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Biopsy Diagnosis of Peripheral Neuropathy

Second Edition

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Foreword

Neurologists specializing on the peripheral nervous system like to boast that theirs is the only area of neurology with no "black box": All connections are known and there is a good understanding of the correlation between the pathological processes, electrophysiological findings, and clinical signs and symptoms. However, prior to the 1995 publication of Midroni and Bilbao "Biopsy Diagnosis of Peripheral Neuropathy" pathological diagnosis was a muddy field obscured by the absence of guidance on how to handle and examine the biopsy, scarcity of high quality images, and over-emphasis on morphometry and nerve fiber teasing. Their book allowed those of us entering the field from different disciplines to decide when to order a nerve biopsy, and to fully understand the clinical implications of the findings on the one hand, and provided us with the intellectual tools to reach a pathological diagnosis on the other. Almost 20 years have gone by, and molecular genetics is now a major player in the diagnosis of peripheral neuropathy. While the number of nerve biopsies has decreased, their importance has not. Furthermore the detailed understanding of the underlying pathological process is critical to the care of patients with peripheral neuropathy, even those who do not undergo a biopsy.

In the new edition, Drs. Bilbao and Schmidt have updated the book, incorporating the molecular advances into the fully integrated contributions of the clinical data and histology to the diagnostic process. They also introduce a condensed review of current developments in the use of skin biopsy for the study of peripheral neuropathy. The book is consistently thorough in its review of the literature, clear in its exposition, and balanced in its assessment of the value of procedures. It is, however, the superb quality of the images that will remain in the minds of most readers as the main vehicle through which understanding of the pathological processes discussed in the book is achieved. The convenience of this monograph lies in that it is also a work of reference, since much of it consists of detailed descriptions of the various clinical and physiopathological manifestations of diseases. I highly recommend this book to any neurologist or pathologist seeking guidance in the study of the peripheral nervous system. It will be as useful to those using a microscope as to those who do not.

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Preface

This book represents a thorough revision of the text (with the addition of new cases) and illustrations of the monograph entitled *Biopsy Diagnosis of Peripheral Neuropathy* published in 1995 and authored by Gyl Midroni and Juan M. Bilbao (Butterworth and Heinemann, publisher). We designed this second edition to present current comprehensive knowledge of the pathology and pathogenetic mechanisms of peripheral nerve disease for pathologists involved in the interpretation of nerve biopsies as well as neurologists, neuropathologists and neuroscientists in training. The first eight chapters present the normal anatomy of peripheral nerve and its cellular constituents, assessment of the biopsy (how to blow away the cobwebs of artefacts), followed by discussions of basic pathologic processes, and of the role of whole nerve biopsy in diagnosis of peripheral neuropathy compared to other techniques of nerve analysis. Following this introduction, the traditional categories of nerve diseases are organized in 13 chapters and the spectrum of microscopic pathology is illustrated profusely.

Over the past 20 years, the impact of molecular genetics on the diagnostic accuracy and understanding of the pathogenesis of peripheral neuropathy has been enormous. The first and still major causal genetic defect for inherited peripheral neuropathies, the CMT1A duplication of the *PMP-22* gene, was discovered in 1991. Since that time, our knowledge of the molecular genetic groundwork of this group of diseases has grown considerably. By applying next-generation genetic techniques, well over 70 causal genes are now identified in patients affected with Charcot-Marie-Tooth disease and cognate disorders alone. Similarly, direct DNA sequencing permits the diagnosis of most of the hereditary amyloidoses that are associated with peripheral neuropathy. Consequently, the indications for nerve biopsy in these suspected entities have sharply declined.

Nonetheless, nerve biopsy represents the gold standard to which other test results and pathogenetic mechanisms have been and are compared. Valid requests for whole nerve biopsy include a search for an interstitial pathological process (vasculitis, and amyloid infiltration in non-secretory myeloma), diagnosis of a hereditary polyneuropathy when genetic-molecular studies are not helpful and the phenotype is atypical, confirmation of the diagnosis of Guillain-Barre syndrome or chronic demyelinating inflammatory polyneuropathy with atypical findings, and diagnosis of a protracted polyneuropathy with negative or incongruous lab studies results (i.e. sarcoidosis, diabetic neuropathy with prominent motor findings). In Chaps. 1 and 17, we discuss the role of skin biopsies in the evaluation of small fiber neuropathy, a technique which may obviate the need for sural nerve biopsy. This method permits visualization and quantification of intraepidermal (unmyelinated) nerve fibers. Furthermore, glabrous skin biopsy has recently been established to be useful for the assessment of dermal myelinated fibers and for the detection of inflammation and Ig deposits. Quantification of the myelinated endings and mechanoreceptors in glabrous skin can expand the role of skin biopsy to include all distal sensory axonopathies. Nonetheless, it remains unproven if the results of skin biopsy alone will provide the overall diagnostic insight possible with whole nerve biopsy.

We are grateful to Ms. Sandra Cohen for providing many of the electron photomicrographs, and to Dr. Gyl Midroni for advice. Dr. Charles Kassardjian gave valuable suggestions in updating Chaps. 1 and 8. We are especially indebted to Drs. Maria Nolano, William Kennedy, and Jun Li for providing many elegant and informative confocal photomicrographs of skin

preparations showing normal and abnormal innervation (see Chap. 1). Drs. Shinji Ohara, Mitsunori Yamada and Hitoshi Takahashi provided us their material for the AMAN case presented. We would also like to thank Karen Green, Chris Dunham, Toral Patel, Connie Marshall and William Kraft, our EM colleagues at Washington University. Ms. Jordana Stewart provided secretarial assistance. Ms. Martina Himberger, who has been in charge of the work for Springer, has been infinitely helpful. Dr. Schmidt would like to thank his wife Pam for her patience and counsel during the preparation of this book and dedicate his work to his children Andrea and David.

Toronto, ON, Canada — Juan M. Bilbao
St. Louis, MO, USA — Robert E. Schmidt

Preface to First Edition

This monograph is directed at neurologists and pathologists who require a work that combines a practical approach to nerve biopsy interpretation in peripheral neuropathy with an overview of the progress that has accumulated over the past two decades in the understanding of peripheral nerve pathobiology. Also covered are clinical features of polyneuropathy and the microscopic anatomy of nerves, both essential to the interpretation of pathological change. In this book attention is on the usefulness and limitations of nerve biopsy as a diagnostic tool, and for a broad perspective, the literature has been extensively reviewed. We hope that our efforts to provide a rich pictorial exposition of peripheral nerve histology and pathology will be helpful to the initiate.

Our material consists of nearly 700 consecutive nerve biopsies collected over a 22-year period at St. Michael's Hospital in Toronto. In the study of this tissue we employ techniques of classical histology, resin histology, immunohistochemistry, electron microscopy, and, to a much lesser degree, morphometry and fiber teasing.

We acknowledge that biopsy of a subcutaneous nerve provides only a window into the rnorphological alterations in peripheral neuropathies; thus throughout the text we refer to other works for a more complete view of the pathology of the human peripheral nervous system.

This work was made possible through the support of the Sisters of St. Joseph, the St. Michael's Hospital Department of Pathology, and Dr. Alan Hudson. Our editor Susan Pioli, who supervised this work from inception, was a constant source of encouragement.

We are grateful to the following pathologists who sent us specimens from conditions not available in our collection: Dimitris Agamanolis (Ohio), George Davidson (Toronto), John Deck (Toronto), Venita Jay (Toronto), Edward Johnson (Edmonton), Jacques Lamarche (Sherbrooke, Quebec), John Maguire (Hamilton), Jean Michaud (Monheal), and David Munoz (London, Ontario).

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Dr. Peter Ashby, Dr. Kalman Kovacs, and Dr. James Perry gave valuable suggestions for the monograph. We thank Dr. Rob Macaulay for his many contributions to this work. Daniel A. Hunter, RT, of Washington University School of Medicine, St. Louis, Missouri, generously performed morphometry. The librarial services at St. Michael's Hospital were critical to the collection of reference material: The authors wish to thank Leica Canada and the Mount Sinai

Hospital for permission to use their equipment. Lianne Friesen and George Trogadis created the original artwork. Dr. Maja Steinlin and Dr. Steffen Albrecht provided German translations.

Sandra M. Cohen wishes to thank Steven M. Doyle of the department of microbiology at the University of Toronto for his invaluable technical assistance and generosity in the loan of a diamond knife.

One of us is a clinical neurologist with a special interest in peripheral neuropathy; the other is a neuropathologist with remote training in clinical neurology and a special interest in the pathology of nerves. Juan M. Bilbao is especially indebted to the late Dr. Morrison Finlayson.

Toronto, ON, Canada Juan M. Bilbao

Contents

Conventions

AMAN	Acute motor axonal neuropathy
CIDP	Chronic inflammatory demyelinating polyradiculoneuropathy
EM	Electron microscopy
GBS	Guillain-Barré Syndrome
H&E	Hematoxylin and eosin
HMSN	Hereditary motor and sensory neuropathy
LCA	Leukocyte common antigen
LM	Light microscopy
MF	Myelinated fiber
NMSC	Non-myelinating Schwann cell
OB	Onion bulb
PAS	Periodic acid-Schiff
PNS	Peripheral nervous system
SC	Schwann cell
ScSu	Schwann cell subunit
UF	Unmyelinated fiber

Peripheral Neuropathy and the Role of Nerve Biopsy

1

1.1 Epidemiology of Peripheral Neuropathy

Diseases of the peripheral nerves represent a significant neurological problem, with the incidence of polyneuropathy in the USA estimated at 40 per 100,000, an incidence comparable to that of epilepsy or parkinsonism (Kurtzke 1982), and an overall prevalence of about 2.4 % in the general population (Martyn and Hughes 1997). Because not all patients are investigated, data regarding the etiologic composition of polyneuropathies is difficult to obtain. For example, Dyck et al. estimated that only 10 % of affected patients in kinships with hypertrophic hereditary motor and sensory neuropathy (HMSN-1 or Charcot–Marie–Tooth disease (CMT) type 1) seek medical attention as a direct consequence of symptoms produced by this disease (Dyck et al. 1993). Many individuals with mild or subclinical neuropathy, whether genetically determined or acquired, may not require investigation or treatment. The largest and best-documented peripheral neuropathy series originate from specialized centers or are part of selected biopsy series and thus are not representative of neuropathy in the general population.

1.1.1 Etiologies of Peripheral Neuropathy

It is helpful to classify the major etiologies of peripheral neuropathy into acquired toxic or metabolic, inflammatory or infectious, neoplastic and paraprotein associated, and genetically determined (see Table 8.4 for details). Acquired toxic or metabolic factors probably account for the majority of neuropathies, and common causes include diabetes, alcoholism, nutritional deficiencies, and pharmaceutical neurotoxins. The most frequent inflammatory and infectious neuropathies are Guillain–Barré syndrome (GBS), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), vasculitic neuropathies, HIV-associated neuropathies, and leprosy. Malignancy is associated with neuropathy through several mechanisms including paraneoplastic phenomena, metabolic derangement, and direct infiltration of nerve by neoplasm. The most common familial neuropathies are CMT types 1 and 2. In the literature, 10–20 % of neuropathies remain cryptogenic (vide infra). Reported series based on consecutive patients from well-defined geographic areas give some insight into the relative frequency of these neuropathies. Prineas (1970) reviewed 278 consecutive English patients hospitalized with a diagnosis of polyneuropathy between 1957 and 1966, excluding 49 patients with questionable or inadequately investigated neuropathies. Approximately 30–35 % of patients had disorders associated with toxic or metabolic causes, with nutritional polyneuropathy and alcoholism forming the largest group. Smaller groups had vitamin B12 deficiency (7 %), drug-induced neuropathies (7 %), malignancy associated (7 %), and diabetes (5 %). Retrospective examination of the data suggests that GBS accounted for 20–25 % and CIDP 7–15 %. Workup of Mayo Clinic patients ($n = 205$) with an undiagnosed peripheral neuropathy eventually resulted in a diagnosis in 76 % (Dyck et al. 1981). CIDP was diagnosed in 21 %, hereditary neuropathy in 42 %, and the remainder (diabetes, toxins, and neoplasia) accounted for 13 %. It is likely that the chronic idiopathic group included some genetically determined neuropathies for which a family history was not obtained.

A very large series (>5,000 cases) including autopsy and nerve biopsy examination resulted in specific diagnoses in only 23 % (Schröder 1998); common diagnoses included vasculitis (64 %), Guillain–Barré (10 %), and amyloid (4 %). In a report on 120 consecutive patients with polyneuropathy seen over a 3-year period in an EMG clinic in Israel, diabetic neuropathy accounted for 22 %, while 20 % had GBS, CIDP, or vasculitis; 13 % were genetically determined, 13 % metabolic, 6 % drug induced, 4 % associated with malignancy, 8 % miscellaneous, and 12 % remained undiagnosed (Argov et al. 1989). A 380-patient prospective peripheral neuropathy series from France revealed alcohol or drug toxicity in 23 % of patients, diabetic neuropathies in 16 %, GBS and CIDP in 16 %, hereditary neuropathies in 10 %, and neuropathies associated with collagen diseases in 10 % (Vallat et al. 1983).

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A large (nearly 1,000 consecutive cases) series of nerve biopsies performed at the University of Toronto established a specific diagnosis in approximately 29 % of cases (Bilbao 2004). Barohn's (1998) experience of 402 consecutive patients referred to the University of Texas neuromuscular outpatient clinics differed, with the most frequent diagnoses being hereditary (30 %), cryptogenic sensory polyneuropathy (23 %), diabetes (15 %), and inflammatory demyelinating polyneuropathy (13 %). Diabetic neuropathy is consistently underestimated in such studies because many diabetic patients are not referred to neurologists, are rarely admitted to hospital because of neuropathy, or are only biopsied if they have an atypical presentation or course.

Thus, despite the absence of information about the types and frequency of polyneuropathy in the general population, a first estimate suggests that in the developed world metabolic (including diabetes), toxic, and nutritional causes account for 50 % or more of neuropathies, inflammatory neuropathies (mainly GBS, CIDP, and vasculitis) for 10–20 %, familial neuropathy for 10–20 %, and neoplasia-associated neuropathy for 5–10%, and approximately 10–20 % remain idiopathic. A large variety of rare neuropathies make up the remaining cases. In pediatric neuropathy, the etiologic spectrum is different, with familial neuropathies predominating (Ouvrier et al. 1990).

Geographic factors are pertinent in determining the relative frequency of neuropathies in a given population. In a large Indian series, 66 % of neuropathies were diabetic, 14 % leprous, and 11 % GBS (Wadia 1984). Leprous, nutritional, and familial neuropathies were underrepresented and GBS overrepresented in this hospital inpatient series. In a 1980 review, Osuntokun (1980) estimated that in Africa 25–40 % of neuropathies seen by neurologists were nutritional, with 5–10 % each due to leprosy, alcoholism, and the Guillain–Barré syndrome. Diabetes was accorded equal ranking with porphyria at about 3 % each! However, this author noted that when all patients are considered, including those not coming to neurologists' attention, leprosy was by far the most important cause of neuropathy, and diabetic neuropathies became more numerous. HIV has become an increasingly important cause of peripheral neuropathy worldwide, with an estimated prevalence of between 10 and 60 % (Schifitto et al. 2002).

1.1.2 Cryptogenic Peripheral Neuropathy

Despite widely variable selection criteria, the proportion of neuropathies which remain cryptogenic is remarkably consistent and ranges from 10 to 25 % (Argov et al. 1989; Corvisier et al. 1987; Grahmann et al. 1991; McLeod et al. 1984; Vallat et al. 1983; Wolfe et al. 1999). Series from the 1950s and 1960s reported 56–70 % of polyneuropathies as cryptogenic (Matthews 1952; Rose 1960), but several currently accepted "etiologies" of peripheral neuropathy were not included, such as GBS, CIDP, paraprotein-associated neuropathy, paraneoplastic neuropathy, as well as the notion of genetically determined neuropathies without a family history (Dyck et al. 1981).

When patients with a chronic idiopathic neuropathy are restudied, an etiologic diagnosis can often be made. McLeod et al. (1984) reported that about 1/3 of their patients with chronic idiopathic neuropathies could be subsequently diagnosed at a 1-year interval, and Grahmann et al. (1991) reported similar numbers. In the previously mentioned review of 205 unclassified patients, an etiologic diagnosis was eventually made in 76 % (Dyck et al. 1981); however, this group was probably not as well studied prior to referral as the cryptogenic patients in the series of McLeod et al. (1984). In contrast to these reports, Jann et al. (2001) found that an etiology could not be determined over a 4-year follow-up period for 40 consecutive patients with idiopathic sensorimotor polyneuropathy. Similarly, Notermans et al. (1994) found an etiology in only 4 out of 75 cases of cryptogenic neuropathy over a 5-year follow-up. These authors suggest that in patients with a minimally progressive polyneuropathy of undetermined cause despite extensive workup, regular repeated investigations may not be warranted.

The terminology for cryptogenic neuropathies has evolved, and the current preferred term is chronic idiopathic axonal polyneuropathy (CIAP) (Singer et al. 2012). Recent data suggest that after a detailed assessment, patients ultimately diagnosed with CIAP tend to have a slowly progressive course, with minimal weakness or disability (Jann et al. 2001; Notermans et al. 1994; Wolfe et al. 1999). Patients with CIAP tend to present in their 50s or 60s with an insidious onset of predominantly sensory symptoms of numbness and paresthesias, occurring in a length-dependent manner. Power is relatively preserved, and reflexes are reduced or absent. Indeed, based on consistent clinical features and course, diagnostic criteria have been proposed to identify CIAP as a distinct clinical entity (Wolfe et al. 1999; Singer et al. 2012).

These studies emphasize the importance of careful history-taking in diagnosis, especially for evidence of a familial neuropathy or of toxic exposure (Dyck et al. 1981; Jann et al. 2001; McLeod et al. 1984). Examination of family members, even if not known to be symptomatic, can prove extremely helpful. In other patients, the etiology becomes clear when malignancy or other systemic conditions emerge, and reassessment is necessary if the clinical course deviates from what is expected in CIAP.

1.2 Usefulness of Nerve Biopsy in Evaluation of Peripheral Neuropathy

For several classes of patients, nerve biopsy is rarely necessary: patients with an established cause of toxic or metabolic neuropathy, patients with a familial neuropathy, those with the Guillain–Barré syndrome, or in patients who are known to have a circulating paraprotein or systemic malignancy. In addition, patients with suspected CIDP may not require biopsy (vide infra). Nevertheless, biopsy continues to have a significant role in the diagnosis of peripheral nerve disease (Table 1.1).

Table 1.1 Specific diagnoses that can be made by nerve biopsy

Inflammatory/infectious
Neuropathy with macrophage-mediated demyelination (CIDP, GBS)
Vasculitic neuropathy
Leprous neuropathy, especially primary neuritic leprosy
Sarcoidosis or granulomatous neuropathy
CMV neuritis in immunosuppressed patients
Neoplasm/paraprotein associated
Non-amyloid paraprotein-associated neuropathies
IgM anti-MAG paraprotein (widely spaced myelin)[a]
POEMS syndrome (uncompacted myelin)[a]
Immunoglobulin deposition disease
Primary amyloidosis
Neoplastic infiltrative neuropathy
Lymphomatoid granulomatosis
Metabolic/toxic
Amiodarone neuropathy
Hexacarbon neuropathy
Genetically determined
Hereditary neuropathy with liability to pressure palsies[b]
Amyloid neuropathy, familial
Giant axonal neuropathy
Neuroaxonal dystrophy
Polyglucosan body disease
Hereditary sensory and autonomic neuropathies
Storage diseases
Metachromatic leukodystrophy[b]
Adrenoleukodystrophy[b]
Globoid cell leukodystrophy[b]
Niemann–Pick disease[b]
Fabry disease[b]
Tangier disease[b]
Neuronal ceroid lipofuscinosis

[a]Strongly but not invariably associated
[b]Noninvasive biochemical or genetic tests may obviate need for tissue examination

1.2.1 Review of Experience at St. Michael's Hospital

1.2.1.1 Histological Material and Clinical Data

Since 1972, the peripheral nerve pathology laboratory at St. Michael's Hospital has processed nearly 700 nerve biopsies. These consist almost exclusively of sural nerves, with infrequent utilization of cutaneous branches from the radial or ulnar nerves, or the lateral peroneal nerve. During this period of time, the same pathologist (JMB) has used a consistent analytic protocol (see Chap. 7) to evaluate all of these cases. The reports of 267 consecutive nerve biopsies examined in the years 1986–1993 were reviewed and the histological changes described classified as either nonspecific (axonal degeneration, segmental demyelination, mixed axonal/demyelinating, and inflammatory or noninflammatory) or findings that permit a specific diagnosis in the absence of clinical information (e.g., vasculitis, amyloid). The clinician most involved in the case used all available information including biopsy findings and follow-up to make the final diagnosis. Based on the available clinical material, the nerve biopsy was classified as being of no value, helpful, or essential for optimal patient management.

A biopsy of no value did not modify management other than providing an impression of the severity and activity of the disease. Helpful biopsies (a) supported the diagnosis of a suspected etiology for which treatment or genetic counseling is available (e.g., CIDP, CMT), (b) ruled out a working diagnosis (e.g., finding of prominent active Wallerian changes in suspected CMT-2), and (c) distinguished between two alternative diagnoses with therapeutic implications (e.g., leprous vs. vasculitic neuropathy in a patient from a leprosy-endemic region). Biopsies essential to management revealed abnormalities that (a) permitted definitive diagnosis of the cause of the neuropathy and (b) revealed suggestive but not completely diagnostic findings, resulting in altered management (e.g., non-caseating granulomata suggesting sarcoidosis or findings typical of CIDP in an "axonal" neuropathy).

1.2.1.2 Results

In a review of nerve biopsies and clinical records for 267 patients with a mean age of 57.5 years (Fig. 1.1), 234 patients had sufficient clinical material to permit accurate clinicopathological correlation. In these 234 patients, a final etiologic diagnosis was reached in 76 % (Table 1.2). Diagnostic findings were seen in 16 % of all nerve biopsies (Table 1.3).

Nerve biopsy was interpreted as essential for management in 48 patients (21 %). In most of these cases, a specific diagnosis was made (e.g., vasculitis, amyloidosis, leprosy). On three occasions, biopsy revealed a picture typical of previously unsuspected CIDP. In one patient clinically diagnosed with CMT-1, biopsy revealed unequivocal

inflammatory features suggestive of CIDP. In two patients with a clinical diagnosis of CMT-2, biopsy revealed additional pathological findings that led to specific disease-modifying treatment; in one patient a toxic medication was discontinued, and in another, steroids were given.

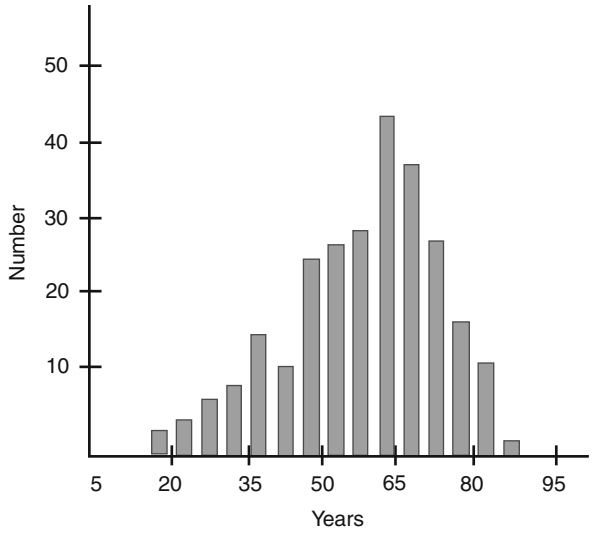

Fig. 1.1 Age distribution of patients for 267 consecutive nerve biopsies at Saint Michael's Hospital 1986–1993

We considered nerve biopsy to be helpful in 52 patients (22 %). In most of these cases, biopsies supported a diagnosis of CIDP, CMT-1, or CMT-2, in other instances as distinguishing between paraneoplastic and chemotherapy-related neuropathy by showing prominent inflammation or confirming the diagnosis of GBS in a patient with atypical history and physical findings. In one patient, nerve biopsy revealed selective small myelinated fiber loss suggestive of amyloidosis, but no amyloid was identified; a persistent search in rectal and muscle tissue indeed confirmed this suspicion.

The 267 consecutive patients studied were identified in the pathology laboratory and thus biased towards diagnostically challenging neuropathies and towards etiologies for which a biopsy is used to confirm a suspected treatable diagnosis. This circumstance presumably accounts for the overrepresentation of CIDP and vasculitis in the final diagnoses and for the relatively high rate of cryptogenic neuropathies (24 %) (Table 1.2). Approximately half of the patients with cryptogenic neuropathy suffered from a minimally disabling and nonprogressive or slowly progressive neuropathy, as has been previously documented (Jann et al. 2001; Wolfe et al. 1999; Grahmann 1991; McLeod et al. 1984).

Analysis of this material suggests that no difference exists in the diagnostic value of nerve biopsy between neuropathies with axonal and demyelinating or mixed electrophysiological

Table 1.2 Clinicopathological correlations in 267 nerve biopsies

		Specific etiology		Nonspecific histology			
				Noninflammatory		Inflammatory	
Histology	*N*	Mixed	Axonal	Mixed/demyel	Axonal	Mixed/demyel	Normal
Final diagnosis obtained (*N* = 177)							
CIDP/GBS)	51	(4)[c]	5	12	7	27	1
Vasculitis	19	19	0	0	0	0	0
Paraprotein	12	2	3	3	1	2	1
NASID	11	0	3	1	4	2	1
Diabetes	10	0	5	2	2	1	0
Toxic/nutritional[a]	10	3	6	1	0	0	0
HMSN-1	5	0	0	5	0	0	0
HMSN-2	7	0	7	0	0	0	0
Amyloid	5	4	1	0	0	0	0
Uremia	4	0	3	1	0	0	0
Paraneoplastic	4	0	2	0	2	0	0
Others[b]	13	10	2	0	1	0	0
Not neuropathy	25	0	7	3	0	0	15
Final diagnosis not obtained (*N* = 90)							
Cryptogenic	52	0	29	10	7	2	4
Insufficient data	33	1	19	2	6	2	3
Random	5	0	2	1	0	0	2
Total	267	39	94	41	30	36	27
		15 %	35 %	15 %	11 %	13 %	10 %

[a]Alcohol (3), amiodarone (3), INH/ethambutol (1), Dilantin (1), propafenone (1), severe malnutrition (1)
[b]Hereditary neuropathy with pressure palsies (3), granulomatous neuropathy (2), leprosy (2), infiltrating lymphoma (2), Fabry disease (1), idiopathic sensory ganglionitis (1), spinocerebellar degeneration (1), hypothyroid (1)
[c]Four biopsies demonstrated macrophage-mediated demyelination on EM. See footnote for Table 1.3

Table 1.3 Specific findings in 43 biopsies

Necrotizing vasculitis	20
Amyloidosis	4 (2 familial TTR, 2 primary)
CIDP (MMD)[a]	4
Tomaculous neuropathy	3
Amiodarone	3
Leprosy	2
Paraprotein[b]	2
Granulomatous (sarcoid)	2
Lymphoma (infiltrative)	2
Fabry disease	1

[a]MMD: macrophage-mediated demyelination. This finding is specific to CIDP and GBS and much more common than 4 of 52 cases. However, electron microscopic search for MMD is unnecessary when the clinical data and light microscopic pictures are congruent

[b]Paraproteinemic neuropathy does not usually have characteristic findings. However, one of these biopsies demonstrated widely spaced myelin strongly associated with circulating IgM paraprotein having activity against myelin-associated glycoprotein. The other demonstrated endoneurial effacement with massive deposits of kappa light chain

Table 1.4 Usefulness of biopsy according to results of electrophysiological studies

	Axonal	Demyelination/mixed	Normal
No benefit	64	21	31
Helpful	21 (19 %)	30 (46 %)	0
Essential	24 (22 %)	14 (22 %)	2 (6 %)

N = 207

features (Table 1.4). Indeed, nerve biopsies in patients with axonal neuropathy reveal important and often unsuspected pathology such as vasculitis and amyloidosis. In addition, biopsies in three patients with axonal electrophysiology showed a picture typical of CIDP.

1.2.2 Literature Data on the Usefulness of Nerve Biopsy

Fifty-three of one hundred and twenty consecutive patients presenting to an EMG clinic underwent nerve biopsy (Argov et al. 1989). Thirty-eight percent of the 53 biopsies yielded diagnostic or contributory information that altered management in half (19 %) of the patients. In another series of 56 sural nerve biopsies, 27 % provided information considered crucial for diagnosis. In an additional 37 %, biopsy findings were nonspecific but supported the suspected clinical diagnosis and excluded elements of the differential diagnosis (Neundorfer et al. 1990). Reviewing personal experience with 385 biopsies, Oh (1990) was able to make a specific diagnosis in 24 % of the cases. Chia et al. (1996) found a high incidence of vasculitis in nerve biopsies (one-third of 100 biopsies) of elderly patients with disabling neuropathy, concluding that nerve biopsy should be considered in all elderly patients with multifocal neuropathies of unknown cause. In another study Deprez et al. (2000) analyzed the clinical features that seemed to increase the diagnostic value of nerve biopsy. These authors found that the biopsy yielded helpful or essential information in 50–60 % of cases with a pre-biopsy diagnosis of vasculitis or CIDP. Additional features that have been shown to improve the usefulness of nerve biopsy include multifocal clinical or electrophysiological findings and performance of the biopsy within 6 months of symptom onset (Schweikert et al. 2007; Deprez et al. 2000). Finally, in a series of 50 consecutive cases, sural nerve biopsy changed the diagnosis in 14 %, altered management in 60 %, and caused persistent increased pain after short-term follow-up in 33 % (Gabriel et al. 2000).

Thus overall, 20–30 % of nerve biopsies provide information that is either critical to diagnosis or may alter management. Nerve biopsy yield is higher if the neuropathy is acute, subacute, or multifocal (i.e., the clinical setting in which the suspicion for a vasculitic neuropathy is high).

1.3 Indications for Nerve Biopsy

Indications for nerve biopsy have not been delineated. A 1967 position paper from the Research Group in Neuromuscular Diseases of the World Federation of Neurology indicated that "…nerve biopsy is an investigation which should only be carried out in selected centers where facilities for full and detailed histological studies are available, and the clinical indications for the use of this method of investigation are few" (Thomas 1970). Although we find this a restrictive statement, we agree that interpretation of pathological change in a nerve specimen requires experience. Many problems that beset the beginner relate to artifacts produced by the biopsy procedure (crush, stretch), during processing (rough handling, delayed fixation, hyperosmolar fixative), and embedding (lack of orientation of fascicles, type of resin). However, the techniques necessary for the processing of nerve tissue can easily be adapted from those found in any established histology laboratory; this includes plastic resin embedding, electron microscopy, and immunohistochemistry in paraffin, frozen, and plastic sections. Diagnosis rarely requires the more difficult technique of nerve teasing. With respect to the indications for nerve biopsy, the position quoted above was taken before (A) full appreciation of CIDP as a treatable neuropathy, (B) the establishment of immunostaining techniques (e.g., identification of amyloid major proteins), and (C) the emergence of such entities as nonsystemic peripheral vasculitic neuropathy (Dyck et al. 1987), giant axonal neuropathy (Asbury et al. 1972), and hereditary neuropathy with liability to pressure palsies (HNPP) (Behse et al. 1972). Furthermore, using

plastic-embedded material for light and electron microscopy permits a far more informative examination of axons and myelin than do the standard paraffin-based histological techniques employed in the 1960s and earlier.

Asbury and Gilliatt (1984) emphasized the usefulness of nerve biopsy in asymmetric and multifocal neuropathies and indicated that "… distal symmetrical polyneuropathies, whether subacute or chronic, axonal or demyelinating, are not further clarified by nerve biopsy…." However, in our experience a significantly asymmetric neuropathy occurred in only a third of the instances in which biopsy was classified as essential for management. Significantly, asymmetric neuropathy appeared in approximately 50–70 % of vasculitic neuropathies and in HNPP. A symmetrical and distally predominant pattern was seen in a significant proportion of patients with vasculitis and in all of the patients with amyloid, sarcoid, amiodarone-related, infiltrative, paraprotein-associated, and inflammatory demyelinating neuropathies.

The spectrum and frequency of findings in our series are very similar to those reported in the literature (Argov et al. 1989; Neundorfer et al. 1990; Oh 1990). This suggests that clinicians in widely separated institutions apply similar indications for nerve biopsy despite the absence of accepted guidelines for performing the procedure. As noted above, biopsy reveals essential information in about 20 % of cases and helpful information in a further 20 %. Clearly, nerve biopsy can be a valuable procedure. Unfortunately, 50 % or more of nerve biopsies in these reports have not provided any useful information. Identifying a group of patients who should not undergo nerve biopsy would lower the frequency of uninformative procedures. Although some workers refer to "appropriate selection" of patients for biopsy, there are no concrete guidelines (England and Asbury 2004; Said 1999).

1.3.1 Clinical and Electrophysiological Criteria Cannot Exclude the Need for Biopsy

Although some workers have suggested that axonal neuropathies have a lower diagnostic yield than demyelinating ones (Argov et al. 1989), our experience (Table 1.4), and that of Neundorfer et al. (1990), does not support this assertion. Asbury and Gilliatt (1984) have commented on the low yield of nerve biopsy in distal symmetrical neuropathies, but we have identified vasculitis, amyloidosis, granulomata, and paraprotein deposition in this context.

Most importantly, clinical criteria cannot exclude the possibility of vasculitic neuropathy. Vasculitis was seen in 7 % of our biopsies and in 4–16 % of other literature series (Schweikert et al. 2007; Harati and Niakan 1986; Hawke et al. 1991; Oh 1990). In a group of 100 elderly patients with a disabling neuropathy severe enough to justify biopsy, 33 % were found to have a vasculitic neuropathy, and 30 % of those had a distal symmetrical or distal mildly asymmetric presentation (Chia et al. 1996). Our experience with 32 consecutive biopsy-proven cases where clinical information was available suggests that the most common syndrome seen in vasculitic neuropathy is a distal symmetrical polyneuropathy (46 %). Thirty-eight percent of patients demonstrated mononeuropathy multiplex, and 16 % had an asymmetric polyneuropathy. Notwithstanding the fact that biopsy is more likely to be performed in patients with mononeuropathy multiplex, an acute, subacute, or chronically progressive symmetrical distal sensorimotor polyneuropathy, where vasculitis is not suspected, has been described in 19–76 % of nerve biopsy series. Furthermore, although the classical electrophysiological finding of vasculitis is axonal neuropathy, 3 of 32 patients with necrotizing vasculitis proven by examination of nerve biopsy in St. Michael's Hospital laboratory demonstrated significant conduction velocity slowing or conduction block. Finally, vasculitis may be confined to the peripheral nervous system, as occurred in 4 of our 32 patients. Consequently, in some instances nerve biopsy alone can identify this treatable cause of neuropathy.

The low diagnostic yield (31 of 33 biopsies were of no value) of biopsy in patients with normal nerve conduction studies (NCS) is not surprising. Most of these patients were ultimately found not to have a neuropathy. However, in two instances specific (and in one case completely unexpected) diagnoses were made: Fabry disease and a granulomatous neuropathy in a patient shown to have sarcoidosis only at autopsy 5 years later.

1.3.2 Nerve Biopsy Has Limited Usefulness in Suspected CIDP

In the years 1986–1993, the single most common indication for nerve biopsy in Saint Michael's Hospital laboratory was the clinicians' desire to rule in or out the diagnosis of CIDP. Workers in other laboratories report a similar experience (Solders 1988). However, as discussed in Chap. 9, our data and the literature suggest that biopsy cannot exclude the diagnosis of CIDP unless an alternative specific diagnosis is made. In one study, sural nerve biopsy failed to show significant pathological differences between cases of CIDP and chronic axonal polyneuropathy (Bosboom et al. 2001). Thus, there may be little value in performing a biopsy for suspected CIDP (Krendel et al. 1989). Nerve biopsy can distinguish between CIDP and CMT-1 or identify CMT-1 with superimposed CIDP, but clinical and electrophysiological criteria, as well as genetic testing, usually suffice (Dyck et al. 1982; Gabreels-Festen et al. 1993; Sladky et al. 1986).

More important than confirming already suspected CIDP is detection of this condition in the absence of clinical

evidence. Histological findings typical of CIDP can be seen in electrophysiological axonal neuropathies (Barohn et al. 1989), as occurred in 3 of 109 patients in our series, thus permitting effective treatment.

1.3.3 Suggested Guidelines for Use of Nerve Biopsy

One cannot set rigid criteria for the indications of nerve biopsy. If the clinician cannot arrive at a diagnosis after all clinical and electrophysiological tests are exhausted, including screening for inherited neuropathy in the patient's relatives, the neuropathy is considered to be cryptogenic, as discussed above. Patients with mild and slowly progressive cryptogenic polyneuropathy should undergo a period of observation and repeat evaluation (Jann et al. 2001; Notermans et al. 1994; Grahmann et al. 1981; McLeod et al. 1984). We have also had the experience of making a definite diagnosis of a treatable condition on biopsy in cases where the patients did not subsequently receive treatment. A patient may refuse treatment because of the mildness of the neuropathy, because of the toxicity of the therapy (usually steroid treatment), or because the patient was at the end stage of a severe illness (e.g., HIV).

Nerve biopsy yields little useful information in nearly all toxic/metabolic and many genetically determined neuropathies. Although biopsy may reveal diagnostic findings in a number of these diseases (e.g., axonal swellings in hexacarbon toxicity, inclusion material in amiodarone toxicity, myelin tomaculae in HNPP), the diagnosis can usually be made on history, physical examination, and noninvasive laboratory testing. Since, as discussed above (see also Chap. 9), CIDP and GBS are diseases best diagnosed on clinical and electrophysiological grounds, the clinician may opt to proceed with treatment, reserving biopsy for treatment failure or atypical presentations.

The clearest indication for nerve biopsy is suspected vasculitic neuropathy, in which the diagnosis is uncertain or cannot be made with other available data. In most cases, these will be acute or subacute and asymmetric neuropathies; however, as discussed above, even symmetrical distal neuropathies may warrant biopsy if the clinical suspicion for an inflammatory neuropathy is high. Depending on the degree of clinical suspicion, vasculitic neuropathy may be treated without tissue confirmation, especially when a systemic vasculitic disease is already known to be present. For some neuropathies, consideration should be given to alternative biopsy sides (vide infra). The appropriate chapters provide more specific discussions regarding sensitivity, specificity, and indications for nerve biopsy in a variety of peripheral nerve diseases. Another relatively clear indication for nerve biopsy includes the clinical suspicion of a disorder with a clear treatment or prognostic implication and negative results from biopsy of other tissue (e.g., abdominal fat pad) or the peripheral nervous system being the only tissue involved in the disease process. Examples include amyloidosis, leprosy, or malignancies such as lymphoma. This is especially relevant in light of new treatment options for some of these conditions, such as diflunisal for familial amyloid neuropathy (Berk et al. 2013).

In light of the preceding discussion, we recommend nerve biopsy for all cryptogenic polyneuropathies, with the following important considerations: (1) The patient should receive a thorough workup by noninvasive testing, and when appropriate, biopsy of more-readily accessible tissue should be sought (e.g., conjunctival biopsy for suspected sarcoidosis). (2) The neuropathy should be sufficiently severe and progressive so as to justify immune therapy if an inflammatory process is identified on the biopsy. A biopsy may not be justified in patients who will refuse treatment or in whom treatment is not indicated. (3) The yield of biopsy is very low if there are no electrophysiological abnormalities. However, if the clinical picture is highly suggestive of neuropathy, biopsy might still be considered. (4) It is preferable to avoid biopsy of a nerve innervating an area of skin with normal sensory function, so as not to produce new deficits.

1.4 Site of Biopsy

1.4.1 Alternative Biopsy Sites

In ordering a nerve biopsy, the clinician must take into account the morbidity of the procedure and the likelihood of a positive diagnosis. In amyloid neuropathy, biopsy of abdominal fat pad often allows identification of amyloid, with a sensitivity of over 80 % in cases of systemic amyloidosis (van Gameren et al. 2006). In a patient suspected of having leprosy, examination of skin lesions should be performed first, although nerve biopsy is indispensable in primary neuritic leprosy. Similarly, skin or conjunctival specimens might readily show characteristic findings in storage and axonal dystrophy states, including giant axonal neuropathy (Chap. 19), neuroaxonal dystrophy (Chap. 19), neuronal ceroid lipofuscinosis, Fabry and Niemann–Pick diseases (Chap. 20), as well as sarcoidosis.

We have on occasion examined nerve biopsy specimens from patients without clinical or electrophysiological evidence of neuropathy, where the tissue was taken in an attempt to confirm clinical suspicion of a systemic disease, usually vasculitis. Although some have been diagnostic, the majority have been negative, and we do not advocate nerve biopsy in this setting without at least electrophysiological evidence of neuropathy. The yield will be higher if organs with clinical evidence of involvement are biopsied, such as the kidney,

skin, liver, or lung. Our experience indicates that for a blind biopsy, the muscle has a higher yield and a lower morbidity than the nerve.

1.4.2 Nerve to Be Biopsied

Dyck and Lofgren (1968) listed the considerations governing optimal site of nerve biopsy. Removal of a segment of peripheral nerve as a diagnostic tool should result in minimal morbidity. Consequently, motor nerves are generally not suitable (vide infra). The site of biopsy should avoid areas where local trauma may produce changes that may be mistaken for those of a peripheral neuropathy. A distal nerve is favored because in most neuropathies the longest fibers are most severely affected. The chosen nerve should be accessible to conduction studies and have a predictable anatomical course and fascicular arrangement.

Because the sural nerve fulfills the criteria above better than any other nerve, it is by far the most common biopsy site. The sural nerve conduction studies are routinely performed in most laboratories and allow correlation of clinical and pathological abnormalities. Nearly all of the several thousand nerve biopsies examined in our laboratories have been taken from this site. Most often, the nerve is exposed posterior and superior to the lateral malleolus, in the groove between the malleolus and the Achilles tendon. A length of as much as 6–10 cm can be excised if needed and will contain no significant branches. Far more normative data exists for this nerve than for any other. For details of the procedure, the reader is referred to the writings of Dyck and colleagues (1984) and Asbury and Johnson's book (1978).

Other superficial cutaneous nerves have been used for biopsy, and this subject has been reviewed by Dyck et al. (1984). Particularly in France the superficial peroneal nerve is favored by a number of authors. This technique has the advantage of easy access to muscle tissue through the same incision (Kissel and Mendel 1992). There is also the theoretical advantage that the peroneal nerve tends to be most commonly affected in vasculitic neuropathy, and data from a single retrospective study reports a diagnostic yield of 30 % for identifying vasculitis from combined superficial peroneal nerve and peroneus brevis muscle (Collins et al. 2000). Taken at the wrist, the superficial radial nerve has the disadvantage of causing sensory loss in the hand, but may prove useful for neuropathies affecting predominantly the upper extremities. The index branch of the radial cutaneous nerve was proposed as a convenient site for the study of early leprous neuropathy (Antia et al. 1975). Other cutaneous nerves available for biopsy include the saphenous nerve, the lateral antebrachial cutaneous nerve, and the greater auricular nerve (Dyck et al. 1984).

Since differences exist between cutaneous nerves at different locations, a worker should become comfortable with one biopsy site. For example, the proportion of large myelinated fibers is higher in the superficial radial than in the sural nerve (Tackmann et al. 1976), and the severity of age-related loss of large fibers is greater in the sural nerve (O'Sullivan and Swallow 1968). These nerves also differ in terms of their fascicular area and fiber density, although literature data are sometimes contradictory (O'Sullivan and Swallow 1968; Tackmann et al. 1976).

Motor nerves are, for the most part, not biopsied because of the muscle weakness that follows. However, situations may arise such as pure motor neuropathy, where motor nerve biopsy alone might reveal diagnostic pathology. Stevens et al. (1973) describe the procedure for obtaining motor branches of the deep peroneal nerves and provide some normative data. Hall et al. (1992) sampled a terminal branch of the musculocutaneous nerve to the long head of the biceps under local anesthesia. In some cases, intramuscular nerves are of sufficient size and myelination to permit some inference regarding involvement of motor fibers in a neuropathic process.

1.4.3 Combined Muscle and Nerve Biopsy

A combined nerve and muscle biopsy is advocated for the diagnosis of vasculitis and amyloidosis, two of the most common indications for nerve biopsy. Muscle biopsy can also prove useful in a search for sarcoidosis (Stern et al. 1985). In necrotizing vasculitis, the addition of muscle biopsy to nerve biopsy may increase the diagnostic yield an additional 15–45 % (Dyck et al. 1987; Hawke et al. 1991; Vincent et al. 1985; Vital et al. 2006). Similarly, a recent meta-analysis found an additional yield of 5–15 % for diagnosing definite vasculitic neuropathy using combined biopsy vs. nerve biopsy alone (Vrancken et al. 2011). In one series of 83 patients who underwent nerve and muscle biopsy, necrotizing arteritis in the muscle alone was found in 45 %, as compared with 20 % in the nerve alone and 30 % in both, including patients with "selective PNS vasculitis" clinically (Said et al. 1988). However, this finding is not universal. In a retrospective study of 53 biopsy-proven cases of vasculitic neuropathy, only one case showed vasculitis in the muscle that was not seen in the nerve (Bennett et al. 2008). Overall, we favor use of the combined procedure when the clinician is considering vasculitis or amyloidosis.

A combination biopsy of the muscle and nerve through a single incision minimizes discomfort to the patient. We obtain good results through a midline incision at midcalf between the heads of the gastrocnemius muscle, where the sural nerve can be found as it emerges through the fascia. An alternative method is biopsy of the superficial peroneal nerve and some of the underlying peroneus brevis muscle (Kissel and Mendel 1992; Said et al. 1988).

1.4.4 Fascicular vs. Whole Nerve Biopsy

We recommend that all nerve biopsies performed for diagnostic purposes comprise the entire thickness of the nerve trunk. The technique for fascicular biopsy was described by Dyck and Lofgren (1966) and proposed as a means of lowering the rate of postoperative sensory loss. However, most workers do not take this approach (Argov et al. 1989; Asbury and Johnson 1978; Behse et al. 1972; Neundorfer et al. 1990; Oh 1990; Pollock et al. 1983). Fascicular biopsy reduces the already small amount of tissue available to make a diagnosis. In diseases where involvement is multifocal, such as vasculitis, amyloidosis, leprosy, or malignant infiltration, the diagnostic yield will undoubtedly be reduced. Peripheral nerve vasculitis is often predominantly epineurial, and little or no epineurium is obtained in fascicular biopsy. Additionally, comparison of fascicle-to-fascicle variation in axonal number and pathology is not possible. While fascicular biopsy of normal nerves is not problematic, the technique becomes more difficult when there is marked neural atrophy, inflammation, or fibrosis. The fascicle is handled intimately, increasing the opportunity for producing artifacts. Finally, although a theoretical advantage of fascicular biopsy is a reduced rate of post-biopsy complications, many studies have found no difference compared to whole nerve biopsy (Pollock et al. 1983; Solders 1988; Gabriel et al. 2000). One possible role for fascicular biopsy is for diagnostic confirmation of focal process affecting a large motor nerve, such as a radiologically identified lesion of the sciatic nerve or brachial plexus (Tracy et al. 2012).

1.5 Sequelae of Nerve Biopsy

Some sensory loss in the area innervated by the sural nerve is likely if a whole nerve biopsy is performed (Flachenecker et al. 1999; Pollock et al. 1983). This result in itself is not ordinarily a problem. Some patients fully recover sensation in the territory of the sural nerve (Solders 1988), while in most the anesthetic area shrinks over months to years to a small region (Neundorfer et al. 1990; Poburski et al. 1985).

1.5.1 Delayed Healing, Wound Infections, and Neuroma Formation

Major complications, such as severe wound infections or neuroma formation requiring resection, occur in 1 % of patients (Asbury and Johnson 1978; Oh 1990; Perry and Bril 1994). Although we have not specifically collected such data for our material, we are aware of only two patients with significant wound infections and one who required resection of a neuroma, out of a total of 267 biopsies. Both of the patients with the severe wound infections had systemic vasculitis and were treated with steroids. Oh (1990) reports a similar experience.

1.5.2 Temporary Discomfort

Thirty to fifty percent of patients experience immediate postoperative pain (Perry and Bril 1994; Solders 1988). At 4 weeks after biopsy, 22 % of 54 patients experienced discomfort due to either pain or paresthesias in one series (Solders 1988). Dyck and colleagues reported that 36 % of patients experienced discomfort of various types at 3 months (Dyck et al. 1984).

1.5.3 Lasting Discomfort

The occurrence of lasting paresthesia, dysesthesia, or pain varies in different series. Oh (1990) reported that no patients suffered from these at 2 years after biopsy. More realistically, a 10 % incidence of significant paresthesia occurring at 6–12 months after biopsy is consistently documented (Dyck et al. 1982; Poburski et al. 1985; Solders 1988), and in the series reported by Perry, 19 % of patients had persistent pain and 50 % had persistent sensory symptoms, although this was never "severe" (Perry and Bril 1994). Similarly, Flachenecker et al. (1999) found that 19 % of patients had persistent dysesthesia and 33 % had persistent pain, with both symptoms becoming less frequent by 2 years post-biopsy. Up to 40 % of the patients studied by Dyck et al. (1984) experienced biopsy-related discomfort at 1 year, but in most cases this was mild, intermittent, and improved with time, and only 10 % had significant levels of discomfort. Despite these sequelae, nerve biopsy remains an invaluable aid to diagnosis in carefully selected patients (King and Ginsberg 2013)

1.6 Future Directions

In past decades, study of biopsied tissues, including the nerve, has rested on the identification of histological patterns that would indicate the presence and etiology of a pathological process. Progressive refinements of these techniques, including electron microscopy and immunohistochemistry, have greatly increased the yield of information obtained from tissue.

1.6.1 Skin Biopsy

Wang and co-workers first demonstrated the extensive innervation of both the epidermis and the dermis in 1990 using an

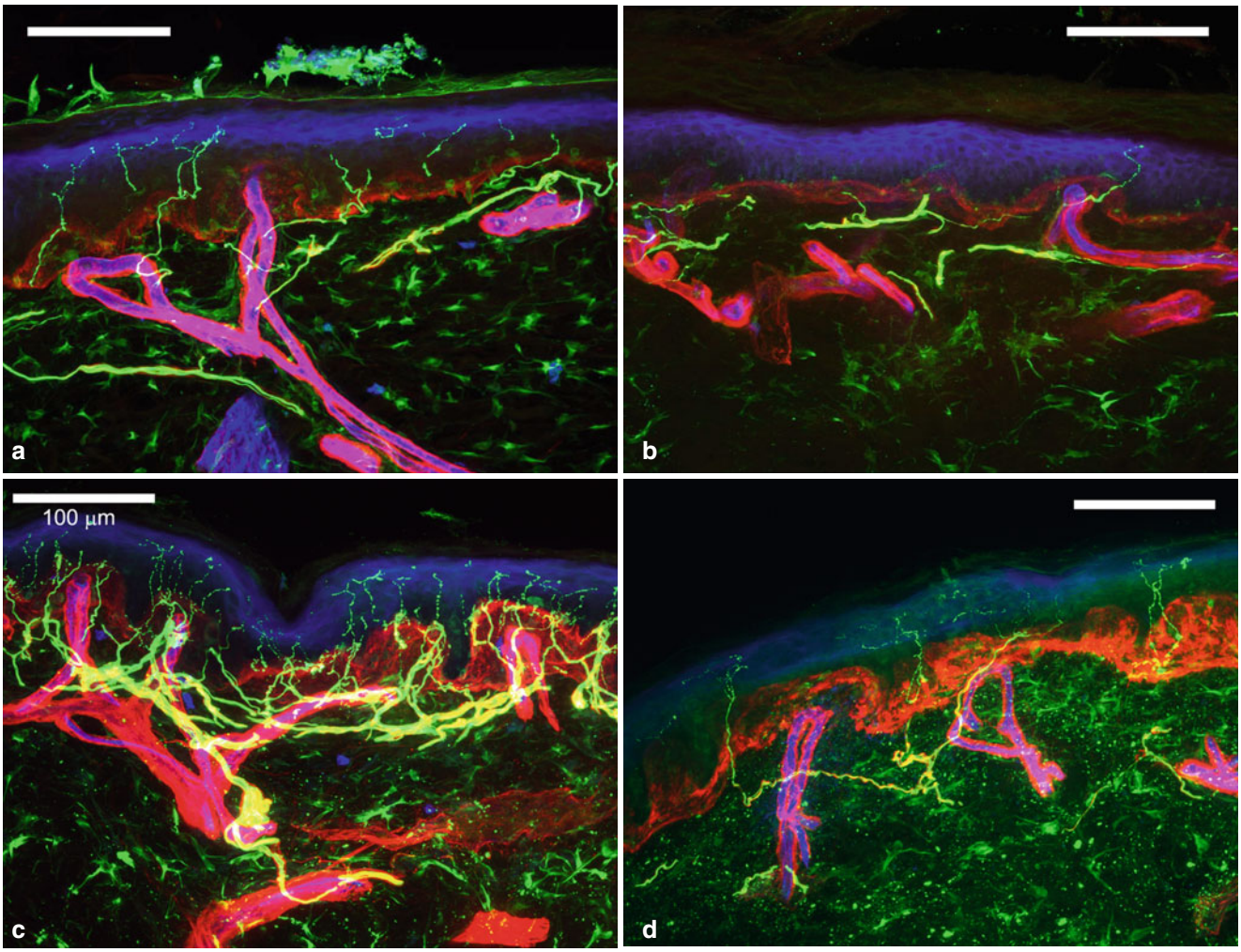

Fig. 1.2 (**a**–**d**) Confocal images of human skin biopsies from normal subjects and patients with neuropathies. Fifty micron-thick sections are immunostained for nerve fibers (*green*, PGP9.5), vascular basement membrane (*red*, type IV collagen), and epidermis (*blue* Ulex europaeus agglutinin type 1). (**a**, **b**) Normal calf skin (**a**) and from a diabetic patient (**b**) showing depletion of intraepidermal axons. (**c**, **d**) Normal back skin (**c**) and a patient with familial dysautonomia (**d**), the latter showing wholesale loss of nerve fibers in both the dermis and epidermis (Magnification: bar = 100 µm)

antibody against protein gene product 9.5. For the subsequent 21 years a steady number of publications have focused research on the use of skin biopsy for the assessment of dermal and intraepidermal nerve fibers (IENF, see Mellgren et al. 2013 for a recent review). Skin biopsy has become an accepted tool for investigating three aspects of skin innervation: quantification of intraepidermal nonmyelinated neurites, pathological features of dermal myelinated nerve fibers, and quantification and morphologic changes of Meissner corpuscles (MCs) (Nolano et al. 2003; Lauria et al. 2009, 2010). Skin biopsy can be performed at any site of the body; thus, in length-dependent neuropathies skin biopsy is taken at the distal site of the leg, about 10 cm above the lateral malleolus (Lauria et al. 2009). The underlying neural structures of the skin comprise a variety of autonomic and sensory organs innervated by the most distal segments of nerve fibers of varying caliber, which originate in the trigeminal nerve and spinal ganglia, and sympathetic ganglion cells. These are not assessed by routine electrophysiology, but their study may provide evidence of abnormality in the PNS in the absence of changes in the sural nerve (Lauria et al. 2009)

In the past, the workup of small fiber neuropathy (SFN) has been hindered by the absence of sural nerve pathological findings in some patients with clear neuropathic symptoms (Bednarik et al. 2009). Microscopic examination of the skin has become a valuable method in the diagnosis of small fiber sensory neuropathy, which preferentially affects intraepidermal unmyelinated fibers (Lauria et al. 2010) (Fig. 1.2a–d). Intraepidermal unmyelinated fibers are more commonly

encountered in the hairy skin of the legs (Ebenezer et al. 2007; Peltier et al. 2013; Myers et al. 2013). Quantitation of intraepidermal nerve fibers using bright-field immunohistochemistry has resulted in normative reference values available in several publications (Lauria et al. 2009).

Punch biopsies (about 3 mm in diameter) of non-glabrous skin from the limbs are immediately fixed in 2 % paraformaldehyde–lysine–periodate for up to 24 h and then kept in a cryoprotective solution at −20°C. Depending on whether bright-field immunohistochemistry or indirect immunofluorescence will be used, sections are prepared of 50 or 100 μm thickness (Lauria et al. 2009).

The immunohistochemical (fluorescent) methods recommended include identification of the pan-axonal protein gene product 9.5 (PGP9.5, which stains all types of axons) (Fig. 1.3a, b), to determine the density of epidermal neurites per millimeter. In addition, myelin basic protein (Fig. 1.3b) labeling highlights myelinated fibers of the dermis, and antibodies against voltage-gated sodium channels assess the nodes of Ranvier (recently reviewed by Mellgren et al. 2013; Peltier et al. 2013; Nolano et al. 2003). To highlight the dermal background (subtended by basement lamina and blood vessels), cryosections are labeled with antibodies against collagen IV to facilitate the visualization of the microanatomical landscape. Nerve fiber density must then be compared to normative data for a particular limb and adjusted to the age of patients. Selective immunolabeling of the dermis for other neuropeptides provides qualitative data for the evaluation of autonomic nerve fibers in the skin comprising studies on sweat gland innervation (labeled with antibodies against beta-unique tubulin and dopamine beta-hydroxylase) and innervation of arrector pilorum (anti-PGP9.5) muscles (vide infra), hair follicles, and blood vessels (Lauria et al. 2009).

Recent studies have begun evaluating dermal cutaneous myelinated fibers in healthy control individuals and in patients with various peripheral neuropathies. Saporta and co-workers (2009) established the normal innervation of the dermis (Fig. 1.3a, b) and, in comparison, reported a consistently shortened intermodal length of dermal myelinated fibers in patients with CMT-1A (Fig. 1.3c, d). These same authors demonstrated short intermodal lengths and paranodal demyelination in skin biopsies from patients with chronic demyelinating polyneuropathy (CIDP, Fig. 1.3e, f). Lombardi et al. (2005) undertook an elegant study using confocal microscopy in double- and triple-stained (anti-MAG, anti-IgM, and anti-PGP9.5) sections from skin biopsies of 14 patients suffering from anti-MAG neuropathy. They found, in all patients, IgM deposits on dermal myelinated fibers, which are the same attributes described with sural nerve biopsy. Provitera et al. (2007) documented degeneration of dermal sensory and autonomic nerve fibers in systemic sclerosis.

Biopsy of normal, non-glabrous, hairy skin (Fig. 1.4a) shows significant differences in comparison with normal glabrous skin (Fig. 1.4b, c) (Provitera et al. 2007; Myers et al. 2013). Biopsy of glabrous skin permits the identification and density assessment of Meissner corpuscles (MCs, arrows, Fig. 1.4b, c) and Merkel complexes (arrowhead, Fig. 1.4b) that are the two mechanoreceptive sensory end terminals of myelinated afferents (Nolano et al. 2003) which label with antibodies against S-100 protein, PGP9.5, and MBP (Garcia-Suarez et al. 2009). Meissner corpuscles (arrow, Fig. 1.4b) and Merkel complexes (arrowheads, Fig. 1.4b) are located in the apex and the base of dermal papillae, respectively, in the subepidermal region immediately below the basement membrane (Fig. 1.4b) (Nolano et al. 2003; Myers et al. 2013). Large myelinated fibers are much more abundant in glabrous skin than in hairy skin reflecting the increased number of mechanoreceptors prevalent in glabrous skin which are found in a decreasing proximodistal gradient. Other mechanoreceptors such as Pacinian corpuscles and Ruffini endings are rarely captured in routine skin punch biopsies because they are irregularly distributed in the deep dermis (Myers et al. 2013; Peltier et al. 2013). In some laboratories, a 2 mm punch biopsy site for glabrous skin is chosen on the lateral aspect of the finger between the first and second interphalangeal joint or on the tip of the third digit. Biopsy of glabrous skin is also useful to quantitatively evaluate axonal loss of myelinated nerve fibers in patients with diabetic peripheral neuropathy (Peltier et al. 2013). Although neuropathic changes in hairy skin consist of axonal loss (Fig. 1.5a) and occasional axonal swellings, alterations in the distribution of myelinated axons in glabrous skin may differ from one dermal papilla to another (Fig. 1.5b–d) and changes in the appearance of individual axons innervating Meissner corpuscles (arrows, Fig. 1.5b, d). The detailed appearance of the nodes of Ranvier and distribution of sodium channels can be determined in glabrous skin biopsies (Fig. 1.6a) and, using immunolocalization of selective markers, allow identification of and alterations in the paranodal apparatus (Fig. 1.6b). A variety of subtle pathological abnormalities of myelinated axons can be identified in glabrous skin biopsies (Fig. 1.7a–d). Skin biopsies may be used to identify autonomic innervation of various skin adnexa including sweat glands (Fig. 1.8a), arteriovenous shunts (Fig. 1.8c) and, exclusively in hairy skin, arrector pili muscles (Fig. 1.8e). Diabetic autonomic neuropathy results in defects in the innervation of sweat glands (Fig. 1.8b), arteriovenous shunts (Fig. 1.8d), and arrector pili muscles (Fig. 1.8f).

The term “small fiber sensory neuropathy” (SFSN) commonly refers to a condition characterized by the damage of unmyelinated axons in the epidermis originating from the C and Aδ fibers, conveying thermal and nociceptive sensation (Lauria and Lombardi 2012). Although idiopathic form of

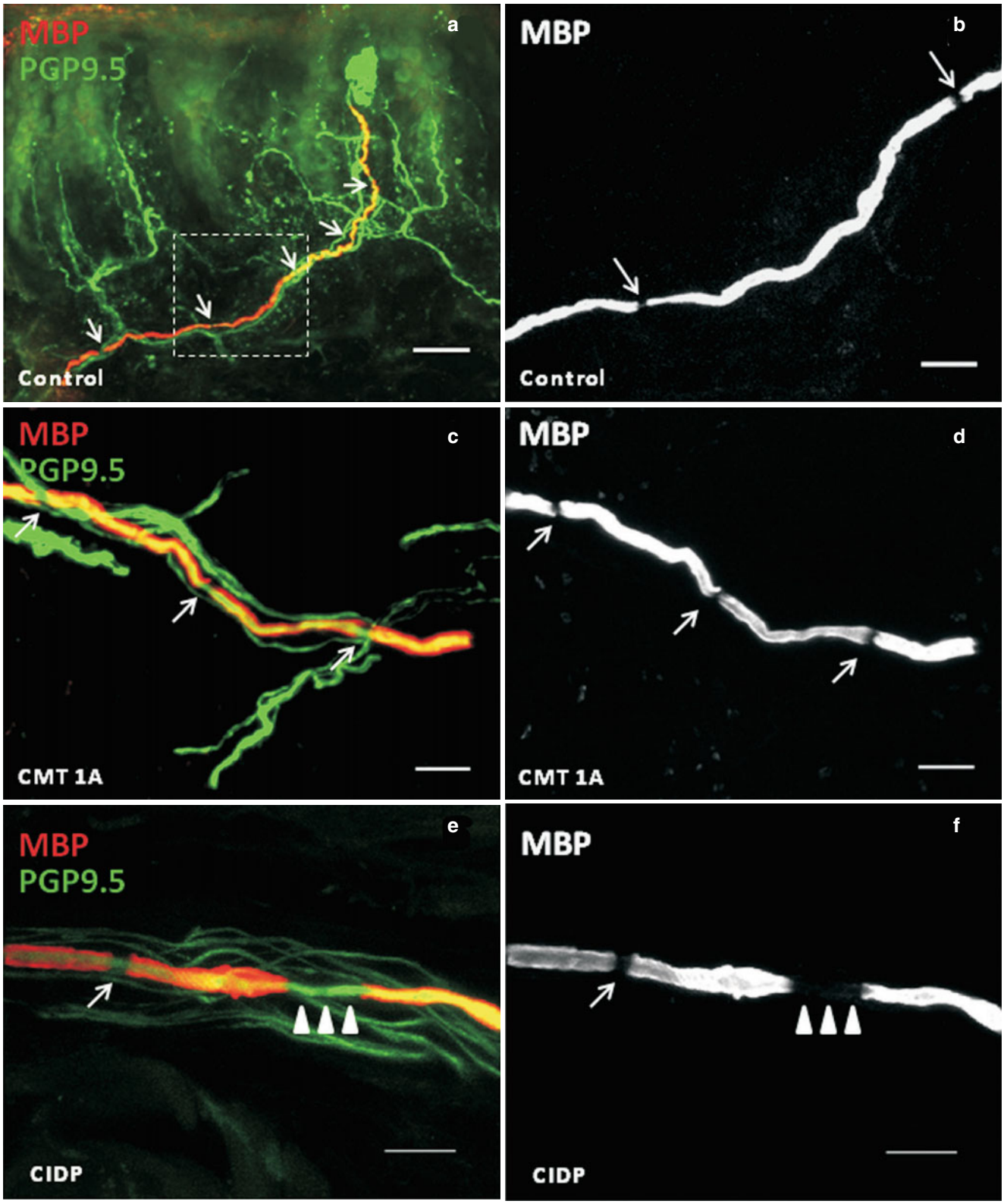

Fig. 1.3 (**a**–**f**) Immunohistochemical analysis of dermal myelinated fibers in CMT-1A, CIDP, and healthy controls. (*Green*, pan-axonal marker PGP9.5; *red*, compact myelin marker MBP; the nodes of Ranvier are identified by the *short arrows*). (**a**, **b**) Skin biopsy from a control subject. Fibers innervating Meissner corpuscles are identified (*arrows*, **a**) and an internode (between *arrows*) shown at higher magnification (**b**). (**c**, **d**) Similar staining was performed in a patient with CMT-1A. Note that internodal length (**d**) is shorter in the CMT-1A patient than that in the control (**b**). (**e**, **f**) Shortened internodes were also identified in patients with CIDP. Segmental demyelination, including the paranodal regions (*arrowheads*), was observed in all patients with CIDP (Magnification bars, (**a**) 50 μm, (**b**–**d**) 20 μm, (**e**, **f**) 10 μm

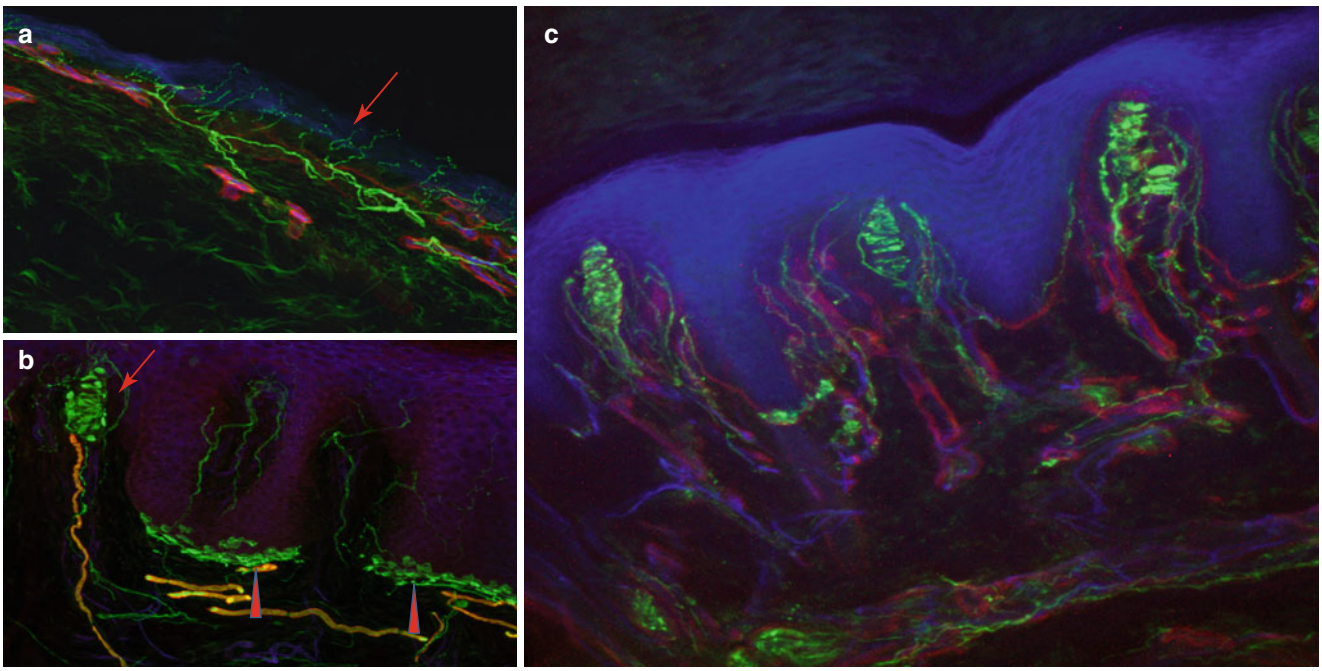

Fig. 1.4 Comparison of normal hairy and glabrous skin innervation. (**a**) Hairy skin shows an extended linear pattern of nerve fibers serving the epidermis (*arrow*) and originating from the subepidermal neural plexus. Epidermal nerve fibers are in *green*, basal membranes and vessels in *red*, and epidermis and endothelium are in *blue*. (*Red*, collagen IV; *green*, PGP9.5; *blue*, Ulex). (**b**) Glabrous skin shows striking Meissner corpuscles (*arrows*) and Merkel complexes (*arrowheads*) prominently innervated by distinctive axonal complexes. (*Red*, myelin basic protein (MBP); *green*, PGP9.5; *blue*, Ulex). (**c**) Normal distribution of Meissner corpuscles at the apex of dermal papillae (*red*, collagen IV; *green*, PGP9.5; *blue*, Ulex)

SFSN is recognized, many histopathological studies have confirmed the high prevalence of degeneration of IENF in patients with chronic diabetes and prediabetes (Bednarik et al. 2009; Lauria and Lombardi 2012) and that the extent of fiber loss correlates with duration of disease. Small fiber pathology in the skin has also been recorded in Friedreich's ataxia (Nolano et al. 2003). Epidermal denervation may be an early feature of Parkinson disease (Nolano et al. 2008). Similarly, skin biopsy has shown degeneration of autonomic nerves in microvessels, sweat glands, and erector pilorum muscles in Parkinson disease (Dabby et al. 2006). Characterization of autonomic denervation in Ross syndrome has been reported (Nolano et al. 2006). Pan and colleagues (2003) detected active nerve fiber degeneration in the epidermis with fragmentation of subepidermal nerve plexuses in skin biopsies of 55 % of 20 patients with the acute demyelinating form of Guillain–Barré syndrome. IENF swellings and decreased density have been confirmed in skin samples from individuals suffering from HIV-induced painful neuropathy (Lauria et al. 2009; Zhou et al. 2007; Polydefkis et al. 2002). Using anti-PGP9.5 bright-field immunohistochemistry in mucosal biopsy specimens, Lauria et al. (2009) found small fiber degeneration in the tongue of patients with burning mouth syndrome. In a study of ten patients with familial dysautonomia (Hilz et al. 2004), the average density of epidermal fibers was greatly reduced; other cutaneous abnormalities included severe loss of fibers in subepidermal neural plexus and reduced innervation density of sweat glands (Fig. 1.2c, d). Recent evidence suggests that the node of Ranvier is the primary target of immune attack in patients with GBS and CIDP (Devaux 2012). This can be unveiled in skin biopsies (Doppler et al. 2013) by immunostaining of the proteins that characterize the node (voltage-gated sodium channels) and paranodal region (neurofascin) (Fig. 1.6b)

Skin biopsy, while offering the ability to sample multiple sites at multiple times, has limitations (Griffin et al. 2001; Kennedy et al. 2005), including variation of the density of epidermal nerve fibers in various parts of the body and the effect of gender and aging (fiber density diminishes with age and is generally lower in males). Studies examining the clinical utility of skin biopsies have suggested that this technique has a high specificity (95–97 %), with sensitivities ranging from 45 to 90 % (for further details, see Hays 2010; Lauria et al. 2010; Myers et al. 2013). Staining for amyloid is also sensitive on skin biopsy specimens. Nonetheless, skin biopsy only infrequently establishes the etiology of an individual peripheral neuropathy.

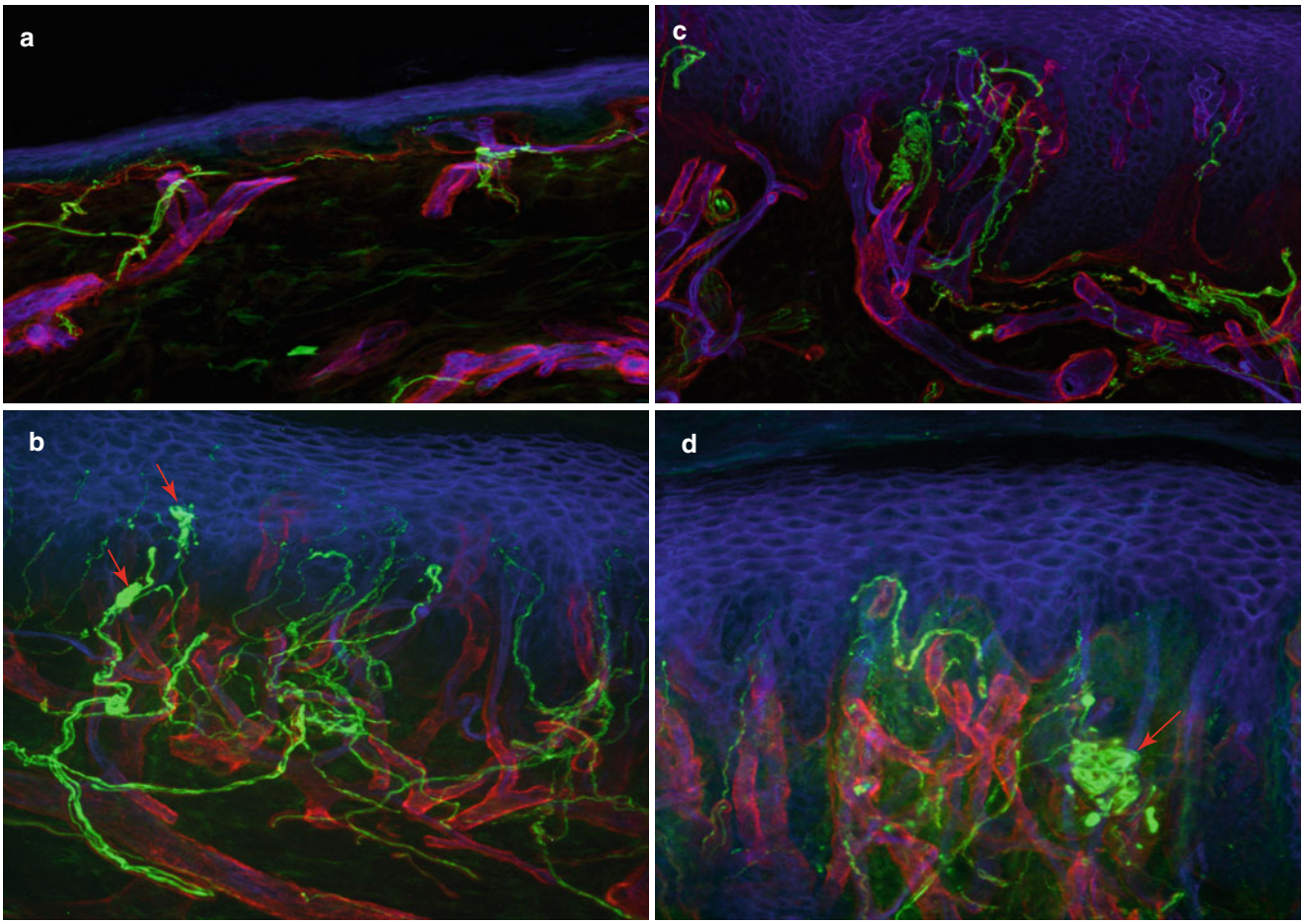

Fig. 1.5 Abnormal, neuropathic innervation of hairy and glabrous skin (*red*, collagen IV; *green*, PGP9.5; *blue*, Ulex). (**a**) Innervation of hairy skin in neuropathic patients shows significant loss of dermal and epidermal innervation. (**b**) Neuropathic innervation of glabrous skin results in patchy loss of the innervation of adjacent papillae and atrophic Meissner corpuscles (*arrows*). (**c**, **d**) Additional patterns of deranged Meissner corpuscles in glabrous skin of neuropathic patients showing abnormalities of morphology and location (lower than the apex in the dermal papillae, *arrow* **d**)

1.6.2 Imaging of the Peripheral Nervous System

Unlike the central nervous system, where magnetic resonance imaging (MRI) is a routine and often first-line investigation, imaging of the peripheral nervous system is not as commonly utilized. Partly this is due to the smaller size of the individual nerves being imaged and the complex and variable anatomy of PNS structures. However, direct imaging of the peripheral nerves with MRI (often called "MR neurography") has been gaining popularity, as it provides useful additional information to the clinical and electrophysiological workup (Amrami et al. 2008; Chhabra 2013). MR neurography may have an advantage over electrophysiology in localization and visualization of proximal nerve segments, nerve roots, or the brachial and lumbosacral plexuses. As an example, MRI can help differentiate between brachial plexopathy due to radiation and tumor invasion. In some clinical scenarios, MRI can make a specific diagnosis, such as compressive mass lesions or peripheral nerve tumors. When a diagnosis cannot be made radiologically, imaging can direct the surgeon where to resect and can be used to follow lesions over time. In some cases, the pattern of post-contrast enhancement can help discriminate between malignant and inflammatory etiologies, although this distinction is often challenging and imperfect (Stoll et al. 2009).

One area of overlap between MR neurography and peripheral nerve biopsy is in guiding the site of biopsy. Identifying a focal abnormality or region of enhancement within a nerve allows a targeted biopsy, potentially increasing the diagnostic yield (Amrami et al. 2008; Tracy et al. 2012). Thus, MR neurography does not replace nerve

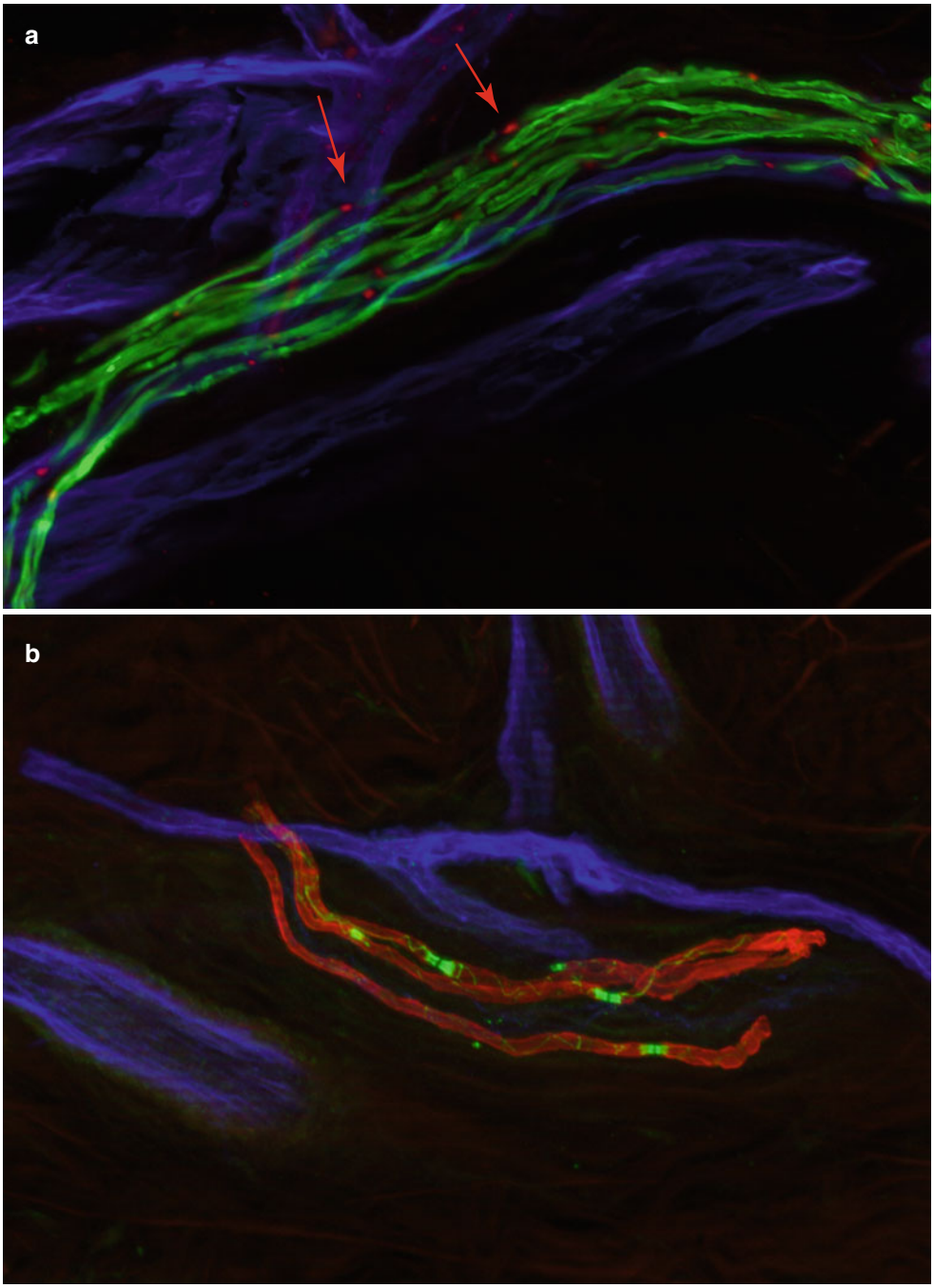

Fig. 1.6 Normal dermal myelinated fibers stained with a variety of antibodies to visualize internodes and nodes. (**a**) Sodium channels at the nodes of Ranvier (*arrows*) separate individual internodes of myelin (*red*, sodium channels; *green*, MBP; *blue*, Ulex). (**b**) Visualization of myelin sheaths and paranodes. (*Red*, MBP; *green*, nodal pan-neurofascin; *Blue*, Ulex)

biopsy, but is a complementary investigation for the evaluation of peripheral neuropathy and is likely to grow in popularity and usefulness as resolution and imaging techniques continue to improve.

Ultrasound has been used to identify patterns of diffuse nerve enlargement which can be used to screen patients suspected of having CMT-1 (Zaidman et al. 2013). Normal, mildly, or regionally enlarged nerves were identified in acquired disorders including GBS, CIDP, and multifocal motor neuropathy; early treatment of CIDP resulted in less nerve enlargement than those treated later.

1.6.3 Molecular Techniques

Advances in molecular biology have caused a revolution in the practice of medicine, and this is apparent in the pathology laboratory as well, diminishing the overall need for nerve biopsy. As a result, new approaches to the study of nerve biopsy have become important.

One likely impact of advances in molecular biology is that biopsy examination will continue to become less important for the diagnosis of genetically determined disease. This is seen in most of the storage diseases, where serum, leukocyte, and fibroblast culture assays of enzyme

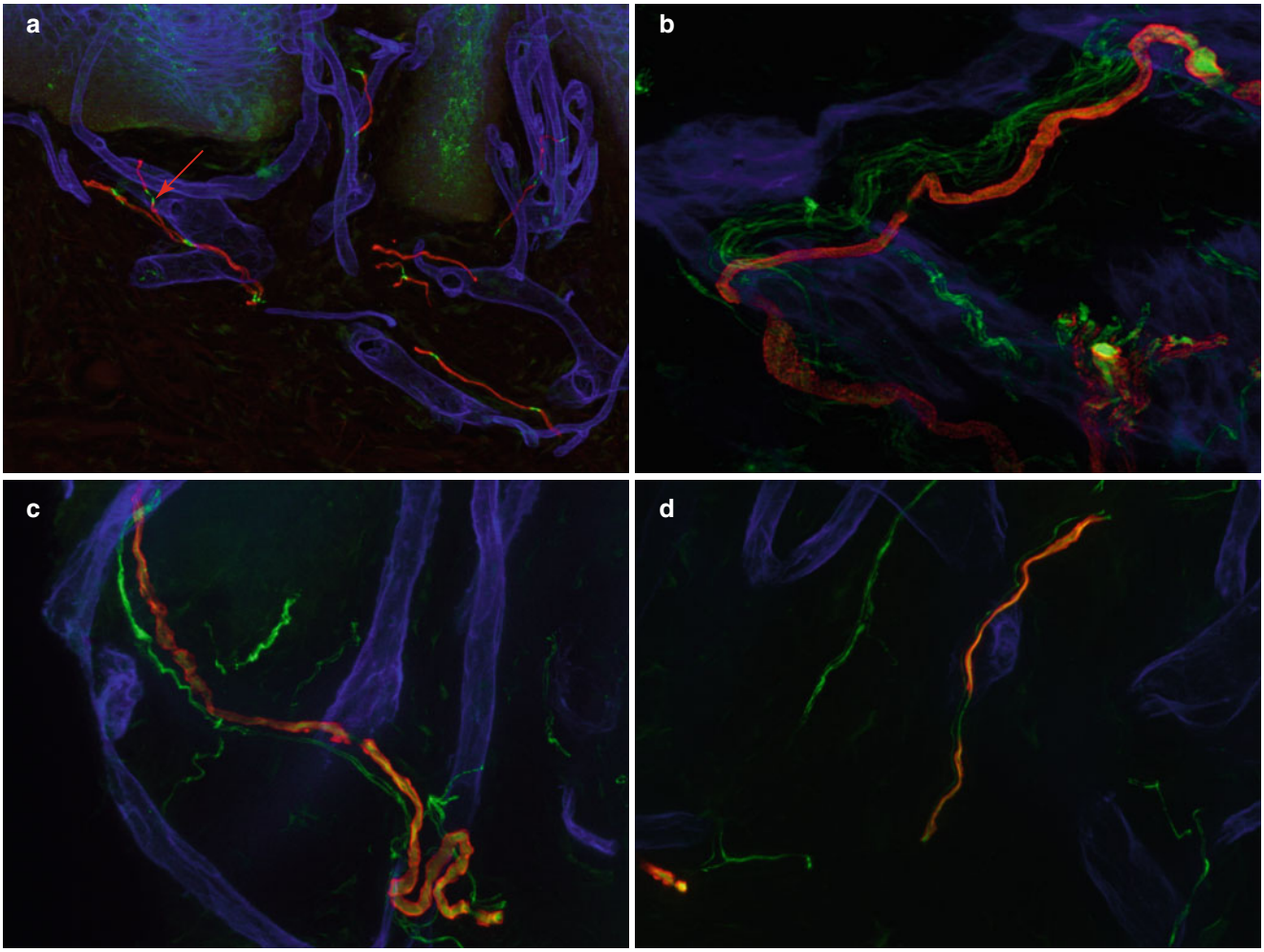

Fig. 1.7 Various morphologic abnormalities involving myelinated axons in sensory neuropathies: (**a**) axonal thinning and enlargement of nodal gap (*arrow*) (*red*, MBP; *green*, pan-neurofascin; *blue*, Ulex). (**b**) Axonal swelling and myelin fragmentation (*red*, MBP; *green*, PGP9.5; *blue*, Ulex). (**c**) Axonal shrinkage and irregular axonal caliber (*red*, MBP; *green*, PGP9.5; *blue*, Ulex). (**d**) Axonal thinning and segmental demyelination (*arrow*) (*red*, MBP; *green*, PGP9.5; *blue*, Ulex)

activity, or genetic analysis for specific DNA mutations, permit precise and noninvasive diagnosis (Chap. 20). In most cases of suspected hereditary neuropathy, genetic testing has supplanted nerve biopsy, as it avoids an invasive procedure and has the potential to provide a specific molecular diagnosis. For hereditary neuropathies such as CMT or HNPP, over 40 genes are now known to harbor causative mutations, and a rational approach to genetic testing based on clinical and electrophysiological criteria has been proposed (Saporta et al. 2011). In a recent large study, a specific genetic mutation was identified in 67 % of CMT cases between 2001 and 2009 (Saporta et al. 2011). Nonetheless, since numerous genes may be involved in hereditary peripheral neuropathies, analysis of nerve lesions can focus the search to mutations in specific genes (Vallat et al. 2009). Correlation of molecular alterations with structural/ultrastructural findings, particularly subcellular correlation, has provided additional insight into pathogenetic mechanisms. These insights occur more frequently in experimental models (e.g., altered mitochondriopathy in some forms of CMT, Baloh et al. 2007) and may correlate the molecular background with ultrastructural abnormalities involving intercellular junctions (Kanda 2009).

In contrast, we expect that emerging techniques permitting amplification and study of minute quantities of DNA will raise the sensitivity and specificity of biopsy in the detection of certain acquired diseases where the limit of conventional histological and immunohistochemical techniques has been reached. If the disease preferentially involves the peripheral nerve, then nerve biopsy may be the best source of tissue. One approach is the use of the polymerase chain reaction (PCR) for detection of organisms in tissues where these cannot be visualized using standard techniques. This has already been applied in

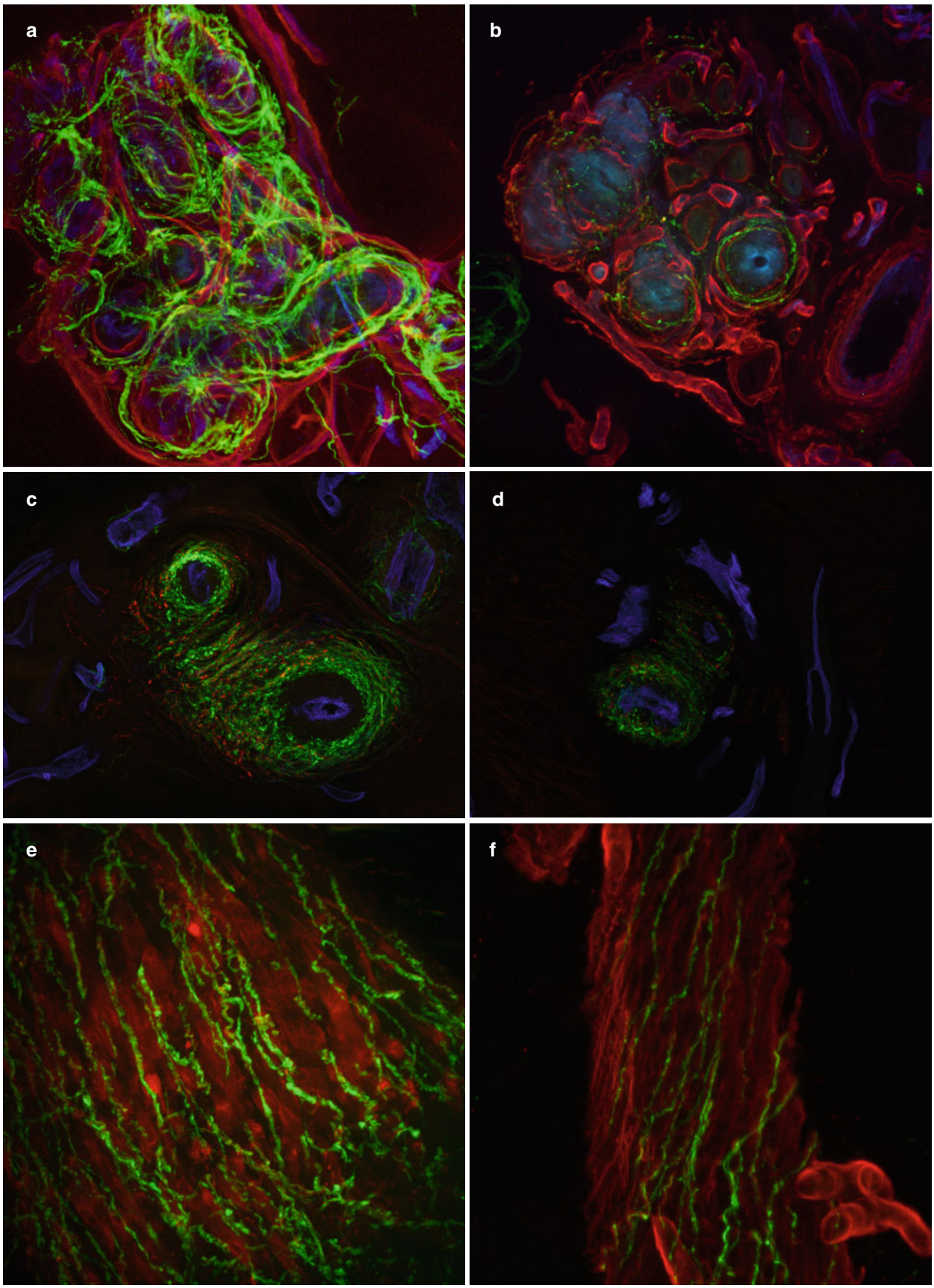

Fig. 1.8 Comparison of the autonomic innervation of skin adnexa in a normal control (**a**, **c**, **e**) and a diabetic patient with autonomic neuropathy (**b**, **d**, **f**). (**a**, **b**). Innervation of a sweat gland (*red*, collagen IV; *green*, PGP9.5; *blue*, Ulex). (**c**, **d**) Arteriovenous anastomosis (*red*, VIP; *green*, Dβ H; *blue*, Ulex). (**e**, **f**) Arrector pili muscle (*red*, collagen IV; *green*, PGP9.5; *blue*, Ulex)

leprosy (DeWit et al. 1991; Nishimura et al. 1994), Lyme disease (Schwartz et al. 1992), HIV (Winer et al. 1992), and CMV (Hughes et al. 1992) infections. Commercially available probes for organism genomes will permit in situ hybridization searching for organism-specific DNA sequences (Vital et al. 1992). In malignant infiltrative neuropathies, the ability of PCR to amplify tiny quantities of DNA is potentially invaluable for determination of monoclonality of lymphoid malignancy through the study of immunoglobulin gene rearrangements (Sleater et al. 1994).

At the time of this writing, these exciting new techniques have emerged and have already impacted significantly on the practice of diagnostic examination of nerve biopsy. One implication of the current rapid evolution of molecular biological technology is that tissue should routinely be taken at the time of the biopsy to permit such analysis if appropriate for the complete analysis of the neuropathy. Although much can be done with formalin-fixed paraffin-embedded material, frozen tissue may be the best substrate for some of the new methods and should be stored whenever possible.

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Normal Anatomy of the Peripheral (Sural) Nerve

2

In this chapter, we review the peripheral nerve anatomy pertinent to interpretation of biopsy material. We discuss the epineurial, perineurial, and endoneurial compartments of nerve, followed by a description of the changes that occur with aging. In subsequent chapters, we will deal with the ultrastructure and pathophysiology of axons, Schwann cells and myelin, the neural vasculature, as well as aspects of quantitative morphometry. Because the sural nerve is almost universally the nerve chosen for biopsy, certain elements of the discussion are specific to this site.

2.1 Normal Peripheral Nerve Structure and Function

2.1.1 Macroscopic Anatomy of the Sural Nerve

The majority of axons that form the sural nerve have their cell bodies in the S1 spinal dorsal root ganglia. However, electrophysiological techniques indicate that L5 and S2 roots also make significant contributions, and, in rare cases, even L3 and L4 contribute as well (Phillips and Park 1993; Liguori et al. 1992). Axons exit through the dorsal roots and travel in the lumbosacral trunk to the sciatic nerve. Most of these fibers travel in the medial division of the sciatic nerve, which goes on to form the tibial nerve (Fig. 2.1). A lesser number travel in the lateral division of the sciatic nerve, which becomes the peroneal nerve. In the popliteal fossa, fibers branch from the tibial nerve to form the medial sural cutaneous nerve. At the same level or slightly below, the peroneal nerve gives rise to the lateral sural cutaneous branch, most of which does not contribute to the sural nerve proper. Rather, the peroneal communicating branch of the lateral sural cutaneous nerve usually joins with the medial cutaneous sural nerve to form the sural nerve proper (Fig. 2.1). The point of union between these two components defines the beginning of the sural nerve. In 80 % of patients, this union occurs 11–20 cm proximal to the lateral malleolus. In 20 %, the sural nerve is entirely derived from the posterior tibial nerve (Ortiguela et al. 1987). Rarely, the sural nerve may arise entirely from the common peroneal nerve (Phillips and Park 1993). In up to 5 % of cases, no junction develops between the medial cutaneous sural nerve and the peroneal communicating nerve, resulting in two separate trunks, both smaller than normal (Behse et al. 1974). The sural nerve subsequently courses laterally and distally, passing 1–1.5 cm posterior to the lateral malleolus adjacent to the lesser saphenous vein. Beginning 2–3 cm distal to the lateral malleolus, cutaneous branches diverge from the main trunk. Two or three cutaneous branches may arise proximal to the lateral malleolus (Ortiguela et al. 1987).

Approximately 20 % of people possess an accessory deep peroneal nerve that supplies motor innervation to the extensor digitorum brevis (Lambert 1969; Infante and Kennedy 1970). When present, this nerve ordinarily runs just anterior to the sural nerve behind the lateral malleolus. Typically, the sural and the accessory deep peroneal nerve fascicles are separate nerve trunks. Singular reports of these motor fibers running in the sural nerve are unconfirmed by larger series (Lambert 1969). On occasion, we have received nerve biopsy specimens containing only a few fascicles and showing an unusual distribution of myelinated fibers. We suspect that in these situations an accessory peroneal nerve was biopsied or, alternatively, that a non-joined medial cutaneous sural nerve or peroneal communicating branch was taken instead of a whole sural nerve (Behse et al. 1974).

The sural is a pure sensory nerve, supplying the skin over the lateral and posterior ankle and lateral foot to the base of the fifth toe, as well as various components of the ankle joint. Although the presence of autonomic fibers is often assumed (e.g., as cutaneous sudomotor or vasoconstrictor fibers), authors who have pursued the issue have not found any evidence to suggest that these fibers make significant contributions to the sural nerve at this location (Chad et al. 1981; Sjo et al. 1976).

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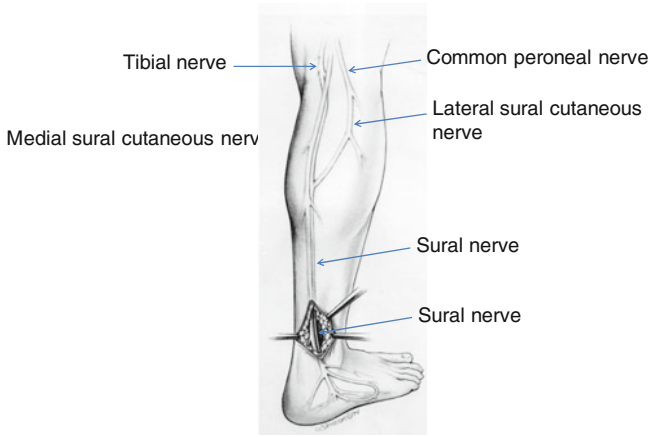

Fig. 2.1 Normal anatomy of the sural nerve, right leg

2.1.2 The Microscopic Architecture of Nerves

2.1.2.1 Epineurium

The epineurium constitutes approximately half of the cross-sectional area of a nerve trunk and consists predominantly of connective tissue that holds together and protects the individual fascicles forming the nerve. Normally, the sural nerve (Fig. 2.2) comprises between 6 and 15 fascicles, but investigators have occasionally observed more than 20. Collagen bundles run predominantly longitudinally along the nerve and are packed more closely immediately adjacent to the perineurium of individual nerve fascicles. Fibrous tissue is also packed more densely at the periphery of the nerve trunk, forming a sheath. Type I collagen predominates over type III in the epineurium (Shellswell et al. 1979), and the collagen fibrils formed are 60–110 nm in diameter. Adipocytes intermingle with the fascicles in variable amounts. Elastic fibers have a longitudinal orientation.

Although the epineurium contains a lymphatic drainage system, from a pathologist's perspective the most important structures found in this site are capillaries, arterioles, and venules. In Chap. 6, we discuss neural vascular anatomy in greater detail. It is noteworthy that occasionally a thin cuff of lymphocytes can be seen around small epineurial vessels in normal nerve and noninflammatory neuropathies of uncertain significance. Other cell types normally seen in the epineurium include fibroblasts and mast cells. Not appreciated on routine microscopy is a neural meshwork (the nervi nervorum) found within the epineurium and perineurium, presumably involved in vasomotor control and some sensory perception (nerve infarction is locally painful) (Beggs et al. 1991). An occasional event is the finding of epineurial (Fig. 2.3a) or endoneurial (Fig. 2.3b) Pacinian corpuscles in the sural nerve. The literature also contains reports of other unusual sensory corpuscles within the endoneurial compartment (Hall et al. 1991).

2.1.2.2 Perineurium

On cross sections, the perineurium (reviewed in detail in Piña-Oviedo and Ortiz-Hidalgo 2008) appears as a lamellated structure that surrounds all nerve fascicles (Figs. 2.2 and 2.4a). If the circular lamellae are "unrolled," perineurial cells will be seen as flattened polygonal cells, attached to each other as in patches of a quilt (Olsson 1990). The number of perineurial lamellae varies directly with the size of the fascicle (Tohgi et al. 1977). Distal nerve fascicles in intramuscular nerves have only one perineurial cell layer, large nerve trunks may have up to 15 layers; typical sural nerve fascicles usually show 5–8 lamellae. The outer perineurial layers merge with the fibroblasts and collagen of the epineurium.

Perineurial cells are invariably surrounded by a basement membrane, typically thicker than that seen around Schwann cells or vessels (Fig. 2.5a–c). Each perineurial layer is one cell wide except at sites where adjacent cells overlap or interdigitate (Fig. 2.5c). A cleft containing collagen separates these layers. In cross section, perineurial cells sometimes appear to branch and join an adjacent layer. The cytoplasm of these cells is rarefied except in the perinuclear region (Fig. 2.5c) where organelles such as mitochondria, glycogen granules, and endoplasmic reticulum occur. The nucleus is elongated and sometimes multilobulated. Perineurial cells also contain filaments reminiscent of those seen in smooth muscle fibers. A prominent feature is a large number of smooth contoured pinocytotic vesicles in various stages of fusion with the cell membrane (Fig. 2.5a). Transmission electron microscopy demonstrates that tight junctions (zonula occludens, arrows, Fig. 2.5a, c) link adjacent perineurial cells, and freeze fracture studies show that these junctions are arranged in numerous long branched arrays (Beamish et al. 1991). This suggests that the perineurium has a barrier property, a hypothesis supported by functional studies of the blood–nerve barrier. Gap junctions may occur between adjacent perineurial cells only rarely (Beamish et al. 1991).

Between the leaves of the perineurium lies a meshwork of collagen fibrils with longitudinal, circumferential, and oblique orientations. At 40–64 nm, the diameter of these fibrils is visibly less than that of epineurial collagen (Fig. 2.5b). The smaller size of these collagen fibrils and immunohistochemical studies suggest that type III collagen is an important constituent, although type I collagen is also present (Lorimier et al. 1992; Shellswell et al. 1979). Tangles of non-branching filaments 10–12 nm in diameter

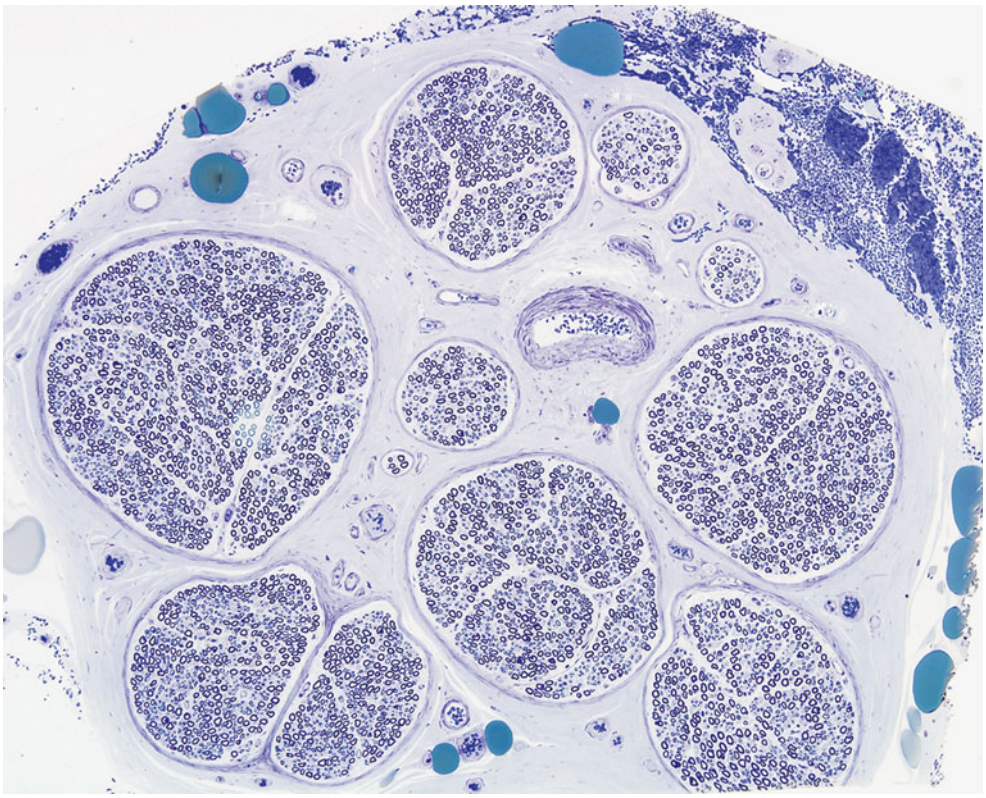

Fig. 2.2 The normal sural nerve: Semithin cross section of entire specimen, composed of ten fascicles surrounded by a thin perineurium which blends imperceptibly into the surrounding epineurium. The *large green epineurial bodies* are adipocytes. (1 μ thick toluidine blue-stained plastic section)

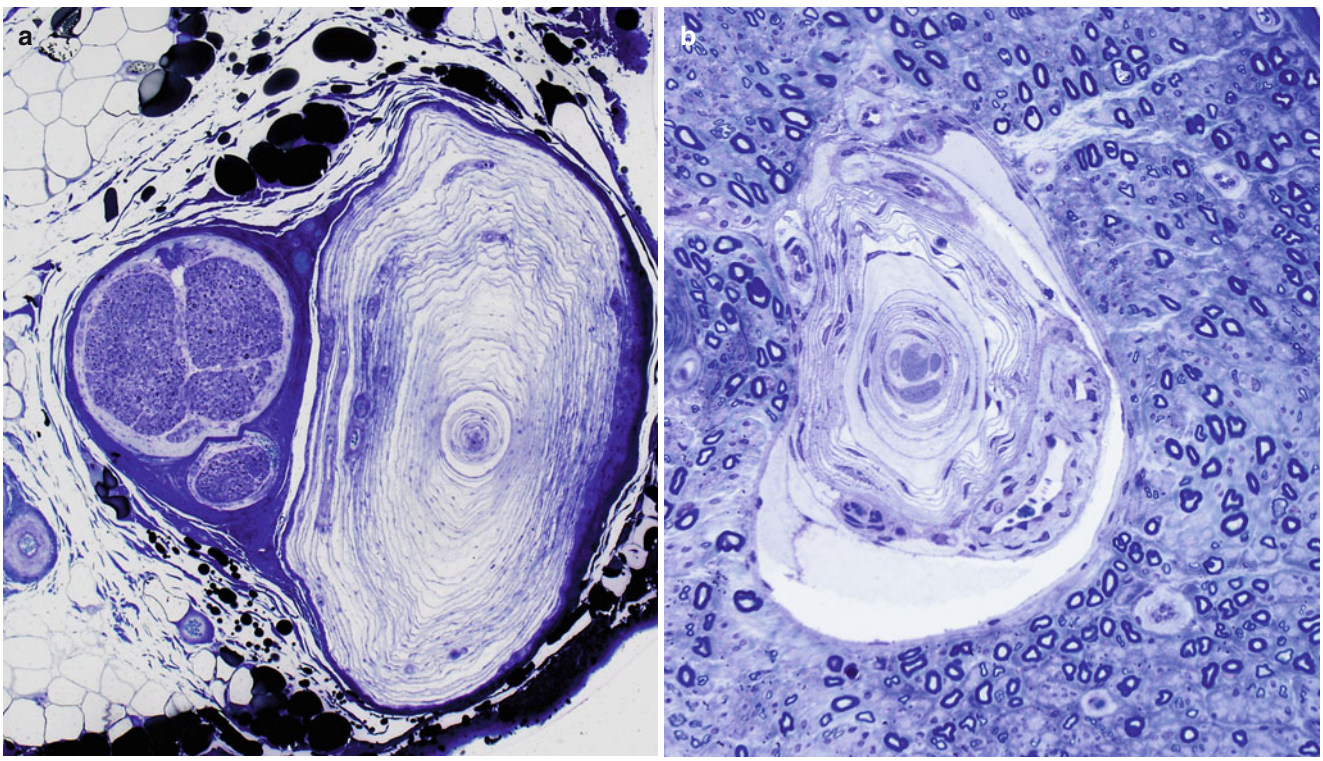

Fig. 2.3 Pacinian corpuscles in the epineurium (**a**) and endoneurium (**b**) are rare findings of no pathological significance. (1 μ thick toluidine blue-stained plastic sections)

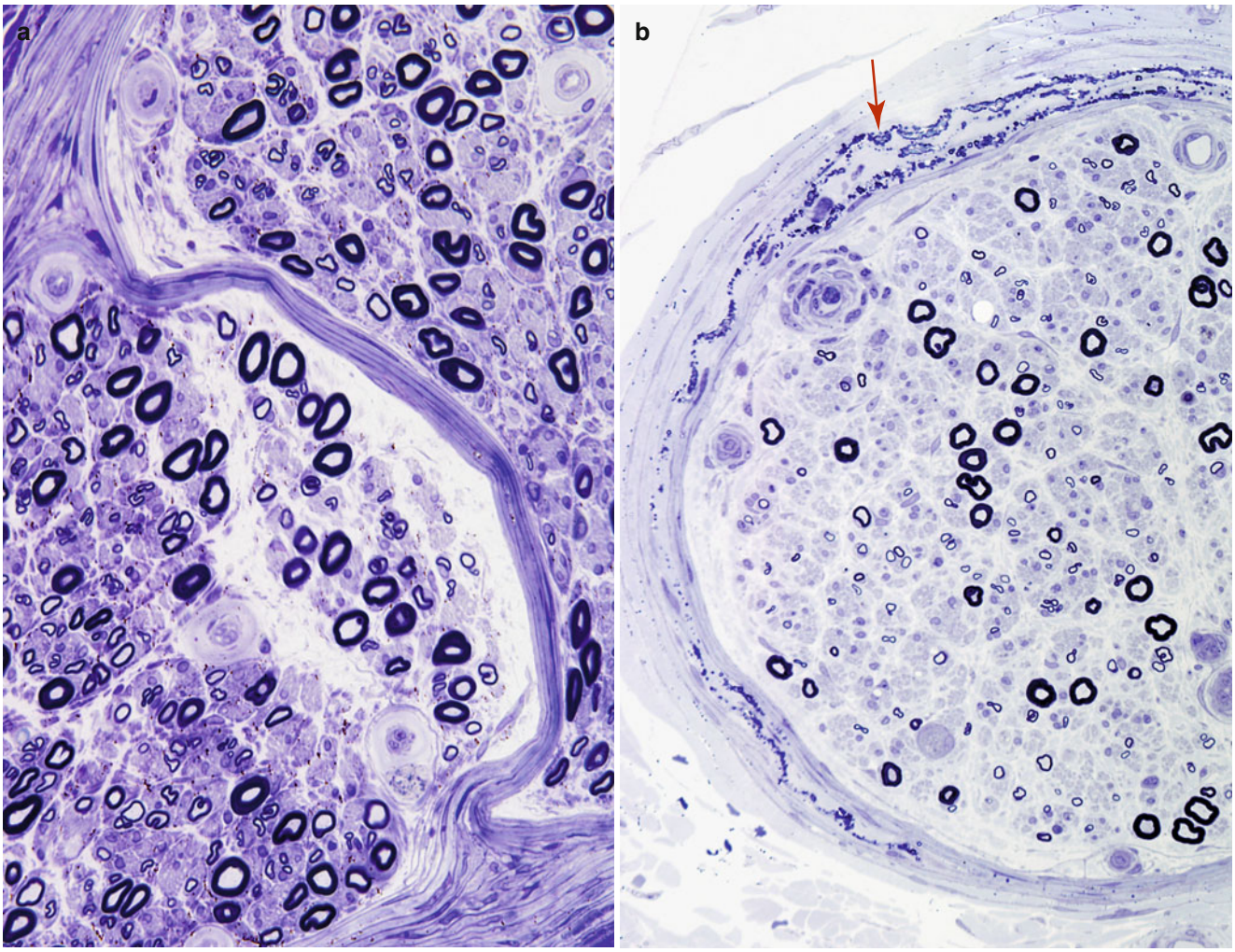

Fig. 2.4 Perineurium: in (**a**) the blending of perineurial layers without intervening epineurium occurs as the two fascicles merge. In (**b**) delicate perineurial calcifications (*arrow*) are shown. (1 μ thick toluidine blue-stained plastic sections)

are also seen, the latter probably representing what has been termed oxytalan, a fibrillar precursor of elastic fibers (vide infra). The occasional fibroblast may be detected. Long-spaced collagen ("Luse bodies") (Fig. 2.6a) and occasionally perineurial calcifications (Fig. 2.6b) can be seen in normal individuals. Such calcifications increase with age and can also be a dramatic but a nonspecific feature of peripheral neuropathies (Figs. 2.4b and 2.6b).

Cross-sectional examination of nerve fascicles often reveals partitions or septae which subdivide the endoneurium. These partitions are made up of perineurial cells, and if followed distally or proximally, these subdivisions of endoneurial contents will be seen to demarcate the sites where fascicles join or branch off (Figs. 2.4a and 6.4).

Perineurial cells are derived from fibroblasts (Bunge et al. 1989), and the perineurial layers which surround nerve fascicles are continuous with the fibroblasts in the pia mater surrounding the nerve roots (Waggener and Beggs 1967; Piña-Oviedo and Ortiz-Hidalgo 2008). It is thought that a chemical mediator, likely desert hedgehog derived from Schwann cells, induces and maintains differentiation of fibroblasts into perineurial cells and directs their organization (Thomas and Bhagat 1978; Piña-Oviedo and Ortiz-Hidalgo 2008) as perineurial cells influence Schwann cell basement membrane structure. Neurofibromin may also play a role in perineurium formation. Perineurial cells have been observed to assume characteristics of fibroblasts when intrafascicular contents are extracted and return to their previous morphology when neural structures regenerate (Thomas and Bhagat 1978). When the perineurium is stripped from living nerve, its regeneration occurs through the migration of endoneurial fibroblasts to the periphery, and their subsequent differentiation into typical perineurial cells (Nesbitt and Acland 1980).

The perineurium is thought to play an important role in regulating the endoneurial milieu in ways similar to the

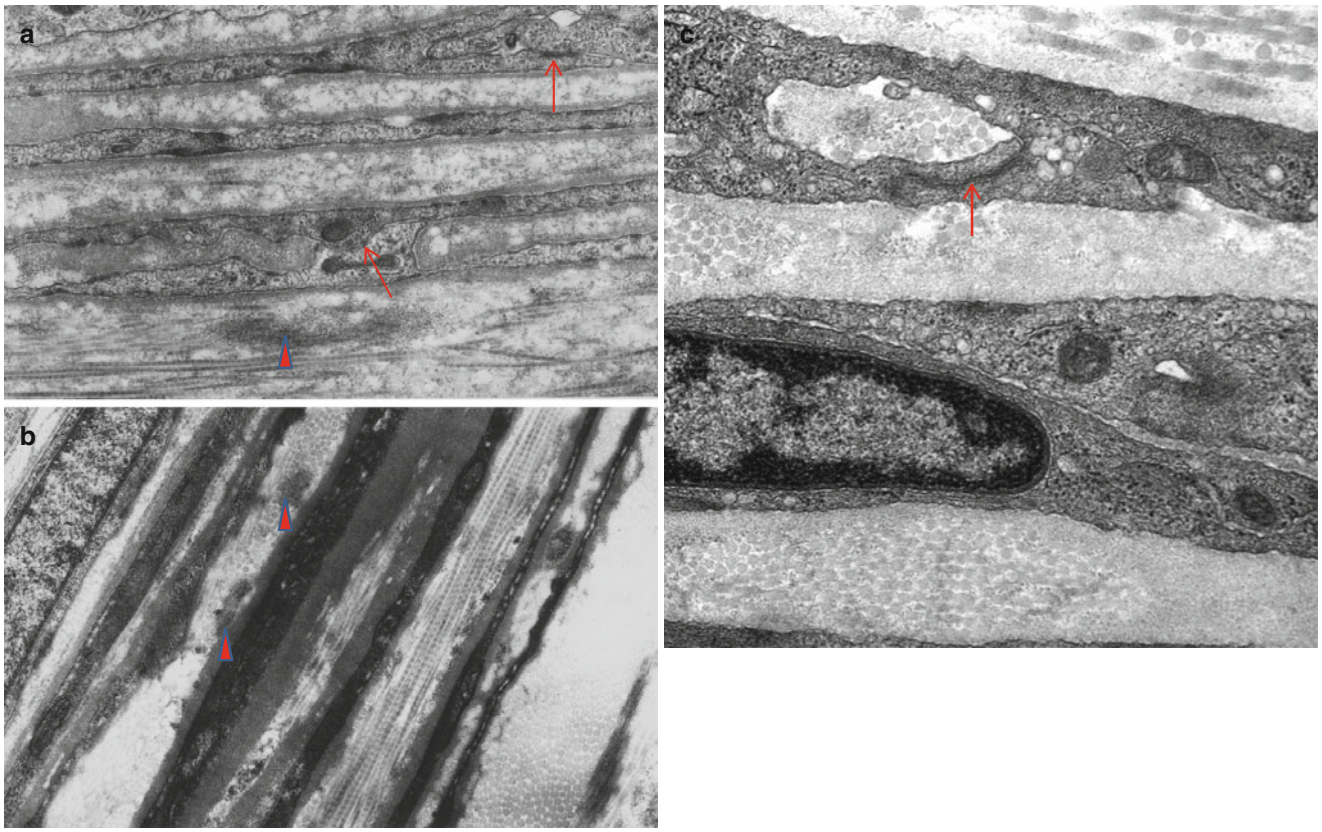

Fig. 2.5 Typical perineurial cells showing tight junctions (*arrows*) and pinocytotic vesicles. Longitudinal and circumferential networks of collagen are seen between the perineurial cell layers. Note clumps of oxytalan (*arrowhead*) (**a**, **b**: 26,400×; **c**: 40,000×)

blood–nerve barrier. Perineurial cells have the necessary complement of enzymes to permit a high degree of metabolic activity (Olsson 1990). Experiments with a variety of dyes and protein tracers have indicated that most macromolecules cannot pass the innermost layer of the perineurium (Olsson 1990), while electrolytes and glucose can pass through slowly. Although investigators have not clearly established the role of the numerous pinocytotic vesicles seen in perineurial cells, the vesicles may act in maintaining the special osmolar and molecular composition of endoneurial fluid. Interestingly, pinocytotic vesicles are more abundant in the external perineurial sheaths with more prominent cell junctions in the inner perineurial layers (Reina et al. 2003). In rapidly evolving neuropathies, debris-filled macrophages often accumulate in the subperineurial area, and debris may appear within perineurial cells in both experimental models (de la Motte et al. 1975), and in human peripheral nerve disease.

2.1.2.3 Endoneurium

The endoneurium consists of the region internal to the innermost layer of the perineurium, excluding perineurial partitions within the fascicle and including all cellular and extracellular components. We will review the fine structure and function of axons, Schwann cells, and the neural vasculature in subsequent chapters.

The endoneurial compartment is under pressure relative to the epineurium, as demonstrated by the tendency of endoneurial contents to herniate out of a perineurial window (Spencer et al. 1975). Technically difficult to accomplish, measurements of endoneurial pressure range from 0.4 to 2.7 mmHg (Low et al. 1977). This expansile tendency and the elastic properties of perineurium create the uniformly circular shape of each fascicle (Sunderland 1978), and any deviation from circularity, except at branching points, indicates either an artifact or a sign of pathology.

The distal sural nerve has a total endoneurial cross-sectional area of 0.65–1.26 mm^2 when Renaut bodies are excluded (Behse 1990), with individual fascicles ranging in size from 0.02 to 0.46 mm^2 (Prineas and McLeod 1976). Myelinated fibers and their Schwann cells account for 24–36 % of this total cross-sectional area, and unmyelinated fibers and their investing Schwann cells for 11–12 % (Behse 1990). The other major component of cross-sectional area is the interstitial compartment which includes densely packed collagen (35–45 %, ranging from 30 to 65 nm) and optically

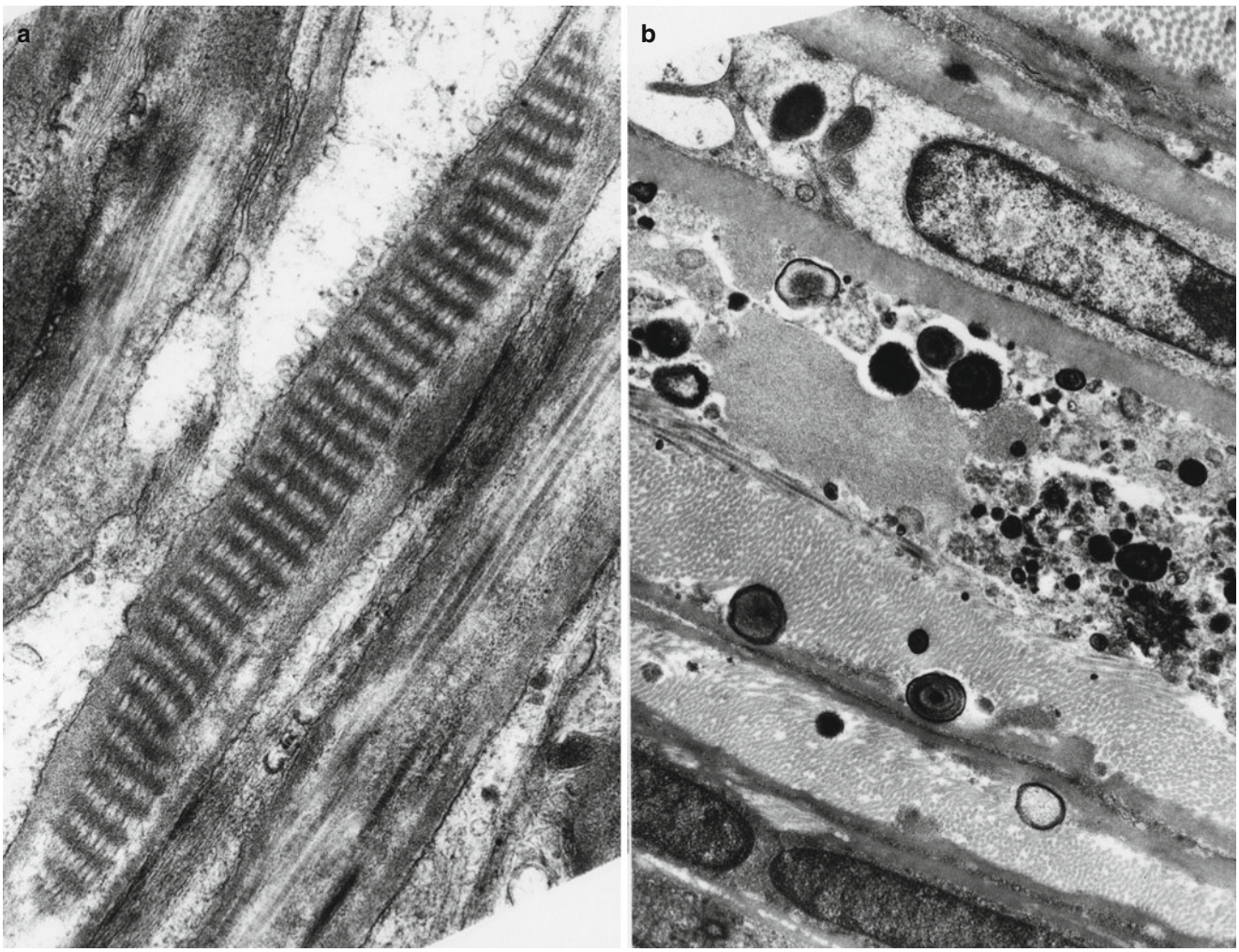

Fig. 2.6 "Raccoon tail" (long-spaced collagen, Luse body) is seen between perineurial cells (**a**). Osmiophilic bodies, some concentrically layered, represent perineurial calcifications (**b**). (**a**: 30,420×; **b**: 12,070×)

empty interstitial space presumably containing water and macromolecules (13–14 %). Vessels account for 2–3 % of the endoneurial area in the rat (Bell and Weddell 1984), although this area may be less than 1 % in man (Behse et al. 1974).

Semithin light microscopic sections (Fig. 2.7a, b) and low-power electron microscopy (Fig. 2.8) best reveal the organization of axons and Schwann cells and their interrelationship in the endoneurium. The superior light microscopic resolution of plastic embedded toluidine blue-stained semithin (1 µm) sections (Fig. 2.7a, b) allows rapid identification of axons and myelin. Even the best paraffin sections cannot rival this technique. Two populations of axons are identified, myelinated fibers (MFs) or unmyelinated fibers (UFs). The latter are best studied with electron microscopy.

2.1.2.4 Myelinated Axons

Apart from a small area just beneath the perineurium that remains cell-free, semithin sections show an even dispersion of myelinated fibers of various calibers throughout the endoneurium. Casual inspection indicates that MFs have a bimodal size spectrum. Tissue sections from human material rarely show the perfectly round outlines of MFs present in specimens from experimental animals after perfusion fixation. Myelin and axons are fragile structures with a liquid-gelatinous consistency. Consequently, the stresses inflicted upon the sural nerve as it is exposed, cut, and fixed by immersion, result in a loss of this circularity. With toluidine blue staining, the axon appears as a sharply demarcated lightly stained area, surrounded by its homogenously dark myelin sheath. A few punctate regions of staining within the axon most often represent mitochondria. Each myelinated axon segment is associated with only a single Schwann cell. This axonal segment and its associated myelin sheath define the "internode," which varies between 200 and 1,800 µm in length, depending mostly on the axon's diameter and whether pathology is present. Most cross sections through an internode show compact myelin surrounding the axon; however, a cut through the center of an internode will reveal the

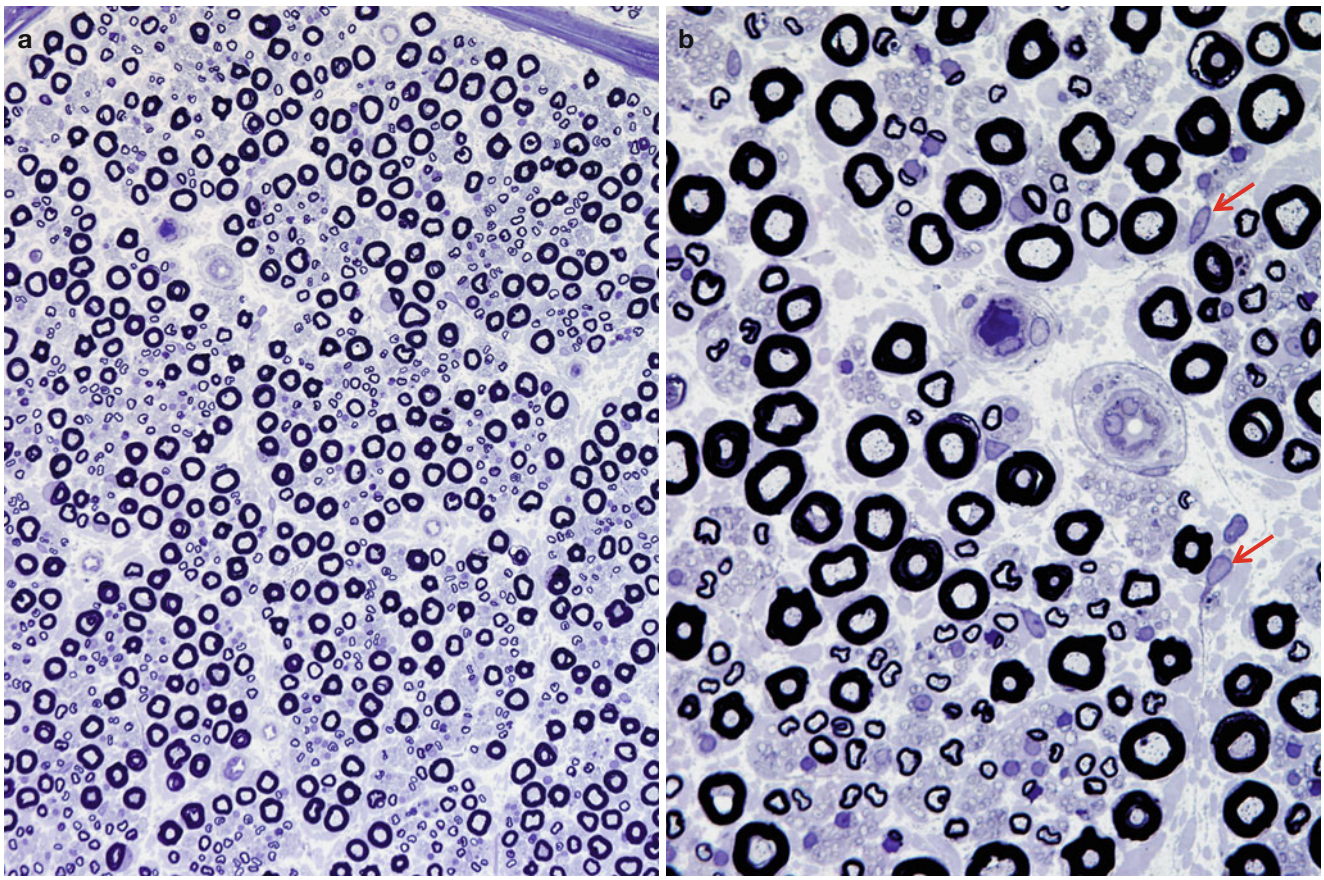

Fig. 2.7 The sural nerve of a young man. Fiber density and myelination are normal. The axons contain punctate densities, which represent mitochondria. Unmyelinated fibers appear in clusters, often associated with MFs of small diameter, and segregated from large MFs. In (**b**), except for occasional fibroblasts (*arrows*), most nuclei belong to Schwann cells. (1 μ thick toluidine blue-stained plastic sections, **a**: 200×, **b**: 1,000×)

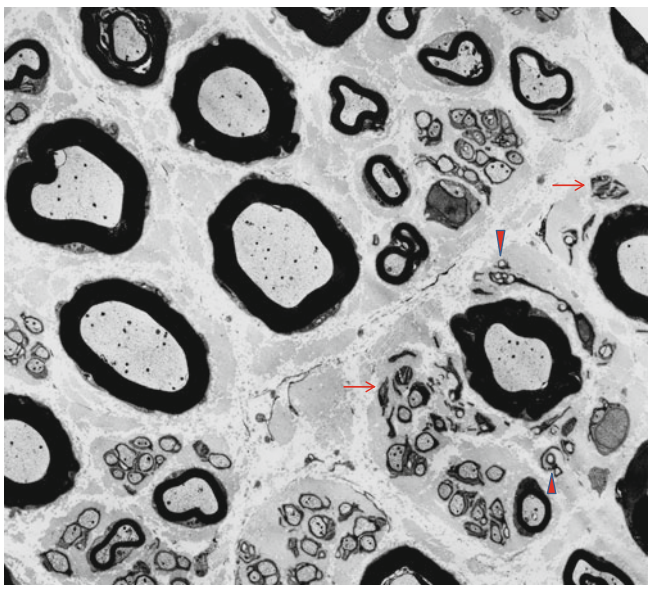

Fig. 2.8 The normal sural nerve of a 25-year-old woman. Denervated Schwann cells (*arrows*) and collagen pockets (*arrowhead*) are normally present (3,520×)

nucleus of the Schwann cell that is associated with the axon and maintains its myelin. In one study, 9 % of small myelinated and 3 % of large myelinated internodes were cut through the nuclear region, reflecting the relatively constant length of Schwann cell nuclei, and the fact that internodes of large myelinated fibers are about three times longer than those of small myelinated fibers (Ochoa and Mair 1969; Behse 1990).

The node of Ranvier, which is the site where adjacent internodes meet, is a 1 μm long region where no myelin surrounds the axon. The node and its molecular constituents have been extensively studied and reviewed (see Scherer and Wrabetz 2008), which is particularly relevant to inherited myelinopathies and CMT. On cross sections, an axon is rarely cut precisely at the node, but when this cut does occur, it should not be mistaken for a demyelinated axon. The increased staining density of axoplasm at the node of Ranvier and paranodal axon is the most reliable clue to this occurrence (Fig. 4.1c, d). The axoplasm lacks the cellular structures associated with protein synthesis, i.e., large numbers of ribosomes (although individual ribosomes have been

reported in normal and pathological axons), rough endoplasmic reticulum, and Golgi apparatus. These features, in conjunction with the length of peripheral nerve axons, have significant consequences for their growth, long-term maintenance, and the mechanisms of axoplasmic transport.

The predominant component of the axoplasm is an electron-lucent matrix within which is suspended a population of filaments and organelles. The smallest constituents consist of microfilaments measuring 5–7 nm in diameter. They contribute approximately 10 % to the total complement of axonal protein. Their numbers are underestimated in conventional transmission electron microscopy studies because the stability of microfilaments is severely compromised by the destructive effects of glutaraldehyde and osmium tetroxide. Neurofilaments, far more obvious common components of axoplasm, consist of longitudinally orientated filaments of 8–11 nm in diameter and indefinite length. The neurofilament density remains constant along the length of individual fibers and is not affected by axonal diameter. Neurofilaments are well preserved after the use of aldehyde fixatives, but they are susceptible to digestion by an intrinsic axonal calcium-activated protease which may account for the effacement of neurofilaments from damaged axons. Clusters of neurofilaments may account for the visibility of axoplasmic and cytoplasmic fibrillar structures which develop after metallic impregnation (e.g., Bielschowsky silver stain). The term microtubules ("neurotubules") defines larger diameter cytoskeletal structures in the axoplasm. Singly or in parallel arrays, they are unbranched longitudinally oriented, hollow structures with a diameter of 23–25 nm. Microtubules consist of globular subunits of the protein tubulin arranged in a lattice and are labile structures with a proclivity to disaggregate when exposed to colchicine and related mitotic spindle inhibitors. The sum of the numbers of the microtubules in the terminal branches of large axons is considerably larger than the number found proximally. A relatively larger density of microtubules is found in unmyelinated axons in comparison to myelinated axons. The axoplasm also contains various types of membranous organelles comprising mitochondria, smooth endoplasmic reticulum, lysosomes, vesicles, and dense-core vesicles of a range in sizes. Coated vesicles occur at the axolemma of myelinated and unmyelinated fibers, particularly at the neuromuscular junction and at the nodes of Ranvier.

Investigators have studied the fiber composition and quantitative morphometry of the sural nerve more intensely than any other nerve and thus quantitative analysis of biopsies from this site is most reliable. However, most of the information pertaining to the general organization of peripheral sensory nerves is widely applicable. The quantitative data provided below is derived from a single laboratory (Behse 1990; Behse et al. 1974); however, these numbers are representative of the literature. Chapter 3 provides a broader discussion of issues surrounding quantitation of nerve biopsy findings.

Myelinated fibers show a bimodal size distribution. When defining axonal size, the investigator needs to specify either the axonal diameter or diameter of the axon plus its myelin sheath. Most workers use the latter approach, and unless otherwise specified, this book will adhere to this convention. Fiber diameters form a spectrum with some overlap between large and small MFs. Typically, small MFs range from 2 to 6 μm in diameter (axon plus myelin), and large MFs range from 8 to 12 μm in diameter, with peaks at 4 and 10 μm, respectively. Using 7 μm as the separation point between large and small MFs, about 32–45 % are large and 55–68 % are small. If one considers only the axonal (i.e., inner myelin) diameters of MFs (for purposes of comparison with unmyelinated fibers), small myelinated axons range in size from 1 to 3 μm, and large axons from 3 to 6 μm, rarely as large as 7–8 μm.

The absolute number of MFs in the sural nerve at the lateral malleolus ranges from 5,200 to 9,460, while the MF density is 5,200–8,000/m^2 (Behse 1990). It is difficult to know whether absolute fiber count or fiber density is the optimal measure. The former is probably subject to greater normal interindividual anatomical variability, while the latter is difficult to interpret in cases where fascicular size alterations are part of the pathology and can vary with fixation technique. In practical terms, however, investigators find it easier to derive a density from counting fibers in several representative endoneurial areas.

The thickness of the myelin sheath is proportional to axon diameter and likely reflects a complex interplay of trophic substances and molecular events (Quintes et al. 2010; Pereira et al. 2012). The parameters governing this relation are somewhat different for large vs. small myelinated fibers. As a rough guide, however, the ratio of axon (without its myelin) diameter to the fully myelinated axon diameter is normally 0.5–0.7. This has been called the "G-ratio" and is best assessed on ultrathin EM sections. Toluidine blue-stained light microscopic sections have a tendency to magnify the myelin sheath thickness relative to the axonal diameter.

Teased fiber preparations allow the measurement of internodal length (IL). As axon diameter increases, so does IL. For large myelinated axons, IL falls in the range of 0.35–1.83 mm, and for small myelinated axons in the range of 0.18–0.66 mm. Clearly, there is substantial variability within normal nerve, and this increases further with pathological processes and with aging.

2.1.2.5 Unmyelinated Axons and Their Schwann Cells

Unmyelinated fibers (UFs) are spread throughout the nerve fascicle, but have a preferential localization adjacent to small MFs, a relationship probably due to their shared developmental history (Figs. 2.7 and 2.8) (Thomas et al. 1993).

Using oil-immersion light microscopy, unmyelinated axons appear as round clear areas within nonmyelinating Schwann cells. A myelin-producing Schwann cell never invests any axon other than the one around which its myelin is wrapped. On the other hand, Schwann cells associated with unmyelinated axons (historically called Remak cells and with their collections of axons, Remak bundles) may have several such fibers under their influence, with the Schwann cell cytoplasm wrapping around and entirely isolating each axon from its neighbors. Sharma and Thomas (1975) defined Schwann cell subunits (ScSu) as any single Schwann cell profile or group of profiles that in transverse section is enclosed by a continuous basal lamina (Sharma and Thomas 1975). This structure can include no axons to several axons. The usual number of clustered cross-sectional profiles per ScSu is 2–5, but as many as 20 profiles may be seen (Behse et al. 1975), and there are usually 1–4 UFs per ScSu. A ScSu lacking axons is referred to as "denervated." ScSu lacking axons or containing collagen pockets are seen in normal nerves (Figs. 2.8 and 2.9) and increase with age and in neuropathy.

Identifying the axon within a ScSu can prove difficult. Sometimes a Schwann cell will seem to partially or fully encircle a bundle of collagen fibers, forming a so-called collagen pocket (Fig. 2.9), which may mimic axons even with oil-immersion light microscopy of 1 μm thick toluidine blue-stained sections, although lacking punctate organelles visible in axons. Ultrastructurally, axons are recognized by their lesser electron density, greater microtubule density, and absence of ribosomes. The axolemma is also somewhat thicker than the Schwann cell membrane. Finally, a distinct mesaxon around a circular profile helps identify that profile as an axon. However, axons are not always completely and individually wrapped by Schwann cell processes, or have an identifiable mesaxon. Isolated projections of Schwann cell cytoplasm surrounded by basement membrane and without an associated axon occur in normal nerves as denervated ScSus or collagen pockets, increasing with age and disease (Behse et al. 1975; Behse 1990).

Unmyelinated fibers outnumber myelinated fibers by about 4:1. The normal variability in UF numbers is even greater than for myelinated axons, with a range of 20,100–51,350 per nerve and a density of 18,000–42,000/mm^2. Unmyelinated axons have a unimodal size distribution, with fibers ranging in size from 0.1 to 3 μm, with a peak at 1–1.5 μm. There is, therefore, a degree of overlap of unmyelinated axon diameters with the smallest myelinated fibers. The only general features that distinguish UFs from MFs are their much higher proportion of axonal microtubules compared with neurofilaments, the frequent presence of dense-cored vesicles in autonomic efferent fibers, and a higher density of sodium channels. Rigorous quantitation and interpretation of unmyelinated fiber counts is complex. Although UFs are not segmented in the fashion of MFs, nonmyelinating Schwann cells do have a typical longitudinal extent of 200–500 μm (Carlsen et al. 1974).

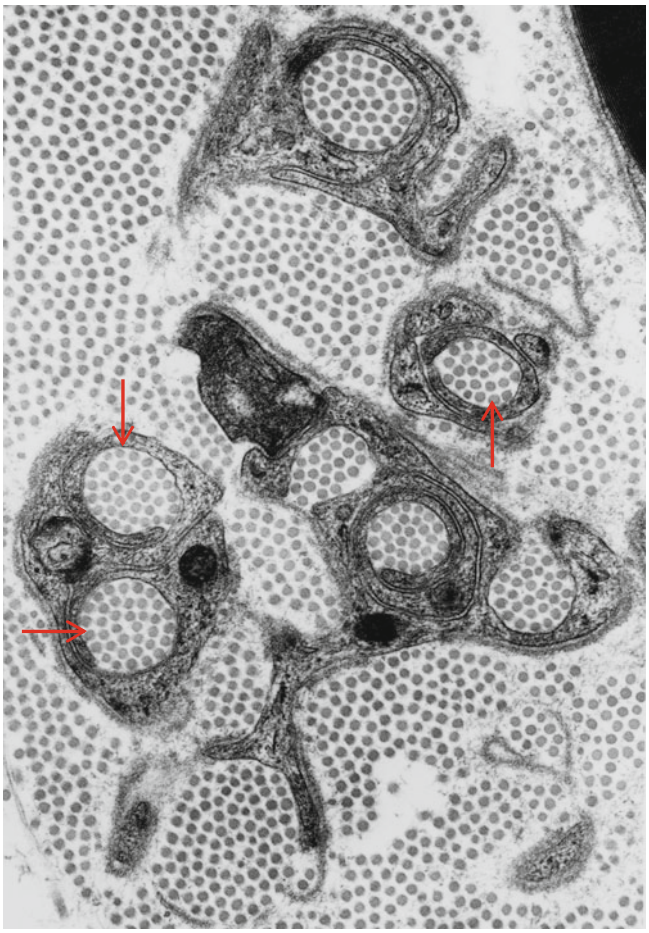

Fig. 2.9 Collagen pockets (*arrows*) surrounded by Schwann cell processes (21,840×)

2.1.3 Other Endoneurial Cell Content

The nucleated cells of the endoneurium are Schwann cells, fibroblasts, vascular endothelial cells, pericytes, smooth muscle cells, macrophages, lymphocytes, and mast cells. Quantitative assays of the numbers of these cells in the human sural nerve indicate that within the endoneurium about 80–90 % are Schwann cells and 10 % are fibroblasts, with nuclei of nonmyelinating Schwann cells outnumbering those of myelinating Schwann cells about 4:1 (Ochoa and Mair 1969). Endothelial cells are excluded from this count, and the other cell types are not present in significant amounts within the endoneurium, excepting perhaps macrophages (vide infra). There is interspecies and internerve variability in such statistics, probably relating to the differences in the unmyelinated fiber composition of the nerves examined (Thomas 1963).

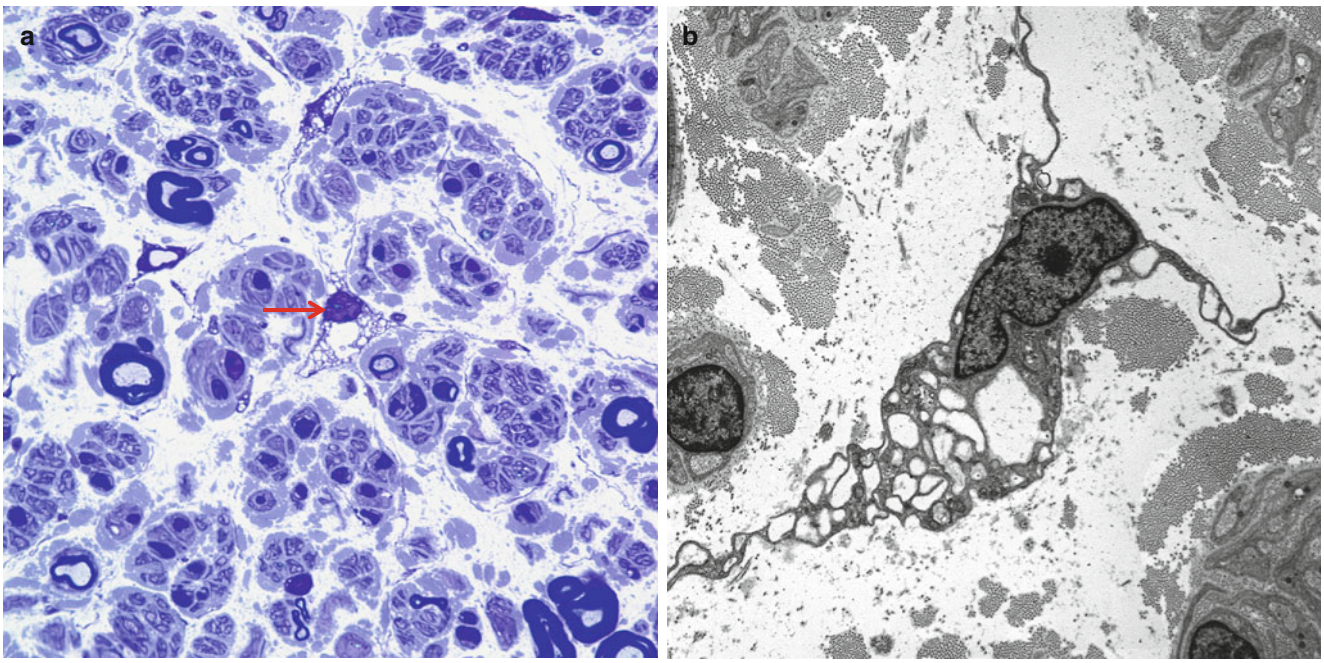

Fig. 2.10 A vacuolated fibroblast is shown in the endoneurium in a one micron thick toluidine blue section (*arrow*, **a**) and by electron microscopy (**b**). (**a**: 1 μ thick toluidine-blue-stained plastic section, 1,000×; **b**: 6,000×)

2.1.3.1 Fibroblasts

Fibroblasts are the second most common nucleated endoneurial cell, accounting for about 10 % of cells, with a density of about 300 nuclei/mm^2 (Ochoa and Mair 1969). These cells are thought to originate from neural crest stem cells and have a potential role in many endoneurial processes including reaction to injury including interleukin secretion, immune surveillance, and a supportive mechanical role (see Richard et al. 2012 for a detailed review). They are more prominent in the vicinity of endoneurial vessels. Use of light microscopy cannot always reliably distinguish fibroblasts from Schwann cells or native endoneurial histiocytes (Ochoa and Mair 1969). With immunohistochemistry, fibroblasts are variably immunoreactive for CD34 (Richard et al. 2012) and fail to stain with S-100 and EMA which distinguishes them from Schwann cells and perineurial cells, respectively. Fibroblasts may possess extremely thin and elongated cell processes (Fig. 2.10a, b). Ultrastructurally, they contain abundant granular endoplasmic reticulum, an irregular nucleus with a dense peripheral chromatin ring, and, most importantly, the absence of a surrounding basement membrane. Focal electron dense zones are sometimes present on the cell membrane and are highly characteristic. Small pinocytotic vesicles can often be seen just beneath the cell membrane. Frequently, fibroblasts occur in intimate contact with the collagen and oxytalan found ubiquitously in the endoneurium. A peculiar feature of some fibroblasts is "giant vacuolation" (Fig. 2.9a, b), especially within Renaut bodies (vide infra) and in regions of "edema"; however, it is uncertain whether these truly represent intracytoplasmic vacuolation or instead extremely convoluted cells caught in cross section.

2.1.3.2 Macrophages

Endogenous macrophages make up 2–9 % of the resident cell population of the endoneurium (Griffin et al. 1993). Their role in the peripheral nervous system has been reviewed (Griffin et al. 1993; Bonetti et al. 1993; Müller et al. 2006). Immunohistochemical techniques identify these cells reliably (Bonetti et al. 1993; Griffin et al. 1990) using antibodies against CD-68, Iba-1, and, even more specifically, CD-163. Quiescent (not activated) endoneurial macrophages are elongated cells which may demonstrate ramified spiny processes with their processes oriented along the longitudinal axis of the nerve. They do not possess a surrounding basement membrane and are often found in a perivascular distribution, stretched out in the longitudinal axis of the nerve (Griffin et al. 1993; Bonetti et al. 1993). Cross sections thus tend to de-emphasize their numbers, estimated at 50/mm^2 in the human sural nerve (Bonetti et al. 1993). They are distinguished from fibroblasts by the presence of an inconspicuous rough endoplasmic reticulum, which is poorly populated with ribosomes, and by coated vesicles and dense granules. Pinocytotic activity is not a feature, although endocytosis into coated vesicles may occur. Residual macrophages are reported to undergo a physiological turnover of 50 % in the sciatic nerve within 12 weeks (Müller et al. 2010).

Investigation of this cell population has suggested several functions. Their location, ability to take up soluble macromolecules, dendritic organization of some of their cell processes, and their constitutive expression of MHC class II and CD4 antigens suggest a possible role as antigen-presenting cells (Griffin et al. 1993). Classic thinking postulated that tissue macrophages were incapable of significant reactive proliferation (Griffin et al. 1993), but endoneurial macrophages do proliferate and participate in experimental Wallerian degeneration (Hann-Bonnekoh et al. 1989; Müller et al. 2006). Consideration of different roles for endoneurial and circulating macrophages is complicated by the lack of unequivocal markers for each; however, studies with chimeric rodents and GFP-labeled cells have established roles for resident macrophages as early responders in Wallerian degeneration, genetic neuropathies, and inflammatory neuropathy (Müller et al. 2006). Further work by this laboratory suggests that resident endoneurial macrophages intrinsically generate the macrophage response in slowly progressive neuropathy, which is supplemented by hematogenous macrophages in distal areas of more pronounced damage (Müller et al. 2008). Nerve regeneration, inflammatory demyelination, and clearance and recycling of myelin lipids are some of the important processes in which the critical role of macrophages is increasingly appreciated (Griffin et al. 1993).

2.1.3.3 Mast Cells

Mast cells occur in the epineurium, endoneurium, and rarely even perineurium. Their characteristic light microscopic appearance is as large oval cells with numerous metachromatic granules (Fig. 2.11) which contain heparin (Olsson 1968, 1971). Mast cells often reside near a blood vessel and can sometimes be identified in intimate contact with Schwann cells. They are found more frequently in the epineurium than endoneurium, usually singly but occasionally in pairs or even tiny clusters (Knorr-Held and Meier 1990; Olsson 1968). Ultrastructural examination reveals that the granules consist of membrane delimited collections of lamellar structures, often rolled up to give a "scroll"-like appearance (Fig. 2.11, inset). These "scroll-bodies" distinguish the cell from a basophil. Under normal circumstances, a few granules may rarely be seen extracellularly adjacent to the mast cell. Some mast cell granules can have particulate contents (Ghadially 1988). Numerous small cell processes protrude from their plasma membrane (Figs. 2.11 and 2.12) and may appear to contact other endoneurial cells (Fig. 2.12), in the case shown, a lymphocyte.

The role mast cells play in peripheral nerve disease is unclear. They synthesize and release proteoglycans, histamine, serotonin, proteases, and cytokines (Kaliner and Metcalfe 1993) and enhance capillary permeability and macrophage infiltration in pathological states. Classically, mast cells release their vasoactive contents following tissue trauma or upon binding and cross-linking of IgE receptors on the cell membrane, but the spectrum of degranulation inducing molecules and settings is broad (Kaliner and Metcalfe 1993). In a study of 31 sural nerve biopsies with various pathologies (but no normals) (Knorr-Held and Meier 1990), mast cell density correlated best with the severity of damage, especially to myelin. The nerve injury did not necessarily have to be very active, as also noted by Olsson (1971). A substantial overlap in mast cell numbers occurred between toxic or familial neuropathies and angiopathic or inflammatory neuropathies, although the latter had the highest values overall. This nonspecific increase in mast cell numbers in peripheral nerve disease is interesting in light of the speculation that chronic piecemeal degranulation of mast cells may be related to the fibrotic process (Claman 1993), which is a very common and nonspecific feature of most chronic peripheral neuropathies.

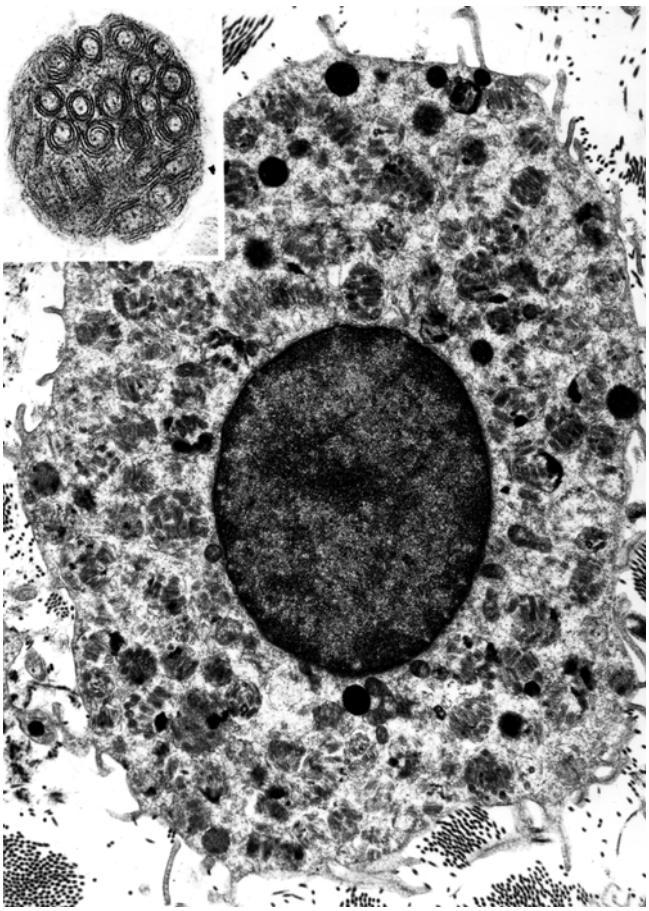

Fig. 2.11 Mast cell with granules which are seen better at higher magnification (*inset*) (12,996, inset 74,400×)

As cells capable of releasing vasoactive amines, mast cells can alter the blood–nerve barrier. The relation of this to neuropathic mechanisms has been considered by Brosnan and colleagues (1990) who demonstrated that pretreatment with the monoamine-depleting drug reserpine can slow the

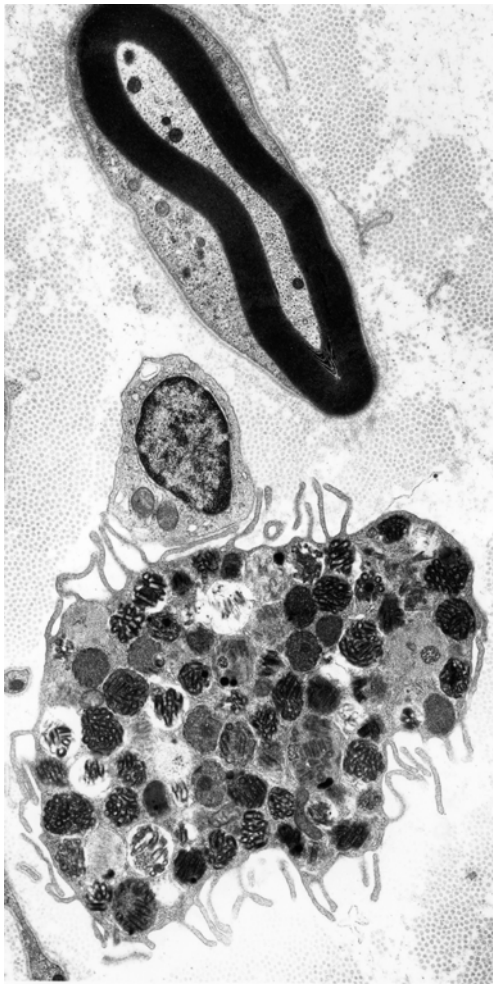

Fig. 2.12 A mast cell in intimate contact with an endoneurial lymphocyte is illustrated (11,000×)

onset of experimental allergic neuritis and that mast cell degranulation takes place in nerve before other alterations. In vitro, mast cells may be degranulated by myelin degradation products, and potentially more importantly, mast cell proteases can directly damage myelin (Johnson et al. 1988). Mast cells proliferate in situ as part of Wallerian degeneration or toxic axonal degeneration (Olsson 1968). Amputation neuromas contain an inordinate number of mast cells (Olsson 1968). Recent studies (Ito et al. 2008; see review by Forsythe and Bienenstock 2012) suggest important bidirectional communication between sensory axons and mast cells, which may involve a novel cell adhesion molecule of mast cells (CADM-1).

2.1.3.4 Leukocytes

Given the importance of inflammation in neuropathy, it is surprising that a consensus does not exist regarding the normal number and distribution of leukocytes in the peripheral nerve. Routine light microscopy is unreliable in the detection and correct identification of scattered lymphocytes. We have found immunostaining with lymphocyte markers in paraffin-embedded material to be helpful in the search for small collections of neural inflammatory cells. This family of cell surface antigens, conventionally identified as CD45 (leukocyte common antigen, LCA), is present on marrow-derived leukocytes including lymphocytes, circulating macrophages, and mast cells (Ellis 1993). Staining with CD45 identifies a population of endogenous neural macrophages, although not as many as would be predicted from the 2 to 9 % of endoneurial cells reported to be native macrophages (Griffin et al. 1993). For further classification of subtypes of lymphocytes, we use CD3 as a T-cell marker and CD20 and CD79a as B cell markers with further subclassification using numerous hematopathologic markers (Lu and Chang 2011).

A few recent studies have examined the presence of leukocytes in "normal" nerve using immunohistochemistry, but results are at times difficult to interpret because of the variability in antigens used and the occasional unpredictability of immunostaining techniques. Honavar et al. (1991) used autopsy material as controls (five patients) and found up to ten LCA-positive cells per mm^2 and six UCHL1 positive (T lymphocytes) per mm^2. Seven of nine sural nerve controls used by Kerkoff and co-workers (1993) had less than 10 T lymphocytes/mm^2 (as identified by CD3 immunostaining), and the highest number detected in normals was 30/mm^2. Pollard et al. (1987) examined four control sural nerve biopsy specimens from patients without neuropathy found T cells "only rarely." De la Monte and colleagues found up to 2.8 epineurial and 4.1 endoneurial LCA-positive cells per mm^2 in four normal nerves, but no T or B lymphocytes (de la Monte et al. 1988). Kerkoff et al. considered the issue of whether axonal degeneration itself elicits a lymphocytic infiltrate and found that there was no difference in lymphocyte numbers between nerves with and without axonal degeneration (Kerkoff et al. 1993).

Our experience suggests that a few CD45-positive cells randomly dispersed throughout the endoneurium are not necessarily abnormal. However, cuffing around an endoneurial vessel is always regarded as a significant marker of inflammation. This is different from the epineurium, where we have seen a few (up to three or four) perivascular leukocytes in normal nerves.

2.1.3.5 The Endoneurial Matrix

With a diameter of 50–60 nm, endoneurial collagen fibrils are smaller than those in the epineurium, perhaps reflecting a greater relative contribution of type III collagen to their composition (Salonen et al. 1987; Junqueira et al. 1979). Just external to the basal lamina of Schwann cells associated with myelinated axons, and to a lesser extent nonmyelinating Schwann cells, lies a tightly packed sheath of longitudinally arrayed collagen fibrils, with a smaller number showing

oblique or circumferential orientation (Friede and Bischhausen 1978; Thomas 1963). The remainder of the endoneurial collagen is less tightly packed, sometimes organized into large longitudinal bundles, other times forming small pockets fully or partially encircled by nonmyelinating Schwann cells. Close examination reveals that these collagen pockets actually lie outside the Schwann cells' basement membrane (Fig. 2.9). Collagen pockets in small numbers are a normal finding, but increase with age and in neuropathy. Collagen fibrils are also more densely concentrated around endoneurial vessels and beneath the innermost layer of the perineurium.

Technical difficulties and contradictions have hampered immunohistochemical efforts to determine the precise composition and localization of the macromolecular components of the endoneurial interstitial space (Lorimier et al. 1992; Shellswell et al. 1979). Types I and III collagen are seen in the endoneurium as in the epineurium, but type III is probably more important in the former than in the latter. Immunohistochemical (Lorimier et al. 1992) and morphologic (Salonen et al. 1987) considerations suggest that type III collagen is a particularly important contributor to the collagenous sheath that invests Schwann cells. Type IV collagen and laminin are ubiquitous components of the basal laminae found around perineurial cells, Schwann cells, endothelial cells, and pericytes (Lorimier et al. 1992).

Collagen in the endoneurium is believed to originate from both fibroblasts, synthesizing predominantly type I collagen, and Schwann cells, producing type III collagen. Other collagen types are minor in quantity. An increase in endoneurial collagen is a common feature of chronic neuropathy. In nonneural tissues, fibroblasts have been identified as the key player in fibrosis and scar formation after injury. Potentially important contributors to endoneurial fibrosis include T cells, mast cells, and macrophage products (Claman 1985). Schwann cells may also produce collagen as part of the regenerative response to injury (Salonen et al. 1987).

Mature elastic fibers are not found in the endoneurium (Ferreira et al. 1987). However, fibrillar material with a diameter of 10–12 nm is seen throughout, particularly in a subperineurial and perivascular disposition (Fig. 2.13a–d), and in Renaut bodies (vide infra). Morphology (Ghadially 1988) and immunostaining (Weis et al. 1993) indicate that this substance is oxytalan, a component of the elastic fiber system originally identified based on its light microscopic staining properties (Ghadially 1988). It is believed that oxytalan serves as the scaffold upon which amorphous elastin is laid during the formation of elastic fibers (Ghadially 1988; Mecham and Heuser 1991). At present, the term "oxytalan" is usually applied in reference to the ultrastructural appearance of this fibrillar material (Ferreira et al. 1987). In some publications, oxytalan has been mistaken for amyloid deposition, but notwithstanding its different histochemical properties and the wavy appearance of oxytalan fibrils contrasts with the rigid appearance of amyloid (Fig. 15.1). We have on occasion observed a more mature stage of elastic fiber formation, elaunin (arrows, Fig. 2.13b), in the endoneurium, but only in abnormal nerves.

There remains an optically empty component to the interstitium, composed of water and various macromolecules. The total endoneurial protein content is about half that of plasma, and the most important constituent is albumin (Low et al. 1982). Other macromolecular components of the endoneurial interstitium include hyaluronic acid, chondroitin sulfate, fibronectin, and versican (Lorimier et al. 1992).

2.1.3.6 Renaut Bodies

Renaut bodies are whorled structures, usually located in the subperineurial area. In cross sections, they are round or ellipsoid (Fig. 2.14a, b), and longitudinal sections reveal that they are elongated and run parallel to the long axis of the nerve (Fig. 2.14c). Renaut bodies contain no axons and few vessels, a rare mast cell, and their predominant cellular content consists of fibroblasts. With glutaraldehyde and B5 fixation, Renaut bodies are seen as round or ellipsoid in cross section, 30–200 μm in diameter, and lightly eosinophilic and are lightly stained with trichrome (Fig. 2.14d) and Alcian blue (Fig. 2.14e), but not with PAS or Congo red. Formalin fixation tends to result in a collapse of the structure. Occasionally, a Renaut body may be found more centrally within the nerve fascicle, surrounded by axons, or may have a circumferential disposition around the entire fascicle, mimicking the so-called subperineurial edema (Fig. 2.15a, b). Vessels adjacent to the Renaut body often show a degree of hyalinization. Electron microscopy reveals a very loosely textured structure with whorls of collagen intermingled with a fine extracellular fibrillar component consisting of filaments 10–12 nm in diameter (Fig. 2.16). Fibroblasts with very long attenuated processes surround and intermingle with this interstitial material, sometimes showing prominent vacuolation (Figs. 2.16 and 2.17a, b). A discontinuous basal lamina may be seen investing some of the "fibroblasts," suggesting a degree of perineurial differentiation, an impression supported by frequent immunostaining positivity for EMA (Weis et al. 1993). Immunohistochemical techniques reveal collagen types I, III, V, and VI in the interstitium, as well as positive staining for oxytalan (Weis et al. 1993). The latter presumably corresponds to the 10–12 nm diameter filaments mentioned above, found ubiquitously throughout the endoneurium, but particularly frequently in Renaut bodies, in a subperineurial location, and around vessels (Weis et al. 1993).

Lack of familiarity with the appearance of Renaut bodies may result in diagnostic errors. Because of their amorphous and focal nature, and the presence of filaments vaguely similar to amyloid fibrils (i.e., oxytalan), they have been mistaken

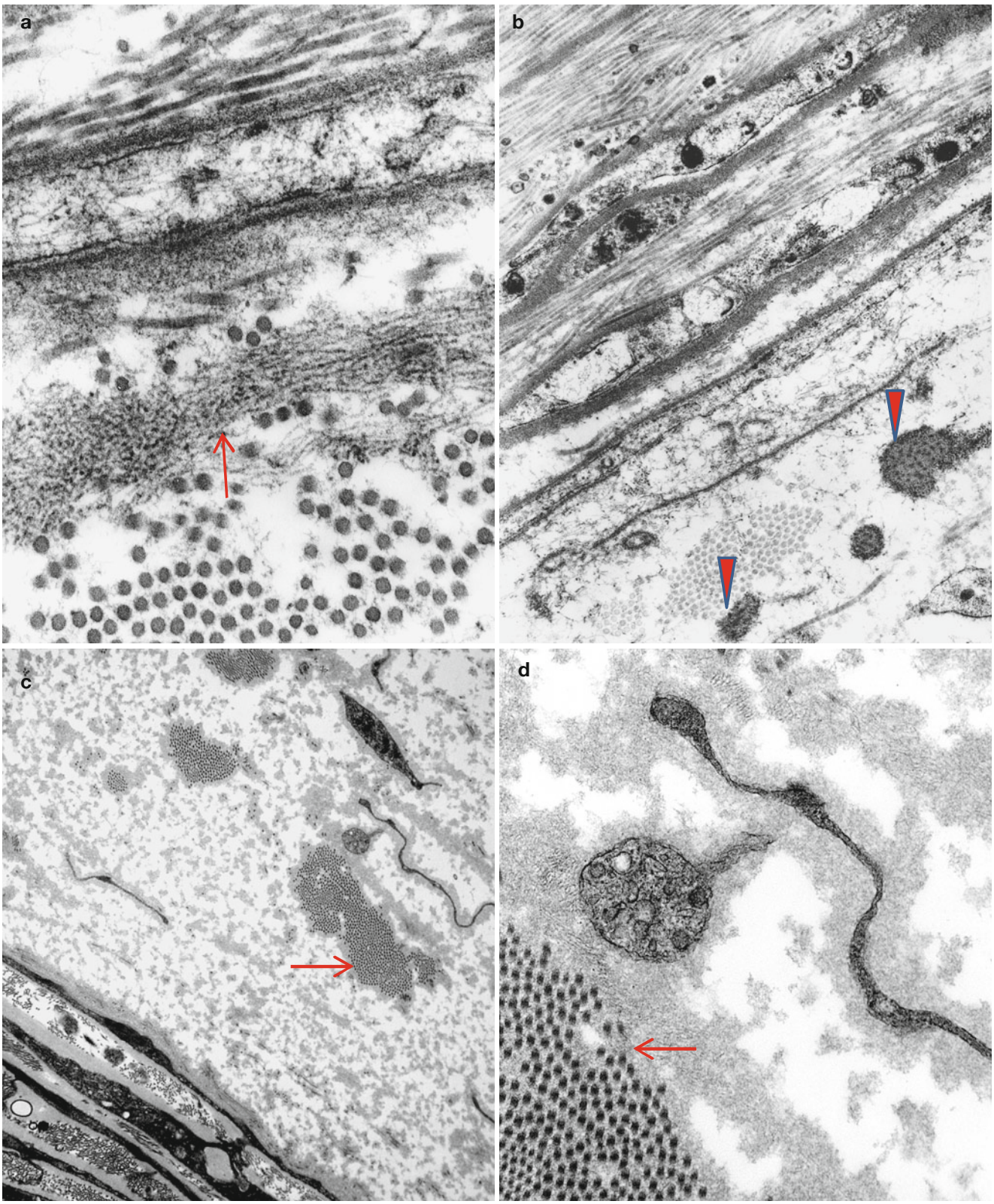

Fig. 2.13 Aggregates of oxytalan are often seen in the subperineurial region (*arrow*, **a**), whereas elaunin formation (**b**) is uncommon. Both collagen and oxytalan are delicately mixed (*arrows*, **c**, **d**) in the endoneurium of this patient with endoneurial edema (**a**: 43,400×; **b**: 7,920; **c**: 6,000×; **d**: 30,000×)

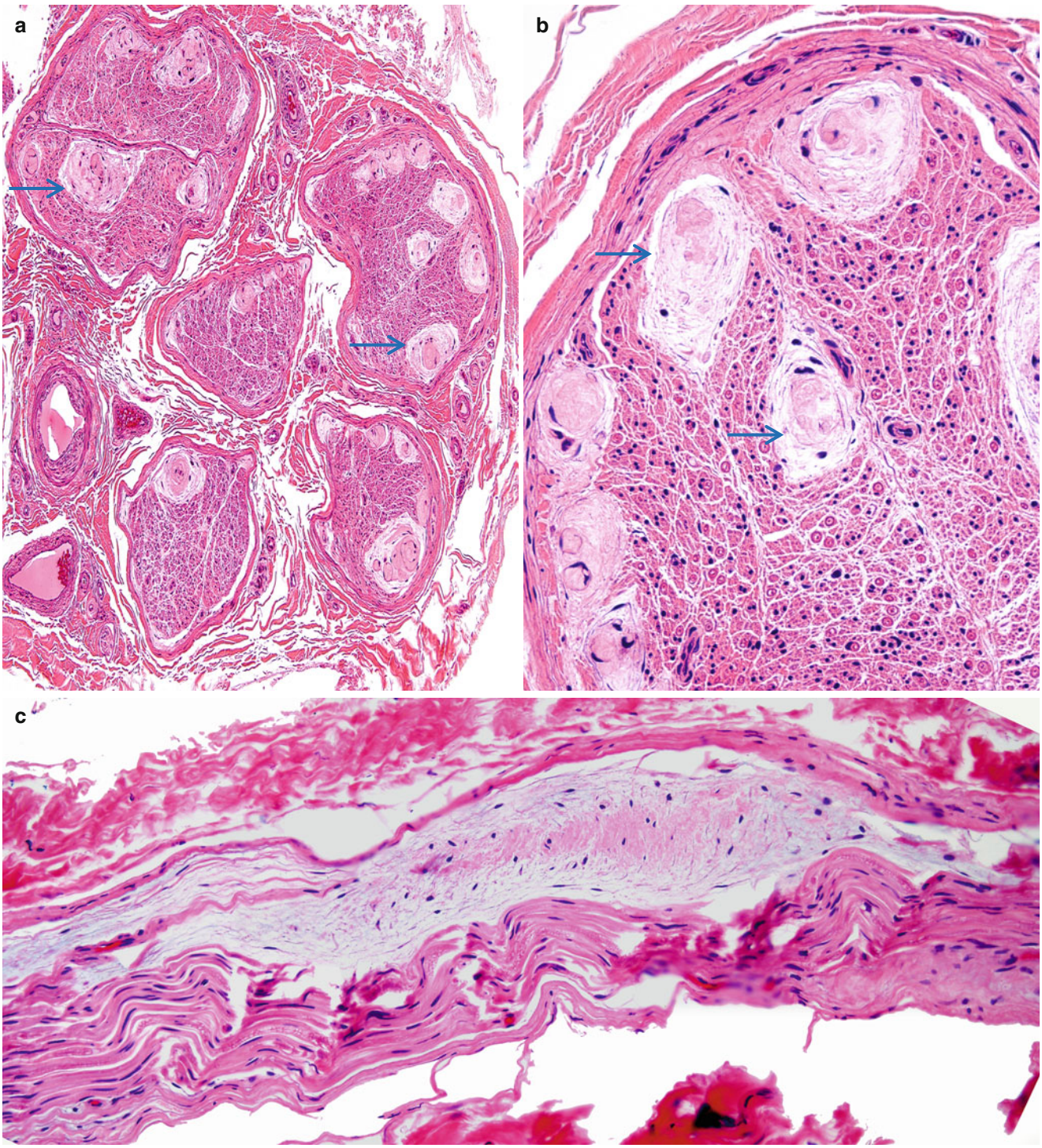

Fig. 2.14 Renaut bodies are shown in cross (*arrows*, **a**, **b**) and longitudinal sections (**c**). Trichrome (**d**) and Alcian blue (**e**) stains demonstrate their collagenous and ground substance/mucopolysaccharide constituents. (H&E-stained paraffin sections: **a** 100×; **b** 400×; **c** 400×; trichrome: **d** 400×; Alcian blue: **e** 400×)

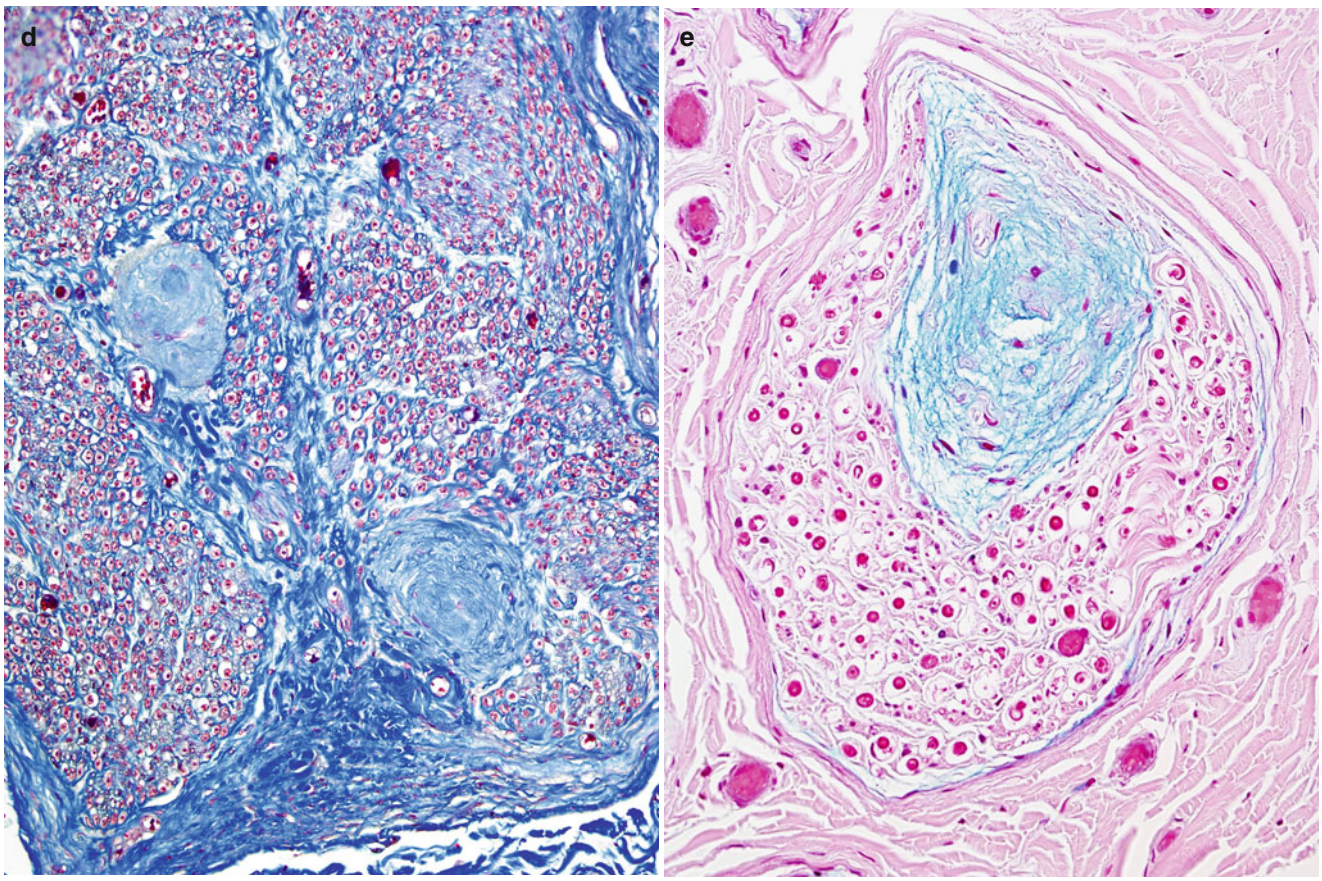

Fig. 2.14 (continued)

for amyloid or immunoglobulin deposits. They have also been misinterpreted as endoneurial edema or as nerve infarcts. We have seen prominent Renaut bodies in about 2 % of 600 sural nerve biopsies, in keeping with literature values which range from 2 to 7.5 % (Meier and Bischoff 1977; Bergouignan and Vital 1984; Weis et al. 1993). Renault bodies occur in otherwise normal nerves. Where there is nerve pathology, it does not seem to be increased in severity adjacent to the Renaut body. Their incidence is unquestionably increased in focal nerve compression (Schroder et al. 1978; Neary et al. 1975; Mackinnon et al. 1986; Jefferson et al. 1981) and may also increase with age (Bergouignan and Vital 1984). It has been suggested that Renaut bodies are more commonly seen in hypothyroid neuropathy, but in the two largest modern reviews of these structures, none of 33 patients were said to have hypothyroid neuropathy (Bergouignan and Vital 1984; Weis et al. 1993).

Experimental production of Renaut bodies through chronic compression demonstrates an evolution from foci of endoneurial "edema," with gradual accumulation of fibroblasts and various fibrillar materials, until a mature Renaut body is formed (Ortman et al. 1983). No correlation exists between degeneration of endoneurial capillaries or nerve fibers and formation of Renaut bodies. This suggests that their formation is a response to mechanical stress. However, in an experimental model of axonal transection without regeneration, at 1 year after transection, the denervated nerve fascicle was filled with fibroblasts and fibrillar configurations vary similar to those of Renaut bodies (Roytta and Salonen 1988).

2.2 Age-Related Changes

The appearance of a normal peripheral nerve varies with age, from the rapid developmental evolution that occurs during the first 5 years of life to the slow degeneration of aging.

