Culture and Phagocytic Characteristics of Schwann Cells *In Vitro*. A Possible Model Substrate for Cultivation of *M. leprae*[1]

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Tissue culture offers a unique opportunity for obtaining insight into living processes of cells under controlled conditions. A tumor arising from Schwann cells, such as the acoustic schwannoma, provides ideal material to confirm and study the phagocytic activity and relationship between mycobacteria and Schwann cells *in vitro*. In these past two decades, both light and electron microscopy have clearly shown the predominant bacillation of Schwann cells in leprous neuritis of any type ([4, 5, 8-10, 16, 17, 20, 26, 32]). It also became obvious that the Schwann cell could serve as a reservoir for possible later dissemination of *M. leprae* ([18]). At the same time, acoustic schwannomas constitute a fairly common type of intracranial tumor, comprising 7.8% of intracranial space-occupying lesions verified histologically at our Unit ([7]).

Cultivation and origin of Schwann cells from embryonal ganglionic nerve tissue has been known to tissue culturists and its behavior well documented, mainly by Lumsden and his colleagues ([21, 22, 24]). This source of Schwann cell has always been of limited experimental use. Acoustic schwannomas, on the other hand, are easy to grow and provide adequate cell culture for the study of morphology, behavior and experimental infection with *M. leprae* ([20, 23]). The present paper reports the culture characteristics and phagocytic activity of Schwann cells *in vitro*. A somewhat fuller account has been given in a thesis by one of us ([19]).

MATERIALS AND METHODS

During neurosurgery on five patients with acoustic schwannomas and four others with spinal schwannoma, biopsies were collected aseptically in tissue culture medium with added antibiotics. The cultures were set up within four hours in plasma clots and on glass-slips, and transferred to flat Leighton tubes ([28]). The cultures were fed 1.5 ml growth medium consisting of Eagles' Minimum Essential Medium (MEM) + 20% human serum, and penicillin (100 IU) and streptomycin (100 UG) per ml. The cultures were examined daily. When desired they were fixed in acetic alcohol (1:3) or 10% formol saline, and stained with hematoxylin and eosin.

When the Schwann cell cultures showed good spreading, generally between the fourth and ninth days, they were inoculated with acid-fast bacilli. The organisms constantly used were ICRC bacilli which were originally derived (at the Indian Cancer Research Centre) from lepromatous nodules ([1, 29]). The ICRC bacilli served as a model for *M. leprae*. In two instances some of the culture preparations were inoculated with *M. leprae* obtained from fresh nodules of untreated lepromatous patients. The inoculated cultures were incubated at 37° and fixed at 30 minutes, 1 hour, 1½ hours and 2 hours in formol saline. These cultures were stained by the Ziehl-Neelsen method for demonstration of acid-fast bacilli (AFB). The cells containing bacilli were counted under oil immersion, and the phagocytic index of Schwann cells *in vitro* was calculated at ½, 1, 1½ and 2 hours. Gomori's method for acid-phosphatase reaction in cells ([15]) was carried out in three instances on Schwann cell cultures which were not inoculated with the bacilli.

In addition to the schwannoma cell cultures, the tissues from two spinal neurofibromas from patients with Von Recklinghausen's disease were cultured to serve as

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TABLE 1. In vitro *behavior of Schwann cells from schwannomas.*

Serial No.	TC No.	NP No.	Histologic Picture (paraffin sections, H&E stained)	Migration of Cells	Morphology of Cells *in vitro* (living phase or fixed, H&E stained)	Period of maintenance (days)
1.	HBT—4 (Spinal)	G-714	Elongated Antoni type A cells forming bundles, and vacuolated Antoni type B cells forming sheets.	4th day	Spindle-shaped cells with nuclei bearing one or two nucleoli; round cells resembling microglia.	10
2.	HBT—6 (Acoustic)	G-830	Both type A and type B cells, type A with large ovoid nuclei.	48 hours	Both spindle-shaped and round cells.	11
3.	HBT—8 (Spinal)	G-846	Predominantly type A cells in sheets, very vascular.	5th day	More of spindle-shaped cells.	12
4.	HBT—15 (Acoustic)	G-937	Both type A and type B cells, latter vacuolated and in discrete groups.	48 hours	Both types, but more of spindle-shaped cells. Multinucleate cells by the 8th day.	16
5.	HBT—16 (Acoustic)	G-978	Both types of cells but predominantly type A, forming typical interweaving bundles.	48 hours	Both spindle-shaped and round cells. Multinucleate cells by the 8th day.	15
6.	HBT—18 (Spinal)	H-53	Predominantly type A cells, forming typical interweaving bundles.	3rd day	More of spindle-shaped cells.	12
7.	HBT—19 (Acoustic)	H-72	Both type A and type B cells intimately mixed and bearing round nuclei.	48 hours	More of round cells.	22
8.	HBT—21 (Acoustic)	H-146	Both types of cells, but more of type B in sheets.	48 hours	More of round cells.	11
9.	HBT—23 (Spinal)	H-525	Predominantly type A cells with typical interweaving bundles, hyalinized vessels +	3rd day	More of spindle-shaped cells.	15

