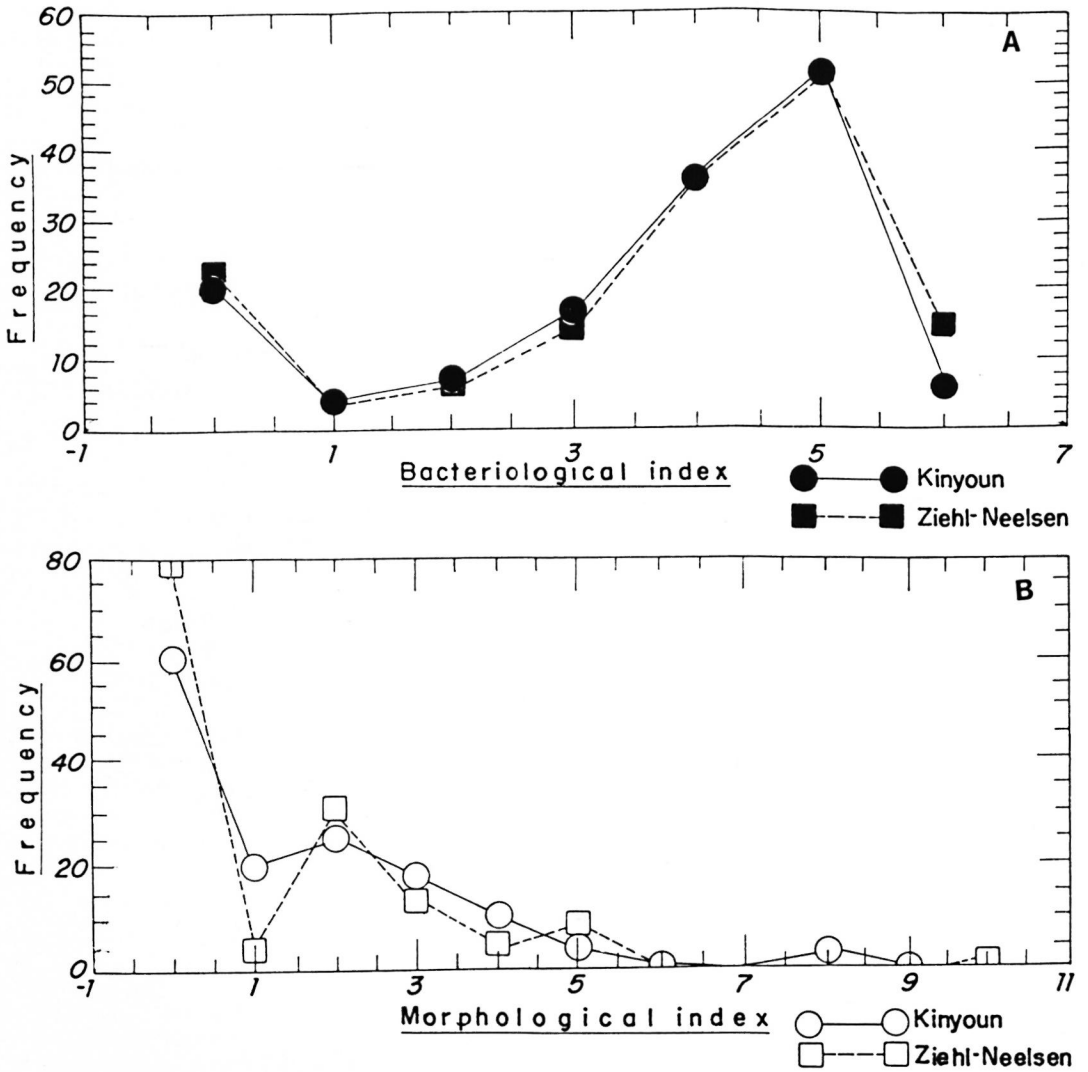

FIG. 1. Frequency polygon showing the frequencies of (**A**) the bacterial index (BI) and (**B**) the morphological index (MI) results usings Ziehl-Neelsen (■ and □) and Kinyoun (● and ○) staining procedures. BI significance level 0.488 for $\alpha = 0.05$; MI significance level 0.403 for $\alpha = 0.05$. Consequently, do not reject the null hypothesis that there are no differences between the two methods (N = 145).

