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Growth Experiment of Mycobacterium Leprae in Cultured Mouse Peritoneal Macrophages*

2. In vivo infection and in vitro cultivation of trypsin-purified Myco. leprae.

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—國文抄錄—

組織培養된 마우스腹腔巨喰細胞에서의 人癩菌增殖實驗*

2. trypsin-精製人癩菌을 使用한 in vivo infection-in vitro cultivation 實驗

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組織培養을 利用한 마우스腹腔巨喰細胞內 人癩菌增殖實驗으로서 trypsin-精製人癩菌을 사용하여 1) 人癩菌의 마우스腹腔內接種에 依하여 惹起되는 腹腔巨喰細胞의 生體內 人癩菌感染에 對한 動力學的樣狀, 2) in vivo infection-in vitro cultivation에 依한 人癩菌의 腹腔巨喰細胞內 增殖態度 그리고 3) 人癩菌의 腹腔內接種으로 因한 마우스脾臟組織의 病理學的變化를 究明코저 本硏究를 實施하였으며 아래의 結論을 얻었다.

1. 人癩菌의 마우스腹腔內接種後 腹腔巨喰細胞培養實施까지의 期間이 延長됨에 따라 培養된 腹腔巨喰細胞에 있어서 細胞質內에 喰菌된 抗酸菌數와 抗酸菌을 喰菌한 巨喰細胞數가 각각 繼續的으로 顯著하게 減小되어감이 觀察되었다.

2. 人癩菌의 腹腔內接種後 5個月에 이르기까지 生體內에 存在하는 마우스腹腔巨喰細胞에 있어서의 人癩菌增殖을 觀察할 수 없었다.

3. 人癩菌의 腹腔內接種後 24時間 및 1週만에 實施된 腹腔巨喰細胞培養을 2 乃至 3個月間 그 培養狀態를 維持하였던바 培養된 巨喰細胞의 染色標本에서 巨喰細胞內 抗酸菌數의 뚜렷한 增加樣狀을 觀察할수 있었다.

4. in vivo infection-in vitro cultivation 手技로서 培養된 腹腔巨喰細胞를 使用한 總抗酸菌數測定實驗에서 組織培養開始 9 및 11週에 이르러 培養된 巨喰細胞內 抗酸菌數增加에 대한 量的證據를 얻을수 있었다.

5. 人癩菌의 腹腔內接種으로 야기되는 마우스脾臟組織의 病理學的所見은 주로 赤髓部位에 나타난 變性變化이었으며, 菌接種後 5個月에 이르기까지 마우스脾臟內에 있어서의 人癩菌의 增殖樣狀을 觀察할 수 없었다.

ABSTRACT

To grow Myocbacterium leprae in cultured mouse peritoneal macrophages, studies were made with trypsin-purified Myco. leprae on 1) the dynamics of infection of mouse peritonal macrophages in vivo with Myco. leprae by intraperitoneal inoculation, 2) growth experiment of Myco. leprae in cultured mouse peritoneal macrophages by in vivo infection and in vitro cultivation and 3) the observation of pathological changes in spleens of mice

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induced by intraperitoneal inoculation of Myco. leprae. Results are summarized as follows:

1. Continuing and significant decreases were observed in the numbers of both acid-fast bacilli in cultured macrophages and of macrophages harboring acid-fast bacilli by the length of intervals between the time of intraperitoneal inoculation of Myco. leprae and the time of initiation of macrophage culture.

2. No evidence of multiplication of Myco. leprae in the peritoneal macrophages in vivo was found up to 5 months after intraperitoneal inoculation.

3. With cultures of macrophages made 24 hours and 1 week after intraperitoneal inoculation of Myco. leprae and maintained in vitro up to 2 to 3 months, microscopic examination of the stained preparations of cultured macrophages indicated that an apparent increase in the number of acid-fast bacilli in the macrophages did occur.

4. Quantitative experiment with in vivo infected-in vitro cultured macrophages revealed certain features of increase in the number of total acid-fast bacilli in the cultured macrophages 7 and 9 weeks after initiation of the cultures.

5. Pathological changes in the spleens mice inoculated with Myco. leprae were of mainly degenerative nature in the red pulp. No multiplication of Myco. leprae was observed in the spleens of mice up to 5 months after intraperitoneal inoculation.

INTRODUCTION

Following Shepard's observations (1960 a[1], 1960 b[2]) that a consistent but limited growth of Myco. leprae occurred in the foot pad of mice, a large amount of experimental works on animal transmission of Myco. leprae has been made. It is now widely accepted that Myco. leprae can be grown in a variety of rodents including mice (See review by Yang and Lew, 1971 a[3]).

Since the growth characteristics of Myco leprae, i.e., 1) failure of growth on bacteriological lifeless media and 2) nature of obligate intracellular growth in vivo, resembled closely to those of viruses, many attempts have been made to cultivate Myco. leprae in tissue culture cells. However, owing to the unusually long generation time of Myco. leprae(Shepard and McRae, 1965[4]; Garbutt, 1965[5]) a large proportion of experimental works on the growth of Myco. leprae in tissue culture system has provided negative results.

As a experimental model for the growth of Myco. leprae in tissue culture, mouse peritoneal macrophage has been used for in vitro cultivation of Myco. lepraemurium (Chang and Neikirk, 1965[6]; Lee et al., 1967[7]; Yang and Lew, 1968[8]; Yang et al., 1968 a[9]), and quantitative evidences for the actual growth of Myco. lepraemurium in cultured mouse peritoneal macrophage have been reported (Chang et al., 1967[10]; Yang and Lew, 1968[8]).

