RSSDI Oration

DIABETIC PERIPHERAL NEUROPATHY

A Brief review of the Pathology and Pathogenesis

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Peripheral neuropathy caused by Diabetes (DM) was recognised only in 1864 by Marchel de Calvi.[1] Till then it was assumed that diabetes was caused by disease of the nervous system. However, once the relationship was rightly recognised, much documentary evidence soon emerged regarding the various clinical manifestations occuring in diabetic peripheral neuropathy. Thus, the loss of tendon reflexes in the legs was described by Bouchard (1887),[2] similarities to tabes stressed by Althaus (1885)[3], spontaneous pain and hyperesthesia by Pavy (1885)* (1904)[6] and motor manifestations by Bruns (1890)[6] and Charcot (1890)' and cranial nerve involvement by Ogle (1896).[8]

While Leyden (1893)[9] and Pryce (1893)[10] set out a classification of the different manifestations of the disease, is was Rundles[11] who in 1945 first drew attention to the autonomic nerve involvement in diabetes. Later, scientists turned their interest to the aetiopathogenetic mechanisms resulting in peripheral neuropathy. This in turn gave impetus to the experimental production of diabetic neuropathy in order to understand the evolution of the disease. Though a large volume of work has been carried out in this regard and many problems solved, many questions remain unanswered as yet, such as for instance the exact relationship between the control of glycemia and the development of neuropathy.

Epidemiology

Definitive statistics on the incidence of DN are not available in many countries for various reasons. Numerous variations exist varying from one extreme i.e., less than 5% (Rundles, 1945).[11] Michon and associates, (1961)[12] to nearly 60% (Fagerberg, 1959)[13] and 90% by Skillman et al (1961).[14] Further, Pirarts' (1978)[15] extensive studies have shown increased prevalence of neuropathy with increasing duration of diabetes from 7.5% at the time of diagnosis to 50% after 25 years of disease. Additionally, while one recent survey (Boulton et al, 1985)[16] has indicated a prevalence of symptomatic diabetic neuropathy in 10.7% of insulin treated diabetic patients, in asymptomatic patients, demonstrable signs of nerve damage could be seen in 19.6%. In addition, if electrophysiological

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studies are to be taken as positive indication of early nerve damage, then, abnormalities of electrical nerve conduction appear to occur in approximately 80% of all diabetic subjects (Ward 1985).[17] However, in clinical practice, consideration is given only to subjects who present with symptoms or in whom physical signs of nerve damage are demonstrable and this generally works out to nearly 20% of all diabetics. It is pertinent to add that the prevalence of neuropathy in diabetics treated with oral drugs or insulin appears remarkably different. Thus in a case study series in Austria (Derfler et al, 1984)[18] where 13.4 and 12.2% of patients based in rural and urban areas exhibited neuropathy it was interesting to record that, while 9% patients treated with oral agents had neuropathy, 49% of patients on insulin developed neuropathy.

In South India, a frequency of 32% was found in patients with tropical pancreatic diabetes (Mohan et al 1984).[19]

Sex incidence: The incidence of neuropathy appears to be similar in both sexes.

Age incidence: Increasing age appears to favour development of neuropathy. Thus Martin (1953)[20] recorded neuropathy in 85% of diabetics over the age of 40. One has to be cautious however in interpretation of neuropathy in the aged since absence of ankle jerks and loss of vibrations sense may be seen in normal aging as well.

In children and adolescents with diabetes, neuropathy appears to be uncommon: a rate of 2% has been recorded by Jordon (1936)[21] Rudy and Epstein (1945),[22] Martin (1953)[20] and Hoffman (1964)."

Classification: Due to the variations in signs and symptoms which develop in diabetic peripheral neuropathy, various different types of classifications have been attempted from time, e.g., Leyden (1893),[9] Pryce (1893),[10] Sullivan (1958)[2]* Fry, Hardwiek and Scott (I962)[25] Gillian (1965)[26] Bruyn and Garland 1970.[27] One main reason for the lack of consistency in classifications, is "the failure to demonstrate the regular occurrence of constellations of clinical manifestations" as observed by Pirart (1965).[28] However, Thomas (1973),[28] advocated a broad subdivision into symmetrical polyneuropathies on the one hand and mononeuropathies and multiple mononeuropathies on the other,

Symmetrical polyneuropathy

Sensory polyneuropathy

Autonomic Neuropathy

Mononeuropathy and multiple mononeuropathy

Cranial nerve lesions
Isolated peripheral nerve lesions
Diabetic amyotrophy

This classification had won wide support for long. However, a more recent classification has been suggested by Ward (1985)[1,] which is as follows:

