Comparison of Heaf and Mantoux Tests in the Cali Area of Colombia

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IN DECEMBER 1959, the Arden House Conference on Tuberculosis emphasized the need for a simple and accurate tuberculin test that could be applied and read by nonprofessional health personnel (*1*). This need is greatest in developing countries where tuberculosis is often a major public health problem and professional health personnel are in short supply. In Colombia, although the exact magnitude of the tuberculosis problem is still unknown, substantial evidence indicates that the disease is a major cause of disability and death.

In 1951, Dr. Frederick Heaf (*2*) published his technique for a multiple-puncture tuberculin test that seemed to have several advantages over the routine Mantoux procedure: not only did it prove relatively consistent, it was also cheap, it could be administered rapidly, and results were easy to read. The Heaf test was subsequently tried out in Great Britain and the Commonwealth with encouraging results. This method is not widely used in the United States, however, despite the publications of Robins and Daly (*3*), Andersen and Smith (*4*), Coulter and Martin (*5*), and Hsu (*6*).

In February 1961, during a morbidity survey in the rural town of Candelaria, Valle, Colombia, we carried out a series of Heaf tests on a random sample of the population that included all ages. The tests were included in the survey at the suggestion of Dr. Robert M. Lennox, then visiting professor of pediatrics at the Universidad del Valle and now professor of child health, Tulane University School of Medicine. Shortly thereafter, we tested a small group of school children in Candelaria with both Heaf and Mantoux techniques. The results encouraged us to carry out a more extensive field trial of the two methods in 1962 and 1963. The primary objective of this study was to compare Heaf and Mantoux tests in individual subjects. For our purposes, diagnostic implications of the test results were a side issue.

Subjects for the field trial, in addition to the school children of Candelaria, were from the cities of Cali, Yumbo, and Palmira. Cali (population about 700,000) is located at an altitude of 3,300 feet in the Cauca Valley, between the Central and Western Cordilleras; Yumbo (population 11,500) and Palmira (population 150,000) are both within 15 miles of Cali and at the same elevation. Candelaria is a nearby rural town (population 22,000) at the same elevation. The subjects included adult and pediatric patients at a university hospital, a general hospital, three tuberculosis sanatoriums, and also medical and nursing students, interns, residents, and staff nurses of the university hospital.

Materials and Methods

Tuberculin for the Heaf test was a 50 percent glycerine solution of purified protein derivative (PPD), prepared by the Connaught Medical Research Laboratories, Toronto, Canada,