2.2.1 Aged Individuals

The role of aging in the development of neuropathic changes has been difficult to study because of the large variation seen in normals even within a single age group. However, changes do occur with aging, and the pathologist must be aware of them, most importantly to avoid overinterpretation of

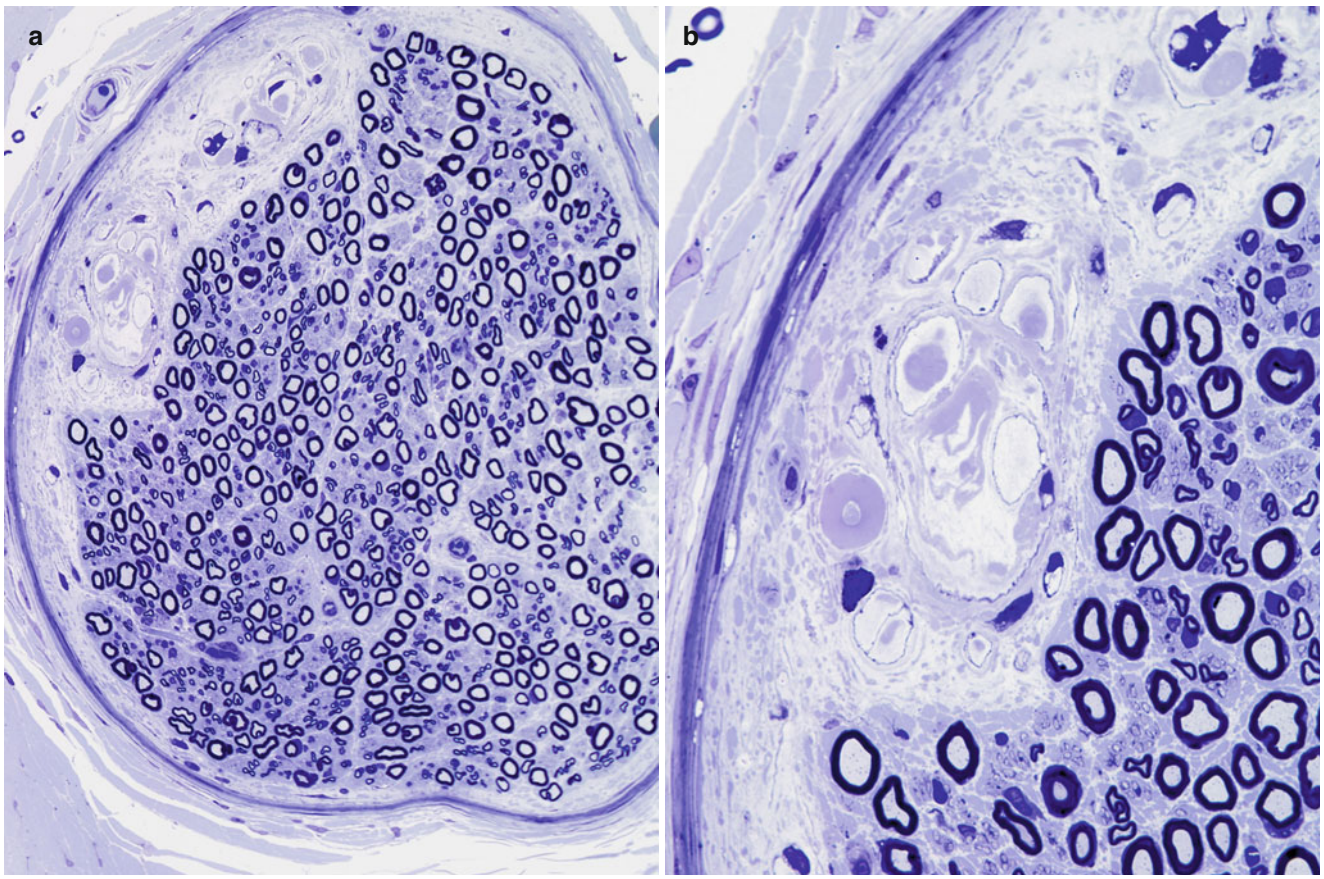

Fig. 2.15 Renaut bodies may have a circumferential subperineurial location (**a**, **b**) as well as both solid and wispy constituents (**a**: 400×; **b**:1,000×, 1 μ thick toluidine blue-stained plastic section)

minimal alterations. Clinically, there is an increasing incidence of absent reflexes and impairment of distal sensation in healthy elderly (Bouche et al. 1993). Such impairment is most pronounced in distal sensory fibers, and electrophysiological techniques indicate that loss of amplitudes is more prominent than reduction of conduction velocities, suggesting that distal axonopathy underlies age-related peripheral nerve changes (Bouche et al. 1993), although functional changes may be amplified by the common occurrence of dystrophic nerve terminals in sensory endings in the gracile (and to a lesser degree cuneate) nucleus.

2.2.2 Early Life

Changes occurring during the first years of life deserve a separate discussion because these reflect normal maturational processes. The issue of peripheral nerve development is an important and complex one which is not specifically considered in the current work (Webster 1993; Mezei 1993). Nerve conduction velocities rise from approximately half the adult value at birth to the adult range by age 3–5 (Kimura 1989).

Researchers have performed several quantitative studies on developing human sural nerves (Jacobs and Love 1985; Gutrecht and Dyck 1970; Ouvrier et al. 1987; Ferriere et al. 1985; Schellens et al. 1993). Onset of myelination is thought to occur at about 18 weeks of gestation (Ochoa 1971). It is likely that by the time of birth nearly all fibers destined to be myelinated have begun this process, but data in human sural nerves are not conclusive. The number of myelinated fibers in the sural nerve increased from 4,000 at birth to 7–12,000 by the age of 5 years in the work of Gutrecht and Dyck (1970), but did not change significantly after birth in the data provided by Ferriere et al. (1985) and Schellens et al. (1993), and showed a trend towards increasing after birth in a fourth study (Jacobs and Love 1985). Fiber density however is clearly highest at birth because of a much smaller endoneurial area: 0.2–0.5 mm^2 at birth vs. 0.6–1.2 in adult life. Typical values are 12–20,000 MF/mm^2 at birth, falling to the adult range by about age 10 years. With maturation an increase in both size and separation of the axons occur (Schellens et al. 1993). Axon diameter and myelin thickness are below adult values at birth and infancy (Fig. 2.18), but the g-ratio is above normal, indicating relative hypomyelination. By the age of 5 years

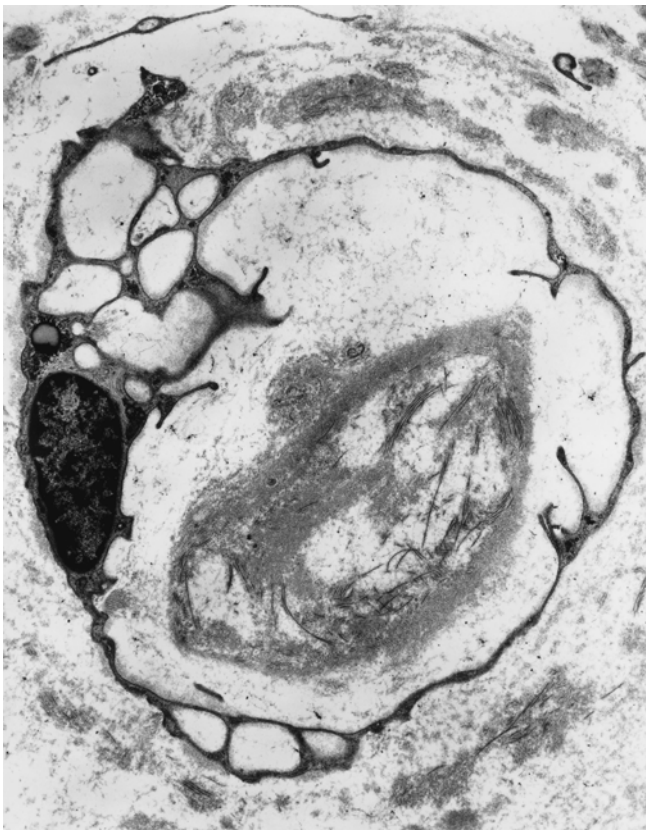

Fig. 2.16 A vacuolated fibroblast encircles a core of oxytalan mingled with collagen fibers (7,200×)

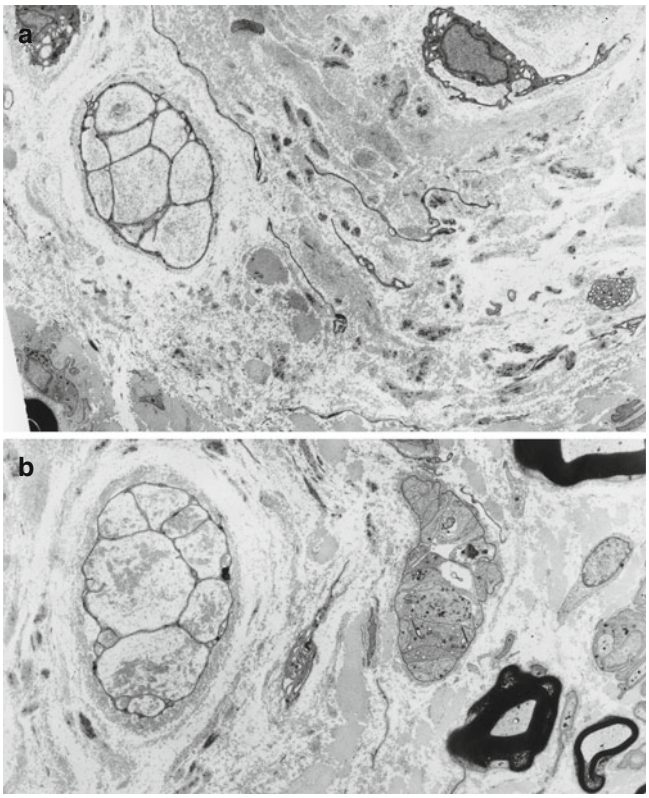

Fig. 2.17 The "honeycomb" configuration of fibroblast processes encircling oxytalan is characteristic of Renaut bodies. Interface between Renaut body and endoneurial contents is shown in (**b**). (**a**: 3,200×; **b**: 3,840×)

axon diameters reach adult values, but final adjustments in myelin thickness continue for 10 more years (Schroder et al. 1978; Ferriere et al. 1985). The fiber diameter–frequency histogram is unimodal at birth and bimodal in adult life, the transition occurring at about 1 year of age (Schellens et al. 1993). With increasing age there is a tendency for ever greater overlapping of the two peaks in the histogram (Schellens et al. 1993). Internodal length is typically 200–300 μm at birth and increases to adult values (200–1,800 μm) in parallel with somatic growth (Gutrecht and Dyck 1970; Schlaepfer and Myers 1973; Friede et al. 1981). Overall, one might consider 10 years of age as a convenient (but rough) guideline for the age at which the human sural nerve reaches histological "maturity."

2.2.3 Age-Related "Degenerative" Changes

Several age-related alterations in the human adult sural nerve have been documented (Jacobs and Love 1985; Tohgi et al. 1977; Ouvrier et al. 1987; Schellens et al. 1993; Takahashi 1966). The value of quantifying many of these changes is dubious because of the large variation in normal at any age. Interpretation of age-related neuropathy is complicated by the increased incidence of comorbidities with aging (Suzuki 2013). Qualitative observations are discussed below, while in Chap. 3 we present quantitative data:

1. Endoneurial area and endoneurial collagen increase with age.
2. Myelinated fiber densities decrease continuously through adult life. However, because of the wide variability seen in normals, and the very slow decrement that occurs with aging, it is not possible to distinguish a normal "old" nerve from a normal "young" nerve based purely on this parameter. The depletion is most pronounced for large myelinated fibers (Tohgi et al. 1977; Takahashi 1966). In one study of 79 sural nerves, the average large myelinated fiber density decreased by almost 50 % from the second to the eighth decade, but there was still overlap in the range of normal between these two age groups (Tohgi et al. 1977).
3. Plots of internode length vs. axon diameter also give evidence of increasingly frequent axonal degeneration and regeneration in as many as 25 % of fibers (Arnold and Harriman 1970; Dyck et al. 1993). However, the incidence of active degeneration in teased fiber studies (i.e.,

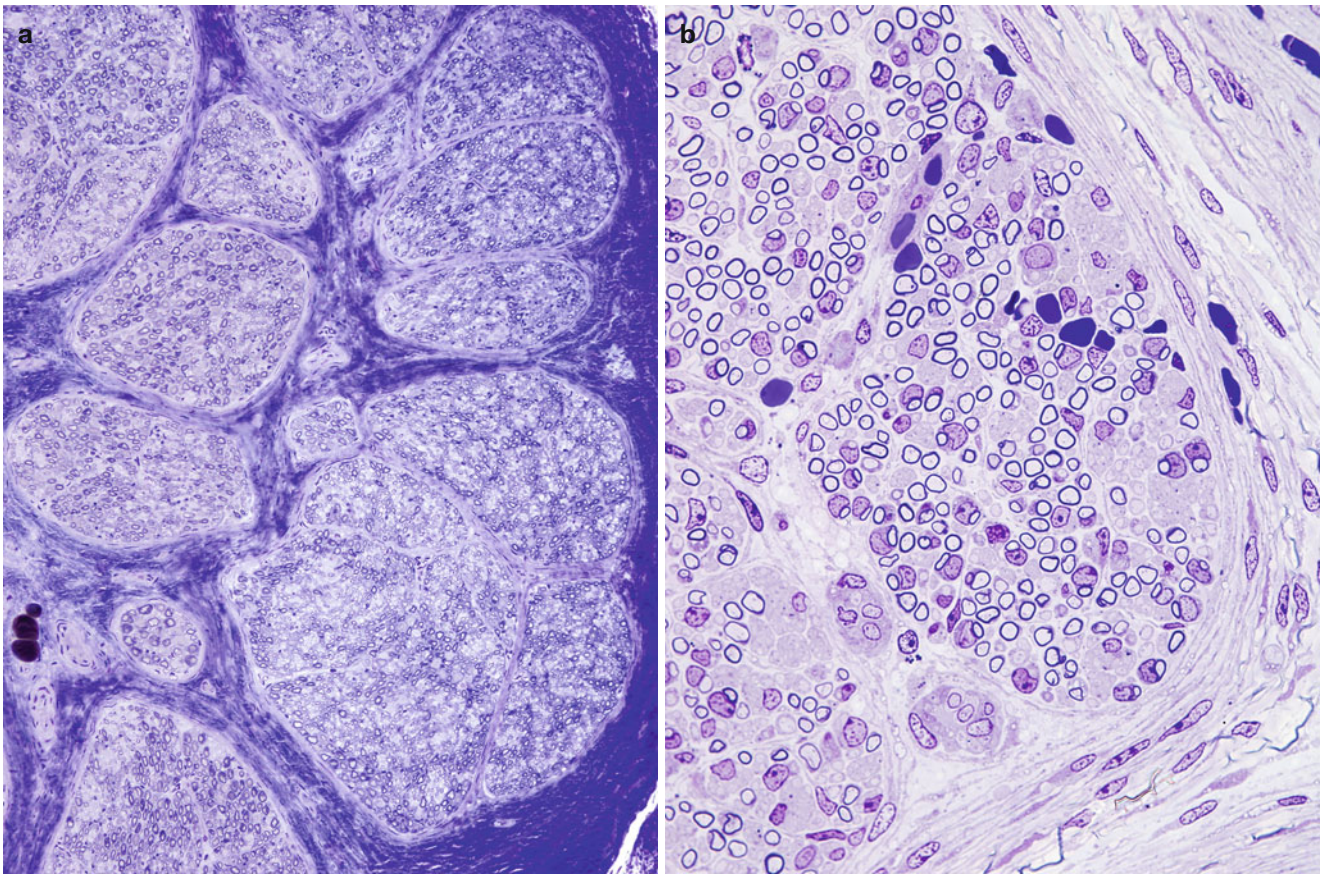

Fig. 2.18 Peripheral nerve from a 33-week preterm infant surviving 16 additional weeks (thus, effectively a 9-week-old) shows a monomorphic population of myelinated axon, at higher density, and including axons with thinned myelin for axon caliber. (1 μ thick plastic section, **a**: 200×; **b**: 1,000×)

rows of myelin ovoids) also probably increases with age, but remains uncommon (Arnold and Harriman 1970). Regenerating clusters are also probably seen more frequently in the elderly, but quantitation of this has not been performed.

4. With increasing age, the number of profiles per ScSu increases, as does the density of denervated ScSus, collagen pockets, and single Schwann cell protrusions (Kanda et al. 1991).
5. Plots of myelin thickness or internodal length vs. fiber diameter demonstrate an increasing scatter, as does the G-ratio (Jacobs and Love 1985; Lascelles and Thomas 1966). These changes are indicative of increasingly frequent segmental demyelination and remyelination. Whether this is secondary to axonal atrophy or a primary change is unclear.
6. In contrast to the reduction in axon densities, which might at least partially be due to a gradual increase in endoneurial area with age, the nuclear density remains stable or increases slightly with age (Tohgi et al. 1977).
7. Endothelial capillaries show increasing hyalinization and duplication of endothelial and pericytic basement membrane (Jacobs and Love 1985), but fenestration does not normally occur with age. Muscular vessels are not significantly altered (Tohgi et al. 1977). The perineurial basement membrane also increases in thickness with age, most prominently at the outer layers (Tohgi et al. 1977; Jacobs and Love 1985). It has been suggested that nutrient artery atherosclerotic changes are correlated with age-related loss of axons (Takahashi 1966).

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Quantitative Techniques

3

Special techniques can sometimes maximize the information obtained from nerve biopsy. The teasing apart of nerve fascicles to free single myelinated fibers for individual study, which has a long history dating to the days of Gombault (1886), has contributed greatly to understanding the pathological processes affecting peripheral nerve. In recent years, image analysis methods have permitted automated measurement of various parameters that occasionally prove helpful in characterizing the process afflicting the nerve under study. Although we do not rely heavily on these techniques, this chapter provides a brief discussion. The references will guide those seeking more detailed information.

3.1 Fiber Teasing

Fiber teasing allows assessment of the pattern of nerve disease along several internodes of the same myelinated fiber. The most sensitive method for detecting segmental myelin changes, in principle, allows workers to determine whether these changes are primary or secondary to axon disease.

Dyck and colleagues provide the most comprehensive discussion of fiber teasing methodology and interpretation (Dyck et al. 1984; Dyck and Giannini 1993). We do not routinely employ nerve teasing in the assessment of biopsy specimens. Rather, we reserve this technique for special situations or research applications; some, but by no means all, workers share this approach (Logigian et al. 1994; Oh 1990; Schaumburg et al. 1992). Teasing is a difficult and tedious work and, as Dyck indicates, even an experienced technologist can prepare only 150–300 fibers a day (Dyck and Giannini 1993). Since the information gained from teased fiber examination is nonspecific, economic considerations mandate that diligent and experienced technologists may best utilize their energy in other areas.

When teasing is not performed properly, the information derived may be unreliable. Examining too few fibers risks drawing conclusions from a nonrepresentative sample. This result is especially likely when the technologist is not highly experienced and causes excessive fiber trauma, for even the most gentle teasing produces myelin artifact (Williams and Hall 1971). Large myelinated fibers are easiest to tease free, and as a result, a biased selection may thus be obtained. Dyck and colleagues have emphasized the need for appropriate sampling, suggesting that at least 100 fibers are necessary (Dyck and Giannini 1993). Assessment of demyelination "clustering" for deciding whether demyelination is primary or secondary requires at least five internodes per teased fiber. The literature, however, abounds with papers reporting results and even "normative data" based on as few as 20–50 teased fibers. Many papers even neglect to indicate how many fibers were studied.

As reviewed in Chap. 1 (Table 1.1), sural nerve biopsy may be a diagnostic procedure for many diseases, foremost among them vasculitis, amyloidosis, and leprosy. These three diagnoses, and indeed all others on the list of diseases for which sural nerve biopsy may be a diagnostic procedure, are not made on the basis of fiber teasing results. Workers most often use teased fibers to detect segmental myelin changes. In addition, teased fibers may permit distinction between primary and secondary demyelination. However, examining teased fibers does not lead to a specific diagnosis. In contrast, electron microscopy in demyelinating neuropathy can reveal macrophage-mediated myelin stripping, widened myelin lamellae, or various inclusions, all of which permit an etiologic diagnosis. Indeed, the long-standing belief based on teased fiber studies that segmental demyelination and remyelination in CMT-1 is secondary to axonal disease and the recent demonstrations that defects may be in either myelin or axonal proteins leads one to wonder how meaningful the identification of demyelination as "secondary" really is.

The degree to which teased fibers surpass semithin cross sections in revealing subtle myelin changes is unclear. In a seminal study of chronic inflammatory demyelinating polyneuropathy (CIDP, Prineas and McLeod 1976), all nerves in which 20 % or more of fibers showed segmental demyelination had evidence of the same on electron microscopy. Of 12

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cases in which 1–10 % of fibers showed segmental demyelination, only 2 had EM changes. Only rarely will EM demonstrate segmental demyelination when teased fibers do not (McLeod et al. 1973). While such data suggests a considerably greater sensitivity of teased fibers, it is important to appreciate that the incidence of segmental myelin changes in controls is not zero. Research criteria for the diagnosis of CIDP require that at least 12 % of teased fibers should show segmental myelin changes (Ad Hoc Subcommittee 1991). Other authors have reported that 3.9–20 % of internodes are abnormal in control nerves on average (Behse 1990; Dyck and Giannini 1993; Tsakuda et al. 1987) with some normals showing even more frequent changes. Furthermore, the incidence of pathological alterations increases in aged controls (Dyck and Giannini 1993). By itself, paranodal myelin retraction, which teased fiber studies very easily detect, is difficult to interpret at any time, because this condition can occur in the earliest stage of segmental demyelination, in axonal degeneration (Williams and Hall 1971), and in normals (Arnold and Harriman 1970).

Evidence of axonal degeneration by demonstration of linear rows of myelin ovoids or of degeneration and regeneration by way of uniformly shortened internodes can also be seen in "normal" nerve, the incidence increasing with age (Arnold and Harriman 1970). Workers cannot regard as unequivocally abnormal up to 4 % of teased fibers showing rows of myelin ovoids (Arnold and Harriman 1970; Dyck and Giannini 1993). In addition, up to 16 % of fibers can show evidence of axonal regeneration in "normal" nerve (Behse 1990). In patients over 60 years of age, Arnold and Harriman (1970) reported that at least 6 of 24 teased fibers should show evidence of axonal degeneration or regeneration before a nerve could be considered abnormal.

Thus, the amount of "pathology" seen in normal nerves limits the usefulness of teased fiber studies in detecting subtle changes, especially with increasing age. Teased fiber abnormalities other than "wrinkled" myelin were seen in 7.5–37.5 % of 9 controls used by Behse (1990), the eldest only 54 years of age. Indeed, most normative data are based on the younger age group even though several studies (Jacobs and Love 1985; O'Sullivan and Swallow 1968; Vital et al. 1990) have shown a dramatic increase in nonspecific changes with age. Approximately half of nerves assessed in our laboratory come from patients over 60 years of age (Fig. 1.1).

A recent study of correlation between nerve conduction studies and sural nerve biopsy reported that in 11 of 52 instances (21 %) when both cross-sectional and teased fiber (minimum of 50 fibers) examinations were employed, the histological diagnoses were discordant (Logigian et al. 1994). However, in 48 % of these biopsies, three independent observers could not agree on the interpretations of the pathology. Thus, the subjective nature of nerve biopsy interpretation represents a greater source of error than whether or not fiber teasing is performed. Notably, the discordance between electrophysiological findings and biopsy results (63 % concordant, 14 % minimally discordant, and 23 % discordant) did not change when only teased fiber results were considered (Logigian et al. 1994).

In conclusion, light microscopic examination of 20 or more semithin cross sections, supplemented by careful electron microscopic review of selected nerve fascicles, leaves little need for teased fiber preparations. In some diseases, such as tomaculous neuropathies, teased fibers provide an elegant and convincing means of illustrating the pathology; however, we cannot identify a situation where our approach would lead to the loss of diagnostically useful information. We recognize the value of fiber teasing for research in peripheral nerve disease, especially when workers can study large numbers of fibers in multiple nerves. Yet, in the daily practice of peripheral nerve pathology, this approach seems insufficiently informative to justify its cost.

3.2 The Morphometric Study of Nerve Biopsies

Several quantitative analytic tools are available to those seeking to describe nerve pathology with greater precision than a subjective assessment of morphology allows. These "morphometric" techniques have become a sometimes useful adjunct to morphology. In our routine practice, other than an estimate of the severity of MF and UF loss, we only infrequently perform morphometric analysis, because morphology, not morphometry, gives specific diagnostic information.

Morphometry has, nevertheless, been important in the evolution of understanding of peripheral nerve function and disease. Quantitative techniques allow presentation of large amounts of data in compact graphs or scatter plots. Informed reading of the peripheral nerve literature also requires a certain familiarity with the various quantitative measures in use. Thus, the discussion below reviews some of the more important morphometric techniques but is by no means comprehensive. For more detailed information, we refer the reader to studies by Behse (1990), Dyck and co-workers (1984), Gibbels (1989), Hunter et al. (2007), and Thomas (1970).

To maximize the usefulness of fiber density measures, each laboratory should ideally use its own normals, prepared with a standard technique. The effect of tissue shrinkage resulting from use of different fixatives should be considered when comparing data from different laboratories. Workers should compare abnormal nerves only to age-matched normals. Some literature control data includes patients with stroke or ALS, on the assumption that these processes do not cause alterations in peripheral sensory nerves. Pollock and colleagues (1984) have shown that there may be alterations in the sural nerve of a hemiplegic limb. Moreover, a growing

literature suggests that alterations of sensory fibers can occur in motor neuron diseases (Isaacs et al. 2007). Paid normal volunteers, where a full neurological history and physical examination can be performed along with electrophysiological studies, represent the best source of control data (Dyck and Giannini 1993). In the absence of such data, the rigorous approach described by Stoebner et al. (1989) is commendable. Their normal nerves came mostly from patients in acute (≤48 h) coma with no previous history of neuropathy, with normal electrophysiological testing of peripheral nerves, and with the specimen removed during organ harvest for transplantation. Because severely cachectic or bed-bound patients are at risk for nutritional and pressure palsies, workers should not consider them to have normal peripheral nerves.

3.2.1 Fiber Counts and Histograms

3.2.1.1 Myelinated Fibers

Investigators have invested much time and effort in studying the absolute number and density of fibers of various diameters in peripheral nerve (Tables 3.1 and 3.2). Myelinated fiber density (MF/mm^2 of endoneurial cross-sectional area) is the standard measure, typically determined using toluidine blue-stained semithin (1 μm) sections. Modern image analysis technology has largely automated such measurements (da Silva et al. 2007; Hunter et al. 2007), although some interaction between technologist and computer still must occur. Dyck and colleagues have considered methodological issues in detail (Dyck et al. 1984).

The total number of fibers in the whole sural nerve varied by nearly a factor of two in one study, from 5,060 to 9,460 fibers (Behse 1990). Although favored by some authors, such measurements are more difficult to perform on nerve biopsy specimens than fiber density. Fiber density is more commonly used despite its dependency on several factors including fixation (10–36 % discrepancy) (Behse 1990; Tohgi et al. 1977), proper sampling (Behse et al. 1974; Dyck et al. 1984), and, in neuropathic nerves, hypertrophy of endoneurial contents (Behse et al. 1974). Fiber density decreases with age, one study showing values in the eighth decade falling to 70 % of those in the third decade (Tohgi et al. 1977). Most observers report similar data (Jacobs and Love 1985; O'Sullivan and Swallow 1968). At least part of this drop results from an increase of endoneurial area with age (Jacobs and Love 1985). Large MFs are more severely influenced in this regard than small MFs. However, the intrinsic variability of MF density does not allow distinction of "old" from "young" nerves on an individual basis.

In children, absolute sural nerve MF numbers probably increase in the first few years of life (Gutrecht and Dyck 1970; Jacobs and Love 1985), although not all investigators have observed this (Ferriere et al. 1985). Fiber density drops dramatically from values of about 20,000 MF/mm^2 at birth to adult values by the age of 10 (Gutrecht and Dyck 1970; Jacobs and Love 1985; Ouvrier et al. 1987). This decrease occurs in parallel with an increase in endoneurial area from birth to the second decade, as axons enlarge and the interstitial space increases.

Myelinated fibers in the sural nerve range from 3 to 14 μm in diameter, defined as the combined width of axon and myelin. Histograms of MF numbers vs. diameter are bimodal, one mode clustering around a diameter of 4 μm, another around a peak of 10 μm, with an overlap between the two groups (Fig. 3.1a, b). Using a cutoff of 7 μm, Behse (1990) found that in normal nerves, 32–45 % fall above and 68–55 % below this value.

MF loss that affects large diameter fibers more than small diameter fibers, such as shown for CMT-1 (Fig. 3.1c), is a common and entirely nonspecific pattern of axon depletion. The opposite pattern, with more severe relative depletion of small myelinated fibers (Fig. 3.1d, Table 7.7), occurs infrequently but can provide a diagnostic clue with a limited number of potential etiologies.

When regenerating clusters are frequent, the relative proportion of small myelinated fibers increases, potentially creating a false impression of relative loss of large myelinated fibers. A shift of both peaks of a bimodal histogram to the left provides evidence of axonal atrophy (Ohi et al. 1985).

3.2.1.2 Unmyelinated Fibers

Unmyelinated fibers (UFs) range between 0.1 and 3.0 μm in diameter (although an UF 2–3 μm in diameter is rare), in a unimodal distribution with a peak at 1.0–1.3 μm (Behse 1990). Workers perform unmyelinated fiber quantitation on electron micrographs, although Johnson and colleagues (1994) have advocated immunocytochemical identification of axons by light microscopy as a rapid means of estimating UF density. Morphometry is technically more difficult for UFs than for MFs because of the greater number of sources of error when working with UFs; particularly distinguishing axons from other circular profiles within Schwann cells can prove difficult.

Unmyelinated fiber density varies even more than MF density (Table 3.1). Selective loss of UFs occurs only rarely and has a similar differential diagnosis to that of selective small MF loss (Table 7.7). With axonal degeneration and regeneration, the number of unmyelinated fibers can increase above the normal range, and the distribution becomes bimodal, with an increased number of very small ("miniature") unmyelinated axons (Behse et al. 1975). When attempting to quantify UFs for purposes of assessing damage to this population of axons, one must decide what proportion of the counted axons are regenerating sprouts: these sprouts can be quite numerous and lead to the false conclusion that unmyelinated fibers are not affected. Thus, the process of counting

Table 3.1 Quantitative data: normal sural nerve

Author	*N*	Age range (years)	Endoneurial area (mm^2)	MF/mm^2	UF/mm^2
Pediatric					
Gutrecht	5	5 weeks premature to 6 years, 9 months	0.16–0.69	12,145–24,732	N/A
Jacobs	13	3 weeks premature to 10 years	0.19–0.54	9,400–25,890	35,800–193,200
Ouvrier	21	0–10	0.24–0.83	7,110–18,340	N/A
Adult					
Behse	12	14–54	0.65–1.25	5,200–8,000	18,000–42,000
Jacobs	14	21–77	0.5–1.2	3,310–7,950	17,300–41,600
Kanda	26	25–89	N/A	N/A	19,040–42,520
Ochoa	6	15–59	N/A	6,604–10,129	21,755–33,859
O'Sullivan	27	17–71	N/A	3,500–7,000[a]	N/A
Ouvrier	6	13–59	N/A	3,810–6,420	N/A
Pollock	6	37–54	N/A	6,078–9,943	21,242–34,155
Tackmann	10	14–61	0.54–0.87	6,030–9,350	N/A

[a]Estimated from figures

Table 3.2 Quantitative data: other nerve biopsy sites

Nerve	Author	*N*	Age range (years)	MF/mm^2	UF/mm^2
Medial sural cutaneous n. (midcalf level)	Ferriere	7	0–10	15,300–37,000	58,500–21,9200
Superficial peroneal	Fujimura	2	12–17	10,300–12,400	44,100–45,000
		5	63 ± 2.5	7,600–9,670	31,780–40,870
Superficial peroneal	Stevens et al. 1973	6	6 days to 10 years	9,877–32,837	N/A
Deep peroneal	Stevens et al. 1973	11	15–71	4,052–9,995	N/A
		3	2–10	9,183–11,414	N/A
		15	14–70	5,141–12,026	N/A
Radial n. (wrist)	O'Sullivan	27	17–71	4,900–10,000[a]	N/A
	Tackmann et al. 1976	6	48–61	5,370–8,350	N/A

[a]Estimated from figures

unmyelinated fibers can be quite complex. Gibbels (1989) and Ochoa (1969b) provide comprehensive discussions.

3.2.2 Usefulness of Fiber Counts and Histograms in Assessment of Nerve Biopsy

As Tables 3.1 and 3.2 indicate, MF and UF densities vary widely in normal nerves, and decline with age, partially due to an increased endoneurial area. With axonal degeneration and regeneration, the formation of regenerating clusters may mask axonal loss. The substantial variability seen within normals makes fiber counts of little value for detecting mild neuropathy. With unmyelinated axons, direct visualization of axonal degeneration or indirect evidence such as denervated Schwann cell bands or excess collagen pocket formation (*vide infra*) is likely to testify to pathology before the actual UF count or density drops low enough to unequivocally indicate abnormality (Behse and Carlsen 1978).

An even more telling argument against the utility of fiber counts, detecting a subtle reduction of axon numbers puts the pathologist no nearer an etiologic diagnosis. The same considerations apply to interpreting changes in the fiber size–frequency histogram. With the exception of selective small fiber loss, even severe changes are nonspecific.

Consequently, we do not believe that precise quantitation of myelinated and unmyelinated axons has great value in the practice of diagnostic sural nerve pathology. Morphometry is undoubtedly valuable in research applications, especially when combining data from many nerves, permitting detection of important trends and leading to understanding of peripheral nerve pathophysiology (Behse 1990). In most instances, however, a quick count of myelinated axons on several representative sections using an eye piece reticule of known dimensions provides sufficient quantitative information.

3.2.3 The G-Ratio

The ratio of the diameter of an axon without its myelin to the diameter of the axon with its myelin is called the "G-ratio." A high G-ratio indicates a thinner myelin sheath. Theoretical considerations suggest that a value of 0.6–0.7 is optimal for rapid conduction (Waxman 1980), and indeed normal nerves

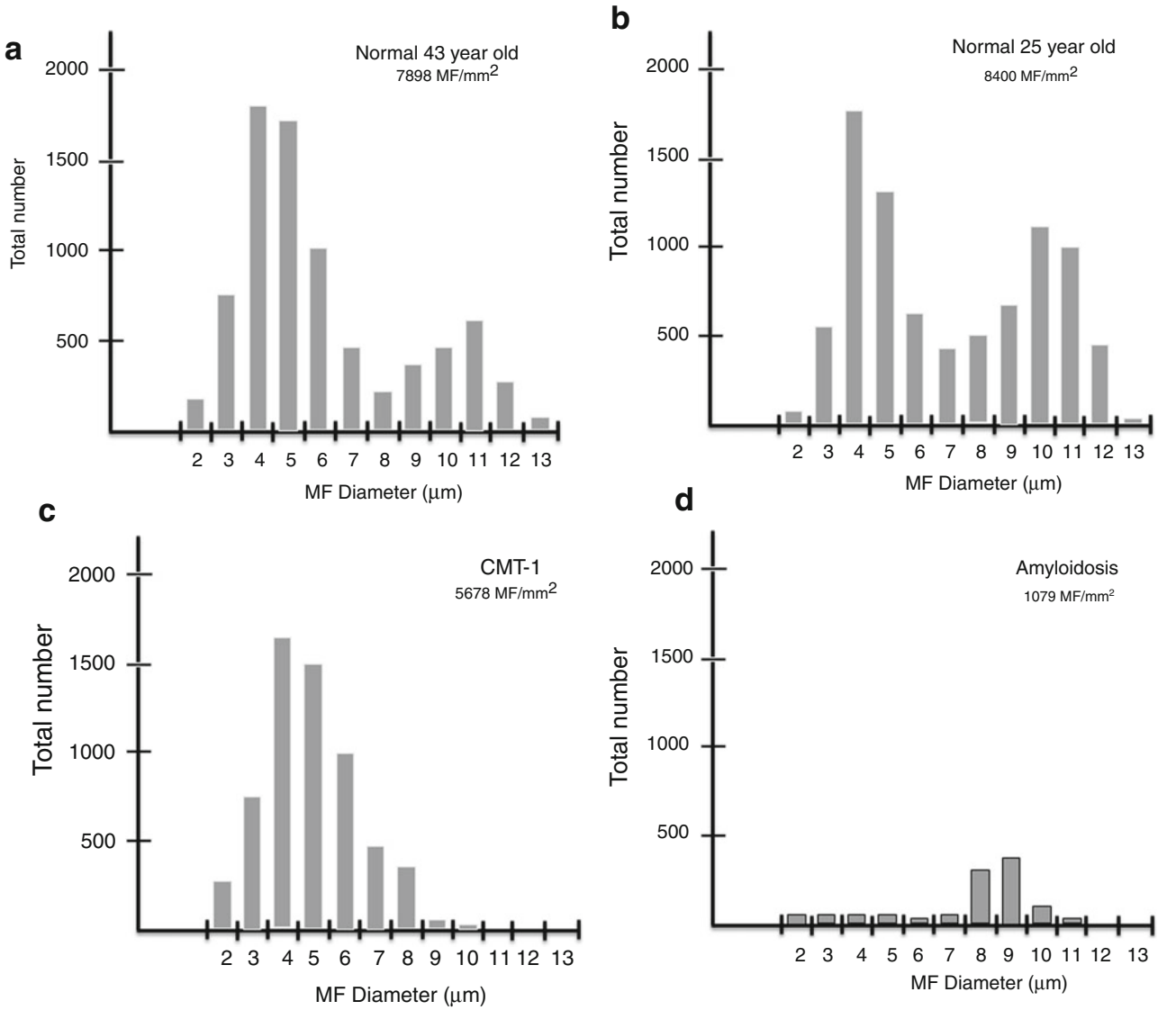

Fig. 3.1 (**a**–**d**) Myelinated fiber diameter–frequency histograms. Note unimodal histogram with predominantly large fiber loss typical of CMT-1 (**c**) and relative sparing of large MFs in amyloid polyneuropathy (**d**)

have a G-ratio close to this range. The G-ratio is somewhat higher (i.e., relatively thinner myelin) for small myelinated axons but generally varies between 0.5 and 0.8 for all fibers (Behse 1990; Jacobs and Love 1985). Because of relative hypomyelination at birth and during the first and second decades of life (Schroder et al. 1978), this age group has a higher G-ratio (Jacobs and Love 1985). Beyond 60 years of age, the scatter in G-ratio values increases greatly. This increased scatter presumably results from recurrent demyelination and remyelination causing some axons to have high G-ratios and axonal atrophy causing some fibers to have low G-ratios (Jacobs and Love 1985).

An increased G-ratio may indicate demyelination and remyelination, axonal regeneration (regenerating axonal sprouts are thinly myelinated), or primary hypomyelination. A uniformly high G-ratio, well beyond the range seen in normals or other neuropathies, is the hallmark of Dejerine–Sottas (CMT-3) syndrome. A low G-ratio may reflect hypermyelination, axonal atrophy, or both.

3.2.4 Endoneurial Area and Cell Counts

Normal endoneurial area of the sural nerve (excluding Renaut bodies and the subperineurial space) measures approximately 0.6–1.2 mm^2 (Behse 1990). This area can increase in a nonspecific manner with neuropathy, although hypertrophic neuropathies bring about the greatest increases

of up to fourfold or more (Behse et al. 1974). The enlarged endoneurial area results from collagen deposition, "empty" interstitial space, and increased cellularity (Behse et al. 1974) and leads to a spurious reduction in fiber density.

Investigators have counted Schwann cells and fibroblast nuclei in both normal and abnormal sural nerves. Nuclei of nonmyelinating Schwann cells ranged in number from 700 to 3,900/mm^2 in 28 normals (Kanda et al. 1991), while Ochoa and Mair (1969a) observed between 1,700 and 2,700 Schwann cell nuclei of UF or MF type per mm^2 in six normals. Schwann cell nuclei outnumber fibroblast nuclei by about 10:1 (Ochoa and Mair 1969a).

3.2.5 Regenerating Clusters

Regenerating clusters are hallmarks of axonal degeneration with regeneration (Fig. 4.24). These clusters are defined by the presence of three or more closely packed myelinated fibers and may be seen in normal nerves (Behse et al. 1975). Twenty, perhaps 40, clusters per mm^2 probably represents the upper limit of normal, but usually, normal nerves have a considerably smaller number (Behse and Carlsen 1978; Fujimura et al. 1991). The myelinated fiber "cluster ratio," which corrects for alterations in endoneurial area and for reductions in number of myelinated fibers, is defined as the number of regenerating clusters per 1,000 MFs (Gabreels-Festen et al. 1991). With an upper limit of 20 clusters per mm^2, a "typical" nerve with MF density of 10,000/mm^2 and endoneurial area of 1 mm^2 would give a cluster ratio of two as an upper limit of normal.

3.2.6 Assessment of Unmyelinated Fiber Loss

We have discussed above the insensitivity of fiber counts for detection of UF depletion. Up to 1.8 % of unmyelinated axons can demonstrate active degeneration in normals, although the value is typically 0.5 % or less (Behse et al. 1975; Gibbels 1989). However, indirect measures may show greater sensitivity in this regard (Behse et al. 1975). Schwann cell subunits (ScSus) refer to unmyelinated fibers only and are defined as a group of cross-sectional profiles surrounded by a common basement membrane. A ScSu usually has two to five interdigitating or apposed profiles but can have up to 20 (Behse and Carlsen 1978). The profiles represent Schwann cell processes or their associated unmyelinated axons. The number of unmyelinated axons per ScSu ranges from 1 to 4 and decreases with age and neuropathy (Behse et al. 1975; Kanda et al. 1991). A ScSu without axons is referred to as "denervated." Such denervated ScSus may result from loss of unmyelinated axons (Ochoa and Mair 1969b) or may reflect proliferation of Schwann cell processes (Kanda et al. 1991). Denervated ScSu are distinguished from bands of Büngner, which are conglomerations of Schwann cells previously associated with myelinated axons (Table 7.10).

Some ScSu alterations are sensitive indicators of UF loss. Denervated ScSu can appear in normals and increase in number before a drop in the UF density can be detected. In normal adult nerves, 4–37 % of ScSus (up to 10,000/mm^2) are denervated, but the frequency of this finding increases with age and nonspecifically with neuropathy (Behse et al. 1975; Behse 1990; Gibbels 1989; Low et al. 1978). Ohnishi and colleagues (1974) and Pollock et al. (1984) obtained much lower values of 40–219 denervated Schwann cell "clusters" per mm^2 but did not provide the criteria used to identify these structures.

The number of profiles per ScSu also increases with neuropathy. Investigators usually do not observe more than six profiles in a ScSu (<5 %), but 50 % or more of ScSus in patients with neuropathy can have this number of profiles (Behse et al. 1975). In normal nerves, collagen pockets occur in 10–24 % of ScSus (3,000–20,000/mm^2), and this number increases with age or neuropathy (Behse 1990; Gibbels 1989; Low et al. 1978; Pollock et al. 1984). Solitary Schwann cell profiles number from 10 to 30,000/mm^2 in normal nerves and also increase in number with age and neuropathy (Behse et al. 1975; Behse 1990; Gibbels 1989).

Only scanty pediatric normative data exist. Three children 2 to 13 years of age had 0–90 denervated ScSus/mm^2 and 250–1,220 collagen pockets/mm^2 (Ouvrier et al. 1981). Ochoa and Mair (1969b) found fewer than 1,000 denervated ScSus/mm^2 in 2 children aged 15 and 16 years and 3,700 denervated ScSus/mm^2 in an adult aged 59, illustrating how dramatically the frequency of this finding increases with age.

3.2.7 Internode Length

The length of a normal myelinated internode is a function of axon diameter and somatic growth. Teased fibers permit measurement of internode length for axons of various internodal diameters. A plot of internode length vs. fiber diameter for 20–50 teased fibers captures this relationship in an elegant fashion (Fullerton et al. 1965). Some variability in internode length is normal, but in peripheral neuropathy, certain patterns of alterations can occur. When a regenerating axon is remyelinated, the internodes tend to be a uniformly short length of about 200–400 μm. Not coincidentally, this range is similar to the internode length at birth (Jacobs and Love 1985). Thus, in axonal degeneration and regeneration, the histogram demonstrates numerous fibers of various diameters with uniformly short internodes. In contrast, random segmental demyelination results in residual long original internodes intermingled with short remyelinated internodes, producing a wide range of internode lengths along the same

fiber. This technique can thus provide information about axon and myelin alterations in a peripheral nerve. Unfortunately, internode length varies by factor of two or more even in normals (Behse 1990), and axonal and myelin changes are increasingly prominent with normal aging (Arnold and Harriman 1970; Jacobs and Love 1985). Thus, as with most of the other morphometric techniques discussed above, pathologists often cannot distinguish mild or moderate pathology from the variability seen in normal, and especially older, patients.

3.3 Summary: The Utility of Morphometric Analysis

The data gathered by morphometric analysis can be displayed with fiber size–frequency histograms, G-ratio plots, and internode length vs. axon diameter displays. These approaches will give an elegant presentation of considerable information about the nature of the axonal and myelin changes in the nerve under study. However, from the perspective of a pathologist trying to make an etiologic diagnosis, we see little essential information emerging from the effort and expense that these methods entail; none of the various morphometric parameters give information about the specific cause of a neuropathy. Because of a large intrinsic variability in peripheral nerve parameters, quantitation does not permit detection of subtle changes. Too often, an impressive array of computer-generated plots and histograms is substituted for thorough review of many nerve cross sections. As a result, a focal but critical finding may be missed, such as a single vessel showing vasculitis, infiltration with atypical cells, or an epineurial granuloma. In practice, of the various measures reviewed above, we regard the presence of increased numbers of regenerating clusters as a valuable sign of myelinated fiber degeneration and regeneration. We also employ a subjective impression of increased numbers of denervated ScSus as an indicator of unmyelinated axon disease. We use diameter–frequency histograms and teased fiber study for research or education.

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The Axon: Normal Structure and Pathological Alterations

4

The most common nerve biopsy findings are nonspecific axonal alterations such as axonal degeneration, depletion, and regeneration. The pathologist can assess the severity and chronicity of the damage, but infrequently can find an unequivocal structural indication of its underlying cause. Two major patterns of axonal disease can be identified: those changes indicating a disturbance of axonal metabolism (axonopathy) and those changes indicating axonal degeneration (Wallerian degeneration). Although electron microscopy provides a more detailed view of normal and pathological features than light microscopy, the stereotyped changes of Wallerian degeneration or axonopathy represent one final common pathway of disease and do not permit specific diagnosis of the underlying etiology. Only rarely do axonal alterations have specific implications, for example, giant axonal swellings with filamentous accumulations or the classic features of neuroaxonal dystrophy.

We shall review the normal ultrastructure of axons; certain aspects of axonal physiology necessary to facilitate understanding of pathogenic mechanisms throughout this book; and the two main variants of axonal injury, Wallerian degeneration, and distal axonopathy. The chapter concludes with a discussion of axonal regeneration.

4.1 The Normal Axon

The axon is a cell process specialized for the conduction of electrical impulses to or from the cell body. While other important functions exist, such as bringing back to the cell body (perikaryon) information regarding the biochemical and hormonal milieu of the nerve terminal, the rapid linear propagation of electrical impulses is the feature that makes the axon unique. The cell bodies of cutaneous sensory axons examined by nerve biopsy lie in spinal ganglia with the axonal diameter roughly proportional to cell body size. Because of the great length of peripheral nerve fibers, the volume of cytoplasm in the axon exceeds that of the perikaryon by several orders of magnitude.

4.1.1 Contents of the Normal Axon

Although recent studies have shown the existence of scattered ribosomes in axons which are likely to have an important role in normal axonal function (Koenig et al. 2000) and in pathological processes (Court et al. 2008), the most essential macromolecules must be synthesized in the cell body and transported centrifugally. An elaborate architecture and physiology have evolved in order to carry out this process (recently reviewed in Brown 2013). The normal axon contains microtubules, neurofilaments, mitochondria, membrane-bound tubulovesicles, and various dense bodies and granules (Fig. 4.1a, b). Many of these organelles are involved in the axonal transport process.

4.1.1.1 Neurofilaments and Microtubules: The Cytoskeleton

Classified as intermediate filaments, neurofilaments measure 10 nm in diameter. These structures, which are the most abundant axoplasmic organelles, align parallel to the axon and are evenly distributed with a density of about 100–300 filaments per μm^2 in cross sections of myelinated and unmyelinated axons of various species (Berthold 1978). In myelinated fibers of cat (Table 2 in Berthold 1978) and mouse (Table 1 in Reles and Friede 1991), the filament density falls into an even narrower range of 125–186 neurofilaments per μm^2. Neurofilaments help to regulate the axonal diameter (Sect. 4.1.4).

Found singly or in groups and aligned in parallel to the axon, microtubules measure 25 nm in external diameter and may extend as far as 350 μm. Their cross-sectional density diminishes in larger axons (Friede and Samorajski 1971; Ohnishi et al. 1976): 10–40 tubules per μm^2 in myelinated fibers and 50–100 tubules per μm^2 in unmyelinated fibers (Berthold 1978; Ohnishi et al. 1976). Microtubules are a fundamental component of the fast axonal transport system, serving as the "track" along which material moves (vide infra). This function probably relates to the close association of tubules with cytoplasmic aggregates and membrane-bound organelles.

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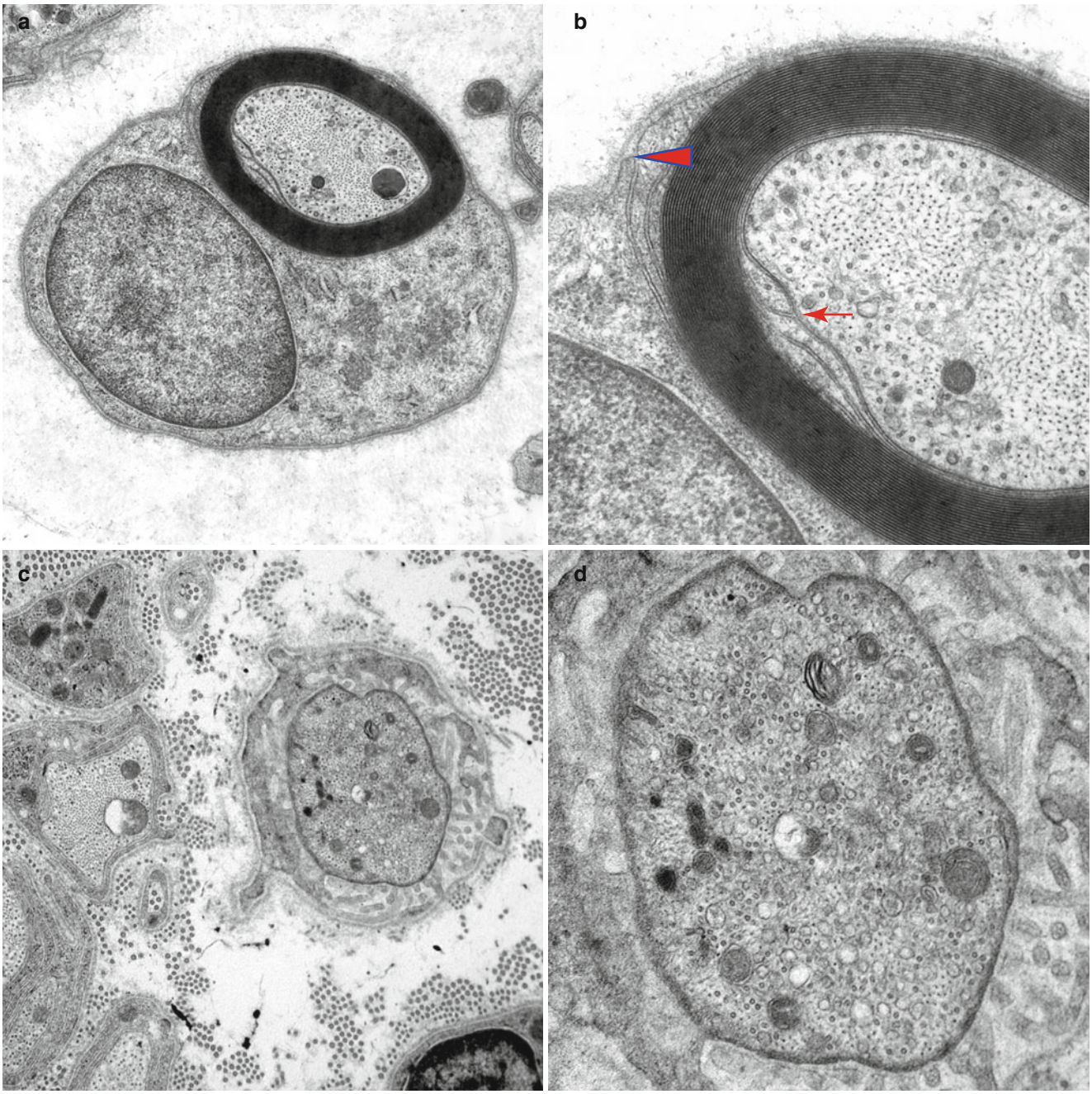

Fig. 4.1 Normal axon. Transverse section at internode (**a**, **b**) showing the internal (*arrow*, **b**) and external (*arrowhead*, **b**) mesaxons with central neurofilaments and peripheral microtubules. (**c**, **d**) Node of Ranvier with increased organelle density. (**e**) Longitudinal section through the node of Ranvier (Magnification, **a** 20,000×; **b** 50,000×; **c** 20,000; **d** 50,000×; **e** 12,000×)

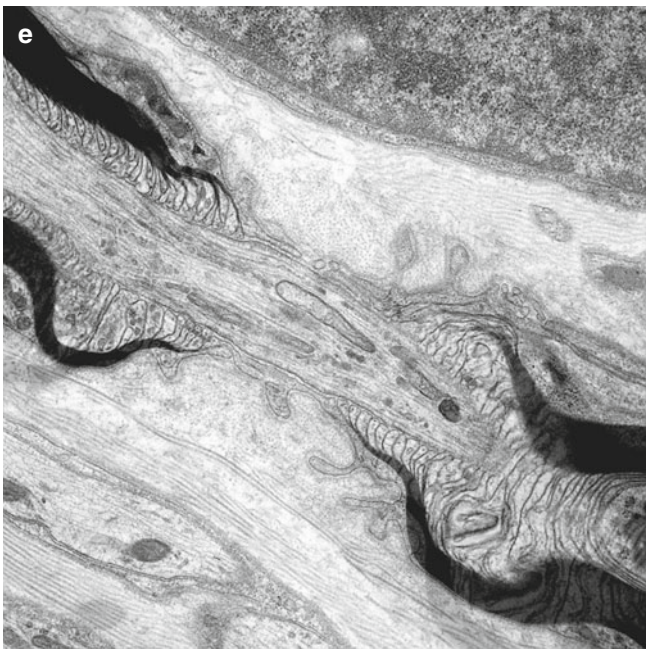

Fig. 4.1 (continued)

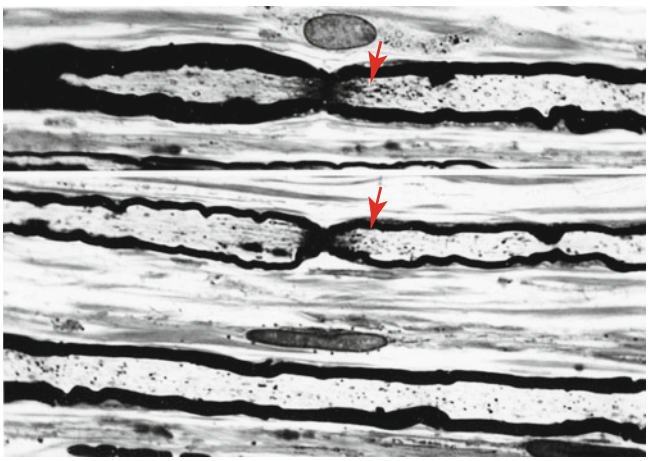

Fig. 4.2 Normal axon. Longitudinal sections of sural nerve show three MFs. Note condensation of axoplasm at nodal and paranodal regions (*arrows*). (One micron thick plastic section, 1,000×)

Microtubules and neurofilaments form the cytoskeleton of the axon. Although previously thought to be largely fixed in position within the axoplasm (Nixon 1992) and interconnected by a lattice of trabeculae and bridges aligned perpendicular to their long axis (Hirokawa 1982; Schnapp and Reese 1982), recent studies (Brown 2013; Brown and Jung 2013) suggest that neurofilaments are dynamic. Motion in axons along microtubules is bidirectional (but anterograde movement predominates); neurofilaments cycle repeatedly between moving and pausing states throughout their journey along the axon (Brown 2013).

4.1.1.2 Mitochondria

Axonal mitochondria are 0.1–0.3 μm in diameter and 0.5–8 μm in length, with basic ultrastructural features no different than those of mitochondria elsewhere in the body. As with microtubules, the mitochondrial density decreases (in cross sections) with increasing axon diameter. One might expect to see 1–2 mitochondria per cross section in an ordinary unmyelinated fiber and 0.1–0.7 mitochondria per μm^2 of axon cross section in myelinated fibers. Density is increased severalfold in the nodal and paranodal segments.

4.1.1.3 Vesicles, Cisternae, and Membranous Tubes

The axon contains various membrane-bound structures: some seemingly empty or multivesicular, some containing granular material, and others having osmiophilic lamellated contents. Most of this membranous material is the axoplasmic reticulum, a meshwork of interconnected tubulovesicular structures contiguous with, but different from, the endoplasmic reticulum of the cell body (Lindsey and Ellisman 1985; Tsukita and Ishikawa 1976). Other vesicles and tubular cisternae, ranging from 50 to 250 nm in diameter, originate from the perikaryal Golgi apparatus and endoplasmic reticulum and are involved in the transport of materials along the axon (Lindsey and Ellisman 1985; Schnapp and Reese 1982; Tsukita and Ishikawa 1976). Most concentrated at the distal paranodal region, dense lamellar bodies and multivesicular bodies probably represent lysosomal or pre-lysosomal organelles in retrograde transport towards the cell body (Lasek and Katz 1987) (Figs. 4.2 and 4.3b).

4.1.1.4 Granular Material

Glycogen appears as approximately 30 nm electron-dense granules. The amount normally found within the axons is scanty and varies between species (Berthold 1978; Zelena 1980). A much finer granular material sometimes accumulates in the nodal segment of the axon and gives it a characteristic darker staining on both light and electron microscopic examination (Fig. 4.3b).

4.1.2 Organization of Axonal Structure

Although the axon is a single, sometimes branched, continuous tube extending from cell body to nerve terminal, myelinated axons do have a periodic architecture as a result of their intimate interaction with segmentally organized Schwann cells. Axon internodal diameter (excluding the myelin sheath) ranges from 1 to 7 μm for myelinated axons and 0.1–3 μm for unmyelinated axons in the human sural nerve

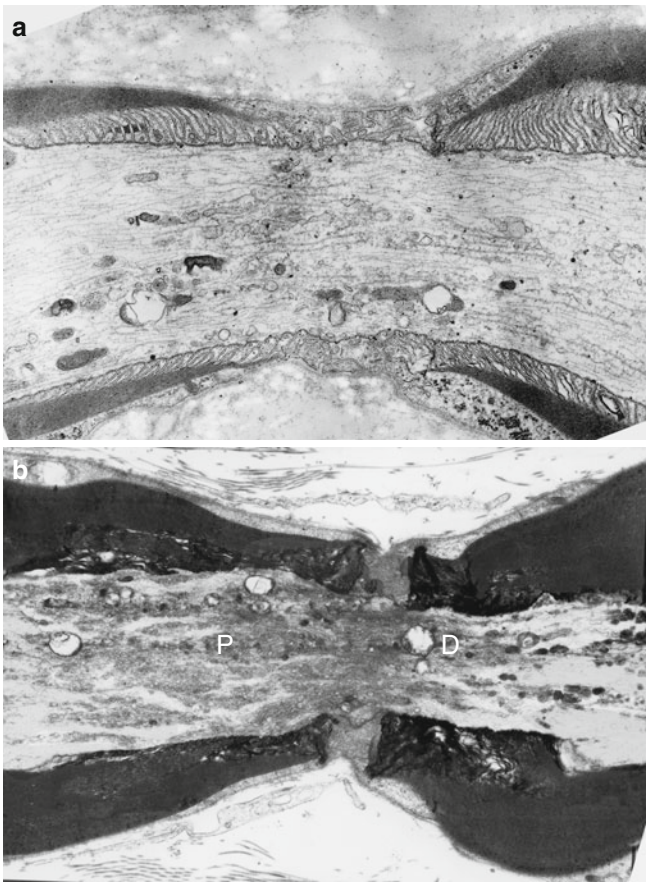

Fig. 4.3 Normal axon. Longitudinal section through node and paranodes of thin (**a**) and thickly (**b**) myelinated fibers. Note condensation of axoplasm and clustering of organelles. In (**b**) dense bodies accumulate in the distal paranodal area (*D*), while tubulovesicular organelles accumulate in the proximal paranodal area (*P*) (Magnification **a** 12,840×; **b** 11,000×)

(Behse 1990). The composition and organization of axonal contents vary depending on whether nodal or intermodal regions are examined.

The internodal organization of axonal contents is relatively simple: Neurofilaments and microtubules align longitudinally and relatively homogenously and intermingle with tubulovesicular structures and the occasional mitochondrion (Fig. 4.1a, b). A normal internode is 200–1,800 µm in length, roughly proportional to axon diameter.

The node of Ranvier (Fig. 4.1c–e) is the unmyelinated axon segment between two Schwann cells and their myelin sheaths, which is typically 1 µm in length. On either side of the node are the paranodal segments, 10–75 µm in length. Approaching the node, an axon loses its rounded contour and develops grooves that appear imposed by the invagination of the overlying myelin (Fig. 5.5b). These grooves taper towards the nodal segment, where the axon diameter decreases to 50 % or less of its value at the internode (Fig. 4.2). The degree of nodal constriction is proportionally greater for large fibers than for small fibers (Reles and Friede 1991). The constricted axon segment is 5–10 µm in length, with the central 1 µm being the unmyelinated true node of Ranvier (Rydmark and Berthold 1983) and the peripheral 3–5 µm on either side being the site where myelin loops attach to the axon (Figs. 4.1e and 4.3a).

In the nodal–paranodal region microtubules and membrane-bound organelles become concentrated centrally, the former at a density several times greater than that seen in internodal cross sections. Neurofilament density does not change significantly along the axon (Berthold 1978; Reles and Friede 1991). Tubulovesicular profiles assume a longitudinal rather than reticular pattern (Fig. 4.3a, b). Retrogradely transported organelles (dense lamellated bodies and multivesicular bodies) tend to accumulate at the distal half of the nodal and paranodal axon, while anterogradely transported organelles (especially tubulovesicular elements) accumulate at the proximal end (Berthold et al. 1993) (Fig. 4.3b). Both types of organelles occur at far greater concentrations at the node of Ranvier than in the internode. Light microscopy reveals a "veil" of toluidine blue-staining substance in approximately 50 % of nodal–paranodal segments (Figs. 4.1e and 4.2). The ultrastructural correlate of this is a diffuse electron-dense granular material composed of particles 5 nm in diameter (Berthold et al. 1993).

In contrast to the segmental organization of myelinated fibers, axons of unmyelinated fibers have same appearance throughout their length, containing neurofilaments, microtubules, approximately one mitochondrion per cross-sectional view, and relatively infrequent tubulovesicular elements and dense bodies.

4.1.3 Axonal Transport

The bulk of neuronal protein synthesis occurs in the perikaryon rather than the axon. Recent studies have, however, shown that ribosomes, initiation and elongation factors, transfer and messenger RNAs, as well as proteins and microRNAs involved in the regulation of mRNA stability and translation are located in the axon. Locally synthesized axonal proteins include cytoskeletal proteins, protein chaperones, metabolic enzymes, some membrane proteins, and secreted proteins. It is likely that local axonal synthesis has an important and dynamic role in local structure and maintenance, synaptic function, and reactions to injury; however, by itself, local synthesis is insufficient for axonal maintenance. Although mRNA was once thought to be excluded from the axonal compartment, the existence of protein synthesis in growing or regenerating axons in culture is now generally accepted. However, its extent and functional importance remains a subject of intense investigation. Furthermore, evidence of mRNA axonal transport and local gene translation in vivo has been shown in axons of the

developing zebra fish (*Danio rerio*) embryo, with frequent accumulation at the growth cones. Additionally, while supporting cells might provide some of the axon's metabolic needs (Gainer et al. 1977), this contribution is insufficient by itself, requiring that most axonal material must be transported centrifugally from the cell body over long distances. For several decades investigators have appreciated the existence of a special mechanism for this purpose (Griffin and Watson 1988; Ochs and Brimijoin 1993), recently reviewed in detail (Brown 2013).

The purpose of axonal transport is not always to move a cargo from the cell body directly to a destination at the most distal end of the axon, rather some cargos (e.g., mitochondria) are functionally active during their transit providing long stretches of axons with an ongoing energy supply. Axonal transport functions dynamically, recruiting and redistributing these cargos in response to the changing physiologic and metabolic needs of the axon.

Axonal transport mechanisms have traditionally been organized on the basis of their direction (anterograde and retrograde) and speed (fast or slow) (Brown 2013; Lasek et al. 1984; Guzik and Goldstein 2004). The prototype examples include:

1. *Fast anterograde transport*: (50–400 mm/day) carrying Golgi-originating vesicles containing membrane-spanning/membrane-anchoring proteins including neurotransmitters to the nerve terminal and axon.
2. *Fast retrograde transport*: (up to 200 mm/day) carrying organelles typical of endocytic, lysosomal, and autophagosomal pathways to the perikaryon. Many are multivesicular bodies containing a variety of cytosomes which may be re-outfitted or destroyed in the perikaryon. Other activated complexes (e.g., Trk/neurotrophin aggregates) are internalized by endocytosis at nerve terminals and sorted into signaling endosomes, which function as carriers to the nucleus where they modulate gene expression. Local synthesis of specific signaling and regulatory proteins in response to axonal injury may initiate a damaged neuron's injury response.
3. *Slow anterograde transport* (possibly as macromolecular complexes) of which there are two main types:
   Slow component a (*SCa*): (0.2–1 mm/day) SCa is composed primarily of neurofilament proteins, tubulin, spectrin, tau protein, and calcium/calmodulin-dependent protein kinase IIb (Brown 2013).
   Slow component b (*SCb*): (about 2–8 mm/day) SCb contains hundreds of non-membranous cytosolic proteins including cytoskeletal proteins (actin, tubulin, cofilin), motor proteins (dynein, dynactin, and myosin), metabolic enzymes (aldolase, creatine phosphokinase, enolase, etc.), and chaperone proteins such as heat shock protein hsp70 (Brown 2013).

Investigators have clarified much of the machinery of fast axonal transport in recent years (Sheetz et al. 1989; Brown 2013). Vesicles can be directly imaged moving along paths demarcated by microtubules at fast anterograde and retrograde rates (Kachar et al. 1987). Deprivation of energy or calcium prevents fast transport, as do agents that depolymerize microtubules. Axoplasmic motor proteins important in axonal transport have been identified: kinesin for fast anterograde and dynein for fast retrograde transport. These proteins have a hinge region and bind to both cargo organelles (tail regions) and microtubules (head region) and, using their ATPase activity, move along microtubules in the presence of ATP (Sheetz et al. 1989; Vallee et al. 1989). Experimental data thus provides strong evidence that fast axonal transport is performed using a microtubule railway upon which a cytoplasmic peptide motor carries vesicles and their contents to the appropriate destination via a calcium- and energy-dependent mechanism. In support of this, mutations in a critical region of β-tubulin (TUBB3) or a motor adaptor protein KIF1Bβ both result in neuropathy (Niwa et al. 2013; Brown 2013). Although the retrograde and anterograde fast transport mechanisms have much in common, specific inhibitors can affect them differently (Edstrom et al. 1988; Guzik and Goldstein 2004).

Slow axonal transport carries cytoskeletal components (neurofilaments predominantly anterogradely) and probably governs the rate of axonal growth and regeneration (Black and Lasek 1979). The classic "structural hypothesis" of slow transport paints a picture of the axonal cytoskeleton composed of tubules and filaments, assembled in the cell body and moving ponderously forward as a whole at a constant rate (Lasek et al. 1984). A competing "unitary hypothesis" suggested that the cytoskeleton is stationary and that its components are transported distally in an unassembled state and assembled locally (Ochs et al. 1989; Bamburg 1988). Recent work (detailed in Brown 2013) suggests that fast and slow transport both take place in association with microtubules; however, they differ in the time the cargo stays in contact with the highway, which has been called the "duty ratio" (the proportion of the time that the cargo spends moving). Neurofilament movement is fast but intermittent, with each filament spending most of its time pausing between short bursts of rapid movement (Tang et al. 2013). Rather than cross-linked neurofilaments and microtubules, the axonal environment may more accurately behave as a polymer solution. Microtubules (all orientated with their minus ends pointing proximally) and microfilaments (relatively flexible, two-stranded, 5–7 nm filamentous polymers of actin capable of multidirectional orientation) serve as the tracks for long-range axial movements in axons and short-range motion, respectively. Neurofilament polymers themselves move along microtubule tracks. However, microfilament myosin motors may influence the long-range transport behavior of mitochondria by delivering these organelles to and from their microtubule tracks. Most motors interact with their cargos

via adapter proteins which bind to receptors on the cargo. A single motor may often be called on to interact with multiple different cargoes.

4.1.4 Regulation of Axonal Diameter

Neurofilaments play a central role in determining axonal diameter (Gold et al. 1985; Hoffman et al. 1984, 1987). This conclusion correlates with the observation that localized accumulation of neurofilaments constitutes the substrate of focal axonal swelling and that impairment of neurofilament translocation to the distal axons underlies at least some types of axonal atrophy (vide infra). It is now thought that sidearms produced by NFM and NFH proteins present on neurofilaments preserve space between adjacent filaments and help maintain axonal size, which is critical for conduction velocity (Brown 2013). Additionally, a rigid cross-linked network of neurofilaments would likely retard the movement of other axonally transported cargos. In mutant animals lacking neurofilaments, axons fail to attain their normal caliber and have delayed conduction velocities.

4.2 Axonal Degeneration

4.2.1 Distal Axonopathy

4.2.1.1 Evolution of the Concept

Neuropathy resulting in degeneration of the most distal parts of the axon with progression proximally towards the cell body, i.e., "dying-back" neuropathy, is a common pattern in neuropathies accompanying acrylamide, diabetes, acquired immunodeficiency syndrome (AIDS), alcohol, arsenic, thallium, uremia, isoniazid, and many other medications and organic solvents (Spencer and Schaumburg 1977; Cavanagh 1979; Raff and Whitmore 2002). Superficially, the concept is a simple one: "[If] the neuron were diseased, its trophic function might be impaired, a condition that could result in damage to those regions furthest removed from the source of their trophic input, that is, the distal nerve" (Spencer and Schaumburg 1977). One corollary of this idea is that larger diameter axons, which have a greater volume of axoplasm, and thus presumably higher metabolic demands, would show changes earliest. A second theoretical consequence is that the most distal central nervous system projections of neurons might show similar alterations to those seen in distal peripheral nerve fibers (central–peripheral distal axonopathy).

Clinically, it is noteworthy that the longest peripheral nerve fibers are sensory, i.e., those providing sensation to the most distal aspect of the lower limbs. Moreover, dissociation occurs between the sensory modalities carried by fibers of various sizes: Small myelinated and unmyelinated axons carry sensations of pain and temperature, and large myelinated fibers carry vibration and kinesthetic sensation. Thus, one might expect that in a "dying-back" process the earliest manifestations result from large fiber sensory loss that a "stocking–glove" symmetrical neuropathy would be the typical pattern and that histological studies would demonstrate a relatively more severe loss of large myelinated fibers. Indeed this is the most common clinical and histological disease pattern seen in axonal polyneuropathies.

The excellent correlation between the theory and reality of clinical and pathological observations served to give "dying-back" axonopathy a special place among categories of peripheral nerve disease. In uremic neuropathy a clear proximodistal gradient of nerve disease severity takes place: atrophy appears as the primary manifestation of axonopathy, with degeneration of the nerve fibers occurring in the most distal segment (Dyck et al. 1971). Similar changes have been demonstrated in other neuropathies.

In some models of toxin-induced axonal degeneration, however, careful ultrastructural and teased fiber studies revealed that the pathological changes do not always occur in a "dying-back," axon length- and diameter-dependent pattern and that axonal atrophy does not necessarily take place. Instead, axonal swelling and myelin retraction occurred in multifocal sites, more prominent in but not necessarily confined to the most distal and largest axons (Spencer and Schaumburg 1977; DeRojas and Goldstein 1990). In some studies axonal swellings tended to occur in the proximal paranodal segment of a given internode. Teased fiber studies suggest that focal axonal swelling might cause demyelination and then resolve, allowing remyelination and thus permitting secondary demyelination/remyelination (Griffin and Price 1981).

Originally suggested by Spencer and Schaumburg (1977) as a replacement for the term "dying-back" neuropathy, "distal axonopathy" encompasses both the typical (uremia-like) and less typical (hexacarbon intoxication-like) varieties of length-dependent axonal degenerative processes. The term can probably apply to nearly all axonal metabolic polyneuropathies, whether due to toxins, endocrine disturbances, organ failure, inherited defects, or nutritional deficiency. Ischemic neuropathies and neuropathies resulting from local damage to nerves (inflammation, amyloid) are not considered distal axonopathies. However, even in these settings similar features occur (Said et al. 1984). The axonopathic ultrastructural changes discussed below are a reflection of the fact that the axon's metabolic machinery is disturbed and represent nonspecific indicators of a "sick" axon or neuron.

4.2.1.2 Pathogenetic Mechanisms

The pathophysiological mechanisms underlying distal axonopathy have been a subject of intense research for a number

of years (Spencer and Schaumburg 1977). The classic notion has been that metabolic function in the cell body, responsible for production of substances essential for survival of the axon, is impaired in some unspecified fashion. Even in normal nerves many transported substances show a proximodistal decreasing gradient in their concentration, presumably on the basis of ongoing degradation and/or utilization of transported materials as they proceed distally (Miller and Spencer 1985). Thus, a defect in production of vital macromolecules would show up first in the distal axon. Attempts to study cell body metabolic activity by assessment of amino acid uptake yielded equivocal results (Spencer and Schaumburg 1977). One hypothesis, derived from the acrylamide toxic neuropathy model, is that interference with glycolysis caused depletion of high-energy groups needed for axonal maintenance (Spencer et al. 1979). Subsequent investigation, however, has called into question the significance of these changes in axonal metabolism (Miller and Spencer 1985).

One alternative to the concept of the cell body as the site of initial disturbance in distal axonopathy is the possibility that it results from a local axonal defect. For example, a circulating toxin might cause a deficiency of, or demand for, a critical macromolecule throughout the length of the axon. Since such a macromolecule would likely arrive from the perikaryon and may occur in lesser concentrations more distally in the fiber (Miller and Spencer 1985), distal parts of the axon would, in this paradigm, suffer the greatest effects (Spencer and Schaumburg 1977).

Both of the above proposed mechanisms for distal axonopathy depend on the notion of transport of essential substances from the cell body to the distal axon. In recent years, attention has increasingly focused on defects of axonal transport as a cause of distal axonopathy. Axonal swellings, a characteristic feature of some human neuropathies including hexacarbon toxicity and giant axonal neuropathy, result from focal accumulations of neurofilaments. This accumulation suggests that impairment of filament transport might underlie the neuropathy. Similarly, distal axonal atrophy, a ubiquitous component of numerous inherited, toxic, and metabolic neuropathies, might be related to impaired production or centrifugal transport of neurofilaments (Hoffman et al. 1984, 1987). Data have at times been conflicting (Spencer and Schaumburg 1977), but potential specific examples of how axonal transport impairment leads to neuropathy include alterations in slow transport (iminodipropionitrile, Griffin et al. 1978) or fast transport (hexacarbons, Mendell et al. 1977) or a disturbance of the microtubules that form the backbone of the transport system (vincristine, colchicine, podophyllotoxin) (Paulson and McClure 1975; Sahenk et al. 1987). Failure of retrograde transport or of the normal mechanism by which a proportion of the membrane and protein delivered distally returns proximally ("turnaround defect") might result in distal accumulation of abnormal, possibly toxic, materials. This situation has been demonstrated in an acrylamide neuropathy model where focal accumulation of particulate organelles constitutes a prominent early feature (Miller and Spencer 1985; Chretien et al. 1981).

Recent studies have proposed additional mechanisms for distal axonopathy, in this case in the setting of diabetic neuropathy. Dying-back axonal degeneration has been proposed to reflect the activation of a self-destruct program in the distal parts of an axon in response to a neuronal insult (Raff and Whitmore 2002) providing a neuron with several choices. One choice is to prevent neuronal death using a controlled self-protective reaction to a metabolic burden, conserving resources so that the preterminal axon and axon terminals can be regenerated at a later time when the neuropathic stress has abated (Raff and Whitmore 2002). Mitochondrial pathology, perhaps reflecting injury secondary to local oxidative stress to the mitochondrial genome or difficulty with mitochondrial calcium homeostasis, may underlie distal axonopathy. In diabetic neuropathy Fernyhough and colleagues (Chowdhury et al. 2013) proposed that the distal axon has a higher and more fluctuating demand for ATP resulting from collateral sprouting and synaptic plasticity (possibly involving cycles of degeneration/regeneration), not paralleled in the perikaryon. Abnormal mitochondria with diminished spare respiratory capacity in diabetic animals are thought to be less capable of adapting to high peaks of ATP demand within the distal axon and result in its degeneration.

Although experimental observations are often based on the study of neurotoxins, similar alterations in axonal transport contribute to a wide variety of neuropathies. Even the "neuropathy of aging" might result from a gradual age-related reduction in transport effectiveness (McQuarrie et al. 1989).

4.2.2 Pathological Alterations in Axonopathy

Studies in animal models and human pathology have provided important insights into the spatiotemporal sequence of distal axonopathy (Spencer and Schaumburg 1977; Prineas 1969a, b; Sahenk and Mendell 1980). Most changes of distal axonal degeneration are nonspecific, but some etiologies have specific patterns. For example, acrylamide and the hexacarbon neuropathies tend to produce neurofilamentous accumulations and axonal swelling; TOCP and zinc pyridinethione produce tubulovesicular aggregates; and thallium intoxication results in mitochondrial abnormalities. However, we cannot catalog all the various combinations of ultrastructural changes that investigators have observed in the numerous animal toxic models and human ultrastructural examinations conducted to date. Such an undertaking would not prove diagnostically useful in the practice of peripheral

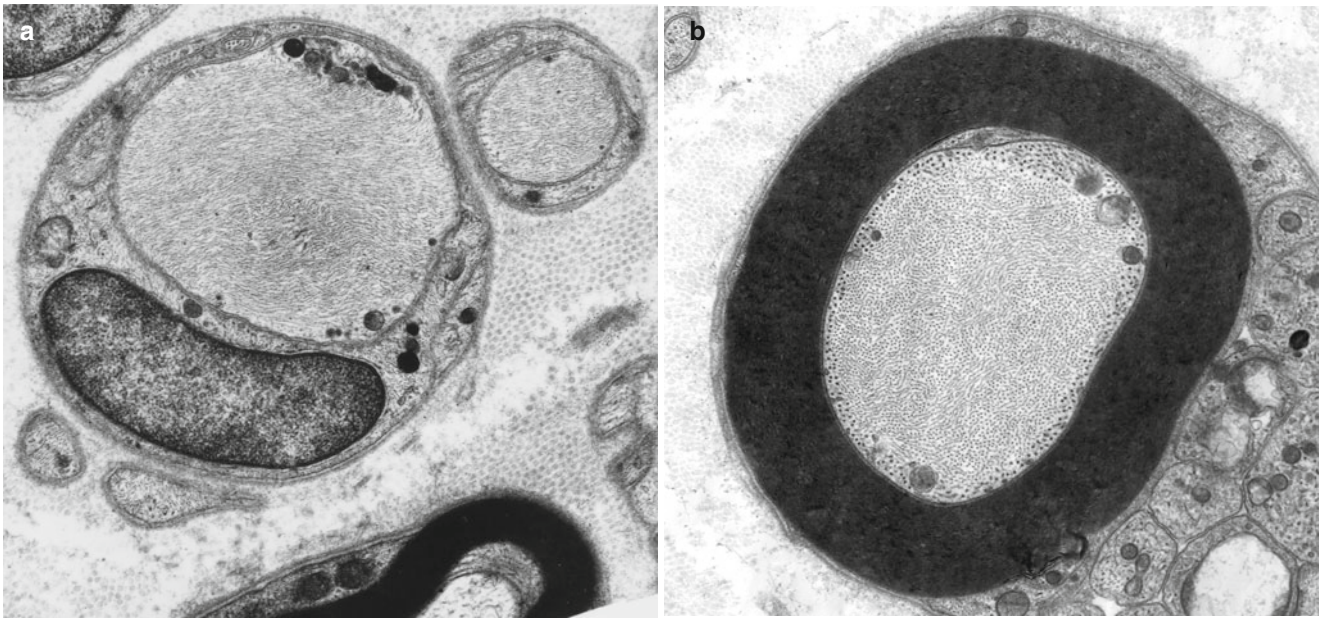

Fig. 4.4 Axonopathy. Intra-axonal filamentous aggregates are nonspecific findings in some chronic neuropathies. Note segregation and marginalization of microtubules in **b** (Magnification, **a** 15,390×; **b** 20,000×)

nerve pathology. On the contrary, the discussion below will emphasize the nonspecificity of ultrastructural changes seen in axonal degeneration. Similar changes occur in axonal degeneration in the CNS (Lampert 1967). No single change is diagnostic, or even necessarily indicative of pathology, as the wide variation of normal appearance, age-related changes, and artifacts induced by the trauma of nerve biopsy and fixation can be misleading. Many of the same alterations are seen in Wallerian degeneration following mechanical or ischemic nerve transection.

4.2.2.1 Axonal Swelling and Filamentous Accumulation

Neurofilament accumulation is the ultrastructural correlate of axonal swellings described to varying degrees in a wide range of human and experimental neuropathies, including giant axonal neuropathy, hexacarbon intoxications, disulfiram, acrylamide (Davenport et al. 1976; Prineas 1969b), iminodipropionitrile (Griffin et al. 1978), carbon disulfide (Gottfried et al. 1985), and one CMT-2 pedigree (Vogel et al. 1985). Investigators also have observed filament accumulations in a case of B12 deficiency (Schochet and Chesson 1977), in amyloidosis (Hanyu et al. 1989; Jedrzejowska 1977), and in misonidazole toxicity (Urtasun et al. 1978). Typically, axons demonstrate a fusiform swelling to several times their normal diameter, and electron microscopy reveals accumulations of neurofilaments arrayed in swirling masses in various planes of orientation. Other organelles are diminished in numbers or displaced. The focal swellings often involve only part of an internode. In hexacarbon neuropathies, these swellings often occur just proximal to a node of Ranvier, their frequency increasing in a proximodistal gradient along the nerve fiber (Spencer and Schaumburg 1977). Myelin overlying the axonal swellings may become thinned, and paranodal myelin retraction and focal demyelination may occur, which is seen best with teased fiber analysis. This axonal change may at some stage still be reversible. As the axon dilates and narrows, it may shed its myelin, resulting in secondary segmental or subsegmental demyelination and remyelination and, if the process is chronic, onion-bulb formation (Spencer and Schaumburg 1977; Griffin and Price 1981).

A rare swollen axon with filamentous accumulations is an infrequent but nonspecific finding that we have seen in cases with diagnoses as varied as CIDP, CMT-2, or granulomatous neuropathy (Fig. 4.4a, b). However, when such axons are found in greater numbers, typically several per fascicle, this finding has diagnostic implications (Table 7.12). Increased neurofilament density without prominent axonal enlargement can occur in vincristine and cisplatin toxicity (Gastaut and Pellisier 1984; Wulfhekel and Dullmann 1972). We have examined a biopsy in porphyric neuropathy where focal swellings of unmyelinated axons with filament accumulations appeared frequently (Thorner et al. 1981).

The pathological significance of axonal swellings in intramuscular nerves is uncertain (Barron and Heffner 1978; Wolfhart 1957) given that swelling occurs in a high proportion of normal human intramuscular nerves and in neuropathies presently thought to be not of the distal axonopathy variety (Alderson 1992).

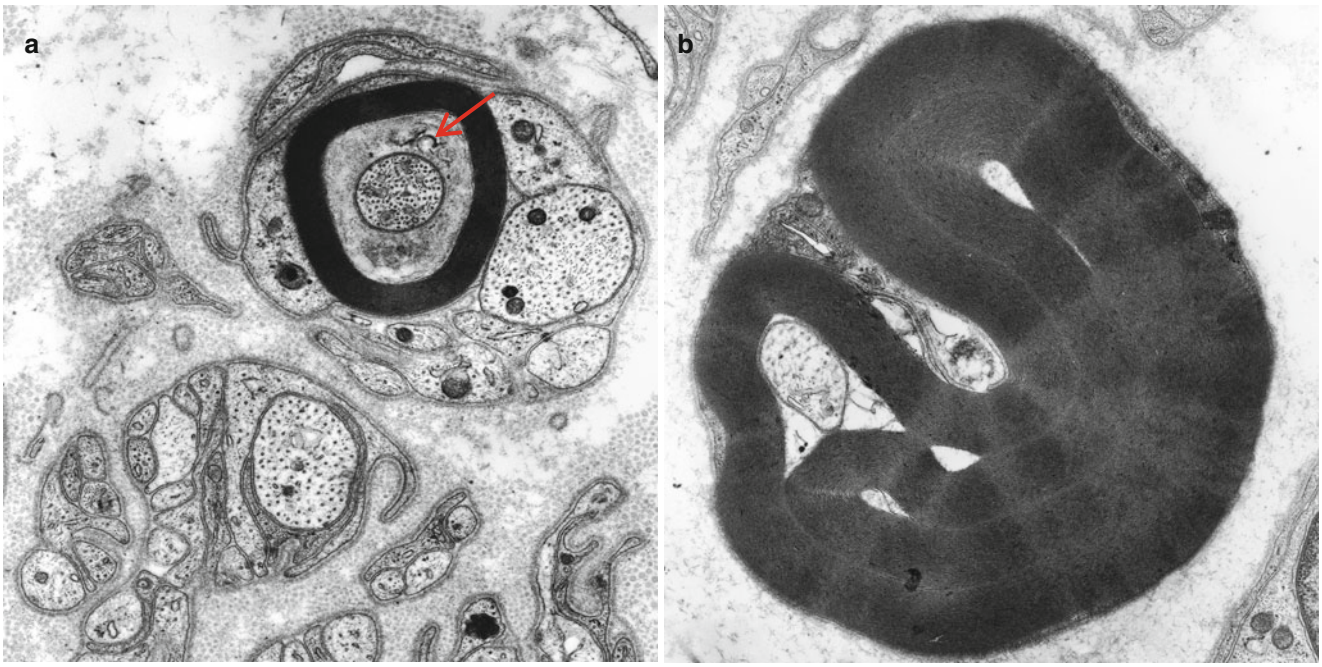

Fig. 4.5 Axonopathy. Axonal "atrophy" seen in a case of nonspecific chronic neuropathy (**a**) and a case of HNPP (**b**). Note organelles in adaxonal space (*arrow*, **a**) (Magnification, **a** 20,000×; **b** 12,000×)

4.2.2.2 Axonal Atrophy

Axonal atrophy is more difficult to recognize than axonal swelling because the changes may be subtle, require quantitative techniques for adequate analysis, and can resemble an artifact. An axon which is clearly inappropriately small for its myelin sheath, often lying in the center of an apparently empty space surrounded by myelin (Fig. 4.5a, b), indicates evidence of atrophy. It is hard to see how the myelin sheath, ordinarily in such intimate anatomical and physiologic contact with the axon, could maintain its structural integrity when separated from its axon in the fashion such micrographs suggest. In some instances, organelles are identified suggesting that the "empty" space actually represents a swollen adaxonal Schwann cell cytoplasmic process (Fig. 4.5a). Convincing demonstration of atrophy requires an intensive quantitative study. Dyck and colleagues have provided such studies for a variety of neuropathies including those of uremia, Friedreich's ataxia, some paraproteinemic neuropathies, and permanent axotomy (Dyck et al. 1971, 1981; Dyck and Lais 1973; Ohi et al. 1985). Suggestive data also exists for some toxic neuropathies in humans and animals including ethylene oxide (Schroder et al. 1985), cisplatin (Gastaut and Pellisier 1984), hereditary motor and sensory neuropathy (CMT) types 1 and 2 (Dyck et al. 1993b; Yasuda et al. 1990), hereditary sensory and autonomic neuropathies (HSAN) (Dyck 1993), and HIV-associated neuropathy (Fuller et al. 1990).

Conclusive identification of axonal atrophy requires demonstration of a shift of fiber diameter–frequency histograms to the left and a decrease in the slope of axonal area/diameter vs. myelin thickness plots, showing that the axon is inappropriately small for its myelin thickness (Ohi et al. 1985). Myelin remodeling often follows axonal atrophy and manifests as excessively wrinkled myelin with numerous infolded loops, bubbles, or fissures (Dyck et al. 1984). Secondary demyelination, as demonstrated by teased fiber studies, often follows.

Neurofilament numbers largely regulate axonal diameter, and neurofilament density is relatively constant within different nerves (vide supra). Thus, a demonstration that this density is not significantly altered would further support the claim that the observed axonal shrinking is not an artifact (Friede 1971; Yasuda et al. 1990). On the other hand an "atrophic" myelinated axon with a markedly increased neurofilament density should be regarded with skepticism. Other organelles, including mitochondria, tubulovesicular profiles, and microtubules, increase in density when an axon undergoes atrophy (Friede 1971).

4.2.2.3 Mitochondrial Abnormalities

Mitochondria may show a variety of changes including focal accumulations (Fig. 4.6a), enlargement, excessive or disorganized cristae, increased electron density of the matrix, accumulation of coarse granular (probably glycogen) or fine granular osmiophilic material, varying degrees of loss of structural integrity, and paracrystalline or amorphous electron-dense inclusions. These changes have been

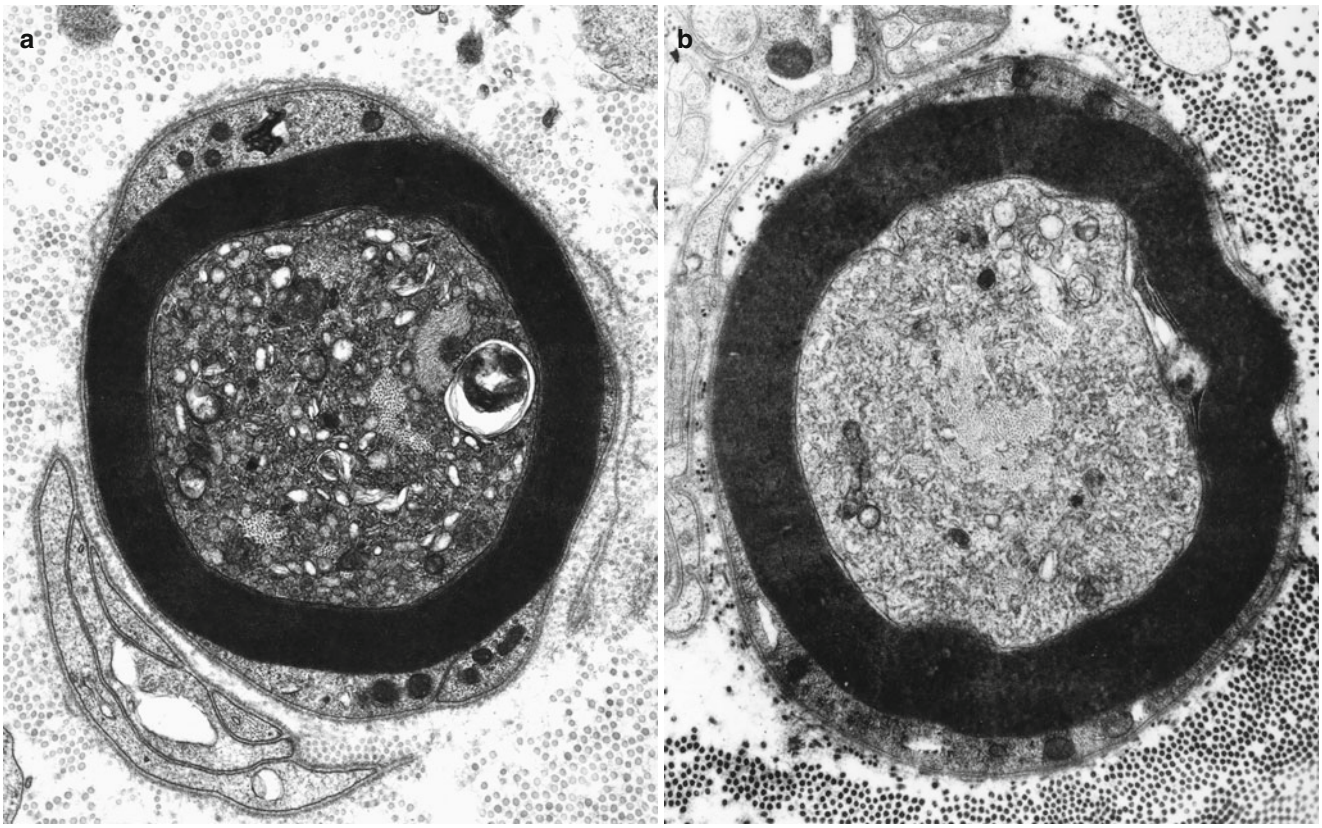

Fig. 4.6 Axonopathy. Alteration of axoplasm with segregation of neurofilaments and pleomorphic vesicular accumulations. In (**b**) most of the axoplasm displays tubulovesicular profiles with a bundle of neurofilaments at the center. Note similarity to neuroaxonal dystrophy (Figs. 19.26 and 19.27). (Magnification, **a** 21,546×; **b** 13,760×)

described in thallium intoxication (Spencer and Schaumburg 1977), uremia (Dyck et al. 1971), alcohol abuse (Tredici and Minazzi 1975), vitamin E deficiency (Schochet 1971), Tangier disease (Dyck et al. 1978), ataxia-telangiectasia (Gardner and Goodman 1969), and numerous toxic agents. These toxic substances include acrylamide, hexacarbons, INH, cisplatin, and vincristine (Bradley et al. 1970; Schlaepfer and Hager 1964; Spencer and Schaumburg 1977; Prineas 1969b; Gastaut and Pellisier 1984). Mitochondrial alterations are also seen in mitochondrial cytopathies (Chap. 21) but more prominently in Schwann cells than in axons. Specific changes such as crystalline inclusions are vanishingly rare.

4.2.2.4 Aggregation of Membranous Organelles and Dense Bodies

In experimental organophosphate intoxication in the cat, Prineas (1969a) observed accumulation of abnormal membrane-bound spaces, arranged as vesicles, tubules, and flattened sacs. These spaces also occur in dimethylaminopropionitrile toxicity (Pestronk et al. 1980), and they are a nonspecific feature of axonal neuropathies, including familial hereditary motor and sensory neuropathy (Yasuda et al. 1990), thiamine deficiency (Takahashi and Nakamura 1976), isoniazid toxicity (Ochoa 1970), amyloidosis (Jedrzejowska 1977), and Tangier disease (Dyck et al. 1978) (Fig. 4.6a, b). Similar considerations apply to accumulation of "dense bodies" (Prineas 1969b; Dyck et al. 1978; Meier and Bischoff 1977; Tredici and Minazzi 1975). Organelles may appear segregated, with domains containing masses of neurofilaments adjacent to regions containing only membranous organelles (Fig. 4.6a, b). With focal nerve ischemia, axonal swelling and accumulation of organelles frequently appear in regions proximal, and to a lesser extent distal, to the site of axonal disruption (Korthals et al. 1978). In neuroaxonal dystrophy, the essential pathological alteration is the accumulation of tubulovesicular profiles with focal congregations of mitochondria, filaments, glycogen and various dense bodies, granules, and vacuoles (Figs. 19.26 and 19.27).

4.2.2.5 Schwann Cell–Axon Networks

Schwann cells participate in the clearance of damaged organelles and removal of axonal debris (Spencer and Thomas 1974). A ridge of Schwann cell cytoplasm evaginates into the axon near a collection of axonal organelles which often show atypical features (enlarged mitochondria, clear vesicles, multivesicular or dense membranous bodies, or collections of neurofilaments). This ridge progressively elongates and thins

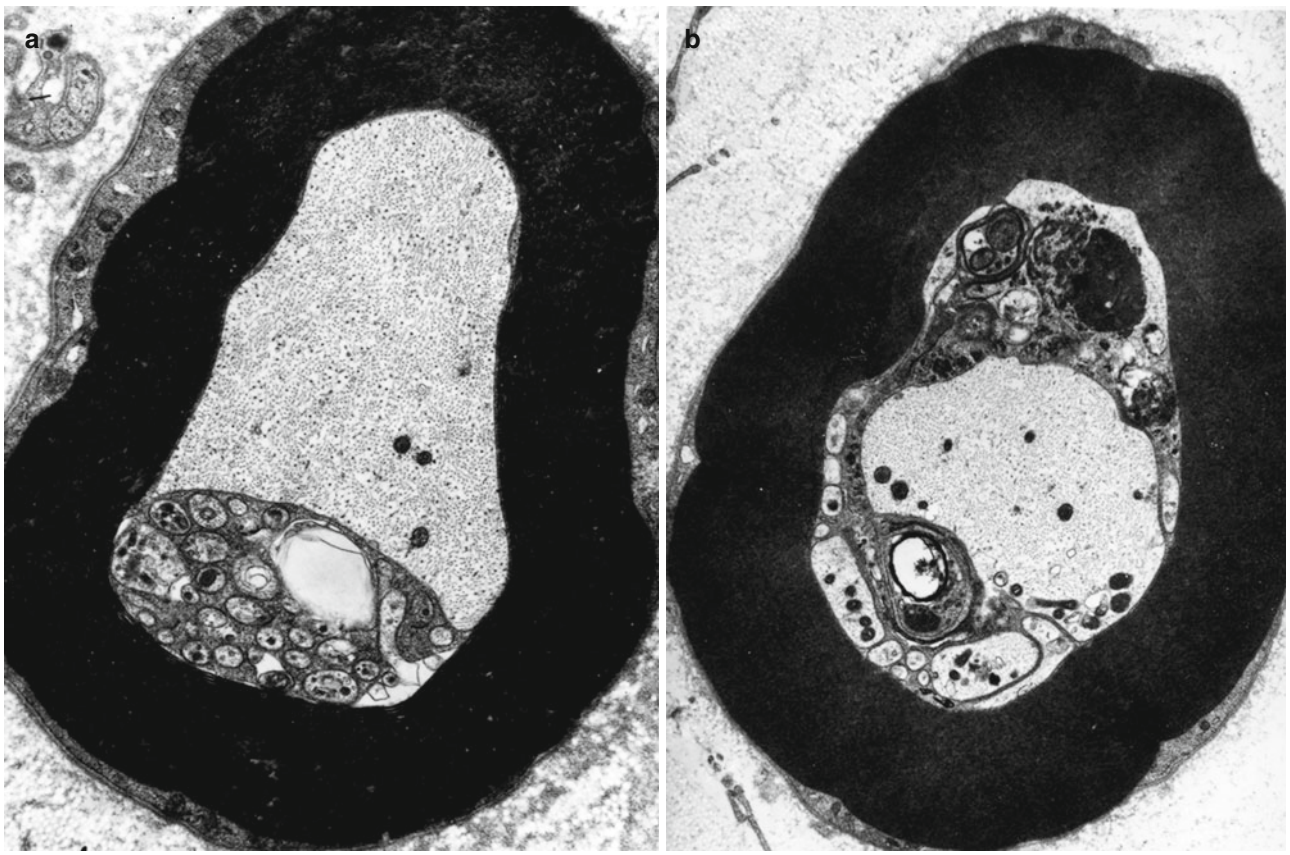

Fig. 4.7 Axonopathy. Schwann cell–axon networks are shown in two MFs. Pockets of axoplasm containing degenerate organelles are incorporated into an inner evagination of Schwann cell cytoplasm (Magnification, **a** 10,944×; **b** 7,820×)

out, tending to surround the abnormal organelles. It then folds and fuses within itself, sequestering aggregates of abnormal axoplasmic contents. The outer sheath of this Schwann cell evagination is maintained, but inner subdivisions fade away, so that the sequestered axoplasmic debris enters the Schwann cell. In cross-sectional anatomical studies, this process results in a honeycombing appearance at the periphery of the axon, with aggregates of granular debris trapped within the individual honeycomb cells (Fig. 4.7a, b). These networks usually appear in paranodal regions, most frequently in large myelinated fibers and only rarely in unmyelinated fibers (Spencer and Thomas 1974).

Normal axons may demonstrate such Schwann cell–axon networks, almost exclusively in small numbers in the paranodal area and not in association with abnormal myelin configurations. Hexacarbon, acrylamide, and thallium intoxications, this observation occurs in axonopathy of various etiologies, including CMT-2 (Yasuda et al. 1990), isoniazid intoxication, and thiamine deficiency in rats (Collins et al. 1964; Schlaepfer and Hager 1964); experimental hexacarbon toxicity, acrylamide and thallium intoxication (Spencer and Thomas 1974); cisplatin (Gastaut and Pellisier 1984), lead (Schlenska and Spalke 1975), and sodium cyanate (Ohnishi et al. 1975) toxic neuropathies; and porphyria-, uremia-, or lymphoma-associated neuropathy (Thorner et al. 1981; Ahonen 1981; Vital and Vallat 1987) in humans. Schwann cell–axon networks may also occur proximal to a focal nerve lesion (Spencer and Thomas 1974) and in the neuropathy associated with chronic vascular insufficiency (Farinon et al. 1984). This finding likely represents a nonspecific mechanism by which the Schwann cell clears debris and helps maintain the integrity of the axon under normal and pathological situations (Gatzinsky and Berthold 1990). Overall, this ultrastructural feature is an uncommon one, which has theoretical importance but little role in the differential diagnosis of human peripheral nerve pathology.

4.2.2.6 Other Nonspecific Changes

Axons may show a reduction in the various organelles normally present or an accumulation of membrane-bound granular debris. Workers have observed a host of filamentous, granular, and lamellar inclusions in axons, none of which is specific to any particular disease or even necessarily a sign of pathology (Figs. 4.8a–d, 4.9a, b, and 4.10). Excessive axonal glycogen has been emphasized in hypothyroidism and in diabetes but is a nonspecific finding (Fig. 4.11a, b).

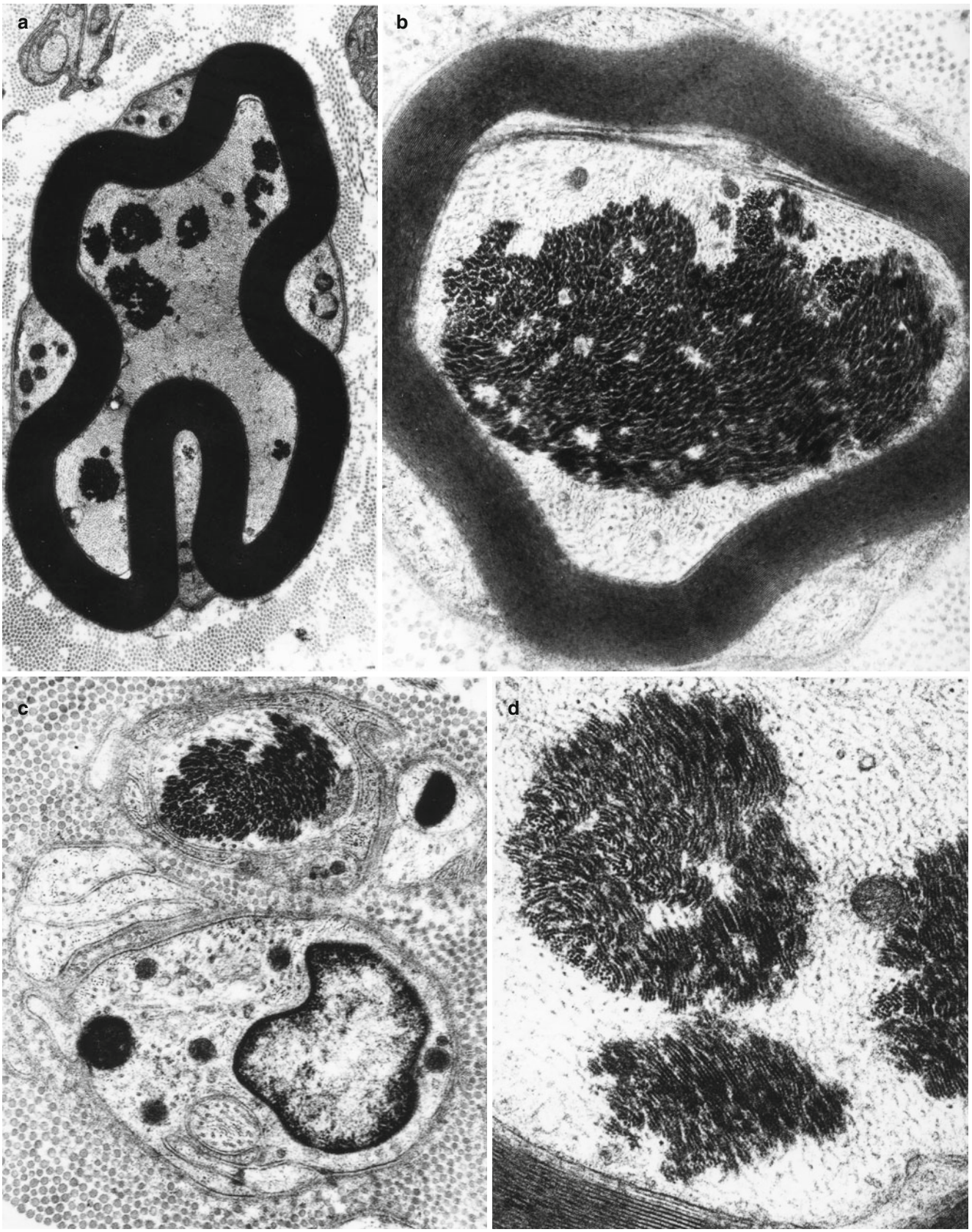

Fig. 4.8 Axonopathy. Intra-axonal aggregates of filaments appear as paracrystalline structures (Magnification, **a** 8,360×; **b** 22,344×; **c** 23,800×; **d** 42,600×)

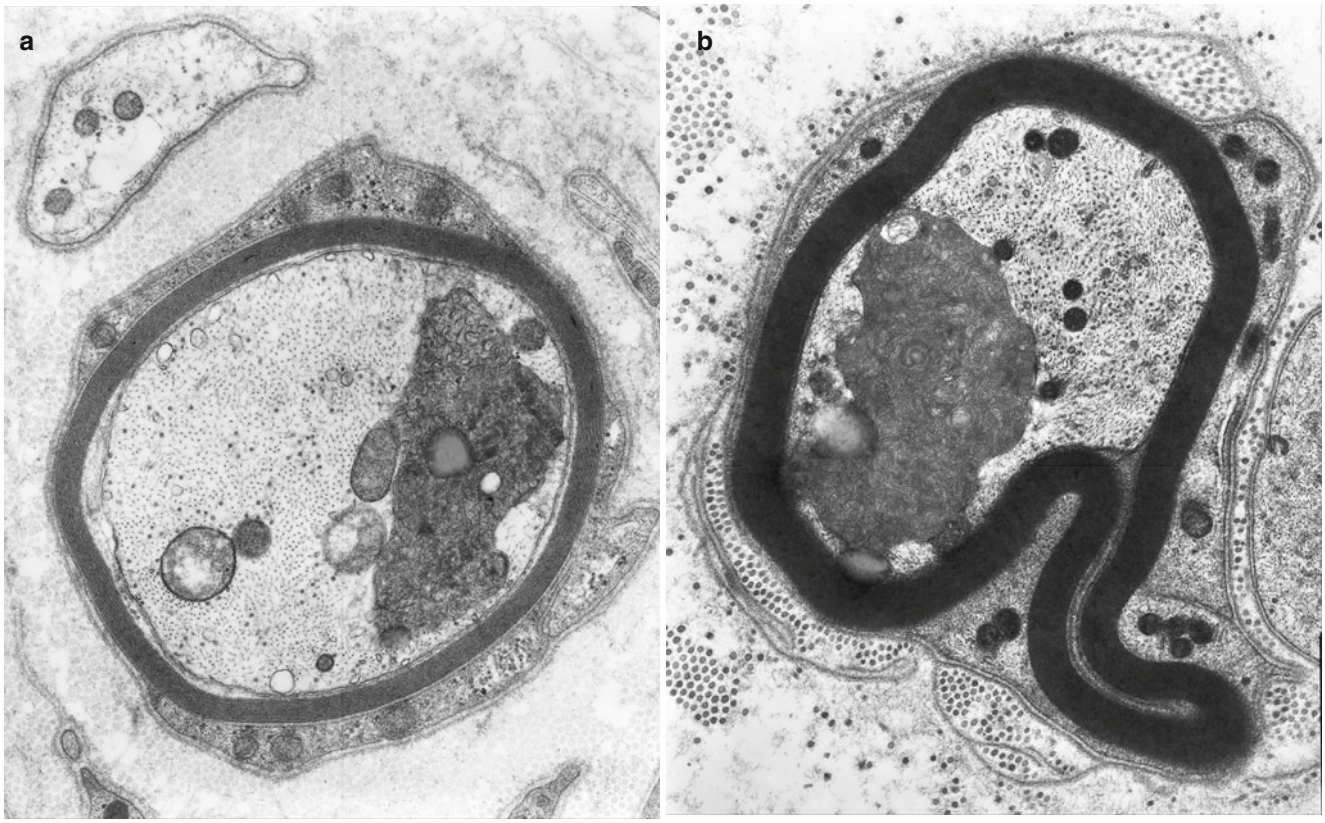

Fig. 4.9 Axonopathy. Intra-axonal compact membranolamellar structures are nonspecific (Magnification: **a** 25,048×; **b** 26,600×)

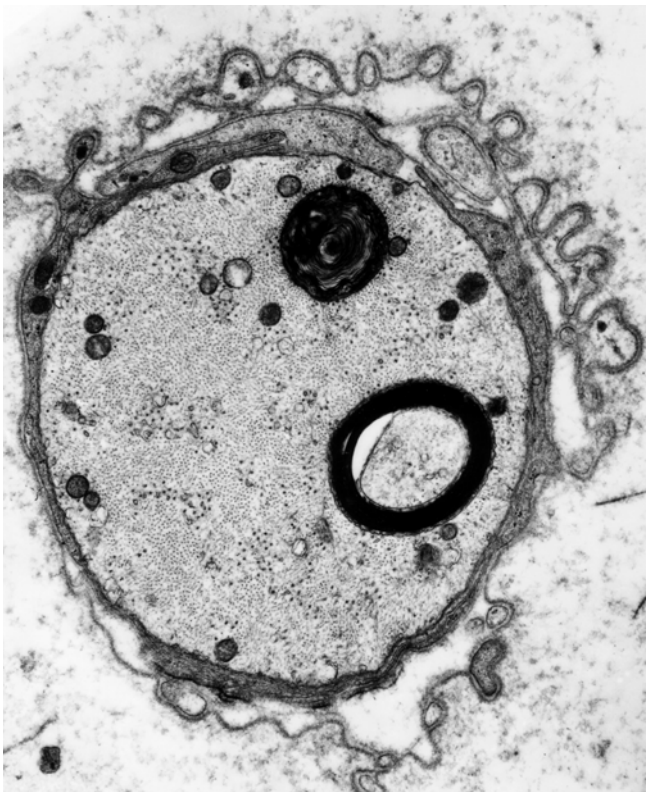

Fig. 4.10 Axonopathy. Unusually large intra-axonal myeloid bodies are seen in a chronic axonal neuropathy (Magnification, 15,000×)

4.3 Wallerian Degeneration

When an axon is transected, most often by trauma or ischemia, a well-studied sequence of events follows as the nerve segment distal to the site of transection degenerates. This process has been named Wallerian degeneration after the pathologist who offered early morphologic observations first emphasized the importance of distal degeneration (Waller 1850). Physiologic studies in man indicate that distal to a transection the axon does not degenerate immediately. Rather, it remains electrically excitable and capable of impulse conduction for as long as 4 or more days, depending on nerve length, with longer distal stumps surviving a few more days (Chaudry et al. 1992). In small experimental animals, the duration of distal stump survival is less, typically 1–2 days. Recent development of animal models of Wallerian degeneration has resulted in rapidly increasing body of literature revealing the complexity of the process (vide infra).

Although the terms "axonal degeneration" and "Wallerian degeneration" are often used interchangeably and may have shared pathogenetic mechanisms, Wallerian degeneration specifically constitutes the microscopic reactions of a nerve segment distal to a site of crush or transection injury. In Wallerian degeneration, histological changes are stereotyped

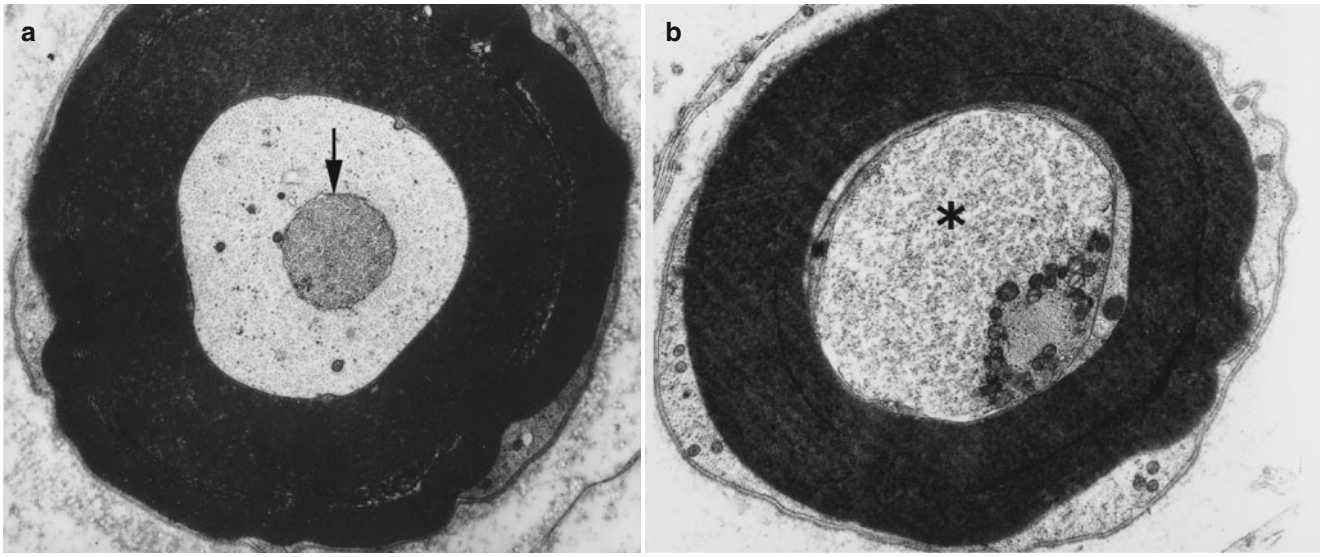

Fig. 4.11 Axonopathy. Nonspecific findings in a nerve biopsy include intra-axonal aggregates of glycogen, either membrane bound (*arrow*, **a**) or diffusely distributed (*, **b**) (Magnification, **a** 9,712×; **b** 10,260×)

and synchronized compared to neuropathies in which axonal degeneration and regeneration often occur simultaneously.

4.3.1 Changes Distal to Transection

4.3.1.1 Axonal Changes

Impulse transmission is maintained for as long as 48 h distal to a site of axonal transection, even though a variety of structural and biochemical changes begin almost immediately. Distal to the site of transection, the earliest morphologic axonal change is an accumulation of abnormal mitochondria, membranous organelles, and glycogen, with disorganization of microtubules and neurofilaments. Histochemical techniques have demonstrated accumulation of lysosomes at 2 h after the injury (Holtzman and Novikoff 1965). These changes, which occur largely in the first few centimeters distal to the injury, are prominent at 12–24 h after transection (Ballin and Thomas 1969; Donat and Wisniewski 1973; Zelena 1980). About 1–2 days after the injury, more distal axon segments begin to show "granular disintegration" with loss of the normal cytoskeletal components, organelles, and axolemma (Figs. 4.12a–d and 4.13a–c). Granular disintegration results in a watery axoplasm containing granular and amorphous debris and surrounded by an intact myelin sheath. Investigators have had difficulty determining whether the axon's "granular disintegration" moves very rapidly centrifugally or occurs simultaneously throughout the length of an axon (Chaudry et al. 1992). In a few days immunohistochemical techniques can no longer identify intact neurofilaments (Bignami et al. 1981) and degenerating axons are seen. Proximal to the site of transection, the axon forms a bulbous swelling, which consists of a circumferential band filled with organelles of various types and a central collection of filaments (Donat and Wisniewski 1973). This region or immediately rostral areas will form the site of future regeneration, the growth cone (vide infra).

4.3.1.2 Myelin Degradation

Widening of the Schmidt–Lanterman incisures occurs within minutes immediately distal to the site of injury and within 24–36 h further below this site (Williams and Hall 1971a). Schwann cell cytoplasm appears to increase in volume, seemingly pressing inwards on the axoplasm (Lubinska 1977). Degenerating axons (and their myelin sheaths) are seen in light microscopic H&E-stained sections (Figs. 4.14a, b and 4.15) and in plastic sections (Fig. 4.14c, d). The process of myelin ovoid formation (Fig. 4.14e) begins at widened Schmidt–Lanterman incisures, typically 1–2 days after injury in rats (Ghabriel and Allt 1979; Williams and Hall 1971a). Myelin degenerative changes evolve in a proximodistal direction and may occur in small fibers before large (Lubinska 1977). Paranodal myelin retraction is an even earlier change and may be accompanied by vesicular myelin degeneration at the node of Ranvier (Ballin and Thomas 1969). Schwann cell cytoplasm and internodal myelin collapse into the space previously occupied by the axon, the Schwann cells "rejecting" their myelin (Beuche and Friede 1984). This collapsed myelin forms "ovoids" (Fig. 4.14e), typically six to ten per internode. Schwann cell cytoplasm separates the ovoids (Fig. 4.14e) at the sites of the former Schmidt–Lanterman incisures. In vivo microscopy reveals

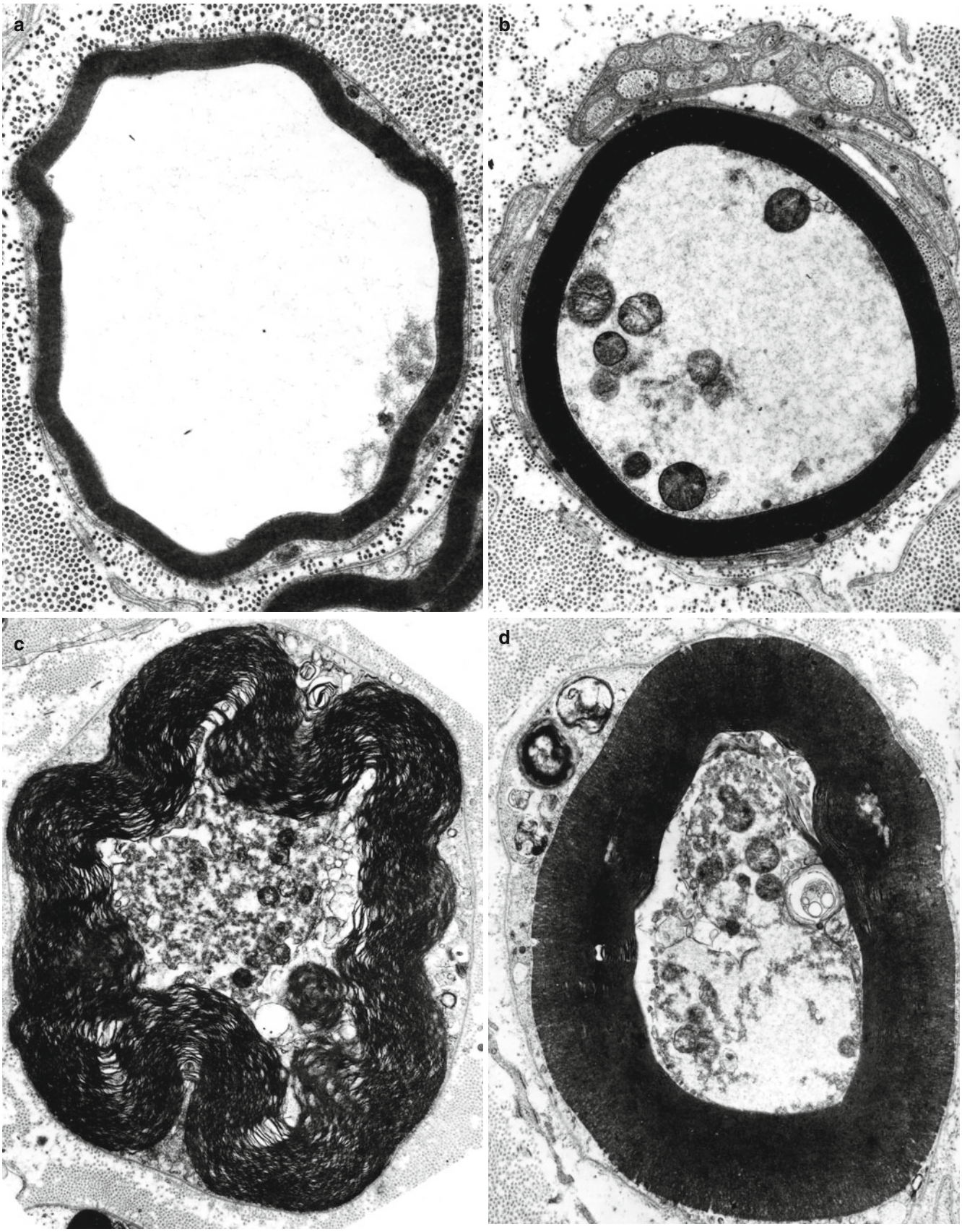

Fig. 4.12 Axonal degeneration. In the early stages, changes range from dissolution of axoplasm to granular osmiophilic accumulations and early fragmentation of myelin (Magnification, **a** 13,579×; **b** 12,141×; **c** 6,841×; **d** 8,892×) (**d**, From Bilbao 1995)

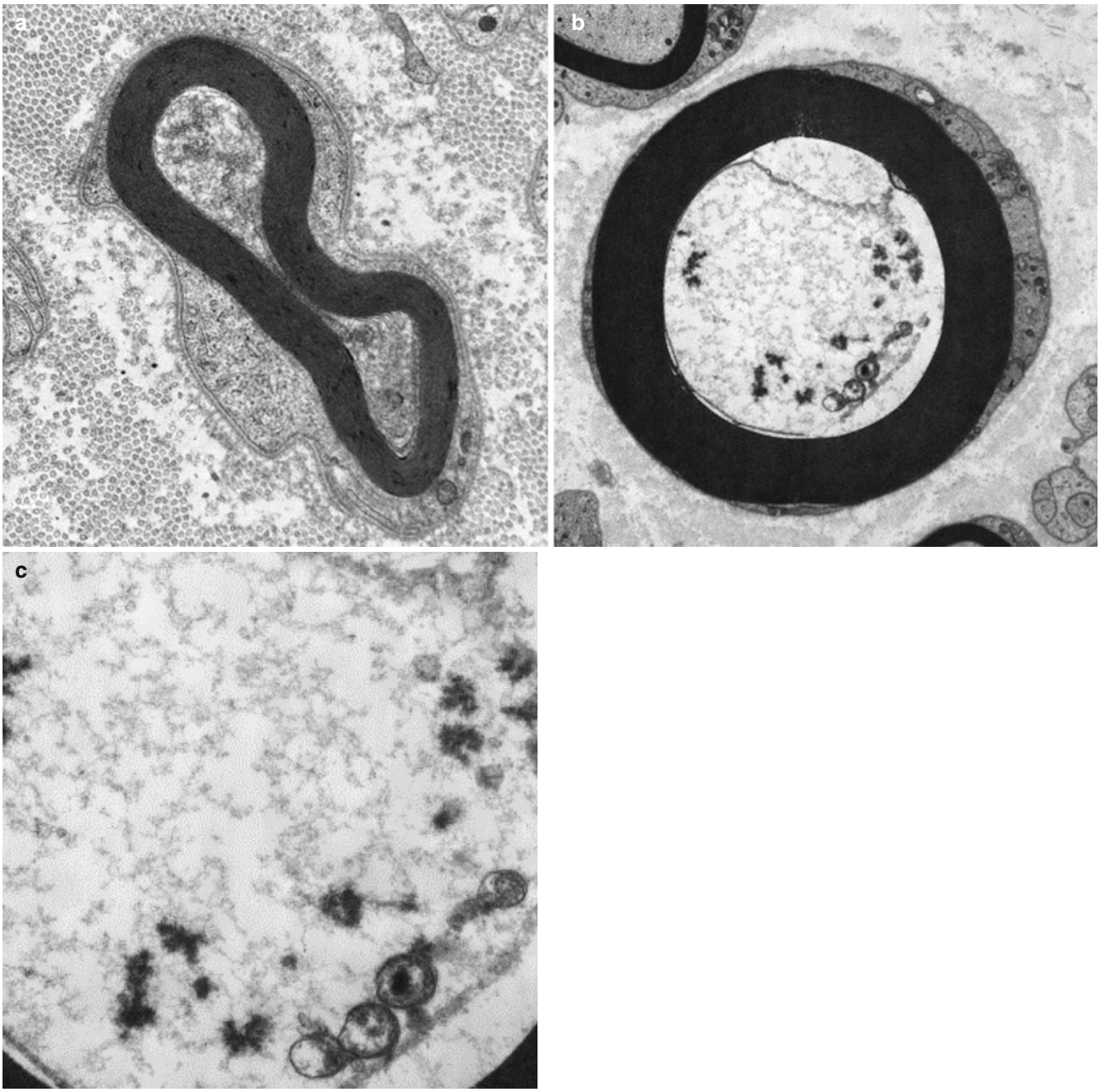

Fig. 4.13 Early axonal degeneration. (**a**–**c**) Early axonal degeneration is characterized by granular, flocculent, pale axoplasm with degenerating axonal organelles in the absence of myelin degeneration (Magnification, **a** 25,000×; **b** 7,500×; **c** 20,000×)

that over the subsequent days and weeks, these ovoids become progressively smaller and more widely separated, as small lipid globules are pinched off and removed (Williams and Hall 1971b).

At the earliest point in the degenerative process, myelin maintains a degree of structural and biochemical integrity, as evidenced by the presence of intracellular debris that has myelin-like periodicity and does not stain with Sudan red. Some myelin debris and evidence of its active degradation appear in Schwann cells (Fig. 4.16a–c) prior to the arrival of macrophages (Holtzman and Novikoff 1965; Stoll et al. 1989). However, at 3–4 days after transection, a wave of invading phagocytes enters the nerve. There may be a role for the greater participation of endogenous macrophages in the slower pace of axonal degeneration found in chronic neuropathies (Muller et al. 2008). The macrophages adhere to and penetrate the Schwann tube (Fig. 4.17a–d), accumulating large amounts of amorphous or lamellar debris.

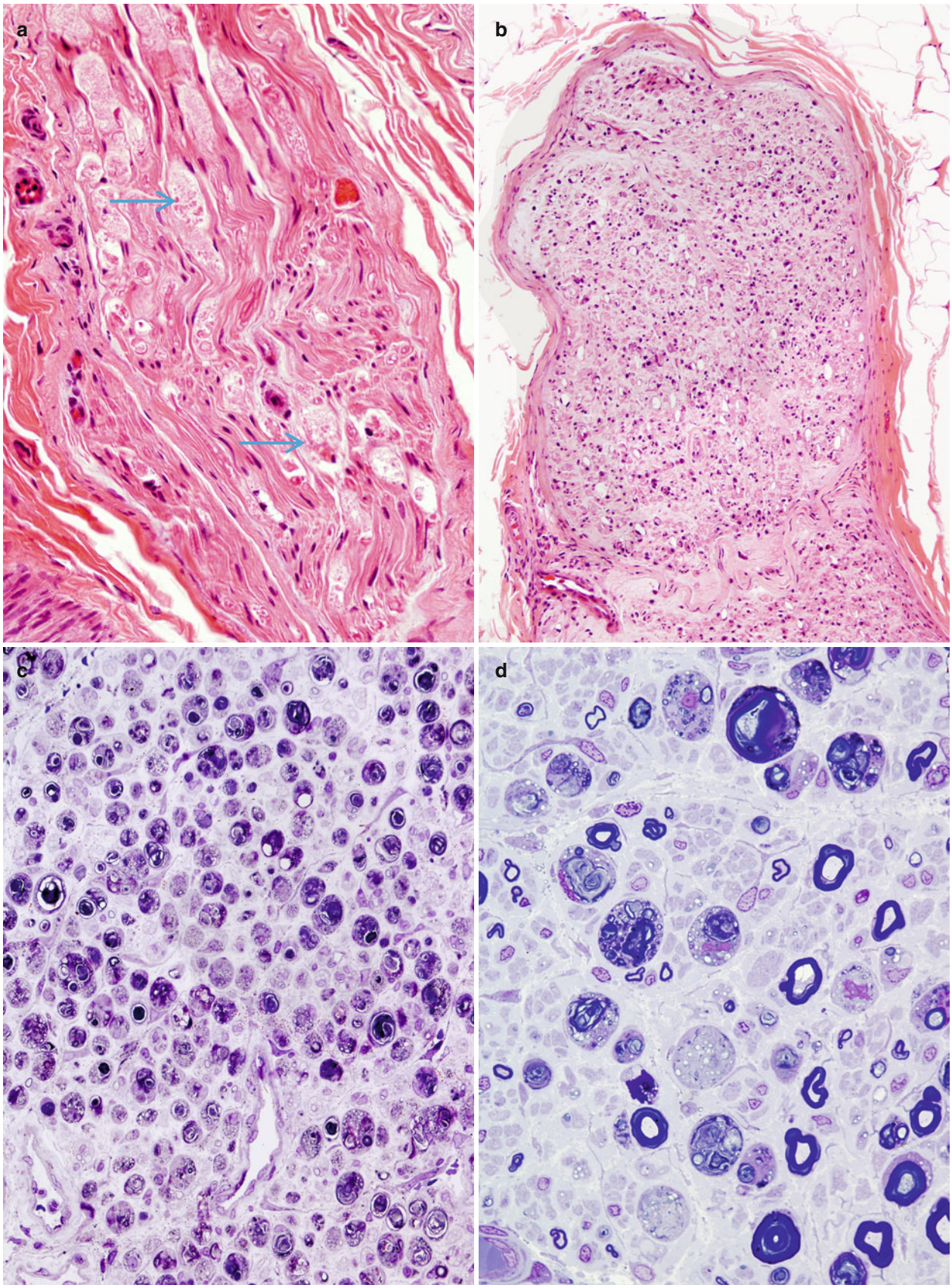

Fig. 4.14 Axonal degeneration, acute. (**a**) Light microscopic appearance of axonal degeneration shows flocculent debris (*arrows*). (**b**) Sural nerve swollen fascicle with increased cellularity. (**c**) Wallerian degeneration characterized by numerous Schwann cells and macrophages containing axonal and myelin debris. Note that all axons appear at the same stage of degeneration in this patient with severe arm trauma. (**d**) Axonal degeneration consists of an admixture of actively degenerating axons and residual maintained normal myelinated axons. (**e**) Longitudinal section of nerve fascicle with ovoids of degenerating myelin and axonal debris. (**a**, **b** paraffin, H&E; **c**, **d**, **e** 1 μ plastic sections) (Magnifications, **a** 400×; **b** 200×; **c**, **d** 1,000×; **e** 400×)

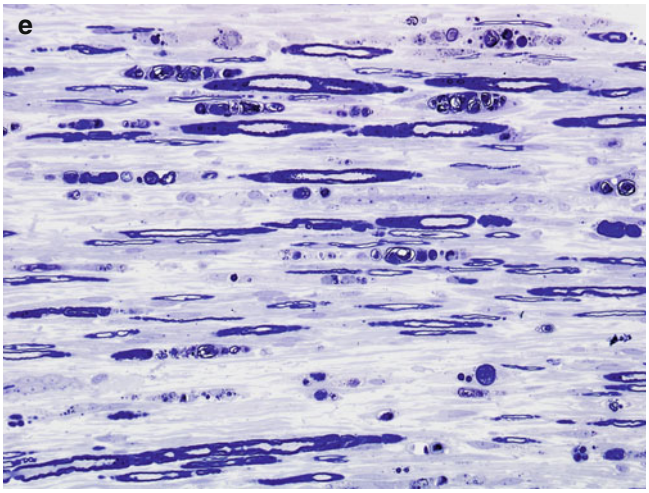

Fig. 4.14 (continued)

At this point the debris often shows sudanophilia, reflecting the degradation of myelin into neutral lipid and cholesterol esters. In the endoneurium light microscopy shows numerous foamy cells (Fig. 4.18a–e) that correspond ultrastructurally to cells with an electron density greater than that of Schwann cells, no basement membrane, containing large amounts of amorphous debris and many lysosomes, as demonstrated by CD68 immunohistochemistry (Fig. 4.18a). In contrast to the association of intact myelin and macrophages in the early phase of primary macrophage-mediated demyelination, macrophages in Wallerian degeneration occur only in association with degenerating axons (Stoll et al. 1989; Williams and Hall 1971a). With further progression, foamy lipid accumulation appears in all cell types, including fibroblasts, endothelial cells, Schwann cells, and even the inner perineurial cell layers (Williams and Hall 1971a). In rodents, myelin debris largely clears within 2 months of the onset of degeneration (Williams and Hall 1971b), but in humans endoneurial foamy cells may persist up to 7 months after nerve transection (Griffin et al. 1992).

4.3.1.3 Unmyelinated Fibers

Unmyelinated fibers degenerate in much the same way as myelinated fibers (Dyck and Hopkins 1972; Thomas and King 1974) (Fig. 4.15). Of course no myelin ovoids form, but within 2–4 days of transection (or earlier using ultrastructural methods) a swelling appears just distal to the site of injury. Accumulation of organelles and granular disintegration accompany the swelling. Schwann cells and macrophages take up axonal debris (dense bodies, degenerate organelles). Multiple flattened Schwann cell processes encircle the degenerating axon. Long after the axon has degenerated, the persistence of flattened Schwann cell processes aligned side by side, the so-called denervated bands, serves as an indicator of unmyelinated fiber loss (Fig. 7.14b).

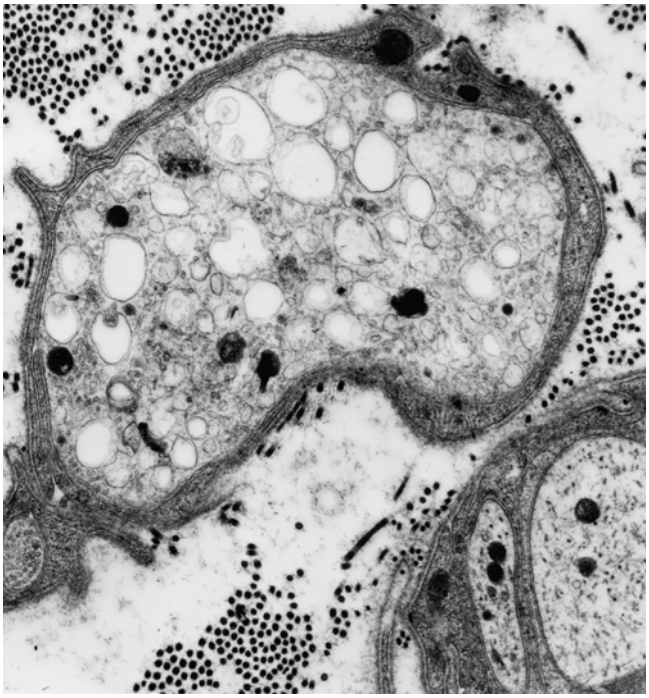

Fig. 4.15 Axonal degeneration. Axoplasm of a degenerating unmyelinated fiber is occupied by smooth-walled vesicles and dense bodies (Magnification, 13,750×)

4.3.1.4 Endoneurial Contents

A great increase in endoneurial cellularity occurs a week after nerve transection, due not only to the invasion of circulating mononuclear phagocytes but also to proliferation of most cell types in the endoneurium. Schwann cells of both myelinated and unmyelinated axons multiply, the former resulting in formation of "bands of Büngner," arrays of Schwann cells and their interdigitating processes within a space circumscribed by the basement membrane (the Schwann tube). Nerve regeneration, outlined later in this chapter, can then occur. If it does not, the Schwann cells become atrophic and diminish in number accompanied by collagen deposition (Roytta and Salonen 1988; Weinberg and Spencer 1978). Unless local trauma has destroyed the tissue, the original Schwann cell basement membrane remains intact at the beginning of Wallerian degeneration. Early during the process, some inward collapse of the basement membrane occurs, but for the most part the Schwann tube remains a continuous identifiable structure (Fig. 4.18). During subsequent months if reinnervation does not occur, the bands of Büngner atrophy and the surrounding basement membrane may fragment and partially disappear (Giannini and Dyck 1990). Such events render future prospects for organized regrowth of axons through the region very dim.

A proliferation of fibroblasts also occurs during the first week, followed by migration of these cells adjacent to degenerating nerve fibers. As a result the Schwann cell columns

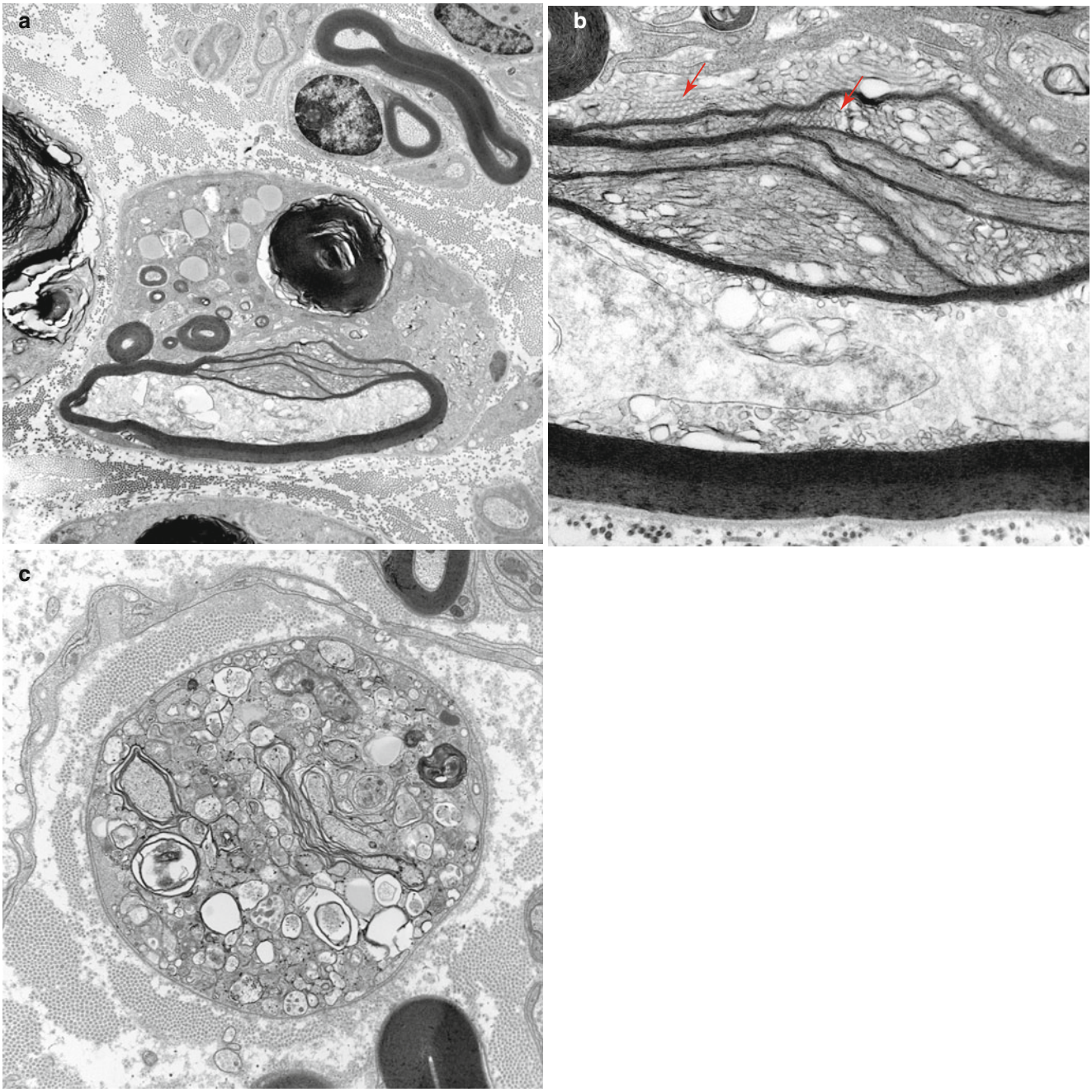

Fig. 4.16 Later axonal degeneration. (**a**, **b**) Later in axonal degeneration, granular axoplasm is accompanied by superimposed myelin degeneration; in this case, vesicular myelin change (*arrows*, **b**). (**c**) Digestion of myelin and axonal debris within the original Schwann cell basal lamina (Magnification, **a** 7,500×; **b** 30,000×; **c** 12,000×)

often become encircled with long thin processes. Collagen of only half the thickness (23–30 μm) of normal endoneurial collagen (50–60 μm) appears adjacent to the basal lamina of the Schwann cells and is probably secreted by them (Salonen et al. 1987). If regeneration occurs, the endoneurial changes reverse with the thin collagen fibers enlarging to a normal diameter, and nerve architecture returns to normal (Roytta et al. 1987). If, however, axons do not reenter the Schwann tube, the fibroblasts encircling the Schwann cell columns may begin to assume some characteristics reminiscent of perineurial cells, forming partial basement membranes, demonstrating numerous pinocytotic vesicles, and seeming to divide the endoneurium into microfascicles (Roytta et al. 1987; Salonen et al. 1987) (Fig. 7.10).

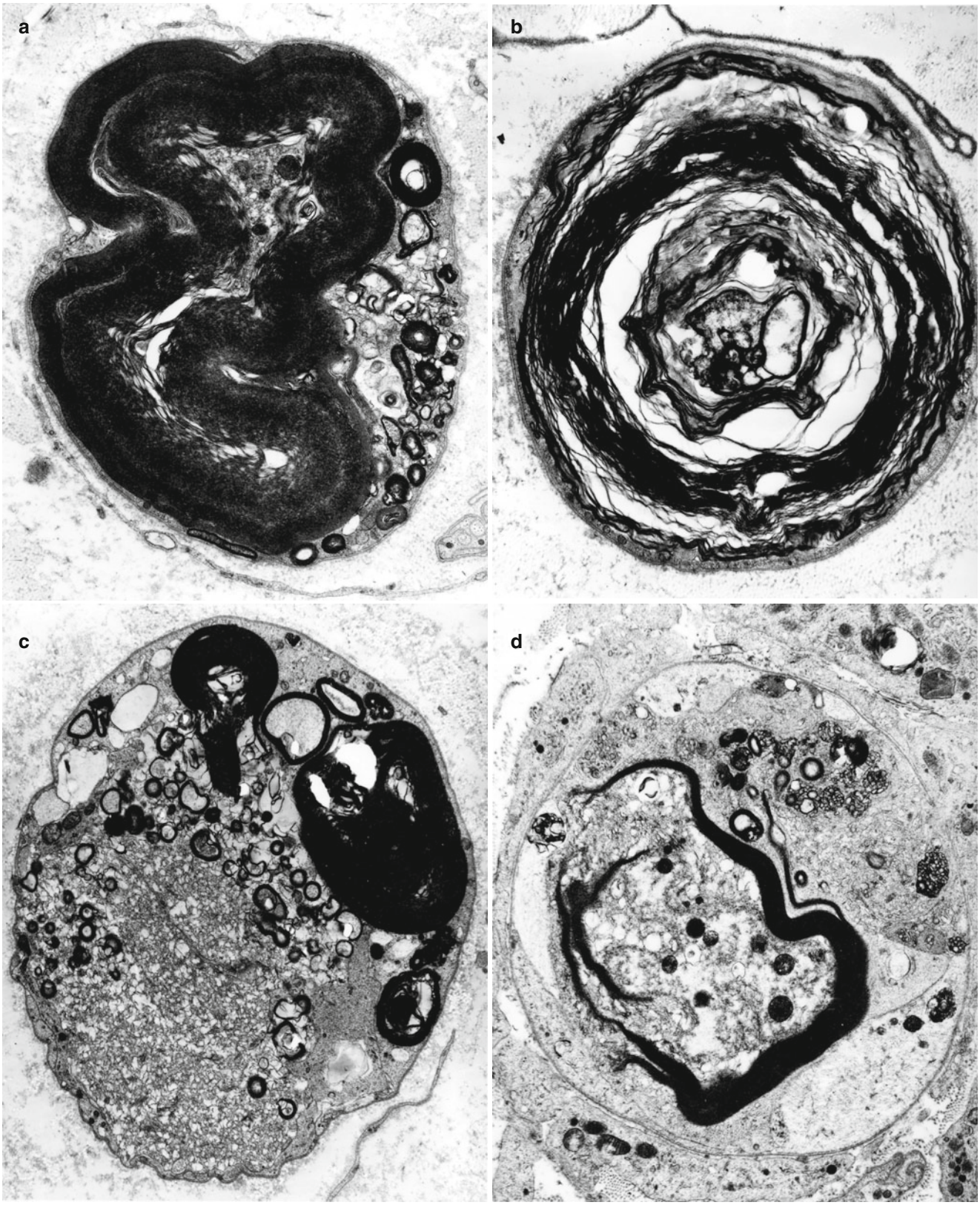

Fig. 4.17 Later axonal degeneration. In later stages axonal disappearance is associated with collapse and fragmentation of myelin. In (**d**) several cytoplasmic profiles of macrophagic or Schwann cell origin occupy the Schwann tube, and an adherent macrophage has penetrated the basal lamina (*arrow*) (Magnification, **a** ×8,379, **b** ×8,379, **c** ×6,589, **d** ×5,928)

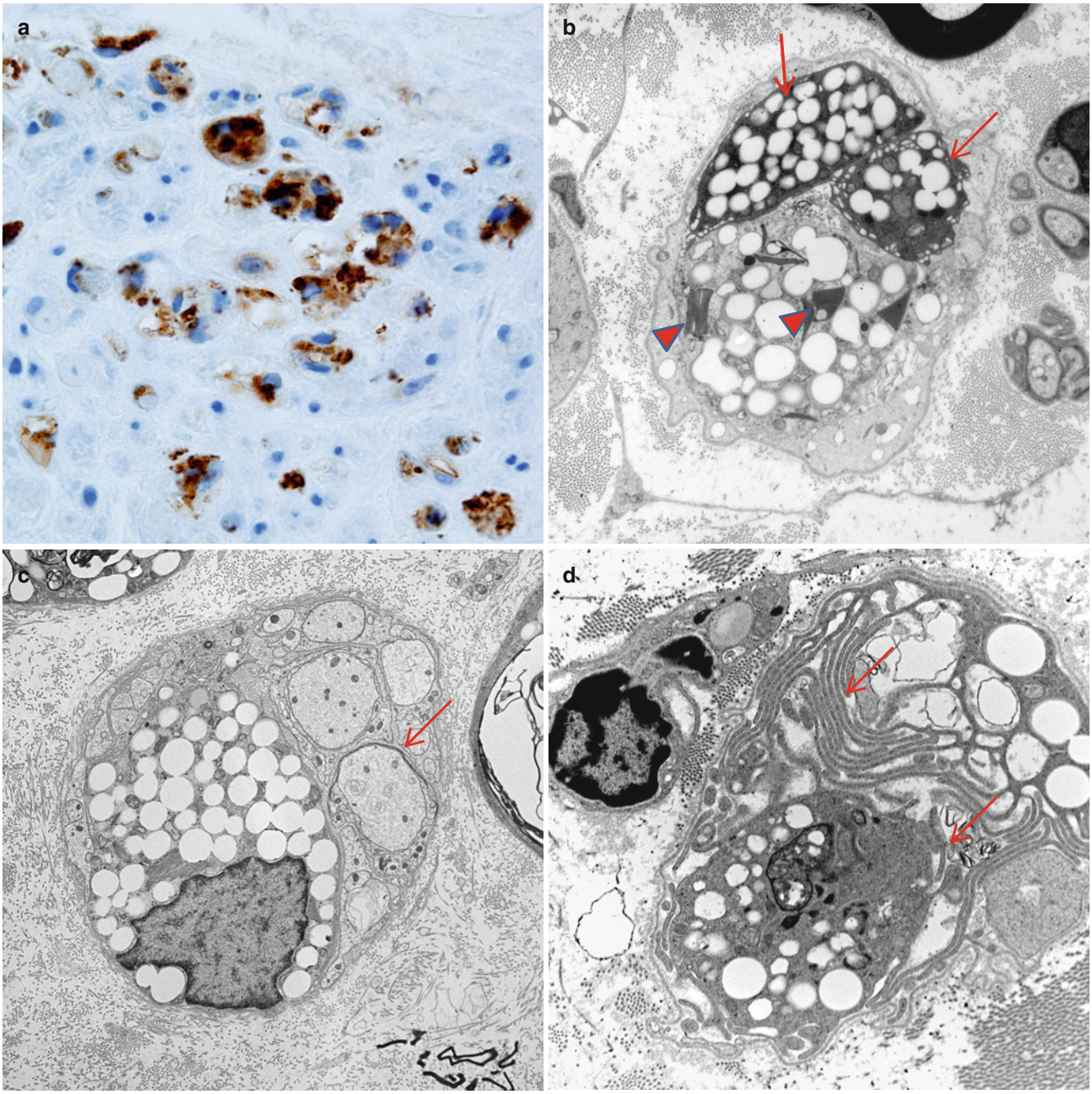

Fig. 4.18 Macrophages and axonal degeneration. (**a**) Frequent macrophages in the endoneurium of a fascicle with numerous degenerating axons as demonstrated by CD68 immunohistochemistry. (**b**) Two macrophages (*arrows*) within the original basal lamina of a degenerated previously myelinated axon. Some debris remains in the cytoplasm of a Schwann cell which continues to have Pi granules (*arrowheads*). (**c**) A macrophage with lipid droplets (extracted during processing) leaving a Schwann cell which contains regenerating axons, one of which is beginning to myelinate (*arrow*). (**d**) A macrophage containing lipid debris and its processes (*arrows*) within the basal lamina of a Schwann cell which is no longer visible. (**e**) Macrophage with lipid debris and Pi granules is shown adjacent to two small venules. (**f**) Numerous macrophages gathering around two endoneurial venules. Note the number of regenerating axons in some Schwann cells (*arrows*) (Magnification, **a** paraffin, CD68 immunohistochemistry, 1,000×; **b** 7,500×; **c** 6,000×; **d** 12,000×; **e** 10,000×; **f** 1 μm plastic section, 1,000×)

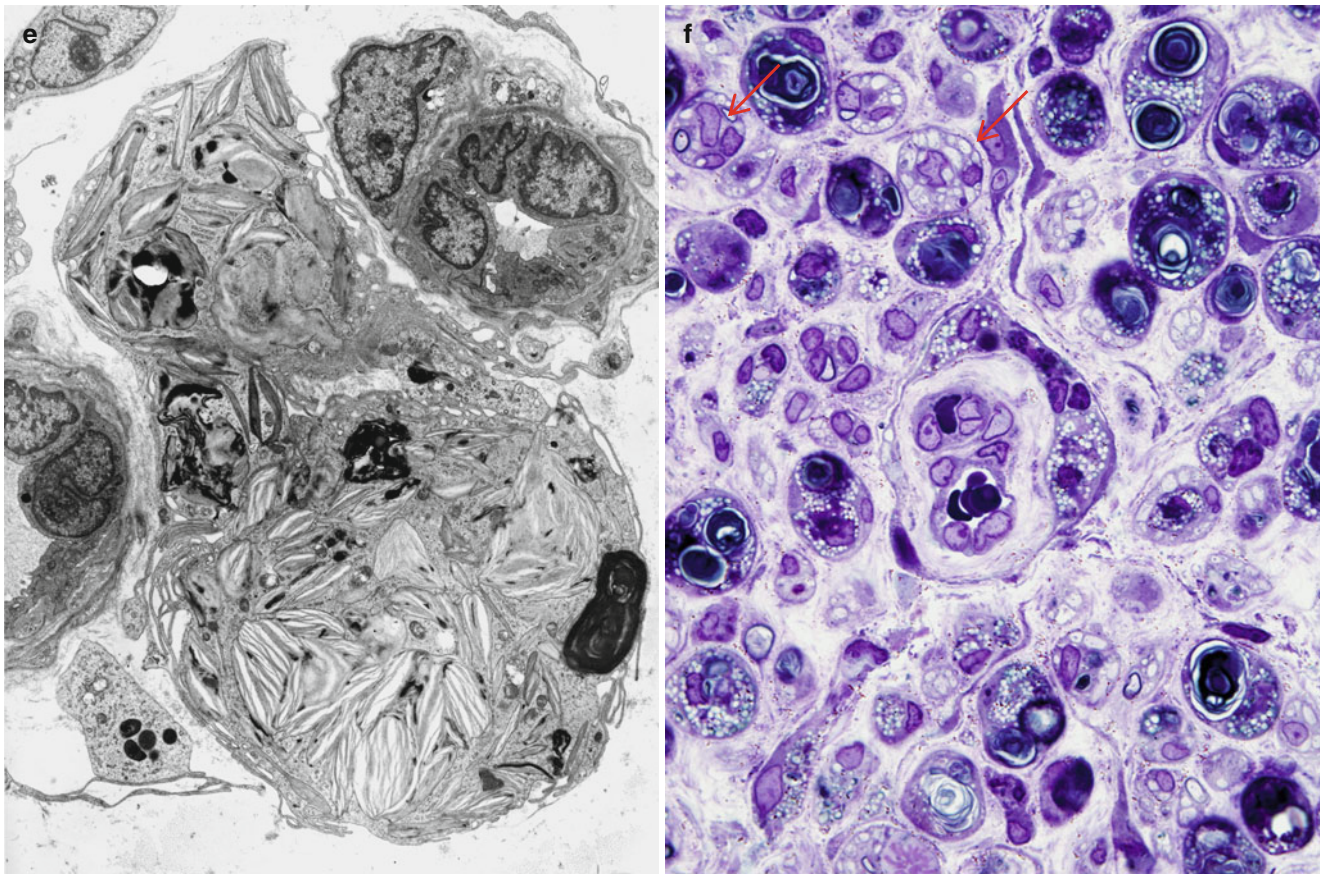

Fig. 4.18 (continued)

4.3.2 Mechanisms of Wallerian Degeneration

4.3.2.1 Axons

Although much is known about the classical pathological changes in Wallerian degeneration, this area remains a fertile field for investigation (see Wang et al. 2012 for review), particularly with the use of animal models. Experimental analysis has provided insights into axonal physiology, axon–Schwann cell interaction, and nerve regeneration (Chaudry et al. 1992).

The *Wlds* (slow Wallerian degeneration) mutant mouse maintains axonal function post-transection and delays Wallerian degeneration up to 3 weeks, suggesting distal axonal degeneration does not simply reflect loss of perikaryal synthetic support (Raff and Whitmore 2002; Wang et al. 2012). Recent studies ascribe these changes to increased expression of a WldS fusion protein, increased expression of nicotinamide mononucleotide adenylyltransferase (Nmnat2), increased nuclear nicotine adenine dinucleotide (NAD) levels, and, subsequently, increased activity of the NAD-dependent deacetylase SIRT1 (Araki et al. 2004). Wlds mice are also resistant to experimental toxic neuropathies (Glass 2004). Additionally, axonal neurofilaments and myelin gene expression are degraded more slowly, and the injury-induced increases in NGF, p75, and tenascin-C mRNA levels do not occur (Stoll and Muller 1999). Recent studies show that dual leucine kinase (DLK), a Drosophila protein highwire (and its mammalian homologue PHR1), and a Toll-like receptor adapter protein promote degeneration of severed axons in Drosophila and mice via a JNK-dependent mechanism (Miller et al. 2009; Xiong et al. 2012; Babetto et al. 2013; Osterloh et al. 2012).

Current thought (reviewed in Wang et al. 2012) proposes that axonal degeneration involves at least three morphologically discernible phases:

Phase (1): Acute non-apoptotic degeneration stage resulting in extracellular calcium influx, calpain (Griffin et al. 1992; Schlaepfer and Hasler 1979), and proteasome–ubiquitin activation. Activated calpain participates in degrading the neurofilaments and microtubules producing watery unstructured axoplasm. Both proximal and distal stumps undergo changes immediately (5–60 min) upon injury (Kerschensteiner et al. 2005), a process inhibited by expression of the WldS transgene (vide infra).

Phase (2): A latency period during which the distal axon remains morphologically intact and electrically excitable for brief periods and via axonal transport forms dystrophic bulbs at terminals and transected ends (Tsao et al. 1994).

Phase (3): An abrupt granular degeneration (GDC) phase resulting in fragmentation of the entire axonal cytoskeleton distal to the injury site. Focal, acute, severe injuries (i.e., axotomy) result in a proximodistal direction of axon degeneration; in contrast, chronic injury results in a retrograde pattern (Beirowski et al. 2005). Calpains are causally responsible for GDC (Ma 2013) and application of a calpain inhibitor cocktail to the transection site completely prevents this fragmentation (Kerschensteiner et al. 2005).

4.3.2.2 Macrophages

Macrophages are an important part of Wallerian degeneration and subsequent axonal regeneration (Beuche and Friede 1984; Hann-Bonnekoh et al. 1989). Activated endogenous endoneurial macrophages are joined by hematogenously derived macrophages in response to recruitment by Schwann cells (using stromal cell-derived factor 1, leukemia inhibitory factor (LIF), tumor necrosis factor (TNF-α), and IL-1β) (Fenrich and Gordon 2004) and in conjunction with mast cell release of vasoactive substances facilitating trans-endothelial migration of macrophages into the endoneurium. Myelin catabolism in Schwann cells is thought to be lectin mediated and opsonin independent, compared to macrophage-mediated myelin phagocytosis which is opsonin dependent, requiring complement receptor type 3 (CR3) expression on macrophages and complement component C3 on degenerating myelin sheaths (Bruck and Friede 1990). Schwann cell myelin debris can be engulfed by macrophages as it is released into the extracellular space (Hirata et al. 1999); directly removed from the Schwann cell in an intimate, poorly understood process; or degraded in situ with residua maintained in the Schwann cell. Macrophages also participate in the synthesis and secretion of apolipoprotein E for reutilizing lipids.

4.3.2.3 Schwann Cells

An elaborate system of signals presumably governs interactions between the degenerating axon, its Schwann cell, incoming macrophages, and other endoneurial cells. The myelin alterations that follow nerve transection are not simply a reaction of the Schwann cell. Rather, they result, at least in part, from a loss of maintenance signals provided by an intact axon (LeBlanc and Poduslo 1990; Lubinska 1977). Proliferation of endoneurial fibroblasts, endothelial cells, and Schwann cells results with macrophage influx (Beuche and Friede 1984). Changes in the endoneurial matrix are also involved in supporting and guiding axonal regrowth (Tona et al. 1993). Previously myelinating Schwann cells dedifferentiate to a pre-myelinating/unmyelinating Schwann cell phenotype, proliferate (maximally at 3–4 days), and form collections of processes ("bands of Büngner") within the original basal lamina of the axon–Schwann cell unit. Intriguingly, Schwann cells composing Remak bundles proliferate in response to degeneration of neighboring myelinated fibers but not in response to unmyelinated axon degeneration (Murinson and Griffin 2004). Schwann cells are thought to initially respond to axonally derived neuregulin concerning axon size and myelin thickness; hypomyelination and hypermyelination result from reduced and overexpression of neuregulin, respectively (Michailov et al. 2004).

In these earliest stages of the process (first 3 days in rats), before macrophages enter the nerve in large numbers, Schwann cells begin the process of myelin collapse and fragmentation along the Schmidt–Lanterman incisures and in the paranode, with small amounts of debris sequestered within the Schwann cell (Stoll et al. 1989). Lysophosphatidylcholine (LPC) generated by the action of Schwann cell-derived phospholipase A2 (PLA2) expressed by Schwann cells likely initiates myelin breakdown early in Wallerian degeneration. The role of native endoneurial macrophages, which make up 2–9 % of the endoneurial cell population, is unclear (Griffin et al. 1993; Hann-Bonnekoh et al. 1989) but likely participates with blood-derived macrophages. Degenerating myelin, not axoplasm, probably provides the most important signal summoning macrophages to their debris-clearing task which appears to require complement (Bruck and Friede 1991; Griffin et al. 1992). Macrophages release IL-1, the major stimulus for Schwann cell production of nerve growth factor (NGF), important for the process of regeneration that will follow.

The blood–nerve barrier (BNB) normally loses its integrity during the early phases of degeneration and regeneration (Ohara and Ikuta 1985; Olsson 1990), but the barrier is gradually reestablished over several months. Whether recovery of the integrity of the BNB requires the return of axons and their myelin sheaths remains controversial (Bouldin et al. 1991; Latker et al. 1991; Seitz et al. 1989). This loss of BNB integrity is a nonspecific response to nerve injury of various etiologies.

4.4 Axonal Pathology in Nerve Biopsy Specimens

This chapter provides a description of axonal degenerative and regenerative processes based mostly upon experimental data; however, this description is presumed to be valid for human peripheral nerve disease. Experience indicates that the ultrastructural changes described in experimental models

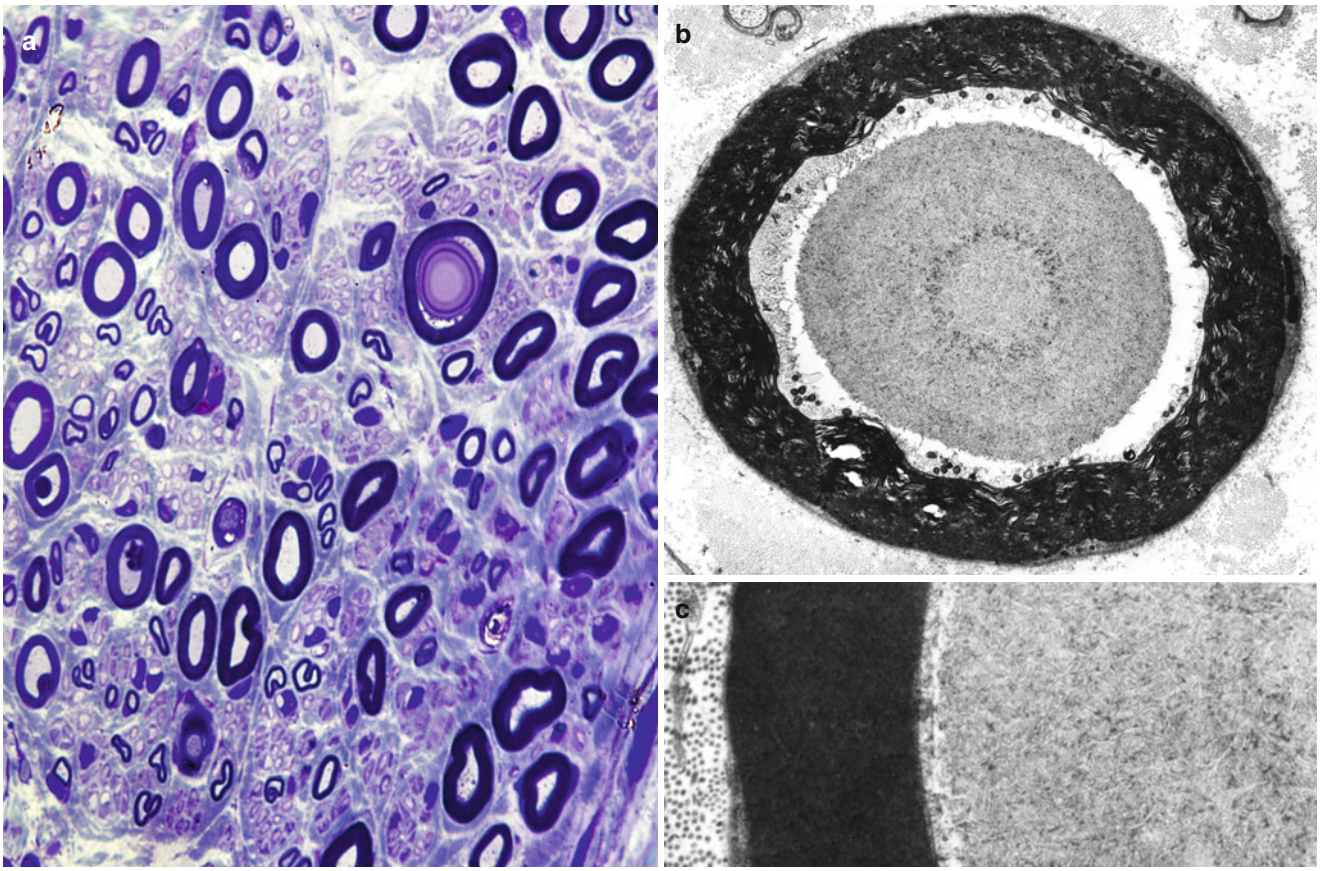

Fig. 4.19 Polyglucosan body. (**a**) A distended fiber with attenuated myelin shows an intra-axonal round structure composed of concentric bands of different density. One micron thick plastic section. (**b**, **c**) Tightly packed randomly oriented short branched filaments are typical of this inclusion. The surrounding halo is probably artifactual (Magnification, **a** 1,000×; **b** 11,856×; **c** 24,282×)

also occur in human neuropathy. Investigators have studied Wallerian degeneration exclusively in rodent models of nerve crush or transection. In human disease seen on biopsy, acute nerve ischemia is most likely to resemble transection. Other causes of axonal death in humans are distal axonopathy or local endoneurial disturbances such as focal amyloid deposits (Sobue et al. 1990) or toxic products of inflammation (Said and Hontebeyrie-Joskowicz 1992). Clearly, the nature of the insult and the effects on endoneurial contents in a nerve crush differ from those in metabolic axonal degeneration, suggesting that the processes elucidated in experimental Wallerian degeneration might not necessarily apply to other types of axonal disease.

Fortunately, experimental data suggest that while changes observed in true Wallerian degeneration differ from those of other causes of axonal degeneration in rapidity and severity, these processes have fundamental similarities (Singer and Steinberg 1972; Stoll et al. 1989). Thus, when a nerve specimen shows the classic appearance of ovoid formation and axonal granular degeneration, the axon is actively degenerating and the cause may include acute ischemia or trauma, injury to the cell body or distal axonopathy, or local axonal damage due to products of inflammation or deposits of amyloid.

4.4.1 Axonal Inclusions

4.4.1.1 Polyglucosan Bodies

Polyglucosan bodies (PGBs) are cytoplasmic inclusions measuring 10–70 μm in cross-sectional diameter. They occur in various locations in the nervous system and in a variety of situations. Researchers consider corpora amylacea, Lafora bodies, and Bielschowsky bodies as polyglucosan bodies and cannot differentiate them microscopically (Robitaille et al. 1980).

PGBs are basophilic with H&E and metachromatic with toluidine blue. In addition, they stain positively with a variety of histochemical stains including PAS (diastase resistant), iodine, silver proteinate, and Alcian blue (Fig. 4.19a–c). Although PGBs appear round in cross section, longitudinal cuts through the nerve may show them to be elongated.

Electron microscopy demonstrates that the PGB has no limiting membrane and consists of randomly arranged granules and 6–8 nm wide branched filaments. An electron-lucent region often surrounds the branched filaments (Fig. 4.19). Occasionally, especially in the larger polyglucosan bodies, the center is denser and composed of more tightly aggregated filaments. Glycogen granules may be prominent at the periphery (Yagishita et al. 1977) or conspicuous by their absence (Robitaille et al. 1980).

Chemical analysis of corpora amylacea and Lafora bodies, presumed to be identical to other types of polyglucosan bodies (Robitaille et al. 1980), indicates that they are composed almost entirely of abnormally branched glycogen (amylopectin) with small amounts of protein, sulfate, and phosphate groups (Cafferty et al. 1991; Stam and Roukema 1973; Steyaert et al. 1990).

Polyglucosan bodies most commonly occur within myelinated axons, but they can occur in unmyelinated axons, Schwann cells, perineurial cells, macrophages, and endothelial cells. In normal nerves, especially with increasing age, one or two PGBs may be seen in as many as 15 % of nerve biopsy specimens (Averback and Langevin 1978). The polyglucosan bodies occur in even greater numbers in intramuscular nerves (Averback and Langevin 1978; Bernsen et al. 1989). However, finding several PGBs on nerve biopsy is unusual and should alert the pathologist to several diagnostic possibilities which include adult polyglucosan body disease, Lafora body disease, and type IV glycogenosis.

4.4.1.2 Paracrystalline Filamentous Aggregates

We have observed single or multiple osmiophilic paracrystalline structures in myelinated and unmyelinated axons in a variety of axonal and demyelinating neuropathies of inflammatory, genetically determined, or metabolic/toxic origin and in nerves showing little or no pathology (Fig. 4.8a–d). These inclusions may also occur in giant axonal neuropathy, perhaps formed by aggregation of neurofilaments (Donaghy et al. 1988). We do not consider these structures to have any pathological significance except perhaps as nonspecific indicators of axonal distress.

4.5 Axonal Regeneration

4.5.1 Mechanisms of Regeneration

Within 2 days of injury, formerly myelinating Schwann cells downregulate steady-state mRNA levels of myelin-directed proteins (Raff and Whitmore 2002; LeBlanc and Poduslo 1990). Subsequently, they dedifferentiate and acquire a phenotype with features similar to pre-myelinating/unmyelinating Schwann cells and switch from neuregulin dependence (presumably an axonal factor) to dependence on endogenously produced non-neuregulin factors. Schwann cells not associated with axons will eventually undergo apoptosis, while those in contact with axons will proliferate and migrate along the axon before synthesizing myelin (Carroll et al. 1997).

However, Schwann cell denervated bands are not equivalent (Hoke et al. 2006). Schwann cells from denervated and reinnervated cutaneous sensory nerves show upregulation of mRNA for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor 1, but similar changes were not found in ventral root. In contrast, mRNA for pleiotrophin (PTN) and glial cell line-derived neurotrophic factor was upregulated to a greater degree in ventral root than in cutaneous nerve. Even as the axon undergoes the changes of Wallerian degeneration, regeneration can begin (Fig. 4.20) and proceed simultaneously. The proliferating Schwann cells lying within the old basal lamina tube (Fig. 4.20b) are stimulated by a variety of cytokine/macrophage products to increase the expression of surface adhesion molecules and nerve growth factor and its receptor (Taniuchi et al. 1986) and a mixture of laminin and other extracellular substances in order to help the regrowing axon survive and find its way (Scarpini et al. 1989; Tona et al. 1993). Regeneration begins at the viable tip of the damaged axon, where a growth cone is formed, and proceeds down the pathway provided by the surrounding environment (Fig. 4.20c). Although important for regeneration, Schwann cell depletion does not interfere with axon elongation when the original basal lamina remains in continuity (Fugleholm et al. 1994).

The growth cone is a terminal enlargement of the distal axon containing numerous vesicular structures of different morphology and content, with a central core and perhaps a thin peripheral rim of filaments (Yamada et al. 1971) (Fig. 4.21a–c). The vesicles probably originate at the perikaryon and appear to interact with the plasma membrane of the growth cone, one of their roles being able to provide membrane material for the growing axon (Lasek and Katz 1987). Among other proteins, the filament protein actin occurs in the growth cone in large amounts and provides a structural framework for the filopodia (motile elongated pointed processes) and lamellipodia (broad sheets) which project from the tip of the cone and lead the axon forward in exploring the environment. Recent studies indicate that regenerating axons receive ribosomes from Schwann cells which may support local axonal protein synthesis by transferring protein synthetic machinery and mRNAs (Court et al. 2008, 2011). A particular growth direction is selected, presumably based on chemotactic interactions between the external milieu and the individual cell projections (Bray 1989). Growth cones may sometimes reach gigantic proportions (Fig. 4.22a), perhaps representing a failure of the normal regenerative processes (Griffin et al. 1977).

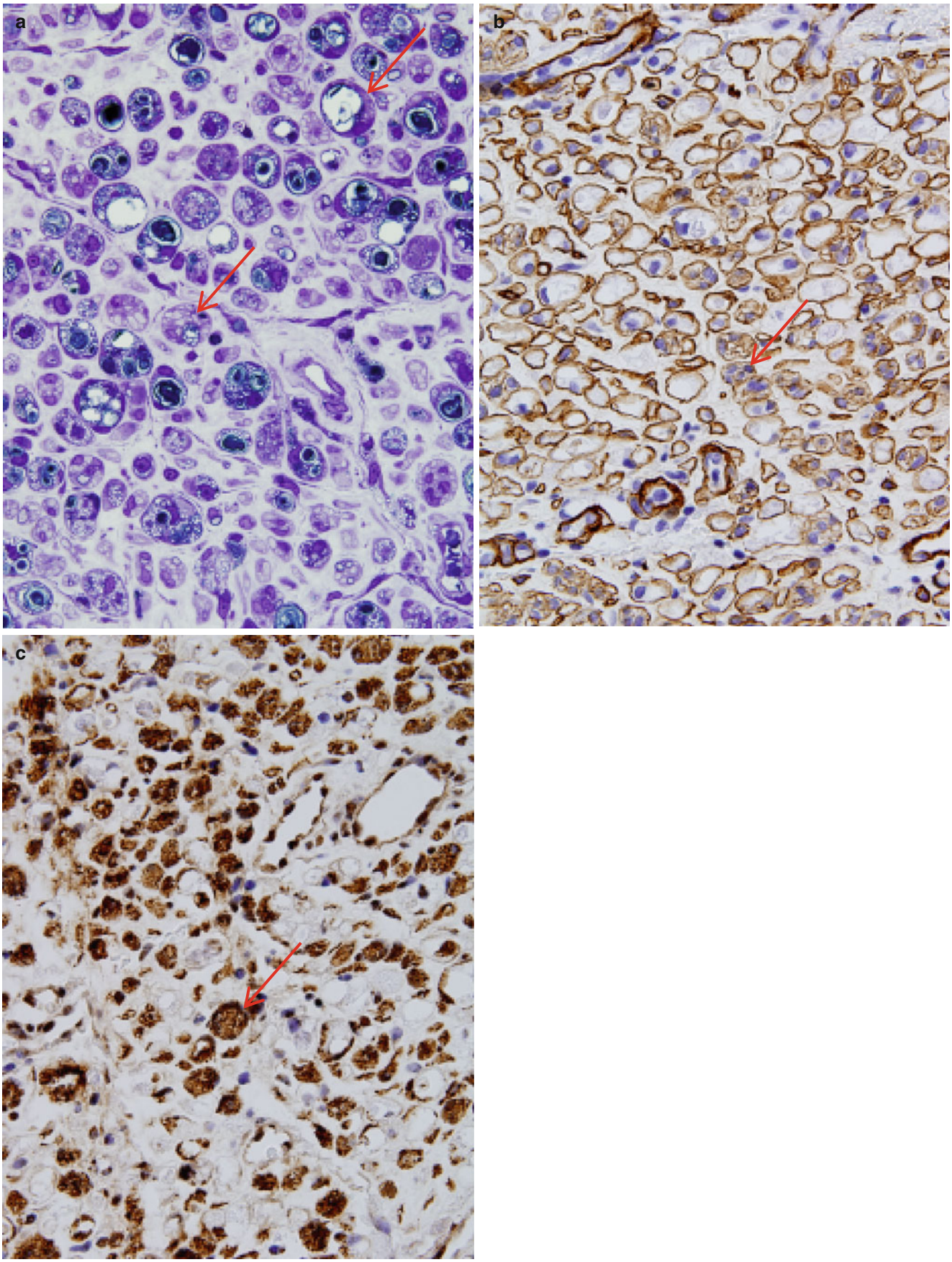

Fig. 4.20 Regenerating axons, light microscopy. (**a**) Following Wallerian degeneration, many axons show regenerating axons within Schwann cell units, many of which still contain debris. (**b**, **c**) Light microscopic image of regenerating axons within Schwann cell tube basal laminae which may have one or more intratubal nuclei (*arrow*, **b**). An adjacent section shows collections of tiny regenerating axons (**c**). (**a** 1 μ Plastic section; **b** paraffin section, collagen IV stain; **c** paraffin section, neurofilament immunohistochemistry; magnification, **a**–**c**, 1,000×)

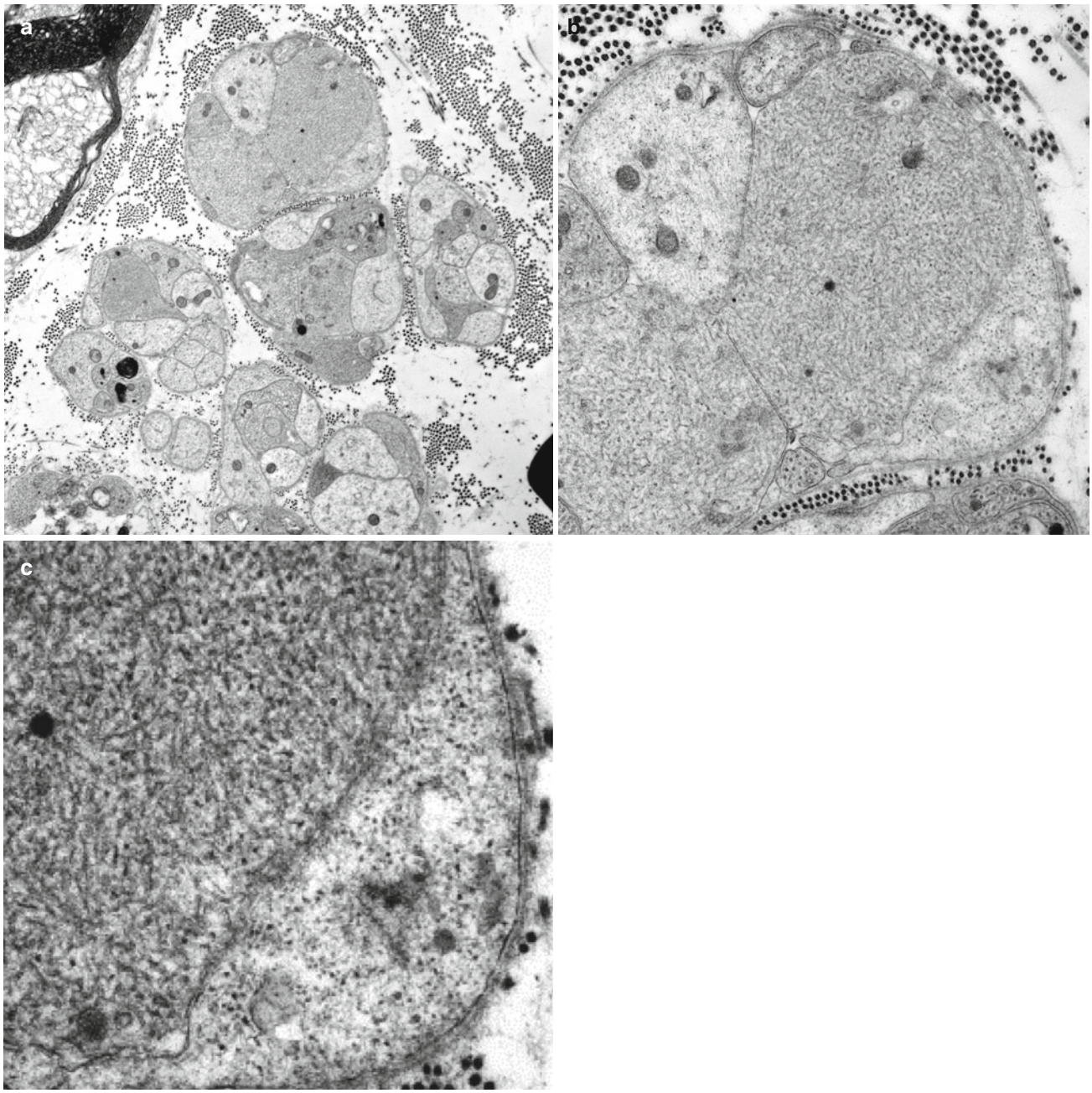

Fig. 4.21 Regenerating axons, ultrastructure. (**a**–**c**) Collections of regenerating axons, likely unmyelinated sprouts, which contain numerous tubulovesicular elements, seen best at higher magnification (**b**, **c**) (Magnification, **a** 10,000×; **b** 30,000×; **c** 60,000×)

Axonal regrowth occurs at rates of 3–5 mm day, decreasing with age (Black and Lasek 1979). This rate exceeds the SCa transport of neurofilaments but correlates closely with the rate at which actin and other peptide components of the microfilament network in the growth cone move distally via SCb (Wujek and Lasek 1983). Already visible by as few as 5 days after initial injury, the newly forming axonal sprouts contain microtubules and few neurofilaments. Schwann cells begin to surround the new axon sprouts. Subsequently, a gradual process of maturation, with enlargement of axonal diameter, appearance of normal cytoskeletal components, and early myelination, begins. Successful regeneration aligns motor axons with motor pathways as a result of guidance molecules of the L2/HNK-1 family, which are not found in dorsal roots and sensory cutaneous nerves (Martini et al. 1994) using a process of collateral branching into appropriate and inappropriate branches with subsequent pruning of inappropriate branches.