Duplicate smears were stained at the Instituto Nacional de Pesquisas da Amazonia (INPA), and were read and classified at INPA. The readings and classifications of the smears were done blindly and independently in the participating laboratories. The results were checked only after complete data were collected.

The two-sample analysis results are shown in Figures 1 and 2. Figure 1 depicts the frequency polygon of the bacterial index (BI) and morphological index (MI) for all the smears (N = 145). The statistics indicated that there were no significant differences between the two staining procedures. Figure 2 shows the correlation analysis of the paired data on the same smears (N = 145). The correlation coefficients were 0.9 and 0.8, respectively, for the BI and MI, also indicating that there were no differences between the two methods.

From the above data, we conclude that the cold method of Kinyoun for acid-fast microscopy is satisfactory for leprosy bacteriology provided that the concentration of the acid in the destaining solution is reduced

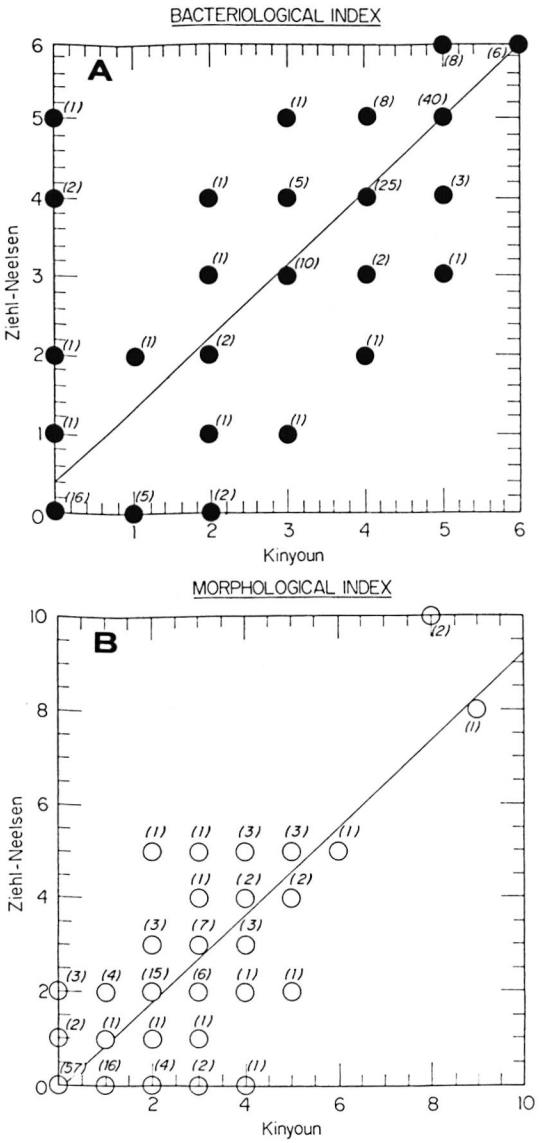

FIG. 2. Regression analysis of paired data for all smears (N = 145). Numbers in parentheses represent how many times each result was equal for the two methods. **(A)** BI correlation coefficient = 0.88; **(B)** MI correlation coefficient = 0.87.

to 1%. We have also observed that smears stained with the Kinyoun method keep better in storage, which might be of interest in preparing teaching materials and in quality control programs. One possible disadvantage of the Kinyoun method over the Ziehl-Neelsen method is that it uses a higher concentration of fuchsin, which may slightly increase the cost of microscopy.

—F. C. O. Fandinho, Biologist

Investigator
Laboratory of Mycobacteriology
Instituto Nacional de
Pesquisas da Amazônia (INPA)
Caixa Postal 478
69.011 Manaus (AM), Brasil

—A. T. Orsi-Souza, M.D.

Chief
Laboratory of Microbiology
Centro de Dermatologia
Tropical Alfredo da Matta

—J. I. Salem, M.D., Ph.D.

Chief
Laboratory of Mycobacteriology
National Research Institute
of the Amazônia (INPA)

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