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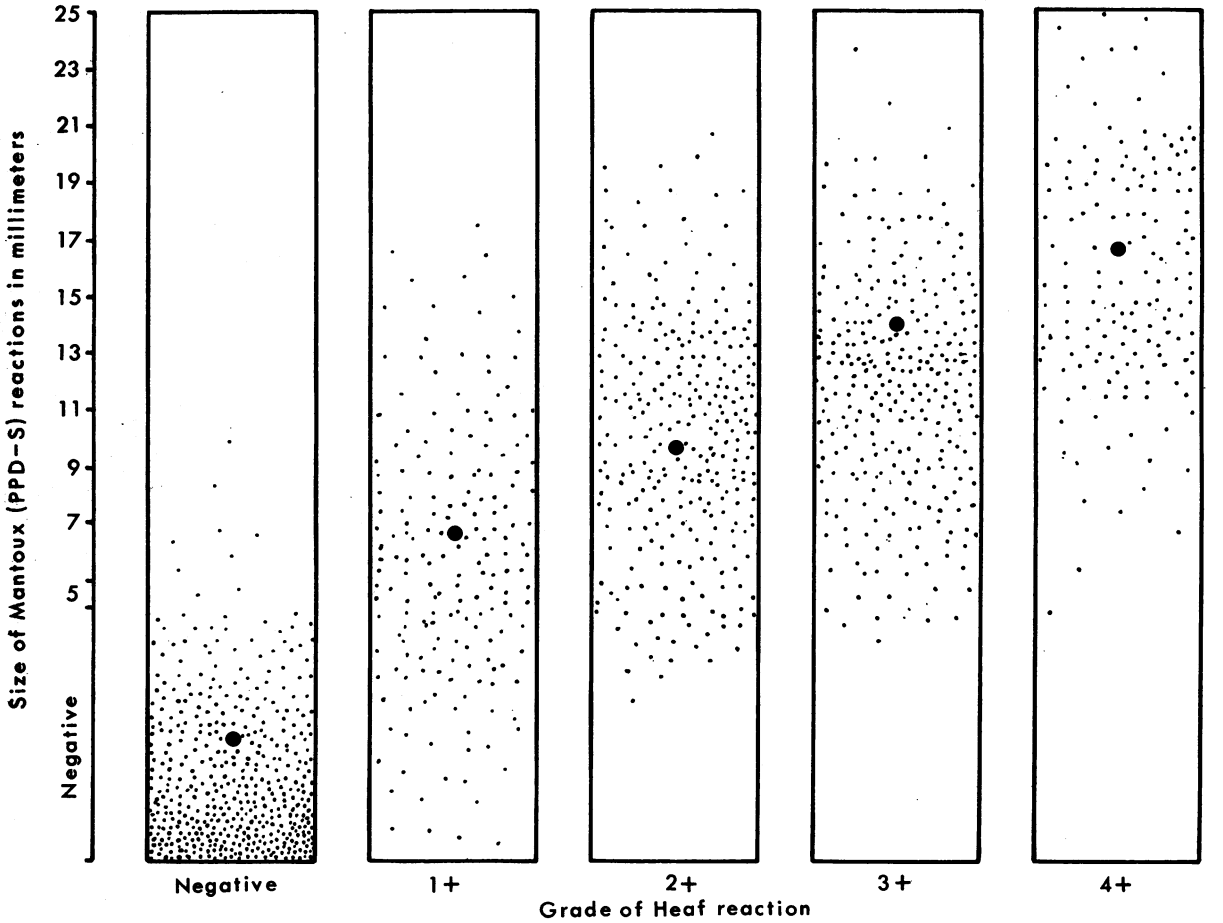
with a concentration of 2 mg. of U.S. standard tuberculin PPD in 1.0 ml. We cleaned the forearm of the subject with acetone. A drop of undiluted Heaf PPD was transferred to the skin of the upper left forearm by means of a toothpick, or preferably a bacteriological loop, and spread over an area roughly 8 mm. in diameter. We made the intracutaneous injection with a Sterneedle gun manufactured by the Panray Corp., Englewood, N.J., using a freshly sterilized cartridge for each patient. The gun with cartridge in place was pressed gently against the skin in the middle of the area where the PPD had been spread. We tensed the skin at the injection site slightly by gripping the under portion of the forearm.

For the Mantoux test we used an intermediate PPD–S prepared by Parke Davis & Co. during the 1962 trials, but in 1963 we shifted to a PPD–S supplied by the Public Health Service. The Service also provided a Battey antigen (PPD–B) that we applied simultaneously with the other two antigens during the 1963 trials. The Parke Davis PPD–S was supplied as tablets which were dissolved in 1 ml. of diluent immediately before use. The PPD–S and PPD–B had been standardized and arrived in liquid form ready for use without further dilution; the vials were stored in a refrigerator at 3–5° C. before testing. For the Mantoux tests we started with new syringes which thereafter were used only with one kind of antigen, and we

Figure 1. Distribution of different sizes in millimeters of Mantoux tests within different grades of Heaf reaction in 1,315 persons of varying age, sex, and tuberculin reactivity [1]



[1] The five large dots indicate the mean of Mantoux reactions (induration) in millimeters for each grade of Heaf reaction.

used sterilized needles of either 25 or 27 gauge, injecting 0.1 ml. of the PPD solution intradermally. This amount was equivalent to 5 TU (0.0001 mg.).

In general, each member of the survey team applied only one test at one predetermined location on the forearm. To avoid confusion we consistently applied the Heaf test to the upper left forearm, PPD–S on the upper right, and PPD–B on the lower right. Many of the subjects received histoplasmin on the lower left forearm (*7*).

Two teams of at least two observers each independently read the skin tests at 48 and 72 hours. In the event of disagreement between the two groups, consensus was reached by joint consultation. Results of the Mantoux tests were recorded in millimeters of induration without regard to the surrounding area of erythema. Heaf test results were noted in accordance with the following classification, modified slightly from Heaf's original criteria (*2*, *8*):

Negative

Absence of reaction at sites of puncture, simple scarring only, or slight erythema without induration.