TC No. = serial number of specimens grown in tissue culture.
NP No. = serial number of specimens received at the neuropathology unit.
H & E = hematoxylin and eosin.

controls. Similarly, human fetal muscle fibroblasts were cultured by the identical method also as controls, This too was infected with ICRC bacilli and the glass-slip preparations stained for AFB or for acid phosphatase activity.

RESULTS

As seen in Table 1, there was consistently good migration of cells from the explants of both acoustic and spinal schwannomas. The tumor cells began migrating within 48 hours. Two distinct types of cells were seen in all cases when examined in the unstained living phase (Fig. 1a). One type was an elongated bipolar cell (Fig. 1b), often with the nucleus bulging to one side (Fig. 2b) corresponding to Antoni type A cell in conventional histologic sections. The other type was a round cell (Fig. 1a), corresponding to Antoni type B cell. While the growth of Schwann cells from explants of spinal schwannomas was slightly slower than that of cells from acoustic schwannomas (Table 1), they too showed good migration by the third to fifth days, and with cell characteristics identical to those of the latter (Figs. 2a-b). By the end of eight days all the Schwann cell cultures showed large multinucleate cellular aggregates (Fig. 3).

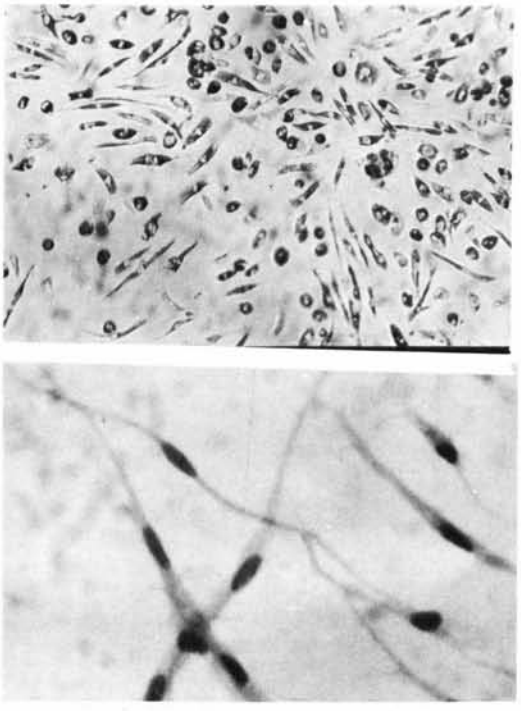


FIG. 1. (HBT-6): a) Culture of acoustic schwannoma cells, showing two clearly distinguishable cell types: Antoni type A—spindle-shaped or elongated with a bulging central nucleus, and Antoni type B—circular or globoid with a central nucleus. (Unstained living phase, photographed while in Leighton tube, × 250); b) Closer view showing narrow elongated cells, each with a plump prominent nucleus. Fixed culture, hematoxylin and eosin (H & E), × 625.