1. Metabolic reversible neuropathy (newly diagnosed diabetes)
2. Insidious, unremitting sensory neuropathy
3. Acute painful neuropathy
4. Progressive motor neuropathy
5. Insulin neuritis (The proximal muscle)
6. Amyotrophy of Garland (A syndrome)
7. Numb Neuropathic foot & leg
8. Focal mononeuropathies
9. Atypical claudication
10. Impotence
11. Autonomic neuropathy

1. *Early reversible metabolic neurophathy:* In 35% of newly diagnosed patients with tingling, weakness, cramps, shooting pains etc all symptoms suggestive of nerve dysfunction may be demonstrated as per Wards observations. It appears that at this stage, adequate blood glucose control ensures reversion back to normal indicating the presence of a reversible metabolic abnormality such as for instance in the sorbitol and myo-inositol metabolism.

2. *Insidious, unremitting sensory neuropathy:* appears to be the most common clinical picture. The symptoms include very severe shooting pain, tingling, hyperaesthesia and muscle cramps worsening in the night. These persist for long periods of time for even more than 5 years and there appears to be no satisfactory treatment for this.

3. *Acute painful neuropathy:* This is rare and rather dramatic in onset. There is often a sudden onset of very severe pain in the legs usually the thighs associated with depression and loss of weight. Recovery occurs within a year in the majority, especially with adequate control of blood glucose levels.

4. *Progressive motor neuropathy:* Here, there is generalised muscle weakness and wasting developing within a few months. No correlation appears to exist with control of sugar levels. Small vessels in the sural nerve have shown occlusion with endothelial cell swelling and platelet plugs.

5. *Insulin neuritis:* Here neuritic symptoms occur soon after the control of blood glucose levels. Ward suggests that in this situation a nerve exposed to high levels of glucose and sorbitol with myo-inositol depletion over many years facing a sudden drop in glucose level cannot compensate adequately and other pathological changes intervene.

6. *Amyotrophy of Garland:* The proximal leg syndrome. This appears to occur during times of poor sugar control when there is sudden onset of extreme pain and wasting in one or both thighs and severe weakness.

7. *Numb Neuropathic foot and leg:* Warm insensitive leg, or ulcerated and infected foot may be seen. Some while insensitive in the foot, appear to have severe pain *the painful painless foot.* There may be an interaction of peripheral nerve damage, autonomic dysfunction and abnormal vascular factors.

8. *Focal mononeuropathies:* One single nerve may undergo damage in some cases. This may be the result of local pressure upon the nerve such as for instance the carpel tunnel syndrome. However vascular occlusions have been demonstrated in many patients.

9. *Atypical claudication:* In treated cases of diabetes with totally normal blood flow, pain in the calves may occur on exercise and this may be similar to that seen in intermittent claudication. In some patients, leg pain and discomfort may occur for the first time after diabetic ketoacidosis and in these patients small vessel occlusions have been demonstable after the onset of symptoms.

10. *Impotence:* 50% of diabetics males over the age of 40 develop impotence. This could be due to both peripheral and autonomic nerve damage, decreased blood flow locally, aggravated especially by psychological factors.

11. *Autonomic neuropathy:* In a number of patients, abnormalities of autonomic function tests, both sympathetic and parasympathetic may be demonstrable. However, the clinical syndromes are rare.

Pathology

The most characteristic lesions occur in the peripheral nerves and the blood vessels supplying them. Further, changes can be seen to occur in the myelin, axon, dorsal root ganglion and even in the spinal cord. Peripheral nerve

degeneration has been recorded by Auche (1890),[30] Eichorst (1892)[81] Pryce (1893)[10] Marinesco (1903)[3]* Wolfman and Wilder (1929)'[8] Changes in the dorsal root ganglia or the spinal cord were recorded by Williamson (1894)[35] (1904)[36], Kraus (1922)[37], lower motor and first sensory neuron by Greenbaum (1964)[38] Segmented demyelination predicted by Gilliatt (1965)[26] was found to be present on teased fibre examination by Thomas and Lascellets (1965)[39] (1966)[40] Chopra et al (1969)[41] and Chopra and Fennin (1971).*[2] Hypertrophic change* in the form of concentric Schwann cell proliferation can also occur as recorded by Thomas and Lascelles (1966)[40].

Axon loss is a conspicuous feature as noted by Greenbaum et al (1964)[38] Chopra et al (1969)[41] though axon atrophy has not been documented. Axonal degenerative changes are conspicuous in the periphery, but can also occur in the spinal roots, especially in the dorsal roots as demonstrated by Olsson et al (1968).[43]

Loss of dorsal root ganglion cells and anterior horn cells can also occur as shown by Alderman (1930)[44] Bosanquet and Henson (1954)[46], Greenbaum et al (1964).[88]

Degeneration of the posterior column of the spinal cord has been demonstrated by Olsson[43] and is thought to be secondary to loss of dorsal root ganglion cells.