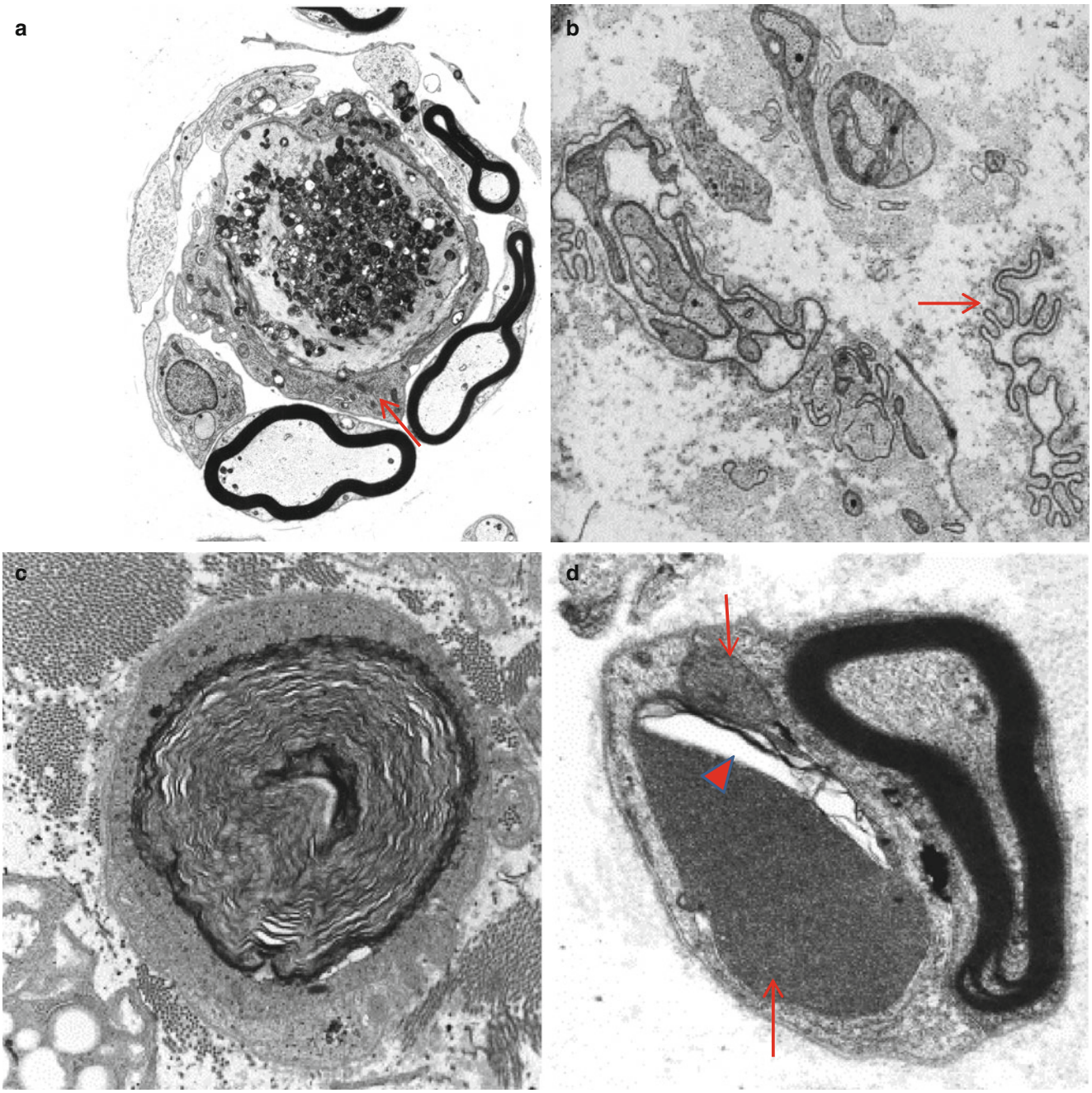

Fig. 4.22 Axonal regeneration. (**a**) Growth cone within a regenerating cluster. Note active Schwann cell cytoplasm surrounding the axonal swelling (*arrow*). (**b**) Very late features include denervated Schwann cell bands and "cast-off" basal laminae (*arrow*), some continuous and others fragmented. (**c**) Lipid debris in a Schwann cell in the absence of regenerative axons. (**d**) Three axons in a cluster, one regenerated and the other two (*arrows*) with dystrophic collection of compacted tubulovesicular elements and a cleft (*arrowhead*) (Magnification, **a** 7,200×; **b** 13,400×; **c** 15,000×; 20,000×)

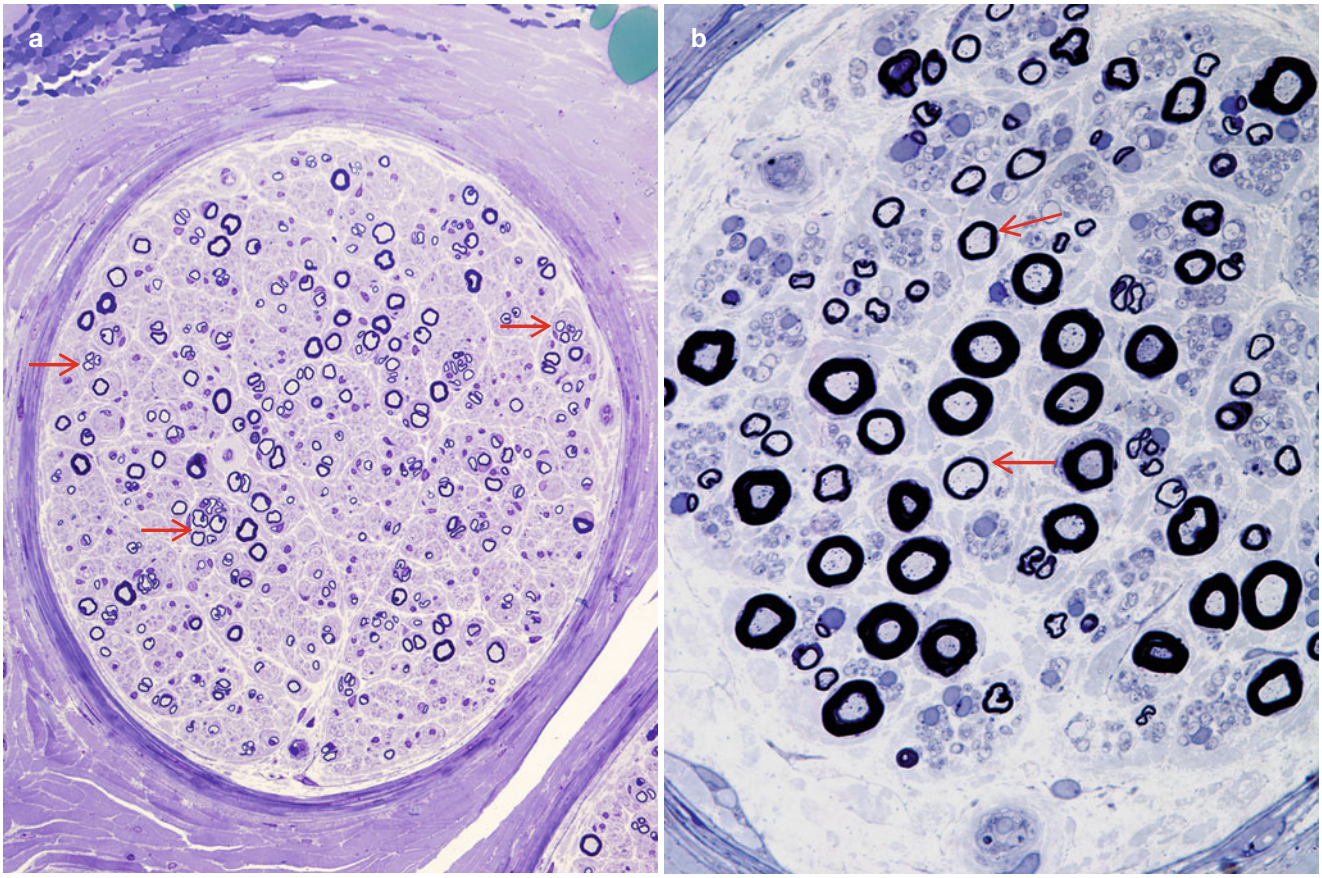

Fig. 4.23 Regenerating axons. (**a**) A fascicle with numerous regenerative clusters of axons (*arrows*). (**b**) End stage of normal regeneration resulting in axons with myelin which is too thin for axon caliber (*arrows*) (Magnification, **a**, **b**, 1 μ plastic sections, 1,000×)

4.5.2 Histological Hallmarks of Regeneration

Multiple axon sprouts often emerge from a single growth cone with several small myelinated axons occupying the space previously taken by a single larger myelinated axon. Such formations represent "regenerating clusters" and are operationally defined by the presence of three or more closely apposed myelinated axons (Fig. 4.23a, b). A single basal lamina belonging to the degenerated myelinated fiber may surround the regenerating cluster (Fig. 4.24a–c), but this lamina will eventually disintegrate and each of the myelinated axons will have its own basement membrane. Ultrastructural examination of a regenerating cluster often reveals the presence of nearby unmyelinated axons and their associated Schwann cells. Some of these are probably sprouts from the same growth cone which did not myelinate. The myelin sheath around regenerating axons appears inordinately thin at first (Fig. 4.23b), but returns to near-normal thickness with maturation of the fiber.

Internodal length normally measures 200–300 μm at birth. Many workers believe that the internode reaches adult values of up to 2,000 μm in length as a consequence of gradual elongation during somatic growth. In nerve regeneration, uniformly shortened internodes approximately 200–400 μm in length characterize the newly formed axons (Fullerton et al. 1965). As no somatic growth occurs, the internodes retain this length as perpetual evidence that the axon has degenerated and regenerated.

In some instances, there is failure of regeneration or atypical regeneration in which marked swelling of growth cones occurs (Fig. 4.22a). Lipid debris in Schwann cells (Fig. 4.22c) or Schwann cell processes with empty non-innervated basal laminae (Fig. 4.22b) occur in chronic neuropathies in which regeneration never occurs. A very unusual appearance of frustrated regeneration occurs in regenerative clusters in which distinctive dystrophic changes are found (Fig. 4.22d).

Normal nerve biopsies contain few (<20/mm^2) regenerating clusters and rare teased fibers with uniformly short

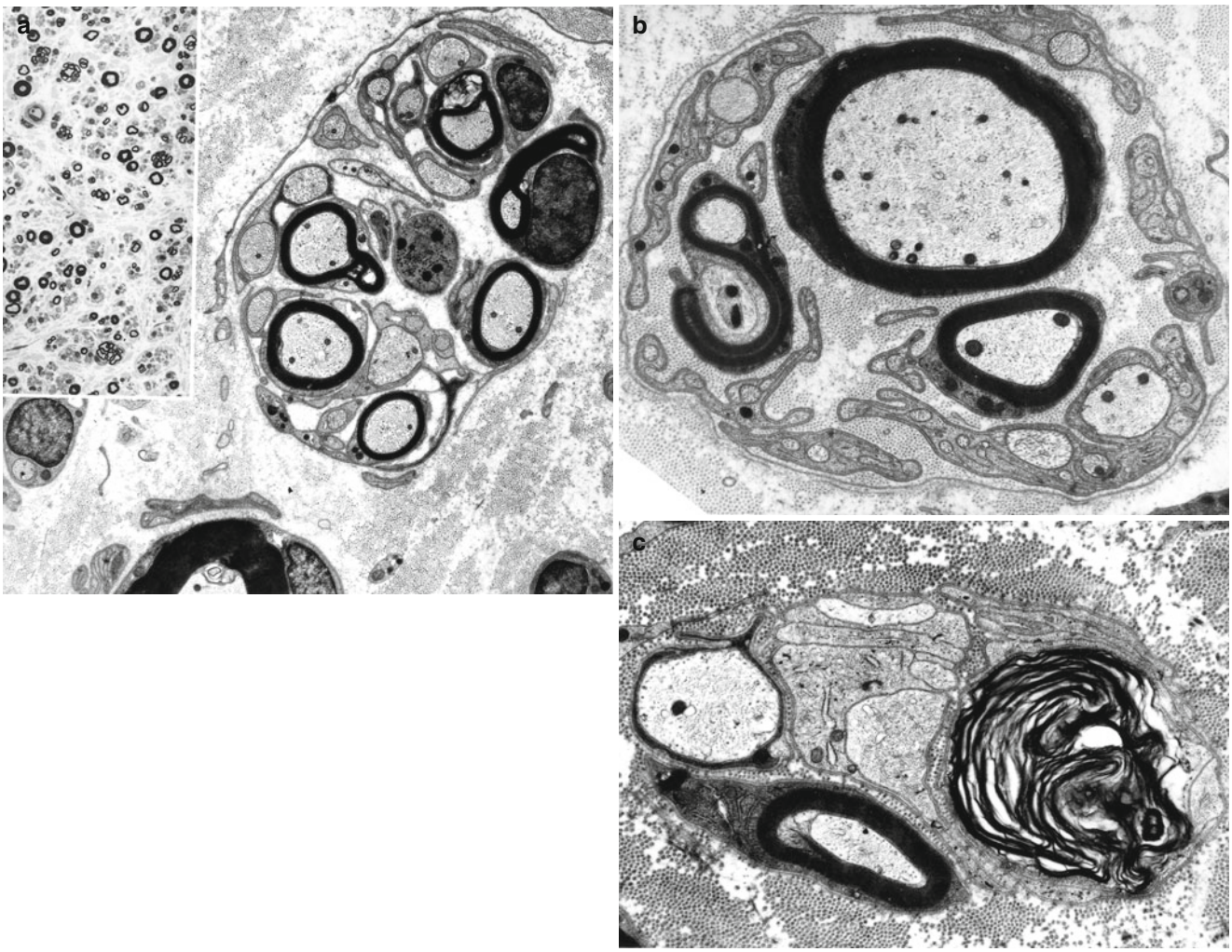

Fig. 4.24 Axonal regeneration. (**a**) Regenerating cluster consists of a group of myelinated and unmyelinated fibers of complementary shape; inset shows a corresponding plastic section. (**b**) Continuous basal lamina encircles a regenerating cluster containing three MFs and numerous UFs. (**c**) This complex consists of regenerating axons, one fully myelinated, a denervated Schwann cell band, and myelin debris surrounded by pockets of Schwann cell cytoplasm (Magnification, **a** 9,257×, inset plastic (1,000×); **b** 7,752×; **c** 14,523×)

internodes, indicating that some degree of axonal degeneration and regeneration is within normal limits. These changes increase with age, but the causes are unknown. Perhaps the etiology is chronic compression or age-related disturbances of axonal metabolism. In some neuropathies, clusters may become so numerous that the fiber diameter histogram demonstrates an absolute increase in small myelinated fibers. Regenerating clusters do not occur in neuronopathies, since regenerative efforts are impossible for a dying cell body.

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Schwann Cells and Myelin in the Peripheral Nervous System

5

5.1 Normal Structure and Function of Schwann Cells and Myelin

Schwann cells (SCs) are derived from the neural crest (Webster 1993) and encircle all peripheral nerve axons. Functionally, two populations of Schwann cells can be identified, those producing myelin and those associated with unmyelinated axons. Because this distinction is fundamental to the appearance and physiology of SCs, the two types will be discussed separately. Schwann cells associated with unmyelinated fibers have been called Remak cells, but we will refer to them as nonmyelinating Schwann cells (NMSCs) because whether an SC produces myelin or not depends not on some critical and irreversible decision made in the process of differentiation, but on signals (likely neuregulin; Birchmeier and Nave (2008)) originating from its axon and related to its caliber (Weinberg and Spencer 1976; Aguayo et al. 1976; Pereira et al. 2012; Quintes et al. 2010). Axonal neuregulin signals information about axon size to Schwann cells; hypomyelination and hypermyelination result from reduced and overexpression of neuregulin, respectively (Michailov et al. 2004). Rarely, an unmyelinated axon develops one or more short myelinated internodes along its course, a phenomenon described particularly in aged animals (Heath 1982). Schwann cell biology has been the subject of a series of reviews collected in a special issue of Glia (Woodhoo and Sommer 2008).

5.1.1 Myelinating Schwann Cells

A myelinated fiber consists of an axon and its longitudinally arrayed series of SCs contained within a continuous basement membrane tube (the "Schwann tube"). Defined as that segment of the nerve fiber myelinated by a single SC, an internode ranges from 200 to 2,000 μm in length (Scherer 1999). The node of Ranvier represents the region where the territories of two Schwann cells meet and is an approximately 1 μm long axon segment not covered by myelin. If viewed longitudinally, the diameter of the myelinated fiber is much the same throughout the internode, excepting some swelling at the region of the nucleus and terminally at the paranodal region (Thomas et al. 1993). The nucleus of a myelinating SC is elongated and about 50 μm long, and thus, on cross sections about 3 % of large myelinated fibers and 9 % of small myelinated fibers are cut through the SC nucleus (Ochoa and Mair 1969).

5.1.1.1 Internodal Structure of Myelinated Fibers

Cross-sectional profiles at the internode reveal a compact myelin sheath surrounded at its internal (adaxonal) and external (abaxonal) aspects by thin rims of SC cytoplasm (Fig. 5.1). Conceptually, if the spiral myelin sheet is unrolled, most of it is seen to consist of compact myelin, with inner and outer semi-compact belts which contain thin layers of Schwann cell cytoplasm. The Schwann cell nucleus, elongated parallel to the axis of the fiber and found at mid-internode, is associated with an expansion of the cytoplasm where organelles such as elongated mitochondria, endoplasmic reticulum, a Golgi apparatus, 10 nm thick filaments, microtubules, the occasional centriole, and a number of inclusions can be identified. Variable amounts of free cytoplasmic glycogen are present. The vast majority of SC organelles are concentrated in the perinuclear region. Plasmalemmal vesicles (caveolae) fuse with the outermost layer of Schwann cell membrane in regions of cytoplasmic expansion (Mugnaini et al. 1977).

The first turn of the myelin sheath overlaps itself at the inner mesaxon. A tight junction seals this site of overlap and leads into the first intraperiod line, formed by the apposition of two outer faces of Schwann cell plasma membrane. Separating the innermost aspect of the Schwann cell sheath and the axolemma is an extracellular gap with a constant width of 12–14 nm. The outer mesaxon is the site where the last turn of Schwann cell cytoplasm overlaps itself and has a tight junction just before opening up to the extracellular space. A basement membrane invariably encloses the outermost portion of Schwann cell cytoplasm. This extracellular

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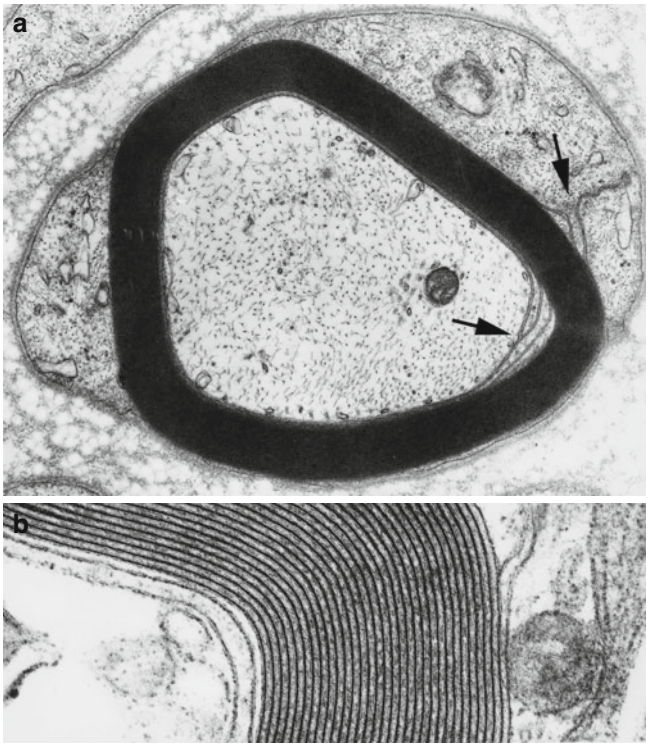

Fig. 5.1 Normal myelinated fiber. Note proximity of inner and outer mesaxons (*arrows*, **a**). High-magnification view allows resolution of the double layer of the intraperiod line (**b**) (**a** 32,200×; **b** 131,520×)

layer, composed of laminin, collagen types IV and V, heparan sulfate, and other macromolecules, forms a continuous "Schwann tube" around the nerve fiber, extending from cell body to nerve terminal (Bunge 1993). Cell culture studies have demonstrated that Schwann cells synthesize their own basement membrane (Bunge 1993).

5.1.1.2 Schmidt–Lanterman Clefts

The adaxonal and abaxonal semi-compacted and uncompacted Schwann cell cytoplasmic regions communicate through channels that run transversely across the spiraled compact myelin layers. These are the Schmidt–Lanterman (SL) clefts (Ghabriel and Allt 1981) (Figs. 5.2 and 5.3). Schmidt–Lanterman incisures are distinguished from compact myelin by the presence of Schwann cell cytoplasm (i.e., the major dense lines are not fused).

Because Schmidt–Lanterman clefts are fragile structures, a fully preserved cleft is rare in ordinary biopsy material. Typically, one sees a partial or full circumferential myelin "split" representing the disrupted SL cleft region (Fig. 7.1). When an intact SL cleft is seen in cross sections, it appears as partial or full circumferential array of stacked Schwann cell cytoplasmic pockets formed by focal separation of myelin at the major dense line (Fig. 5.2). On longitudinal cuts these pockets are revealed to be conically arranged and form an angle of about 9° to the long axis of the nerve fiber (Fig. 5.3a). The apex of the SL cleft "cone" does not point in a definite direction, and adjacent clefts may point in the same or opposite direction (Fig. 5.3a).

The linear density of SL clefts increases with the number of myelin lamellae present. In large myelinated fibers of human sural nerve, Buchthal et al. (1987) found about 35 clefts per mm, with each cleft having a length of about 13 μm. In small myelinated fibers of human sural nerve, these authors found about 8 clefts per mm of internode, each cleft measuring about 9 mm in length. Thus, with cross-sectional cuts one might expect to see clefts in about a 33–50 % of large myelinated fibers and 5–10 % of small myelinated fibers. Schmidt–Lanterman clefts can be seen in even the thinnest myelinated fibers (Hall and Williams 1970; Ghabriel and Allt 1981).

The cytoplasm within SL clefts often has a granular appearance and typically contains a single microtubule and less often membrane-bound organelles including mitochondria (Fig. 5.3). Incisure membranes are similar to paranodal loop membranes in that they lack compact myelin membrane proteins and contain myelin-associated glycoprotein (MAG). Immunohistochemical studies have shown preferential localization of connexin 32 to Schmidt–Lanterman incisures. The outermost few turns of the SL cleft may show a desmosome-like region of electron density. In addition to typical SL clefts, the myelin sheath contains some transverse cytoplasmic channels which terminate blindly and do not connect the inner and outer cytoplasmic belts. The myelin sheath also has longitudinal channels that run parallel to the axis of the nerve fiber (Mugnaini et al. 1977).

The function of SL clefts is unknown. They were previously considered by some to be artifactual, and indeed a distortion of the SL clefts is responsible for most myelin splits seen in cross sections (Fig. 7.1). However, in vivo studies conclusively prove their existence (Hall and Williams 1970). They may serve as a pathway of intracellular communication between the inner and outer Schwann cell cytoplasmic compartments. Some investigators have suggested that SL clefts give the myelin sheath a site into which newly synthesized cell membrane can be incorporated (Celio 1976) and may allow nerve fibers to tolerate bending and stretching better (Buchthal et al. 1987).

In Wallerian degeneration, early alterations occur at the SL clefts and the initial myelin ovoid consists of the myelin between two clefts (Williams and Hall 1971; Ghabriel and Allt 1979). In uncompacted myelin, a pathological alteration most often seen in paraproteinemic neuropathies with the POEMS syndrome, loss of myelin compaction, often begins at the Schmidt–Lanterman clefts; indeed it is sometimes difficult to distinguish between early loss of myelin compaction and normal variation in the form of the SL cleft (vide infra). Schroder and Himmelmann (1992) have studied pathological

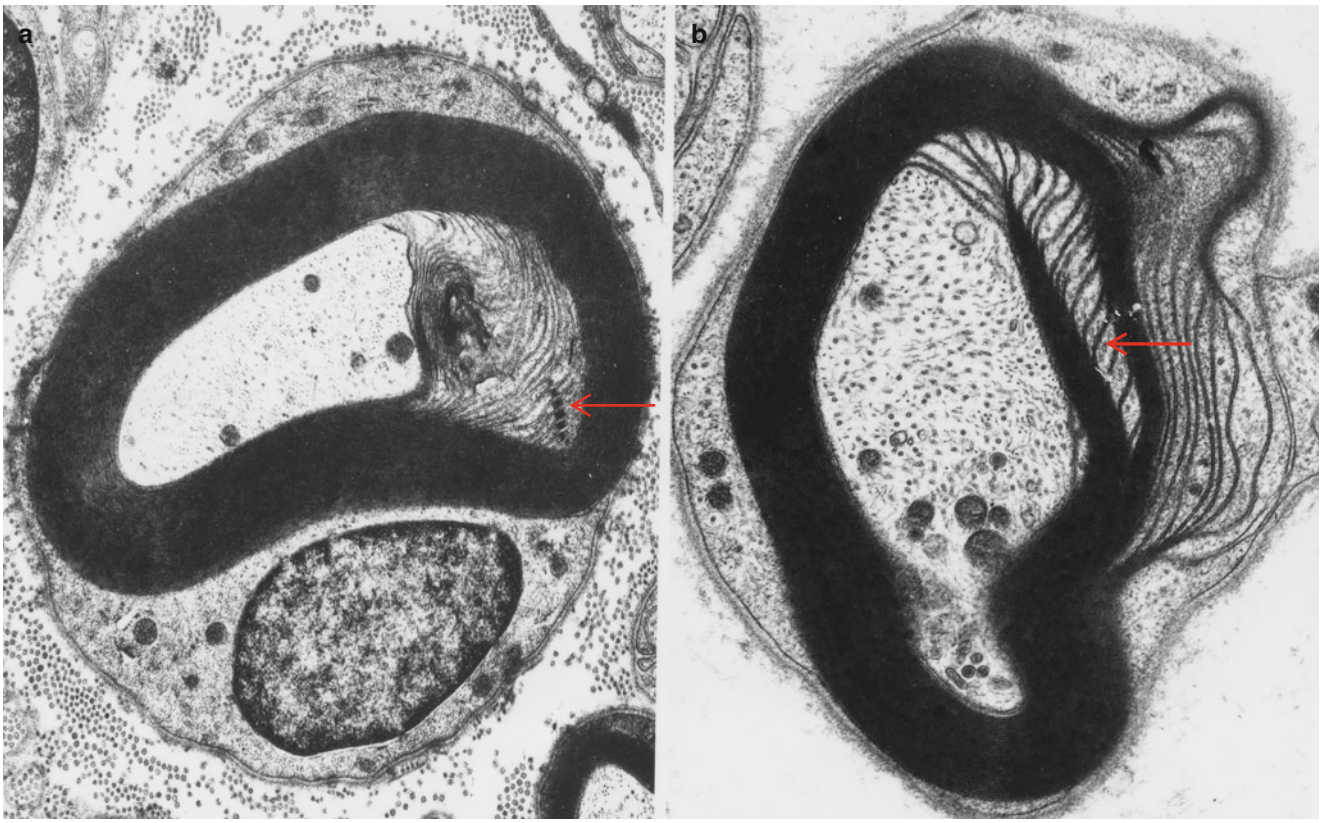

Fig. 5.2 Schmidt–Lanterman incisures. Note hemidesmosome-like areas of attachment in (**a**, *arrow*). Microtubules are present in the cytoplasm of the incisures (**b**, *arrow*) (**a** 13,307×; **b** 21,888×)

alterations at the SL cleft. Their work describes described granular degeneration, vesiculation, formation of membranous whorls, and accumulation of inclusions at the SL clefts as early but nonspecific findings in neuropathy, although more often seen in demyelinating processes.

5.1.1.3 The Nodal and Paranodal Structures

The paranodal segments are 5–7.5 μm in length at either side of the internode, depending on axon diameter (Berthold and Rydmark 1983). As the fiber approaches the node (Fig. 5.4a), it loses its rounded contour and develops grooves in both the myelin and the underlying axon, creating a crenated cross-sectional appearance (Fig. 5.4b). Within each crenation are cytoplasmic accumulations of membranous organelles; mitochondria are especially numerous (Figs. 5.3b and 5.4b). The appearance of the axon at this region is described in detail in Chap. 4. Further structural and molecular details regarding nodal and paranodal organization are provided in a variety of publications (Berthold and Rydmark 1983; Salzer et al. 2008; Scherer 1999; Scherer and Wrabetz 2008; Thomas et al. 1993; Buttermore et al. 2013; Kidd et al. 2013). The last 3–5 μm of the internode is the site of myelin loop termination. Seen longitudinally, the innermost myelin lamella terminates first, as the major dense line opens up to form a small "terminal cytoplasmic pocket." This pocket attaches to the axolemma by a gap junction-like complex (Fig. 5.3b). The periaxonal space at this point of attachment measures 3–5 nm, in contrast with the 12–14 nm seen in the internodal region. The juncture of myelin loops with the underlying axolemma is a complex arrangement involving contactin, CASPR, and an isoform of neurofascin, NF155 (see Kidd et al. for review). Subsequent lamellae behave similarly, but in thickly myelinated axons there is insufficient room for all the lamellae to attach to the axons, so 80–90 % of the terminal myelin loops are coiled on top of one another in sets of 10–25, creating a picture reminiscent of "ears of barley" (Thomas et al. 1993). Junctions attach terminal myelin loops to one another and to the axolemma where possible. Freeze fracture studies reveal a complex membrane particle structure, some of which represents ion channels essential for the electrical conducting function of the axon (Wiley and Ellisman 1980).

A space of about 1 μm separates the terminal myelin loops of one paranode from those of the one opposing it. Through the center of this space runs the axon (Fig. 5.3b). Slightly overlapping extensions of the Schwann cell of each internode roof over the nodal space. These extensions project numerous radially arrayed microvilli into the nodal gap, to

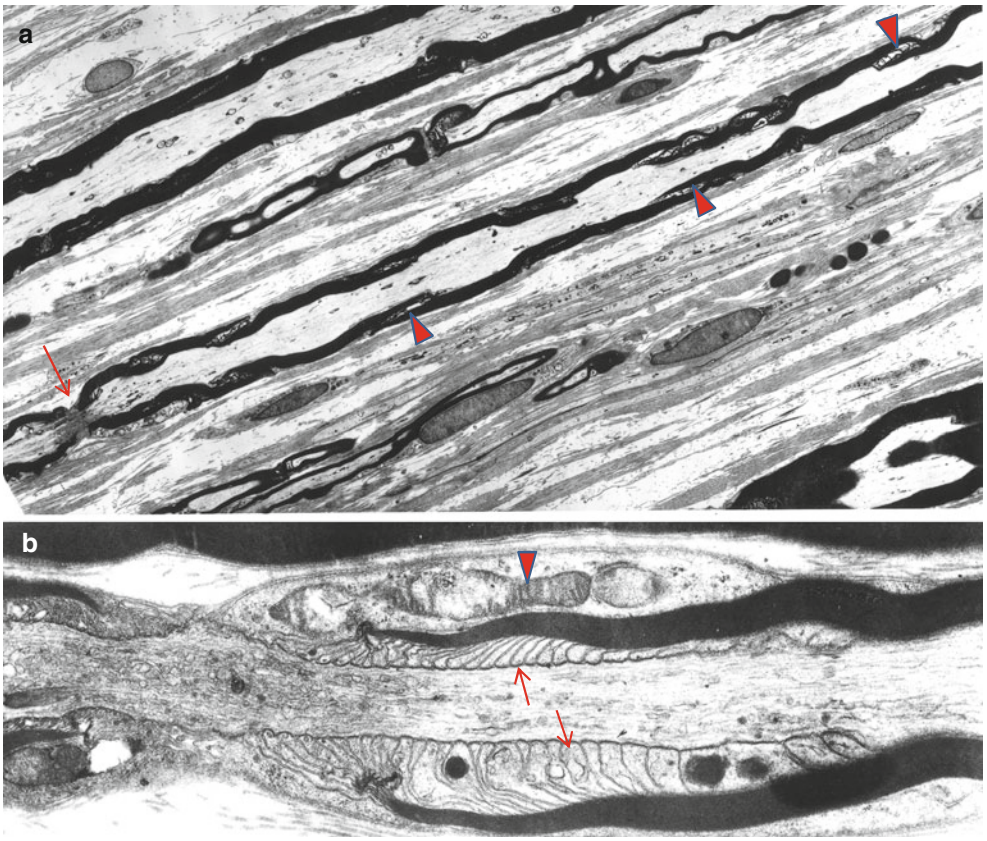

Fig. 5.3 Longitudinal view shows myelinated fibers and a node of Ranvier (*arrow*). Note that incisural cones do not have the same orientation (*arrowheads*). In (**b**) the paranodal area shows terminal cytoplasmic pockets (*arrows*) attaching to the axon and condensation of the nodal and paranodal axoplasm. The paranodal area displays a pocket of Schwann cell cytoplasm containing mitochondria (*arrowhead*) (**a** 1,450×; **b** 17,040×)

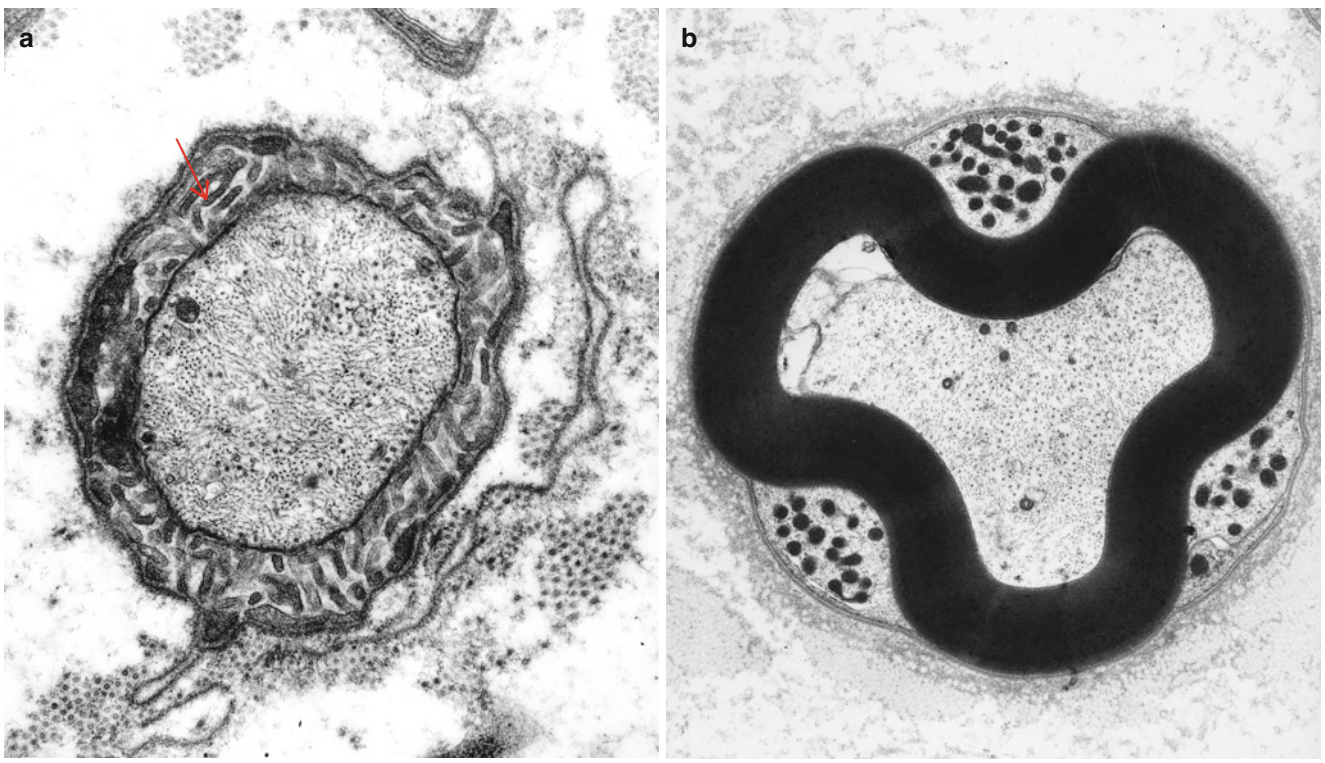

Fig. 5.4 Cross section through the node of Ranvier (**a**) and the paranodal area (**b**). In (**a**) Schwann cell microvilli (*arrow*) surround the axon. In (**b**) the crenated appearance of myelin conforms to three mitochondria containing pockets (**a** 16,302×; **b** 9,230×)

terminate within 5 nm of the axon (Fig. 5.4a). There is a gap substance around microvilli filling the space between the Schwann cell nodal processes and the basal lamina. This gap substance contains glycosaminoglycans with cation-binding and exchange properties maintaining an osmotically inactive concentration of sodium ions and limiting diffusion of K+ away from the node. The microvilli are enriched in actin filaments and actin-associated proteins. The Schwann tube (basal lamina) that surrounds all Schwann cells bridges over the nodal and paranodal structures and has equally complex constituents consisting of collagens, laminins, nidogen (entactin), fibronectin, and proteoglycans such as glypican and perlecan (Aszodi et al. 2006).

5.1.2 Myelin

Myelin is a multilamellated membranous substance produced through structural and biochemical modification of Schwann cell plasma membrane (reviewed in Webster 1993; Mezei 1993; Scherer 1999; Scherer and Wrabetz 2008; Svaren and Meijer 2008). Schematic drawings invariably illustrate mature myelin as a smooth cylindrical structure, but in reality serial sections reveal occasional infoldings and outpouchings, which in cross sections may appear as isolated "ovoids" or "loops" of myelin. Such alteration is more common in large myelinated axons, particularly near the node (Webster and Spiro 1960). The appearance can be misinterpreted as artifactual or as an indicator of pathology, as emphasized by Webster and Spiro (1960). Elzholz bodies, and myelin "wrinkling," are probably related to this myelin irregularity.

5.1.2.1 Determinants of Internode Parameters

The three principal parameters that govern conduction in a myelinated fiber are axonal diameter, myelin thickness, and internode length (Waxman 1980). Maximal internodal lengths are about 200–300 μm at birth and increase to adult values in close parallel with somatic growth (Gutrecht and Dyck 1970; Schlaepfer and Myers 1973; Friede et al. 1981). The actual site of a node of Ranvier is determined prior to the onset of myelination by specializations of the axolemma which presumably interact with the Schwann cell to limit its longitudinal growth (Wiley-Livingston and Ellisman 1980; Waxman 1980). Somatic elongation from onset of myelination to adult life is the main factor governing internode length (Friede et al. 1981). Larger-diameter axons have longer internodes, perhaps because these fibers myelinate earlier and are thus subject to a relatively greater degree of somatic growth (Thomas and Young 1949; Schlaepfer and Myers 1973). However, this explanation is incomplete, as the total complement of large and small myelinated fibers in human nerve is probably obtained very early in life (Jacobs 1988).

Myelin thickness is proportional to axon diameter, and in a first approximation, a single linear relationship provides a good degree of correlation across all fiber sizes (Behse 1990). However, small myelinated fibers are relatively more thickly myelinated than large myelinated fibers (Behse 1990; Friede and Bischhausen 1982). For axons of a given diameter, myelin thickness seems to increase slightly with internode length, a finding that is congruent with theoretical considerations (Friede and Bischhausen 1982; Friede and Beuche 1985).

5.1.2.2 The Structure of Compact Myelin

The periodicity of compact PNS myelin in vivo is 18 nm, although fixation usually reduces this to 15 nm, mostly through dehydration (Kirshner and Ganser 1984). The major dense line is about 6 nm thick and is made up of the fused cytoplasmic aspects of the Schwann cell membrane. In vivo the major dense line is actually composed of two membrane leaflets separated by about 2 nm of intracellular space. However, glutaraldehyde fixation and osmium tetroxide postfixation remove water and some membrane lipids, resulting in fusion of the two layers (Kirshner and Ganser 1984). The intraperiod line (IPL) is made up of two thinner lines, each representing the outer leaf of the Schwann cell plasma membrane, separated by about 4 nm (Fig. 5.1b). Thus, the region between the two leaves of the IPL is contiguous with the extracellular environment, and the space within the major dense line (in vivo) is contiguous with the Schwann cell cytoplasm.

Although the space within the IPL is contiguous with the extracellular matrix, junctional complexes at the outer and inner mesaxons and at the nodal area somewhat restrict flow of molecules and ions in this space (Chernousov et al. 2008). Because the cell membranes lining the IPL space have a negative charge (Inouye and Kirschner 1988), cations find their way into it more readily than anions or neutral molecules (Ropte et al. 1990). The presence of cations within this space is important for maintenance of the normal periodicity, as removal of positive charges results in widening of the space, presumably due to repulsion from unmasked opposing negative charges (Ropte et al. 1990).

5.1.2.3 Composition of Myelin

Myelin is a modification of the Schwann cell membrane. It contains 45 % water by weight, and the composition of dehydrated human myelin is 71 % lipid and 29 % protein (Inouye and Kirschner 1988; Norton and Cammer 1984). The important lipid components are phospholipids (55 % of total lipids), glycolipids (cerebrosides, sulfatides, and gangliosides representing 22 % of total lipid), and cholesterol (23 % of total lipid). Physiologically and clinically important proteins include P_0, P_1, and P_2 glycoproteins, peripheral myelin protein (PMP)-22, and myelin-associated glycoprotein (MAG).

Glycolipids occur only on the external layer of the Schwann cell membrane and thus are localized to the

intraperiod line (Inouye and Kirschner 1988). Immunization of experimental animals with galactosylceramide can produce experimental allergic neuritis (EAN), an animal model for Guillain–Barré syndrome (Stoll et al. 1986). Monoclonal and polyclonal antibodies to gangliosides have been identified as potential causative agents of human demyelinating and axonal neuropathies. Metachromatic leukodystrophy and Krabbe leukodystrophy are inherited diseases with defects in glycolipid metabolism which produce a demyelinating neuropathy, although the mechanism of the neuropathy is uncertain.

P_0 is a 30 kDa transmembrane glycoprotein which makes up about 60 % of all PNS myelin protein by weight, but is not present in CNS myelin or in nonmyelinating Schwann cells (Giese et al. 1992). It may function in maintaining the compaction of myelin at both the intraperiod and major dense lines (Lemke et al. 1988; Filbin and Tennekoon 1991). Immunization with P_0 protein can produce EAN (Milner et al. 1989), and antibody against P_0 protein has been associated with a severe childhood hypertrophic neuropathy (Ben Jelloun-Dellagi et al. 1992). Mice with defective P_0 protein expression show loss of most myelin compaction. However, some degree of myelin organization remains, indicating that proteins other than P_0 are important in this role (Giese et al. 1992). Mutations in P_0 protein have been implicated in the human familial neuropathies CMT-1B and Dejerine–Sottas syndrome (CMT-3).

P_1 protein (identical to CNS myelin basic protein) has been extensively studied in its role as the major neuritogenic antigen of experimental allergic encephalitis (Linington and Brostoff 1993). This is a glycoprotein with multiple isoforms, of which the 18 kDa variant is the most important. It accounts for a species variable 2–16 % of PNS protein. P_1 is found on the cytoplasmic aspect of the Schwann cell membrane, but its function in PNS myelin is largely unknown (Linington and Brostoff 1993; Peterson and Bray 1984).

P_2 is a 14.8 kDa glycoprotein found predominantly in the PNS where it makes up 2–15 % of myelin protein (Linington and Brostoff 1993). It is located in the cytoplasmic layer of Schwann cell membrane, particularly in regions where myelin is not compacted (Trapp et al. 1979). The physiologic function of P_2 protein is uncertain. P_2 glycoprotein is the major antigenic stimulus for production of EAN by immunization with peripheral nerve myelin (Rostami 1993).

Peripheral myelin protein-22 (PMP-22) is a more recently discovered 18 kDa integral membrane protein found primarily in PNS myelin (Suter et al. 1993). Although it is localized to compact myelin, its physiologic importance is unknown. Mutations in the PMP-22 gene in mice produce the Trembler phenotype. In humans, there is a strong association between defects involving the PMP-22 gene and CMT-1A hypertrophic neuropathy as well as hereditary neuropathy with liability to pressure palsies (HNPP).

Myelin-associated glycoprotein (MAG) is a 100 kDa transmembrane protein found in both the CNS and the PNS. This protein has an immunoglobulin-like domain on the outer face of the Schwann cell/oligodendrocyte membrane. Quantitatively, it makes up only 0.1 % of PNS myelin protein (Trapp 1990). In PNS myelin, MAG is found in the periaxonal space, paranodal regions, Schmidt–Lanterman clefts, and inner and outer mesaxonal membranes (Trapp 1990; Kidd et al. 2013). All membranes containing MAG appose other membranes by 12–14 nm (Trapp 1990). MAG is a member of the family of cell adhesion molecules and may play a role in maintaining the anatomical and physiologic integrity of the myelin sheath–axon unit. It may also be important in initiating the process of myelin formation and in controlling which parts of the Schwann cell cytoplasm become compacted (Leblanc and Poduslo 1990; Trapp 1990). IgM paraproteins with specificity against MAG have been strongly implicated in the causation of a common human demyelinating neuropathy.

5.1.3 Nonmyelinating Schwann cells

In humans sural nerve nuclear counts show that nonmyelinating Schwann cells (NMSCs) outnumber myelinating SCs by a factor of 4:1 (Ochoa and Mair 1969). Significant differences exist in the biology of nonmyelinating Schwann cells compared to myelinating Schwann cells (reviewed in detail by Griffin and Thompson 2008). In contrast to the strict 1:1 relationship between axons and Schwann cells seen in myelinated fibers, single or multiple NMSC profiles may be seen without associated axons, or a single NMSC may be seen to support one, two, or more axons. A Schwann cell subunit (ScSu) has been defined as any single Schwann cell profile or group of profiles that in cross section is enclosed by a continuous basal lamina (Sharma and Thomas 1975). Schwann cells surrounding a collection of unmyelinated axons have been designated as Remak fibers, a term which refers only to unmyelinated fibers. Remak Schwann cells have territories that extend longitudinally for 50–100 μm and ensheathe different axon modalities. Unmyelinated axons exchange frequently among different Remak bundles during their proximodistal courses (Kidd et al. 2013). Quantitative aspects of Schwann cell subunits are reviewed elsewhere. The discussion here will focus on ultrastructural features.

Schwann cell cytoplasm completely encircles most unmyelinated axons, although occasionally one may see a segment of axon that is in direct contact with the surrounding basement membrane. The formation of individual mesaxons for each unmyelinated axon is thought to be the result of secretion of neuregulin 1 type III (Taveggia et al. 2005). Nonmyelinating Schwann cell cytoplasm contains

the same complement of organelles as myelinating Schwann cells. The NMSC nucleus is elongated, 10–20 μm long, with only about 1 in 14 Schwann cell profiles showing the nucleus in cross section (Carlsen et al. 1974); the typical length of an NMSC has been estimated at 200–500 μm (Carlsen et al. 1974).

It may be difficult or impossible to distinguish a circular NMSC profile from an axon when the cross section contains no nucleus. Some criteria assist in making the distinction (Dyck 1969; Gibbels 1989). Axons are more likely to be round and tend to be less electron dense than Schwann cell processes. Neurofilaments and neurotubules are found in roughly equivalent numbers in unmyelinated axons, but tubules are far less prominent than filaments in Schwann cells. The formation of a mesaxon around a circular profile favors the possibility that the circular profile is an axon. The axolemma tends to have a greater electron density than the Schwann cell plasma membrane.

Occasionally, several Schwann cell processes may surround an axon. This occurrence usually relates to regions where the longitudinal extent of one Schwann cell ends and the next begins. These junctional points are not as clearly demarcated as the nodes of Ranvier in myelinated fibers. Studies by Eames and Gamble (1970) demonstrate how Schwann cell processes form variably complex regions of overlap and interdigitation at the junction point. Often the cytoplasm of the innermost Schwann cell at the site of overlap is particularly electron dense (Eames and Gamble 1970). Bundles of collagen may be partially or fully encircled by Schwann cell cytoplasm. These "collagen pockets" (Fig. 2.12) increase in prominence with age and in neuropathy. Denervated ScSus are identified by their lack of axons. This condition also occurs in normals, but increases with age or pathology.

5.1.3.1 Control of Myelination Phenotype

Myelination requires a balance of transcriptional programs (reviewed in Mirsky et al. 2008) with positive regulators of this process, including Krox-20, Sox-10, and Oct-6, and negative regulators (Notch, Sox-2, Pax-3, Id2, Krox-24, and Egr-3) which are expressed prior to myelination, downregulated as myelination starts, and reactivated as Schwann cells dedifferentiate following injury which is required for effective nerve regeneration.

5.1.4 Schwann Cell Inclusions

5.1.4.1 Reich Pi Granules

These inclusion bodies are typically found adjacent to the nucleus of myelinating Schwann cells (arrows, Fig. 5.5a, b). They can be seen by light microscopy and may number in the dozens within a perinuclear region (Fig. 5.6a, b). Reich identified their metachromasia with aniline dyes and noted that they had different staining properties than mast cell granules – a point of controversy at the time (Reich 1903). Pi granules are metachromatic with toluidine blue (Fig. 5.5a), Hirsch–Peiffer, and methylene blue stains, are refractile under polarized light (Fig. 5.5b), and will stain positively with Sudan black and PAS on frozen material (Noback 1953; Olsson and Sourander 1969). Their positive staining for acid phosphatase identifies them as lysosomes (Weller and Herzog 1970). Pi granules are best seen with the electron microscope, where they appear as single membrane-bound organelles, typically 1 μm in size, whose contents are often partially or totally removed in processing. However, when a structure can be discerned, there is usually a lamellar pattern with a periodicity less than that of myelin (5 nm vs. 12–17 nm) (Fig. 5.6b). At time they may take a "zebra body"-like appearance or contain globules of amorphous variably osmiophilic material. Their shape may be elongated, oval, or polygonal. In nerves undergoing active degeneration, we have seen pi granule-like inclusions within macrophages, perhaps representing the indigestible remains of Schwann cells. Thomas et al. (1993) have indicated that pi granules are not found in nonmyelinating Schwann cells. Although pi granule-like inclusions can rarely be seen in NMSCs, such configurations may represent denervated, formerly myelinating, Schwann cells.

Reich pi granules normally appear in myelinating Schwann cells, but are mostly restricted to the perinuclear area, so most fibers seen in cross sections do not show them. Rarely, they can be encountered in the paranodal Schwann cell cytoplasm. They are said to not be present in nerves of young people (<8 years of age, Robson 1951); Reich himself (1903) pointed out that the inclusions were not seen in the first years of life. Most authors have indicated, without formal quantitation, that pi granule numbers increase with age, suggesting that they are a "wear-and-tear"-related accumulation (Babel et al. 1970, p 48; Weller and Herzog 1970; Leibowitz et al. 1983; Asbury and Johnson 1978; Thomas et al. 1980; Robson 1951). It is noteworthy that they are not seen in recently remyelinated fibers (Weller and Herzog 1970).

Pi granules can be more prominent in neuropathy (Evans et al. 1965; Dyck and Lambert 1970; Shetty et al. 1988), but this is nonspecific. They may also be seen in increased numbers in some storage diseases (Goebel et al. 1976), but workers should exercise care not to confuse them with zebra bodies or other abnormal storage material (Olsson and Sourander 1969).

No major advances in the understanding of the nature of pi granules have taken place since Noback provided histochemical evidence for earlier suggestions that the granules were composed of sulfatides and/or phosphatides in the 1950s (Noback 1953, 1954).

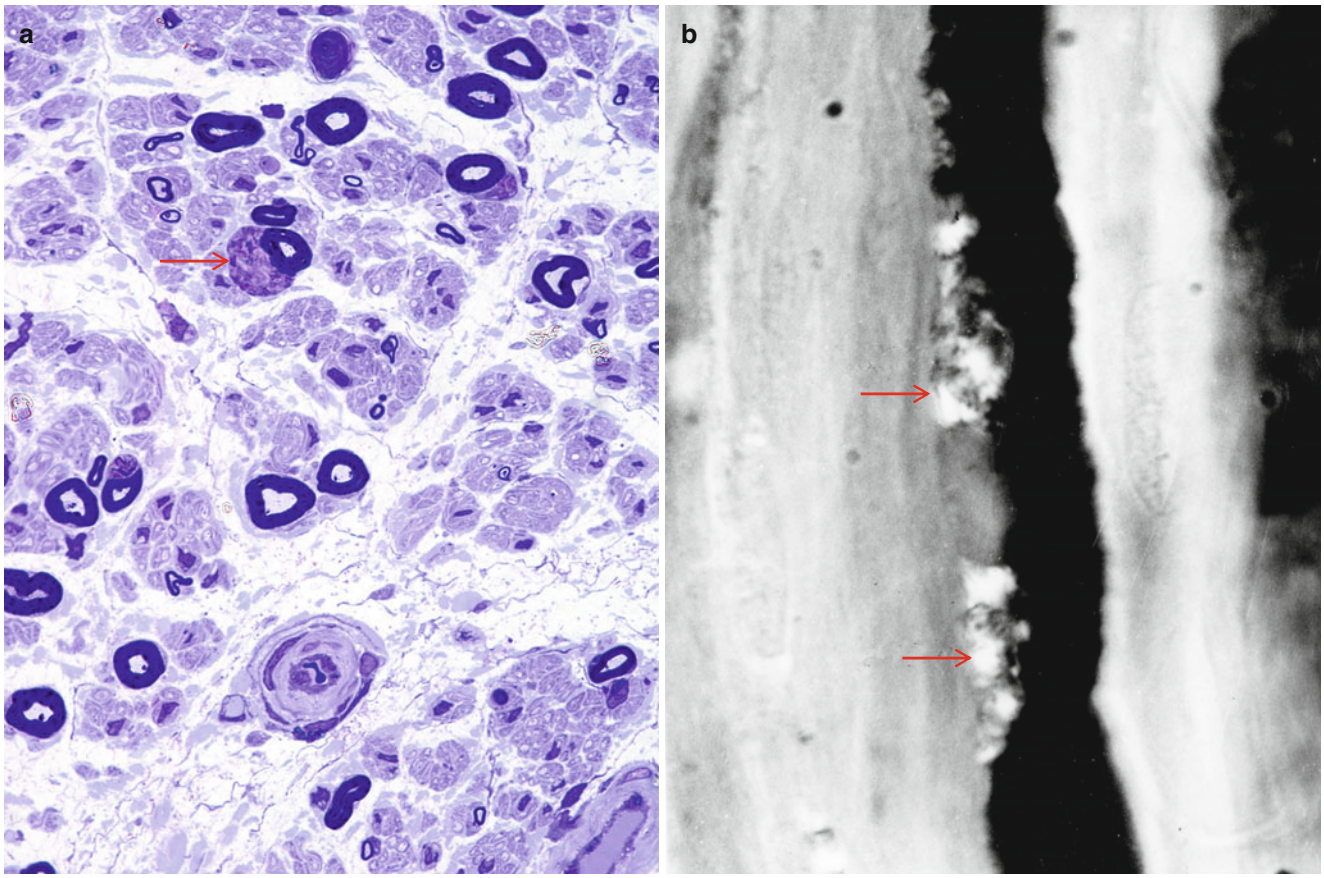

Fig. 5.5 Reich pi granules. These osmiophilic and metachromatic structures (*arrow*, **a**) are usually found in the perinuclear Schwann cell cytoplasm. In (**b**) a teased fiber preparation demonstrates refractile pi bodies (*arrows*) adjacent to the nucleus (**a** 1 μ plastic, 1,000×; **b** teased fiber photographed under half-crossed polarizing filters)

5.1.4.2 Elzholz Bodies (Mu Granules)

Elzholz bodies are rounded, osmiophilic, Marchi-positive bodies containing unsaturated lipid seen in the cytoplasm of myelinating Schwann cells, external to compact myelin, and most frequently in the perinuclear and paranodal regions (Schroder and Himmelmann 1992; Dyck et al. 1984a) (Fig. 5.7). Electron microscopy reveals a periodicity similar to that of myelin. No pathological significance has been attached to Elzholz bodies, and they may be seen in normal nerves. Most likely their formation is related to myelin remodeling and to the loops and redundant myelin folds that are seen in normal and abnormal myelinated fibers (Webster and Spiro 1960; Schroder and Himmelmann 1992).

5.1.4.3 Lipofuscin

Lipofuscin is an insoluble intracellular pigmentary accumulation seen in a variety of tissues throughout the body. Intracellular storage of lipofuscin results from the gradual accumulation of indigestible residues of autophagy and likely reflects the "wear and tear" of aging. In the PNS, the main site of accumulation of lipofuscin is in nonmyelinating Schwann cells, where the substance appears as autofluorescent single membrane-bound material, most of which is electron-dense amorphous or granular, intermingled with lightly or moderately electron-dense lipid droplets (Fig. 5.8). In one study the number of lipofuscin granules increased by a factor of 4 from age 17 to age 69 in unmyelinated fibers of human vagus nerves (Sharma and Thomas 1975). Although lipofuscin is normally not demonstrable in myelinating Schwann cells (Thomas 1993), such occurrence has been noted in Refsum disease (Thomas 1993), adrenoleukodystrophy, and Niemann–Pick disease. We have also examined a biopsy from a patient with Dejerine–Sottas syndrome in which myelinating Schwann cells contained abundant lipofuscin.

5.1.4.4 Other Inclusions

Some Schwann cell inclusions are of great value in the diagnosis of storage diseases and certain intoxications; these are discussed elsewhere (Table 7.9; Chap. 20). Other dense membrane-bound

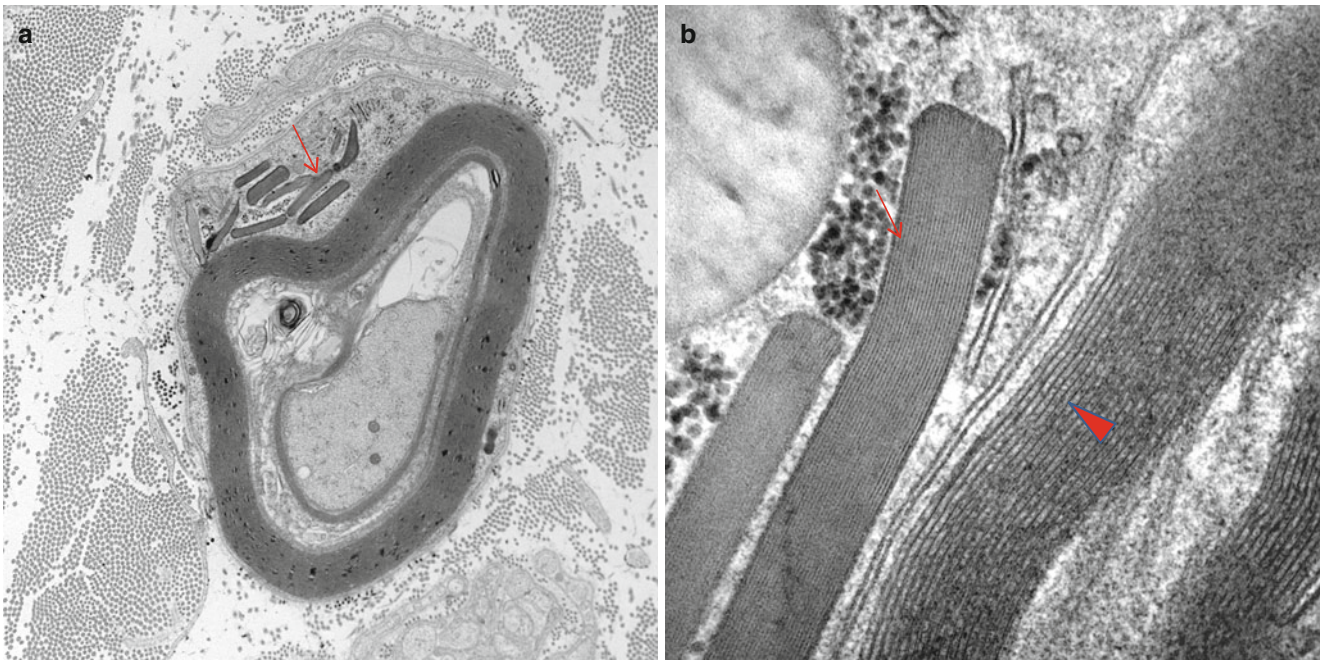

Fig. 5.6 Reich pi granules. Membrane-bound pleomorphic inclusions of variable osmiophilia are seen in the perinuclear region. Some rodlike granules (*arrow*, **a**) have a fine periodicity different from adjacent myelin (*arrowhead*, **b**) (**a** 12,000×; **b** 100,000×)

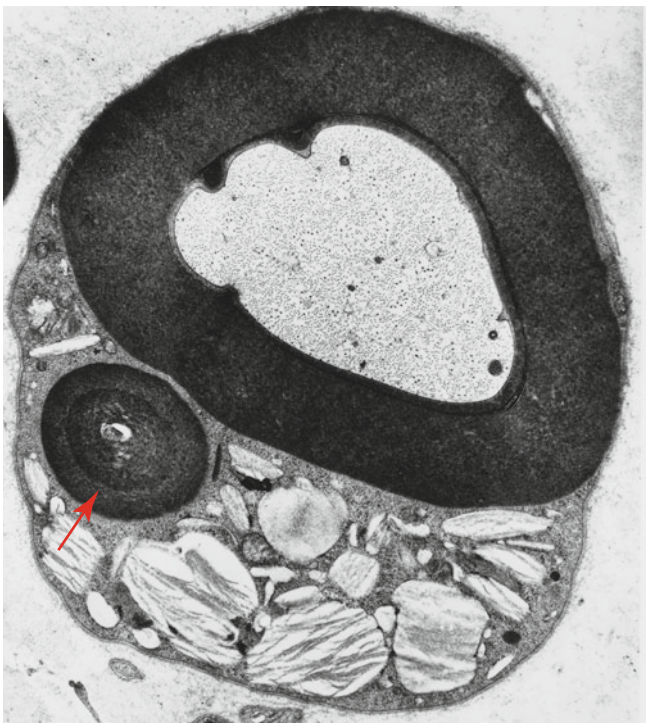

Fig. 5.7 Elzholz body. In contrast to pi bodies, the Elzholz body (*arrow*) is rounded and has the periodicity of myelin (11,400×)

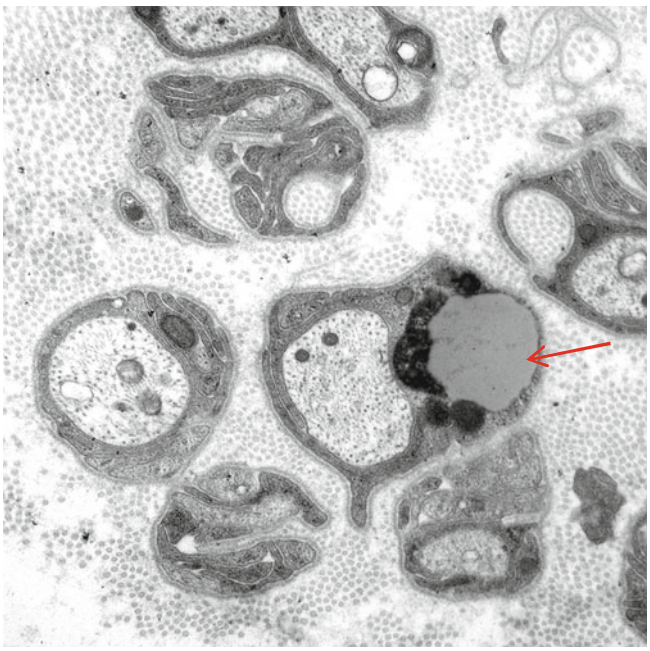

Fig. 5.8 Schwann cell inclusions. Lipofuscin (*arrow*) is ordinarily only found in nonmyelinating Schwann cells (magnification, **a** 20,235×; **b** 8,915×)

inclusions of uncertain significance may be seen in Schwann cells. Some of these probably would have been identified as "Elzholz bodies" by some workers. Osmiophilic inclusions seem to be more numerous in "sick" or stressed Schwann cells and probably represent autophagic vacuoles. Glycogen accumulation in the occasional myelinating and nonmyelinating Schwann cell can be striking, but is nonspecific. Increased Schwann cell glycogen may be particularly prominent in glycogenoses and hypothyroidism (Dyck and Lambert 1970).

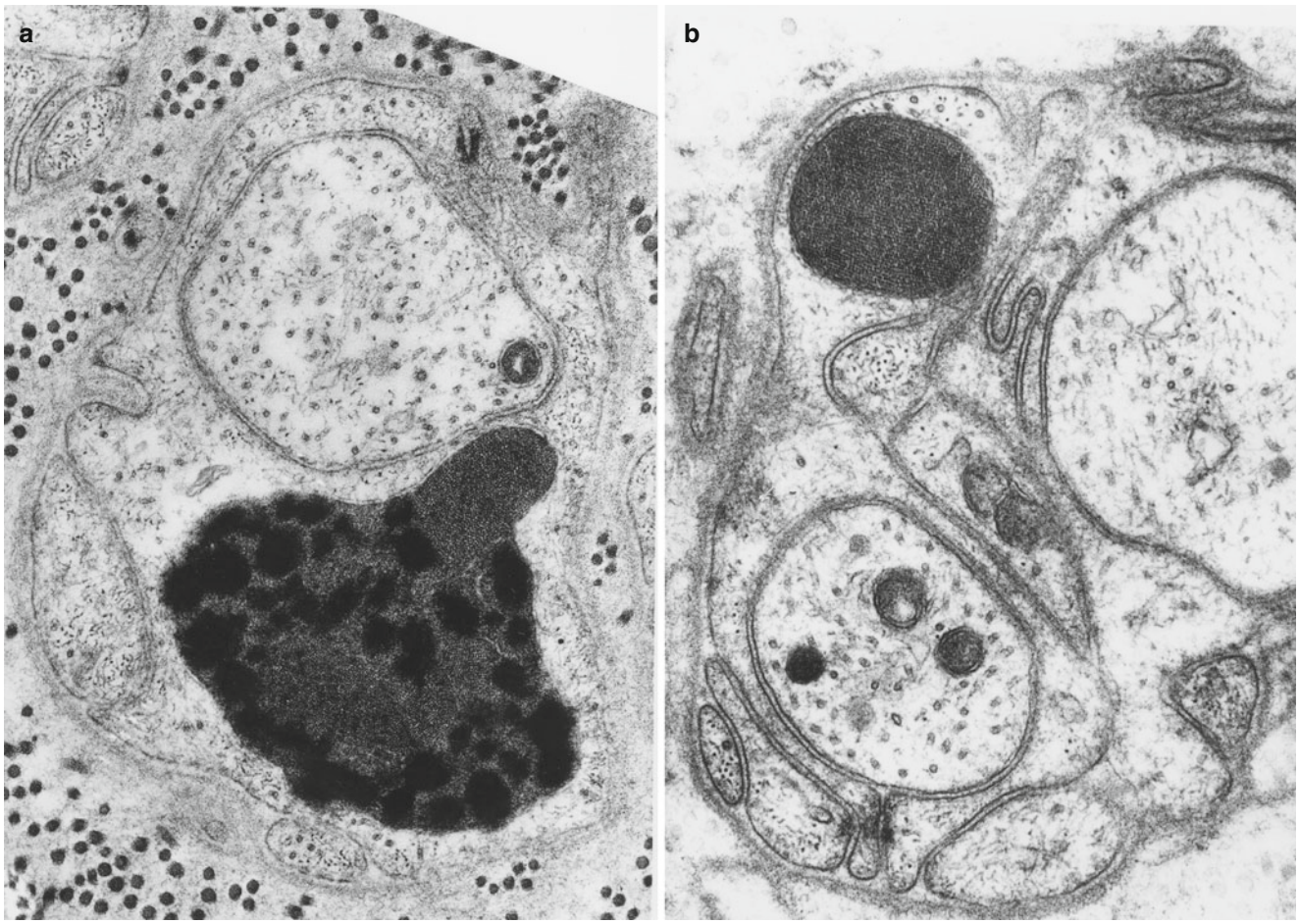

Fig. 5.9 Schwann cell inclusions. Paracrystalline inclusions are not uncommon in chronic neuropathies. These are probably of mitochondrial origin and may contain lipid (**a** 34,884×; **b** 35,700×)

One may observe double membrane-bound paracrystalline inclusions with small amorphous electron-dense regions in nonmyelinating Schwann cells (Fig. 5.9a, b). Such inclusions were initially emphasized in Refsum disease in which the authors hypothesized that they might be modified mitochondria (Fardeau and Engel 1969). Subsequently, workers identified similar inclusions in a host of toxic, metabolic, and genetically based neuropathies (Thomas and King 1974; Lyon and Evrard 1970; Vallat et al. 1973; Schroder and Sommer 1991). These inclusions have no diagnostic specificity and were found in 25 of 280 unselected peripheral neuropathies, in up to 10 % of NMSC mitochondria (Schroder and Sommer 1991). We have observed such inclusions in situations including CIDP, sarcoid neuropathy, disulfiram neuropathy, diabetic neuropathy, idiopathic axonal neuropathies, and a nerve showing only minimal changes attributable to aging. Similar inclusions have also been emphasized in mitochondrial diseases (Yiannikas et al. 1986; Schroder and Sommer 1991), may be very prominent in a variety of cell types in amiodarone neuropathy, and can be produced experimentally by treating rats with inhibitors of cholesterol synthesis (Hedley-White 1973; Suzuki and DePaul 1972).

5.2 Demyelination

When studying the pathology of a neuropathic disease, one must decide whether the process is fundamentally axonal or demyelinating, as this is of great diagnostic and prognostic importance. The distinction may not be obvious, as the Schwann cell and axon are highly interdependent; a diseased axon results in disturbance of Schwann cell metabolism and myelin structure, and myelin alterations affect axon morphology. Processes involving the nerve (toxins, trauma, ischemia, inflammation) seldom damage only myelin or only the axon.

Demyelination may occur as a consequence of Schwann cell or myelin defects, in which case it is primary demyelination. However, the typical changes of demyelination can also be seen in diseases known to primarily act upon the axon. This is termed secondary demyelination. Primary demyelination may be a consequence of Schwann cell dysmetabolism with loss of ability to maintain the myelin sheath or may result from direct attack on myelin itself. The processes leading to myelin damage are many, but often the appearance of the resultant demyelination is

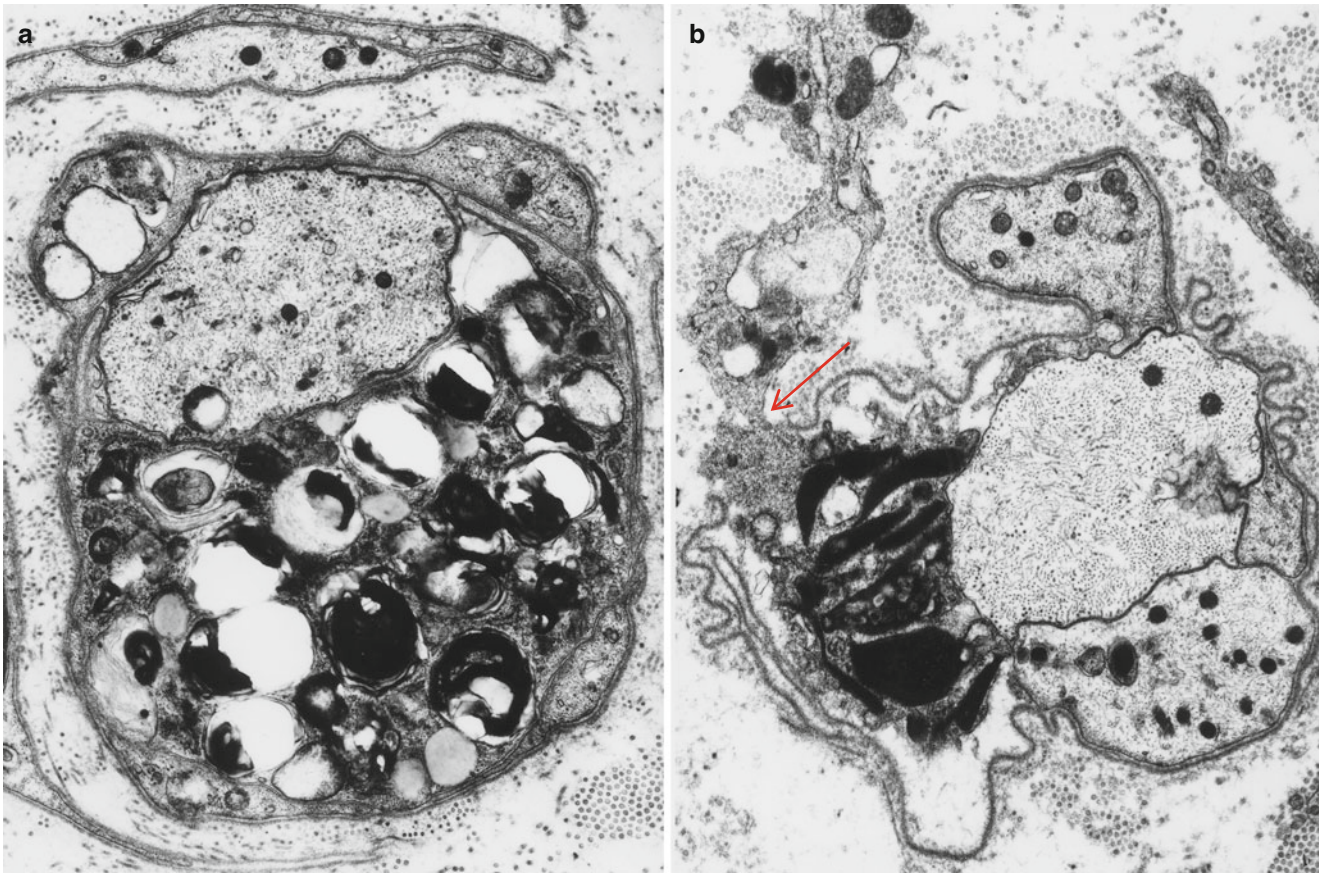

Fig. 5.10 Demyelination. In (**a**) only one of the intratubal cell processes contains debris and likely belongs to a macrophage. In (**b**) a macrophage process is captured exiting through a wide gap in the basal lamina (*arrow*) (**a** 13,760×; **b** 12,070×)

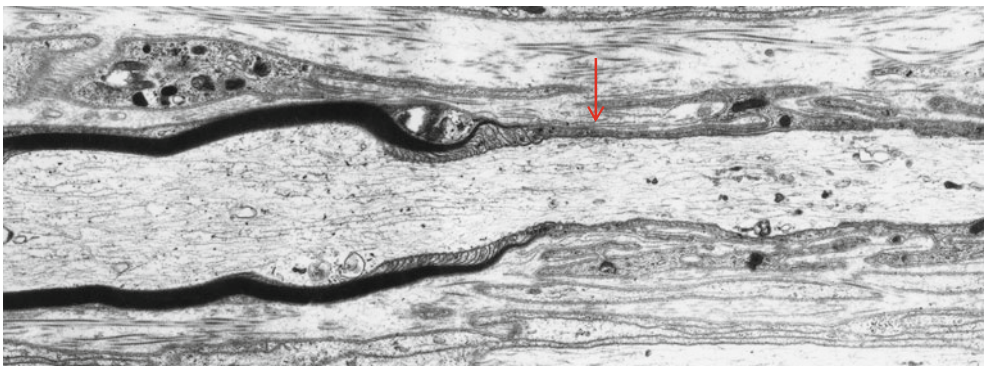

Fig. 5.11 Demyelination. Note interface (*arrow*) between remyelinated internode and a demyelinated axon (7,800×)

nonspecific. Below, we review both nonspecific and specific features of demyelination with emphasis on elements of differential diagnosis.

5.2.1 Nonspecific Features of Demyelination

A normal large diameter axon either surrounded by degenerating myelin or entirely denuded constitutes clear-cut light microscopic evidence of ongoing demyelination (Figs. 5.10a, b and 5.11). Myelin debris, consisting of osmiophilic material contained in cells around the axon or elsewhere in the endoneurium, indicates that the process is actively proceeding, as exogenous phagocytes clear debris within days to weeks. Although Schwann cells are capable of taking up myelin debris in nonphysiologic situations, they seem to leave this task to macrophages in vivo (Friede and Bruch 1993; Griffin et al. 1993).

Early myelin changes can include splitting or vacuolation of the compact myelin sheath, focal areas of vesicular

degeneration, and the appearance of myeloid bodies in Schwann cell cytoplasm. Alterations in terminal myelin loops at the node of Ranvier, small myelin "ovoids" in the paranodal regions, vesicular degeneration, and retraction of Schwann cell processes with resultant widening of the nodal gap are considered early changes in demyelination of all varieties (Asbury et al. 1969, 1978; Lampert and Schochet 1968; Masurovsky et al. 1967; Allt 1983), including that secondary to axonal disease (Dyck et al. 1981), and in axonal degeneration (Ballin and Thomas 1969a). Thus, paranodal myelin alterations should not be interpreted as evidence of a primary demyelinating process.

While a degenerating or absent myelin sheath around an intact formerly myelinated axon directly indicates demyelination, a thinly myelinated axon is a sign of remyelination. The hallmark of recurrent demyelination and remyelination is onion-bulb formation, consisting of a central axon (myelinated or otherwise) surrounded by concentric attenuated Schwann cell lamellae. Onion bulbs are described and illustrated elsewhere, and remyelination is considered below.

Buckling and splitting of myelin, inpouching and outpouching of myelin folds, and focal separation of the axon from its myelin sheath are commonly observed in nerve biopsies. Such changes can be seen in otherwise normal nerves but are clearly more prominent in neuropathy and correspond to myelin "wrinkling" seen on teased fibers (Dyck et al. 1971, 1984b; Webster and Spiro 1960). This alteration increases in frequency with the normal aging process (Dyck et al. 1993, Table 30.4) and is particularly common with myelin remodeling in response to axonal atrophy (Dyck et al. 1984b). Mechanical or chemical trauma during the biopsy procedure might also produce some of these alterations.

5.2.2 Specific Myelin Changes in Primary Demyelinating Neuropathies

5.2.2.1 Macrophage-Mediated Myelin Stripping

Destructive stripping of myelin by macrophages is the central mechanism of demyelination in the inflammatory demyelinating neuropathies, GBS and CIDP, and is generally regarded as specific to these entities (Prineas 1981; Brechenmacher et al. 1987). The macrophage penetrates the Schwann cell basement membrane and the cell membrane, separates Schwann cell cytoplasm from its myelin, and insinuates cytoplasmic fingers into the intraperiod lines of the myelin sheath. Most often this occurs in idiopathic CIDP and GBS; however, macrophage-mediated demyelination is also seen when these clinical syndromes occur in the setting of other illnesses, such as HIV infection (Cornblath et al. 1987), a circulating IgG (Pollard et al. 1983; Bleasel et al. 1993) or IgM (Vital et al. 1991) paraprotein, or lymphoma (Sumi et al. 1983). Macrophage-mediated demyelination is seen in EAN, the animal model of GBS (Wisniewski et al. 1969), and several other veterinary diseases (Marek's disease, coonhound paralysis). It also appears in recurrent lysophosphatidylcholine-induced demyelination, probably as a consequence of "self-immunization" against myelin following repeated episodes of iatrogenic demyelination (Hall 1984). Macrophage-mediated myelin stripping has rarely been described in familial hypertrophic neuropathy (Vital et al. 1992; Madrid et al. 1977) or uremia (Said et al. 1983), but we have never observed this and find the illustrative figures to be unconvincing, perhaps simply showing scavenger macrophages which have entered the Schwann tube to clear out degenerating myelin. An alternative interpretation is that these cases represent instances of inflammatory demyelinating neuropathy superimposed on another neuropathy.

Macrophage-mediated demyelination is likely the final common pathway of a variety of dysimmune phenomena. Complement and immunoglobulin deposits have been found on the Schwann cell membrane in a variety of inflammatory neuropathies (Hays et al. 1988). In theory, these substances can opsonize myelin and promote phagocytosis via the immunoglobulin and complement receptors normally found on macrophages (Stoll et al. 1991). Indeed, intact myelin seemed to be taken into coated pits in a study of inflammatory demyelination in the CNS (Epstein et al. 1983), and this process may be initiated by IgG binding to the myelin sheath (Wayne Moore and Raine 1988). However, the finding of myelin-binding antibodies or complement has been inconsistent in both CIDP and GBS (see discussion Chap. 9), and these deposits may be an epiphenomenon relating to the alteration in vascular permeability often seen early in the course of inflammatory neuropathy.

Investigators generally regard the insertion of cell processes and removal of myelin debris as discussed above and in more detail elsewhere as unique to macrophages. However, Schwann cells of unmyelinated fibers displayed identical behavior when serum from GBS patients was introduced into a neural cell culture line in a macrophage-free medium (Birchem et al. 1987). There are also reports of malignant lymphocytes in CLL apparently carrying out myelin stripping (Vital et al. 1975; Sumi et al. 1983).

5.2.2.2 Vesicular Demyelination

Myelin can sometimes disintegrate around an apparently intact axon through splitting at the major dense line, creating a netlike pattern of vesicles 80 nm in diameter (see Fig. 7.4a–c) (Arnason and Soliven 1993; Brechenmacher et al. 1987). In a different plane of section, these are seen as parallel lines, suggesting that in three dimensions the structure is composed of parallel cylinders (Carpenter 1972). Detached pieces of vesicular myelin are often seen as debris within

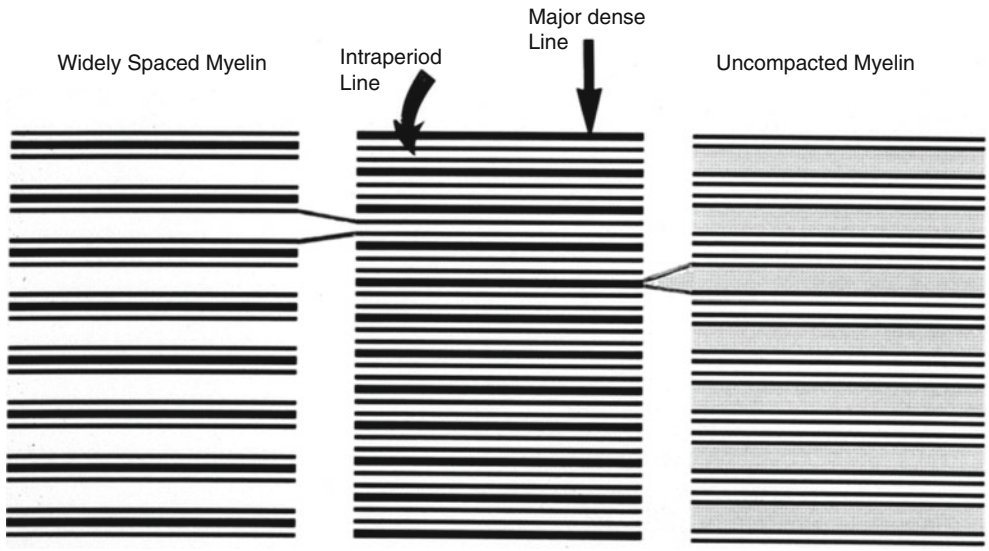

Fig. 5.12 Alterations in myelin periodicity. Widely spaced myelin (*left*) is formed by separation at the intraperiod line (*curved arrow*), while uncompacted myelin (*right*) results from separation at the major dense line (*arrow*)

macrophages. Some have noted a close correlation of this change with the postmortem state or have found similar alterations in control autopsy nerves. These workers consequently dismiss this change as an artifact of delayed fixation (Honavar et al. 1991). Indeed, in GBS there is a tendency for this finding to be reported on autopsy studies. However, vesicular demyelination has also been observed in nerve biopsy specimens (Brechenmacher et al. 1987; Vital et al. 1985; Prineas 1972; Sumi et al. 1983). Although our experience confirms that this alteration is most often an artifact of delayed fixation or accompanying crush artifact, we have observed a number of examples in well-preserved nerve biopsy material, usually in diabetic neuropathy. Injecting sera from GBS patients into nerve culture or animal peripheral nerve produces vesicular demyelination (Hirano et al. 1971; Birchem et al. 1987; Saida et al. 1982; Brown et al. 1987). In some experimental allergic neuritis (EAN) models, vesicular demyelination takes place in close association with macrophage invasion (Dal Canto et al. 1975) or prior to the appearance of any inflammatory cells (Rosen et al. 1990). The latter authors offered the hypothesis that vesicular myelin degeneration is produced by humoral factors, most likely antibodies to myelin antigens, whereas cell-mediated immunity was involved in macrophage-mediated myelin stripping (Rosen et al. 1990). Vesicular demyelination has also been observed in experimental situations where immune attack does not operate, such as lead (Lampert and Schochet 1968) or tellurium (Lampert and Garrett 1971) toxicity and radiation injury (Masurovsky et al. 1967). Incubating peripheral nerve with calcium and a calcium ionophore results in the rapid occurrence of severe diffuse vesicular demyelination without axonal damage (Smith and Hall 1988), suggesting that vesicular myelin disintegration is a nonspecific consequence of any process that causes calcium leakage into the Schwann cell, with subsequent activation of endogenous phospholipase A_2 (Smith and Hall 1988).

5.2.2.3 Abnormal Periodicity of Myelin

As discussed above, normal myelin periodicity is 18 nm in the fresh state and 12–17 nm in fixed nerves. Some peripheral nerve diseases and experimentally induced nerve lesions demonstrate regularly increase myelin periodicity. King and Thomas (1984) emphasized the importance of distinguishing between uncompacted myelin (UCM) and widely spaced myelin (WSM) (Fig. 5.12). Both these alterations are rare, but when detected have important diagnostic implications.

Uncompacted Myelin

In uncompacted myelin (UCM), the cytoplasmic aspects of the Schwann cell membrane fail to appose, leaving considerable quantities of cytoplasm between the two halves of the major dense line (Fig. 5.13a, b). Often the outer faces of the Schwann cell membrane also fail to appose, and the intraperiod line fails to form. This pathological alteration occurs in several human neuropathies and experimental models (Table 5.1), most immune related. Membranous whorled bodies can be seen within the cytoplasm at the site of uncompaction. The intracytoplasmic uncompacted space is contiguous with the mesaxon, which may also be enlarged (Vital et al. 1983). Loss of compaction can involve part or all of the myelin sheath thickness. Because uncompacted myelin often shows an intimate relation with Schmidt–Lanterman clefts, apparent changes must be interpreted with caution. Some of the pictures used to illustrate "uncompacted myelin" seem to us to represent minimally

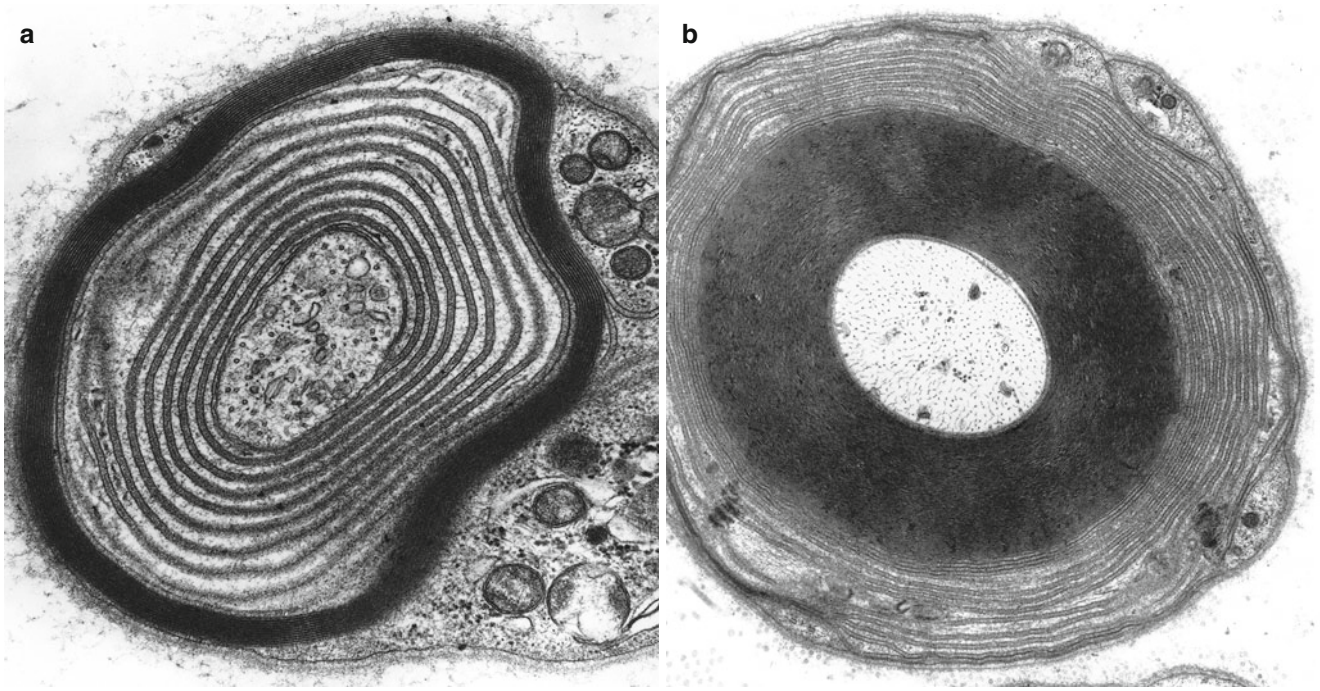

Fig. 5.13 Uncompacted myelin. The innermost (**a**) and outermost (**b**) layers of Schwann cell cytoplasm are uncompacted – POEMS (**a** 12,000×; **b** 10,000×)

Table 5.1 Neuropathies with uncompacted myelin

Human disease	
POEMS syndrome	Vital et al. (1994)
IgG or IgA paraprotein-associated neuropathy not meeting criteria for POEMS	Vital et al. (1983)
Hereditary neuropathy with liability to pressure palsies	Yoshikawa and Dyck (1991)
GBS	Brechenmacher et al. (1987)
CIDP	Vital et al. (1990), Fig. 18
Acute demyelinating neuritis with portosystemic shunt	Vital et al. (1978)
Paraneoplastic	Lamarche and Vital (1987)
Congenital dysmyelinating neuropathy	Asbury and Johnson (1978), p. 135; Lyon (1969)
Vincristine neuropathy	Vital and Vallat (1987), Fig. 89
Experimental data	
Experimental allergic neuritis	King and Thomas (1984)
Irradiation	Masurovsky et al. (1967)
Trembler mouse	Ayers and Anderson (1975)

altered Schmidt–Lanterman clefts of little significance (reference Vital et al. 1994 Fig. 4 vs. our material).

Most reports of uncompacted myelin in humans have occurred in the setting of the POEMS syndrome, where UCM was said to occur in 1–16 % of internodal cross sections (Vital et al. 1994). Ohnishi and Hirano (1981), who first described this alteration, noted that UCM often appeared to arise from abnormal terminal myelin loops, with an appearance similar to that illustrated by Allt (1969) while studying remyelination. Indeed, some authors have suggested that UCM results from disruption of remyelination, not as a primary change of compact myelin (Vital et al. 1983). Immunoglobulin deposits have not been detected on the involved membrane (Vital et al. 1994). Yoshikawa and Dyck (1991) studied uncompacted myelin in hereditary neuropathy with liability to pressure palsies (HNPP) and suggested an abnormality of a myelin protein involved in lack of compaction. P_0 protein, thought to be important for compaction of both the major dense line and the intraperiod line, was considered a good candidate, and experiments involving disruption of the myelin P_0 protein have resulted in production of some uncompacted myelin (Giese et al. 1992). The Trembler mouse, in which uncompacted myelin is a prominent ultrastructural feature (Ayers and Anderson 1975; Low 1976), has a point mutation in the PMP-22 gene (Suter et al. 1992). This mutation seems worthy of note given that deletion of a part of chromosome 17 which

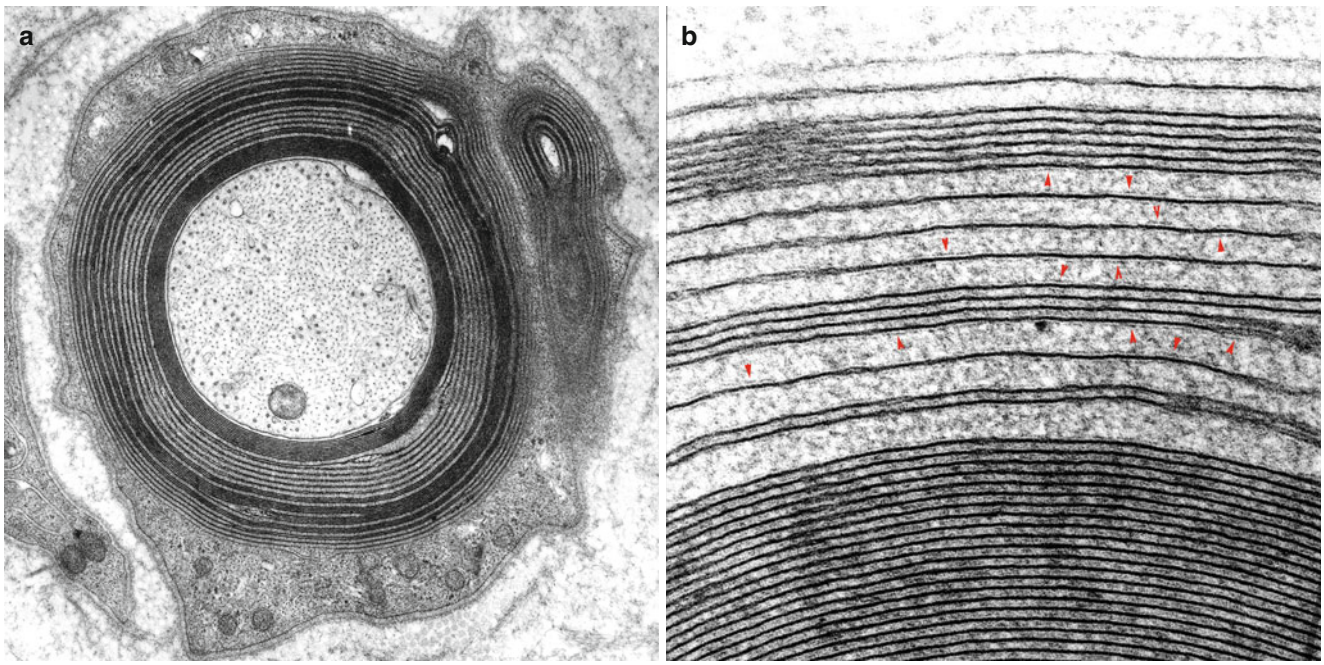

Fig. 5.14 Widely spaced myelin. (**a**) Typical appearance in a patient with circulating IgM paraprotein and demyelinating neuropathy. (**b**) WSM is caused by separation of the intraperiod line (*arrowheads*) (**a** 25,000×; **b** 137,000×)

Table 5.2 Neuropathies with widely spaced myelin

Human disease	
Paraproteinemic neuropathy, IgM≫IgG or IgA	
CIDP	King and Thomas (1984), Vital et al. (1986)
GBS	Vallat et al. (1994)
Experimental material	
Soaking in hypotonic solution prior to fixation	King and Thomas (1984)
Experimental allergic neuritis	King and Thomas (1984), Lampert et al. (1977)
Injection of GBS or EAN serum into peripheral nerve or nerve culture	Hirano et al. (1971), Raine and Bornstein (1979)
Irradiation	Masurovsky et al. (1967)
Silver nitrate-induced cerebral edema	Hirano et al. (1965)

includes the PMP-22 gene that occurs in HNPP results in a human disease where uncompacted myelin may be seen (Yoshikawa and Dyck 1991).

Widely Spaced Myelin

Widely spaced myelin (WSM) results from the separation of the intraperiod line (IPL), which normally comprises two thin lines 2–4 nm apart; in WSM this distance increases to 20–30 nm, causing the normal major dense lines to be separated by a space contiguous with the extracellular compartment (Fig. 5.14a, b). The striking regularity of this increased myelin periodicity distinguishes it from artifacts and from nonspecific degenerative changes. Widely spaced myelin is most often seen in the outermost myelin lamellae. Alternatively, WSM may remain confined to inner layers or occur throughout the entire thickness of the sheath. The abnormality may begin at the external mesaxon and continue into the myelin lamellae, and the external mesaxon itself may be dilated or misshapen. A moderately electron-dense granular material is often present in the widened extracellular space. Anywhere from a few fibers to every visible myelinated axon may show WSM in a biopsy specimen, and small myelinated axons often seem prominently involved (Pollard et al. 1985). An increased separation can also be seen between layers of the Schmidt–Lanterman cleft, between adjacent terminal myelin loops at the paranodal area, and between the Schwann cell and its basement membrane, all areas corresponding to the extracellular space (Jacobs and Scadding 1990). The normal adaxonal gap, normally 13 nm with narrowing to 4 nm at junctional sites, may be increased in width with apparent disruption of the junctions (King and Thomas 1984).

The vast majority of cases displaying WSM in humans have been associated with a circulating paraprotein, usually (but not invariably) with activity against myelin-associated

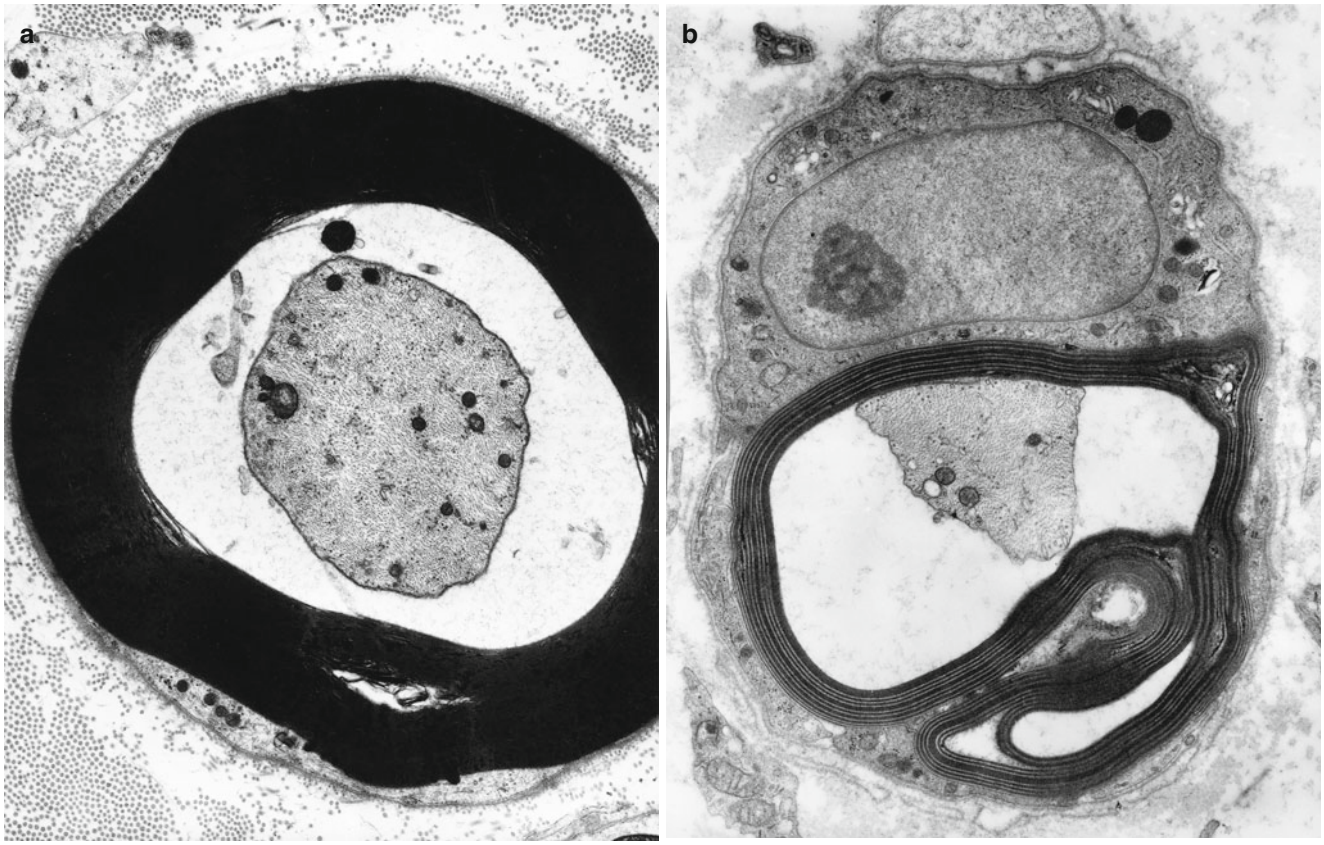

Fig. 5.15 Intramyelinic edema is seen in uremic neuropathy (**a**) and IgM paraprotein (**b**) (**a** 8,000×; **b** 12,780×)

glycoprotein (MAG) (Table 5.2). Indeed the association with IgM paraprotein is so strong that one wonders if the rare cases of WSM occurring outside this setting were also due to an occult IgM paraprotein or polyclonal immunoglobulin with anti-MAG or other myelin antigen activity. Myelin-associated glycoprotein is an outer cell surface antigen thought to be operative in cell adhesion and separation. Perhaps interference with this function results in the separation of myelin (Attia et al. 1989). The paraprotein has been conclusively immunolocalized to the extracellular space within the widely spaced myelin (Lach et al. 1993), adding weight to this hypothesis. In a model of experimental allergic encephalitis, it was possible to demonstrate unequivocally that widely separated myelin lamellae were associated with deposition of immunoglobulin at the outer aspect of both half-membrane leaflets making up the intraperiod line (Johnson et al. 1979). Immunoglobulins are positively charged molecules that will be strongly attracted to the negatively charged membranes lining the extracellular space within the intraperiod line. Separation of the myelin lamellae may result from interference with the electrostatic forces that maintain normal compaction (Ropte et al. 1990; Thomas et al. 1993).

King and Thomas (1984) produced WSM simply by soaking the nerve in hypotonic solution prior to fixation, indicating that abnormal hydration of the myelin sheath can cause this change. Other authors who were able to produce WSM without immune manipulations, for example, radiation (Masurovsky et al. 1967) or silver nitrate impregnation (Hirano et al. 1965), also postulated the occurrence of "intramyelinic edema."

"Loosened myelin" is a different myelin alteration that has been illustrated in the literature and which should not be mistaken for WSM. In loosened myelin the myelin lamellae are irregularly separated and wavy. This condition has been detected in leprous neuropathy and metachromatic leukodystrophy, but also can be seen in other neuropathies. We share the opinion of Vital and Vallat that loosened myelin may be artifactual (Vital and Vallat 1987, p 71).

Intramyelinic Edema

Although at first glance myelinated axons in which axons are smaller than expected for the overall MF size were thought to represent atrophic axons, the current explanations of this ultrastructural appearance is that it represents intramyelinic edema involving the periaxonal lamellae (Fig. 5.15a, b).

Table 5.3 Demyelinating neuropathies with Schwann cell inclusions

Storage diseases (Chap. 20)
Metachromatic leukodystrophy
Krabbe leukodystrophy
Niemann–Pick disease
Farber disease
Adrenoleukodystrophy
Cerebrotendinous xanthomatosis
Amiodarone, perhexiline, chloroquine
Leprosy (Chap. 12)
CMV neuritis

5.2.3 Schwannopathy

Many of the demyelinating neuropathies can be thought of as "schwannopathies," where the Schwann cell metabolic machinery is unable to maintain the myelin sheath. Schwann cell inclusions may be a critical clue to this situation (Table 5.3). Nonspecific evidence of a "sick" or "stressed" Schwann cell may be seen, such as a proliferation, or conversely a loss, of endoplasmic reticulum, vacuolation, accumulation of glycogen, dysmorphic mitochondria, accumulations of electron-dense amorphous or lamellated inclusions, and watery distention of the normally thin mesaxons and rims of Schwann cell cytoplasm surrounding the myelin sheath.

5.2.3.1 Alterations in Myelin Sheath Thickness and Form

"Tomaculous" alterations refer to fibers with a myelin sheath which is thickened circumferentially or eccentrically and may show prominent focal outfoldings and infoldings. This abnormality is discussed elsewhere and most often is associated with HNPP. Conversely, hypomyelinating neuropathy shows a diffuse picture of myelin sheaths too thin for axonal diameter. Diffuse hypomyelination may be congenital or may result from widespread demyelination and remyelination. Rare congenital neuropathies have been reported with "unstable" myelin showing a striking tendency to fragment and form globular debris in association with demyelination.

5.2.4 Secondary Demyelination

More than a century ago, Gombault (1886) considered that demyelination can occur as a consequence of axonal disease. In the 1970s, Dyck and colleagues (1984b) studied a number of clinical and experimental models of peripheral nerve disease and demonstrated convincingly that the myelin changes seen are actually caused by axonal abnormalities. Prior to the clear delineation of this concept, some neuropathies now recognized as primarily axonal had been misinterpreted as predominantly demyelinating, including diabetes, porphyria, and uremia.

5.2.4.1 Experimental Data Supporting Secondary Demyelination

Dyck and colleagues (1971) conducted the first quantitative study of secondary demyelination in uremic neuropathy when they examined biopsied sural nerve fascicles from ankle and midcalf levels in two patients with renal failure and neuropathy. These biopsies provided unequivocal evidence of demyelination, but this occurred in a nonrandom fashion along certain axons. In more proximal nerve segments, the most common abnormality was myelin wrinkling, whereas the distal nerve segment demonstrated predominantly axonal degeneration and segmental demyelination. Individual teased fibers showing segmental myelin change were then cut in sequential cross section along their length; the axons found within were extremely atrophic. Such data, and similar results in Friedreich's ataxia nerves (Dyck and Lais 1973), suggested that in these situations a distal axonal atrophy resulted in segmental demyelination. Although convincing, the possibility that the Schwann cell was still somehow defective in both uremia and Friedreich's ataxia could not be entirely dismissed. Dyck and colleagues provided conclusive evidence for secondary demyelination in their permanent axotomy model, taking advantage of the fact that after nerve transection the proximal segment becomes somewhat atrophic (Dyck et al. 1981). With time, myelin wrinkling evolved into frank demyelination without significant axonal loss proximal to the site of axotomy. In the current conception of secondary demyelination, axonal atrophy results in myelin wrinkling, followed by paranodal or segmental demyelination, then remyelination. Why the myelin wrinkles when the axon atrophies remains unclear. Is it a simple mechanical problem, with loss of support to the inner myelin layers or even traction on them (Dyck et al. 1984b)? Or is there an interruption of crucial physiologic interactions between the axon and Schwann cell?

5.2.4.2 Pathological Alterations in Secondary Demyelination

Distinguishing secondary demyelination from primary demyelination can prove difficult. In practice, one simply weighs the balance of axonal disease vs. that of myelin change. Occasional thinly myelinated fibers are likely to be a secondary alteration if axonal dropout or active degeneration is very prominent. With abundant evidence of active or chronic demyelination in the presence of minimal axonal dropout and active degeneration, one suspects that the demyelination is primary. The difficulty arises when significant amounts of both axonal and myelin alterations are present, a common situation in practice.

Some observations may provide helpful clues. Onion bulbs and naked axons can occur in secondary demyelination, but in general should lead one to favor a primary demyelinating process. If some of the specific alterations of

primary demyelination discussed above are seen (Schwann cell inclusions, macrophage-mediated myelin stripping, widely spaced myelin, etc.), then one would reasonably diagnose a primary disease of myelin or Schwann cells. Electron microscopic examination can prove very helpful in deciding whether demyelination is occurring around normal axons or around axons which show signs of pathology, such as atrophy, accumulations of organelles, or even disintegration. Light microscopy and nerve teasing do not provide this information.

After weighing these various factors, it is usually possible to decide whether the process is likely a primary demyelination or a primary axonopathy. In those cases where the distinction is still not possible, more involved teased fiber and quantitative techniques are very helpful. A fiber size histogram demonstrating a bimodal peak with a shift towards to the left is suggestive of the presence of axonal attenuation (Ohi et al. 1985). Demonstration that the myelin internode length is inappropriately long for the axon diameter allows a similar inference (Ohi et al. 1985). However, the most conclusive way of making the distinction involves the use of teased fiber preparations. In secondary demyelination, "sick" axons with many abnormal internodes along their length are intermingled with healthy axons which have a full complement of normal internodes. Thus, there is a clustering of abnormal myelin segments along certain axons (Dyck and Lais 1973). In primary demyelination the abnormal segments are spread randomly through the fibers, as some experimental lead neuropathy models demonstrate (Windebank and Dyck 1984).

While teased nerve preparations are necessary in research situations, we almost never make use of them in practice. For satisfactory teasing studies, at least 100 fibers with 5 intact internodes must be obtained to derive statistically useful data (Dyck et al. 1971). Routinely performing such analysis is beyond the reach of most pathology laboratories, including our own. Either there is a lack of expert technologists who can carry this meticulous task or the procedure proves too expensive and time consuming. We do not believe that the information gained is enough to justify the cost of maintaining the ability to provide this service. Deciding whether the demyelination is secondary or primary may help in narrowing the differential diagnosis, but never gives a final etiologic diagnosis. In those instances where an etiologic diagnosis is possible, it is almost always reached by identification of specific changes in the nerve interstitium using cross-sectional views. Moreover, the appearance of myelin and axons is very well visualized on appropriately stained plastic-embedded material.

Some authors do not try to make the distinction between primary or secondary demyelination or call the demyelination "primary" if myelin loss exceeds axon loss (Honavar et al. 1991). Another approach involves labeling as "predominantly axonal" those biopsies where axonal changes are more prominent than demyelinating changes and as "predominantly demyelinating" those biopsies where myelin alterations are more prominent. Intermediate features are labeled as either mixed or indeterminate (Barohn et al. 1989; Logigian et al. 1994). We recognize that these approaches are subjective and prone to error in inexperienced hands, but nevertheless feel that they are a necessary concession to pragmatism and adequate for routine clinical work.

5.2.4.3 The Clinical Significance of Secondary Demyelination

Conditions in which secondary demyelination has been conclusively shown to be important include uremia (Dyck et al. 1971), Friedreich's ataxia (Dyck and Lais 1973), some paraproteinemic neuropathies (Ohi et al. 1985), and thiamine deficiency (Ohnishi et al. 1980). Conditions in which secondary demyelination is likely to be important include some paraneoplastic neuropathies (Schlaepfer 1974), hexacarbon toxicity (Chap. 18), and Tangier disease (Pollock et al. 1983). However, there is probably some secondary demyelination in any axonal process including amyloidosis (Said et al. 1984), vasculitis (Nukada and Dyck 1987), and all distal axonopathies. Interestingly, an abundance of experimental data suggests that CMT-1 is also a distal axonopathy with secondary demyelination, yet alterations in peripheral myelin protein (PMP-22) are believed to somehow cause the disease in most patients. Clearly, the complexities of axon–Schwann cell interactions make determining which one is primarily responsible for a neuropathy a difficult task when done purely on morphologic grounds.

5.2.5 Mechanisms of Demyelination

A few of the mechanisms by which demyelination can occur have been discussed previously including macrophage- and antibody-mediated attack, demyelination secondary to axonal atrophy, and the role of calcium influx with activation of phospholipases (Smith and Hall 1988) or endogenous proteases (Koski 1992; Banik 1992). Such calcium influx might result from activation of the complement membrane attack complex (Koski 1992). Myelinotoxic products of the inflammatory response may be important in granulomatous and inflammatory neuropathies. Macrophage-derived neutral proteases can produce selective demyelination in vitro (Said and Hontebeyrie-Joskowicz 1992; Cammer et al. 1978). In diphtheria, historically a cornerstone of the study of demyelinating neuropathies, the toxin does not "attack" the myelin, but rather inhibits Schwann cell protein synthesis (Pappenheimer and McGill 1973) interfering with the normal turnover of myelin P_0 and basic proteins (Pleasure et al. 1973). Similarly, lead neuropathy is probably caused by an impairment of Schwann cell metabolism, but the molecular mechanisms are unclear (Windebank and Dyck 1984).

5.3 Remyelination

5.3.1 Normal Remyelination

The experimental models from which the discussion below is derived are based on a variety of demyelinating mechanisms, including recurrent immunization with myelin (Pollard et al. 1975), injection with lysophosphatidylcholine (Hall 1983), exposure to diphtheria toxin (Allt 1969), intoxication with iminodipropionitrile (Griffin et al. 1987), and mechanical pressure (Dyck 1969). The process of remyelination appears to be independent of the mechanism of demyelination. Whether originally macrophage mediated or not, debris from demyelinating fibers is removed by macrophages, attracted to the site by as yet unclear signals (Griffin et al. 1993).

In experimental animals, the process of regeneration of the myelin sheath begins as soon as 2 days after demyelination, with a proliferation of SCs (Griffin et al. 1987). Schwann cells that have lost their myelin sheath, and perhaps neighboring nonmyelinating Schwann cells, provide the pool of proliferating cells (Griffin et al. 1990). At the onset of remyelination, several SCs may be visible within the basal lamina of one axon (Ballin and Thomas 1969b; Pollard et al. 1975; Dyck 1969). Only one Schwann cell, however, succeeds in capturing and myelinating the axon, while the others are displaced peripherally, undergo involutional changes, and diminish in number with time (Fig. 5.17). Necrosis of these supernumerary Schwann cells can be observed (Pollard et al. 1975). One characteristic of an axon that has become demyelinated and remyelinated is the presence of folds of redundant basement membrane, probably left behind by the now-obsolescent SCs that were produced in the early stages of the remyelinating process (Fig. 5.16a) (Dyck 1969).

Remyelinating SCs often have an abundance of RER, mitochondria, Golgi membranes, and free ribosomes and are rich in cytoplasm (Fig. 5.17). As successive turns of myelin are laid down, the most superficial lamellae are the first to reach that part of the axon destined to form the new node of Ranvier (Prineas 1972). Initially the myelin lamellae are poorly compacted but this resolves as successive turns are laid down (Fig. 5.16b). By four weeks after the demyelinating event, axons have a thin new compact myelin sheath which continues to grow for several months thereafter and eventually reaches a thickness which does not quite equal that of the original (Pollard et al. 1975).

The mechanism of remyelination depends to a certain extent on the size of the demyelinated segment. If only a small paranodal segment of myelin is lost, it may be replaced by a single, new, "intercalated" internode (Allt 1969; Griffin et al. 1987). Where the paranodal demyelinated segment is very short (<15 μm), an extension of the already present but retracted Schwann cell cytoplasm might recover the denuded

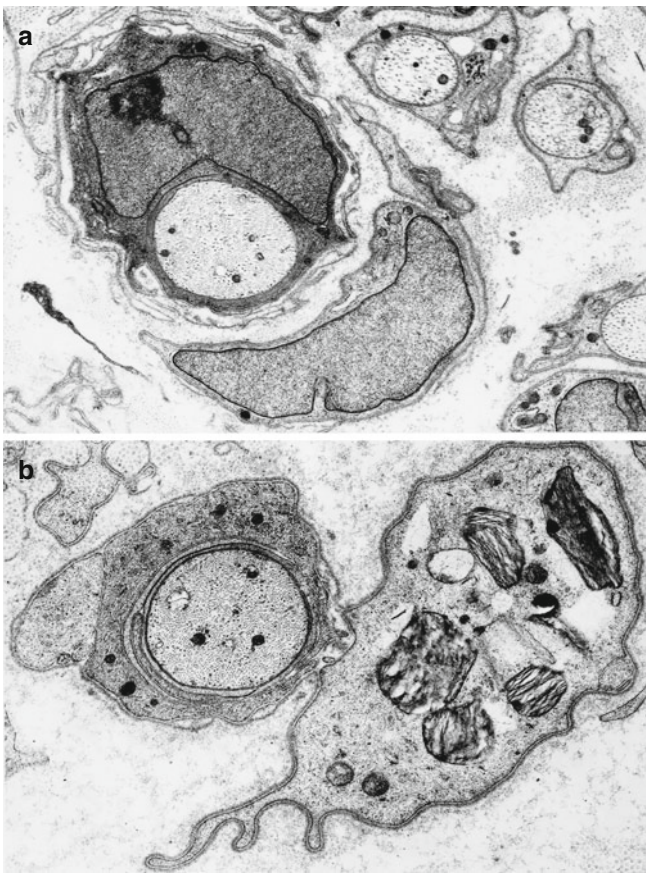

Fig. 5.16 Remyelination. Remyelinating fibers show 2–3 spirals of uncompacted Schwann cell cytoplasm. Redundant basal lamina and a displaced denervated Schwann cell ("S") are shown in (**a**). In (**b**) a displaced Schwann cell process containing pi granules is invested by basal lamina which is continuous with that of the remyelinating fiber (**a** 13,680×; **b** 16,416×)

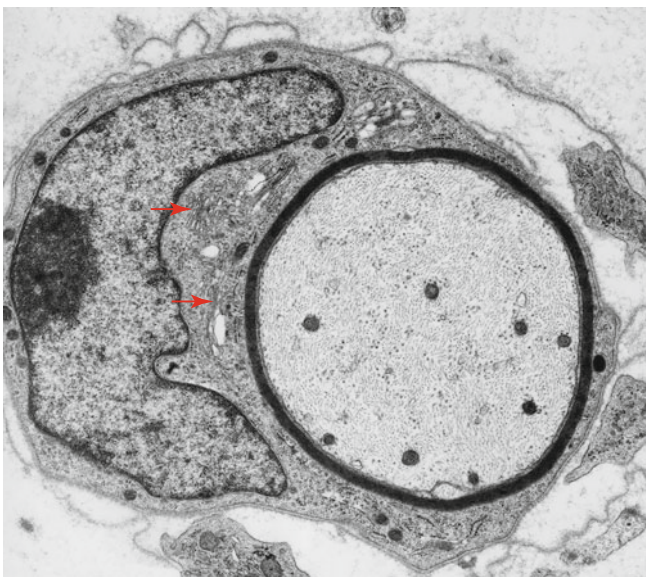

Fig. 5.17 Remyelinating fiber with thin compacted myelin sheath. The Schwann cell displays prominent Golgi complex (*arrows*), abundant RER, and a redundant basal lamina (18,616×)

axon segment, without formation of a new internode (Allt 1969). If a whole internode is demyelinated, it is typically repaired through the formation of several much smaller internodes. This process results in an increased variability of internodal length, as short intercalated nodes intermingle with longer normal undisturbed internodes.

Nearly all detailed descriptions of remyelination have commented on the association of unmyelinated axons with the supernumerary Schwann cells around a remyelinating axon (Pollard et al. 1975; Dyck 1969; Hall 1983). Such unmyelinated fibers probably correspond to those routinely incorporated into the Schwann cell layers of onion-bulb formations. The origin of these axons is unclear. They may be seen even when there is no evidence of axonal degeneration (Hall 1983). It is possible that these unmyelinated axons are collateral sprouts emerging from a demyelinated but intact axon. The observation that such unmyelinated axonal sprouts decrease in number with time favors this hypothesis, since this finding would not be expected if these profiles represented stable, previously existing, unmyelinated fibers (Pollard et al. 1975). This hypothesis receives further support from the observation that these unmyelinated axons can sometimes be found within the original basal lamina (Pollard et al. 1975). However, the work of Griffin and colleagues (1987) indicates that nonmyelinating Schwann cells nearby a demyelinated axon can become involved in the process of remyelination, suggesting that this involvement may be the origin of some unmyelinated axons associated with remyelinating fibers.

Remyelination may occur aberrantly as shown (Fig. 5.18) in which a central unmyelinated axon and its surrounding Schwann cell are themselves surrounded by a myelin sheath. In some conditions, uninjured Schwann cells nearby demyelinated or degenerating axons can be stimulated to proliferate, which may underlie a very rare mitosis (Fig. 5.19) in an otherwise normal appearing Remak bundle.

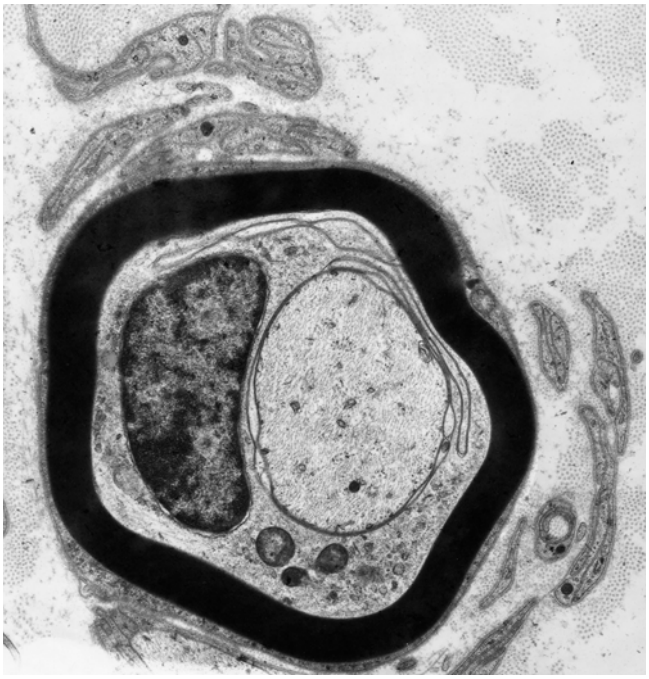

Fig. 5.18 Aberrant remyelination. A myelinated sheath surrounds a Schwann cell nucleus adjacent to an unmyelinated axon which is partially surrounded by a few turns of uncompacted Schwann cell membrane (12,000×)

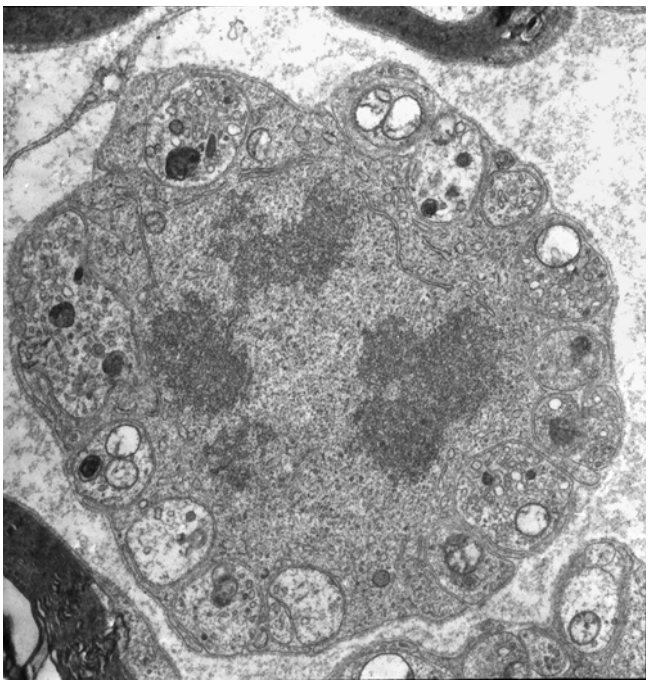

Fig. 5.19 Demonstration of a mitotic figure in an otherwise normal Schwann cell composing a Remak bundle is a very rare occurrence (12,000×)

5.3.2 Onion-Bulb Formation

Animal models have demonstrated that a single demyelinating/remyelinating event is insufficient to create onion bulbs, but recurrent or continuing demyelinating insults can produce onion bulbs in great numbers (Pollard et al. 1975; Dyck 1969). However, if after one complete cycle of demyelination and remyelination the myelinated axon completely returns to normal (except for a slightly thinner myelin sheath), it is unclear how recurrent events produce the onion bulb. Certainly in some models of recurrent demyelination, it has been quite difficult to create these formations (Raine 1977; Hall 1983).

Ultrastructural observations suggest that with each cycle of demyelination, Schwann cell processes that do not succeed

in capturing an axon become atrophic and are displaced centrifugally by the new Schwann cell that remyelinates the axon. Ordinarily these supernumerary cells become obsolete so that only one Schwann cell is associated with the axon. One explanation for onion-bulb formation would be that if demyelination occurs again before the orderly process of elimination of supernumerary Schwann cells is complete, excessive numbers of surviving Schwann cells remain around the axon and create the onion bulb (Pollard et al. 1975). Repetitive cycles build up the concentric array of flattened Schwann cell processes and their basement membranes that makes up an onion bulb. Diseases in which demyelination is occurring concurrently with remyelination for prolonged periods should produce the most dramatic onion bulbs, for example, the inherited hypertrophic neuropathies or CIDP. Authors who have had difficulty producing "true" onion bulbs despite exposing the nerve to recurrent episodes of demyelination have suggested that other factors in addition to recurrent demyelination/remyelination are required to produce onion bulbs, for example, hereditary or acquired metabolic defects of the Schwann cell (Hall 1983; Raine 1977).

Observations in human disease (Pleasure and Towfighi 1972) and experimental models (Pollard et al. 1975) suggest that the presence of onion bulbs indicates a demyelinating process that has been active for at least several months, and the longer the duration of the demyelinating process, the more onion bulbs are seen. Although onion bulbs are far more likely to be seen in diseases causing primary demyelination, demyelination secondary to axonal disease may also cause onion-bulb formation. This occurrence presumably depends on the chronicity of the process and on how many times the axon sheds and rebuilds its myelin sheath before finally dying (Griffin and Price 1981).

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The Peripheral Nerve Vasculature

6

6.1 Normal Structure and Function

6.1.1 Vascular Anatomy

Nutrient arteries arise from major vessels and penetrate the nerve trunk roughly perpendicular to its axis. These vessels are highly variable in caliber (up to 1 mm) and in location, both between and within (from side to side) individuals. The median and ulnar nerves, for example, have 1–11 (mean 3.24) and 2–19 (mean 7.75) such nutrient arteries in their forearm segments, respectively (Sunderland 1978). After entering the nerve trunk, the nutrient vessels divide into longitudinal ascending and descending branches and supply several arrays of vessels which run along the nerve and interconnect via oblique or laterally directed channels.

Lundborg (Lundborg 1970; Lundborg and Branemark 1968) has divided the neural vascular system into extrinsic and intrinsic parts. The extrinsic system consists of the "… regional nutrient arteries which supply the Intraneural vascular bed at varying intervals." The intrinsic system is contained within the nerve and consists of longitudinal arrays of vessels running along the nerve, with abundant anastomosing channels between them. This system includes both epineurial and endoneurial vessels. More recently, some authors have used the term "intrinsic" to mean the intrafascicular vascular plexus (McManis et al. 1993; Olsson 1972).

The epineurial (extrafascicular) intrinsic vessels include arterioles which run longitudinally at the most superficial aspects of the epineurium. These arterioles are sometimes visible on the surface of the nerve. The epineurial intrinsic vessels (Fig. 6.1a) also include an array of arterioles deeper within the nerve, located between individual fascicles and vasculature within perineurial septa (Fig. 6.1b). A cross section of the nerve trunk may show several arterioles, but one usually dominates (arrowhead, Fig. 6.1a). The lack of an elastic lamina distinguishes venules which outnumber arterioles (Figs. 6.1a and 6.2a, b).

An endoneurial capillary system runs longitudinally with numerous oblique and transverse anastomoses among vessels (Figs. 6.1a and 6.3). The literature is somewhat unclear about whether arterioles are found in the endoneurium. Bell and Weddell (1984) described endoneurial arterioles having one or two layers of smooth muscle cells and an incomplete internal elastic lamina, but Sunderland (1978) has stated that arterioles are not seen in the endoneurium. This disagreement may simply be a nosologic issue, depending on the definition of endoneurium and of arterioles. Beggs et al. (1991) noted that true arterioles, when found inside a fascicle, are confined to intrafascicular perineurial septa and are not in the endoneurium, strictly speaking (Fig. 6.4). Moreover, the presence of arterioles may vary depending on the nerve trunk and species under study (Beggs et al. 1991), for the studies of Bell and Weddell (1984) were performed on sciatic nerves of several species including humans. We have never found an intrafascicular arteriole outside a perineurial septum.

The endoneurial capillary density has been measured at 60–100 per mm^2 in human sural nerve, decreasing somewhat with age (Dyck et al. 1985; Giannini and Dyck 1993). These endoneurial microvessels differ from those of other organs (Bell and Weddell 1984). The presence of strong alkaline phosphatase activity suggests their essential capillary nature. However, most vessels are associated with a periendothelial cell with cytoplasm extending over 50 % or more of the circumference and are thus reminiscent of postcapillary venules. Furthermore, endoneurial microvessels are larger than those of other tissues, with a mean diameter of 9 μm vs. 5.2 μm in muscle, for example (Bell and Weddell 1984). Giannini and Dyck (1993) found endoneurial microvessel diameters ranging from 5 to 22 μm. Endoneurial capillaries are lined by a single layer of thin endothelial cells, about five per vessel cross section, linked by tight junctions at points of contact (Giannini and Dyck 1993) (Figs. 6.5, and 6.6a). Junctions are associated with a large variety of molecules (claudins 1,2,5,12,19; zona occludens (ZO) 1,2; and other adhesion molecules, Ubogu

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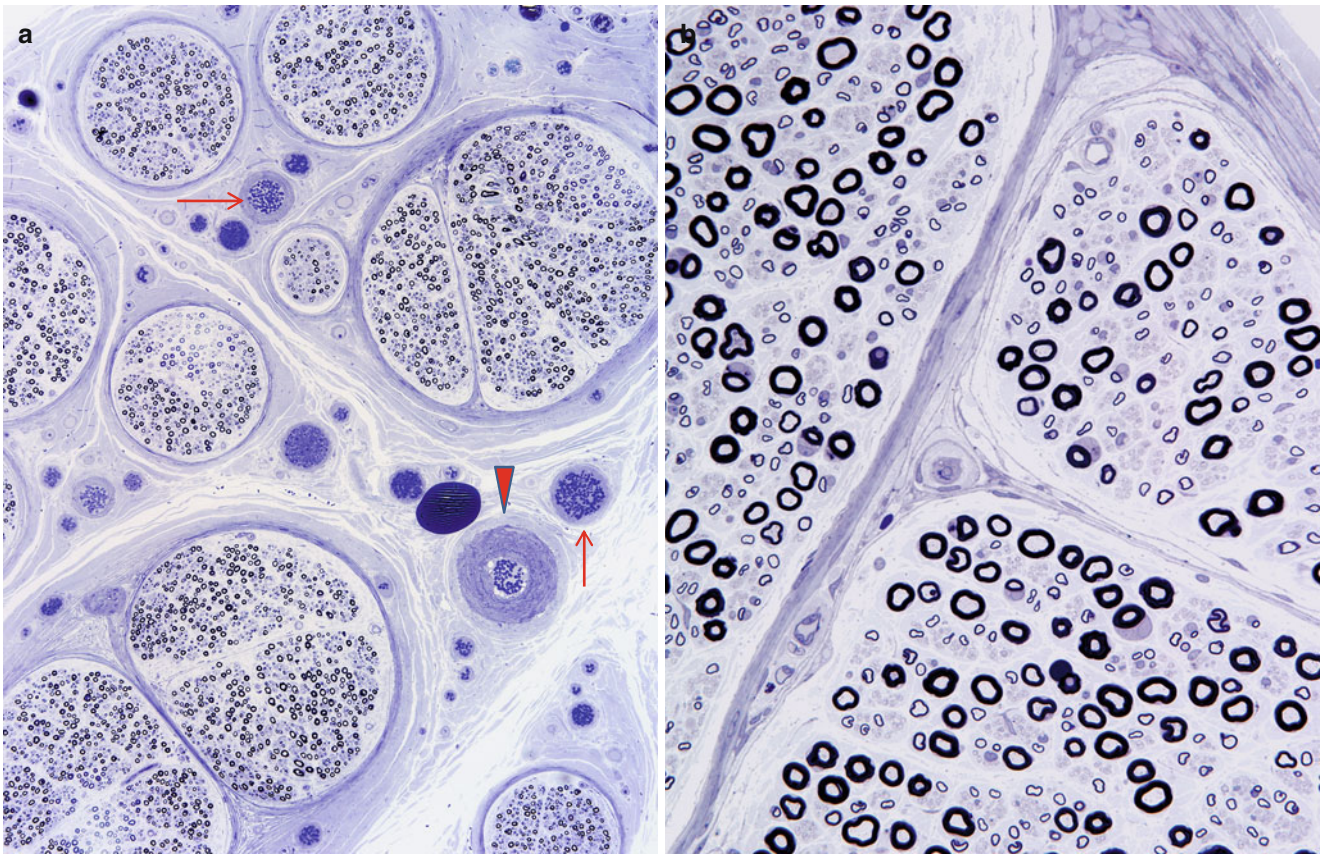

Fig. 6.1 One arteriole (*arrowhead*) and several venules (*arrows*) are shown in the epineurium (**a**). In (**b**) note vessels within perineurial septum (1 μm thick toluidine blue-stained plastic section, 1,000×)

2013). Capillary endothelial cells contain Weibel–Palade bodies (Fig. 6.6a) which are thought to produce factor VIII-related antigen (Rondaij et al. 2006). Endothelial cells may also express numerous chemokines, cytokines, and growth factors, which may participate in neuropathy. Normal endothelial cells of the epineurium and perineurium may demonstrate fenestrae, but those of the endoneurium do not. Immediately around the capillary is a condensation of the collagen found throughout the endoneurium. However, in contrast to vascular structures in the central nervous system, there is a generous extracellular space around vessels, and no "glial" cells invest the walls of the blood vessel.

Transperineurial arterioles connect the epineurial and endoneurial vessel arrays. Beggs and colleagues (1991) have defined transperineurial arterioles as vascular segments exhibiting a continuous smooth muscle coat and confined to the perineurial compartment, including all arterioles in the perineurium proper and arterioles within perineurial septa in the endoneurium (Fig. 6.5). These arterioles, which measure 10–25 μm in diameter, travel an oblique or transverse route through the perineurium (Beggs et al. 1991). The presence of axon terminals on the smooth muscle cells of these vessels suggests a potential for neurogenic regulation of their caliber (Beggs et al. 1991).

An incomplete smooth muscle cell layer and endothelial cells that are somewhat thinner than those of arterioles identified endoneurial venules (Bell and Weddell 1984). The endoneurial vessels drain into epineurial venules, which outnumber epineurial arterioles, and ultimately flow into large vessels which exit the nerve trunk along with the nutrient arteries.

A lymphatic drainage system has been described in the epineurium, but not in the endoneurium (Sunderland 1978). We have found that some large vessels in the epineurium demonstrate focal aggregations of cytoplasmic filaments in endothelial cells adjacent to focal condensation of basal

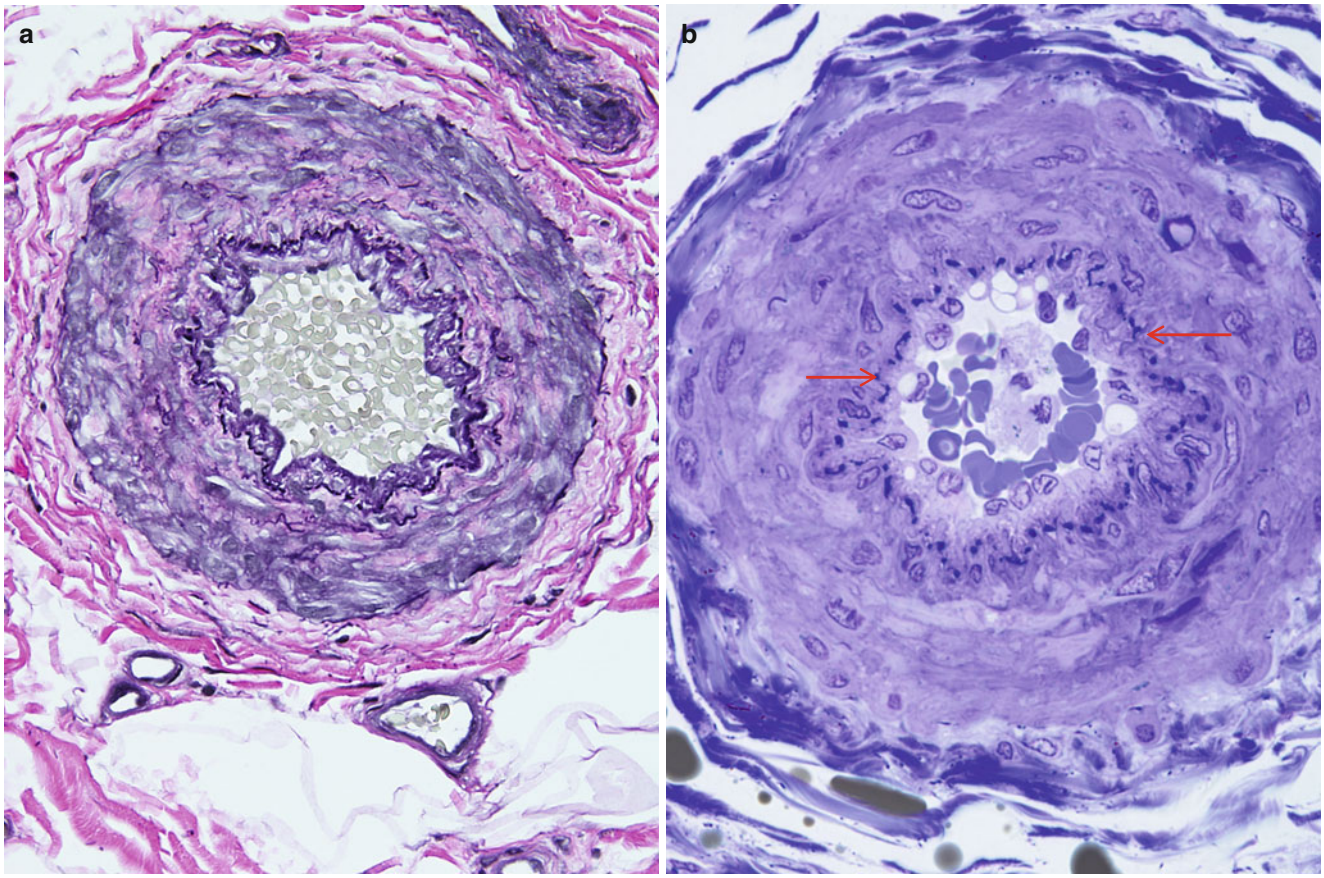

Fig. 6.2 (**a**) Epineurial arteriole is stained with Verhoeff–Van Gieson elastica. In semithin sections the internal elastic lamina (*arrow*) is less conspicuous (**b**) (**a**: paraffin, **b**: 1 μm thick toluidine blue-stained plastic section)

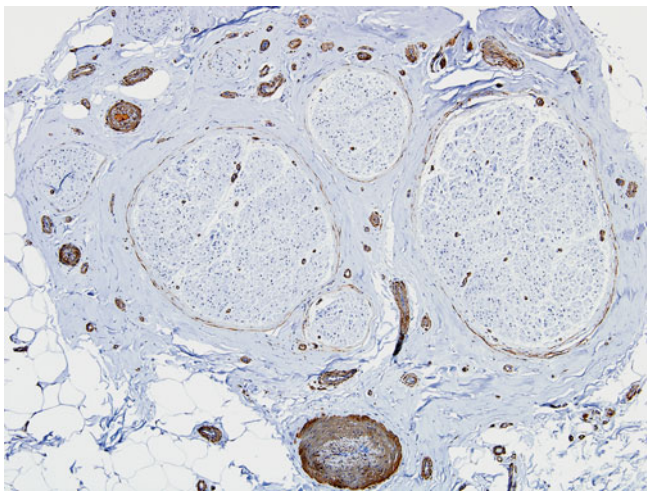

Fig. 6.3 Smooth muscle actin immunostaining highlights the epineurial and endoneurial microvasculature (paraffin, 100×)

lamina (Fig. 6.6b, c). The frequency with which these complexes are seen suggests that they are lymphatic vessels and a normal finding.

The endoneurium and subperineurial interstitial spaces are contiguous along the length of the nerve; consequently, material injected into the endoneurial compartment can be detected at considerable lengths up and down the nerve trunk, although it does not spread into the epineurium. Indeed, the endoneurial interstitial compartment is contiguous with the subarachnoid space (Olsson 1990).

6.1.2 Resistance of Peripheral Nerve to Ischemia

Overall then, there are four longitudinal arrays of vessels; superficial epineurial, deep epineurial, perineurial, and

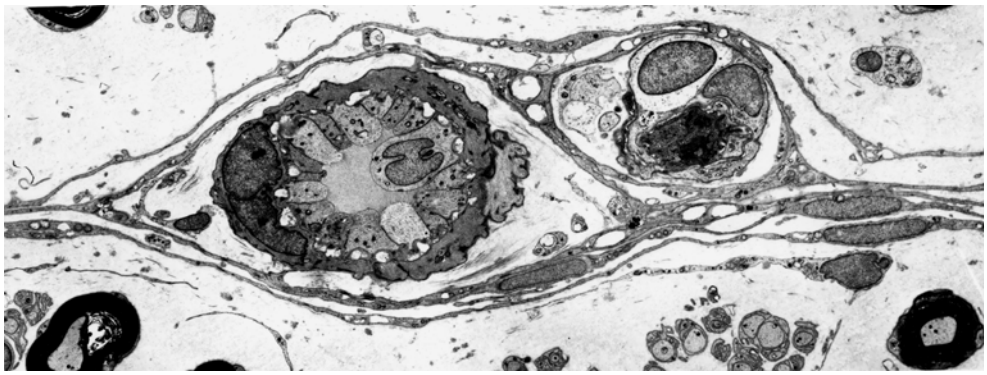

Fig. 6.4 Terminal arteriole in perineurial partition (2,070×)

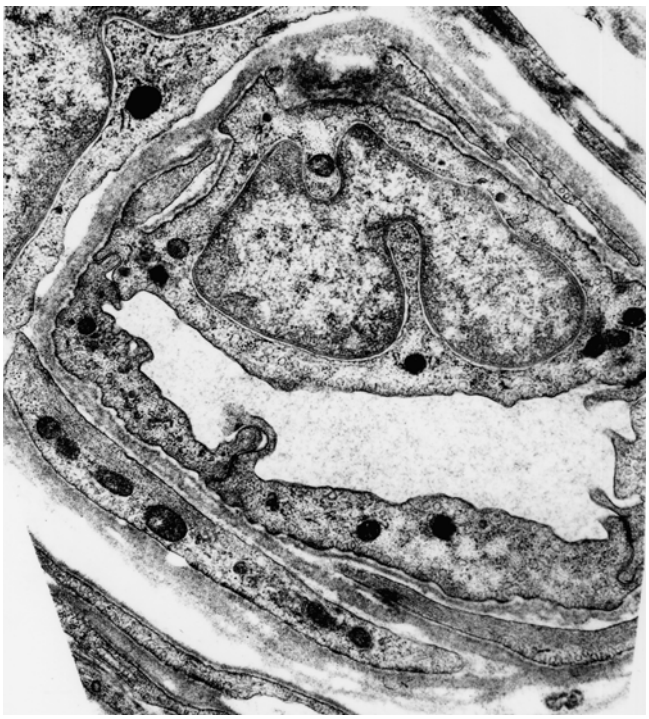

Fig. 6.5 Typical endoneurial microvessel displays intense pinocytotic activity, tight junctions, and pericytic component (1,3794×)

endoneurial, each of progressively lesser caliber. Typical lumina are 75–250 μm for epineurial arterioles, 10–25 μm for transperineurial arterioles, and 5–20 μm for endoneurial capillaries (Beggs et al. 1991; Dyck et al. 1972, 1985, 1987; Giannini and Dyck 1993). Numerous interconnections exist within and between the intra- and extrafascicular systems, providing the nerve with a high resistance to focal ischemia.

In vivo studies using rabbit tibial nerve demonstrate that occlusion of all nutrient vessels over a several centimeter segment does not cause visible change in blood flow, nor does the additional stripping of all epineurial vessels (Lundborg and Branemark 1968). Presumably, longitudinal flow within the intrafascicular circulation is able to compensate. Conversely, sectioning a nerve 3 cm above and below the observation point, thus eliminating the longitudinal intrafascicular blood flow, does not result in visible ischemia. Hypothetically, blood flow is maintained because the nutrient arteries feed the epineurial vessels, which interconnect readily with the intrafascicular capillaries. Indeed, two nutrient arteries are sufficient to maintain a nerve segment in the absence of the longitudinal intrafascicular circulation (Lundborg and Branemark 1968). Recent studies of peripheral nerve ischemia verify that endoneurial blood flow is dependent on both regional anastomoses with epineurial vessels through transperineurial arterioles and on longitudinal intrafascicular flow (Myers et al. 1991).

Peripheral nerve vasculature demonstrates an impressive functional reserve capacity, with numerous channels capable of opening in response to only a slight increase in nerve temperature or the sectioning of the other vessels (Lundborg 1970). Sympathetic activity also modulates the lumen of muscular vessels (epineurial, perineurial, transperineurial) and hence neural blood flow (Beggs et al. 1991; Kihara and Low 1990; Lundborg 1970).

6.1.3 The Blood–Nerve Barrier

The endoneurial compartment has a specialized ionic and macromolecular milieu (Ubogu 2013) thought to be important in nerve function, especially impulse propagation (Olsson 1972). Endoneurial fluid is under hydrostatic pressure greater than that of epineurial fluid, is hypertonic to plasma, and has less protein than plasma (Low et al. 1977; Myers et al. 1983). Maintenance of this unique composition requires isolation of the endoneurium from the vascular compartment and from the epineurium. Such isolation is a function of the blood–nerve barrier (BNB) and the perineurial barrier (PB) reviewed by Olsson (1990) and Ubogu (2013).

Early studies showed that, when injected into the experimental animal's circulation, various markers would penetrate minimally, or not at all, into the endoneurial compartment (Olsson 1972, 1990). Microscopic studies suggested that the

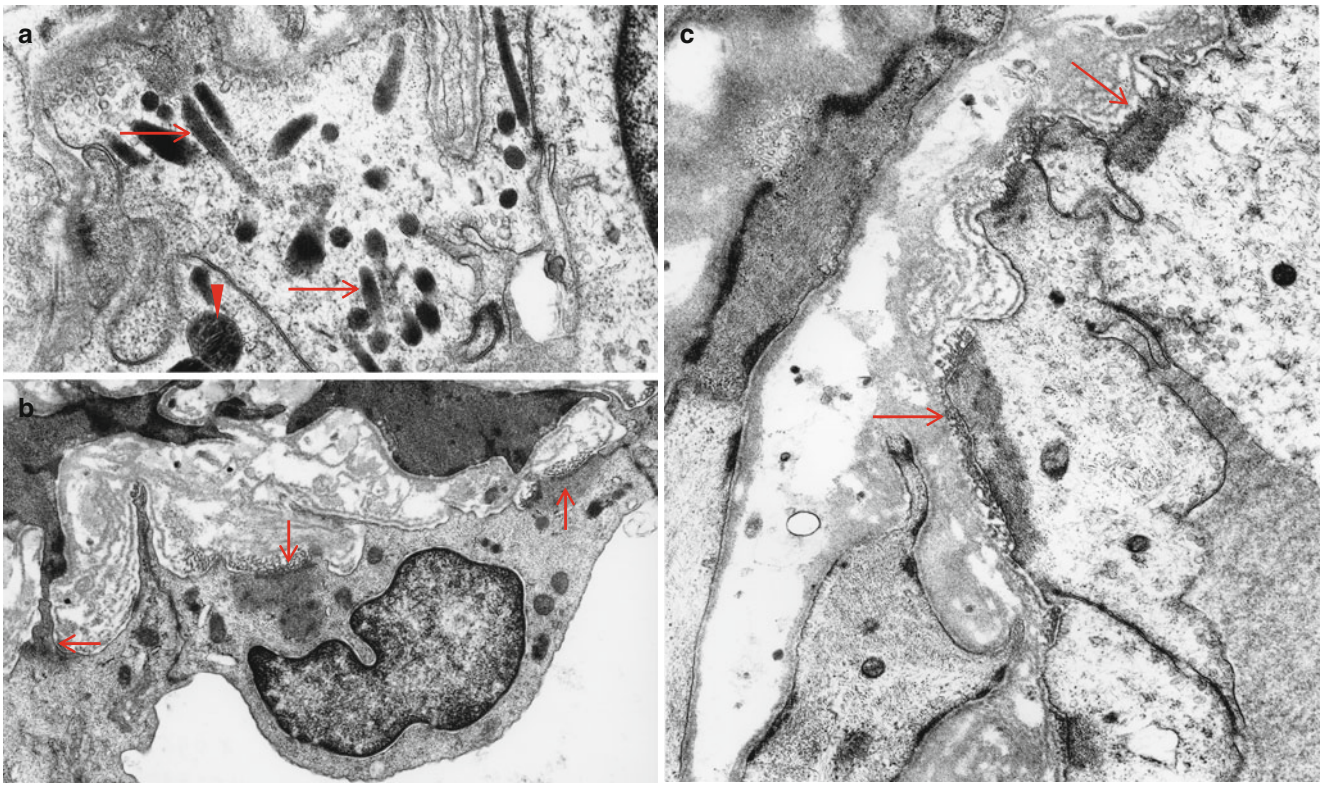

Fig. 6.6 (**a**) Large number of Weibel–Palade bodies (*arrows*) in endoneurial capillary endothelium. This patient suffered from Fabry disease: a single lipid inclusion is also seen (*arrowhead*). In (**b**, **c**) a complex of basal lamina condensation and anchoring filaments opposite focal filamentous accumulations (*arrows*) identifies these vessels as lymphatics (**a** 37,180×; **b** 19,760×)

site at which passage was blocked or markedly slowed was the innermost layer of the perineurium and the normally nonfenestrated endothelial cells of the endoneurium; the electron-dense tight junctions found at both of these sites form the anatomical basis of the PB and BNB, respectively. Vessels within the epineurium and perineurium contain numerous fenestrations and lack tight junctions. Studies using a mast cell degranulation promoting agent given intravascularly to experimental animals demonstrated that this structural barrier was also a functional barrier. Mast cell contents were liberated in the epineurium but not in the endoneurium (Olsson 1972). Substantial interspecies variation exists in the BNB (Olsson 1972). Consequently, the extent to which one can apply data derived from animals to human tissues in health and disease is uncertain.

Albumin can enter the endoneurium (Poduslo 1993), and immunohistochemical studies on normal nerve biopsy specimens suggest that IgG and to a lesser extent IgA also can penetrate this compartment, while IgM and complement (C3) probably cannot enter in significant amounts (Liebert et al. 1985; Schenone et al. 1988; Takatsu et al. 1985). These observations indicate that the BNB is a relative barrier, meaning that one macromolecule (e.g., IgA) might have a much slower diffusion or transport rate into the endoneurial compartment than another (e.g., IgG), but given sufficient time and a large enough concentration gradient between the two compartments, a certain amount of both macromolecules will accumulate (Mata et al. 1987). Accumulation of macromolecules in the subperineurial area is a common finding in normal and abnormal nerves (Graham and Johnson 1985; Liebert et al. 1985; Schenone et al. 1988; Van Lis and Jennekens 1977) and should not be ascribed undue significance.

This blood–nerve barrier is leaky in the spinal nerve roots and in the dorsal root and autonomic ganglia allowing access to exogenous tracers, some toxins, and plasma proteins. This has been attributed to a lack of tight junctions between the capillary endothelial cells of the endoneurial compartment at these sites.

6.2 Pathological Alterations

6.2.1 Alteration in the Blood–Nerve Barrier in Neuropathy

Many peripheral neuropathies alter the BNB. Diabetes, Guillain–Barré syndrome, paraproteinemic neuropathy, leprosy, and lead exposure are examples of extensively studied peripheral nerve diseases where careful observation of the

BNB indicates that it may be abnormal at times and that it potentially plays an important part in the pathogenesis of the neuropathy (see respective chapters for details). Whether such alterations in the BNB are primary or secondary to the neuropathy is a central question that has not been resolved in most cases (vide infra). In theory at least, an altered BNB can permit toxic substances to enter nerve and may cause increased endoneurial pressure with resultant reduced blood and nutrient flow, resulting in changes in the composition of the endoneurial milieu. These changes in turn may induce pathological processes such as fibrosis (Olsson 1990). An influx of vascular fluid into the endoneurium may result in alterations of the electrolyte concentration which permit the normal electrical function of peripheral nerve axons (Myers et al. 1983).

Immunohistological techniques permit study of alterations in the BNB in various human neuropathies (Liebert et al. 1985; Neuen et al. 1987; Van Lis and Jennekens 1977). IgM, ordinarily excluded from the endoneurium, frequently appears within it in inflammatory and vasculitic neuropathies. On the other hand, endoneurial IgM does not typically appear in metabolic, hereditary, or toxic neuropathies. An increase in the endoneurial content of smaller macromolecules such as IgG and albumin occurs in some noninflammatory neuropathies, representing perhaps a more subtle change in the BNB than the one which permits IgM to enter. In one study, the severity of disruption in the BNB correlated better with the presence of inflammation than with the severity of myelin or axon damage (Neuen et al. 1987).

An accumulation of macromolecules beneath the inner perineurial layer is commonly seen, perhaps due to entry via the endoneurial capillaries but blockage at the perineurium (Van Lis and Jennekens 1977). This accumulation of macromolecules seems to be particularly prominent in hypertrophic neuropathies (Van Lis and Jennekens 1977) and may correlate with the frequent finding of subperineurial edema in this setting.

Endoneurial vessel fenestration is always abnormal (Fig. 6.7). While more commonly associated with leprosy (Boddingius 1977, 1984), CIDP (Johnson 1977), and paraproteinemic neuropathy (Lach et al. 1993), this alteration is also found in diabetic neuropathy (Powell et al. 1985) and Wallerian degeneration (Ohara and Ikuta 1985). In addition to these settings, we have also found endoneurial fenestration in chronic axonal neuropathies of unknown etiology.

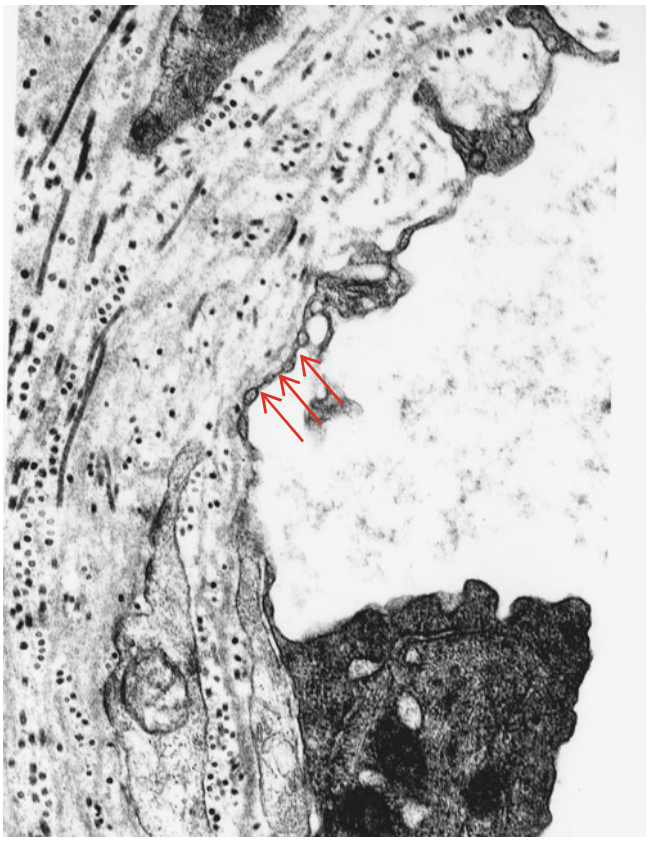

Fig. 6.7 Fenestration of endoneurial endothelium is shown (*arrows*) in a patient with CIDP (25,536×)

6.2.2 Endoneurial "Edema"

An increase in the amorphous or clear interstitial space of the endoneurium and subperineurial area is a common and nonspecific light microscopic finding. However, this finding is most closely associated with the hypertrophic neuropathies, familial or acquired (Behse et al. 1974; Matthews et al. 1970) (Fig. 6.8). The increased separation between nerve fibers and widening of the subperineurial space has been referred to as "endoneurial edema" and "subperineurial edema," respectively. This "edema" is lightly eosinophilic, stains weakly with Alcian blue or toluidine blue, and does not stain with PAS or Congo red (Asbury et al. 1971). Electron microscopy shows no specific features except for dispersal of normal elements, although at times a fine granular substance accumulates. Possibly this substance represents "acid mucopolysaccharide" material in keeping with the histostaining properties described above. Such granular material has not been a significant finding in biopsies we have examined; perhaps the granular appearance is an artifact related to fixation or preparation.

The tendency of macromolecules to accumulate in the subperineurial area in both normal and pathologically altered nerves (Van Lis and Jennekens 1977) may relate to the frequent observation of subperineurial "edema." Watanabe and Ohnishi (1979) demonstrated a significant enlargement of the subperineurial space in idiopathic polyradiculoneuropathy and thiamine deficiency-associated neuropathy. No correlation was found between prominence of subperineurial "edema" and severity of fiber damage or duration of illness (Watanabe and Ohnishi 1979). Such subperineurial space enlargement has also been described in a variety of inflammatory, toxic, metabolic, and inherited neuropathies.

Regions of endoneurial "edema" may be related to Renaut bodies. A fibrillar precursor of elastic fibers, oxytalan, is

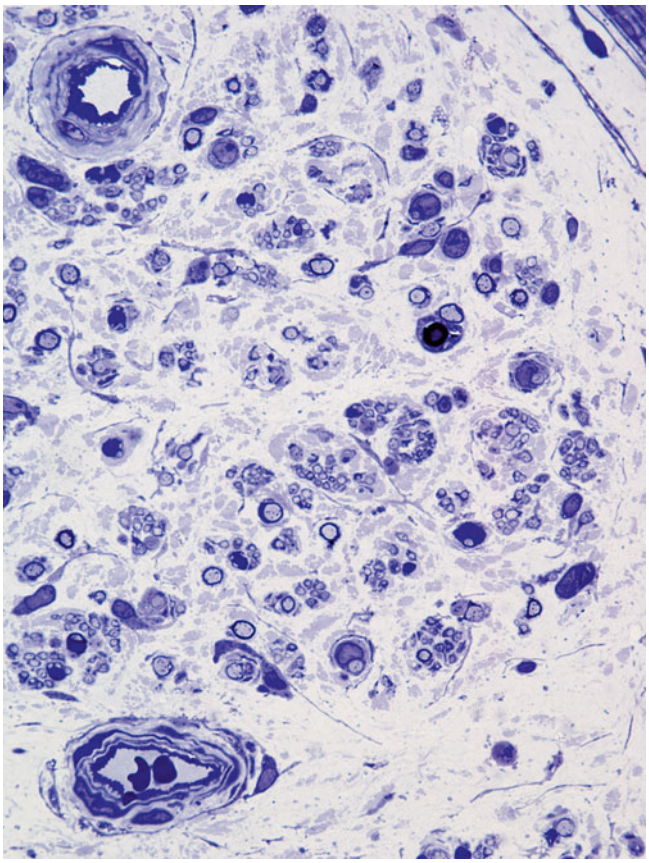

Fig. 6.8 Dispersion of endoneurial contents by increased intercellular matrix in CIDP (1 μm thick toluidine blue-stained plastic section, 1,000×)

frequently seen at sites of "edema" and in Renaut bodies. Fibroblasts with giant vacuoles are also often detected in both of these locations. Enlarged subperineurial regions devoid of axons and containing few or no cellular elements are often described as "subperineurial edema," but when fibroblastic configurations typical of Renaut bodies (Figs. 2.13c, d and 2.15a, b) are seen at this site, the distinction between "edema" and Renaut bodies becomes blurred. Certain ill-defined environmental conditions induce structural and functional changes in fibroblasts that result in the giant vacuolated appearance and in the production of acid mucopolysaccharide material and oxytalan (i.e., "edema"). In some instances, this process may proceed further to the formation of Renaut bodies. Indeed, precisely such a sequence of events has been reproduced experimentally in chronic nerve compression (Ortman et al. 1983).

While nerve "edema" may reflect deposition of acid mucopolysaccharides or oxytalan, or may represent a fixation artifact, it is also possible that this appearance may result from an osmotic increase in interstitial fluid. A balance of hydrostatic and osmotic pressures regulates endoneurial water content (Olsson 1990) and an increase in the macromolecular content of the interstitial compartment will cause an influx of fluid. This increase may result from pathological vascular permeability permitting an influx of albumin and other macromolecules, but can also theoretically occur without alteration in the BNB. For example, an influx of fluid could occur as a consequence of the release of macromolecules by endoneurial cells or the entrapment of macromolecules within the endoneurium by biochemical modification (Olsson 1990).

An increase in endoneurial interstitium is not always due to "edema." We have seen cases of IgM paraproteinemic neuropathy where massive buildup of IgM in the endoneurium was present and caused the neuropathy. In this setting the acellular spaces were PAS positive.

6.2.3 Significance of Alterations in the Blood–Nerve Barrier

A central question is whether the increase in BNB permeability seen in neuropathy is secondary to axon and/or myelin damage or whether the permeability change is primary, with resultant nerve damage. Studies of Wallerian degeneration and toxic neuropathy demonstrate that the BNB normally loses its integrity during the early phases of axonal degeneration or demyelination and gradually recovers over several months, usually in parallel with return of axons and their myelin sheaths (Bouldin et al. 1991; Ohara and Ikuta 1985; Seitz et al. 1989). Latker et al. (1991) showed that the BNB recovered in the absence of nerve fiber regeneration, while the PB did not. In a lead neuropathy model, permeability and fluid contents changed only after substantial quantities of lead had entered the endoneurial compartment (Poduslo 1993). Although such data suggests the primacy of axon and Schwann cell changes, alterations in vascular permeability and endoneurial fluid composition have frequently been postulated to be contributors to the severity of neuropathy, in diseases ranging from diabetes and inflammatory neuropathies to leprosy and amyloidosis (Boddingius 1977; Brosnan et al. 1990; Hahn et al. 1985; Koski 1992; Poduslo 1993). Computer models and in vivo experimental work suggest that transperineurial vessels, especially venules, will become distorted and compressed when endoneurial pressure is increased, possibly resulting in reduced blood flow and injury to neural elements (Kalichman and Myers 1991; Myers et al. 1986, 1991).

6.3 Focal Ischemic Injury of Peripheral Nerves

The vascular architecture of peripheral nerves results in a high degree of resistance to focal ischemia, as reviewed above. Furthermore, the metabolic demands of peripheral nerve are low relative to other neural tissues (Kihara and Low 1990). Nevertheless, acute ischemia is clinically

important (Olsson 1972), most obviously in the setting of vasculitic neuropathy and possibly in diabetic neuropathies. Numerous experimental models have explored nerve ischemia, but the interspecies variability in vascular structure and function should raise a flag of caution when extending such data to human ischemic neuropathy (Olsson 1972). Conversely, the possible confounding factor of nerve damage due to inflammatory mediators cannot be excluded in the human vasculitic neuropathies. Thus, a combination of human and experimental data is reviewed below in order to construct a picture of the effect of focal ischemia on peripheral nerve.

6.3.1 Fascicular Geography of Nerve Damage

6.3.1.1 Human Material

Dyck et al. studied limb nerves in autopsy material of patients with rheumatoid arthritis. With over 15,000 sections examined in one case, this work has yielded excellent insight into the distribution of nerve lesions in focal ischemia in human disease (Dyck et al. 1972). Various patterns of axonal injury occurred. In more proximal nerve segments, centrofascicular degeneration of fibers was the most common observation, but as the examination moved more distally, there was increasing admixture of intact and degenerating fibers. This increase presumably resulted from the inter- and intrafascicular intermingling that normally occurs as a nerve trunk progresses distally. Coagulative necrosis was not seen, and there was no correlation between regions of axonal degeneration and presence or absence of vascular occlusion. Similarly, in our experience with nerve biopsy in vasculitic neuropathies, the most typical pattern is multifocal Wallerian degeneration of variable severity in adjacent fascicles (Fujimura et al. 1991; Hawke et al. 1991). Much less frequently we have observed centrofascicular injury or wedge-shaped regions of axon loss that extend towards the perineurium. This observation is expected because the sural is a distal nerve trunk.

6.3.1.2 Experimental Material

In experimental animal models of large vessel occlusion, the centrofascicular pattern of axon degeneration has been produced most consistently, typically in large proximal nerve fascicles. On the other hand, distal nerve segments showed a more randomly disposed multifocal pattern of axonal degeneration (Hess et al. 1979; Korthals and Wisniewski 1975). A zone of more tenuous blood flow in the center of the nerve fascicle was shown to be models of ischemia produced by large vessel ligation (Sladky et al. 1985), thus providing an explanation for selective centrofascicular injury. Another possibility is that in endoneurial ischemia a simple transperineurial diffusion of oxygen supplies some of the nerve's requirements, thus protecting the subperineurial area (McManis et al. 1993). Alternatively, Nukada and Dyck (1984) found a higher capillary density in the subperineurial area than in the centrofascicular region. The studies of McManis and Low (1988) favor the importance of transperineurial oxygen diffusion. During acute ischemia there was no significant gradient between the subperineurial and centrofascicular regions in blood flow, but oxygen tension was clearly higher in the subperineurial area, presumably as a consequence of direct oxygen diffusion from the pool of oil in which the nerve fascicle was maintained (McManis and Low 1988). This explanation agrees with the observation that smaller fascicles are less severely affected than large fascicles (Korthals et al. 1978), since diffusion through the perineurium reaches a greater proportion of the fascicular contents in the former. A wedge-shaped or subperineurial region of axonal degeneration has been produced less frequently (Nukada et al. 1993). Occlusion of transperineurial arterioles, perhaps by a rise in endoneurial pressure, may result in subperineurial ischemia and axonal degeneration (Nukada et al. 1993).

To study microvessel occlusion Nukada and Dyck (1984) injected 15 μm microspheres into the arterial supply of rat sciatic nerve, producing blood flow deficits restricted to the capillary level. Obstruction of blood flow in individual microvessels did not induce fiber degeneration, but when sufficient vessels were occluded, a centrofascicular core of degenerating fibers emerged. Parry and Brown (1982) injected arachidonic acid into the femoral artery of rats, producing ischemia through "… platelet aggregation and occlusion of vasa nervorum." Again, a centrofascicular pattern of axonal degeneration was often identified.

Consequently, experimental and clinical data suggest that regardless of whether large or small vessels are occluded, the centers of nerve fascicles are more vulnerable to ischemia than the subperineurial area. However, this appearance is usually seen only at proximal "watershed" regions, and the intermingling of nerve fibers as the trunk courses distally results in the appearance of multifocal axonal degeneration as the consequence of more proximal ischemic events.

6.3.2 Involvement of Endoneurial Contents

6.3.2.1 Human Material

Reports on human biopsy material in vasculitic neuropathy are in conflict regarding the issue of whether there is preferential injury of any fiber type. Said, Fujimura, and colleagues demonstrated that large myelinated axons are more severely involved than small myelinated axons and that unmyelinated fibers are relatively spared except in the most severe cases (Fujimura et al. 1991; Said et al. 1988). However, other workers have not made this observation (Hawke et al. 1991;

Vital and Vital 1985). Segmental demyelination has been observed in vasculitic neuropathy and was thought to be secondary to axonal damage (Fujimura et al. 1991) or a primary effect of ischemia on Schwann cells (Vital and Vital 1985).

While axons and, to a lesser extent, Schwann cells are the principal targets of peripheral nerve ischemia, other cell types may be involved. The perineurium, in particular, may show focal damage and proliferation suggestive of reaction to a localized insult. Formation of a microneuroma is a rare but highly suggestive observation in vascular neuropathies (Figs. 17.2 and 17.4).

6.3.2.2 Experimental Material

Animal experiments have also been in conflict. No evidence of selective myelinated fiber loss was seen in the vessel ligation studies of Hess et al. (1979), but unmyelinated fibers were most often relatively spared. Parry and Brown (1982) used an arachidonic acid injection model and made the surprising observation that small myelinated and unmyelinated fibers were the most severely affected.

Intra-axonal accumulations of organelles are seen at the boundaries of ischemic regions, suggesting the presence of alterations in axoplasmic transport (Korthals et al. 1978; Nukada and Dyck 1987). In another experimental model of ischemia, the internodes just proximal to sites of axonal degeneration sometimes showed demyelination and remyelination, and less often this was seen even with no evidence of axonal degeneration (Hess et al. 1979). Similarly, the microsphere model of Nukada and Dyck (1987) demonstrated demyelination in association with accumulations of axonal organelles in the regions bordering severe ischemia. Such observations suggest that demyelination is secondary to axonal injury.

6.4 Chronic Vascular Insufficiency

In contrast to the clear-cut relation between acute ischemia and nerve damage, the importance of chronic ischemia due to vascular disease is difficult to quantify, reproduce, and interpret. Yet, this has been postulated as a causative factor in various neuropathies including diabetes, amyloidosis, leprosy, dysproteinemia, and in the "neuropathy of aging." Ischemic neuropathy may also follow aortic atherosclerosis, use of an intra-aortic balloon pump, abdominal aortic aneurysms or their repair, overall low cardiac output, hypercoagulable/hyperviscosity states, use of vasoconstrictors like methysergide, or prolonged tourniquet time in surgery (Laghi Pasini et al. 1996).

A study of 32 patients with severe symptomatic vascular disease of the legs and no other identifiable cause of neuropathy exemplifies the possible importance of chronic vascular insufficiency. The majority of patients suffered paresthesias and sensory loss, while half had weakness or wasting (Eames and Lange 1967). Ankle reflexes were reduced or absent in 13, and the presence of this finding correlated with the severity of vascular compromise in that limb. It appears that revascularization does not remedy the problem (Hunter et al. 1988).

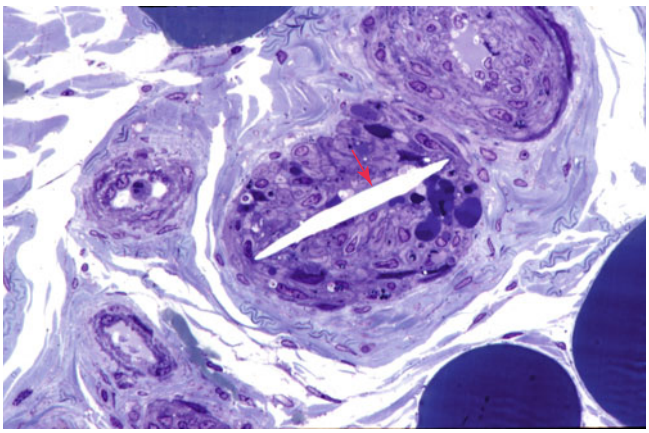

Fig. 6.9 Lucent cholesterol shard (*arrow*) within the lumen of a small arteriole in patient with cholesterol embolism (1 μm toluidine blue-stained plastic section, 1,000×)

Peripheral neuropathy has been described in a population of patients with a history of vascular catheterization or in patients with severe aortic atherosclerosis. In some of these patients, cholesterol clefts have been found in the lumina of small epineurial arteries (arrow, Fig. 6.9), cholesterol emboli syndrome, Bendixen et al. 1992), only occasionally resulting in necrotizing arteritis, but with chronic axonal degeneration involving peripheral and intramuscular nerves.

Investigators ascribe a broad spectrum of histological changes to chronic arterial insufficiency (Eames and Lange 1967; Farinon et al. 1984; Hunter et al. 1988; Vital et al. 1986). Variably severe active and chronic axonal loss has been described, ranging from no significant difference from controls (Chopra and Hurwitz 1969) to 50 % loss (Farinon et al. 1984; Hunter et al. 1988). Large myelinated fibers may be more severely involved, but this is not always the case (Vital et al. 1986). An increase in denervated Schwann cell bands and numerous dystrophic unmyelinated axons suggests that unmyelinated fibers are not spared (Vital et al. 1986). The severity of axonal loss is not correlated with the severity of vascular disease (Farinon et al. 1984). Regenerative activity may be very prominent (Gemignami et al. 1989). Nonspecific axonal changes including atrophy, organelle accumulations, and Schwann cell–axon networks are observed. A wide spectrum of segmental myelin changes has also been seen, including onion bulbs (Farinon et al. 1984).

Endoneurial capillaries show thickening and reduplication of basement membranes, hyperplasia and hypertrophy of endothelial cells, and projection of small endothelial cell

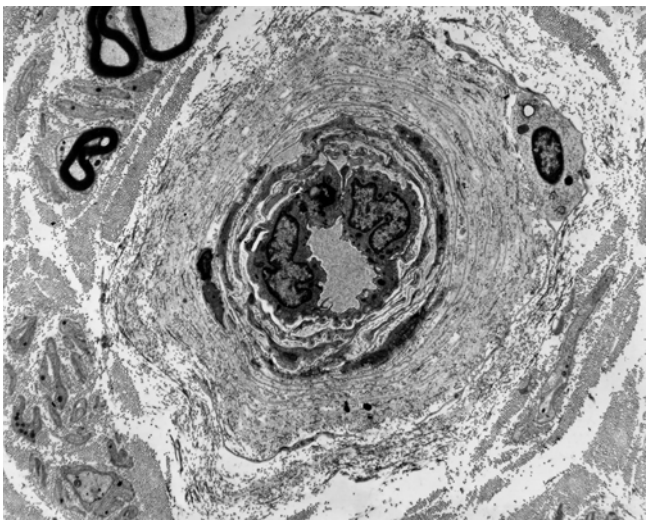

Fig. 6.10 Reduplication of basal lamina with intervening collagen in an endoneurial microvessel (3,830×)

processes into the vessel lumen (Fig. 6.10). Occasional sites of microvascular occlusion may be identified (Eames and Lange 1967; Hunter et al. 1988). Muscular vessels in the epineurium may show medial smooth muscle proliferation and disorganization of the layers composing the vessel wall.

The nonspecificity of such changes makes interpretation difficult. Most of the patients studied are over 60 years of age, and such alterations are also seen with normal aging. Indeed, chronic ischemia has been proposed to contribute to the "neuropathy of aging" and to diabetic neuropathy, with similar histological findings. Often, arteriopathic patients have concomitant small vessel disease, even in the absence of diabetes. Thus, at present there is little hard evidence to suggest that large vessel ischemia causes obvious peripheral nerve injury. In an animal model of chronic endoneurial ischemia, the predominant morphologic abnormality was axonal swelling and retraction with remodeling of myelin at the node of Ranvier, but without significant axonal loss (Sladky et al. 1991). This correlated with slowing of conduction.

6.5 Chronic Hypoxemia

Patients with chronic hypoxemic lung disease are sometimes found to have a mild neuropathy that cannot be ascribed to other causes (Appenzeller et al. 1968; Malik et al. 1990; Paramelle et al. 1986). Symptoms include mild sensory complaints, and objective signs are minimal, largely confined to distal sensory abnormalities or diminished ankle jerks. Electrophysiological tests reveal a distal predominantly axonal process.

The nerve biopsy alterations in these patients have correlated best with duration and severity of hypoxia (Appenzeller et al. 1968; Malik et al. 1990; Paramelle et al. 1986). Although Malik and co-workers (1990) found no significant alterations in myelinated fiber number and distribution, other workers have documented prominent chronic and active axonal degeneration (Appenzeller et al. 1968; Paramelle et al. 1986). An increase in denervated Schwann cell subunits and concurrent decrease in unmyelinated axons may occur (Paramelle et al. 1986). Teased nerve fibers have shown predominantly paranodal demyelination and a slight increase in the frequency of axonal degenerative changes (Malik et al. 1990). Significant perineurial thickening may be seen. Endoneurial vessels show hyperplasia and hypertrophy of endothelial cells and increased basement membrane thickness, resulting in narrowing of the vessel lumen (Malik et al. 1990; Paramelle et al. 1986; Stoebner et al. 1989). The significance of such changes is uncertain as the same caveats that were noted above for neural changes in chronic vascular insufficiency are applicable to the neural changes of chronic hypoxemia.

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Examination of the Peripheral Nerve Biopsy

7

This chapter provides a brief sketch of methodology for the preparation of peripheral nerve tissue, followed by a systematic approach to histological examination of a peripheral nerve biopsy, with an emphasis on differential diagnosis.

7.1 Methods

Specimen preparation is fundamental, and four methods should be available for the study of peripheral nerve pathology. Immediately upon excision of the sural nerve, the technologist receives the specimen on a piece of dental wax and removes a 5 mm segment for freezing. The rest of the specimen is placed straightened and slightly stretched on a wooden stick and immediately immersed in cooled 2.5 % glutaraldehyde in 0.025 M cacodylate buffer at pH 7.4 and an osmolarity between 300 and 330 mOsm. After 5 min, portions of tissue are taken for paraffin embedding and fiber teasing; then the specimen is returned to fresh glutaraldehyde.

7.1.1 Paraffin-Embedded Sections

The Saint Michael's Hospital lab obtains better results with B5 as the main fixative. After about 3 h, the tissue is post fixed in formalin, and segments are selected for cross and longitudinal sectioning. Routine stains include H&E and Congo red (amyloid). Other commonly used stains are Verhoeff–Van Gieson (elastin), Perl's Prussian blue (iron), fite (acid-fast bacilli, AFB), Martius Scarlet blue, and phosphotungstic acid hematoxylin (PTAH) (fibrin).

