CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Criteria for Establishing *O*-Diphenoloxidase Activity

TO THE EDITOR:

Dr. Skinsnes (IJL **45** [1977] 187) states that his alleged *M. leprae* cultures oxidized DOPA, and questions the specificity of *o*-diphenoloxidase in *M. leprae*. Dr. Skinsnes did not carry out the reaction according to our procedure for testing enzymatic oxidation of DOPA by mycobacteria (W. F. Kirchheimer and K. Prabhakaran. IJL **36** [1968] 162-165). DOPA would undergo auto-oxidation in the presence of metal ions, on prolonged incubation, at higher temperatures and at alkaline pH. What Dr. Skinsnes observes is nonenzymatic oxidation of DOPA stimulated by metal ions in the culture medium (K. Prabhakaran. Lepr. India **48** [1976] 268-271). It may be mentioned that, not only DOPA, but many biochemicals used in enzyme chemistry are highly unstable substances. That is why they have to be stored in the cold, and the experimental conditions properly controlled to exclude nonenzymatic degradation of the substances. All substrates undergo slow degradation; what enzymes do is to accelerate the process by their catalytic action. What is important is to find out whether the bacteria by themselves can convert DOPA to quinone enzymatically. Our criteria for establishing *o*-diphenoloxidase activity are as follows.

1. We purify the bacteria used in our reactions to remove host tissue elements or culture media components that might stimulate auto-oxidation of the substrates. We carry out the reaction at pH 6.8 and at 37°C, for a duration of 30-120 minutes. When fresh DOPA solution is used, there is very little auto-oxidation of the substrate under these conditions, while there is rapid enzymatic oxidation. This was established by determining the ultraviolet spectrum of DOPA before and after incubation, and by comparing oxygen-uptake with and without added enzyme in the incubation mixture.

2. *M. leprae* preparations do not lose *o*-diphenoloxidase activity after treatment with alkali, proteolytic enzymes and acetone and ether, indicating that superficially adsorbed materials are not responsible for the reaction.

3. When *M. leprae* is heated, it loses the activity, proving the enzymic nature of the reaction, and excluding stimulation by inorganic ions.

4. The enzyme activity of *M. leprae* can be released from the bacterial particles as a copper-containing protein, by detergent-treatment.

5. The *o*-diphenoloxidase of the bacilli was demonstrated using a variety of technics: measuring oxygen-uptake manometrically and polarographically, determining the quinones produced spectrophotometrically, and assaying the labeled water formed with tritiated DOPA as substrate.

6. The enzyme is present not only in the bacilli separated from skin nodules, but also from spleen, liver, testes and lymph nodes which do not contain *o*-diphenoloxidase.

7. Mammalian *o*-diphenoloxidase is relatively specific for L-DOPA and has no activ-

ity towards epinephrine or norepinephrine. *M. leprae* oxidizes both L- and D-DOPA at the same rate, and acts on a variety of diphenols including epinephrine and norepinephrine.

8. *M. leprae* retains *o*-diphenoloxidase activity in the passage of the bacillus from the human to the animal (mouse as well as armadillo) host, indicating that it is a constitutive enzyme of the bacillus.

Precautions.

1. *M. leprae* suspensions used in enzyme assays have to be obtained from fresh tissues or tissues kept at 0°C or below, to avoid denaturation of *o*-diphenoloxidase. The entire separation procedure also has to be carried out in the cold. If the bacterial preparations have little activity to start with, both heated and unheated samples would give similar results. Denatured enzymes often retain a residual activity.

2. Many tissues contain inhibitors of *o*-diphenoloxidase. As such, testing the enzyme activity in crude homogenates of lepromatous tissues would yield no definitive results. Adequate amounts of purified organisms have to be used.

3. It is important to have heated samples as controls. We tested several cultivable mycobacteria as well as *M. lepraemurium* (separated from mouse spleen), and two unidentified mycobacteria (obtained from the liver and skin tissues of two other species of mammals). In presence of added DOPA, both heated and unheated suspensions of these bacteria gave similar results, proving that the organisms have no enzyme activity.

4. The mere demonstration of any enzyme in a host-derived organism is of little significance, especially if the enzyme is ubiquitously present in the host tissues as well. Acid-fast staining alone is a poor criterion for excluding host-tissue materials adsorbed on the bacilli. It has to be established that the enzyme is an inherent property of the organism itself.

Anyone who is interested is welcome to visit our laboratory. We would be willing to demonstrate that metabolically active preparations of *M. leprae* convert DOPA to quinone enzymatically and that this activity is distinct from mammalian tyrosinase.

—K. Prabhakaran, Ph.D.

U.S. Public Health Service Hospital
Carville, LA 70721