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## Ultrastructural Changes in the Myelinated Nerve Fibers of the Sciatic Nerve in Galactose Intoxication in Rats

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**Abstract:** The objectives were to study the ultrastructural changes in the myelinated nerve fibers in an animal model of galactosaemia. The study was done in the Anatomy Department, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. Twenty-four adult male albino rats were used (6 control and 12 experimental animals). Galactosaemia was induced by adding 40% D-galactose to the rats' diet for 2 months. Sciatic nerves of the control and experimental animals were removed and processed for electron microscopic study. Four months following galactosaemia, the myelinated nerve fibers showed cytoplasmic vacuoles in Schwann cells, myelin degeneration, axonal retraction and destruction of the myelinated axons. 6 months after induction, some myelinated nerve fibers showed disruption of myelin sheaths with marked shrinkage of the axons. The endoneurial edema was prominent and some regenerating nerve fibers were reported. 8 months latter: the endoneurial and intra-axonal oedema accumulated more. Schwann cells showed cytoplasmic degenerated myelin, fat vacuoles and accumulation of fine glycogen granules in the axoplasm. It could be concluded that in galactose intoxication induced degenerative changes in the myelinated nerve fibers. It showed also decrease in diameters of the myelinated nerve fibers and axons.

**Key words:** Sciatic nerve, galactose intoxication, schwann cells, Endoneurial oedema

### INTRODUCTION

Specific sequence of histopathological events occurs following peripheral nerve injury, which eventually results in the full or partial regeneration of the injured nerve. In order to achieve a successful nerve repair, the injured tissue needs to be cleaned and the axonal growth-inhibiting myelin debris must be removed Grados-Munro and Fournier (2003). Schwann cells are the main glial cells of the peripheral nervous system. They are responsible for protection and support of the axons and for the synthesis of myelin. Consequently they are important for nerve regeneration following nerve injury (Marcol *et al.*, 2003). They play a crucial role in the process of axonal regeneration where nerve injury stimulate proliferation and activation of Schwann cells in the injured nerve fibers and synthesis of S-100 protein (Hu *et al.*, 2003; Mimura *et al.*, 2004).

In humans, mutations in the galactokinase gene can lead to the diseased state referred to as Type II galactosaemia (Liu *et al.*, 2003). Galactokinase deficiency is a rare autosomal recessive inborn error of galactose metabolism Thoden and Holden (2003) resulting in the accumulation of galactose or galactose-1-phosphate in the blood and tissues (Lai *et al.*, 2003a). The use of galactosaemia as a model for some aspects of diabetic polyneuropathy allows the influence of glycation to be studied independently of other effects (Lai *et al.*, 2003b). There are well-studied abnormalities of the peripheral nerves in galactosaemic rats, one of which is that the efficiency of regeneration is initially reduced (King *et al.*, 2002). One possible cause could be that glycated myelin debris in macrophages is less

degradable and interferes with macrophage function. Macrophage recognition and ingestion of myelin glycosylated in vitro increases with the duration of incubation in a sugar-rich medium (Arn, 2003). Galactosemia has good prognosis, if detected in neonatal period or early infancy where elimination of milk from diet is quite simple and effective treatment modality (Afzal, 2003).

Hyperglycemia results in reductions in nerve conduction velocity probably due to accumulation of polyol pathway metabolites (Gabbay and Snider, 1972; Thomas *et al.*, 1981). There are several reports of decreased nerve blood flow (Cameron *et al.*, 1991) and reduced caliber of nerve fibers (Nukada *et al.*, 1986) in hyperglycaemic animals. It was also reported that ischemia is characteristic of hyperglycaemic models (Cameron *et al.*, 1991; Nukada *et al.*, 1986). They suggested that the reduced nerve blood flow is associated with decreased caliber and the caliber reductions are responsible for the decreased nerve conduction velocity, or that the conduction deficits are caused by similar mechanisms. The aim of the present study was to investigate the ultrastructural changes in the myelinated nerve fibers in galactose intoxication and consequently, their possible roles in delayed nerve regeneration in hyperglycaemia.

