Exchange of Information so Important

Dr. M. Aoki, Director

First of all, it was a great pleasure for us to have received so many letters from people in various countries responding to the last issue of the newsletter from Kiyose. As the number of tuberculosis cases has decreased recently in developed countries, tuberculosis tends to be neglected in main public health care efforts. So the exchange of information is becoming more and more important for the promotion of tuberculosis control programme in various countries.

Seminar in Egypt

In February of this year I visited Egypt with Dr. Ishikawa to conduct a tuberculosis seminar with exparticipants and other doctors involved there. Actually, as many as 40 doctors and laboratory technicians had participated in the Institute’s training course over the past 27 years since 1963. This was the first tuberculosis seminar sponsored in Egypt by the JICA. We were delighted to welcome nearly 70% of the Egyptian exparticipants to directly discuss the tuberculosis control programme, and in addition I had a pleasant time with many good friends. JICA-sponsored tuberculosis seminars were also held in Bangladesh and Myanmar in 1987, in Thailand and Nepal in 1989, and in Tanzania in 1990. Unfortunately I missed out on the Tanzania seminar. We feel that this kind of meeting with exparticipants is very important and productive, and we expect to have other chances in the future.

New Move in the World

After returning from Egypt, I have been to the Netherlands to attend the First European Workshop/Course in TB Control in Low Prevalence Countries—the Last Fight against Tuberculosis until Elimination organized by the WHO and European Region of IUATLD. In the U.S., a report entitled “A Strategic Plan for the Elimination of Tuberculosis in the United States” was published in April 1989. So in this Workshop/Course the attitude of the Europeans was to adopt the U.S. Plan. There is a considerable difference between the TB situation faced in developing countries and in developed countries. If we do not make any efforts to change present TB control programmes, this gap will become much wider. I believe that now is the time to start improving and strongly promoting modern tuberculosis control programme in the developing countries.
Course Director Changes

Dr. Umemura retired on March 31 after working 5 years for the international training courses, taken over by Dr. Ishikawa assisted by Dr. Matsuda.

Dr. Norihiro Umemura

After 5 years in clinical service at a teaching hospital in Nagoya University, 25 years of service in tuberculosis control in a local government of the Aichi Prefectural Government, 5 years of medical cooperation in Tuberculosis Control in Western Nepal and the last 5 years of service to the Institute as a course coordinator, I have decided to retire, after already exceeding the Association’s normal retirement age. From now on I will be a free lance agent working against tuberculosis!

Since I have worked in tuberculosis control in developing countries such as Nepal and Myanmar for several years, I would very much like to contribute to tuberculosis control in developing countries in response to your requests. At first I plan to visit Bolivia and Peru after the IUATLD meeting in Boston this May. I would like to see the actual tuberculosis situation in these countries. Without knowledge of the actual tuberculosis situation, suitable solutions can hardly be found. In Bolivia, I am going to observe the anti-tuberculosis activities in the Oruro area where exparticipants are working. Silico-tuberculosis is prevalent due to the mining industry in this area.

An old Italian proverb says “Vedi Napoli, e poi mori!” which means “See Naples and then die.” I learned this saying during my visit to Rome, attending the “fight against tuberculosis” course conducted a long time ago, and I still clearly remember it now. I can not wait to visit every place where course exparticipants are serving. When you have difficulties in tuberculosis control and ask me to come, I will be very happy to come as my schedule permits and cooperate voluntarily with you. Without first seeing the prevailing tuberculosis condition, no one can say what you have to do to improve or solve the tuberculosis problem. So, do not hesitate to ask me about your problems in detail. I will try my best to fight against tuberculosis with you as long as I am active.

I can be reached through the Institute. I plan to enjoy a rewarding retirement life, cherishing warm memories of our courses and maintaining my zeal for tuberculosis control until tuberculosis is banished from the world. I am looking forward to meeting you right where you are working and renewing our friendship face to face!

Thank you and see you again! Gracie e arrivederci! Arigatou, soshite Sayonara!