Among the experiments of the cultivation of Myco. leprae in tissue culture system, Chang and Neikirk (1965[6]) observed some features of growth of Myco. leprae in cultured mouse peritoneal macrophages, and Garbutt (1965[5]) reported the growth of Myco. leprae in cell lines of 14 pf rat fibroblast and human embryo lung but failed to observe the growth of Myco. leprae in the cultures of mouse peritoneal macrophages. Nevertheless, exact biological characteristics of so-called tissue culture-grown Myco. leprae have not been fully elucidated.

In the first part of this investigation (Yang et al., 1972[11]), a simple and effective purification of viable Myco. leprae became possible by trypsinization of minced lepromatous nodules

followed by high-speed centrifugation. Therefore, studies were made on 1) the dynamics of infection of mouse peritoneal macrophages in vivo with trypsin-purified Myco. leprae by intraperitoneal inoculation 2) growth experiment of purified Myco. leprae in cultured mouse peritoneal macrophages by in vivo infection-in vitro cultivation technique and 3) the observation of pathological changes in spleens of mice induced by intraperitoneal inocualtion of purified Myco. leprae.

MATERIAL AND METHODS

1. Trypsin purification of Myco. leprae from lepromatous nodules:

The biopsied nodules were obtained from untreated lepromatous leprosy patients at the World Vision Speical Skin Clinic, Seoul, Korea (Table 1).

Table 1. Biopsied nodules from lepromatous leprosy patients.

OPD No. *	Sex	Age	Skin bacteriology (Wade's method)		Date of biopsy
			BI**	SFG***	
4837	M	35	5+	0—2—0	Jan. 1971
4901	M	34	5+	0—2—0	May 1971
4913	F	17	4+	0—2—0	June 1971

*: OPD No. at the World Vision Special Skin Clinic, Seoul.
**: Bacterial index (Ridley, 1964 a[12]).
***: SFG value (Ridley, 1964 b[13]).

The nodules were processed for trypsin purification of Myco. leprae by the method of Yang et al. (1972[11]) either immediately after the biopsy or kept frozen at-15°C before use.

2. In vivo infection-in vitro cultivation technique:

One-tenth milliliter of PBS suspension of purified Myco. leprae was injected intraperitoneally into adult mice of a random bred CFW strain ($1.6-4.6\times10^7$ bacilli/mouse), and the cultures of peritoneal macrophages were made at certain intervals after inoculation by the procedure reported previously (Yang and Lew, 1968[8]; Yang and Lew, 1971 b[14]; Chang et al., 1972[29]) For each culture experiment, 4 to 5 mice were used.

The numbers of macrophages in peritoneal washings were approximately 1.0×10^6 cells/ml. The cultures of macrophages were made by inoculating 1 ml of peritoneal washings into a glass Leighton tube or plastic petri dish (35× 10mm Style, Falcon Plastics) and incubating at 34°C under 5% CO_2 atmosphere.

Growth mdium for macrophage culture consisted of double strength NCTC 135 (Difco, U. S. A); 5parts, heat-inactivated bovine serum; 4 parts and diluted (1:5) bovine embryo extract; 1 part. The medium was changed at 2 to 3 days interval. Only penicillilin was added to NCTC 135, PBS and trypsin solution(0.25% in PBS) at the concentration of 200 to 300 units per ml.

3. Microscopic observation of acid-fast stained preparation of cultured macrophages:

After the initiation of macrophage cultures by in vivo infection-in vitro cultivation technique, the macrophage grown on cover slip in the Leighton tube was stained for acid-fast bacilli (AFB) by the method of Yang et al. (1968 b[15]), and examined microscopically to follow grossly the changes in the number of Myco. leprae inside cultured macrophages.

4. Enumeration of numbers of acid-fast bacilli per macrophage culture:

The numbers of AFB in PBS suspension of trypsin-purified Myco. leprae and in the ultrasonicate of cultured macrophages in Leighton tube or plastic dish were determined by the pin head method of Hanks (1968[16]).

For ultrasonic disruption of cultured macrophages in a tube or dish, old medium was decanted and 1.5ml of prewarmed (37°C) trypsin solution was added. Then, the macrophages were completely detached from glass or plastic surface with rubber policeman and the cell suspension was treated by ultrasonic distintergrator Ultrasonic Probe, Fisher, Model BP-2) at 20 KC for 4 minutes.

5. Pathological findings in the spleens of mice inoculated intraperitoneally with trypsin-purified Myco. leprae:

At the time of culture of peritoneal macrophages by in vivo infection-in vitro cultivation technique, spleens were removed, fixed with 10% formalin and hematoxylin-eosin (H-E) and acid-fast (A-F) stained preparations were made.

RESULTS

1. Changes in the numbers of AFB inside cultured macrophages in relation to the time of tissue cultures after intraperitoneal inoculation of purified Myco. leprae into mice.

About 30 mice were inoculated with purified Myco. leprae and tissue culture of peritoneal macrophages from 4 to 5 mice were made 24 hours, 1 week, 4 weeks, 6 weeks, and 5 months after inoculation.