Blood vessel changes:

While atherosclerotic changes, absolutely indentical to those occurring in the non diabetic can occur in the diabetic, other changes occur characteristically and consistently. Thus there is narrowing of the vessel lumina due to thickening and hyalinization of the walls. The thickening is seen in the capillary basement membrane and occurs in many tissues such as blood capillaries, renal glomeruli and tubules, alveoli, cornea, lens capsule, retina and muscle among others. Siperstein (1972)[4]' showed presence of PAS positive material in them and this was at one time thought to be specific for diabetics but is now known to be present in some other conditions as well.

At the ultra structural level, reduplication of the basal lamina around capillaries has been shown to occur by Williamson and colleagues (1969).[47] Kilo et al (1972)"[18] have reported that muscle capillary basement membrane thickness correlated with the duration of glucose intolerance and the age of the patient.

Danowski et al (1972)[49], Pardo et al (1972)'°, Siess et al (1979)[51] have also confirmed the association of capillary basement membrane thickening and duration of diabetes.

Pathogenesis

Though voluminous work has been recorded ever since the first documentation on diabetic neuropathy, many challenges still remain unanswered. Thus till date, no conclusive understanding has been obtained in regard to the pathogenetic mechanisms setting into motion the changes of neuropathy. Various theories have been proposed from time to time to correlate signs and symptoms with lesions. The lesions which predominantly occur in the peripheral nerves include demyelination, axon degeneration and vessel wall degeneration. Which among these lesions could be deemed to have occured first is the question waiting to be answered. The following theories have been propounded none of which appear satisfactory till date.

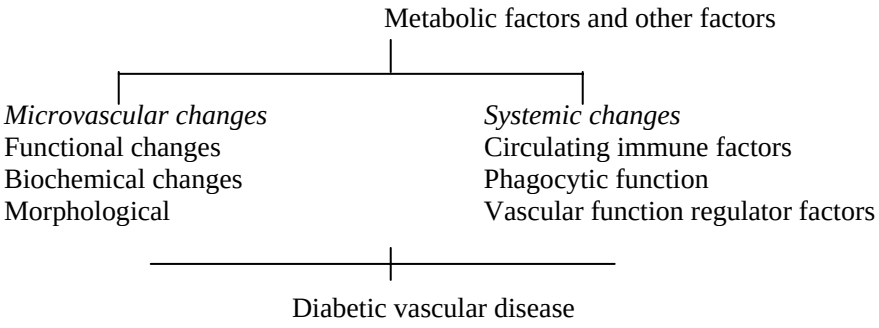
1. Vascular Hypothesis
2. Myelin theory
3. Axon theory
4. Biochemical theories
    - i) Enzymatic deficiencies
    - ii) Osmotic effects
    - iii) Abnormalities in Transport mechanism
5. Spinal ganglion theory

1. *Vascular Theory:* It was suggested that the histological features seen in the blood vessels of affected nerves were severe enough and were almost always demonstrably present to cause ischaemia, suggesting a vascular pathogenesis (Dreyfus et al 1957," Raff etal!968[63] Asbury et al 1970)[54]. Thus a vascular theory could explain the neuropathy in the form of ischaemia or occlussive vascular disease. The endothelial cell proliferation associated with the PAS positive material could cause narrowing of lumina resulting in ischaemia. However though delayed motor conduction could be demonstrated in diabetic patients, symmetrical distribution of lesions is unlikely to occur as a result of single blood vessel occlusion. To quote Dyke et al, "The selective involvement of sensory and autonomic nerve fibres in the symmetrical form of diabetic poly-neuropathy mimics a "system degeneration" and argues in favour of a metabolic basis. Further, the early involvement of autonomic function is evidence against a vascular basis, since unmyelinated axons are relatively resistant to ischaemia.

2. *Schwann cell theory:* The accumulation of much complex lipid in the cytoplasm of the Schwann cells (Bischoff, 1967)[68] gave rise to the hypothesis that this material could interfere with the normal metabolism of the Schwann cell,

Further it was thought that there could perhaps be an abnormality in the lipid metabolism of Schwann cell with resulting lipid accumulation and subsequent breakdown. However, no definite evidence has accumulated so far as to how this occurs and what the abnormality is, if any. This awaits further confirmation.

3. *Biochemical Theories:* These suggest that metabolic factors together with other factors lead on the one hand, to microvascular changes and on the other, to systemic changes and these are probably responsible for the development of diabetic vascular disease.