Most immunohistochemical reactions are carried out on paraffin-embedded tissue. Commercially available immunohistochemical stains are frequently used including antibodies to CD-3 (T cells), CD-20 and CD79a (B cells), CD68 and CD163 (macrophages), neurofilaments, Ki67 (proliferating cells), CD34 and smooth muscle actin (endothelial cells, vascular wall integrity), collagen IV (cells with basement membranes), epithelial membrane antigen (perineurial cells), and others.

7.1.2 Plastic-Embedded Sections

In the assessment of a nerve specimen, examination of toluidine blue-stained plastic-embedded sections is critical. After 2 h in glutaraldehyde, the nerve is examined under a dissecting microscope. Using a new razor blade and biological forceps, the specimen is subdivided into 3–4 mm long segments; four or five of these are retained intact as large blocks, representing the entire cross-sectional area of the nerve. The rest of the segments of nerve are sectioned in the longitudinal plane which optimally results in 20–30 blocks, each having two to three fascicles. The shape of the blocks, namely, slabs having a recognizable fascicular arrangement, enables technicians to align the nerve bundles properly for transverse and longitudinal sectioning. We believe that tissue from a nerve biopsy should not be diced or cut indiscriminately into small cubes, for such blocks cannot be oriented by the technologist. Penetration of solutions is consistently adequate even for large blocks. This method allows for sections of a useful size for light microscopy and permits examination of the entire cross section of the nerve at multiple levels. A balance must be struck between trimming away sufficient epineurial tissue to facilitate dehydration and infiltration of resin and preserving enough epineurial elements for ultrastructural examination. All blocks are again immersed in new glutaraldehyde overnight or over the weekend. Then, the tissue is post fixed in 1 % OsO_4 in Millonig's buffer at pH 7.4 for 3–5 h at room temperature, followed by dehydration, infiltration with resin, and embedding in Epon-Araldite (Hayat 1989). We have used Embed medium effectively since some constituents used for Epon-Araldite plastic embedding are no longer available. When necessary the processing can be interrupted in the dehydration stage; the tissue can be kept in 70 % alcohol overnight at 4 °C without deleterious effects.

From each biopsy, semithin (approximately 1 μm thick) sections (optimally) from 20 blocks (including all large transversely oriented specimens) are obtained, mounted on glass slides, and stained with toluidine blue. We perform

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ultrastructural studies of thin sections stained with uranyl acetate and lead citrate from selected blocks in nearly one-half of cases, including those containing all fascicles. The use of a diamond knife facilitates sectioning of large blocks, but similar results can be obtained with standard glass knives. Mounting of sections on wide-mesh grids or formvar-coated slot grids permits a better appreciation of endoneurium on low-power EM examination.

7.1.3 Frozen Sections

Tissue frozen at the time of biopsy may be used for biochemical studies, stained for lipids, and used in immunofluorescent studies.

7.1.4 Fiber Teasing

Although fiber teasing is not routinely performed at our institutions, a 1–1.2 cm nerve segment taken immediately after biopsy is fixed in formalin, post fixed in osmium tetroxide in Millonig's buffer at pH 7.4 for 3–5 h at room temperature, and passed through graded glycerin from 30 to 100 %, where it may be kept for several weeks before dissection.

7.2 Approach to Specimen Examination

7.2.1 Biopsy Examination

Biopsy examination includes:

1. Assessment of the fascicular anatomy and specimen quality
2. Light microscopy
   - Epineurium
   - Perineurium
   - Endoneurium
     - Neuropathic process
     - Other pathology
3. Ultrastructural examination (selected tissue blocks)
4. Immunohistochemical examination (specific circumstances)
5. Fiber teasing, morphometry (rarely used)

7.2.2 Essential Points to Be Addressed by the Examination

Essential points to be addressed by the examination are:

1. Is there evidence of peripheral neuropathy?
2. Is the pathological process axonal, demyelinating, or both?
3. Is the pathological process acute or chronic?
4. Are there any features permitting a specific etiologic diagnosis?

In over half of the cases, no specific features are found. In such instances, we provide a differential diagnosis and our impression of the most likely etiology. We make every effort to secure clinical, laboratory, and electrophysiological information with each nerve biopsy. This makes tissue interpretation more meaningful and will sometimes prompt special procedures. For example, if vasculitis, amyloid, or leprosy is strongly suspected and only nonspecific findings are seen on the initial tissue examination, the entire specimen may be sectioned and examined using special stains looking for a diagnostic lesion. Conversely, our observations regarding striking discrepancies between clinical and histological findings may prompt the clinician to reassess the patient, and further enquiry may reveal important information such as a positive family history or a toxic exposure. When indicated (e.g., suspected vasculitis), one can examine frozen sections following biopsy for a rapid diagnosis.

7.3 Assessment of Specimen Quality

Peripheral nerve is highly susceptible to artifacts, which should not be misinterpreted as pathological changes (Table 7.1). Nerve fascicles are round in vivo, a consequence of the elastic properties of perineurium and the pressure gradient between the endoneurial and epineurial compartments. Any deviation from this is likely to be an artifact, although a severely diseased fascicle may also become distorted. Similarly, in vivo myelinated internodes are thought to be round in cross section, excepting the normal crenated appearance at the paranodal area. Another clue to the presence of artifact is the observation of intrafascicular geographic regions of abnormal structure, without evidence of cellular reactive change.

Partial or full circumferential splits in the myelin sheath are commonly seen (Fig. 7.1a–d). These correspond to Schmidt–Lanterman (SL) clefts, which are extremely fragile structures, enlarged and distorted by the trauma of biopsy. In normal large myelinated fibers, SL clefts can be seen in up to 33–50 % of cross sections, while in small myelinated fibers, they are present in 5–10 %. This difference explains why the myelin splits are so much more common in large myelinated fibers. "Neurokeratin" artifact refers to the herringbone appearance of myelinated fibers on formalin-fixed longitudinal sections or wagon wheel appearance in cross sections (Fig. 7.2a, b). Asbury and Johnson (1978) suggest that this is a result of autolytic myelin vacuolation. Fixation for several hours with B5 prior to formalin markedly reduces the occurrence of this alteration, and it is not seen in glutaraldehyde-fixed material.

Table 7.1 Artifacts in nerve biopsy specimens (paraffin and resin histology)

Procedure	Appearance	Cause
Biopsy	Acute inflammatory cells in vessels (Fig. 7.9)	Excessively long procedure
	Hemorrhage	Trauma
	Split of myelin sheath at SL clefts (Fig. 7.1)	Stretch trauma
	Focal distortion and ballooning of myelin and axon	Stretch trauma
	Invagination or rupture of vessels or perineurium (Fig. 7.3)	Crush trauma
	Vacuolation of tissue, loss of stain affinity (Fig. 7.7)	Electrocoagulation
Fixation	"Neurokeratin" artifact (Fig. 7.2)	Formalin fixation
	Shrinkage, with crescent-shaped fascicles and loss of fiber circularity (Fig. 7.8)	Hyperosmolarity of primary fixative
	Swelling of cells, loss of resolution, dissolution of membranes	Delayed fixation, autopsy material
Trimming	Oblique myelin profiles	Malorientation
	Perineurial wrinkles	Incomplete trimming of epineurium
		Incomplete heat stabilization of sections on hot plate
Embedding	"Popping" of sections, usually at sites of lipid accumulation or degradation, when examined under high-intensity electron beam	Type of resin
Sectioning	Chatter	Dull knife
	Large wrinkles in tissue	Excessive heat on hot plate
Staining	Stain precipitate, contamination	Stain not filtered
	Blotchy staining	Vaporization of solution due to excessive heat on hot plate
	Dark staining with loss of contrast	Sections too thick, excessive staining time

The nerve biopsy procedure has been described in detail (Dyck et al. 1993; Asbury and Johnson 1978). Meticulous technique and delicate handling are essential. Electrocoagulation should not be used during any part of the procedure. While the pathologist is always requesting longer sections of nerve, the surgeon naturally wishes to minimize the size of the incision and we continually are challenged by short segments of nerve with significant superimposed artifact. One unfortunate approach is to pull the nerve down through a small incision, cut a long section of nerve, and then allow the cut end of the nerve to retract deeply into the tissue layers! While this is reasonable for harvesting nerve graft material, it is unacceptable for diagnostic nerve biopsy.

Recognition of artifact is an important part of interpreting nerve biopsies, particularly when contributed by outside institution without our control of fixation and handling. Distorted nerve fascicles and crushed vessels are typically seen near the ends of biopsy specimen (Figs. 7.3a–d); the vessels telescope internally and may superficially appear to be scarred (Fig. 7.3c). Myelin can sometimes disintegrate around an apparently intact axon through splitting at the major dense line, creating a net-like pattern of vesicles 80 nm in diameter. This alteration is seen both as artifact and as part of a variety of neuropathies. The case shown (Fig. 7.4a–c) represents delayed fixation in an autopsied patient and, thus, most assuredly artifact. Delayed fixation may also cause swelling and clearing of the cytoplasm of Schwann cells (Fig. 7.4d). An unusual artifact is creation of "two-toned myelin" (Fig. 7.5a–c) in which there is uneven osmication of the myelin sheaths, likely a result of problems with osmium penetration (King 1999). A common alteration is shown in Fig. 7.6a, b resulting in focal apparent loss of myelin structure which is thought to represent problems with dehydration of the specimen during processing. Thermocoagulation of the nerve produces a vacuolated appearance (arrow, Fig. 7.7a) and makes interpretation of pathology almost impossible and mimics the appearance of active axonal degeneration (Fig. 7.7b).

The importance of optimal fixation for accurate morphometry has been repeatedly demonstrated. The endoneurial area of fixed nerve is reduced by 10–43 % relative to snap-frozen nerve (Behse et al. 1974; Dyck et al. 1979). The most important determinant of the degree of shrinkage is the osmolarity of the fixative solution, with a 10 % shrinkage in iso-osmolar fixative (~300 mOsm) and 43 % shrinkage in hyperosmolar fixative (~640 mOsm) (Fig. 7.8a, b). Whether the increased osmolarity results from higher glutaraldehyde concentration or the addition of sucrose is of no consequence (Dyck et al. 1979). Separation between myelin lamellae is decreased by about 17 % with iso-osmolar fixation (Behse et al. 1974). Axonal area and circularity similarly decrease with increasing fixative osmolarity; a difference of 10–18 % was seen with osmolarity of 900 mOsm compared to 410 mOsm, depending on axon diameter (Ohnishi et al. 1976; Holland 1982). Increasing duration of glutaraldehyde fixation is claimed to have a similar impact: axonal area was reduced by 17 % when fixation for 1 and 12 h was compared (Ohnishi et al. 1974). This was not accompanied by a reduction

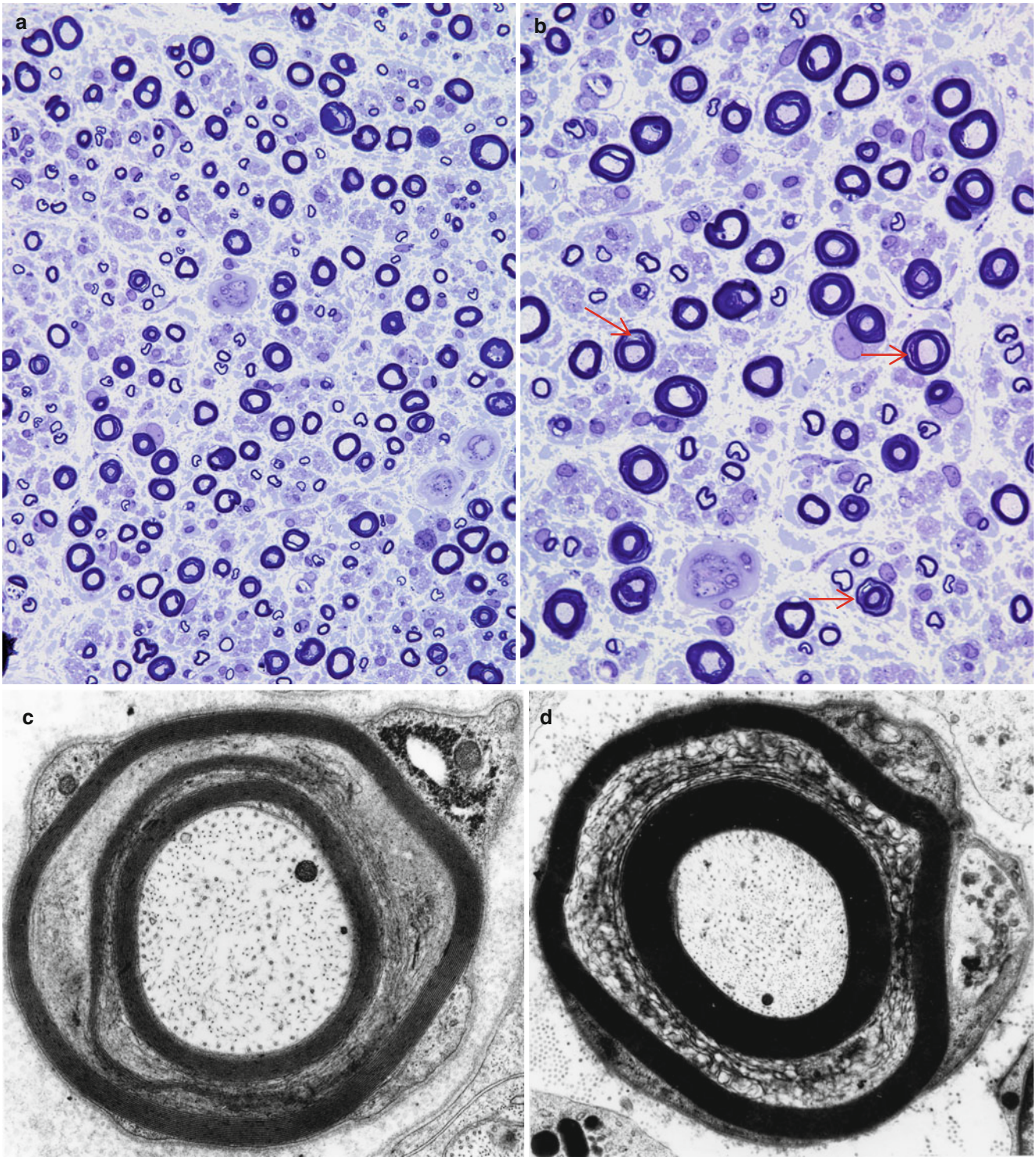

Fig. 7.1 Stretch artifact. Myelin splits (*arrows*, **b**) show a vague resemblance to uncompacted myelin. Large MFs bear the brunt of the insult (**a**, **b** 1 μ thick toluidine blue-stained plastic sections, **a** 400×; **b** 1,000×; **c**, **d** electron micrographs, **c** 21,600×; **d** 11,440×)

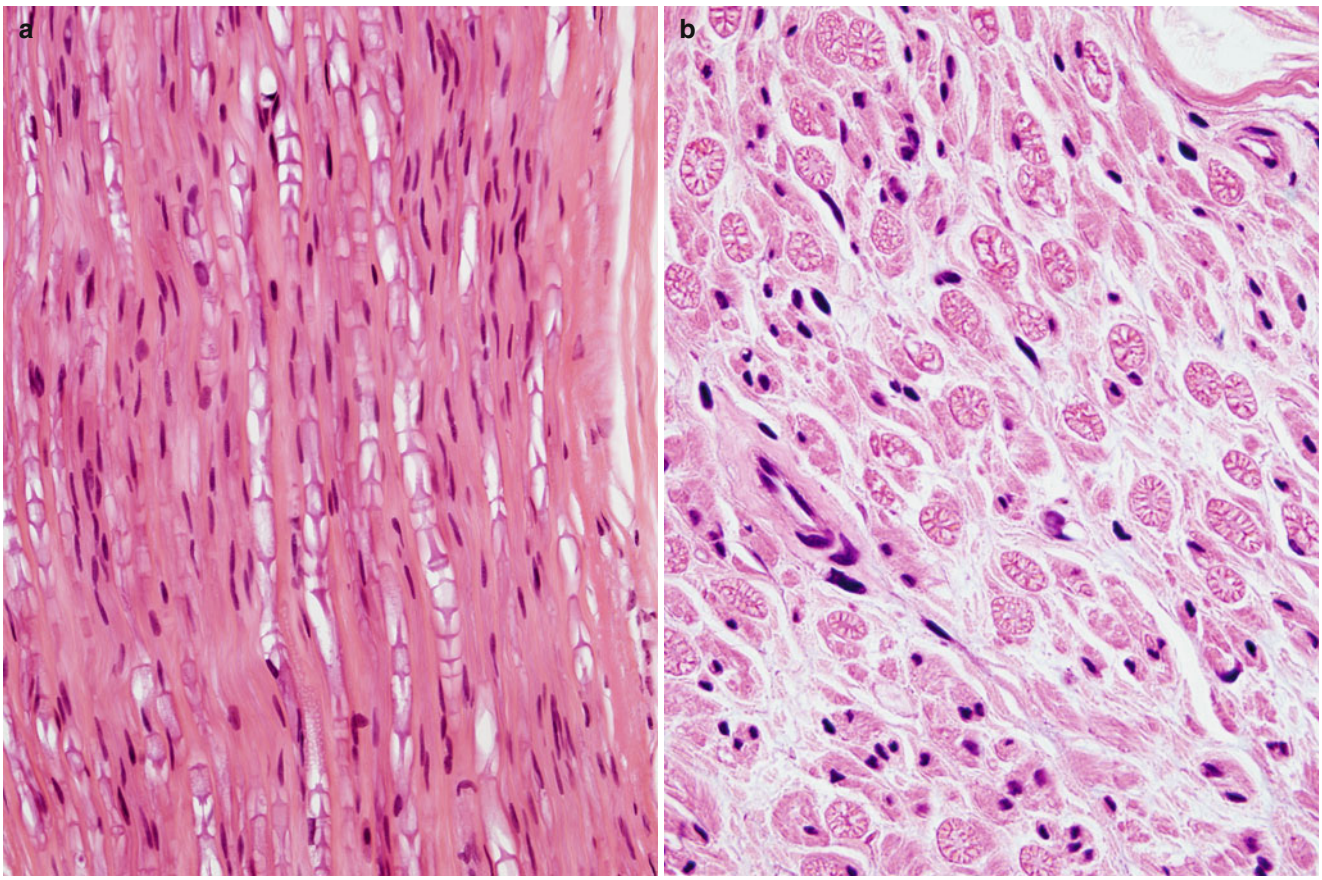

Fig. 7.2 Neurokeratin artifact shown in longitudinal ("herringbone," **a**) and cross section ("wagon wheel," **b**) is commonly seen after formalin fixation (paraffin, 600×)

in fascicular area, and the extra space was said to fill by clefts in the endoneurium (Ohnishi et al. 1974). However, we have noticed only minimal changes in the nerve specimen even when nerves are left in glutaraldehyde over a weekend.

7.4 Light Microscopy

7.4.1 Examination of the Epineurium

7.4.1.1 Fascicular Anatomy

It is worthwhile to record the number and shape of fascicles and quantity of epineurium provided by the biopsy. The typical sural nerve contains 6–15 fascicles, but we have received specimens with only two or three fascicles, perhaps representing an accessory peroneal nerve or the non-fused posterior tibial or peroneal components of the sural nerve. The sensitivity of the biopsy for detection of vasculitis, amyloidosis, malignant cell infiltrations, or granulomata is proportional to the amount of tissue available for examination.

Visibly enlarged nerves suggest a number of diagnoses (Table 7.2). Obliteration of the epineurial and fascicular architecture can be seen in tuberculoid or borderline leprosy.

7.4.1.2 Epineurial Vessels

Arterioles, venules, variable amounts of adipose tissue, and occasional elastic fibers are recognized in the epineurium. Adipose cells appear as optically empty spaces on paraffin sections, whereas in plastic sections, they exhibit varying degrees of osmiophilia; osmium tetroxide fixation prevents dissolution of fat in the alcohol bath. Toluidine blue staining of semithin sections results in green staining of fat when it has not been extracted during processing. Blood vessels in

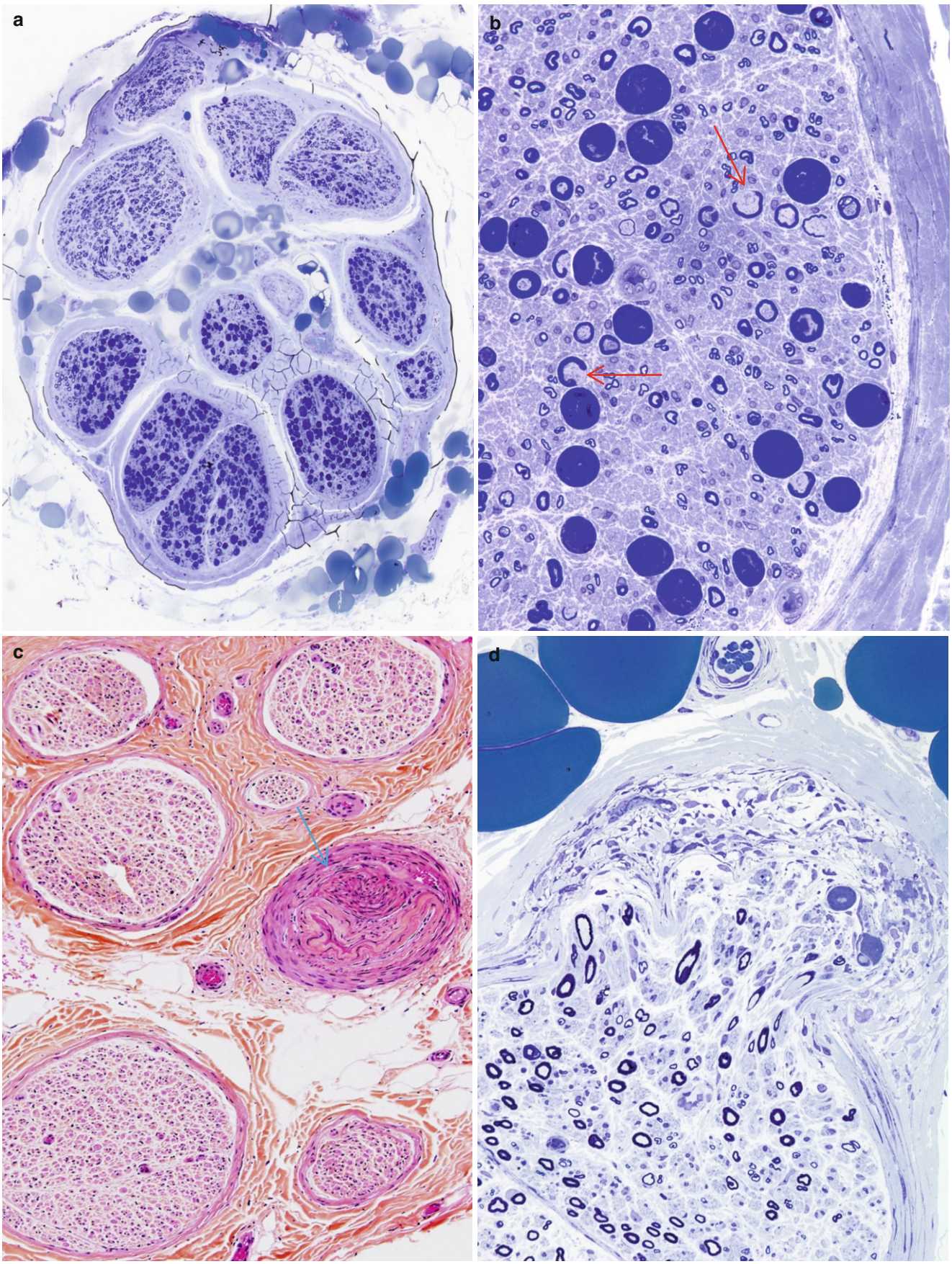

Fig. 7.3 Crush artifact. (**a**, **b**) Note collections of dark staining myelin involving the inferior fascicles of nerve (**a**) which consists of artifactual swellings of MFs with extrusion of axoplasm through artifactual gaps in the myelin sheath (*arrows*, **b**). (**c**) An artifactually telescoped epineurial arteriole (*arrow*) may be misinterpreted as healed vasculitis. In (**d**) disruption of the perineurium with extrusion of endoneurial contents is seen (**a**, **b** 1 μ thick toluidine blue-stained plastic sections, 400×; **c** hematoxylin phloxine saffron, HPS trichrome, 200×; **d**, 1 μ thick toluidine blue-stained plastic sections, 600×)

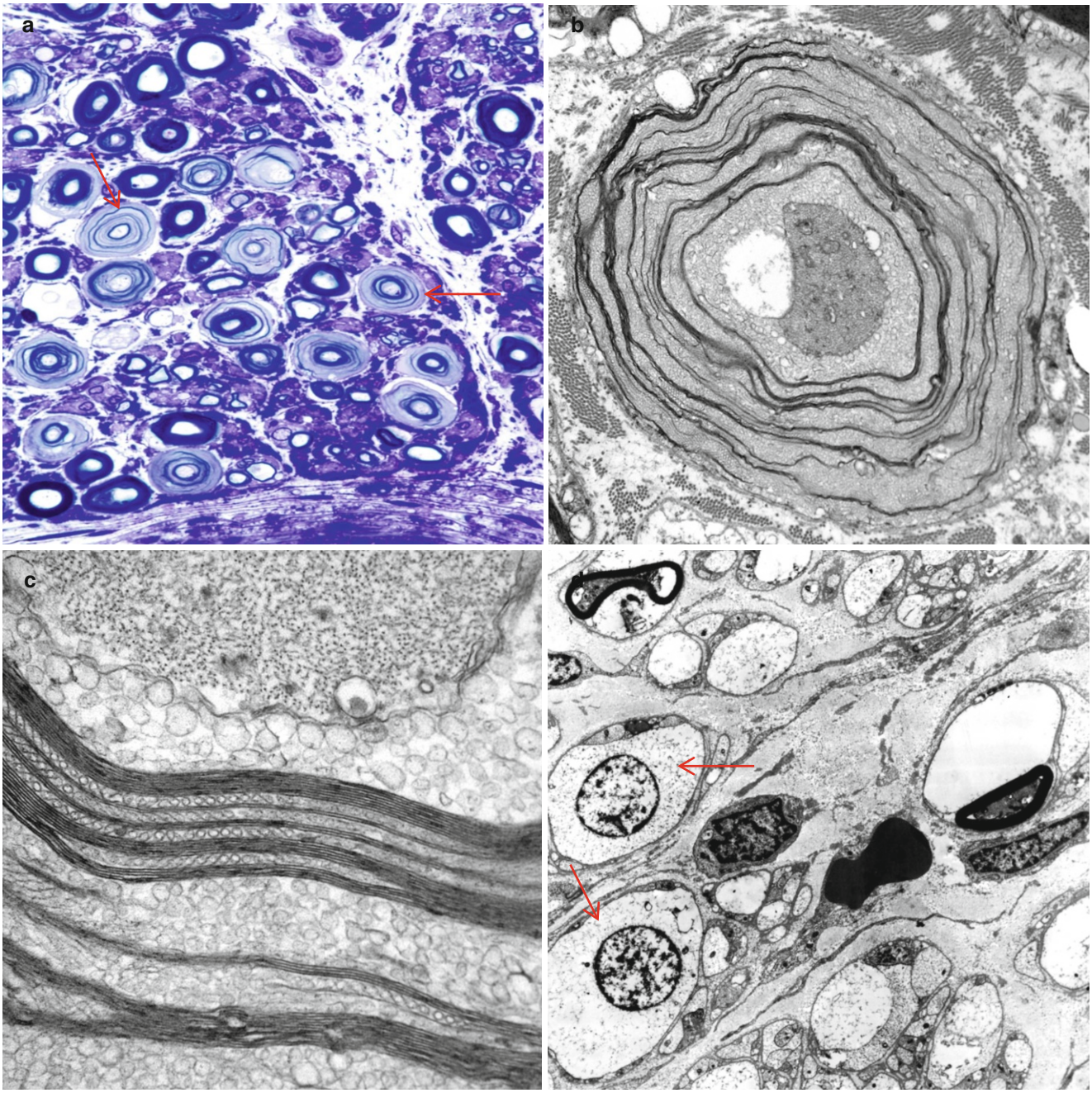

Fig. 7.4 Delayed fixation artifact. (**a**) toluidine blue stains show replacement of normal myelinated axon appearance by pale expanded sheaths (*arrows*). (**b**, **c**) Ultrastructural examination shows that pale sheaths represent myelin which has been completely replaced by vesicular myelin (**b**), seen at higher magnification in (**c**). (**d**) Marked swelling and vacuolation of Schwann cell cytoplasm (*arrows*) in autopsied patient (**a** 1 μ plastic section, 1,000×; **b** 12,000×; **c** 50,000×; **d** 3,000×)

the epineurium are predominantly oriented longitudinally, although some transverse and oblique profiles can be detected at points of branching. Assessment of vessels should begin on paraffin sections with a search for evidence of acute or remote vasculitis (Fig. 7.10, Table 13.2). The caliber, location, and type of vessels most involved should be assessed: epineurial, perineurial, or endoneurial or arterioles or venules. One must avoid overcalling nondiagnostic vascular alterations (Fig. 7.11a, b), even in a patient with appropriate history. When an active inflammatory infiltrate is detected, the cell types should be identified (Table 7.3).

Perivascular cuffing with mononuclear cells is a common observation and is more readily recognized with leukocyte common antigen (LCA, CD45) immunohistostaining or

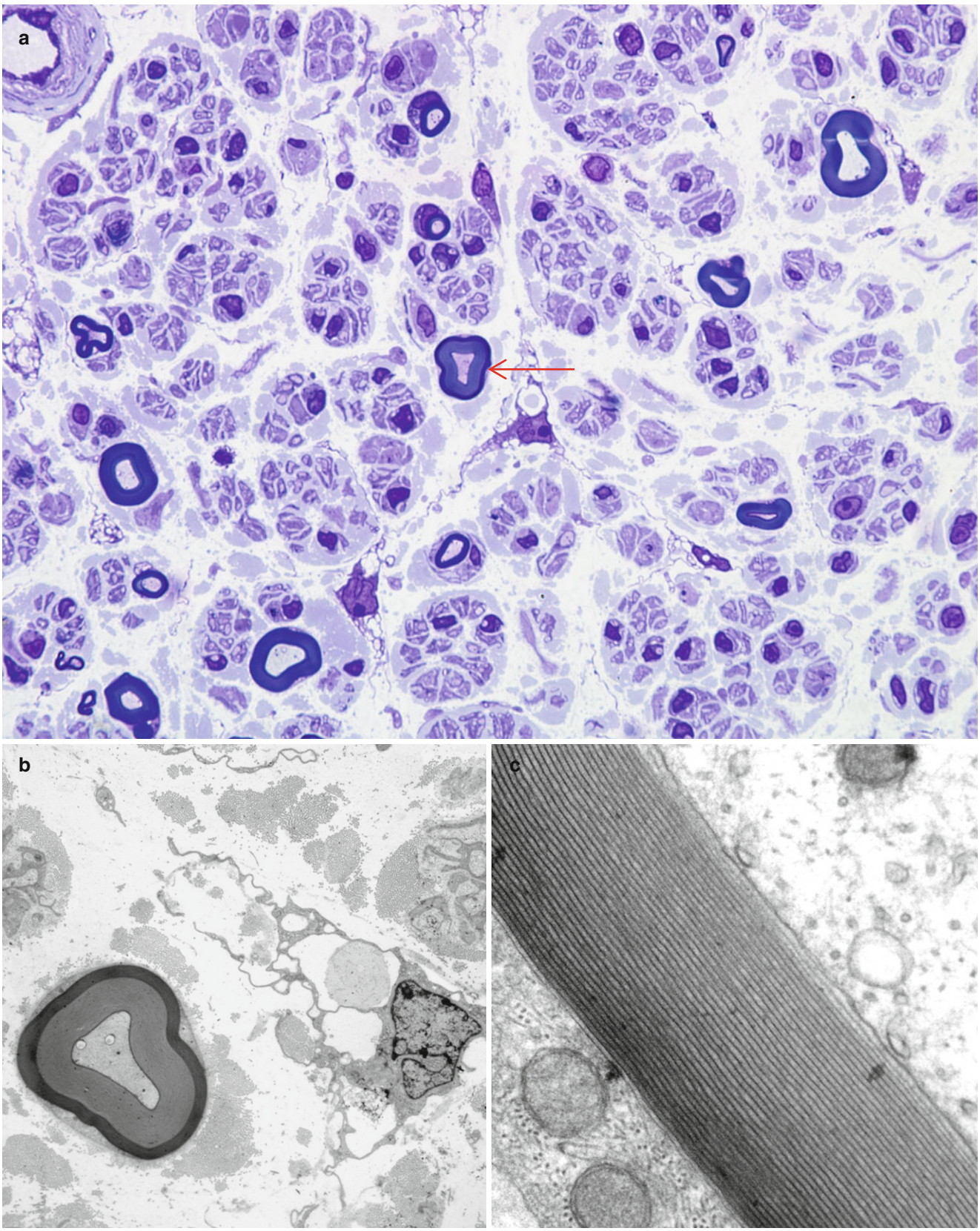

Fig. 7.5 Uneven myelin staining ("two-tone myelin"). Altered density of the lamellae thought to reflect uneven osmication (*arrow*, **a**). (**b**, **c**) Ultrastructural examination of the same axon shown in (**a**) mimics changes in myelin periodicity, a result which is not confirmed at higher magnification (**c**) (**a** 1 μ toluidine blue-stained plastic section, 1,000×; **b** electron micrograph, 5,000×; **c** 100,000×)

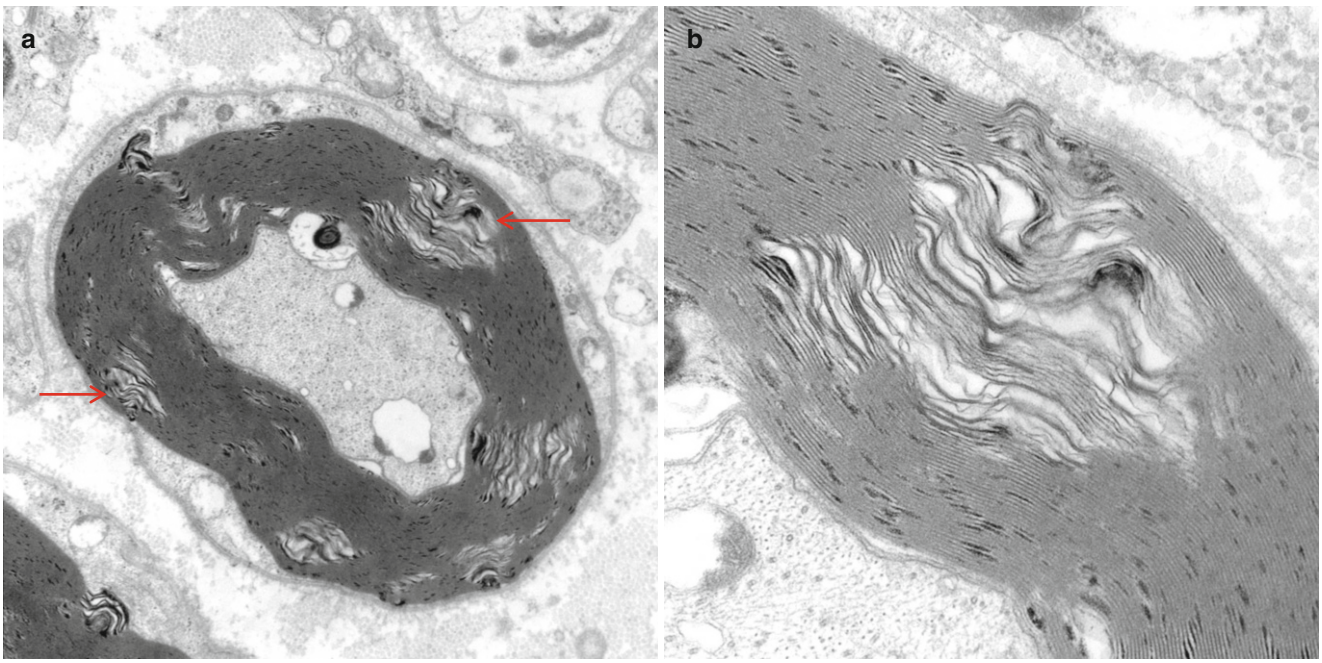

Fig. 7.6 Intramyelinic gaps. These pale separations of myelin seen at low (*arrows*, **a**) and high magnification (**b**) are thought to represent dehydration artifact during processing (King 1999) (**a** 15,000×; **b** 50,000×)

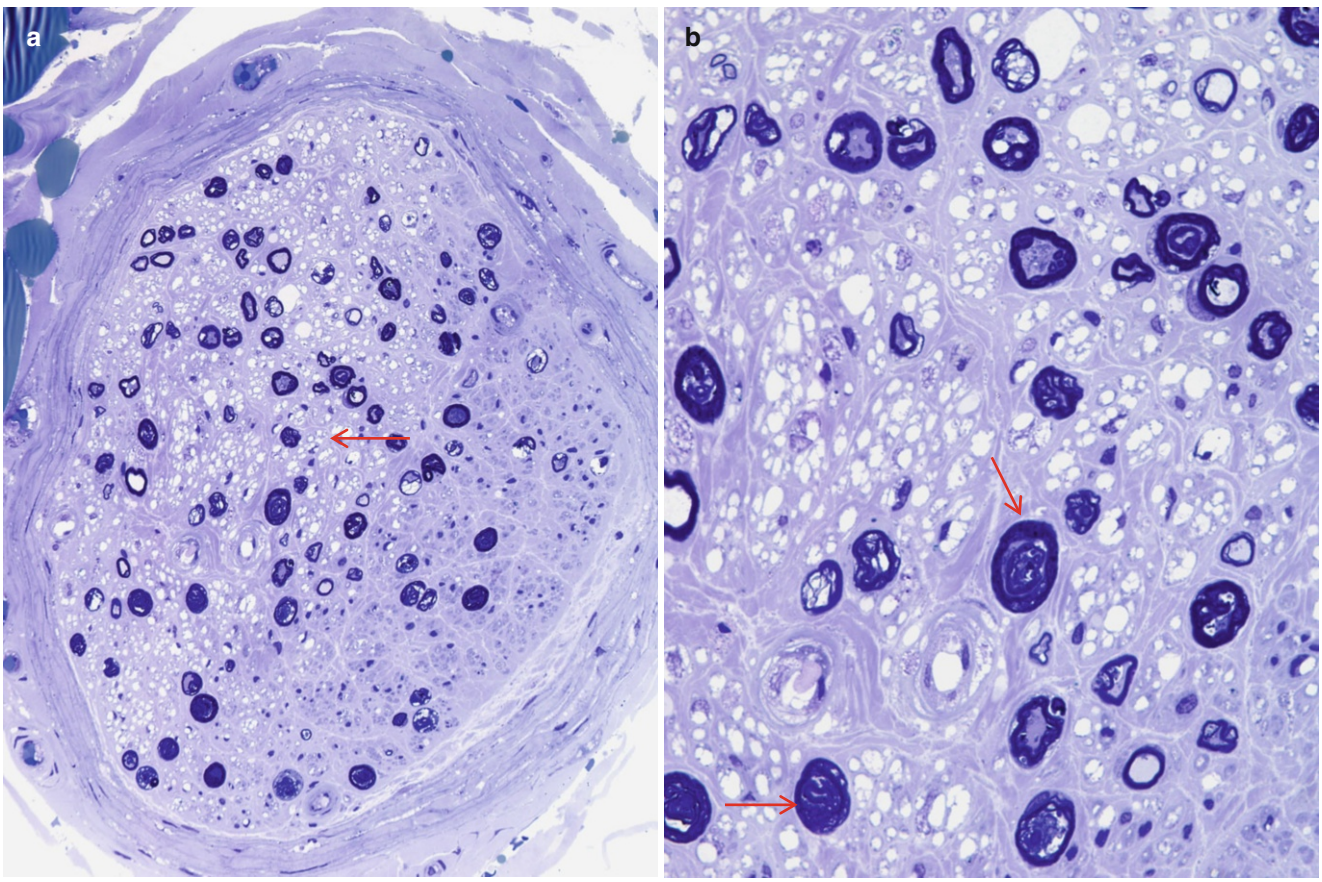

Fig. 7.7 Electrocoagulation artifact. Note vacuolation and pallor of endoneurium (*arrow*, **a**). At higher magnification, the distortion and ballooning of large MFs (*arrows*, **b**) mimics Wallerian degeneration (1 μ thick toluidine blue-stained plastic sections) (**a** 400×; **b** 1,000×)

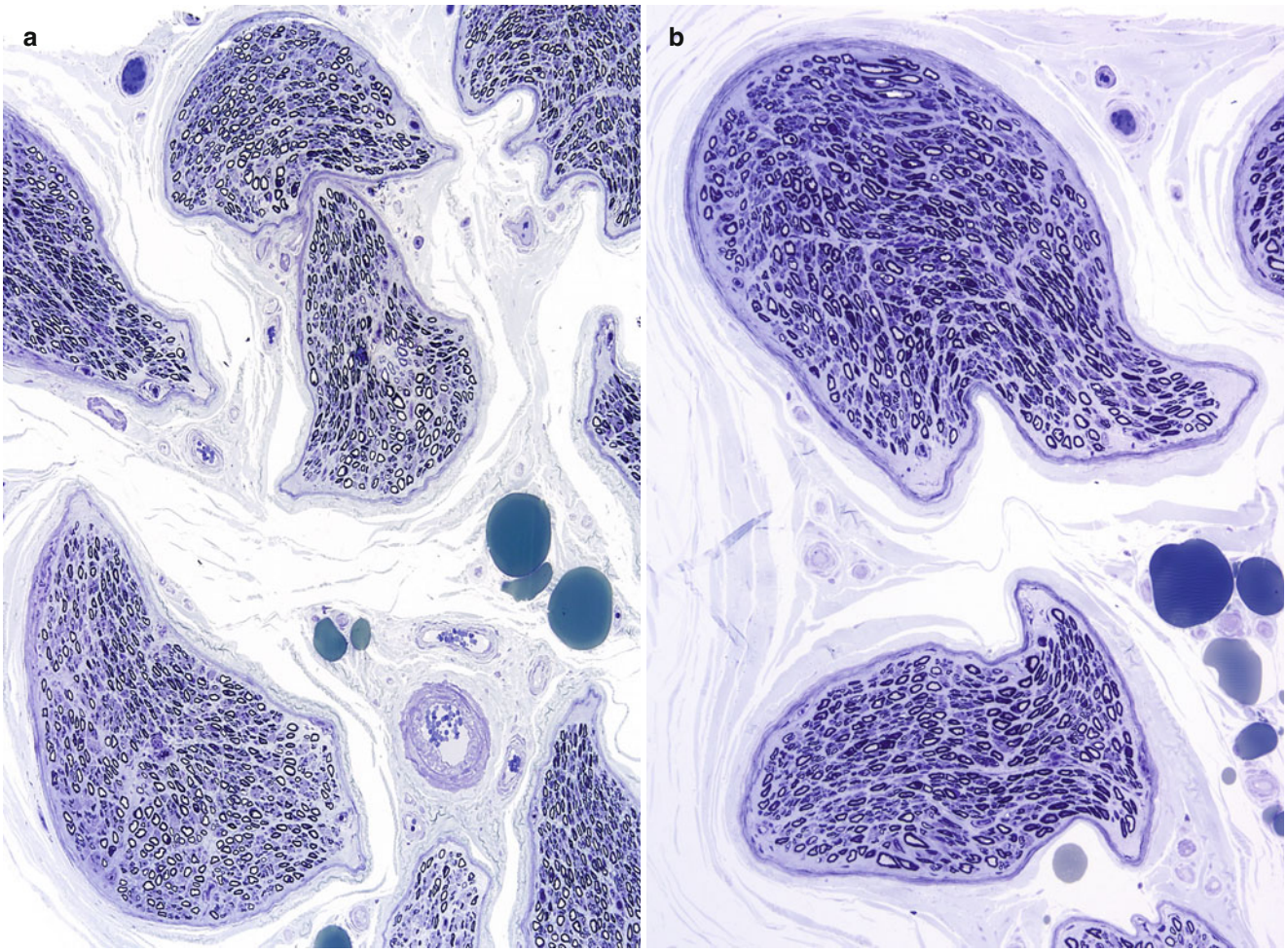

Fig. 7.8 Hyperosmolar artifact. (**a**, **b**) Note shrunken crescentic fascicles and loss of fiber circularity (1 μ thick toluidine blue-stained plastic sections [**a** 100×; **b** 400×])

Table 7.2 Diseases causing nerve enlargement

Leprosy
"Onion-bulb" neuropathies
CMT-1, Dejerine–Sottas (CMT-3)
CIDP
Refsum disease
Interstitial enlargement
Amyloidosis
Acromegaly
Nerve neoplasm

more specifically using immunomarkers for T (CD3) or B (CD20) cells. Several mononuclear inflammatory cells around the occasional epineurial vessel may be seen in noninflammatory neuropathies and in normal nerves and should not be over-interpreted. A more significant infiltrate is abnormal but nonspecific (Table 7.4). A major problem is deciding where brisk perivascular cuffing ends and vasculitis begins, especially in very small vessels and capillaries. Prominent perivascular cuffing around small vessels has sometimes been called "microvasculitis" and has led to some difficulty in interpretation of the literature. We believe the diagnosis of vasculitis requires evidence of vessel injury; otherwise "perivascular cuffing" is a better description, even if occasional inflammatory cells are found within the vessel wall. Examination of serial sections alternately stained with LCA, Verhoeff–Van Gieson, Martius Scarlet blue, and Perl's ferrocyanide is helpful in this regard. Diffuse or patchy inflammatory cell accumulation, particularly containing atypical cells, in the nerve should suggest the diagnosis of an infiltrative lymphoid malignancy but can also be seen in

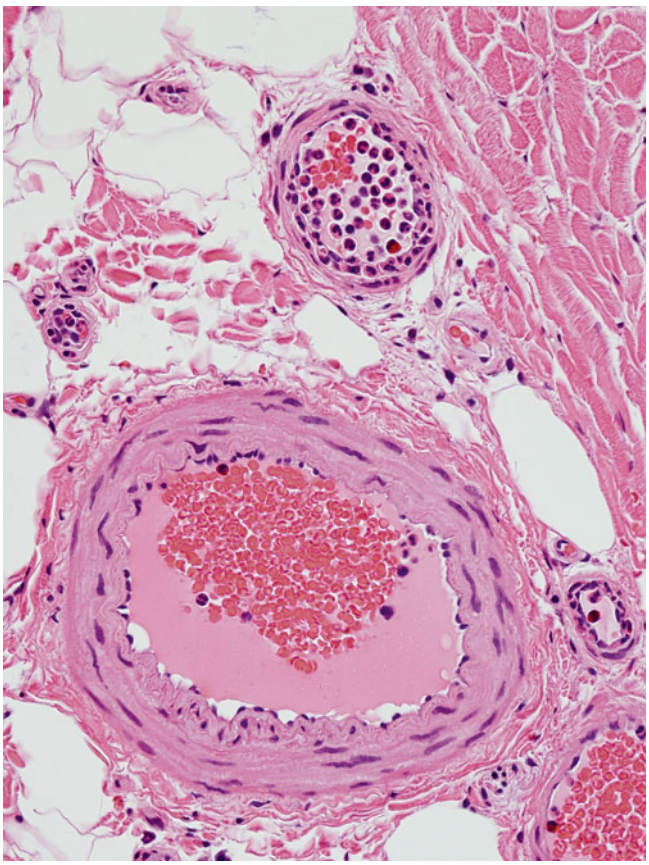

Fig. 7.9 Neutrophil margination and migration through vessel wall is seen in prolonged surgical procedures (paraffin, H&E, 400×)

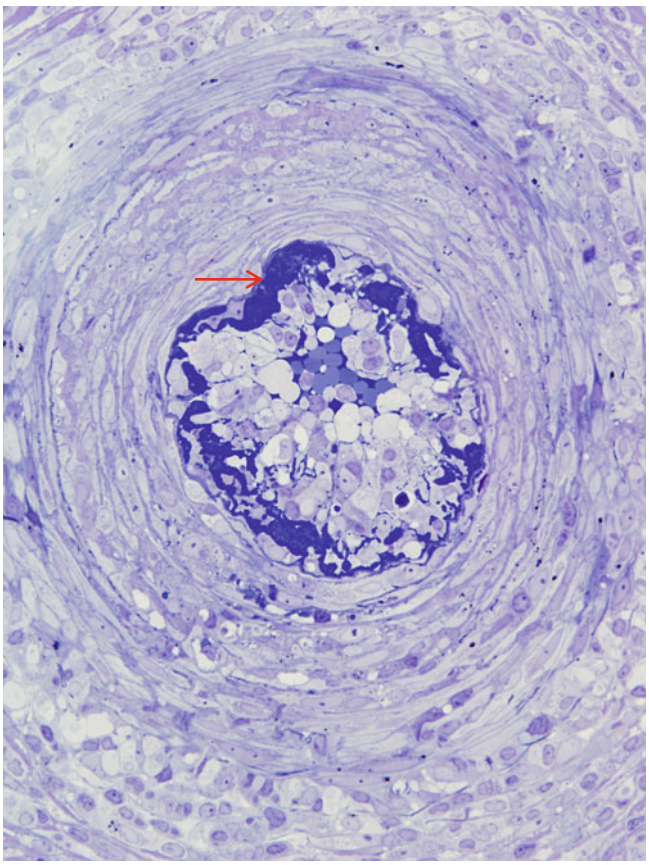

Fig. 7.10 Acute necrotizing vasculitis: In semi-thin sections fibrinoid necrosis is osmiophilic and dark blue (*arrow*). (1 μ thick toluidine blue stained plastic sections)

lymphomatoid granulomatosis, leprosy, and vasculitic neuropathy.

Epineurial vascular proliferation provides indirect evidence of vascular compromise and is seen most commonly in remote vasculitis and diabetes but has also been described in Castleman disease and leprosy (Schroder 1986; Donaghy et al. 1989; Jacobs et al. 1993). Very rarely, the intravascular contents themselves can provide a specific diagnosis, such as intravascular lymphoma (Vital et al. 1989) or cholesterol embolus syndrome (Bendixen et al. 1992).

7.4.1.3 Other Elements of the Epineurium

We routinely examine a Congo red-stained cross section of the full nerve under polarized light and/or thioflavin-S by immunofluorescence. We will review multiple sections through the entire block if amyloid is specifically considered by the clinician. Amyloid deposits may be subtle, and in one instance, we observed them only in association with the epineurial adipose tissue. Proliferation of epineurial connective tissue is nonspecific but if particularly massive, may be suggestive of neuropathy associated with scleroderma (Richter 1954). Focal conglomerates of epithelioid cells or frank granulomata suggest the diagnosis of sarcoidosis, but by definition, there should be evidence of a multisystem process (Chap. 10), and additional diagnoses should also be considered (Table 7.5). The rare observation of Pacinian corpuscles and other sensory nerve organs in the epineurium and perineurium is a normal finding that should not be given pathological significance (Fig. 2.3).

7.4.2 Examination of the Perineurium

Some diseases affecting peripheral nerves have a disproportionate impact on the perineurium, usually in the form of perineurial inflammation. The epineurial and endoneurial compartments may be entirely spared, but more often, there is mild but definite inflammation in these regions also, typically around vessels. This is a nonspecific picture, but suggestive of certain diagnostic possibilities (Table 7.6). Leprosy in

Table 7.3 Significance of neural infiltrates

Cell type	Significance
Lymphocytes	Suggest vasculitis if within vessel wall but otherwise nonspecific (see text)
Neutrophils	Almost always suggest necrotizing vasculitis; however, may represent artifact of excessively long biopsy procedure
Eosinophils	May appear in any necrotizing vasculitis, but favor Churg–Strauss angiitis if very prominent; may occur in hypereosinophilic syndrome, toxic oil, and eosinophilia–myalgia syndromes
Foamy cells	Leprosy, late Wallerian degeneration, lipid storage disease
Epithelioid histiocytes	Sarcoidosis, leprosy
Polymorphic inflammatory infiltrate	Lymphomatoid granulomatosis, any necrotizing vasculitis, leprosy
Plasma cells	Plasma cell dyscrasia, syphilis, vasculitis, Lyme disease, leprosy
Atypical mononuclear cells (monoclonal immunophenotype)	Infiltrative lymphoproliferative disorders, lymphomatoid granulomatosis

Table 7.4 Inflammatory neuropathies

Necrotizing vasculitis
Non-necrotizing inflammatory neuropathy in systemic inflammatory disease (Sjogren's, SLE, etc.)
Inflammatory demyelinating neuropathy (GBS, CIDP)
Paraneoplastic neuropathies
Sensorimotor neuropathy
Vasculitic neuropathy
Subacute sensory neuropathy
Paraproteinemic neuropathy
Perineuritis
Neuropathy with graft vs. host disease
Sarcoidosis
Infectious diseases
Leprosy
HIV DSPN
Lyme disease
CMV neuritis
Chagas disease (Chimelli and Schieber 1994)
Non-Lyme neuritis associated with insect sting
Toxic exposure
Adulterated rapeseed oil intoxication
Eosinophilia

Table 7.5 Granulomatous neuropathies

Sarcoidosis
Noncaseating granulomatous neuropathy (no evidence of systemic disease)
Leprosy (TT, BT, BB)
Churg–Strauss angiitis
Wegener granulomatosis
Lymphomatoid granulomatosis
Angioimmunoblastic lymphadenopathy

particular is notorious for its perineurial involvement, and a search for the organism should be conducted with step sections through the specimen using special stains (fite or auramine rhodamine).

Table 7.6 Relatively selective perineurial inflammation

Leprosy
Idiopathic perineuritis
Toxic oil syndrome, eosinophilia–myalgia syndrome
Cryoglobulinemia (unusual)
Sarcoidosis (unusual)
Lyme disease (unusual)
Lymphoma (unusual)

Calcification of the perineurium is a nonspecific finding in chronic neuropathy (Figs. 2.4b and 2.6b). It is often not detected because tissue preparation methods result in its removal (Van Lis et al. 1979). We have seen perineurial calcification in all three cases of amiodarone neuropathy examined in our laboratory, and this alteration was also significant in a patient with renal failure (Paetau and Haltia 1985). The calcium deposits often have a target-like configuration and are found interspersed between perineurial lamellae. "Ghost bodies" are circular, well-circumscribed, interstitial structures which demonstrate irregular electron density; their presence has been described in a variety of tissues undergoing calcification (Anzil and Palmucci 1983).

Following some nerve injuries, focal proliferation and endoneurial invasion of perineurial cells causes a "microfasciculation." This is a nonspecific response of perineurial cells to injury and has been described in leprosy (Fig. 7.12a, b), diabetes, and sarcoid neuropathy (Pearson and Weddell 1975; Vallat et al. 1991; Thomas and Bhagat 1978; Nesbitt and Acland 1980; Johnson and Kline 1989). The occasional finding of a few disorganized nerve fibers that invade the perineurium and even epineurium, forming a microneuroma, is a response to focal injury of neural and perineurial elements, and we have on occasion observed this in diabetic neuropathy (Figs. 17.2 and 17.4b).

Lipid-filled cells infiltrating the perineurium can be seen in lepromatous leprosy and were particularly prominent in a case of idiopathic perineuritis we have examined (Fig. 10.7) and have also been described in primary biliary cirrhosis

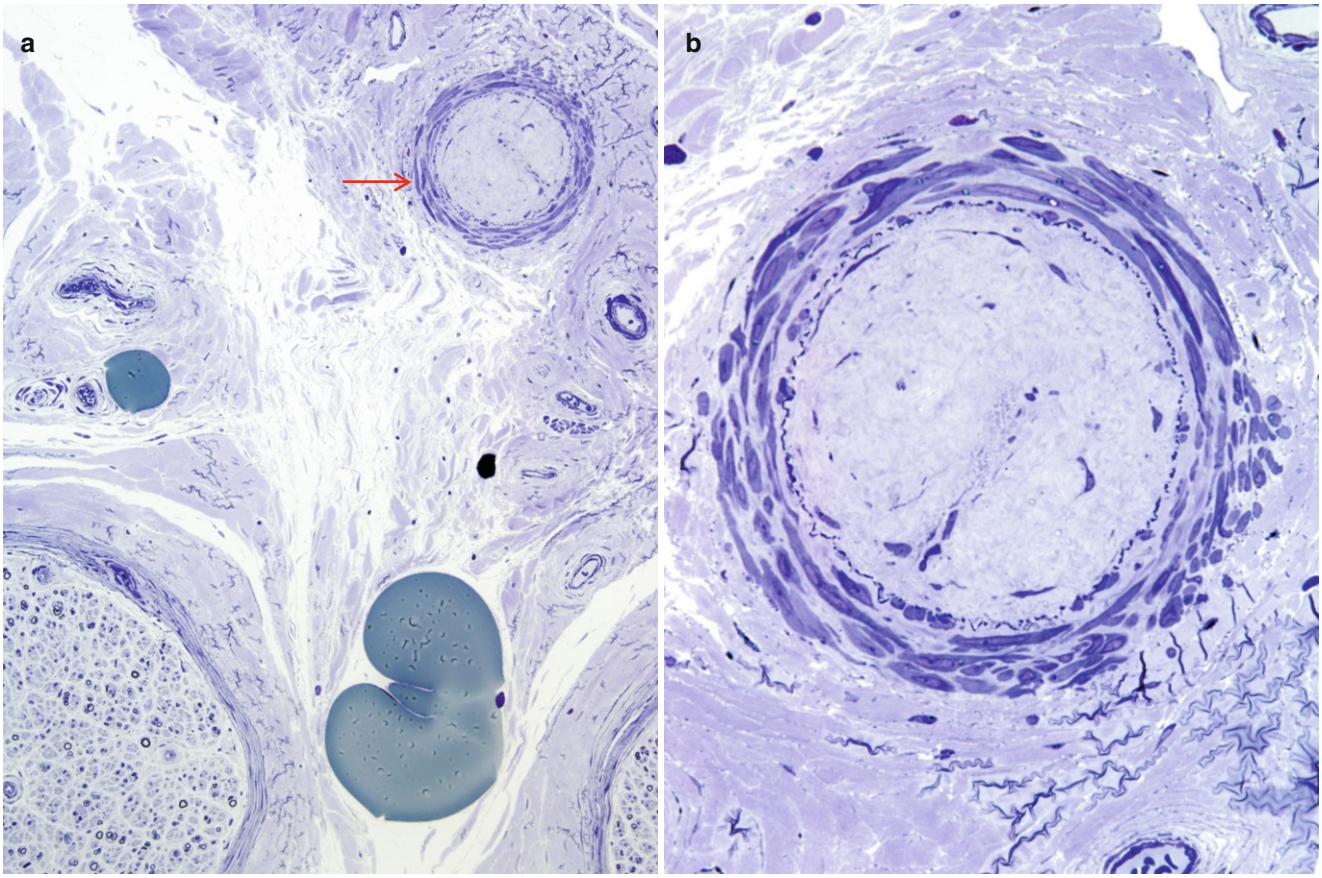

Fig. 7.11 Non diagnostic vascular alterations (**a**, **b**): This non-diabetic presented with mononeuropathy multiplex. An epineurial arteriole (*arrow*, **a**) shows hyaline luminal obliteration with preservation of internal elastica. Although suggestive this lesion is insufficient for a diagnosis of vasculitis. These were the only vascular alterations seen in the entire specimen. (1 μ thick toluidine blue stained plastic sections [**a**, 200×; **b**, 600×])

(Thomas and Walker 1965). There may be an association with high serum cholesterol. Perineurial cells may accumulate lipid in Fabry and Niemann–Pick diseases and in the neuropathy seen with amiodarone and other amphiphilic cationic toxins. Polarized light examination of fresh frozen tissue demonstrates a "Maltese cross" pattern in some lipid storage diseases (Fig. 20.10), but this is a nonspecific property of anisotropic cholesterol esters (Weller 1967; Hirsch and Peiffer 1957).

7.4.3 Examination of the Endoneurium

7.4.3.1 Light Microscopy: Characterization of the Neuropathic Process

The pathologist must decide if a neuropathy is present and, if so, identify the process involved.

Is There a Loss of Myelinated Axons? Are Large or Small Fibers Selectively Affected?

With toluidine blue sections, at a magnification of 20×, we achieve a quick estimation of whether there is severe, moderate, mild, or no discernible axon loss. Given the wide range of normal, it is not possible to detect anything less than 25 % axonal depletion, although a fiber frequency–diameter histogram may show a significant alteration in large to small myelinated fiber number ratio even with a normal total myelinated fiber number (see Chap. 3 for additional discussion of quantitative morphometry). Even without morphometry, an impression of the relative proportion of large and small myelinated fiber loss should be obtained, the former being more commonly affected, regardless of etiology. A selective loss of small myelinated fibers (Fig. 7.13a) confirmed by morphometry (Fig. 7.13b) is a finding of considerable importance, with a limited differential diagnosis (Table 7.7). Light microscopy does not readily permit assessment of unmyelinated fiber numbers on routine sections, even under oil immersion, although early workers used silver impregnation to derive good estimates of unmyelinated fiber counts and even diameter (Thomas et al. 1993). Johnson and colleagues (1994) have recently advocated the use of light microcopy with immunocytochemical labeling of unmyelinated axons for rapid estimation of UF density.

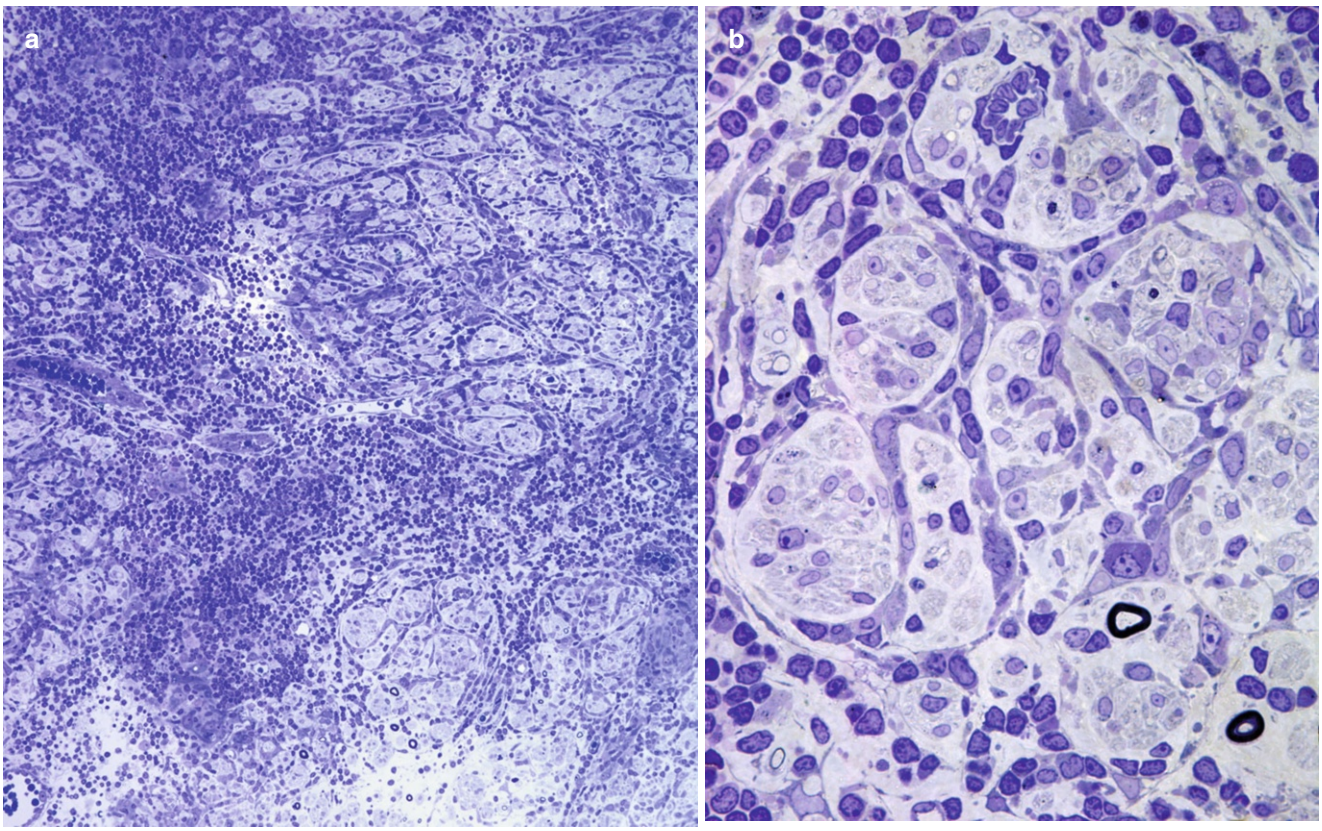

Fig. 7.12 Leprous neuropathy **a**, **b** Microfasciculation is caused by a reactive proliferation of perineurial cells. (1 μ thick toluidine blue stained plastic sections [**a** 400×; **b**, 1000×])

Table 7.7 Selective small fiber/unmyelinated fiber loss

Amyloidosis
Small fiber diabetic neuropathy
Fabry disease
Tangier disease (pseudosyringomyelic form)
Hereditary sensory and autonomic neuropathies
Alcoholic neuropathy (rare)
Idiopathic acute pandysautonomia (Kita et al. 1984, Pavesi et al. 1992
Idiopathic chronic anhidrosis (Case Records MGH 1994)
Dimethylaminopropionitrile toxic neuropathy (see Table 18.2)

What Is the Tempo of Axonal Loss?

If axonal degeneration is identified, its temporal course should be assessed. The earliest changes of acute axonal degeneration discernible on light microscopy are pallor of the normally lightly staining axoplasm and loss of the punctate densities which represent mitochondria (Fig. 4.13). A more advanced stage of Wallerian degeneration (the term "active axonal degeneration" is used synonymously) is identified by the presence of myelin ovoids (Fig. 4.14). In longitudinal sections or teased fibers, the ovoids can be readily seen as longitudinal arrays of myelin debris aligned within the former axonal space (Fig. 4.14e). On cross sections, a single myelin ovoid per average-sized fascicle may be normal, especially if the total population is acceptable and no other changes are seen. In older patients (>60), one may accept two or three degenerating axons per fascicle as within normal limits or consistent with the "neuropathy of aging." It is also important to be familiar with artifactual changes that mimic axonal degeneration (Figs. 7.5, 7.6, 7.7, 7.8, and 7.9).

The process is obviously chronic when a reduction in axon numbers is present with little or no ongoing Wallerian degeneration. Acute massive axonal degeneration is frequently observed in vasculitis and in toxic neuropathies, while an indolent process is seen in most genetically determined neuropathies. Acute degeneration superimposed on chronic axon loss is often seen in vasculitic neuropathy.

Is There Axonal Regeneration?

The presence of three or more closely apposed myelinated fibers (regenerating clusters) is an indicator of myelinated fiber regeneration. Morphometric studies indicate that up to 20 clusters per mm^2 is a normal finding and up to 40 may be present. Thus, as a rough guideline, one or two such clusters per average-sized nerve fascicle are not necessarily

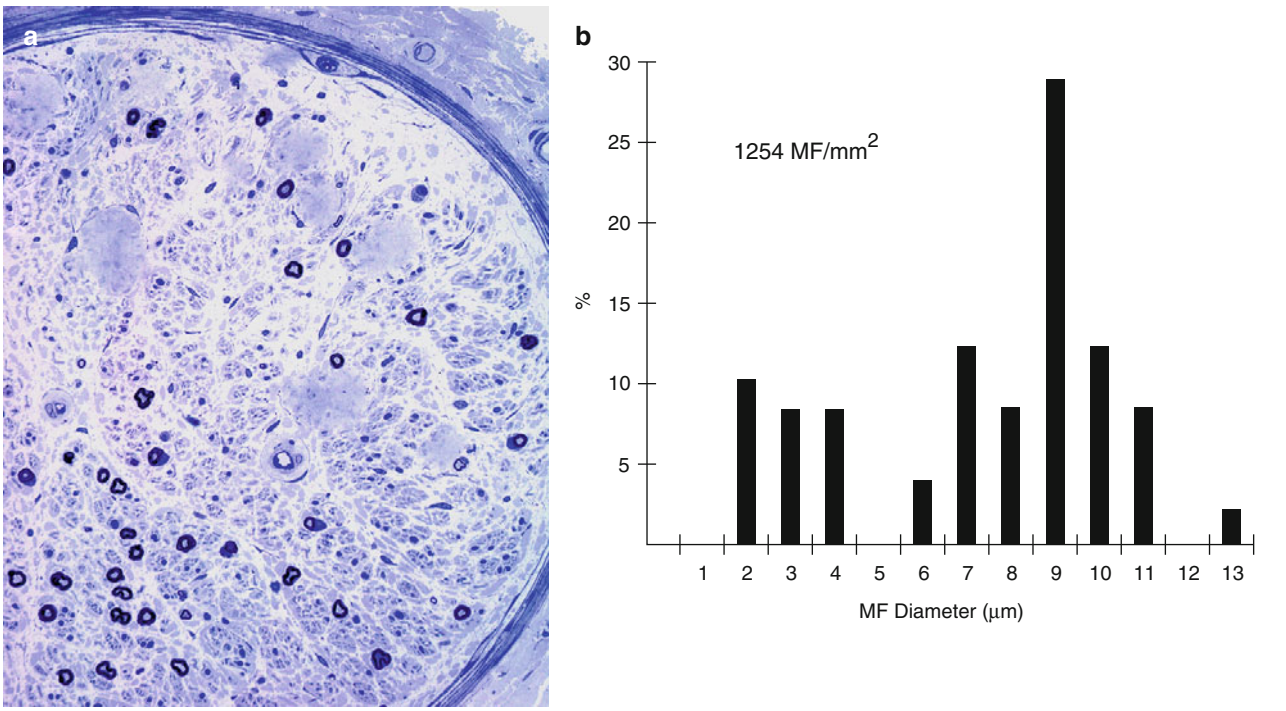

Fig. 7.13 Amyloid neuropathy Note severe axonal depletion with relative sparing of large MFs which is confirmed by morphometric analysis (**b**). (**a**: 1 μ thick toluidine blue stained plastic section [400×])

abnormal. When there is severe axon loss, it is better to estimate the number of clusters per 1,000 myelinated fibers, 2.0 representing the upper limit range of normal. Regenerative activity may occur in any axonal neuropathy and is thus nonspecific. Nevertheless, we have consistently been impressed by the prominence of regenerating clusters in CMT-2. In any axonal neuropathy, lack of regenerating clusters suggests a neuronopathy, such as that seen with sensory ganglionopathies, some spinocerebellar degenerations, and perhaps amyloid neuropathy.

Is There Active Demyelination?

Active demyelination is evidenced by the finding of previously myelinated axons whose myelin is absent or degenerating, often in the presence of myelin debris-filled macrophages. Even a single actively demyelinating axon is probably abnormal and suggestive of a demyelinating neuropathy, but more is needed to permit a confident diagnosis. This may be very obvious on light microscopy, especially with oil immersion, but is best assessed ultrastructurally, where preservation of the axon can be proven, and the suspected demyelination examined more closely. It is essential to determine whether adjacent debris-filled cells are passive clearers of degenerate myelin or are causing myelin destruction. The latter observation ultimately requires electron microscopic confirmation (vide infra). The rare cross section of an MF through the node of Ranvier should not be taken for a demyelinated axon; axoplasm at this site is denser than normal (arrow, Fig. 7.14).

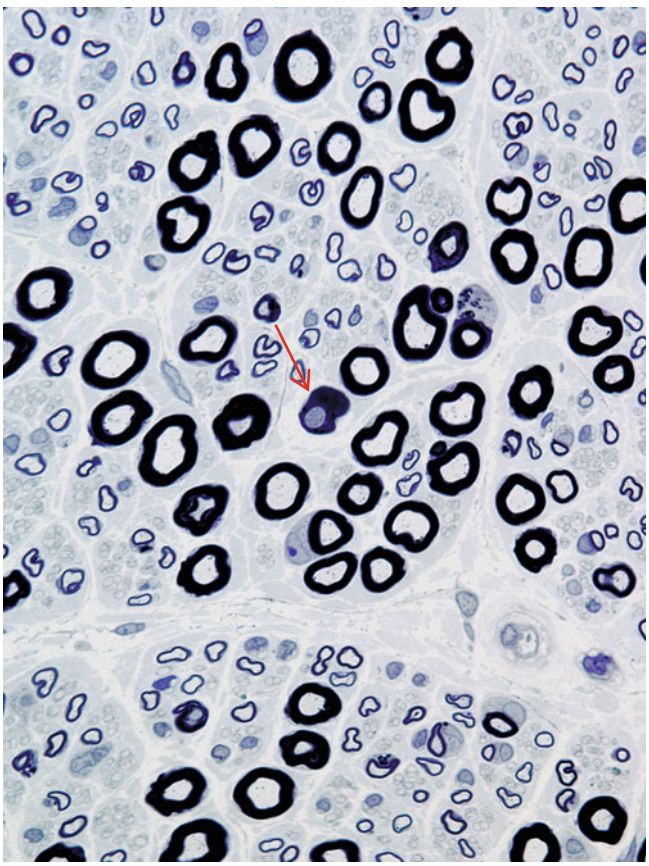

Fig. 7.14 Increased density of axoplasm and abnormal configuration of myelin (*arrow*) represent a section through the nodal-paranodal region (1 μ thick toluidine blue-stained plastic sections)

Is There Demyelination or Remyelination?

Axons with an attenuated myelin sheath suggest remyelination after demyelination. However, this may also be seen in primary hypomyelination or around regenerating axons. Thickness of the myelin sheath is best assessed on electron micrographs but can be reliably determined with semithin (1 μm thick) plastic-embedded sections. Thicker sections may result in overestimation of myelin thickness if the section is not precisely perpendicular to the long axis of the nerve fiber (Dyck et al. 1968). The G-ratio provides a graphic illustration of the state of myelination. The finding of universal hypomyelination is strongly suggestive of an inherited demyelinating or dysmyelinating neuropathy, the classic example being Dejerine–Sottas syndrome (CMT-3). We have also seen universal hypomyelination, albeit not as severe (i.e., lower G-ratio) in metachromatic leukodystrophy (Fig. 20.1), Niemann–Pick disease (Fig. 20.14), and infrequently in CIDP. More typically in CIDP and in CMT-1, myelin thickness varies widely, some fibers falling within the normal range and others showing excessively thin myelin.

Are Onion Bulbs Present?

The hallmark of recurrent demyelination with remyelination is onion-bulb formation. Paraffin-embedded material is very poor at showing these structures; plastic sections are much more reliable. Onion bulbs are never seen in normal nerve. Electron microscopy may detect rudimentary onion bulbs overlooked by light microscopy and helps exclude "pseudo"-onion bulbs (vide infra). Although onion bulbs have been described in many neuropathies (Table 7.8), the differential diagnosis of neuropathies where onion bulbs are a dominant feature is limited; in practice, the differential diagnosis is quickly narrowed to CMT-1 vs. CIDP. Detection of rare "early" onion bulbs, with only one circumferential layer of redundant Schwann cell processes, while still suggestive of a demyelinating process, is of lesser differential diagnostic importance as these can be seen in neuropathies not primarily characterized by demyelination, presumably examples of demyelination secondary to axonal influences.

We have observed unequivocal demyelination with numerous onion bulbs in a patient who ultimately was determined to have a tethered cord. Surgical treatment resulted in clinical improvement. While it is possible that this patient suffered from both a tethered cord and an acquired hypertrophic neuropathy (improvement would not be expected in a familial neuropathy), there was no clinical evidence for this, and we suspect that chronic traction on the nerve roots produced repeated episodes of demyelination secondary to axonal changes.

Decision on the Nature of the Neuropathic Process

The information gathered by considering the above questions should be used to construct an overall view of the nature of the process causing the neuropathy: axonal, demyelinating or mixed, and acute, chronic, or both. When seen in isolation, axonal and demyelinating changes are usually obvious. When both are present, however, the central issue is deciding whether, in the presence of axonal abnormalities, demyelinating features are primary or secondary. This is important because primary demyelination has a better prognosis than axonal degeneration and a limited differential diagnosis. Florid demyelinating changes such as naked or actively demyelinating axons favor a primary demyelinating process. Unless prominent, onion bulbs do not necessarily indicate a primary demyelinating process, but their presence favors this possibility.

The most troublesome, and not uncommon, situation is a biopsy which shows chronic axonal loss with a variable number of inappropriately thinly myelinated axons. The relative severity of axonal vs. myelin changes is considered.

Table 7.8 Onion-bulb neuropathies

Genetically determined
CMT-1 (invariable) (Figs. 19.1, 19.2, 19.3, 19.4, and 19.5)
Congenital dysmyelinating neuropathies (frequent) (Figs. 19.14, 19.15, and 19.16,)
Hereditary neuropathy with pressure palsies (frequent) (Figs. 19.14, 19.15, 19.16, 19.17, and 19.18)
Mitochondrial diseases (variable)
Refsum disease (very frequent)
Tangier disease (variable)
Metachromatic leukodystrophy (variable)
Globoid cell leukodystrophy (variable)
Adrenoleukodystrophy (infrequent) (Figs. 20.25, 20.26, and 20.27)
Niemann–Pick disease (infrequent) (Figs. 20.16, 20.17, 20.18, 20.19, and 20.20)
Cerebrotendinous xanthomatosis (frequent)
Xeroderma pigmentosum
Cockayne syndrome
Acquired
CIDP (20 % of biopsies) (Figs. 9.11, 9.12, 9.13, 9.14, 9.15, 9.16, 9.17, 9.18, 9.19, 9.20, 9.21, 9.22, 9.23, 9.24, 9.25, 9.26, 9.27, 9.28, 9.29, and 9.30)
Multifocal neuropathy with persistent conduction block (variable) (Figs. 9.31, 9.32, and 9.33)
IgM paraprotein-associated neuropathy (common) (Figs. 14.2, 14.3, 14.5, 14.6, 14.9, 14.10, 14.11, 14.12)
Non-IgM paraprotein-associated neuropathies (uncommon)
Paraneoplastic demyelinating neuropathies (variable)
Neuropathy of acromegaly (frequent)
Diabetes (infrequent) (Figs. 17.1, 17.2, 17.3, 17.4, 17.5, 17.6, 17.7, 17.8, and 17.9)
Hypothyroid neuropathy (infrequent)
Toxic neuropathies
Amiodarone (infrequent)
Eosinophilia–myalgia syndrome (infrequent)

This list excludes neuropathies where rare "early" onion bulbs have been described

Numerous thinly myelinated axons with little axonal depletion suggest primary demyelination, while seeing the occasional thinly myelinated axon in the face of significant axonal loss suggests secondary demyelination or incomplete regeneration. Between these two extremes, the process is described as "mixed" without further distinction. Elaborate morphometric and teased fiber techniques might resolve the issue of primary or secondary myelin change conclusively, but are time consuming, and the diagnostic value is questionable. Ultrastructural examination may later settle the issue and provide crucial diagnostic information (vide infra).

Assessment of the Distribution of Pathology

Determination of whether the nerve involvement is diffuse, focal, or multifocal is helpful. Diffuse involvement is suggestive of an inherited or toxic/metabolic process, while focal or multifocal pathology is typical of vascular, inflammatory, and infectious disorders. In some hypertrophic neuropathies, this may be the most useful means of distinguishing between CIDP (focal, multifocal) and CMT-1 (diffuse), although the separation is not absolute (Gabreels-Festen et al. 1993). Similarly, a nerve showing multiple areas of active axonal degeneration should always suggest the presence of vasculopathy, even if no specific vascular abnormalities can be identified in the available sections.

7.4.3.2 Light Microscopy: Other Pathological Features in the Endoneurium

Alterations of Myelin and Axons

Giant axonal swellings can be readily seen on LM and if present in sufficient numbers are diagnostic of genetic or toxin-related giant axonal neuropathy (Figs. 19.19, 19.20 and 19.21) (Table 7.12). Electron microscopic confirmation of filament accumulation is necessary. An occasional swollen filament-filled axon is a nonspecific sign of axonopathy or may represent a swollen growth cone (Fig. 4.22a). Although a rare intra-axonal polyglucosan body is a nonspecific finding, especially in elderly individuals, the detection of multiple intra-axonal polyglucosan bodies is suggestive of polyglucosan body disease, Lafora's disease, or type IV glycogenosis.

A nerve biopsy showing several massively hypermyelinated axons with other fibers showing redundant loops is sufficient for a diagnosis of tomaculous neuropathy provided artifact is excluded. While this nearly always implies hereditary neuropathy with liability to pressure palsies, other diagnoses might be considered; genetic analysis may be helpful.

Other Cellular Alterations

The presence of endoneurial mononuclear infiltrates is always significant; LCA immunostaining will highlight these cells. The distinction between inflammatory and neoplastic mononuclear infiltrates relies on other immunohistochemical markers and cytological evidence of atypia. Our experience with immunostaining suggests that one or two scattered lymphocytes (T cells) within a nerve fascicle may be seen in normal nerves. In contrast to the epineurium where an occasional small cuff of lymphocytes around a vessel may be insignificant, we would regard such a finding in the endoneurium as indicative of inflammatory neuropathy. Whether perivascular or randomly dispersed in the endoneurium, it is important to characterize the nature of an infiltrate (Table 7.3). Vasculitis can involve endoneurial vessels, although this is much less common than epineurial involvement. In our experience, the most common cause of selective endoneurial perivascular cuffing is CIDP. Lymphocytes can be scanty in GBS, where macrophages usually make a greater contribution to the endoneurial hypercellularity.

Atypical cells suggest an infiltrating neoplasm, most often lymphoma, but may be seen in paraproteinemic neuropathies without lymphoma (Chap. 14). Cytomegalic cells have been detected in peripheral nerve of HIV patients with mononeuritis multiplex and CMV, and endothelial cells may contain CMV inclusions (Fig. 11.3). Granulomata and giant cells can be present in endoneurium or epineurium and suggest a number of diagnoses (Table 7.5). Increased endoneurial cellularity may also be due to Schwann cell or fibroblast proliferation, a nonspecific response to peripheral nerve injury. Although mast cells increase in number in neuropathy, this is nonspecific. Tenfold enlargement of mast cell granules has been described in Chediak–Higashi syndrome (Chap. 19).

Intraneurial hemorrhage is most often a consequence of vasculitic neuropathy but has also been described in patients with a hemorrhagic diathesis, such a leukemia, immune thrombocytopenia, or hemophilia (Brun et al. 1964; Greenberg and Sonoda 1991; Katz et al. 1991).

Inclusions

Storage material in various endoneurial cell types may be seen in semithin sections, particularly under oil immersion (Table 7.9). Some preservation of metachromasia to toluidine blue may be seen in semithin sections in metachromatic leukodystrophy. However, such observations should be pursued with electron microscopy, where pathological storage material can be distinguished from normal inclusions such as Reich Pi granules (vide infra and, for more detail, in Chap. 20).

Interstitial Components

A clear and widened acellular compartment in the endoneurium or subperineurial area has been called "edema," but the biochemical nature and significance of this observation are uncertain. Typically, this "edema" stains lightly with

Table 7.9 Neuropathies with diagnostically useful inclusions

Infectious
Leprosy
CMV
Toxic
Amphiphilic cationic drugs (amiodarone, chloroquine, perhexiline)
Genetically determined
Sphingolipidoses
Metachromatic leukodystrophy
Globoid cell leukodystrophy
Fabry disease
Niemann–Pick disease
Farber disease
GM1 and GM2 gangliosidosis
Adrenomyeloneuropathy/adrenoleukodystrophy
Batten–Kufs disease
Tangier disease
Cerebrotendinous xanthomatosis
Giant axonal neuropathy
Neuroaxonal dystrophy
Others
Polyglucosan body disease

toluidine blue or Alcian blue and contains a few fibroblasts with very tenuous processes. If no such staining and no cells are seen in this space, it might well represent an artifact of fixation. Nonetheless, true "edema" is most prominent in hypertrophic neuropathies (inherited or acquired) but is also seen in other neuropathies and does not have differential diagnostic utility. Renaut bodies share some features of endoneurial "edema," are seen in about 5 % of nerve biopsies, and have no diagnostic value, although they are associated with chronic compression. Structures that we regard as circumferential Renaut bodies have been described as "subperineurial edema" (Fig. 2.15). Endoneurial fibrosis is a nonspecific finding in many biopsies whose only value is to suggest chronicity.

Amorphous deposits in endoneurium raise the possibility of amyloid or non-amyloid immunoglobulin deposition. Amyloid is highlighted by Congo red staining with polarization microscopy, while non-amyloid immunoglobulin deposition, though PAS positive, is non-refractile. Others have used thioflavin-S to detect amyloid, although occasionally an artificially increased background staining interferes with its utility. Immunohistochemical techniques conclusively demonstrate the immunoglobulin nature of the deposits.

7.5 Electron Microscopy

We carry out ultrastructural examination in about half of nerve biopsies. Appropriate plastic-embedded sections are initially selected by oil immersion light microscopy.

7.5.1 Assessment of Unmyelinated Fibers

Unmyelinated fibers (UFs) are difficult to assess with precision, and we rarely perform UF counts. However, a rough impression of whether UF disease is present can be gained by examination of Schwann cell subunit (ScSu) composition, as discussed elsewhere in this book. Ochoa (1978) and Gibbels (1989) offer authoritative reviews. One may examine the frequency of ScSu denervation, the ratio of Schwann cell profiles to axon profiles, the total number of profiles in a ScSu, and the frequency of collagen pockets and isolated Schwann cell projections. "Denervated" ScSus and collagen pockets are seen normally but increase in number with age and in neuropathy (Kanda et al. 1991: Behse et al. 1975; Ochoa 1978). A sequence of alterations in unmyelinated fibers with increasing severity of neuropathy has been postulated (Behse et al. 1975). The earliest change is an increased number of cytoplasmic processes projecting from Schwann cells, followed by an increase in Schwann cell profiles per ScSu and an increased number of denervated Schwann cell subunits. Measurable reduction in unmyelinated axon numbers is not an early finding (Behse et al. 1975). Unmyelinated fiber regeneration may later lead to a normalization of axon numbers and a decrease in the numbers of denervated ScSu (Behse et al. 1975). "Denervated" ScSus may not simply be a consequence of loss of unmyelinated axons but could result from a proliferation of nonmyelinating Schwann cells and/or increased number of projections from each cell (Kanda et al. 1991).

It is obviously difficult to assess such alterations by inspection alone, and the practical value of making a quantitative effort to do so is dubious; changes in UFs are almost always nonspecific (Behse et al. 1975). We rely upon a subjective impression of denervated ScSu numbers as an indicator of unmyelinated fiber loss. Unfortunately, the presence of this finding varies widely in normals: up to 25 % of ScSus have no axons in some adults and probably even more in older patients (Behse 1990; Kanda et al. 1991). An increased number of denervated profiles per ScSu are helpful, controls showing only 5 % of ScSus with more than six denervated profiles, while in neuropathy 50 % or more of ScSus may have more than 6 denervated profiles (Behse et al. 1975).

Another difficulty is that not all denervated Schwann cell profiles are caused by loss of unmyelinated fibers. Collections of Schwann cells (bands of Büngner) resulting from loss of myelinated axons may be difficult to distinguish from denervated ScSus, but some criteria are useful (Ochoa 1978; Behse et al. 1975) (Figs. 7.15, 7.16, 7.17) (Table 7.10).

Active degeneration of unmyelinated fibers is an unusual observation, occurring in <0.5 % of such axons in normals (Behse et al. 1975). An increased population of tiny (diameter < 0.8 μm) unmyelinated axons may be indicative of unmyelinated axon degeneration and regeneration (Ochoa 1978), and a UF histogram is the best way of assessing this issue.

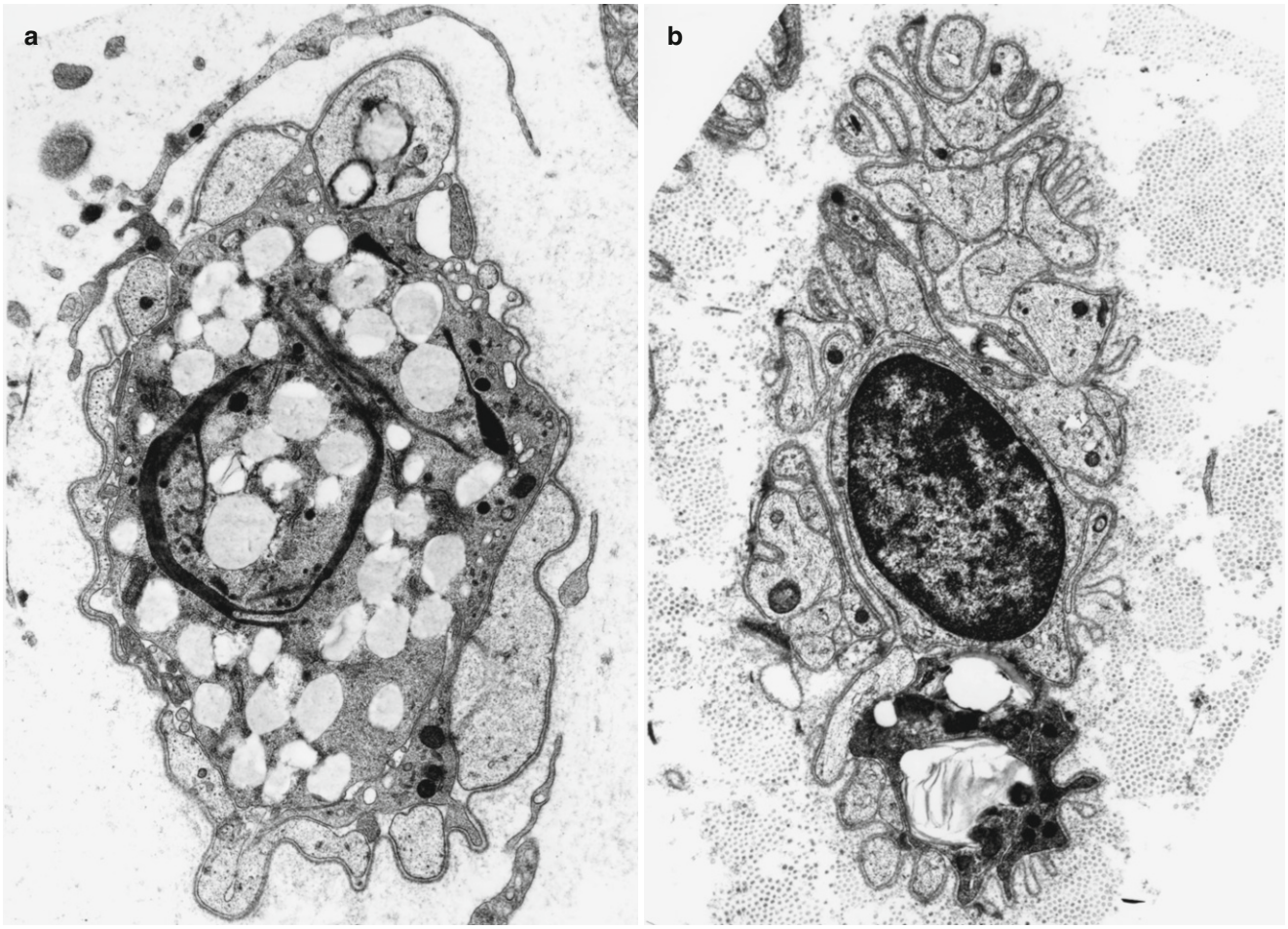

Fig. 7.15 Denervated Schwann cell bands. Residual lipid debris indicates origin from a previously myelinating Schwann cell (**a** 10,055×; **b** 11,332×)

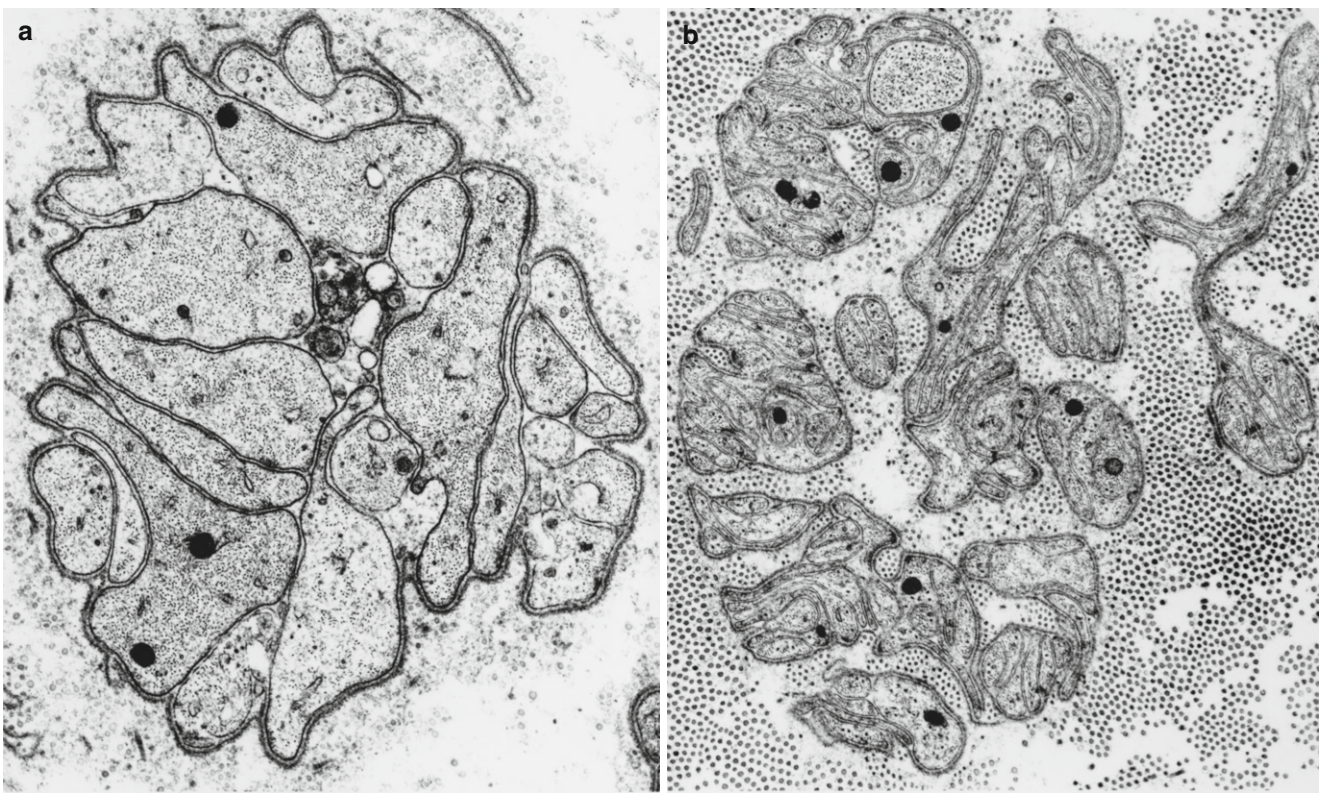

Fig. 7.16 Denervated Schwann cell band from myelinated (**a**) and unmyelinated (**b**) axons (**a** 16,302×; **b** 13,760×)

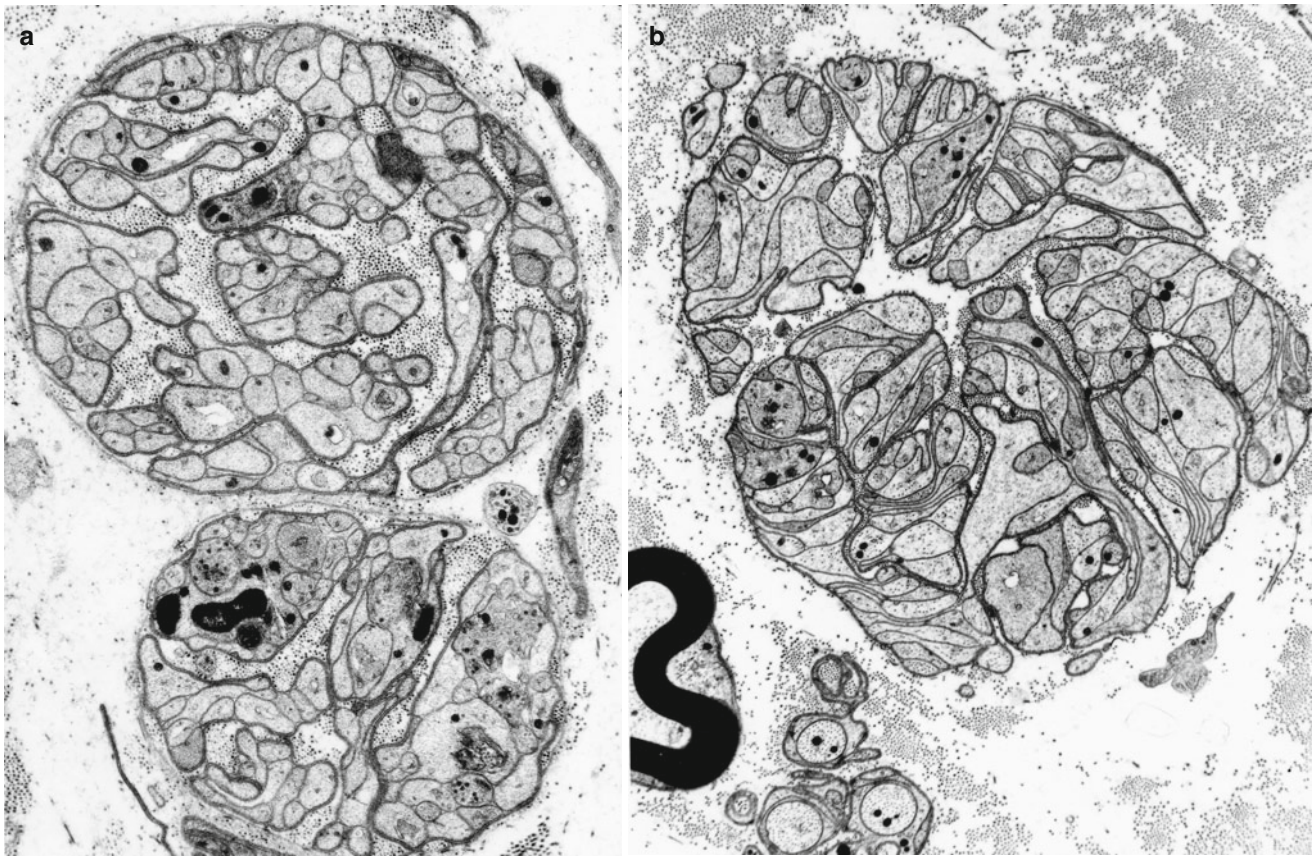

Fig. 7.17 Denervated Schwann cell bands. The origin of these formations, whether from myelinating or nonmyelinating Schwann cells, is uncertain. They may represent denervation of a regenerated cluster (**a** 5,928×; **b** 6,202×)

The study of UF disease is clearly a difficult task, and even if significant unmyelinated axon disease is present, this has minimal diagnostic value unless myelinated axons are relatively spared (Table 7.7).

7.5.2 Electron Microscopy: Assessment of Myelin and Demyelination

A valuable application of ultrastructural examination of peripheral nerves is in assessment of myelin alterations. Electron microscopy can provide confirmation that demyelination is occurring in the absence of ongoing axonal disease (i.e., primary), can show specific patterns of demyelination and myelinopathy, and may identify Schwann cell inclusion material with diagnostic specificity. Alterations suggesting the presence of a schwannopathy are discussed elsewhere.

7.5.2.1 Findings Favoring Secondary Demyelination

When myelin alterations are seen on EM, consistent observation of simultaneous axonal pathology, generally nonspecific (Table 7.11), might suggest that the axonal disease is primary. Particularly, suggestive findings are frequent profiles showing axonal atrophy or focal axonal swellings with organelle or filament accumulations. Redundant and blind myelin loops and intramyelinic splits correspond to the myelin "wrinkling" that is seen in type B teased fiber change (Dyck et al. 1984). This is nonspecific but has been seen in experimental and human models of axonal disease with secondary myelin change.

7.5.2.2 Findings Favoring Primary Demyelination

The myelin itself may show diagnostic alterations, for example, the finding of widely spaced myelin (Figs. 14.5 and 15.4), strongly but not exclusively associated with IgM paraproteinemic neuropathy (Table 5.2). Uncompacted myelin is a less common finding with several diagnostic implications, foremost among them the POEMS syndrome and osteosclerotic myeloma (Table 5.1). The most common and important ultrastructural observation in demyelinating neuropathies is the detection of macrophage-mediated demyelination. When seen in its classical form, with macrophage processes penetrating and destroying a myelin sheath around an intact axon, this is diagnostic of CIDP or GBS and thus has therapeutic

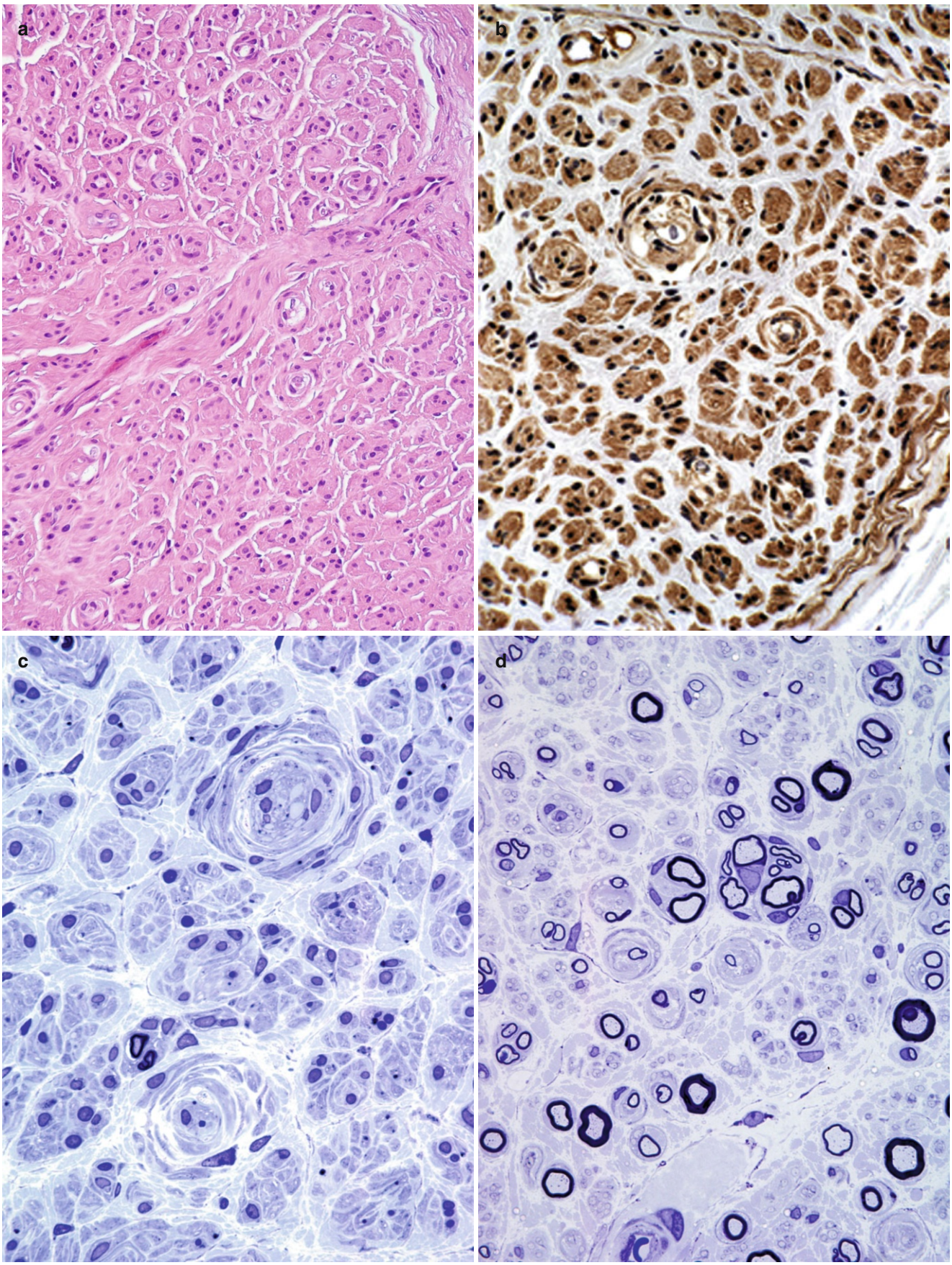

Fig. 7.18 Pseudo-onion bulbs. Using light microscopy, pseudo-onion bulbs closely mimic genuine onion bulbs by both H&E stain (**a**) and with collagen IV (**b**). (**c**) Even this 1 μ thick plastic-embedded nerve section shows large concentric apparent onion bulbs, although the nerve section shown in (**d**) is more convincingly the result of axonal degeneration with thinly myelinated axonal clusters (**a**, **b** paraffin sections [**a**, **b** 400×]; **c**, **d** 1 μ thick toluidine blue-stained plastic sections [**c**, **d** 1,000×])

Table 7.10 Denervated Schwann cell bands

Criterion	Unmyelinated	Myelinated (bands of Büngner)
Size (least useful)	1–4 μm	3–8 μm
Contour of BM	Smooth, regular	Irregular
Myelin debris	No	Sometimes
Other features	Conglomerates of platelike processes	

Table 7.11 Nonspecific ultrastructural changes of axonopathy

Focal accumulation of vesicles (dense core, lamellar, empty, tubulovesicular)
Small aggregates of tubules or filaments not enlarging the axon
Abnormal numbers or morphology of mitochondria
Accumulations of glycogen
Axonal atrophy
Schwann cell–axon networks

and prognostic implications. Vesicular demyelination around an intact axon has also been associated with inflammatory demyelinating neuropathy, but we have not found this to be frequent except in autopsy material. The presence of a scavenger macrophage around an intact axon should be distinguished from macrophage-mediated demyelination.

7.5.2.3 Onion Bulbs

Electron microscopy complements high-power light microscopy in the study of onion bulbs. Their morphologic features almost never help with etiologic diagnosis or even distinguish between acquired or inherited disorders. Parallel arrays of basal laminae can be seen in the onion bulbs of any hypertrophic neuropathy but when very prominent suggest a diagnosis of congenital dysmyelinating neuropathy (including Dejerine–Sottas syndrome). We have also observed this in an atypical adult-onset familial neuropathy clearly not belonging to the Dejerine–Sottas group (Fig. 19.8). Nerves showing very focal regions containing massive bizarre onion bulbs have rarely been seen in multifocal neuropathy with persistent conduction block (Figs. 9.33, 9.34, and 9.35).

Whether the structure observed is a true onion bulb, or simply a regenerating cluster with numerous unmyelinated collateral sprouts forming a "pseudo"-onion bulb, is sometimes difficult to determine (Figs. 7.18, 7.19, and 7.20). Light microscopic studies often are not adequate for separation of true and pseudo-onion bulbs (Fig. 7.18a–d); however, the clinical and family history, labs, and distribution of pathology may contribute significantly to the interpretation of the nerve biopsy. It is normal to see a few unmyelinated axons in a typical onion bulb, but if most of the concentric Schwann cell processes contain axons, the structure under examination is more indicative of degeneration and regeneration than of demyelination and remyelination. Also it is important to know the company the pathology keeps, e.g., a gamut of partly demyelinated forms, their distribution from fascicle to fascicle, etc. Ultrastructural examination may resolve plastic section confusion by demonstrating wholesale loss of axons and numerous Schwann cell bands (Fig. 7.20a, b). Some cases are difficult to assign confidently by pathology alone (Fig. 7.20c), although again history may contribute to assign the pathology to pseudo-onion bulbs as it did in this case.

7.5.3 Axonal Ultrastructural Changes

A variety of nonspecific axonal ultrastructural changes (Table 7.11) have no diagnostic specificity and serve only to confirm the impression of axonal disease if present more prominently than in normals. Only a few specific axonal alterations allow the pathologist to make a diagnosis or at least narrow the differential rather than releasing the unsatisfying report: "nonspecific axonal degeneration."

7.5.3.1 Axonal Swelling with Filamentous Accumulations

This should suggest the possibility of certain toxic neuropathies or giant axonal neuropathy (Table 7.12). The toxic giant axonal neuropathies do not have the focal osmiophilic aggregates seen in typical inherited giant axonal neuropathy. A pedigree with clinical features suggestive of CMT-2 has shown giant axonal change (Vogel et al. 1985). Occasional cross sections showing filamentous accumulation without axon swelling are nonspecific, as we have seen these in cases ranging from CIDP (with myelin sheath intact) to sarcoidosis. Demyelinated/hypomyelinated/dysmyelinated axons may display axonal atrophy with resulting increased filament density (Fig. 9.27).

7.5.3.2 Neuroaxonal Dystrophy

The term "neuroaxonal dystrophy" is often used descriptively in reference to nonspecific alterations in the numbers and morphology of axonal organelles and includes most of the alterations described in Table 7.12. In the specific disease infantile neuroaxonal dystrophy (INAD, Seitelberger disease), these alterations are seen in association with a more specific accumulation of small tubulovesicular elements, occasional prominent clefts, and aggregates of loosely packed parallel linear or circular membranes (compare Figs. 19.26, 19.27 and 4.6).

7.5.3.3 Axonal Inclusions

Inclusions with diagnostic specificity are far less common in axons than in Schwann cells. Amiodarone toxicity (and presumably other amphiphilic cationic toxins), leprosy, and polyglucosan body disease all may demonstrate

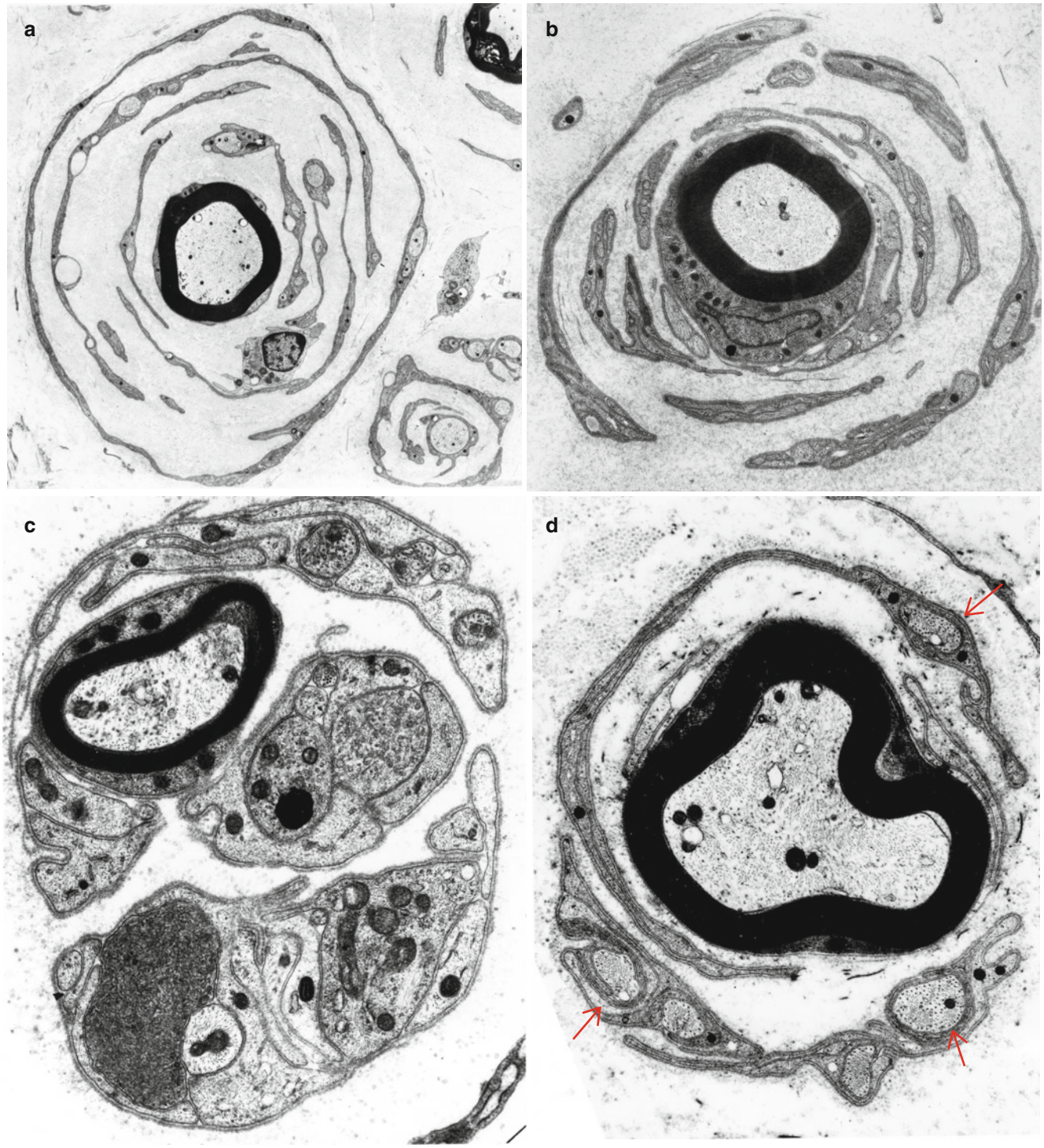

Fig. 7.19 Pseudo-onion bulb ultrastructure. Pseudo-onion bulbs typically show Schwann cell processes which are frequently populated by small axons (**a**–**c**); however, judgment has to be suspended in (**d**), as the configuration seems typical of a genuine onion bulb, yet nearly all surrounding Schwann cell processes enclose unmyelinated axons (*arrows*) (**a**, **b** 10,000×; **c** 15,390×; **d** 10,522×)

pathognomonic axonal inclusions, but in the first two, these are found far more reliably in other endoneurial or perineurial cells (vide infra). Polyglucosan bodies may be found in otherwise normal nerves, but the presence of several, in the appropriate clinical setting, has definite differential diagnostic implications (Chap. 21). Polyglucosan bodies increase in frequency with age (Fig. 21.2); their observation in the pediatric population is always a matter for concern. In some storage

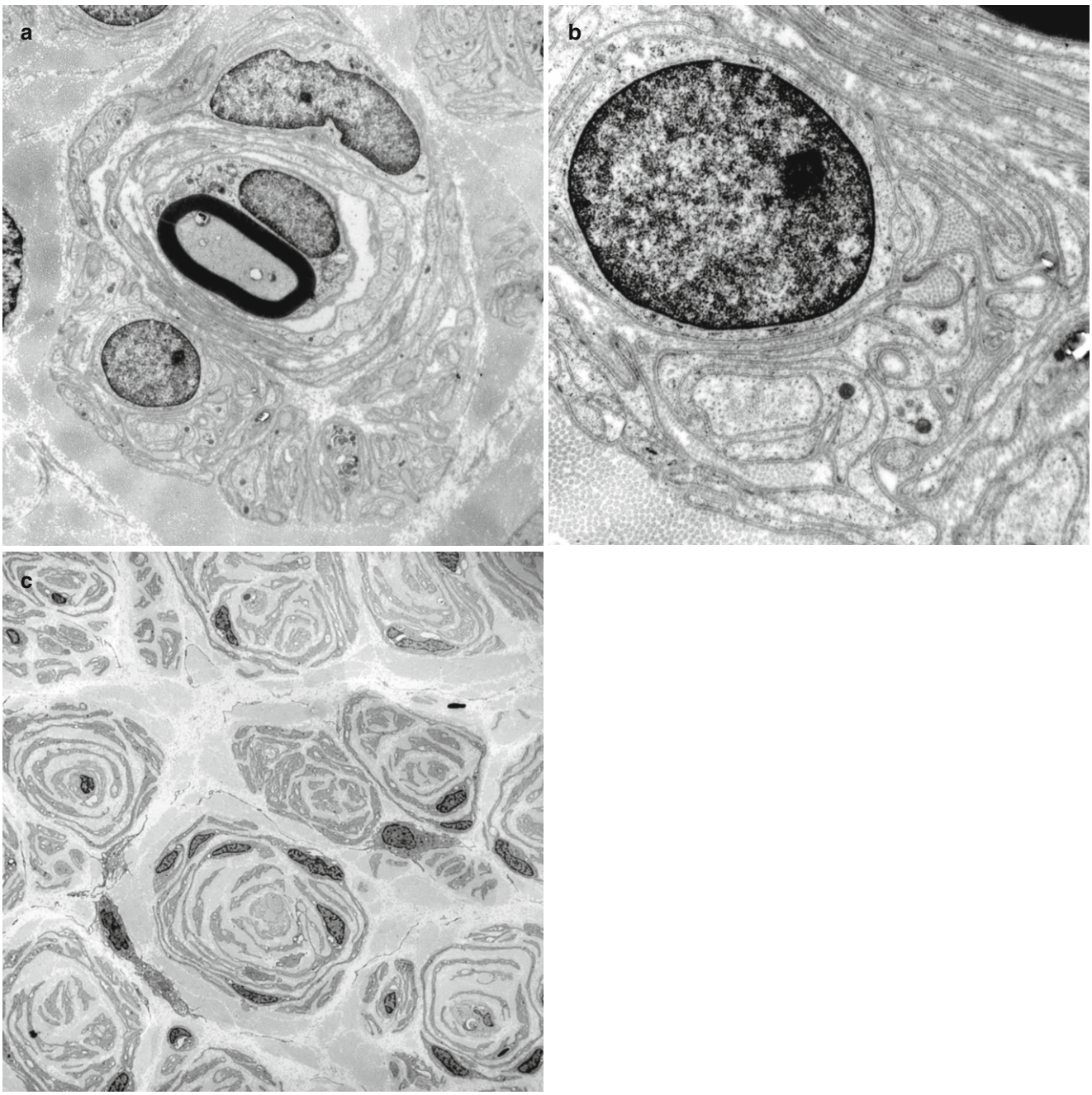

Fig. 7.20 Unusual pseudo-onion bulbs (**a**, **b**) represent collections of concentric processes surrounding a well-myelinated axon; however, the pseudo-onion bulb also contains several denervated bands of Schwann cell processes typical for axonal degeneration; (**c**) a remarkable field without a single remaining axon. In other fascicles, however, there were scattered myelinated axons without clear demyelinative forms, supported by clinical history (**a** 6,000×; **b** 20,000×; **c** 4,000×)

diseases (Chap. 20), typical cytosomes have rarely been illustrated within axons in some of the storage diseases, but invariably these are more prominent in other cellular elements (MLD, Fabry disease, GM1 and GM2 gangliosidosis). We have seen axonal paracrystalline filamentous accumulations in a variety of circumstances and doubt that these have any diagnostic utility. They have been observed in "normal" nerves (Ochoa and Mair 1969).

7.5.4 Ultrastructural Examination of Inclusions in Endoneurial Cells

The reader is referred to Chap. 20 for details regarding ultrastructure, cell distribution, and frequency of storage cytosomes characteristic of some neuropathies.

Inclusions normally found in peripheral nerve include the Pi granules of Reich, seen in myelinating Schwann cells,

Table 7.12 Neuropathies with axonal swelling and filament accumulation

Giant axonal neuropathy
Toxic exposure
N-hexane
Methyl *N*-butyl ketone
Disulfiram and carbon disulfide
Acrylamide
Others in which this has been an infrequent feature
"CMT-2" (one pedigree)
B12 deficiency[a]
Amyloidosis[a]

[a]Not present to the degree seen in typical giant axonal neuropathies

and lipofuscin pigment in nonmyelinating Schwann cells. These are considered in more detail elsewhere. Both of these inclusions increase with aging and probably in a nonspecific fashion with neuropathy. The inclusion material of Niemann–Pick disease may be indistinguishable from lipofuscin, and the realization that a metabolic problem is present depends on appreciating the massive accumulation of storage material in both myelinating and nonmyelinating Schwann cells, as well as other endoneurial cells. Giant Schwann cell lysosomes may be seen in Chediak–Higashi syndrome. Giant multilobulated hyperchromatic Schwann cell nuclei are a feature of ataxia telangiectasia.

Tubuloreticular inclusions (TRI, tubuloreticular structures, and lupus inclusions) are subcellular structures 0.1–3 μm in size, described in a variety of disease states but not in normal human tissue (Grimley and Schaff 1976). They appear as aggregates of branching membranous tubules located within the endoplasmic reticulum and less often within the perinuclear envelope. The tubules are 20–30 nm in diameter and may be organized into compact honeycombs or loose tangles. Tubuloreticular inclusions are most often found in endothelial cells, lymphocytes, and macrophages and have been described in a variety of cell types, but not in neurons or Schwann cells. Originally thought to be viral in origin, it is now felt that the membranous tubules are somehow produced by invagination of endoplasmic reticulum in response to high levels of interferon (Luu et al. 1989). As a marker of human disease, TRIs are nonspecific and have been described in most collagen diseases, neoplasms, viral infections, and several neurodegenerative and storage diseases (Grimley and Schaff 1976), as well as in patients treated with intravenous interferon (Grimley et al. 1983). Their main importance in peripheral nerve pathology is as potential clues to collagen disease- or HIV-associated neuropathy (Figs. 11.2 and 13.16). TRIs have not been described in typical GBS or CIDP.

7.5.5 The Interstitial Compartment

Electron microscopy reveals that a fine fibrillar material is found scattered throughout the endoneurium, most prominently around vessels, in the subperineurial area, and within Renaut bodies. This material is oxytalan and has occasionally been mistaken for amyloid. An ultrastructural distinction can be made as oxytalan fibrils show a slightly greater width and a less "rigid" appearance than amyloid fibrils (Fig. 15.1). We have been impressed by deposition of endoneurial elaunin (immature elastin) in two cases of paraproteinemic neuropathy (Fig. 2.13b). Accumulations of oxytalan are often associated with vacuolated fibroblasts, but this has no diagnostic utility.

The giant vacuolated fibroblasts described by Schoene et al. (1970) in a case of hereditary sensory neuropathy have subsequently been described in patients with hypertrophic neuropathy (Asbury et al. 1971), in CMT-3 with basal lamina onion bulbs (Joosten et al. 1974), and in CMT-1 showing "focal mucoid degeneration" (Meier 1979). Vacuolated fibroblasts are seen in Renaut bodies, and in an experimental model of Renaut body formation, vacuolated fibroblasts were demonstrated to arise during evolution of regions of endoneurial "edema" into Renaut bodies (Ortman et al. 1983). The presence of vacuolated fibroblasts in these instances is associated with a focal or diffuse accumulation of amorphous endoneurial material with a mucoid staining pattern on LM and a granular and fibrillar (oxytalan) structure on EM. Joosten et al. (1974) observed that the same fine granular material found in the endoneurial deposits was found in the fibroblast "vacuoles," and Meier (1979) found morphologic evidence to suggest that the fibroblasts were secreting the endoneurial mucoid substance (Meier 1979). It is possible that the "vacuoles" are actually extracellular spaces enclosed by an extremely convoluted and attenuated fibroblast. We have observed fibroblasts with this appearance, usually in a region of endoneurial "edema," in a wide variety of acquired and genetically determined neuropathies, and attach no special significance to them.

Several nonspecific vascular alterations are commonly seen. Reduplication of perivascular basal lamina is a nonspecific finding in chronic neuropathies but reaches its most dramatic appearances in diabetic neuropathy. This appearance is also increasingly prominent with aging. Fenestration of endoneurial endothelial cells, discussed elsewhere, is always abnormal, and we have seen this most frequently with CIDP. "Ghost" vessels are obsolete vessels within a scarred endoneurium, usually seen in end-stage diabetic neuropathy or "burned-out" vasculitic neuropathy.

7.6 Immunohistochemical Techniques

The increasing availability and reliability of immunohistological technology has been very helpful in the examination of nerve biopsy material. We routinely use leukocyte common antigen (LCA) immunostain to detect inconspicuous

cellular infiltrates. As our experience with this technique has evolved, we have recognized that no significance can be attached to the detection of a few epineurial lymphocytes, even if they are cuffing a small vessel. In contrast, we regard one or two lymphocytes within a fascicle as within normal limits but consider an endoneurial perivascular inflammatory cuff to be abnormal. More rigorous efforts at quantitation have not yielded consistent results.

Detection of excessive endoneurial or epineurial LCA positive cells does not necessarily indicate an inflammatory neuropathy. Infiltrative neoplastic neuropathy, almost always with lymphoma, usually appears as a massive infiltrate of the nerve, but we have examined nerves with surprisingly subtle focal cellular accumulations. Thus, if the cells demonstrate any degree of atypia on routine LM or EM examination, a search for monoclonality, using available B cell, T cell, and immunoglobulin markers, is indicated.

More than any other entity, the diagnosis of amyloid neuropathy has been revolutionized by the availability of immunostains permitting identification of amyloid major proteins (Chap. 15). Immunostaining for TTR and kappa and lambda light chains must be undertaken when a diagnosis of amyloid neuropathy is made. We have found the TTR reaction to be highly reliable, but the light chain immunostains are sometimes unpredictable, perhaps due to absence or masking of the light chain C region in the amyloid deposit. Examination of step sections alternately stained with Congo red and the relevant immunostain is mandatory, in order to verify that the focal immunostain abnormality corresponds to an amyloid deposit. Otherwise, non-amyloid light or heavy chain deposition might be mistaken for amyloid, although these are different diseases (Chap. 15).

The peripheral nerve pathology literature abounds with reports of immunohistological studies indicating neural deposition of IgM, IgG, and IgA, complement, and other macromolecules, and some reports have suggested that these techniques may have diagnostic utility. However, a number of studies focusing on this issue have been published which make it clear that almost all findings of this sort are nonspecific (Van Lis and Jennekens 1977; Schenone et al. 1988; Liebert et al. 1985; Graham and Johnson 1985; Takatsu et al. 1985; Neuen et al. 1987). Perineurial or subperineurial immunoglobulin deposition can occur in numerous inflammatory and noninflammatory neuropathies, as well as in normals. Similarly, diffuse endoneurial staining with IgG and probably IgA is of no significance, as the blood–nerve barrier is partially permeable to these macromolecules. IgM is not normally found in the endoneurium, and thus staining with IgM is suggestive of pathology, especially an IgM paraproteinemic neuropathy. The single important exception to the general lack of utility of such techniques is the finding of IgM deposits on myelin sheaths, using either immunohistochemical or immunofluorescent techniques. This latter observation suggests the possibility of IgM paraproteinemic neuropathy with antibodies against myelin-associated glycoprotein, although it is neither 100 % sensitive nor specific for this diagnosis. However, given the localization of Fc-γ and complement receptors on normal human nerve (Vedeler et al. 1989), the importance of this finding remains unclear. We do not recommend use of direct immunofluorescence or immunohistochemical techniques for immunoglobulins, complement, or other macromolecules in the routine nerve biopsy.

Human immunodeficiency virus (HIV)-infected patients with an atypical neuropathy and severe immunosuppression may have a cytomegalovirus-associated vasculitic neuropathy, and immunostaining can be diagnostic (Fig. 11.3d).

7.7 Fiber Teasing

We do not carry out fiber teasing on most specimens, and the interested reader is referred to the writings of Dyck et al. (1993). In tomaculous neuropathies, teased fibers are an elegant means of demonstrating the redundant myelin formations. The sensitivity and reliability of teased fibers in detecting segmental demyelination and remyelination is probably superior to that of cross sections, even with EM, but there is difficulty in separating mild pathology from the normal range of findings, and the results do not routinely lead to specific diagnoses.

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Clinical Aspects of Peripheral Neuropathy

8

A brief discussion of some of the clinical issues involved in the diagnosis of peripheral neuropathy will be helpful to pathologists who receive clinical data from their neurological colleagues and wish to correlate this with biopsy findings. More information will be extracted from the biopsy when there is close contact between clinician and pathologist. Discrepancies between the clinical and histological impressions should result in reevaluation of both types of data. For example, in a case where the biopsy shows a nonspecific picture suggesting great chronicity and prominence of regenerating clusters, the pathologist might offer the possibility that the neuropathy is genetically determined, and this may lead to a revealing reevaluation of the patient's family history or greater emphasis placed on examination finding of foot deformity or scoliosis. Conversely, when the clinician suspects vasculitis or amyloidosis, the pathologist may examine additional sections with special stains if the initial assessment of the biopsy is unrevealing.

8.1 Clinical Approach to Peripheral Neuropathy

8.1.1 Clinical Questions

When assessing a patient with a suspected peripheral neuropathy, it is useful to consider several questions to help localize the problem and narrow the list of possible etiologies (Bromberg 2010). First, it is useful to ask if the clinical and examination features support a peripheral neuropathy. If present, what is the anatomical distribution of nerve involvement? What fiber types are involved? What is the temporal course? Is the primary pathological process demyelinating or axonal? This section will address these clinical questions in turn.

8.1.1.1 Is a Peripheral Neuropathy Present, and Is It the Cause of the Patient's Symptoms?

It is not always easy to decide if a peripheral neuropathy is present and clinically significant, especially with predominantly sensory symptomatology or when additional nervous system pathology is present. Common motor and sensory symptoms can be due to many other peripheral or central nervous system causes. Of 267 consecutive biopsies from the St. Michael's laboratory (1987–1993), 33 were found to be normal or insignificantly abnormal; a diagnosis other than peripheral neuropathy was ultimately made in most, including plexopathy, radiculopathy, myelopathy, neuromuscular junction disorder, or myopathy. Others report similar findings (Prineas 1970). Electrophysiological tests are extremely important in resolving this problem but are not 100 % sensitive (vide infra). Neurological examination is also helpful in excluding a central nervous system etiology. Since many neuropathies are distal and symmetric, muscle bulk in the hands and feet should be assessed. Foot deformities, such as hammer toes and pes cavus may be seen in chronic neuropathies. Hyporeflexia or areflexia in the anatomical distribution of symptoms should be present in most peripheral neuropathies. Sensory examination should be guided by the differential diagnosis derived from history, looking specifically for sensory loss in a pattern suggesting a lesion of an individual nerve, multiple nerves, a nerve root, or in a distal symmetrical (glove and stocking) distribution. Small fiber (pain, temperature) and large fiber (vibration, proprioception) modalities should be assessed.

8.1.1.2 What Is the Distribution of the Peripheral Nerve Disease?

History, physical examination, and electrophysiological testing clarify the spatial pattern of the patient's peripheral nerve problem. A mononeuropathy affects one nerve only.

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A mononeuropathy multiplex involves several individual nerve trunks, including nerve roots or cranial nerves. A polyneuropathy is a generalized disorder of peripheral nerves, with clinical findings usually symmetrical and length dependent (more pronounced distally). Making this syndromic diagnosis is important, for it narrows the diagnostic possibilities considerably and directs further testing. One would not, for example, biopsy a sural nerve if there was no evidence of disease anywhere except for isolated radial or common peroneal nerve palsy. Electrophysiological tests are critical for detection of subclinical polyneuropathy or multifocal nerve disease.

The boundary between mononeuropathy multiplex and polyneuropathy is often unclear. As the number of individual nerves involved increases, deficits become confluent and the clinical picture may be similar to that of a distally predominant polyneuropathy (Waxman et al. 1976). Again, electrophysiological testing may reveal evidence that the abnormalities are asymmetric or that there is prominent proximal involvement, both favoring a confluent mononeuropathy multiplex (Vavra and Rubin 2011; Olney 1992).

8.1.1.3 What Is the Fiber Type Affected?

Most neuropathies impair both motor and sensory functions. Early, patients usually complain of distal sensory disturbances (paresthesias, numbness), as they are unlikely to notice distal intrinsic foot muscle weakness. In such cases, careful examination and electrophysiological testing are critical to clarify the fiber types involved. Clinically, it is important to determine if predominantly small or large sensory fibers are affected. Loss of pain and temperature sensation (small fiber) with relative sparing of joint position and vibration modalities (large fiber) is evidence of a small fiber neuropathy. This pattern is uncommon but extremely useful if found, for the differential diagnosis is limited (Table 7.7). More common and nonspecific are nonselective fiber loss or more prominent involvement of large fibers. Notably, deep tendon reflexes and routine electrophysiological testing do not assess small fiber function and thus may be entirely normal in this setting. Small fiber neuropathies often involve the autonomic nervous system, and testing of autonomic reflexes provides some information about unmyelinated fiber pathology.

Pure sensory or pure motor neuropathies have specific differential diagnostic implications (Tables 8.1 and 8.2). Neuropathies with predominant small fiber and/or autonomic involvement are listed in Table 8.3.

8.1.1.4 Temporal Course

In neurological parlance, an acute process evolves over less than 2–4 weeks, a chronic process evolves over years, and a subacute process lies in between (usually months to a year or two). The distinction is important, since the differential diagnosis of acute neuropathy is considerably more limited (Table 8.4). In general, an acute presentation should make one think of Guillain–Barre syndrome (GBS) or vasculitis and more rarely infections, porphyria, or some toxins (e.g., arsenic). A relapsing course is often associated with inflammatory conditions such as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) or vasculitis but can also occur with porphyria.

Table 8.1 Predominantly sensory neuropathies*

Inflammatory
Inflammatory polyganglionopathy (IPG) not associated with malignancy
Sjogren's syndrome (can be associated with IPG)
Predominantly sensory Guillain–Barre syndrome (unusual)
Predominantly sensory CIDP (unusual)
Vasculitic neuropathy (unusual)
Sensory perineuritis
Infection associated
HIV distal predominantly sensory neuropathy (DSPN)
Leprosy
Lyme disease
Metabolic
Diabetes
Hypothyroidism
Uremia
Liver disease (including primary biliary cirrhosis)
Nutritional
B12 deficiency
Pyridoxine excess
Thiamine deficiency (atypical cases)
Vitamin E deficiency
Strachan syndrome (Cuban neuropathy)
Toxins
Cisplatin
Chloramphenicol
Metronidazole, misonidazole
Isoniazid, ethionamide
Nitrous oxide
L-tryptophan
Thalidomide
Ethylene oxide
Chlorpyrifos (organophosphate insecticide)
Neoplasia associated
Paraneoplastic sensory ganglionopathy
Paraprotein associated
Infiltration with lymphoma/leukemia
Genetically determined
Associated with spinocerebellar degeneration, especially Friedreich's ataxia
Hereditary sensory and autonomic neuropathies (HSAN)
Fabry disease
Tangier disease

*Neuropathies can manifest in a predominantly sensory fashion, but do not always do so

Table 8.2 Predominantly motor neuropathy

Inflammatory/demyelinating
GBS or CIDP with motor predominance (common)
Multifocal motor neuropathy with conduction block
Infection associated
Diphtheria
Metabolic
Porphyria
Hypoglycemia-associated neuropathy
Toxic
Lead, mercury
Dapsone
Organophosphate poisoning
Neoplasm associated
Lymphoma-associated motor neuropathy
Paraprotein-associated motor neuropathy
POEMS syndrome
Genetically determined
CMT-1
CMT-2

Also need to consider: spinal muscular atrophies (SMA), distal SMA (distal hereditary motor neuropathies)

Table 8.3 Predominantly small fiber neuropathy

Inflammatory
GBS
Autoimmune autonomic ganglionopathy
Infectious
HIV associated
Lepromatous leprosy
Metabolic or systemic disease
Diabetes (most common etiology)
Amyloidosis
Porphyria
Sarcoidosis
Alcohol
Neoplasm associated
Paraneoplastic neuropathy
Genetically determined
Hereditary sensory and autonomic neuropathies (HSAN)
Fabry disease
Tangier disease
Amyloidosis (familial)

8.1.1.5 Is the Nerve Damage Caused by Axonal Degeneration or Demyelination?

Differentiating a primarily demyelinating neuropathy from a primarily axonal neuropathy can considerably narrow the differential diagnosis. Since many demyelinating neuropathies are inflammatory, this distinction also has profound implications for prognosis and treatment. Electrophysiological testing (vide infra) is invariably

Table 8.4 Syndromic classification of neuropathy

Focal neuropathy/mononeuropathy
Compression/trauma
Diabetes
Vasculitis
Herpes zoster
Neoplasm
Radiation
Leprosy
Sarcoidosis
Hypothyroidism, acromegaly, cachexia: predispose to mononeuropathies (e.g., median neuropathy at the wrist)
Multifocal neuropathy
Demyelinating
Multiple compression
CIDP
GBS
Multifocal motor neuropathy
Hereditary neuropathy with pressure palsies (HNPP)
POEMS syndrome
Radiation plexopathy
Tangier disease (some cases)
Axonal
Vasculitis
Primary vasculitis: polyarteritis nodosa, granulomatosis with polyangiitis (Wegener's), Churg–Strauss
Secondary to connective tissue disease or systemic disease: rheumatoid arthritis, SLE, HIV, cryoglobulinemia (often associated with hepatitis C)
Isolated PNS vasculitis
Diabetes
Sarcoidosis
Leprosy
Lyme disease
Perineuritis
Bacterial endocarditis
Neoplastic nerve infiltration
Hemorrhagic diathesis
Primary nerve tumors (e.g., neurofibromatosis)
Axonal polyneuropathy
Acute
Axonal GBS
Porphyria
Critical illness polyneuropathy
Toxic exposures
Misonidazole, nitrofurantoin
Arsenic, thallium
Alcohol/nutritional
Acquired subacute/chronic
Diabetes
Toxic exposures (alcohol, drugs, industrial)
Vasculitis
Collagen diseases without vasculitis
Nutritional defects (B12, B6, B1, vitamin E)
Uremia

(continued)

Table 8.4 (continued)

Hypothyroidism
HIV, HTLV-I
Lyme disease
Paraproteinemia
Paraneoplastic
Neoplastic infiltration
Sarcoidosis
Hypereosinophilic syndrome
Perineuritis
Genetically determined chronic
CMT-2
CMT-X (in females)
Hereditary sensory and autonomic neuropathies (HSAN)
Amyloidosis (acquired or familial)
Spinocerebellar degeneration
Fabry disease
Mitochondrial cytopathies
Adrenoleukodystrophy/adrenomyeloneuropathy
Tangier disease (some cases)
Polyglucosan body disease
Demyelinating polyneuropathy
Acute
GBS
Diphtheria
Subacute/chronic – nonuniform demyelination
CIDP
Paraprotein-associated neuropathy
POEMS syndrome
Hereditary neuropathy with pressure palsies (HNPP)
Toxins (perhexiline, amiodarone, solvents, chloroquine)
L-tryptophan toxicity (eosinophilia–myalgia)
Oxalosis
Chronic – uniform demyelination
CMT-1
CMT-X (in males)
Dejerine–Sottas syndrome
Refsum disease
Metachromatic leukodystrophy
Globoid cell leukodystrophy (Krabbe disease)
Adrenoleukodystrophy (usually primarily axonal)
MNGIE syndrome
Niemann–Pick disease
Tangier disease (some cases)

Modified from Schaumburg et al. (1992), Fig. 3.2

required in this regard, as the distinction is often not easy to make clinically. Clues to a demyelinating process on examination include early loss of reflexes out of proportion to weakness, motor greater than sensory deficits, and palpably enlarged nerves.

8.2 Classification of Peripheral Neuropathies

A syndromic classification of peripheral neuropathy is provided in Table 8.4. It is also possible to formulate a classification of neuropathy along etiologic (as best understood) lines (Table 8.5). The discussion of neuropathy in the second part of this book is organized according to Table 8.5.

Table 8.5 Etiologic classification of peripheral neuropathy

Acquired demyelinating neuropathies
Guillain–Barre syndrome
Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)
Secondary CIDP (associated with connective tissue disease, paraprotein, HIV)
Multifocal motor neuropathy with conduction block
Other inflammatory (noninfectious) neuropathies
Sarcoid neuropathy
Connective tissue diseases: rheumatoid arthritis, systemic lupus erythematosus, Sjogren syndrome, mixed connective tissue disease, scleroderma
Perineuritis
Neuritis associated with insect stings/bites (excluding Lyme disease)
Infectious- or infection-associated neuropathies
Leprous neuropathy
HIV-associated neuropathies
CMV neuritis in immunosuppressed patients
HTLV-I associated neuropathy
Lyme neuritis
Diphtheric neuropathy
Neuropathy in Creutzfeldt-Jakob disease
Chagas disease
Hepatitis C (with or without cryoglobulins)
Vasculitic neuropathy
Primary vasculitis: polyarteritis nodosa, granulomatosis with polyangiitis (Wegener), Churg–Strauss
Secondary vasculitis: associated with connective tissue diseases
Isolated PNS vasculitis
Dysproteinemia-associated neuropathy (excluding primary amyloidosis)
IgM paraprotein (MGUS, Waldenstrom's macroglobulinemia, lymphoma, anti-MAG syndrome)
IgG or IgA MGUS or multiple myeloma
POEMS syndrome and osteosclerotic myeloma
Cryoglobulinemia
Amyloidosis
Primary amyloidosis
Familial amyloid polyneuropathy
Transthyretin
Apolipoprotein A
Gelsolin

(continued)

Table 8.5 (continued)

Neuropathy associated with neoplasia (excluding paraproteins)
Paraneoplastic neuropathy
Infiltrative neuropathy
Lymphoma
Leukemia
Castleman's disease-associated neuropathy
Metabolic neuropathies
Endocrine
Diabetes
Hypothyroidism
Acromegaly
Organ disease
Renal failure
Liver disease
Chronic hypoxia
Celiac disease
Nutritional
B vitamin deficiency (B12, B1, B5)
Vitamin E deficiency
Pyridoxine excess
Toxic neuropathy
Alcohol
Numerous toxins (see Chap. 18)
Genetically determined neuropathies
Charcot–Marie–Tooth disease and variants[a]
CMT-1
CMT-2
CMT-X
CMT-4
Dejerine–Sottas syndrome
Hereditary neuropathy with pressure palsies
Hereditary sensory and autonomic neuropathies
Autosomal dominant: HSAN1
Autosomal recessive: HSAN 2, 3, 4, 5
Storage diseases
Sphingolipidoses
Metachromatic leukodystrophy
Globoid cell leukodystrophy
Fabry disease
Niemann–Pick disease
Farber disease
Adrenoleukodystrophy
Cerebrotendinous xanthomatosis
Abetalipoproteinemia
Familial amyloidosis
TTR mutations, apolipoprotein A mutations, gelsolin mutations
Neuropathies in hereditary ataxias
Friedreich's ataxia
Other autosomal recessive ataxias: ataxia-telangiectasia, abetalipoproteinemia, ataxia-oculomotor apraxia
Autosomal dominant ataxias (spinocerebellar ataxias)
Mitochondrial cytopathies
MNGIE syndrome
NARP syndrome
MELAS syndrome
Other inherited causes of neuropathy
Porphyria
Tangier disease
Polyglucosan body disease
Giant axonal neuropathy
Other causes of neuropathy
Critical illness polyneuropathy
Neuropathy associated with mitochondrial disease
Non-paraneoplastic inflammatory polyganglionopathy
Neuropathy of the hypereosinophilic syndrome
Neuropathy in Madelung's disease

[a]See Table 20.1 for more detailed listing

8.3 Laboratory Testing in Peripheral Neuropathy

Prior to obtaining a nerve biopsy, all reasonable noninvasive or less invasive diagnostic modalities should be exhausted. It bears repeating that nerve biopsy is associated with more short- and long-term sequelae than skin biopsy, bone marrow biopsy, or abdominal fat pad aspiration. Routine and specialized diagnostic tests that are useful in peripheral neuropathies are listed in Table 8.6.

8.3.1 Electrophysiological Testing

The value of electrophysiological testing in the assessment of peripheral neuropathy cannot be overstated. These investigations are simply an extension of the physical examination, allowing the clinician to answer the questions listed in part I above with greater sensitivity and accuracy than would otherwise be possible. In particular, nerve conduction studies (NCS) can verify if the primary pathological mechanism is demyelination or axonal degeneration. A detailed discussion is beyond the scope of this text, and we will only review NCS. A pathologist interested in peripheral nerve examination should understand the information provided by NCS, which explore regions of the peripheral nervous system not routinely accessible to biopsy.

Electromyography (EMG), not discussed further, permits assessment of the status of motor units. Insertion of a needle electrode into a muscle allows recording of an electrical activity that can suggest recent denervation or chronic denervation with collateral sprouting and reinnervation of motor units. Interested readers are referred to several excellent texts (Kimura 2001; Preston and Shapiro 2005).

Table 8.6 Diagnostic tests useful in peripheral neuropathy

Routine laboratory testing in peripheral neuropathy
CBC
ESR
BUN, creatinine, electrolytes
Liver enzymes, bilirubin, albumin
Glycosylated hemoglobin or glucose tolerance test
Thyroid function test
ANA, rheumatoid factor
Serum and urine immunoelectrophoresis and immunofixation
Serum B12
Other commonly used laboratory tests
Chest X-ray (sarcoid, malignancy, systemic disease)
CSF examination (malignant cells, lymphocytes, increased protein)
Skeletal survey (solitary or multiple plasmacytoma despite negative immunoelectrophoresis)
Lyme serology (in endemic areas or if clinical picture suggestive)
HIV testing (if high-risk group or suggestive features)
Hepatitis C serology
Fat aspirate (for amyloid)
Skin or conjunctival biopsy (for sarcoidosis)
MRI with contrast (root or nerve enhancement or enlargement as seen in CIDP)
Noninvasive tests based on clinical suspicion
DNA blood testing for CMT mutations
Hair or urine heavy metal analysis (if suspect metal exposure)
Anti-ganglioside (GM1, sulfatide), anti-MAG serum activity
Anti-RO, anti-LA antibodies (if suspect Sjogren's syndrome)
Anti-neutrophil cytoplasm antibodies – ANCA (if suspect vasculitis)
Paraneoplastic antibodies (e.g., anti-Hu in sensory ganglionopathy)
Serum cryoglobulins
Anti-gliadin antibodies
Abdominal ultrasound or CT (for occult malignancy)
Cholesterol and lipid assays, lipoprotein electrophoresis (Tangier disease, abetalipoproteinemia)
Vitamin E levels (malabsorption syndromes, abetalipoproteinemia)
Lipid metabolism testing (if clinical suspicion)
Serum or leukocyte arylsulfatase (metachromatic leukodystrophy)
Quantitated urinary sulfatide excretion (metachromatic leukodystrophy)
Serum or leukocyte β-galactosidase activity (Krabbe's leukodystrophy)
Serum or leukocyte α-galactosidase A (Fabry disease)
Serum or cell culture sphingomyelinase activity (type I Niemann–Pick)
Serum very long-chain fatty acids (adrenoleukodystrophy)
Serum phytanic acid (Refsum disease)

8.3.1.1 Nerve Conduction Studies

Nerve conduction studies are performed by stimulating a nerve at a designated site and recording an electrical response at a second site. Motor NCS involve stimulation of a nerve and recording over a muscle innervated by that nerve. The recorded waveform is the compound muscle action potential (CMAP), produced by a combination of the individual muscle fiber action potentials. The CMAP is an easily visualized waveform with amplitudes in the millivolt range. Sensory NCS involve stimulation of the nerve at one site and recording of the sensory nerve action potential (SNAP) in the nerve as it passes by a second site. The SNAP has a much smaller amplitude, in the microvolt range. It is possible to calculate the velocity with which the nerve action potential propagates by physically measuring the distances involved (Fig. 8.1a). Different stimulus and recording techniques permit measurement of conduction in proximal (root and plexus) nerves, termed the F-wave.

The primary determinant of the amplitudes measured during NCS (motor or sensory) is the number of axons propagating the impulse. For sensory potentials, the amplitude is essentially a reflection of the number of intact large myelinated fibers and receives little contribution from small myelinated and unmyelinated fibers (Behse and Buchthal 1978).

The measured conduction velocity reflects the velocity of the fastest conducting elements, i.e., the largest myelinated fibers. Determinants of conduction velocity are more complex. The most important element is myelinated fiber diameter, which is proportional to the maximum conduction velocity (Waxman 1980). The total myelinated fiber diameter includes the diameter of the axon and the thickness of the surrounding myelin sheath. An increase in axon diameter alone reduces the axoplasmic resistance to current (increases conduction velocity) but has the opposing effect of increasing membrane conductance (decreases conduction velocity). Increased myelin sheath thickness in turn reduces membrane conductance, increasing conduction velocity. Thus, in the largest myelinated fibers, a combination of larger axon diameter and thicker myelin sheath combines to contribute to higher conduction velocities (Kimura 2001). Internode length is a less significant determinant of velocity under ordinary circumstances, as should be evident by the observation that remyelinated nerves achieve a conduction velocity almost as high as that prior to demyelination, even though the internodes are much shorter (Gilliatt 1966; Waxman 1980).

8.3.1.2 Interpretation of NCS Results

Nerve conduction studies with normal amplitudes but slowed conduction velocities indicate either a loss of myelin (demyelination) or an overall decrease in the myelinated fiber diameter, i.e., axonal atrophy (Fig. 8.1b). Based on the basic principles above, NCS showing preserved conduction velocities but a reduced amplitude indicate a loss of axons, i.e., an axonal neuropathy (Fig. 8.1c).

Interpretation of NCS is more complex in practice. A loss of axons can cause a reduction in conduction velocity, and in turn, demyelination can cause reduction in amplitude. The first situation occurs because in axonal neuropathies, there is a loss of fibers, including (and often relatively

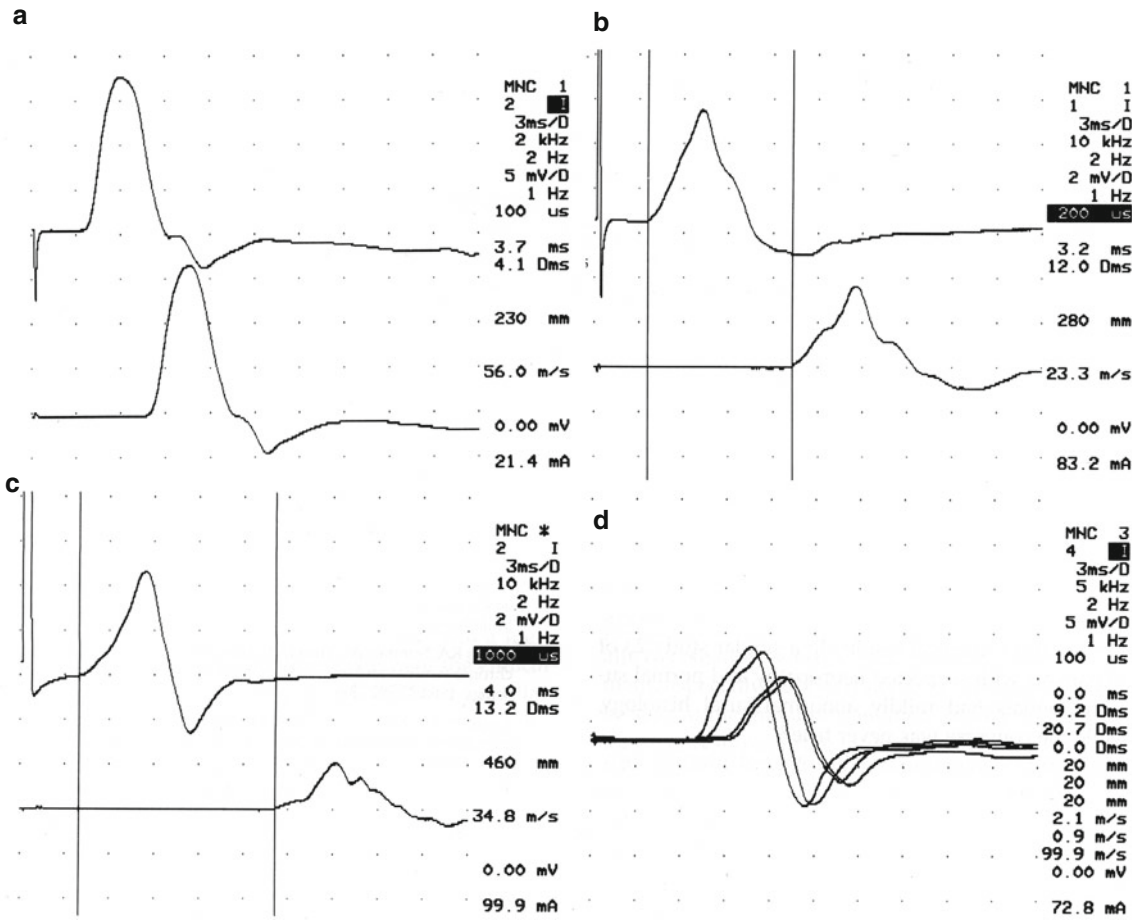

Fig. 8.1 Motor nerve conduction studies: (**a**) normal patient, (**b**) conduction slowing (23.3 m/s) with relative preservation of amplitude in CMT-1, (**c**) severe reduction in CMAP amplitude sufficient to make a conduction velocity of 34.8 m/s consistent with an axonal neuropathy, (**d**) focal conduction block note 30 % reduction in amplitude between second and third stimuli (Recordings courtesy of Mr. G. Trogadis)

selectively) the large myelinated fibers that contribute to the conduction velocity measurement (Fig. 8.1c). A reduction in the measured maximum conduction velocity results from loss of these fastest-conducting fibers. In axonal lesions, the conduction velocity should not be markedly reduced (into the demyelinating range) because some of the smaller, more slowly conducting myelinated fibers are still present. The second situation, reduced amplitudes due to demyelination, occurs because the normal CMAP or SNAP is a superposed summation of signals carried along many individual axons. Even slight desynchronization of the signals may result in simultaneous negative and positive voltages tending to cancel each other out. Because the SNAP is of lower amplitude and shorter duration than the CMAP, it is more sensitive to this problem. This explains why the amplitude of the CMAP or SNAP is normally reduced when the same nerve is stimulated proximally vs. distally. The greater distance from the proximal stimulation site to the distal recording site amplifies the difference in conduction velocity between the fastest and slowest myelinated fibers (temporal dispersion).

Another manner in which demyelination can reduce amplitude is via conduction block. A demyelinated set of axons cannot conduct action potentials, and thus there is a block of conduction at the site of demyelination. If the nerve is stimulated proximal to the site of demyelination, then the demyelinated (blocked) axons do not contribute to the distally recorded CMAP, resulting in a smaller amplitude (Preston and Shapiro 2005). Stimulation distal to the site of demyelination results in a normal CMAP (because the axons are intact). The finding of unequivocal conduction block between proximal and distal stimulation sites is an indication of demyelination.

Thus, amplitudes and velocities must be interpreted together, and the relative importance of alterations in these parameters weighed, much as the pathologist needs to consider the relative weight of axonal vs. demyelinating changes on the biopsy in order to decide whether the demyelination is primary or secondary to axonal alterations (Fig. 8.1c). An example of these is seen in several of the proposed diagnostic criteria for CIDP (Ad Hoc Committee 1991; Nicolas et al.

2002). In these criteria, reduction of conduction velocity to 80 % or less of the lower limit of normal (LLN) suggests demyelination only if the amplitude is at least 80 % of LLN. If the amplitude is less than 80 % of LLN, velocity should be reduced to less than 70 % of LLN to be suggestive of demyelination.

The pattern of conduction velocity abnormalities also provides useful information. In many genetically determined neuropathies, there is a diffuse slowing of similar severity in all nerves examined, presumably because the process is very chronic and involves all fibers (Gabreels-Festen et al. 1993; Kaku et al. 1993). By comparison, in acquired demyelinating neuropathies, the slowing is widely variable between proximal and distal nerve segments and even between different nerves in the same limb. This occurs because the process is multifocal (Lewis and Sumner 1982). Conduction block (Fig. 8.1d) usually indicates focal demyelination and is important in some acquired demyelinating neuropathies (Chap. 9).

8.3.1.3 Comparison and Correlation of NCS and Biopsy Examination

Nerve conduction studies permit assessment of the peripheral nervous system at many sites, including very proximal nerve segments such as roots or plexus, and allow examination of motor and sensory nerves. This is clearly an advantage over nerve biopsy examination, which assesses PNS pathology in a 3–6 cm region of a single pure sensory nerve. However, nerve biopsy has the potential to reveal the cause of the neuropathy, such as vasculitis, amyloid, leprosy, metachromatic leukodystrophy, etc., while NCS allow only description of the pattern of disease.

Clinicians rely upon NCS to determine if a neuropathy is present in situations where symptoms and signs are subtle or hard to interpret. However, nerve biopsy is far more sensitive in detecting mild abnormalities. Review of our own experience revealed that 17 of 32 patients with normal NCS had an abnormal biopsy while only 4 of 19 patients with a normal biopsy had an abnormal NCS (Table 8.7). Almost identical results were reported by Logigian et al. (1994), and in a similar study, 23 of 33 patients with suspected neuropathy and normal sural nerve potentials had mildly abnormal histology, while the converse was never true (Behse and Buchthal 1978). Schweikert et al. (2007) found evidence of mild axonal damage in six patients with normal electrophysiology who underwent nerve biopsy.

Electrophysiological and histological findings usually correlate moderately well (Table 8.7); 146 of 207 patients showed concordant results on NCS and biopsy in a recent review of our material, and other authors report similar data (Schweikert et al. 2007; Logigian et al. 1994). In the study by Schweikert et al. (2007), in 14 of 38 cases where a definitive diagnosis could be made after nerve biopsy, the biopsy and electrophysiology agreed in 11 cases. However, instances in which nerve pathology does not correlate with the clinical or electrophysiological picture are not rare. Bosboom et al. (2001) compared sural nerve biopsy findings of 21 patients with CIDP and 13 patients with an idiopathic axonal polyneuropathy. Although there were pathological differences between the groups, there was also significant overlap in pathological findings between CIDP and axonal neuropathy. A neuropathy with significant conduction slowing may reveal only mild demyelinating changes on biopsy, or conversely, a neuropathy with mildly slowed conductions may show prominent hypertrophic features histologically (Gherardi et al. 1983; McLeod et al. 1973). The former situation is more common and presumably occurs because fibers in the distal nerve segment examined on biopsy have been insulted more proximally, with only axonal degeneration seen in the distal portions.

Table 8.7 Correlation between NCS and biopsy results

	Electrophysiology		
	Axonal	Demyelination/ mixed	Normal
Biopsy			
Axonal	86	19	14
Demyelination/ mixed	20	45	3
Normal	3	1	15
Total	109	65	33

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The Inflammatory Demyelinating Neuropathies

9

9.1 Guillain–Barré Syndrome

Guillain–Barré syndrome (GBS, reviewed in Arnason and Soliven 1993; Ropper et al. 1991; McFarlin 1990; and, most recently, in Rinaldi 2013), the most common cause of acute–subacute neuropathy, has an incidence of 1.5–2 cases per 100,000 throughout the world (Arnason and Soliven 1993). GBS is characterized by the development of a rapidly progressive paralytic syndrome, and although often thought of as a motor disease, sensory and autonomic symptoms may occur before motor dysfunction. Despite the presence of several variants, most cases are so typical that the diagnosis can be made clinically with a high degree of confidence.

9.1.1 Clinical Manifestations

Clinical GBS may be subclassified into several clinicopathological categories (Winer 2011), the most common of which are acute inflammatory demyelinating polyneuropathy ("classic GBS," AIDP) and acute motor axonal neuropathy ("axonal GBS," AMAN).

9.1.1.1 Acute Inflammatory Demyelinating Polyneuropathy (AIDP)

Persons of any age may be afflicted with AIDP. In about two-thirds of cases, an antecedent event precedes the illness by 1–3 weeks. Most commonly, this is a nonspecific upper respiratory illness, but organisms associated with AIDP include cytomegalovirus, Epstein–Barr virus, *Mycoplasma pneumoniae*, and human immunodeficiency virus (HIV). Other possible antecedent events are vaccination, pregnancy, insect bites, and surgery (Arnason and Soliven 1993).

The most important clinical features of GBS (Arnason and Soliven 1993; Ropper et al. 1991 [an exhaustive clinical review], Ropper 1992) are:

1. Progression to peak deficit over a few hours to 4 weeks, with a monophasic course
2. Progressive motor and usually sensory deficits in more than one limb
3. Hypo- or areflexia

Paresthesias often herald the onset, together with or soon followed by weakness. Proximal and distal muscles can be involved, usually symmetrically. The "ascending paralysis" pattern is seen in about half of patients, but upper extremity weakness may predominate from the beginning. Sensory signs are usually overshadowed by motor deficits. Cranial nerve involvement, especially the facial nerve, is common and may be present at the initial presentation. Bulbar and respiratory paralysis requiring intubation occurs in about one-third of patients. Clinical variants of this form of GBS include pure motor or predominantly sensory syndromes, the Miller Fisher syndrome (ataxia, ophthalmoplegia, hyporeflexia), and a dysautonomic picture. Papilledema is seen in up to 5 % of patients.

Elevated CSF protein with a normal cell count (i.e., albuminocytologic dissociation) has historically been very important in delineating GBS but is not seen in as many as 30 % of patients and is often absent in the first weeks of illness (Ropper et al. 1991). Electrophysiological tests are more sensitive, but early in the course, abnormalities may be subtle (Ropper et al. 1991). Typically, there is prolongation of the most proximal and the most distal conduction times, followed by diffuse slowing of conduction velocities, conduction block, and dispersion. Axonal features are frequently found but generally do not predominate.

Cases that progress beyond 8 weeks are best classified as CIDP, and intermediate cases pose a nosologic conundrum. Following maximal deficit, there is usually a stage of stabilization, lasting days to weeks, followed by gradual improvement over weeks to months. The ultimate outcome is good in 80 %, various degrees of deficit remain in 15 %, and death occurs in 5 % of patients (Ropper et al. 1991). Advanced age, a need for mechanical ventilation, and evidence of significant axonal degeneration on electrophysiological tests predict a poor outcome.

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Supportive measures, especially for patients requiring respiratory assistance, are the cornerstone of treatment. Plasma exchange (French Cooperative Group on Plasma Exchange in Guillain-Barre Syndrome 1987; Guillain-Barre Syndrome Study Group 1985) and intravenous immunoglobulin (van der Meche et al. 1992) benefit many patients, while the consensus is that steroid therapy is of no value (Ropper 1992).

9.1.1.2 "Axonal" GBS (Acute Motor Axonal Neuropathy, AMAN, and Acute Motor Sensory Axonal Neuropathy, AMSAN)

The definition of GBS provided above does not indicate whether an axonal or demyelinating pathology underlies the syndrome. In Europe and the USA, GBS has come to be synonymous with acute inflammatory demyelinating neuropathy (AIDP), its most common form, and axonopathy as a bystander effect (Arnason and Soliven 1993). Some time ago, the existence of a primary "axonal" form was proposed on the basis of a few cases (Feasby et al. 1986). It is characterized by a more aggressive course, poorer outcome, electrical inexcitability and other "axonal" findings on electrophysiological testing, and a stronger association with *C. jejuni* infection (Yuki et al. 1991; Vriesendorp et al. 1993; Ropper et al. 1991). The existence of this primary axonal process (Feasby et al. 1986) with clinical and electrophysiological features of GBS was not initially accepted as a separate syndrome, and early cases have shown diffuse demyelinating or mixed pathology on autopsy (Fuller et al. 1992; Berciano et al. 1993; Kanda et al. 1989). There was early resistance to the separation of axonal GBS from AIDP since the presence of axonal degeneration in distal nerves is not proof of a primary axonal insult (Feasby et al. 1993). Conversely, distal motor nerve demyelination can account for "axonal" findings on conduction studies (Hall et al. 1992; Triggs et al. 1992) and early studies debated if this entity truly existed or actually represented a GBS variant with primary inflammatory demyelination and superimposed "bystander" axonal damage (vide infra, Cros and Triggs 1994; Fuller et al. 1992; Yokota et al. 1992; Triggs et al. 1992; van der Meche et al. 1991; Brown et al. 1993; Dyck 1993; Feasby et al. 1993; Vallat et al. 1990).

Perhaps the strongest argument for the existence of an "axonal" variant of GBS was provided by the description of Chinese paralytic syndrome, an acute motor neuropathy seen mostly in rural northern Chinese children and having a predilection for the summer season (McKhann et al. 1991, 1993). Autopsy material revealed an axonal noninflammatory neuropathy in some of these patients (McKhann et al. 1993). The observation that 85 % of these patients have serology indicating recent *C. jejuni* infection (Griffin 1994) has proven of great interest, as the same association was present in some forms of severe GBS and was thought to reflect a common pathogenesis. This pattern of acutely evolving paralytic injury, clinically consistent with GBS, is not confined to China and comprises a significant number of cases of GBS in Asia and Central and South America in which it accounts for 30–65 % of patients. There are clinical differences between AIDP and AMAN, e.g., areflexia is a clinical criterion required to make a diagnosis of GBS, but patients with AMAN have normo- or hyperreflexia. Electrodiagnostic criteria suggest that in Europe and North America, a diagnosis of AMAN was made in only 3–17 % of cases of GBS, whereas the AIDP subtype accounted for 69–90 % (Kuwabara and Yuki 2013).

Currently, AMAN/AMSAN or axonal GBS is accepted as a separate form of GBS different from AIDP (reviewed in Kuwabara and Yuki 2013). AMAN/AMSAN exhibits prominent electrophysiological and pathological evidence of axon loss, with little demyelination, and, frequently, a more aggressive course than AIDP. Antigenic epitopes of infectious agents, such as the lipopolysaccharides of *C. jejuni*, a Gram-negative poultry-associated organism causing gastroenteritis, are shared with peripheral nerve antigens. The association of *C. jejuni* with AMAN is more established than with AIDP, and incidence of *C. jejuni* enteritis may account for incidence variations in forms of GBS in different parts of the world. In addition, population differences in HLA epitopes suggest an immunogenetic component (Ho et al. 1995; Magira et al. 2003).

9.1.2 Pathology

9.1.2.1 General Considerations

Most cases of GBS need not undergo biopsy, as the typical syndrome is easily recognized. The most important alternative peripheral nerve cause of rapidly progressive weakness is vasculitic neuropathy, and there may be times when GBS is so asymmetric or prominently axonal that vasculitis needs to be excluded.

The histological hallmarks of AIDP are a mononuclear inflammatory infiltrate and macrophage-mediated demyelination with a variable amount of axonal damage. Affected nerve may be unremarkable on histological examination (Ropper et al. 1991; Hughes et al. 1992), even when autopsy documents ongoing demyelination elsewhere, usually the nerve root (Kanda et al. 1989; Berciano et al. 1993). Involvement can extend from the spinal root to intramuscular twigs and may result in damage to distal sensory axons and spinal and sympathetic ganglia (Asbury et al. 1969). The sensitivity of peripheral nerve microscopic examination cannot be assessed from the literature because not all patients undergo biopsy; probably more than half of the specimens show specific abnormalities if electron microscopic examination is undertaken (Brechenmacher et al. 1987; Hughes et al. 1992), but a significant minority is normal and another

group nonspecific. When sural nerve conduction is normal, biopsy is also likely to be unrevealing (Ropper et al. 1991). In such cases, if it is truly necessary, consideration should be given to biopsy of an electrically involved superficial sensory nerve, or even a motor nerve (Hall et al. 1992). The latter is also a consideration in the uncommon "pure motor" cases of GBS.

9.1.2.2 Light Microscopy

AIDP

An endoneurial mononuclear cell infiltrate composed of macrophages and lymphocytes (chiefly $CD4^+$) is often present (Fig. 9.1), and although the predominant cell type may depend on the stage of evolution of nerve damage, macrophages are usually most numerous (Figs. 9.1, 9.2, and 9.3) (Wisniewski et al. 1969; Brechenmacher et al. 1987; Pollard et al. 1987). The prominence of lymphocytes varies widely in the literature. Asbury and colleagues commented on the "primacy of lymphocytic lesions in idiopathic polyneuritis" (Asbury et al. 1969), but most studies have shown few or no lymphocytes even with immunohistochemistry (Honavar et al. 1991; Mancardi et al. 1988; Hughes et al. 1992; Kanda et al. 1989; Berciano et al. 1993). It is worth noting that only 5 of 57 specimens in the largest GBS nerve biopsy study reported to date demonstrated an increase in endoneurial lymphocytes (Brechenmacher et al. 1987), despite the presence of active demyelination in most of these specimens. It has been argued that the absence of lymphocytes in sural nerve biopsies is due to the limited sampling inherent therein, but several autopsy studies included a close examination of the nerve roots (Honavar et al. 1991; Kanda et al. 1989; Berciano et al. 1993) and did not support this notion. When present, lymphocytes may be perivascular or randomly distributed in the epineurium and endoneurium (Fig. 9.1). Debris-filled macrophages can be seen in association with axons (vide infra), in a perivascular or subperineurial distribution, or randomly dispersed throughout the nerve fascicle (Fig. 9.3). Schwann cells are typically free of myelin debris in AIDP, which differs from typical demyelination in which Schwann cells are the initial degradative site. Schwann cells separate from their myelin sheaths, reenter the cell cycle, produce daughter Schwann cells outside the original basal

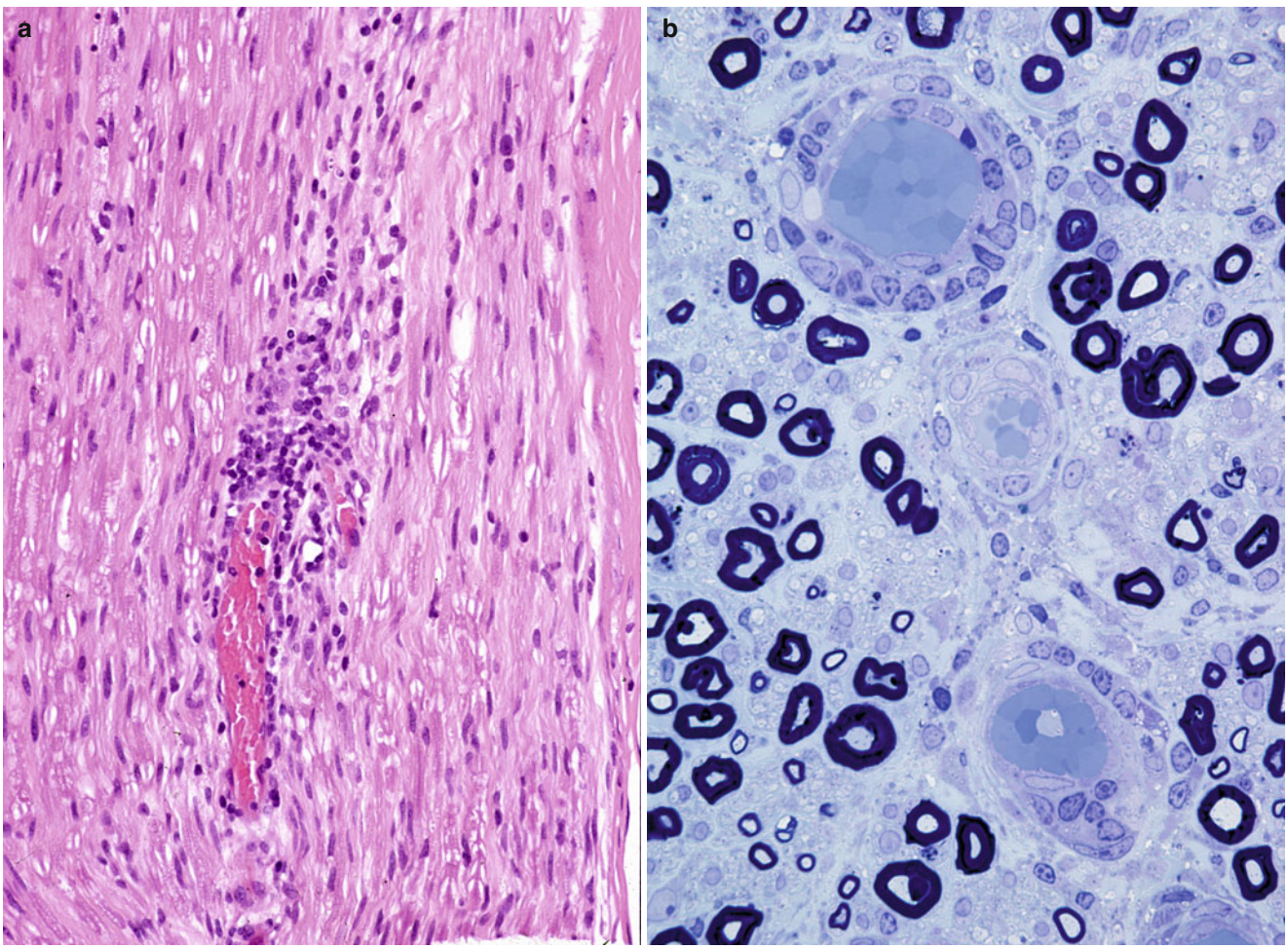

Fig. 9.1 GBS: note endoneurial perivascular cuffing with mononuclear cells in H&E and 1 μ plastic sections (**a**, paraffin 400×; **b**, plastic 1,000×)

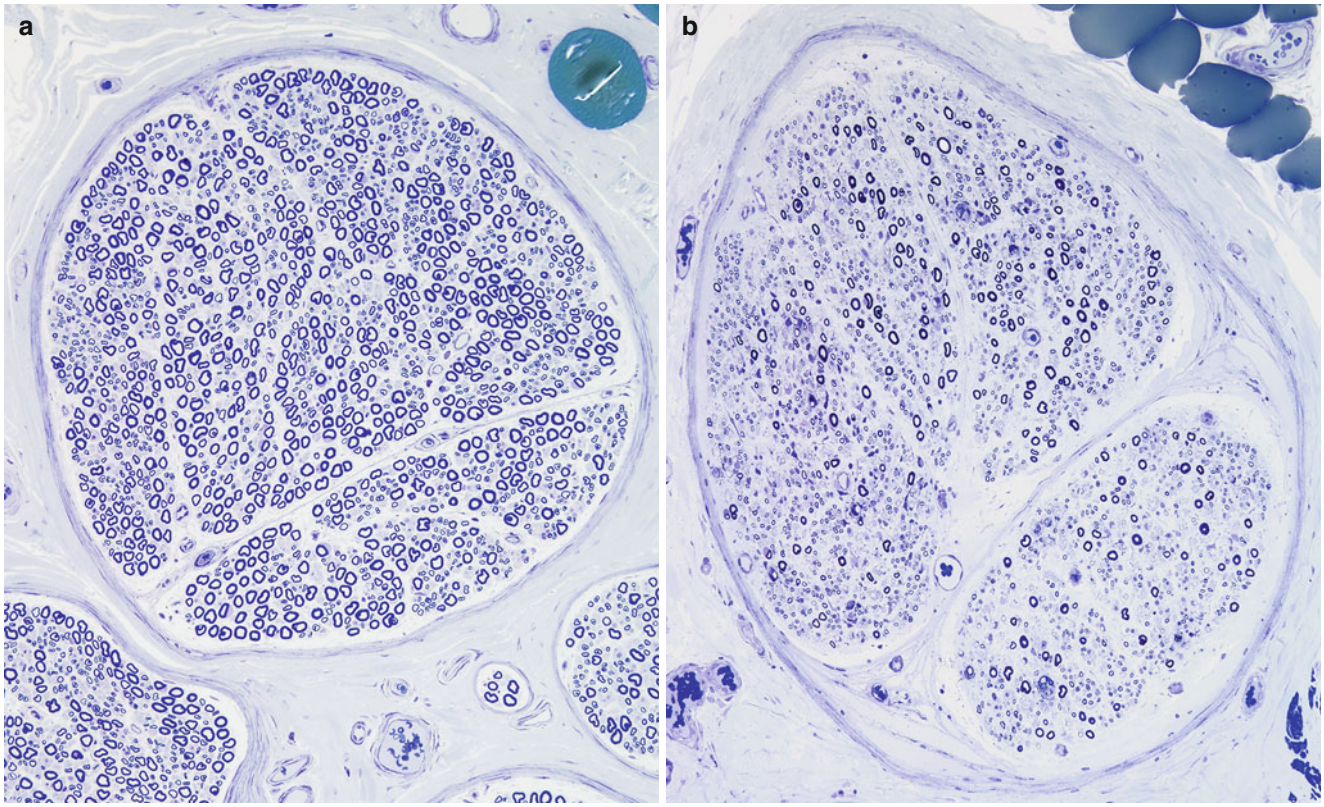

Fig. 9.2 Fiber density and endoneurial cellularity are contrasted in normal (**a**) and GBS, AIDP subtype (**b**) sural nerves (**a**, **b**: 1 μ plastic sections, 200×)

lamina, and reenter and remyelinate the denuded internode. Axonal loss or axonopathy may represent a "bystander effect" due to endoneurial toxic cytokines (e.g., TNF-α) or edema-induced local ischemia and compression. It is surprising for a demyelinative condition that AIDP often shows considerable loss of axons (Fig. 9.2). Schwann cell proliferation begins at 1–2 weeks after the onset of disease (Asbury et al. 1969) and contributes to endoneurial hypercellularity. Polymorphonuclear infiltrates have been reported in early fulminant disease (Asbury et al. 1969), but we have not observed this in the sural nerve nor has it been noted in other large series (Prineas 1972; Brechenmacher et al. 1987). Plasma cells are infrequently seen. Endoneurial edema is often present but we have never seen it in the absence of other pathology, contrary to the initial reports of Haymaker and Kernohan (1949). Months and even years after the acute illness, inflammatory cells may still be seen in peripheral nerve (Asbury et al. 1969).

The predominant pathological process in AIDP is segmental demyelination and remyelination. The entire spectrum of normally myelinated, thinly myelinated, and denuded axons can be seen (Figs. 9.2 and 9.3). A longitudinal section shows a long expanse of naked axon (arrow, Fig. 9.3d) surrounded by multitudes of closely applied, myelin-containing macrophages. The severity of these changes often depends on the duration of the disease. Some cases may show selective involvement of large myelinated fibers. Active axonal degeneration is not uncommon, but this is usually minor relative to the demyelinating changes. A demyelinated axon with an adjacent macrophage filled with myelin debris may be mistaken for a myelin ovoid of axonal degeneration if the attenuated axon is not visualized by light microscopy. As Schwann cells do not accumulate large amount of debris, any cell laden with myelin breakdown products is likely to be a macrophage. Axonal degeneration should not dominate the picture if a diagnosis of "primary demyelination consistent with GBS" is to be made (however, exceptions exist, vide infra).

Widening of the nodal gap, best seen on teased fiber preparations, is an early but nonspecific alteration, whether in GBS (Asbury et al. 1969) or in other demyelinating or axonal neuropathies. Onion bulbs have been seen in cases reported as GBS (Prineas 1972), but their presence suggests chronicity and that the patient more likely suffers from CIDP presenting with an acute exacerbation of previously subclinical disease.