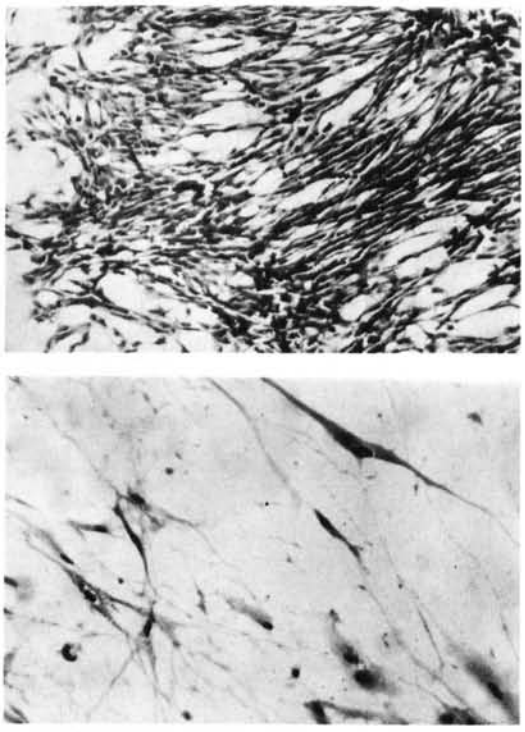


FIG. 2. (HBT-8): a) Eight day old: Spinal schwannoma cell culture showing good growth and migration of fusiform cells; b) Another part of the same culture with more elongated and discrete cells with a central bulging nucleus, typical of Schwann cells. Both H & E: a) × 100; b) × 250.

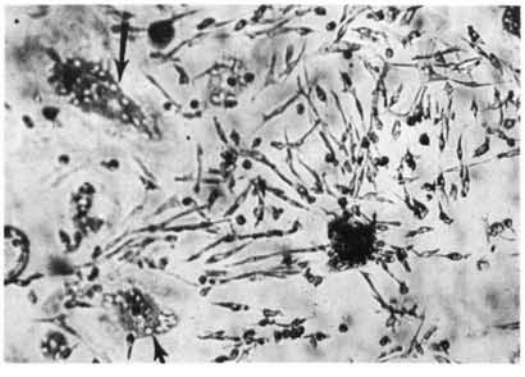
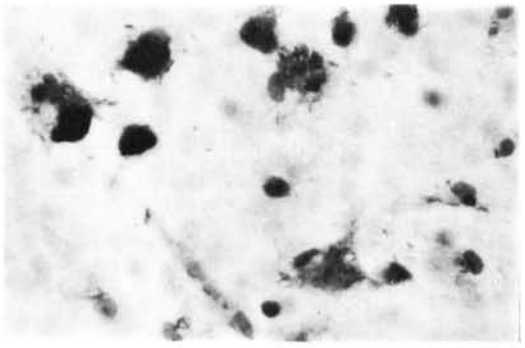


FIG. 3. (HBT-16): Four day old culture of acoustic schwannoma; note A and B types of Schwann cells, the former predominating, and the multinucleate giant cells or syncytia (arrows). As in Figure 1a, × 100.



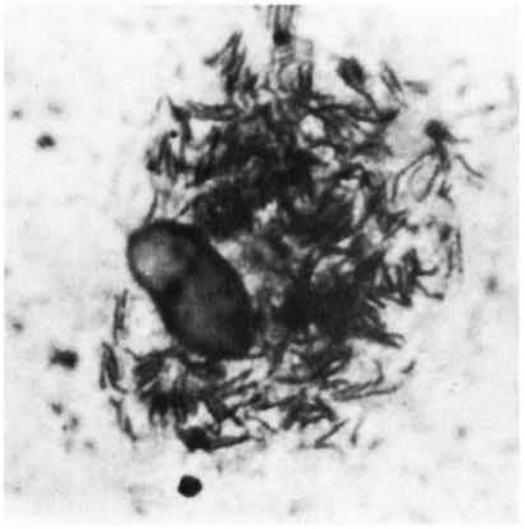


FIG. 4. Same culture showing: a) All Schwann cells packed with ICRC bacilli (see text); note globus formation in two of the cells (arrows); the extracellular bacilli are those remaining unwashed; b) Closer view of another cell enormously distended with the bacilli; the nucleus is pushed to one side. Ziehl-Neelsen stain, a) × 625; b) × 1,500.