While no conclusions can be drawn as yet, further work is necessary in the form of studies on experimentally produced diabetic neuropathy to understand the pathogenesis of neuropathy.

Examination of the nerves obtained from diabetic patients of long standing diabetes, either controlled or uncontrolled have disclosed various changes involving the myelin, axon, peripheral ganglion, spinal cord and even the blood vessels supplying the nerve. However, these represent the result of metabolic changes of long standing diabetes and extensive studies of these human nerves may be insufficient to allow conclusions in regard to the onset and evolution of the disease. Thus experimental induction studies of chronic diabetic states in the laboratory animals with subsequent scientific indepth studies carried out at planned intervals would appear to be important sources of meaningful information. It is with such an intention that experiments were planned and undertaken with the commonly available Wistar rats. It is understood at the same time that man is no mean mouse and therefore the results obtained cannot be extrapolated to the human, yet one can perhaps obtain some insight into the problem of pathogenesis.

Material and Methods

Groups of wistar strain albino rats of both sexes weighing between 100-120 gms each and of approximately identical age namely 11-12 weeks were taken up

for study. While one group was made diabetic by intravenous injection of alloxan monohydrate in a dose of 40 mg/kg body wt, the other group was set aside to serve as control. Both groups were given standard lab diet. The diabetic animals were sacrificed at intervals of 1 week from the first to 25th week and the tissues were obtained from the sciatic and tibial nerves and spinal rat ganglia, for light and electron microscopy.

Human Material

Sural nerves removed for biopsy from the patients of long standing diabetes with fair control of their diabetic state were received for both light and electron microscopy. The specimens were received in 2% glutaraldehyde at 3 degree centigrade and were sectioned off for optical and electron microscopy. The specimen for optical microscopy were processed in the standard manner and the latter as per methodology described earlier (Sarasa Bharati and S. Arumugam).[56]

Light Microscopy Appearances

Rat Nerve: The nerve as a whole showed reduction in width though no definite morphometric studies were undertaken. There appeared to be an increase in the space between the axons. This could especially be appreciated in the thin cross sections. In rats of upto 15-20 weeks, an increase in the fibro-collagenous tissue could be seen. Single fibre teasing showed demyelination of varying degrees at varying intervals. It appeared to be of a segmental type in many areas.

In some cases, Schwann cell hyperplasia of varying degrees though never striking enough to form 'onion bulbs' could be seen.

In the human, demyelination often of a segmental type could clearly be demonstrated (Fig 1). In addition areas of what appeared to be remyelination

Fig 1. Demyelination. LFB PASX600.

could also be seen. Further, in most cases, there was active Wallerian type degeneration as well.

Axons: In the rats, axon loss was not impressive though present. Most of the loss could be seen to have occurred in the periphery, though some loss could be seen in the region of the spinal roots as well.

Spinal cord: Degeneration of the posterior column could be seen in 3 animals sacrificed after 25 weeks.

Changes in the anterior horn cell

Though absolute cell loss could not be definitely be made out, mild non specific change such as chromatolysis could be seen occasionally. This could be due to the fact that no morphometric studies were undertaken.

Posterior root ganglia: Here again, the changes were minimal and nonspecific in most cases. Complete loss of ganglion cells could however be seen in 3 cases.

Blood vessels: These did not show much change in the experimental animal. However in the human, a variety of changes were seen. While almost all vessels appeared narrowed with thickening of their walls, near occlusion could be demonstrated in 2 cases, PAS stains showed presence of PAS positive material in the walls.

Ultrastructural changes: Changes were demonstrable in almost all the parts of the tissues examined in the rat. Changes were seen in the Schwann cell cytoplasm, myelin, axon and its exoplasmic organelles, blood vessels, basement membrane and the endothelial cell.

Schwann cell: Changes in the Schwann cell were very remarkable. There was a varying degree of enlargement of the cytoplasm which could be seen from small protrusions to remarkable sizes. These were of an irregular nature and contained a variety of organelles and inclusions (Fig 2). While some showed presence of large amount of granular material of low electron density others showed presence of lipid of varying configurations (Fig 3). The granular material was seen in some cases to occupy even the mitochondria. The lipid was seen in two forms either as grey or dense areas surrounded by unit membranes or as irregular myelin figures. These latter had a periodical lamellar structure comparable to that seen in myelin.