Dr. N. Ishikawa, Course Director

I am convinced that in the worldwide battle against tuberculosis, long working, enthusiastic and dedicated persons are necessary. I feel the basic role of our international training courses is a manpower development in this sense. On the basis made by predecessors, I want to develop the courses more to meet the needs in the working fields, and welcome all the exparticipants, honorable members of the RIT to feed back their experiences on the front line. I am very lucky to have Dr. Matsuda, competent assistant who will practically lead the courses.

Dr. M. Matsuda, Assistant Course Director

After my graduation in 1977 from the School of Health Sciences, Faculty of medicine, Tokyo University, I took Master by the thesis on “Development of PHC in Thailand”. My doctoral thesis was “Basic unity on Community health care system provided by health professions and inhabitants - natural history of health care”. From 1986 to 87, I worked in Thailand at ASEAN Training Center for PHC Development of Mahidol University as a JICA expert and a visiting professor on public health administration. After taking the course at RIT (I am also an exparticipant!) in 1988, I joined the JICA tuberculosis project in North Yemen for 6 months. As a coordinator for the courses, I hope to facilitate the participants to learn in the courses and I myself also want to learn particularly how tuberculosis programme is integrated at PHC level.
I am quite happy to receive the "Newsletter from Kiyose" to know that all of my old teachers have been in good health, especially of Dr. Iwasaki. It is moving that the RIT still remembers the old participants since 1972. From 1971 to 1975, I had worked for National TB Control Programme of South Vietnam and I am grateful to the training course in Japan in 1972, which had provided me many interesting and practical information concerning TB control. After 1975, South and North Vietnams were reunited. I returned to TB Control Programme in 1978. I am now working at TB Control Programme Section of Phamngochach TB and Lung Disease Center at Hochiminh City (ex Saigon).

With much regret, I inform you that among five southern Vietnamese doctors having attended the course of Kiyose, four of them have already left Vietnam.

In October 1988, I was very glad to see Dr. Aoki and Dr. Mori at Dubrovnic conference. In my country, according to the survey in Hochiminh City from 1986 to 1989 conducted by ITSC at Amsterdam, the risk of infection was 3.1% (for group from 5 to 19 years old). From the beginning of 1989, with the help of Medish-Co of Netherlands, we have applied the short course chemotherapy (2HRZ/6HR or 6H3T1) at 6 districts at Hochiminh City.

After discussing with Prof. Huong, director of National Institute of Tuberculosis and Lung Disease at Hanoi, I will send the available data on items listed in your Newsletter.

Dr. Le Ba Tung, VIETNAM ('73 A & '72 C)

Many thanks for your Newsletter from Kiyose which content I found extremely interesting. Congratulations and be sure it will promote communication between our loved Institute and former participants.

Thanks to the training on tuberculosis control provided by the Institute, we are improving our regional programme in La Paz, transmitting knowledge and skills to other health professionals, mainly to those working in remote rural areas through short courses, seminars, meetings, examining with them our performances and restrictions in order to achieve best results and success in Tuberculosis control.

Dr. Oscar Lanza V., BOLIVIA ('88 C)

I was more than happy to hear that Dr. Iwasaki is still active and amused by Dr. Azuma's comments. Since my lovely stay in Kiyose-shi, I was the head of the National Tuberculosis Control for nearly 4 years. I was then a consultant to the Epidemiology Department of the Ministry of Health. During 1988/1989, I was in London for the Epidemiology MSC course. I am now the head of the National Leprosy Control Programme and am working on the integration of leprosy and tuberculosis. This in part is due to the training I had at the RIT.

Dr. Befikadu Sissay, ETHIOPIA ('80 C)

We have received many other letters from our exparticipants. But because of limitation of space, we list only the names of correspondents (as of April 15) as follows.