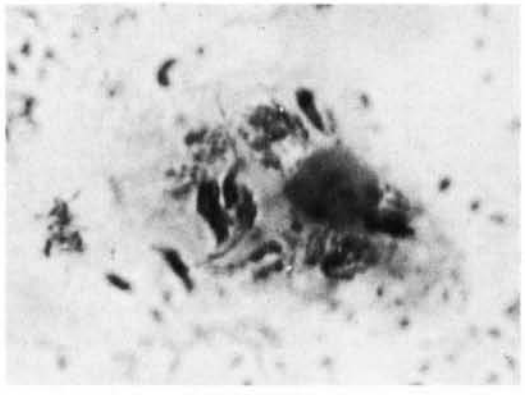


FIG. 5. (HBT-21): Cell similar to that in Fig. 4b, with cytoplasm containing globi of *M. leprae*, and homogenous nucleus with nucleoli. Ziehl-Neelsen, × 1,500.

When the Schwann cell cultures were inoculated with mycobacteria (ICRC bacilli or separate isolates of *M. leprae*) the neoplastic Schwann cells exhibited avid phagocytosis (Figs. 4a-b, 5), at times with formation of globi. The control cultures of human fetal fibroblasts failed to show any phagocytic activity at the end of two hours. The phagocytic index at the end of two hours was seen to be over 90% (Fig. 7). The difference between the phagocytic index of

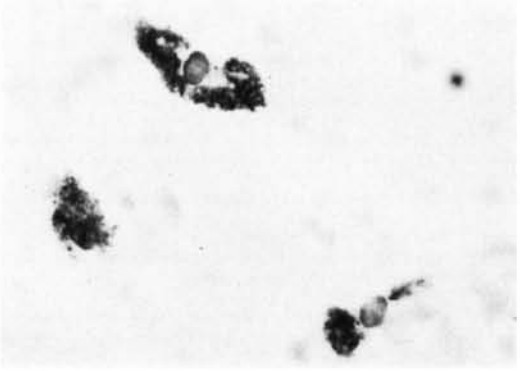


FIG. 6. Another glass-slip preparation of same culture, showing three Schwann cells with granular reaction product of acid phosphatase filling the cytoplasm, except for a small perinuclear zone; note central nucleus. Gomori's acid phosphatase, × 625.

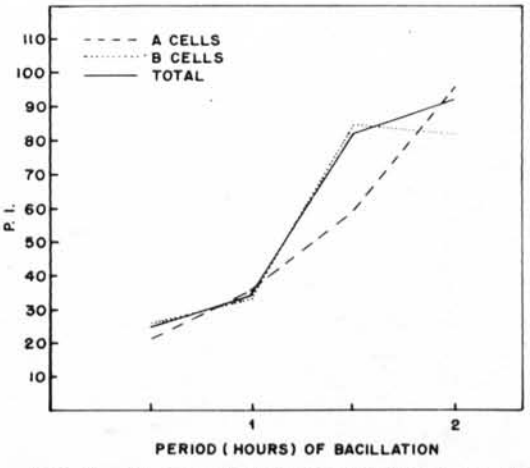


FIG. 7. Phagocytic index of Schwann cells treated *in vitro* with mycobacteria (ICRC bacilli) showing 32% cells bacillated at 1 hour, 82% at 1½ hours, and 92% at 2 hours. Note lack of appreciable difference between Antoni type A and Antoni type B cells.

type A cells and that of type B cells was found to be negligible.

Acid phosphatase stain revealed intense granular activity in the cytoplasm of the neoplastic Schwann cells (Fig. 6), while the control cultures of human embryonic fibroblasts failed to show either acid phosphatase activity or any phagocytic capacity.

In contrast, in one of the two neurofibromas which were put up for culture, there was only scanty migration of cells after eight days. Most of the cells had morphologic characters of fibroblasts, and very few were slender and spindle-shaped like Schwann cells.

DISCUSSION

Sharenberg and Liss [30] described three types of cells in acoustic schwannomas, the first cell type being slender and spindle-shaped, the second arrow-headed and the third resembling fibroblasts. Cravioto and Lockwood [2] recorded the following four types of cells: 1) ameboid microglia-like cells, 2) slender, spindle-shaped cells, 3) racket-shaped cells, and 4) large kite-shaped cells. The first two types were Schwann cells and the last type were throught to be fibroblasts. They did not observe any transformation of one cell type to another. Lumsden [22, 23] gives a detailed account of Antoni type A cells which are spindle-shaped, Antoni type B cells which are ameboid and a transitional type. In the present study two distinct types of cells were seen constantly, one being spindle-shaped and the other being round and resembling microglia, along with a few fibroblasts.