As shown in Fig. 1, 2 and 3, a clear difference was noted in the numbers of both AFB per macrophage and macrophages harboring AFB between the cultures made at different times post-inoculation. Marked decreases in the number of AFB per cultured macrophage and of macrophage harboring AFB were apparent in stained preparations of the cultures made at 4 weeks and 5 months (Fig. 2 and 3).

In support of these microscopic findings of stained preparations of cultured macrophages made at different times following intraperitoneal inocluation of Myco. leprae into mice, the enumeration of a total number of AFB per macrophage culture (following ultrasonic treatment of the cells) demonstrated sharp decreases in the numbers of AFB in cultured macrophages by the time of tissue culture after intraperitoneal inoculation of purified Myco. leprae as shown in Table 2.

Table 2. AFB counting of macrophage cultures after ultrasonic treatment.

Time of tissue culture made after inoculation	Total mumber of AFB/plate*
24 hours	1.8×10^{5}**
	9.2×10^{4}
	2.8×10^{5}
1 week	9.2×10^{4}
	1.4×10^{5}
	none***
4 weeks	none
	none
	none
6 weeks	5.4×10^{4}
	none
	none
5 months	none
	none
	none

*: Plastic petri dish, 36mm diameter.
**: The number represents a total number of AFB from each of 3 plates randomly selected from the cultures.
***: Undetected by the pin head method of Hanks (1968[16]).

The numbers of AFB in macrophage cultures made 4 and 6 weeks and 5 months after inoculation were all below the level of 3.0×10^{4}

that is the minimum number of AFB countable by the pin head method of Hanks (1968[16]) except 1 plate among the 6 weeks culture. It became evident that the longer the state of in vivo infection was, the smaller were the numbers of AFB in the cultured macrophages countable at the time of tissue culture, and that there was no indication of intracellular growth of Myco. leprae inside peritoneal macrophages in vivo up to 5 months following intraperitoneal inoculation.

2. Micrescopic observation and quantitative assessment of growth of trypsin-purified Myco. leprae in cultured macrophages.

When the macrophage cultures made either 24 hours or 1 week after intraperitoneal inoculation of purified Myco. leprae were maintained in vitro at 34°C under 5% CO_2 atmosphere for a prolonged period of time up to 2 to 3 months, the features of significant increases in the number of AFB inside individual macrophages became manifest (Fig. 4a-4f). At the same time, most of the cultured macrophages exhibited morphological alterations similar to those of "activatied" macrophages.

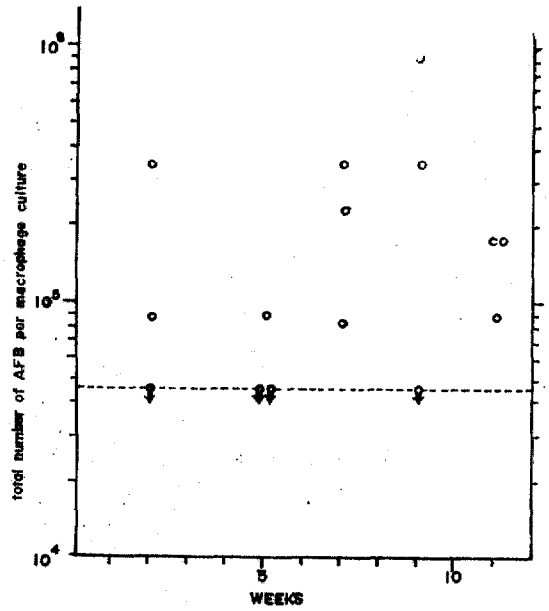

Fig. 5. Changes in total number of AFB per macrophage culture during prolonged in vitro culture.

In an effort to obtain a quantitative evidence of actual increase in total number of AFB per macrophage culture, about 40 of Leighton tube cultures of in vivo-infected peritoneal macrophages were set up 24 hours after intraperitoneal inoculation of purified Myco. leprae, and during prolonged period of in vitro culture of the macrophages 3 tubes of macrophage cultures were randomly selected and the counting of total number of AFB per culture was made after ultrasonic disintegration of dispersed macrophages. The results of this quantitative experiment was shown in Fig. 5.

Considerable fluctuation was apparent in total numbers of AFB per macrophage culture in each counting. The numbers of AFB counted 7 and 9 weeks after the initation of macrophage culture indicated a substantial increase in the numbers of intracellular acid-fast bacilli at this time of prolonged incubation. This finding coincided well with the results of microscopic observation of stained preparation of cultured macrophages maintained in vitro for similar period of time (Fig. 4c, 4d, 4e and 4f).

Taking into account the numbers of macrophages that might have been detached from the glass surface during such long period of in vitro culture and of corresponding decrease in the number of AFB from the macrophage culture, the countings of increased numbers of AFB per macrophage culture at 7 and 9 weeks of in vitro culture appeared to indicate a limited but substantial multiplication of trypsin-purified Myco. leprae in this experimental model.

3. Pathological findings of the spleens of mice inoculated with trypsin-purified Myco. leprae.

Macroscopically, a few of the spleens

appeared to be considerably enlarged when examined at the time of macrophage cultures after intraperitoneal inoculation. Microscopic observation of stained preparation of the spleens revealed characteristic changes as shown in Fig. 6 a and 6 b. A predominant pathologic involvement was the wide-spread hyalinization in the red pulp of the organ. There was a slight indication of lymphoid hyperplasia and extramedularly hematopoietic activity in the white pulp, but in general the white pulp appeared to be shrunken.