AMAN/AMSAN

In the initial case of axonal GBS (AMAN) studied at autopsy, peripheral nerve showed only axonal degeneration and scanty inflammation (Feasby et al. 1986) which overlaps with some cases of clinically typical GBS (Prineas 1972;

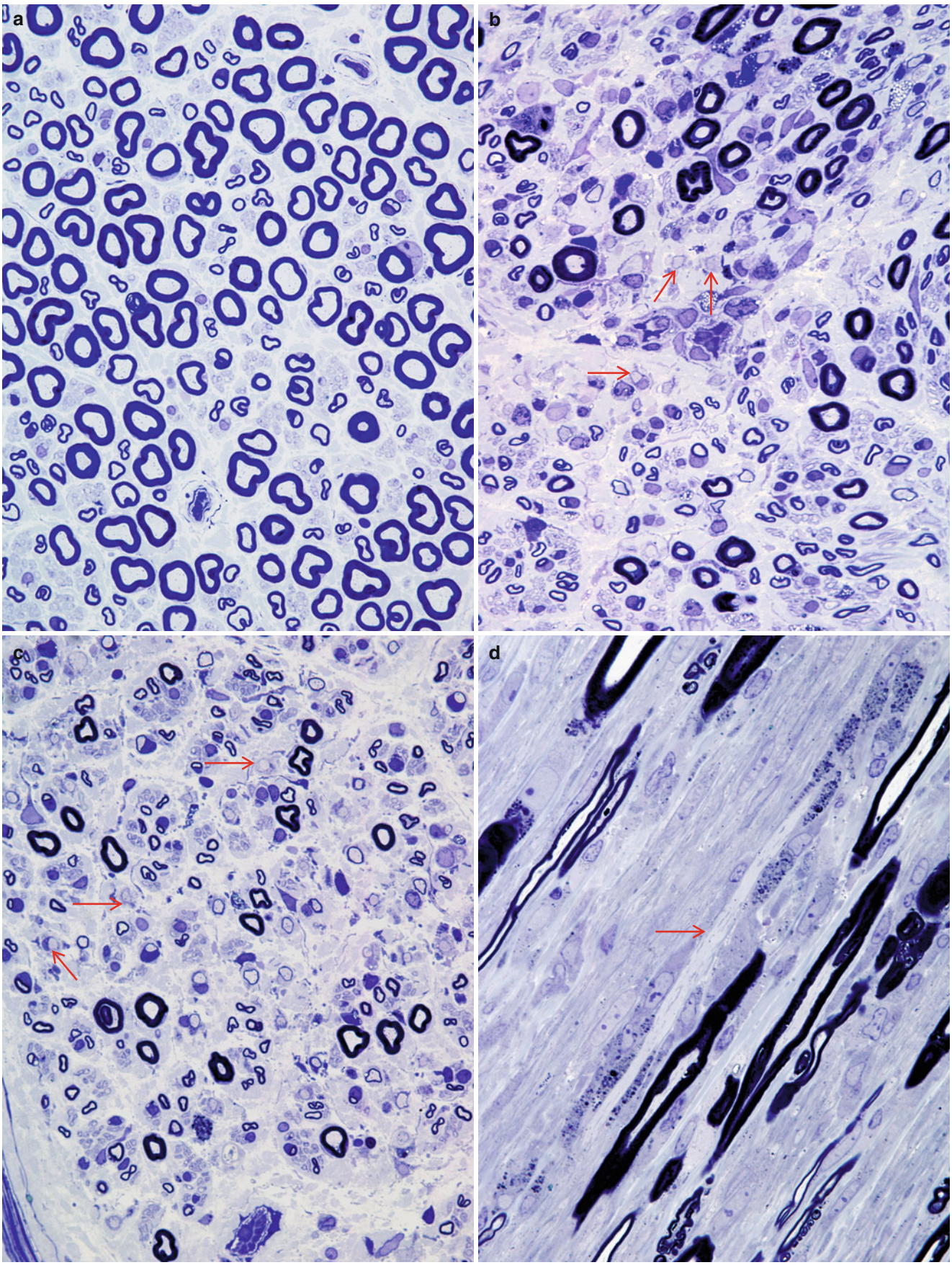

Fig. 9.3 GBS, AIDP subtype: normal sural nerve (**a**) compared with the typical appearance of Guillain–Barré syndrome (**b**–**d**). Note the large number of elongated and irregular cells in the endoneurium, loss of MF number, and myelin breakdown with numerous denuded axons (*arrows*, **b**, **c**). (**d**) Longitudinal section shows a naked axon (*arrow*) surrounded by multiple Schwann cells and macrophages with myelin debris (**a**, **b**, 1 μ plastic section 600×; **c**, **d** 1 μ plastic section 1,000×)

Hughes et al. 1992; Vallat et al. 1990). Although typical cases of AMAN show mild sensory axon degeneration, some cases of axonal GBS may show extensive sensory changes which are designated acute motor sensory axonal neuropathy (AMSAN). These cases are thought to represent a spectrum of a single type of immune attack on the axon differing in severity, immunologic presentation, or distribution of a critical epitope (Griffin et al. 1996, vide infra). Since the recognition of additional cases of AMAN/AMSAN, it has become clear that the most striking differences between AIDP and AMAN/AMSAN are the paucity of lymphocytic inflammation and the lack of demyelination and a variable increase in the number of degenerating axons in AMAN/AMSAN. Axonopathy ranges from diffuse axonal degeneration to distal axonal damage, with denervated neuromuscular junctions; the latter changes may be reversed rapidly.

Case 9.1

We have seen an illustrative case of AMAN of a 66-year-old Japanese woman who experienced a flu-like illness approximately 3 weeks prior to the initial onset of paresthesias of distal hand and feet with weakness which soon dominated her relatively minor sensory symptoms. During the course of a single day, she rapidly progressed to a condition where she could not stand without support and had difficulties swallowing and speaking, resulting in hospitalization. At that time, she was awake, alert, and fully oriented with normal pupillary light reflex and extraocular movement but mild weakness of facial muscles and tongue protrusion. Her arms and legs were weak bilaterally, and tendon reflexes were diminished or absent throughout. On the next hospital day, she was completely tetraplegic with absent tendon reflexes. On the third hospital day, she suddenly developed circulatory collapse, was resuscitated, but remained in persistent coma thereafter. The patient died with a total duration of illness of 41 days. Autopsy was performed and her anterior and posterior roots were sampled extensively.

Her lumbar anterior roots were most heavily involved. The proximal portion of her L4 anterior root showed occasional degenerating axons (Fig. 9.4a), accompanied by little inflammatory or macrophage infiltration. The distal portion of the same L4 anterior root, however, showed extensive axonal degeneration (Fig. 9.4b) with macrophage infiltration. Another portion of L4 anterior root showed residual degenerating axons but well-established regenerating axonal clusters (arrows, Fig. 9.4c), resembling the minifascicles of a neuroma. Sampled thoracic anterior roots also show ongoing axonal degeneration, although at that level, dorsal roots appeared minimally involved (Fig. 9.4d). Macrophages are found adjacent to the axons, in an expanded node of Ranvier (Fig. 9.5a) or within intact myelin sheaths (Fig. 9.5b, c) (case provided by Drs. Shinji Ohara, Mitsunori Yamada, and Hitoshi Takahashi).

9.1.2.3 Electron Microscopy

AIDP

Ultrastructural examination shows a complex collection of pathological findings (Fig. 9.6) including naked axons, edema separating axons, a background of macrophages with debris, demyelination (arrowhead, Fig. 9.6), and remyelination (arrows, Fig. 9.6). Ultrastructural analysis is critical for establishing the diagnosis of macrophage-mediated demyelination (Figs. 9.7, 9.8, and 9.9), the hallmark of inflammatory demyelinating neuropathy, whether acute (GBS) or chronic (CIDP). The classic picture is of a macrophage penetrating the Schwann cell basement membrane (Fig. 9.7a, b, arrows), separating Schwann cell cytoplasm from its underlying intact myelin (Fig. 9.7c) and insinuating cytoplasmic fingers into the intraperiod lines of the myelin sheath. Myelin destruction may seem to occur by dissolution along the site of contact with macrophage processes or through the separation, engulfment, and removal of large segments of myelin off the axon. The invading macrophage is rich in organelles including smooth ER, mitochondria, Golgi, and ribosomes, but the processes inserted into the myelin lamellae are largely free of these. All the while, the axon maintains a relatively normal appearance. Sometimes several processes may attack the myelin sheath at once, and it is unclear if they belong to one or several macrophages. Debris may appear as pieces of normally compacted myelin, vesicular myelin, lamellar disorganized osmiophilic material, or amorphous globules (Figs. 9.8, 9.9, and 9.10). Rarely, debris that seems to be extracellular may be detected (Prineas 1972).

The picture described above is not always easy to demonstrate. A more common observation is the presence of supernumerary cell processes sharing the Schwann tube with a residual Schwann cell and a demyelinated axon (Fig. 9.8); further afield, a macrophage may be present but separated from the affected fiber. Presumably the site of macrophage penetration has occurred outside the plane of section. As Schwann cells do not commonly accumulate myelin debris in AIDP, one may infer that the cell processes harboring phagocytosed lipid debris are those of a macrophage (Prineas 1972). The Schwann cell may be absent altogether, with the axon sharing a Schwann tube only with macrophages. Demonstration of segmental demyelination alone cannot establish macrophage-mediated demyelination. In summary, what is characteristic about macrophage-mediated demyelination is separation or peeling off of normal appearing myelin by fingerlike macrophage extensions; this process cannot be visualized after myelin disintegration is complete, and in such a situation, it cannot be determined whether the macrophage is the primary effector of the damage or simply a scavenger cell (Fig. 9.10). Alterations of myelin periodicity have very rarely been reported in GBS, including both uncompacted myelin (Brechenmacher et al. 1987) and widely separated myelin involving the two or three outermost turns of the myelin sheath (Vallat et al. 1994).

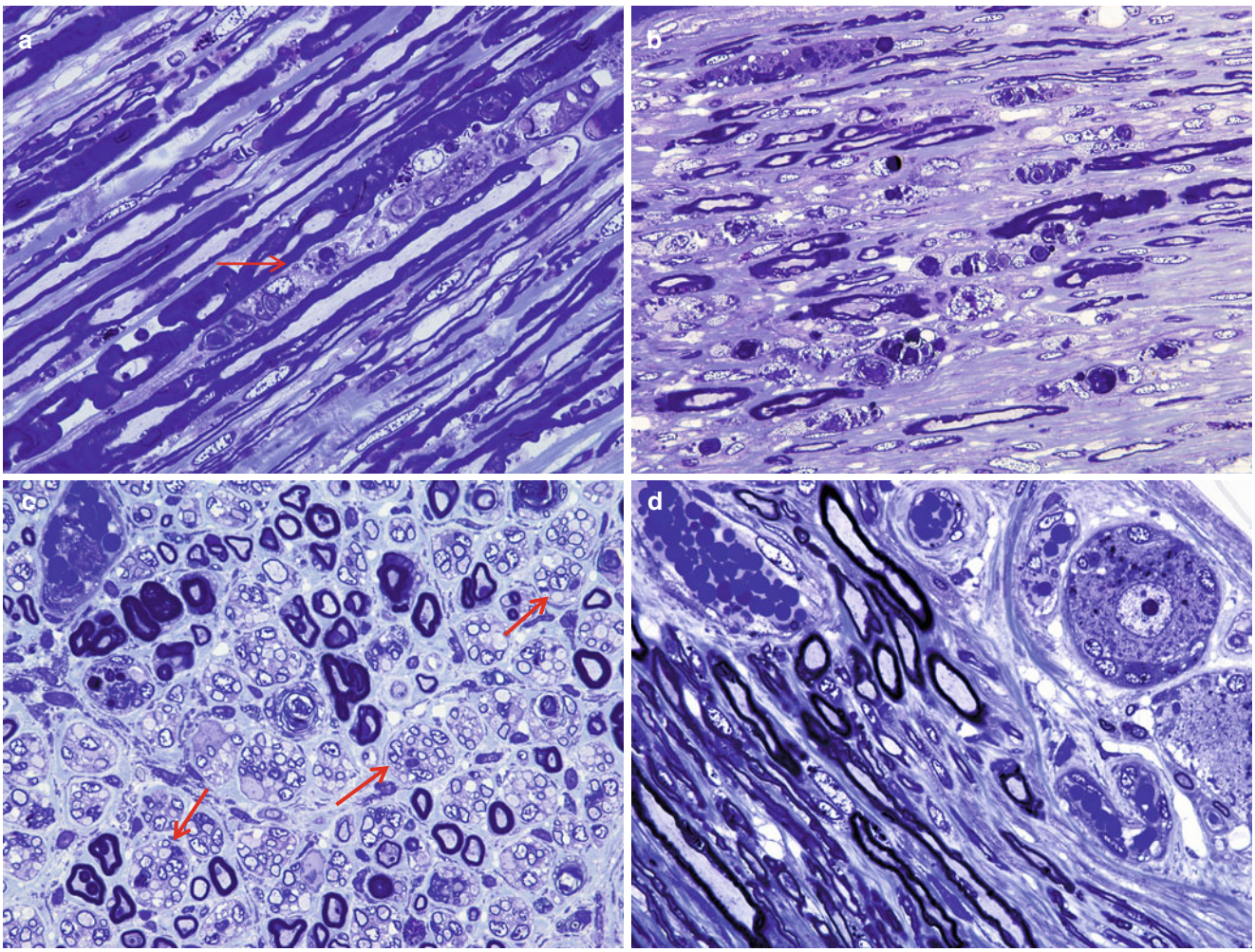

Fig. 9.4 GBS, AMAN subtype: lumbar (L4) proximal anterior roots showing individual axonal degeneration (*arrow*, **a**) or, in its more distal segments, extensive degeneration (**b**). (**c**) Rare degenerated axon surrounded by numerous clusters of regenerating axons (*arrows*). (**d**) Proximal segment of thoracic posterior root shows a DRG neuron and no axonal degeneration (1 μ thick plastic sections; magnification: **a**–**d**, 400×) (Case provided by Drs. Shinji Ohara, Mitsunori Yamada, and Hitoshi Takahashi)

Schwann cells can appear normal as they are being separated from their myelin or may show reactive or degenerative changes, with dilated ER, loss of organelles, and watery distension. Little or no myelin debris accumulates within them. Most axons appear unperturbed in the face of the myelin destruction surrounding them, but some show various stages of degeneration, ranging from loss of electron density to Wallerian degeneration. Naked axons have been said to shrink by as much as 50 % in volume as a consequence of the myelin loss, with a resulting increased density of tubules and filaments, and wrinkling of the axolemma (Prineas and McLeod 1976; Carpenter 1972; Raine et al. 1969). Evidence of recent remyelination is found in the presence of axons surrounded by very thin, poorly compacted myelin sheaths and Schwann cells rich in organelles.

A loss of unmyelinated axons may be seen but is rarely prominent and never predominant (Prineas 1972). Tubuloreticular inclusions have rarely been seen in endothelial cells and macrophages in GBS associated with HIV infection (Fuller and Jacobs 1989) but never in classic GBS. On two occasions, we have seen mononuclear cells, free of debris, within a seemingly intact myelin sheath without any detectable axon. These were cases with a particularly aggressive course and prominent axonal degeneration, but the observation has been too infrequent to allow generalization. We have never observed this finding outside of GBS. Similar observations were reported by Brechenmacher et al. (1981). A single report claims to document "viral particles" within the nerve in classic GBS (Sibley 1972), but in the absence of any corroborating observations, this must be regarded with skepticism. PCR studies have failed to demonstrate evidence of CMV genome in nerve specimens of patients with GBS (Hughes et al. 1992).

Although myelinated axon numbers are clearly decreased in GBS (Fig. 9.2b), ongoing axonal degeneration may be

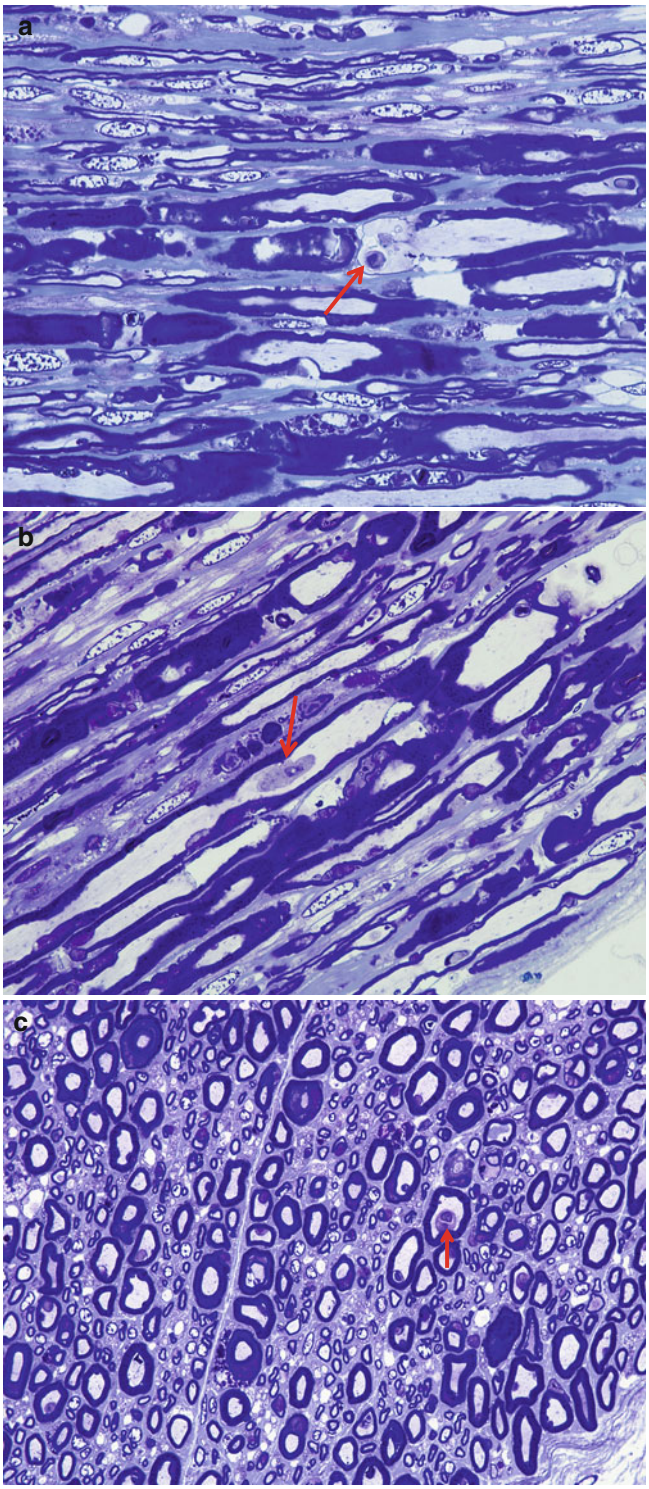

Fig. 9.5 GBS, AMAN subtype: L4 anterior roots show paranodal swelling with infiltrating macrophages (*arrow*, **a**). Longitudinal and cross sections show macrophages (*arrows*, **b**, **c**) within intact myelin sheaths adjacent to intact axons (1 μ thick plastic sections; magnification: **a**–**c**, 1,000×)

infrequent at any one time. Nonetheless, some cases of GBS exhibit so much axonal degeneration (Fig. 9.11a) that they can be mistaken for pure axonal degeneration. The case

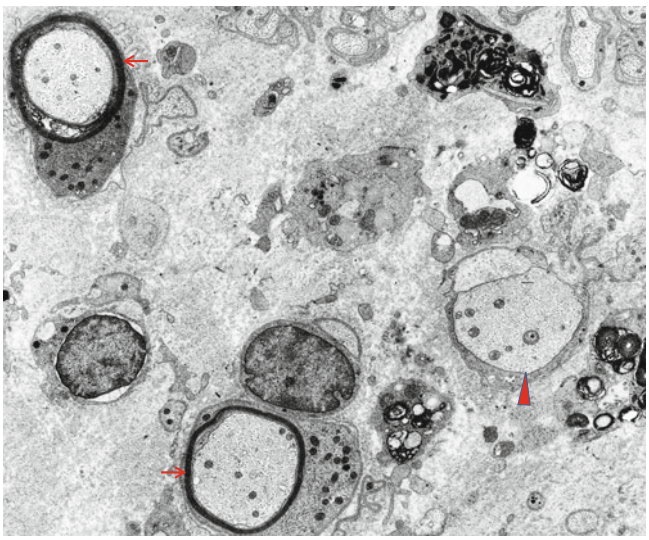

Fig. 9.6 GBS, AIDP subtype at 5 weeks: the endoneurium contains macrophage processes laden with myelin debris, a demyelinated axon (*arrowhead*), and two remyelinating fibers (*arrows*) (×9,256, from Bilbao 1995)

illustrated in Fig. 9.11 was initially thought to represent fulminant axonal degeneration alone; however, examination of other fascicles where myelinated axons were still present demonstrated macrophage-mediated demyelination (Fig. 9.11b, c).

AMAN/AMSAN

Ultrastructural changes include penetration of macrophage processes into the periaxonal space at the paranode or more distantly (Fig. 9.12a) and demonstration of an entire macrophage within myelin sheaths adjacent to intact axons (Fig. 9.12b, different case). Altered nodal structure with widening of the nodes and distortion of paranodal myelin may be followed by degeneration of outermost myelin terminal loops, eventually causing local degeneration of Schwann cell cytoplasm but sparing the internodal myelin sheath.

9.1.2.4 Immunohistochemistry

As in other inflammatory neuropathies, the use of immunohistochemical stains for leukocytes may bring out subtle infiltrates. Increased cellularity of the endoneurium can be due to macrophages, Schwann cells, or lymphocytes which can be separated by immunohistochemistry. However, the range of normal is not well established, and anything less than perivascular cuffing is of uncertain significance, especially in the epineurium.

An immunohistochemical and immunofluorescence search for IgG, IgM, or complement deposition on Schwann cells and myelin is usually (Brechenmacher et al. 1987; Hughes et al. 1992; Honavar et al. 1991) but not always (Luijten and Baart de la Faille-Kuyper 1972; Nyland et al. 1981; Vallat et al. 1994) negative in AIDP. Immunohistochemistry may be useful in characterizing the binding of anti-ganglioside antibody at the node in AMAN/AMSAN.

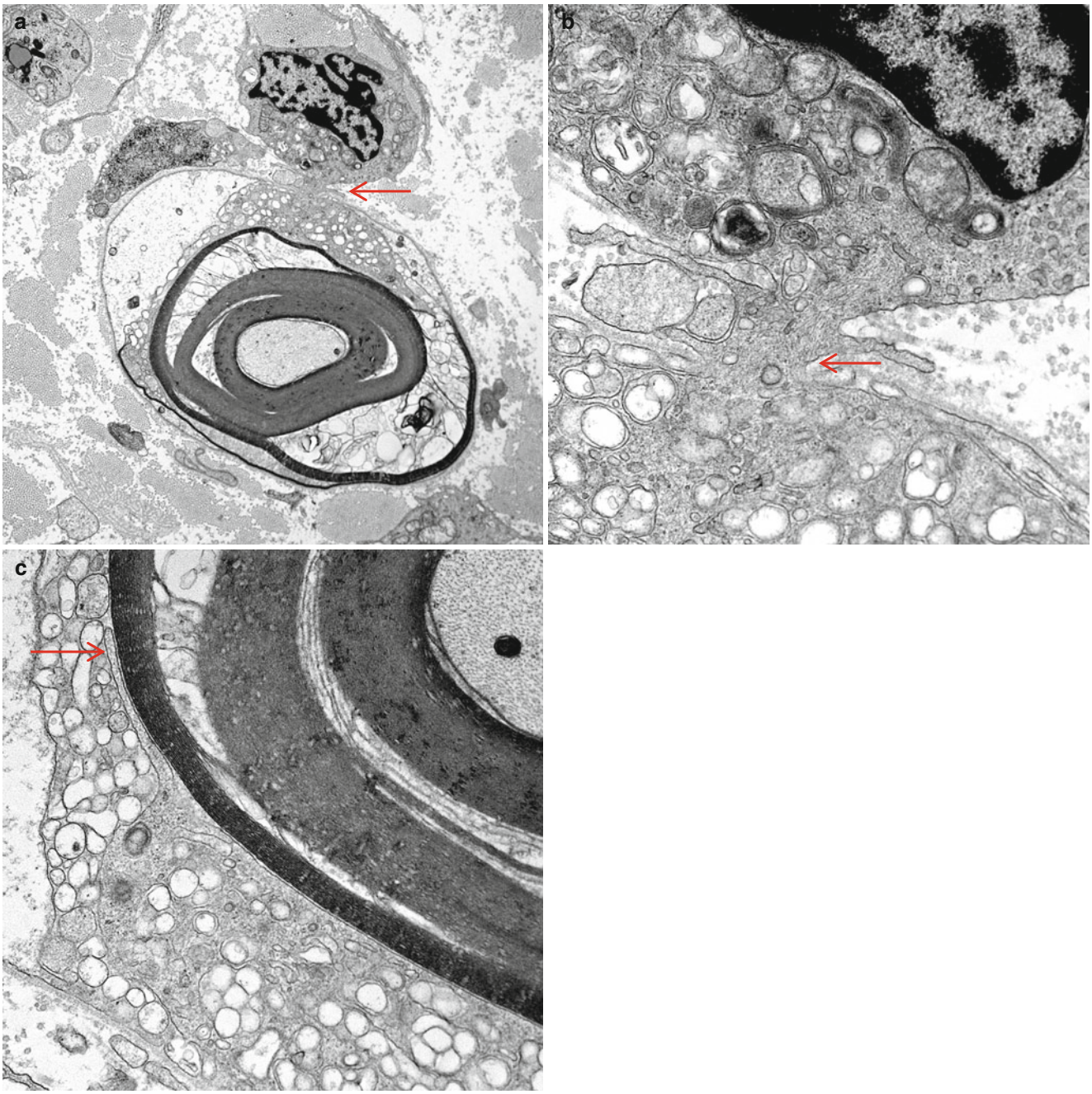

Fig. 9.7 GBS, AIDP subtype: a macrophage has begun to insinuate processes into the Schwann cell/axon complex at the *arrow* (**a**), seen at higher magnification in (**b**). The myelin has begun to disintegrate in response to the macrophage which contains relatively little digested myelin. At the site indicated (*arrow*, **c**), higher magnification shows a macrophage process adjacent to the outermost myelin lamella (Magnification: **a**, 6,000×; **b**, 40,000; **c**, 25,000×)

9.1.3 Pathogenesis

In the AIDP form of GBS, the fundamental process is primary demyelination, and axonal degeneration is variable in severity. Pathological alterations occur throughout the peripheral nervous system, not necessarily most prominently at the spinal nerve roots (Asbury et al. 1969). The experimental literature pertaining to pathogenesis has focused at various times on lymphocytes, macrophages, humoral factors, and alterations in the blood–nerve barrier. In AMAN, the primary target of the immune attack is the axon (particularly nodal/paranodal gangliosides) rather than the Schwann cell or myelin, with a predilection for motor axons. Recent evidence suggests that the node of Ranvier is the primary target

of immune attack in patients with both forms of GBS and CIDP (Devaux 2012), an interpretation which has been proposed and supported by skin biopsies (Doppler et al. 2013) using immunolocalization of the proteins that characterize the node (voltage-gated sodium channels) and paranodal region (neurofascin).

9.1.3.1 Lymphocytes

Emphasis on the role of lymphocytes began largely with an extensive autopsy review in 1969 which showed many of these cells in peripheral nerve at the earliest stages of GBS (Asbury et al. 1969). The importance of lymphocytes in pathogenesis has subsequently been supported by studies of animal models for GBS (vide infra). Interestingly, authors of autopsy and sural nerve biopsy reports have commented on the relative paucity of lymphocytes in their material (Honavar et al. 1991; Brechenmacher et al. 1987; Mancardi et al. 1988; Kanda et al. 1989). This may indicate that more than one pathophysiological mechanism may cause the same clinical picture or, more likely, that the disease is being examined at different stages.

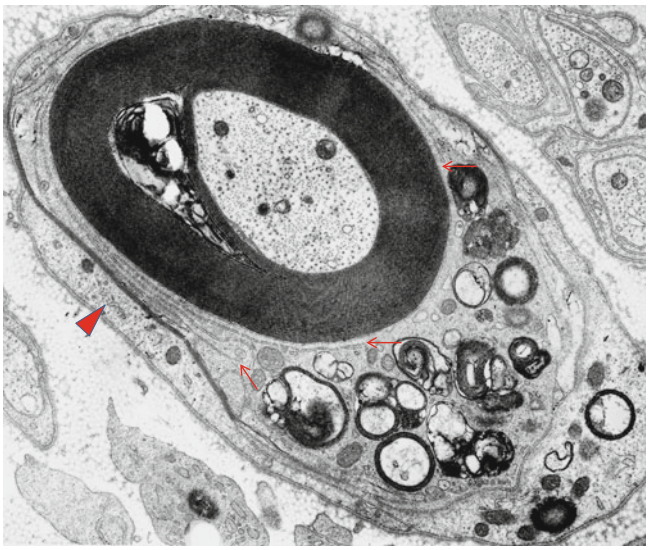

Fig. 9.8 GBS, AIDP subtype: an intratubal macrophage process strips myelin. Note interleaving of myelin lamellae and tongue-like macrophage extensions (*arrows*). The Schwann cell has been pushed peripherally (*arrowhead*) (Magnification: 20,800×)

The experimental allergic neuritis (EAN) animal model, in its numerous permutations, has been the cornerstone of research in GBS (Waksman and Adams 1955; Raine 1985; Hartung and Toyka 1990; Hahn et al. 1990). Experiments suggest that EAN is a T-cell-mediated autoimmune disease (Hartung and Toyka 1990). Starting 8–12 days after injection, lymphocytes and macrophages migrate across endothelial capillaries into the interstitium (Hahn et al. 1985), and demyelination soon follows. The most important antigen inciting EAN appears to be P2 protein (Rostami et al. 1984). CD4+ T cells sensitized to whole myelin, P2 protein, and even certain neuritogenic fragments of P2 protein can transfer the disease (Linington et al. 1984; Rostami et al. 1985; Rostami 1993). The severity of EAN is markedly diminished in T-cell-depleted recipient animals (Brosnan et al. 1987, 1988; Strigard et al. 1988). Administration of antibody to interleukin-2 receptor blocks activation of T cells and prevents development of the adoptive disease (Hartung et al. 1989).

In human material, some authors have emphasized infiltration with lymphocytes as the earliest change (Asbury et al. 1969; Iqbal et al. 1981). Serum IL-2 and its receptor are

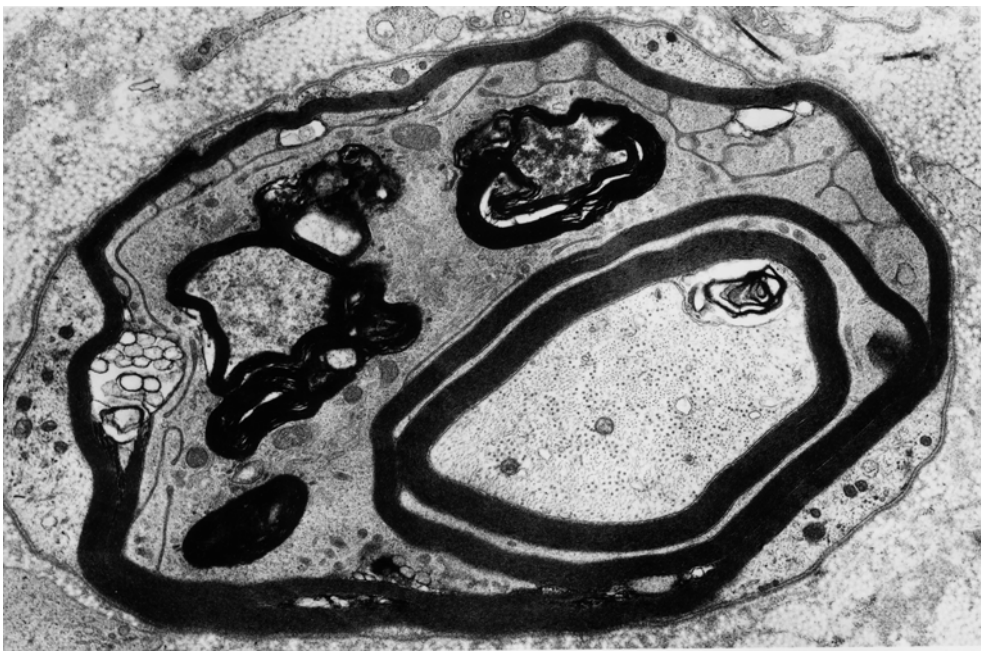

Fig. 9.9 GBS, AIDP subtype: intramyelinic macrophage process displays mitochondria and vacuoles containing myelin fragments. Adjacent myelin is being "peeled off" (15,620×)

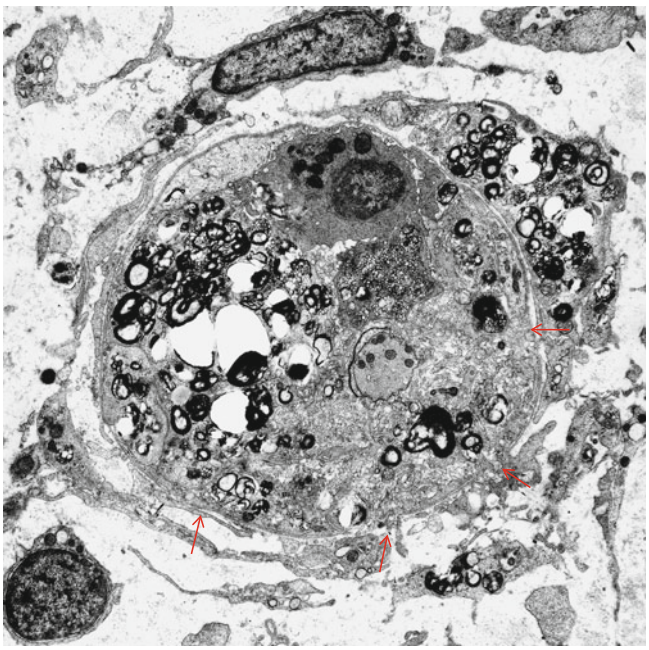

Fig. 9.10 GBS, AIDP subtype: advanced stage of demyelination in a fiber whose shrunken axon is at the center. The Schwann cell basal lamina is penetrated in at least four sites (*arrows*). Macrophage processes and a lymphocyte are seen outside the fiber (8,914×, from Bilbao 1995)

increased in patients with GBS relative to controls (Hartung and Toyka 1990) and correlate with disease activity (Hartung et al. 1991). Detection of specific sensitization of GBS patients' lymphocytes to P2 protein has been inconsistent (Taylor et al. 1991; Sheremata et al. 1975; Iqbal et al. 1981; Burns et al. 1986), and treatment with anti-lymphocyte antibodies has had no effect on the disease (Feasby 1991).

The pathogenesis of AMAN may initially involve antibody targeting of a constituent of the node of Ranvier or paranodal axolemma, most likely a ganglioside, to which it binds, fixes complement, and secondarily recruits macrophages; these events are thought to culminate in axonal dysfunction or, in some cases, axonal degeneration. In support of this hypothesis, rabbits immunized with gangliosides or GM1 may develop weakness, high titers of anti-GM1 antibodies, and pathological changes similar to human AMAN, with axonal degeneration confined to motor roots largely in the absence of inflammation (Susuki et al. 2003, 2007). Some people immunized with gangliosides have developed axonal forms of GBS.

9.1.3.2 Macrophages

Macrophages play a central role in inflammatory demyelination. At the height of disease activity, they form the predominant inflammatory cell (Iqbal et al. 1981; Brechenmacher et al. 1987). Macrophages are the dominant infiltrating cell type after the earliest stages of EAN (Lampert 1969; Raine 1985), and suppression of their activity diminishes severity of demyelination (Hartung et al. 1988). Ultrastructural studies in both human and animal inflammatory demyelination demonstrate that myelin destruction begins when a macrophage inserts a process through the basement membrane surrounding a myelinated fiber. Myelin lamellae are split, and resulting debris is carted away in the macrophage. In human disease, macrophage-mediated myelin stripping is only seen in CIDP and GBS. Reasons for the specific attack on myelin remain unclear. Complement and immunoglobulin deposits have been described on Schwann cells in AIDP (Luijten and Baart de la Faille-Kuyper 1972; Nyland et al. 1981) and may serve to opsonize the myelin sheath and promote macrophage adhesion and attack. However, this finding is inconsistent (Brechenmacher et al. 1987; Hughes et al. 1992) and nonspecific (Hays et al. 1988).

Macrophages may also play an important role in axonal damage. It has been noted that in both EAN and human inflammatory demyelinating neuropathies, the severity of axon loss is correlated with the severity of local inflammation and demyelination (Asbury et al. 1969; Feasby et al. 1990; Madrid and Wisniewski 1977). Tumor necrosis factor, a cytokine released by activated macrophages, has been shown to cause axonal degeneration (Said and Hontebeyrie-Joskowicz 1992). Macrophages are directly related to the degree of axonopathy in AMAN/AMSAN in response to periaxonal invasion.

9.1.3.3 Antibodies and Complement

In patients with GBS, circulating antibodies to peripheral nerve tissue are found in higher titers than in noninflammatory neuropathies or controls (Lundkvist et al. 1989; van Doorn et al. 1987). Complement-fixing antibody to peripheral nerve myelin is present in the serum of up to 95 % of GBS patients (Koski 1990). Antibody titers may correlate with clinical (Vriesendorp et al. 1991; Koski et al. 1986) or histological (Koski 1990) disease severity or response to treatment (van Doorn et al. 1987). However, some observers have failed to detect anti-myelin antibodies (Winer et al. 1988), and while IgG can pass the placental barrier from mother to fetus, maternal GBS does not cause neuropathy in the neonate (Ropper et al. 1991). Moreover, the proven efficacy of plasma exchange may result from removal of circulating antibodies, but an alternative explanation is the removal of cytokines (Pollard 1987) or a circulating antigen (Harvey et al. 1988).

Immunoglobulins may mediate myelin damage through activation of complement. Serum from Guillain–Barré AIDP patients injected into neural cultures and animal nerve may cause demyelination (Saida et al. 1982; Feasby et al. 1982; Sawant-Mane et al. 1991), even in the absence of macrophages (Birchem et al. 1987). Terminal complement complex (Koski 1990) is required for this demyelination to take

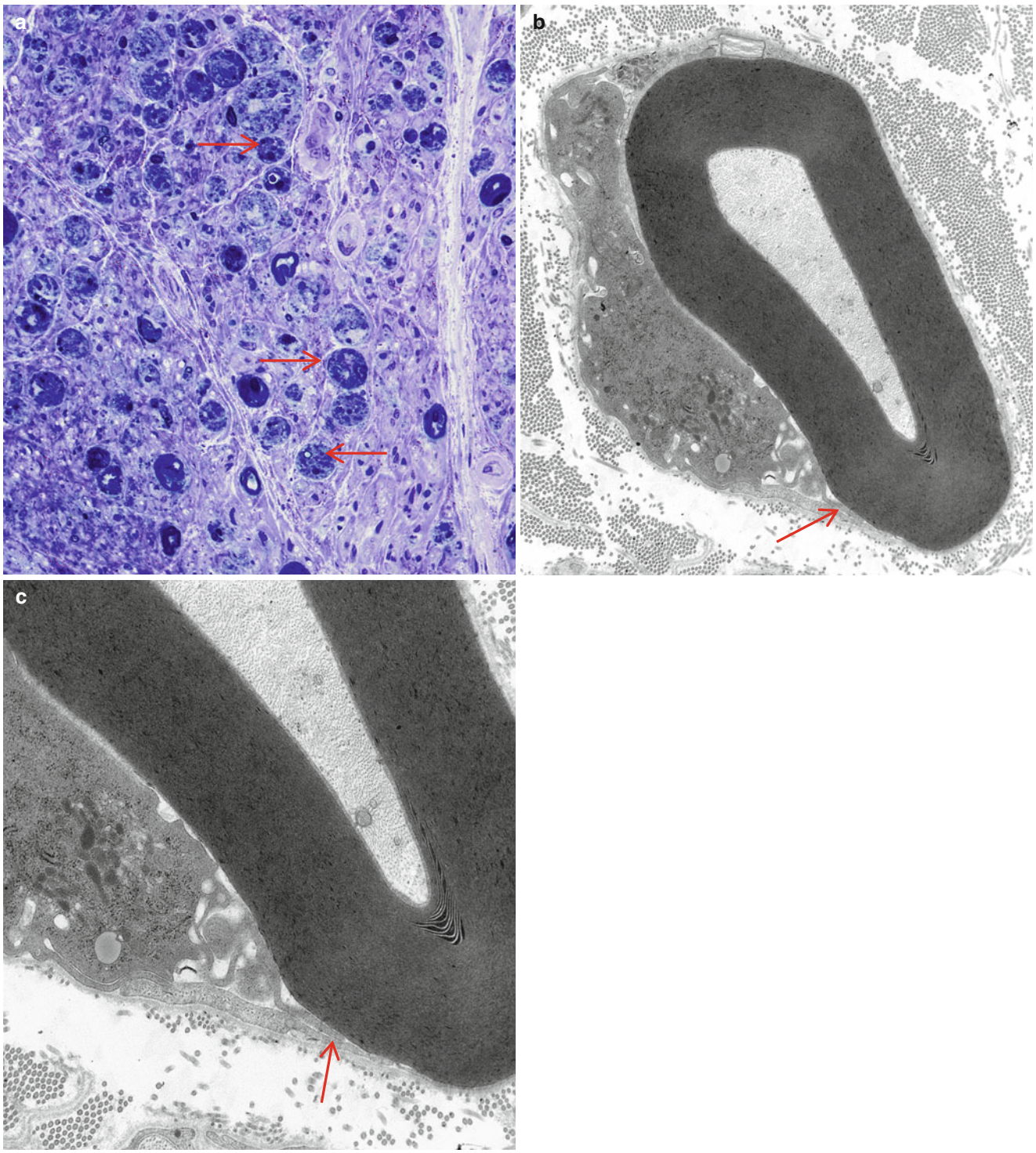

Fig. 9.11 GBS, AIDP subtype, with marked axonal degeneration. Numerous actively degenerating axons (*arrows*, **a**) do not suggest a demyelinative process. Nonetheless, at other sites in an adjacent fascicle, the characteristic macrophage-mediated demyelination (*arrows*, **b**, **c**) identifies this as a case of GBS, AIDP subtype (**a**, 1 μ plastic section, 1,000×; **b**, 12,000×; **c**, 20,000)

place (Sawant-Mane et al. 1991; Feasby et al. 1987). The complement cascade terminates with formation of C5b-9 membrane attack complex, a transmembrane channel which causes cell lysis by allowing ionic influx. The resulting influx of calcium may activate endogenous neutral proteases which can cleave important myelin proteins. Indeed, calcium influx into Schwann cells results in vesicular demyelination and macrophage-mediated myelin stripping (Smith and Hall

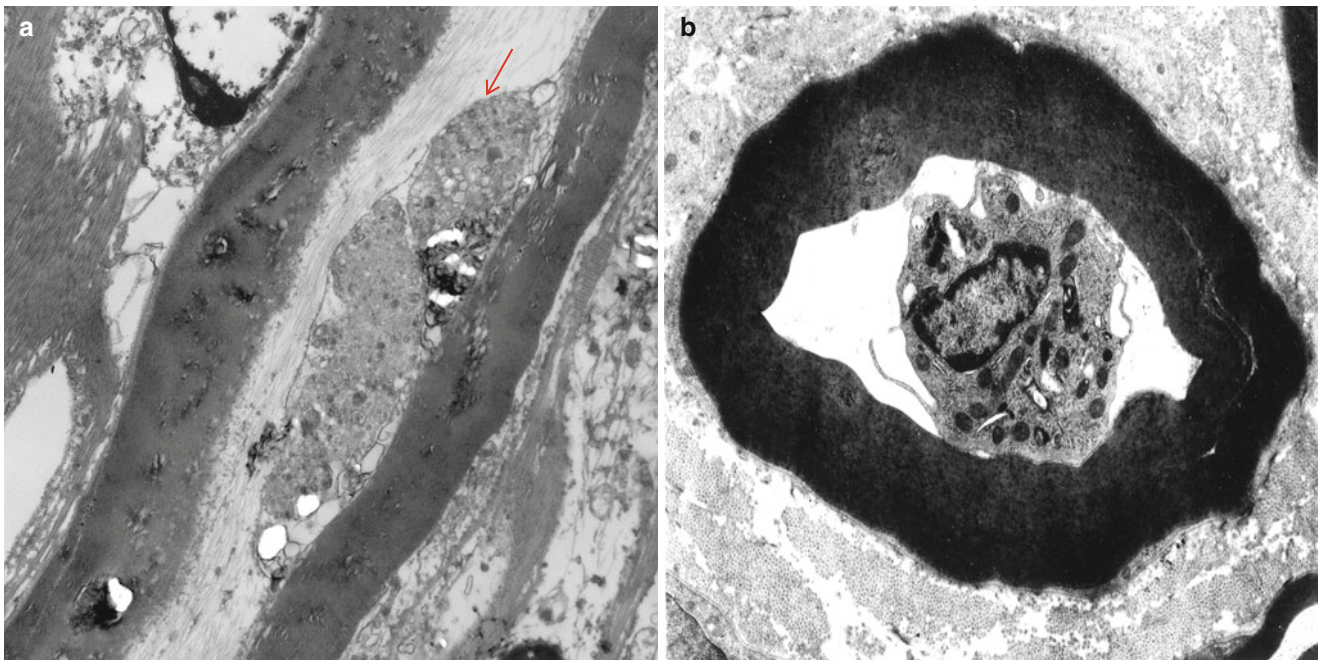

Fig. 9.12 GBS, (axonal type, AMAN): (**a**) a macrophage process (*arrow*) adjacent to an intact axon and within an intact myelin sheath. (**b**) A mononuclear cell, most likely a macrophage, surrounded by an intact myelin sheath (different case) (Magnification: **a**, 12,000×; **b**, 4,560×)

1988). In vivo, markers of complement activation are present both in serum and on spinal nerve roots and peripheral nerves of patients with GBS (Koski et al. 1987; Sanders et al. 1986).

Immunoglobulins may mediate myelin damage through activation of macrophages.

Transient complement deposits on Schwann cell and myelin membrane were seen in peripheral nerve in an EAN model before the onset of disease, and regions of accumulations of complement correlated with sites of macrophage concentration (Stoll et al. 1991). In experimental allergic encephalomyelitis, myelin lamellae are attached to coated pits on the macrophage surface, suggesting receptor–ligand interaction (Epstein et al. 1983). However, the finding of immunoglobulin or complement deposits on myelin or Schwann cell membranes is inconsistent (Luijten and Baart de la Faille-Kuyper 1972; Nyland et al. 1981; Brechenmacher et al. 1987; Hughes et al. 1992; Schenone et al. 1988).

The antigenic specificity of the putative complement-fixing or macrophage-activating anti-myelin antibodies is an area of active research. Several myelin antigens have been implicated including GM1 and a number of neutral and acidic myelin glycolipids. Some of these myelin antigens have cross-reactivity with antigens on organisms which have been epidemiologically associated with GBS: GM1 with *Campylobacter jejuni* and neutral myelin glycolipids with Forssman antigen, seen on a variety of viral agents (Koski 1992). However, these antibodies have been seen in a low percentage of patients with inflammatory demyelinating polyneuropathies and in a high proportion of controls, and there may be no association between the organism and its alleged cross-reactive peripheral nerve antigen (Quarles et al. 1990; Svennerholm and Fredman 1990; Vriesendorp et al. 1993; Enders et al. 1993; Mithen et al. 1992).

AMAN is associated with serum IgG binding to GM1, GD1a, and GalNAc-GD1a gangliosides. The selectivity of AMAN for motor axons may reflect the different affinities of anti-GD1a antibodies for sensory GD1a and motor GD1a with some evidence for preferential staining of motor axons and a subpopulation of small sensory axons (Lunn et al. 2000). Immunoglobulin G (IgG) anti-GD1a antibodies were found in 60 % of Chinese patients with axonal GBS but in only 4 % of patients with AIDP (Lunn et al. 2000), whereas anti-GM1 antibodies were frequent in both variants (Ho et al. 1999). Recently, AMAN and AMSAN with their strong association with antibodies to gangliosides have been classified as nodo-paranodopathy (Uncini et al. 2013). Others have proposed that autoantibodies targeting antigens in the presynaptic membrane of the NMJ (Fewou et al. 2013) represent an additional selective mode of access. Evidence is emerging to show that at least some antibodies ("ganglioside complexes antibodies") can induce pathogenic effects (Rinaldi 2013). Although antibodies against gangliosides GM1, GM2, basal lamina components, and several myelin proteins have been identified in some cases (Griffin and Sheikh 2005), no characteristic pattern of anti-ganglioside antibodies has been established in AIDP (compared with acute motor axonal neuropathy, AMAN, vide infra). Different serotypes of ganglioside-like polysaccharide antigens in *C.*

jejuni may influence the likelihood of AMAN since only a small minority of patients with *C. jejuni* enteritis develop AMAN. Both AMAN and AMSAN are thought to be a part of the spectrum of a single type of immune attack on the axon (Griffin et al. 1996), a contention supported by the presence in both entities of anti-GM1 (64 %), anti-GM1b (66 %), and anti-GalNAc-GD1a (33 %) IgG antibodies (Yuki et al. 1999; Hughes and Cornblath 2005) and the association of both conditions with *C. jejuni* enteritis.

Experimental animal studies have shown immunization with a bovine brain ganglioside mixture, or isolated GM1 produces antibody deposition at nodes, monophasic weakness, and prominent axonal degeneration without lymphocytic infiltration or demyelination. The final product of complement activation, the complement membrane attack complex, is formed at the nodal axolemma. Nodal sodium channel clusters disappear as complement is deposited and the node becomes exposed to the paranodal regions, lowering the safety factor. Autoimmune attack causes the disappearance of adhesion molecules, such as contactin and contactin-associated protein, at the paranodes, and disrupts the axoglial junctions and detachment of paranodal myelin terminal loops, as seen with electron microscopy (Kuwabara and Yuki 2013).

9.1.3.4 Alteration in the Blood–Nerve Barrier

Serum from GBS patients has no demyelinating activity unless it somehow bypasses the blood–nerve barrier (Tandon et al. 1980). In the EAN model, vascular permeability changes coincident with immunoglobulin changes have been observed before or along with other signs of pathology (Powell et al. 1983; Hahn et al. 1985). One group working with the EAN model demonstrated an increase in mast cell degranulation before the appearance of lymphocytes and onset of symptoms and showed that antagonism of mast cell vasoactive amine activity at the right time delayed the onset of EAN symptoms (Brosnan et al. 1990). Thus, early permeability changes in the blood–nerve barrier may play an important role in the pathogenesis of GBS.

9.1.3.5 Summary

Influenced by analysis of the experimental allergic neuritis, AIDP T cells were thought to recognize an autoantigen, perhaps an epitope on an infectious agent ("molecular mimicry"), in the presence of MHC class II and co-stimulatory molecules on the surface of an antigen-presenting cell. Nonetheless, no characteristic pattern of induced antibodies has been identified in AIDP as it has in AMAN.

9.1.4 Differential Diagnosis

Diseases of muscle, nerve root, and spinal cord that superficially resemble GBS are sorted out on clinical grounds. Common in clinical neuropathology are nonspecific findings in a nerve biopsy such as chronic inflammation and axonal degeneration, both being features of GBS and vasculitic neuropathy. Epineurial inflammation, presence of granulocytes, and intense and multifocal axonal damage favor vasculitis, whereas endoneurial inflammation, macrophage infiltration, and milder axonal degeneration argue for GBS.

Nonspecific histological findings that are not out of keeping with GBS can be seen in other neuropathies included in the clinical differential diagnosis. Such neuropathies include paraneoplastic sensorimotor neuropathy, paraprotein-associated neuropathy (Chap. 14), porphyria, Lyme neuritis, alcohol (Tabaraud et al. 1990), arsenic and thallium poisoning (Chap. 18), and neurolymphomatosis (Diaz-Arrastia et al. 1992). We have also seen a patient with granulomatous polyneuropathy evolving at the tempo of GBS (Chap. 10, case 10.1).

Macrophage-mediated demyelination is the only pathognomonic feature of GBS, AIDP subtype (or CIDP). In the search for macrophage penetration of Schwann cells and myelin, we resort to surveying many semithin sections under oil immersion and then selecting suggestive areas for ultrastructural examination. Vesicular degeneration of myelin is less specific. The finding of onion bulbs in the case of putative GBS suggests CIDP.

9.2 Chronic Inflammatory Demyelinating Polyradiculoneuropathy

Patients suffering from what is now called chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) have been described under several names in the past, among them chronic relapsing polyneuritis (Prineas and McLeod 1976), hypertrophic interstitial radiculoneuropathy (Austin 1956), chronic relapsing dysimmune polyneuropathy (Dalakas and Engel 1981), and chronic Guillain–Barré syndrome (Thomas et al. 1969). The clinical and pathological spectrum has been clearly delineated (Dyck et al. 1975; Prineas and McLeod 1976; McCombe et al. 1987; Barohn et al. 1989), and with the appreciation that many of these patients are responsive to therapy and that this is a common peripheral neuropathy (Dyck et al. 1982a), CIDP has become a very important neurological syndrome.

9.2.1 Clinical Manifestations

9.2.1.1 The Typical Syndrome

Criteria for the diagnosis of CIDP have been formulated by several groups (Barohn et al. 1989; Dyck et al. 1975, 1993; Ad Hoc Subcommittee 1991). Clinical features include:

1. Evolution over at least 2 months, often over many months
2. Progressive or relapsing and remitting course

3. Motor and/or sensory (usually both) symptoms and signs
4. Hypo- or areflexia

Onset may be at any age, and involvement is usually symmetrical. Motor manifestations tend to predominate, with proximal and distal muscles being involved. Large fiber sensory deficits can be prominent (vibration, joint position), but usually all modalities are affected. Cranial nerve palsies, frequently cranial nerve VII, occur in 14–43 % of patients (Barohn et al. 1989; Prineas and McLeod 1976; McCombe et al. 1987), and autonomic dysfunction is an uncommon feature. A palpably enlarged nerve is found in 11 % (Dyck et al. 1975; Prineas and McLeod 1976) and unexplained papilledema in as many as 7 % (Dyck et al. 1975).

CSF protein is elevated and cell count is normal in 90–95 % of patients (Dyck et al. 1975; Prineas and McLeod 1976; Barohn et al. 1989). Nerve conductions usually give evidence of a demyelinating process which is most severe proximally, but a variable amount of axonal disease can be seen (Prineas and McLeod 1976; Dyck et al. 1975; McCombe et al. 1987; Barohn et al. 1989). Most patients show conduction slowing in at least two peripheral nerves. Sensory potentials may be lost, and motor distal latencies become prolonged. Because the brunt of the disease falls proximally and standard nerve conduction studies assess the distal nerve better than the proximal nerve, abnormalities may be subtle early in the course. EMG examination may show acute or chronic neurogenic changes.

9.2.1.2 Diseases Associated with CIDP

A circulating paraprotein is seen in 8–30 % of cases (Barohn et al. 1989; Bromberg et al. 1992), but some authors exclude these patients (Dalakas and Engel 1981; Dyck et al. 1975) or classify them as having CIDP associated with a concurrent disease (Ad Hoc Subcommittee 1991). Clinically, there is no major difference between the two (Bromberg et al. 1992), although IgM paraprotein-associated neuropathies may enjoy a special status (Chap. 14). Classically, the pathology of paraproteinemic neuropathy and CIDP differs, but there can be an overlap. It remains to be determined whether CIDP with or without a paraprotein are different pathophysiological entities (Dyck 1990). Similar considerations apply to HIV-positive patients who can develop a CIDP-like syndrome with identical clinical, pathological (Vital et al. 1992), and therapeutic response patterns to those seen with classic CIDP (Cornblath et al. 1987), except for the tendency for CSF pleocytosis associated with HIV infection. Furthermore, a CIDP-like picture has infrequently been described in patients with SLE, chronic active hepatitis, ulcerative colitis, lymphoma (Sumi et al. 1983), and other systemic disease including diabetes, malignancy (e.g., melanoma), Sjögren's syndrome, and hepatitis C, and it is unclear whether there is any distinction between such cases and isolated CIDP (Rechthand et al. 1984; Barohn et al. 1989). In diabetic individuals, CIDP is reported to be more severe, but this is not established.

The distinction between recurrent Guillain–Barré syndrome (GBS) and relapsing CIDP may be difficult (Grand'Maison et al. 1992). Complete recovery between attacks favors the former (Dyck et al. 1993). The relationship between CIDP and multiple sclerosis is tantalizing, as central nervous system white matter involvement is known to occur in cases which are otherwise typical of CIDP (Dyck et al. 1975; Mendell et al. 1987; Uncini et al. 1991; Thomas et al. 1986), and peripheral nerve dysmyelination has been documented in multiple sclerosis (Schoene et al. 1977; Pollock et al. 1977). Whether any or all of these variations represent a process different than the one causing pure isolated CIDP remains to be clarified. A small number of these patients have a malignant plasma cell dyscrasia and an immunoglobulin G (IgG) (or less commonly IgA) paraprotein that causes the POEMS syndrome. Most patients with CIDP-MGUS and an IgG or IgA monoclonal protein have a disorder that is indistinguishable from idiopathic CIDP, including improvement with immune therapies and preferential involvement of the nerve roots.

9.2.1.3 Treatment

CIDP often responds to treatment, underlining the importance of establishing the diagnosis. Reports of improvement with corticosteroids in 39–95 % of patients (Dyck et al. 1975; Prineas and McLeod 1976; McCombe et al. 1987) have withstood controlled testing (Dyck et al. 1982a). Patients can, however, improve spontaneously. Plasma exchange (Dyck et al. 1986) and intravenous immunoglobulin administration (Faed et al. 1989; Van Doorn et al. 1991) appear to be effective, clinical benefit usually becoming apparent within days to weeks.

9.2.2 Pathology

9.2.2.1 General Considerations

Multifocal peripheral nerve disease that predominantly involves the proximal nerve (root, plexus, large nerves) is the substrate of CIDP (Milder et al. 1985; Dyck et al. 1975; Thomas et al. 1969). Examination of the sural nerve only provides a keyhole view of the entire picture. Nevertheless, since the clear delineation of this treatable neuropathy in the 1970s, biopsy has been used to confirm the diagnosis and justify the treatment required. In recent years in our institution, a frequent indication for sural nerve biopsies was for confirmation of a clinical diagnosis of CIDP. Other workers describe a similar experience (Krendel et al. 1989; Solders 1988; Oh 1990).

An understanding of the information to be gained from nerve biopsy in CIDP leads one to question the value of this

Table 9.1 Biopsy results in literature CIDP series

Biopsy shows:	Dyck et al. (1975) (*N*=26)	Prineas and McLeod (1976) (*N*=26)	Barohn et al. (1989) (*N*=56)	Small and Lovelace (1993) (*N*=19)	Krendel et al. (1989) (*N*=14)
Inflammation	54 %	Slight	11 %	11 %	29 % endoneurial
Onion bulbs	15 %	40 %	NA	21 %	36 %
Normal	NA	24 %	18 %	37 %	NA
Axonal	Teased fiber studies show axonal degeneration is overall more common than segmental demyelination	Demyelinating changes predominate over axonal but often are mild and mixed	21 %	16 %	NA
Mixed			13 %	N/A	N/A
Demyelinating			48 %	26 %	50 %

Table 9.2 Biopsy results in 51 personal cases of CIDP

	Inflammatory	Noninflammatory
Normal		1 (2 %)
Axonal	7 (14 %)	5 (10 %)
Demyelinating[a]	3 (6 %)	1 (2 %)
Mixed	23 (45 %)	11 (22 %)

Details of methodology in Chap. 1; three patients had an associated paraprotein

[a]Onion-bulb formations were seen in ten biopsies (20 %)

procedure in this setting. The rationale for biopsy has not been clearly defined but relates to corticosteroids being the mainstay of therapy and the clinicians' desire to have proof of the diagnosis before undertaking a potentially complicated long-term treatment (Krendel et al. 1989). However, an entirely normal sural nerve can be seen in as many as 24 % of cases, predominantly axonal pathology is not unusual, and often nerve biopsy changes are nonspecific (Tables 9.1 and 9.2). Inflammation is seen in two-thirds or less of the specimens, and a characteristic inflammatory predominantly demyelinating picture is probably present about half of the cases (Table 9.1). We have examined some biopsies in which only chronic axonal degeneration was seen despite an extensive search for inflammation or demyelination, where the patient met clinical criteria for CIDP and responded to immune-modulating treatment.

The clinical definition of CIDP is a complex issue (Ad Hoc Subcommittee 1991). The most constant feature is elevation in CSF protein (95 %, Barohn et al. 1989), but the nonspecificity of this finding renders it less useful. The "gold standard" of diagnosis is probably autopsy, where inflammation and demyelination are most consistently found in the proximal nerve segments. However, cases clinically typical of CIDP have shown neither inflammation nor demyelination at autopsy (Julien et al. 1989). Nerve biopsy demonstrates the classic picture of both inflammation and demyelination in about minority of cases, and axonal changes are common. Nerve conduction studies may show little or no evidence of demyelination (Barohn et al. 1989). Thus, the neuropathy may seem to be neither inflammatory nor demyelinating. There is poor correlation between the finding of axonal or demyelinating changes on electrophysiological tests and the finding of axon or myelin disease on sural nerve biopsy (Barohn et al. 1989). Finally, the degree of response to treatment does not correlate with the clinical picture, nerve conduction/EMG findings, or histological changes (Barohn et al. 1989).

When all these issues are weighed, we feel that a sural nerve biopsy should not ordinarily be performed in patients suspected of suffering from CIDP unless an alternative diagnosis is being entertained (i.e., amyloidosis, vasculitis). This invasive procedure cannot exclude CIDP, shows typical findings in 50 % or less of cases, and provides no useful prognostic information. A patient with chronic progressive weakness whose sural nerve shows normal histology or axonal changes may still be suffering from "CIDP" and should be given a trial of therapy if CIDP is suspected. Perhaps the biopsy should be performed only after failure of such a trial. If the differential diagnostic issue is CIDP vs. CMT-1, a not infrequent situation, biopsy can be helpful, but clinical and laboratory criteria are probably more reliable (Gabreels-Festen et al. 1993); a CSF examination is far less invasive than nerve biopsy. Moreover, the increasing availability of noninvasive genetic testing threatens to make this issue obsolete (Chap. 19, Gabreels-Festen et al. 1993).

9.2.2.2 Light Microscopic Findings in Typical CIDP

The pattern of involvement in CIDP is multifocal rather than diffuse, and this represents an important clue in distinguishing CIDP from a familial hypertrophic neuropathy. The histological picture may be so variable that in a single cross section of the sural nerve, one may observe normal fascicles, fascicles with mild segmental myelin changes, and fascicles showing severe demyelination and onion bulbs in various stages of formation (Figs. 9.13, 9.14, and 9.15). When significant involvement occurs, the appearance of the whole

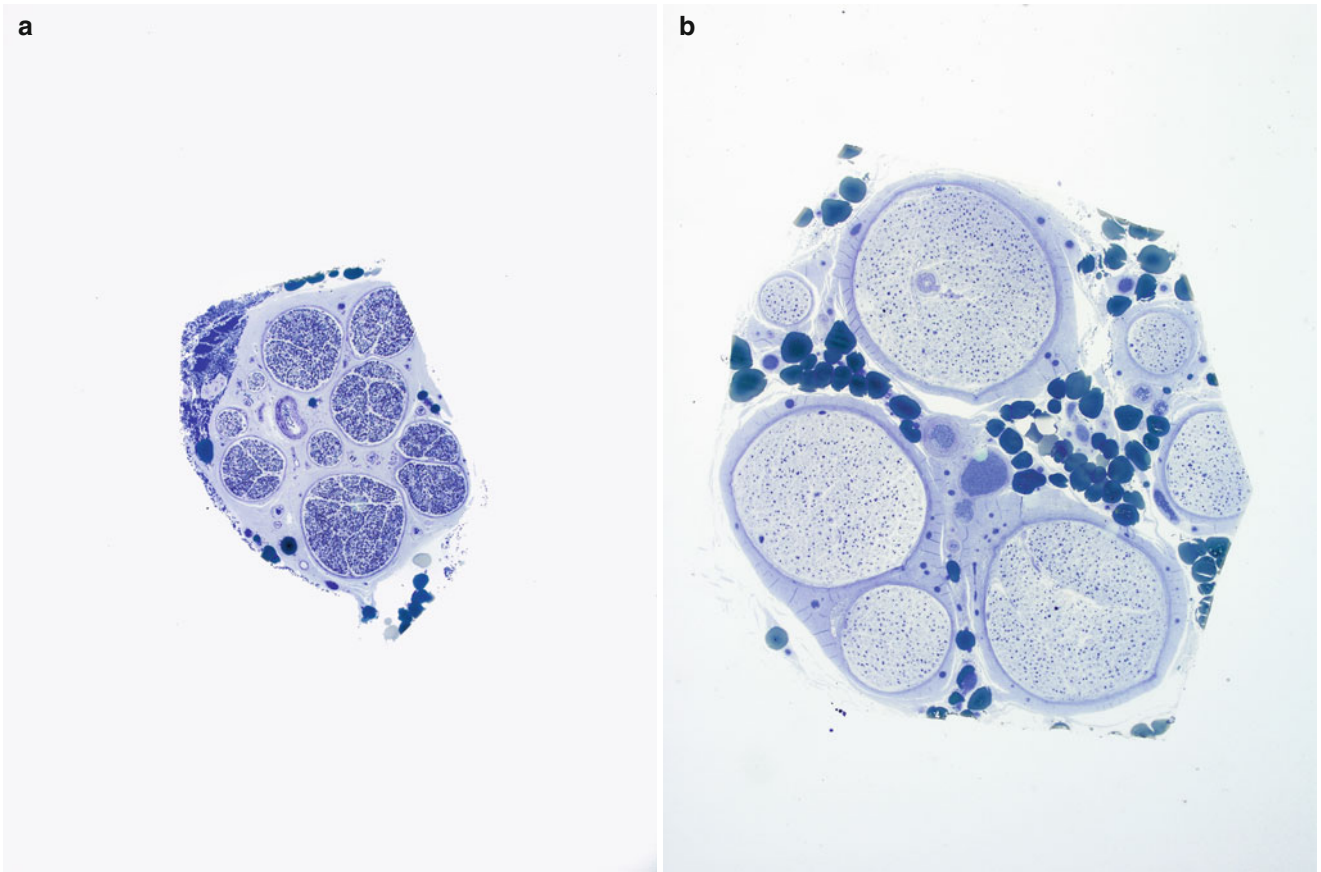

Fig. 9.13 Comparison of normal (**a**) and CIDP (**b**) sural nerves at same magnification (**a**, **b** 1 μ thick plastic sections, 40×)

nerve may be markedly increased in size (compare Fig. 9.13a, b, presented at identical magnification).

An inflammatory infiltrate has been seen in 0–50 % of cases in large series of cases (Dyck et al. 1975; Barohn et al. 1989; Dalakas and Engel 1981; Krendel et al. 1989; Oh 1990; Cornblath et al. 1990; Sluga and Poewe 1983; Small and Lovelace 1993; Gorson and Katz 2013) and is rarely striking. The light microscopic appearance of paraffin-sectioned material shows a variable degree of inflammatory infiltrate often involving the epineurial vasculature (Fig. 9.15a) but also involving the endoneurium as perivascular infiltrates (Fig. 9.15b) or as diffuse infiltration generally increasing the cellularity of the endoneurium (Fig. 9.15a). In our experience, inflammation is more frequent, 65 %, probably relating to our frequent use of immunohistochemistry. The mononuclear inflammatory cells are macrophages and lymphocytes, and the latter can be found in the epineurium or endoneurium. Significant perineurial lymphocytic infiltration has been observed (Poewe et al. 1981). Although endoneurial or epineurial perivascular lymphocytic cuffing may become quite prominent, this has no features of true vasculitis (Fig. 9.15). Some authors (Prineas and McLeod 1976) have not observed a perivascular pattern, and others have denied observing an inflammatory infiltrate altogether (Dalakas and Engel 1981). Plasma cells are uncommon, and the observation of plasmacytoid lymphocytes suggests the presence of a circulating paraprotein (Fig. 14.1). Polymorphonuclear cells are not seen in CIDP, and their presence suggests the diagnosis of vasculitis. Both $CD4^+$ and $CD8^+$ T cells are found in the infiltrate (Gorson and Katz 2013), along with activated macrophages but without significant numbers of B cells.

The early literature emphasizes the presence of eosinophilic subperineurial and endoneurial interstitial "edematous" changes (Fig. 9.16a, b), with deposition of an amorphous material which stains lightly with toluidine blue and Alcian blue and negatively with PAS, Azure A, or Congo red (Prineas and McLeod 1976; Dyck et al. 1975; Matthews et al. 1970). This often creates a subperineurial cell sparse region which should not be confused with Renaut bodies. A correlation between the presence of "endoneurial edema" and a loss of unmyelinated fibers has been suggested (Prineas and McLeod 1976).

A classic CIDP biopsy shows primary demyelination, with numerous thinly myelinated axons, macrophages filled with granular debris, and onion-bulb formations indicative of chronic recurrent demyelination and remyelination ranging from areas which are nearly normal (Fig. 9.14b) and those

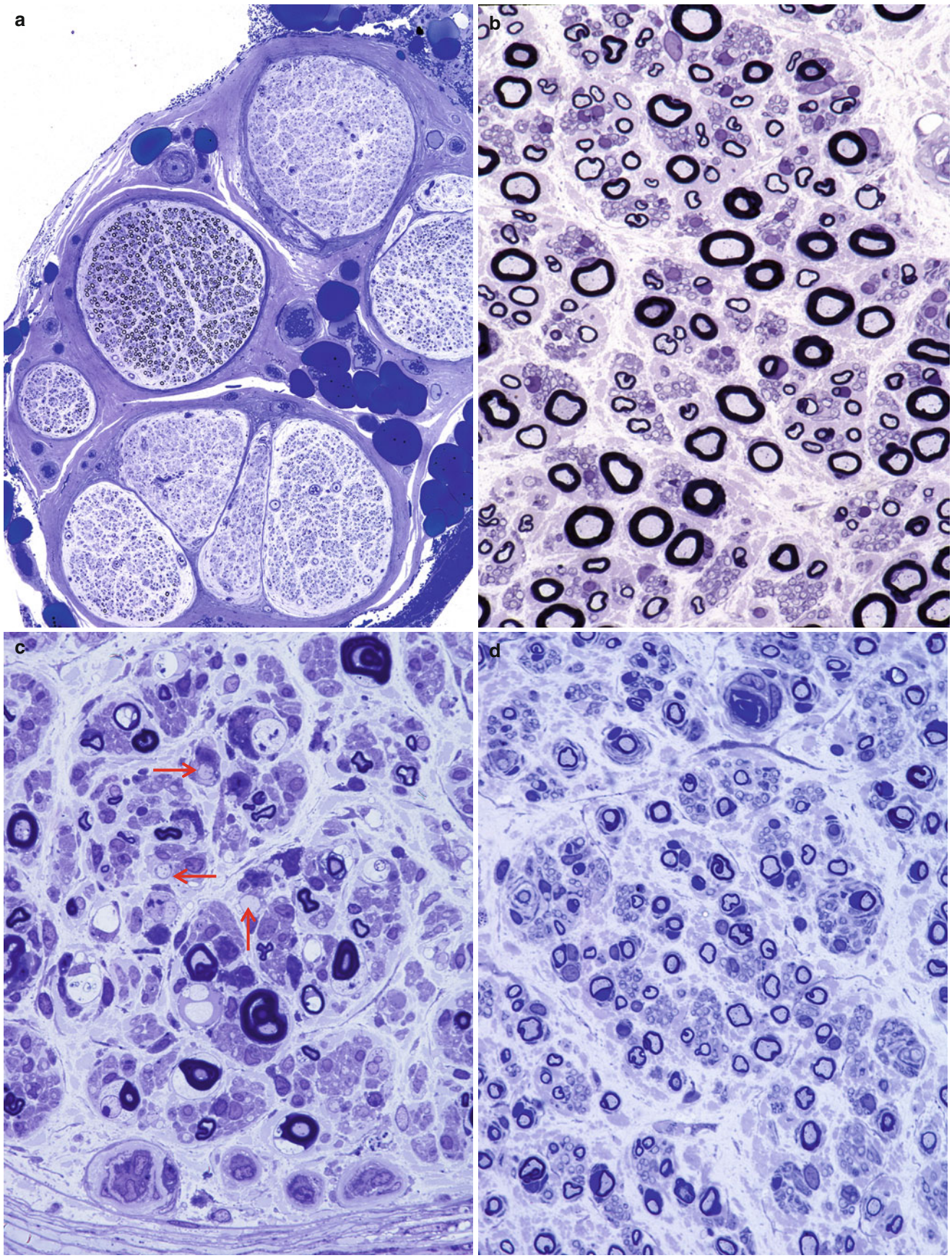

Fig. 9.14 CIDP: 70-year-old woman with a 1-year history of progressive sensorimotor polyneuropathy of moderate severity. After biopsy, treatment with steroids resulted in dramatic improvement. Variation in fiber density between fascicles is significant (**a**). In some areas the population of fibers is almost normal (**b**). Under oil immersion, naked axons are detected (*arrows*, **c**), while other fibers display generally attenuated myelin sheaths (**d**) (**a**, 100×; **b**, 1,000×; **c**, 1,000×; **d**, 1,000× 1 μ thick plastic)

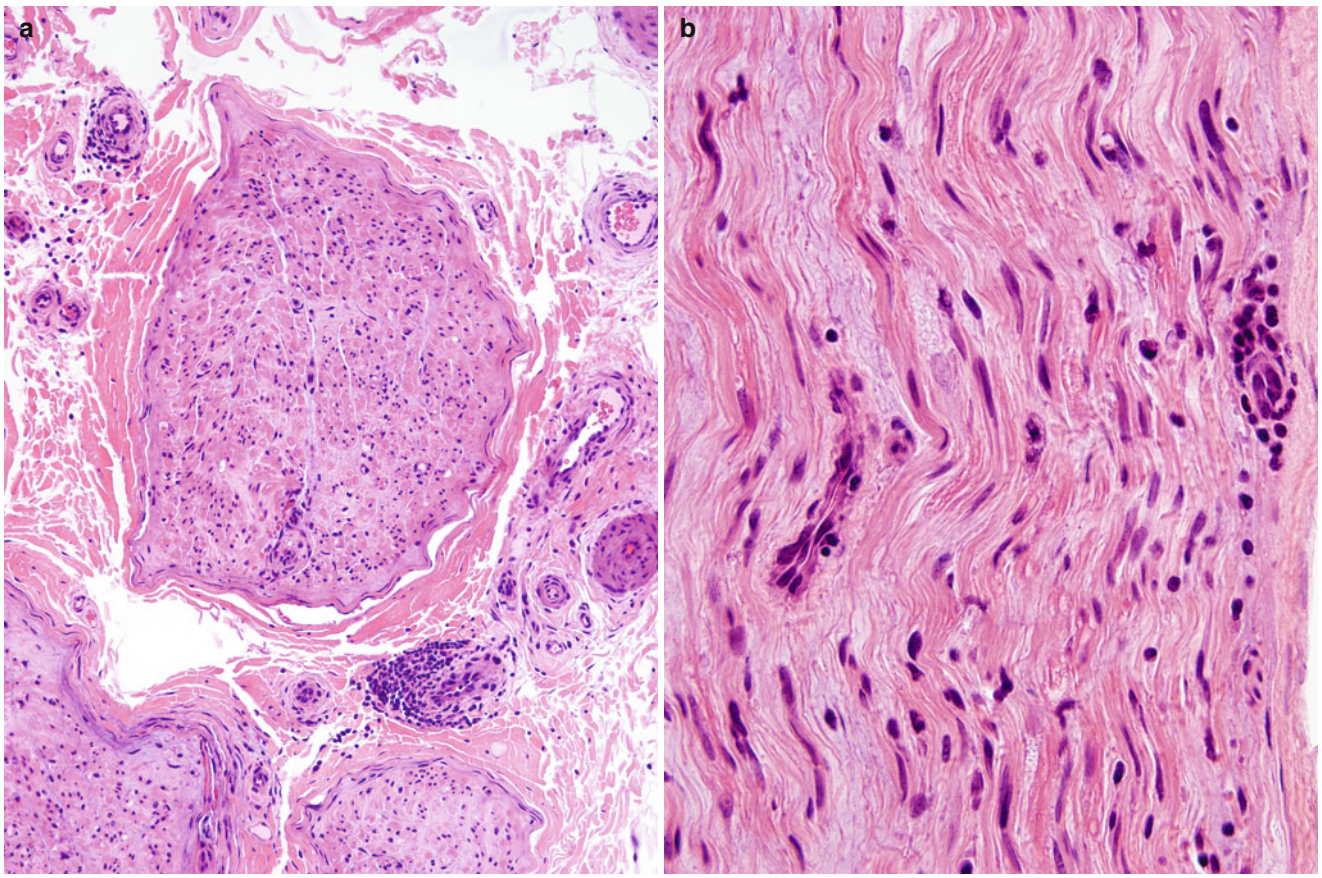

Fig. 9.15 CIDP: inflammation is present in the epineurium (**a**), chiefly perivascular, and accompanies a single fascicle with an overall increase in cellularity. (**b**) Endoneurial perivascular mononuclear infiltrate (paraffin, H&E)

with extensive demyelination and various degrees of onion-bulb formation (Fig. 9.15c, d) and areas in which onion bulbs have become "obsolete" (Fig. 9.16b). In a pure case, axonal loss is minimal. Such a clear-cut picture is present in minority of cases (Table 9.2), and the appearance will depend on the duration of illness. The demyelinating nature of the process may be hard to appreciate if only a few thinly myelinated or naked axons are seen. Additional sections or nerve teasing may be more revealing in such cases. On light microscopy, finding even rare debris-filled cells attached to seemingly intact naked axons is very suggestive of segmental demyelination but should be regarded with a degree of caution, as electron microscopy has shown us that axons that appear intact on light microscopy can be in early stages of degeneration. Onion bulbs are seen in 15–40 % of specimens (Figs. 9.17 and 9.18a, b) (Table 9.1) and can occasionally be so striking as to suggest a familial hypertrophic neuropathy. Hypocellular collagenized denervated onion bulbs represent the fate of some of these structures after several cycles of demyelination/remyelination and loss of the axon (Fig. 9.16b). A detailed discussion of onion-bulb morphology is provided elsewhere. The only morphologic feature that might distinguish the onion bulbs of CIDP from those of any other hypertrophic neuropathy is the occasional presence of inflammatory cells between Schwann cell layers in the former, although even this is no guarantee since some hereditary onion-bulb neuropathies may be associated with a lymphocytic infiltrate.

The presence of axonal damage is variable. Myelinated axon numbers can be normal even in the face of overwhelming primary demyelination (Pollard et al. 1983), but most often, axon numbers are reduced, 50 % of the lower limit of normal being not uncommon (Prineas and McLeod 1976; Dyck et al. 1975). Biopsies showing a predominance of axonal degeneration are commonly seen and large myelinated axons may be selectively affected. The axon loss may manifest as Wallerian degeneration or as diminution of axon numbers, presumably due to more proximal disease. The bimodal axonal diameter–frequency histogram may be retained. Regenerating clusters are not unusual. Unmyelinated axons are also probably damaged, but this is hard to prove and is not a prominent feature (Gibbels and Kentenich 1990; Ingall et al. 1990).

9.2.2.3 Ultrastructural Examination in Typical CIDP

The observation of macrophage processes breaching the basal lamina of a myelinated fiber and insinuating between

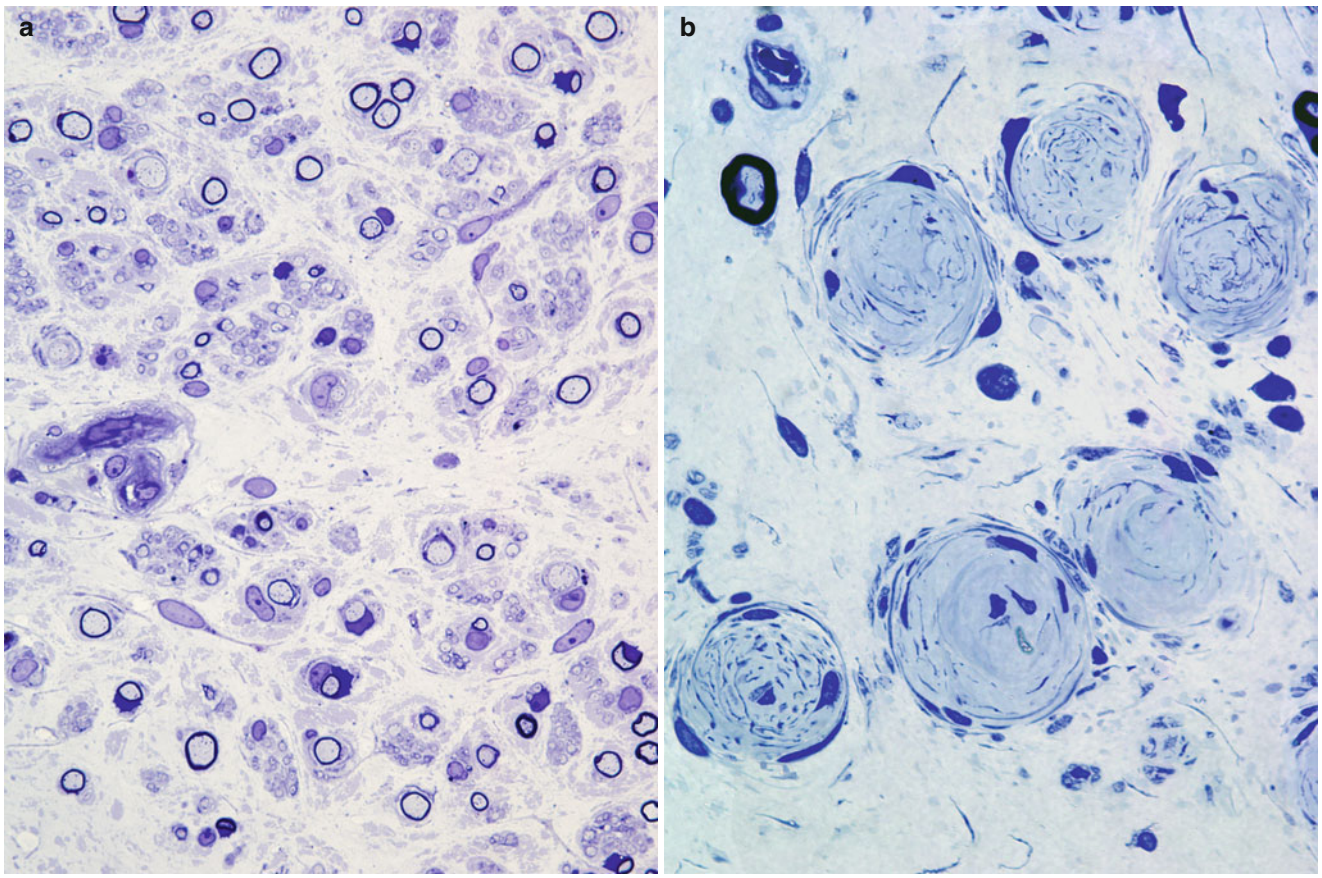

Fig. 9.16 CIDP: the dispersion of intrafascicular contents is due to the accumulation of endoneurial matrix (**a**). Obsolete onion bulbs as well as residual myelinated fibers are shown (**b**) (1 μ thick plastic sections)

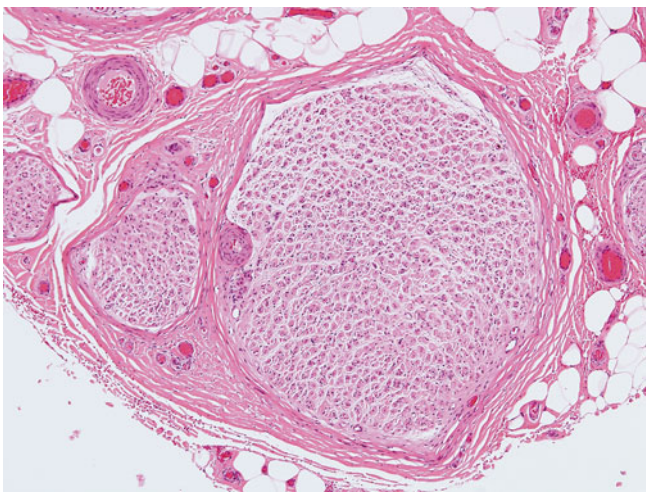

Fig. 9.17 CIDP: onion bulbs are numerous in this case and exaggerate size differences from fascicle to fascicle (paraffin, H&E)

myelin lamellae confirms the presence of macrophage-mediated demyelination, pathognomonic of GBS and CIDP. More often, however, the site of penetration is not obvious. Cross sections may reveal supernumerary cell processes sharing space with the axon, Schwann cell, and some degenerating myelin (Figs. 9.19, 9.20, and 9.21). When the cell processes contain debris, one may infer that these belong to a macrophage. Serial sections may reveal a site of basal lamina penetration. The key to the analysis of macrophage-mediated demyelination is the uptake of normal appearing myelin lamellae (Figs. 9.19 and 9.20). When a macrophage is seen engulfing or removing a myelin that is already extensively damaged, it cannot be determined whether this macrophage is primarily responsible for the demyelination or a scavenger (Griffin et al. 1993), and the diagnosis of macrophage-mediated demyelination cannot be made. Just as in GBS, vesicular demyelination may be present but is infrequent in comparison to myelin stripping (Bonnaud et al. 1974; Prineas and McLeod 1976; Rizzuto et al. 1982).

Vesicular demyelination is a controversial finding. In our experience, and in the literature, this myelin change is most often described in autopsy material (Honavar et al. 1991; Kanda et al. 1989; Carpenter 1972; Hart et al. 1972; Arstila et al. 1971; Mei Liu 1970). However, vesicular demyelination in nerve biopsy material has been described by numerous authors (Brechenmacher et al. 1987; Hall et al. 1992; Hughes et al. 1992; Vital et al. 1985; Fuller et al. 1992), making

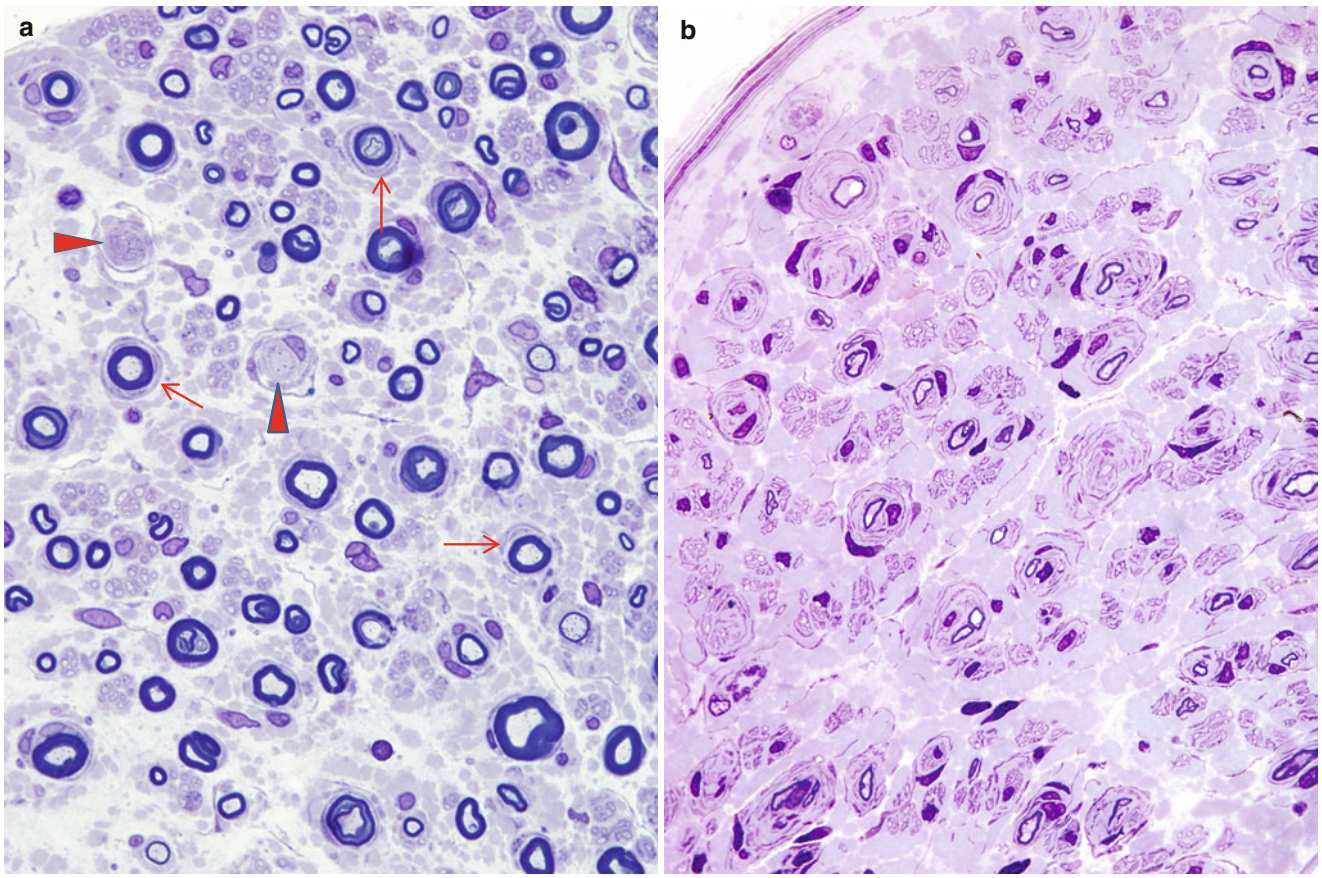

Fig. 9.18 CIDP: numbers of onion bulbs range from a few Schwann cells (**a**, *arrows*) accompanying naked axons (*arrowhead*, **a**) to numerous and well-formed (**b**) examples (1 μ thick plastic sections 1,000×)

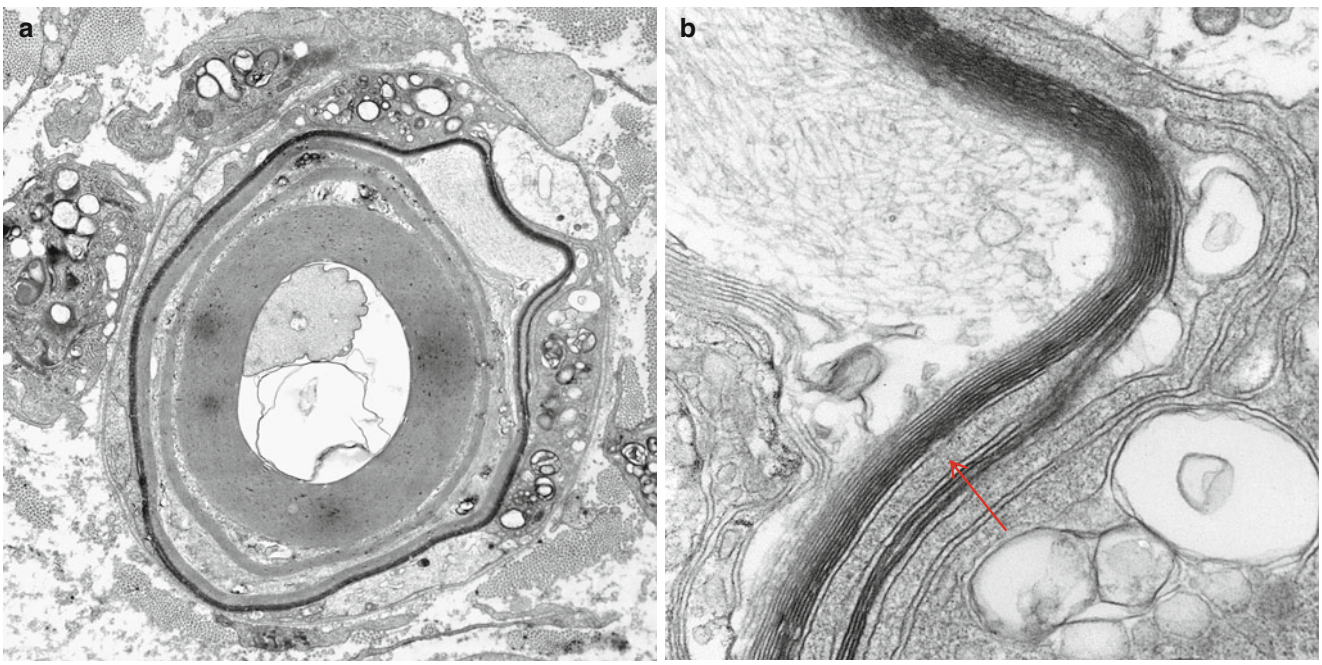

Fig. 9.19 CIDP: multiple macrophage processes surround a myelinated axon undergoing macrophage-mediated demyelination (**a**). An interdigitating macrophage process (*arrow*, **b**) is seen separating individual myelin lamellae (**a**, 7,500×; **b**, 50,000×)

it hard to dismiss this finding as a postmortem or poor fixation artifact. It may be seen in the same section as ongoing myelin stripping (Fig. 9.21a, b) (Prineas 1972). At times, vesicular demyelination may be the most prominent process, and although these seem to be cases with a more fulminant course, this notion has not been systematically studied. While myelin stripping always requires the presence of a macrophage, vesicular change can be observed in the absence of nearby inflammatory cells. Indeed, vesicular myelin degeneration can be seen in noninflammatory axonal neuropathies. Typically, there is focal separation of myelin lamellae at the major dense line, forming vesicles and cylinders that are about 80 nm in diameter. As this process progresses, the myelin degenerates into a meshwork of vesicles and tubes that may be focal or entirely surround the axon (Fig. 9.21b). Myelin in proximity to inflammatory cells may also show other nonspecific degenerative changes, as may Schwann cells. There are also rare reports of widened myelin lamellae or uncompacted myelin, ordinarily considered highly suggestive of dysproteinemia-associated neuropathy.

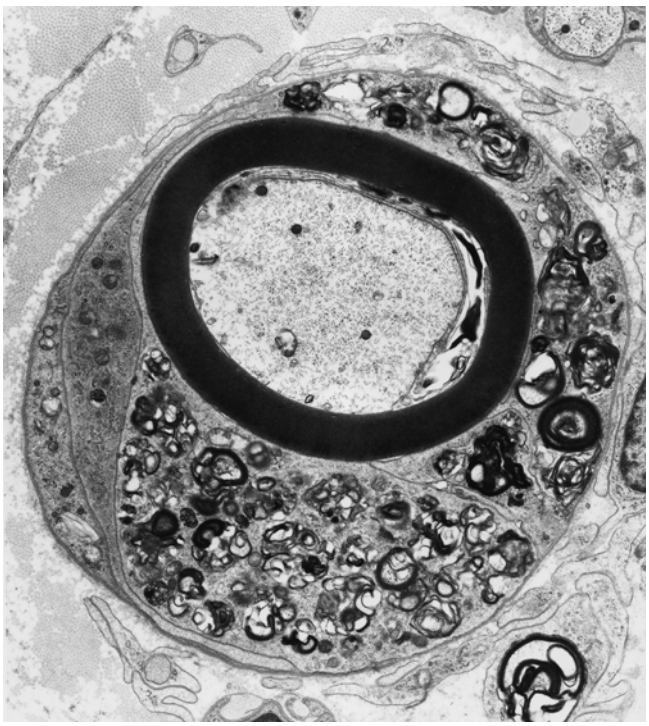

Fig. 9.20 CIDP: Partially demyelinated axon surrounded by multiple pmacrophage processes containing myelin debris (10,000×)

Axons within well-developed onion bulbs may occasionally degenerate resulting in an onion bulb without a central axon (Fig. 9.22a, b). Otherwise, at the end of the demyelinative process, there remains a central, naked axon occupying the intratubal space with one or more debris-laden macrophages (Figs. 9.23 and 9.24). After macrophages depart (arrows, Figs. 9.25 and 9.26), the process is represented by a naked axon surrounded by multiple processes (Fig. 9.25) or none (Fig. 9.26) largely free of debris, some of which likely represent Schwann cells (Fig. 9.25). The remaining naked demyelinated axons may be recognized by shrunken condensed axoplasm with increased neurofilament density and reactive Schwann cell cytoplasm (Fig. 9.27).

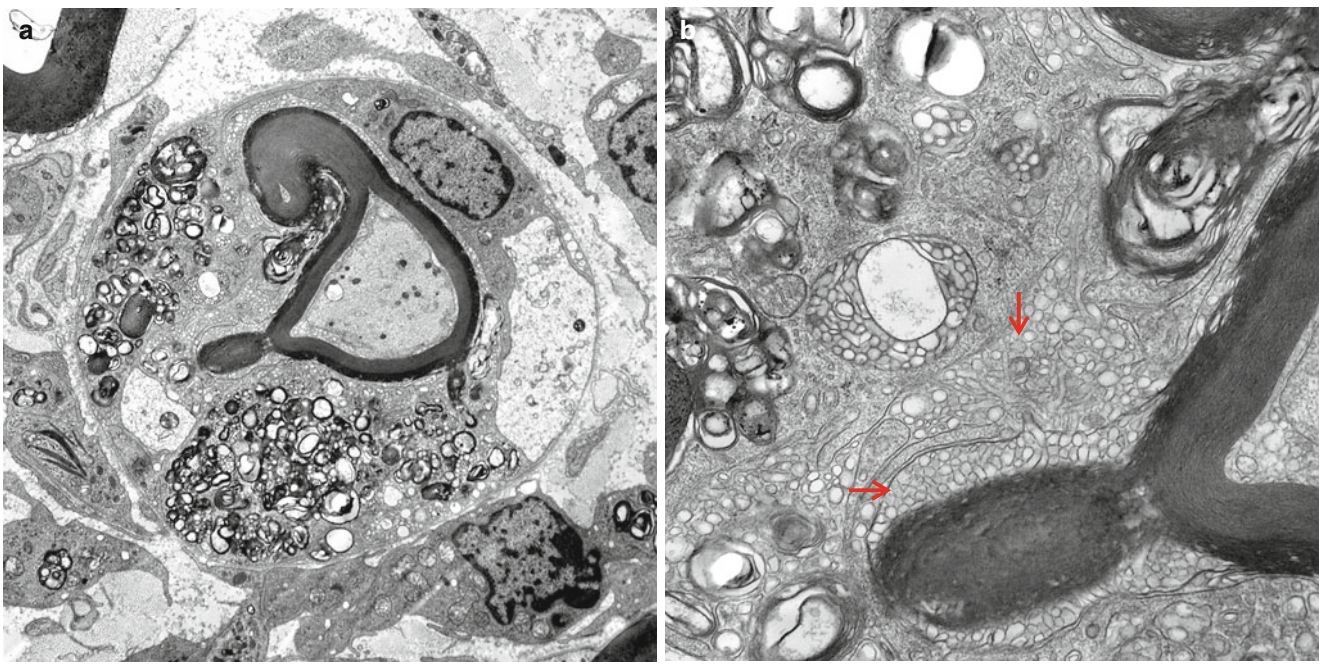

Fig. 9.21 CIDP: vesicular myelin change (*arrows*, **b**) in macrophage-mediated demyelination (**a**, 6,000; **b**, 25,000×)

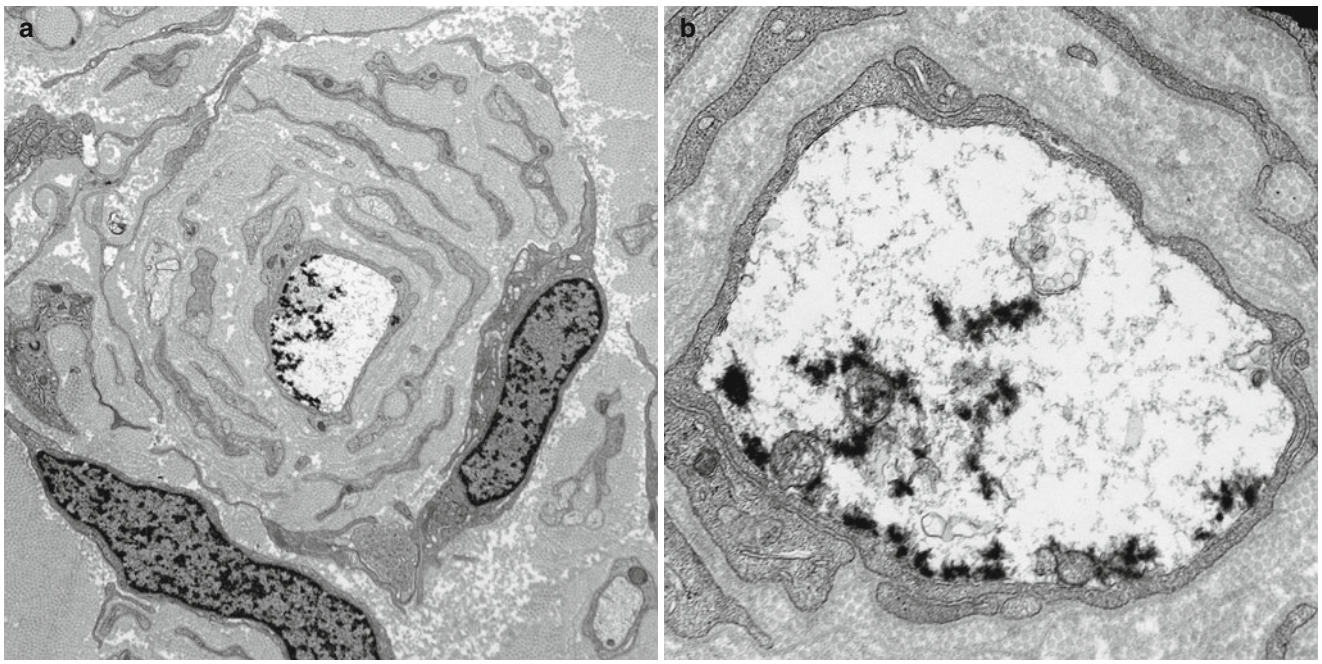

Fig. 9.22 CIDP: an onion bulb in which the central axon is demyelinated and undergoing active degeneration (**a**), seen at higher magnification in (**b**) (**a**, 7,500×; **b**, 25,000×)

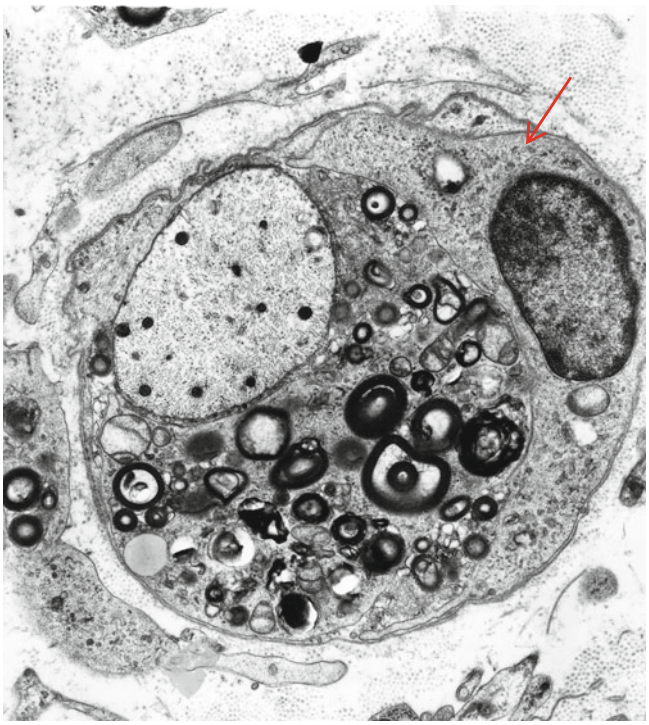

Fig. 9.23 CIDP: late stage of segmental demyelination is shown. A residual Schwann cell (*arrow*) is identified by a lack of myelin debris (9,230×)

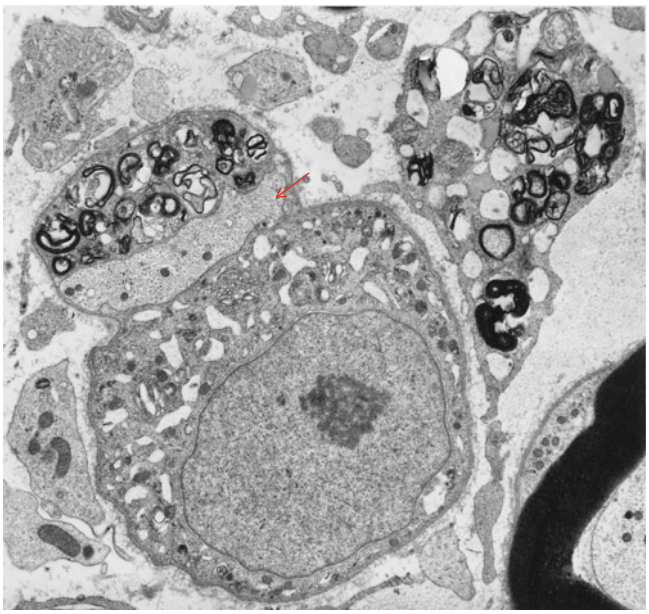

Fig. 9.24 CIDP: end stage of segmental demyelination. Three cellular processes are identified enclosed by Schwann cell basal lamina: a nucleated Schwann cell with prominent cytoplasmic organelles, an intervening denuded axon (*arrow*), and a macrophage process laden with myelin debris (13,406×)

Axons in early stages of remyelination may also be found, recognized by the presence of a few lamellae of loosely compacted myelin and a Schwann cell rich in cytoplasmic organelles (Figs. 9.6 and 9.28). In the earliest stages of remyelination, several Schwann cells may be in contact with a single axon, but by the time remyelination is complete, only one remains. Bands of Schwann cell processes and collagen pockets may give evidence of unmyelinated fiber loss.

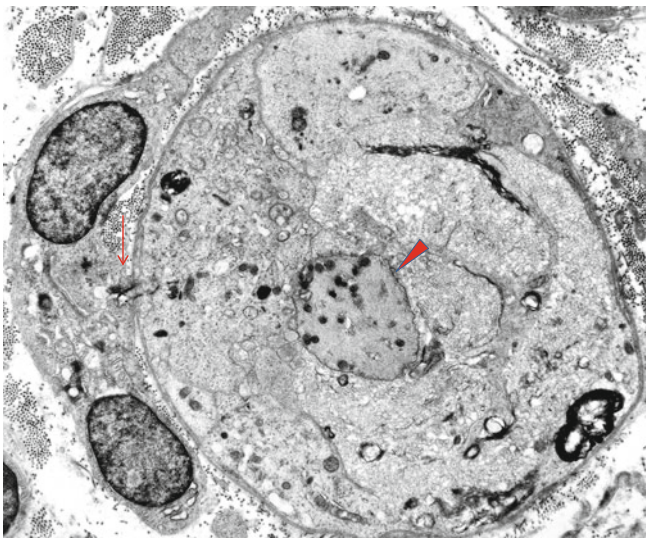

Fig. 9.25 CIDP: numerous cell processes are within a Schwann cell basal lamina. The axon can be visualized at the center (*arrowhead*), and a site of macrophage penetration (*arrow*) is shown (11,240×)

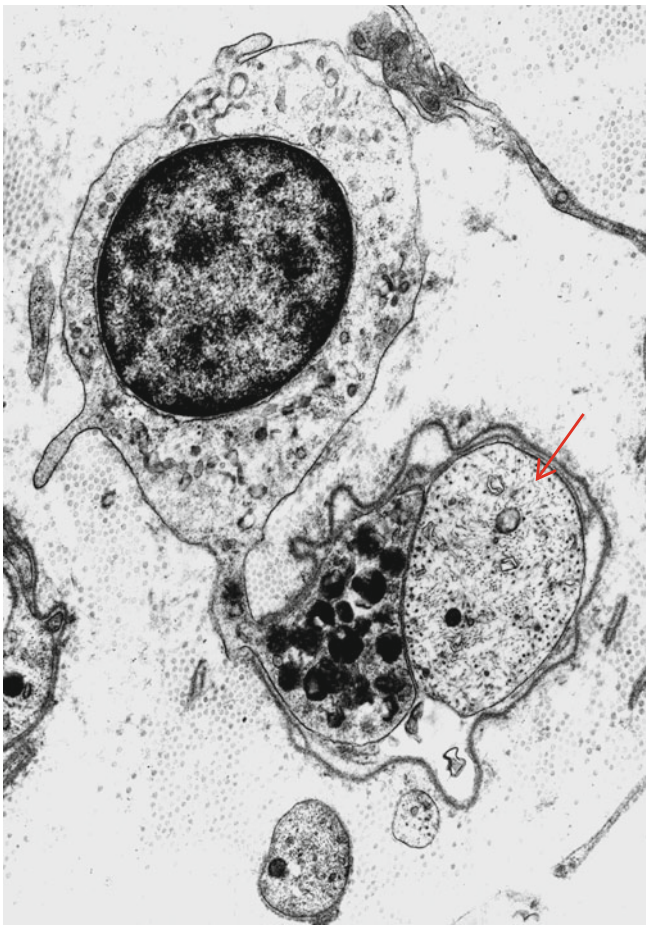

Fig. 9.26 CIDP: final stage of segmental demyelination. The macrophage is exiting the Schwann tube through a gap in the basal lamina. Note that the residual axon (*arrow*) is invested by basal lamina with no intervening Schwann cell process (13,490×)

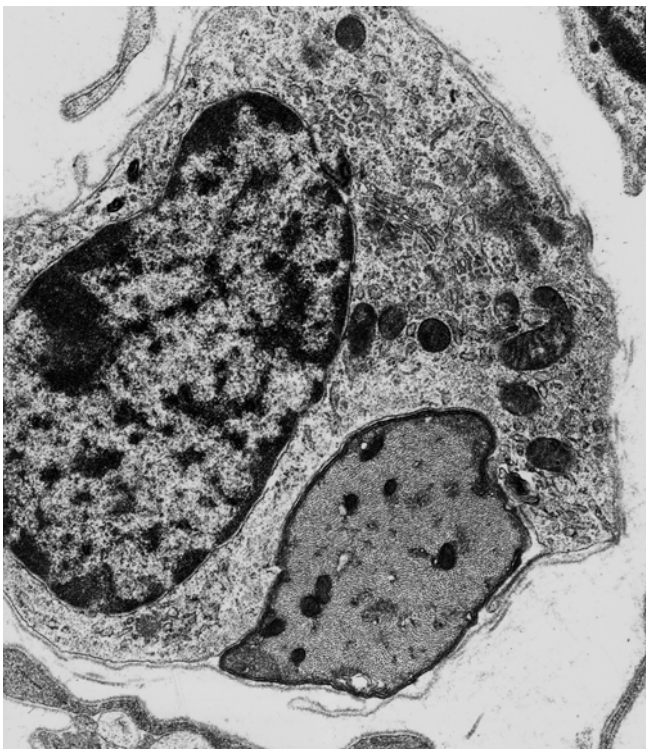

Fig. 9.27 CIDP: condensation of the axoplasm is often seen in demyelinated axons. Schwann cell cytoplasm shows reactive changes. (12,312×)

The ultrastructural correlate of "edema" in the subperineurial and endoneurial matrix has been described as an accumulation of collagen and filaments with a diameter of 8–11 nm (Figs. 9.29 and 9.30) (Watanabe and Ohnishi 1979; Prineas and McLeod 1976). Prineas and McLeod (1976) has also noted 20–50 nm acid mucopolysaccharide-like endoneurial granules. At least some of the fibrillar material is oxytalan. The amorphous deposits emphasized in one published report were Renaut bodies. Swollen endothelial cells are not uncommon, and we have infrequently observed fenestration of endothelial vessels in CIDP (Fig. 9.30). Mononuclear inflammatory cells can be spotted randomly in the endoneurium, perivascularly, or within onion-bulb formations (Figs. 9.31 and 9.32).

9.2.2.4 Nerve Teasing

Nerve teasing identifies segmental demyelination in most cases of CIDP (Prineas and McLeod 1976; Dyck et al. 1975), and if the appropriate statistical studies are performed and demonstrate that the abnormalities are randomly placed, primary demyelination is documented. However, the single most common abnormality seen in teased nerves can be active axonal degeneration (Dyck et al. 1975). Given the uncertain frequency of segmental myelin change in teased fibers of "normal" nerves, one must interpret mild changes with caution: 8 of 17 of Prineas' cases with segmental

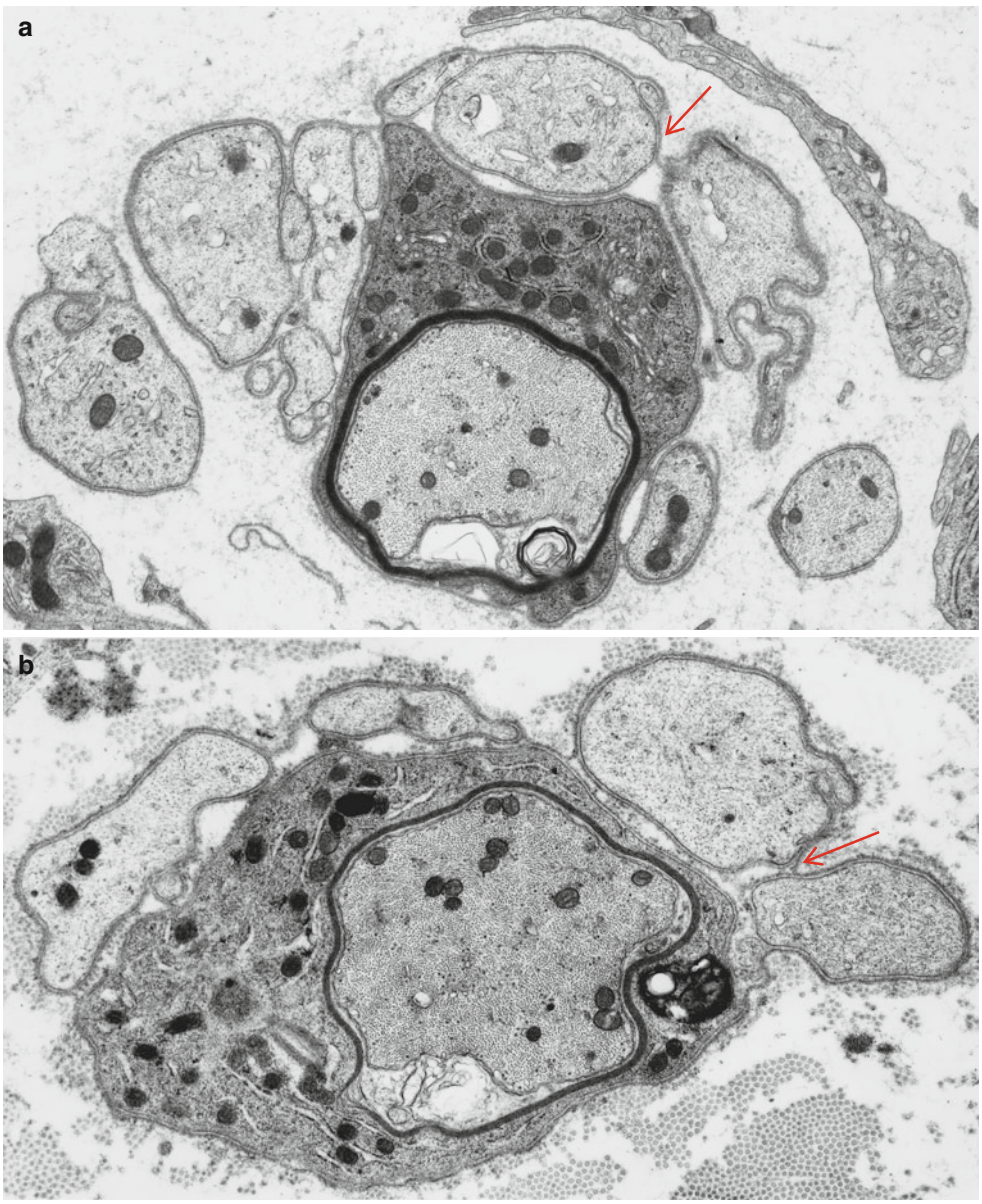

Fig. 9.28 CIDP: remyelination. Two new myelinated axons are associated with Schwann cells whose cytoplasm is rich in organelles. Obsolescent supernumerary Schwann cells are seen at the periphery, still sharing a common basal lamina (*arrows*) (**a**, 15,048×; **b**, 16,997×)

demyelination showed this alteration in 5 % or less of fibers (Prineas and McLeod 1976). Widening of the node of Ranvier with myelin retraction has been hailed as an early and sensitive sign of demyelination but is nonspecific. Immunohistochemical staining for macrophages, such that they might be more easily identified as clinging to intact axons, should be relatively specific for macrophage-mediated demyelination (Krendel et al. 1989; Griffin et al. 1990).

9.2.2.5 Immunohistochemistry

In cases when inflammation is unconvincing with routine stains, we have found the use of LCA immunohistochemistry to be of benefit in detecting subtle collections of leukocytes. In CIDP, the number can range from none to many hundreds per mm^2 (Cornblath et al. 1990). A small number of epineurial or endoneurial lymphocytes is within normal limits (<20/mm^2), and a few lymphocytes may be normally found around epineurial vessels giving the impression of slight cuffing. Endoneurial capillary lymphocytic cuffing is abnormal, and more than one or two leukocytes in an average nerve fascicle should be regarded with suspicion. In CIDP the $CD4^+/CD8^+$ ratio is typically 1–2:1 (Cornblath et al. 1990; Pollard et al. 1986).

Dalakas and Engel (1980) found IgM, IgG, and complement deposits in the walls of endoneurial blood vessels and on the surface of Schwann cells using direct immunofluorescence. The vascular deposits had a granular pattern suggesting deposition of immune complexes, and the Schwann cell deposits showed a linear pattern on otherwise normal cells, suggesting binding to an antigen on the cell membrane.

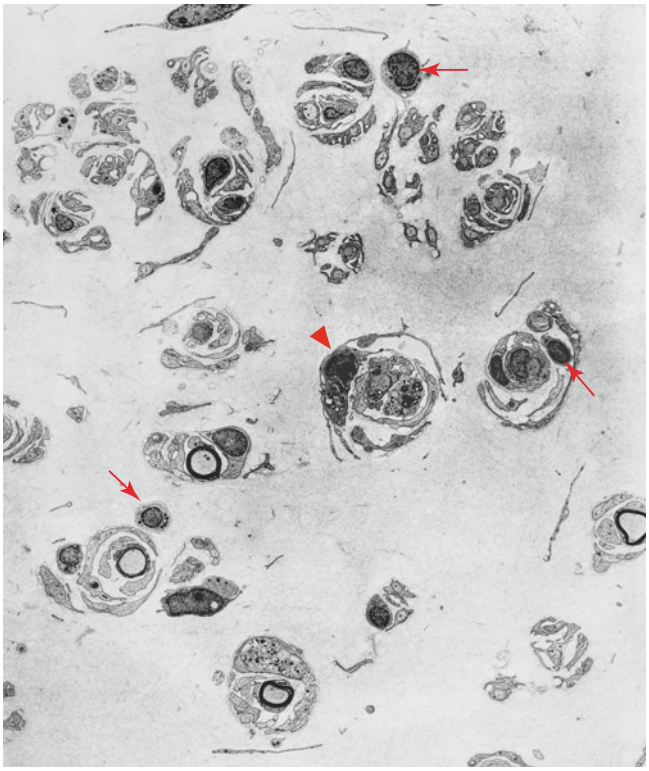

Fig. 9.29 CIDP: low-power electron micrograph shows expansion of the endoneurial matrix. Onion bulbs containing demyelinating and remyelinating fibers are widely separated. Note macrophage (*arrowhead*) and lymphocytes (*arrows*) (2,610×)

These deposits were not found in any of the ten nonimmune-mediated neuropathy or neurologically normal controls. However, subsequent studies have rarely or never described similar alterations (McCombe et al. 1989; Hays et al. 1988; Schenone et al. 1988; Small and Lovelace 1993).

9.2.3 Pathogenesis

As with Guillain–Barré syndrome, it is believed that CIDP results from an immune attack on the peripheral nerve. As poorly as GBS is understood, even less is known about the causes of CIDP. An animal model does exist and is based on repeated immunization with high doses of nerve antigens, much like the experimental allergic neuritis model used to study GBS (Harvey et al. 1987; Pollard et al. 1975). Support for the importance of humoral factors in the pathogenesis of CIDP is provided by the clinical similarity between CIDP and paraprotein-associated neuropathies. The pathogenesis of CIDP has been reviewed (Dyck et al. 1993; Gorson and Katz 2013; Said and Krarup 2013). Recent evidence suggests that the node of Ranvier is the primary target of immune attack in patients with GBS and CIDP (Devaux 2012). This can be unveiled in skin biopsies (Doppler et al. 2013) by immunostaining of the proteins that characterize the node (voltage-gated sodium channels) and paranodal region (neurofascin).

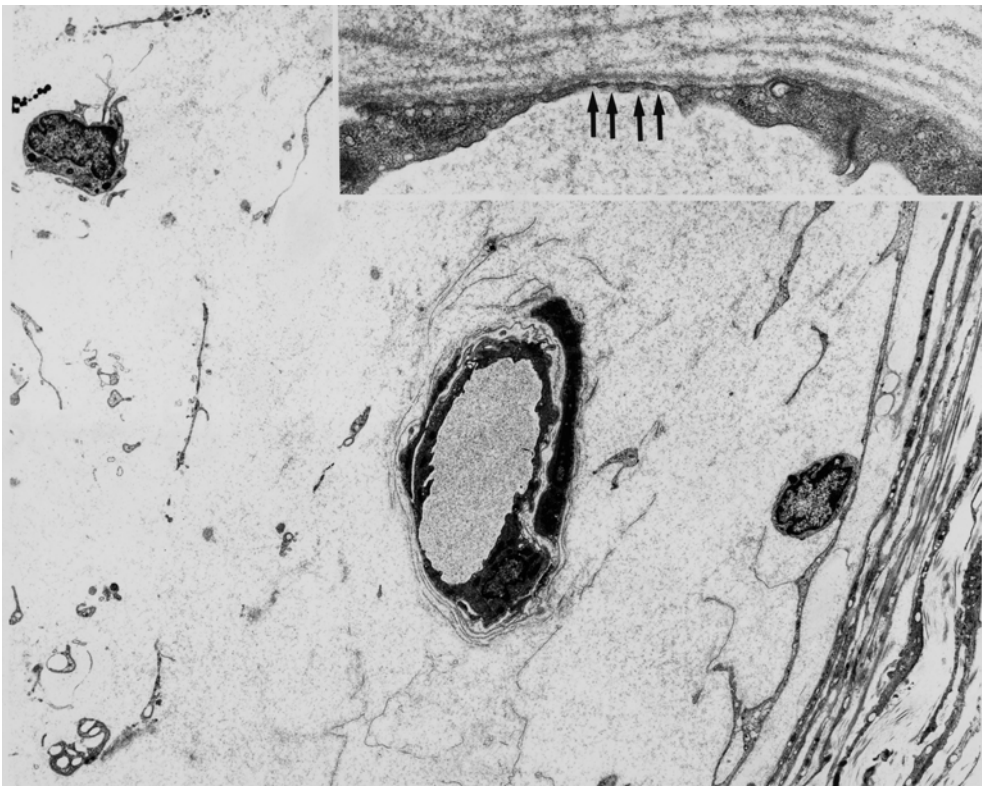

Fig. 9.30 CIDP: endoneurial "edema." Vessel shows fenestrated endothelium (*arrows*, *inset*) (4,000×; *inset*, 26,600×)

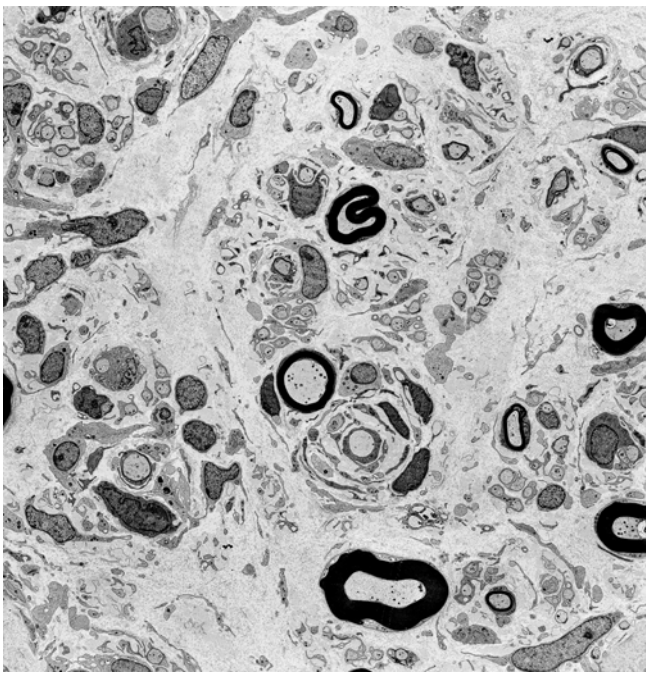

Fig. 9.31 CIDP: hypercellularity of endoneurium is due to nonnative cells, either lymphocytes or macrophages. Note variation in myelin thickness and early onion-bulb formation (1,890×)

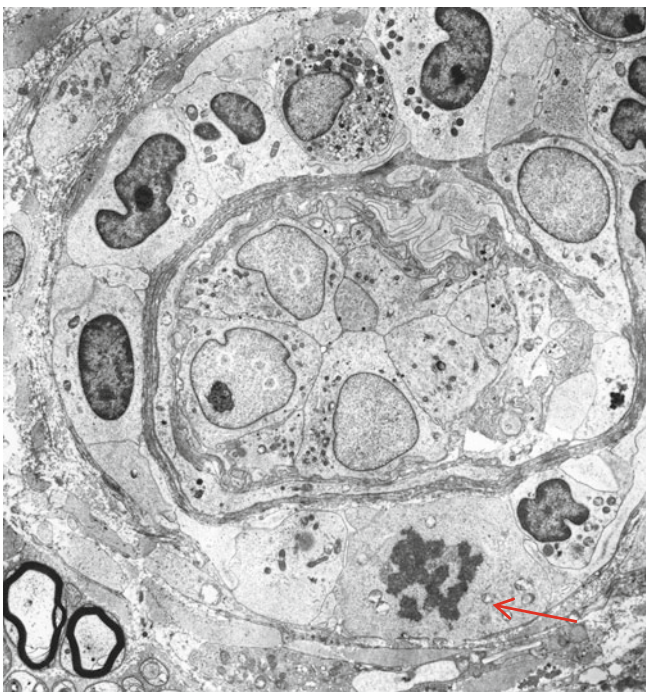

Fig. 9.32 CIDP: this endoneurial microvessel is surrounded by mononuclear cells with abundant cytoplasm. A mitosis (*arrow*) is seen in one of these cells (2,720×)

Many similarities exist between GBS and CIDP, including a shared and highly specific mechanism of demyelination via a macrophage-mediated myelin stripping process, the presence of inflammatory infiltration, and clinical improvement with plasmapheresis and intravenous immunoglobulin. Clearly, there are differences, however, as GBS is a self-limited monophasic illness, while the mechanisms that mediate CIDP must be active continuously or intermittently.

GBS and CIDP patients show an elevation in serum IL-2 (an indicator of T-cell activation), relative to patients with other neuropathies and other noninflammatory neurological diseases. However, the elevation in GBS is far more striking than in CIDP (Hartung et al. 1991). Patients with CIDP have many of the same serum antibodies to peripheral nerve myelin that are found in GBS (see GBS discussion) but less frequently (Koski 1990; Ilyas et al. 1992; McCombe et al. 1989; Quarles et al. 1990; Lundkvist et al. 1989; van Doorn et al. 1987). Complement activation also appears to be similar (Koski et al. 1987). Initial reports of immunoglobulin and complement deposition in peripheral nerve vessels and on Schwann cell membrane (Dalakas and Engel 1981) have been largely unconfirmed by subsequent work (McCombe et al. 1989; Hays et al. 1988; Schenone et al. 1988). Serum from CIDP patients seems to be less effective than GBS serum at causing demyelination when injected into a nerve (McCombe et al. 1989) although Heininger et al. (1984) demonstrated reduction of conduction velocity in monkeys given intramuscular immunoglobulins purified from the serum of patients with CIDP. Recently, 57 % of sera from patients with CIDP possessed IgG or IgM antibodies selective for beta-tubulin, compared with 20 % of GBS patients, and only 2 % of controls with other neuropathies; the significance of this finding is presently unknown (Connolly et al. 1993).

Thus, at present no definite conclusions can be drawn about the mechanisms initiating and maintaining the inflammatory attack on peripheral nerve that is seen in CIDP, although humoral factors are probably important. No major differences have as yet been demonstrated between immune parameters in CIDP and GBS, leaving open the question of a close relationship between these two common neuropathies.

9.2.4 Differential Diagnosis

Lack of a specific diagnosis, or even evidence of pathology, in a nerve biopsy from a patient with CIDP should not preclude treatment if the clinical suspicion is high. A CIDP syndrome in association with a circulating paraprotein (Pollard et al. 1983) or HIV infection (Vital et al. 1992) may show the macrophage-mediated demyelination typical of CIDP. The distinction should be made clinically, not histologically. Similarly, the CIDP cases described in association with inflammatory bowel disease, lymphoma, chronic active hepatitis (Barohn et al. 1989), or SLE (Rechthand et al. 1984) have no distinguishing histological features.

Table 9.3 Histological features that favor CIDP over CMT-1

Nonuniform involvement within and between fascicles
Macrophage-mediated myelin stripping
Perivascular lymphocytic infiltrates, especially endoneurial
Signs of active demyelination (less reliable in children)
Numerous naked axons
Scattered endoneurial macrophages
Schwann cell mitoses
A bimodal MF diameter–frequency histogram

Many neuropathies described elsewhere in this book have histological changes, particularly lymphocytic infiltrates, which resemble CIDP. In such cases, special stains, step sections, immunohistochemistry, and ultrastructural studies may help unmask the hidden vascular involvement of vasculitis, the early granuloma of sarcoidosis, the more specific features of borderline leprosy, the clonal plasmacytoid lymphocyte of paraprotein or lymphoma-associated neuropathy, and the abnormal myelin periodicity of IgM paraprotein-associated neuropathy. Diabetes may present with a combination of distal sensorimotor neuropathy and subacutely evolving plexopathy that may mimic the clinical features of CIDP. A potentially related condition, chronic immune sensory polyradiculopathy (CISP; sensory CIDP), preferentially affecting large myelinated fibers of the posterior roots, may be a restricted form of CIDP (Oh et al. 1992). Other CIDP variants have been described, including Lewis–Sumner multifocal CIDP with predilection for the upper extremities.

Familial hypertrophic neuropathies can usually be distinguished from CIDP clinically and electrophysiologically. However, when the nerve biopsy shows numerous onion bulbs, the question of a familial neuropathy may arise. The diagnosis of CIDP is favored by a number of histological features (Table 9.3). The presence of multifocal (rather than diffuse) involvement, signs of active demyelination in the way of debris-filled cells, or an inflammatory infiltrate strongly favors CIDP. Macrophage-mediated myelin stripping is diagnostic of CIDP in this setting. However, an association between familial hypertrophic neuropathy and CIDP may exist, and the possibility that both are present should be considered in patients of all ages, including infants (Dyck et al. 1982b; Bird and Sladky 1991; Sladky et al. 1986) (Chap. 19). This issue has been considered in detail by Gabreels-Festen et al. (1993). These authors found perivascular lymphocytic cuffs in 5 of 42 biopsies of clear-cut cases of CMT-1, three times in the epineurium and twice in the endoneurium. While we do not ascribe much significance to a few perivascular epineurial inflammatory cells, endoneurial inflammatory perivascular cuffs are, in our experience, always abnormal. The possibility that these patients had a familial neuropathy with superimposed CIDP was felt to be unlikely on clinical grounds. None of the 42 control cases of CMT-1 showed variability of disease severity from fascicle to fascicle, although one of eight "de novo" mutations did (Gabreels-Festen et al. 1993). Twenty-one percent of the CMT-1 cases showed prominent endoneurial edema, and in our experience, this has not been a reliable means of distinguishing between acquired and inherited hypertrophic neuropathy. These workers also verified the observation that in CMT-1, the fiber histogram usually shows a broad single peak with loss of large myelinated fibers more prominent than that of small myelinated fibers (Gabreels-Festen et al. 1993).

Case 9.2

A 28-year-old man was referred for neurological opinion because of paresthesias. Symptoms began at the age of 20, with tingling and shooting pains in the fourth and fifth digits of both hands. At 4 years prior to assessment, the patient noticed a loss of bulk in the small muscles of the right hand. Numbness and tingling also appeared in the toes, usually associated with cross-leg sitting, and the patient observed that deep pressure in the forearm on leg might result in similar sensations. The patient worked as a motor vehicle mechanic, but no specific neurotoxic exposure could be identified. He denied use of vibrating tools. Medical and family histories were unremarkable.

The non-neurological examination was unremarkable. The cranial nerves were normal. There was marked wasting of the interossei bilaterally, with fasciculations on the right side. The extensor digitorum brevis was wasted bilaterally in the lower limbs. Muscle testing revealed moderate bilateral weakness of muscle innervated by the ulnar nerve and lesser weakness of the left hand innervated by hand muscles. All other muscles in the upper and lower extremities were normal. Reflexes were all obtained and symmetrical but were felt to be sluggish in the upper limbs relative to the lower limbs. The plantar responses were flexor. No sensory abnormalities could be demonstrated to vibration, joint position, pinprick, light touch, or two-point discrimination, despite the patient's symptomatology. Both ulnar nerves were enlarged.

Normal investigations included renal function, liver function, immunoelectrophoresis of blood and urine, vasculitis screening tests, urinary porphyrins, and serum phytanic acid. Electrophysiological testing revealed absence of sural and median sensory potentials. Motor conductions showed marked increase in distal latencies (three to four times normal), with velocities at 22–25 m/s in the arms and 40 m/s in the legs. No F-waves were obtainable. Marked dispersion of the CMAP was noted, but "inching" assessment for focal conduction block was not performed. Sural nerve biopsy was performed 8 years after onset of symptoms (Figs. 9.31, 9.32, and 9.33). Treatment with steroids, cytotoxic agents, and plasma exchange did not ameliorate the neuropathy. At 10 years post-biopsy, the patient's condition has not progressed significantly and he is not receiving any therapy (clinical material courtesy of Dr. G. Sawa and Dr. C. Lambert).

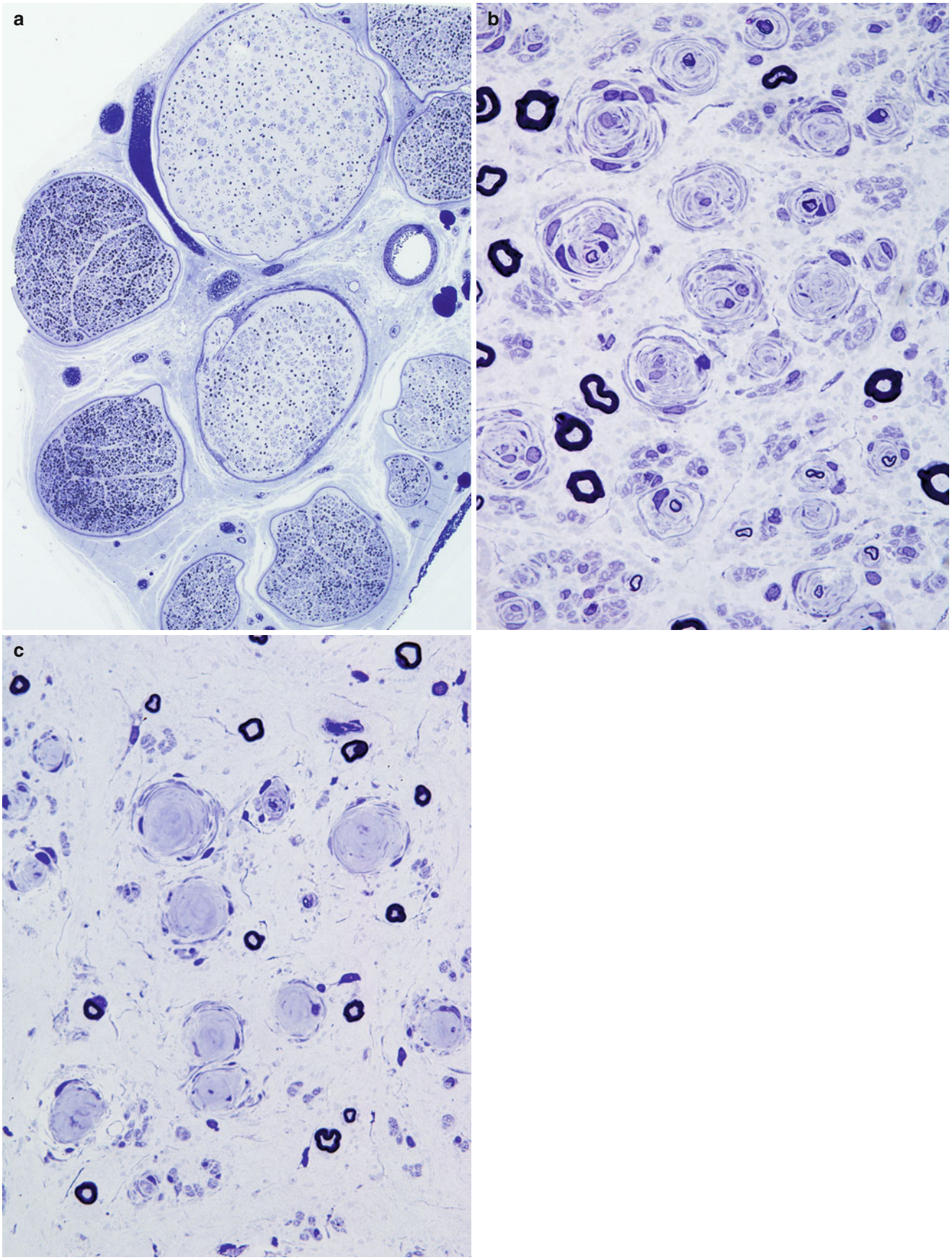

Fig. 9.33 Multifocal neuropathy with persistent conduction block: nerve involvement is nonuniform and affected fascicles are swollen (**a**). Onion-bulb formations vary from well-developed (**b**) to "burned-out" (**c**) (1 μ thick plastic sections, **a**, 100×; **b**, **c** 1,000×)

9.3 Multifocal Neuropathy with Persistent Conduction Block

9.3.1 Clinical Syndrome

This syndrome of multifocal neuropathy is characterized by adult onset, male predominant, and slowly progressive, asymmetric weakness with muscle wasting, cramps, and fasciculations, and persistent conduction block without sensory dysfunction has received considerable attention (Lewis et al. 1982; Adams et al. 1965; Gibbels et al. 1993; Vlam et al. 2011). When the findings are overwhelmingly motor in nature, the designation "multifocal motor neuropathy" has been applied (Parry and Sumner 1992). Typically, there is a very slowly progressive multifocal disease, with deficits often corresponding to individual nerve territories, especially in the upper limbs, rather than showing the typical distal predominance of CIDP. The essential diagnostic criterion is the demonstration of persistent (over months and years) and very focal conduction block. Some believe this syndrome to be a CIDP variant (Parry and Sumner 1992), but the specific association of antibodies against GM1 with multifocal motor neuropathy suggests the presence of distinct entity (Kornberg and Pestronk 1994). Most patients have circulating high-titer anti-ganglioside GM1 (Pestronk and Choksi 1999) or NP-9 IgM antibodies and, to a lesser extent, antibodies to other glycolipids. These antibodies, although not specific for MMN, are not seen in ALS/motor neuron disease.

9.3.2 Findings in Multifocal Motor Neuropathy with Persistent Conduction Block

We have examined the sural nerve biopsy of a patient with typical multifocal neuropathy with persistent conduction block (Case 9.1 below). This biopsy clearly showed an inflammatory hypertrophic neuropathy with multifocal demyelination, epineurial and endoneurial perivascular inflammation, onion bulbs in the area of conduction block, and motor axon degeneration and loss (especially larger axons). However, it was unusual in that it showed a pronounced focality, with enlarged fascicles containing massive onion-bulb formations immediately adjacent to almost normal fascicles (Fig. 9.33a–c). Mononuclear cells were seen in endoneurium, often between layers of Schwann cell processes in onion bulbs, but no macrophage-mediated demyelination was demonstrable (Figs. 9.34 and 9.35). Case 1 of Gibbels et al. (1993) is remarkably similar to our own, and a patient described by Nukada et al. (1989) showed less dramatic but similar findings. Other reports of sural nerve biopsy in neuropathies with persistent conduction block have shown findings ranging from normal to variable combinations of mild axonal loss, inflammation, and demyelination, indistinguishable from the findings of typical CIDP (Lewis et al. 1982; Krarup et al. 1990; van den Bergh et al. 1989; Pestronk et al. 1988; Adams et al. 1965; Gibbels et al. 1993;

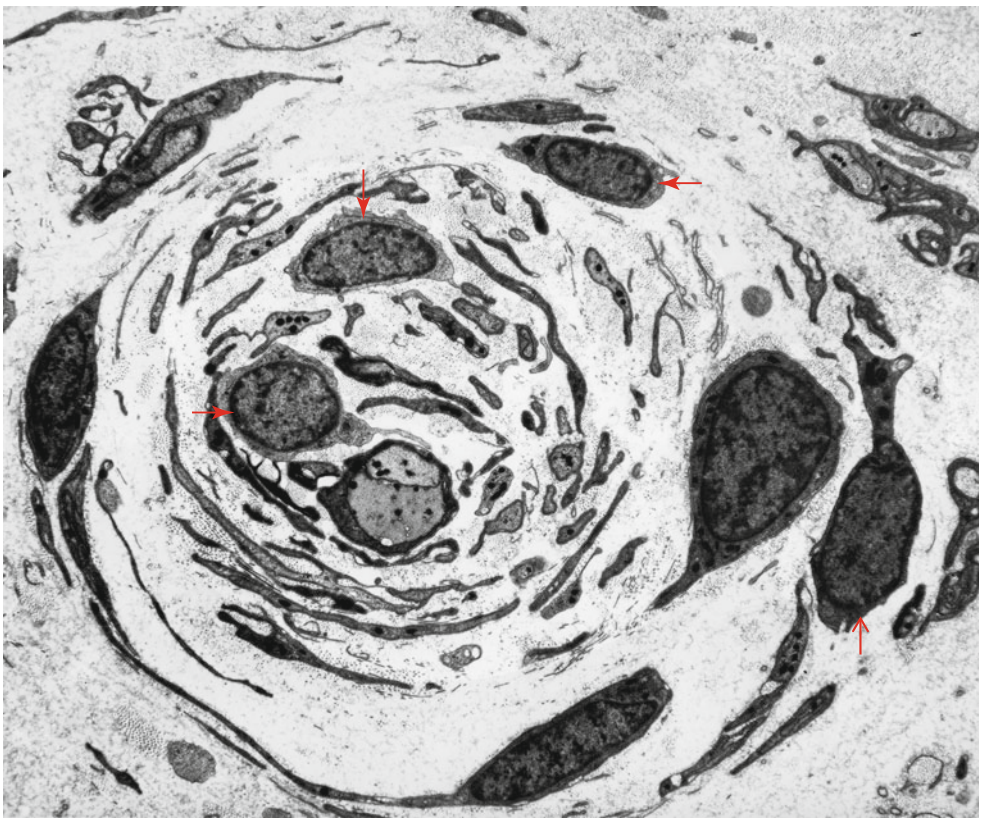

Fig. 9.34 Multifocal neuropathy with persistent conduction block: large onion bulb. Many of the cells seen between layers of Schwann cell processes are devoid of basal lamina and have scanty cytoplasm (*arrows*); most are probably lymphocytes (17,100×)

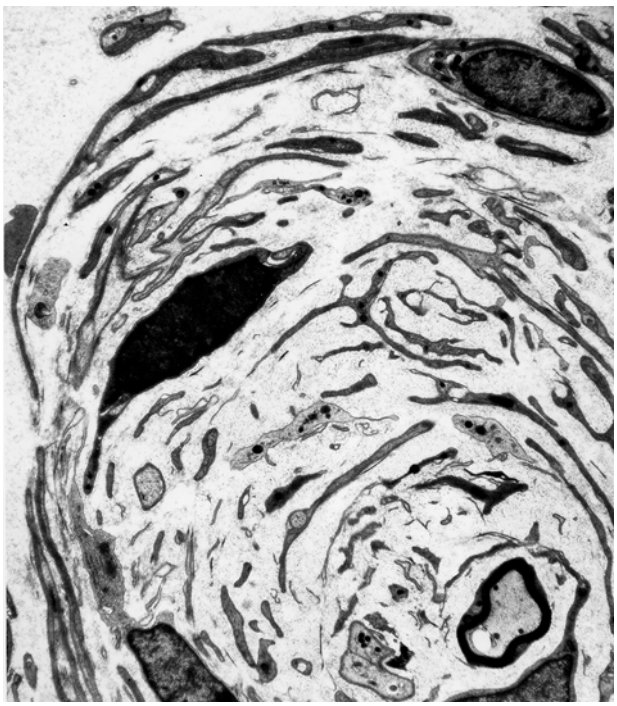

Fig. 9.35 Multifocal neuropathy with persistent conduction block. Giant "cabbage roll" centered by a myelinated fiber (4,788×)

Verma et al. 1990), even in cases with no significant clinical and electrophysiological sensory manifestations. A brief report of ten sural nerve biopsies in multifocal motor neuropathy documents mild demyelinating changes, "minor" onion bulbs, and no significant axonal injury or inflammatory infiltration (Corse et al. 1994). Macrophage-mediated demyelination has not been mentioned in these reports, and we did not observe this in our case despite an extensive search. More recently, a pathological study of seven patients has demonstrated multifocal axonal degeneration without any evidence of demyelination (Taylor et al. 2004).

We have examined surgically excised tissue from two patients who suffered from a relapsing-remitting hypertrophic brachial plexus neuritis. Peripheral nerve from the involved plexus revealed a hypertrophic neuropathy with very large and atypical onion-bulb formations (Fig. 9.36) strikingly similar to the picture we observed in a case of multifocal neuropathy with persistent conduction block (Cusimano et al. 1988) (Figs. 9.34 and 9.35). Review of the literature indicates that hypertrophic brachial plexus neuritis can be seen in multifocal neuropathy with persistent conduction block (Adams et al. 1965; Bradley et al. 1988; Auer et al. 1989; Kaji et al. 1993). Surgically excised brachial plexus branches near a site of conduction block have revealed massive onion bulbs with inflammatory infiltration (Adams et al. 1965; Bradley et al. 1988) or focal regions of demyelination

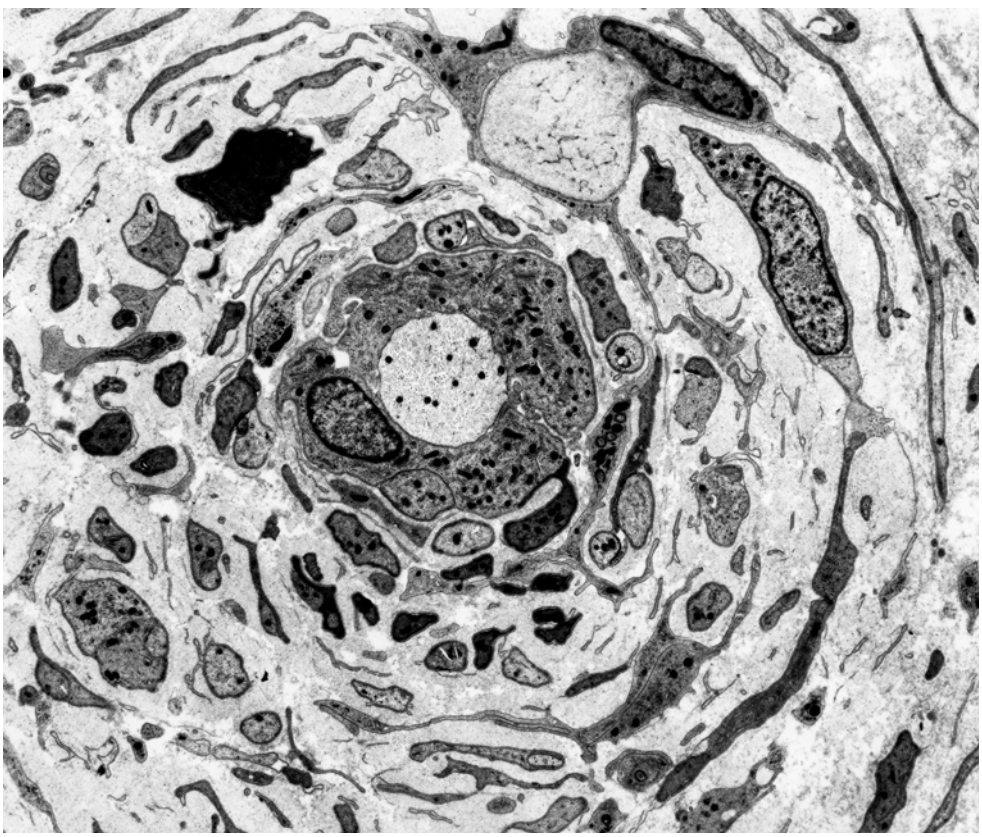

Fig. 9.36 Hypertrophic brachial plexus neuritis: atypical onion bulb similar to that seen in multifocal neuropathy with persistent conduction block (Figs. 9.32 and 9.33) (2,880×)

with less striking onion bulb-formations and no inflammation (Auer et al. 1989; Kaji et al. 1993). In our material, unique features of brachial neuritis histology included massive mononuclear inflammatory infiltration with formation of germinal centers, tubuloreticular inclusions in endothelial cells and lymphocytes, and vasculitis (Cusimano et al. 1988). The relation between CIDP, multifocal neuropathy with persistent conduction block, and hypertrophic brachial plexus neuropathy remains unclear. These findings differ from CIDP in the absence of macrophage-mediated demyelination. Immunoglobulin deposition and inflammatory demyelination were found in motor roots in an autopsy case (Oh et al. 1995).

9.3.3 Pathogenesis

The pathogenesis of MMN is not understood although it is thought to involve immune mediation and the possible participation of anti-GM1 antibodies (recent reviews include Guimaraes-Costa et al. 2013; Galban-Horcajoa et al. 2013; Arcila-Londono and Lewis 2013; Nobile-Orazio and Gallia 2013) and may have some features in common with AMAN (Yuki 2013). Focal conduction block has been induced in vivo and in vitro after intraneural injection of sera from patients with high IgM anti-GM1 antibodies and MMN, but not with purified anti-GM1 antibodies (Harvey et al. 1995). Anti-GM1 antibodies react with the lipopolysaccharide of *C. jejuni*, but only 5 % of patients with MMN had high levels of antibodies to *C. jejuni* (Oh et al. 1995).

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Idiopathic Inflammatory Neuropathies

10

10.1 Sarcoidosis

10.1.1 Clinical Manifestations

Sarcoidosis is a multisystem disease characterized by formation of noncaseating granulomata in many tissues and organs with a predilection for African Americans, Europeans, and, especially, Swedes. Neurological symptoms occur in about 5 % of patients (Delaney 1977) and may be the only manifestation of disease (Chapelon et al. 1990). Any part of the nervous system may be involved, but the single most common clinical feature is a peripheral seventh cranial nerve palsy, seen in about a third of patients with neurosarcoidosis (Delaney 1977; Chapelon et al. 1990; Oksanen 1986). Non-cranial neuropathy occurs in 15–40 % of patients (Delaney 1977; Chapelon et al. 1990; Oksanen 1986) and may exist in the absence of any other symptoms, neurological or systemic (Chapelon et al. 1990). Subclinical neuropathy is even more common (Challenor et al. 1984). Although the older literature emphasizes mononeuritis multiplex, a relatively mild chronic progressive distal polyneuropathy is more common (Chapelon et al. 1990; Zuniga et al. 1991) and was the syndrome seen in nearly every pathologically verified case to date, even those with vascular changes (vide infra). Mononeuropathy may occur (Chapelon et al. 1990), and a Guillain–Barré-like picture has been very rarely reported in association with sarcoidosis (Chapelon et al. 1990; Oksanen 1986; Miller et al. 1989) (Case 10.1). Electrophysiological testing is usually most consistent with axonal neuropathy, either distal symmetrical or multifocal (Challenor et al. 1984; Zuniga et al. 1991), but slowed conduction suggesting demyelination has been reported (Chapelon et al. 1990; Galassi et al. 1984). CSF examination may show increased protein, pleocytosis, or low glucose.

Controlled studies do not exist, but the clinical impression is that treatment with steroids tends to produce improvement or stabilization (Chapelon et al. 1990). Occasionally, the response is dramatic (Godwin and Sahn 1990).

10.1.2 Pathology

10.1.2.1 Role of Nerve Biopsy

Peripheral nerve pathology in sarcoidosis has only rarely been reported, as most often the systemic diagnosis is already known, or diagnosis is obtained from another tissue in a patient with polyneuropathy. Thus, it is impossible to determine the sensitivity of nerve biopsy for the diagnosis. In one autopsy study that explored several peripheral nerves, there were only sparse inflammatory infiltrates without granulomata despite marked axonal loss (Zuniga et al. 1991). In one case we have examined, an initial nerve biopsy was diagnostic, but at autopsy 5 years later, no disease was found in any of the five peripheral nerves studied, even though the disease was disseminated in other tissues. Muscle biopsy may be positive in as many as 50 % of patients without clinical evidence of muscle disease (Stern et al. 1985). Thus, if nerve biopsy is being considered, a muscle specimen should be taken as well since tuberculoid leprosy, often in the differential diagnosis, does not involve the muscle. The discussion below is based on review of 11 adequately described cases in the literature (Brochet et al. 1988; Ide et al. 1984; Gainsborough et al. 1991; Galassi et al. 1984; Krendel and Costigan 1992; Nemni et al. 1981; Oh 1980; Vital et al. 1982; Yakane et al. 1986) and personal experience with two cases (vide infra). Krendel and Costigan (1994) also described a case with granulomatous perineuritis, cutaneous anergy, and abnormal lung diffusing capacity that very likely had sarcoidosis.

10.1.2.2 Light Microscopy

The sarcoid granuloma is a compact mass of epithelioid cells derived from macrophages which reflect the development of secretory and bactericidal capability and loss of some phagocytic capability. With time, fibroblasts appear and perilesional fibrosis develops. The number of lymphocytes surrounding the granuloma varies from moderate (Fig. 10.1a, b) to none ("naked" granuloma) (Fig. 10.2). T-cell lymphocytes may be markedly increased in areas of active granulomatous disease,

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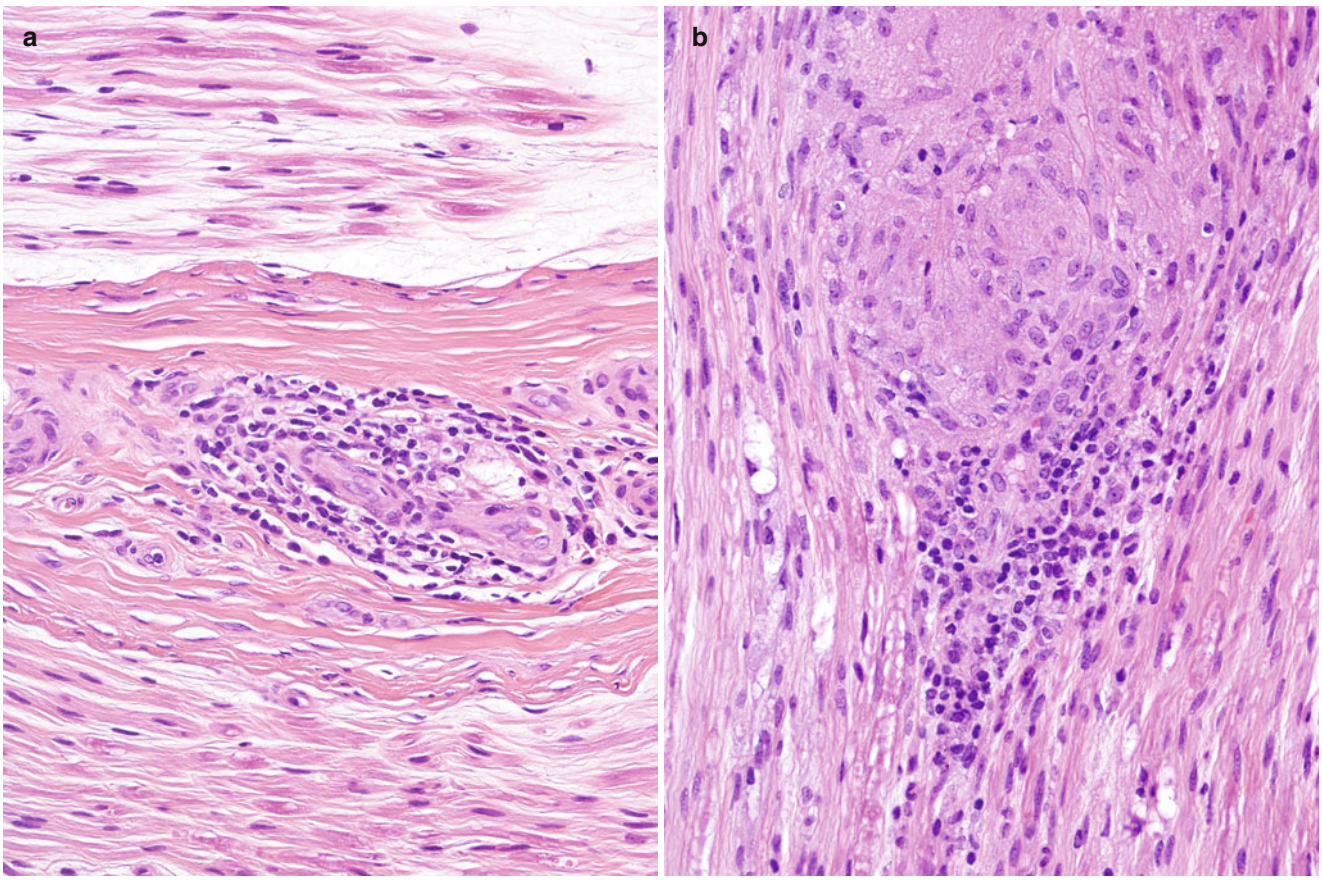

Fig. 10.1 Sarcoidosis: Sural nerve shows nonspecific perivascular lymphocytic collars (**a**) and organized epithelioid cell granuloma (**b**) (paraffin, H&E, 400×)

with an increased ratio of T-helper cells to T-suppressor cells (Said 2013). A Th1-type T-cell response with secretion of IFN-γ, TNF-α, IL-2, and IL-12 is thought to favor the granulomatous response at sites of disease activity (Kataria and Holter 1997). Infrequently plasma cells and eosinophils are seen in the periphery. A cytological evolution from macrophages to epithelioid cells can be seen, and epithelioid cells may fuse into multinucleate giant cells (Soler and Bassett 1976). However, the latter are only rarely seen in the peripheral nerve. Caseation is not present.

Granulomata may be found in the endoneurium or epineurium (Figs. 10.1, 10.2, 10.3, and 10.4). Vascular involvement ranges from complete sparing (Nemni et al. 1981) through periangiitis (Galassi et al. 1984; Ide et al. 1984; Yakane et al. 1986) to true vasculitis with fibrinoid necrosis and luminal occlusion (Vital et al. 1982; Said et al. 2002). Lymphocytic cuffing can be present in the absence of granulomata (Fig. 10.1a). Infiltration of the perineurium by epithelioid histiocytes (Fig. 10.4a) may be so prominent as to be reminiscent of leprosy or idiopathic perineuritis (Brochet et al. 1988; Krendel and Costigan 1994; Oh 1980).

Axonal loss of variable severity predominates and may be multifocal or diffuse or very active or chronic. Regenerating clusters may be seen. Unmyelinated fibers can be relatively spared. Segmental demyelination and remyelination may occur, and in our material this seemed to be most prominent immediately adjacent to the endoneurial granulomata (Fig. 10.5). Some teased fiber studies have demonstrated a pattern consistent with primary demyelination (Nemni et al. 1981), but others showed only axonal damage (Oh 1980) or secondary demyelination (Gainsborough et al. 1991).

10.1.2.3 Electron Microscopy

Epithelioid histiocytes are large elongated cells with long processes that interdigitate with those of adjacent cells (Fig. 10.5). Boundaries between adjacent cells may become indistinct. Nuclei are irregular and convoluted and display prominent nucleoli. The cytoplasm contains numerous mitochondria, active Golgi complexes, and accumulations of RER. Small single membrane-bound vesicles can be abundant, sometimes containing an ill-defined granular material. Non-epithelioid macrophages are distinguished by the presence of lysosomes and relatively few long cellular processes. Nerve fibers in the vicinity of granulomata may appear distorted (Gainsborough et al. 1991), and in one of our cases, axons immediately adjacent to the granuloma showed

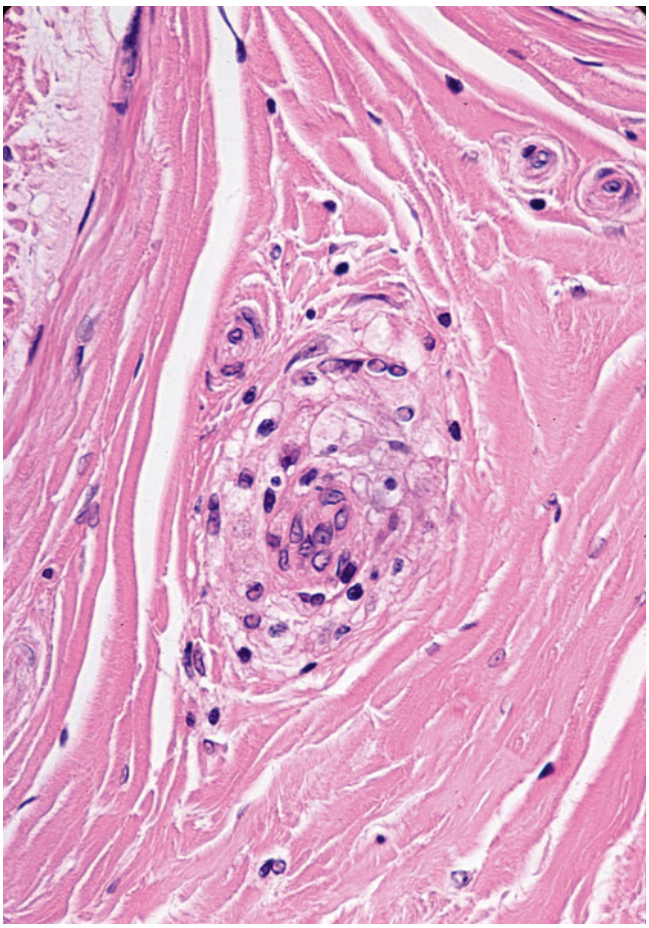

Fig. 10.2 Sarcoidosis: A typical "naked" granuloma is seen in the perivascular space of the epineurium. Multinucleated giant cells are not common in neural sarcoidosis (paraffin, H&E, 400×)

filamentous accumulations and swelling, reminiscent of "giant axonal" change (Fig. 10.5).

10.1.3 Pathogenesis

The lesion in sarcoid neuropathy is predominantly axonal. Because some biopsies have demonstrated marked distortion of nerve fibers by endoneurial granulomata, with no evidence of vascular damage or ischemia, a role for local perinflammatory injury has been postulated. Indeed, histiocytes secrete cytotoxic substances that might have local effects (Said and Hontebeyrie-Joskowicz 1992; Selmaj and Raine 1988). Precisely, such a pattern was observed in our material, with axonal swelling and filament accumulations seen only immediately adjacent to the granulomata. In such a manner, multiple random small endoneurial lesions might summate to produce a distally predominant polyneuropathy (Waxman et al. 1976). Conversely, those authors who have observed vascular lesions (Ide et al. 1984; Oh 1980; Vital et al. 1982)

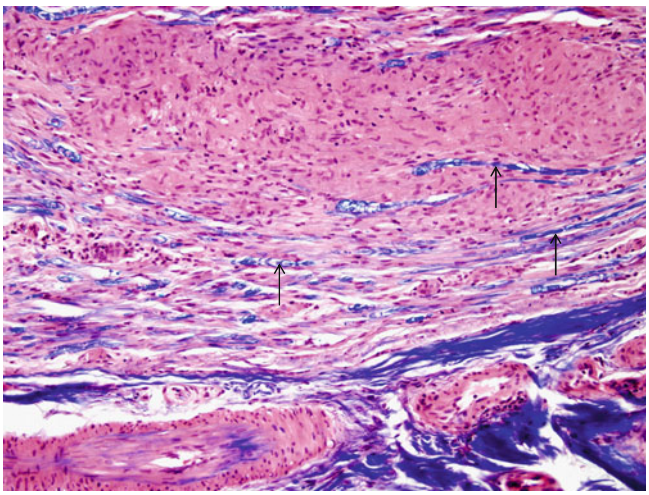

Fig. 10.3 Sarcoidosis: Note displaced MF-stained blue with LFB–H&E (*arrows*) adjacent to an epithelioid cell granuloma (paraffin, 400×)

favor an ischemic pathogenesis, which would fit the mononeuritis multiplex pattern sometimes seen in sarcoidosis. The involvement of nerve roots and dorsal root ganglia must also be considered (Camp and Grierson 1962; James and Sharma 1967; Manz 1983).

No histology is available for the rare reports of a Guillain–Barré-like syndrome in association with sarcoidosis (Oksanen 1986; Miller et al. 1989). Some of the cases reported as GBS were clinically atypical (Strickland and Moser 1967). This may be a chance association (Miller et al. 1989), despite Oksanen's observation (1986) that GBS developed coincident with activation of systemic sarcoidosis in two patients. Case 10.1 reported below initially presented as GBS, but the subsequent clinical course was not typical of the syndrome.

10.1.4 Differential Diagnosis

Sarcoidosis is not a diagnosis that can be made on peripheral nerve biopsy alone, because the disease is defined as "a multisystem granulomatous disorder of unknown etiology…supported by histological evidence of widespread non-caseating epithelioid cell granulomas in more than one organ…" (James et al. 1976). Even so, the presence of granulomata in a nerve biopsy almost always indicates a systemic disease, the exception being primary neuritic leprosy. Interestingly, in Case 10.1 described below, no evidence of sarcoidosis was found, and most reported cases of peripheral nerve sarcoidosis have had normal chest X-rays and minimal evidence of a systemic disease (Krendel and Costigan 1994). As no organisms are likely to be demonstrable in tuberculoid lesions of leprosy, clinical information becomes essential.

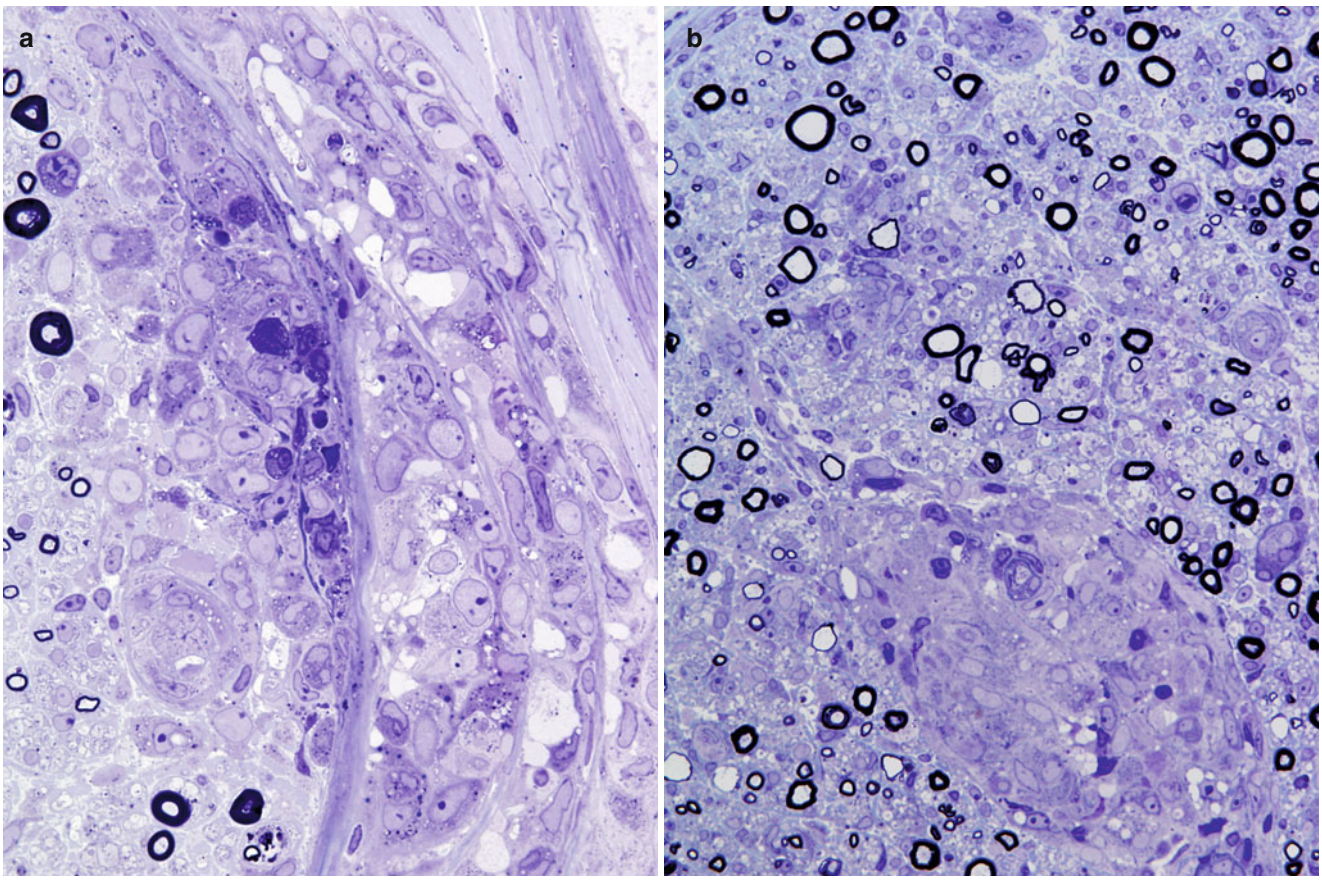

Fig. 10.4 Sarcoidosis: Focal lymphocytic and histiocytic infiltrate in the perineurium (**a**) may accompany endoneurial epithelioid cell granulomata (**b**). Many of the MFs surrounding the granuloma have attenuated myelin sheaths (1 μ thick toluidine-stained plastic sections, **a**, **b**: 600×)

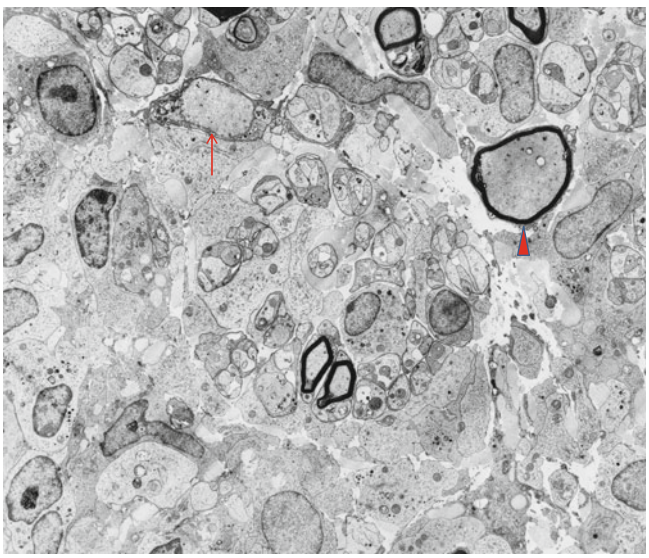

Fig. 10.5 Sarcoidosis: Nerve fibers adjacent to granuloma show accumulation of neurofilaments (*arrowhead*) and segmental demyelination (*arrow*) (4,160×)

In idiopathic perineuritis (vide infra), inflammation and epithelioid cell infiltration are largely confined to the perineurium, but the distinction from sarcoidosis may be difficult (Krendel and Costigan 1994).

Infiltration and even destruction of vessels by epithelioid cells may occur in sarcoidosis. This picture can also be seen in Wegener granulomatosis, lymphomatoid granulomatosis, Churg–Strauss angiitis, polyarteritis nodosa, and giant cell arteritis. Angiocentric neurolymphomatosis should also be considered. The cytology and immunophenotype of the infiltrating cells will assist in the identification of the various entities. Eosinophils are not reliable indicators of the likelihood of Churg–Strauss angiitis.

Case 10.1

A 65-year-old woman had been experiencing GI upset with nausea and abdominal pain for 3 months preceding neurological presentation. Numerous investigations including endoscopy revealed no cause. Following onset with symmetrical paresthesias, the patient progressed over about

2–3 weeks to symmetrical moderate bilateral leg weakness and mild distal leg sensory loss to all modalities. There was no significant history of toxic exposure or travel. Reflexes were diminished and ankle jerks absent. Nerve conduction studies demonstrated a symmetrical axonal polyneuropathy, with minimal fibrillation in distal muscles. CSF examination was normal. The only abnormalities on further investigation were an ESR of 39 (Westergren) and ANA of 1:320. Renal, liver, and thyroid indices were normal. CBC, immunoelectrophoresis, serum calcium, serum ACE, porphyria screen, chest X-ray, pulmonary function tests, and abdominal ultrasound were normal. Nerve biopsy was performed 2 months after the onset of symptoms (Figs. 10.1, 10.2, and 10.3).

Following nerve biopsy, prednisone was initiated but was not tolerated by the patient. The severity of the patient's illness remained unchanged. Prednisone was initiated again 6 months after onset, and over the subsequent year, there was gradual improvement with complete resolution of weakness and most sensory signs. Painful paresthesias became the major management issue. At several months since cessation of steroid therapy, there was no worsening (clinical material courtesy of Dr. C. Zahn).

10.2 Idiopathic Perineuritis

10.2.1 Clinical Manifestations

Idiopathic perineuritis was described in 1972 (Asbury et al. 1972) and remains an uncommon entity (Bourque et al. 1985; Matthews and Squier 1988; Logigian et al. 1993; Simmons et al. 1992; Younger and Quan 1994). Patients present with prominent sensory findings, including paresthesias, pain, and hypersensitivity to touch over affected nerves. Reports have emphasized the sensory involvement, but motor abnormalities are usually present. The condition evolves over weeks to months and may mimic mononeuritis multiplex (Logigian et al. 1993; Simmons et al. 1992), distal sensorimotor neuropathy (Bourque et al. 1985) (Case 10.2), or CIDP (Chad et al. 1986) both clinically and electrophysiologically. By definition, no identifiable disease is present, although nonspecific systemic symptoms may be seen. Improvement with steroid use or plasmapheresis has been reported anecdotally (Bourque et al. 1985; Logigian et al. 1993; Simmons et al. 1992).

Secondary perineuritis, i.e., relatively selective inflammation of the perineurium in the setting of a specific systemic disease, has been seen in neuropathy associated with cryoglobulinemia (Konishi et al. 1982), sarcoidosis (Krendel and Costigan 1994; Oh 1980), ulcerative colitis (Chad et al. 1986), adulterated rapeseed oil exposure (Ricoy et al. 1983), L-tryptophan ingestion (Smith and Dyck 1990), and perhaps with systemic malignancy (Matthews and Squier 1988).

10.2.2 Pathology

The affected nerve shows patchy lesions of varying age predominantly affecting the perineurium (Fig. 10.6a–d). Fascicular involvement lacks uniformity (Fig. 10.6a); one fascicle might be spared while its neighbor shows severe disease (Fig. 10.7a–d). Perineurial hyperplasia may be demonstrated with epithelial membrane antigen (EMA) immunostaining (Fig. 10.6d). Active lesions often involve a segment of the perineurium and consist of infiltration of the perineurial lamellae by epithelioid cells and lymphocytes, sometimes almost to the point of forming granulomata (Fig. 10.7). Cellular formations resembling giant cells have been described, but true multinucleate cells are rare or absent (Asbury et al. 1972; Bourque et al. 1985; Matthews and Squier 1988). Collagen, fibroblasts, and inflammatory cells spread apart the perineurial laminae, creating an "onion skin" appearance (Fig. 10.7b, d). Chronic lesions show prominent perineurial fibrosis and a scantier inflammatory infiltrate. Perineurial blood vessels may be present in increased numbers, and epineurial or endoneurial vessels can show perivascular inflammatory cuffing, but not true vasculitis. Hyalinization of vessel walls may be striking (Fig. 10.7c). Typical perineurial involvement was noted in two cases presented by Logigian et al. (1993) but inflammation also extended into the endoneurium. In addition to a lymphocytic inflammatory infiltrate, the one case we have studied demonstrated striking deposition of cholesterol crystals and foamy changes in perineurial macrophages (Figs. 10.7d, 10.8, and 10.9; Case 10.2).

Myelinated fiber density is decreased, and active axonal degeneration may be seen adjacent to areas of perineuritis. Near obliteration of the endoneurial contents may occur with severe lesions (Fig. 10.6c), and the degree of axon loss seems to parallel the intensity of perineurial inflammation (Fig. 10.7b). However, axonal loss can also be seen in fascicles showing little or no inflammation (Fig. 10.7). There may be evidence of remyelination, with thinly myelinated axons and even small onion-bulb formations.

Electron microscopy may show splitting of perineurial laminae and necrotic perineurial cells (Figs. 10.8 and 10.9). In the case documented below, we did not observe endothelial tubuloreticular inclusions.

Immunohistochemical techniques have demonstrated that at sites of involvement, the perineurium appears to be abnormally permeable, allowing leakage of polyvalent IgG and IgM (Bourque et al. 1985; Chad et al. 1986; Simmons et al. 1992), but this finding has no specificity.

