

## 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.*

## Comments on Dr. Kato's Editorial

Prior to submitting his editorial as presented in this issue, Dr. Kato sent copies of it to a number of correspondents seeking their comments. The replies received up to press time are here presented.—EDITOR

## TO THE EDITOR:

In his editorial appearing in the JOURNAL (45: 175-182) Kato proposed that host grown vs. *in vitro* grown *M. leprae* will show a "Janus-face."

One of the most important characteristics of microorganisms is undoubtedly the great versatility of their protein and other molecular and macromolecular biosynthetic mechanisms. It is believed that only about 15% to 20% of the bacterial genome is transcribed, i.e., expressed in a given situation. It has been demonstrated that a large number of genes coding for the synthesis of enzymes, of certain structural proteins and other cell components are under the control of regulatory genes, whose activity depends partly on the composition of the surrounding medium.

Thus, it becomes evident that a variation of the external environment of the cell, while providing new activators and inhibitors of regulatory genes, will bring about the expression of many hitherto unexpressed genes affecting metabolic pathways, morphologic and antigenic structures and many other physiological aspects.

Examples which illustrate the consequences of a variation of the external environment are numerous in the procaryotic world. To name a few, we have the necessary symbiotic relationship of the *Rhizobium* genus with many leguminous plants for nitrogen fixation to occur. The same microorganisms lose this characteristic when cultured in the test tubes. Sporulation in the *Bacillus* and *Clostridium* groups is marked by profound physiological changes in those bacteria. Transition from autotrophic to heterotrophic growth for many of the non-

obligate autotrophs is characterized by great metabolic changes. Bacteria of the *Sarcina* type as briefly mentioned in Dr. Kato's article, are another example of the changes which occur in microorganisms when set in different environments. Isolated from soil and cultivated firstly at pH 2 and then at pH 8, bacteria of the *Sarcina* type are unable to grow again at pH 2, even if the attempt is made quickly or slowly. In this example, it can be seen that even if the bacterium has all the genetic information to make a cell wall resistant to a high concentration of H<sup>+</sup> ions, a variation of the environment seems to have eliminated this property (Canale-Parola, E.: Bacteriol. Rev. 34 [1970] 82-97).

Thus it can be suggested that *M. leprae*, through its abundant and formerly unexpressed genetic information, as well as on account of the versatility of its biosynthetic apparatus, both properties being inherent characteristics of procaryotes, could without much difficulty grow on a synthetic medium, rather than exist as an "obligate intracellular parasite." The activators and inhibitors of regulatory genes differ considerably in the intracellular cycle from those in the culture medium. Consequently, it is to be expected that host grown and *in vitro* grown *M. leprae* will show quite different metabolic, biochemical, antigenic and pathogenic profiles, thus lending support to the proposed theory of the Janus-face of *M. leprae*.

—Pierre Beaulnes  
—Michel Bourque

Institut Armand-Frappier  
Université du Québec  
Laval-des-Rapides, P. Québec, Canada

TO THE EDITOR:

Dear Laszlo:

I deeply appreciate your 13 May suggestion that I might comment on your proposed editorial to the JOURNAL (45: 175-182). Accordingly, I offer two comments.

1. It would be helpful if the questions you raise were taken up with professional students of taxonomy. Larry Wayne might be a suitable person.

2. It seems to me that you have made an excellent case that *M. leprae* = *M. scrofulaceum*. I, therefore, have concluded that when *M. scrofulaceum* was cultivated from humans in the past, the isolations were from undiagnosed cases of leprosy.

As you say, it is not necessary to take such matters seriously.

With highest regards, I am

Yours sincerely,  
John H. Hanks, Ph.D.

Professor Emeritus of Pathobiology  
John Hopkins School of Hygiene and  
Public Health  
615 North Wolfe Street  
Baltimore, Maryland 21205

TO THE EDITOR:

Dr. Laszlo Kato asked me to make some comments on his manuscript entitled "The Janus-Face of *Mycobacterium leprae*" which will be published as an editorial in the INTERNATIONAL JOURNAL OF LEPROSY (45: 175-182).

As he wrote in the beginning, the objective of this editorial is not to defend the culture of the *M. scrofulacea* species isolated by his group and others as the etiologic agent of leprosy, but I understand that he is going to introduce an unconventional way of thinking as a means to expand the scope of approach towards the cultivation of *M. leprae* and to encourage the cultivators.

Nevertheless, it would not be denied that he attached a great meaning to the isolates belonging to the *M. scrofulacea* species from leprosy lesions, suspecting a possibility that they might be an *in vitro*-adapted form of *M. leprae*. This assumption was derived from his own laboratory experiences and also from his survey of the historical papers which appear closely related with his observations.

His basic idea is well stated by a metaphorical expression that *M. leprae* micro-organism has indeed a Janus-face; one is smiling at the macrophage and the other is grimacing at the culture media.

As far as this working hypothesis is concerned, I have no objections. The rationales on which he constructed this idea are stimulative and full of suggestions. I myself have been carrying an assumption that pathogenic mycobacteria growing *in vivo* may differ metabolically from the same strain growing *in vitro*, and that they can switch the metabolic pattern in response to the environmental condition into which they are placed. If this could be the case in general, it would follow that leprosy bacilli are particular mycobacteria which are very unskillful in this kind of adaptation.

Concerning the evolutionary and taxonomic relation between the *M. scrofulacea* species and *M. leprae* as speculated in the editorial, I do not hold the position to make any authoritative comment. I have no experience in cultivation of leprosy bacilli and *M. scrofulaceum*. Therefore, all I can do on this occasion is to introduce my own observations in experimental tuberculosis which might be related with the underlying concept of the editorial.

1. The inoculum consisting of the *in vivo* grown mycobacteria harvested directly from the infected mouse lungs can initiate multiplication soon after implantation into the mouse tissue, whereas the inoculum of the same strain grown *in vitro* requires a much longer lag period for that. This might be an indirect evidence for the concept that infecting mycobacteria adapt themselves to the *in vivo* environment and may differ metabolically from the corresponding *in vitro* strain.

2. In connection with Dr. Kato's observation that the *M. scrofulacea* species is sensitive to rifampicin and this drug also exerts therapeutic effect on leprosy, our following finding might be interesting. In the chronic stage of infection, tubercle bacilli are persisting in lesions, probably without showing any significant multiplication but keeping a very low level of metabolism required only for their self-maintenance. Such persisting bacilli, which are INH-sensitive at least *in vitro*, do not respond to the treatment with this drug. However, rifampicin can still be

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. scrofulacea* species grown *in vitro* and *M. leprae* separated from the infected tissue, even if his Janus-face theory is correct.

—Koomi Kanai, M.D.

Director  
The First Department of Bacteriology  
National Institute of Health  
Tokyo 141, Japan

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, was two colonies of rapidly growing, scotochromogenic mycobacteria which are an intermediate between *M. vaccae* and *M. parafortuitum* (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.

—Michio Tsukamura, M.D.

National Chubu Hospital  
Obu, Aichi, Japan 474