The earliest of these changes were manifest even 1 week after intraperitoneal inoculation. However, no multiplication of Myco. leprae was observed in the spleens up to 5 months after inoculation.

DISCUSSION

By in vivo infection-in vitro culture technique excellent growth of Myco. leprae-infected mouse peritoneal macrophages were obtained in tissue culture, and the cultures could be maintained in viable, good condition up to 3 months after the initiation of tissue culture without much difficulty. It appeared that the use of glass tubes was far superior to plastic plates for such prolonged in vitro maintenance of cultured macrophages.

Recently, studies have been made on the activation of mouse peritoneal macrophages by infection with certain microbial agents and others (Mallucci, 1969[17]; Mackaness and Blanden, 1967[18]; Gorden and Cohn, 1970[19], 1971[20]; Virolainen and Defendi, 1967[21]; North, 1969 a[22], 1969 b[23]; Blanden et al., 1969[24]), and Godal et al. (1971[25]) observed macrophage activation when the culture of blood-derived macrophages from the tuberculoid leprosy patients were exposed to Myco. leprae in vitro in the presence of lymphocytes.

The appearances of the cultured macrophages (Fig. 3 and 4) exhibited morphological alterations similar to those observed in the activated macrophages (Blanden, 1968[26]; Blanden et al., 1969[24]). However, no study has been made whether the infection of mouse peritoneal macrophages with Myco. leprae could induce per se activation of the macrophages or not.

The numbers of AFB per macrophage and of macrophage harboring AFB declined sharply depending on the interval between the time of intraperitoneal inoculation of purified Myco. leprae and the time of macrophage tissue culture. Quantitative assessment of the numbers of AFB in the macrophages at the time of tissue culture (Table 2) corresponded well to microscopic findings of stained preparations of cultured macrophages (Fig. 1, 2 and 3). Since mouse peritoneal macrohages are considered end cells, derived from circulating monocytes, in a normal steady state situation (van Furth and Cohn, 1968[27]), and turn-over rate of peritoneal macrophage is estimated at about 0.1% per hour and turn-over time (Trasher, 1966[28]) can be estiamted at about 40 days (van Furth and Cohn, 1968[27]), sharp decreases in the numbers of AFB in the macrophage cultures made 4 and 6 and 5 months after intraperitoneal inoculation of trypsin-purified Myco. leprae may reflect the dilution effect elicited by the turn-over of macrophage population in the mouse peritoneal cavity, as have been pointed out Yang and Lew (1971[14]) and Chang et al. (1972[29]). Throughout the experimental period of 5 months following intraperitoneal inoculation of purified Myco. leprae into mice, there observed no evidence of multiplication of Myco. leprae in the peritoneal macrophages in vivo that could be detected either by microscopic observations of stained preparation of cultured macrophages or by quantitative assessment of total numbers of AFB

per macrophage culture.

In this regard, over-all dynamics of infection of mouse peritoneal macrophanges in vivo with trypsin-purified Myco. leprae differed significantly from those with Myco. lepraemurium (Chang et al., 1972[29]).

When the macrophage culture made 24 hours and 1 week after inoculation was maintained in vitro up to 2 to 3 months, a sign of increase in the number of AFB inside individual macrophage became gradually apparent in microscopic observation of stained preparations (Fig. 4a-4f). Earlier experiment to assess the quantitative increases in the numbers of AFB in cultured macrophages by ultrasonic treatment has falied because of the limited period of maintenance of the macrophages cultured in the plastic petri dishes. However, the cultured macrophages can be maintained in glass Leighton tubes for much longer periods of time in a good viable condition than in the plastic dishes. The results of experiment to follow the changes in the total number of AFB in cultured macrophages grown and maintained in glass Leighton tubes indicated that a limited but substantial multiplication of trypsin-purified Myco. leprae did occur in the cultured macrophages under prolonged in vitro maintenance of infected macrophage cultures (Fig. 5).

Garbutt (1965)[5] reported multiplications of Myco. leprae in the cultures of cell lines of 14 pf rat fibroblast and human embryo lung by serial transfer technique, and pointed out the importance of human element-either human cells grown in medium containing non-human serum or rat cells grown in human cord serum. It is planned to determine the effect of the human element on the multiplication of Myco. leprae in our system.

The phenonmenon of a lag phase has been observed in the experimental inoculation of Myco. leprae into the foot pad of mice and of other rodents (Shepard and McRae, 1965[4]; Hilson, 1965[30]; Rees, 1965[31]; Rees and Weddell, 1970[32]; Lew et al., 1970[35]; Yang and Lew, 1971 c[33]), and Garbutt (1965[5]) demonstrated a lag phase of approximately 3 months before Myco. leprae adapted to a new environment(tissue culture cells) and began to multiply actively. In our study, the features of increases in the total numbers of AFB per macrophage culture became manifest 7 and 9 weeks of in vitro culture, and this may reflect the presence of a lag phase in this system too. The presence of such a lag phase will certainly become a limiting factor in experiments to grow Myco. leprae in all of tissue culture systems including mouse peritoneal macrophages.