### MATERIALS AND METHODS

The study was done in the Anatomy Department, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. Twenty-four adult male albino rats weighed (200-250 gm) were used (6 normal control and 18 experimental animals). Galactosaemia was induced by

adding 40% D-galactose to the rats' diet for 2 months. Hyperglycaemia was tested by withdrawal of blood from the tail vein and blood sugar was monitored by gluco-test. The blood sugar in the studied mice should exceed 300 mg/L to be diagnosed as hyperglycemic. After 4, 6 and 8 months from induction of galactosaemia, 6 of the experimental animals in each time were anesthetized by intra-peritoneal injection of a solution containing pentobarbital 12.5 mg/ml and diazepam 1.25 mg/ml in 0.9% saline at a dose of 2 ml/kg. The sciatic nerves of the diabetic and the control animals were removed and transferred to 2.5% phosphate buffered glutaraldehyde. Following at least 24 h of fixation, nerves were put in osmic acid, dehydrated, and embedded in araldite resin. Semithin section (1 micron) were obtained for light microscopic morphometric and statistical studies. Ultrathin sections (0.06 micron) were obtained using ultramicrotome and stained with uranyl acetate and lead citrate for electron microscopic study to obtain the structural changes in the myelinated nerve fibers. Morphometric studies were performed using computerized image analysis system. Three fields of view of per each sciatic nerve section were captured under a 100<sub>oil</sub> immersion objective. The diameters of 75 myelinated nerve fibers and axons (regenerating and original) were measured per animal; their means and standard deviation were calculated. Significance of

difference between the normal and galactosaemic nerves was evaluated using student's t-test. For the multiple comparisons, one way Analysis of Variance (ANOVA) was used. Significant differences were determined (Turkey Simultaneous Tests) using the Minitab 13 computing program.  $p < 0.05$  or  $p < 0.005$  were considered statistically significant.

## RESULTS

**Ultrastructure of normal sciatic nerves:** The endoneurium showed normal perineurium, myelinated nerve axons and Schwann cells. Collagen bundles were compacted in the endoneurium and in the subperineurial space (Fig. 1a).

**Ultrastructural changes in sciatic nerves of the hyperglycemic rats:** After 4 months, Few of Schwann cells showed asymmetric cytoplasmic hypertrophy with increased number of cytoplasmic processes. The endoneurium appeared oedematous, this was indicated by the wide separation of the collagen bundles. Such oedema was more prominent in the subperineurial space (Fig. 1b). Other Schwann cells showed cytoplasmic electron-dense degenerated myelin, electron-lucent fat vacuoles (Fig. 2a). The degenerative changes in Schwann cells and myelinated nerve axons were not uniform throughout the endoneurium but affected sporadic myelinated nerve fibers. Other nerve