- Mr. A.S. Afghanzada (Afghanistan/75L)
- Dr. J. Ordonez Blacutt (Bolivia/85C)
- Dr. L.D. da Silva (Brazil/82C & 81A)
- Dr. Y. El Hawary El Sayed A. (Egypt/77)
- Dr. Abdul Latif Hegazy (Egypt/77C)
- Dr. Nabe M. Nassef (Egypt/83A)
- Dr. M.A.-K. El Gengaiby (Egypt/85C)
- Dr. Demeke Feissa (Ethiopia/83C)
- Dr. Nyatha Louissaint (Haïti/88C)
- Dr. Indira K.S. (India/89A)
- Dr. Hertanto T. (Indonesia/79C)
- Dr. H. Ong Yoo (Korea/72S)
- Dr. Aziah A. Mahayiddin (Malaysia/88A)
- Ms. Shining Moon (Myanmar/70I)
- Dr. N. Kumal (Nepal/88C)
- Dr. Abdullah Jan Pathan (Pakistan/85A)
- Dr. Aida Callier (Peru/82A)
- Dr. M.E. Uypanuco (Philippines/71C)
- Dr. Dina B. Racho (Philippines/82C)
- Ms. Chua Bee Koon (Singapore/74C)
- Dr. Tommy Goh (Singapore/77C)
- Dr. Grace M. Ilmolelian (Tanzania/70I)
- Dr. Juma Daudi Uledi (Tanzania/76C)
- Dr. H.J. Chum (Tanzania/86C)
- Dr. Art Arthorhantrasook (Thailand/69S)
- Ms. Chanpen Tanakehas (Thailand/70I)
- Dr. Tanapan Kitpanpisit (Thailand/75C)
- Dr. A. Wahab A. El Jami (YAR/89C)
- Mr. M.G. Yahua Abdulwahab (YAR/85I)

*ABBREVIATION*
C: Group Training Course in TB Control
A: Group Training Course in TB Control for Administrative Medical Officers
L: Group Training Course in Laboratory Works for TB Control
S: Group Training Course in Chest Surgery
I: Individual Training Course
Application of New Technology in Mycobacteriology

Dr. Chiyoji Abe, Bacteriology & Serology Division

Laboratories today are looking for faster and more efficient systems to provide physicians and patients with faster results and to free laboratory personnel from unnecessary hours of tedious labour.

The BACTEC 460 TB System for Rapid Automated TB Results

Radiometric techniques were first introduced in mycobacteriology by Cummings et al. in 1975. A major advancement was made in 1977, when Middlebrook introduced a liquid 7H-12 medium containing C-labeled palmitic acid for radiometric detection of mycobacterial growth. The BACTEC TB system detects the presence of mycobacteria based on their metabolism rather than on visible growth. When the C-labeled substrate present in the medium is metabolized, CO₂ is produced and is measured by the BACTEC 460 instrument and reported in terms of a Growth Index (GI) value.

BACTEC 7H-12B medium is a liquid medium which is an enriched Middlebrook 7H-9 base supplemented with bovine serum albumin, catalase, casein hydrolysate and C-labeled substrate. This medium is used for isolation, differentiation of Mycobacterium tuberculosis (TB) complex from mycobacteria other than tuberculosis bacilli (MOTT), and for drug susceptibility testing.

The PANTA kit is a modified Mitchison’s combination of the following antimicrobial drugs; polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin. When added to the BACTEC 12B medium, it suppresses the growth of residual normal microbial flora which may have survived during the digestion and decontamination process and which may contaminate the medium.

For primary isolation, a specimen which had been pretreated with NaOH or NaOH + N-acetyl-L-cysteine methods was inoculated onto conventional egg medium (3% Ogawa) and into BACTEC 7H-12 Middlebrook medium with PANTA for detection of mycobacterial growth. Of the 182 specimens processed, 36 (19.8%) were detected as culture positive by conventional methods, and 70 (38.5%) as culture positive by the BACTEC method as shown in Table 1 (Higashitsuetsumi, 1989). There was a significant difference in percentage of positive cultures, and the BACTEC method outperformed the conventional method. One reason for the higher recovery rate in the BACTEC method may have been because specimens were pretreated with 2% NaOH, diluted with phosphate buffer and then concentrated; perhaps less damage occurred to the mycobacteria (especially MOTT) during processing.