Positive

1+: Discrete palpable induration, with erythema, of at least four puncture points. (Induration at fewer than four points was classified as negative.)

2+: Coalescence of the indurated points to form an edematous ring.

3+: Induration more intensive than in a 2+ reaction, involving the center of the ring as well in a so-called coin pattern.

4+: Induration extending well beyond the circumference of the ring or necrosis at any one of the puncture sites or adjacent areas or both.

In rare instances interpretation of the reaction was difficult. Where there was a typical 2+ indurated ring with one or more points of necrosis, by definition we considered this as 4+. Occasionally, however, there was disagreement as to whether or not the center of the ring was indurated. The decision on this point was left to the subjective interpretation of the observer. Although there might have been an argument as to whether a reaction of this sort was 2+ or 3+, the question was largely academic. More rarely, we encountered three indurated puncture points joined together. This result failed to meet the criteria of the 1+ reaction, but for the area that did react the induration was of a type classified as 2+. Such results were assumed to have been caused by faulty technique, such as holding the Sterneedle gun at an angle instead of perpendicular to the skin surface; they were therefore eliminated for purposes of the study.

Composition of study groups. During the 2 years of the study we deliberately selected groups that varied according to age, sex, and the probability of their having active tuberculous disease. We arbitrarily divided the 1,315 subjects into 4 groups. We further subdivided these groups by the calendar year in which they were tested since different sources of PPD–S antigens were used in 1962 and 1963. Sources of the 560 subjects tested in 1962 were as follows:

1962 test subjects	*Number*
Total	560
Group A:	
Primary school children, Candelaria	31
Pediatric patients, Hospital Universitario, Cali	64
Group B:	
Pediatric patients, tuberculosis sanatorium, Yumbo	53
Group C:	
Adult outpatients, Hospital Universitario, Cali	60
Adult ward patients, Hospital Universitario, Cali	115
Adult ward patients, Hospital San Juan de Dios, Cali	173
Group D:	
Adult ward patients, tuberculosis sanatorium, Cali	64

The small number of children from Candelaria are listed with the 1962 group for the sake of convenience, although they were actually tested in 1961.

Following are the sources of the 755 subjects tested in 1963:

1963 test subjects	*Number*
Total	755
Group A:	
Pediatric patients, Hospital Universitario, Cali	44
Group B:	
Pediatric patients, tuberculosis sanatorium, Yumbo	44
Group C:	
Adult ward patients, Hospital Universitario, Cali	127
Medical and nursing students, interns, residents, and staff nurses, Hospital Universitario, Cali	392
Group D:	
Adult ward patients, tuberculosis sanatorium, Cali	78
Adult ward patients, tuberculosis sanatorium, Palmira	70

In making these arbitrary groupings we realized that some of the outpatients and ward patients in both general hospitals in Cali might well have active tuberculosis; conversely, the results indicated that a few of the children hospitalized in the Yumbo tuberculosis sanatorium were probably not tuberculous. For the purpose of this paper, however, this discrepancy in grouping was unimportant since each subject served as his own control for comparison of the test antigens regardless of his sensibility status.

Results

Statistical considerations. Analysis of comparative results of the Heaf and Mantoux tests for 1962 and 1963 indicated no significant difference between the reactions to the two PPD–S antigens; hence we have combined the results for the 2 years. We have similarly divided the Mantoux test results, expressed in millimeters of induration, into five arbitrary groups corresponding roughly to negative and grades 1+, 2+, 3+, and 4+ of the Heaf classification. We felt justified in combining results of all tests, since comparative figures were the same

Figure 2. Comparison of results of simultaneous Mantoux tests with PPD–S and PPD–B antigens in 744 persons of varying age, sex, and tuberculin reactivity

