effective. I imagine that rifampicin, unlike other chemotherapeutic agents, can be effective even against the slowly metabolizing mycobacteria such as leprosy bacilli and dormant tubercle bacilli in chronic lesions.

3. The longer the tubercle bacillus stays in the host tissue, the more prolonged incubation period they demand to appear as a visible colony after they are transferred onto the culture medium. I have a feeling that if tubercle bacilli have completely adapted themselves to the in vivo environment, it would be extremely difficult to retire them into the in vitro environment just like the case of leprosy bacilli. I predict that such a situation may actually exist in clinical tuberculosis.

Finally, I would like to repeat that a broader way of thinking and approach should be recommended for attempts to cultivate leprosy bacilli. Cultivators should be encouraged to do so as Dr. Kato insists, if they are prudent enough in drawing a conclusion from the results. In addition, I hope that Dr. Kato will be successful in finding more common characteristics between the M. scrofulaceae species grown in vitro and M. leprae separated from the infected tissue, even if his Janus-face theory is correct.

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To THE EDITOR:

I have read a copy of the editorial by Professor Kato which is to appear in the Journal (45: 175-182) with great interest. Although I am not a student of the leprosy bacillus, I would like to state as a mycobacteriologist my opinion on this subject.

One should not overlook that several investigators-similarly isolated slowly or rapidly growing, scotochromogenic mycobacteria from leprosy lesions. The idea stated by Kato is very interesting and should be a subject for serious consideration. In respect to this problem, K. Shimizu (Obihiro Veterinary College, Obihiro, Japan) and myself have a little experience. In the lesions of 16 cows, we found many acid-fast bacilli but failed to cultivate these organisms in most cases. Only twice we succeeded in obtaining acid-fast cultures. The first, was four colonies of M. gordonae-like organisms (Shimizu, K. and Tsukamura, M.; Jap. J. Microbiol. 18 [1974] 259-261); and second, were two colonies of rapidly growing, scotochromogenic mycobacteria which are an intermediate between M. vaccae and M. parafortitum (Shimizu, K. et al.; Jap. J. Microbiol. In press). In relation to this the finding recently published by Stanford and Rook (Int. J. Lepr. 44 [1976] 216-221) is interesting. They reported that M. vaccae and in vivo M. leprae share the same antigen structure.

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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 metals 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 \textit{M. leprae} preparations are not made from fresh material or from tissues transported at \(0\,^\circ\text{C}\) or below, the bacilli would have no phenoloxidase activity. Kato \textit{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 aeric acid, the enzyme would be inactivated. They also do not mention the amount of bacilli used in their reactions. Kato \textit{et al} state that we demonstrated DOPA oxidation by \textit{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 \textit{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.

- K. Prabhakaran

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\textit{M. leprae} Does Not Oxidize DOPA

To the Editor:

The claim that DOPA oxidation is a unique property of \textit{M. leprae} has been widely accepted without further critical investigations of the phenolase activity of host grown \textit{M. leprae}. The test has been erroneously used for the identification of human leprosy bacilli, until Skinsnes \textit{et al} (JIL 43 [1975] 193-209) and Kato \textit{et al} (JIL 44 [1975] 435-442) have shown that identification of \textit{M. leprae} is not related to phenolase activity. These investigations included all necessary controls, analysis of the observations were done by qualified investigators, and results are so indisputable that any further debate would just be repetitive.

Since DOPA is one of the most unstable compounds in nature, its chemical or auto-oxidation might be induced by just anything. It has been stated several times by Prabhakaran that DOPA is an unstable compound and is easily auto-oxidized. Any substance which is quite unstable and undergoes rapid auto-oxidation has never been used as a substrate for microorganisms, whereas DOPA has been claimed by Prabhakaran to be the only substrate, so far discovered, for \textit{M. leprae}. There is no evidence whatsoever that \textit{M. leprae} utilizes DOPA oxidation to produce energy for growth or multiplication.

The closing sentences of Prabhakaran's Letter to the Editor are quite unusual language on the pages of the \textit{Journal} and regrettable in academic debates. We therefore do not wish to comment on this and we consider the DOPA debate as closed.

- Laszlo Kato

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