Table 1 shows the time required to detect a positive culture. Of 70 culture positive specimens, 63 (90%) were detected within 2 weeks by the BACTEC method. On the other hand, 4 weeks were required to recover mycobacteria from 90% of culture positive specimens by the conventional method. The recovery time for mycobacteria was remarkably shorter with the BACTEC method. Overall bacterial contamination was 6.0% and 7.7% for the BACTEC and the conventional media, respectively (Higashitsuetsumi, 1989).

Susceptibility to para-nitro-alpha-acetylmethoxypropiolophenone (NAF) is used
in the BACTEC TB system to differentiate *M. tuberculosis* complex from other mycobacteria quickly and easily (2-4 days). The growth of the TB complex is inhibited by NAP which is determined by either a decrease or no increase in the GI ("CO₂ output), while other mycobacteria grow in the presence of NAP and show an increase in GI output. In addition, the NAP test is independent of the conventional niacin test thus facilitating correct identification of niacin positive MOTT and niacin negative *M. tuberculosis*.

For BACTEC TB susceptibility testing, antituberculosis drugs are added to 12B medium (one drug per vial). The rate of growth in the control vial, a 1:100 dilution of the test organisms, is compared to the rate of growth in a vial containing one drug and an undiluted suspension of the test organism. Radiometric antimicrobial susceptibility tests required 4-5 days with over 98% agreement with the susceptibility results obtained by the conventional plate or tube methods. BACTEC TB drug susceptibility testing can be performed from a freshly grown culture on conventional media or directly from smear positive specimens.

The BACTEC 460 TB system offers a major advance for laboratories seeking a standardized, faster and more efficient system for mycobacteriology.

### Rapid Identification of Mycobacteria Using DNA Probes

DNA probe technology facilitates a rapid and specific identification of microorganisms. Commercial kits (Gen-Probe, Inc., San Diego, Calif.) are available for culture confirmation and identification of species belonging to the *M. tuberculosis* complex, the *M. avium* complex and *M. gordonae*. This system is based on the hybridization of ³¹P-labeled DNA probes specific for *M. tuberculosis* complex, *M. avium*, *M. intracellulare* and *M. gordonae* with rRNA from a test organism.

Of the 34 clinical isolates which had been identified as belonging to the *M. tuberculosis* complex by the conventional culture method, all 34 isolates were positive with the *M. tuberculosis* DNA probe (Table 3). With the *M. tuberculosis* complex probe, *M. tuberculosis* complex isolates gave percent hybridization value ranging from 32.51% to 52.88% and the non-*M. tuberculosis* complex species gave percent hybridization value ranging from 0.71% to 1.97%. The diagnostic specificity and sensitivity of the DNA probe test were 100%.

Similarly, there was good correlation between the DNA probe test and the conventional culture and biochemical identification results for the *M. avium* complex isolates, 305 were positive and 3 were negative with *M. avium* complex DNA probes. The diagnostic specificity and sensitivity of the DNA probe test were 100% and 99%, respectively.

The Gen-Probe rapid diagnostic system for the *M. avium* complex can detect as few as 10⁷ *M. avium* or *M. intracellulare* cells per assay and 10⁷ *M. tuberculosis* cells for *M. tuberculosis* complex. However, this method has not been tested for direct application to clinical specimens. The identification of members of the *M. tuberculosis* complex, *M. avium* complex or *M. gordonae* using these tests should be limited to organisms isolated in culture.

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**Table 3**

Percent hybridization of *M. tuberculosis* ³¹P-labeled DNA probe to mycobacteria isolates

<table>
<thead>
<tr>
<th>Isolates (n)</th>
<th>% Hybridization of DNA probe (X ± SE [range])</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em> (34)</td>
<td>46.86 ± 0.61 (32.51 - 52.98)</td>
</tr>
<tr>
<td><em>M. avium</em> complex (7)</td>
<td>1.33 ± 0.22 (0.71 - 1.97)</td>
</tr>
<tr>
<td><em>M. kansasi</em> (1)</td>
<td>1.21</td>
</tr>
</tbody>
</table>

**Table 4**

Percent hybridization of *M. avium* and *M. intracellulare* ³¹P-labeled DNA probes to mycobacteria isolates