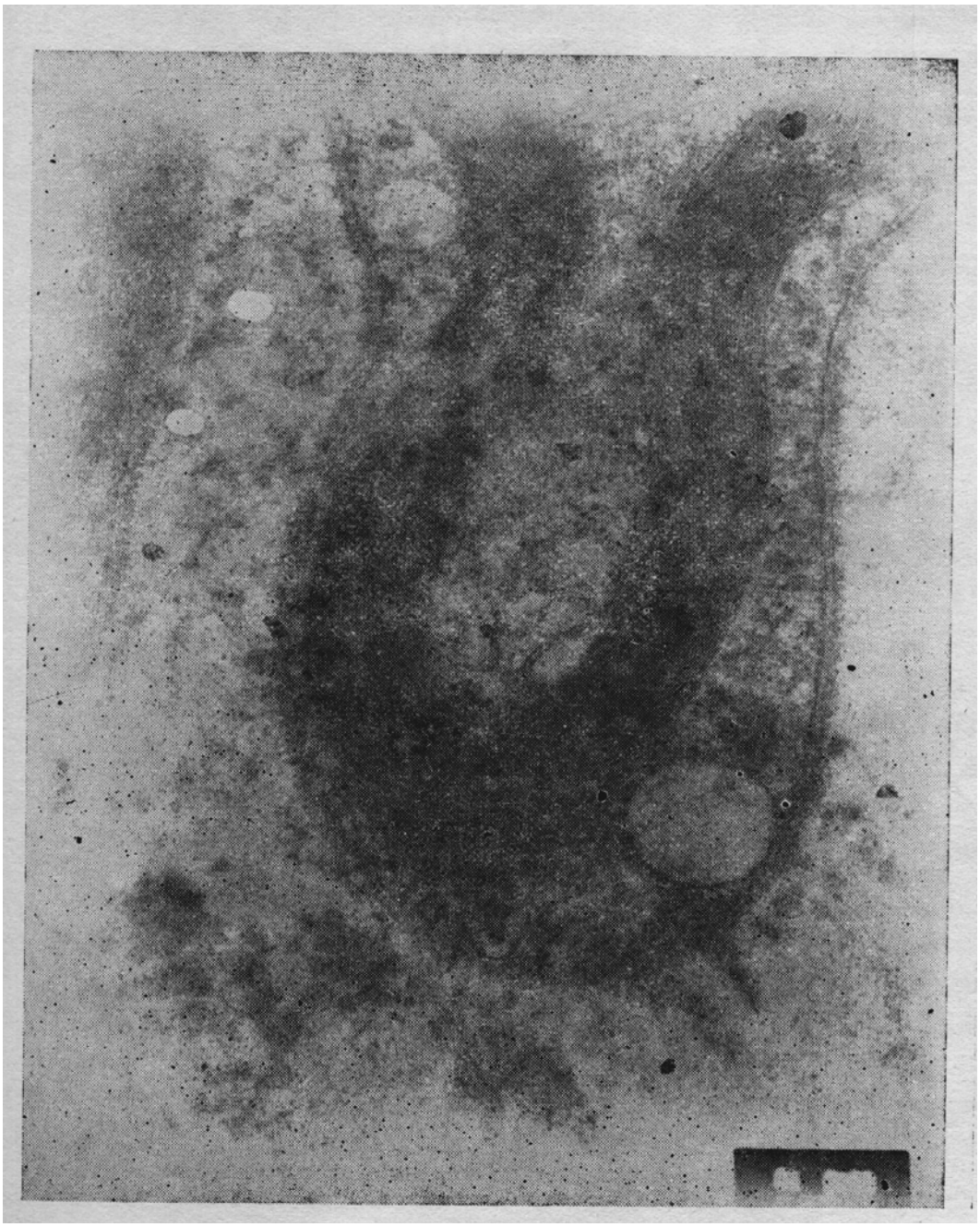


Fig 2. Schwann celicytoplasm-swollen with accumulate material X 11100

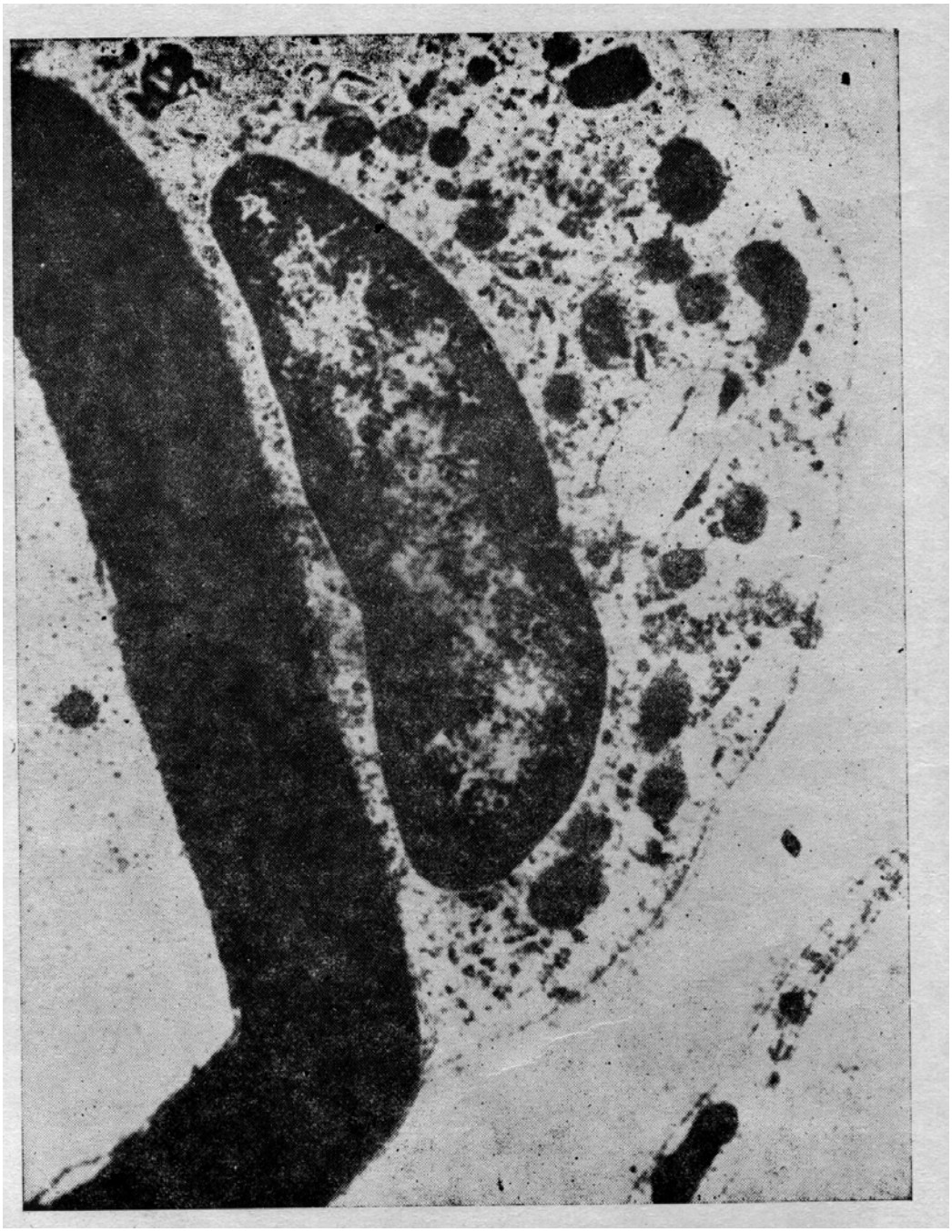


Fig 3. Schwann cell cytoplasm with lipid%11200

In chronic cases Schwann cell hyperplasia in the form of membrane bound slender processes originating from the inner Schwann cell lip and forming honeycomb profiles could be seen in addition (Fig 4). Various irregularities of myelin in the form of retraction, wrinkling, splitting and disruption could also be seen.

