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Letter to the Editor

Enzyme Activity of Hyaluronic Acid

We reported earlier, in the *Leprosy Review* (Prabhakaran, 1974; Prabhakaran *et al.*, 1977) a rapid identification test for *Mycobacterium leprae,* based on the oxidation of D-DOPA by the bacillus. In the *International Journal of Leprosy* (1976) (which appeared in April, 1977), Kato *et al.* published a paper claiming that a polysaccharide, hyaluronic acid, contains DOPA oxidase activity. In an earlier report in *Leprosy in India* (Prabhakaran, 1976), we had shown that hyaluronic acid contains no phenoloxidase. Any one with a basic understanding of biochemistry would recall that all enzymes are proteins. Other substances like polysaccharides do not contain any enzyme activity. No nonprotein enzymes exist. These facts were established early in the century by giants in the field like Sumner and Northrop. In fact, a Nobel Prize was awarded for crystallizing and proving that enzymes are proteins. It is an anachronism to say now that a polysaccharide contains the phenoloxidase enzyme. I am making the following specific comments about this claim, because silence might be misconstrued.

(1) The DOPA oxidation they report is not enzymatic; heated hyaluronic acid also would give similar results. 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 (*Leprosy in India* **48**, 268-271) we used 2 types of hyaluronic acid, prepared from umbilical cord and from vitreaous 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. In the report of Kato *et al.* itself, it may be noted that 10 μg of an enzyme like mushroom tyrosinase gives an absorbance of 0.250-0.350 in 5 min; whereas hyaluronic acid is used in 1-4 mg concentrations. No purified preparation with enzyme activity 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.

(2) The *M. leprae* preparation they used probably had no enzymatic activity to start with. 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 pa[illegible] shown it to be a copper-containing protein. Recently we tested 2 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. Taking g at 2 wavelengths (as Kato *et al.* have done) is not enough to prove that a ar pigment is formed in the reaction. To prove that, the whole spectrum be measured and the absorbance peak characteristic of the pigment has to monstrated. Kato *et al.* have not done so.

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