<table>
<thead>
<tr>
<th>Isolates (n)</th>
<th>% Hybridization of DNA probe (X ± SE [range])</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. avium</em> (244)</td>
<td>45.41 ± 0.86 (20.89 - 65.95)</td>
</tr>
<tr>
<td><em>M. intracellulare</em> (61)</td>
<td>1.42 ± 0.08 (0.64 - 3.08)</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> (8)</td>
<td>1.21 ± 0.13 (0.80 - 1.80)</td>
</tr>
<tr>
<td><em>M. kansasi</em> (4)</td>
<td>1.18 ± 0.13 (0.97 - 1.55)</td>
</tr>
<tr>
<td><em>M. scrofulaceum</em> (1)</td>
<td>1.47</td>
</tr>
<tr>
<td><em>M. nonchromogenicum</em> (1)</td>
<td>1.73</td>
</tr>
<tr>
<td><em>M. fortuitum</em> (1)</td>
<td>1.11</td>
</tr>
<tr>
<td><em>M. simiae</em> (1)</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Bacteriology & Serology Division

We moved to the third story of the new building in 1989. This laboratory is well-equipped; in particular it contains three biohazard rooms equipped with a class II biological safety cabinet, allowing us to safely handle mycobacteria in it. The Bacteriology Division, carries out various studies directed towards the development and improvement of diagnostic methods for mycobacterial infections. The main project is studies on species-specific antigens for mycobacteria by isolation and purification of the antigens using monoclonal antibodies directed to mycobacteria. In this division, three hybridomas have been constructed, which secrete antibodies for specific mycobacteria, one of which shows limited cross-reactivity among the *M. tuberculosis* complex and other two antibodies which are specific for *M. avium* and *M. scrofulaceum*. The gene for the protein antigen recognized by the monoclonal antibody specific for *M. avium* has been cloned, sequenced, and expressed in *Escherichia coli*. Experiments are presently under way to determine epitope of the *M. avium*-specific protein recognized by murine or human T and B lymphocytes, by using a recombinant DNA technique. Studies are also being done on the expression of other mycobacterial antigens in *E. coli*.

Other study subjects are improvement of the media for primary isolation of mycobacteria from various specimens and for drug susceptibility test, identification of mycobacteria using specific DNA probes and production of L-forms from mycobacteria. Also we are working on identification of mycobacteria isolates with the conventional culture tests, and culture collection of mycobacteria. Some of the above studies are carried out in cooperation with other institutions in Japan.

Pathology Division

The pathology division has acquired last year two new staff, Dr. Nakata, clinical immunologist and Dr. Nakano, clinical pathologist and now actively engaging in immunohistochemistry. The research activities have now shifted to more basic, experimental and immunological fields. Simultaneously, a flow cytometry which can differentiate and enumerate each lymphocyte subpopulation by using fluorescent-labeled monoclonal antibodies, was introduced into our institute making possible to analyze the pathogenesis of tuberculosis as well as non-tuberculous diseases from deep immunological insight.

Lung health, which is a main subject of the Boston Meeting held in May, 1990, is also our interest, and health effects of asbestos or automobile exhausts are being studied extensively on human lungs or in animal experiments. Whether and how tuberculous infection may be modified by such air pollutants and harmful lung burdens are the subjects to be clarified. Actual situation of asbestos lung burden in urban and rural residents and its relation to lung cancer incidence in Japanese is another subject to be studied. Lung cancer risk in Japanese due to diesel exhaust smoke were also calculated in order to push the governmental environmental policies in Japan.

Clinico-pathological studies on surgical, autopsy and cytological specimens are also carried out enthusiastically as before. Many demonstrable pathological specimens of various lung diseases including all types of tuberculosis have been selected newly and displayed in showcases in the auditorium for educational purposes.
1989 Laboratory Works Course

This 15th Laboratory Works Course began with 6 participants on 28th October, 1989 and concluded on 9th February, 1990. The total number of exparticipants is presently 98 persons.

During this year’s course it snowed 3 times! There was especially heavy snow at the Tsukuba Science city in Ibaraki prefecture, north of Kiyose which we visited for a study tour. Began in 1970, the Tsukuba Science city is a center for scientific and education. At each institute we visited, the staff greeted our participants with “Welcome, but unfortunately the weather is very bad.” But the participants’ response was “We are very happy, because we can experience the beauty of the snow.”