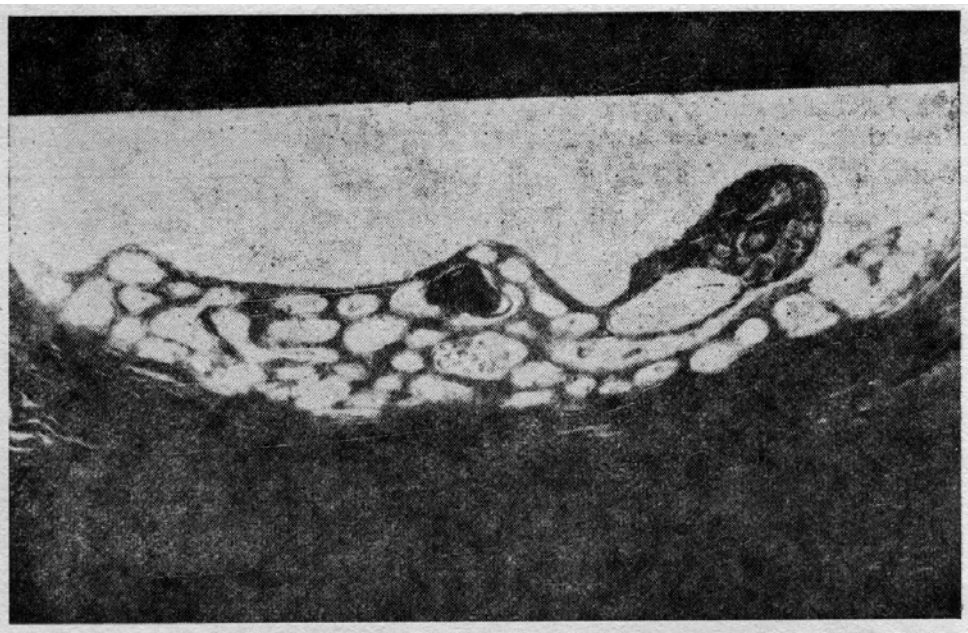


Fig 4. Honey comb profile in Axon × 13200

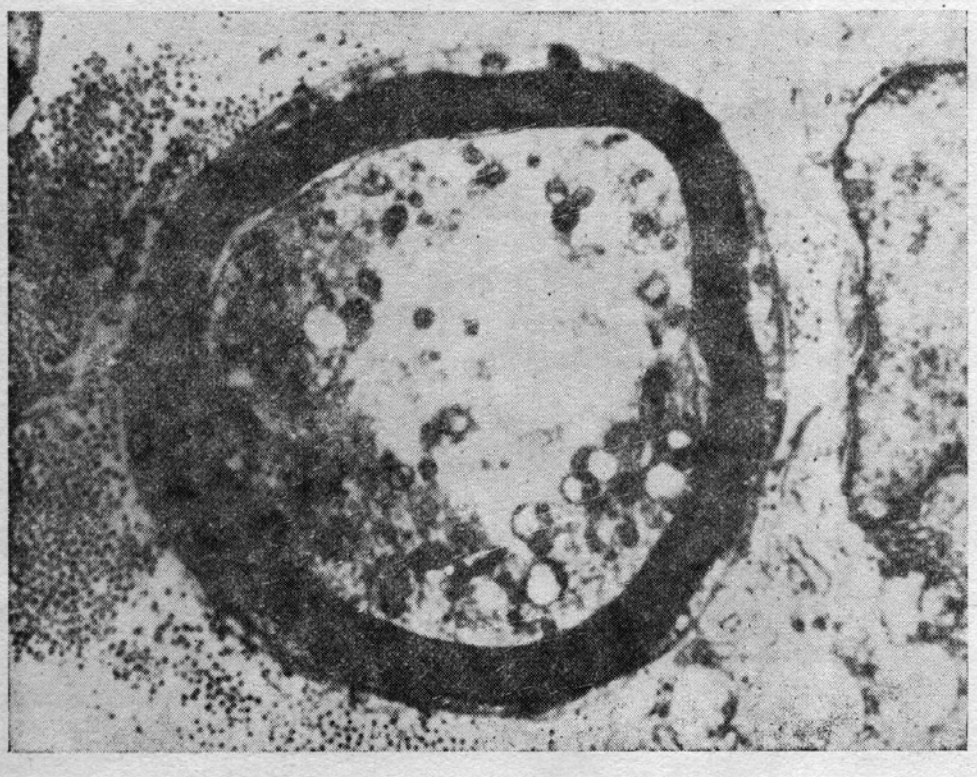


Fig. 5. Axon with degenerated material × 8800

The axons showed a number of changes. Many appeared swollen with ballooned appearance, seen in both longitudinal and cross sections. There appeared to be a variable increase in the number of neurofilaments, microtubules, dense bodies, vesciles and mitochondria. In others, there was granular degeneration of the tubules and filaments. In the early cases, large accumulations of electron dense granular material-glycogen? sorbitol was seen (Fig 5). Later