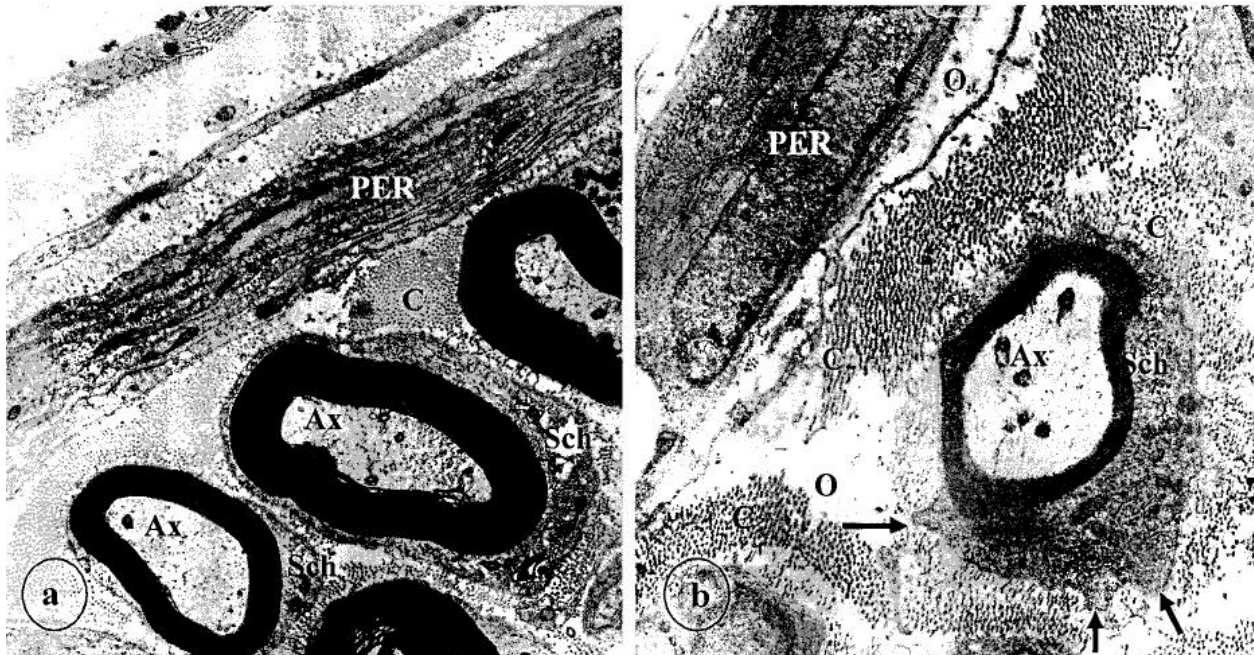


Fig. 1: Electron photomicrographs of transverse sections sciatic nerves. Figure 1a shows normal Perineurium (PER), myelinated nerve axons (Ax) and Schwann cells (Sch). Collagen bundles (C) are compacted in the endoneurium and in the subperineurial space. Fig. 1b - Four months after galactose intoxication showing cytoplasmic asymmetrical hypertrophy of some of Schwann cell (Sch) with multiple cytoplasmic processes (arrows). Collagen bundles (C) are widely separated from the perineurium and from each other indicating accumulation of endoneurial oedema (O). Scale bar: 1  $\mu$ m

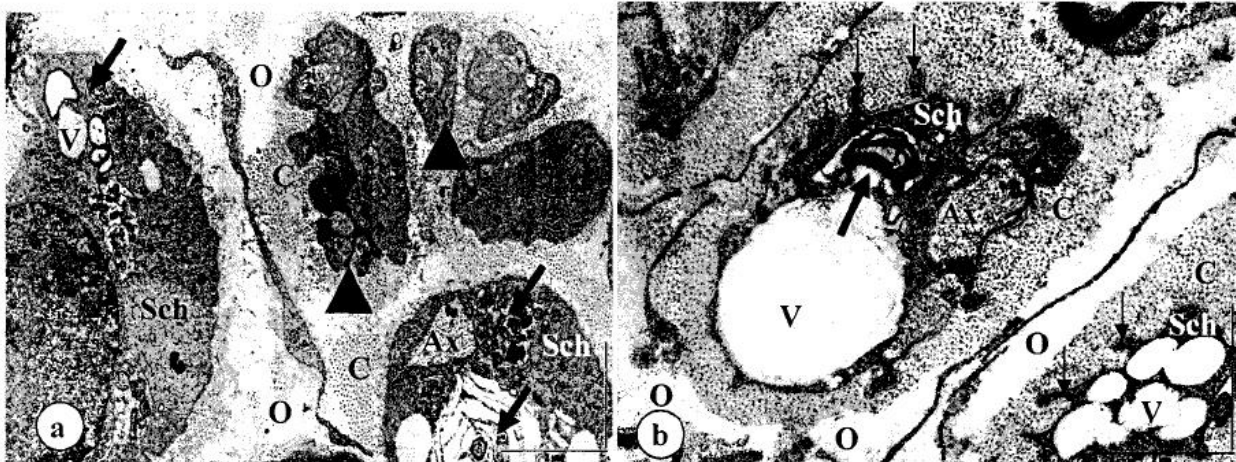


Fig. 2: Fig. 2a - After 4 months of galactose intoxication and Fig. 2b - After 6 months of galactose intoxication) show degenerative changes in Schwann cells in the form of cytoplasmic vacuoles (V) and degenerated myelin (thick arrows). It shows also demyelinated nerve axons (Ax) and endoneurial oedema (O). Figure 2a shows regenerating small diametered nerve fibers. Figure 2b showed multiple cytoplasmic processes of Schwann cell (thin arrows). Scale bars: 1  $\mu$ m