The exact life span of mononulear phagocytes either in the tissues or in vitro remains to be measured. Nevertheless, an ususually long life span of mouse peritoneal macrophages in vivo was indicated by the demonstration of van Furth and Cohn (1968[27]) that about 1% of the mouse peritoneal macrophages were still labeled 5 weeks after 4 pulses of tritiated thymidine, and after 8 weeks an occasional positive peritoneal macrophage was found. Furthermore, by in vitro cultivation Chang (1964[34]) succeeded in the extended maintenance of cultured mouse peritoneal macrophages up to 220 days, averaging 172.5 days, and eventually observed the growth of Myco. lepraemurium in the cultured macrophages (Chang and Neikirk, 1965[6]; Chang et al., 1967[10]). However, the effects of infection of mouse peritoneal macrophages with Myco. leprae (or with Myco. lepraemurium) on the life span of the macrophages are unknown.

The pathological changes observed in the spleens of mice inoculated with purified Myco. leprae were of degenerative nature, involving

mainly the red pulp of the organ. No sign was observed of extensive proliferation of the macrophage(or macrophage-like cells) nor any indication of active multiplication of Myco. leprae in the organ.

The features of pathological involvement in the spleens of mice inoculated intraperitoneally with purified Myco. leprae differed markedly from those of mice inoculated with Myco. lepraemurium (Chang et al., 1972[20]), and this finding may be regarded as one of the important biological distinctions of Myco. leprae from Myco. lepraemurium in the mouse system.

REFERENCES

1) Shepard, C. C. : *Acid-fast bacilli in nasal excretions in leprosy and results of inoculation of mice, Am. J. Hyg., 71:147, 1960 a.*

2) Shepard, C. C. : *The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice, J. Exp. Med., 112:445, 1960 b.*

3) Yang, Y. T. and Lew, J. : *Recent developments in the experimental cultivation of Mycobacterium leprae: A reivew, J. Res. Inst. Med. Sci. Korea, 3:643, 1971 a.*

4) Shepard, C. C. and McRae, D. H. : *Mycobacterium leprae in mice: Minimal infectious dose, relationship between staining quality and infectivity and effect of cortisone, J. Bacteriol., 89:365, 1965.*

5) Garbutt, E. W. : *Studies on M. lepraemurium and M. leprae in tissue culture, Internat. J. Leprosy, 33:578. 1965.*

6) Chang, Y. T. and Neikirk, R. L. : *Mycobacterium lepraemurium and Mycobacterium leprae in culture of mouse peritoneal macrophages. Preliminary results, Internat. J. Leprosy, 33:586, 1965.*

7) Lee, I. J., Hong. S. Y. and Lew, J. : *Cultivation of Mycobacterium leprae murium and Mycobacterium leprae by the tissue culture method, Kor. Leprosy Bull., 4:91, 1967.*

8) Yang, Y. T. and Lew, J. : *Mycobacterium lepraemurium in cultured mouse peritoneal macrophages: A preliminary report, Yonsei Med. J., 9:38. 1968.*

9) Yang, Y. T., Lew, J., Han, S. H. and Kim, S. Y. : *Mycobacterium lepraemurium in cultured mouse peritoneal macrophages, Kor. Leprosy Bull., 5:109, 1968 a.*

10) Chang, Y. T.. Andersen, R. N. and Vaituzis, Z. : *Growth of Mycobacterium lepraemurium in cultures of mouse peritoneal macrophages, J. Bacteriol., 93:119, 1967.*

11) Yang, Y. T., Lew, J., Cho, S. H. and Kim, J. K. : *Growth experiments of Mycobacterium leprae in cultured mouse peritoneal macrophages. 1. Purification of viable Myco. leprae from biopsied lepromatous nodules by trypsinization method, J. Kor. Soc. Microbiol., 7:17, 1972.*

12) Ridley, D. S. : *Bactial index. In "Leprosy in Theory and Practice", Cochrane, R.G. and Davey, T. F. (ed.). Bristol: John Wight and Sons, Ltd., 2nd ed., p. 620. 1964 a.*

13) Ridley, D. S. : *Granularity index. In "Leprosy in Theory and Practice", Cochrane. R. G. and Davey. T. F. (ed.). Bristol: John Wight and Sons, Ltd., 2nd ed., p. 621, 1964 b.*

14) Yang, Y. T. and Lew, J. : *Mycobacterium leprae in cultured mouse peritoneal macrophages: In vivo infection-in vitro cultivation, Yonsei Med. J., 12:1, 1971 b.*

15) Yang, Y. T., Lew, J. and Chang, C. H. : *An attempt to cultivate Mycobacterium leprae in cultured rabbit embryo fibroblast (REF), Kor, Cent. J. Med., 14:293, 1968 b.*

16) Hanks, J. H. : *Microscopic counts of mycobacteria. Conversion factors for the the pin head method, Internat. J. Leprosy, 36:76, 1968.*

17) Mallucci, L. : *T-antigen and DNA synthesis in macrophages infected with polyoma virus. Nature, 223:630, 1969.*

18) Mackaness, G. B. and Blanden, R. V. : *Cellular immunity, Progr. Allergy. 11:89. 1967.*

19) Gordon, S. and Cohn, Z. : *Macrophage-malanocyte heterokaryons. I. Preparation and properties, J. Exp. Med., 131:981, 1970.*

20) Gordon, S. and Cohn, Z. : *Macrophage-mela-*

nocyte heterokaryons, II. The activation of macrophage DNA synthesis. Studies with inhibitors of RNA systhesis, J. Exp. Med., 133: 321, 1971.