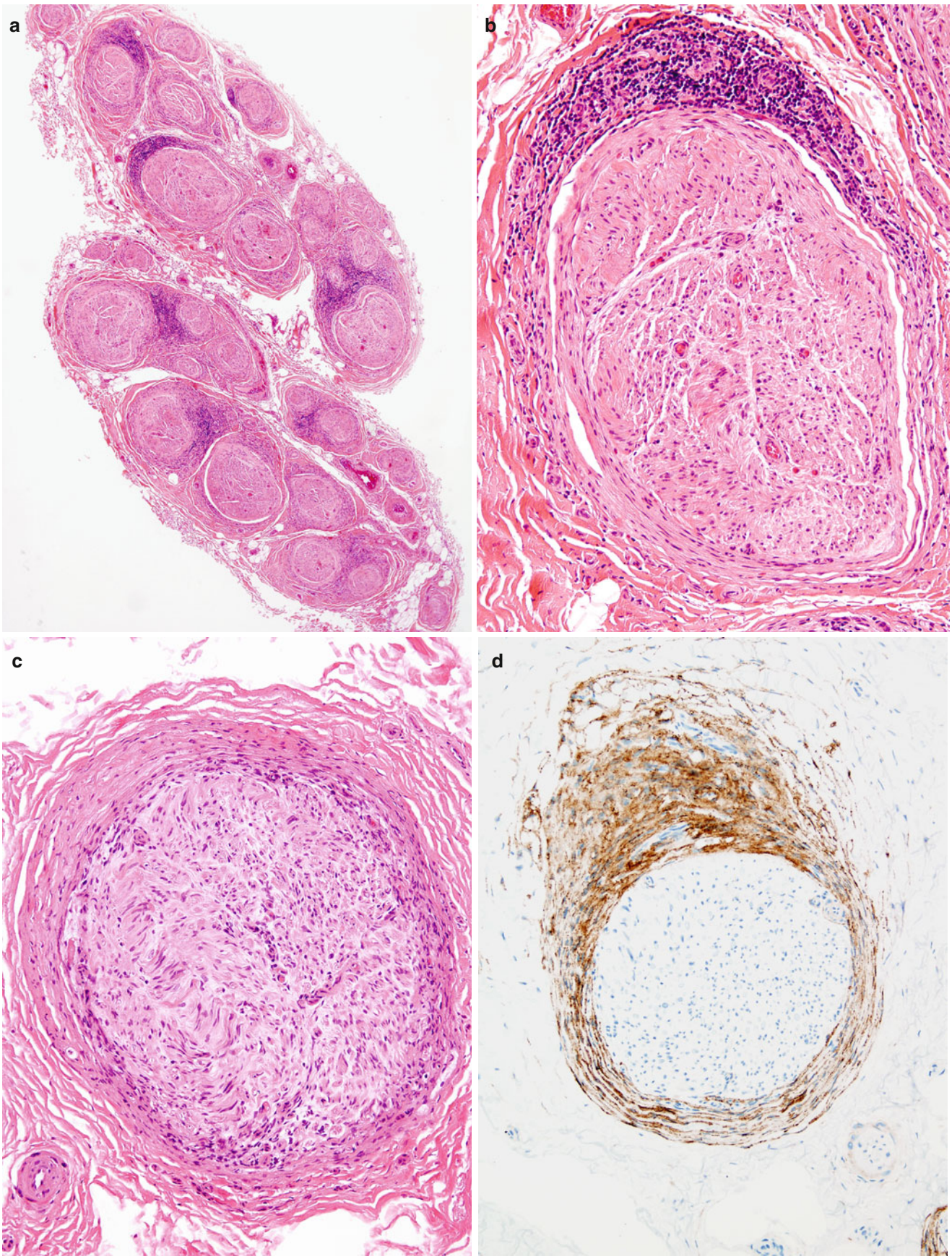

Fig. 10.6 Perineuritis: (**a**, **b**) Patchy perineurial inflammation involves some fascicles and spares others and may asymmetrically affect individual fascicles (**b**). (**c**) Marked perineuritis has resulted in near complete loss of axons. (**d**) Perineurial hyperplasia in response to an inflammatory infiltrate is particularly striking when visualized by EMA immunoreactivity (paraffin; **a**, 40×; **b**, 200×; **c**, **d** 200×)

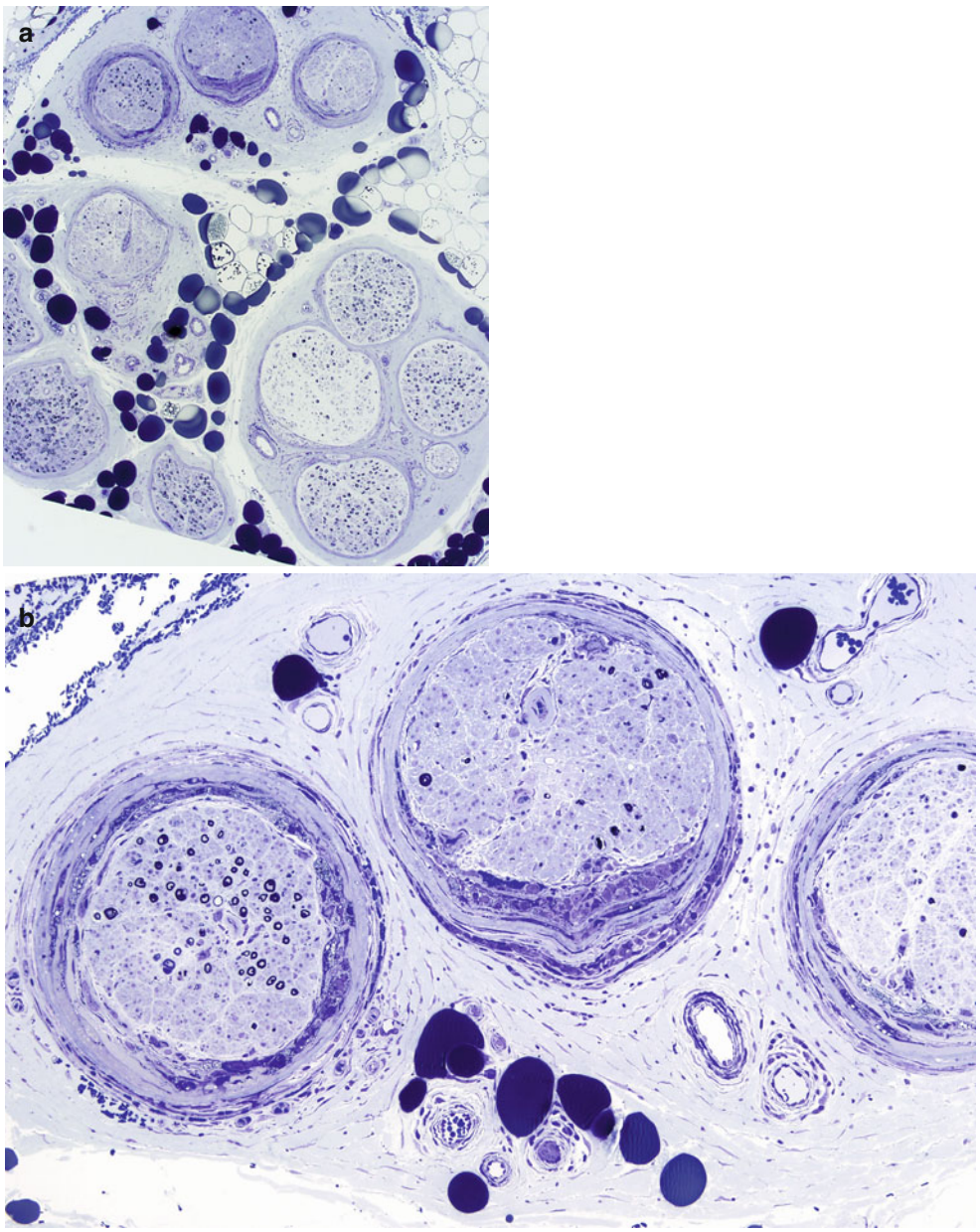

Fig. 10.7 Perineuritis: (**a**, **b**) Multiple fascicles in this case show great variation in extent of perineurial inflammation, fibrosis, and axon loss. (**c**, **d**) One particularly involved fascicle shows markedly thickened hyalinized vascular walls (**c**) and lipid-laden histiocytes within the leaflets of the perineurium (**d**) (1 μ thick toluidine-stained plastic section; **a**, 100×; **b**, 200×; **c**, **d**, 1,000×)

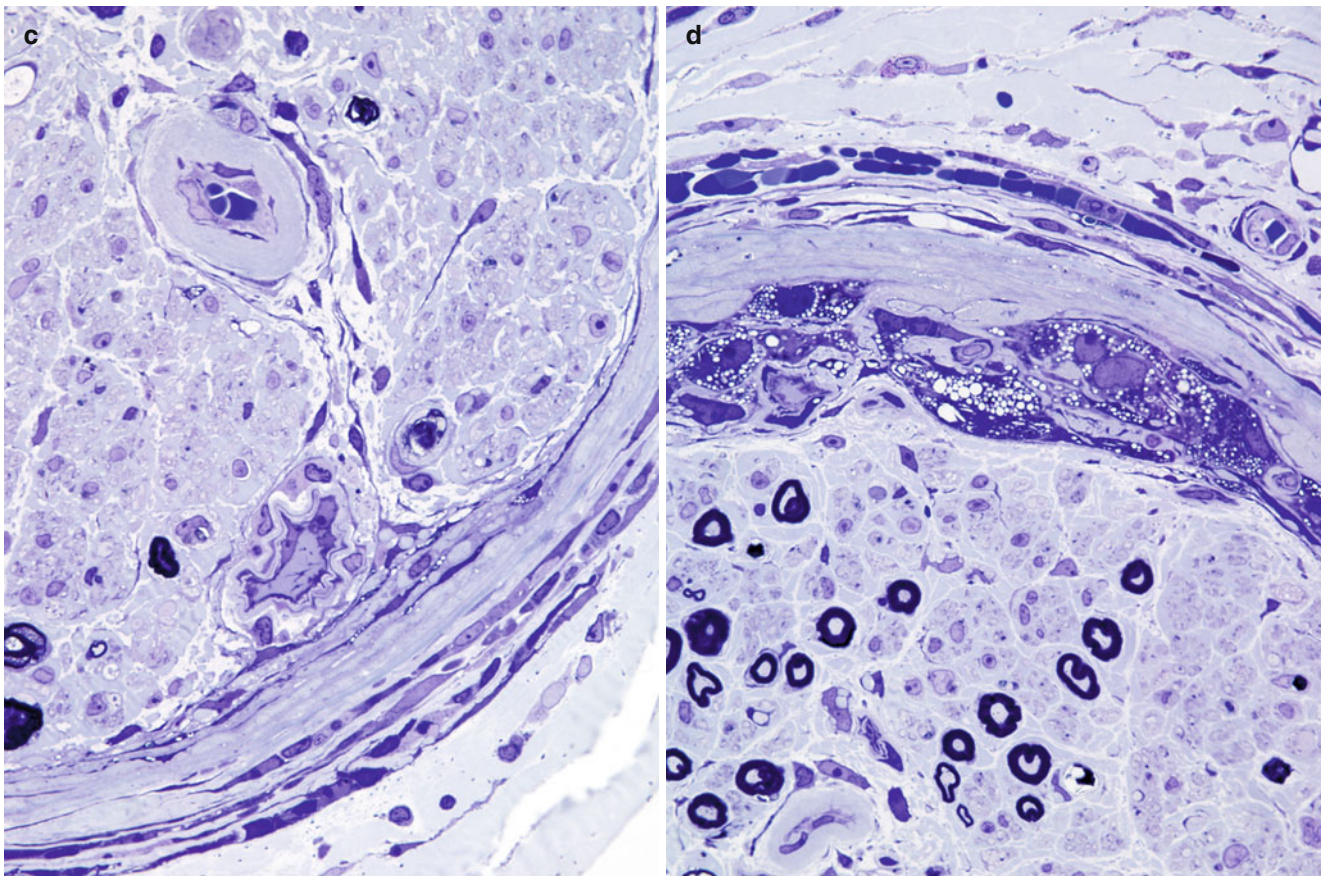

Fig. 10.7 (continued)

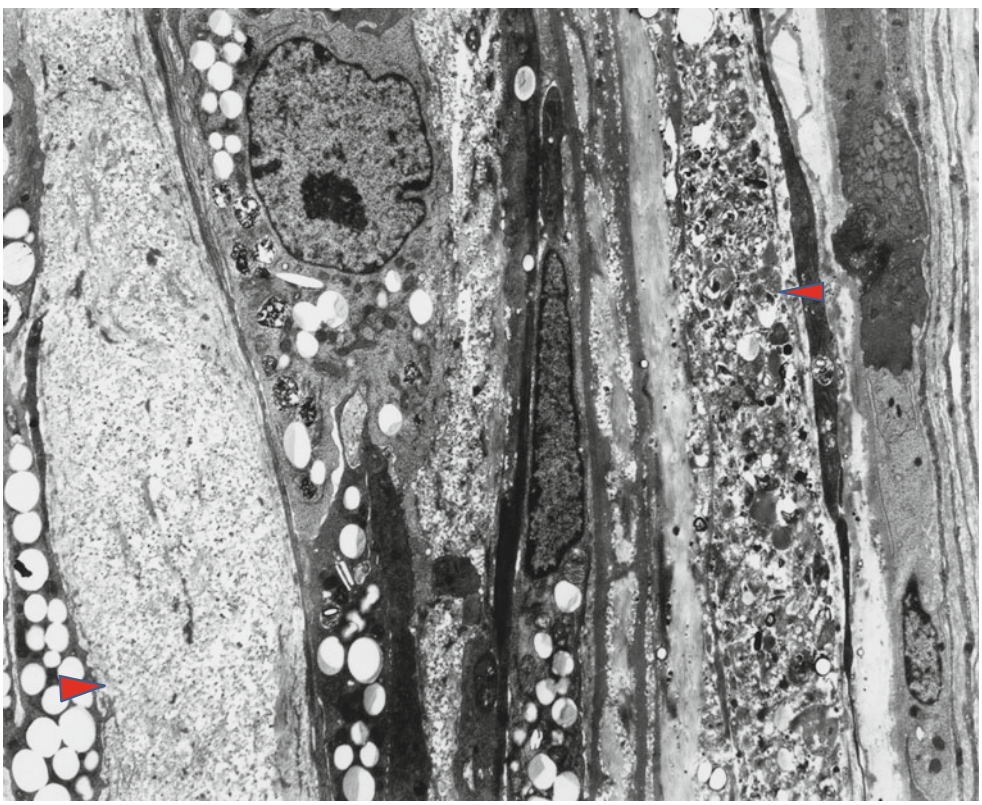

Fig. 10.8 Perineuritis: Note interspersion of perineurial cells, macrophages, and two intervening necrotic cells (*arrowheads*) (6,240×)

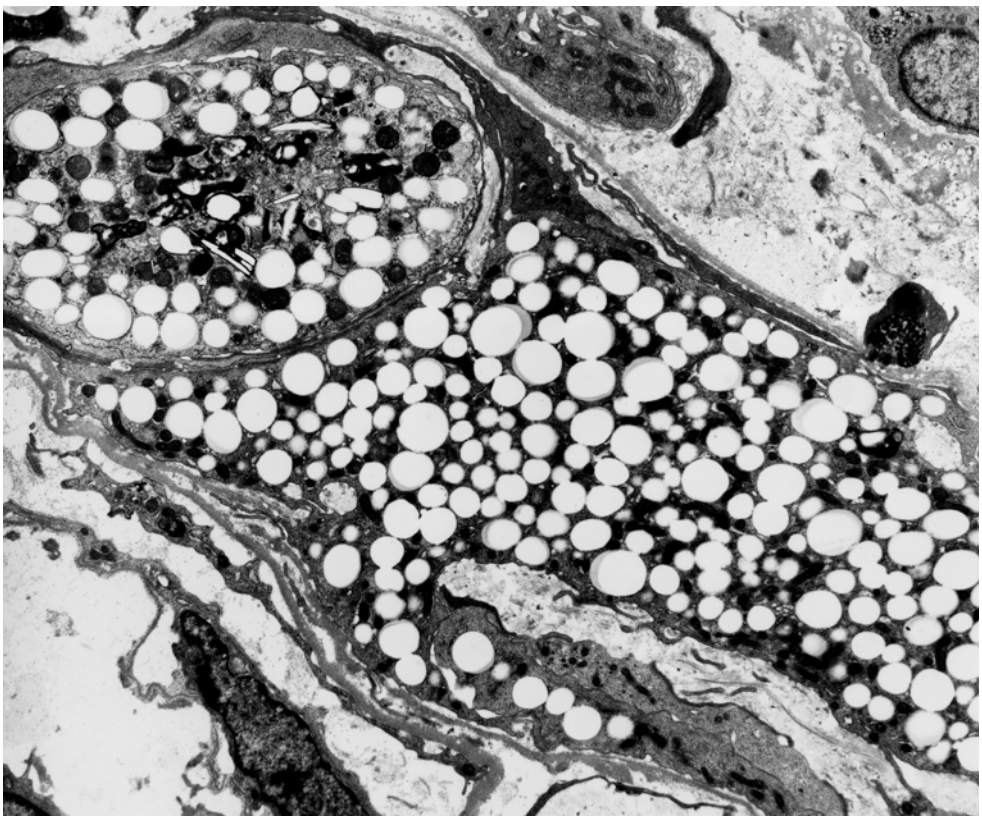

Fig. 10.9 Perineuritis: Foamy macrophages occupy the space outlined by perineurial cell basal lamina (6,300×)

10.2.3 Pathogenesis

Cases of perineuritis have been too infrequent to allow analysis of possible mechanisms. Although perineurial immunoglobulin deposition has been emphasized, this is a nonspecific finding and may simply reflect the injury to the perineurial barrier at this location. Asbury et al. (1972) offered the hypothesis that the thickened perineurium "strangles" the endoneurial contents. Alternatively, as the endoneurial blood supply partially passes through transperineurial vessels, perineuritis might result in endoneurial ischemia. A relationship to sarcoidosis is speculative (Krendel and Costigan 1994).

10.2.4 Differential Diagnosis

As leprous neuritis, both lepromatous and borderline, enters into the differential diagnosis, a careful search for bacilli is indicated, and if Fite or fluorescent stains are negative, PCR techniques offer the highest sensitivity (Nishimura et al. 1994). Sarcoidosis should also be carefully considered, as evidence of systemic disease may be inconspicuous (Krendel and Costigan 1994). Certain toxic agents have been reported to cause a perineuritis, namely, adulterated rapeseed oil or L-tryptophan, but these are associated with characteristic epidemic syndromes, and no new cases are being reported. Lyme disease may show prominent perineurial involvement but also shows widespread perivascular inflammation (Vallat et al. 1987). Literature from the first half of the twentieth century anecdotally refers to syphilitic peripheral nerve perineuritis, but no modern reports are available, and Merritt and Adams did not believe in the existence of this entity (Merritt et al. 1946). The xanthomatous neuropathy in primary biliary cirrhosis reported by Thomas and Walker (1965) did not demonstrate inflammation, only lipid deposition, in the perineurium.

In summary, idiopathic sensory perineuritis is a diagnosis of exclusion. The histological finding of perineuritis should prompt a search for leprosy and sarcoidosis and consideration of the possibility of certain toxic exposures, cryoglobulinemia, Lyme disease, systemic vasculitis, and ulcerative colitis. Matthews (Matthews and Squier 1988) has suggested the possibility of an association with neoplasia.

Case 10.2

A 56-year-old man experienced the onset over about 6 weeks of bilateral progressive numbness and paresthesias of feet and hands, in a stocking–glove distribution, along with bilateral knee pain. Over the same period, mild symmetrical weakness of the hands and feet evolved. Subsequently, symptoms remained stable. The patient had a history of "heavy" alcohol intake until the onset of his symptoms but subsequently stopped his alcohol intake. In addition to the

bilateral knee pain, he had also experienced a swollen wrist which resolved over a period of 2 weeks.

Physical examination 6 months after onset showed mild wasting and weakness in distal hand and leg muscles, with stocking–glove diminishment of pinprick sensation and relative sparing of vibration and joint position sensation. Ankle jerks were diminished, but otherwise reflexes were normal. A rheumatological consultant documented 21 actively inflamed joints, particularly in the distal small joints of the upper extremities, but could not identify a specific rheumatological disease. No other abnormalities were noted on examination.

Biochemical, hematologic, and serological investigations (including liver function, cholesterol, ESR, ANA, RF, immunoelectrophoresis, circulating immune complexes, cryoglobulins, and VDRL) were normal. Chest X-ray was normal as was CSF examination. Nerve conductions and EMG showed a mild mixed but predominantly axonal sensorimotor polyneuropathy. Nerve biopsy was performed approximately 9 months after onset of symptoms. Muscle, liver, and skin biopsies were performed and showed no abnormalities.

Treatment with corticosteroids over the subsequent year was associated with slight improvement. Steroids were discontinued after 1 year, and subsequent repeated physical examination and electrical testing showed no significant changes.

About 3 years after onset of his neuropathy, at the age of 59, the patient developed gradually diminishing level of alertness over several days and was admitted to the hospital in a near-comatose state. In the hospital, he became pancytopenic and died within a few days of admission. Autopsy demonstrated intracerebral hemorrhage, systemic sepsis, and early alcoholic cirrhosis. Examination of the peripheral nervous system was not performed (clinical material courtesy of Dr. P. Ashby).

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Infectious Diseases Causing Neuropathy

11

Peripheral nerves are affected by a small number of infectious diseases in comparison to other organs. Nevertheless, the impact of these diseases is great, as evidenced by the frequency of leprous neuropathy in the underdeveloped world and HIV-related neuropathies globally. The spectrum of pathogens affecting the peripheral nervous system ranges from prions and viruses to spirochetes and parasites; some of these are reviewed in this chapter. Leprosy is discussed separately in Chap. 12. Parasitic diseases of the peripheral nervous system are discussed by Connor and Manz (1993).

Table 11.1 Peripheral neuropathies associated with HIV infection

Distal symmetrical (predominantly sensory) polyneuropathy
Inflammatory demyelinating polyneuropathy (GBS, CIDP)
HIV-associated mononeuropathy multiplex (MM) syndromes
Cytomegalovirus-associated neuropathies (lumbosacral polyradiculomyelopathy, CMV vasculitis)
Herpes zoster reactivation
Neuropathy associated with ddI/ddC
Infiltrative (lymphomatous) neuropathy
Diffuse infiltrative lymphocytosis
Autonomic neuropathy

11.1 HIV Infection

As understanding of the spectrum of disease associated with HIV infection has evolved over the last decade (Table 11.1), it has become clear that peripheral nerve pathology is present in nearly all patients by the end stage of their illness (i.e., AIDS), although it is often asymptomatic (Fuller et al. 1991, 1993; Gastaut et al. 1989; de la Monte et al. 1988). In the early stages of HIV infection, symptomatic neuropathy is seen in as few as 0.5 % of patients, but this increases dramatically as patients develop the full syndrome (Fuller et al. 1993; Barohn et al. 1993; So et al. 1988; Hall et al. 1991). Lange (1994), Gabbati et al. (2013), and Centner et al. (2013) provide a comprehensive review of the neuromuscular manifestations associated with HIV infection.

The neuropathic syndromes seen in association with HIV infection tend to occur at specific phases in the evolution of the disease (Cornblath and McArthur 1988). During seroconversion or the subsequent asymptomatic stage, a neuropathy identical to Guillain–Barré syndrome (GBS) or chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is sometimes seen. Mononeuropathy multiplex is an uncommon feature of the early symptomatic stage (ARC, CDC classes III and IVA) of HIV infection (Figs. 11.1 and 11.2). A distal symmetrical polyneuropathy (DSPN) is the most prevalent syndrome in patients with severe immunodeficiency (AIDS), who are also at risk of developing a neuropathy as the result of antiretroviral therapy with ddI (2′3′-dideoxyinosine) and ddC (2′3′-dideoxycytidine) (Table 18.1). Cytomegalovirus polyradiculoneuritis is increasingly recognized as a complication of HIV infection (Figs. 11.3 and 11.4), and lymphomatous neuropathies may also occur (Gold et al. 1988; Cohen et al. 1993; Fuller et al. 1993). Finally, autonomic neuropathy has been reported, typically characterized by orthostatic intolerance and secretomotor and gastrointestinal complaints, late in the course of HIV (Compostella et al. 2008).

Inflammation with CD8+ prominence has been described as "diffuse infiltrative lymphocytosis" syndrome (DILS) and may be confused with lymphoma in some cases. The former condition is characterized by hyperlymphocytosis with multivisceral CD8+ T-cell infiltration, particularly involving the salivary glands resulting in xerostomia. Additionally, there is marked angiocentric inflammation of nerve without angionecrosis (Moulignier et al. 1997). Mycobacterium avium intracellulare (MAI) has been reported within macrophages in peripheral nerve on two occasions (Cornblath et al. 1993), but whether it played a pathogenic role is unclear. Patients with underlying infectious neuropathy may develop IRIS (immune reconstitution inflammatory syndrome, Beishuizen and Geerlings 2009) which may present as acute inflammatory demyelinating polyneuropathy, i.e., as Guillain–Barré syndrome.

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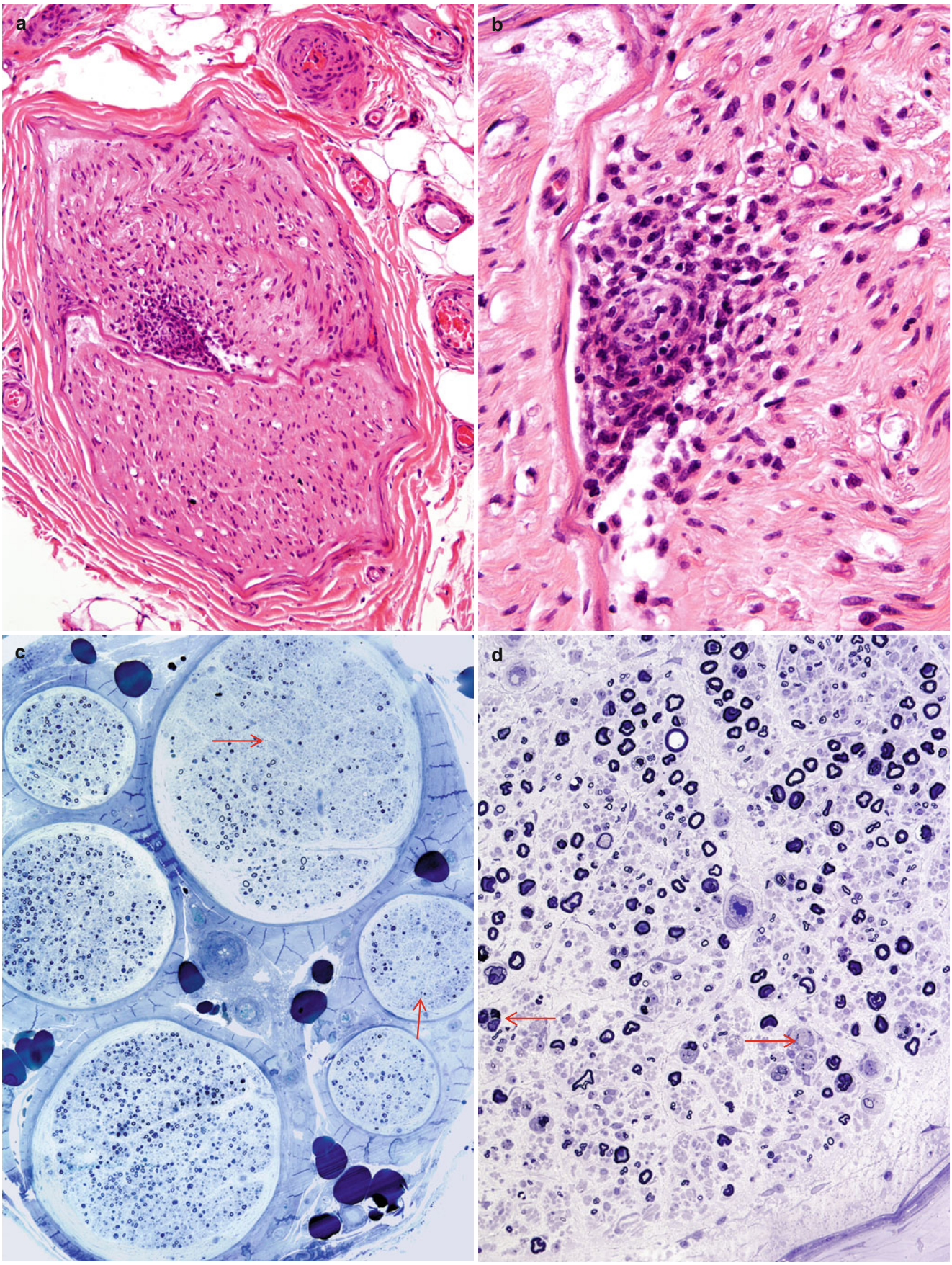

Fig. 11.1 HIV-associated vasculitis: An endoneurial microvessel is obliterated and surrounded by mononuclear cells. Note karyorrhexis in inflammatory infiltrate (**b**). Massive axonal degeneration with fascicle-to-fascicle variability in axon loss with intrafascicular loss (*arrows,* **c**) and numerous actively degenerating axons (*arrows,* **d**) is consistent with ischemic injury (**c**). Rows of myelin ovoids are seen in (**d**) (**a**, **b**, 400×, 1,000×; **c**, 200×; **d**, 400×) (**a**, **b**, paraffin H&E; **c**, 1 μ thick plastic section 400×; **d**, 1 μ thick plastic section 400×)

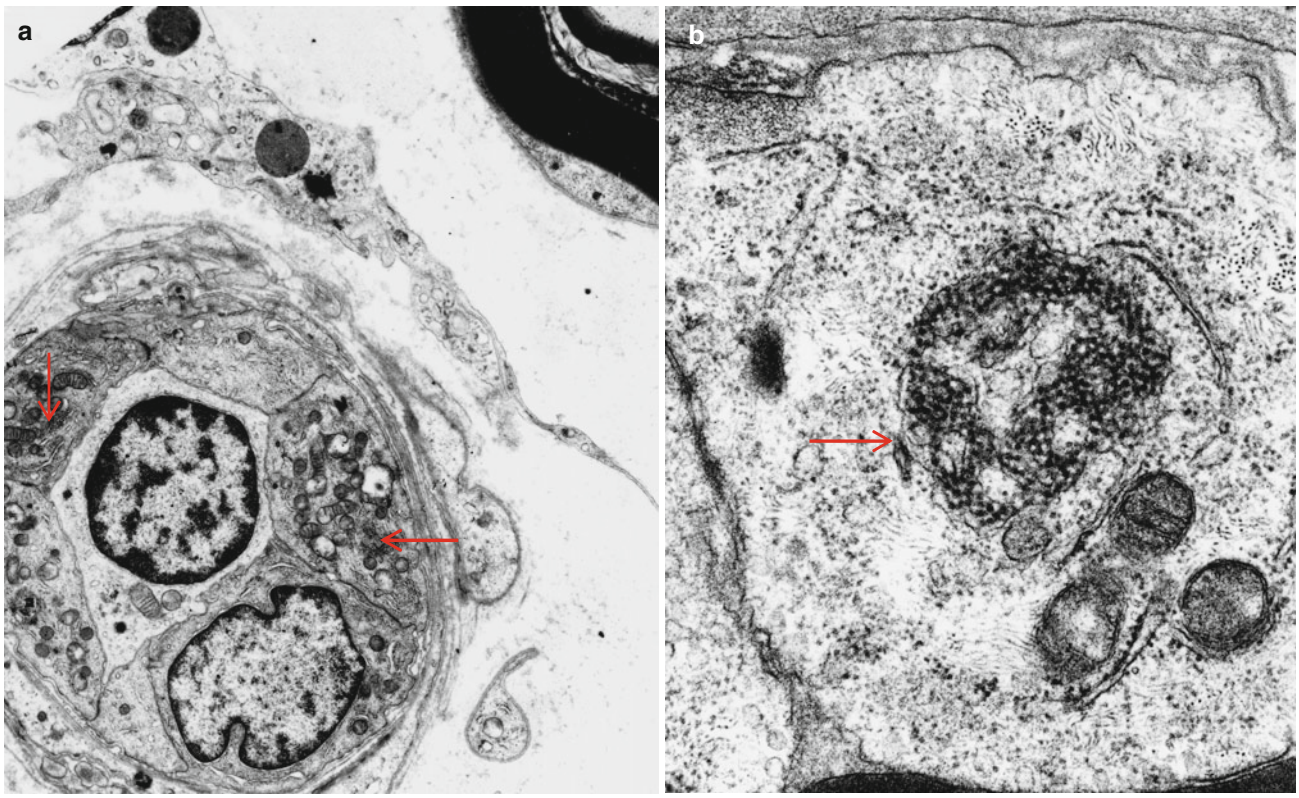

Fig. 11.2 HIV-associated vasculitis: Tubuloreticular inclusions (TRIs) (*arrows* **a**, **b**) are commonly found in endothelial cells of patients with AIDS, even in uninvolved vessels (**a**, 5,720×; **b**, 30,420×)

11.1.1 Distal Symmetrical Polyneuropathy (DSPN)

11.1.1.1 Clinical Manifestations

DSPN is seen in 10–35 % of patients in the late stages of HIV disease (Fuller et al. 1993; Barohn et al. 1993; Hall et al. 1991; Cornblath and McArthur 1988; So et al. 1988) but may occur in any stage of HIV infection (Vital et al. 1992; Hall et al. 1991; Winer et al. 1992). Initial complaints usually involve the legs distally, with paresthesias, sensory deficit, and dysesthetic pain sometimes so severe that patients are unable to walk because of discomfort rather than weakness. Motor involvement, if present, is usually confined to the distal lower extremity. Patients often have concomitant brain and spinal cord disease. The CSF may show a mild nonspecific elevation in cell count or protein, and electrical testing demonstrates diffuse distal axonal dysfunction with variable conduction slowing. Therapy is limited to symptom control (Cornblath et al. 1993).

11.1.1.2 Pathology

Although DSPN is by far the most common cause of neuropathy in this group of patients, other neuropathies must be considered, especially in patients experiencing weight loss and nutritional deficits. Patients with a clinical syndrome resembling DSPN may have necrotizing peripheral nerve vasculitis and are discussed separately below.

Light Microscopy

Epineurial or endoneurial inflammatory infiltrates may be seen. When present they are usually perivascular, range from minimal to very prominent, and do not correlate with the clinical severity, or even presence, of the neuropathy (Bailey et al. 1988; Chaunu et al. 1989; de la Monte et al. 1988; Fuller et al. 1993; Kiprov et al. 1988; Gastaut et al. 1989; Vital et al. 1992; Winer et al. 1992). Absence of inflammation may be related to systemic depletion of lymphocytes, as DSPN during AIDS-related complex (ARC) is more likely to show lymphocytic infiltration than DSPN during AIDS (Chaunu et al. 1989; Leger et al. 1988). Perivascular cuffing has been referred to as "microvasculitis" (Chaunu et al. 1989; Leger et al. 1988; Leport et al. 1987), but in our opinion, this is overstated in the absence of vessel necrosis or endothelial damage. Winer and colleagues (1992) observed frequent positivity for iron (Perls' stain) in endoneurial and perineurial macrophages of patients with neuropathy. Such a finding might suggest a vasculitic process (Adams et al. 1989), but similar positivity was seen in relatively normal nerves, making this finding hard to interpret. Overall, although perivascular inflammation can be very prominent in DSPN, true vasculitis does not appear to be a significant factor.

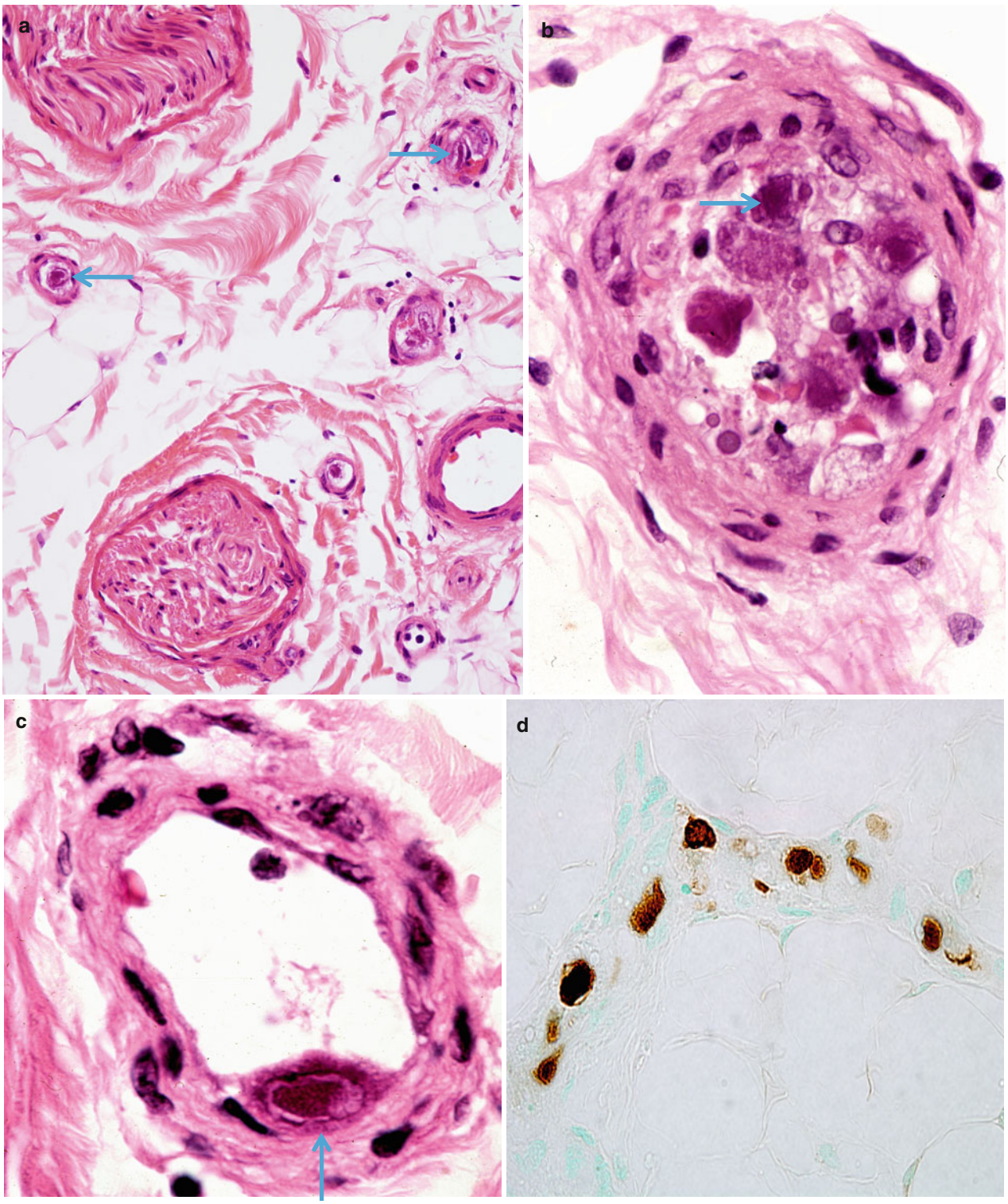

Fig. 11.3 CMV neuritis: Epineurial vessels (**a**: *arrow*) display typical amphophilic inclusions in endothelium (*arrows*, **a–c**). Lack of inflammation is attributed to immune suppression. (**d**) CMV is identified by immunostaining (**a**, 400×; **b**, **c**, 1,000×; **d**, 600×)

Typically, the neuropathic process is a variably severe acute and chronic axonal degeneration (Fig. 11.5), with less prominent segmental demyelination (Gastaut et al. 1989; Kiprov et al. 1988; Winer et al. 1992; Bailey et al. 1988; Vital et al. 1992). Large myelinated fibers may be more involved (Gastaut et al. 1989), although this could reflect axonal atrophy rather than true selective loss of large axons (Fuller et al. 1990a). Unmyelinated fibers may be relatively

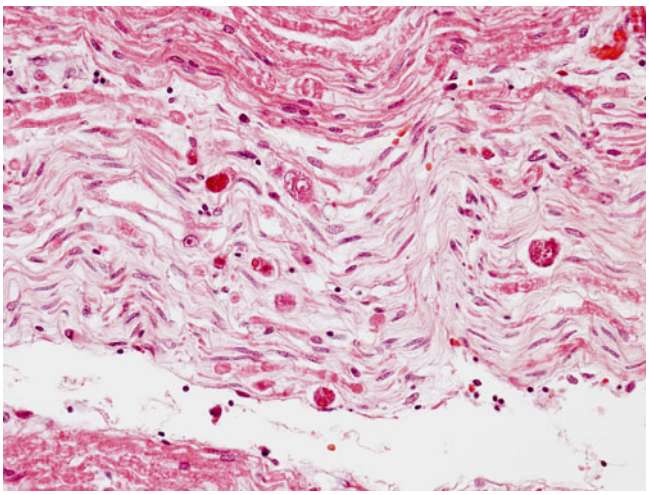

Fig. 11.4 Lumbosacral polyradiculomyelopathy: Extensive involvement of proximal portion of nerve root adjacent to lumbosacral cord represents an additional site of involvement (paraffin section, 600×)

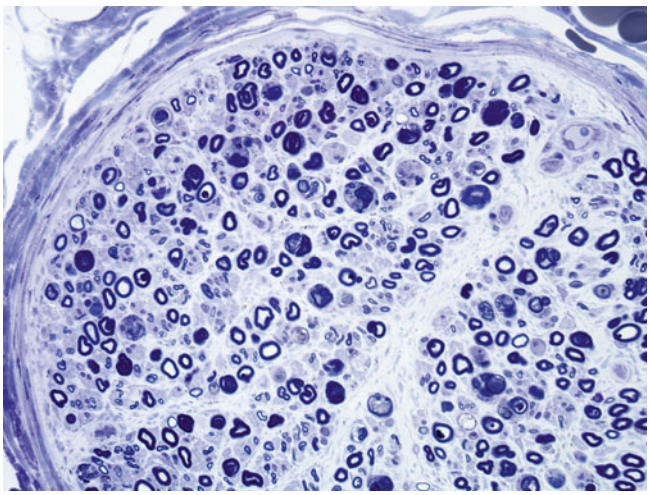

Fig. 11.5 HIV-associated Distal sensory polyneuropathy (DSPN): Extensive loss of myelinated axons with ongoing axonal degeneration may present with sensory loss and pain, often with increased numbers of macrophages (1 μ thick plastic section 400×)

spared (Fuller et al. 1990a). Regenerating clusters are occasionally seen. Segmental demyelination has not been a significant feature in studies employing morphometric techniques or electron microscopy (Mah et al. 1988; Cornblath et al. 1993; Vital et al. 1992), and the one report suggesting that segmental demyelination occurred in DPSN was performed without semithin sections, electron microscopy, or fiber teasing (de la Monte et al. 1988).

Vital et al. (1992) found positive in situ hybridization for HIV in 2 of 12 patients with DSPN, but the cell containing the signal was not identified and could simply have been a circulating leukocyte. An immunohistochemical search for HIV antigens in peripheral nerve of patients with DSPN has been unrewarding (de la Monte et al. 1988; Grafe and Wiley 1989).

Electron Microscopy

Tubuloreticular inclusions (TRIs, vide infra) are probably detectable in most patients with DSPN (Mezin et al. 1991; Vital et al. 1992; Fuller and Jacobs 1989). TRI numbers seem to correlate with the duration and severity of HIV disease, as they were found in only 1 of 10 patients with ARC or HIV seropositivity and in 17 of 18 patients with AIDS (Mezin et al. 1991; Vital et al. 1992). TRIs are also detectable in macrophages and perineurial cells (Mezin et al. 1991). Mezin et al. (1991) observed necrosis of capillary endothelial cells in 5 of their 12 specimens, but no nearby inflammatory cells were identified (Mezin et al. 1991). Nonspecific basal lamina thickening may be seen.

Intra-axonal "retroviral-like" particles which were felt to be mature HIV nucleocapsids have been reported in nerve in only one case (Bailey et al. 1988). In autopsy studies, Schwann cells containing CMV inclusions were seen, but clinical details of the peripheral neuropathy were not provided (Grafe and Wiley 1989; Guarda et al. 1984). Inclusions consistent with CMV have not been described in typical cases of DSPN. Fuller and co-workers found axonal atrophy to be a prominent feature of painful DSPN (Fuller et al. 1990a).

11.1.1.3 Pathogenesis

Examination of the peripheral nervous system at autopsy in 27 patients dying of AIDS, with and without neuropathy, demonstrated axonal loss and Wallerian degeneration in all, with a proximodistal gradient (Cornblath et al. 1993). Distal degeneration of the fasciculus gracilis in patients with DSPN and AIDS has also been demonstrated (Rance et al. 1988). Such observations provide evidence for a distal axonopathy. Spinal ganglion disease cannot be implicated as the sole explanation for DSPN (Fuller et al. 1993) because there is a motor component to the neuropathy and because inflammation and cell loss at this site do not correlate with peripheral nerve axon loss (Henin et al. 1990; Cornblath et al. 1993). The significance of perivascular cuffing seen in many patients with DSPN is unclear because this is a nonspecific finding which can be prominent in asymptomatic patients (de la Monte et al. 1988).

Fuller and colleagues report a higher incidence of active or recurrent CMV infection in AIDS patients with DSPN (80 %) than AIDS patients without DSPN (37 %), suggesting a possible contributory role of CMV to DPSN (Fuller et al. 1993). However, other workers have not detected this correlation (Winer et al. 1992). That this form of neuropathy is not seen in immunosuppressed transplant patients suggests that the HIV virus itself plays a role. AIDS patients with and without DSPN have a similar nutritional state (Fuller et al. 1993).

The evidence for necrotizing vasculitis is weak in DPSN, but the observation of capillary endothelial cell necrosis in association with TRIs (Mezin et al. 1991) is intriguing. We

have examined biopsies in HIV-associated and SLE-associated vasculitic neuropathy where numerous TRIs and necrotic endothelial cells were present (Case 11.1). Interferon administration, known to induce formation of TRIs, may cause a peripheral neuropathy, but its pathological substrate is not known (Gastineau et al. 1989).

The HIV has on rare occasions been isolated from nerve homogenates (Ho et al. 1985; de la Monte et al. 1988) or detected by in situ hybridization (Vital et al. 1992), most likely originating from infected leukocytes or macrophages circulating through the peripheral nerve (Gherardi et al. 1989). Indeed, viral antigen was not detected by immunohistochemistry in the same nerve specimens from which it was cultured (de la Monte et al. 1988). Nevertheless, it is possible that DSPN results from HIV infection (Cornblath et al. 1993), perhaps through neurotoxic substances secreted by HIV-infected phagocytes (Giulian et al. 1990) or interference with nerve function by binding of HIV gp120 glycoprotein to peripheral nerve or sensory neuron antigens (van den Berg et al. 1992; Apostolski et al. 1993).

11.1.2 Inflammatory Demyelinating Polyneuropathy in HIV Infection

11.1.2.1 Clinical Manifestations

Early in the HIV epidemic, GBS and CIDP received considerable attention (Cornblath et al. 1987). However, more recent prospective studies suggest that the incidence of these neuropathies in HIV infection is small (Barohn et al. 1993; Hall et al. 1991; Fuller et al. 1993). While these neuropathies tend to occur in seroconverting or asymptomatic HIV seropositive patients, they have been described in patients with ARC (Cornblath et al. 1987; Vital et al. 1992) and AIDS (Chaunu et al. 1989). The disease manifestations, including course and response to treatment, are identical to those seen in HIV seronegative patients, excepting only the presence of a CSF pleocytosis in many cases (Cornblath et al. 1987; Miller et al. 1988).

11.1.2.2 Pathology

The cardinal histological findings, mononuclear inflammation, macrophage-mediated myelin stripping, and a variable amount of axonal damage, are identical to those seen in inflammatory demyelination not associated with HIV (see Chap. 9) (Vital et al. 1992). The only observation that suggests an inflammatory demyelinating neuropathy is associated with HIV infection is visualization of tubuloreticular inclusions (TRIs) on electron microscopy (Fuller and Jacobs 1989). TRIs have not been reported in GBS or CIDP in the absence of HIV infection. Gibbels and Diederich (1988) described unusual "onion-bulb" formations in which the surrounding Schwann cell processes were thick and electron dense, and occasionally multinucleated! However, similar observations have not been reported elsewhere. As with DSPN, the virus has rarely been isolated from, but not directly visualized in, nerve in patients with this syndrome.

11.1.2.3 Pathogenesis

HIV has not been localized to endoneurial cells in these patients, and as the neuropathy tends to improve, it seems unlikely that the virus is directly related. Complement fixing anti-peripheral nerve antibodies similar to those described in classic GBS have been described in HIV seropositive patients with a GBS-like syndrome (Mishra et al. 1985). Kiprov et al. (1988) also reported patients with circulating anti-peripheral nerve antibodies, but identical findings were seen in HIV-infected patients with mononeuritis multiplex and DSPN. Patients are usually not severely immunosuppressed at the time of onset of their inflammatory demyelinating neuropathy, and most likely the pathogenesis of GBS and CIDP in HIV seropositive patients is similar to that of HIV seronegative patients (Chap. 9). The virus may play a role comparable to CMV or EBV in "precipitating" the onset of GBS.

11.1.3 Mononeuropathy Multiplex and Vasculitic Neuropathy in HIV Infection

11.1.3.1 Clinical Manifestations

Mononeuropathy multiplex (MM) has been described in HIV patients, most often during the early symptomatic (ARC) phase. The pathological substrate includes vasculitis associated with CMV infection (discussed separately below), vasculitis associated with HIV infection, and patients in whom no evidence of vasculitis is detected. Whether these groups truly differ or simply reflect the error inherent in sampling a nerve biopsy is unknown.

The patients with MM reported by Lipkin et al. (1985) did not have evidence of necrotizing vasculitis in nerve biopsy specimens, and often improved spontaneously. So and Olney (1991) also indicated that patients with mononeuritis multiplex tend to improve spontaneously or stabilize, but the histology in these patients was not specified.

Histologically verified non-CMV-related necrotizing vasculitis is a well-recognized complication of HIV infection (Calabrese et al. 1989) and can present neurologically as mononeuritis multiplex or distal symmetrical polyneuropathy (Case 11.1 below; Said et al. 1987; Dalakas and Pezeshkpour 1988; Gherardi et al. 1989; Kiprov et al. 1988; Conri et al. 1991; Lange 1994). Steroids or plasma exchange may be of benefit (Conri et al. 1991).

11.1.3.2 Pathology

Vasculitis involving endoneurial or epineurial vessels has been documented on many occasions in patients with HIV infection (Calabrese et al. 1989; Gherardi et al. 1989; Dalakas and Pezeshkpour 1988; Said et al. 1988a; Vinters et al. 1988; Weber et al. 1987; Lange 1994) and is probably underdiagnosed because these patients tend not to undergo biopsy. Issues relating to necrotizing vasculitis are discussed in detail in Chap. 13. In the one case of HIV-associated vasculitis we have examined, the vascular involvement took the form of endoneurial vascular and perivascular inflammation with karyorrhexis (Fig. 11.1a, b) with accompanying fascicle to fascicle variability in axon number and presence of large numbers of degenerating axons (Fig. 11.1d). Tubuloreticular inclusions were very prominent in this specimen (Fig. 11.2a, b), a finding which should always raise the alert for HIV infection, but we have also detected these in a patient with SLE and predominantly endoneurial vasculopathy (see Case 13.1).

The patients with mononeuropathy multiplex reported by Lipkin and colleagues (1985) showed perivascular inflammatory infiltrates, axonal degeneration, and demyelination of variable severity. No definite vasculitis was seen, but this does not rule out necrotizing vasculitis, which may be seen only on examination of another tissue or on angiography as demonstrated in other HIV-positive patients (Lange et al. 1988; Conri et al. 1991).

The HIV virus itself has been found in perivascular cells in vasculitis, probably macrophages, by techniques ranging from electron microscopy to RNA probes (Gherardi et al. 1989).

11.1.3.3 Pathogenesis

HIV antigens have been found in vessel walls and perivascular inflammatory cells in patients with vasculitis (Gherardi et al. 1989; Said et al. 1987). Circulating immune complexes to the HIV are present in seropositive patients (McDougal et al. 1985), a relevant observation in light of the known relationship between immune complexes and polyarteritis nodosa (Cornblath et al. 1993). Patients infected with HIV have also rarely been shown to suffer from angiocentric lymphoproliferative diseases which range from prominent polyclonal perivascular inflammation, through lymphomatoid granulomatosis, to malignant angiocentric lymphoma (Calabrese et al. 1989).

Case 11.1

A 34-year-old man was seen because of worsening paresthesia. He had tested positive for HIV infection 5 years earlier and had experienced intermittent diarrhea and a 40 lb weight loss a year prior to the neurological assessment. During the 6 months before nerve biopsy, the patient complained of paresthesias and numbness, involving first the left hand in a median nerve distribution, followed by the lateral left foot and shin, and then the right hand. Weakness of right hand muscles then ensued. Examination disclosed motor weakness in the right hand consistent with anterior interosseous nerve palsy. Discrete areas of diminished pin and temperature sensation were present in both the hands and feet, with bilateral mild impairment of distal vibration sensation at the toes. Reflexes were easily obtained and symmetrical. The patient had previously been treated with AZT, DDI, and DDC, but these had been discontinued prior to the onset of his symptoms due to adverse effects other than neuropathy.

At the time of neurological assessment, the absolute CD4 count was 34. ESR, ANA, immunoelectrophoresis, and routine biochemical and hematologic indices were normal. Electrophysiological tests performed on two occasions were interpreted as essentially normal, although a suspicion of bilateral carpal tunnel syndrome was entertained because of a slight disparity between distal latencies of the ulnar and median motor conductions. Electromyography was normal.

Nerve biopsy was performed 6 months after the onset of symptoms (Fig. 11.1). At this time, the patient was receiving low-dose steroid treatment for palliation of other symptomatology. Following nerve biopsy, no further treatment was offered, and the neuropathy continued to progress (clinical material courtesy of Dr. J. R. Wherrett).

11.1.4 Cytomegalovirus-Associated Neuropathy

The presence of CMV in peripheral nerve has been frequently documented, almost invariably in the setting of late-stage HIV infection (Bishopric et al. 1985; Eidelberg et al. 1986; Said et al. 1991; Fuller et al. 1990b; Morgello and Simpson 1994; Lange 1994). The clinical picture is usually fulminant polyneuropathy, polyradiculopathy, or mononeuritis multiplex. Some of these patients have improved with specific anti-CMV therapy (Said et al. 1991; Fuller et al. 1990b). CMV neuritis is probably part of the spectrum of CMV myeloradiculoneuritis (Cohen et al. 1993).

11.1.4.1 Pathology

The significance of finding CMV in any tissue in an AIDS patient is uncertain because this organism is ubiquitous by the end stages of the HIV infection (de la Monte et al. 1988; Guarda et al. 1984).

We have seen several cases of CMV-related vasculitic neuritis in an AIDS patient and another with immunosuppression in a cardiac transplant patient. The literature documents several patients with biopsy-proven CMV-associated

peripheral nerve vasculitis, all with late-stage HIV infection (Said et al. 1991; Fuller et al. 1990b; Eidelberg et al. 1986). Nerve biopsy reveals necrotizing endoneurial and epineurial vasculitis with a prominent neutrophilic infiltrate, but in some cases, the tissue reaction is minimal, depending presumably on the host's immune state (Fig. 11.3a). Cytomegalic cells, 30–50 μm in diameter, and cells containing intranuclear and intracytoplasmic amphophilic inclusions characteristic of CMV infection are observed, with immunostains confirming the presence of CMV (Fig. 11.3b–d). CMV virions can be identified on electron microscopy in endoneurial cells, especially endothelial cells. Although segmental demyelination can be present, it is overshadowed by active axonal degeneration.

CMV infection may also produce peripheral nerve disease through infection of Schwann cells. In one report, Schwann cell infection with CMV was demonstrated in four of seven patients with focal peripheral nerve inflammation; the infected cells were seen in association with regions of inflammation (Grafe and Wiley 1989). Clinical details were unfortunately not provided. Other reports document patients with a fulminant neuropathy and CMV inclusions in Schwann cells and other endothelial cells, associated with inflammation, demyelination, and cellular "necrosis" (Bishopric et al. 1985; Budzilovich et al. 1989). Rare cytomegalic Schwann cells containing intranuclear inclusions were associated with nerve root segmental demyelination in the case reported by Moskowitz et al. (1984). Morgello and Simpson (1994) reported a patient with multifocal demyelinating neuropathy where autopsy material demonstrated no evidence of vasculitis, but rather a mixed axonal and demyelinating neuropathy with evidence of Schwann cell necrosis. Intraneural mixed inflammatory infiltrates were associated with cytomegalic cells and "owl's eye" inclusions, and herpesvirus capsids were demonstrated within Schwann cells.

Patients presenting with lumbosacral polyradiculomyelopathy, typically a late complication of CMV infection in AIDS, may have extensive involvement in the roots (Fig. 11.4) and adjacent spinal cord.

11.1.4.2 Pathogenesis

CMV neuritis seems to occur only in the setting of severe immunosuppression and can affect the peripheral nerve either by necrotizing vasculitis or through injury to Schwann cells and other endoneurial components.

11.1.5 Role of Nerve Biopsy in HIV Patients with Neuropathy

Little justification exists for nerve biopsy in typical cases of HIV-associated DSPN. The inflammatory demyelinating polyneuropathies (IDP) and vasculitic neuropathy seen in HIV disease may be responsive to specific treatment (vide supra) and should be identified. However, while biopsy might support the diagnosis of HIV-associated CIDP or GBS, histological examination is probably not very sensitive (Chap. 9), and in HIV infection, neural inflammation is nonspecific. Thus, clinical and electrophysiological criteria are probably more reliable than biopsy in making a diagnosis of CIDP or GBS in HIV patients.

The possibility of vasculitic neuropathy, often but not invariably associated with CMV infection, provides the strongest impetus for nerve biopsy in the HIV-positive patient, as this represents one of the few treatable neuropathies of HIV disease. CMV can also cause a demyelinating neuropathy (vide supra). The clinical picture may not always suggest the underlying process, because a predominantly sensory and symmetrical neuropathy, similar to DSPN, can be seen in this setting (Gherardi et al. 1989; Said et al. 1987; Calabrese et al. 1989). If mononeuritis multiplex is present, vasculitic neuropathy is suspected clinically, but the differential diagnosis includes HIV vasculitis, CMV vasculitis, or multiple peripheral nerve or root lesions due to lymphoma (Gold et al. 1988), CMV, or herpes zoster.

Suggested guidelines for nerve biopsy in HIV-positive patients include:

- No biopsy in typical inflammatory demyelinating neuropathy or DSPN patients
- Biopsy in patients with mononeuritis multiplex in whom aggressive treatment would be considered
- Consideration of biopsy of patients with atypical (i.e., very severe or rapidly evolving) DSPN or demyelinating neuropathy

Accordingly, only a small number of patients will undergo nerve biopsy because the vast majority of peripheral neuropathies in HIV disease are typical DSPN.

11.1.6 Differential Diagnosis

Aside from TRIs, usually seen in late-stage HIV infection, no histological finding in nerve biopsy of patients with CIDP or GBS can predict HIV status in the patient. However, in an HIV-positive patient with a demyelinating neuropathy, the observation of macrophage-mediated demyelination is important because it indicates a relatively good prognosis with appropriate treatment. Macrophage-mediated myelin stripping is never seen in DSPN. The finding of axonal loss and lymphocytic infiltration, although typical of DSPN, is entirely nonspecific and can be seen in any of the HIV-associated neuropathies. Inflammation may occur as frequently in asymptomatic as in symptomatic AIDS patients (de la Monte et al. 1988).

A patient with mononeuritis multiplex may show vasculitis that is indistinguishable from classic PAN, rheumatoid

arthritis, or other connective tissue disease. HIV-associated vasculitis probably involves endoneurial vessels more often than typical PAN, but there is much overlap. Detection of CMV in nerve in such a case should be attempted since specific treatment may be of benefit (Cohen et al. 1993; Said et al. 1991). Even if CMV is not demonstrated, the presence of endoneurial vasculitis, prominent polymorphonuclear infiltrates, with a very low CD4 count, should suggest the same possibility (Said et al. 1991). Infiltrative lymphoma involving nerve should always be considered in an HIV-positive patient (Gold et al. 1988; Fuller et al. 1993).

11.2 Neuropathy Associated with HTLV-I Infection

11.2.1 Clinical Features

HTLV-I infection has been linked to adult T-cell leukemia and tropical spastic paraparesis (Gessain and Gout 1992), and the relation of HTLV-I to neuropathy has been recently reviewed by Nascimento and Marques (2013). Neuropathy is present in 16–32 % of these patients (Bhigjee et al. 1993), although other workers have failed to find a significant association between HTLV-I seropositivity and peripheral neuropathy (Vallat et al. 1993). Worldwide, HTLV-I is a rare pathogen except in Japan, the Caribbean, and parts of Africa HLTV-I (Gessain and Gout 1992). A pseudo-ALS form of the disease is uncommon (Roman et al. 1991) and is not discussed further. It may be difficult to discern the presence of a neuropathy in the face of a prominent myelopathy, but the absence of distal tendon reflexes and abnormalities on nerve conduction studies allow the distinction.

11.2.2 Pathology

Most biopsy reports have indicated an absence of neural inflammation (Said et al. 1988b; Bhigjee et al. 1993; Sugimura et al. 1990) although others have commented on an inflammatory, occasionally vasculitic, process (Nascimento and Marques 2013). The predominant process is axonal degeneration of variable severity, with regenerating clusters and occasional thinly myelinated fibers. Unmyelinated axons may be less severely involved. Some biopsies have demonstrated demyelination that appeared out of proportion to axon loss (Bhigjee et al. 1993), suggesting primary myelin injury. With one exception (vide infra), electron microscopy has failed to demonstrate viral particles (Bhigjee et al. 1993; Sugimura et al. 1990).

Focal paranodal myelin swellings were noted in two reports (Bhigjee et al. 1993; Sugimura et al. 1990)) but were not found in a third study (Vallat et al. 1993). Ultrastructural examination of the myelin swellings showed redundancy and excessive folding of the myelin, frequently with globular degeneration.

HTLV-I is associated with adult T-cell lymphoma/leukemia (ATLL). This may in itself cause an infiltrative neuropathy. In one reported case of ATLL infiltrative neuropathy, scattered malignant cell infiltrates were seen in the nerve. Some of these contained vacuoles which when examined at a high magnification were seen to contain particles that resembled HTLV-1 virions (Vital et al. 1993). PCR confirmed that these cells were infected with HTLV-1 virus.

11.2.3 Pathogenesis

Wayne-Moore et al. (1989) found viral material in the CNS and postulated a T-cell-mediated immune attack on infected cells; a similar process may occur in the peripheral nervous system. However, viral DNA has not been detected in nerve specimens (Bhigjee et al. 1993). Furthermore, whereas the myelopathic changes are associated with perivascular inflammation (Wayne-Moore et al. 1989), this is not usually in evidence on nerve biopsy, and there is little evidence for an immune-mediated phenomenon (Bhigjee et al. 1993). The bulk of peripheral disease may be active at the nerve root level and inaccessible to biopsy (Akizuki et al. 1988). The distal symmetrical neuropathies seen in HTLV-I infection is similar to that of HIV infection, and a common, as yet undefined, mechanism may be at work. It is only fair to note that some authors have found no evidence to suggest that HTLV-I is causally associated with peripheral neuropathy (Vallat et al. 1993).

11.3 Neuropathy of Lyme Disease (Lyme Neuroborreliosis)

The neurological and systemic manifestations of Lyme disease are diverse and have been summarized in reviews by Reik (1991, 1993) and Hansen et al. (2013). Lyme disease is caused by a spirochete, *Borrelia burgdorferi*, which is inoculated into man via tick bite. It is increasingly recognized as a cause of human neurological disease, and the peripheral nervous system is a major site of involvement. Lyme disease has been reported in all inhabited continents but is most prevalent in the Northeastern USA and Western Europe including Sweden. In endemic areas, *B. burgdorferi* is a major cause of peripheral nerve disease (Reik 1993).

11.3.1 Clinical Manifestations

Lyme disease has been subdivided into early and late stages. Within days to weeks after inoculation by tick bite,

a skin lesion (erythema migrans) appears at the site, resolving after several weeks. Dissemination and subsequent organ-specific manifestations occur weeks to months after the initial infection, the most vulnerable organs being the nervous system, the heart, and the joints. Late Lyme disease manifests a year or more after the initial infection.

In the second stage of early Lyme disease, cranial neuropathies and polyradiculoneuropathies are the most common disease manifestations. The latter are often acute and painful, with focal or multifocal weakness predominating over objective sensory abnormalities. The pattern of disease includes radiculopathy, plexopathy, mononeuritis multiplex, and symmetrical polyneuropathy. Peripheral nerve manifestations are more frequent in the European than in the North American form of Lyme borreliosis (Reik 1991). Electrophysiological testing shows abnormalities most often suggestive of axonal neuropathy, but a demyelinating component can be present. CSF examination usually reveals pleocytosis and elevated protein (Reik 1991). Rarely, the clinical picture may be suggestive of GBS (Bouma et al. 1989; Sterman et al. 1982).

In late Lyme disease, peripheral nerve manifestations are more indolent, with intermittent paresthesias or radicular pain seen more commonly than definite weakness and sensory loss. Acrodermatitis chronica atrophicans (ACA) is a bluish-red discoloration and swelling of the distal extremities that occurs in some patients with late Lyme disease in Europe and which is often associated with neuropathic symptoms (Reik 1991; Kristoferitsch et al. 1988; Hansen et al. 2013). Electrophysiological studies reveal symmetrical or asymmetric axonal neuropathy (Kristoferitsch et al. 1988; Halperin et al. 1990). As often as not, the CSF examination is normal (Reik 1991).

Diagnosis of Lyme disease can be made by visualizing or culturing the organism in biopsy material, especially the skin. These direct techniques have a low yield, and most often the diagnosis rests on clinical suspicion and confirmatory Lyme serology. However, in the earliest (<4 weeks) and latest (years) stages of the disease, such assays may be falsely negative (Reik 1993). Antibiotic treatment is highly effective in both early- and late-stage disease (Reik 1993; Halperin et al. 1987).

11.3.2 Pathology

The discussion below is based on a review of the nerve biopsy literature in Lyme disease (Meurers et al. 1990; Vallat et al. 1987; Meier et al. 1989; Camponovo and Meier 1986; Kristoferitsch et al. 1988; Halperin et al. 1987; Tezzon et al. 1991; Hansen et al. 2013). Lyme organisms have never been reported in peripheral nerve. Duray et al. (1985) describe a modification of the Dieterle stain for those who wish to search for the spirochete. PCR techniques will probably prove more sensitive in the future (Schwartz et al. 1992).

The histological picture in Lyme neuropathy has a similar profile regardless of disease stage (Kristoferitsch et al. 1988). An inflammatory and axonal process has almost invariably been described, although no inflammation was present in two patients, one who was biopsied 4 weeks after the beginning of antibiotic treatment (Meurers et al. 1990) and another with a minimal neuropathy who also was treated prior to biopsy (Halperin et al. 1987). The infiltrate centers around epineurial (most often), perineurial, and endoneurial vessels, and a few randomly scattered lymphocytes may be seen within the fascicle. Focal perineurial inflammation may occur. The inflammatory cells include lymphocytes, plasma cells, and histiocytes and may form very prominent perivascular cuffs, occasional infiltrating vessel walls. Camponovo and Meier (1986) described thrombosed vessels and extensive damage to the epineurium in one case, initially reporting this as an example of Lyme "vasculitis." However, in a subsequent report, it was pointed out that disruption of the internal elastic lamina was never seen in this biopsy (Meier et al. 1989). Indeed, although the appearance has frequently been suspicious, necrotizing vasculitis has been described only very rarely (Tezzon et al. 1991). The axon loss, which may be dramatic, affects myelinated and unmyelinated fibers. Segmental demyelination may be seen but does not dominate the picture (Meier et al. 1989; Halperin et al. 1987). The few described cases of North American late-stage polyneuropathy (Halperin et al. 1987) seemed to have milder pathology than that described in the more numerous European cases, whether late or early stage. However, they had been treated, and numbers are too small to permit generalizations.

Immunohistochemical or immunofluorescent techniques for detection of *B. burgdorferi*-specific antigen performed on most of the biopsies discussed above were negative. A search for immune complex deposition in vessel walls has also been unrewarding (Meier et al. 1989; Kristoferitsch et al. 1988).

11.3.3 Pathogenesis

Vascular inflammation incited by organism invasion is associated with many of the extraneural manifestations of Lyme disease (Duray 1989). Immune complex deposits and organisms are not seen sufficiently frequently to directly explain this inflammatory response (Duray 1989). Organisms persist in untreated patients at all disease stages (Garcia-Monaco and Benach 1989; Duray 1989), and antibiotic treatment reverses most manifestations (Reik 1993). Vasculopathy has been seen in various tissues including the brain, most often as an endarteritis obliterans of small vessels during the chronic late phase of the disease (Garcia-Monaco and Benach 1989; Duray 1989).

Although frank necrotizing vasculitis is generally not present in peripheral nerve or other tissues in Lyme disease

(Duray 1989), a picture of mononeuritis multiplex or painful radiculopathies is suggestive of ischemic neuropathy. However, in late Lyme disease, the clinical, electrophysiological, and scanty histological data have been interpreted as suggestive of a distal axonopathy (Halperin et al. 1987). Halperin et al. (1990) found similarities in electrophysiological data and response to treatment in various types of Lyme neuropathy and postulated that multifocal ischemia is the common cause. If not ischemia, a variety of cytokines released by inflammatory cells can potentially damage axons or myelin (Said and Hontebeyrie-Joskowicz 1992).

Circulating antibodies against *B. burgdorferi* which cross-react with axonal or myelin antigens have been detected by some workers (Sigal 1993). However, this is hard to reconcile with the multifocal nature of the pathological changes and with the rapid clinical improvement that often occurs with treatment. Minor antigenic differences between European and North American spirochetes may underlie clinical differences in the two forms of the disease (Duray 1989).

11.3.4 Differential Diagnosis

Perivascular inflammation and active axonal degeneration are nonspecific. The possibility of vasculitic neuropathy should be considered, and the wide differential diagnosis of inflammatory axonal neuropathies is discussed elsewhere (Tables 7.3 and 7.4). The severity of the perivascular inflammation in Lyme neuritis seems to be beyond that seen in most of the other conditions in the differential diagnosis of inflammatory axonal neuropathies. Determination of atypia and immunophenotype of the cellular infiltrate will help exclude a lymphoproliferative process affecting the nerve. Meier et al. (1989) were impressed by the prominence of plasma cells in their Lyme neuritis material and thought that this was an important clue to the diagnosis. If perineurial inflammation is very prominent, it may be difficult to distinguish idiopathic perineuritis from Lyme neuritis, and leprosy should always be ruled out.

11.4 Non-Lyme Peripheral Neuropathy with Arthropod Stings

Peripheral nerve syndromes have been described following insect bites or stings (Creange et al. 1993). The offending insects include bees, hornets, wasps, fire ants, and spiders, and the peripheral nerve manifestations include mononeuritis multiplex, radiculopathies, polyneuropathies, and a GBS-like picture. Lyme disease must be excluded. Electrophysiology usually reveals an axonal neuropathy, although a mixed picture may also be seen (Creange et al. 1993). Prognosis for recovery is good.

In nearly all biopsied cases, the neuropathy had a strong inflammatory component with lymphocytes and plasma cells surrounding epineurial and endoneurial vessels in thick cuffs, and sometimes infiltrating the vessel wall (Creange et al. 1993; Means et al. 1973; Bachman et al. 1982). This was sometimes described as a "vasculitis," but no fibrinoid necrosis was shown, and the illustrations provided do not demonstrate thrombosis or disruption of vessel walls. Thus, we would classify these as strongly inflammatory but not necrotizing vasculitic neuropathies. Wallerian degeneration was noted in most cases, although one case with a GBS-like picture demonstrated prominent segmental demyelination (Bachman et al. 1982).

The mechanisms underlying these neuropathies are unknown, and the infrequency of neuropathy with insect bite in general suggests that some of these case reports represent a coincidence, especially in cases with a GBS-like picture. With the presence of prominent perivascular inflammation and massive Wallerian degeneration, vascular compromise must be suspected but remains unproven. The insect may elaborate a toxin that directly damages nerve, or there may be antigenic cross-reactivity between insect and peripheral nerve antigens leading to an autoimmune injury (Creange et al. 1993).

11.5 Other Infection-Associated Neuropathies

11.5.1 Diphtheritic Polyneuropathy

11.5.1.1 Clinical Features

Although of great historical importance in the understanding of mechanisms in peripheral nerve disease, diphtheritic polyneuropathy has now all but disappeared in the developed world due to immunization programs. The clinical picture is highly characteristic (McDonald and Kocen 1993): in about 20 % of patients, 3 or 4 weeks after the faucial infection, paralysis of the palate appears, followed by the pharynx, ocular muscles, larynx, diaphragm, and ultimately limbs by 8–12 weeks after symptom onset. Limb involvement is sensorimotor and distally predominant. Polyneuropathy can occur in extrafaucial diphtheria and may involve the limbs initially (McDonald and Kocen 1993). Diphtheria antitoxin given in the first 48 h of infection reduces the incidence of complications, but otherwise, the physician's role is to provide supportive treatment while spontaneous recovery, usually complete, occurs (McDonald and Kocen 1993).

11.5.1.2 Pathology

The definitive description of the pathology of diphtheritic polyneuritis in humans was provided by Fisher and Adams

(1956). The brunt of disease fell upon the spinal ganglia and nerve roots. Debris-filled macrophages and other mononuclear cells created a hypercellular picture, but an inflammatory infiltrate was generally not seen. In regions of greatest disease activity, strikingly focal segmental demyelination was present, with almost complete sparing of axons. The impression of the authors was that demyelination occurred before the influx of macrophages. In the peripheral nerves, there were often no demonstrable abnormalities, although discrete foci of myelin degeneration were sometimes seen.

In the only case of which we are aware where plastic-embedded semithin sections and electron microscopy were used to study a peripheral nerve biopsy, a slightly reduced number of myelinated fibers were seen, distributed in a normal size–frequency histogram (Solders et al. 1989). Myelinated fibers showed rare segmental and paranodal myelin alterations. Unmyelinated fibers were said to be normal, but quantitative studies were not performed.

11.5.1.3 Pathogenesis

Diphtheria toxin inhibits Schwann cell synthesis of myelin proteolipid and basic protein by inactivation of elongation factor 2, required for polypeptide synthesis in eukaryotic ribosomes (Jørgensen et al. 2006). The demyelination of diphtheritic neuropathy results from a failure of normal myelin protein turnover (Kaplan 1980).

11.5.2 Herpes Zoster ("Shingles")

Herpes zoster presents as a painful vesicular rash, often corresponding to a cutaneous sensory dermatome innervated by a single dorsal root or trigeminal ganglion. The varicella/zoster virus initially gains access to ganglia by parasitizing the cutaneous nerves at the time of childhood chickenpox infection and traveling to dorsal root ganglia via axonal transport. At this time the virus becomes latent in the neuron as the result of a nonintegrated (episomal) circular/concatemeric form of latency-associated gene (Steiner 2013). Latent virus is not visible by electron microscopy but the viral genome can be demonstrated as well as a mild lymphocytic infiltrate. For unclear reasons, which may include immune defect and/or advancing age, the virus emerges from latency and infects adjacent neurons, satellite and Schwann cells, and vasculature in the parent DRG, producing hemorrhagic ganglionitis (Fig. 11.6a). Necrotic neurons and satellite cells are admixed with angionecrosis, hemorrhage, and an inflammatory mononuclear cell infiltrate involving dorsal root ganglia and adjacent dorsal roots. Cowdry type A inclusions are seen in neurons and, more frequently, within the nuclei of satellite cells (arrow, Fig. 11.6b). Virus undergoes axonal transport to the skin and results in pustular eruption in the dermatomal distribution of the involved DRG or cranial ganglion dermatome. Occasional spread of the process along the dorsal roots to the spinal cord is a recorded, but relatively rare, complication (Fig. 11.6c). Varicella may reactivate without a rash (zoster sine herpete). Ramsay Hunt syndrome is characterized by a herpetic eruption in the external auditory canal accompanied by facial nerve palsy and ear pain.

11.5.3 Syphilitic Neuropathy

More typically, a radiculopathy syphilis is a rare cause of peripheral neuropathy in the sural nerve which is dominated by an epineurial, perineurial, and commonly perivascular, plasma cell infiltrate (Fig. 11.7a, b).

11.5.4 Neuropathy in Creutzfeldt–Jakob Disease

Generally regarded as a central nervous system disease, three reports describe peripheral nerve pathology in CJD (Neufeld et al. 1992; Sadeh et al. 1990; Vallat et al. 1983). The incidence of peripheral neuropathy in patients with CJD is unknown, for it is overshadowed by the CNS manifestations. Given the rarity of the association, the possibility remains that reports of neuropathy in CJD represent a coincidence between two different diseases. A stocking–glove sensory loss has been described and may be the presenting manifestation (Neufeld et al. 1992; Sadeh et al. 1990). Amyotrophy is a well-recognized feature of CJD. Electrophysiology may show conduction slowing and EMG evidence of denervation (Neufeld et al. 1992; Sadeh et al. 1990).

Peripheral nerve was said to show a widespread demyelinating neuropathy of variable activity in three biopsies (Neufeld et al. 1992; Sadeh et al. 1990; Vallat et al. 1983). Onion bulbs, some made up of only basement membrane arrays, were described in a patient with familial panencephalopathic CJD (Vallat et al. 1983). Axonal changes were minimal, and reductions in myelinated fiber number mild or absent. Inflammation and storage material were not present. In three other biopsies, the peripheral nerves were entirely normal histologically (Vallat et al. 1983; Neufeld et al. 1992). Recently, a PRNP Y163X truncation mutation and adult-onset prionopathy was described (Mead et al. 2013) which is characterized by a phenotype of chronic diarrhea with autonomic failure and a length-dependent axonal, predominantly sensory, peripheral polyneuropathy. Prion protein amyloid was found throughout peripheral organs, including the bowel and peripheral nerves and in end-stage brain tissue.

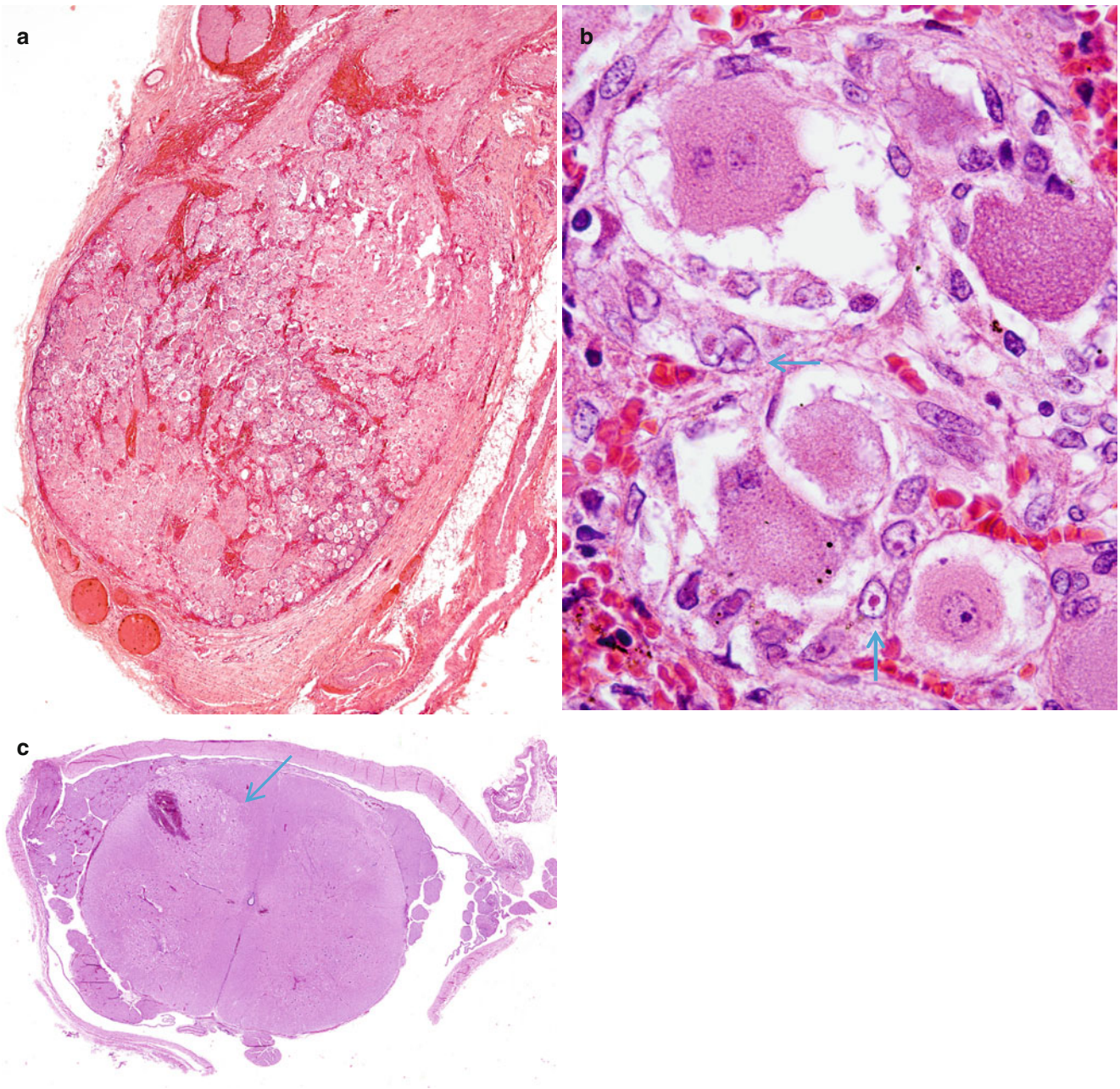

Fig. 11.6 Herpes zoster ("shingles"): Reactivation typically results in hemorrhagic ganglionitis (**a**) in which intranuclear Cowdry type A inclusions can be seen in neurons and, more commonly, in satellite (*arrows*, **b**) and Schwann cells. Myelitis with focal hemorrhage (*arrow*, **c**), one rare complication of Herpes zoster, results from transport of virus centrally rather than peripherally (paraffin) (**a**, 100×; **b**, 400×; **c**, 2×)

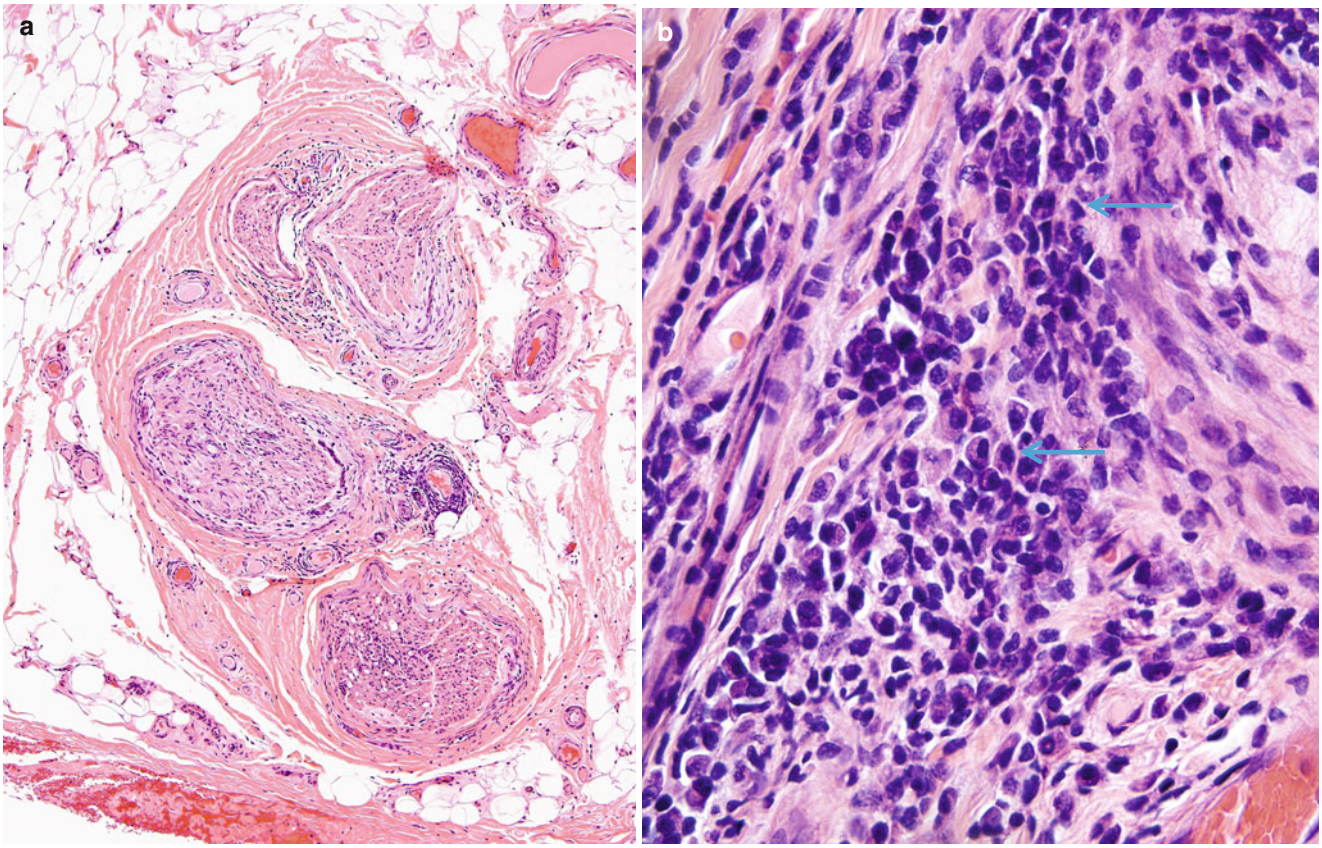

Fig. 11.7 Syphilitic neuritis: An uncommon cause of peripheral neuropathy, syphilis may occasionally result in inflammatory involvement of nerve (**a**) in which plasma cells predominate (*arrows*, **b**) (paraffin) (**a**, 200×; **b**, 1,000×)

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Leprous Neuropathy

12

Leprosy is the most common cause of neuropathy in the underdeveloped world (Misch et al. 2010). Notwithstanding a spectacular decrease in global prevalence since 1982, leprosy consistently remains a public health problem in 32 countries, mostly in Africa, Asia, and South America. The detection rate (figures from 2010) for leprosy is about 250,000 new cases being registered each year. The rare cases originating in North America are confined to certain regions in Louisiana, Texas, California, and Hawaii. With present immigration patterns in North America, clinicians can expect to see more patients with this disease, with the Philippines, Southeast Asia (Vietnam, Cambodia, and Laos), South America, and the Caribbean being the particularly high-risk regions of origin. In North America secondarily transmitted cases are exceedingly rare. Exceptionally, the disease is identified in patients who seem to have no reason to be so afflicted (Mastro et al. 1992). In the St. Michael's Hospital nerve biopsy experience, leprosy has been the fifth most common specific diagnosis, with the patients all emigrating from endemic areas in Southeast Asia or India.

Unless an individual is identified as being at high risk for leprosy, the diagnosis of leprous neuropathy depends on histological examination of the skin or nerve. The diagnosis is usually easily made by skin biopsy when cutaneous lesions are present. However, nerve biopsy is essential for the diagnosis of primary neuritic leprosy, which exhibits no skin lesions (vide infra). The identification of *Mycobacterium leprae* (ML) in a sample can be obtained by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) of the heat shock 65 gene (*hsp65*), which is ML specific (Martiniuk et al. 2007).

Mycobacterium leprae is a fastidious, acid-fast, Gram-positive, slightly curved bacillus measuring 1–8 μm in length and 0.2–0.5 μm in diameter (Carpenter and Miller 1964). Functionally the bacterium is nonmotile and aerobic and cannot form spores. It is an obligate intracellular parasite and survival is unfavorable outside the host cell. Reproduction of the organism occurs by binary fission at approximately 10–14 day intervals assuming optimal growth conditions of 27–30° Celsius (by comparison, doubling time for virulent strain of *M. tuberculosis* is about 20 h). The only confirmed methods of interindividual disease transmission and spread occur by aerosol droplets expelled through the upper airway mucosa and shedding of bacilli from skin lesions by infected patients having the lepromatous form of the disease, the organisms then entering contacts by the nasal mucosa (Ridley 1988; WHO 1988). The occurrence of acid-fast bacilli (AFB) in the skin (Figueredo and Desai 1949; Chatterjee et al. 1976) and nasal mucosa of apparently healthy subjects (Chacko et al. 1979) has been reported. ML can survive outside the human body for 45 days; this raises the possibility of indirect transmission of the bacillus from soil contamination (Lavania et al. 2008; Turankar et al. 2012). The local development of skin leprosy following the inoculation of bacilli from a contaminated tattoo needle is rare, but not exceptional (Ghorpade 2002). Only about 5 % of individuals exposed to ML will progress to an infected state (Newell 1966). The incubation period is between 2 months and 10 years (Ridley 1988). Vulnerability to ML appears to be selective, and there is evidence of an inherited component to this susceptibility (Shields et al. 1987; Misch et al. 2010). The circular genome sequence of *M. leprae* (TN strain, Tamil Nadu, India) was unraveled in 2001 by Cole's research group (Eiglmeier et al. 2001; Monot et al. 2005). Recent epidemiologic studies in Colombia have shown that multiple different strains of ML vary in distribution from Andean and Atlantic locales (Cardona-Castro et al. 2013).

While human beings are the principal reservoir of the infection, in the Americas the armadillo provides a reservoir. Consequently, in the latter part of the twentieth century, the nine-banded armadillo was introduced as an experimental animal model for human leprosy. The proportion of human cases of leprosy attributable to the armadillo remains unknown, but some are well documented (Hamilton et al. 2008; Sharma et al. 2013). Studies are in progress in the estimation that two-thirds of acquired human cases in southern USA have armadillo-derived ML in the lesions. In 1960, Shepard was able to reproducibly induce granulomata

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containing acid-fast bacilli in the footpads of mice after inoculation with bacteria harvested from nasal passages (22/22 "takes") or biopsies (12/16"takes") of human leprosy cases (Shepard 1960). The footpads of the athymic nude mouse are susceptible to ML infection serving as experimental models of leprosy and biological sources of abundant *M. leprae* (Alter et al. 2011).

In leprosy, neural involvement occurs early and invariably (Job 1989), the human Schwann cell and endoneurial endothelium having a particular affinity for the bacillus (Ridley 1988). This predilection of *M. leprae* for Schwann cells is likely determined by the organism selectively binding to the G domain of the laminin-alpha 2 chain, which is a unique component of the Schwann cell basal lamina (Rambukkana et al. 1997). Clinical leprosy lies in a spectrum between two extremes: tuberculoid and lepromatous according to the Ridley–Jopling classification, which is based on skin lesion type and bacterial load. At the tuberculoid pole a brisk cell-mediated immune reaction is generated against the organism, while those patients with lepromatous leprosy are anergic toward ML, have multiple lesions, and are pluribacillary. Most patients can be classified along this spectrum based on histology (vide infra). The disease does not remain static, but evolves spontaneously or in response to therapy. Workers refer to a transition toward the tuberculoid pole as upgrading and one toward the lepromatous pole as downgrading. While some researchers report spontaneous resolution of tuberculoid and indeterminate infections, spontaneous regression does not occur in lepromatous leprosy patients (Misch et al. 2010). Associated with the initiation of treatment types 1 and 2, immune-mediated reactions continue to be major complications. An upgrading of the host's immune response can result in acute neuritis, as can formation of immune complexes to ML antigens (vide infra: acute neuritis). Two types of immune-mediated reactions are observed in leprosy that affects around 30 % of patients with multibacillary disease during and after treatment (Rodrigues and Lockwood 2011). Type 1 or reversal reactions (RR) represent the sudden activation of a Th1 inflammatory response to ML antigens. RR often occurs after the initiation of treatment in patients at the borderline or toward the lepromatous pole of the leprosy spectrum (LL, BL, BT, or borderline [BB] category) and reflects a switch from a Th2-predominant cytokine response toward a Th1-predominant cytokine response (Britton and Lockwood 2004; Scollard et al. 2006). Risk factors for RR intrinsic to the host include age (Ranque et al. 2007) and some genetic variants, although the latter have not been intensively investigated. Type 2 reaction erythema nodosum leprosum (ENL) is an acute systemic inflammatory condition involving tumor necrosis factor (TNF), tissue infiltration by $CD4^+$ cells (Kahawita and Lockwood 2008), and deposition of immune complexes and complement (Britton and Lockwood 2004). ENL also occurs in LL or BL patients and is more commonly seen in patients with a high bacterial index (multibacillary disease). The host factors that regulate the immunoclinical phenotypes of ENL and RR are poorly understood (Sapkota et al. 2010).

12.1 Clinical Manifestations

There are no sensitive serological tests to routinely detect leprosy cases resulting from ML infection. Consequently, the current diagnosis relies on clinical observations combined with invasive procedures to confirm acid-fast bacilli in slit skin smears or immunopathological changes in biopsies of skin lesions or nerve for determining the presence of the disease and its characterization (Geluk 2013). Although the presence in sera of IgM antibodies against phenolic glycolipid-I (PGL-I) is positive in nearly all leprosy patients with high bacillary loads, most paucibacillary leprosy patients do not develop detectable antibodies against PGL-I. Furthermore, almost 50 % of individuals with positive anti-PGL-I IgM responses never develop leprosy and many of those who develop leprosy do not have PGL-I antibodies. Thus, the detection of asymptomatic *M. leprae* infection, allegedly a principal source of infection, remains elusive. The clinical hallmarks of leprosy are sensory loss and skin lesions. The specific manifestations depend on the host's ability to react against the organism. Reviews are provided by Sabin and co-workers (1993), Pearson and Ross (1975), and, most recently, de Freitas and Said (2013).

12.1.1 Lepromatous Leprosy

In lepromatous leprosy cell-mediated immunity to the organism is absent, bacteria disseminate hematogenously throughout the tissues. Microscopic examination of affected regions reveals a striking number of organisms located within a variety of cells, often with a minimal local inflammatory response. Disease progression occurs slowly and insidiously, with late and symmetrical neuropathic manifestations. Because *M. leprae* proliferates more effectively in a cool environment, nerves in certain characteristic areas are affected early: the pinnae, dorsum of the hand, elbow, dorsum of the foot, and anterolateral leg. This pattern may mimic a stocking-glove peripheral neuropathy, but the palms, soles, and skin between the digits remain unaffected. The initial manifestations are sensory because sensory nerves run a more superficial course, but as the disease progresses it affects mixed nerves, with the ulnar nerve at the elbow being particularly vulnerable. Infiltration with massive numbers of bacteria and inflammatory cells, along with fibroblast proliferation (Tzourio et al. 1992), results in

fusiform enlargement of superficial nerves, typically including the greater auricular, ulnar, and common peroneal. Reflexes are spared until the late stages, because the reflex arc involves deep nerves.

12.1.2 Tuberculoid Leprosy

In pure tuberculoid leprosy, the host's cell-mediated immune response is relatively well preserved and features a Th1 T-cell cytokine response, vigorous T-cell reaction to *M. leprae* antigen, and containment of the infection in well-formed granulomata (Scollard et al. 2006). Spread occurs locally along neurovascular structures (Ridley 1988). Symptomatic neuropathy develops early and is focal and asymmetric, and temperature dependence of involved sites is not prominent. The skin lesions show epithelioid cell granulomata that invade through cutaneous layers, and organisms are not detected. Small sensory nerves as well as larger mixed nerve trunks are damaged as they pass through local regions of inflammation. Thus, in addition to loss of sensation over the skin lesions, this form may present as a mononeuritis multiplex. Evolution of neurological deficits occurs more rapidly than in lepromatous leprosy. Nerve enlargement (Fig. 12.1a–c) takes place as a result not of massive infiltration with bacteria as in the lepromatous form, but of the exuberant inflammatory response.

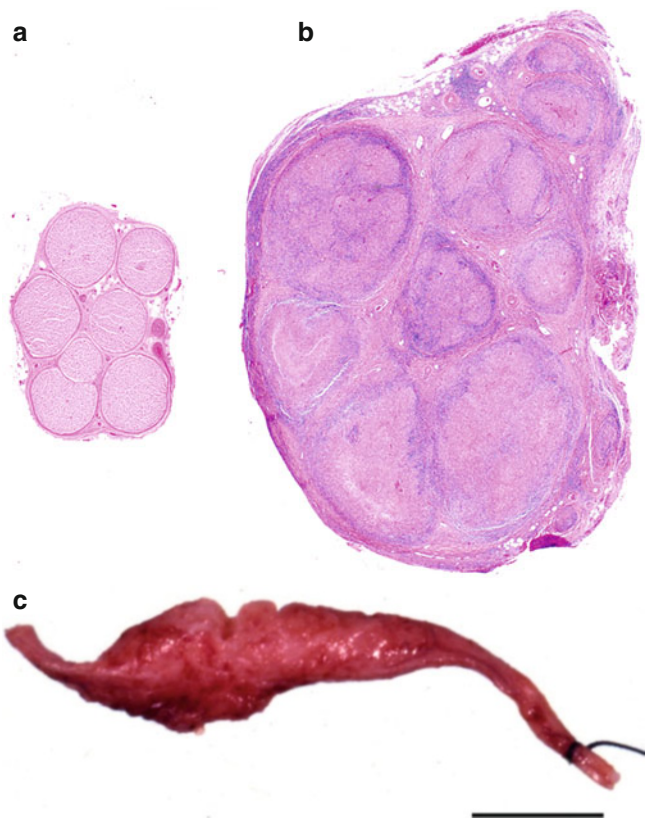

Fig. 12.1 Cross sections of sural nerve. Normal sural nerve (**a**) and tuberculoid leprosy (**b**). Note the massive enlargement of the nerve and presence of multiple granulomata with effacement of the microscopic anatomy. (**c**) Gross specimen of tuberculoid leprosy. (**a**, **b**: paraffin, H&E, 20×; **c**, surgical specimen, bar = 1 cm)

12.1.3 Borderline Leprosy

Borderline (BB, dimorphous) leprosy takes the middle ground between tuberculoid (TT) and lepromatous (LL), with intermediate clinical and pathological features. The lesions can show substantial numbers of organisms as well as a histiocytic infiltrate. Classification as borderline tuberculoid (BT) or borderline lepromatous (BL) depends on which side of the spectrum dominates. Clinically, the nerve involvement tends to favor low-temperature areas as in the lepromatous form, but brisk local reaction in these areas produces more severe nerve damage. If the host immune response is insufficient to prevent hematogenous dissemination, and a cell-mediated response still exists, a particularly devastating neuropathy may result.

12.1.4 Pure Neuritic Leprosy

In leprosy, damage to nerves can occur before, during, and after treatment and can result in disability and long-term disfigurement (Rodrigues and Lockwood 2011). A cohort study in Ethiopia showed that 47 % of 594 new cases already had established nerve function impairment at the time of diagnosis (Saunderson 2000). Pure neuritic leprosy refers to involvement of peripheral nerves in the absence of skin lesions and was seen in 4.3 % of 11,000 patients with leprous neuropathy in one series (Osuntokun 1980). The clinical picture does not predict the histological subtype (vide infra). Nerve involvement may range from a small superficial nerve twig with isolated skin patch of anesthesia to infection of several nerves. Widespread neuropathies may ensue with greater areas of sensory deficits, sometimes preceded by pruritus, paresthesia, and rarely pain. The nerves more likely to be involved are the superficial mixed nerves trunks of upper and lower extremities. This includes the ulnar nerve at the elbow, the median nerve above the carpal tunnel, the radial nerve at the spiral groove, the radial cutaneous nerve in the lower forearm, the peroneal nerve above the fibula head, the tibial nerve above the ankle, and the facial nerve at the region of the zygomatic bone (Ooi and Srinivasan 2004). The sensory nerves, such as the superficial peroneal, posterior auricular, and sural nerves, may also be involved.

12.1.5 Acute Neuritis

Although leprous neuropathy is usually chronic, leprosy reactions can cause an acute neuritis. In an upgrading reaction the host's immune status improves, and areas where the organism previously lay dormant become damaged by a cell-mediated inflammatory response. An acute neuritis is also seen with erythema nodosum leprosum, believed to be due to immune complex formation and deposition, particularly at sites where large numbers of organisms are located. Both types of acute neuritis tend to occur within the first year after initiation of therapy, but may develop spontaneously (Ridley 1988).

12.1.6 Treatment of Leprosy

It is important to establish histological type before initiating pharmacotherapy. The WHO recommends multidrug therapy with rifampicin and dapsone for paucibacillary disease and with rifampicin, dapsone, and clofazimine for patients with multibacillary leprosy. These regimens will effectively eradicate *M. leprae* in most patients (Rodrigues and Lockwood 2011). Vaccination with BCG protects some people from developing leprosy.

12.2 Pathology

12.2.1 General Considerations

The histological classification of leprosy is largely based on the examination of skin lesions. Ridley's text (1988) provides an excellent review. Of note is that the skin and nerve histology are often incongruous (Ridley and Ridley 1986). Generally, the viable bacterial load is higher in the nerve, probably because it is a relatively protected site: Organisms within Schwann cells tend not to incite an inflammatory response, and neural architecture hinders the influx of lymphocytes (Pearson and Ross 1975; Ridley 1988). For example, Nilsen et al. (1989) found that 8 of 11 patients with multibacillary leprosy in the nerve had paucibacillary leprosy in the skin. This observation might give rise to concern regarding the usefulness of skin biopsy in characterizing the nature of the disease and providing a rational approach to treatment. However, Ridley and Ridley (1986) point out that such data simply demonstrates the "protected site" behavior of the leprosy organism, that skin responses are more indicative of the general tissue response, and that consequently skin biopsy is still the best guide for classification and treatment of the patient.

Falsely negative histology in nerve biopsy for leprous neuropathy probably occurs (Jacob and Mathai 1988), but the frequency is unknown. Nerve biopsy is likely more sensitive than skin biopsy (Nilsen et al. 1989). To minimize the incidence of false negatives, it is best to biopsy clinically involved nerves rather than proceed with blind sural nerve biopsy. Some investigators use the index branch of the radial cutaneous nerve (Antia et al. 1975) and others use the radial cutaneous nerve itself (Nilsen et al. 1989). A biopsy report indicating the absence of bacilli implies that the specimen has been embedded in toto and that appropriately stained step sections through the entire tissue block have been examined.

Even when the diagnosis of leprosy is strongly suspected, atypical neuropathy can justify a nerve biopsy. In a study of primary neuritic leprosy from an endemic area (Jacob and Mathai 1988), only 38 of 77 patients with neuropathy had leprosy histologically; nineteen of 54 clinically enlarged nerves demonstrated no evidence of leprosy. False negatives were undoubtedly present, but a number of alternative diagnoses, including polyarteritis nodosa, hereditary neuropathy, and inflammatory demyelinating neuropathy, were made. These results suggest that in certain situations there may be a need for histological confirmation of the diagnosis even in endemic areas. Moreover, there are cases when the disease appears to be inactive based on examination of skin scrapings and subcutaneous nerves, yet the patient develops new neurological deficits. Nerve biopsy may show that the neural component of the disease is still active (Enna et al. 1970; Liu and Qiu 1984; Srinivasan et al. 1982), again reflecting the potential dissociation between cutaneous and neural disease activity. Leprous neuropathy was a surprise diagnosis on two of our surgical intraoperative consultation sections for peripheral nerve tumor.

12.2.2 Lepromatous Leprosy

12.2.2.1 Light Microscopy

In lepromatous leprosy, there is an uneven involvement of fascicles with relative preservation of the overall architecture (Fig. 12.2). Macrophages and Schwann cells filled with organisms and debris (foamy cells) appear in the epineurium, endoneurium, and perineurium, accompanied by a strikingly mild local reaction (Fig. 12.3a). In the perineurium, foamy macrophages infiltrate and separate individual layers, fibroblasts and perineurial cells proliferate, and collagen is deposited, producing a striking "onion skinning" of the nerve fascicles (arrow, Fig. 12.3b). Perineurial cells often demonstrate swelling and foamy changes and the bacillary macrophages predominating. Lymphocytes are not

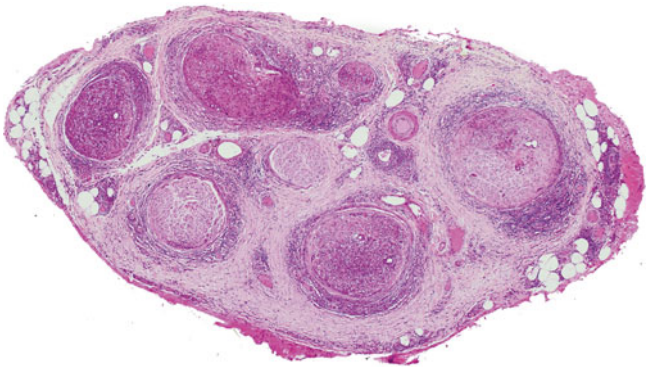

Fig. 12.2 Lepromatous leprosy. While the fascicular arrangement is preserved, there is prominent inflammation in the epineurium, perineurium, and endoneurium. Some fascicles are more involved than others. (Paraffin, HPS, 27×)

numerous in polar lepromatous (LL) leprosy, but may occur in subpolar forms. Perivascular cuffing is common but never reaches the level of true vasculitis, except in acute reactions (vide infra).

Bacilli abound (Fig. 12.4a) and are found within macrophages ("lepra cells") as intracellular "globi" containing dozens or even hundreds of bacteria as seen in H&E-stained nerve (Fig. 12.4b) and plastic sections (Fig. 12.4c). Even in heavily involved nerves, some fascicles may be relatively spared (arrow, Fig. 12.4d). Toluidine blue (in plastic resin-embedded sections) will demonstrate *M. leprae* in Schwann cells, perineurial cells, fibroblasts, endothelium, and infrequently axons (Fig. 12.5a–c). Fite (Fig. 12.5d) or auramine rhodamine stains for acid-fast organisms often demonstrate impressive numbers of organisms. Any cell type, but most often Schwann cells or macrophages, can show this foamy appearance, representing cellular reaction to the accumulation of living and dead organisms. Macrophages often display prominent perivascular localization. Giant cells, granulomata, and massive lymphocytic infiltration are not features of lepromatous leprosy.

Initially, despite the presence of innumerable organisms, the neural architecture and axon numbers are relatively preserved. Segmental myelin changes may predominate early on, with thinly myelinated and naked axons and even occasional onion bulbs (Job 1971). With progression of the disease, axonal degeneration becomes significant and involves both myelinated and unmyelinated fibers. Regenerating clusters may be present and can feature prominently in successfully treated patients (Jacobs et al. 1993). If untreated, most nerve fibers will ultimately degenerate.

With progression of the disease, collagen is laid down in ever increasing amounts, sometimes to the point where the nerve is entirely replaced with fibrous and hyaline material. Bacilli are often demonstrable, albeit in small numbers, even in the nerve that has been totally replaced by collagen and large vacuolated cells containing lipid and bacterial debris can be prominent long after treatment has finished (Fig. 12.6a–c).

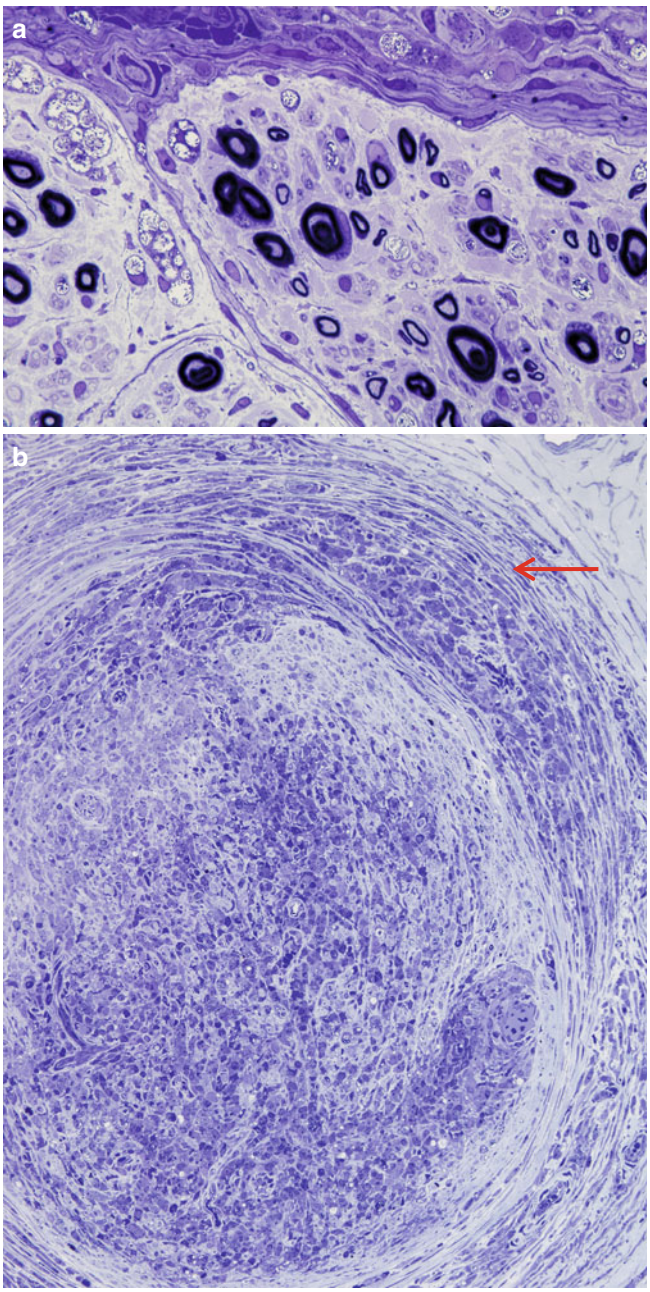

Fig. 12.3 Lepromatous leprosy. (**a**) Heavy intracellular bacterial load exists with no overt inflammatory response. (**b**) Note separation of perineurial leaves by inflammation (*arrow*) and heavy infiltration of the endoneurium by mononuclear cells. (1 μ toluidine blue-stained plastic sections; magnification, **a**, 1,000×; **b**)

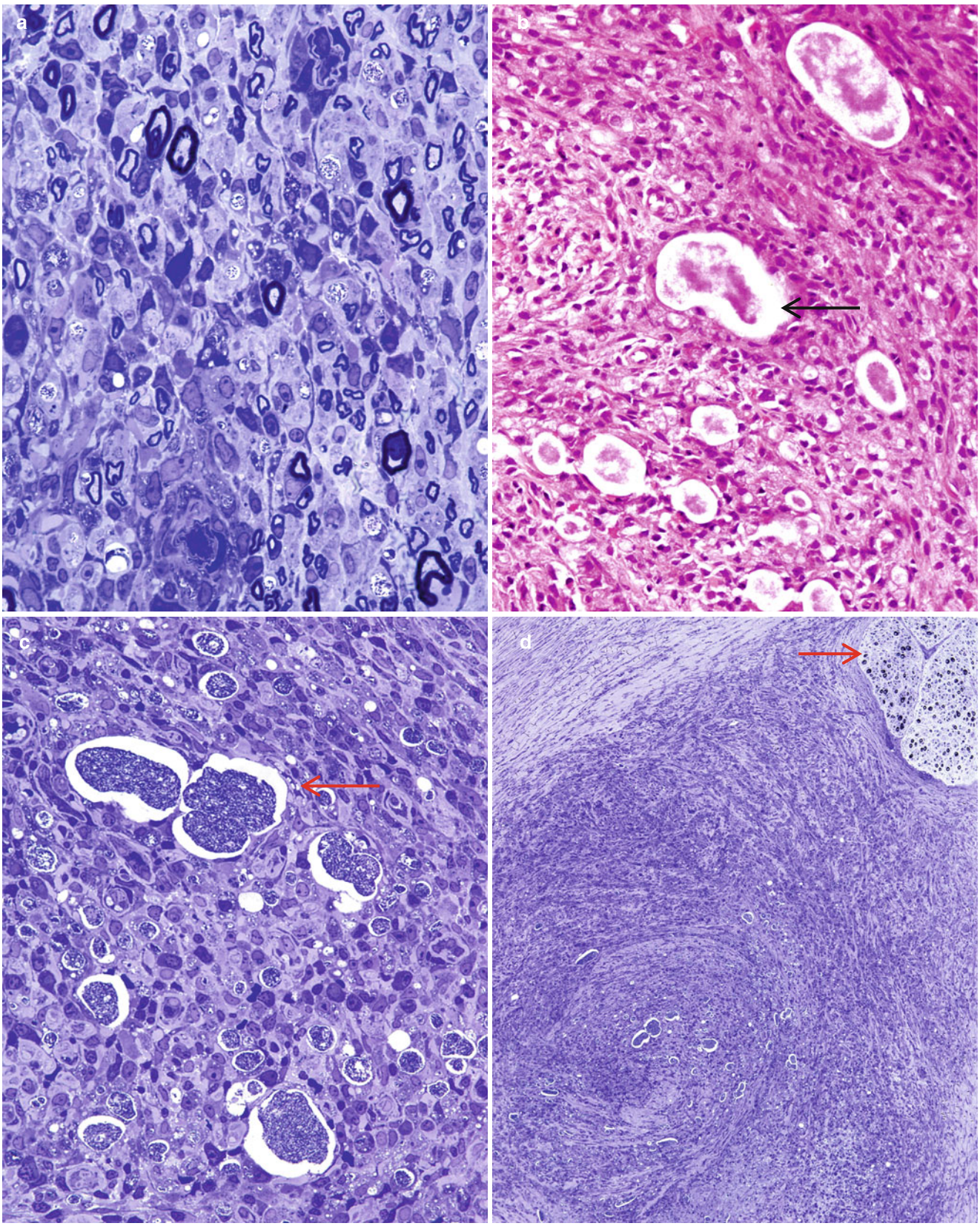

Fig. 12.4 Lepromatous leprosy. (**a**) Numerous organisms are noted in the endoneurium intermingled with intact MFs. (**b**, **c**) Organism-laden macrophages or globi (*arrows*) are seen by H&E (**b**) and toluidine blue stains (**c**), frequently without visible nuclei. The endoneurial damage here is more severe, with no recognizable myelinated axons. (**d**) Extensive endoneurial and epineurial inflammation largely spares an adjacent fascicle (*arrow*) (1 μ toluidine blue-stained plastic section; magnification, **a**, 400×; **b**, **c** 600×; **d**, 100×)

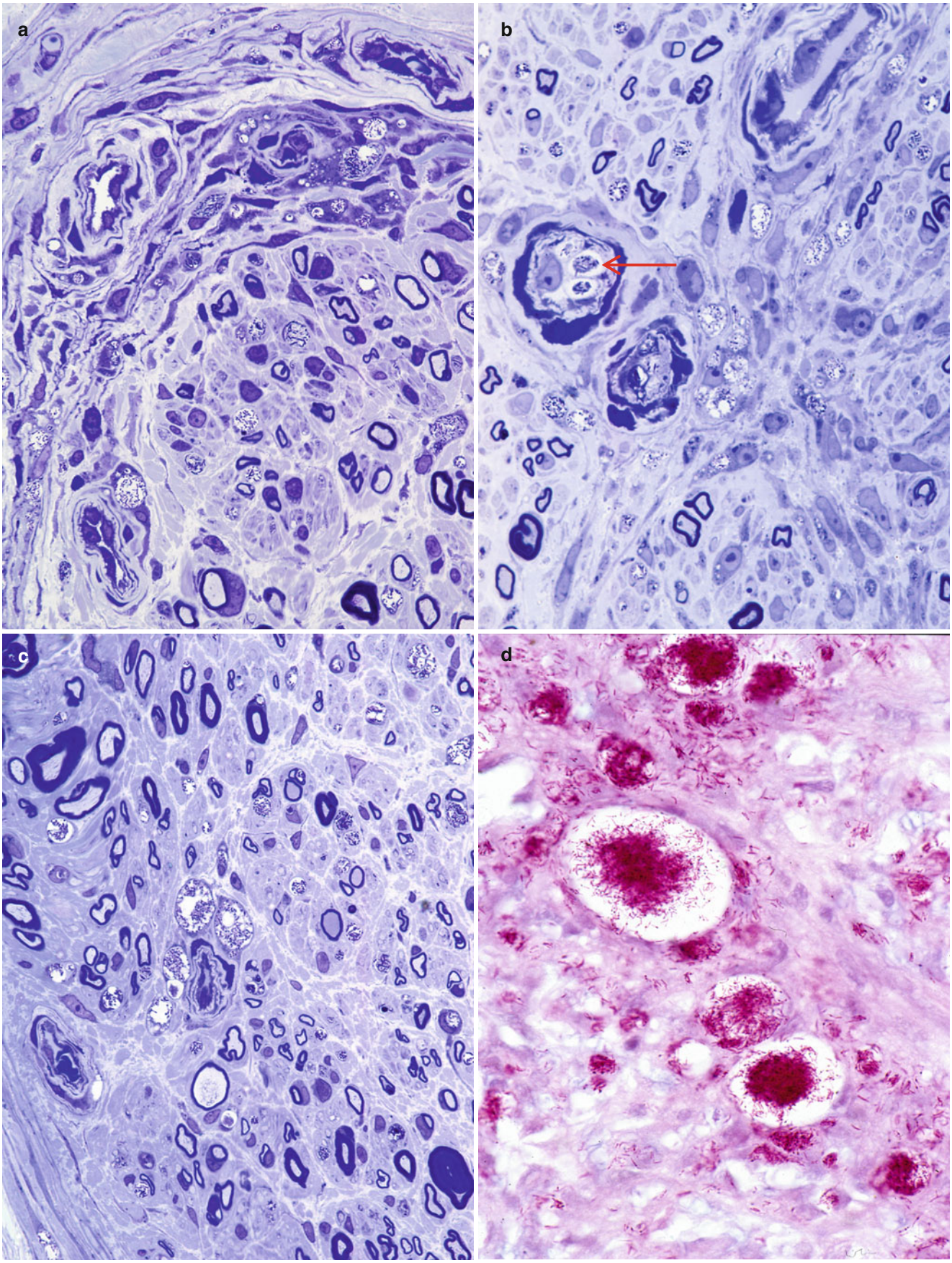

Fig. 12.5 Lepromatous leprosy. Numerous bacilli are demonstrated within perineurial (**a**) and endothelial (*arrow*, **b**) cells. Bacilli-laden macrophages collect adjacent to endoneurial vasculature (**c**). Fite stain for organisms demonstrates the extent of endoneurial bacilli (**d**) (**a**–**c**, 1 μ toluidine blue-stained plastic section; magnification, 1,000×; paraffin section, Fite stain, 1,000×)

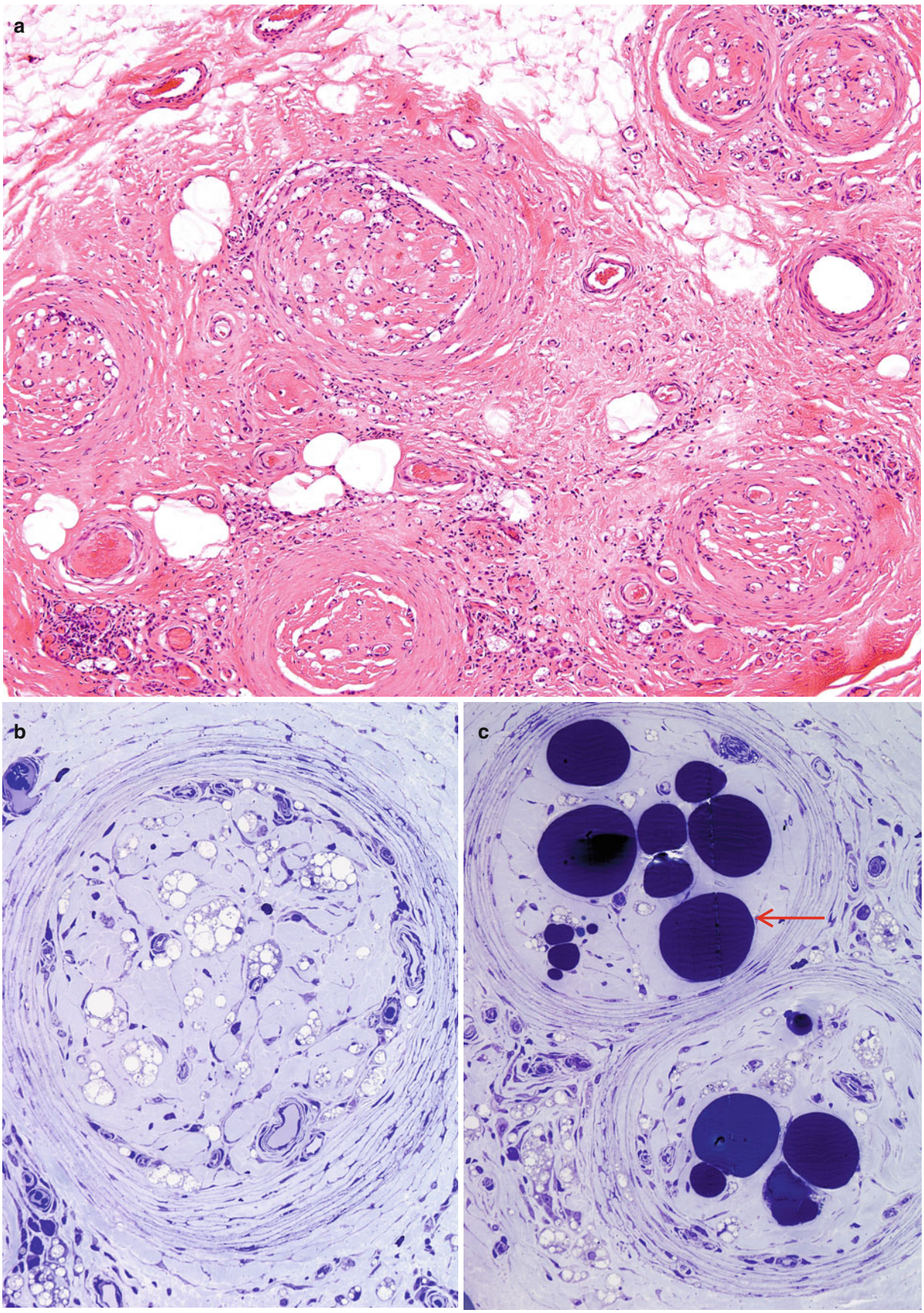

Fig. 12.6 Lepromatous leprosy, burnt out stage. No residual bacilli are present on Fite, fluorescent, or toluidine blue preparations. (**a**, **b**) The endoneurium is devoid of any nervous elements and contains foamy histiocytes and hyaline connective tissue. (**c**) The large osmiophilic globules (*arrow*) are adipocytes in the endoneurium. (**a**, Paraffin section, H&E, 100×; **b**, **c**, 1 μ toluidine blue-stained plastic section, 400×)

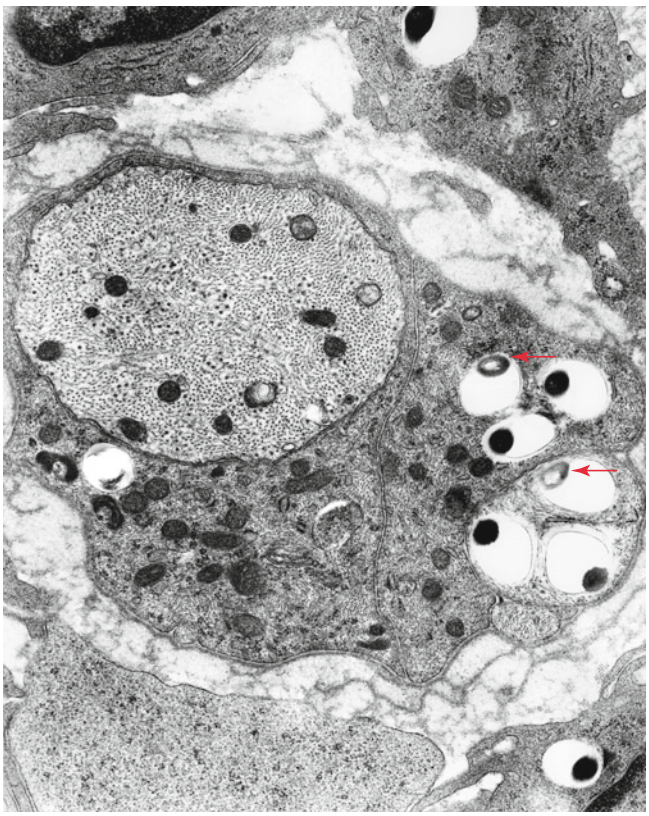

Fig. 12.7 Lepromatous leprosy. Segmental demyelination. Bacilli within Schwann cell exhibit characteristic electron-lucent halo. Some are degenerating (*arrows*), as shown by loss of uniform staining (26,000×)

12.2.2.2 Ultrastructural Examination

Electron microscopy shows the organisms as membrane-bound round or rod-shaped electron-dense structures surrounded by a clear halo (Fig. 12.7) (possibly partially composed of bacterial metabolites and/or denatured host cytoplasmic components) (Imaeda 1965; Job 1971). Degenerating bacilli show loss of osmiophilic staining (arrow, Fig. 12.7) or irregularities in their cell wall (arrows Fig. 12.8a, b) (Rees and Valentine 1964). Bacteria are easily found in macrophages and Schwann cells of unmyelinated fibers and less frequently in Schwann cells associated with myelinated fibers. Bacteria do not appear extracellularly (Figs. 12.8, 12.9a, b, and 12.10). Initially, the Schwann cell shows no reaction, but with increasing numbers of organisms, signs of degeneration appear. Macrophages can contain large numbers of bacilli, most in a degenerating state.

Diseased endothelial cells may appear swollen, with loss of integrity of cell junctions and other signs of damage to the blood–nerve barrier (fenestrae, increased pinocytotic activity). Multilayering and thickening of basement membrane around vessels is seen in all types of leprosy (Boddingius 1977) but is a nonspecific change seen in many chronic neuropathies.

When many nerve fibers still remain in the fascicle, ultrastructural examination readily demonstrates naked or thinly myelinated axons, suggesting primary demyelination (Fig. 12.7). Macrophage-mediated demyelination does not occur. Jacobs and colleagues (1987b) describe a circumferential

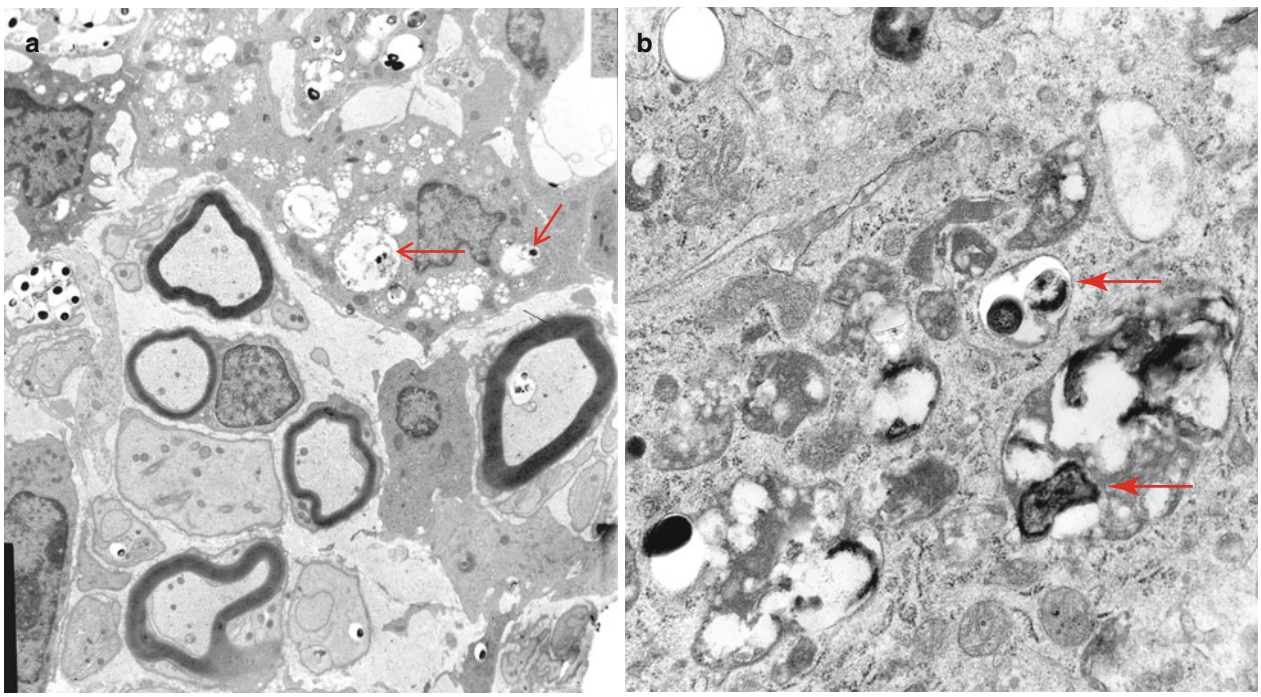

Fig. 12.8 Lepromatous leprosy. Bacilli in nonmyelinating Schwann cells and within macrophages. (**a**, **b**) Organisms within Schwann cells appear intact, whereas those in histiocytes (*arrows*) are degenerating. (**a**, 9,690×; **b**, 25,000×)

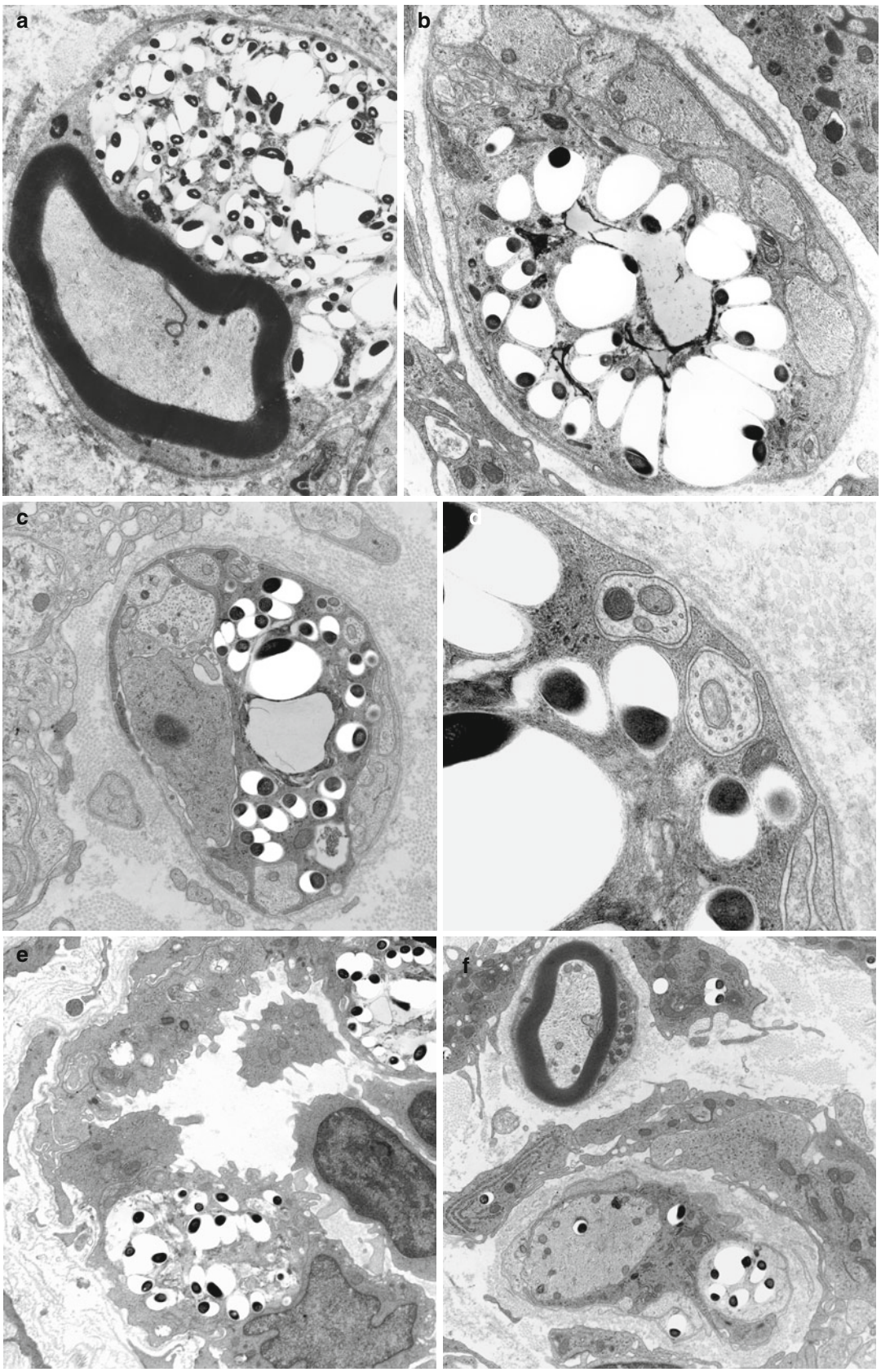

Fig. 12.9 Lepromatous leprosy. (**a**–**f**) Myelinating and nonmyelinating Schwann cells harboring numerous organisms. In (**a**), the Schwann cell remains viable and its myelin sheath is maintained. In (**b**) several unmyelinated axons are displaced but intact. (**c**, **d**) Unmyelinated intact axons are admixed with organisms. (**e**) Several endothelial cells contain numerous organisms. (**f**) Some demyelinated axons show intra-axonal bacilli (**a**, 6,144×; **b**, 8,520×; **c**, 15,000×; **d**, 50,000×; **e**, 10,000×; **f**, 10,000×)

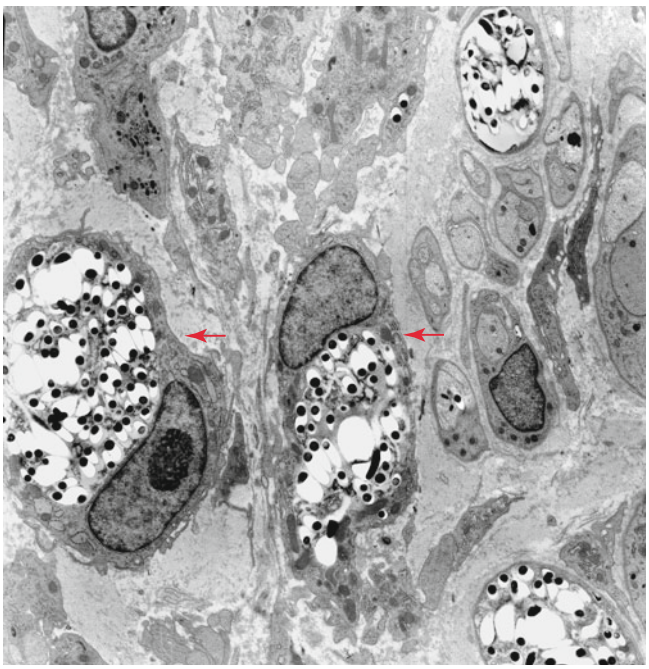

Fig. 12.10 Lepromatous leprosy. Several macrophages (*arrows*), identified by absence of basal lamina and presence of cytoplasmic processes, are seen laden with organisms, intermingled with surviving axons (7,350×)

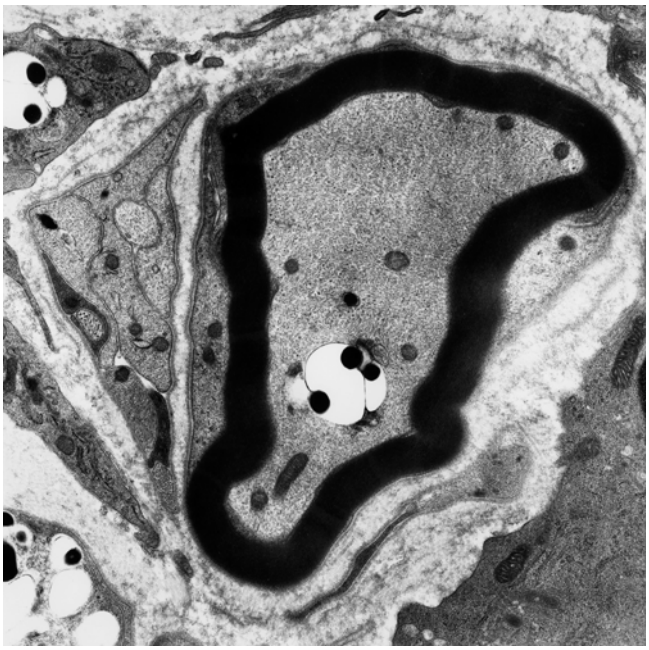

Fig. 12.11 Lepromatous leprosy. Intra-axonal organisms within myelinated axons are rare (17,000×)

separation of myelin lamellae at the intraperiod line, creating a markedly increased lamellar periodicity similar to widely spaced myelin. However, these myelin lamellae are much more irregularly separated than those of widely spaced myelin. Vital and Vallat illustrate similar pictures without commenting on the myelin abnormality (Vital and Vallat 1987, Figs. 168 and 169). We have never observed this alteration.

Axonal pathology develops at a later stage than Schwann cell and myelin changes. In rare cases the axoplasm of myelinated axons contains organisms (Fig. 12.11), and thus intact axons surrounded by heavily parasitized degenerating Schwann cells, naked axons, and segmental demyelination may be seen (Fig. 12.7). Rarely, organisms are identified in the axoplasm (Figs. 12.9f and 12.11). Unmyelinated axon loss may feature prominently, even early in the disease (Shetty et al. 1988).

12.2.3 Tuberculoid Leprosy

The hallmark of peripheral nerve involvement in tuberculoid leprosy is an exuberant inflammatory reaction that severely damages the microscopic anatomy. In biopsy of skin lesions, dermal nerves are often afflicted by a process so destructive that fascicular pattern is obliterated and recognition of peripheral nerve components is possible only with perineurial and Schwann cell immunomarkers. The disease is often multifocal, and damaged fascicles or parts of fascicles lie adjacent to normal ones. The architecture of subcutaneous nerve trunks is also barely recognizable (Fig. 12.12a).

The perineurium is markedly thickened, often fused with the epineurium into a thick fibrotic mass, and infiltrated by inflammatory cells and small vascular channels. Pearson and Weddell (1975) emphasize the distinction between the fibrotic organism-free lymphocyte-infiltrated perineurium of TT or BT leprosy and the thick, cytoplasmic perineurial widening seen in LL or BL leprosy, which displays infiltration by histiocytes and plasma cells, an obvious bacillary load, and a variable number of lymphocytes.

Granulomata consisting of epithelioid histiocytes, multinucleated giant cells, and variable amounts of lymphocytes and plasma cells (Fig. 12.12a–c) may replace the endoneurial compartment (Fig. 12.12b). Caseation is often seen (Fig. 12.12c) and axon loss may be extensive (Fig. 12.12d). The multinucleate cells may be of the Langhans type or the less distinctive foreign body type. Although foreign body giant cells usually predominate, Ridley has indicated that classifying the lesion as TT requires the presence of some Langhans-type giant cells (Ridley 1988). Organisms are not detectable (Fig. 12.12e) in polar tuberculoid leprosy; making this determination requires a thorough examination of serial sections through the tissue block. Axonal damage is prominent in tuberculoid leprosy, presumably as a bystander effect of the aggressive tissue reaction. With time the lesion becomes less active, leaving behind a hyalinized and fibrotic fascicle with few or no intact axons.

Ultrastructural features of epithelioid histiocytes suggest that these cells are not phagocytic: They contain few lysosomes and little debris. Rather, proliferation of rough endoplasmic reticulum and Golgi membranes and accumulation of mitochondria occur, giving the appearance of a cell involved in secretory activity (Fig. 12.13).

12.2.4 Borderline Leprosy

True borderline leprosy is uncommon, as the disease displays a tendency to drift toward the poles: tuberculoid if treated, lepromatous otherwise. Midway between these extremes, the genuine borderline lesion (BB) is characterized by diffusely spread epithelioid histiocytes, no foamy cells or giant cells, and easily demonstrable organisms. In the presence of occasional giant cells, naked granulomata, or lymphocytes surrounding foci of epithelioid histiocytes, the lesion would be classified as borderline tuberculoid (BT) (Fig. 12.14a, b). On the other hand, the absence of epithelioid histiocytes, the presence of occasional foamy cells, numerous organisms, and diffuse lymphocyte infiltration favor the diagnosis of borderline lepromatous (BL) leprosy. While some pathologists set out classification of lesions in great detail (Ridley 1988), in practical terms the distinction blurs because incongruous features are present in the same section, or histology may differ from one area to another of the same specimen (Fig. 12.15a, b). We find organism numbers (Fig. 12.14b) to be the most reliable way of subdividing the borderline leprosies. Absence of organisms is only compatible with TT or BT leprosy.

In borderline leprosy, the perineurium may bear the brunt of the disease, with perineurial splitting, edema, thickening,

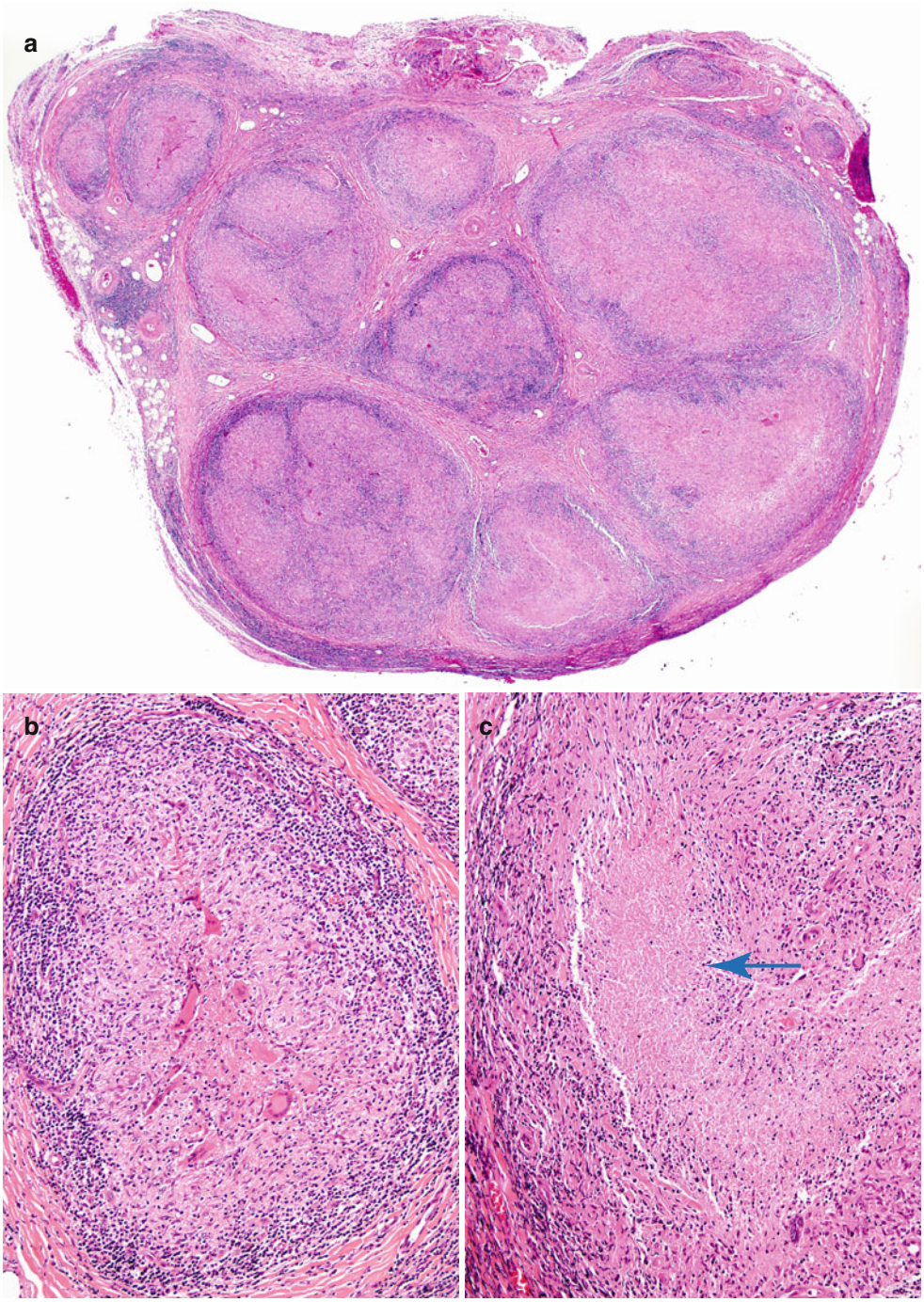

Fig. 12.12 Tuberculoid leprosy. (**a**) The cross-sectional configuration of the sural nerve is overrun by granulomata, although basic outlines of fascicles are discernible. (**b**) This fascicle is entirely replaced by a granuloma. (**c**) Caseation necrosis (*arrow*) is present at the center of this fascicle. (**d**) The destruction of nearly all myelinated axons is striking. (**e**) No organisms are visible in a section stained with the Fite method for organisms. (**a**–**c**, **e** Paraffin; **a**, 20×; **b**; **d**, 1 micron thick section of plastic-embedded nerve, 400×; (**e**) paraffin, Fite stain)

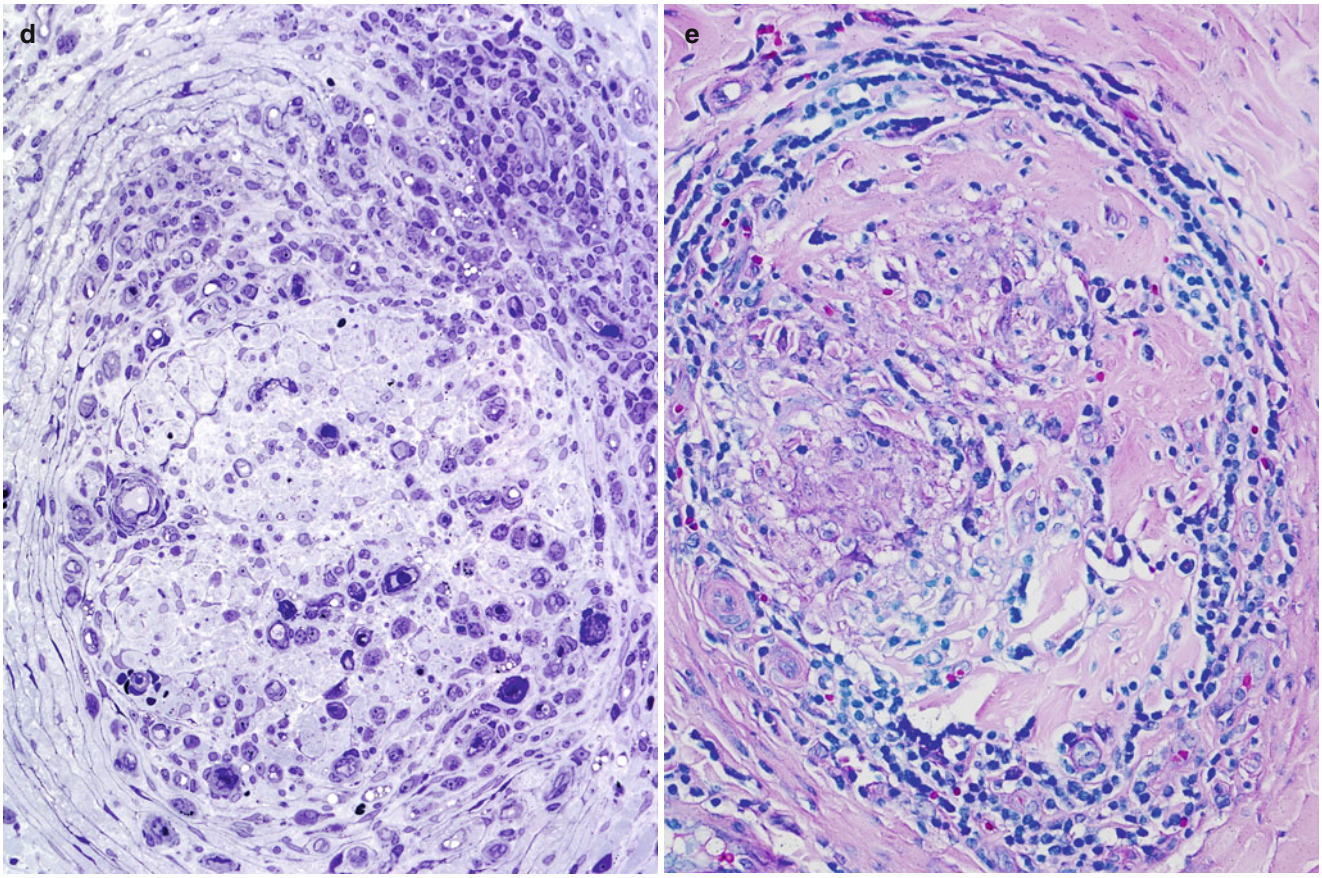

Fig. 12.12 (continued)

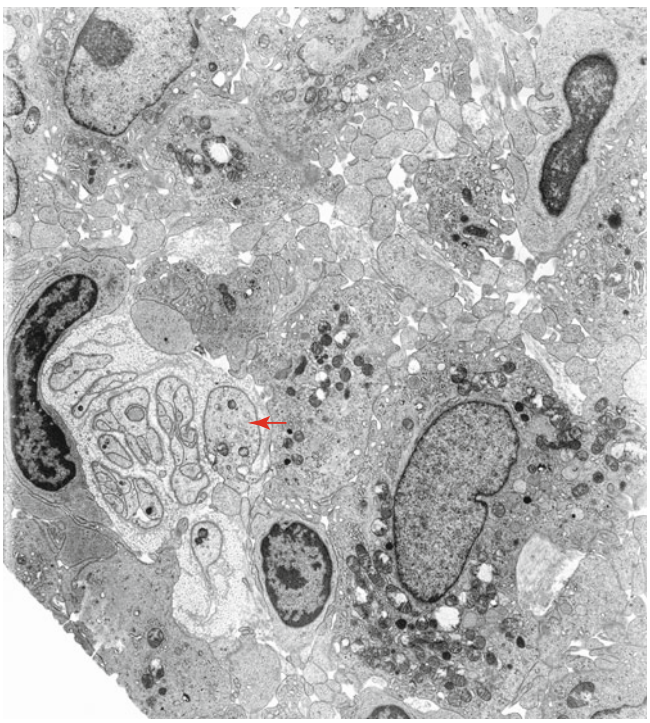

Fig. 12.13 Tuberculoid leprosy. Epithelioid histiocytes filled with organelles and endoplasmic reticulum are molded against one another. Note the residual denervated band and a single unmyelinated axon (*arrow*) (5,520×)

and infiltration with inflammatory cells and histiocytes occurring (Weddell and Pearson 1975). When present, the epithelioid response becomes more marked near the perineurial margin of the nerve fascicle. Myelin and Schwann cell changes appear earlier than axonal changes, as in pure lepromatous leprosy (Finlayson et al. 1974).

Pearson and Weddell (1975) described a peculiar tendency for perineurial cells to "invade" and subdivide the adjacent endoneurium into multiple microfascicles circumscribed by perineurial cells (Fig. 7.12a, b). This invasion was initially described in a few cases of borderline range leprosy, but Vallat et al. (1991) observed similar changes in various types of leprous neuritis. Perineurial cells respond in similar fashion to other insults, and this response is nonspecific.

12.2.5 Indeterminate Leprosy

Indeterminate leprosy represents early disease that is not amenable to be classified. Characteristics include lymphocytic infiltration, often perivascular, without epithelioid cells or foamy macrophages. One may detect only subperineurial edema, slight perineurial thickening, and perhaps a subtle increase in the numbers of endoneurial macrophages and

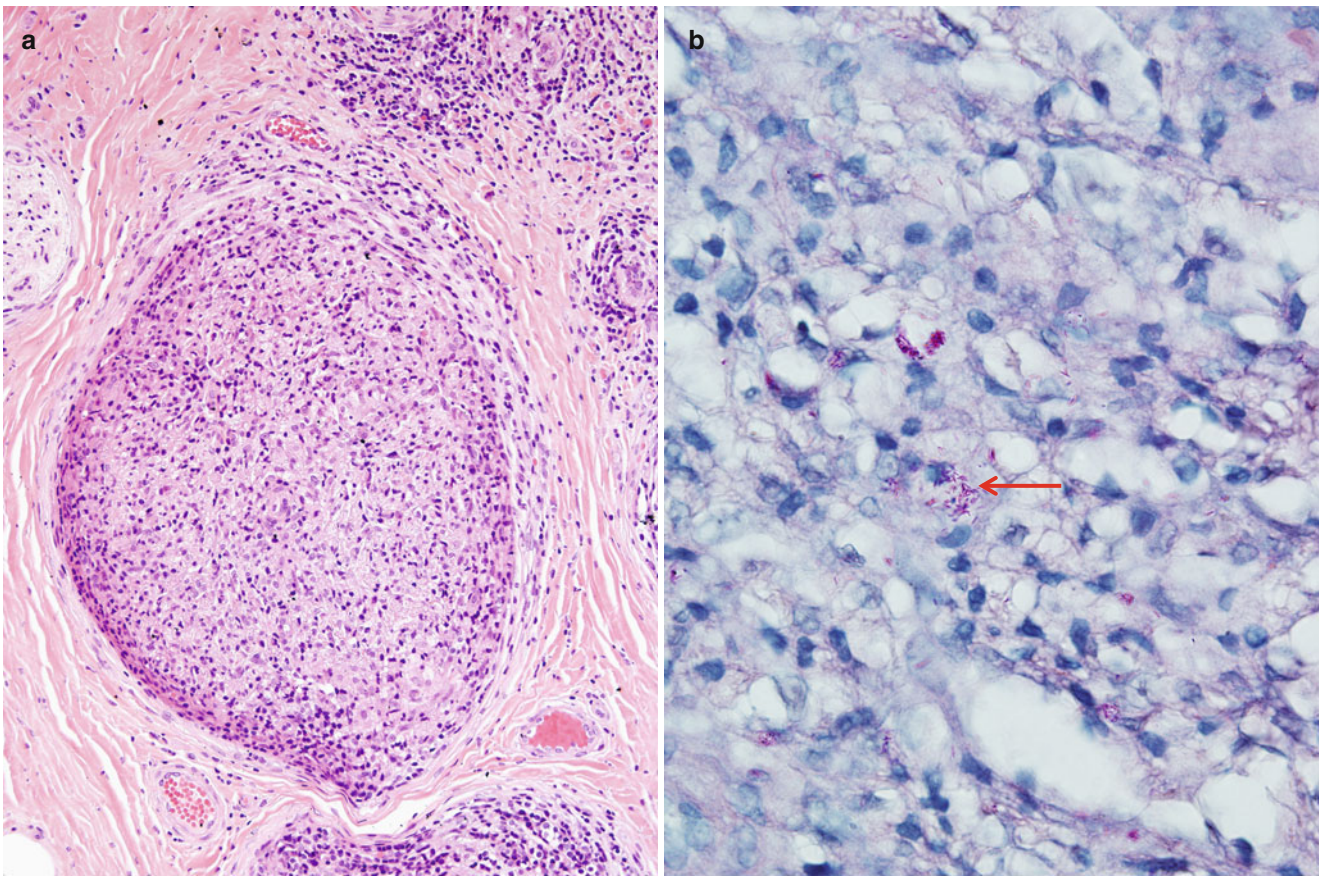

Fig. 12.14 Borderline tuberculoid leprosy. (**a**) One fascicle is shown which shows inflammatory small cell infiltration and scattered epithelioid histiocytes. (**b**) Special stains for organisms show a modest number (*arrow*). (**a**, H&E 400×; **b**, Fite stain, 1,000×)

fibroblasts (Shetty et al. 1988). Acid-fast stains will show few or no organisms, but *M. leprae* may also be unveiled in the absence of any inflammatory infiltrate (Kaur et al. 1991). In time this lesion develops into a classifiable lesion, although persistence in an indeterminate form may last for years (Ridley 1988).

12.2.6 Primary Neuritic Leprosy

In two studies of patients with primary neuritic leprosy, a total of 77 cutaneous nerves were biopsied (Jacob and Mathai 1988; Kaur et al. 1991). The pathology was consistent with LL/BL in 24, TT/BT in 20, and indeterminate or borderline in 17. The case illustrated in Fig. 12.15a–d represents a borderline tuberculoid response with infiltrates of small lymphocytes and developing granulomata (arrows, Fig. 12.15b–d). Some lesions could not be classified. Findings on physical examination did not predict the histological type.

12.2.7 Acute Neuritis

In acute neuritis associated with erythema nodosum leprosum (ENL, Fig. 12.16), the nerve becomes infiltrated with granulocytes, with the formation of intraneural microabscesses (Job 1971). The inflammatory response damages myelin and axons alike. True vasculitis is not a feature of the typical sural nerve biopsy in leprosy, but in ENL perivascular inflammation and true vasculitis occur, with transmural vessel wall inflammatory infiltrates and fibrinoid necrosis (Fig. 12.16). This vasculitis is assumed to be on the basis of a hypersensitivity reaction, that is, an immune response to large amounts of leprous antigen and deposition of immune complexes resembling an Arthus reaction.

In an upgrading reaction (increased host immune response, usually associated with treatment of borderline or lepromatous leprosy), lymphocytes and edema fluid infiltrate the affected nerve segment, causing a sudden onset of focal palsy, usually reversible if treated rapidly with corticosteroids (Weddell and Pearson 1975).

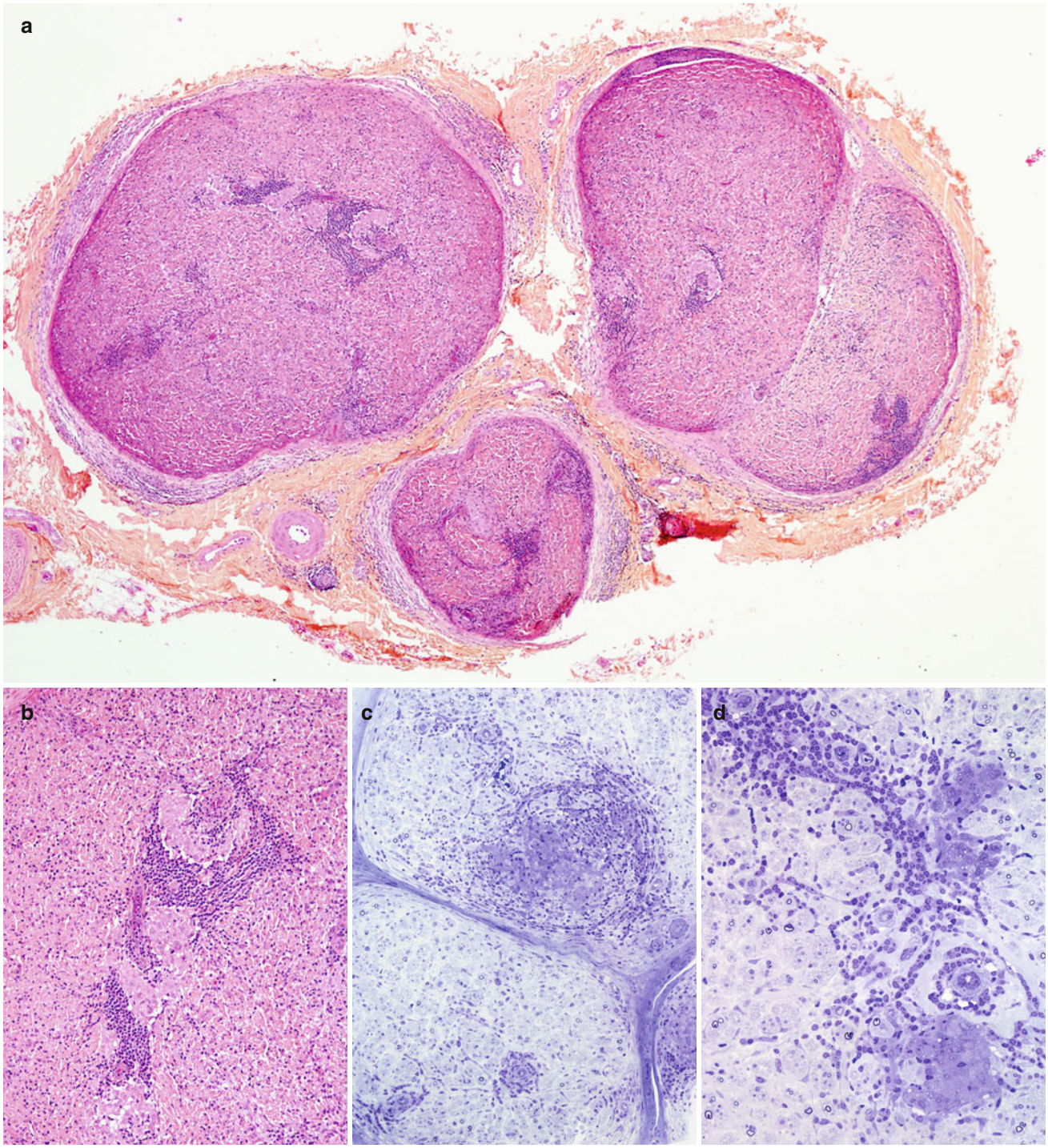

Fig. 12.15 Pure neuritic leprosy, borderline tuberculoid type. A 20-year-old Vietnamese immigrant to Canada had a long history of patchy dermoanesthesia in all limbs and groin area and a palpable right ulnar nerve. No skin lesions were present. (**a**) Sural nerve biopsy disclosed preservation of fascicular anatomy, endoneurial epithelioid granulomata, and perivascular chronic inflammation. No giant cells or organisms are demonstrable. In (**b**), ill-defined granulomata are arising perivascularly. Well-defined microgranulomata in the endoneurium are also shown on cross section (**c**, **d**) (**a**, paraffin, HPS 40×; **b**, paraffin, HPS, 200×; **c**, 1 μ toluidine blue-stained plastic section, 200×; **d**, 1 μ thick plastic section, 400×)

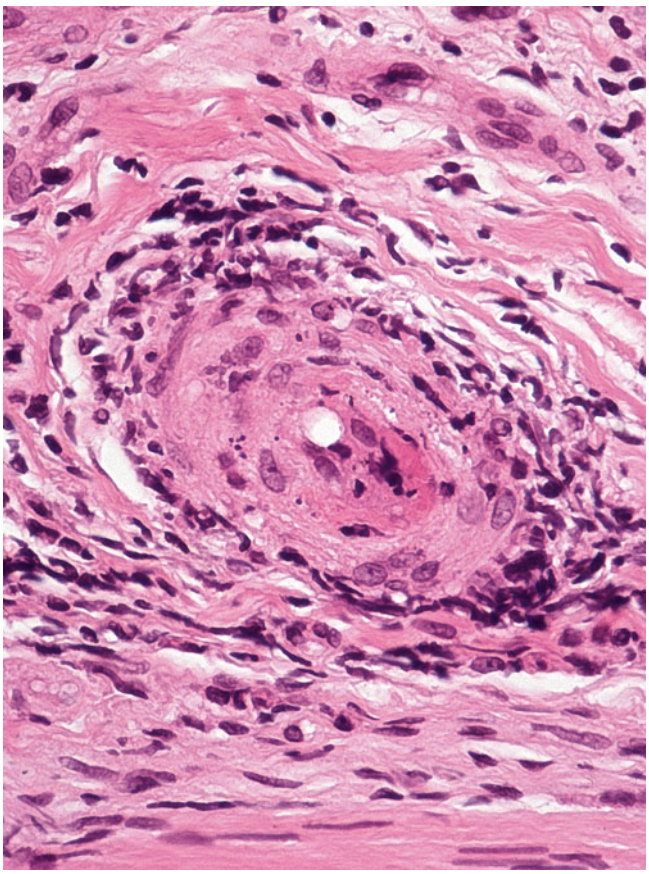

Fig. 12.16 Erythema nodosum leprosum. This lesion typically shows angionecrosis unlike more typical forms of leprous neuropathy (H&E, 600×)

12.3 Pathogenesis

Only a small proportion of people exposed to ML develop clinical leprosy. Susceptibility to the organism has been associated with various socioeconomic factors, host immune state (genetic and acquired), and age at exposure. Patients who develop lepromatous leprosy have a defect in cell-mediated immunity that is specific to the leprosy organism. They demonstrate highly selective anergy to *M. leprae* (negative lepromin test), with normal skin response even to related mycobacteria. The humoral immune response is intact (Cohn and Kaplan 1991). Blood monocytes from these patients become normally activated in response to lymphokines (Kaplan 1993) and have normal ability to present antigens and eliminate organisms (Ridley 1988). The selective defect appears to be in T-helper lymphocytes, which fail to proliferate or release lymphokines in response to *M. leprae* antigens (Cohn and Kaplan 1991). In the tuberculoid pole of leprosy, there is predominance of the HLA-DR2 and HLA-DR3 phenotypes, a Th1 cytokine response, vigorous T-cell responses to ML antigen, and containment of the infection in well-formed granulomata. Lepromatous leprosy is characterized by a predominance of the HLA-DQ1 phenotype, by a Th2 immune response and poor containment of the bacillus.

12.3.1 Mechanism of Organism Spread

Whether lepromatous or tuberculoid, the mechanism of initial bacillary entry and dissemination through peripheral nerve has remained controversial, particularly in the relative role played by axons and Schwann cells. The leading hypothesis involves primary distal cutaneous nerve infestation (Boddingius 1981), perhaps at skin lesions, where the organism can pass through a weakly protected nerve terminal. Either Schwann cells (Mukherjee and Antia 1986) or axons (Boddingius 1981) may be primarily infected, but in vitro studies strongly support the former. Proximal neural movement of the organism follows, most likely through cell to cell infection and spread, although axoplasmic transport has been proposed (Boddingius 1981). Bacillated macrophages may carry the organisms proximally by traveling in the subperineurial space (Boddingius 1977; Finlayson et al. 1974).

In lepromatous leprosy the scattered location of bacterial colonies (Job and Desikan 1968; Liu and Qiu 1984), as well as the observation of bacilli in endothelial cells (Boddingius 1977; Dastur et al. 1973; Mukherjee 1990), suggests that hematogenous spread of the organism and entry through endoneurial capillaries is the main mechanism of nerve involvement. After entering the peripheral nerve, the organism becomes relatively inaccessible to the immune system.

12.3.2 Mechanism of Nerve Injury

In tuberculoid leprosy, the destructive effects of TNF-alpha generated in the inflammatory response cause axonal damage (Lockwood and Saunderson 2012). Intrafascicular ischemia due to a marked increase in endoneurial pressure may be an important factor (Job 1981).

In lepromatous leprosy the mechanism of nerve damage is more complex. Clinically, massively enlarged and colonized nerves may retain function. Electrical studies often demonstrate conduction slowing across involved nerve segments in lepromatous leprosy even in the absence of sensory loss (Tzourio et al. 1992), suggesting that demyelination occurs early. However, the loss of sweating and pain sensation before light touch sensation suggests that unmyelinated axons are affected first (Antia et al. 1975), and nerve conduction studies

do not assess these fibers. Histological data has been conflicting, with some authors reporting evidence of early primary demyelination, degeneration of unmyelinated axons, or axonal atrophy with secondary demyelination (Antia et al. 1975; Dastur et al. 1973; Jacobs et al. 1987a; Shetty et al. 1988; Tzourio et al. 1992). Schwann cells are much more heavily colonized and show pathological alterations earlier than axons (Boddingius 1981; Finlayson et al. 1974; Job 1971); however, axonal changes with secondary demyelination may also be present early in the disease (Jacobs et al. 1987a; Shetty et al. 1988).

Mechanisms of Schwann cell damage are also controversial in lepromatous leprosy. Some have assumed that the bacillary load directly causes Schwann cell injury (Job 1981; Finlayson et al. 1974). In cell culture, a marked alteration in Schwann cell DNA synthesis is associated with bacillary infestation (Mukherjee and Antia 1986). The early demyelination is hard to reconcile with the observation that the number of bacilli within Schwann cells of myelinated fibers is small in comparison to that seen in Schwann cells from unmyelinated axons (Boddingius 1981). Possibly myelinotoxic macrophage products (Said and Hontebeyrie-Joskowicz 1992) bring about demyelination and such secreted substances may also stimulate the fibrosis inevitably seen in long-standing disease.

Authors observing subperineurial edema and subtle microvascular changes before organisms are demonstrable in the nerve suggest that humoral factors may contribute to the pathogenesis of neuropathy in lepromatous leprosy (Shetty et al. 1988; Shetty and Antia 1989). Boddingius (1981) indicates that early demyelination may be due to an immune attack on Schwann cells or myelin or perhaps changes in the local environment. In support of the latter are the observations that very early in the disease, at a time when nerves might be otherwise normal, endothelial cells show loss of tight junctions and multilayering of pericapillary basement membrane (Boddingius 1977). These changes may cause a defect in diffusion of oxygen and other nutrients into the endoneurium (Boddingius 1977). As the disease progresses, perineurial thickening and fibrosis combined with intrafascicular cell proliferation and edema may raise the endoneurial pressure to damaging levels (Boddingius 1981, 1984; Job 1981; Myers et al. 1986). Organisms are unlikely to cause nerve fiber loss directly since they are infrequently found intra-axonally.

Endothelial cells can be parasitized and perivascular mononuclear infiltrates are common; however, vasculitis is not a feature of nerve involvement in leprosy, except in acute neuritis. Little reason exists to implicate focal ischemia in the pathogenesis of leprous neuropathy, except in cases where edema and infiltration cause compression within a tight fibrotic perineurium, either acutely (upgrading reaction, Weddell and Pearson 1975) or chronically as discussed above.

12.4 Differential Diagnosis

In tuberculoid leprosy no organisms are detectable, and other granulomatous conditions involving the nerve, especially sarcoidosis (which can present with skin lesions and neuropathy), may be entertained as possibilities. In the absence of demonstrable mycobacteria, there is no certain histological means of distinguishing the two. PCR detection of *M. leprae* targeting the *M. leprae*-specific 16S ribosomal RNA is specific and more sensitive than conventional methods and can contribute to early and accurate diagnosis of leprosy (DeWit et al. 1991; Nishimura et al. 1994; Turankar et al. 2012). Other mycobacterial organisms do not cause neuropathy. We are unaware of any adequately documented cases of neuropathy in tuberculosis in which the organism itself has been directly implicated. Rare cases of *Mycobacterium avium-intracellulare* in the nerve have been reported in the setting of HIV infection, but the detail provided is scanty (Cornblath et al. 1993; Wrzolek et al. 1989). Pavie et al. (2010) report a case of severe peripheral neuropathy following HAART initiation in an HIV-infected patient with leprosy. Leprosy is now considered a new unmasked infectious disease associated with the immune reconstitution inflammatory syndrome (IRIS).

The perineurial involvement in leprosy has led to differential diagnostic consideration of idiopathic perineuritis and other conditions which mimic it (Chap. 10). In lepromatous leprosy the perineurial thickening is dramatic, and organisms are readily demonstrated, thus resolving the issue. In tuberculoid leprosy no organisms are likely to be seen, but a nonspecific fibrotic thickening and multilayering can be present, with lymphocytes and epithelioid cell infiltrating the perineurial leaves. In such a case the presence of obvious endoneurial granulomata makes the diagnosis of tuberculoid leprosy almost certain, although sarcoidosis cannot be absolutely excluded without the demonstration of organisms. Lymphomatoid granulomatosis involving the peripheral nerve may superficially resemble tuberculoid leprosy (Chap. 13), but the immunophenotype, pleomorphism, and angiocentricity of the infiltrating cells allow differentiation of these two entities. Similar considerations apply to infiltrative lymphoma where perineurial involvement can be prominent.

In indeterminate, or very early, leprosy the main abnormalities may be subperineurial edema and subtle lymphocytic infiltration, with no organisms (Shetty et al. 1988). These are nonspecific findings, and if leprosy is not suspected clinically and a careful search for bacilli performed, the diagnosis may be missed entirely. This situation is more likely to be found in a dermal nerve twig rather than in a subcutaneous nerve.

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Vasculitic Neuropathy

13

Vasculitis of the peripheral nerve (VPN) can occur as an isolated process or more commonly as the manifestation, initial or late, of a systemic disease (recently reviewed Vrancken and Said 2013; Collins et al. 2013). Inflammation affects endoneurial and epineurial microvessels, arterioles, and venules; thrombosis and necrosis are often present resulting in ischemia of nerve with varying degrees of axonal degeneration. The vasculitic processes detected in routine nerve biopsy specimens (sural, peroneal, median) occur in medium- and small-sized vessels as the diameter of these structures range from 10 to 350 μm.

With some exceptions the vasculitides found in nerve biopsies (Vital et al. 2006) in many diseases and syndromes are histologically similar; distinction is usually dependent on clinical criteria or biopsy at other sites. In classifying peripheral nerve vasculitis, it is helpful to separate the infectious etiologies and subdivide the remainder into those diseases in which inflammation selectively affects vessels in nerve and those in which vascular involvement is part of a systemic process, acknowledging that sometimes this separation is difficult to establish. Polyarteritis nodosa (PAN), ANCA-related microscopic polyangiitis, Churg–Strauss angiitis (CSA), Wegener granulomatosis (WG), and rheumatoid vasculitis account for about 50 % of cases with systemic vasculitis neuropathy. Attempts to classify vasculopathic diseases, on the basis of the size and type of vessels involved or through putative pathogenic mechanisms, have been unsatisfying (Collins et al. 2010a). Several schemes are in use and we will draw, for the discussion in this chapter, from the nomenclature of the Chapel Hill Consensus Conference (Jennette et al. 2013) and from the classifications proposed by Vrancken and Said (2013) and Gwathmey et al. (2014). We will review the most important types of peripheral nerve vasculitis, followed by a general discussion of the pathological spectrum of peripheral nerve vasculitis and the differential diagnosis. Table 13.1 classifies vasculitis into isolated

Table 13.1 Peripheral neuropathy due to vasculitis

Isolated PNS vasculitis
Primary systemic vasculitis
Polyarteritis nodosa (PAN)
Churg–Strauss syndrome
Microscopic polyangiitis
Wegener granulomatosis
Essential mixed cryoglobulinemia
Behçet's disease
Henoch–Schönlein purpura
Collagen vascular diseases
Rheumatoid arthritis
Systemic lupus erythematosus (SLE)
Sjögren syndrome
Scleroderma
Other vasculitis and vasculopathies
Giant cell arteritis
Paraneoplastic[a] (hematological or solid malignancy)
Hypersensitivity vasculitis
Essential mixed cryoglobulinemia
Sarcoidosis[a]
Lymphomatoid granulomatosis[a]
Cholesterol embolus syndrome[a] (Bendixen et al. 1992)
Infection-associated vasculitis
Leprosy in ENL reaction
HIV infection
CMV related
Unknown basis
Lyme disease
Various arthropod stings[a]
Bacterial endocarditis[a] (Jones and Siekert 1968; Pamphlett and Walsh 1989)
Tuberculosis[a] (Stubgen 1992)
Other
Eosinophilia–myalgia syndrome[a]
Toxic oil syndrome[a]

[a]Necrotizing vasculitis not proven

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vasculitis of peripheral nerves, primary systemic vasculitis, vasculitis of connective tissue disease, and less common vasculopathies.

13.1 Clinical Manifestations

13.1.1 Mononeuritis Multiplex

Mononeuritis multiplex is regarded as the classic manifestation of vasculitic neuropathy, as the multifocal process affects nerves throughout the body (more often peroneal, ulnar, tibial, and the sural) including the cranial nerves. Patients experience an abrupt onset of pain, paresthesia, and paralysis in the distribution of a single nerve trunk evolving over hours to days, with newly involved nerves appearing over days to months. Atypical forms of presentation include pure sensory ataxia and radiculoplexopathy (Vrancken and Said 2013). When the lesions are disseminated, and their territories overlap, the presentation is in the form of an asymmetric polyneuropathy. Notwithstanding that biopsy is more likely to be performed in patients with mononeuritis multiplex, an acutely, subacutely, or chronically progressive symmetric distal sensorimotor polyneuropathy, where vasculitis is often not suspected, has been described in 19–76 % of biopsy-proven vasculitic neuropathies (Harati and Niakan 1986; Dyck et al. 1987; Hawke et al. 1991; Wees et al. 1981; Kissel et al. 1985; Panegyres et al. 1990; Said et al. 1988). This indicates that progressive symmetric distal polyneuropathy is a common clinical presentation of vasculitic neuropathy. Experience with vasculitic neuropathy at St. Michael's Hospital includes 39 cases, for which adequate clinical information was available in 32. Fourteen (44 %) presented with a distal symmetrical pattern, 12 (38 %) with obvious mononeuritis multiplex, 5 (16 %) with asymmetrical polyneuropathy, and 1 was asymptomatic. Evolution can occur rapidly enough to result in an initial clinical diagnosis of GBS (Suggs et al. 1992).

Nerve conductions give evidence of axonal abnormalities and are invaluable for demonstrating the asymmetry and multifocality that are most suggestive of a vasculitic neuropathy (Hawke et al. 1991; Kissel et al. 1985; Olney 1992). Although demyelination is generally not a significant element of the histologic picture, conduction block has occasionally been documented as part of an ischemic or vasculitic neuropathy (Hughes et al. 1982; Jamieson et al. 1991; Kaku et al. 1993). Significant demyelinating electrophysiological features were observed in 3 of 32 adequately documented cases of vasculitic neuropathy identified in our laboratory.

In patients known to have a systemic vasculitic illness, nerve biopsy is of questionable value. Of greater interest to the pathologist are those situations where a diagnosis of vasculitis is unsuspected, either because the disease seems confined to the peripheral nervous system, or because it is not yet fully evident

13.1.2 Nonsystemic (Isolated) Peripheral Nervous System Vasculitis (NSVN)

Vasculitis involving the peripheral nerves can be seen in isolation and results in a peripheral nerve syndrome indistinguishable from that of vasculitic neuropathy in multisystem disease. Approximately 25 % of cases with vasculitis neuropathy are diagnosed with primary vasculitis neuropathy. These patients usually present with a subacute or chronic neuropathy, often with a mononeuritis multiplex, and have few or no systemic abnormalities on history, physical, or laboratory investigation. Cases fulfilling these criteria are found in all large series of vasculitic neuropathy (Dyck et al. 1987; Hawke et al. 1991; Vincent et al. 1985; Harati and Niakan 1986; Kissel et al. 1985; Panegyres et al. 1990), and the pathogenesis is probably heterogeneous, as the pathology seems to be (vide infra). It has been argued that these cases do not represent a truly isolated PNS disease because a very high incidence of concurrent vasculitis was found in muscle biopsy (Said et al. 1988). The apparent selectivity of peripheral nerve involvement may reflect an increased vulnerability of this tissue to multifocal microvascular insults, and the presence of a milder disease sufficient to clinically affect nerve but no other tissues might explain the better outcome observed in these patients (Said et al. 1988; Said 1989; Dyck et al. 1987). Some authors have suggested that NSVN should be considered a low-grade systemic vasculitis that is symptomatic in nerves only (Said and Lacroix 2005).

13.1.3 Primary Systemic Vasculitis

13.1.3.1 Classic Polyarteritis Nodosa (PAN)

Classic polyarteritis nodosa (PAN) is a necrotizing arteritis associated with fibrinoid change. It is not uncommonly seen in patients suffering from hepatitis B infection (70 % of cases) and, less often, in patients with hepatitis C or HIV infections (Siva 2001). Although in its presentation PAN may appear limited to skin, muscles or nerves, PAN is a primary systemic vasculitis. Mononeuritis multiplex is recognized in 50–67 % of patients, and it may be the presenting manifestation in most (Hawke et al. 1991; Guillevin et al. 1988; Chumbley et al. 1977; Frohnert and Sheps 1967; Vrancken and Said 2013). Positivity for pANCA is exclusionary for classic PAN. Involvement of medium-sized vessels in epineurium is the domain of classic PAN.

13.1.3.2 Churg–Strauss Angiitis (CSA)

Churg–Strauss angiitis (CSA) is also designated eosinophilic granulomatosis with polyangiitis. In a large series of VPN, CSA accounted for the largest number of cases followed by PAN and WG (Mathew et al. 2007). This disease affects small- and medium-sized vessels. Patients display prominent pulmonary symptoms and eosinophilia, and vasculitic neuropathy develops in 20–65 % of cases, which can be the initial manifestation (Hattori et al. 2002; Uchiyama et al. 2012; Vrancken and Said 2013), CSA is a member of the family of ANCA-associated vasculitides that also includes WG and MPA. Perinuclear ANCA/MPO-ANCA is the pattern most often detected in CSA. The necrotizing vasculitis exhibits PAN-like fibrinoid change and an eosinophilic-rich lymphocytic and histiocytic infiltrate. Extravascular necrotizing granulomata are a feature of CSA, but not PAN (Chumbley et al. 1977).

13.1.3.3 Wegener Granulomatosis (WG)

Wegener granulomatosis (WG) (also termed *granulomatosis with polyangiitis*) is not a primary vasculitis, but rather a systemic disease characterized by necrotizing granulomata and granulomatous cytoplasmic-ANCA/PR3-ANCA-associated vasculitis (Gross and Csernok 2008; Suppiah et al. 2011) of small- and medium-sized vessels. Paranasal sinuses, lungs, and kidneys (resulting in necrotizing glomerulonephritis) are most severely involved. Peripheral neuropathy, most often with a mononeuritis multiplex pattern due to vasculitis, is the most common neurological manifestation, seen in 10–22 % of patients (Fauci et al. 1983; Drachman 1963; Nishino et al. 1993; Mahr 2009).