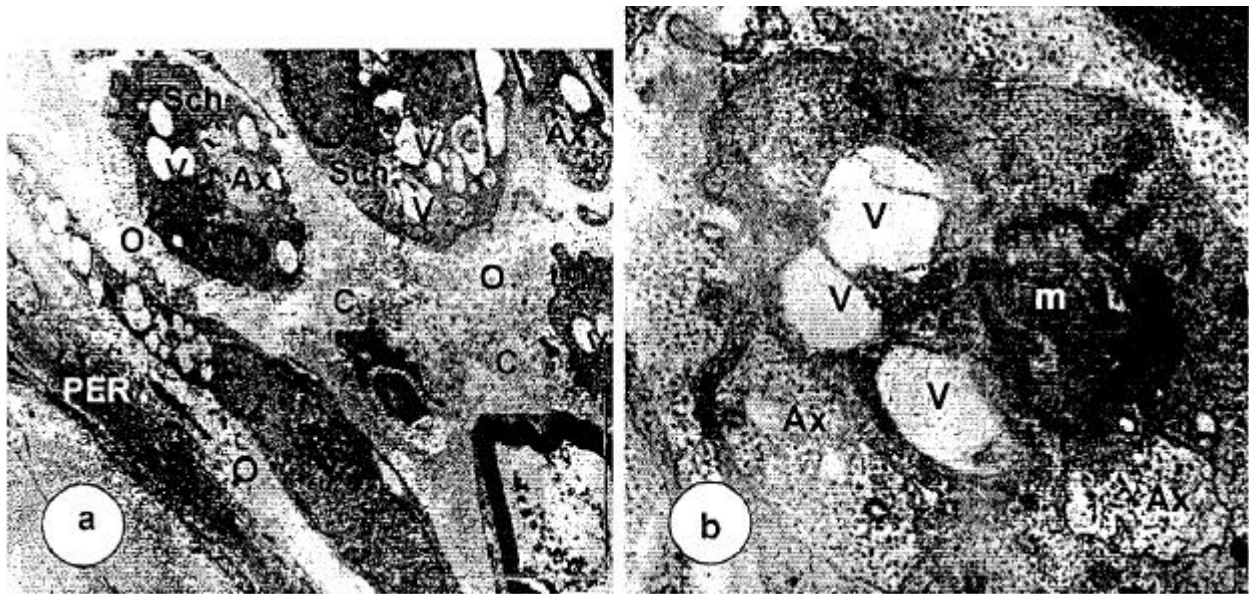


Fig. 3: 6 months after galactose intoxication. Figure 3a shows degenerative changes in Schwann cells (Sch) of myelinated nerve fibers in the form of several electronlucent vacuoles (V). It shows the wide separation of collagen bundles (C) that indicates accumulation of endoneurial oedema. Figure 3b shows Schwann cells containing cytoplasmic electronlucent vacuoles (V), degenerated myelin (m). It wraps demyelinated nerve axons (Ax). Scale bars: a, 5  $\mu$ m; b, 2  $\mu$ m

fibers appeared normal. There was also increased demyelinated nerve axons and regenerating myelinated nerve fibers of smaller diameters (Fig. 2a). After six months of galactose intoxication, some of the myelinated nerve axons showed demyelination. Schwann cells showed also marked degenerative changes in the form of multiple cytoplasmic electronlucent fat vacuoles and electrondense

degenerated myelin lamellae. They showed also multiple cytoplasmic processes (Fig. 2b, 3a and 3b). There was retraction of the axoplasm of some myelinated nerve axons with accumulation of periaxonal oedema (Fig. 4 and 5). Accumulation of endoneurial oedema that was more prominent in the subperineurial space and in the endoneurium among the nerve fibers. Such oedema contained electrondense remnants of the

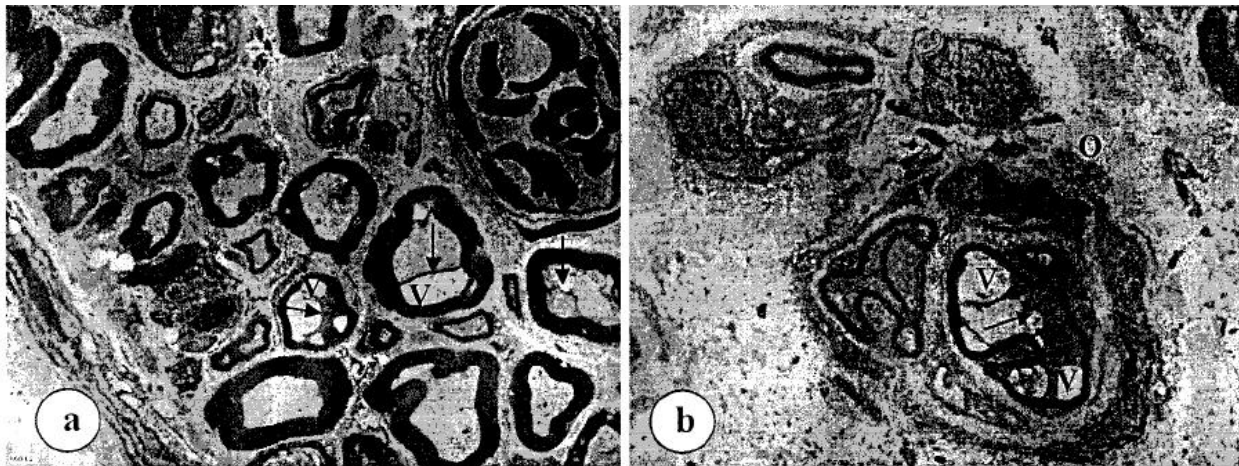


Fig. 4: (4a and 4b) 6 months after galactose intoxication. They show retraction (arrows) of myelinated nerve axoplasm (A), there are electronlucent areas containing electrondense particles separating the axoplasm from the myelin indicating periaxonal oedema (V). O: endoneurial oedema. Scale bars: 5  $\mu$ m