21) Virolainen, M. and Defendi, V. : *Dependence of macrophage growth in vitro upon interaction with other cell types, Wistar Inst. Symp. Monogr., 7:67, 1967.*

22) North, R. J. : *Cellular kinetics associated with the development of acquired cellular resistance, J. Exp. Med., 130:229, 1969 a.*

23) North, R. J. *The mitotic potential of fixed phagocytes in the liver as revealed during the development of cellular immunity, J. Exp. Med,, 130:315, 1969 b.*

24) Blanden, R. V., Lefford, M. J. and Mackaness, G. B. : *The host response to Calmette-Guérin bacillus infection in mice, J. Exp. Med., 129: 1079, 1969.*

25) Godal, T., Rees, R. J. W. and Lamvik, J. O. : *Lymphocyte-mediated modification of blood-derived macrophage function in vitro: Inhibition of growth of intracellular mycobacteria with lymphokines, Clin. exp. Immunol., 8:625, 1971.*

26) Blanden, R. V. : *Modification of macrophage function, J. Reticuloendoth. Soc., 5:179, 1968.*

27) van Furth, R. and Cohn, Z. : *The origins and kinetics of mononuclear phagocytes, J. Exp. Med., 128:415, 1968.*

28) Trasher, J. D. : *Analysis of renewing epithelial cell populations. In "Methods in Cell Physiology", Prescott, D. M. (ed.), Academic Press, New York, 2:323, 1966.*

29) Chang, I. K., Yang, Y. T. and Lew, J. : *A study of the pathogenesis of murine leprosy infection of mice, Yonsei J. Med. Sci., 5:209, 1972.*

30) Hilson, G. R. F. : *Observations on the inoculation of M. leprae in the foot pad of the white rat, Internat. J. Leprosy, 33:662. 1965.*

31) Rees, R. J. W. : *Recent bacteriologic, immunologic and pathologic studies on experimantal leprosy in the mouse foot pad, Internat. J. Leprosy, 33: 646, 1965.*

32) Rees, R. J. W. and Weddell, A. G. M. : *Transmission of human leprosy to the mouse and its clinical implication, Trans. Roy. Soc. Med. & Hyg., 64:31, 1970.*

33) Yang, Y. T. and Lew, J. : *Studie on the cultivation of Mycobacterium leprae. 1. Mycobacterium leprae in foot pads of mice and Korean squirrels. Presented at Spring Meeting of Korean Society for Microbiology, 1971 c.*

34) Chang, Y. T. : *Long-term cultivation of mouse peritoneal macrophages, J. Nat. Cancer Inst., 32:19, 1964.*

35) Lew, J., Choi, T. K,. Hong, J. P. and Yang, Y. T. : *Inoculation of Mycobacterium leprae into foot pad and ear lobe of Korean squirrels II. Presented at the Annual Convention of Society of Korean Leprologists, 1970.*

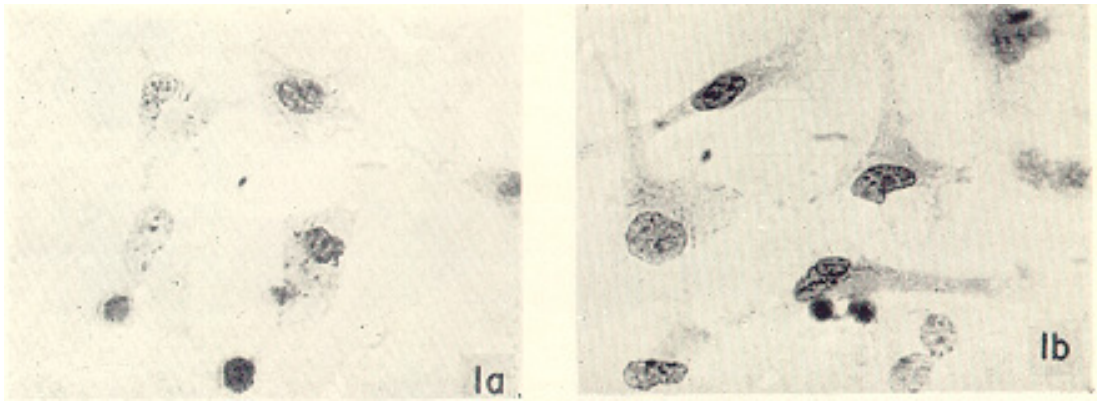

Fig. 1a & 1b. Intracellular acid-fast bacilli observed in the macrophage cultures made 24 hrs after intraperitoneal incoculation of trypsin-purified M. leprae into mice. A-F stain, 1000×.