13.1.3.4 ANCA-Associated Microscopic Polyangiitis (MPA)

ANCA-associated microscopic polyangiitis (MPA) (formerly designated as microscopic periarteritis nodosa, Wohlwill 1923) is a necrotizing vasculitis affecting predominantly small-sized vessels, associated with antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase (MPO-ANCA) with a perinuclear pattern and sparse immune deposits. The most characteristic clinical feature is rapidly progressive glomerulonephritis, pulmonary involvement, and palpable purpura due to cutaneous vasculitis. Peripheral neuropathy (with frequent features of mononeuritis multiplex) occurs in about 57 % of patients (Guivellin et al. 1999; Mahr 2009; Chung and Seo 2010). Frequently affected nerves include the peroneal, ulnar, and median nerves. Cranial nerves are rarely involved (Vrancken and Said 2013). MPA may develop insidiously with nonspecific constitutional signs and symptoms or may be disguised as polymyalgia rheumatica.

13.1.3.5 Henoch–Schönlein Purpura (HSP)

Henoch–Schönlein purpura (HSP) is a systemic vasculitis of unknown etiology that involves the small vessels, most notably those in the skin (palpable purpura), gastrointestinal tract, and glomeruli, accompanied by arthralgia or arthritis. HSP is seen predominantly in children and its main histopathological features are leukocytoclastic vasculitis (LCV) mainly in papillary dermis associated with the deposition of IgA immune complexes in small vessels. Peripheral nerves are rarely affected (Mathew et al. 2007; Linskey et al. 2012). The prognosis of HSP is excellent as progressive renal disease occurs only in a minority of patients.

13.1.3.6 Behçet's Disease (BD)

Behçet's disease (BD) is a systemic disorder of unknown etiology. The criteria for diagnosis require the presence of oral ulceration plus any two of the following manifestations: genital ulcerations, papulopustular lesions, erythema nodosum-like nodular lesions, positivity of skin pathergy reaction, and uveitis (Walker et al. 1990, Melikoglu et al. 2008). BD also features a systemic vasculitis with a predilection to affect large veins and arteries including vena cava and aorta and its branches. Pseudoaneurysm formation in affected vessels is common. Involvement of small vessels is rare and manifestation of mononeuritis multiplex is exceptional (Takeuchi et al. 1989; Walker et al. 1990).

13.1.3.7 Connective Tissue Diseases

Vasculitis is suspected when a patient with multisystem disease develops symptoms and signs of peripheral nerve dysfunction. However, nerve biopsies in many such patients show nonspecific changes and normal vessels (Olney 1992). Clinicopathological review of our material revealed that non-vasculitic neuropathy-associated with systemic inflammatory disease (NASID) was the fourth most common diagnosis (Table 1.2), and the histological correlates of this included any combination of axonal, demyelinating, and inflammatory changes (Table 1.3). It is impossible to determine whether this represents a sampling error or whether a non-vasculitic pathologic process is at work.

Vasculitis and vasculitic neuropathy are typically seen in rheumatoid arthritis (RA) only after many years of disease activity, in the presence of erosive joint disease, cutaneous nodules, and high titers of rheumatoid factor (Scott et al. 1981; Hawke et al. 1991). Vasculitic neuropathy can rarely precede the diagnosis (Peyronnard et al. 1992; Chang et al. 1984). The development of vasculitis in a patient with RA indicates a poor prognosis (Vollertsen et al. 1986; Hawke et al. 1991). In rheumatoid arthritis a mild predominantly sensory neuropathy is more frequent than the more severe sensorimotor neuropathy associated with vasculitis

(Olney 1992). In rare biopsied cases the underlying pathology has been mild axonal loss with segmental demyelination in the absence or paucity of vascular changes, but whether this is primary or secondary demyelination has not been determined (Weller et al. 1970; Beckett and Dinn 1972). Patients suffering from rheumatoid arthritis are also prone to developing other types of neuropathy including entrapments (e.g., median mononeuropathy at the wrist and digital nerve compression secondary to tenosynovitis and arthritis of the carpal bones and digits (Pallis and Scott 1965; Gwathmey et al. 2014). The most devastating type of neuropathy is a progressive multifocal neuropathy, with features similar to those of PAN.

Sjögren syndrome is an autoimmune disease caused by inflammation of the salivary and lacrimal glands. Patients develop the sicca complex (dry eyes and dry mouth), and peripheral neuropathy may be the presenting manifestation (Mellgren et al. 1989; Peyronnard et al. 1992). The clinical picture is usually not a mononeuritis multiplex, but rather a distal sensorimotor neuropathy. Pure sensory neuropathy is also seen and has features suggestive of dorsal root ganglionitis (Pavlakis et al. 2012). Vasculitis (with no other systemic manifestations) has been reported to account for about 15 % of Sjögren-related neuropathies. Important to the diagnosis of Sjögren syndrome is the presence of SS-A (Ro)/SS-B (La) antibodies (Theander and Jacobsson 2008). In Sjögren syndrome a pure sensory neuropathy is occasionally seen and is likely due to spinal ganglionitis (Chap. 21)

Peripheral neuropathy develops in about 10–20 % of patients with systemic lupus erythematosus (SLE) (Chalk et al. 1993; Richardson 1982; Wallace and Metzger 1993; Collins and Periquet 2008; Florica et al. 2011). A distal sensorimotor polyneuropathy, a mononeuritis multiplex, and rarely a CIDP-like picture (Richardson 1982; Rechthand et al. 1984) may be seen. The neuropathy is usually seen after the disease is well established, but can be the presenting manifestation (McCombe et al. 1987; Hughes et al. 1982). The vasculitis affects small vessels and has leukocytoclastic characteristics. PAN-like necrotizing vasculitis may be observed in medium-sized blood vessels. In SLE, CIDP has been infrequently reported (Rechthand et al. 1984; Richardson 1982). Some of the vasculitides encountered in mixed connective tissue disease are similar to the morphologic vascular changes in SLE-associated vasculitis.

Peripheral neuropathy (excluding trigeminal sensory neuropathy and carpal tunnel syndrome) is present in 1–10 % of patients with Scleroderma (Olney 1992; Lee et al. 1984; Averbuch-Heller et al. 1992; Dierckx et al. 1987; Hietaharju et al. 1993). Histological data is scanty, but there are several reported cases of biopsy-proven vasculitic neuropathy in progressive systemic sclerosis (PSS), most often in the presence of Sjögren syndrome (Oddis et al. 1987; Dyck et al. 1987; Vincent et al. 1985). A neuropathy in which vasculitis cannot be implicated, and which can precede generalized clinical manifestations, may be seen in PSS (Di Trapani et al. 1986). Several histological reports have documented prominent epineurial and perineurial collagenization and microangiopathic but non-vasculitic changes similar to those seen systemically with this disease (Richter 1954; Di Trapani et al. 1986; Corbo et al. 1993).

13.1.4 Other Vasculitides

A peripheral neuropathy is said to occur in as many as 14 % of patients with giant cell (temporal) arteritis and frequently precedes the diagnosis by several months (Caselli et al. 1988). Isolated reports exist documenting necrotizing vasculitis, with or without giant cells, involving neural vessels of all sizes in cases of clear-cut temporal cell arteritis (Bridges et al. 1989; Torvik and Berntzen 1968; Merianos et al. 1983; Pons et al. 1987; Nesher et al. 1987). Sites involved include brachial plexus, peroneal nerve, mononeuropathies, and polyneuropathies. Histopathologic features consist of transmural lymphohistiocytic inflammation in the absence of fibrinoid necrosis. Multinucleated giant cell formation occurs associated to degeneration of internal elastica.

A group of nonsystemic, localized vasculopathies termed diabetic lumbosacral radiculoplexus neuropathy and nondiabetic lumbosacral radiculoplexus neuropathy (LRPM) are characterized by intense pain and weakness in the thighs and progressing to affect lower extremities including feet and toes. The clinical course is monophasic but results in protracted morbidity (Gwathmey et al. 2014). Histopathological studies revealed reduction in the number of nerve fibers, perineurial thickening, neuroma formation, neovascularization, and half of the cases featured changes suggestive of microvasculitis.

A heterogeneous group of diseases termed hypersensitivity vasculitis or cutaneous small-vessel vasculitis may present as an idiopathic condition, or secondary to infections, adverse reaction to pharmaceuticals, in the setting of malignancy or autoimmune diseases. Peripheral neuropathy occurs only rarely, except in patients suffering from autoimmune disorders. The hypersensitivity vasculitis that results from the administration of heterologous sera has received the most interest in the past (Iqbal and Arnason 1984).

Paraneoplastic Vasculitic Neuropathy. The most common paraneoplastic vasculitis is leukocytoclastic vasculitis, 75 % of which are caused by hematological malignancies. Second in frequency is small-cell carcinoma of the lung followed by malignancies of the colon, breast, and kidney (Solans-Laqué et al. 2008). In a review of 14 cases, nine cases involved microvasculitis and five cases involved necrotizing vasculitis in medium-sized vessels (Oh 1997; Naka et al. 1991; Choi et al. 2013; Paul 1996).

The most frequently described neuropathy in chronic hepatitis C is a vasculitic neuropathy in the setting of essential cryoglobulinemia (Ferri 2008; Gwathmey et al. 2014; Ramos-Casals et al. 2006). Peripheral neuropathy is more common in essential mixed cryoglobulinemia, but reports of pathological studies are rare (Vrancken and Said 2013).

Vasculitic neuropathy is rare in sarcoidosis, but it is well documented (Said et al. 2002; Vital et al. 2008), including epineurial necrotizing vasculitis in the vicinity of granulomata. Our experience with this disease indicates the presence of naked granuloma in perivascular spaces of epineurial medium-sized vessels, with intrusion into the vessel wall. Some patients suffering from sarcoidosis exhibit the clinical syndrome of mononeuritis multiplex.

VPS may develop in association with leprosy (ENL), HIV, CMV, HCV, and Lyme disease (see Chaps. 11 and 12).

13.2 Pathology

13.2.1 General Considerations: Sensitivity of Biopsy

Nerve biopsy is critical for the diagnosis of vasculitis in two clinical situations: "atypical" presentation of systemic vasculitis masquerading as a cryptogenic polyneuropathy and isolated vasculitis of the peripheral nervous system.

The sensitivity of nerve biopsy in the detection of vasculitis is not known because of the variability of selection criteria in published series and the absence of a "gold standard" against which to measure the biopsy. In a study which identified 35 consecutive patients with mononeuritis multiplex from a hospital EMG laboratory, 11 had a previously known rheumatic disease, 9 had the simultaneous onset of systemic disease, and 15 showed only peripheral nerve manifestations (Hellmann et al. 1988). Biopsies showed definite vasculitis in 3 of 5 patients strongly suspected of having polyarteritis nodosa clinically, but in none of 7 biopsies in patients with isolated PNS disease. In the latter group, however, nerve "infarction" was seen in 5 of 7. Dyck et al. (1987) noted that of 45 patients with a biopsy-proven systemic vasculitic illness with neuropathy, nerve biopsy was positive in 58 % in patients, suggestive in 29 %, and nondiagnostic in 23 %. It is possible to find necrotizing vasculitis in an electrically normal nerve (Kissel and Mendel 1992), as has been our experience with 3 patients who underwent nerve and muscle biopsy for suspected systemic vasculitis despite normal nerve conductions studies and EMG. However, the yield is lower than in clinically or electrically involved nerves (Wees et al. 1981). Such data do not make possible estimation of the sensitivity of sural nerve biopsy for the diagnosis of vasculitic neuropathy.

Epineurial vascular involvement usually predominates over endoneurial involvement and is often exclusive; thus, fascicular biopsy is not appropriate when vasculitis is suspected (Dyck et al. 1972; Oh 1990); this is the strongest argument against the utility of fascicular nerve biopsy for diagnostic purposes.

If muscle biopsy can be added to the procedure, the yield may increase an additional 15–45 % (Hawke et al. 1991; Vincent et al. 1985; Dyck et al. 1987). In one series of 83 patients who underwent nerve and muscle biopsy, necrotizing arteritis in the muscle alone was found in 45 %, as compared with 20 % in the nerve alone, and 30 % in both, including patients with seemingly isolated PNS vasculitis (Said et al. 1988). We thus advocate combined nerve and muscle biopsy whenever vasculitis is being considered.

13.2.2 Some Pathological Considerations

Perivascular inflammation is a common and nonspecific finding in peripheral nerve pathology and should be differentiated from "vasculitis." Throughout this book, the term "vasculitis" indicates inflammation of the vessel and evidence of destruction such as fibrinoid necrosis, thrombosis, hemorrhage, or disruption of the endothelium (Fig. 13.1a, b). Transmural inflammation accompanied by karyorrhexis has substantially the same value as fibrinoid necrosis (Fig. 11.2), whereas the presence of leukocytes within the vessel wall is suggestive but not diagnostic of vasculitis. At times, what seems to be prominent perivascular cuffing around small vessels (Fig. 13.2) has been called "microvasculitis" by some (Oh et al. 1991; Vincent et al. 1985), but in our experience this lesion does not have the same diagnostic specificity as necrotizing vasculitis. For example, some of the patients reported by Leger et al. (1988) as showing neural "vasculitis" likely suffered from CIDP. Vasculitis can involve small-sized endoneurial and epineurial vessels for which the designation of microvasculitis is preferred. The anatomy of peripheral nerve vasculature is reviewed in Chap. 6.

13.2.2.1 Light Microscopy

The hallmark of acute vasculitis is destruction and disorganization of muscularis and endothelial layers of the vessel, with deposition of fibrinoid material in the presence of transmural mononuclear or polymorphonuclear inflammatory cells and thrombosis. The damage is often focal, involving only a segment of the vessel wall (Fig. 13.3a, b). Hemorrhage into the surrounding tissue may be seen (Fig. 13.4a), sometimes in a perineurial or subperineurial crescentic pattern. Perl's ferrocyanide stain (Fig. 13.4b) will highlight old hemorrhages (Adams et al. 1989), but the specificity of this finding is uncertain (Winer et al. 1992). The use of MSB or PTAH staining (Fig. 13.5a, b) may bring out inconspicuous fibrinoid change. The pattern of involvement in vasculitis is often patchy, with unscathed vessels

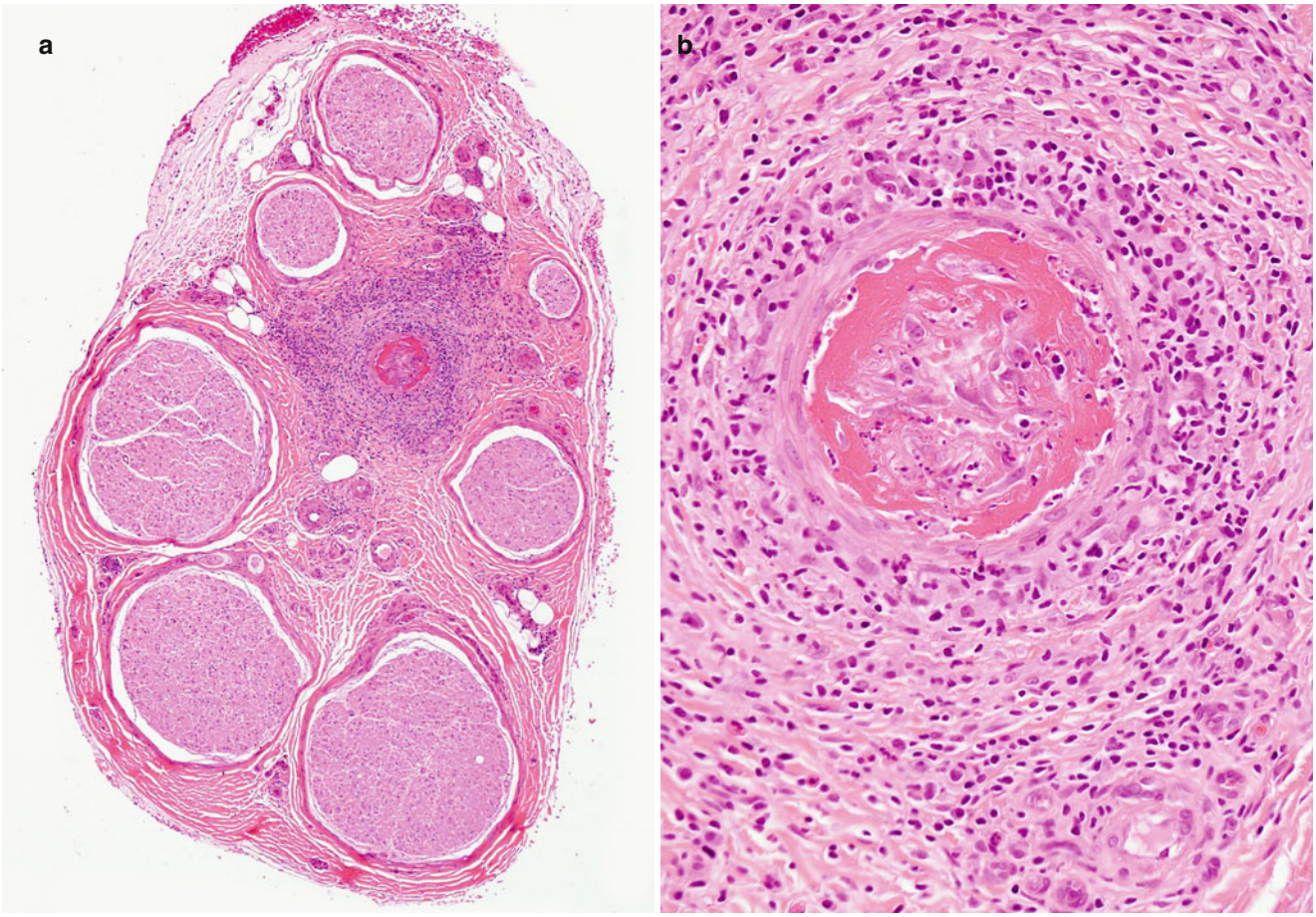


Fig. 13.1 Vasculitic neuropathy: (**a**) recent fibrinoid necrosis and thrombosis of an epineurial vessel, with perivascular hemorrhage. (**b**) Transmural inflammation and karyorrhexis are associated with perivascular accumulation of polymorphonuclear leukocytes and mononuclear cells (**b**) (paraffin, H&E stain; magnification: **a**, 100×; **b**, 400×)

and nerve fascicles adjacent to severely damaged ones (Fig. 13.6a, b). Epineurial vessels, predominantly arterioles, are much more frequently damaged than endoneurial vessels (Fujimura et al. 1991). The size of vessels affected has diagnostic implication (Table 13.2). Step sections encompassing the entire thickness of the tissue block may be necessary to arrive at the correct diagnosis if initial examination shows nonspecific features such as lymphocytes in the form of large aggregates or perivascularly (Fig. 13.2). The internal elastic lamina is fragmented, and the use of elastic stains alternating with H&E is helpful in the search for vascular damage.

In the acute stage of vasculitis, polymorphonuclear leukocytes can be prominent (Fig. 13.1b and 13.7a–e), but usually T lymphocytes predominate in the vessel wall and perivascular area, with variable numbers of macrophages (Kissel et al. 1989). Any nerve biopsy showing extravasation of neutrophils should be regarded as suspicious for vasculitis, as polymorphonuclear infiltrates are almost never seen in any other cause of neuropathy. However, neutrophils may also be seen in any biopsied tissue if an inordinate amount of time is taken to perform the procedure (30 min or more) (Fig. 7.13). The presence of inflammatory cells within the vessel wall is also suggestive but not diagnostic of vasculitis (vide supra). The inflammatory cells may include epithelioid elements, which can be loosely clustered, palisading, or tightly packed in association with multinucleated giant cells. Eosinophils commonly and plasma cells less frequently contribute to the cellularity. Two of four patients with Churg–Strauss angiitis whose nerve biopsies we have studied had large numbers of eosinophils within the vessel wall in perivascular spaces of epineurium (Fig. 13.8a–e). Conspicuous in one case were Charcot–Leyden crystals within the inflammatory infiltrate (Fig. 13.8d, e). The inflammatory damage

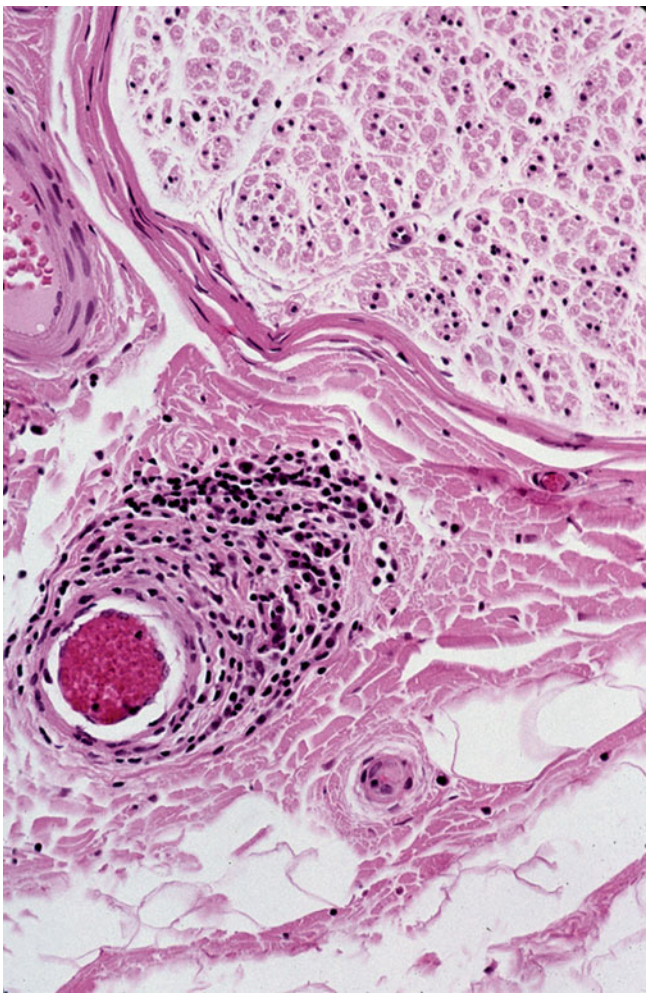


Fig. 13.2 Epineurial perivascular inflammation in the absence of necrosis does not meet the gold standard of angionecrosis, although additional adjacent sections may meet that criterion (paraffin, H&E, 400×)

to the vessel usually results in narrowing and thrombotic occlusion (Figs. 13.5b and 13.8c). Accumulation of mucoid "edema" in endoneurium, subperineurium, and around vessels is occasionally seen.

Findings indicative of the disease underlying the vasculitis are rarely present, but include such observations as CMV inclusions in endothelial cells (Fig. 11.4), detection of intravascular cholesterol crystals in cholesterol embolus syndrome (Bendixen et al. 1992), or the presence of an atypical cellular infiltration in lymphomatoid granulomatosis (vide infra).

With time, the inflammation subsides or disappears and vessels may show marked narrowing (Fig. 13.9a), fibrous obliteration or asymmetric mural fibrosis (Fig. 13.9b), calcification (Fig. 13.9c), recanalization (Fig. 13.9d–f), fragmentation of the internal elastica (Fig. 13.9g), or increased numbers of small vessels (Fig. 13.9h). This should be distinguished from vessel retraction and invagination caused by surgical trauma (Fig. 13.10a–c). A specimen displaying both acute and healed vascular lesions is typical of the polyphasic course of polyarteritis nodosa (Lie 1990). Hemosiderin-laden macrophages indicative of past hemorrhage may be found clustered in a periadventitial location (Fig. 13.4a). We have occasionally observed miniature bundles of aberrantly regenerating axons, much like a traumatic neuroma, growing into the perineurium, and regard this as suggestive evidence of a previous infarction. Schroder (1986) has drawn attention to the reactive proliferation of capillaries that can occur in the epineurium after a vascular insult (Fig. 13.9a), although this is not specific to vasculitis.

Vasculitis of endoneurial vessels is uncommon and would theoretically be classified with hypersensitivity vasculitides (Fig. 13.11). Involvement of capillaries and postcapillary venules is seen as infiltration by polymorphonuclear leukocytes and leukocytoclasis. Fibrinoid necrosis, thrombosis, and hemorrhage may also be present (Figs. 13.11, 13.12, 13.13, and 13.14). In skin biopsies the inflammatory process may show mononuclear, neutrophilic, or eosinophilic predominance and even granulomatous characteristics (Lie 1990), but whether this wide spectrum of pathology is also seen in nerve biopsy material is uncertain. In ischemic neuropathy, nerve fiber degeneration ranges from none at all to universal and from chronic to hyperacute (Fig. 13.15a, b). The geography of endoneurial damage is variable; centrofascicular or perineurial-based wedge-shaped regions of nerve fiber loss are infrequently seen in biopsy material (Fig. 13.12a, b). More typically there is multifocal axonal degeneration, and a striking variability in the severity of involvement may be seen between different fascicles (Fig. 13.6a, b) (Fujimura et al. 1991). The fibers may all be in the same stage of degeneration, suggesting a single massive insult, but more commonly evidence of both acute and chronic axonal changes is seen. Large myelinated axons can be selectively affected, but in severe lesions, all fiber types are involved (Vital and Vital 1985; Fujimura et al. 1991). Regenerating clusters will appear in the recovery phase, but may be less prominent with more severe insults, perhaps because loss of Schwann cells impairs the regenerative process (Fujimura et al. 1991).

While segmental demyelination and/or remyelination may occur, it is always subordinate to prevailing axonal degeneration (Panegyres et al. 1990; Vital and Vital 1985). Remarkably, Harati and Niakan (1986) observed segmental demyelination/remyelination in a majority of patients with vasculitic neuropathy using teased fibers, but most other workers (Said et al. 1988; Fujimura et al. 1991; Dyck et al. 1987; Hawke et al. 1991) have reported minor segmental

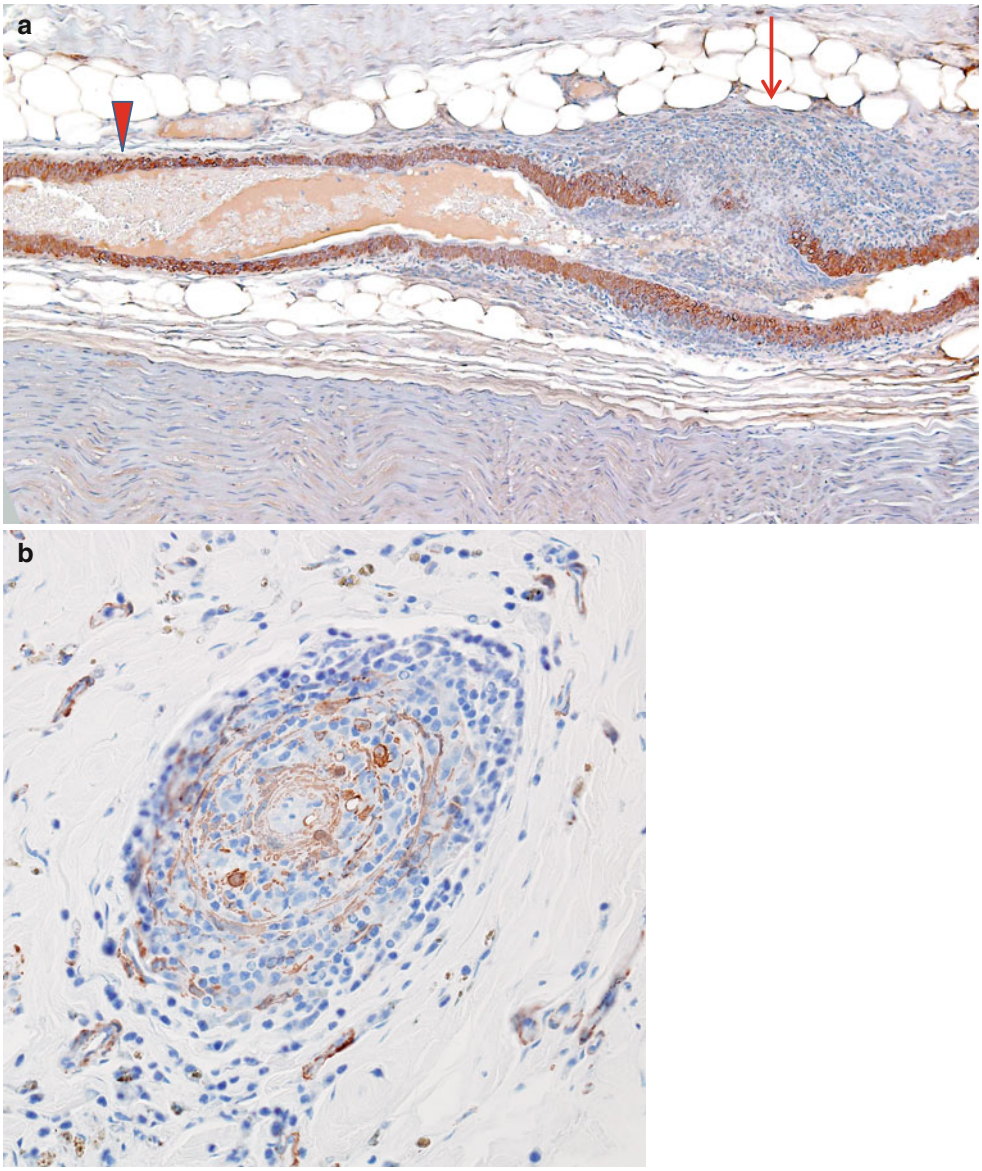


Fig. 13.3 (**a**) Vasculitis: longitudinal section of epineurial artery shows the focal nature of vasculitis. At the distalmost aspect shown (*arrow*) marked inflammation has resulted in complete thrombosis and mural destruction only a few hundred microns from a portion of the vessel exhibiting only a perivascular lymphocytic infiltrate (*arrowhead*). (**b**) Vascular cross section shows destruction of the entire smooth muscle component of the vessel wall (paraffin, anti-smooth muscle actin (anti-SMA) immunohistochemistry; magnification: **a**, 100×; **b**, 200×)

myelin pathology in a distribution suggestive of a process secondary to axonal disease, as has been amply demonstrated in experimental material.

13.2.3 Electron Microscopy

The superior resolution of electron microscopy may reveal endothelial cell necrosis and disruption of basal laminae in cases where light microscopy showed inflammation only. It has been suggested that hypertrophied endothelial cells with prominent intraluminal projections may be a helpful clue (Nemni et al. 1988), but we find this to be nonspecific.

Nonspecific axonal degenerative changes may be seen. Vesicular "demyelination" has been observed in neuropathy associated with necrotizing vasculitis, but in contrast to the picture in GBS or CIDP, the axons showed concomitant damage (Hughes et al. 1982; Vital and Vital 1985). In ischemic damage unmyelinated fibers may be as prominently involved as myelinated fibers or may show relative sparing (Said et al. 1988). Segmental demyelination may be detected, as has been shown in human material and in experimental models of ischemic neuropathy. Other than in the setting of HIV infection, tubuloreticular inclusions are a very rare finding in peripheral nerve, but we have observed them in vasculitis associated with SLE (Fig. 13.16a, b) (Case 13.1).

13.2.4 Immunohistochemistry

The predominant inflammatory cell in any vasculitis is the lymphocyte, of which 95 % or more are CD4+/helper and

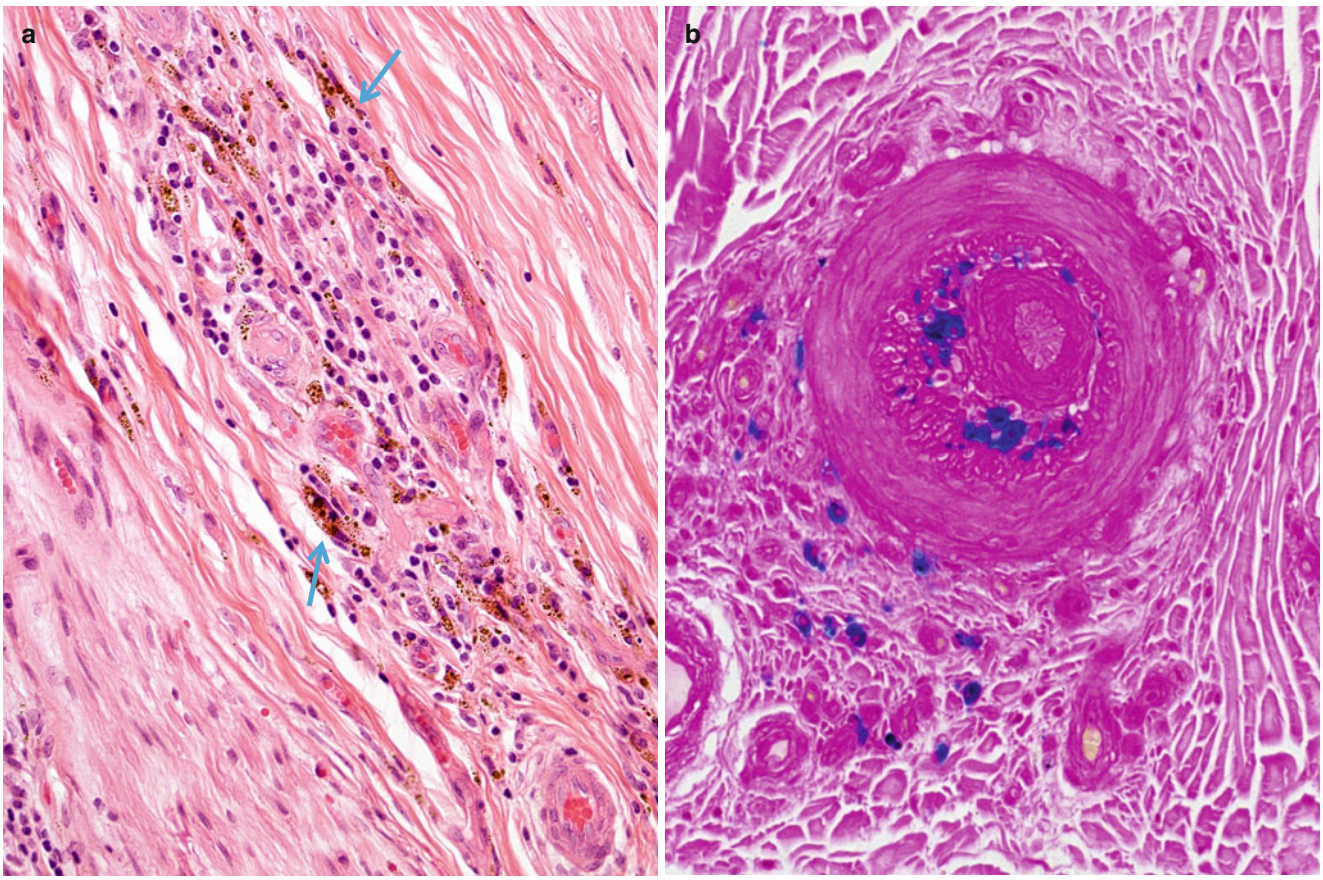


Fig. 13.4 Vasculitis, hemorrhagic residua: perivascular inflammation and hemosiderin deposition (*arrows*, **a**) are highlighted by Prussian blue stain (**b**) for iron (paraffin, **a**, H&E; **b**, Perls' iron stain; magnification: **a**, **b**, 200×)

CD8+/cytotoxic T cells (Fig. 13.17a, b) and macrophages. Depending on the acuteness of the process, variable number of neutrophils and eosinophils may also be present. Collins et al. (2010b), using combined peroneal nerve/peroneus brevis muscle biopsies, direct immunofluorescence revealed immunoglobulin, complement, or fibrinogen (DIF, IgG, IgM, and complement 3) deposits in epineurial vessel walls in 70–80 % of nerve biopsies in patients with suspected peripheral nervous system vasculitis and diabetic radiculoplexus neuropathy (Collins et al. 2010b). These authors concluded that epineurial/perimysial vascular deposits of immunoglobulin/C3 by DIF are a specific marker of vasculitic neuropathy. The less sensitive DIF may be more specific for identifying vasculitis-relevant immune deposits by not labeling low concentrations of vascular immunoglobulin and complement found in "normal" and non-vasculitic cases.

13.3 Pathogenesis

Nerve dysfunction is presumed to occur on the basis of ischemia secondary to vessel destruction, with the areas of maximal clinical involvement (mid-arm and mid-thigh), representing vascular watershed regions, as demonstrated in a remarkable study by Dyck et al. (1972). In patients with a symmetrical sensorimotor neuropathy, the mechanism is probably multiple random minor lesions which will summate to affect the longest nerve fibers more severely (Waxman et al. 1976). Axonal destruction is invariably the dominant process, but segmental demyelination attributable to ischemia is shown by the occasional finding of conduction block in biopsy-proven vasculitic neuropathy (Ropert and Metral 1990) and experimental studies of nerve ischemia.

Epineurial arterioles are in the 75–350 μm diameter size range, while perineurial and endoneurial vessels fall well below 75 μm in diameter. It is thought that this sharp size separation between the two classes of vessels relates to the far more frequent finding of epineurial damage, as most vasculitic process are vessel size specific (Dyck et al. 1972).

Activated complement and immunoglobulin deposits are found in 63–100 % of inflamed vessel walls regardless of etiology (Kissel et al. 1989; Hawke et al. 1991; Panegyres et al. 1990), and immune complexes have long been known to be associated with vasculitis (Smiley and Moore 1989). In animal models deposition of these complexes within vessel walls follows the introduction of a foreign antigen, and

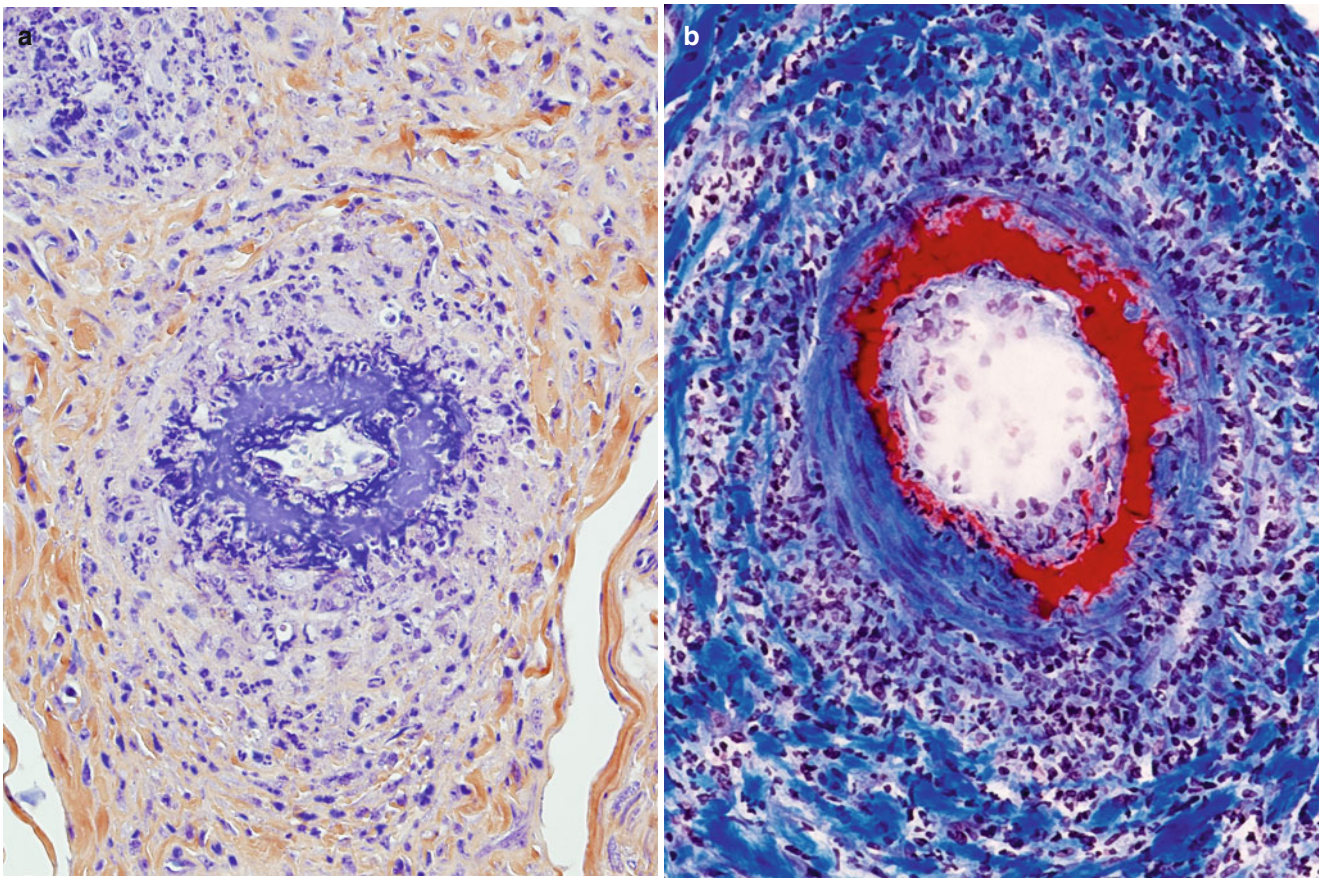


Fig. 13.5 (**a**, **b**) Fibrinoid necrosis/fibrin deposition in vasculitis (**a**) phosphotungstic acid (PTAH) stain; B, Landrum stain for fibrin (magnification: **a**, **b** ~400×)

results in activation of complement, attraction of neutrophils and other inflammatory cells, and release of toxic substances that can cause vessel wall necrosis. In human disease, a putative antigen source is sometimes found, as exemplified by hepatitis B antigen in PAN (Guillevin et al. 1988). Serum sickness and amphetamine-associated hypersensitivity vasculitis are examples of a clear-cut source of antigen for the formation of antigen–antibody complexes. It is unlikely that the immunoglobulins identified in vessel walls are generated at the site of the lesion, as B cells are very infrequent in the vascular lesion (Kissel et al. 1989). Despite the lack of a source of antigen–antibody complexes, immunoglobulin and complement deposits are also seen in patients with isolated PNS vasculitis (Kissel et al. 1989).

Immunohistochemical studies of the cells involved in vasculitis suggest that other mechanisms may be at play. Activated T cells form a major component of the inflammatory infiltrate regardless of underlying disease (Kissel et al. 1989; Panegyres et al. 1990), and the observation that they are predominantly of the CD8 subtype suggests an important role for cytotoxic T-cell-mediated damage, perhaps directed at a vascular antigen (Panegyres et al. 1990), or an antigen presented by endothelial cells (Kissel and Mendel 1992; Panegyres et al. 1992). It may be that the deposition of immunoglobulin and complement is not a primary cause of the vasculitis, but follows the cell-mediated attack (Kissel and Mendel 1992).

Mechanisms of vasculitis are probably heterogeneous, disease specific (Panegyres et al. 1990), and perhaps tissue specific. Excellent reviews in the pathogenesis of vasculitic neuropathy are available (Younger 2004; Pagnoux and Guillevin 2005; Gwathmey et al. 2014).

13.3.1 Significance of Size of Involved Vessels

Epineurial vascular involvement is said to be typical of PAN, CSS, WG, and isolated PNS vasculitis (Dyck et al. 1987; Marazzi et al. 1992; Fujimura et al. 1991). Epineurial arterioles are usually 75–350 μm in diameter, and there may be a tendency towards involvement of vessels in the lower part of this size range in isolated PNS vasculitis (Dyck et al. 1987). Involvement of veins is more common in WG and CSS than in PAN (Lie 1990). Endoneurial vessels are usually <30 μm in size, and arterioles, venules, and capillaries of this caliber in both endoneurium and epineurium are the typical

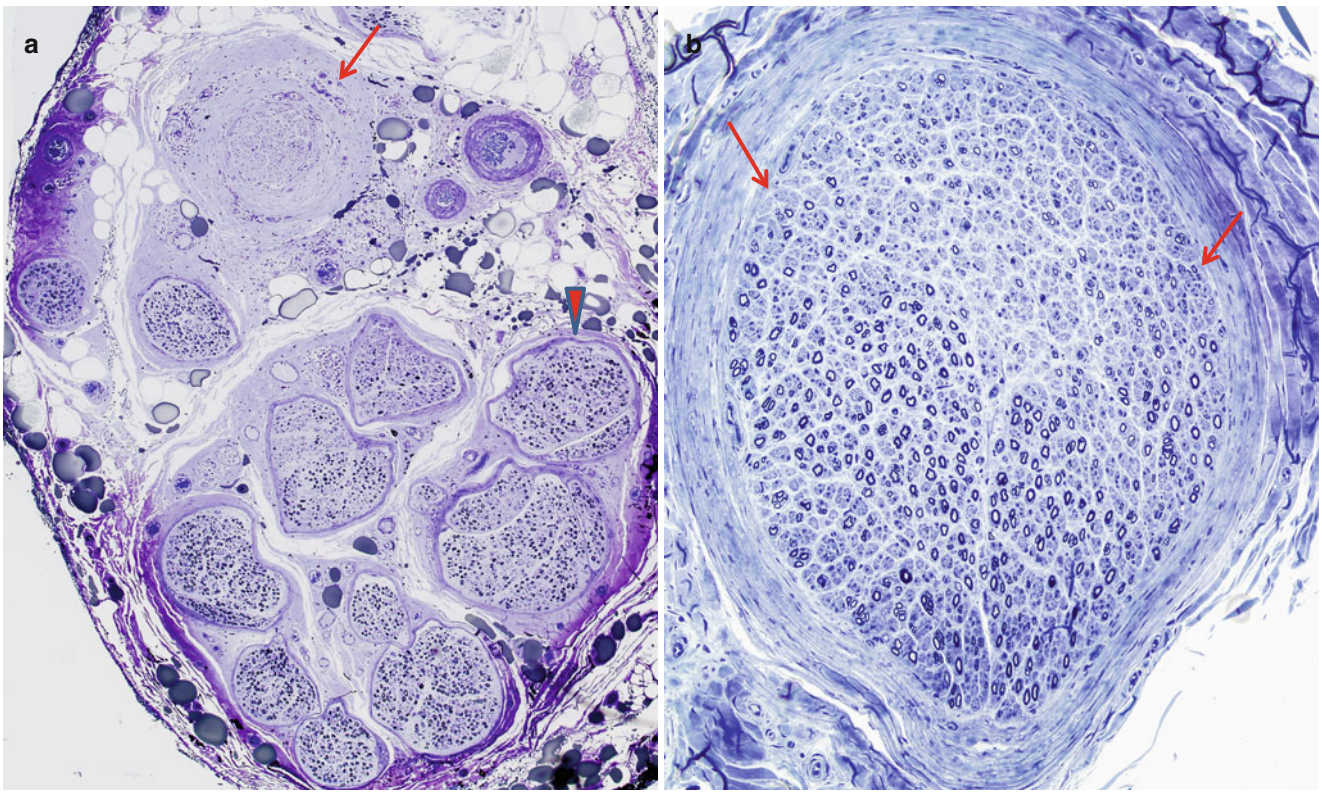


Fig. 13.6 Ischemic pattern of axon loss: (**a**) typical pattern of fascicle-to-fascicle variability of axon loss is characterized by marked axon loss (*arrow*) compared to more modest loss (*arrowhead*). Note that none of the vessels in the cross section demonstrate vasculitis. (**b**) Individual fascicles show intrafascicular variability with patches of axon loss (delimited by *arrows*) (1 μ thick plastic sections; magnification: **a**, 100×; **b**, 200×)

Table 13.2 Clues to the presence of remote vasculitis

Luminal narrowing or thrombosis
Disorganization of vessel: intimal hyperplasia, thinning or proliferation of the media, breakup of circumferential ring of internal elastic lamina
Vessel sclerosis, recanalization
Proliferation of epineurial capillaries
Old hemorrhage in nerve (hemosiderin, positive Perl's Prussian blue)
Nonuniform fascicular or multifocal axonal degeneration
Vascular immunoglobulin and complement deposition
Focal calcification of or near vessel walls
Focal perineurial damage with aberrant regenerating nerve bundles

site of injury in SLE, hypersensitivity vasculitis, Henoch–Schönlein purpura, and essential mixed cryoglobulinemia. Vessels in either size range can be involved in the collagen diseases. While these guidelines are useful in the differential diagnosis, any conclusion drawn must consider that overlap syndromes, in which vessels of all sizes are involved, are not uncommon. Review of the literature on vasculitis, based on histology of nerve or other tissues, indicates that PAN, Wegener's granulomatosis, Churg–Strauss angiitis, rheumatoid arthritis, SLE, Sjögren syndrome, cryoglobulinemia, and scleroderma may all show involvement of vessels in a size range considered atypical for that disease (Bouche et al. 1986; Dyck et al. 1987; Vincent et al. 1985; Lie 1989; Fauci et al. 1978; Leavitt and Fauci 1986; Vincent et al. 1985). Because of this overlap, the caliber or type of vessels involved does not point to a specific diagnosis. Vincent and colleagues reviewed 40 biopsies where only vasculitis involving arterioles, venules, and capillaries less than 70 μm in diameter was present (Vincent et al. 1985). The clinical diagnoses include PAN, RA, CSA, and SLE, despite the absence of arteriolar necrotizing vasculitis. Eleven patients had an isolated neuropathy in association with "microvasculitis," contradicting the suggestion that only epineurial vessels are involved in isolated PNS vasculitis (Dyck et al. 1987).

13.3.2 Significance of Inflammatory Cell Types

Eosinophils are seen in vasculitis of various etiologies and are especially well demonstrated by Giemsa histostain. Their presence does not necessarily correlate with peripheral eosinophilia (Vincent et al. 1985; Lie 1990; Ijichi et al. 1991). The finding of eosinophils in the vasculitic infiltrate has little diagnostic specificity, but if they are very prominent Churg–Strauss angiitis (CSA) should be considered more

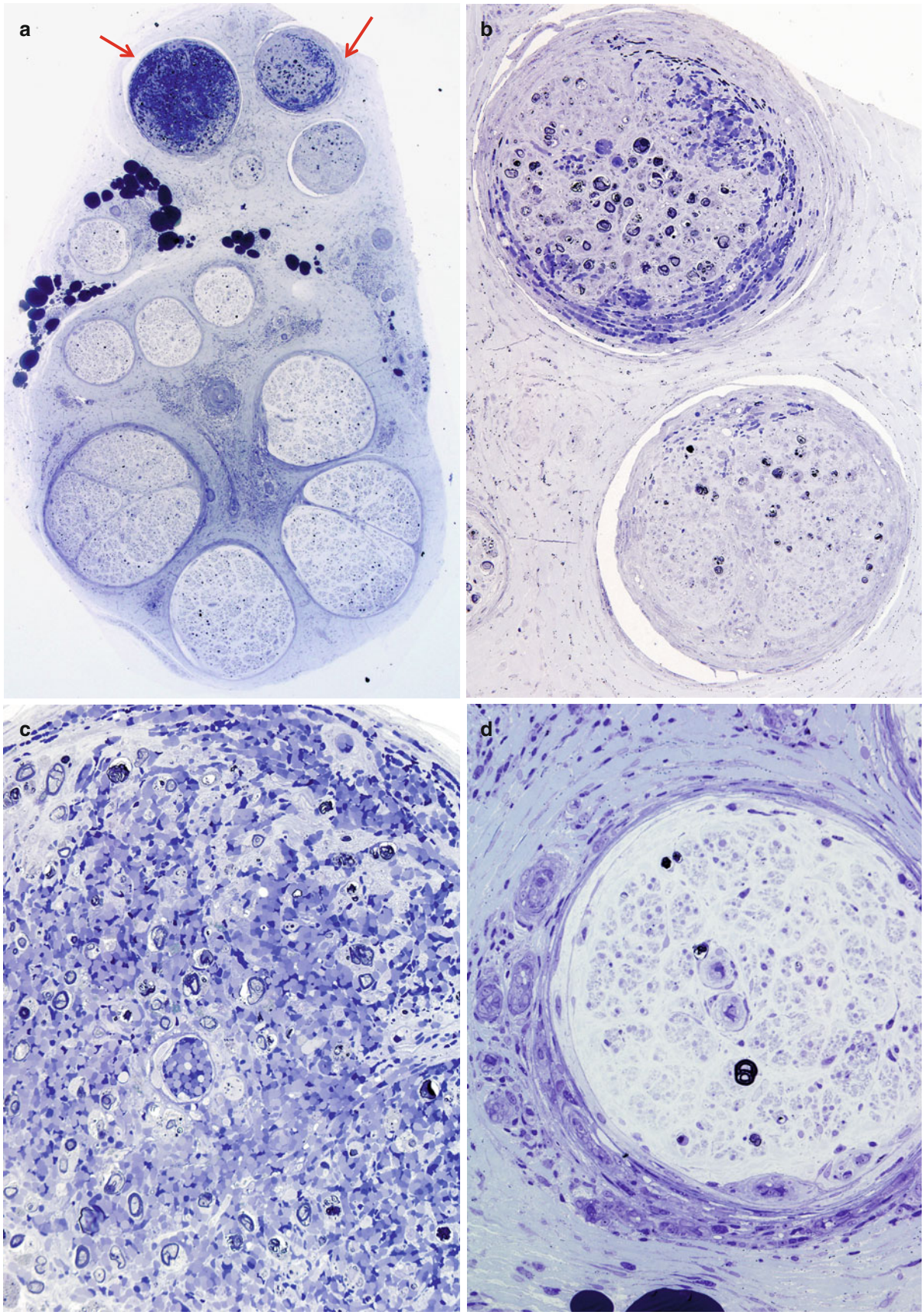
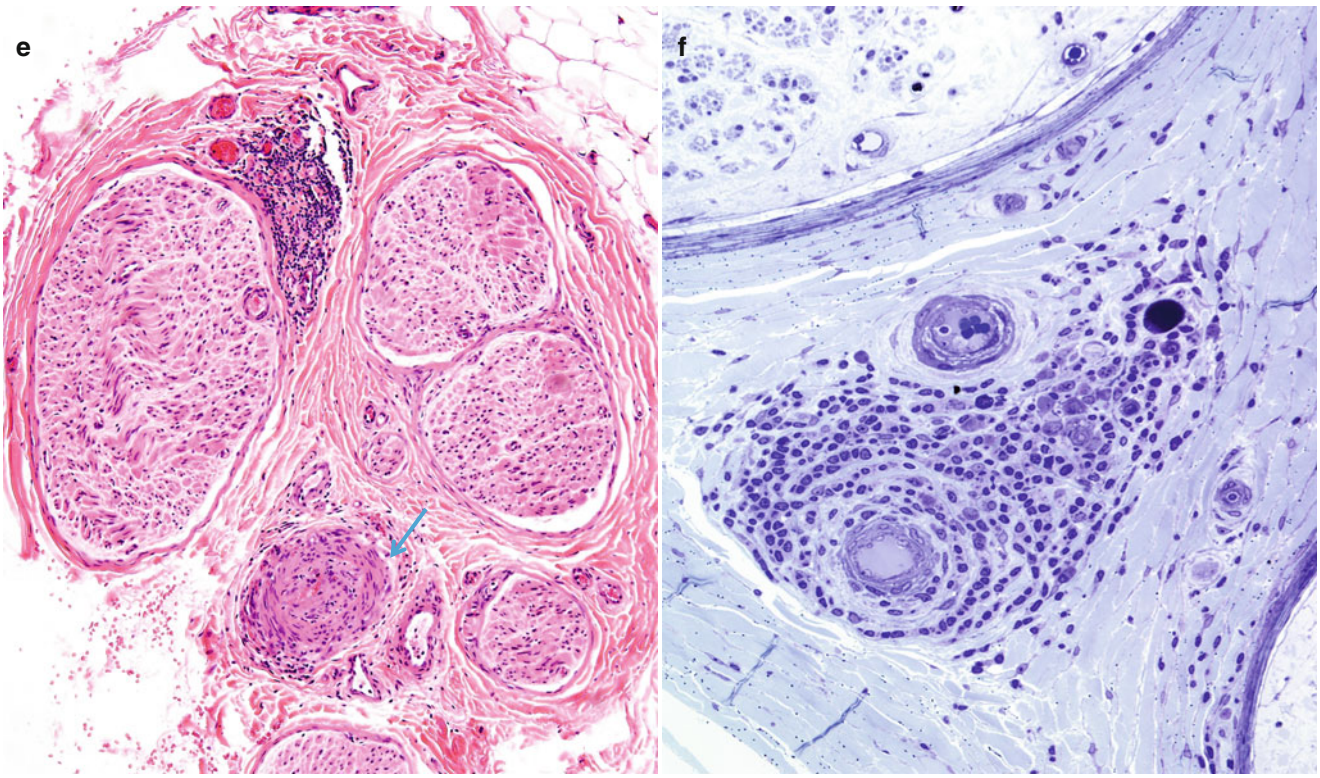


Fig. 13.7 Polyarteritis nodosum (PAN): (**a**) two fascicles show hemorrhagic necrosis (*arrows*). (**b**, **c**) Higher magnification of involved fascicles show hemorrhage and axonal degeneration. (**d**) Axonal depletion is the cardinal feature in all other fascicles. (**e**) Epineurial perivascular chronic inflammation is accompanied by a vessel showing resolving vasculitis (*arrow*). (**f**) Perivascular inflammation in the absence of angionecrosis is also common. (**a**–**f** 1 μ thick plastic sections, **f** paraffin, H&E; magnification: **a**, 40×; **b**, 200×; **c**, **d** 600×; **e**, 100×; **f**, 400×)



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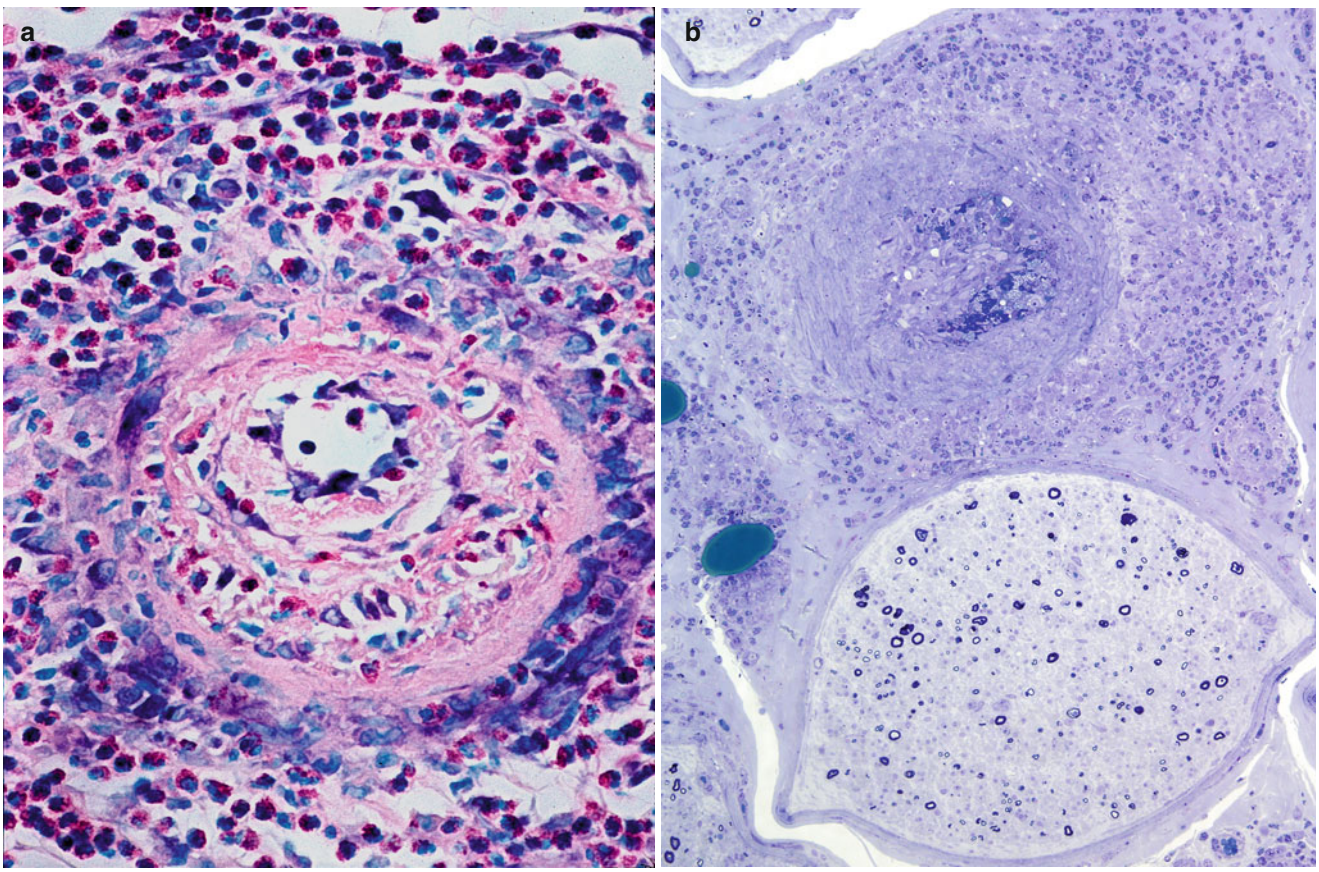
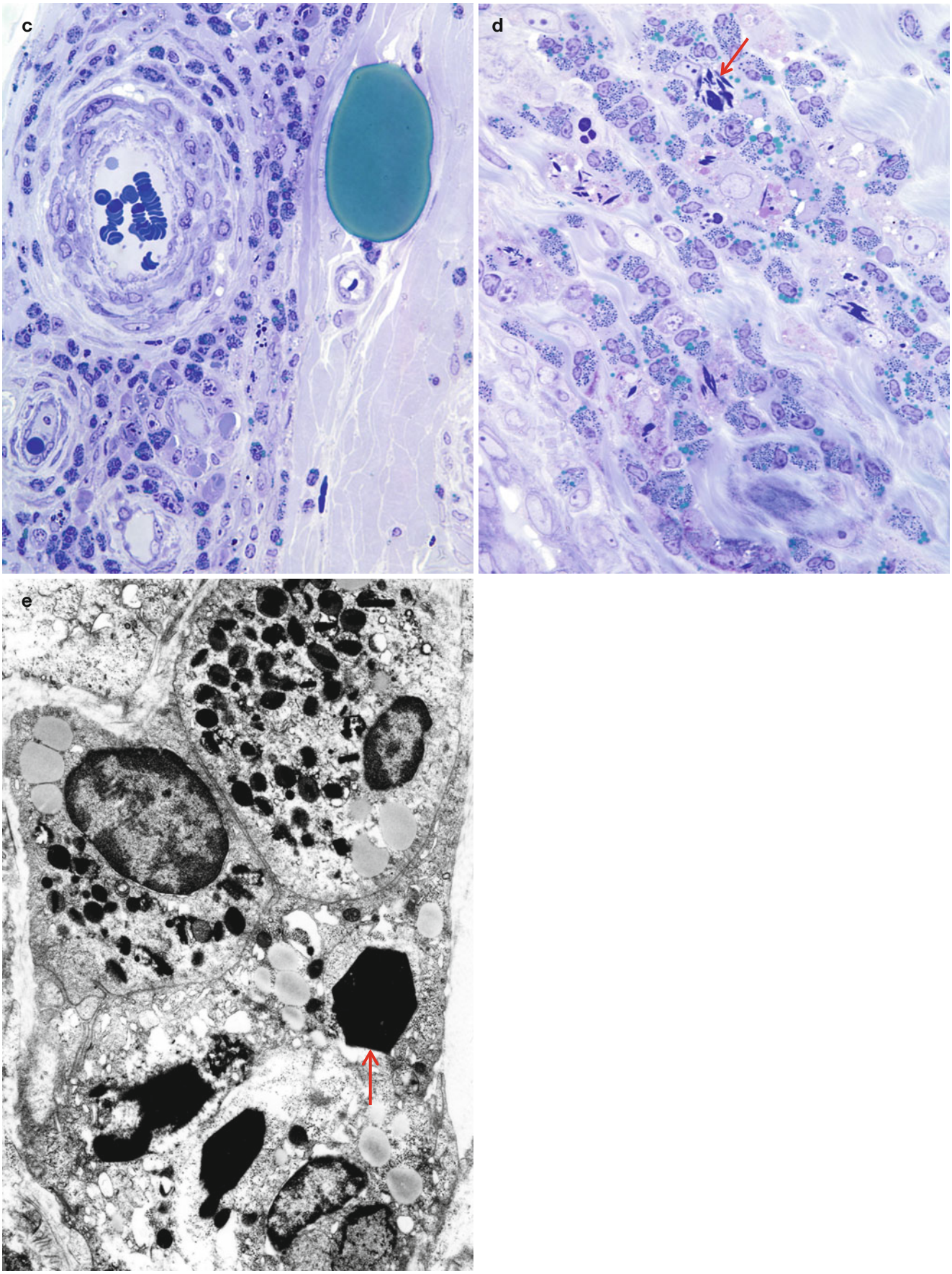


Fig. 13.8 Churg–Strauss angiitis: (**a**) eosinophils predominate in vascular and perivascular inflammation, which ranges from fibrinoid necrosis/thrombosis (**b**) to marked inflammation lacking angionecrosis. (**c**) Note Charcot–Leyden crystal formation (*arrows*, **d**, **e**) (**a**, paraffin, H&E; **b**–**d** 1 μ thick plastic sections; **e**, electron micrograph) (magnification **a**, **c**, 400×; **b**, 300×; **d**, 600×; **e**, 6,000×)



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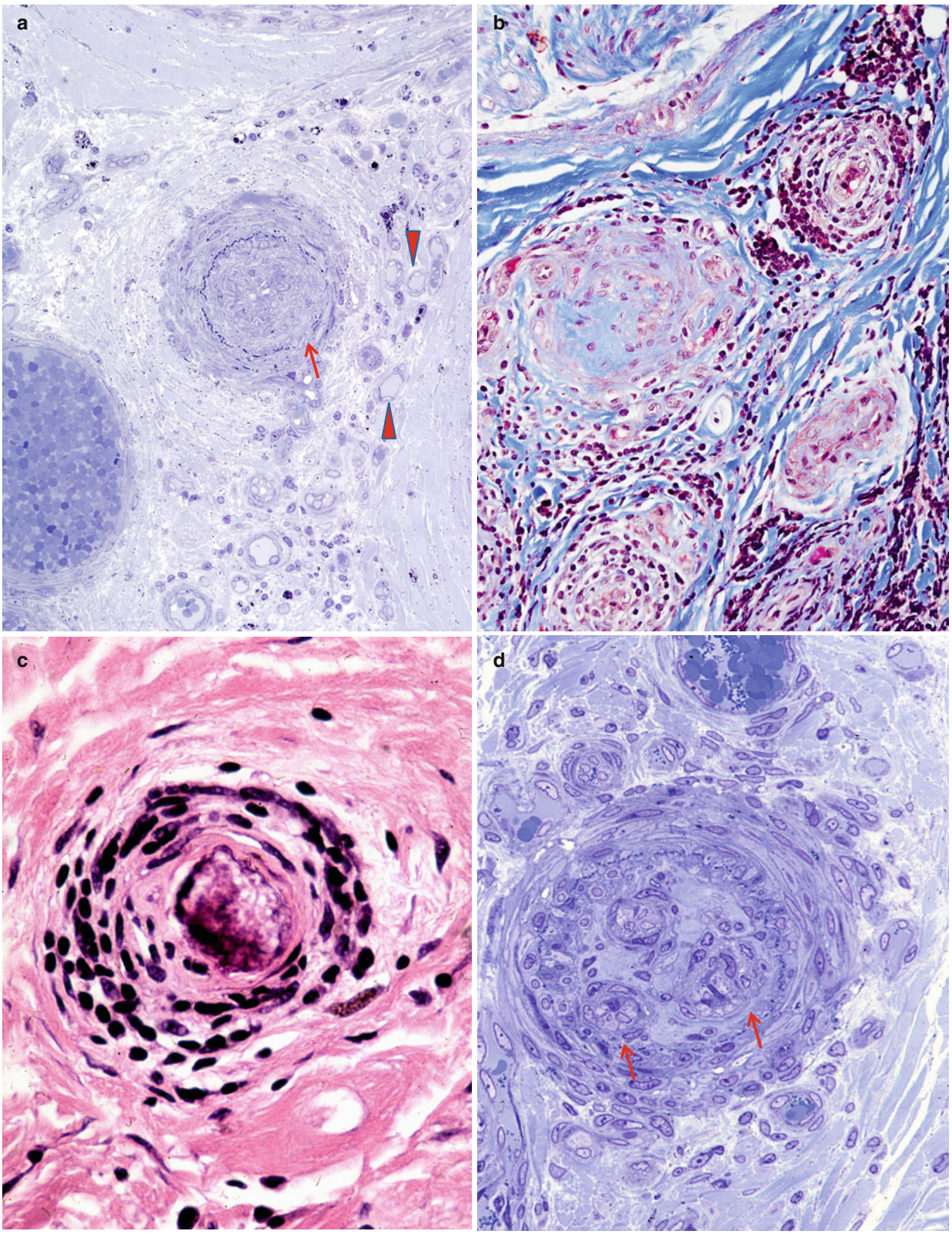

Fig. 13.9 Remote vasculitis. (**a**) A small epineurial vessel shows luminal thrombosis, loss of part of the internal elastic lamina (*arrow*) and increased numbers of small telangiectatic vessels (*arrowheads*). (**b**) One destroyed vessel shows occlusion and patchy fibrosis in this trichrome stain for collagen in the presence of damage to adjacent inflamed vessels. (**c**) Chronic inflammation surrounds a calcified epineurial vessel. (**d**) An obstructed vessel has recanalized to form small channels (*arrows*). (**e**, **f**) Recanalized small vessel has formed multiple channels resembling an intraneural vascular malformation, within its original adventitia as seen in trichrome (**e**) and smooth muscle actin immunostain (**f**). (**g**) Elastin stain shows recanalization within the remnants of the internal elastic lamina (*arrow*). (**h**) Proliferation of reactive vessels is highlighted by CD34 immunohistochemistry and elastin stain (**g**) (**a**, **d** 1 μ thick plastic sections; **b**, **c**, **e**,**f**, **g** paraffin) (magnification: **a**, **b** 400×;**c**–**f**, 400×)

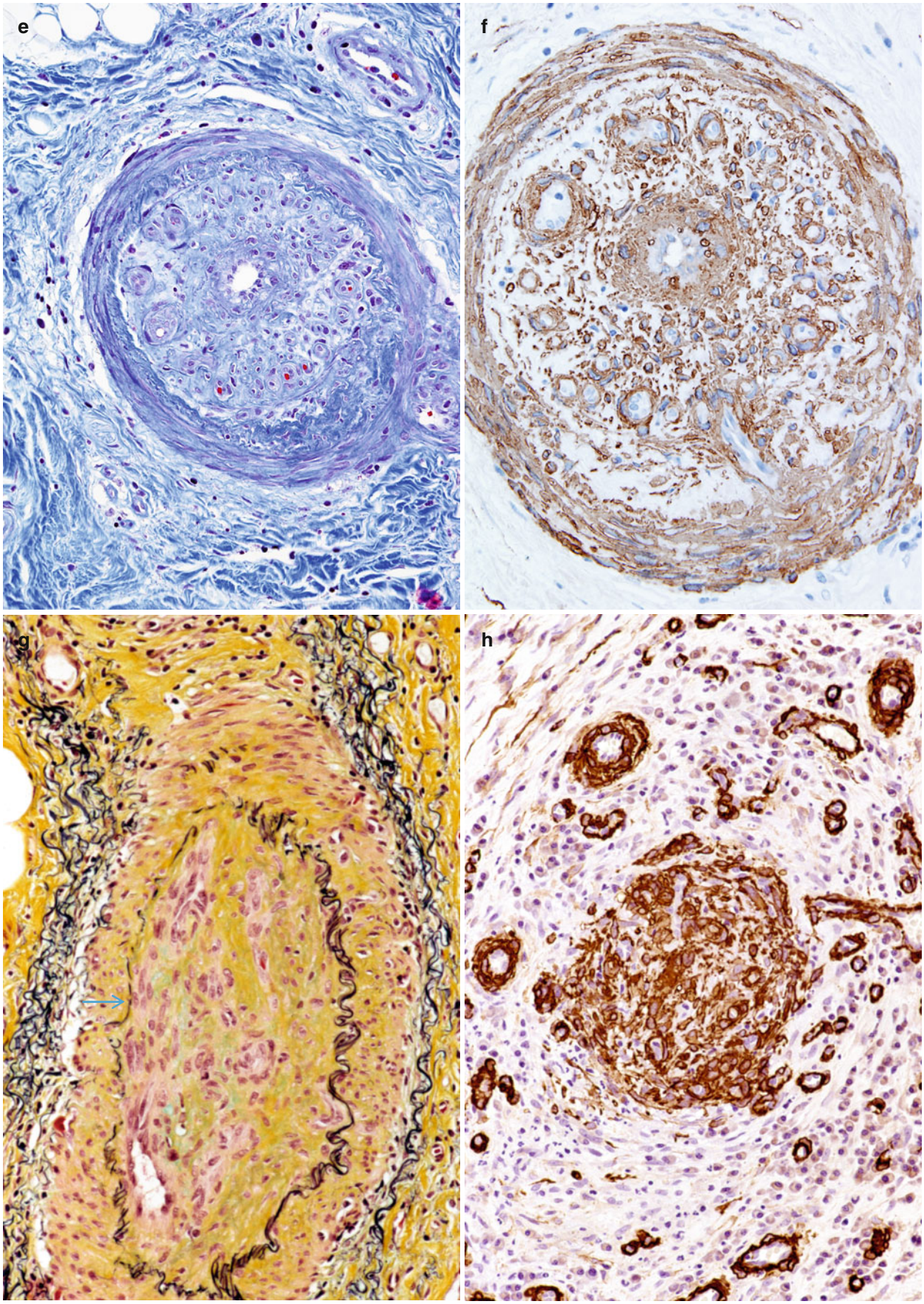

Fig. 13.9 (continued)

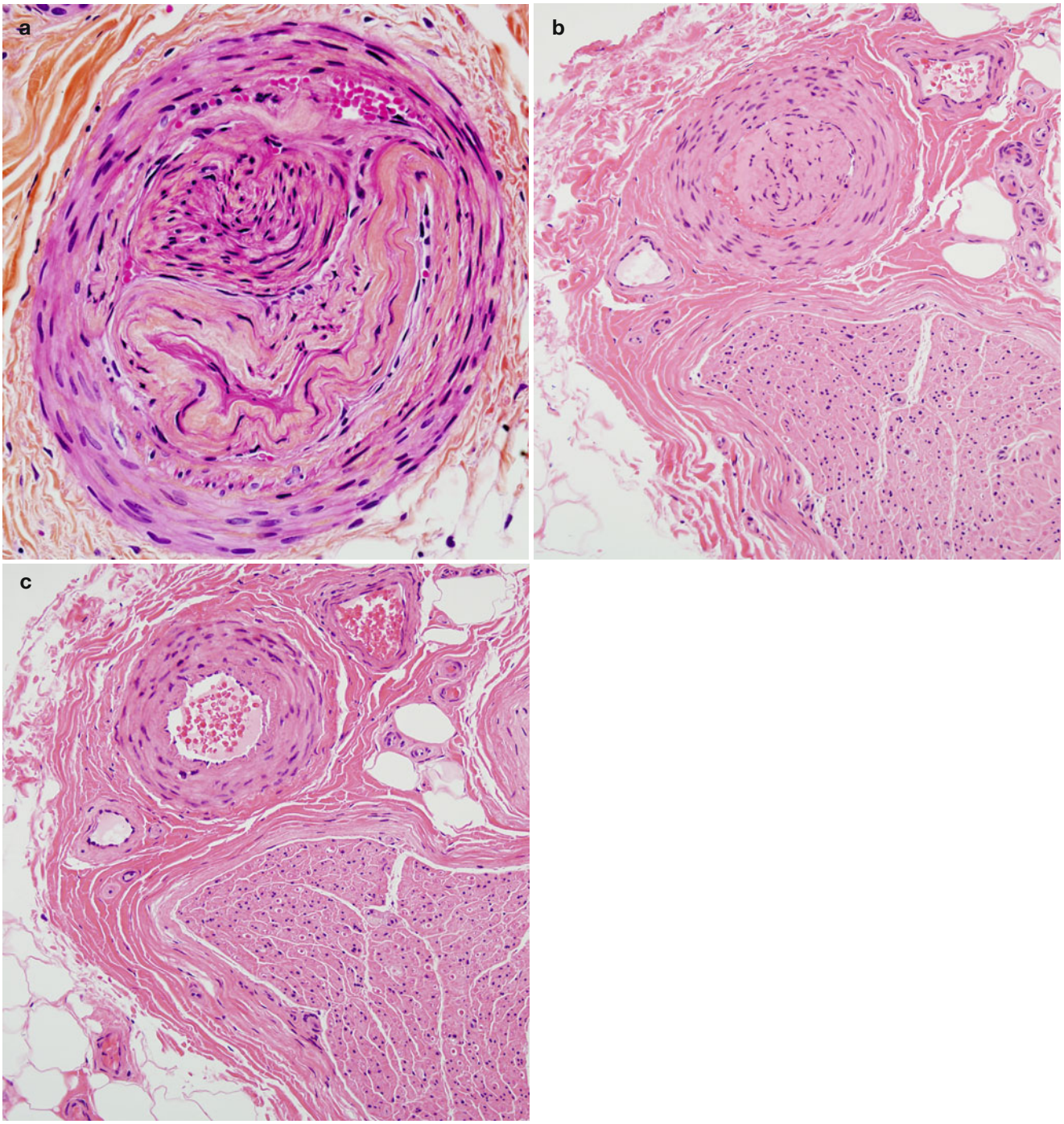

Fig. 13.10 (**a**) Retraction and invagination of arterial wall near resection site is a crushing artifact which should not be confused with healed vasculitis. Epineurial artery shows the effect of artifactual crush injury (**b**) which disappears in deeper section (**c**) (paraffin, **a** HPS trichrome, **b**, **c**: H&E) (magnification 400×)

strongly (Oh et al. 1986). This has also been our experience: eosinophils were prominent in the inflammatory infiltrate in only two of the four cases of vasculitic neuropathy in CSA that we have examined. In a study of 7 nerve biopsies in patients with neuropathy and CSA, only nonspecific perivascular inflammatory infiltrates and one perineurial granulomatous lesion were seen (Inoue et al. 1992). In three cases reported by Marazzi et al. (1992), epineurial necrotizing vasculitis was seen in 2, but eosinophils did not form a significant part of the inflammatory infiltrate, and were only occasionally seen in the necrotizing vasculitis described by other authors (Weinstein et al. 1983; Aupy et al. 1983). Eosinophils in perivascular infiltrates and peripheral nerve "inflammatory angiopathy" have also been described in eosinophilia myalgia syndrome (Smith and Dyck 1990). The idiopathic hypereosinophilic syndrome only rarely demonstrates inflammatory infiltrates, and the distinction between this syndrome and CSA may be unclear.

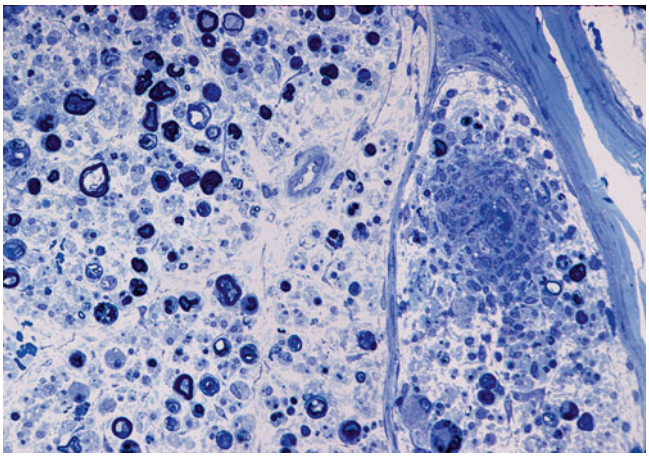

Fig. 13.11 Churg–Strauss angiitis: against a background of acute axonal degeneration, an endoneurial vessel shows transmural and perivascular inflammation (1 μ thick plastic sections, 600×)

The cellular content and characteristics of the vascular inflammatory infiltrate does not distinguish reliably between the most important disease processes: PAN, CSA, WG, and isolated PNS vasculitis (Dyck et al. 1972, 1987; Stern et al. 1965). Frankly granulomatous angiitis is infrequently seen in peripheral nerve, but the literature on systemic vasculitis suggests that granulomatous angiitis can be seen in PAN, SLE (Lie 1989,1990), or RA (Yoshioka et al. 1989), although it is more typical of Wegener's granulomatosis and CSA (Aupy et al.1983; Stern et al. 1965; Evans et al. 1992). Conversely, granulomatous angiitis is not always seen in vasculitis associated with CSA or WG (Marazzi et al. 1992; Lie 1990). Thus, classification of a systemic vasculitis cannot be based solely on peripheral nerve biopsy findings (Evans et al. 1992). The differential diagnosis of granulomatous angiitis also includes leprosy, sarcoidosis, and lymphomatoid granulomatosis.

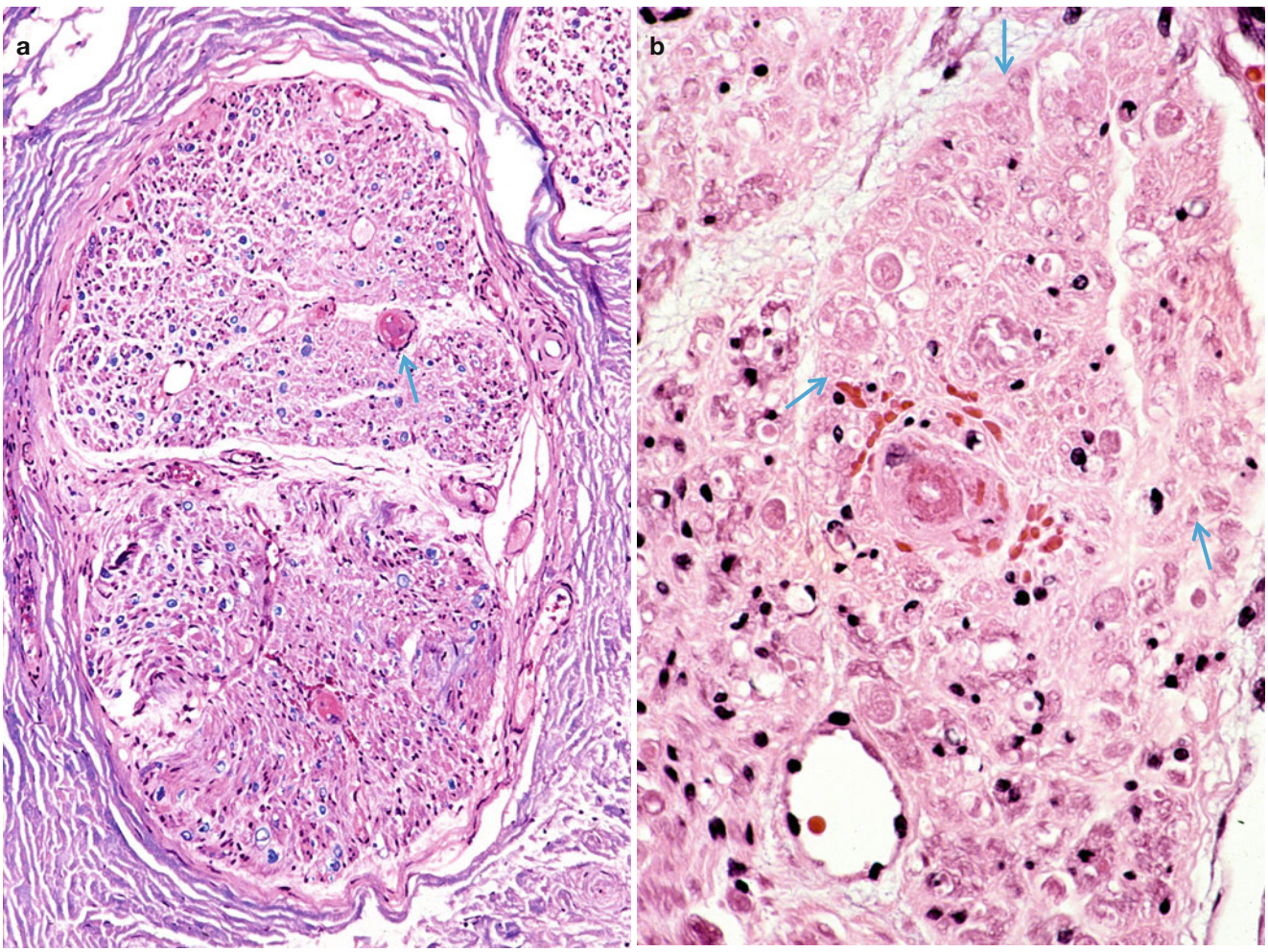

Fig. 13.12 SLE. Ischemia appears as pallor around a thrombosed microvessel (*arrow*, **a**) at the center of fascicle resulting in confluent area of necrosis (*arrow*, **b**) (paraffin, H&E, ~400×)

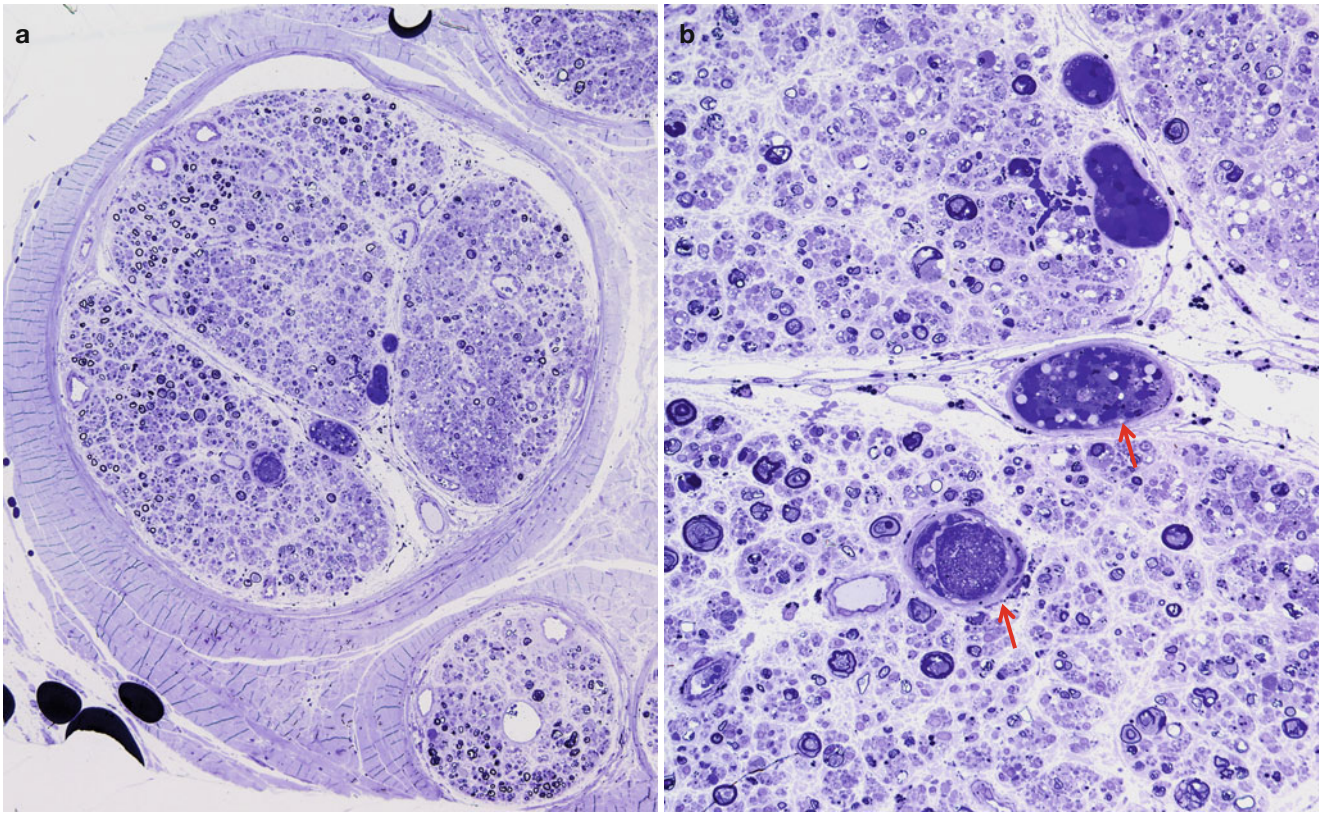

Fig. 13.13 SLE. Plastic sections demonstrate axon loss at the center of the fascicle (**a**, **b**) and thrombosis of the adjacent microvasculature (*arrows*) (1 μ thick plastic sections, magnification: **a**, 400×; B, 1,000×)

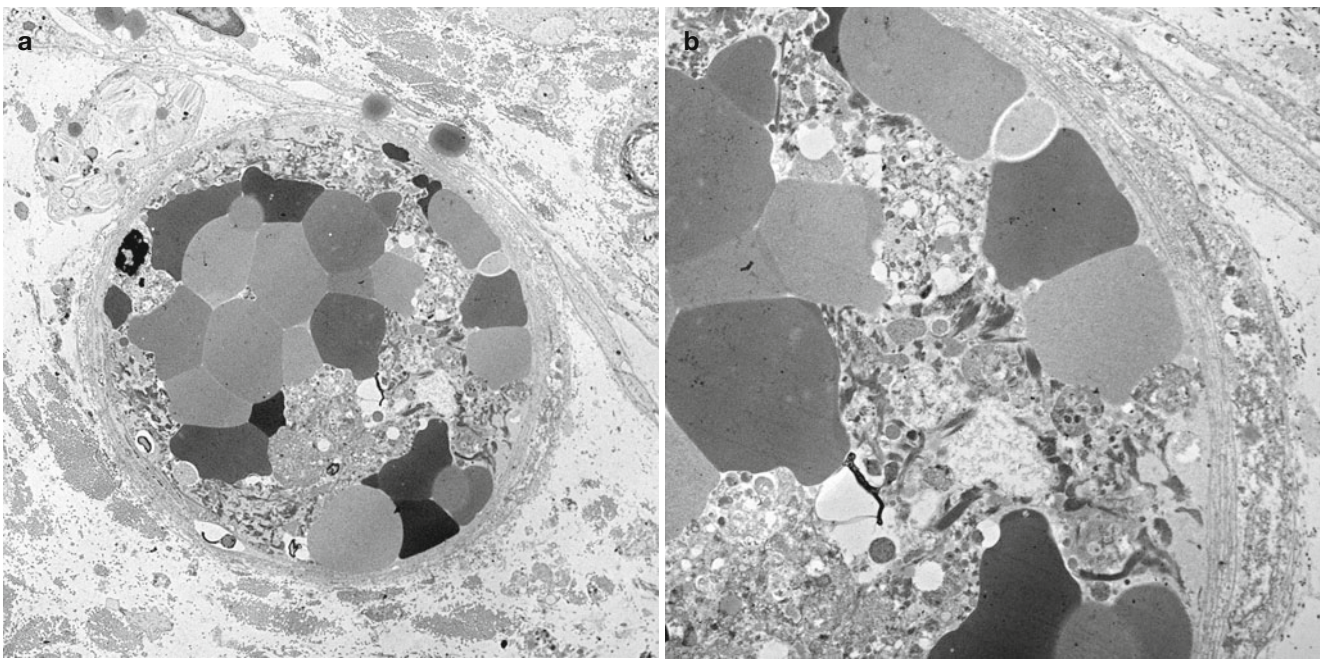

Fig. 13.14 SLE. (**a**, **b**) Ultrastructural appearance of thrombosed vessels shows an admixture of red cells, platelets, and fibrin leading to dissolution of the endothelium (magnification: **a**, 3,000×; **b**, 7,500×)

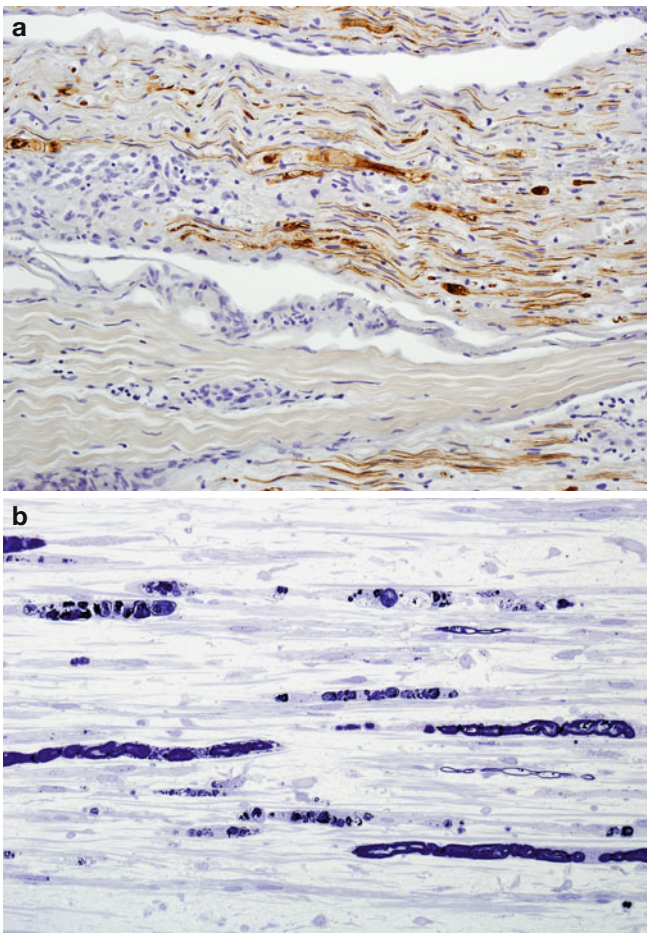

Fig. 13.15 Vasculitis. (**a**, **b**) Actively degenerating axons shown in longitudinal section as seen with neurofilament immunohistochemistry (**a**) and in plastic section (**b**) (magnification: paraffin section, **a**, 400×; **b**, 1 μ thick plastic sections, 600×)

Hemorrhage in vessel wall can be used to support a diagnosis of vasculitis, but other evidence of vasculitis should be seen, as neural hemorrhage can also be seen in hemorrhagic diathesis, which can present with a mononeuritis multiplex (Greenberg and Sonoda 1991).

13.3.3 Other Elements of Differential Diagnosis

Primary PNS vasculitis may be difficult to distinguish from CIDP if only perivascular inflammation, without typical vascular lesions, is seen. Acute axonal degeneration favors the former, while prominent segmental demyelination is characteristic of the latter. Inflammation is typically predominantly endoneurial in CIDP, but epineurial in most vasculitides. Florid perivascular inflammation is quite uncommon in CIDP.

Neural vasculitis may be seen in infectious causes of vasculitis, including leprosy in acute erythema nodosum leprosum reaction, CMV- or HIV-associated vasculitis, and Lyme neuritis. Usually the clinical information provides the necessary information, but appropriate histochemical and immunostaining can provide confirmation.

Case 13.1
Ten years prior to nerve biopsy, a 49-year-old woman was diagnosed as having SLE when she presented with fever of unknown origin, arthralgia, serositis, epilepsy, hematological abnormalities, and positive ANF. A sister also was said to suffer from SLE. Long-term treatment with steroids (5 mg/2.5 mg, alternate days) was instituted. Three years prior to nerve biopsy, the patient developed burning paresthesia in the left foot, progressing over 2 years to numbness and paresthesia of both feet. Initial neurological assessment disclosed wasting of foot muscles, no weakness, and absence of the ankle jerks only. All sensory modalities were reduced in a stocking distribution to the ankles. Electrophysiological tests revealed a mild symmetrical sensorimotor neuropathy, with borderline normal conduction velocities and reductions in CMAP and sensory amplitudes. EMG disclosed mild denervation and evidence of chronic re-innervation in foot muscles. Following sural nerve biopsy, the patient's steroid dose was increased to 30 mg/day, with reduction in ESR from 55 to 10, but little change in her symptoms. The neuropathy did not progress further and remained a relatively insignificant part of her general medical illness for the next 10 years. Following death due to cardiac arrest at the age of 68, autopsy examination revealed multisystem chronic disease consistent with longstanding SLE.

Discussion of pathology: We are aware of no other cases in the literature where tubuloreticular (undulating tubules) inclusions were detected in peripheral nerve in association with a collagen disease (discussion of TRI's: Sect. 7.5.4), although this finding is commonly seen in endothelial cells of HIV-infected patients (clinical material courtesy of Dr. D. McGillivray)

13.4 Lymphomatoid Granulomatosis

Lymphomatoid granulomatosis (LYG) is a rare, angiocentric lymphoproliferative disease EBV driven, most often affecting the lungs of affected individuals (Katzenstein et al. 2010; Dunleavy et al. 2012). Most LYG patients are middle aged with male predominance, and most do not have a history of immunodeficiency. Multisystem involvement is often seen, including peripheral neuropathy in 7–15 % (Liebow et al. 1972; Katzenstein et al. 1979). Tissues involved in LYG show an infiltrative lymphocytic, angiodestructive,

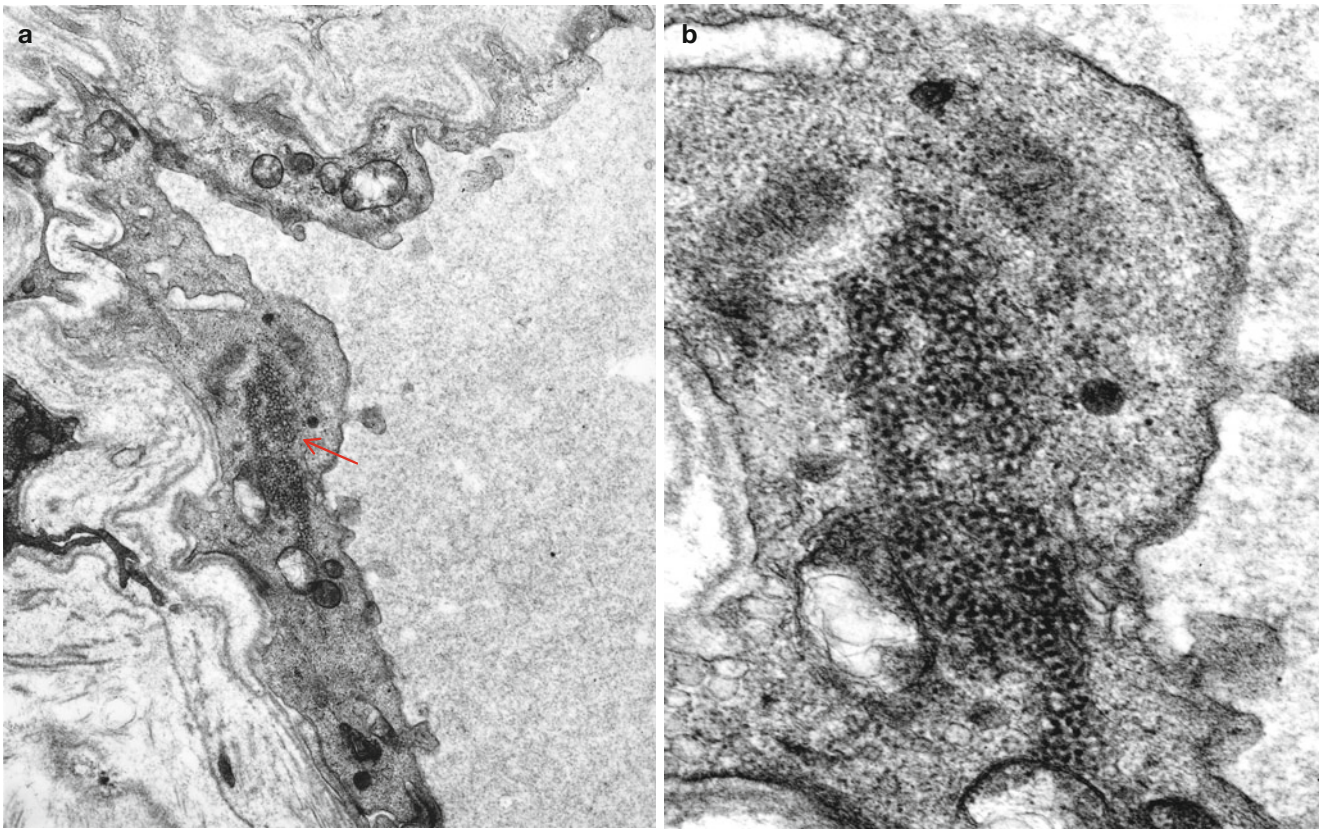

Fig. 13.16 SLE. (**a**, **b**) Endoneurial endothelial cell contains intracytoplasmic inclusion (*arrow*, **a**) also shown at higher magnification (**b**) (electron micrographs, **a**, magnification 10,000×; **b**, 39,100×)

non-leukocytoclastic process with varying numbers of large, often atypical, CD20 and CD30 and EBV-positive B lymphocytes accompanied by numerous CD3-positive small T lymphocytes and scattered admixed plasma cells and histiocytes but lack giant cells or epithelioid cell palisading. Intimal thickening of blood vessels and accompanying necrosis are demonstrable in many cases. LYG can be divided into three grades according to the prevalence and proportion of large atypical EBV-positive B cells and necrosis against a background of reactive lymphocytes (Peiper 1993; Katzenstein et al. 2010). Dunleavy et al. (2012) hypothesize that progressive oncogenic events transform lower-grade to higher-grade disease: grades I and II are polyclonal or oligoclonal, and grade III is polyclonal.

It has been suggested that LYG with increased atypia (grades 2 and 3) be classified as LYG-derived lymphoma either T-cell-rich large B cell lymphoma or diffuse large B cell lymphoma (Katzenstein et al. 2010). We discuss LYG in this chapter because vascular necrosis is part of the pathology (Dunleavy et al. 2012).

Peripheral nerve material is scanty, with Liebow's original article mentioning peripheral nerve examination in three patients, revealing atypical lymphoid cell infiltration, loss of myelin, and fibrosis, but without specific mention of vasculitis or discussion of the clinical picture in these patients (Liebow et al. 1972). Henson and Urich (1982) illustrate an example of a LYG in a woman with "unclassified lymphoma" who late in the course of her illness developed mononeuritis multiplex. Biopsy showed a necrotizing arteritis with massive lymphoplasmacytoid infiltration (Henson and Urich 1982, p 246). In another report of LYG with mononeuritis multiplex, thickened perineurium and massive diffuse and perivascular inflammatory infiltrates without necrosis of epineurial vessels were described. The two cases mentioned in this latter report mimicked tuberculoid or borderline leprosy clinically and histologically (Garcia et al. 1978). In a case we have examined (Fig. 13.18, Case 13.2), low-power views were indeed reminiscent of leprosy, but higher magnification revealed atypical mononuclear cells infiltrating all compartments of the peripheral nerve, with an angiocentric predominance (Fig. 13.18a–e). Fibrinoid necrosis was not seen, but the infiltrating cells obliterated epineurial vessels which at times showed recanalization (Fig. 13.18d, e). The atypical cellular infiltrate immunostained positively for T-cell markers (UCHL-1, CD43).

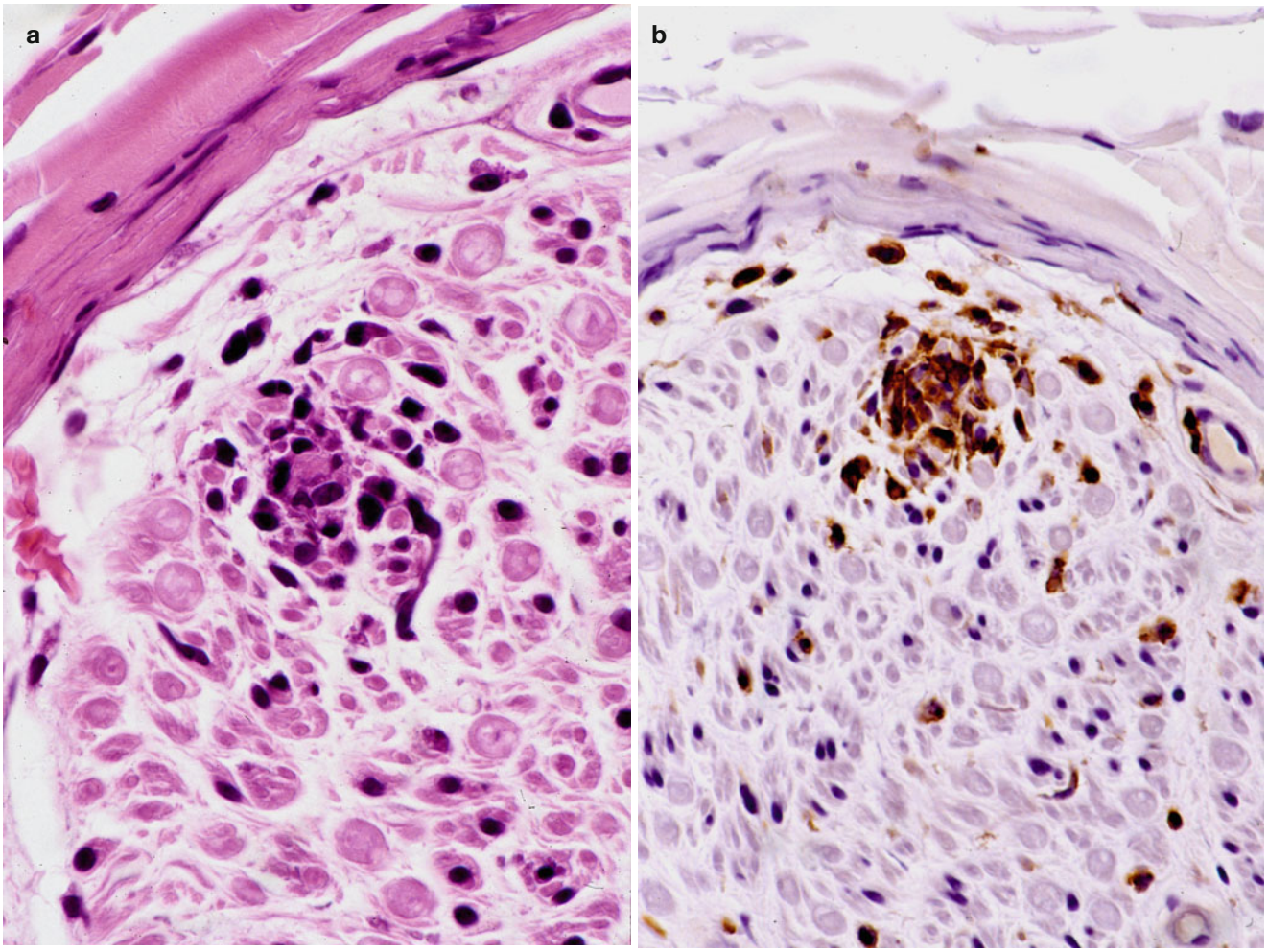

Fig. 13.17 The predominant inflammatory cell in any vasculitis is the lymphocyte (**a**), in this case involving a small endoneurial venule, of which 95 % or more are T cells (**b**) (paraffin, **a**, H&E, 600×; **b**) CD3 immunohistochemistry, 600×)

Case 13.2

A 30-year-old nickel worker experienced painful paresthesias initially involving the forearms, progressing over 3–4 months to affect almost the entire body in a multifocal distribution. At this time he began to note worsening hand weakness and wasting, more prominent on the right than on the left. A multifocal macular rash appeared, affecting his forearms most severely. During this period he experienced generalized malaise, but no fever or respiratory symptoms. Physical examination revealed a well-looking man with no evidence of pulmonary disease, organomegaly, or lymphadenopathy. No abnormalities were detected on examination of the cranial nerves. In the limbs striking atrophy and weakness were seen in the distribution of the ulnar nerves, more severe on the right. Reflexes were easily obtainable and symmetrical. There was a glove-stocking distribution of light touch and pinprick deficit, extending to mid-thigh and elbows.

Electrophysiological testing revealed a multifocal axonal neuropathy superimposed on a mild diffuse axonal sensorimotor neuropathy. CSF examination revealed a normal cell count and slightly elevated protein at 0.57 g/L. ESR, ANA, RF, C3, C4, ANCA, immunoelectrophoresis, cryoglobulins, chest X-ray, and abdominal ultrasound were normal. Bone marrow biopsy was unremarkable. Sural nerve biopsy revealed findings suggestive of lymphomatoid granulomatosis or an angiocentric lymphoma (Fig. 13.18). Because of the marked angiodestruction, the former diagnosis was favored despite the absence of pulmonary findings. Treatment with oral cyclophosphamide and prednisone resulted in a dramatic improvement of sensory symptoms within a month. Over a period of several years, he also showed clinical and electrophysiological evidence of recovery in his ulnar nerves. A second nerve biopsy performed 1 year after the first revealed only nonspecific chronic degenerative changes without cellular infiltration.

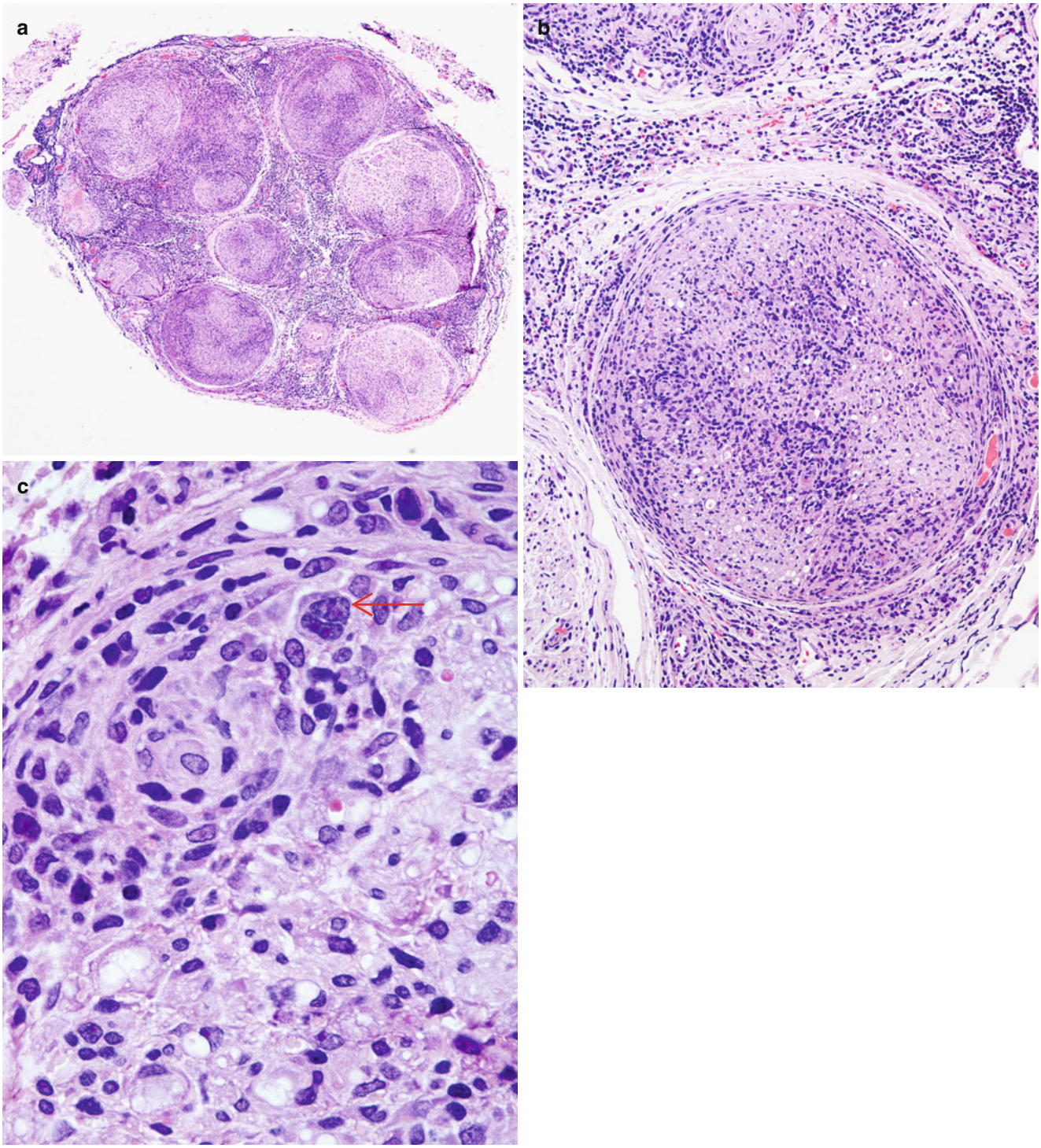

Fig. 13.18 Lymphomatoid granulomatosis. (**a**) Pleomorphic mononuclear cell infiltrate involving all compartments of sural nerve (**a**). (**b**, **c**) Note angiocentricity and the atypical appearance of scattered cells (*arrow*, **c**). (**d**, **e**) The epineurial vasculature is damaged and overrun by the inflammatory infiltrate, with recanalization (*arrows*, **d**), as shown in H&E (**d**) and PAS (**e**) (magnification: **a**, 40×; **b**, 200×; **c**, 1,000×; **d**, **e**, 400×)

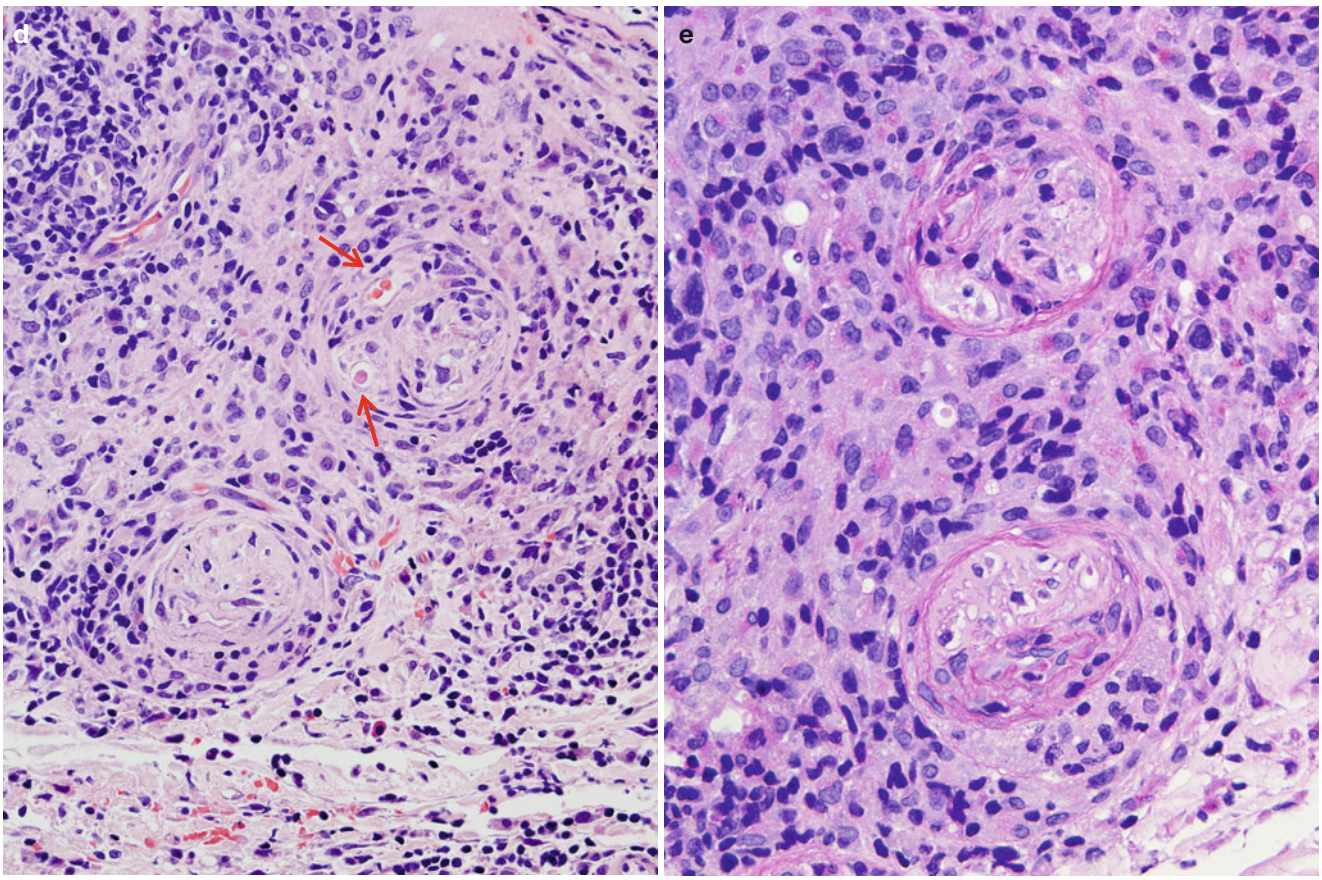

Fig.13.18 (continued)

Neuropathy has been described as preceding pulmonary findings for as long as 5 years (Katzenstein et al. 1979). This patient has now been followed for 10 years and has not developed pulmonary symptomatology or frank lymphoma (tissue courtesy of Dr. J Deck).

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Dysproteinemic Neuropathies

14

14.1 Neuropathy Associated with Paraproteinemia

A significant association exists between the presence of circulating monoclonal immunoglobulins and peripheral neuropathy (NAP, neuropathy associated with paraproteinemia, is used throughout this chapter). In large series a paraprotein has been found in 5–10 % of otherwise idiopathic neuropathies; conversely, a neuropathy is seen in up to 58 % of patients with paraproteinemia (Read et al. 1978; Kelly et al. 1981a; Osby et al. 1982; Isobe and Osserman 1971; Vrethem et al. 1993; Walsh 1971; Smith et al. 1983; Ramchandren and Lewis 2012). In some cases the clinicopathological features of NAP suggests a causal relationship, while in others the significance of the association remains to be determined. The circulating paraprotein can be seen as part of several well-defined diseases, or may be present in isolation, with the frequency of neuropathy depending on the underlying disease, but, more importantly, on the particular type of paraprotein heavy or light chain. Table 14.1 lists the dysproteinemias associated with neuropathy (Kyle 1992).

Paraproteins may consist of the entire immunoglobulin molecule or only the heavy or light chain. While there are some notable exceptions, clinical features of the paraproteinemic neuropathies are determined by the type of paraprotein (IgM vs. IgG or IgA, light chain vs. whole immunoglobulin) rather than with the associated disease. IgG is the most common class of protein in patients with benign paraproteinemia; however, IgM is more common in those patients with neuropathy (60 %, Luegetti et al. 2012), followed by IgG (30 %) and IgA (10 %). A small number of IgD paraprotein-associated neuropathies have been reported (Hansen et al. 1989; Merelli et al. 1986), but the infrequency of this finding and lack of adequate pathological study does not allow meaningful discussion of the "typical" syndrome or pathology. Thus, the individual diseases will be discussed briefly below, but clinical and pathological features will be discussed without reference to the underlying disease. Primary amyloidosis is discussed in Chap. 15.

14.1.1 Clinical Manifestations

14.1.1.1 Monoclonal Gammopathy of Unknown Significance (MGUS)

MGUS refers to a clonal expansion of B-cell lineage cells (lymphocytes and plasma cells) that secrete a monoclonal immunoglobulin (Caers et al. 2013). This is the most frequent dysproteinemia associated with peripheral neuropathy (recently reviewed in Ramchandren and Lewis 2012; Nobile-Orazio 2010; Kyle and Rajkumar 2005). "Benign" paraproteinemia is found in 1–3 % of unselected patients over the age of 50 and increases in an age-dependent fashion (Kohn 1976). The diagnostic criteria of MGUS comprise a level of monoclonal protein below 30 g/L, normal renal function, normal calcemia and hemoglobin level, bone marrow plasmacytosis below 10 %, and absence of bone osteolytic lesions, or systemic disease. The most common type of M-protein is IgG followed by IgM and then IgA. Monoclonal IgM can later transform into lymphoproliferative disorders such as Waldenström macroglobulinemia (WM), B-cell non-Hodgkin lymphoma, and chronic lymphocytic leukemia, while IgA and IgG paraproteinemias transform into MM (17 % in 10 years and 33 % by 20 years) (Luegetti et al. 2012). Light chain MGUS is a unique subtype of MGUS in which the secreted protein lacks the immunoglobulin heavy chain component (Caers et al. 2013; Lubimova et al. 2012).

Epidemiological evidence suggests an association between MGUS and neuropathy. Peripheral neuropathy is seen in 5–50 % of patients with MGUS (Kahn et al. 1980; Osby et al. 1982; Isobe and Osserman 1971; Nobile-Orazio et al. 1992). Conversely MGUS is found in 3.2–5.7 % of patients with otherwise cryptogenic peripheral neuropathy, severalfold the age-matched population prevalence (Sherman et al. 1984; Kelly et al. 1981a). Neuropathy is frequently the first manifestation of MGUS (Johansen and Leegaard 1985). Patients with MGUS and neuropathy demonstrate an IgM paraprotein far more frequently than patients with MGUS without neuropathy (Gosselin et al. 1991; Kahn et al. 1980; Yeung et al. 1991; Vrethem et al. 1993; Johansen and

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Table 14.1 Diseases associated with paraproteinemia and neuropathy

Monoclonal gammopathies of unknown significance (MGUS)
Multiple myeloma
Waldenström macroglobulinemia
Osteosclerotic myeloma and the POEMS syndrome
Primary amyloidosis (can be seen in any of the above)
Other lymphoproliferative diseases:
Lymphoma
Leukemia (CLL, hairy cell, myelomonocytic, others)
Non-amyloid immunoglobulin deposition diseases
Heavy chain disease
Cryoglobulinemia (discussed separately)
CANOMAD

Leegaard 1985; Nobile-Orazio et al. 1992). About 50–90 % of IgM paraproteins associated with neuropathy have specificity for myelin-associated glycoprotein (MAG) and/or sulfate-3-glucuronyl paragloboside (Gosselin et al. 1991; Nobile-Orazio et al. 1992; Kelly 1990; Vital et al. 1989; Yeung et al. 1991). The majority of patients with anti-MAG antibody have no associated malignancy. The association between IgA and IgG paraproteins and neuropathy is not as impressive and often may represent the chance co-occurrence of two not-uncommon situations in the elderly (Smith et al. 1983; Kelly et al. 1981a; Nobile-Orazio et al. 1992; Read et al. 1978).

14.1.1.2 Multiple Myeloma

Multiple myeloma (MM) is distinguished from MGUS by the presence of a malignant proliferative clone of plasma cells, as demonstrated by bone marrow biopsy, high or rising paraprotein levels, or multisystem disease (renal, bone marrow). Symptomatic neuropathy (IgG kappa the most commonly associated paraprotein) has been described in 3.5–10 % of patients (Silverstein and Doniger 1963; Walsh 1971). As many as 40 % of such cases may be accounted for by primary amyloidosis (Kelly et al. 1981b), and others are caused by malignant myeloma nerve infiltration (Barron et al. 1960). However, in the majority of cases the cause of the neuropathy is not identified. Subclinical neuropathy is present in up to 40 % of patients (Silverstein and Doniger 1963; Walsh 1971; Kelly et al. 1981a). When it occurs, neuropathy is often an initial manifestation of the disease (Kelly et al. 1981a; Ramchandren and Lewis 2012). IgM paraprotein rarely occurs with MM.

14.1.1.3 POEMS Syndrome and the Osteosclerotic Myeloma

Osteosclerotic myeloma (OSM) is distinguished from multiple myeloma by the absence of anemia or marrow plasmacytosis and the presence of osteosclerotic bone lesions. A strong but not invariable association is seen with the POEMS syndrome of polyneuropathy, organomegaly (liver and spleen), endocrinopathy (thyroid, gonadal), M-protein, and skin changes (hyperpigmentation, hypertrichosis, edema). The diagnosis is confirmed by demonstrating clonal proliferation of plasma cells at the site of a sclerotic bone lesion (Kelly et al. 1983). Circulating paraproteins (typically IgG or IgA lambda), found in 93 % of patients with OSM, almost invariably have a λ light chain (Kelly et al. 1983). Osteosclerotic myeloma is unique in its strong association with neuropathy, which is found in at least 50 % of patients and is almost always the presenting manifestation, with subsequent investigation leading to the correct diagnosis (Kelly et al. 1983). OSM is also unique in that removal or irradiation of a localized lesion may result in clinical improvement (Kelly et al. 1983). Most reported cases of solitary plasmacytoma with neuropathy have shown osteosclerotic bone lesions (Read and Warlow 1978).

Depending on how the syndrome is defined, 5–45 % of patients with POEMS have no bone lesions, and 14 % of those who do have bone lesions have no sclerotic changes (Nakanishi et al. 1984; Miralles et al. 1992). Similarly, a paraprotein is not found in 13–25 % of patients with POEMS, although it may appear after a follow-up period (Nakanishi et al. 1984; Miralles et al. 1992). An association also exists between OSM, POEMS, and angiofollicular lymph node hyperplasia (Castleman disease), an uncommon nonmalignant disorder that occurs as either a localized or a multicentric form, which may include other systemic signs with features of POEMS. The complexity of associated lesions has resulted in redefinition and updating the syndrome (Dispenzieri 2011).

It has been shown that an elevation of serum vascular endothelial growth factor (VEGF) levels and a variety of proinflammatory cytokines is a consistent feature of the POEMS syndrome (Scarlato et al. 2005; Nobile-Orazio 2013).

14.1.1.4 Waldenström Macroglobulinemia

WM is a B-cell malignancy characterized by a lymphoplasmacytic infiltration into the bone marrow and a monoclonal immunoglobulin M (IgM) protein in serum (Hodge and Ansell 2013). It differs from IgM MGUS on the amount of circulating paraprotein (>30 g/L in WM) and by the finding of either a 6q21 deletion or a mutation in the MYD88 gene. While some patients with WM have no symptoms at diagnosis, others present with anemia, retinopathy, bleeding, or neurological complaints. Neuropathy has been reported in 10–50 % of these patients and may precede or follow the systemic manifestations (Nobile-Orazio et al. 1987; Meier et al. 1984; Ramchandren and Lewis 2012). The circulating paraprotein (most commonly IgM kappa) in WM patients is associated with or without anti-MAG antibodies. Electrophysiological studies are indicative of demyelination.

14.1.1.5 Immunoglobulin Deposition Disease

Light chain deposition disease (LCDD) and light and heavy chain deposition disease (LHCDD) are rare conditions in which monoclonal light and/or heavy chains deposit diffusely in peripheral nerves. The light microscopic features are identical to amyloid but lack congophilia (Randall et al. 1976; Buxbaum 1992; Figueroa et al. 2012; Luigetti 2010). This amyloid-like deposition polyneuropathy occurs in patients having an IgM, kappa or lambda, monoclonal gammopathy, established WM, or MM. IgG or isolated light chains are most commonly seen in the systemic form of the disease.

14.1.1.6 CANOMAD

The constellation of chronic ataxic neuropathy with ophthalmoplegia, M-protein, cold agglutinins, and anti-disialosyl antibodies constitutes a rare disorder designated CANOMAD. The IgM proteins form antibodies against disialylated gangliosides.

14.1.1.7 Other Paraproteinemias and Lymphoproliferative Diseases

A few cases of solitary plasmacytoma have been associated with neuropathy, but most are best considered with osteosclerotic myeloma (Read and Warlow 1978). A circulating paraprotein, most often IgM, is seen in 3–18 % of patients with lymphoma, more frequently in cases with diffuse rather than nodular histology (Kyle and Garton 1987).

14.1.1.8 Neuropathic Manifestations

The neuropathy associated with paraproteinemia (NAP) is usually distal sensorimotor, symmetrical, of variable severity, and progresses over months to years. However, the syndrome can resemble polyradiculopathy or even mononeuritis multiplex (this may be a prominent picture in some patients with WM), may have predominantly motor or predominantly sensory presentations, and can progress in a subacute, stepwise, or relapsing and remitting fashion (Mamoli et al. 1991; Kusunoki et al. 1989; Rowland et al. 1982; Lamarca et al. 1987; Case records MGH 1993; Yeung et al. 1991; Gosselin et al. 1991). Reflex loss is usually prominent, CSF protein is most often increased, and nerve conduction studies usually reveal demyelinating or mixed features (Gosselin et al. 1991; Yeung et al. 1991; Meier 1985; Kelly 1983). The neuropathy associated with osteosclerotic myeloma and the POEMS syndrome is somewhat distinct in its motor predominance, greater severity, and more consistent demyelinating features (Kelly 1983). The clinical picture of the chronic and progressive polyneuropathy of CANOMAD is marked by sensory ataxia and areflexia, with sparing of distal motor strength and function (Ramchandren and Lewis 2012).

From the study of the small number of immunoglobulin deposition disease cases published, one discerns two clinical syndromes of peripheral nerve involvement: a progressive (distal to proximal), painful, asymmetric sensorimotor axonal polyneuropathy and a mononeuropathy multiplex-like picture (Leschziner et al. 2009; Luigetti et al. 2010). The relation between typical chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) without a paraprotein (Chap. 9) and a CIDP-like picture associated with a circulating paraprotein, seen in 8–30 % of "CIDP" cases (Barohn et al. 1989; Bromberg et al. 1992), is problematic. Some authors exclude the latter group of patients from the definition of CIDP (Dalakas and Engel 1981; Dyck et al. 1975; Kyle 1992) or classify them separately (Ad Hoc subcommittee 1991). Clinically the two syndromes are far more similar (manifestations, electrophysiology, CSF parameters, and response to treatment) than they are different, regardless of paraprotein immunoglobulin class (Bleasel et al. 1993; Bromberg et al. 1992; Yeung et al. 1991; Kyle 1992; Gosselin et al. 1991), and the histological distinction between the two is also potentially difficult (vide infra).

14.1.1.9 Treatment

Treatment for patients with circulating paraprotein-associated neuropathy includes plasma exchange (Sherman et al. 1984; Dyck et al. 1991; Meier et al. 1984), intravenous immune globulin (Cook et al. 1990) steroids, and cytotoxic alkylating drugs (Dalakas and Engel 1981; Smith et al. 1987; Yeung et al. 1991). All have been reported to improve clinical status, but results are unpredictable. In patients with a localized plasmacytoma or osteosclerotic myeloma, there are reports of benefit with resection or local irradiation, bortezomib, and stem cell therapy, although "cure" is unlikely (Broussolle et al. 1991; Nakanishi et al. 1984; Miralles et al. 1992; Kelly 1983; Read and Warlow 1978; Ramchandren and Lewis 2012). Plasmapheresis is more effective with IgG/IgA paraproteinemic neuropathies. Monoclonal antibody (rituximab) against the CD20 surface antigen is the most promising drug for anti-MAG neuropathy (Dalakas 2010).

14.1.2 Pathology

14.1.2.1 General Considerations

The pathological alterations observed in most cases of IgG or IgA paraprotein and some cases with an IgM paraprotein are generally nonspecific, except for increased periodicity of myelin lamellae; widely spaced myelin is highly suggestive of a subset of IgM paraproteins with activity against MAG and other myelin antigens, while uncompacted myelin is strongly associated with the POEMS syndrome. Full investigation of a patient with neuropathy may reveal the presence of a paraprotein, cryoglobulins, or a sclerotic bone lesion. In such cases, biopsy results will not modify management or give useful prognostic information unless a different diagnosis is found, perhaps vasculitis or amyloidosis. Thus we do

not recommend nerve biopsy for patients with a known paraprotein unless the picture is atypical and suggestive of a different diagnosis. A simultaneous muscle biopsy will increase the diagnostic yield in both vasculitis and amyloidosis.

The pathological findings in NAP depend more on the heavy chain type (IgG and IgA vs. IgM) than on the underlying disease (MGUS, MM, OSM, or WM). The discussion below is organized accordingly. Cryoglobulinemia is discussed separately later in this chapter.

14.1.2.2 Light Microscopy

In NAP, nerves not uncommonly show focal, epineurial, and endoneurial, infiltrates of lymphocytes and macrophages and, less frequently, plasmacytoid cells are observed (arrows, Fig. 14.1a, b) (Vital et al. 1982; Julien et al. 1984a; Luigetti 2012), although multiple myeloma can cause massive nerve infiltration (Barron et al. 1960). Some reports have documented perineurial widening due to thickening and vacuolation of perineurial cells (Iwashita et al. 1974) or excessive spacing between perineurial layers (Smith et al. 1983). A circulating paraprotein is rarely associated with necrotizing vasculitis (Gherardi et al. 1989; Panegyres et al. 1990).

The spectrum of axonal and demyelinating changes seen in IgG, IgA, and IgM paraprotein-associated neuropathies overlaps considerably. Demyelinating changes such as active myelin breakdown, denuded axons, and thinly myelinated fibers are, on average, more prominent in IgM paraprotein-associated neuropathy, especially if the IgM paraprotein has activity against MAG (Meier 1985; Smith et al. 1987; Yeung et al. 1991; Walsh 1971; Vital et al. 1989; Simmons et al. 1993; Powell et al. 1984). Active axonal degeneration and debris-filled macrophages can be present, but more common is an overall reduction in the number of fibers. Examination of a patient with IgM NAP early in her disease (Fig. 14.2a, c and 16 years later (Fig. 14.2b, d) shows the progression of axonal loss, presented as axon number vs. size distribution (Fig. 14.3) and development of onion bulbs. Onion-bulb formations, as well as redundant myelin loops and/or abnormally thick myelin, are most commonly observed in IgM paraprotein-associated neuropathies (Vital et al. 1985a, b, 1989; Nardelli et al. 1981; Meier et al. 1983; Smith et al. 1983; Rebai et al. 1989; Jacobs and Scadding 1990; Lach et al. 1993). Regenerating clusters may be seen. Unmyelinated fibers may be depleted, but this is usually overshadowed by

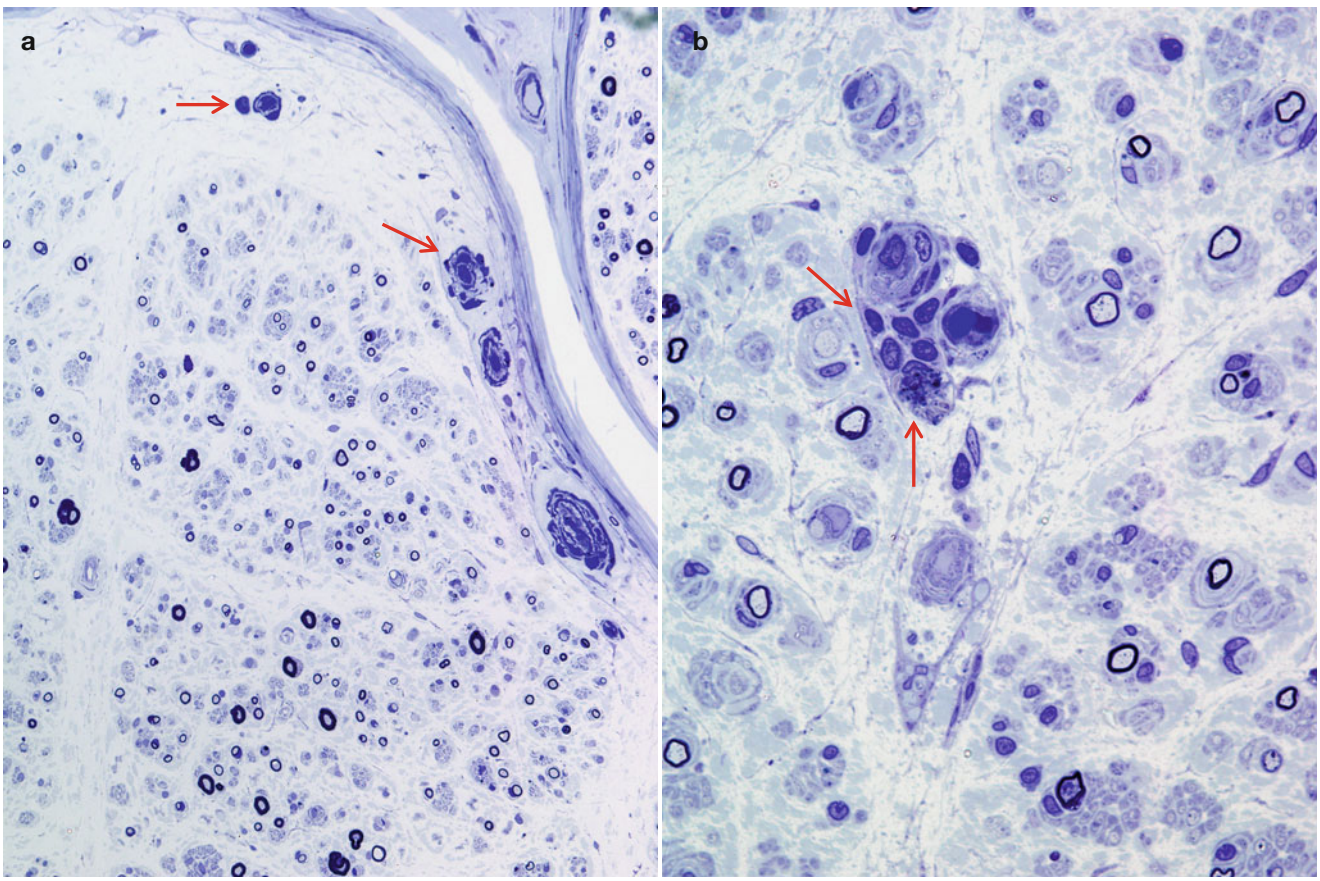

Fig. 14.1 Lambda chain MGUS NAP: semithin sections reveal rare endoneurial perivascular mononuclear cells (*arrows*, **a**, **b**), moderate dropout of fibers, and numerous thinly myelinated elements (**a**, **b** 1 μ thick plastic sections, 1,000×)

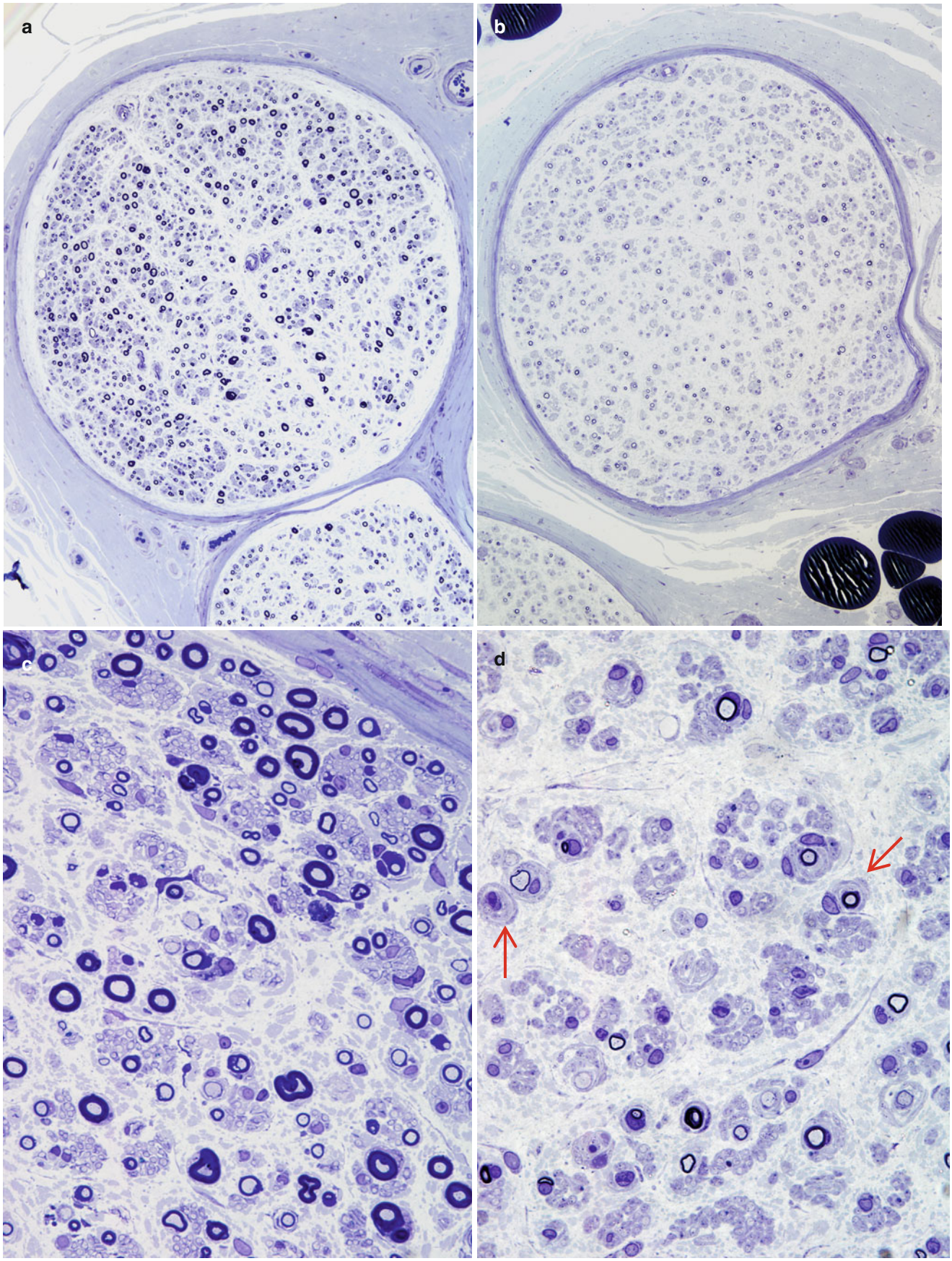

Fig. 14.2 IgM NAP: monoclonal gammopathy of 16 years' duration. Sural nerve biopsies in 1978 (**a**, **c**) and in 1993 (**b**, **d**). Note progression in severity of axonal loss and myelin alterations, with onion-bulb formation (*arrows* **d**) (1 μ thick plastic sections, **a**, **c** 100×; **b**, **d** 600×)

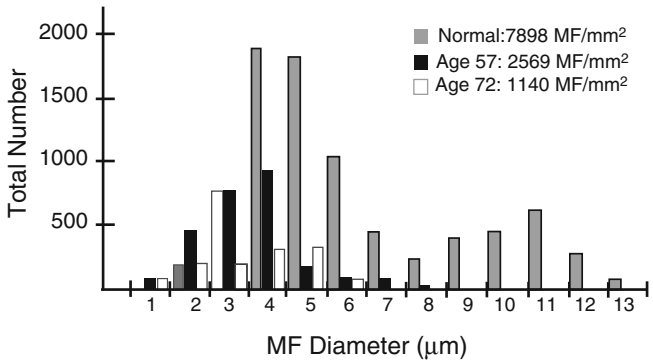

Fig. 14.3 IgM NAP: fiber diameter-frequency histograms chronicle the slow but relentless loss of axons in this acquired neuropathy of 16 years' duration

the loss of myelinated axons (Walsh 1971; Vital et al. 1989). Strong evidence exists that segmental demyelination, at least in cases not associated with an IgM paraprotein, is secondary to axonal atrophy (Ohi et al. 1985).

Nerve biopsies in the POEMS syndrome have revealed axonal degeneration, segmental demyelination, or both, with little or no inflammatory infiltration (Vital et al. 1994; Nakanishi et al. 1984; Kelly et al. 1983). Axonal degeneration predominated in the one case we have examined. Very few neuropathological studies of CANOMAD have been undertaken. In an autopsied case (McKelvie et al. 2013), there was severe dorsal column degeneration, dropout of ganglion cells in spinal ganglia, and endoneurial infiltration of clonal B-lymphocytes in cranial and peripheral nerves, dorsal roots, and cauda equina.

In *immunoglobulin deposition disease*, endoneurial, perivascular, and subperineurial deposition of PAS-positive (diastase-resistant) Congo red negative amorphous material has been reported (Dubas et al. 1987; Iwashita et al. 1974; Lamarca et al. 1987). The proteinaceous aggregates appear as hyaline eosinophilic pools associated with blood vessels and are bluish in toluidine blue-stained semithin plastic sections (Fig. 14.4a–c). While many cases exhibit immunoreactivity for kappa light chain, labeling for lambda is also on record (Leschziner et al. 2009). Using mass spectrometry of microdissected tissue, a recent paper document in three patients that the amyloid-like endoneurial amorphous aggregates result from the deposition of the whole IgM molecule (Figueroa et al. 2012). This contrasts with primary amyloidosis, where the intraneural deposits consist entirely of Ig light chains.

14.1.2.3 Electron Microscopy

An increase in myelin periodicity has been the most discussed histological feature in paraproteinemic neuropathies. The distinction between uncompacted myelin (UCM) and widely spaced myelin (WSM) is characterized by alterations in the patterns of periodicity (vide infra). Additional details of the ultrastructural features and possible mechanism of formation are provided in Chap. 5. Trauma during nerve handling, especially traction, can cause splitting of the delicate myelin sheath, which may mimic alteration in myelin periodicity. This usually manifests as a wavy, irregular separation of myelin lamellae which should not be difficult to distinguish from abnormalities of myelin periodicity (Fig. 7.1).

WSM has been described more frequently and is due to lack of apposition of the outer (extracellular) aspects of the Schwann cell membrane. The intraperiod line can no longer be identified, as the normal 2–4 nm separation between paired membranes is increased to 20–30 nm (Fig. 14.5a–d) and represents a virtual extracellular space. Intrusion of anti-MAG antibodies opens the intraperiod line, producing a wide space between the myelin lamellae (20–30 nm) resulting in myelin breakdown and remodeling (Kawagashira et al. 2010). Although there is a tendency for WSM to be seen in the outer layers of the myelin sheath, it has been described in the inner layers or throughout the entire thickness of the sheath. WSM is seen in 50–90 % of an IgM paraprotein-associated neuropathies, and anti-MAG antibody activity is usually, but not invariably, found in these cases (King and Thomas 1984; Vital et al. 1989; Yeung et al. 1991). Unusual configurations of redundant myelin (Fig. 14.6) may be found involving WSM. Two cases with WSM have been reported in the setting of IgG or IgA paraprotein-associated neuropathy (Powell et al. 1984; Vital et al. 1989).

A different pattern of altered myelin periodicity has been designed uncompacted myelin (UCM). In UCM the major dense line is not formed because the inner (cytoplasmic) aspects of the Schwann cell membrane do not fuse (Fig. 14.7a–c). This results in an axon wrapped in spirals of Schwann cell cytoplasm. Vital et al. (1994) found this alteration in 1–16 % of internode cross sections in 19 of 22 biopsies in patients with POEMS syndrome, while Ohnishi (1984) indicated its presence in 3–8 % of cross sections in "over half" of nine cases of POEMS syndrome. A circulating paraprotein is usually, but not invariably, present, usually IgG or IgA (Bergouignan et al. 1987; Gherardi et al. 1988; Ohnishi and Hirano 1981; Vital et al. 1985b, 1994). Macrophage-mediated myelin stripping, generally regarded as the hallmark of the inflammatory demyelinating neuropathies GBS and CIDP, is occasionally seen in IgM (Vital et al. 1991b) and IgG (Bleasel et al. 1993; Pollard et al. 1983) paraprotein-associated neuropathies.

Cases of IgM paraprotein-associated neuropathy having the features of immunoglobulin deposition disease show focal or diffuse deposition of granular material, lacking the distinctive "rigid" fibrillar ultrastructure of amyloid, in the endoneurium or around endoneurial vessels (Fig. 14.8a, b) (Dubas et al. 1987; Iwashita et al. 1974; Lamarca et al. 1987; Meier et al. 1984; Vital and Vital 1993). This material does

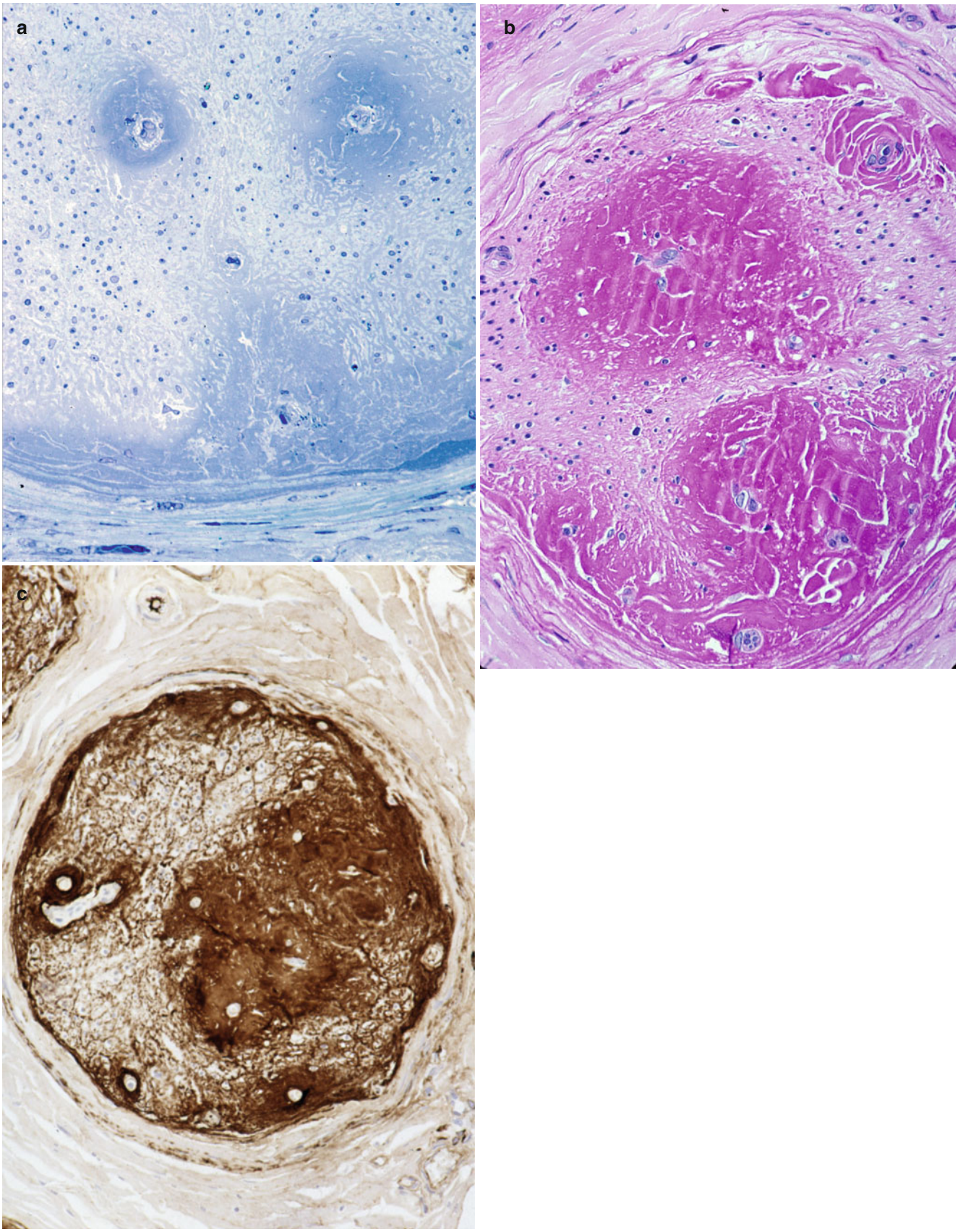

Fig. 14.4 Kappa chain NAP: (**a**) This fascicle shows a desolate endoneurium with no residual nerve fibers. Note subperineurial and perivascular deposition of amorphous material which is PAS positive (**b**) and Congo red negative. Intense immunoreactivity for kappa light chain is obtained (**c**) (**a**: 1 μ thick plastic sections 200×; **b**, **c**: paraffin 100×)

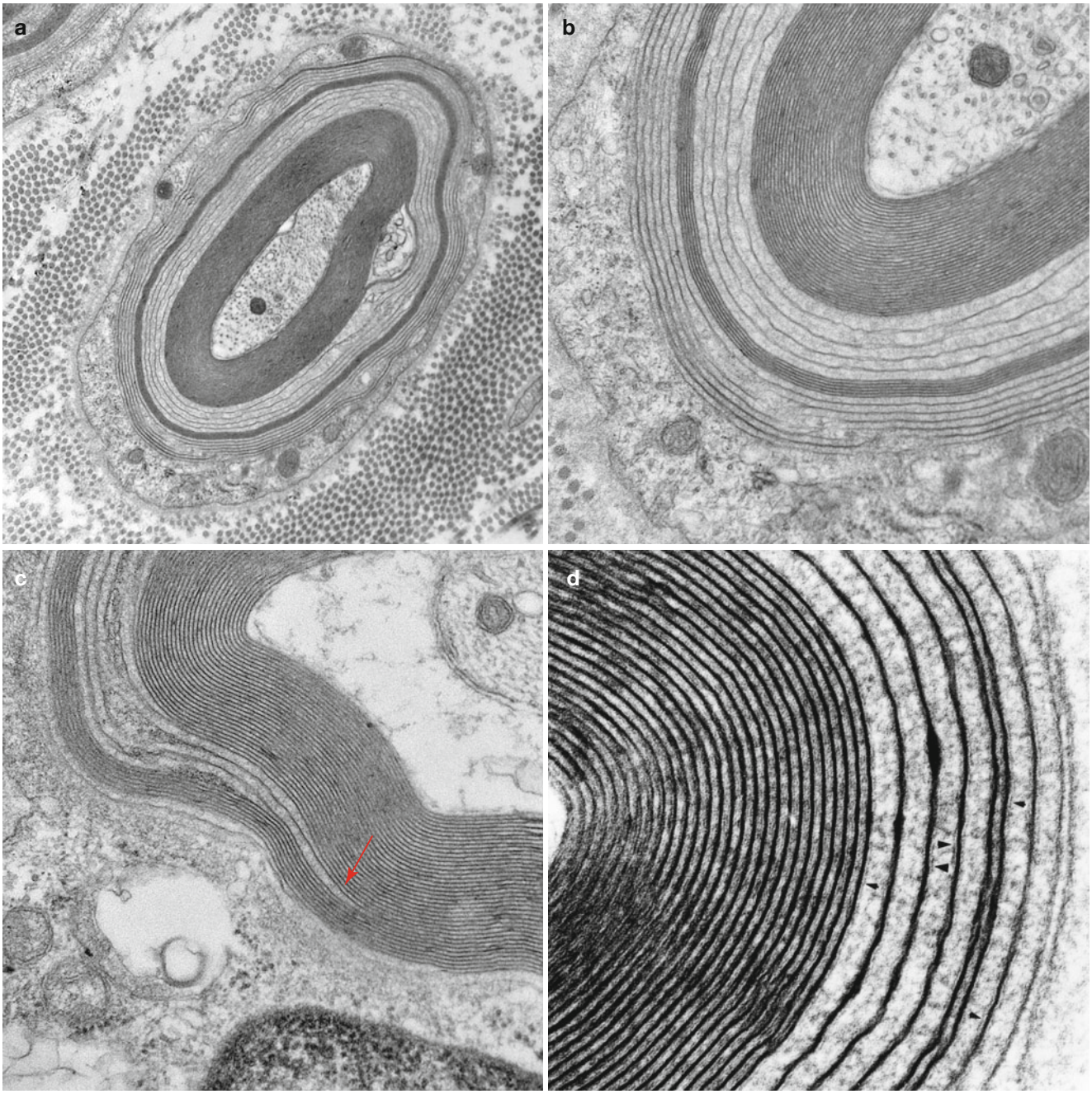

Fig. 14.5 IgM NAP: (**a**–**d**) Widely spaced myelin (WSM). (**a**, **b**) This myelinated axon shows the classic features of widely spaced myelin involving some of the lamellae, seen at higher magnification in (b). (**c**) Point of myelin separation (*arrow*, **c**). (**d**) Residual fragments of the intraperiod line are seen in the areas of WSM (*arrowheads*, **d**) (**a**, 20,000×; **b**, 50,000×; **c**, 60,000×; **d**, 137,000×)

not stain histochemically for amyloid, but will immunostain for light and/or heavy chain of the circulating paraprotein. At times this deposited material seems to cause Schwann cell and vascular injury (Lamarca et al. 1987; Meier et al. 1984; Vital and Vital 1993). Endoneurial accumulation of amorphous or granular electron opaque material and small bundles of 10–18 nm diameter filaments has been also reported in IgM paraproteinemia with anti-chondroitin sulfate specificity (Yee et al. 1989; Sherman et al. 1983). Subperineurial and endoneurial elaunin collections have at times been mistaken for endoneurial immunoglobulin deposition (Fig. 14.9a) (Chazot et al. 1976; Carrier et al. 1978; Fitting et al. 1979). A patient with axonal neuropathy and an IgG kappa paraprotein whose peripheral nerve showed "immunotactoid-like" material intra-axonally and in endoneurium (Moorhouse et al. 1992) has been reported.

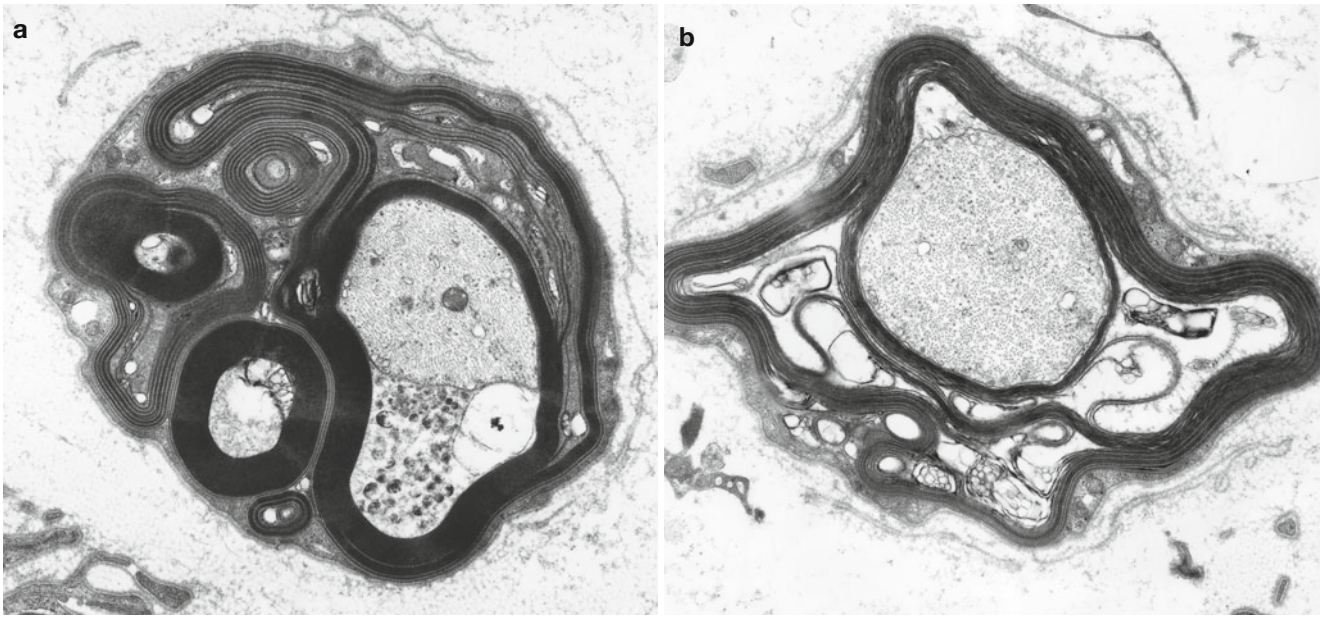

Fig. 14.6 IgM NAP: Unusual configurations of redundant myelin with WSM are shown (**a**). Note intramyelinic splitting and vesicular degeneration (**b**) (**a**, 14,910×; **b**, 22,800×)

This material was amorphous on light microscopy but stained positively for IgG. Ultrastructurally, the deposits were composed of straight or slightly curved single, bi-, or tri-layered tubular structures 50–300 nm in diameter. The neural microvasculature appeared damaged.

Microangiopathy has been emphasized by Powell et al. (1984) in neuropathy associated with IgG and IgM paraproteinemia. These authors demonstrated swollen capillary endothelial cells filled with microfilaments. A similar finding was seen in 1 of 31 biopsies in IgM gammopathy and neuropathy (Vital et al. 1989). However, a degree of endothelial cell hypertrophy, reduplication of basement membrane, and filamentous accumulations in endothelial cells are all common nonspecific findings of chronic neuropathy. Definitive evidence of an impaired blood nerve barrier, such as fenestration, gaps between endothelial cells, or excessive pinocytotic vesicle activity, has also been observed (Fig. 14.9b) (Lach et al. 1993; Powell et al. 1984; Meier et al. 1984).

Ultrastructural examination allows close assessment of axonal viability and provides evidence of primary demyelination when degenerating myelin is seen around healthy axons. Some authors (Jacobs and Scadding 1990; Ohi et al. 1985; Mendell et al. 1985) have emphasized axonal atrophy as evidenced by the finding of myelin inappropriately wide for axon diameter or retraction of the axons from the myelin sheath (Fig. 14.10). However, the former change is hard to separate from hypermyelination (commonly seen in IgM paraproteinemic neuropathy), while the latter may be artifactual. Such an atrophic appearance is currently thought to reflect intramyelinic edema. Quantitative studies by Ohi et al. (1985) support the hypothesis that axonal atrophy is present, as do autopsy findings in another case (Mendell et al. 1985). Evidence of unmyelinated fiber loss, with stacks of Schwann cell processes (Bands of Büngner) and collagen pockets, can often be seen, indicating that unmyelinated axons are not always spared (Vital et al. 1989).

14.1.2.4 Immunohistochemistry

Immunohistochemistry for the diagnosis of paraproteinemic neuropathy may give results that are inconsistent and difficult to interpret. Direct immunofluorescence of frozen tissue and immunoperoxidase staining of frozen or fixed tissue have been used in NAP to search for paraprotein binding to nerve (Fig. 14.11). Similarly, immunogold staining has conclusively demonstrated that IgM is found in the regions of widened myelin (Fig. 14.12) (Lach et al. 1993). Many reports demonstrate localization of the clonal immunoglobulin (heavy or light chain) to the myelin sheath (Mendell et al. 1985; Propp et al. 1975; Stefansson et al. 1983; Vital et al. 1982, 1989; Meier et al. 1983; Dellagi et al. 1983; Takatsu et al. 1985; Smith et al. 1983; Schenone et al. 1988; Dubas et al. 1987; Johansen and Leegaard 1985; Pollard et al. 1985; Yeung et al. 1991). When neuropathy is associated with an IgM paraprotein, this technique is positive in 40–80 % of cases (Dellagi et al. 1983; Dubas et al. 1987; Yeung et al. 1991), usually in the setting of a paraprotein with anti-MAG activity (Nobile-Orazio et al. 1987; Vital et al. 1989; Yeung et al. 1991; Takatsu et al. 1985). Myelin binding can be to the periphery of the sheath (Mendell et al. 1985; Pollard et al. 1985) or to its entire thickness (Smith et al. 1983).

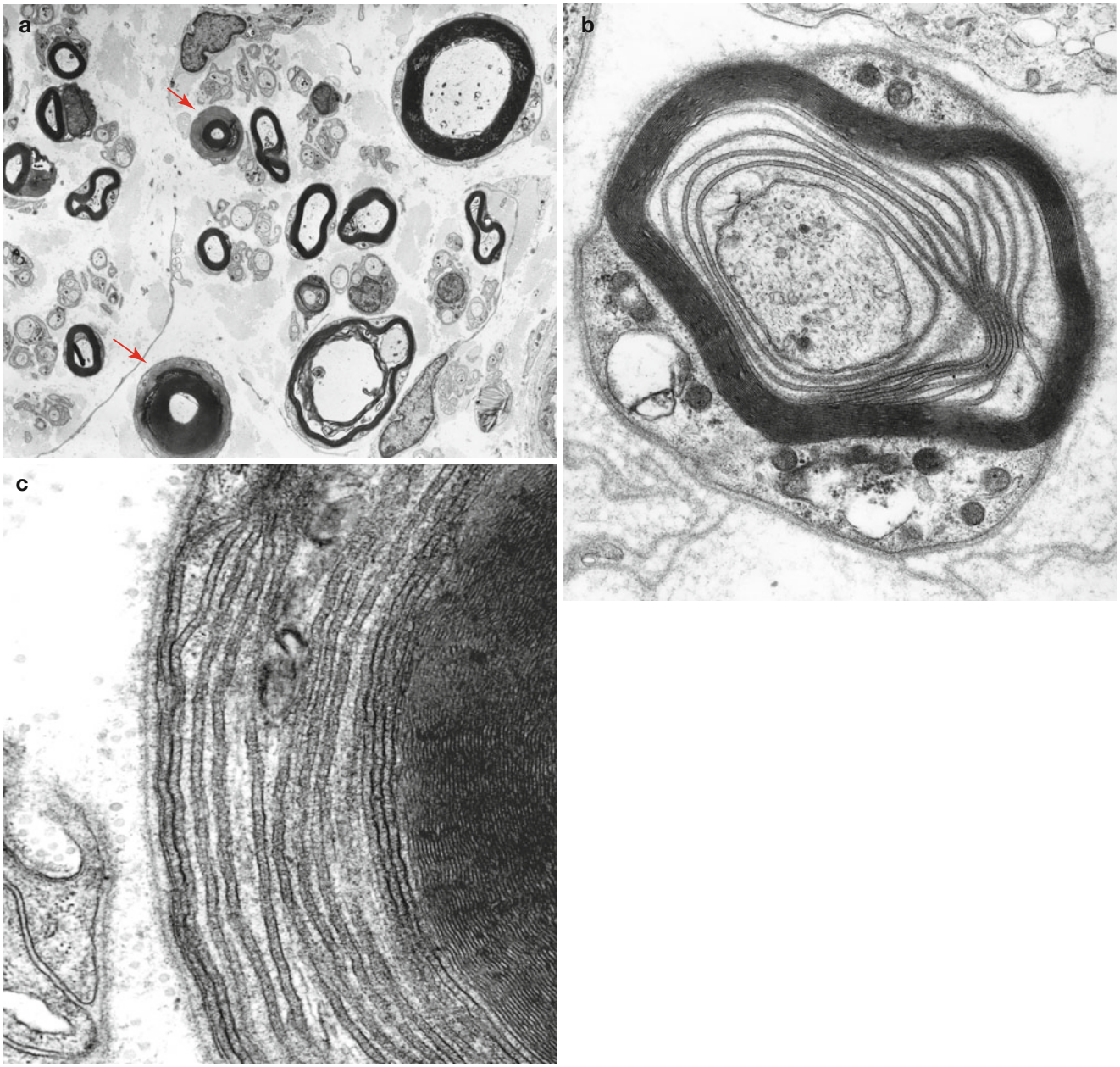

Fig. 14.7 Uncompacted myelin (UCM) two axons show uncompacted myelin (*arrows*, **a**). At higher magnification loss of myelin compaction is seen which shows separation at the major dense line

In IgG and IgA NAP, this finding is uncommon (Yeung et al. 1991; Dalakas and Engel 1981; Bailey et al. 1986; Bleasel et al. 1993; Sewell et al. 1981). Similar binding may be present in asymptomatic patients with a paraprotein (Dellagi et al. 1983) and in CIDP (Dalakas and Engel 1980). Indirect immunofluorescence using patient serum and animal or human nerve is often positive and can be performed on fixed or frozen material (Dellagi et al. 1983; Takatsu et al. 1985).

Positive perineurial anti-IgM immunostaining is a common but nonspecific finding (Propp et al. 1975; Johansen and Leegaard 1985; Yeung et al. 1991; Nobile-Orazio et al. 1987). However, positive endoneurial immunostaining for IgM is an important observation (Vital et al. 1989; Takatsu et al. 1985; Jonsson et al. 1988; Chazot et al. 1976; Vital and Vital 1993). A few such cases have been associated with anti-chondroitin sulfate IgM paraprotein-associated neuropathy (Sherman et al. 1983; Yee et al. 1989), typically presenting with an axonal sensory neuropathy and showing diffuse staining of endoneurial connective tissues. Focal areas of positivity likely correspond to the local immunoglobulin deposits discussed above (Iwashita et al. 1974; Chazot et al. 1976; Vital and Vital 1993). Light chain deposits may also be

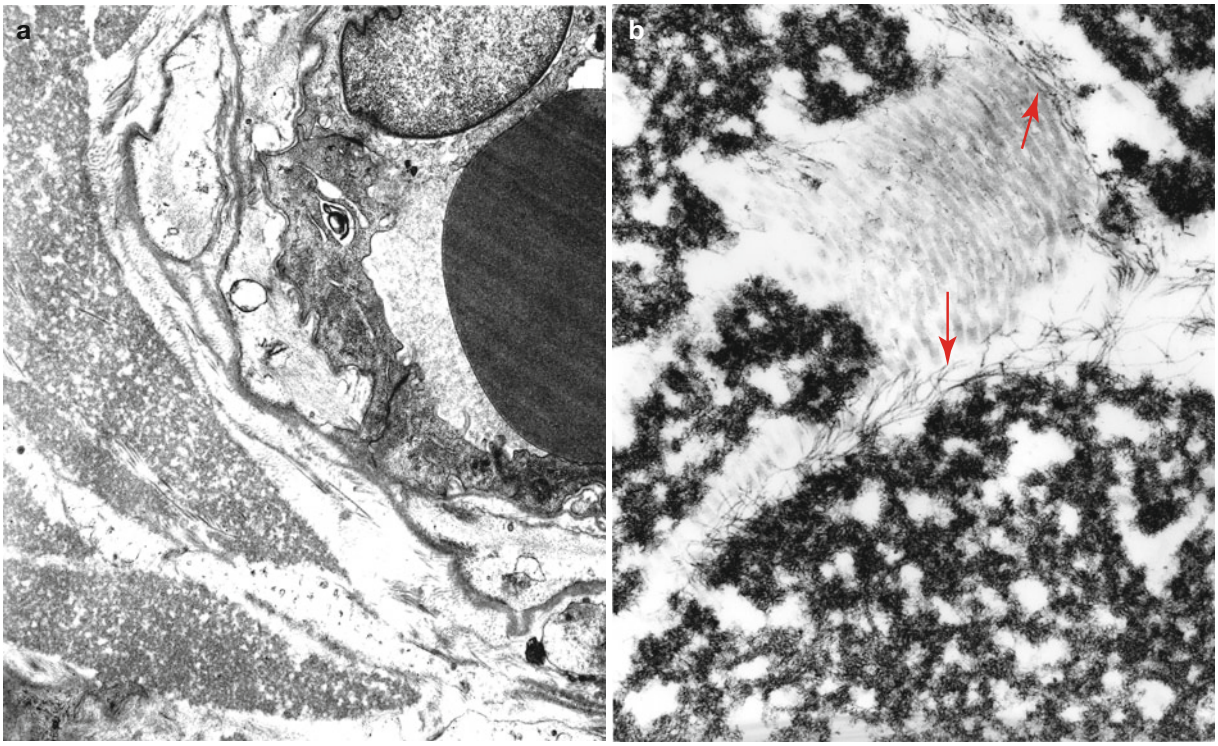

Fig. 14.8 Kappa chain NAP: (**a**) Ultrastructure demonstrates a perivascular granular deposit without features of amyloid. (**b**) Endoneurium shows collagen fibers, oxytalan filaments (*arrows*), and granular osmiophilic material considered typical of non-amyloid light chain (same case as Fig. 14.4). (**a**: 15,000×; **b** 33,800×)

detected (Fig. 14.13). In IgG and IgA paraprotein-associated neuropathies, immunohistochemistry is of little value because IgG and, to a lesser extent, IgA can pass through an intact blood nerve barrier, so their presence in endoneurium in the setting of increased serum levels would not be surprising.

14.1.3 Pathogenesis

Although the clinical syndromes and pathology of the various paraprotein-associated neuropathies overlap, the IgM-associated neuropathies are somewhat distinct clinically and pathologically and will be considered separately. Only in IgM paraprotein with activity against MAG and related antigens has a causal relation between paraprotein and neuropathy been convincingly demonstrated.

14.1.3.1 IgM "Anti-MAG" Paraprotein-Associated Neuropathy

Reactivity toward MAG is seen in 50–90 % of IgM paraproteins associated with neuropathy (Nobile-Orazio et al. 1989; Quarles 1989; Kelly 1990; Vital et al. 1989; Yeung et al. 1991), and there is a strong correlation between the presence of anti-MAG IgM in myelin and the presence of a demyelinating neuropathy with widened myelin lamellae (Vital et al. 1989; Mendell et al. 1985; Monaco et al. 1990; Yeung et al. 1991). The use of immunogold technique has conclusively demonstrated the localization of IgM and light chain to the separated myelin lamellae in one case (Lach et al. 1993). The central question is whether presence of the antibody in myelin is an epiphenomenon or a primary event in the neuropathy.

The cellular localization and possible importance of MAG is discussed elsewhere. Myelin-associated glycoprotein is a member of the family of cell adhesion molecules and may play a role in maintaining the ultrastructural integrity of the myelin sheath– axon unit (Quarles 1989; Trapp and Quarles 1982). Interference with this function may result in demyelination and the widened myelin lamellae often seen in IgM-associated neuropathies (Trapp and Quarles 1982). Lombardi et al. have demonstrated IgM deposits on dermal myelinated fibers in all their patients with anti-MAG neuropathy (Lombardi et al. 2005). A convincing anti-MAG IgM passive transfer experiment, with chronic intraperitoneal administration of human anti-MAG IgM to rats, produced a neuropathy with morphologic similarity to the human disease (Tatum 1993).

Despite compelling evidence for the causal role of anti-MAG IgM in neuropathy, unresolved questions do exist. More detailed studies have revealed that the anti-MAG specificity of the antibody is actually directed against an antigenic carbohydrate epitope present on MAG as well as other glycoproteins and glycolipids, some of which are more

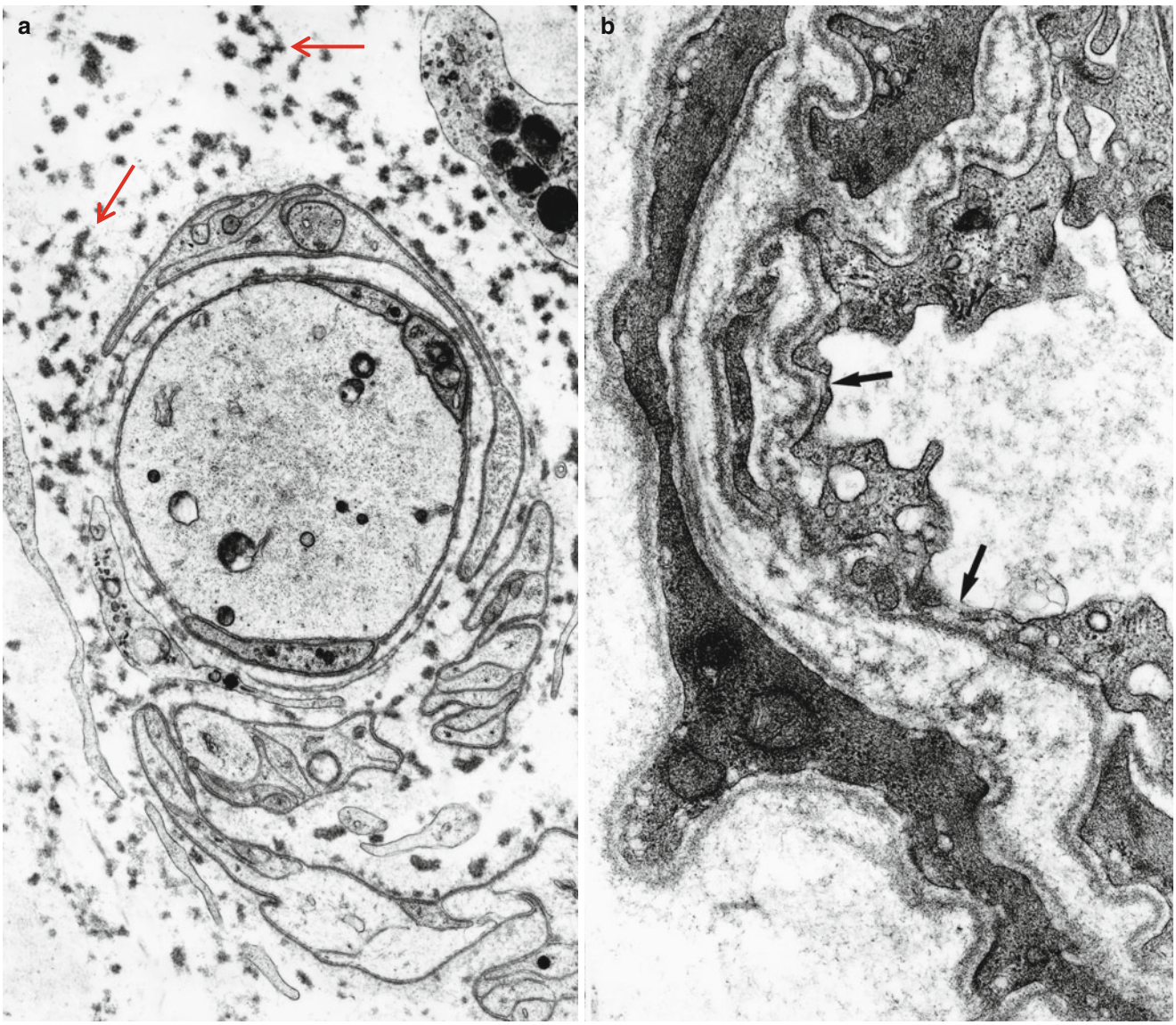

Fig. 14.9 IgM NAP: Endoneurial deposition of elaunin is prominent (*arrows*, **a**). "Porous" endothelium is noted in endoneurial vessels (*arrows*, **b**) (**a**, 8,800×; **b**, 32,200×)

specific for peripheral nerve than MAG itself (Quarles 1989; Nobile-Orazio et al. 1984; O'Shannessy et al. 1986; Steck et al. 1987; Bollensen et al. 1988; Shy et al. 1984; Lieberman et al. 1985; Kusunoki et al. 1987; Monaco et al. 1990). Some IgM paraproteins associated with neuropathy have anti-myelin activity against glycolipids but not MAG. Thus, myelin glycolipids may be a more important antigenic target than MAG (Quarles 1989). Regardless of what the precise antigen is, the blood nerve barrier should prevent entry of IgM into the endoneurium. Direct secretion of paraprotein in the endoneurium may occur with infiltration by plasmacytoid cells (Vital et al. 1982; Julien et al. 1984a). Alternatively, interaction of the paraprotein with endothelial antigens is an early and necessary event, a hypothesis supported by the observation of microvascular changes in this disorder (Lach et al. 1993; Powell et al. 1984). Even if IgM is present in the endoneurium, the putative mechanism of myelin damage is unclear, although a role for complement has been suggested (Monaco et al. 1990; Hays et al. 1988; Jonsson et al. 1988). Finally, IgM paraprotein penetration into the nerve may simply be an epiphenomenon secondary to a primary insult that opens up myelin lamellae and exposes cell surface antigens.