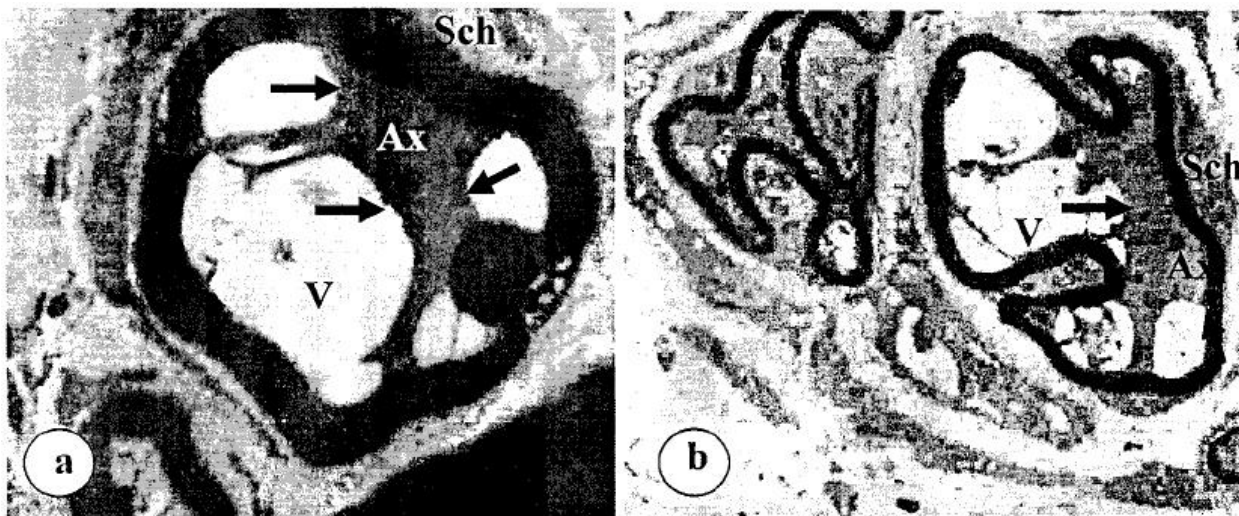


Fig. 5: Figure 5a and b are magnified parts of Fig. 4a and b respectively show retraction (arrows) of the axoplasm (Ax) of the myelinated nerve fibers. The electronlucent areas between the axoplasm and the myelin sheath contain electrondense particles indicating the presence of periaxonal oedema (O). The surrounding Schwann cells (Sch) appear normal

degenerated myelin lamellae. Accumulation of endoneurial and subperineurial oedema was noticed in most of the studied sections (Fig. 6).

After eight months of galactose intoxication, Schwann cells showed cytoplasmic degenerated myelin and multiple electronlucent vacuoles. They wrapped demyelinated small diametered nerve axons (Fig. 6b and 7). Some myelinated nerve axons showed degenerative changes and were replaced by electronlucent fat vacuoles and degenerated myelin lamellae. Even in the same affected nerve fibers, degenerated nerve axons were surrounded by normal Schwann cells (Fig. 8). There was an increase in the number of the small diametered thinly myelinated

regenerating nerve fibers. Endoneurial oedema was more prominent in some of the studied specimens among the nerve fibers and collagen bundles specially in the subperineurial spaces (Fig. 7 and 8).

**Morphometric and statistic study of the myelinated nerve fibers:** There was a significant decrease ( $p < 0.05$  and  $p < 0.005$ ) in the diameters of the myelinated nerve fibers in the diabetic animals which were at a mean of  $7.32 \pm 0.02 \mu\text{m}$  (ranging from 5.26 to 10) compared to those of the myelinated axons of the normal control animals that were at a mean of  $9.23 \pm 0.01 \mu\text{m}$  (ranging from 5.45 to 12.18) which was a significant decrease. In the galactosaemic rats, the diameters of the myelinated