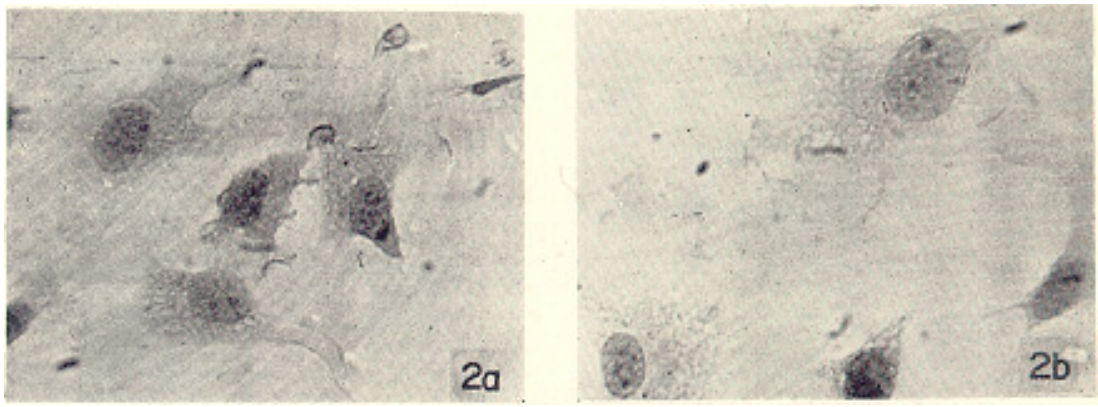

Fig. 2a & 2b. Intracellular acid-fast bacilli observed in the macrophage cultures made 4 weeks after intraperitoneal inoculation of trypsin-purified M. leprae into mice. Notice the apparent decrease in the numbers of both intracellular AFB and of the macrophages harboring AFB. A-F stain, 1000×.

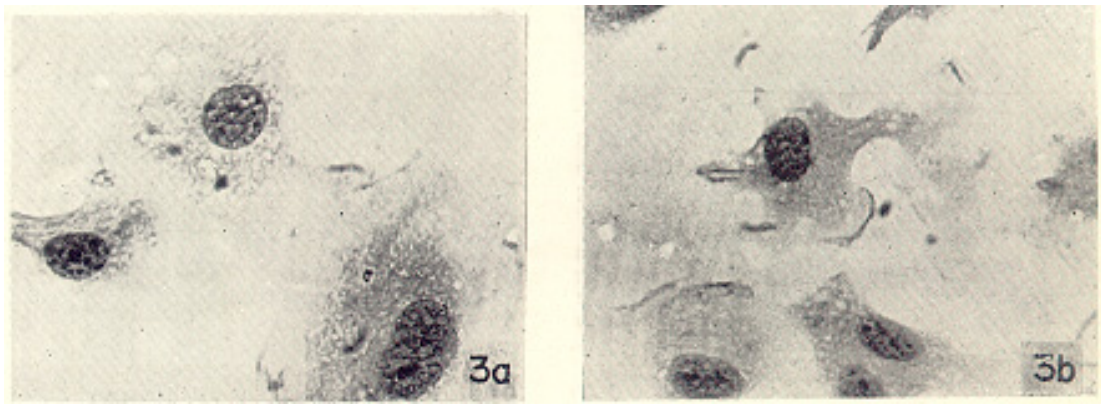

Fig. 3a & 3b. Macrophage cultures made 5 months after intraperitoneal inoculation of trypsin-purified M. leprae into mice. A-F stain, 1000×.

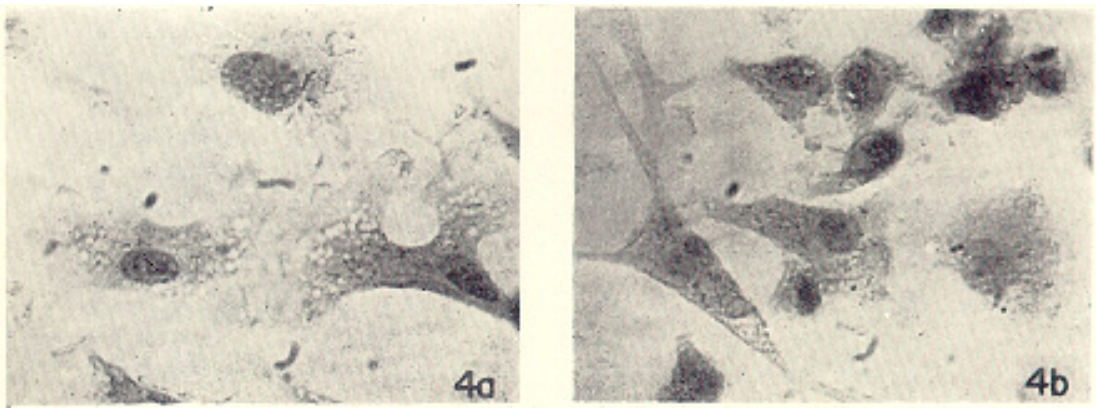

Fig. 4a & 4b. Significant increase in number of AFB inside macrophage of which culture was made 24 hrs after intraperitoneal inoculation of trypsin-purified M. leprae into mice and maintained in vitro for 34 days. A-F stain, 1000×.

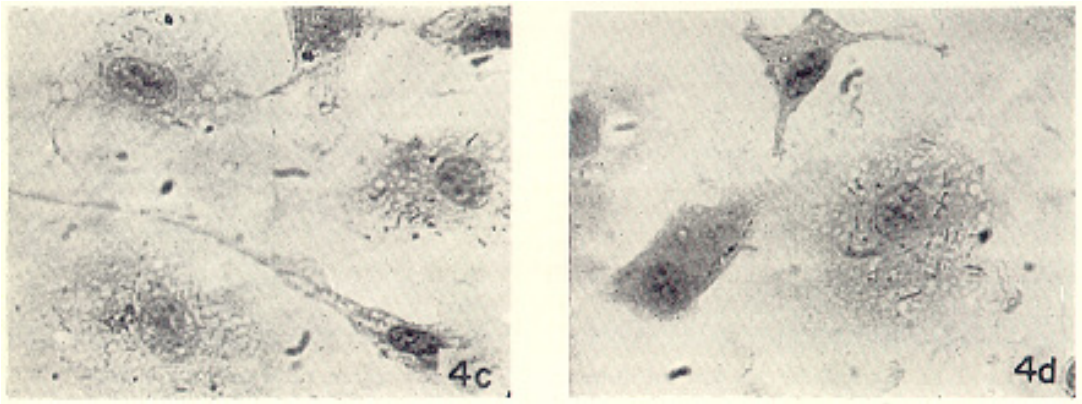

Fig. 4c & 4d. Same culture as in Fig. 4a & 4b and maintained for 49 days. A-F stain, 1000×.

