DOPA Oxidation and *M. leprae*

To The Editor:

Regarding the paper by Kato *et al* on oxidation of DOPA, I wish to make two specific comments: 1) The DOPA oxidation they report is not enzymatic; heated hyaluronic acid also would give similar results. 2) The *M. leprae* preparation they used probably had no enzymatic activity to start with.

Point 1. Excessive amounts of tissue extracts like hyaluronic acid containing metal ions would stimulate the auto-oxidation of DOPA. In the experiments reported by Kato *et al* they have used no controls using heat-inactivated preparations. In the studies we reported (Lepr. India **48:** 268-271) we used two types of hyaluronic acid, prepared from umbilical cord and from vitreous humor. We measured not only quinone formation but also oxygen uptake. Both types of hyaluronic acid showed no enzymatic oxidation of DOPA. Unheated preparations gave the same results as heated samples. It may be noted that 10 μg of an enzyme like mushroom tyrosinase gives an absorbance of 0.250-0.350 in five minutes; whereas hyaluronic acid is used in 1-4 mg concentrations. No purified enzyme has to be used at such high concentrations. What they measure

with hyaluronic acid is not enzymatic activity. When using other tissue extracts and bacterial preparations as well, Kato *et al* do not have heated controls. The readings have to be corrected for those given by the heated samples. It should be recognized that DOPA is an unstable amino acid. Without proper controls, the results obtained are not valid.

Point 2. If the *M. leprae* preparations are not made from fresh material or from tissues transported at 0°C or below, the bacilli would have no phenoloxidase activity. Kato *et al* do not state in what condition the tissues were transported from Dakar. If this was done (as on previous occasions) at ambient temperatures in acetic acid, the enzyme would be inactivated. They also do not mention the amount of bacilli used in their reactions. Kato *et al* state that we demonstrated DOPA oxidation by *M. leprae* in crude preparations. We have treated our bacterial preparations with NaOH, trypsin and also acetone and ether, without loss of enzyme activity. The activity was lost on heating, indicating that the phenoloxidase is an enzymatic process. We have also separated the enzyme from the bacterial particles, and shown it to be a copper-containing protein. Recently we tested two cultures of mycobacteria claimed to oxidize DOPA. When the organisms were thoroughly washed free of the culture media, the bacilli had no DOPA oxidase activity.

When DOPA undergoes auto-oxidation or enzymatic oxidation, there is a general increase in absorbance in the spectrum. Therefore, at whatever wavelength (of the spectrum) the absorbance is measured, there would be an increase. This is not enough to prove that a particular pigment is formed in the reaction. To prove that, the whole spectrum has to be measured and the absorbance peak characteristic of the pigment has to be demonstrated. Kato *et al* have not done so.

To conclude: Anyone with an elementary knowledge of enzyme chemistry would recall that all enzymes are proteins and that polysaccharides do not have enzymatic activity. No nonprotein enzymes exist. These facts were established by giants in the field like Sumner and Northrop early in the century. In fact, a Nobel Prize was awarded for crystallizing and proving that enzymes are proteins.

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