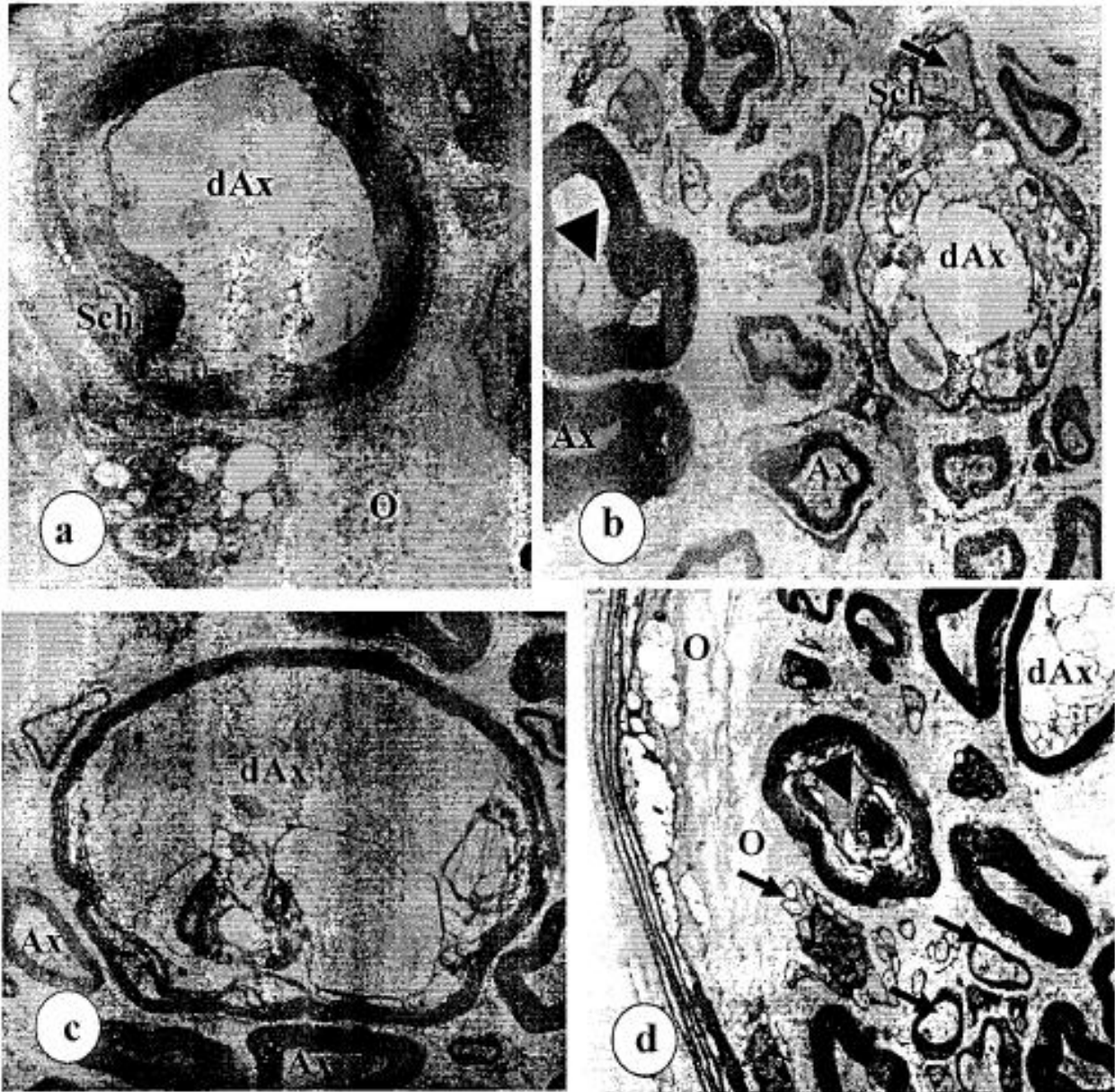


Fig. 8: Eight months after galactose intoxication. It shows degenerated myelinated nerve axons (dAx), retraction of the axoplasm (arrows heads) which are surrounded by normal Schwann cells. It also shows normal myelinated nerve axons (Ax), subperineurial and endoneurial oedema (O) and regenerating nerve axons (arrows). Scale bars: a,b,c, 1 μm; d, 10 μm

Table 1: Diameters of the galactosaemic and normal nerve fibers and axons (in μm)