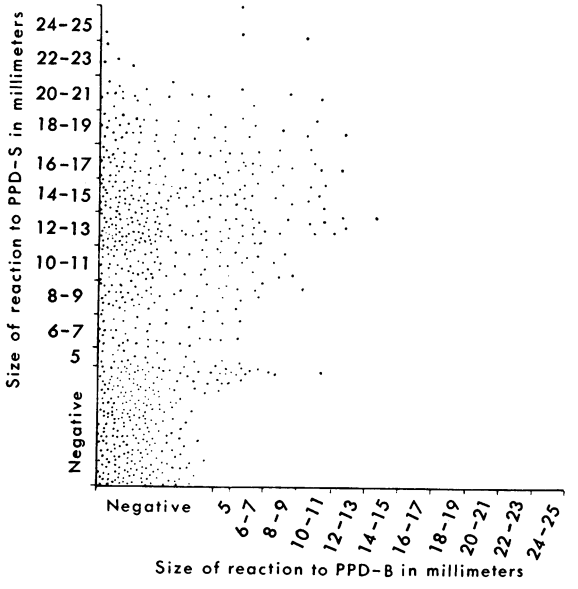
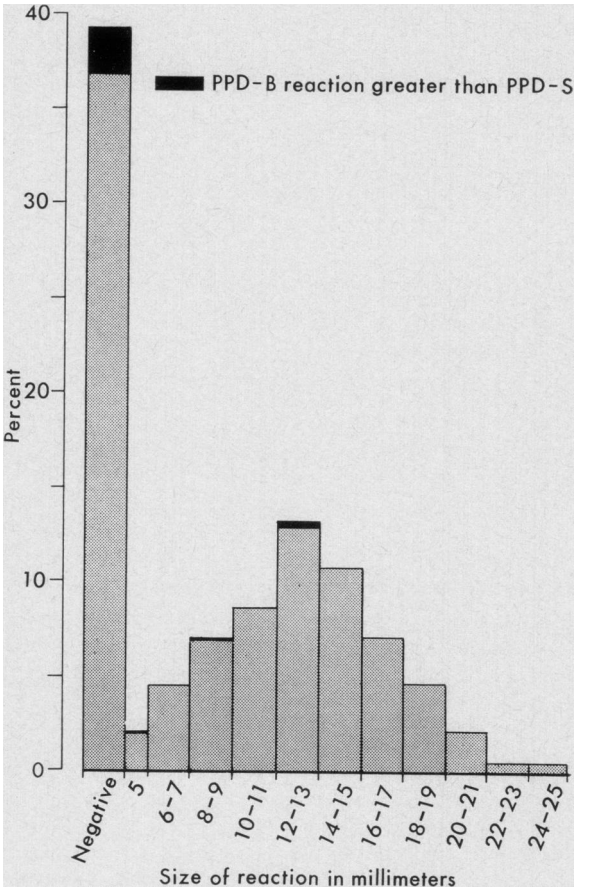


Figure 3. Frequency distribution of size of reactions to PPD–S in 744 persons, showing proportion of persons whose reaction to Battey (PPD–B) antigen was greater than to PPD–S



whether the individuals were adults or children, tuberculous or presumably nontuberculous, male or female. The correlation coefficients for the comparative results of Heaf and Mantoux tests by group for 1962 and 1963 were: group A, 0.94; group B, 0.74; group C, 0.84; and group D, 0.72.

Comparison of Heaf and PPD–S Mantoux tests. We have combined the results of all of the 1,315 Heaf and PPD–S Mantoux tests in a scatter diagram in which the Heaf results are plotted against the PPD–S Mantoux reactions in millimeters of induration (fig. 1). Also shown are the points on a regression curve indicating the mean value of millimeters of induration with the Mantoux test for each degree of Heaf reaction. Although the correlation

coefficient for the comparison of all of the Heaf and respective Mantoux tests was 0.80, the spread appears more marked as the reactions become more severe. The striking thing, however, is that with a criterion of Mantoux positivity commencing at either 5 or 8 millimeters of induration, a large number of 1+ positive Heaf test reactions are found in subjects whose simultaneous Mantoux tests were negative.

Mantoux tests with PPD–S and PPD–B (Battey antigen). The slightly greater sensitivity of the Heaf test as compared with the PPD–S Mantoux technique led us to wonder whether this was merely a matter of dosage or whether there was some additional protein in the Heaf product that was cross-reacting in response to infection with an unknown local strain of atypical mycobacteria. During 1963, therefore, we added skin tests with an antigen prepared from the Battey strain and provided by the Public Health Service.