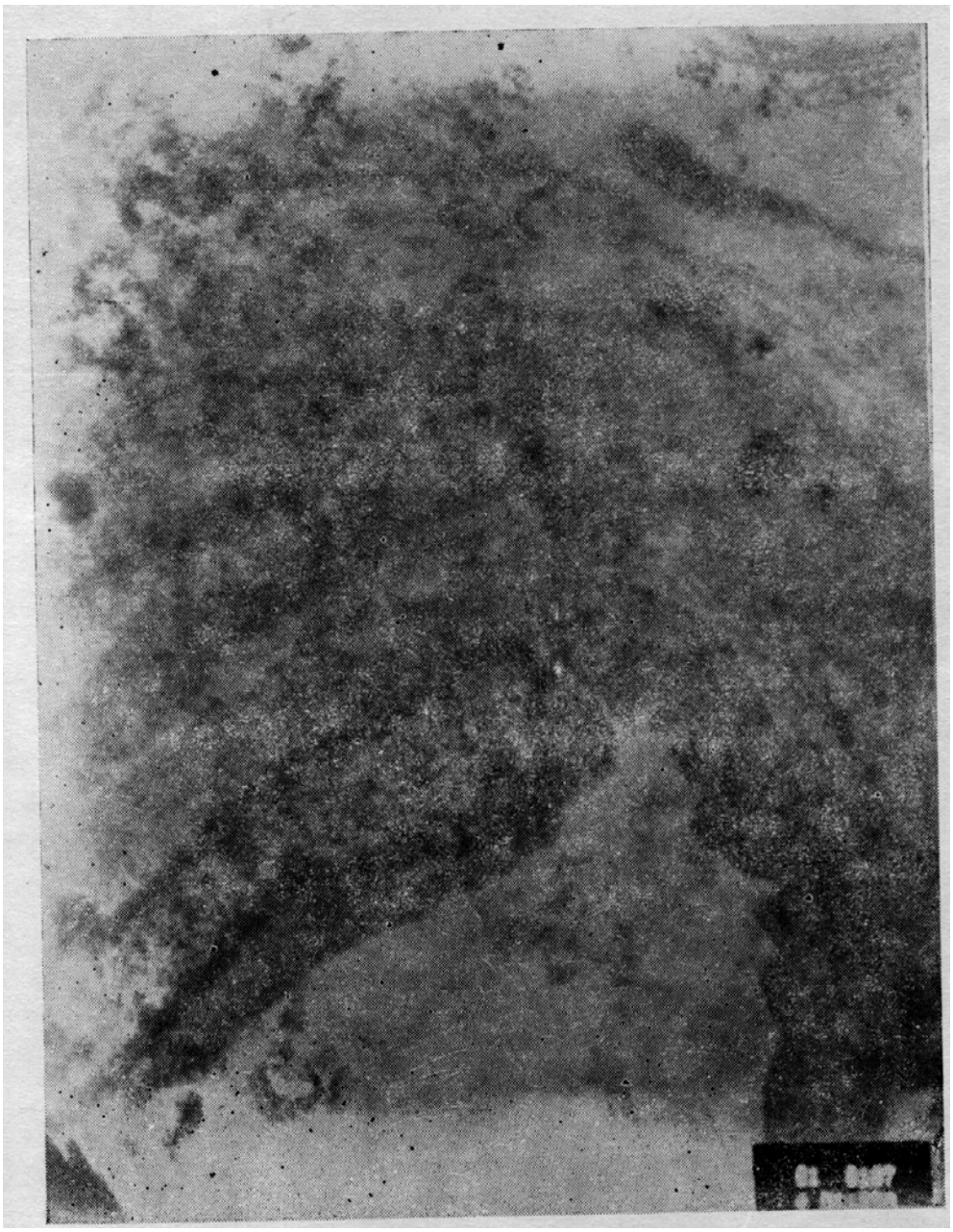


Fig 6. Blood vessel with reduplicated basement membrane material X 8800

cases showed massive aggregates of these electron dense granular materials. Penetration of axon by Schwann cell and degeneration of the sequestrated axoplasm were also evident. Surrounding the axons were thinned out myelin lamellae and macrophages.

Blood vessels . The vessels showed varying degrees of reduplication of basement membrane (Fig 6). Electron dense deposits were seen accumulated between layers of basement membrane material. In addition, the cytoplasm of the endothelial cells were filled with electron dense granular material. Further, proteinacious material was also seen in the lumen of the vessel.

The granular material was sometimes seen to fill almost completely some of the organelles such as the mitochondria.

In the human

Changes in the axons were quite marked. There was varying degree of ballooning of the cytoplasm which however was filled with extremely fine material. Degenerative material could also be seen in varying degrees.

Changes in the myelin: These were striking; varying degree of thinning, retraction, curling, breaking up wrinkling, etc. were very pronounced. Multiple Schwann cell cytoplasmic protrusions were seen, containing swollen organnelles and finely granular material. Further, myelin figures and vast quantities of lipid material could be seen in most areas (Fig 15).

Changes in the blood vessels: Marked thickening of the blood vessel walls, with multiple layers of basement material could be seen. Endothelial cell hyperplasia with degenerate material in the lumina could also be seen. In addition there was accumulation of electron dense material both in the interstices of the basement membrane as well as in the endothelial cell cytoplasm and more importantly in the mitochondria.

From the above it appears that a theory as to the pathogenetic mechanism could be suggested tentatively namely that the accumulation of sorbitol in the organelles could perhaps render normal metabolism impossible which therefore could result in necrosis and degeneration triggering hyperplasia of Schwann cell on the one hand and/or endothelial proliferation on the other, the latter resulting in ischaemia with its resultant ill effects.

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