Galactosemia duration	The diameters of the myelinated nerve fibers (μm)		The diameters of the myelinated nerve axons (μm)		The normal diameters (μm)		
	Number	Mean±SD	Number	Mean±SD	Number	Nerve fibers Mean±SD	Nerve axons Mean±SD
4 months	450	7.87±0.02*	450	7.21±0.01*	150	9.12±0.01	8.72±0.06
6 months	450	7.20±0.03*	450	6.54±0.02**	150	9.54±0.01	7.53±0.06
8 months	450	6.75±0.01*	450	5.56±0.04*	150	8.89±0.01	7.21±0.06
Mean±SD		7.32±0.02*		6.56±0.47*		9.23±0.01	7.88±0.06

(\*p<0.05, \*\*p<0.005). p<0.05 and p<0.005 are considered significant

myelinated nerve axons were significantly reduced compared to the normal control. Such a decrease can be helpful in explanation of the reductions in nerve conduction velocity recorded by several authors (Gabbay and Snider, 1972; Thomas *et al.*, 1981).

According to the current study, some nerve fibers in sciatic nerves of the galactosaemia rats showed degenerative changes such as demyelination or disruption of the myelin of the myelinated nerve fibers. Other Schwann cells showed increased amount of cytoplasmic electronlucent fat vacuoles of different sizes which could be attributed to the degeneration of the myelin sheaths of the myelinated nerve axons. These degenerative changes were reported after one week of galactose intoxication (Mizisin and Powell, 1997) and after 2, 4 and 24 months after galactose intoxication (Kalichman *et al.*, 1998). The cytoplasmic processes of Schwann cells wrapped the regenerating or demyelinated nerve axons. Some of the degenerated Schwann cells wrapped quite apparently normal but demyelinated nerve axons. This might indicate that Schwann cells in galactosaemic nerves are more vulnerable or more affected than the wrapped myelinated axons which may or may not be affected consequently. This was specially apparent 4 and 8 months after galactose intoxication. On the other hand, 8 months after intoxication, degenerated myelinated nerve axons were frequently encountered and were wrapped by apparently normal Schwann cells. Consequently, It could be suggested that axonal injury does not necessarily associate Schwann cell injury while degenerative changes in Schwann cells affect demyelination and re-myelination of the nerve axons. Most of nerve axons wrapped by degenerating Schwann cells were demyelinated axons with small diameters. Schwann cell injury could result in subsequent axonal injury due to stimulation of aldolase reductase enzyme which appeared to be localized to the myelinating Schwann cells (Schroder and Sommer, 1991).

According to the current study Schwann cells of sciatic nerves of galactosaemic animals possessed cytoplasmic processes that wrapped the regenerating nerve axons. This might result in asymmetric hypertrophy of their cytoplasm reported by several authors (Sima *et al.*, 1983). Such recorded asymmetric hypertrophy of Schwann cells in different types of neuropathies could be an evidence of the occurrence of regeneration of the nerve fibers in cases of galactosaemia. However, many of the regenerating nerve fibers showed small diameters which might indicate the slow process of regeneration in galactosaemic peripheral nerves. The degenerative changes recorded in Schwann cells during the current study could explain at least partially such delayed regeneration of the nerve axons and consequently the

reduced diameter of the regenerating myelinated nerve fiber and their axons. It also explain the presence of small diametered demyelinated nerve axons in the degenerated Schwann as seen in the present work. Accumulations of Schwann cell cytoplasmic lipid droplets and degenerated myelin have been reported to occur in a variety of experimental and clinical neuropathies (Feldman *et al.*, 1992; Goodrum *et al.*, 1990). Moreover such degenerative changes in Schwann cells together with swollen mitochondria have been reported in both aging (Choo *et al.*, 1990) and a neurotoxic disorder (Anderson *et al.*, 1994) and in different types of neuropathies (Schroder *et al.*, 1996; Sima *et al.*, 1983).

Consequent to the current study, some myelinated axons specially 8 months after intoxication, showed retraction of their axoplasm which ranged from mild to severe retraction. The spaces between the axolemma and the myelin sheaths were electronlucent containing electrondense particles suggesting the accumulation of periaxonal oedema. Such periaxonal oedema and retraction of the axoplasm could add another explanation for the reduced calibers of myelinated nerve axons in galactose intoxication. Such reduced caliber can explain the reduction in nerve conduction in the nerves of the galactose and streptozotocin models recorded by several authors (Nukada *et al.*, 1986).