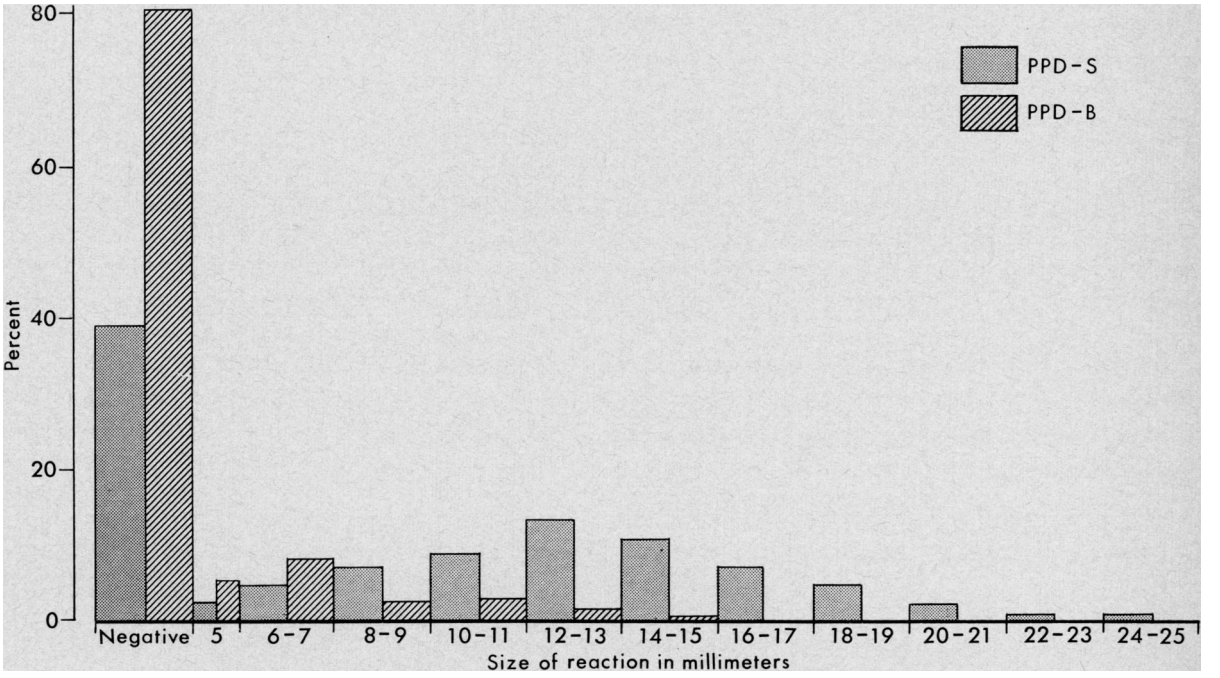
We were able to apply and read tests with this antigen in 744 of the total of 755 subjects. Comparative results of reactions to PPD–S and PPD–B in the same individuals are shown in the scatter diagram in figure 2. In only 23 subjects (3.0 percent), was the reaction to PPD–B greater than that to PPD–S. Of these, 19 had PPD–S reactions of less than 5 mm. (negative) and a maximum of 8 mm. with PPD–B.

These results are also shown in a bar diagram (fig. 3), which indicates the frequency distribution of size of reaction in millimeters to PPD–S and the relative proportion (black shading) of subjects whose reaction was greater to PPD–B than to PPD–S. Whereas roughly 40 percent of this group of 744 individuals were negative to PPD–S (less than 5 mm.), 80.7 percent were negative to PPD–B. This comparison of reactions is shown more clearly in figure 4, which indicates the relative proportions of subjects with negative reactions and those with positive reactions at various levels of intensity to both PPD–S and PPD–B. The maximum reaction to the Battey antigen was 15 mm. and occurred in one subject only.

Discussion

Others have already shown that, in trying to compare two different types of skin tests, one must consider the many factors, other than spe-

Figure 4. Frequency distributions of the sizes of reactions to PPD–S and PPD–B administered subcutaneously to 744 persons of varying age, sex, and tuberculin reactivity



cific sensitivity, that can influence a cutaneous reaction. Among these, perhaps the most difficult to control are human variables due to differences in age, race, and sex, and to the varying capacity of individuals to react. There are also variations according to the cutaneous site selected for the test. Meyer and his collaborators (*9*) found differences in the degree of response to identical doses of PPD injected simultaneously in the same area of a person's two forearms, and Rosenthal and Libby (*10*) demonstrated that in comparable groups the application of one or two antigens injected at the same time resulted in significantly different reactions as a result of what they called "competition of antigens." Better understood are the variations resulting from differences between batches of the same antigen, in age of the antigens, in concentration, and in their relative exposure to heat and light (*11*). Techniques of injection vary from technician to technician and with the same technician as, for example, do the amounts of antigen administered and the depths of injection below the surface of the skin. Interpretation of results of intradermal tests also varies. All these factors, some of which are more subject to control than others, may tend to neutralize each other. On the other hand, their effects may be cumulative.