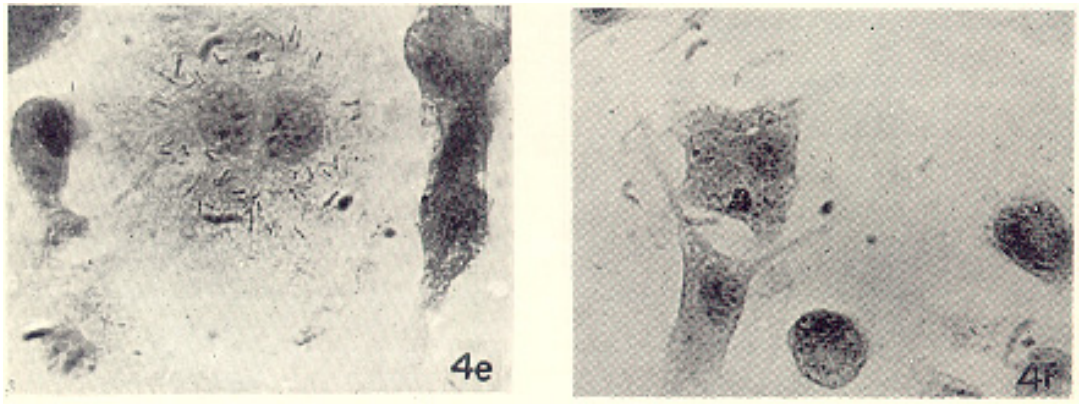

Fig. 4e & 4f. Same culture as in Fig. 4a & 4b and maintained for 60 days. A-F stain, 1000×.

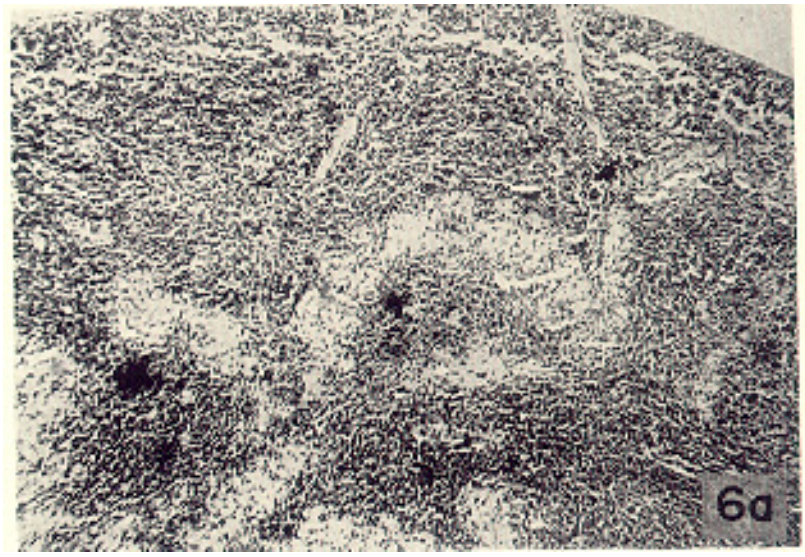

Fig. 6a. Pathological changes in the spleen of mouse 5 months after intraperitoneal inoculation of trypsin-purified M. leprae. H-E stain, 100×.

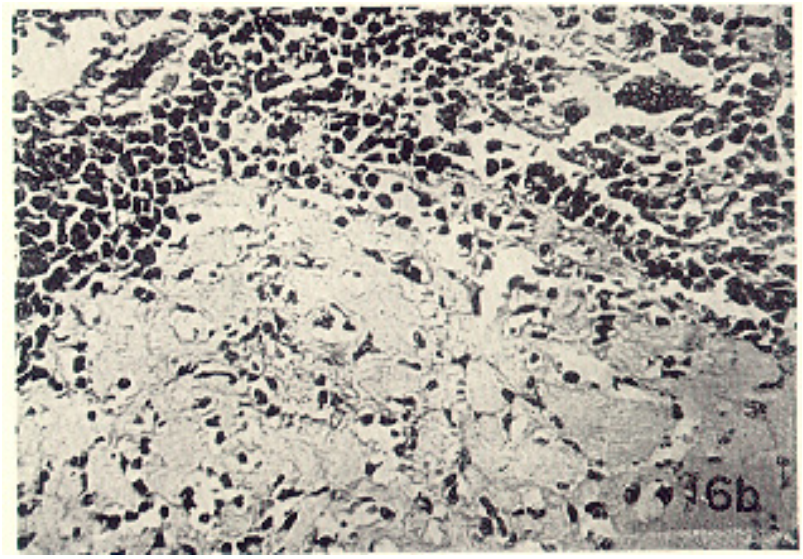

Fig. 6b. Higher magnification of Fig. 6a, indicating hyalinization feature in the red pulp of the spleen. H-E stain, 430×.