The vigorous phagocytic activity of schwannoma cells *in vitro* has been demonstrated by Lumsden [20] in his experiments on *M. leprae* and Schwann cells. He found that the uptake of bacilli by schwannoma cells was far greater than that by HeLa cells and human amnion cells. His observations did not indicate any special affinity of these cells for lepra bacilli and there was no evidence of intracellular bacillary multiplication. It is well known that Schwann cells are capable of engulfing any particulate matter such as myelin debris, hemosiderin, India ink particles injected subperineurially [27], and even melanin [11]. Sharenberg and Liss [30] recorded a larger number of fibroblasts than Schwann cells in the cultures of neurofibromas of Von Recklinghausen's disease, as seen also in two instances in the present study.

In the present study, ICRC bacilli were used because they serve as acid-fast bacilli and were easily available in the same laboratory at the Cancer Research Institute where the current tissue culture procedures were carried out. The schwannoma cells with a phagocytic index of 95% at the end of two hours were noticed to behave like macrophages. Deville [13] observed 80% of macrophages to contain bacilli within 24 hours of inoculation. Our control cultures of fetal fibroblasts did not show any phagocytic activity. This finding is at variance with that of Fildes [14], who reported the presence of *M. leprae,* predominantly in the cytoplasm of macrophages and fibroblasts in the infected organotypic cultures of dorsal root ganglia of rat and mouse, studied under the electron microscope. In our investigation, the period of maintenance of the cultures, up to 22 days, was too short for a consideration of multiplication of *M. leprae in vitro.* However, the avid phagocytosis and clarity for microscopic observation strongly suggests that schwannoma cells provide a useful substrate for cultivation of *M. leprae in vitro,* and for a sequential study if they can be maintained for a few months. Such cultures may also be useful in monitoring the effect of chemotherapeutic agents on *M. leprae.* It is relevant to recall here the *in vitro* cultivation of leprosy bacilli on a hyaluronic acid-based medium [31], the rich content of β-glucuronidase (another lysosomal enzyme) in or around these bacilli and the control of infection by the administration of vitamin C which inhibits this enzyme [25].

It is difficult to comment on the lysosomal activity evidenced by the strong acid phosphatase reaction [12] invariably observed in the Schwann cells grown *in vitro.* The granular appearance and intracytoplasmic location of the reaction product was identical to that seen in Schwann cells and macrophages, in nerves and skin lesions of untreated lepromatous and tuberculoid patients [6]. The two negative features that have now clearly emerged are: a) the acid phosphatase reaction was not related to the presence of bacilli since glass slips unexposed to mycobacteria were stained for this enzyme, which b) was also not detected even in rapidly multiplying

fetal fibroblasts in similar cultures. Similarly in human leprous tissue, fibroblasts at sites of active collagenosis fail to show this enzyme activity. One is led to postulate that cells with a phagocytic potentiality are endowed with lysosomal enzymes, which the marker reaction (acid phosphatase) detects when these cells are metabolically active and even when they have nothing in their milieu requiring engulfment or digestion ([3]).

SUMMARY

Tissue cultures of five acoustic and four spinal schwannomas demonstrated good growth and migration of Schwann cells within two to four days. Two types of cells corresponding to Antoni type A tissue and Antoni type B tissue were clearly recognized. Both these cell types showed avid phagocytosis when the cultures were inoculated with mycobacteria, either ICRC bacilli or *M. leprae*. The phagocytic index was 95% at the end of two hours.

The Schwann cells grown *in vitro* also showed intense acid phosphatase reaction with Gomori's stain, suggesting lysosomal activity. Neither this nor any phagocytosis was evidenced by fetal fibroblasts cultured similarly. Cells from two spinal neurofibromas grew and migrated slowly *in vitro* and were mainly fibroblasts.

RESUMEN

El cultivo de cinco schwannomas acústicos y cuatro espinales mostró buen crecimiento y migración de las células de Schwann entre el segundo y el cuarto día del cultivo. Claramente se observaron dos tipos de células correspondientes, respectivamente, a los tejidos A y B de Antoni. Ambos tipos celulares fueron altamente fagocíticos cuando los cultivos se inocularon con micobacterias, ya fueron bacilos ICRC o *M. leprae*. El índice fagocítico fue del 95% al final de 2 horas.

