Comments on Dr. Chatterjee's Correspondence

To The Editor:

In his very interesting correspondence (IJL **45** [1977] 294-296), Dr. Chatterjee called attention to some forgotten or neglected bacteriological observations in leprosy research.

Pioneers as well as several modern workers thought that cyanophil germs obtained in attempts to cultivate *M. leprae* might be related to the Hansen bacillus. Concepts of dimorphism or hypotheses regarding a life cycle were early considered. Thinking that this hypothesis afforded an explanation of some clinical features in leprosy we adopted it at the beginning of our work.

Patients with the tuberculoid form of leprosy frequently develop lesions wherever even a few acid-fast bacilli are observed. This small number of germs cannot explain the intensity of the lesions. It seemed rational to suppose that *M. leprae* may exist in another form than as classical bacilli, this form being not observable by routine investigations.

Assays were performed to verify the hypothesis of a life cycle for *M. leprae* and other mycobacteria. Results obtained were the following:

1. Prolonged incubation of bacillary suspensions obtained from lepromas have not enabled us to obtain direct cultures of acid-fast bacilli. In the more propitious cases, bacilli elongation or limited multiplication (X 10, X 20) were observed ([5]).

2. In numerous assays prolonged incubation (6-12 months) of bacillary suspensions from lepromas or of banal mycobacteria brought forth some peculiar elements (called form 2) possessing bacillary morphology and able to undergo sporulation ([1,2]).

These microorganisms were regarded by some workers with scepticism and criticism. However, assays in this way were carried on and new results were obtained.

First, it is possible, by using specific media, to induce with various mycobacteria, a rapid and abundant appearance of form 2. Delays of two to six weeks were sufficient whereas in our previous assays 6-12 months were necessary before observing few non-multiplying form 2 organisms (report in preparation).

Second, the successive stages by which sporulated form 2 undergo transformation to acid-fast bacilli were observed with various mycobacteria, in numerous assays ([8]). Furthermore, form 2 types of several mycobacteria reacted respectively to antibiotics, antileprous drugs, as well as to phosphate bromides in the same way as acid-fast bacilli of these mycobacterial species ([7]).

3. With other assays we obtained coccoids only evidenced as cyanophil forms by the Ziehl-Gram technic and further becoming blue-stainable with the Ziehl-Neelsen method.

Coccoid germs were obtained from bacillary suspensions derived from lepromas, crushed leprids, and lepromatous and tuber-

culoid sera filtered and unfiltered through Millipore membranes (0.45 or 0.22 μ). All these leprous materials were inoculated in unclassical media [3,6] (and unpublished data).

4. The obtaining of coccoid microforms after filtration led to examination of the problem of their origin.

Filterable forms of mycobacteria have been the object of controversies in the past. Their existence was finally not admitted because it was never possible to obtain acid-fast bacilli after inoculation of the filtrates in nutritive media. These repeated failures were due to the inability of classical media to allow the evolution of filterable forms into classical acid-fast bacilli.

With our special media used recently and with electron microscopic studies, the stages of development of these inframicroscopic elements (called form 3) were observed until they reached first visible coccoid forms and then became acid-fast organisms (manuscript in preparation).

5. A lot of 34 acid-fast strains issued from coccoid germs were obtained. Biochemical tests provided evidences for a new mycobacterial species ([4]).

Analysis of bacterial cell walls of this species showed that mycolic acid of mycobacterial type with 22-24 carbon chains were present (manuscript in preparation).

If we consider all these facts, it appears that a life cycle characterizes the Mycobacteriaceae. To get and then to observe the characteristic stages of this life cycle, suitable media must be used.

Existence of a life cycle is also well known among various organisms: some bacteria, fungi, protozoans, etc. Numerous workers have showed that environmental variations, i.e., temperature, light, and medium composition, may induce biochemical changes and correlated morphological evolution. These different stages of the biological cycle are the expression of specific portions of the genome.

Particularly, it has been demonstrated that bacterial sporulation is under genetic control and that specific environmental factors are responsible for the activation of sporulative genes.

In mycobacteria it may be considered that inexpressed genes in classical media may be activated under peculiar conditions and thus determine the development of the different stages of the life cycle.

In conclusion, it would be useful to remember that Dr. Wade ([9]) said in 1962: "leprologists in general seem not to have taken very seriously the idea that other than familiar form of *M. leprae* may exist."

The concept of a dimorphism or better of a life cycle in the Mycobacteriaceae, may be a new and beneficial approach for further investigations and leprosy understanding.

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