The endoneurial oedema in hyperglycemic nerves is a matter of debate. Edematous nerves were considered to be characteristic of the galactose model; however, few authors have denied its presence in diabetic neuropathy (Sima *et al.*, 1983). Griffey *et al.* (1987) demonstrated that sural nerves of human diabetics can have elevated water contents and that this elevation is ameliorated by treatment with an aldose reductase inhibitor. Further, Eaton *et al.* (1996) used magnetic resonance image to reveal that sural nerve hydration is significantly increased in both asymptomatic and symptomatic diabetic patients. It is noteworthy that these authors (Griffey *et al.*, 1987; Eaton *et al.*, 1996) found that a subpopulation of asymptomatic patients had water contents in excess of two standard deviations greater than controls.

In the current study, it was observed in the galactosaemic rats that the collagen bundles in the endoneurium of the sciatic nerves were more widely separated than in the control nerves which indicate the accumulation of endoneurial oedema. The latter was more apparent deep to the perineurium in the subperineurial space and to a lesser extend among the nerve fibers. The distribution of endoneurial oedema in the present study was not even in the different parts of the endoneurium. Its accumulation in the endoneurium might lead the reduced subperineurial and to a lesser extent the centrifascicular blood flow. Also It can reduce



the endoneurial blood flow (McManis *et al.*, 1986). Consequently, it can play a major part in the degenerative changes in Schwann cell and myelinated nerve axons recorded during the current study. It also add third explanation to the reduced diameter of the myelinated nerve fibers and axons. It can also be an important factor in the pathogenesis of neuropathy in hyperglycemic rats which supports the reported conclusion of other authors, that ischemia is characteristic of hyperglycaemic models (Cameron *et al.*, 1991; Nukada *et al.*, 1986).

Mizisin and Powell (1997) did not report the presence of the endoneurial oedema 1 week after galactose intoxication but recorded the presence of peri-axonal and mitochondrial swelling. Kalichman *et al.* (1998) reported the occurrence of endoneurial oedema in the diabetic and galactosaemic rats. its accumulation of will reduce the endoneurial blood flow and causes ischemia (McManis *et al.*, 1986).

The degenerative changes in the myelinated nerve fibers of the peripheral nerve of galactosemic rats could be due to the accumulation of galactose and galactose metabolites. The galactose metabolites Gal-1-P, galactitol and UDPgal play an important role in this toxicity and proposed mechanisms include interference with cells apoptosis (Bhat, 2003). Other authors (Bhat, 2003; Lai and Elsas, 2000; Lai *et al.*, 2003a,b; Slepak, 2005) postulated that the molecular link between defective GALT enzyme, which result in classic galactosemia and the cerebroside galactosyl transferase, which is responsible for galactosylation of cerebroside is dependent on the cellular concentrations of UDP-galactose. They further hypothesized that a threshold concentration of UDP-galactose exist below which the integrity of cerebroside galactosylation suffers.

It was reported that the above-mentioned disorders are ameliorated by treatment with inhibitors of aldose reductase, the first enzyme of the polyol pathway (Tilton *et al.*, 1989). Schwann cell has been further implicated in diabetic neuropathy because it is the primary intrafascicular location for the first enzyme of the polyol pathway, aldose reductase, which appears to have a role in modulating a variety of complications of diabetes, including diabetic neuropathy (Klinchman *et al.*, 1998; Goodrum *et al.*, 1990). Based on fine-structural observations in nerves seen in galactose-fed rats and these were reported also in adult-onset diabetic patients emphasizes the role of flux through aldose reductase in the complex pathology of diabetic neuropathy and points to the utility of galactose intoxication in helping to understand this metabolic changes (Bhat, 2003; Liu *et al.*, 2000; Wehrli *et al.*, 2007).

Patients with an inherited deficiency of Galactokinase (GALK) do not manifest either the acute toxicity

syndrome or chronic complications seen in galactosaemic patients (Schroder *et al.*, 1996; Lebea and Pretorius, 2005; Segal and Berry, 1995). Since GALK-deficient patients do not accumulate gal-1-p in their tissues (Schroder *et al.*, 1996), researchers have suggested that gal-1-p plays a significant role in the pathogenesis of Galactosaemia (Bhat, 2003; Ideker *et al.*, 2001). It could be a cause of the recorded small diametered regenerating nerve fibers in the current study specially 8 months after galactose intoxication.

It could be concluded that galactose intoxication resulted in degenerative changes in both Schwann cells and the myelinated nerve axons. Retraction of some myelinated nerve axons, the small diametered regenerating nerve and demyelinated axons can explain the decrease in their diameters in galactose intoxication. Accumulated endoneurial oedema may have an impact on the endoneurial blood flow and can delay nerve fibers regeneration together with degenerations of Schwann cells. All these observations can explain the reduced nerve conduction reported in hyperglycemic patients.

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