Las células de Schwann crecidas *in vitro* también mostraron una intensa actividad de fosfatasa ácida con la tinción de Gomori, sugestiva de actividad lisosomal. Los fibroblastos fetales cultivados de manera similar no mostraron evidencias ni de fagocitósis ni de actividad lisosomal. En el cultivo de 2 neurofibromas espinales, las células crecieron y migraron lentamente. Estas células fueron principalmente fibroblastos.

RÉSUMÉ

Les cultures de tissus préparés à partir de cinq schwannomes acoustiques et de quatre schwannomes médullaires ont montré une croissance excellente, ainsi qu'une migration des cellules de Schwann dans les deux à quatre jours. Deux types de cellules ont pu être clairement mises en évidence, qui correspondent au tissu d'Antoni type A et au tissu d'Antoni type B. L'un et l'autre de ces types cellulaires ont montré une phagocytose intense lorsque les cultures sont inoculées avec des mycobactéries, qu'ils s'agissent de bacilles ICRC ou de *M. leprae*. L'index phagocytaire était de 95% après deux heures.

Les cellules de Schwann poussées *in vitro* ont également montré une réaction forte à l'acide phosphatase avec le colorant de Gomori, ce qui suggère une activité lysosomiale. Cette coloration, pas plus qu'une phagocytose, n'ont pu être mises en évidence dans des cultures de fibroblastes foetaux cultivés de manière similaire. Les cellules provenant de deux neurofibromes médullaires ont poussé et migré lentement *in vitro;* il s'agissait essentiellement de fibroblastes.

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REFERENCES

1. Bapat, C. V., Ranadive, K. J. and Khanolkar, V. R. Growth characteristics of an acid-fast mycobacterium isolated from human lepromatous leprosy. Int. J. Lepr. **29** (1969) 329-342.
2. Cravioto, H. and Lockwood, R. The behavior of acoustic neuroma in tissue culture. Acta Neuropathol. **12** (1969) 141-157.
3. Dastur, D. K. Leprosy—An infectious and immunological disorder of the nervous system. *In:* Handbook of Clinical Neurology, P. J. Vinken and G. W. Bruyn, eds., Amsterdam: North Holland Publishing Co., vol. 21, 1977, in press.
4. Dastur, D. K. The motor unit in leprous neuritis: a clinicopathological study. Neurol. India **4** (1956) 1-27.
5. Dastur, D. K. The peripheral neuropathology of leprosy. *In:* Symposium on Leprosy, N. H. Antia and D. K. Dastur, eds., Bombay: Bombay University Press, 1967, pp 57-71.