Course Participant Comments:

Dr. Patrick Lans T. Amara, Liberia

Thank you for permitting us to express our views on the role laboratories play in the control of tuberculosis in developing countries. It may sound as if we are preaching, but it is worth the effort to point out facts which will have an impact on human lives.

Unlike the developed countries where health care facilities are readily available, tuberculosis control programmes in developing countries have to rely greatly on laboratory test results. Therefore, there is an acute need for many simple laboratories. Such laboratories need only be concerned with direct smear examination of sputum. The required equipments are simple and portable, but yet the results are reliable. If developing countries utilize these laboratories in their tuberculosis control programmes, case-detection will become easier and there will be far fewer people suffering from this disease.

Thanks to modern Japanese techniques in tuberculosis bacteriology, JICA, through The Research Institute of Tuberculosis annually trains laboratory workers from the developing world in modern techniques of detecting mycobacteria. The Institute also offers courses necessary for Laboratory methodology teaching, organization and evaluation. Highly-trained technologists and researchers help the participants to learn the essential things that are lacking in their TB control system. This year, five laboratory technicians and a medical doctor were trained.

In the treatment of tuberculosis, doctors make their diagnosis based on laboratory test results. They prescribe drugs for the patient based on laboratory analysis. It is then crystal clear that tuberculosis is a problem which concerns the laboratory worker.

We from the developing world cannot afford to remain passive in this noble effort. The Japanese people have set the pace. All that we need is to actively move with them. We are hopeful that when we return to our countries we will be able to put our new knowledge into practice for global benefit. If we do this, we are sure our tuberculosis problems will be as less a burden to us as is presently the case in Japan.
In Remembrance of Dr. Kihara

We are grieved to inform you that Dr. Kazuro Kihara died suddenly of bronchial asthma on January 2 at the age of 63. He worked 30 years for RIT and was previously head of the Education and Training Department. His presence with us will be missed, and his service will be fondly remembered.

Follow-up Visits

Two teams made follow-up visits recently to exparicipants. Indonesia and Malaysia were covered in the first visit and Egypt and Tanzania in the second. The first visit was made from January 14 to 24 by the RIT team of Dr. T. Mori, Dr. N. Ishikawa and Mrs. Tanaka who met exparicipants at Bali and Yogyakarta in Indonesia and at Kuala Lumpur in Malaysia. It was again felt that meeting at the participants’ worksite was very useful for both side. Evaluative discussions on the course were exchanged based on the experiences of the exparicipants. The second visit was a Tuberculosis Seminar organized by JICA for exparicipants in both of Egypt and Tanzania. Lectures on the latest TB control information were given by Dr. Aoki, Dr. Ishikawa and other members from the Ministry of Health & Welfare and JICA. Both of these seminars were very successful and stimulating in exchanging the information. Contents of discussions conducted in these visits will be summarized in the next issue.

Individual Training

Mrs. Milan Karanjeet from Nepal has been studying public health nursing at RIT since January for 6 months under JICA’s individual training scheme. She is a staff nurse of the National Tuberculosis Centre in Kathmandu.

Advanced Course Starts

The group training course in TB Control for administrative medical officers ('Advanced Course') of this year starts from 14th May for 6 weeks. Ten participants from Bangladesh, Brazil, Indonesia, Malaysia, N. Yemen, Philippines, Paraguay, Thailand and Japan are participating. To strengthen the administrative capacity, evaluation methodology is a focused subject, and more workshops and country evaluation report writing are given importance in this course.

Jun Tamura left

Mr. J. Tamura who have worked for the international courses for four years left the institute to take other work. Mr. N. Nukui has taken his place as an administrative clerk.

Staff News

Welcome:
Mr. N. Doi (Bacteriology & Serology Div.)
Mr. N. Nukui (Administration Dept.)

Farewell:
Dr. S. Shishido
Dr. N. Umemura
Mr. J. Tamura

Your news and voices are always welcome!

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