Results of our application of the Heaf test in Colombia, like those of Heaf, of Robins and Daly, and of Andersen and Smith, indicate that the test is a good measure of sensitivity to tuberculin; it is also slightly more sensitive than the Mantoux test with PPD–S of intermediate strength since, although the techniques of application are different, the Heaf antigen is far stronger. With the exception of the weakly positive reactions, results of the two types of tests correlate well at various levels of reaction. Of the 1,315 subjects tested, we found only 10 instances of a negative Heaf test with a PPD–S reaction of 5 millimeters or more of induration, whereas there were 126 individuals with Heaf tests of 1+ or greater and a PPD–S reaction of less than 5 millimeters' induration.

We consider the Heaf technique for testing tuberculin sensitivity preferable to the Mantoux test. It is easier to apply, more acceptable to the subjects (particularly children), and its results except in rare instances are easier to read. It is cheaper and faster, particularly when applied to groups, and thus is especially well adapted for surveys. The usually clear distinction between a negative and a 1+ result that this test affords is an added advantage.

As for atypical strains of mycobacteria, we have thus far been able to carry out field tests with only one antigen, the Battey strain. The results indicate that this strain does not now play a significant role in the Cali environment. Our curve of the frequency distribution of sizes of reactions to PPD–S is unlike that obtained by Palmer and Edwards and co-workers (*12–14*) in geographic areas in which the Battey strain figures prominently as a sensitizing agent. Rather than try to carry out further field tests with antigens from other atypical strains isolated in the United States and elsewhere, it seems more appropriate for us to amplify the facilities for culture and isolation of tubercle bacilli from local sources. If atypical strains are important locally in the epidemiology of the disease, this fact should become apparent from the proportion and type of such strains as may be isolated. Tuberculin tests could then be performed with antigens prepared from these strains. As efforts to control tuberculosis expand, atypical strains may become increasingly important.

Summary

In the area of Cali, Colombia, 1,315 subjects of varying age, sex, and status with regard to active tuberculous infection were tested simultaneously with the multiple-puncture Heaf method and the Mantoux technique. Intermediate-strength standard PPD was used. The results indicated that the Heaf test is more sensitive than the Mantoux, particularly in the range where reactions are less marked.

Parallel studies with the Battey antigen in slightly more than 50 percent of the group indicated that this strain of atypical mycobacterium is, for the moment at least, unimportant in the epidemiology of tuberculosis in the local environment.

The Heaf method for tuberculin tests has a number of technical and administrative advantages over the Mantoux test, particularly in surveys and with large groups. It is cheaper, faster and easier to apply, more acceptable to subjects, and provides results easier to read.

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Advertising Campaign on Mental Retardation

Mental retardation has been selected by the Advertising Council as the subject of its 1965 national health campaign. Endorsed by President Lyndon B. Johnson, the campaign is conducted as a public service for the President's Committee on Mental Retardation. It is being financed jointly by the Department of Health, Education, and Welfare and the Joseph P. Kennedy, Jr., Foundation.

Advertisements, which will be appearing in publications throughout the year, are designed to acquaint readers with the problem of mental retardation, what can be done to prevent mental retardation, and what can be done to help the retarded become productive members of society. The advertisements point out that 85 percent of the retarded could help support themselves if they had proper training.

Most advertisements in the Advertising Council's series will list the following six steps that can be taken now to help prevent mental retardation and bring new hope to the 5½ million people whose minds are retarded:

1. If you expect a baby, stay under a doctor's or a hospital's care. Urge all expectant mothers to do so.

2. Visit local schools and urge them to provide special teachers and special classes to identify and help mentally retarded children early in their lives.

3. Urge your community to set up workshops to train retardates who are capable of employment.

4. Select jobs in your company that the mentally retarded can fill and hire them.

5. Accept the mentally retarded as American citizens. Give them a chance to live useful, dignified lives in your community.

6. Write for the free booklet to the President's Committee on Mental Retardation, Washington, D.C.