6. DASTUR, D. K. and DABHOLKAR, A. S. Histochemistry of leprous nerves and skin lesions. Acid phosphatase. J. Pathol. **113** (1974) 69-77.
7. DASTUR, D. K. and LALITHA, V. S. Pathological analysis of intracranial space-occupying lesions in 1000 cases including children. Part 3. Vascular tumors and hamartomas; hemangiomas; schwannomas. J. Neurol. Sci. **11** (1970) 501-535.
8. DASTUR, D. K., PANDYA, S. S. and ANTIA, N. H. Nerves in the arm in leprosy. II. Pathology, pathogenesis and clinical correlations. Int. J. Lepr. **38** (1970) 30-48.
9. DASTUR, D. K., RAMAMOHAN, Y. and DABHOLKAR, A. S. Some neuropathologic and cellular aspects of leprosy. *In:* Progress Research, E. Jucker, ed., Basel: Birkhauser Verlag, vol. 18, 1974, pp 53-75.
10. DASTUR, D. K., RAMAMOHAN, Y. and SHAH, J. S. Ultrastructure of lepromatous nerves. Neural pathogenesis in leprosy. Int. J. Lepr. **41** (1973) 47-80.
11. DASTUR, D. K., SINH, G. and PANDYA, S. K. Melanotic tumor of the acoustic nerve. Case report. J. Neurosurg. **27** (1967) 166-170.
12. DE DUVE, C. Lysosomes—a new group of cytoplasmic particles. *In:* Subcellular Particles, T. Mayashi, ed., New York: Ronald Press, 1959, p 128.
13. DEVILLE, J. *In vitro* behavior of macrophages from healthy persons against *M. leprae* and other mycobacteria. Int. J. Lepr. **39** (1971) 329-339.
14. FILDES, C. Organized nerve tissue cultures infected with *Mycobacterium leprae* and *Mycobacterium lepraemurium*. Int. J. Lepr. **42** (1974) 154-161.
15. GOMORI, G. The lead nitrate method for acid phosphatase. *In:* Histochemistry. Theoretical and Applied, by A. G. E. Pearse, 3rd edit., London: J. & A. Churchill, Ltd, vol. 1, 1968, p 728.
16. JOB, C. K. *Mycobacterium leprae* in nerve lesions in lepromatous leprosy. An electron microscopic study. Arch. Pathol. **89** (1970) 195-207.
17. JOB, C. K. and DESIKAN, K. V. Pathologic changes and their distribution in peripheral nerves in lepromatous leprosy. Int. J. Lepr. **36** (1968) 257-270.
18. KHANOLKAR, V. R. Pathology of leprosy. *In:* Leprosy in Theory and Practice, R. G. Cochrane and T. F. Davey, eds., Bristol: John Wright & Sons, 1964, pp 125-151.
19. LALITHA, V. S. Space-occupying lesions of human central nervous system. Part 2. Ph.D. Thesis, University of Bombay, 1975, pp 223-244.
20. LUMSDEN, C. E. Leprosy and the Schwann cell. *In:* Leprosy in Theory and Practice, R. G. Cochrane and T. F. Davey, eds., Bristol: John Wright & Sons, 1964, pp 221-250.
21. LUMSDEN, C. E. New horizons in the physiology of the glia. *In:* Livre Jubilaire du Dr. Ludo van Bogaert, Brussels: Acta Medical Belgica, 1962, p 515.
22. LUMSDEN, C. E. The study by tissue culture of tumors of the nervous system. *In:* Pathology of Tumors of the Nervous System, 3rd edit., London: Edward Arnold, 1971, pp 334-420.
23. LUMSDEN, C. E. Tissue culture of brain tumors. *In:* Handbook of Clinical Neurology, P. J. Vinken and G. W. Bruyn, eds., Amsterdam: North Holland Publishing Co., vol. 17, part 2, 1974, pp 42-103.
24. LUMSDEN, C. E. and POMERAT, C. M. Normal oligodendrocytes in tissue culture. Exp. Cell Res. **2** (1951) 103-114.
25. MATSUO, E., SKINSNES, O. K. and CHANG, P. H. C. Acid mucopolysaccharide metabolism in leprosy. 3. Hyaluronic acid mycobacterial growth enhancement, and growth suppression by saccharic acid and vitamin C as inhibitors of β-glucuronidase. Int. J. Lepr. **43** (1975) 1-13.
26. NISHIURA, M. The electron microscopic basis of the pathology of leprosy. Int. J. Lepr. **28** (1960) 357-400.
27. PALMER, E., REES, R. J. W. and WEDDELL, G. The phagocytic activity of Schwann cells. *In:* Cytology of Nervous Tissue. Rep. Ann. Mtg. Anatomical Soc. (1961) 49.
28. PAUL, J. *Cell Tissue and Culture,* 4th edit., London: E & S Livingstone, 1970, pp 172-202.
29. RANADIVE, K. J. Experimental studies on human leprosy. *In:* The Pathogenesis of Leprosy, G. E. W. Wolstenholme and M. O'Connor, eds., London: J & A Churchill, 1963.
30. SHARENBERG, K. and LISS, L. *Neuroectodermal Tumors of the Central and Peripheral Nervous System,* Baltimore: Williams & Wilkins, 1969, pp 69-77.
31. SKINSNES, O. K., MATSUO, E., CHANG, P. H. C. and ANDERSSON, B. *In vitro* cultivation of leprosy bacilli on hyaluronic acid based medium. Preliminary report. Int. J. Lepr. **43** (1975) 193-203.
32. WEDDELL, G., PALMER, E., REES, R. J. W. and JAMISON, D. G. Experimental observations related to the histopathology of leprosy. *In:* The Pathogenesis of Leprosy, G. E. W. Wolstenholme and M. O'Connor, eds., London: J & A Churchill, 1963, pp 3-15.