Clinical experience provides contradictory information. The clinical syndromes of anti-MAG IgM paraproteinemic neuropathy and of other IgM paraproteinemic neuropathies are not always distinct (Gosselin et al. 1991; Nobile-Orazio et al. 1987). Furthermore, MAG is found in higher quantities in the CNS than the PNS, suggesting that the CNS should be involved in IgM anti-MAG paraprotein-associated neuropathies, both clinically and histologically. This is not the case (Mendell et al. 1985), probably because of the more effective blood–brain barrier. The correlation between

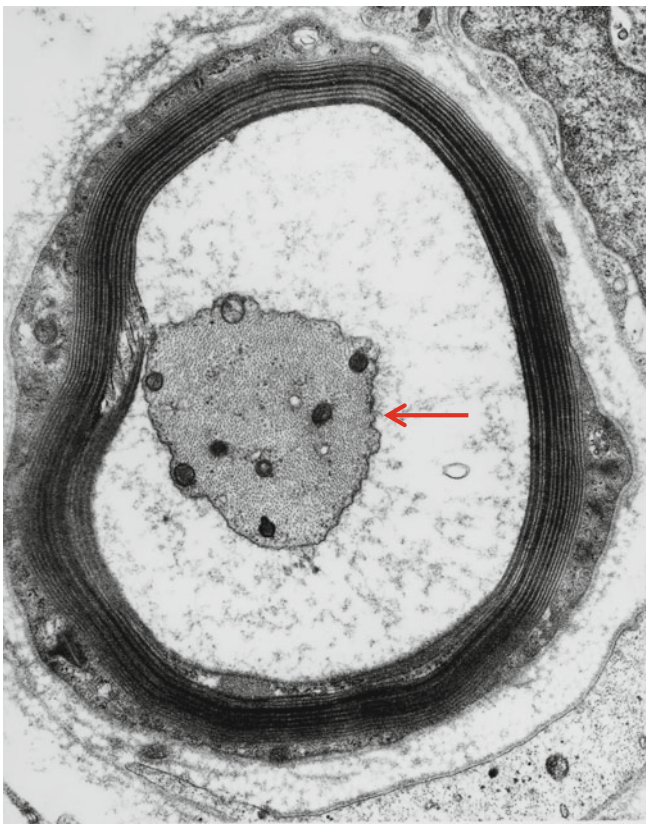

Fig. 14.10 IgM NAP: An adaxonal space resulting from intramyelinic edema separates a shrunken atrophic axon (*arrow*) from its myelin (14,560×)

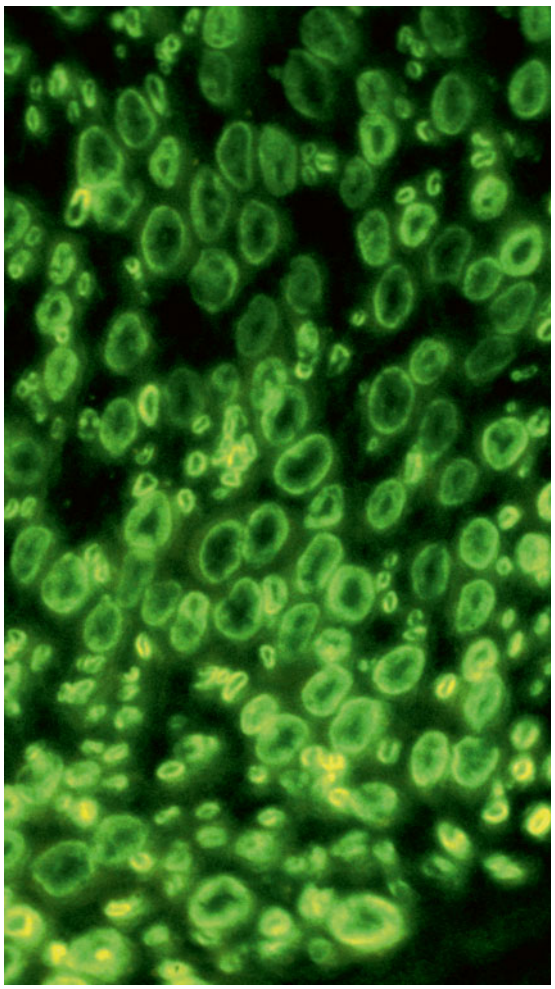

Fig. 14.11 Fluorescent anti-IgM antibody demonstrates immunolocalization of bound IgM to myelin sheaths of large and small myelinated axons (1,000×)

titers of anti-IgM paraprotein and disease activity is inconsistent (Dellagi et al. 1983; Ernerudh et al. 1992; Harbs et al. 1987; Gosselin et al. 1991; Nobile-Orazio et al. 1988; Sherman et al. 1984; Pollard et al. 1985), although this might be explained by methodological problems in the ELISA assay used to measure serum anti-MAG activity (Pestronk et al. 1994).

Chondroitin sulfate, a mucopolysaccharide component of neural cells and peripheral nerve ground substance (Margolis et al. 1979; Tona et al. 1993), and Schwann cell intermediate filaments (Dellagi et al. 1983) have been identified as target antigens in dysproteinemic neuropathy. The former usually presents with an axonal sensorimotor neuropathy (Sherman et al. 1983; Yee et al. 1989; Quattrini et al. 1991; Mamoli et al. 1991; Nemni et al. 1993). A paraprotein with activity against GM1 ganglioside has been associated with pure motor neuropathy (Kusunoki et al. 1989).

14.1.3.2 Non-IgM Paraproteins

The epidemiological and experimental data connecting IgA and IgG paraproteinemia to neuropathy is far weaker than that available for IgM paraproteins. Immunostaining studies, which have played such an important role in the understanding of IgM paraprotein-associated neuropathy, are not nearly as useful with IgG and IgA paraproteins. However, one passive transfer experiment has provided some evidence for a causal role (Besinger et al. 1981), as has the observation that neuropathy may regress with resection or irradiation of a solitary lesion (Read and Warlow 1978). Studies by Ohi et al. (1985) suggest that the neuropathy of multiple myeloma and of osteosclerotic myeloma and the POEMS syndrome are distal axonopathies with secondary demyelination, perhaps due to unspecified toxic effects on nerve. An axonal neuropathy has been reported with a paraprotein seemingly directed against neurofilament (Nemni et al. 1990; Jakobsen et al. 1986) and uptake of myeloma proteins into neural cells, presumably via distal terminals outside the blood nerve barrier, has been described (Borges and Busis 1985).

14.1.4 Differential Diagnosis

The neuropathies of MGUS, MM, WM, osteosclerotic myeloma, or the POEMS syndrome are usually not

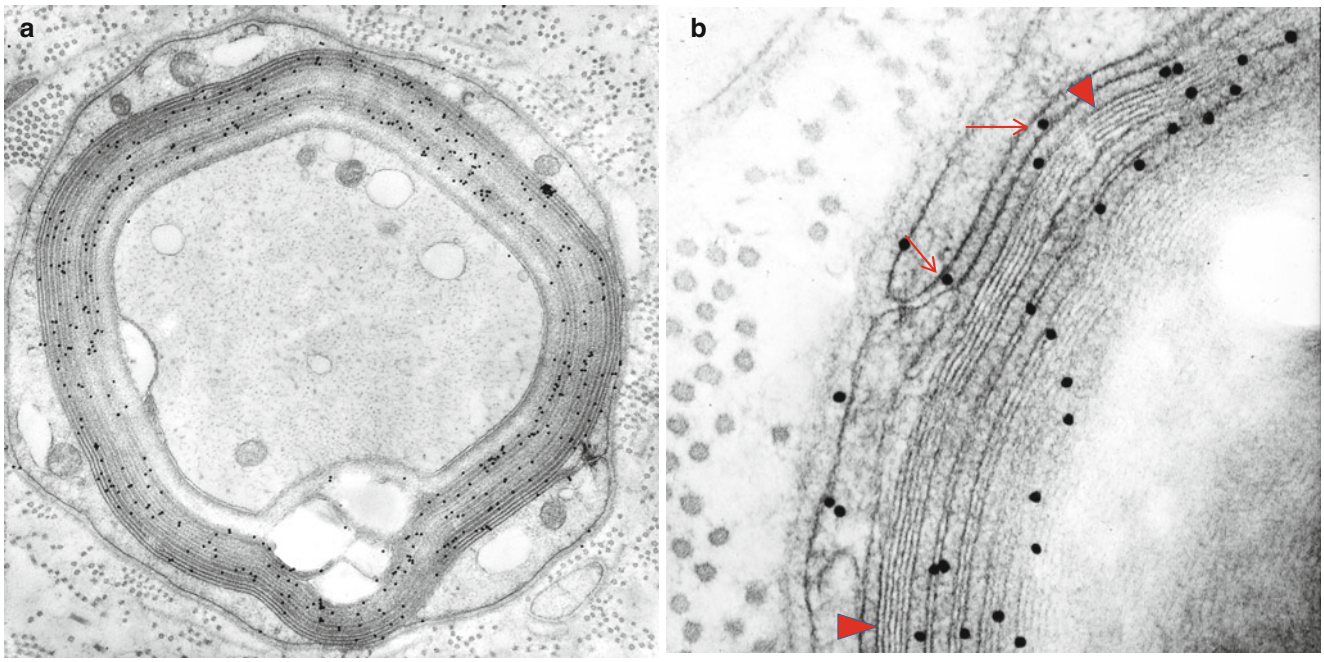

Fig. 14.12 IgM NAP: (**a**, **b**) Bound IgM is immunolocalized to WSM areas (*arrows*, **b**) and spares compacted myelin (*arrowheads*, **b**)

distinguishable histologically. Specific features, including focal immunoglobulin deposits, and alterations in myelin periodicity might be helpful. Otherwise, the typical finding in NAP is a nonspecific, minimally inflammatory, demyelinating, mixed, or less often purely axonal neuropathy.

Increased myelin periodicity is a useful finding if present. A detailed discussion of alterations in myelin periodicity is provided in Chap. 5). The only convincing example of uncompacted myelin that we have ever personally seen, which could not be simply dismissed as an early stage of remyelination or a slightly disorganized Schmidt–Lanterman cleft, was found in a single case of non-paraprotein-associated CIDP. Nevertheless, the literature suggests that this myelin alteration should lead to strong consideration of osteosclerotic myeloma and the POEMS syndrome, where it is present in over half of cases, even without a circulating paraprotein (Vital et al. 1994; Ohnishi 1984). Uncompacted myelin has also been reported in IgM paraprotein-associated neuropathy (Vital et al. 1985b), hereditary neuropathy with pressure palsies (Yoshikawa and Dyck 1991); GBS (Brechenmacher et al. 1987), congenital dysmyelinating neuropathy (Asbury and Johnson 1978; Lyon 1969), "paraneoplastic" neuropathy (Lamarche and Vital 1987), a case of probable CIDP in the setting of portocaval anastomosis (Vital et al. 1978), and vincristine neuropathy (Vital and Vallat 1987, Fig. 89).

Widely spaced myelin is specific for IgM paraprotein-associated neuropathy, with only 2 cases reported in IgG paraproteinemia, and rare reports in CIDP (King and Thomas 1984; Vital et al. 1986). This alteration usually suggests the presence of IgM paraprotein with anti-MAG activity (vide supra). Reports exist of cases in which WSM was the only clue to the presence of an IgM paraprotein which was too small in amount to detect unless special techniques or repeated testing were performed (Julien et al. 1984a; Vital and Vallat 1987; Nobile-Orazio et al. 1984; Valldeoriola et al. 1993). Thus, if no paraprotein is found in a patient with WSM, an intensified search is indicated, perhaps using immunofixation rather than the less sensitive immunoelectrophoresis (Kyle 1992) or even bone marrow biopsy.

It is usually not possible, and perhaps not meaningful, to distinguish the CIDP-like paraprotein-associated neuropathies from CIDP without a paraprotein. Widely spaced myelin suggests the former, while macrophage-mediated myelin stripping favors the latter. However, typical macrophage-mediated myelin stripping can be seen in the setting of a circulating paraprotein (Bleasel et al. 1993; Pollard et al. 1983; Vital et al. 1991b), and CIDP without a detectable paraprotein may demonstrate WSM (King and Thomas 1984) and deposition of IgM on myelin (Dalakas and Engel 1980). Macrophage-mediated myelin stripping and widely spaced myelin can even be seen in the same specimen (Vital et al. 1991b). Fortunately, therapy for both conditions is similar.

Nerves demonstrating deposition of non-amyloid granular material that stains for heavy or light chain antigens probably should be classified under the spectrum of non-amyloid monoclonal immunoglobulin deposition diseases (Buxbaum 1992). Curiously, this systemic disease is generally associated with a light chain or IgG and IgA paraprotein, but nearly all cases of monoclonal immunoglobulin deposition in nerve are due to IgM paraproteinemia, as part of WM or IgM

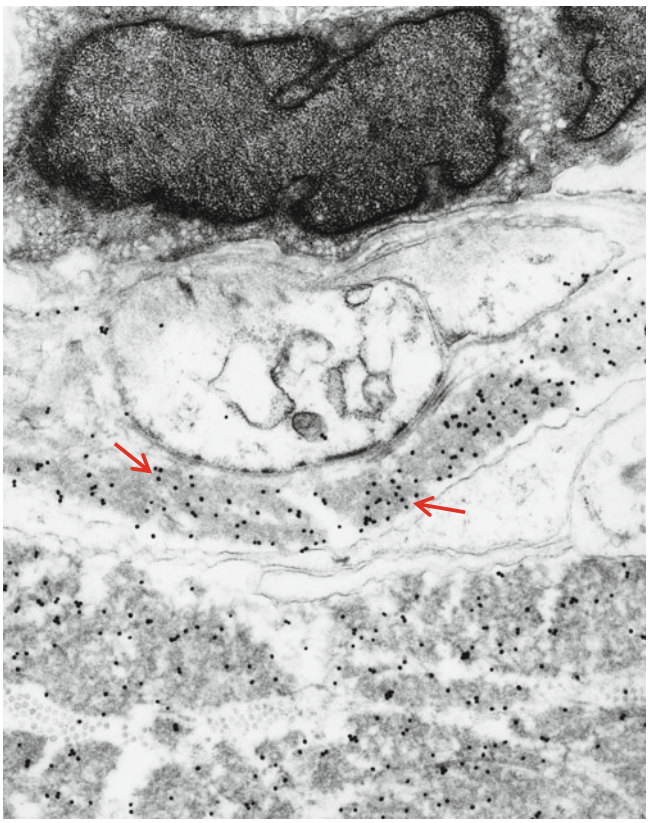

Fig. 14.13 Kappa chain NAP: Immunoelectronmicroscopy. Gold particles label kappa light chain composition of granular endoneurial deposits (same case as Fig. 14.4) (19,170×)

MGUS (Randall et al. 1976; Iwashita et al. 1974; Vital and Vital 1993; Meier et al. 1984; Dubas et al. 1987; Lamarca et al. 1987). Case 14.1 reported below is a rare exception to this rule. IgM paraprotein with anti-chondroitin sulfate activity may account for some of these cases (Yee et al. 1989; Sherman et al. 1983). Congo red staining and electron microscopic appearance allow distinction between immunoglobulin and amyloid deposition, an important distinction given than response to therapy is probably better in non-amyloid deposition disease (Kelly et al. 1979; Meier et al. 1984). Biopsy from a patient with biclonal gammopathy (IgG, IgM) displayed both intraneural amyloid deposits and widely spaced myelin (Julien et al. 1984b).

With the marked hypermyelination seen in some cases of IgM paraprotein-associated neuropathy, the diagnosis of tomaculous neuropathy might be suggested (Rebai et al. 1989; Lach et al. 1993; Vital et al. 1985a) but the distinction, both clinically and histologically, is straightforward.

Case 14.1

A 70-year-old woman suffered from progressively worsening bilateral hand and foot dysesthesia and aching over a period of 1 year prior to nerve biopsy. Over the last few months of this period, she developed progressive bilateral upper and lower extremity weakness to the point where she was bed bound. Examination disclosed distal foot and hand muscle atrophy and diffuse weakness, which was roughly symmetrical. While the arms were diffusely weak at about grade 4 MRC, the legs showed grade 3–4 proximal strength and grade 0–1 distal strength. Deep tendon reflexes were easily obtained at all sites excepting both ankles, where they were absent. The sensory examination revealed impairment of all sensory modalities in a glove and stocking distribution.

Investigations included normal CBC and ESR. Immunoelectrophoresis showed a normal IgA and IgG concentration, but increased IgM at 6.6 g/L. Cryoglobulins were absent. Bone marrow biopsy demonstrated focal aggregates of plasmacytoid lymphocytes, but all cell lines were normal in number and appearance. Skeletal survey was negative, and no splenomegaly was noted on a radionuclide scan. Electrophysiological tests demonstrated a severe axonal neuropathy with no sensory or motor responses obtainable in the lower extremities and only motor responses obtainable in the upper extremities. Sural nerve biopsy was performed 1 year after the onset of symptoms.

Clinical material courtesy of Dr. C. Sawka.

Case 14.2

A 57-year-old man presented with numbness of the hands and feet which seemed to resolve spontaneously after several weeks. A postural tremor then appeared, with a return of numbness and tingling in both legs, followed by bilateral distal leg weakness, and subsequently similar symptoms in the arms. Family history was negative for neurological disease. Nerve conduction studies showed a generalized demyelinating sensorimotor neuropathy, with motor conduction velocity of 23 and 18 m/s in the median and ulnar nerves, respectively, and unobtainable sensory responses in the arms and legs. Investigations showed hypothyroidism, and replacement therapy was instituted. An IgM-kappa paraprotein was also detected. Sural nerve biopsy was performed several months after onset of symptoms (Fig. 14.2a, c). Symptoms progressed very slowly over several years, with worsening numbness and an increasingly unsteady wide-based gait. Several months of treatment with prednisone did not dramatically alter the disease severity and the patient improved slightly over subsequent years.

At the age of 69, examination of the patient revealed no significant weakness, postural tremor, areflexia excepting barely obtainable triceps jerks, and loss of vibration sensation to the iliac spine, lower extremity ataxia, and an unsteady wide-based gait. No sensory responses were obtainable. Motor conduction velocity was 15 m/s in the median nerve. No significant changes were noted on repeat examination 2 years later.

At the age of 72, the patient was found to be anemic, and bone marrow biopsy revealed malignant lymphoma, lymphocytic small cell type. Examination revealed bilateral postural tremor, normal strength, absent reflexes, and loss of

vibration to the knees. A walker was necessary for walking. Laboratory investigations revealed a gamma-globulin fraction of 60 g/L, and because of symptoms and signs of hyperviscosity syndrome, plasma exchange was performed. Treatment with chlorambucil and prednisone was also instituted, with apparent remission of the lymphoma. The patient noted a marked subjective improvement in all symptoms with this regimen, and examination revealed a reduction in his tremor and improved walking. Nerve biopsy was performed 16 years after the onset of his original symptoms and showed no evidence of malignant infiltration, but progression of axonal loss and persistence of widely spaced myelin in most myelinated fibers (Fig. 14.2b, d).

Clinical material courtesy of Dr. F. Tyndel.

14.2 Cryoglobulinemic Neuropathy

14.2.1 Clinical Manifestations

Cryoglobulins are circulating proteins which can reversibly precipitate when cooled. Analysis of the precipitated substance permits subdivision into three groups. In type I cryoglobulinemia, there is a single monoclonal immunoglobulin, usually IgM. In types II and III cryoglobulinemia, there is a mixture of immunoglobulins, some of which have rheumatoid factor activity. Type II cryoglobulinemia is present if there is a monoclonal component, almost always IgM; otherwise type III cryoglobulinemia, most common of the three, is diagnosed (Frankel et al. 1992; Gorevic et al. 1980; Brouet et al. 1974; Feiner 1988). Hepatitis C virus appears to be the most common cause of mixed cryoglobulinemia (Nemni et al. 2003). These disorders can be seen in isolation, termed "essential," in about a third of cases (Gorevic et al. 1980; Valli et al. 1989). More commonly there is an association with a well-defined systemic illness, such as collagen vascular, chronic inflammatory, or lymphoproliferative diseases (Gorevic et al. 1980). Clinical features include cutaneous vasculitic lesions, joint disease, along with varying severity of hematological, renal, hepatic, respiratory, and other organ dysfunction. Mortality and morbidity are substantial but may be modified by immune suppressive therapy (Frankel et al. 1992; Gorevic et al. 1980).

Peripheral neuropathy is most prevalent in mixed cryoglobulinemia (Garcia-Bragado et al. 1988; Brouet et al. 1974). It has been described in 7–62 % of large series and takes the form of a distal sensorimotor neuropathy with varying degrees of asymmetry, less frequently mononeuritis or mononeuritis multiplex. In essential mixed cryoglobulinemia, neuropathy is the presenting sign of the disease in 12–19 % of patients (Gemignani et al. 1992; Gorevic et al. 1980). Severity ranges from mild to disabling, and sensory features often predominate (Frankel et al. 1992; Ferri et al. 1992; Cavaletti et al. 1990; Gemignani et al. 1992; Garcia-Bragado et al. 1988; Nemni et al. 1988; Valli et al. 1989). As with other vasculitides, a distal sensorimotor polyneuropathy may be seen rather than the classical picture of mononeuritis multiplex (Garcia-Bragado et al. 1988). Electrical testing shows a predominantly axonal neuropathy, although demyelinating features may be present in a minority of cases (Gemignani et al. 1992; Garcia-Bragado et al. 1988; Ferri et al. 1992).

Treatment with a variety of immune suppressive agents has yielded inconsistent results in terms of recovery from neuropathy (Garcia-Bragado et al. 1988; Cavaletti et al. 1990), but there does seem to be a role for steroid and cytotoxic therapy based on present anecdotal information. Plasma exchange may also be of benefit (Betourne et al. 1980; Frankel et al. 1992).

14.2.2 Pathology

14.2.2.1 Light Microscopy

Cryoglobulinemic neuropathy has several faces. Evidence of typical active or remote epineurial arteriolar necrotizing vasculitis has been detected in 0–75 % of biopsies (Garcia-Bragado et al. 1988; Gemignani et al. 1992; Nemni et al. 1988; Tredici et al. 1992; Vital et al. 1988; Valli et al. 1989). In the absence of vasculitis, a nonspecific mononuclear inflammatory infiltrate may be present, perhaps depending on whether there has been recent treatment with leukocyte-depleting agents. Rarely, focal deposits of cryoglobulin in endoneurium and microvessels are seen, staining positively with PAS (Prior et al. 1992). Perineurial inflammation can be very prominent (Konishi et al. 1982). Immunostaining for IgG or IgM may show deposition of immunoglobulin in vessel walls, but staining of myelin sheaths is not seen (Cavaletti et al. 1990; Gemignani et al. 1992; Nemni et al. 1988).

Axonal loss is usually present, sometimes with relatively selective involvement of large myelinated fibers (Cavaletti et al. 1990; Gemignani et al. 1992; Nemni et al. 1988). Active (Wallerian) degeneration may be prominent and multifocal, a finding suggestive of vasculitic neuropathy even in the absence of arteriolar changes. Unmyelinated fibers are relatively spared, regenerating clusters are common, and onion bulbs can rarely occur (Gemignani et al. 1992; Thomas et al. 1992).

14.2.2.2 Electron Microscopy

Ultrastructural examination usually reveals ongoing axonal degeneration and variable demyelination, but little else of specific interest. Nonspecific endoneurial vascular alterations can be seen, with multilayering and reduplication of basement membrane, and swelling of endoneurial cells with filament accumulations to the point of microvascular obliteration (Cavaletti et al. 1990; Gemignani et al. 1992; Tredici et al. 1992; Vital et al. 1988).

Precipitated cryoglobulin is very rarely seen in endoneurium and microvasculature and has a unique fingerprint-like appearance composed of tubules with a diameter of 30–50 nm (Prior et al. 1992; Vallat et al. 1980; Vital et al. 1991a). The pattern of deposition in one study seemed to suggest movement from the intravascular compartment into the endoneurium via pinocytotic activity of endothelial cells (Vallat et al. 1980). The three cases where this has been described showed little or no inflammatory infiltration, and the predominant pathology was diffuse axonal degeneration.

14.2.3 Pathogenesis

Vasculitis is a prominent feature of cryoglobulinemia, as is depletion of early complement components (Gorevic et al. 1980; Frankel et al. 1992). Although numerous target antigens have been implicated, the final common pathway of damage in cryoglobulinemia probably involves deposition of the offending immune complexes in vessel walls, activation of complement, and destruction of vessels by inflammatory mediators and proteolytic enzymes, much the same as postulated for the immune complex model of other types of necrotizing vasculitis (Frankel et al. 1992; Gorevic et al. 1980). However, there is poor correlation between cryoglobulin levels and clinical disease severity (Frankel et al. 1992), and although plasma exchange removes cryoglobulins and has been correlated with clinical improvement, the duration of benefit is longer than might be explained by removal of the cryoglobulin (Frankel et al. 1992).

While vasculitis probably accounts for most cases of peripheral neuropathy in cryoglobulinemia, especially types II and III, there are times where there is no evidence of arteriolar damage, and the primary pathological process is demyelination or diffuse axonopathy (Lippa et al. 1986; Chad et al. 1982; Valli et al. 1989). Analysis of the available literature is hampered by the difficulty of determining what type of cryoglobulinemia (I, II, III) was present and whether it was essential or secondary. The very rare reported cases of type I cryoglobulinemic neuropathy have not demonstrated vasculitis or inflammation and have shown diffuse axonal degeneration, and in two instances "fingerprint-like" cryoglobulin deposits were detected in distended peripheral nerve microvasculature (Vallat et al. 1980; Vital et al. 1991a). These cases probably have a distinct pathogenesis relating to an occlusive microangiopathy. A study comparing nerve biopsies in type II cryoglobulinemia in patients with and without neuropathy demonstrated that the essential difference was endoneurial microvascular damage, not necrotizing vasculitis (Tredici et al. 1992). Thus, microangiopathy may play an important role in some cryoglobulinemic neuropathies.

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Amyloid Neuropathy

15

Amyloidosis is among the best known of the gain-of-toxic-function protein misfolding diseases. They are characterized by the accumulation and aggregation of nonfunctional and toxic proteins that damage cells and tissues (Blancas-Mejia and Ramirez-Alvarado 2013). Amyloid is detected as an extracellular proteinaceous substance that appears amorphous by light microscopy and fibrillar under the electron microscope. Amyloid fibrils differ from other protein aggregates because they show apple green-yellow birefringence when stained by Congo red and viewed with polarized light. Amyloid consists of beta-pleated sheet micelles forming matted linear non-branching fibrils, 7–10 nm in width. Each fibril comprises two filamentous subunits that run in parallel and twist about each other. The common constituents of all amyloid types are amyloid P component, an endogenous glycoprotein produced by the liver, and various mucopolysaccharides (Pepys 1986, 1990). Different amyloid types are distinguished by their major protein, which is the largest constituent of the deposit. To date there are 30 known extracellular proteins that fulfill the criteria for the designation of amyloid (Sipe et al. 2012), of which five affect the peripheral nervous system (AmTTR, AApoA, AGel, AL, and APrP). Amyloid is insoluble and highly resistant to proteolysis. Its gradual accumulation in a variety of locations ultimately results in clinical disease by mechanisms that have not been fully elucidated.

Amyloidosis can be divided into primary, secondary, familial, and tissue-specific forms (see Adams 2001; Buxbaum 2004; Plante'-Bordeneuve and Kerschen 2013). Primary (AL) amyloidosis refers to amyloid whose major protein is derived from immunoglobulin light chains, predominantly the V region (Feiner 1988). AL implies the presence of plasma cell dyscrasia, whether obvious or occult. Patients with familial amyloidosis have an inherited tendency for deposition of amyloid in various tissues, very often with prominent involvement of peripheral nerve, as seen in several of familial amyloid polyneuropathies (FAPs). Secondary amyloidosis, with serum protein A as the major protein, may occur as part of a chronic systemic inflammatory disease and does not ordinarily cause a clinical polyneuropathy (vide infra). Although there are other forms of amyloidosis (Kyle and Dyck 1993), including one associated with dialysis where the major protein is beta-2 microglobulin and carpal tunnel syndrome is seen (Gertz and Kyle 1989), these have little relevance to peripheral polyneuropathy and nerve biopsy and are not considered further. In 2013 Mead et al (2013) reported a novel prion disease phenotype that is associated with chronic diarrhea and hereditary sensory and autonomic neuropathy caused by a novel *PRNP* mutation. Deposition of prion protein amyloid was demonstrable systemically and in peripheral nerves.

15.1 Clinical Manifestations

15.1.1 Primary Amyloidosis

Primary amyloidosis is usually a multisystem disease with neural involvement in 20–34 % of patients, and 7–12 % of all primary amyloidosis patients present with an isolated neuropathy months to years before other manifestations of the systemic disease are evident (Buxbaum 1992; Duston et al. 1989; Gertz and Kyle 1989; Trotter et al. 1977). Age at diagnosis of peripheral nerve involvement and disease duration are 57.9 ± 8.7 years and 14.7 ± 7.2 months, respectively (Matsuda et al. 2011). The polyneuropathy is length dependent with a sensory-dominant impairment, early involvement of lower limbs, loss of all sensations, and late development of motor weakness, painful paresthesia, and frequent autonomic dysfunction. This pattern has many similarities to the polyneuropathy manifested by patients afflicted with mTTR amyloidosis.

Traditionally, once amyloid deposition has been documented, the diagnosis of primary amyloidosis is made when evidence for a plasma cell dyscrasia is found, most commonly in the way of a paraprotein. This approach is prone to error because patients with a familial amyloid neuropathy, where no family history is found in as many as

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50 % (Li et al. 1992), are as likely as anyone else to have an incidental paraprotein. Systemic amyloid light chain (AL) amyloidosis, the most common systemic amyloidosis, is characterized by a plasma cell disorder in which deposits of the N-terminal region of light chains cause progressive organ failure. More importantly a serum or urine paraprotein is absent in 13 % or more of patients with primary systemic amyloidosis and in almost half of patients with primary amyloidosis presenting with an isolated neuropathy (Buxbaum et al. 1990; Gertz and Kyle 1989). In such cases diagnosis has to be pursued by bone marrow biopsy, skeletal survey, and repeated search for abnormal proteins with immunofixation of serum and urine, and immunoglobulin-free light chain kappa and lambda testing. Monoclonal immunoglobulin-free light chain (Ig FLC) assay is essential in the diagnosis and monitoring of light chain only secreting multiple myeloma (Bence Jones myeloma), and it has allowed the detection of monoclonal protein in some patients with nonsecretory myeloma that were previously undetectable (Uchida et al. 2012). If immunofixation of serum and urine is negative and the Ig FLC (kappa/lambda) ratio is normal (0.26–1.65), AL amyloidosis is unlikely and further evaluation should not be undertaken (Gertz 2013; Lubimova et al. 2012). In Sweden, the median survival time after diagnosis of AL is 3 years.

15.1.2 Hereditary Amyloid Neuropathies

Several distinct familial amyloid polyneuropathies (FAP) have been described, all autosomal dominant. For practical purposes, the FAPs can be classified according to the precursor protein of amyloid (Blancas-Mejia and Ramirez-Alvarado 2013). Clinically, a family history is initially obtained in as few as 50 % (Li et al. 1992), but with intense scrutiny, this may increase substantially (Gertz et al. 1992; Li et al. 1992). Patients typically present with a peripheral neuropathy, although multisystem disease, especially cardiac, is found in many and, uncommonly, there is no clinical nerve involvement at all (Gertz et al. 1992; Ikeda et al. 1987; Meretoja and Teppo 1971; Reilly and King 1993). Three circulating proteins have been identified to date as being the source of a major protein in FAP:

15.1.3 Transthyretin

Hepatocyte-derived transthyretin (TTR) is a serum transport protein, previously called pre-albumin, which serves as a carrier for several substances, including thyroxin and vitamin A. It is encoded by a single copy gene on chromosome 18. Over 113 different missense point mutations in the TTR gene have been associated with deposition of circulating transthyretin as the amyloid major protein (Reilly and King 1993; Plante'-Bordeneuve and Kerschen 2013) in peripheral nerves, heart, gastrointestinal tract, and kidneys. First discovered in 1952 and still the most common pathogenic substitution is the Val30Met mutation (Andrade/Portuguese variant, FAP type I) seen in Portuguese, Japanese, and Swedish patients. Gene penetrance ranges from 69 % in Sweden to 85 % in Portugal (Plante-Bordeneuve and Said 2011). Of interest is the high prevalence of the Val122Ile mutation (most often associated with cardiac disease) in African Americans. Most patients are heterozygous for the TTR mutations, and the amyloid deposits consist of mutant and nonmutant transthyretin. Transthyretin is also synthesized by the retina (retinal pigment epithelium) and choroid plexus, which can lead to vitreous and leptomeningeal accumulations (Coelho et al. 2013)

Although a family history may be negative, it is usually presumed that this is due to incomplete penetrance or inability to examine the entire family. Patients with amyloid neuropathy in which the major protein is TTR and which are truly sporadic (i.e., new mutations in the *TTR* gene as verified by molecular biological techniques) do seem to exist (Murakami et al. 1992), but their frequency is as yet unknown. In addition normal, nonmutant wild-type transthyretin (wtTTR) is amyloidogenic, and transthyretin amyloid deposits are present in many tissues in at least 10 % of persons over the age of 80 years. This syndrome of systemic senile amyloidosis does not feature deposits in endoneurium or evidence of nerve fibre damage.

In a large study of patients with idiopathic carpal tunnel syndrome, 34 % of tenosynovial tissue obtained at surgery showed amyloid deposition characterized as wtTTR by direct DNA sequencing (Sekijima et al. 2011).

15.1.4 Apolipoprotein A1

Familial amyloid neuropathy (FAP) type III (Van Allen, Iowa variant) is due to amyloid deposits derived from a mutant apolipoprotein A1, encoded on chromosome 11 (Reilly and King 1993). Apolipoprotein A1 is a plasma protein with an extensive α-helical structure synthesized by the liver and the small intestine. The neuropathic pattern of symptoms is associated with the Gly26Arg mutation. The clinical features include length-dependent peripheral neuropathy of variable severity but never prominent, peptic ulcer disease, liver disease, nephropathy, and death from renal failure.

15.1.5 Gelsolin

Gelsolin is an actin-modulating protein encoded on the long arm of chromosome 9q32-34. A point mutation in this protein

has been associated with FAP IV (Finland, Meretoja variant, Maury 1991; Sunada et al. 1993). The preferred name is gelsolin amyloidosis (Pihlamaa et al. 2012; Kiuru-Enari and Haltia 2013). The same mutation is seen in unrelated Finnish and Japanese families. A point mutation in the gelsolin gene results in the production of an amyloidogenic circulating degradation product of this protein (Maury and Rossi 1993). Clinically, the neuropathy starts in the third or fourth decade of life and is distinctive by virtue of prominent cranial nerve involvement with facial paralysis, although amyloid deposits are seen throughout the PNS (Meretoja and Teppo 1971) and are accompanied by distinctive corneal lattice dystrophy (this is diagnostic for this disease, sometimes noted years before the appearance of clinical symptoms of neuropathy) and skin changes (cutis laxa) (Kiuru-Enari et al 2002).

15.1.6 "Sporadic" Amyloid Neuropathy

Up to 39 % of patients with amyloid neuropathy have no family history and show no evidence for plasma cell dyscrasia (Dalakas and Cunningham 1986). These may be patients with primary amyloidosis but no detectable paraprotein, or "familial" (usually mTTR) amyloidosis with an incomplete family history, incomplete penetrance, or new mutations (Adams et al. 1992). Such patients pose a clinical challenge in terms of investigation and treatment. Recent advances in immunohistochemistry and Ig-free light chain determination (Lubimova et al. 2012) can play a major role in the management of this group (vide infra).

15.1.7 Clinical Features

The first signs and symptoms of MTTR-FAP develop most commonly from the third to the fifth decade of life (Val30Met variant, patients with other mutations experience a later age of onset). The disease affects both men and women equally (Coelho et al. 2013). In its classical form (Val30Met mutation), patients display two patterns of sensorimotor deficit associated with variable autonomic dysfunction (orthostatic hypotension, bladder or sexual dysfunction, and constipation) and extra-neurological manifestations (nephrotic syndrome or renal failure, hepatomegaly, peripheral neuropathy, orthostatic hypotension, and macroglossia Kyle and Gertz 1995; Plante-Bordeneuve and Said 2011). One pattern consists of a length-dependent sensorimotor polyneuropathy. Symptoms start with discomfort in the feet with impaired thermal sensitivity and decreased pinprick sensation with preservation of light touch, proprioception, and tendon reflexes; the other type begins with focal deficits resulting from intraneural amyloid aggregates followed by a bilateral sensory motor polyneuropathy. An ataxic phenotype was recently reported in France in up to 26 % of TTR-FAP patients (Adams et al 2014). At the outset the patients display foot numbness and imbalance. On examination there is vibration and position sense loss and diffuse areflexia. The course is rapid with progressive ataxia. Patients are often misdiagnosed as having CIDP. Different TTR mutations (Val93Met) may present with an ALS phenotype (Adams et al 2014).

Once the length-dependent neuropathy is established, it progresses relentlessly proximally to thighs. Motor deficits occur in the same fashion from distal to proximal. Upper limbs are then affected, there is loss of pain sensation distally, and the development of ulcers in feet and foot arthropathy occurs in the course of few years. By electrophysiological tests, in the early stages quantitative sensory testing and sympathetic skin tests confirm small fiber involvement (Heldestad and Nordh 2007). Later, EMG shows active and chronic neurogenic changes. In the natural course, the disease is progressive and invariably fatal after an average duration of 10–13 years.

15.1.8 Treatment

Correct diagnosis of familial amyloidosis is important for both genetic counseling and the avoidance of unnecessary invasive testing. Liver transplantation has been shown to stabilize disease progression because it eliminates the production of mutant protein, particularly if performed early in the disease (Holmgren et al. 1993; Adams 2001; Yamamoto et al. 2007). However, additional deposition of wtTTR on the template of preexisting amyloid occurs after transplantation, leading to cardiomyopathy and worsening of polyneuropathy. Liver transplantation has no effect on ocular complications or eventual CNS symptoms of amyloidosis caused by the persistent synthesis of mTTR by retinal epithelial cells and the choroid plexus (Hara et al. 2010). Tafamidis, a small chaperone molecule that binds to transthyretin and prevents tetramer dissociations into monomers (the rate-limiting step in amyloidogenesis), slows deterioration of peripheral nerve function and improves quality of life (Coelho et al. 2012). New techniques of lowering m*TTR* gene expression with TTR-specific lipid-based siRNAs are in development (Love et al. 2010). Coelho et al. (2013) reported the use of a lipid nanoparticle envelope to facilitate the safe delivery of the siRNA to hepatocytes, which resulted in dramatic and sustained specific knockdown of transthyretin production. There is no specific treatment for either gelsolin amyloidosis or for apolipoprotein A-1 FAP.

Once the amyloidosis is confirmed to be of light chain origin, treatment options include stem cell transplant or trials of chemotherapy, which include corticosteroids, alkylating agents (melphalan, cyclophosphamide), immunomodulatory

drugs (thalidomide, lenalidomide), and proteasome inhibitors (bortezomib) (Gertz 2013). Alternative therapeutic strategies to liver transplant include tafamidis, diflunisal, antisense oligonucleotides, and small interfering RNA (Adams et al 2014).

15.1.9 Secondary Amyloidosis

In general, polyneuropathy is not considered to be a feature of secondary (AA) amyloidosis (Benson et al. 1975). However, there are reported cases of secondary amyloidosis associated with autonomic, cranial, and perhaps sensorimotor neuropathy (Horn et al. 1991; McGill et al. 1986; Nordborg et al. 1973; Tsunoda et al. 1994). The autonomic features can be explained by AA amyloid deposits in sympathetic ganglia (McGill et al. 1986). However, secondary amyloid can also be deposited in the endoneurium (McGill et al. 1986; Tsunoda et al. 1994). Of two such reported instances, no polyneuropathy was seen in the first case (McGill et al. 1986), and the presence of a non-amyloidogenic circulating paraprotein might have explained the neuropathy in the second case (Tsunoda et al. 1994).

15.2 Pathology

15.2.1 Utility of Nerve Biopsy

It is important to emphasize that there are alternatives to nerve biopsy. Biopsy of abdominal fat pad or rectal mucosa is a high yield, low morbidity option if primary or familial amyloidosis is suspected (Gertz et al. 1988, 1992). In one large study skin biopsy was as sensitive as nerve biopsy for detection of amyloid in Portuguese FAP (Guimaraes et al. 1987). Muscle biopsy probably has a higher yield than nerve, at least in primary amyloidosis (Dalakas and Engel 1979); we favor a combined biopsy of muscle and nerve.

In familial amyloid neuropathy nerves from asymptomatic/presymptomatic known carriers may appear normal histologically or show abnormalities similar to those of patients with a neuropathy (Guimaraes et al. 1987). Skin and nerve biopsies detect amyloid in about half of asymptomatic carriers, with skin biopsy being somewhat more sensitive (Leite et al. 1987). This contradicts the results of previous work where no amyloid was found in asymptomatic or presymptomatic carriers (Carvalho et al. 1976; Harats et al. 1989). In symptomatic patients, data regarding sensitivity of the sural nerve biopsy is hard to extract from the literature, because the diagnosis of amyloid neuropathy has historically been based on histological findings in nerve alone. One large study of familial amyloid neuropathy (TTR 30) demonstrated neural amyloid deposits in 42 of 44 clinically affected patients (Guimaraes et al. 1987). The two whose nerve biopsies did not contain amyloid did show moderate–severe fiber loss in a typical pattern (unmyelinated more than myelinated fibers), and their skin biopsies were positive for amyloid. In another study of a variety of familial amyloid neuropathies, 3 out of 23 patients had negative nerve biopsies, but the presence or absence of clinical peripheral nerve involvement was not stated (Gertz et al. 1992). These data suggest a high but not 100 % sensitivity for sural nerve biopsy in clinically affected patients with FAP, and a role for skin or other tissue biopsy if the clinical picture and pattern of fiber loss suggest amyloid neuropathy but no amyloid is noted. Data on asymptomatic/presymptomatic FAP patients suggest that nerve biopsy is not very sensitive in revealing amyloid, as only 23 % of asymptomatic patients with histological neuropathy (i.e., loss of fibers) actually showed amyloid deposits (Leite et al. 1987). Whether this relates to sampling error or the fact that amyloid deposition does not directly cause the neuropathy remains unknown.

The sensitivity of nerve biopsy in primary amyloidosis with peripheral neuropathy has been considered to be high. Amyloid was detected in 86–100 % of sural nerve biopsies (Kelly et al. 1979; Kyle and Dyck 1993), but a selection bias obviously confounds these numbers. In another report, only 2 of 8 nerve biopsies were amyloid positive in patients with a neuropathy consistent with that of amyloidosis and who were ultimately diagnosed as having primary amyloidosis (Simmons et al. 1993). We have seen three patients with systemic amyloidosis (two primary, one indeterminate) and neuropathy in whom sural nerve biopsy did not reveal amyloid, although in one the neuropathy might well have been due to the circulating IgM paraprotein, not amyloid deposition. In another, the diagnosis was first suspected after biopsy showed a selective loss of small myelinated fibers, but abdominal fat pad biopsy was necessary to arrive at the diagnosis, while in the third the diagnosis was revealed at autopsy. Thus, if clinical suspicion is high and nerve biopsy is unrewarding, the clinician should consider another biopsy site.

15.2.2 General Considerations

Our experience with eight cases (3 familial TTR, 3 light chain, and 2 not characterized) and that of some workers (Li et al. 1992) suggests there is no significant difference between the histopathology seen in familial amyloid neuropathy and in primary amyloidosis and the two are discussed together below.

Physical methods (autoclaving) and histochemical techniques (potassium permanganate, alkaline guanidine) exist which can distinguish between some of the different types of amyloid (Elghetany and Saleem 1988), but immunohistochemistry currently represents the most useful method, as it

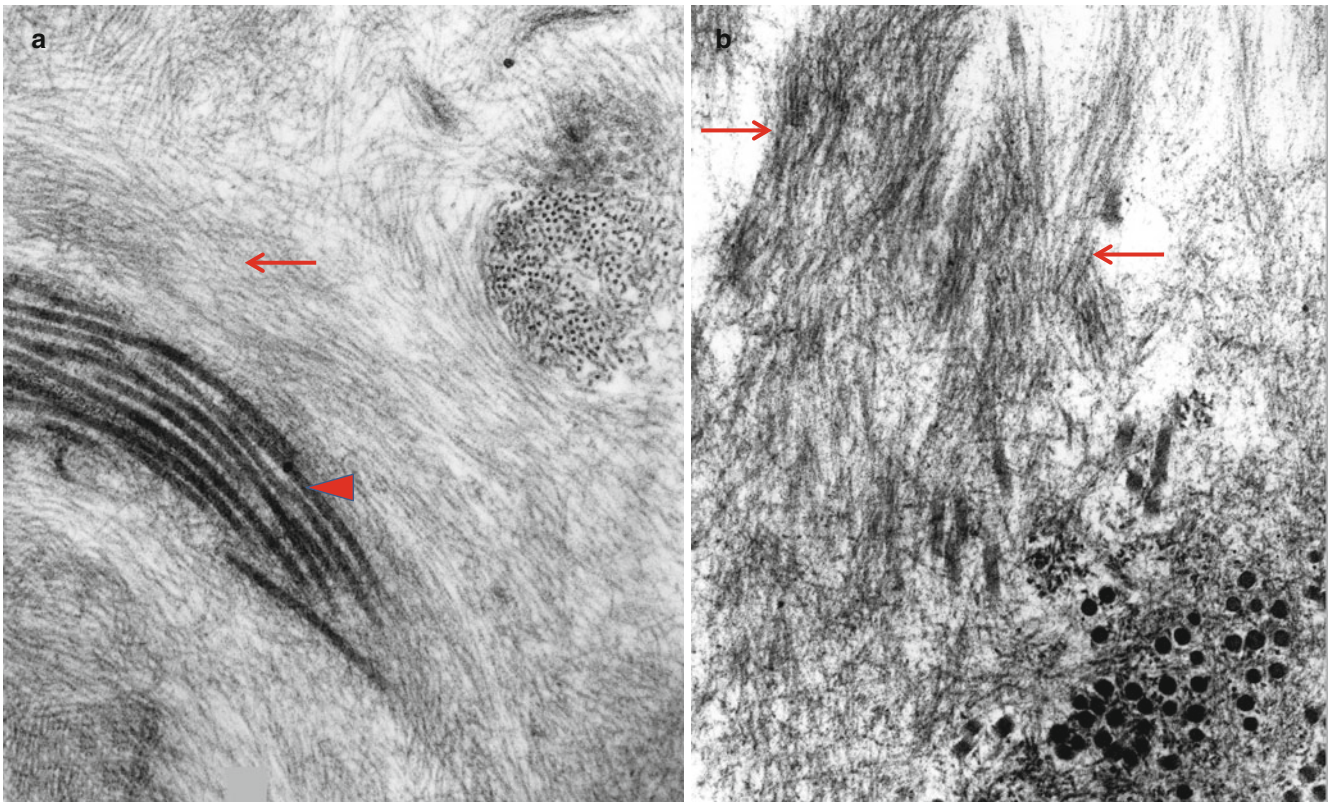

Fig. 15.1 The ultrastructural differences between oxytalan (*arrow*, **a**), collagen (*arrowhead*, **a**), and amyloid fibrils (*arrows*, **b**) are the slightly greater thickness of oxytalan and the apparent rigidity of the amyloid fibril as compared with curved or wavy appearance of oxytalan (**a**, **b**: 65,100×)

reliably distinguishes familial from light chain amyloid proteins (Linke et al. 1986). The amyloid of FAP and primary amyloidosis is resistant to potassium permanganate while that of secondary amyloidosis is not (Sommer and Schroder 1989). This is of minimal value because secondary amyloidosis does not cause a peripheral polyneuropathy (vide supra).

Using strain-free lenses, amyloid displays birefringence under polarized light even when unstained, but the classic apple-green-yellow birefringence is demonstrated best with Congo red staining in 5–10 μm thick sections. Fresh-frozen sections exhibit the highest affinity for Congo red. A number of non-amyloid substances stain positively with Congo red but show no birefringence (fibrin, elastic fibers, mast cell granules) while others even show the birefringence (fungi, plant cell walls, cotton fibers, starch). For details and additional technical considerations, see Elghetany and Saleem 1988; Francis 1990. The sensitivity of Congo red staining for amyloid is excellent, but the affinity for this stain is gradually lost with prolonged formalin fixation. We have seen cases where small amyloid deposits were detected only on EM, presumably due to sampling error, and if clinical suspicion is high, one should not abandon the search for amyloid if Congo red staining is negative. The use of fluorochromic dyes (thioflavin T, thioflavin S, and Phorwhite BBU) is also a reliable method for the screening of amyloid in fresh-frozen and in paraffin sections (Francis 1990; Waldrop et al. 1972).

Renaut bodies have been mistaken for amyloid deposits because of their amorphous light microscopic appearance and the presence of fibrillar material intermixed with collagen on EM. The microfibrillar component, typically slightly wider (8–13 nm vs. 7–10 nm) and not as long and straight as amyloid fibrils, is probably oxytalan, a constituent of elastic fibers (Ghadially 1988a; Weis et al. 1993) (Fig. 15.1a, b). The absence of Congo red staining and the characteristic location and shape of Renaut bodies should clarify the situation. Similar microfibrils are often seen in subperineurial, endoneurial interstitial, and a perivascular location and should not be mistaken for amyloid. There should be no difficulty distinguishing endoneurial "edema," which is Congo red negative and Alcian blue positive, from amyloid.

15.2.3 Light Microscopy

Amyloid accumulates in epineurium, perineurium, and endoneurium. One case we examined showed amyloid deposition

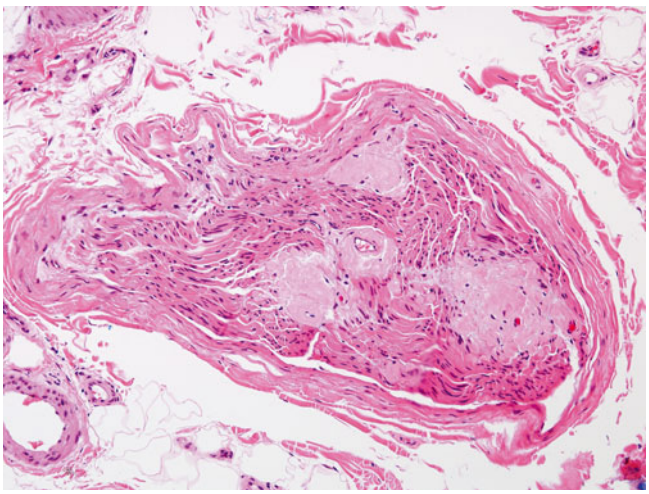

Fig. 15.2 Amyloidosis: clumps of pink amorphous amyloid are found within the endoneurium, adjacent to the perineurium and perivascularly (H&E, paraffin)

mostly in the epineurial fat and could have been missed with a fascicular biopsy. When seen in the endoneurial interstitial compartment, amyloid may be found in large pools or distributed diffusely (Figs. 15.2, 15.3a–c, and 15.4a–d). Amyloid masses can be seen focally in the vessel wall or forming perivascular collars (Figs. 15.4a–d and 15.5). In the latter case the resulting appearance may be dismissed as nonspecific hyaline thickening of the vessel unless Congo red-stained sections are examined routinely under polarized light. It has been suggested that in primary, as contrasted with familial, amyloidosis there is a tendency for the deposits to be more prominent in and around vessels, particularly the vasa nervorum in the epineurium (Asbury and Johnson 1978), but we have not observed such a distinction (Li et al 1992). Perineurial and subperineurial deposition is common and often prominent (Figs. 15.3c and 15.4c, d). In secondary amyloidosis there may be epineurial, but not endoneurial amyloid deposition (Belokrenitzky 1911). Inflammatory cells are not a feature of amyloid neuropathy.

The predominant pathological process is axonal degeneration. The degree of fiber loss depends on the evolution of the disease. In early stages there is usually selective loss of unmyelinated and small myelinated fibers, but as the disease progresses, fibers of all sizes are severely affected (Fig. 15.3a–c). Sometimes the selective loss of unmyelinated fibers is very striking (Fig. 15.3b) (Dyck and Lambert 1969). A spectrum ranging from moderately active Wallerian to chronic axonal degeneration with regenerating clusters may be seen, although we have not found the latter to be prominent in our material. Although amyloid neuropathy is typically axonal, several authors have reported the presence of segmental myelin changes using teased fibers (Dyck and Lambert 1969; Hanyu et al. 1989; Jedrzejowska 1977; Said et al. 1984; Thomas and King 1974). We have never seen prominent segmental demyelination in amyloid neuropathy.

15.2.4 Electron Microscopy

Electron microscopy shows extracellular deposits composed of 7–10 nm wide straight unbranched fibrils in an irregular matted or radial arrangement (Figs. 15.6a, b, 15.7, 15.8a, b, 15.9a, b, and 15.10). Very high magnification will demonstrate that the fibril is made up of two parallel subunits with a thin separation. Lateral aggregation of several fibrils can result in fibers up to 40 nm in diameter (Ghadially 1988b). The amyloid masses are extracellular, often surrounded by and in intimate contact with collagen. Fibrils may make contact with endothelium basement membrane (Figs. 15.7 and 15.10) and that of the Schwann cell seemingly damaging the cells they encroach upon. At the interface between amyloid and cell (especially macrophages), a hemidesmosome-like electron-dense thickening of the cell membrane has been described (Sommer and Schroder 1989), with amyloid fibrils oriented perpendicular to the membrane. Small fibrillar amyloid masses can be dispersed among endoneurial interstitial material and may be missed on light microscopy. A nonfibrillar granular homogenous background matrix composed of acid mucopolysaccharides (Coimbra and Andrade 1971b; Hanyu et al. 1989) may be seen in the endoneurium and is an entirely nonspecific change seen in many chronic neuropathies. Swollen axons due to accumulation of neurofilaments have been occasionally described (Hanyu et al. 1989; Jedrzejowska 1977), but this is probably a nonspecific axonal degenerative change. In advanced cases of amyloid neuropathy, the endoneurium presents a desolate picture with depletion of myelinated fibers and residual denervated bands even in areas with no amyloid deposition (Fig. 15.9a, b).

Although amyloid is generally considered to be an extracellular substance, it has been described within intracellular coated and uncoated vesicles. In one such study the intracellular material was shown to be light chain immunoglobulin (Sommer and Schroder 1989); this remains a controversial issue (Ghadially 1988a, b). Nonspecific evidence of compromise of the blood–nerve barrier can be seen in the form of fenestration and increased pinocytotic activity of endothelial cells.

15.2.5 Immunohistochemistry

Advances in immunohistochemistry have made characterization of the amyloid protein possible, and this should now be considered a standard practice (Fig. 15.11a, b). Pretreatment of paraffin sections with formic acid enhances immunoreactivity of amyloid (Kitamoto et al. 1987). Antisera to kappa and lambda light chains (Fig. 15.11a), gelsolin, and TTR (Fig. 15.11b) are available commercially. Light chain amyloid may be detected in patients without other evidence of light chain disease, and TTR amyloid may be demonstrable in the nerves of patients with no family history. In one study (Dalakas and Cunningham 1986) of 39 amyloid neuropathy patients, 15

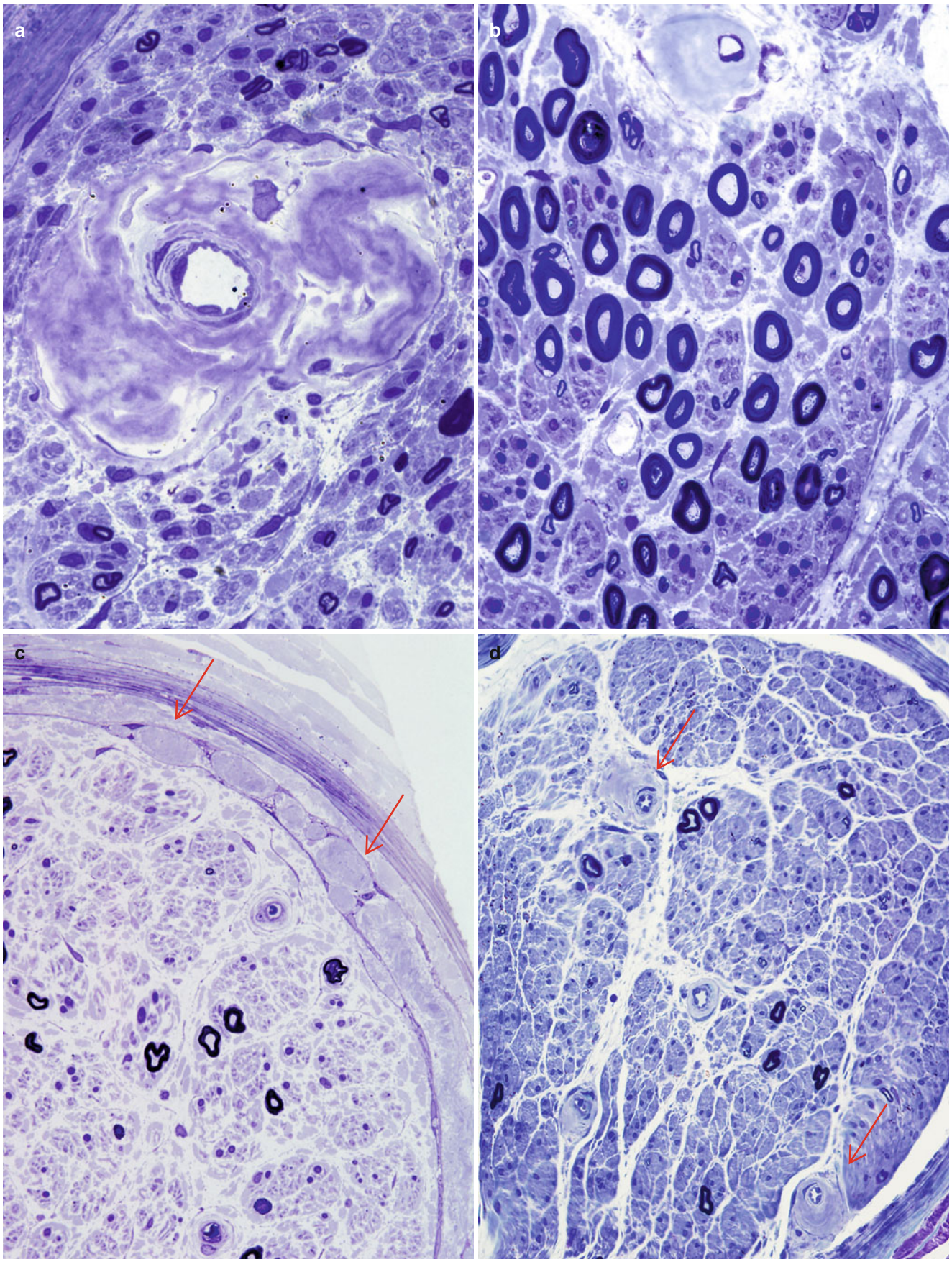

Fig. 15.3 Amyloidosis: (**a**) irregular perivascular clumps of amyloid. (**b**) Although large myelinated axons appear well maintained in this plastic section, small myelinated axons are greatly diminished in number. Note again the asymmetry of amyloid deposition around the vessel. (**c**) Expansion of the subperineurial and intraperineurial spaces by clumps of amyloid (*arrows*, **c**). (**d**) More modest irregular perivascular aggregates (*arrows*) (**a**–**c**: 1 **μ** thick toluidine blue-stained plastic section, 1,000×; D 400×)

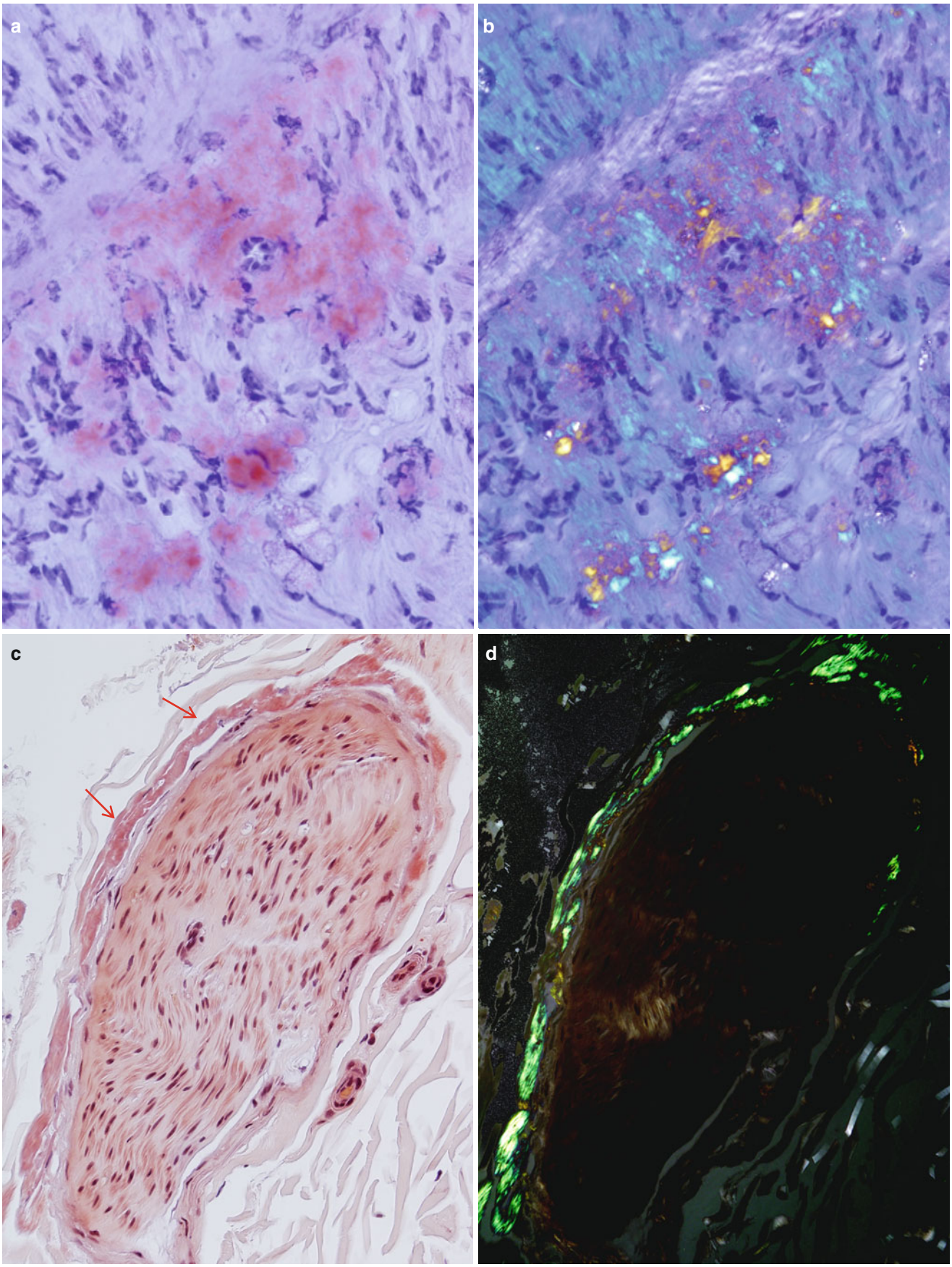

Fig. 15.4 Amyloidosis: delicate wispy amyloid expands the endoneurium as shown by Congo red (CR) stain (**a**) which, upon polarization, results in apple-green fluorescence (**b**). Perineurial deposition is demonstrated by CR staining (*arrows*, **c**) and with polarization (**d**) (paraffin sections, **a**–**d** 400×)

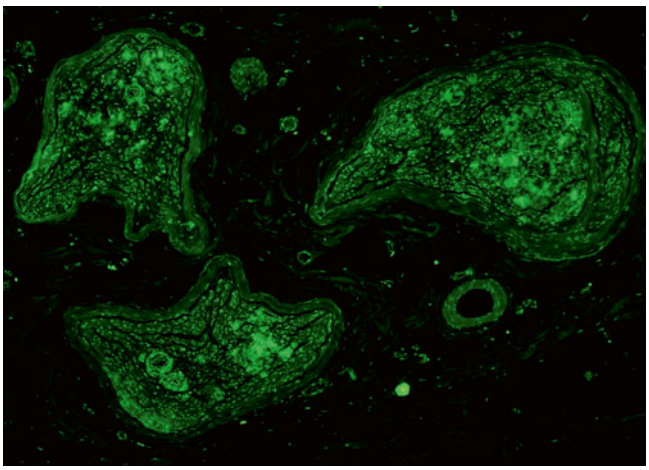

Fig. 15.5 A large endoneurial accumulation of amyloid is demonstrated with thioflavin S fluorescence (paraffin, 200×)

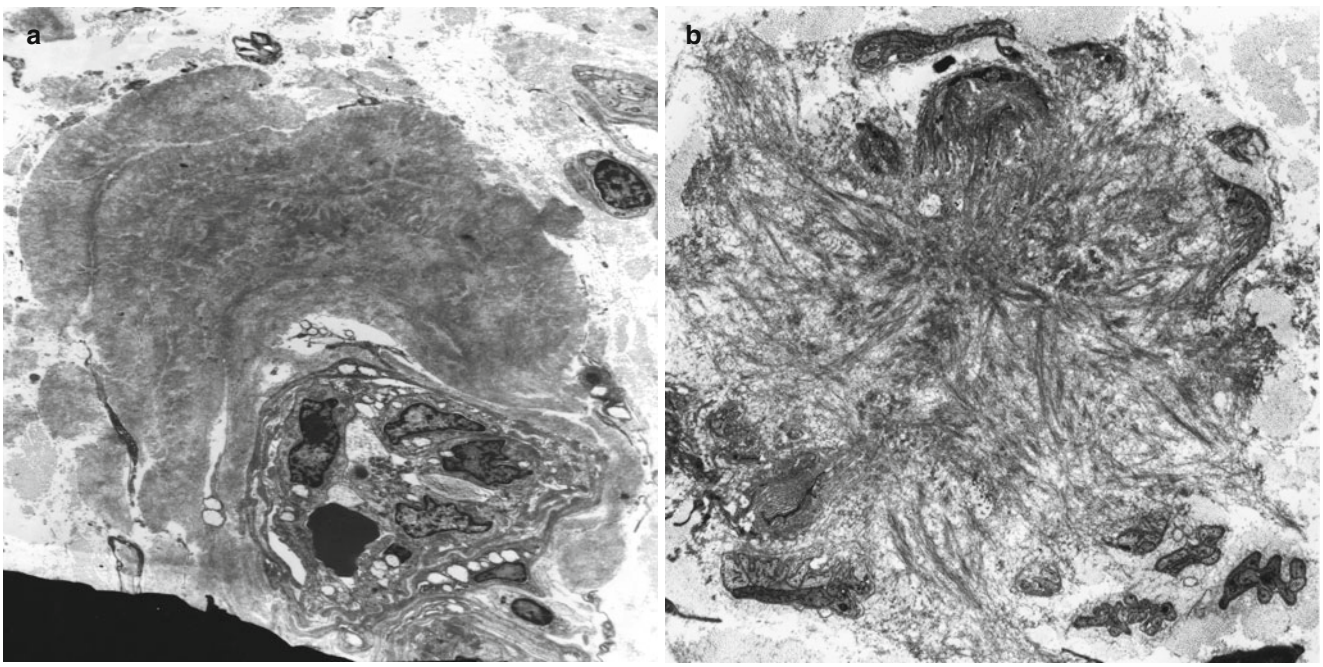

Fig. 15.6 Clumps of amyloid surround an endoneurial venule (**a**) and at higher magnification coarse whorls of amyloid are seen (**b**) (electron micrographs)

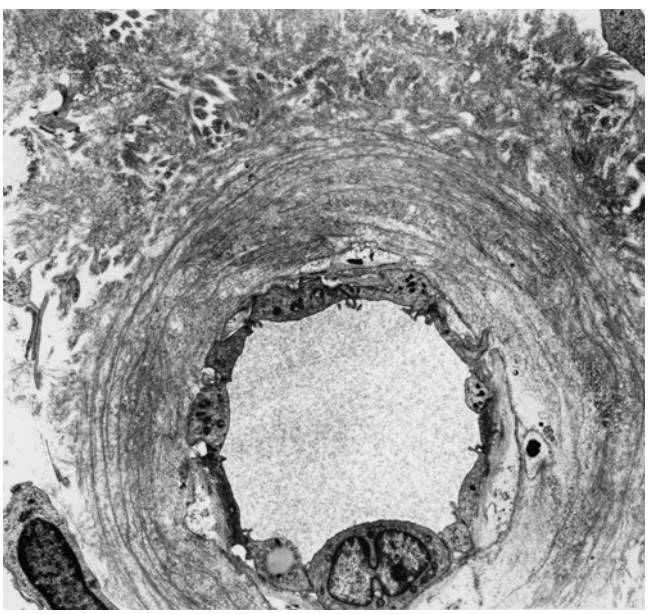

Fig. 15.7 FAP (TTR): electron micrograph of perivascular amyloid deposition. Note imbrication of amyloid fibrils and basal lamina (6,900×)

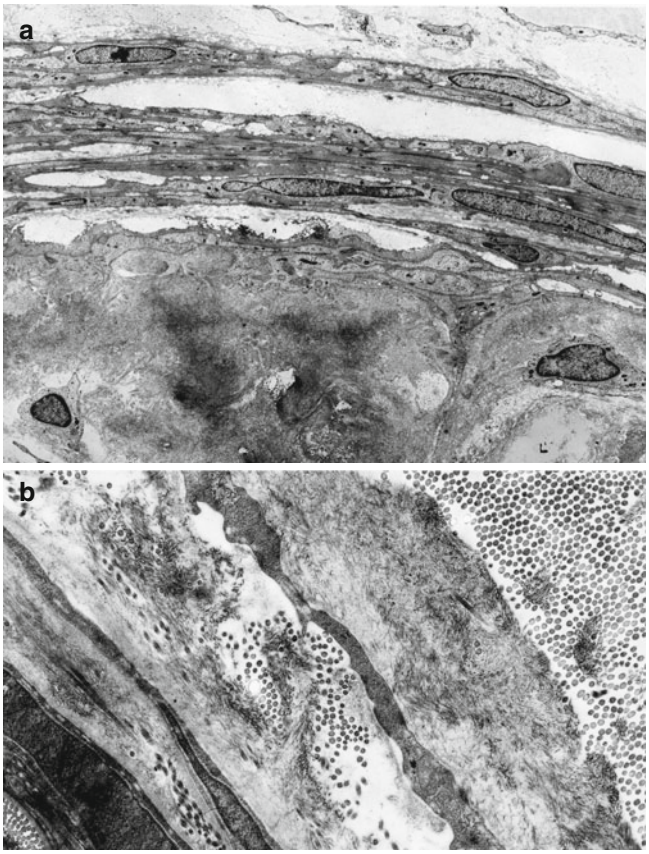

Fig. 15.8 FAP (TTR): (**a**) large subperineurial amyloid deposits are viewed on low-power EM. (**b**) The close association of amyloid and perineurial cells, with effacement of the basal lamina, is shown (**a**, 3,360×; **b**, 15,480×)

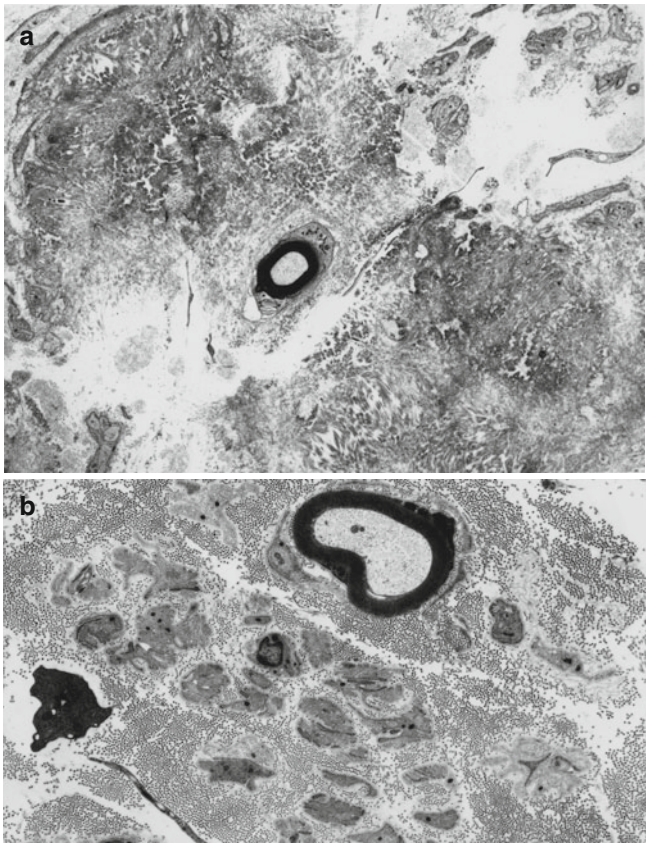

Fig. 15.9 These pictures demonstrate the classic pattern of established amyloid neuropathy with relative sparing of large MFs and severe loss of unmyelinated fibers as evidenced by numerous denervated bands. Compare the massive local deposition of amyloid in **a** with its absence in **b** (**a**, 3,200×; **b**, 9,496×)

had "sporadic" amyloidosis. The amyloid was characterized using immunohistochemistry in muscle. Eleven of the specimens immunostained positively for light chain and three for TTR. In another study of sural nerve biopsy in 39 patients (Li et al. 1992), of 11 found to have positive reactivity for TTR, 3 would not have been suspected of having a familial neuropathy. Of 15 found to have a monoclonal light chain paraprotein (8 Lambda, 7 Kappa), 2 had no evidence of a circulating paraprotein. Thus, immunostaining can be critical in diagnosis. Patients with primary amyloidosis need further hematologic investigation and perhaps chemotherapy. Patients with TTR amyloidosis may be spared aggressive investigation and therapy and may benefit from genetic counseling or liver transplant.

Caution is warranted in interpretation of immunostaining. Alternating sections of Congo red and immunostains are needed for verification that the localization of the immunostain corresponds to sites of amyloid deposits, as non-amyloid immunoglobulin deposition will immunostain positively and is Congo red negative. Amyloid deposits can also be distinguished from immunoglobulin deposits by use of an antiserum amyloid P immunostain, but routine use of this technique

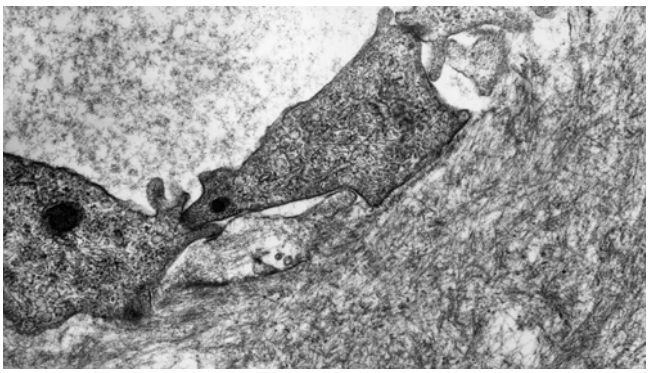

Fig. 15.10 FAP (TTR): the intimate association of amyloid fibrils with endothelial cells is shown. Focally the basal lamina of the affected cell is not discernible (32,110×)

is unnecessary. Sensitivity approaching 100 % has been reported for immunostaining of amyloid (Dalakas and Cunningham 1986; Linke et al. 1986), but those studies were probably done on tissues with uncommonly large amyloid deposits. A more realistic estimate of the failure rate of this

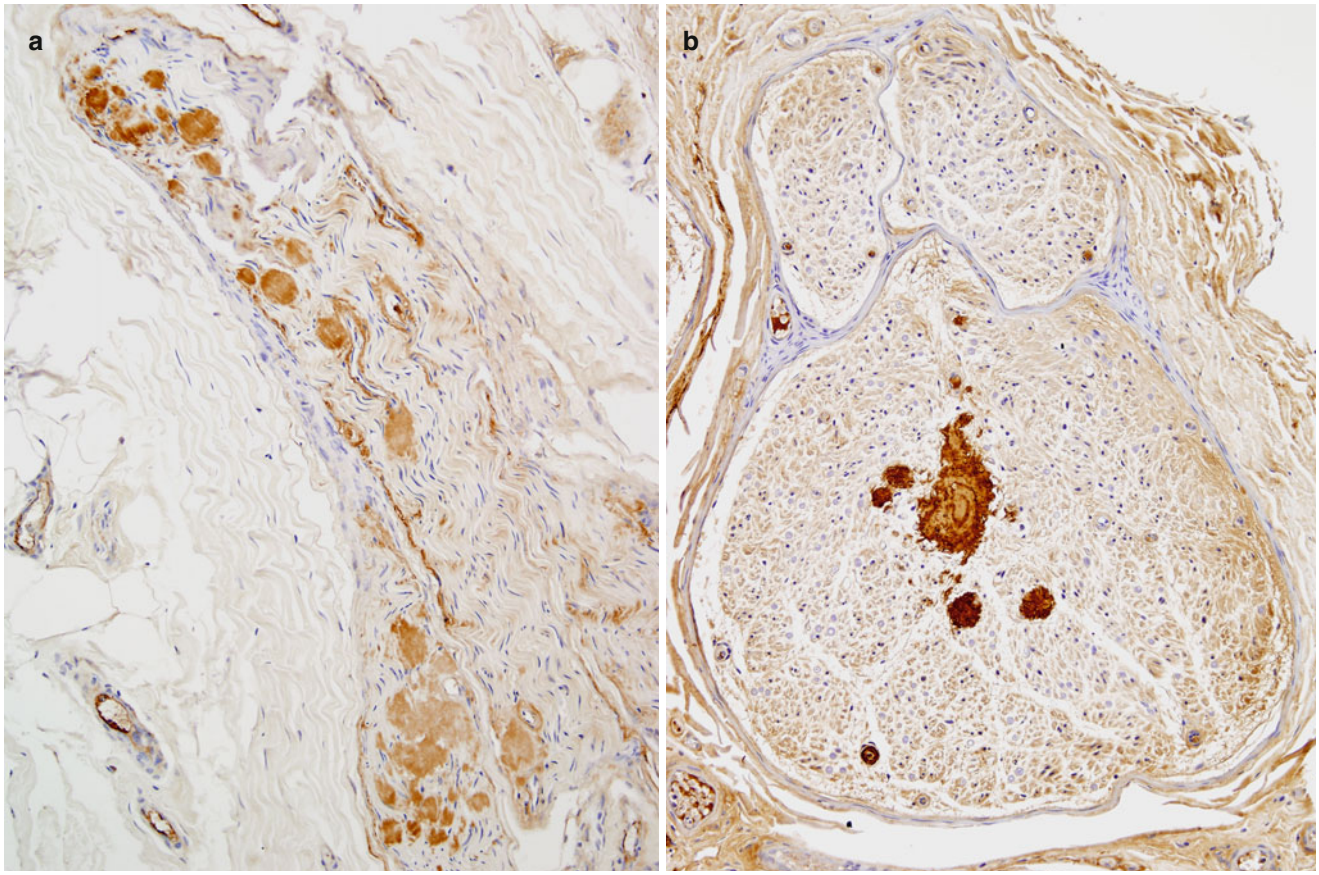

Fig. 15.11 Light chain kappa (**a**) and transthyretin (**b**) deposition is demonstrated with peroxidase immunolocalization (paraffin)

technique in nerve is 10–30 %, perhaps due to the absence of the C region of the light chain in the amyloid deposit, as generally the V region makes a greater contribution to the formation of amyloid (Buxbaum 1992; Feiner 1988; Li et al. 1992). Staining with both kappa and lambda chains should be regarded as uninterpretable, although it could in theory be due to a biclonal gammopathy (Julien et al. 1984). Although not in general use, a promising and very accurate new technique to identify the amyloid main protein in biopsied nerve is laser microdissection and mass spectrometric-based proteomic analysis (Klein et al. 2011)

15.3 Pathogenesis

The mechanisms of amyloidogenesis remain unknown. Kappa light chains outnumber lambda 3:1 in patients with a paraprotein, yet this ratio is reversed in primary amyloidosis (Dalakas and Cunningham 1986; Feiner 1988). Light chains of the λVI subtype have a particular tendency to form amyloid (Solomon et al. 1986). However, no specific explanation exists as to why some paraproteins form amyloid while others do not (Buxbaum 1992; Feiner 1988). The process of creation of amyloid itself remains a much-studied mystery (Buxbaum 1992; Sipe 1992). Neurotoxicity is currently thought to depend on binding of nonfibrillar soluble oligomers to membrane receptors such as the receptor for advanced glycation endproducts (RAGE) resulting in intracellular stress, release of calcium from endoplasmic reticulum, and generation of reactive oxygen species leading to cell death (Hou et al. 2007). Mechanical occlusion of endoneurial capillaries by amyloid deposits is not thought to result in ischemia (Said 2003).

It is of interest that the spinal ganglion is a major site of involvement in amyloidosis (presumably due to the absence of a blood–nerve barrier) and that axonal loss may be ascribed to disease at this location. Selective loss of small neurons in the sympathetic and spinal ganglia has been observed and correlates very well with the early clinical features of amyloid neuropathy (Sobue et al. 1990). As this would not produce motor involvement, it is only a partial explanation. Heavy deposition in proximal nerves with relative sparing of the sural nerve has also been shown to occur (Hanyu et al. 1989; Ikeda et al. 1987; Sobue et al. 1990; Verghese et al. 1983).

A study comparing deposition patterns in primary amyloidosis patients with and without neuropathy noted very little or no endoneurial interstitial or pericapillary amyloid in

patients without neuropathy (Yamada et al. 1984). In both groups there was epineurial and perineurial amyloid deposition. This can be interpreted as evidence for a localized metabolic or cytotoxic effect of amyloid as it comes into contact with endoneurial contents. Furthermore, selective areas of axon and Schwann cell damage may be seen in the immediate vicinity of deposits of amyloid, and distortion of nerve fibers by amyloid masses can be seen (Dyck and Lambert 1969; Jedrzejowska 1977; Said et al. 1984; Sobue et al. 1990). However, in familial amyloid neuropathy, many authors have commented on the absence of correlation between amount of sural nerve endoneurial amyloid and clinical severity or nerve fiber abnormalities in familial neuropathy (Carvalho et al. 1976; Coimbra and Andrade 1971a, b; Guimaraes et al. 1987; Leite et al. 1987).

The importance of epineurial vascular insufficiency has been emphasized by some authors (Asbury and Johnson 1978). Hanyu and colleagues proposed that damage to the blood–nerve barrier by endoneurial capillary amyloid deposits results in edema and increased endoneurial pressure, culminating in nerve fiber ischemic injury (Hanyu et al. 1989). However, standing against this theory is the fact that small fibers are not thought to be more vulnerable to ischemia than large fibers, yet they are affected preferentially in amyloidosis. Epineurial amyloid is probably of limited importance, as the presence of neuropathy correlates best with endoneurial deposits (Yamada et al. 1984). However, these authors also correlated the presence of epineurial vascular amyloid deposits with a loss of large axons (Yamada et al. 1984). In secondary amyloidosis there is epineurial but not endoneurial amyloid deposition, and yet no polyneuropathy develops (Belokrenitzky 1911). Thus, the available evidence suggests that if ischemia is a factor, it most likely occurs at proximal levels not seen on a sural nerve biopsy (Asbury and Johnson 1978; Hanyu et al. 1989), and epineurial vascular damage plays a questionable role except perhaps in late stages of the disease (Sobue et al. 1990).

The presence of a circulating toxin associated with amyloidosis has been postulated (Dalakas and Engel 1979; Trotter et al. 1977). This hypothesis cannot be entirely dismissed because of the observation that amyloid deposition may not correlate with the severity of clinical or pathological involvement (Carvalho et al. 1976; Coimbra and Andrade 1971b; Dalakas and Engel 1979; Guimaraes et al. 1987; Leite et al. 1987). Indeed, the clinical progression and at times the electrophysiological features of amyloid neuropathy may be consistent with a dying-back axonopathy (Said et al. 1984; Sales Luis 1978). However, no specific toxin or metabolic defect has been identified.

The segmental demyelination seen in some biopsies does not correlate with most electrophysiological studies and probably represents secondary demyelination of injured axons (Said et al. 1984). However, there are reports of amyloid neuropathy with significant conduction slowing (Sunada et al. 1993) and of early Schwann cell changes in familial amyloid neuropathy (Carvalho et al. 1976; Coimbra and Andrade 1971b). It may be that different pathogenetic mechanisms are at play in different stages of the disease (Verghese et al. 1983) and in primary vs. familial amyloidosis.

15.4 Differential Diagnosis

The finding of true amyloid deposits within a peripheral nerve is always abnormal, and a diagnosis of amyloid neuropathy can be made. However, circulating paraproteins most commonly cause a neuropathy without amyloid, and sometimes non-amyloid immunoglobulin deposition occurs (immunoglobulin deposition disease – IDD (Buxbaum 1992), Chap. 14). Non-amyloid immunoglobulin deposition may respond to treatment (Meier et al. 1984), while primary amyloidosis has a uniformly poor prognosis (Kelly et al. 1979). The number of cases of IDD presently available for review does not permit meaningful assessment of this issue. The distinction can be made on several criteria. Congo red and other amyloid stains are negative in IDD and deposited immunoglobulin has an ultrastructural granulofibrillar appearance that is different from the straight filaments of amyloid (Figs. 14.4a–c and 14.8a, b). Immunohistochemical techniques may be helpful because IgM has been implicated in almost all well-documented cases of non-amyloid immunoglobulin deposition in nerve, in contrast with the light chain deposition typical of primary amyloidosis.

Oxytalan fibers, the fibrillar constituent of elastic fibers, can be found in a subperineurial, endoneurial, and perivascular locations and may be confused for amyloid. This material differs ultrastructurally from amyloid in that the fibrils are not nearly as straight and long as amyloid fibrils and in being slightly wider in diameter (Fig. 15.1) (Ghadially 1988a; Weis et al. 1993). Moreover, the inner face of the perineurium is a potential site of accumulation of polyclonal immunoglobulin and other macromolecules in pathological and nonpathological settings. Thus, Congo red staining, positive immunostaining for a monoclonal immunoglobulin, and a characteristic ultrastructural appearance are necessary if one is to assert confidently that a fibrillar deposit is amyloid.

The detection of amyloid in a peripheral nerve biopsy specimen should always be followed by the identification of the amyloid major protein by immunohistochemistry. Should the tissue be unsuitable for further studies, biopsy at another site must be considered given the importance of determining whether the patient has a plasma cell dyscrasia or a familial disorder. Skin biopsy has excellent sensitivity in familial amyloid neuropathy (Guimaraes et al. 1987; Leite et al. 1987) and a case can be made for using skin biopsy before nerve biopsy in diagnosis of amyloid neuropathy, especially if a familial cause is suspected (vide supra). Amyloid can

also be visualized in muscle specimens, abdominal fat, and salivary glands. DNA sequencing for specific mutations has become essential noninvasive means for diagnosing familial neuropathies (Adams et al. 1992, 2014; Li et al. 1992; Reilly and King 1993).

Deposition of prion protein amyloid was demonstrable systemically and in peripheral nerves.

Case Report

A 30-year-old male was referred for assessment of recurrent episodes of myoglobinuria and renal failure which had been occurring since the age of 5. Functional enquiry revealed that for the previous 2 years the patient had been experiencing paresthesias below the knee, with difficulty determining temperature of water with his feet. No significant weakness and no autonomic symptomatology were present. The patient was of Portuguese ancestry, and family history was positive for neuropathy. The patient's father had presented at the age of 45 with diminished sensation from the knee down and biopsy proven peripheral nerve amyloid deposits, without any evidence of plasma cell dyscrasia. A paternal uncle and the paternal grandfather were similarly afflicted. The father ultimately died 15 years after the onset of symptoms, being in a bed-bound state at that time.

At initial examination, there was mild (4/5 MRC) symmetrical distal leg muscle and minimal (4+/5) small hand muscle weakness, with minimal distal atrophy. Ankle jerks were absent, knee jerks reduced, and upper extremity reflexes normal. Sensation was diminished to pin and temperature below the knee, with relative sparing of joint position and vibration sensation. Nerve conduction studies revealed absent sensory potentials and diminished amplitudes in the lower extremities. EMG showed a severe loss of motor units, but minimal active distal denervation in the legs. Electrophysiological studies in the upper extremities were normal. Routine screening tests for other causes of peripheral neuropathy were unremarkable. Nerve and muscle biopsy were performed mostly for investigation of the patient's episodic myoglobinuria, which remained ultimately undiagnosed (Figs. 15.8a, b and 15.11b)

The subsequent course was one of slow progression. Four years after initial assessment, the patient had progressed to bilateral foot drop and more disabling hand weakness. Autonomic symptomatology, including impaired bladder control and orthostatic hypotension, became problematic. Examination continued to show relative preservation of reflexes and large fiber sensory modalities.

Case material courtesy of Dr. P. Ashby and Dr. R. McDonald.

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Neuropathy Associated with Neoplasia

16

Systemic malignancy is often associated with polyneuropathy. In this chapter we discuss neuropathy due to tumor infiltration of the peripheral nerve and neuropathy associated with the "paraneoplastic" remote effects of malignancy. We exclude peripheral nerve disease caused by clearly identified systemic metabolic disturbances such as renal failure or malabsorption or occurring as a complication of chemotherapy (see Chap. 18). The neuropathy associated with circulating paraproteins or cryoglobulins is discussed in Chap. 14.

16.1 Paraneoplastic Neuropathy

Paraneoplastic neuropathy has been the subject of recent, extensive reviews (Giometto et al. 2010; Grisold et al. 2013; Graus and Dalmau 2013; Koike and Sobue 2013). Formally defined, paraneoplastic neurological syndromes are rare neurological complications occurring in less than 1 % of people with malignancy which are not due to direct tumor infiltration or metastases, ischemia, metabolic deficits, nutritional deficits, or side effects of chemotherapy (Graus et al. 2004). Paraneoplastic conditions involving the central and peripheral nervous systems are thought to result from an autoimmune response to onconeural antigens shared by the cancer and the peripheral or central nervous systems. A large study (979 patients) performed by the Paraneoplastic Neurological Syndrome Euronetwork (PNSE) (Giometto et al. 2010) has developed criteria for the diagnosis of paraneoplastic neurological syndromes based on the earlier classification of Graus et al. (2004). A definite paraneoplastic syndrome is that arising in a patient with idiopathic neurological symptoms and seropositivity for any well-characterized autoantibodies (anti-Hu, Yo, CV2/CRMP-5, Ri, Ma2, or amphiphysin), regardless of whether an underlying malignancy is found. Other schemes require the cancer to develop within 5 years of the diagnosis of the neurological disorder. However, some cases may not, at the time of diagnosis, be definitively associated with an identifiable onconeural antibody (Rudnicki and Dalmau 2005; Molinuevo et al. 1999) and, as a result, are typically considered nonclassical or "possible" paraneoplastic syndrome. Using these criteria, classical paraneoplastic syndromes of the peripheral nervous system include two major entities: subacute sensory neuronopathy and chronic gastrointestinal pseudo-obstruction (Graus et al. 2004), both often associated with anti-Hu antibodies. In a small study of 20 patients with paraneoplastic neuropathy due to anti-Hu antibodies, neuropathy was classified as sensory in 70 % of patients, sensorimotor in 25 %, and motor in 5 % (Camdessanché et al. 2002). A variety of malignancies have been associated with paraneoplastic peripheral neuropathies, the lung most commonly (vide infra).

Thus, peripheral neuropathies associated with neoplasia include acute, subacute, and chronic sensory and sensorimotor neuropathies, neuropathy with vasculitis, and autonomic neuropathies. Vasculitis is more frequent with hematologic malignancies than with solid malignancies (Fain et al. 2007) and may respond to immunosuppressive therapy (Oh et al. 1991; Antoine et al. 1999).

16.1.1 Paraneoplastic Subacute Sensory Neuropathy (SSN)

According to the PNSE database (Giometto et al. 2010), one-third of patients with paraneoplastic syndromes show primary involvement of the peripheral nervous system, and, of those, subacute sensory neuropathy is the most common form (Graus et al. 2004; Giometto et al. 2010) and may anticipate the detection of neoplasia (Dalmau et al. 1992; Graus et al. 2001). Most commonly (66–76 % of cases), small cell lung cancer (SCLC) is the associated malignancy, but squamous and anaplastic lung cancers, Hodgkin's disease, breast or ovarian cancer, sarcoma, lymphoma, thymoma, and adenocarcinomas have also been described, and single case reports document other malignancies in this setting (Dalmau et al. 1992; Smith 1992; Horwich et al. 1977; Gozzard and Maddison 2010).

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16.1.1.1 Clinical Manifestations

Subacute sensory neuropathy (SSN) is the most common paraneoplastic peripheral neuropathy (24 % of patients in a large European series, Giometto et al. 2010) and usually precedes diagnosis of the associated tumor, sometimes for as long as several years (Dyck et al. 1958; Dalmau et al. 1992; Chalk et al. 1992). Patients present with combinations of sensory ataxia, paresthesias, and limb pain, often with early involvement of the upper extremity, but with little or no weakness or wasting (although involvement of motor functions does not exclude SSN, Graus et al. 2004). Oki et al. (2007) divided paraneoplastic sensory neuropathy into patients with ataxia vs. those with pain which may reflect the size of targeted DRG neurons. Autonomic symptoms, such as urinary retention, orthostatic hypotension, or intestinal pseudo-obstruction, the last often a prominent early feature, may also occur in patients with subacute sensory neuronopathy (Oki et al. 2007). Autonomic features, present in 5 % of a large European series (Giometto et al. 2010) may herald a worse prognosis. A number of patients with autonomic involvement may have anti-Hu, anti-CV2/CRMP-5, or anti-Ach receptor antibodies (Lucchinetti et al. 1998; Yu et al. 2001; McKeon et al. 2009; Graus and Dalmau 2013). Progression occurs over days to weeks, often to a severity which precludes limb use. Concurrent paraneoplastic CNS involvement is common, including encephalopathy, cerebellar syndrome, or myelopathy. The CSF protein is typically elevated and the cell count usually normal. Electrical testing shows features of an axonal sensory neuropathy with normal or minimally altered motor conductions and EMG.

Survival from onset of neurological symptoms averages 2–3 years (Horwich et al. 1977), and death results as often from the neurological disease as from the underlying tumor (Dalmau et al. 1992). With a few exceptions, treatment appears to be ineffective, including that directed at the associated malignancy, but several patients with Hodgkin's disease have experienced improvement with successful treatment of their malignancy (Smith 1992).

A clinically similar inflammatory sensory ganglionopathy can occur in the absence of systemic malignancy (Smith 1992). However, central nervous system features are absent, and the anti-Hu antibody (vide infra) is not detected.

16.1.1.2 Pathology

Most cases of SSN result from damage to the spinal dorsal root ganglia (DRG, Fig. 16.1a). Autopsy studies have shown a loss of DRG neurons and focal chronic inflammatory infiltrates, chiefly CD8-positive cytotoxic T cells (Wanschitz et al. 1997; Ichimura et al. 1998), with resultant degeneration of the dorsal columns (Horwich et al. 1977). Large neurons may be preferentially affected (Ohnishi and Ogawa 1986). These patients should not undergo sural nerve biopsy, as their syndrome is characteristic, biopsy pathology is nonspecific, and serological tests for the associated onconeural antibodies are available. However, diseases with characteristic histology which might mimic SSN include sarcoidosis, paraprotein-associated neuropathies, and CIDP.

Nonetheless, pathological reports on a few patients are available (Lamarche and Vital 1987; Horwich et al. 1977; Dalmau et al. 1992; Dyck et al. 1958; Chalk et al. 1992). Sural nerve biopsy in patients with subacute sensory neuronopathy and marked sensory ataxia shows loss predominantly of large myelinated fibers, differing from preferential loss of small myelinated and unmyelinated axons in patients with pain predominant symptoms (Oki et al. 2007). Axon depletion in SSN ranges from mild to near total, with active degeneration a variably prominent feature (Fig. 16.1b, c). Axonal regenerative activity is usually minimal or absent, as expected in a neuronopathy (Lamarche and Vital 1987; Koike and Sobue 2008).

The majority of nerve biopsies reported in SSN have not shown inflammation, although one of our cases has shown significant sural inflammatory infiltration (Fig. 16.2a, b). However, prominent perivascular and vessel wall inflammation has occasionally been noted (Vallat et al. 1986; Plante-Bordeneuve et al. 1994) and, rarely, even vasculitis has been described (Johnson et al. 1979; Eggers et al. 1998). Such cases seem to reflect disease of the peripheral nerve rather than the spinal ganglion and may have a better prognosis in some patients (Plante-Bordeneuve et al. 1994) or may reflect a role for CV2/CRMP-5 which may occur with anti-Hu and be more targeted to peripheral nerve vs. dorsal root ganglia (vide infra).

16.1.1.3 Pathophysiology and Pathogenesis

The Paraneoplastic Neurological Syndrome Euronetwork (Giometto et al. 2010) has defined a number of antibodies (anti-Hu, Yo, CV2/CRMP-5, Ri, AChR, Ma2, Tr, and amphiphysin) which are thought to have a role in various paraneoplastic syndromes and may result in different clinical syndromes. Paraneoplastic SSN is most strongly associated with circulating antineuronal antibodies (anti-Hu, also called antineuronal nuclear antibody type 1, ANNA-1), which exhibits specificity for neuronal nuclear antigens (Smith 1992) and is most typically found in association with small cell lung cancer, much less frequently in other malignancies (Dalmau et al. 1992). It is thought that patients with anti-Hu antibodies alone most often present with subacute sensory neuronopathy, those with anti-CV2/CRMP-5 antibodies develop mixed axonal and demyelinating sensorimotor neuropathy, and those with both antibodies exhibit subacute sensory neuronopathy superimposed on demyelinating sensorimotor neuropathy (Antoine et al. 2001; Koike and Sobue 2013). Although patients with anti-Hu antibodies show a wide range of paraneoplastic neurological manifestations, including cerebellar ataxia, limbic encephalitis,

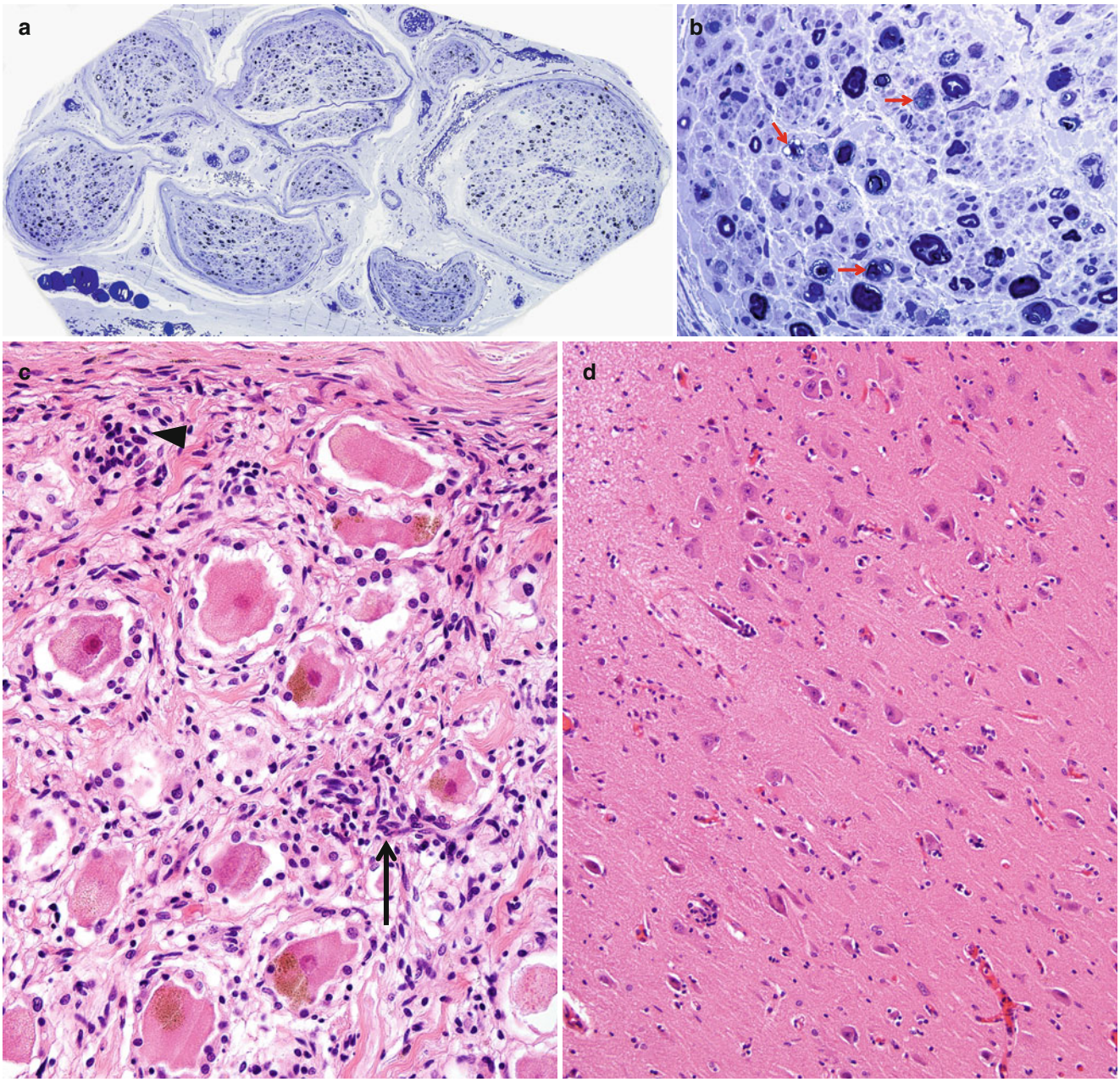

Fig. 16.1 Paraneoplastic subacute sensory neuropathy, radial nerve taken at the anatomical "snuffbox." This 43-year-old woman was diagnosed as having small cell cancer of the lung following presentation with subacute sensory neuropathy involving the upper extremities. Note axon loss involving all fascicles (**a**) and marked active axonal degeneration affecting all fiber sizes (*arrows*, **b**). Regenerative axonal clusters are not seen. (**c**) Dorsal root ganglion of a different patient with small cell lung cancer and laboratory proven anti-Hu antibody. Inflammation at sites of neuron degeneration (*arrow*, **c**) and a developing residual nodule of Nageotte, a tombstone of prior neuronal degeneration (*arrowhead*, **c**) are shown. This patient also had limbic encephalitis with inflammatory changes in the hippocampus (**d**) (magnification: **a**, 40×; **b**, 1,000× 1 μ thick plastic sections; **c** 400×; **d**, 200×)

myelopathy, Lambert–Eaton syndrome, and myopathy, peripheral neuropathy is the most common manifestation and present in 60–80 % of patients with SSN (Dalmau et al. 1992; Lucchinetti et al. 1998; Graus et al. 2001; Sillevis Smitt et al. 2002). The SSN DRG shown in Fig. 16.1c was accompanied by limbic encephalitis in the hippocampus (Fig. 16.1d). The anti-Hu antibody reacts with neoplasm antigens (Dalmau et al. 1992), leading to the assertion that the clinical syndrome is caused by antibodies produced against the tumor which cross-react with neuronal antigens. However, some of the lymphocytes infiltrating nervous tissue also appear to be sensitized to the Hu antigen, and cell-mediated damage may also play a role (Dalmau et al. 1992). In a previous study of 162 consecutive patients with anti-Hu

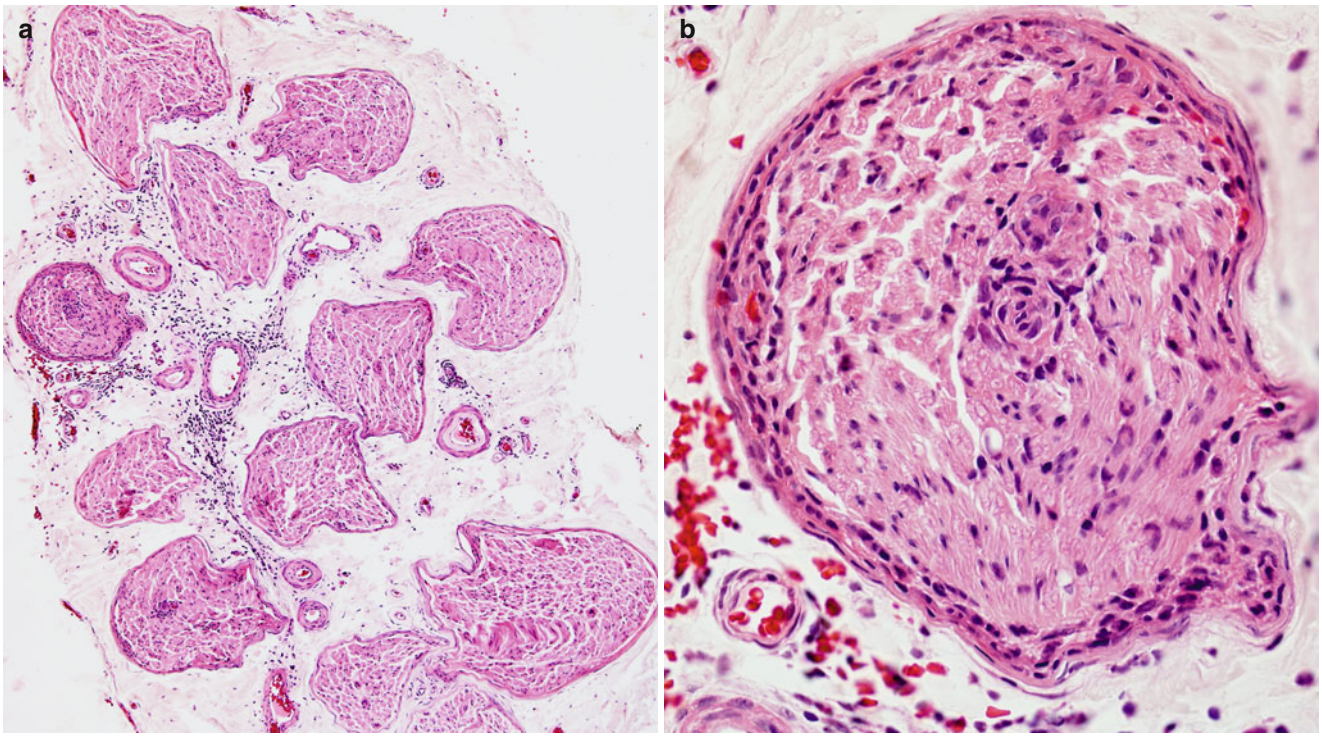

Fig. 16.2 Paraneoplastic neuropathy: sural nerve biopsy which led to the discovery of a small cell lung tumor in a 54-year-old woman. (**a**) Somewhat atypically, inflammation involves the epineurium, both perivascular and diffuse. (**b**) Higher magnification demonstrates prominent inflammatory infiltrate involving the perineurium and cuffing a small endoneurial vessel (paraffin, H&E, **a**, 100×; **b**, 600×)

antibodies, malignancy was present in 88 % of patients, and small cell lung cancer was subsequently identified in 93 % (Lucchinetti et al. 1998). $CD8^+$ T cells in the DRG of SSN include both cytotoxic $CD8^+$-positive T cells and atypical noncytotoxic $CD8^+$-positive T cells with an uncertain dynamic (Roberts et al. 2009). It has been proposed that small cell lung cancer may evade surveillance by skewing tumor antigen-specific $CD8^+$ T cells to an unusual noncytotoxic phenotype, providing an explanation for variation of clinicopathological features in anti-Hu antibody-associated paraneoplastic neurological syndromes (Roberts et al. 2009). Anti-CV2/CRMP-5 is also strongly associated with lung carcinoma (Honnorat et al. 1996; Yu et al. 2001), mostly small cell type, which was ultimately found in 77 % of patients with anti-CV2/CRMP-5 antibodies. Unlike anti-Hu antibodies, anti-CV2/CRMP-5 antibodies cross-react with antigens in the peripheral nerve trunk rather than DRG neurons (Antoine et al. 2001). Findings suggestive of demyelination and axonal degeneration have also been reported in biopsy specimens from patients with anti-CV2/CRMP-5 antibodies (Antoine et al. 2001). Somatic neuropathy is the most common manifestation in patients with anti-Hu and anti-CV2/CRMP-5 antibodies, while antiganglionic AchR antibody is associated with autonomic neuropathies (Graus et al. 2004). However, passive transfer of these antibodies or immunization has failed to reproduce the disease in animals (Sillevis Smitt et al. 1995).

A small proportion of patients with the antibody and the clinical syndrome have had no malignancy detected, even at autopsy (Dalmau et al. 1992). It is unclear whether the tumor was simply too small to be detected (Chartrand-Lefebvre et al. 1998; Lucchinetti et al. 1998) or the immune reaction to Hu antigen was caused by another factor. Fluorodeoxyglucose positron emission tomography (FDG-PET) may reveal the malignancy even if small (Rees et al. 2001; Younes-Mhenni et al. 2004; Titulaer et al. 2011).

Patients presenting with subacute sensory neuronopathy tend to show a better response to immunomodulatory treatment compared to patients with other classic paraneoplastic neurological syndromes (Uchuya et al. 1996; Keime-Guibert et al. 2000; Graus et al. 2001; Vernino et al. 2004).

16.1.2 Paraneoplastic Sensorimotor Neuropathy

16.1.2.1 Clinical Manifestations

Whereas SSN is a rare but characteristic paraneoplastic syndrome, a more nondescript sensorimotor neuropathy is seen in 5–16 % of cancer patients, with as many as 30–40 % showing subclinical electrophysiological abnormalities (McLeod 1993; Croft and Wilkinson 1965; Hawley et al. 1980; Walsh 1971; Graus et al. 1983; Paul et al. 1978). Small cell lung cancer is not as uniformly detected with these

neuropathies as it is with SSN, and every type of malignancy has been associated with sensorimotor neuropathy, most often lung, breast, ovarian, stomach, and prostate cancers, as well as lymphoma (Croft and Wilkinson 1965; Walsh 1971; Dayan et al. 1965). Although the existence of this nonspecific sensorimotor "paraneoplastic" neuropathy is accepted, it is less precisely defined than SSN. Nonetheless, there is an association of patients with anti-CV2/CRMP-5 antibodies and the development of mixed axonal and demyelinating sensorimotor neuropathy, and those with anti-Hu and anti-CV2/CRMP-5 antibodies exhibit subacute sensory neuronopathy superimposed on demyelinating sensorimotor neuropathy (Antoine et al. 2001; Koike and Sobue 2013).

The paraneoplastic sensorimotor neuropathies are not homogenous, and classification is difficult (Corbo and Balmaceda 2001) because the pathophysiology of these entities is not understood and clinical features overlap. One approach that has been commonly used (Croft et al. 1967; McLeod 1993) distinguishes three groups on a clinical basis. Most frequently seen is a mild sensorimotor neuropathy that appears after the diagnosis of malignancy, sometimes called "mild terminal neuropathy"; the only abnormality may be hypoactivity of distal reflexes. The second group shows a more rapidly evolving and severe polyneuropathy that as often as not presents before the malignancy is diagnosed. The third and smallest group is composed of patients with a relapsing–remitting sensorimotor neuropathy. Regardless of where the patient falls into this classification, the neuropathic manifestations are typically sensorimotor, symmetrical, and predominantly distal, although proximal wasting and weakness can suggest a "neuromyopathy" (Croft and Wilkinson 1963). Motor neuron degeneration in the spinal cord at autopsy has been reported (Dalmau et al. 1992; Verma et al. 1996) and may be the cause of the motor component of paraneoplastic sensorimotor neuropathy in patients with anti-Hu-associated paraneoplastic neuropathy.

CSF protein is often elevated in the rapidly evolving and relapsing groups, but rarely in patients with "mild terminal neuropathy." In this latter group, the electrophysiological assessment usually demonstrates axonal features, but in the more severe sensorimotor and the relapsing/remitting groups, a demyelinating or mixed pattern is often seen (Croft et al.1967; Paul et al. 1978; Graus et al. 1983).

As a rule, treatment of the tumor does little for the neuropathy, but exceptions have been reported, especially for lymphoma and seminoma (Evans and Kaufman 1971; Littler 1970; Enevoldson et al. 1990). Anecdotal reports indicate improvement with steroid or plasma exchange (Croft et al. 1967; Valbonesi et al. 1985).

16.1.2.2 Pathology

Autopsy studies have not shown disease of the spinal ganglia in paraneoplastic sensorimotor neuropathy except when SSN was superimposed (Croft et al. 1967). Inflammatory infiltrates have also been sparse in proximal nerve trunks (Croft et al. 1967). The literature on the peripheral nerve pathology of paraneoplastic neuropathy is scanty, since patients with a mild "terminal" neuropathy are unlikely to undergo a nerve biopsy. Moreover, some authors have not distinguished between the various sensorimotor neuropathy groups.

Mild Terminal Neuropathy

Sural nerve biopsy of patients with "mild terminal neuropathy" and lung cancer or lymphoma (Graus et al. 1983; Walsh 1971; Schlaepfer 1974) has revealed minimal reduction in total myelinated fiber density, but a significant decrease in large myelinated axons. At times active axonal degeneration and regenerating clusters have been present. Axons may appear thinly myelinated, and small onion bulbs can occasionally be seen. Schlaepfer (1974) suggested that the myelin alterations were secondary to axonal disease. Unmyelinated fibers are relatively or entirely spared (Walsh 1971). Inflammatory infiltrates are not a feature of "mild terminal neuropathy." Immunofluorescence did not reveal immunoglobulin or complement deposition in the cases studied by Graus and co-workers (1983).

Severe Rapidly Evolving or Relapsing Sensorimotor Neuropathies

Croft and colleagues (1967) examined the peripheral nerve obtained at autopsy in 10 patients, 8 with subacutely evolving neuropathy and 2 with relapsing neuropathy. Slight lymphocytic infiltration was seen in 3 of 10 cases. Segmental demyelination and axonal degeneration were present, the former often predominating. Lamarche and Vital (1987) described nerve biopsies in six patients, most of whom had an acute or subacute neuropathy (Lamarche and Vital 1987). Axonal and demyelinating features were seen, the former usually predominating. Onion bulbs and regenerating clusters were often present. Inflammation was only identified in one case. Ultrastructural examination revealed that unmyelinated fibers were also affected, and uncompacted myelin was described in one instance. Graus et al. (1983) studied two patients with lymphoma and a severe neuropathy, one showing prominent demyelination and the other a mixed picture; inflammation was absent in both cases. The case of lymphoma reported by Sumi et al. (1983, case 1) showed typical features of macrophage-mediated demyelination and a course consistent with CIDP.

Although the demyelinating features of rapidly evolving paraneoplastic neuropathy have been emphasized in the literature, axonal neuropathies with no inflammatory features have also been described in this setting (Schlaepfer 1974; Enevoldson et al. 1990). In our experience, severe sensorimotor neuropathies associated with systemic malignancy may be axonal or demyelinating and inflammatory or noninflammatory. No specific features are present, and the diagnosis is made on clinical grounds.

Immunofluorescent studies have shown IgM and complement deposits in a subperineurial and perivascular localization (Ongerboer de Visser et al. 1983); however, this is a nonspecific finding.

Paraneoplastic "Microvasculitic" Neuropathy

A number of patients have been described who presented with a sensorimotor polyneuropathy in association with systemic malignancy and demonstrated "microvasculitis" on nerve biopsy (Vincent et al. 1986; Oh et al. 1991; Johnson et al. 1979; Younger et al. 1994). In the case reported by Younger et al. (1994), there was an associated anti-Hu antibody, but whether this was a causal factor or simply a coincidence is unknown. The descriptions and figures in these reports indicate an axonal neuropathy with intense perivascular cuffing and mononuclear infiltration of the vessel wall.

Use of the term "vasculitis" requires some evidence of vessel wall destruction, preferably fibrinoid necrosis and at the very least some evidence of disruption of the endothelium. While infiltration of the vessel wall is suggestive, it is not in itself sufficient to be considered vasculitis. Intramural lymphocytes may be in the process of migration into the endoneurium (Vallat et al. 1986). We too have seen axonal inflammatory neuropathies in which no etiology was found but a systemic malignancy was present, and we prefer to regard these as inflammatory neuropathies rather than "microvasculitis." In lymphoid malignancies, nonspecific nonneoplastic inflammatory infiltrates seem to be a common finding (Dickenman and Chason 1958; Jellinger and Radiaszkiewicz 1976). The presence of prominent inflammation in "paraneoplastic neuropathy" may have therapeutic implications (Oh et al. 1991).

16.1.2.3 Pathogenesis

In contrast to paraneoplastic subacute sensory neuropathy, the cause of paraneoplastic sensorimotor neuropathy remains more obscure. Although previously no tumor-related immunologic alteration or toxin has been found to affect peripheral nerve (McLeod 1993; Ongerboer de Visser et al. 1983), more recent studies have identified onconeural antibodies. Specifically, patients with anti-CV2/CRMP-5 antibodies are now known to develop mixed axonal and demyelinating sensorimotor neuropathy, and those with both antibodies exhibit subacute sensory neuronopathy superimposed on demyelinating sensorimotor neuropathy (Antoine et al. 2001; Koike and Sobue 2013). In some studies, the frequency of neuropathy was correlated with weight loss (Hawley et al. 1980; Graus et al. 1983; Hildebrand and Coers 1967), but this is not consistent even for "mild terminal neuropathy" (Croft et al. 1967).

In the rapidly evolving or relapsing paraneoplastic neuropathies, segmental demyelination and onion bulbs have been seen, although not usually to the extent seen in CIDP or other hypertrophic neuropathies. This suggests an important role for myelin injury. Indeed, CIDP may be "precipitated" by immunologic disturbances associated with the tumor (Sumi et al. 1983). However, segmental demyelination in paraneoplastic sensorimotor neuropathy may be secondary, as suggested by some teased fiber studies (Schlaepfer 1974; Lamarche and Vital 1987; Walsh 1971).

Some paraneoplastic sensorimotor neuropathies may be "dying-back" axonopathies. Intramuscular nerve axonal swelling has been described as paraneoplastic phenomenon by several authors (Barron and Heffner 1978; Brownell and Hughes 1975; Hildebrand and Coers 1967; Awad 1968) and may represent the earliest stages of a distal axonopathy as seen first in the longest motor fibers (Barron and Heffner 1978); similar changes are seen in typical toxic distal axonopathies (Spencer and Schaumburg 1977). However, the specificity and significance of this finding are suspect given its high frequency in normal intramuscular nerves and in neuropathies which are not believed to be due to a "dying-back" process (Alderson 1992).

16.1.3 Other Associations Between Neuropathy and Malignancy

16.1.3.1 Necrotizing Peripheral Nerve Vasculitis

The co-occurrence of vasculitis and malignancy is well recognized (Sanchez-Guerrero et al. 1990), but peripheral nerve involvement seems to be rare. Nonetheless, peripheral nerve necrotizing vasculitis has been described in association with malignancy. Patients may present with a sensorimotor polyneuropathy (Harati and Niakan 1986; Torvik and Berntzen 1968; Naka et al. 1991) or as pure sensory syndrome (Johnson et al. 1979). Deposition of immunoglobulin or complement in the vessel has not been detected (Johnson et al. 1979). Given the infrequency of this association, the possibility of a chance co-occurrence of peripheral nerve vasculitis and systemic malignancy must be considered.

16.1.3.2 Acute Demyelinating Neuropathy

Guillain–Barré syndrome has been infrequently reported in association with Hodgkin's disease (Lisak et al. 1977; Ropper et al. 1991; Julien et al. 1980; Vital et al. 1990). No special features characterized the few cases studied pathologically, and typical macrophage-mediated demyelination has been observed (Julien et al. 1980).

16.1.4 Differential Diagnosis

Identification of paraneoplastic neuropathy may lead to early detection of malignancy. A paraneoplastic basis should be considered in any sensorimotor axonal or mixed neuropathy,

inflammatory or not, when no other etiology can be determined. As lung cancer and lymphoma account for a large fraction of the associated malignancies, a thorough physical exam and a chest X-ray would go a long way toward excluding the possibility of an occult malignancy. Unfortunately, nerve biopsy does not reveal any characteristic histological features to suggest the diagnosis and should only be performed when an alternative and treatable diagnosis is under consideration.

16.2 Neuropathy Due to Neoplastic Infiltration

Diffuse infiltration of nerve with malignant cells is an uncommon cause of neuropathy. This is essentially confined to hematologic malignancy, including lymphoma, leukemia, myeloma, and a few other rare entities. Solid organ cancers, such as those of the ear, nose, and throat (ENT), pancreas, and prostate, often produce peripheral nerve dysfunction by compression or gross infiltration of roots, plexus, or focal segments of nerve but do not produce a diffuse infiltrative neuropathy (Grisold et al. 2013) and are thus not considered.

16.2.1 Lymphomatous Neuropathy

16.2.1.1 Clinical Manifestations

Lymphomatous neuropathy is diagnosed when malignant lymphoma infiltrates and damages the peripheral nerve. This has been called neurolymphomatosis by some (Diaz-Arrastia et al. 1992), although others have taken nosologic exception (Guberman 1984; Baehring et al. 2003; Grisariu et al. 2010). Diaz-Arrastia et al. (1992) have recently reviewed the subject and examined a total of forty cases from the literature. Clearly the disorder is much more common than such numbers would indicate, as we have seen 4 cases in our own laboratory. About half of patients are not known to have systemic lymphoma at the time of diagnosis. Even at autopsy, 7 of 22 patients had lymphoma restricted to the nervous system alone, 2 had a focal lymphoma with dissemination to peripheral nerves only, and disseminated lymphoma was present in 13 (Diaz-Arrastia et al. 1992). The lymphoma is almost invariably of the non-Hodgkin (NHL) type (98 %), and 75 % of appropriately studied cases were of the B-cell malignancies (Diaz-Arrastia et al. 1992). Neuropathy is usually diffuse and sensorimotor in type and can evolve acutely mimicking Guillain–Barré syndrome, in a relapsing–remitting fashion, or most often progressing relentlessly over weeks to months (Diaz-Arrastia et al. 1992). One of the cases we have studied progressed slowly over 2 years. Pain may be a prominent feature. Electrophysiological testing can demonstrate axonal, demyelinating, or mixed features, and CSF examination often reveals elevated protein or CSF pleocytosis, with malignant cells in about half of cases (Diaz-Arrastia et al. 1992). Steroids, chemotherapy, or radiation therapy may be helpful. While the prognosis is usually poor (Diaz-Arrastia et al. 1992), in some patients, appropriate treatment may result in survival for many years (Ince et al. 1987).

Also intravascular lymphoma, formerly named "Tappeiner" disease or neoplastic angioendotheliomatosis, has been added to the concept of lymphomatous involvement (Glass et al. 1993). It can present as multiplex neuropathy, as Guillain–Barré syndrome (GBS) (Jiang et al. 2010), and as chronic inflammatory demyelinating polyneuropathy (CIDP) (Grisold et al. 2007; Briani et al. 2009, 2011). Intravascular lymphoma (Glass et al. 1993) is typically of B-cell origin and is thought to reflect lack of CD29 (β-1 integrin) and CD54 (ICAM-1) adhesion molecules on the neoplastic lymphoma cells interfering with transvascular migration (Ponzoni et al. 2000). Cauda equina syndrome, ascending polyradiculopathies (Levin and Lutz 1996; Patel et al. 2006; Jiang et al. 2010), multiple mononeuropathies (Roux et al. 1995), isolated mononeuropathies (Vital et al. 1989), symmetrical neuropathies (Oei et al. 2002), and muscle involvement (Fallon et al. 2002) have been observed (reviewed in Grisold et al. 2013).

16.2.1.2 Pathology

Because only half of patients are known to have systemic lymphoma, neurolymphomatosis is one instance where nerve biopsy may be essential in making the diagnosis (Diaz-Arrastia et al. 1992). Even if malignancy is known to be present, infiltration of the nerve may be the only visible manifestation of recurrence after treatment (Shoenfelt et al. 1983; Krendel et al. 1991). Descriptions below are based on literature material (Barron et al. 1960, case 5; Gherardi et al. 1986; Guberman et al. 1978; Ince et al. 1987; Krendel et al. 1991; Thomas et al. 1990; Vital et al. 1990; Diaz-Arrastia et al. 1992; Zuber et al. 1987; Kohut 1946; Stack 1991; Atiq et al. 1992; Shoenfeld et al. 1983; Gold et al. 1988) and our experience with 4 cases – two at autopsy and two at nerve biopsy.

Autopsy reviews provide an inconsistent picture of the frequency of peripheral nerve involvement. In two autopsy series of Hodgkin's disease, peripheral nerve tumor infiltration was detected in 0/30 and 5/30 patients, but far more often there were nonspecific nonneoplastic inflammatory infiltrates (Dickenman and Chason 1958; Jellinger and Radiaszkiewicz 1976). In the study of Grisariu et al. (2010), neurolymphomatosis was due to non-Hodgkin's lymphoma in 90 % of cases and to acute leukemia in 10 %. In up to 26 % of the cases, it occurred as an initial manifestation of malignancy. We have found only one report of Hodgkin's lymphoma causing a histologically proven infiltrative clinical

neuropathy (Barron et al. 1960). In NHL the incidence of detected peripheral nerve infiltration by tumor cells in autopsy material varied from 0/20 to 41/98 (Dickenman and Chason 1958; Jellinger and Radiaszkiewicz 1976), but the quantity and location of peripheral nervous tissue studied was not clearly specified. A wide variety of NHL types have been present, including cutaneous and non-cutaneous T-cell lymphomas and high- and low-grade B-cell lymphomas. No particular subtype emerges as particularly likely to infiltrate peripheral nerve. Analysis of the literature is limited by small numbers and lack of the modern immunohistochemical and cytological techniques now routinely used to categorize lymphoma.

Light Microscopy

In our experience, a diffuse massive infiltration of all peripheral nerve compartments is most typical of lymphoma, although fascicles may be differentially involved (Figs. 16.3 and 16.4). The infiltrate may demonstrate epineurial predominance or prominent perineurial and subperineurial localization (Fig. 16.3). Selective exclusive endoneurial involvement is rare and has been described after treatment with chemotherapy (Schoenfeld 1983; Krendel et al. 1991), but may also occur in untreated patients (Atiq et al. 1992). The illustrated case of neurolymphomatosis showed small epineurial perivascular foci of atypical cells in only 2 of 38 tissue blocks (Fig. 16.4b). This underscores the importance of examining the maximum amount of tissue possible.

A tendency toward perivascular cuffing is commonly seen, and sometimes a striking angiocentricity of the tumor cells is present. The infiltrating cells may be found within vessel walls but do not cause vascular necrosis. Reticulin stains will demonstrate the formation of a delicate network around vessels and within collections of tumor cells. Proliferation of epineurial or endoneurial vessels may be seen (Gherardi et al. 1986; Zuber et al. 1987). Mitotic figures, pleomorphism, and atypia of the infiltrating cells are usually immediately suggestive of the diagnosis (Figs. 16.3, 16.4 and 16.5). However, well-differentiated lymphoma cells might be difficult to distinguish from mature lymphocytes (Fig. 16.5).

The neuropathic process is a variable mixture of acute and chronic axonal degeneration and segmental demyelination with remyelination, the former usually predominating. At times the co-localization of myelino-axonal changes and focal endoneurial tumor deposits may be impressive (Barron et al. 1960). In one case of a severe diffuse demyelinating neuropathy, the degree of myelin damage was disproportionate to the density of the neural infiltrate, and the infiltrating tumor was shown to produce an IgM paraprotein (Ince et al. 1987).

Angiotropic lymphoma is rare and unique in its histological features (Fig. 16.6a–f). Multisystem manifestations result from obstruction of microvascular blood flow by aggregates of lymphoma cells. Nerve biopsy has typically shown a mild degree of axonal degeneration and occlusion of endoneurial and epineurial vessels by malignant lymphocytes with little or no spillover into the endoneurium (Vital et al. 1989) which may reflect lack of elements needed for homing, binding, and extravascular migration.

Immunohistochemistry

Demonstration of clonality is best performed with flow cytometry on large volumes of tumor tissue. If atypical cells are seen in a nerve biopsy specimen, confirmation and characterization of the lymphoid malignancy can be done using available B- and T-cell markers. The presence of a monoclonal population of infiltrating cells is inferred when the vast majority of the cells belong to a single lymphocyte subset. A few reactive lymphocytes not belonging to the malignant clone are usually present. Numerous markers are available to characterize various lymphocyte subtypes and could be used to characterize the lymphoma if no other tissue is available.

16.2.2 Leukemic Infiltration of the Nerve

16.2.2.1 Clinical Manifestations

The most frequent type of neoplastic involvement ("neuroleukemiosis") is multiple nerve root involvement, including in particular cranial nerves by CSF spread. The extraleptomeningeal infiltration of either cranial (Hiraki et al. 1997) or peripheral nerves is rare. However, the myelomonocytic type (FAB M4/5) tends to have a propensity to leukemic infiltration of peripheral nerves (Grisold et al. 1985; Krendel et al. 1987). Chronic lymphatic leukemia rarely affects the peripheral nerves (Lange 2007). Clinically significant infiltration of nerves in leukemia occurs less frequently than in lymphoma. We have identified approximately 20 cases in the literature with adequate peripheral nerve histology, including 6 of chronic lymphocytic leukemia (CLL) (Grisold et al. 1990; Thomas et al. 1990; Vital et al. 1975; Sumi et al. 1983; Haberland et al. 1987; Rowland and Schneck 1963), 6 of acute lymphocytic leukemia (ALL) (Alajouanine et al. 1949; Barron et al. 1960, case 8; Harris 1921; Liu et al. 2007; Aregawi et al. 2008), two of adult T-cell leukemia/lymphoma (ATLL) (Kuroda et al. 1989; Vital et al. 1993a), two of acute monoblastic leukemia (Henson and Urich 1982; Krendel et al. 1987), two of acute myelomonoblastic leukemia (Vital et al. 1993b), 4 of acute myelogenous leukemia (Lekos et al. 1994; Platten et al. 2007; Reddy et al. 2012), and one each of acute megakaryoblastic (Nishi et al. 1991), chronic myelogenous leukemia (Barron et al. 1960, case 4), natural killer cell (Bobker and Deloughery 1993), and erythroleukemia (Barron et al. 1960). Autopsy data indicates that the incidence of peripheral nerve

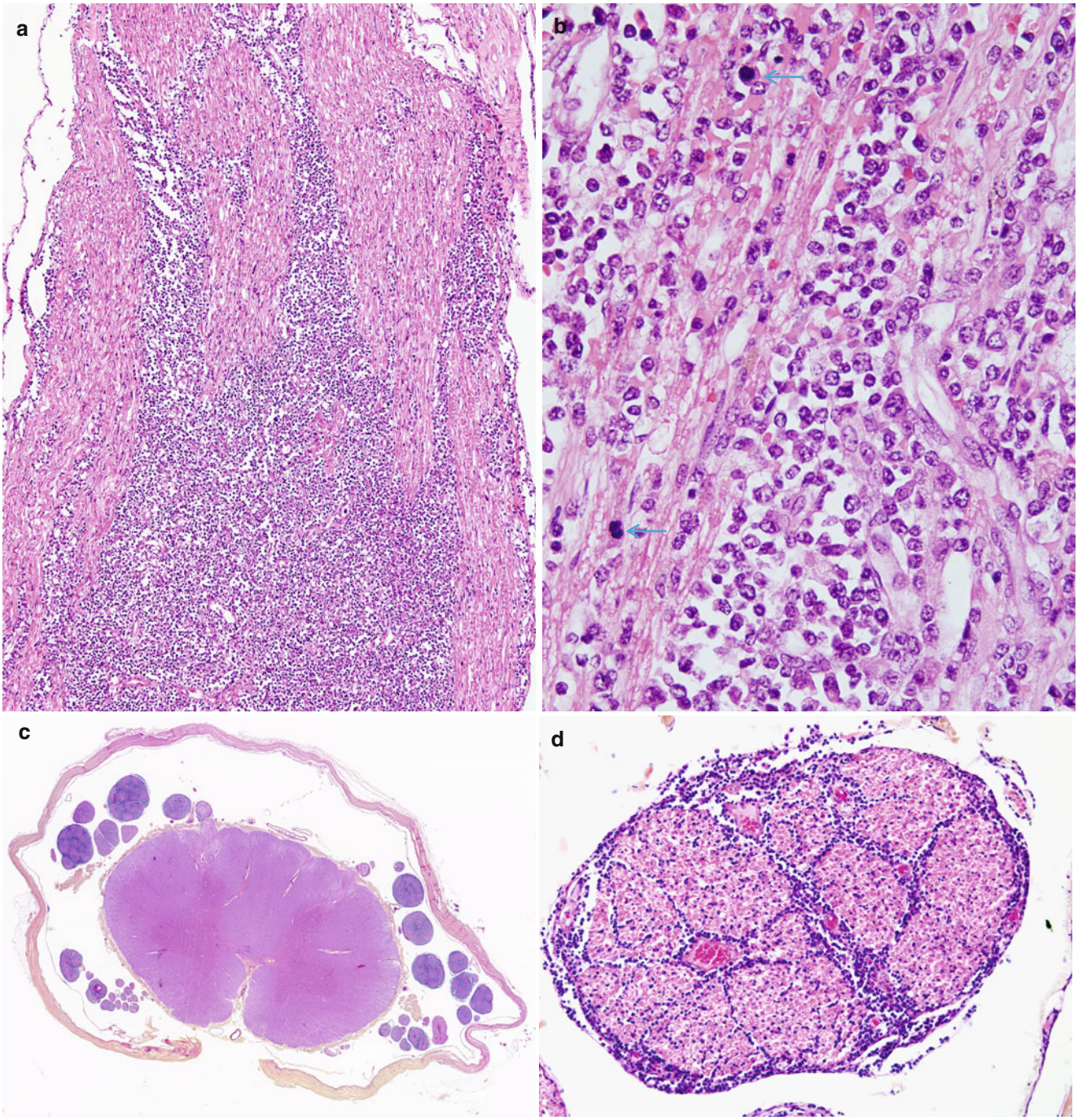

Fig. 16.3 Neurolymphomatosis: longitudinal view demonstrates streaming of lymphomatous infiltrate between axons (**a**). The presence of markedly atypical cells and numerous mitoses (*arrows*) indicates a high-grade lymphoma. In addition to sural nerve involvement, there was extensive infiltration of the dorsal and ventral roots (**c**) with extension into the perineurium and endoneurium (**d**) (paraffin)

infiltration in leukemia is higher than these small numbers might suggest (Jellinger and Radiaszkiewicz 1976). A symmetrical sensorimotor polyneuropathy was the most common clinical pattern, and asymmetric polyneuropathy or mononeuritis multiplex could also be seen. Concurrent leukemic meningitis was seen in some cases.

16.2.2.2 Pathology

As with the lymphomatous infiltrations, the tumor cells may show a variety of distribution patterns including massive diffuse involvement, preferential perineurial, and subperineurial location (Fig. 16.7a–c), and in the case illustrated, the peripheral part of the oculomotor nerve is extensively

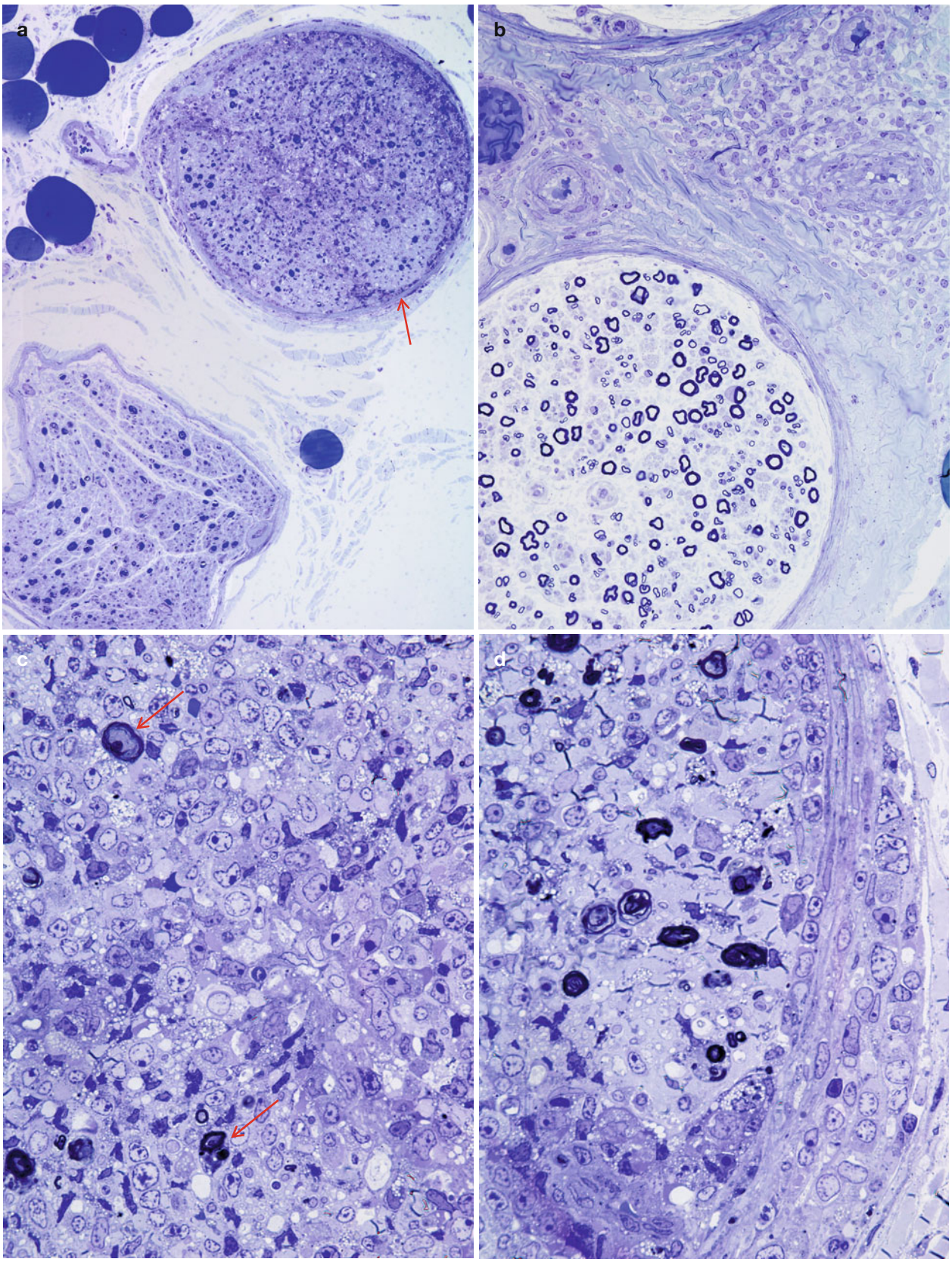

Fig. 16.4 Neurolymphomatosis: (**a**) lymphomatous infiltration involves one fascicle (*arrow*) of sural nerve but spares another. (**b**) Atypical perivascular mononuclear infiltrate in the epineurium. (**c**) Higher magnification of the involved fascicle shows replacement of the endoneurial contents by lymphoma sparing a few myelinated axons (*arrows*). (**d**) Part of the perineurium is disrupted by neoplastic lymphocytes (paraffin, magnification **a**, 200×; **b**, 400×; 1 μ thick plastic sections **c**, **d**, 1,000×)

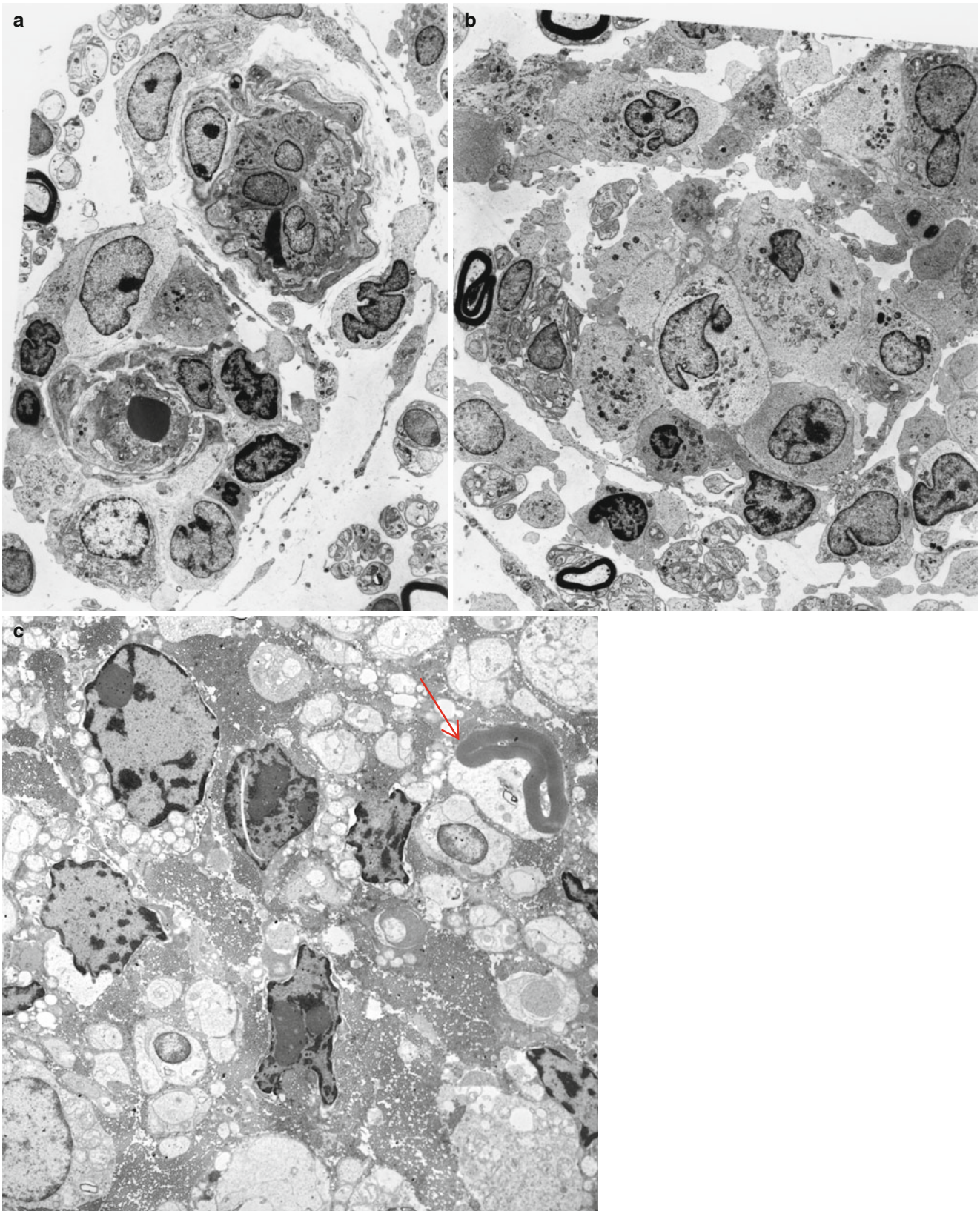

Fig. 16.5 Neurolymphomatosis: ultrastructurally, the endoneurial perivascular cells display features consistent with lymphoma (**a**, **b**) and infiltrate between a few residual myelinated axons (*arrows*, **c**) (magnification: **a**, **b**, 19,200×; **c**, 5,000×)

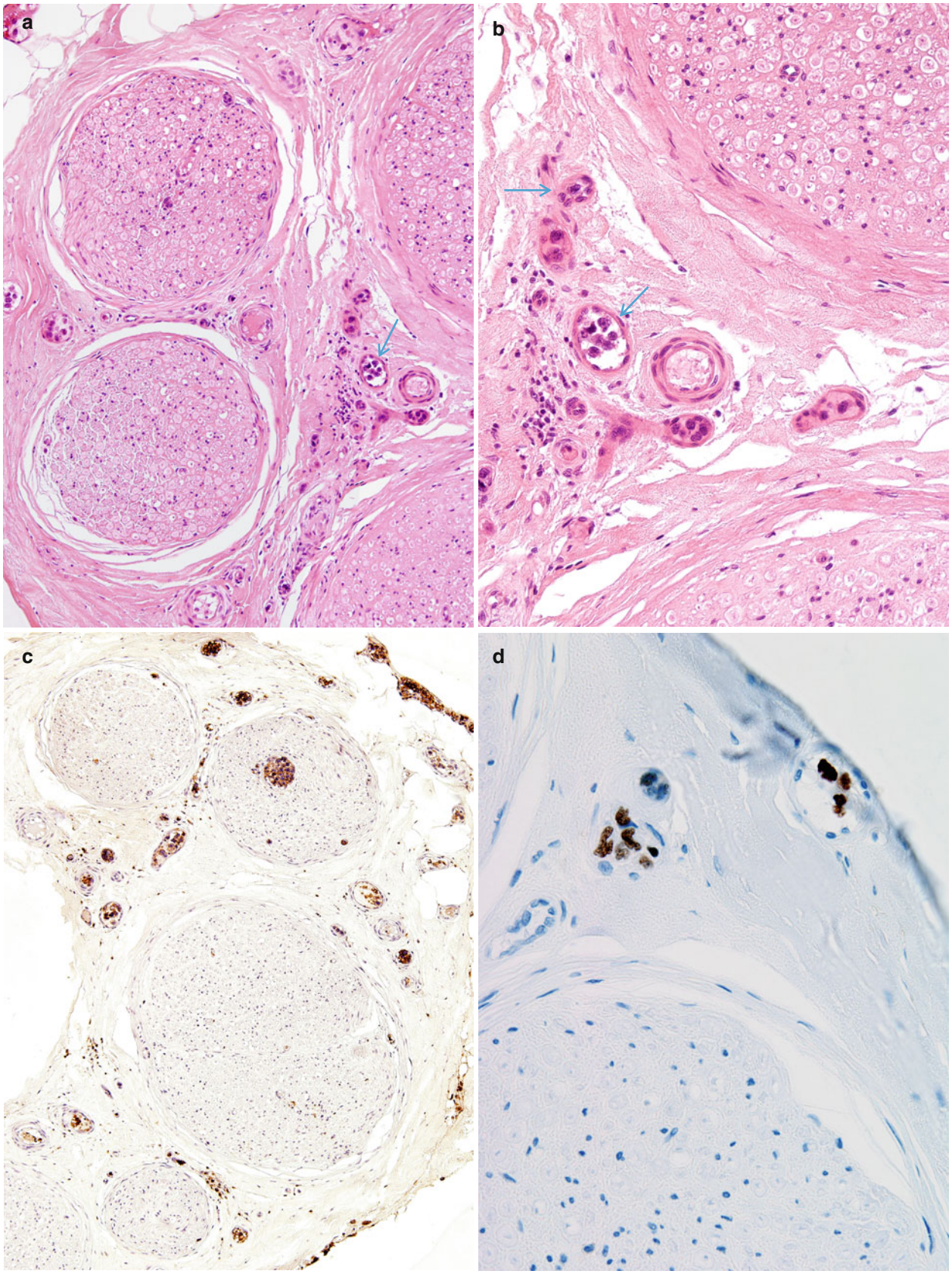

Fig. 16.6 Intravascular lymphoma: this 64-year-old patient with rheumatoid arthritis presented with mild numbness in both hands and nerve conduction defects. The sural nerve shows prominent intravascular growth of neoplastic large B cells as demonstrated by H&E (*arrows*, **a**, **b**), CD45 (leukocyte common antigen) (**c**), the proliferative marker Ki67 (**d**), and endothelial marker Ulex europaeus (**e**, **f**). Note the mitotic figure in **f** (*arrow*). Paraffin sections (magnification: **a**, 200×; **b**, 400×; **c**, 200×; **d**, 400×; **e**, 200×; **f**, 1,000×)

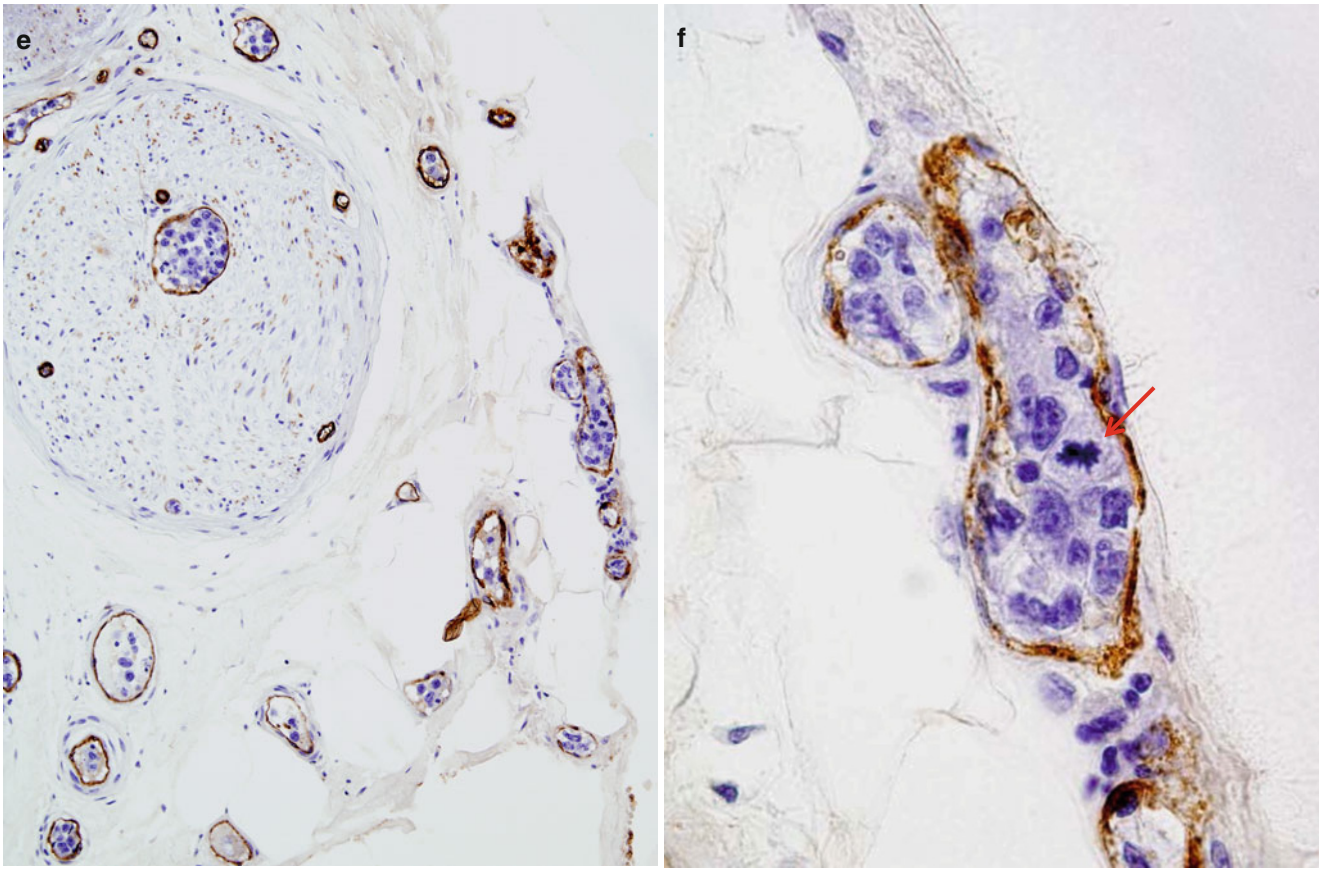

Fig. 16.6 (continued)

involved (Fig. 16.7c) while sparing the more proximal segments with their blood–brain barrier apparently intact (arrow, Fig. 16.7a, b). In one case reported as a recurrence after chemotherapy, malignant cells were seen only within the endoneurium (Krendel et al. 1987). Review of the details provided in literature reports suggests that the pathological process is less severe in CLL than in other leukemic conditions, both in terms of the damage to myelino-axonal elements and in the intensity of neural infiltration. Segmental demyelination may be very prominent (Sumi et al. 1983; Barron et al. 1960), but combined features of axonal degeneration and demyelination are usually present, usually correlating with the local presence of malignant infiltrates.

Brun et al. (1964) reported a case presenting with mononeuritis multiplex where multifocal intraneural lunate subperineurial hemorrhages were seen in the peripheral nerve. Although most of the hemorrhages appeared recent, a few iron-containing macrophages in the perineurium gave evidence of previous hemorrhage. No leukemic infiltrates were detected.

In one report of adult T-cell leukemia, scattered malignant cell infiltrates were seen in the nerve. Some of these contained vacuoles, and when examined at a high magnification, these were seen to contain particles that resembled HTLV-1 virions. PCR techniques confirmed that these cells were infected with HTLV-1 virus (Vital et al. 1993a).

Two reports of CLL document malignant lymphocytes involved in segmental demyelination through invasion of Schwann cell basal lamina and separation of myelin from the axon, much like macrophage-mediated demyelination of inflammatory demyelinating neuropathies (Vital et al. 1975; Sumi et al. 1983). Subsequent analysis has shown one of these cases to be a T-cell leukemia (Vital et al. 1990).

16.2.3 Multiple Myeloma

Barron et al. (1960) described three cases of multiple myeloma with symmetrical sensorimotor peripheral neuropathy in which autopsy revealed malignant myeloma cells diffusely in the perineurium and endoneurium. The neural pathology consisted of focal regions of severe demyelination and axonal degeneration, which in the eyes of the authors clearly seemed to be spatially (and by inference causally) related to foci of malignant cells.

Atypical lymphoplasmacytoid cells are an infrequent finding in peripheral nerve with neuropathy associated with nonmalignant plasma cell dyscrasia (Fig. 14.1) (Vital et al.

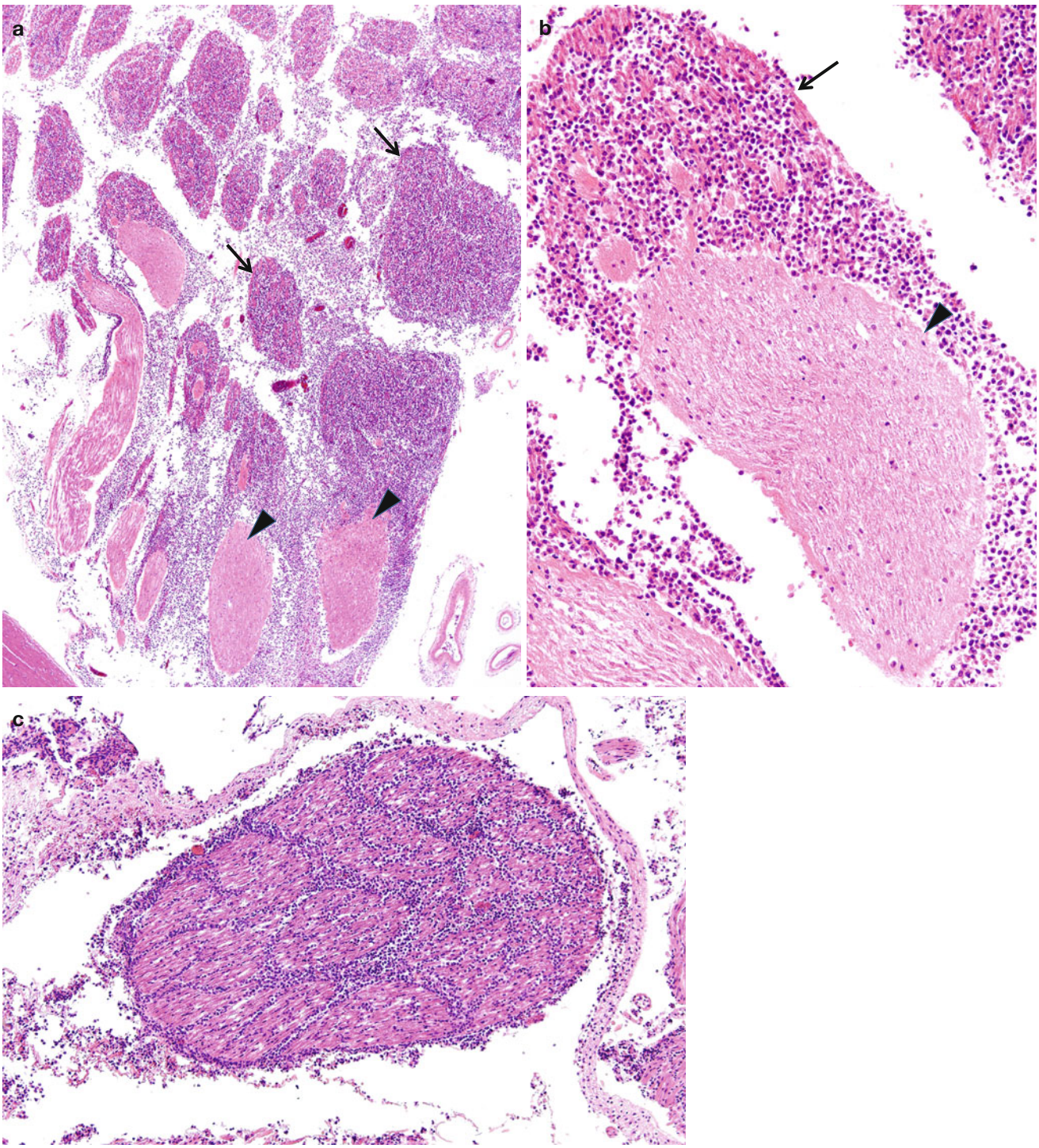

Fig. 16.7 Acute monocytic leukemia diffusely invading the endo- and perineurium of the oculomotor nerve roots (*arrows* **a**, **b**) but sparing the proximal portions of the CNS component of the root junctions (*arrowheads*, **a**, **b**). (**c**) Higher magnification of the peripheral portion of a root shows the extent of involvement (paraffin, H&E stain, **a**, 40×; **b**, **c**, 200×)

1984; Julien et al. 1984). In such instances, it is most likely the paraprotein itself, not the scanty collections of endoneurial malignant cells, which causes the neuropathy, as with the case reported by Ince et al. (1987).

16.2.4 Differential Diagnosis of Infiltrative Neuropathy

In neoplastic infiltrative neuropathies we have examined, the cells were clearly atypical and the possibility that the inflammatory infiltrate was anything other than malignant was not entertained. Although we have not seen such material, some of the literature cases reported as "neurolymphomatosis" would be very difficult to distinguish from neuropathy with prominent but not monoclonal, inflammatory infiltration (Borit and Altrocchi 1971), as might neural infiltrates in CLL. Immunophenotyping can be used to verify monoclonality (Thomas et al. 1990), but the issue is complicated by the possibility that intraneural tumor infiltrate may initiate a secondary autoimmune inflammatory attack on the myelin or axon (Grisold et al. 1990) and the possible occurrence of polyclonal immunocytomas in certain lymphoid malignancies (Grisold et al. 1990).

The angiocentric lymphoproliferative diseases pose an ongoing nosologic dilemma. Angioimmunoblastic T-cell lymphoma, lymphomatoid granulomatosis, and angiocentric malignant lymphoma are entities which seem to be related and reflect the spectrum of "angiocentric lymphoproliferative lesions" (Frizzera et al. 1989). Neuropathy with an angiocentric polymorphic lymphohistiocytic infiltrate has been described in all these entities, and there may be a common causal connection with Epstein–Barr virus infection (Peiper 1993) or HIV infection (Calabrese et al. 1989). Vasodestruction suggests the diagnosis of lymphomatoid granulomatosis, and obvious monoclonality of the infiltrating cells leads to the diagnosis of lymphoma. However, it may be difficult to distinguish between these apparently closely related systemic diseases on the basis of a nerve biopsy alone.

16.3 Castleman Disease

16.3.1 Clinical Manifestations

Castleman disease (CD, angiofollicular lymph node hyperplasia) is a lymphoproliferative disorder with marked follicular capillary proliferation and endothelial hyperplasia associated in a subset of cases with the human immunodeficiency virus (HIV) and human herpesvirus 8 (HHV-8). CD comprises at least two distinct diseases (localized and multicentric). It is also associated with Kaposi's sarcoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma (Weisenburger et al. 1985), and POEMS syndrome. In most cases, Castleman disease is likely due to hypersecretion of the cytokine IL-6. Nosology is controversial and complex, with localized or multifocal disease and "plasma cell" or "hyaline vascular" histological variants (Kessler 1985; Frizzera et al. 1985; Weisenburger et al. 1985). Patients typically present with lymphadenopathy. Neuropathy is uncommon, but may be the presenting manifestation, and is most often seen with the multifocal "plasma cell" variant (Donaghy et al. 1989; Case Records MGH 1984, 1987; Scherokman et al. 1991; Hineman et al. 1982; Yu and Carson 1976; Gherardi et al. 1991; Feigert et al. 1990; Gaba et al. 1978; Gottfried et al. 1986; Bitter et al. 1985). Features of the POEMS syndrome are often present. A circulating paraprotein is seen in only a small fraction of all cases of CD but may be present in a disproportionate number of cases with neuropathy. Polyclonal hypergammaglobulinemia is far more common.

It is of interest that a majority of patients with POEMS syndrome show lymph node pathology typical of Castleman disease, although lymphadenopathy may not be a clinically important part of their illness (Nakanishi et al. 1984; Gherardi et al. 1988). Moreover, the neuropathy of Castleman disease resembles that of osteosclerotic myeloma and the POEMS syndrome, being predominantly motor and associated with significant conduction slowing (Donaghy et al. 1989). At the present time, the relationship between POEMS syndrome and Castleman disease has been noted, but not well understood. There seems to be little reason to differentiate patients who present with neuropathy and POEMS syndrome and show asymptomatic angioproliferative lymph node hyperplasia from patients who present with features of Castleman disease and later develop a neuropathy and features of the POEMS syndrome. Angiofollicular lymph node hyperplasia may simply be a nonspecific response to abnormal immune regulation (Gherardi et al. 1988), likely as the result of IL-6 hypersecretion.

16.3.2 Pathology

A small number of peripheral nerve examinations have been reported, and most have not shown inflammatory infiltration (Donaghy et al. 1989; Gottfried et al. 1986; Scherokman

et al. 1991), although an epineurial infiltrate consisting of macrophages, lymphocytes, and plasma cells, occasionally invading the perineurium, was observed in one case (Case records MGH 1984). Lymphomatous infiltration was not diagnosed in this case, but cell markers were not assessed. Proliferation of epineurial vessels has been emphasized along with hypertrophy of endoneurial capillaries and their endothelial cells (Donaghy et al. 1989). Severe and active degeneration of myelinated and unmyelinated axons has usually been observed, with evidence of regenerative activity (Donaghy et al. 1989; Scherokman et al. 1991). Demyelination is usually also present and may be very prominent (Case Records MGH 1984).

Uncompacted myelin lamellae have been described in POEMS syndrome associated with Castleman disease lymphoid pathology (Vital et al. 1994). Immunoglobulin deposits outlining the axons were described by Gottfried and colleagues (1986), but Donaghy et al. (1989) did not detect such deposition (Gottfried et al. 1986; Donaghy et al. 1989). One patient has been described who presented with mononeuritis multiplex and had plasmacytoma and lymph node changes suggestive of Castleman disease, in which necrotizing hypersensitivity vasculitis was seen in the nerve (Gherardi et al. 1991).

16.3.3 Pathogenesis

Interleukin-6, produced by cells in the germinal centers of lymph nodes in Castleman disease (Yoshizaki et al. 1989), has been proposed as a cytokine which promotes proliferation of plasma cell and vascular endothelial cells (Nakazawa et al. 1992). The etiology of the neuropathy remains undetermined, although the observation of vascular proliferation has raised the possibility of ischemia (Donaghy et al. 1989). Ono et al. (1985) reported finding capillary hyperplasia in numerous tissues including the lymph nodes, kidney, skin, and subarachnoid space, in a patient with POEMS syndrome who temporarily showed CD-type lymph node histology.

16.4 Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL, previously called angioimmunoblastic lymphadenopathy) is a distinct subtype of peripheral T-cell lymphoma characterized by generalized lymphadenopathy and frequent autoimmune-like manifestations (Sakata-Yanagimoto et al. 2014). Peripheral neuropathy has been described and studied histologically in a number of cases (Brunet et al. 1981; Tredici et al. 1979; Cytowic et al. 1982). Most often, a mixed axonal and demyelinating neuropathy is seen, with a nonspecific polyclonal inflammatory infiltrate. Proliferation of endoneurial vessels and hypertrophy of endothelial cells have also been described (Brunet et al. 1981). In one case, a perivascular lymphohistiocytic infiltration of the nerve was seen (Brunet et al. 1981, case 4). Some biopsies have been normal or showed axonal dropout without any local abnormalities.

Case 16.1

A 62-year-old man presented with left cervical adenopathy 6 years prior to the onset of neuropathy. Lymph node biopsies at that time, and 3 years later, revealed changes of Castleman disease, plasma cell variant. A 3rd lymph node biopsy one month prior to the onset of neuropathy was interpreted by the same hematopathologist as showing angioimmunoblastic lymphadenopathy. At this time two bone marrow biopsies with cytogenetic studies showed no evidence of lymphoma or plasmacytoma. Repeated search for a circulating paraprotein was negative. HIV and HTLV-I/II serology were negative.

At the age of 68, the patient presented with symmetrical distal leg paresthesias and worsening weakness, progressing over 2 months to an inability to stand unsupported. Clumsiness and numbness of the hands appeared toward the end of this period. Examination showed diffuse symmetrical weakness, more severe proximally in the arms, and more severe distally in the legs, with complete foot drop. All reflexes were present and normal excepting the ankle jerks, which were absent. Vibration was absent to the knees and wrists, and a stocking-glove sensory deficit to pin was found as high as the mid-leg and wrist. Electrophysiological tests showed borderline low conduction velocities and normal sensory and motor amplitudes. The patient's proximal limb weakness was at least partially explained by an elevated serum calcium (3.69 mmol/L). A circulating paraprotein was not detected. CSF protein and cell count were normal. Bone scan was negative, and abdominal ultrasound showed moderate splenomegaly. Nerve biopsy was performed which showed a markedly atypical polymorphic mononuclear infiltrate consistent with neurolymphomatosis involving some fascicles and largely sparing others (Figs. 16.4 and 16.5).

The patient was treated with prednisone and improved; a large part of this improvement was attributable to correction of hypercalcemia. Four months after admission, he walked with a cane and showed mild distal leg weakness and absent ankle jerks but normal deep tendon reflexes elsewhere. Pinprick sensation remained diminished in a stocking-glove distribution at the mid-leg and base of the fingers.

This patient had a variety of hematologic diagnoses at different times. The initial diagnosis was Castleman disease, and during this period, there was no peripheral neuropathy. Repeated lymph node biopsies performed during the period when neuropathy was present revealed angioimmunoblastic lymphadenopathy (angioimmunoblastic T-cell lymphoma) culminating in lymphomatous invasion of the peripheral nerve (case and hematopathological material courtesy of Dr. D. Sutton and Dr. D. Pantalone, Toronto)

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Neuropathy Associated with Endocrine and Metabolic Disease

17

Endocrine and metabolic disturbances are probably the most common causes of neuropathy (Table 17.1). In these situations, the diagnosis is based on clinical and laboratory findings, examination of peripheral nerve biopsy having no role in management. Nevertheless, the pathologist should be aware of the spectrum of pathology seen in each entity, as biopsy may be performed in such patients on the suspicion that another pathological process is at play. Toxic neuropathies other than alcohol are considered separately in Chap. 18.

17.1 Diabetic Neuropathy

The global prevalence of diabetes is predicted to increase from 366 million in 2011 to 552 million in 2030 (Whiting et al. 2011) and will likely lead to a marked increase in diabetic neuropathy, which is currently the single most common neuropathy in the world and a leading cause of morbidity in this disease. One frequently quoted prospective study of 4,400 patients documented clinically evident neuropathy in 7.5 % of diabetics at the time of initial diagnosis, increasing linearly with time to approximately 50 % at 25 years (Pirart 1978a, b). More recent data indicates that although over half of diabetics have a neuropathy of some sort, it is most often subclinical, and significant symptomatology is seen in only a small fraction (Dyck et al. 1993). Neuropathy develops in both type 1 and type 2 diabetes, is associated with retinopathy and nephropathy, and increases in frequency and severity with duration of diabetes, age, hypertension, smoking (in people with type 1 diabetes), patient height, alcohol consumption, and worsening degree of glycemic control (Llewelyn et al. 2005).

17.1.1 Clinical Manifestations

Diabetic neuropathy is not a single neuropathy; rather, it is a complex spectrum of neuropathies (Table 17.2), including symmetrical, asymmetric (involving cranial and somatic nerves), and autonomic neuropathies (Thomas and Tomlinson 1993; Llewelyn et al. 2005). The first group includes sensory or sensorimotor polyneuropathy, autonomic neuropathy, and symmetrical proximal motor neuropathy. The second group includes cranial neuropathies, trunk and limb mononeuropathies, and asymmetric lower limb motor neuropathy. A number of patients may present very early with painful small fiber neuropathy (detectable with skin biopsy but not as frequently with sural nerve biopsy) at the time they only exhibit an impaired glucose tolerance test (Smith et al. 2001; Novella et al. 2001).

17.1.1.1 Distal Symmetrical Sensorimotor Neuropathy

Symmetrical length-dependent and predominantly sensory neuropathy is the most common, manifesting with numbness, variable severity of pain and paresthesias, impaired vibration sense, and loss of distal reflexes. When sensory loss extends above the knees, it develops in the fingers, spreading to the hands and forearms as it progresses proximally in the lower limbs. The anterior aspect of the trunk can become affected due to the involvement of the distal territory of the intercostal nerves and crown of the head due to the involvement of the longest fibers of the trigeminal nerve (Sabin et al. 1978). Distal motor manifestations can be associated but are rarely dominant. Although usually mild, at its most extreme form, this sensorimotor polyneuropathy may cause a sensory ataxic, acrodystrophic, or arthropathic picture and culminate in amputation. Small fiber neuropathy manifesting with pain, hyperesthesia, and autonomic disturbance can be present in isolation, or associated with the sensorimotor syndrome. Although symptoms usually evolve slowly, an acute painful diabetic neuropathy can appear in the setting of poor diabetic control and weight loss (Asbury et al. 1963), or conversely with initiation of insulin treatment (Llewelyn et al. 1986). Diabetic neuropathy can also present with pain, proximal weakness and tissue wasting, and minimal sensory deficits, evolving insidiously and symmetrically,

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Table 17.1 Endocrine/metabolic neuropathies

Endocrine
Diabetes
Hypothyroidism (?hyperthyroidism)
Acromegaly
Organ disease
Uremic neuropathy
Neuropathy in liver disease
Nerve alterations in pulmonary disease
Vitamin deficiencies
Vitamin B_1 (thiamine)
Vitamin B_6 (pyridoxine)
Vitamin B_{12} (cobalamin)
Vitamin E (α-tocopherol)
Folate

Table 17.2 Classification of diabetic neuropathies

Impaired glucose tolerance and hyperglycemic neuropathy
Generalized (symmetrical) neuropathies
Sensorimotor
Acute painful (including treatment induced)
Autonomic (parasympathetic, sympathetic, enteric, visceral sensory)
Acute motor (unusual)
Asymmetric (focal and multifocal neuropathies)
Cranial nerves (III, IV, VI)
Thoracolumbar
Upper limb
Lumbosacral radiculoplexus (Bruns–Garland syndrome)
Superimposed chronic inflammatory demyelinating neuropathy (CIDP)
Hypoglycemic neuropathy

or acutely and focally (Barohn et al. 1991; Bastron and Thomas 1981). The simultaneous presence of several types of neuropathy is common.

In symmetrical sensorimotor neuropathy, electrophysiological studies reveal a loss of distal amplitudes, especially in sensory fibers. Conduction slowing is common, although rarely to the extent usually present in demyelinating neuropathies such as CMT-1 or CIDP (Behse et al. 1977; Thomas and Tomlinson 1993). EMG in the asymmetric diabetic nerve syndromes reveals multifocal regions of denervation, with involvement of paraspinal muscles often suggesting a nerve root lesion (Bastron and Thomas 1981).

A growing body of data suggests that severity of peripheral nerve manifestations correlates with severity of hyperglycemia over a long-term period and that optimal glycemic control results in amelioration or at least delayed progression of diabetic neuropathies (Committee Health Care Issues ANA 1986; DCCT Research Group 1993). At one time the use of aldose reductase inhibitors in treatment and prevention of diabetic neuropathy held great promise, but clinical results have been largely disappointing (Harati 1992; Tsai and Burnakis 1993).

17.1.1.2 Asymmetric Diabetic Neuropathies

This category (recently reviewed by Younger 2011; Prasnoor et al. 2013) includes diabetic lumbosacral radiculoplexus neuropathy (DLRPN; also known as "diabetic amyotrophy," "femoral neuropathy of diabetes," and "proximal diabetic neuropathy" or "Bruns–Garland syndrome"), cranial nerve palsies (usually cranial nerves III, IV, and VI), truncal radiculopathy, and upper limb mononeuropathy. Early in diabetes, occasional middle-aged and elderly men with type 2 diabetes, often following institution of insulin therapy or associated with significant weight loss, experience sudden or subacute onset of (typically unilateral) motor dysfunction with weakness and muscle wasting, involving large nerves to the lower limbs or cranial nerves. Although the condition usually begins in one leg, spread to the other leg may occur within weeks or months, and, in a third of the cases, proximal arm muscle involvement (cervicobrachial radiculoplexopathy) has been reported. Occasionally, upper limb involvement occurs. Oculomotor nerve palsies are the most common cranial neuropathy observed in diabetic patients which occurs in diabetic patients over 50 years of age, both in insulin- and in noninsulin-dependent diabetic patients. Spontaneous complete recovery invariably occurs within 2–3 months.

17.1.1.3 Diabetic Autonomic Neuropathy

Both sympathetic and parasympathetic neuropathy occur in diabetes resulting in a myriad of autonomic complaints, which may present individually or in groups (Rundles 1945) involving cardiovascular, genitourinary, alimentary, and sudomotor functions. The presence of symptomatic diabetic autonomic neuropathy significantly increases morbidity and mortality (Ewing et al. 1980).

17.1.2 Pathology

Discussion of histological findings is complicated by the co-occurrence of several diabetic neuropathy variants which may have different pathogenic mechanisms. Biopsy findings in diabetic neuropathy are invariably nonspecific, and this invasive procedure should be performed for diagnostic purposes only if a treatable alternative is being considered.

17.1.2.1 Pathology in Diabetic Symmetrical Sensorimotor Neuropathy

Light Microscopy

Sensorimotor diabetic neuropathy is a predominantly axonal process (Behse and Buchthal 1977; Dyck et al. 1986a, b; Johnson et al. 1986; Yagihashi and Matsunaga 1979; Schmidt

and Bilbao (in press)) (Fig. 17.1a, b). Behse and co-workers (1977) observed that in predominantly sensory neuropathies, large and small myelinated fibers were equally depleted, while in sensorimotor neuropathy the large myelinated fibers (MFs) were more severely affected (Behse and Buchthal 1977). However, Dyck et al. (1986b) found no evidence of selective depletion of small or large MFs. Regenerating clusters can be prominent, but with severe neuropathy they often diminish in number. Although the neuropathy can be very chronic, actively degenerating axons may be seen, and the overall appearance is not as indolent as that of neuronal Charcot–Marie–Tooth disease (CMT). Nonetheless, studies of "early" diabetic neuropathy have reported normal myelinated fiber density, axonal area, and G-ratio in the presence of teased fiber studies showing paranodal abnormalities, segmental demyelination, and remyelination without active degeneration of myelinated axons.

We have been impressed by the frequency of focal nerve lesions in these patients, often appearing as perineurial-based regions where myelinated fibers are reduced in number, actively degenerating, or thinly myelinated (Figs. 17.2, 17.3, and 17.4). The perineurium at such sites may appear disrupted, and rarely a few randomly directed myelinated fibers may escape the confines of the nerve fascicle, forming a miniature "traumatic" neuroma (Figs. 17.2 and 17.4) which appears most common in nerves of diabetics. It is intuitive to regard such abnormalities as nerve "infarcts" although this implies an ischemic pathogenesis (vide infra). The epineurial vasculature is typically less severely involved, although vasculopathy has been reported.

Sima and colleagues have suggested a difference in the pattern of axonal pathology depending on patient age and type of diabetes: younger patients with IDDM are less likely to display multifocal nerve degeneration, and the typical axonal alteration on teased fibers is myelin wrinkling, while older patients with NIDDM demonstrate multifocal nerve lesions, with Wallerian degeneration on teased fibers (Sima et al. 1988a). In support of this observation, Llewelyn et al. (1988) found no difference in the nonuniformity of fiber loss between a group of relatively young diabetics and in control nerve taken from patients with CMT-1.

Segmental myelin changes usually take the form of inappropriately thinly myelinated axons indicating remyelination or regeneration, with cross sections revealing ongoing demyelination considerably less frequently (Figs. 17.1, 17.5, and 17.6b). Teased fibers demonstrate the spectrum of myelin alteration from paranodal widening to demyelinated segments. In asymptomatic diabetics, segmental myelin alterations may precede loss of axons (Chopra et al. 1969; Vital et al. 1974). Onion-bulb (OB) formations can be seen in diabetic neuropathy but have probably been overemphasized (Fig. 17.7). The OBs are most often rudimentary and do not dominate the histological picture as with CMT-1 or some cases of CIDP (Ballin and Thomas 1968). Indeed, onion-bulb-like structures (pseudo-onion bulbs) are more likely to be a reflection of axonal regeneration (Fig. 17.7a, b). Thomas has commented that in retrospect, the prominent onion-bulb formations described in a case of "diabetic" neuropathy in his 1966 report (Thomas and Lascelles 1966) were likely due to concurrent CIDP (Thomas and Tomlinson 1993). Some authors have reported that the myelin alterations appeared to be independent of axonal changes (Behse and Buchthal 1977), while others have emphasized clustering along certain fibers indicating secondary demyelination (Dyck et al. 1986b).

Hyalinization of endoneurial microvessels is a typical feature of diabetic nerves, regardless of the presence or absence of neuropathy (Fig. 17.1c). This may reach striking proportions, but these microvascular changes remain a nonspecific finding seen in many chronic neuropathies. Alternatively, the severity of endoneurial microvascular alterations has been correlated with the severity of the neuropathy (Malik et al. 1993). Endoneurial area may be increased (Behse and Buchthal 1977), with expansion of the endoneurial and subperineurial extracellular matrix (Ballin and Thomas 1968). Changes in epineurial microvessels are usually mild by comparison (Malik et al. 1993). A low-grade mononuclear inflammatory infiltrate is infrequently seen.

Electron Microscopy

The ultrastructural correlate of vascular hyalinization is reduplication and thickening of microvascular basement membranes (Fig. 17.8a, b). Other alterations which have been emphasized in diabetic neuropathy include endothelial cell hypertrophy and hyperplasia, reduction in capillary luminal area, and microvessel "closure" (Dyck et al. 1985, 1986b; Malik et al. 1989) (Fig. 17.8c, d). However, not all workers have confirmed these findings, and they may be seen in other chronic neuropathies (Bradley et al. 1990) or be related to age (Sima et al. 1988a). Obstruction of capillary lumina with degenerate cellular material and fibrin has been described (Timperley et al. 1985; Yasuda and Dyck 1987), but is uncommon in our experience (Fig. 17.8d, e).

The full range of nonspecific axonal alterations may be seen. One lesion which has received considerable attention is "axoglial dysjunction," referring to ultrastructural and molecular findings resulting in the disruption of the characteristic junctional complexes joining terminal myelin loops to the axolemma at the paranodal area (Sima 1993; Sima et al. 2000). Swelling of the paranodal axon is often associated with this finding. Axoglial dysjunction seems to be more significant in type 1 (younger) patients than in type 2 (Sima et al. 1988a), but its specificity is uncertain (Sladky et al. 1991). Other differences between types 1 and 2 diabetes in the neuropathologic findings of diabetic somatic nerves have also been described in animals and humans. Type 1 diabetics are

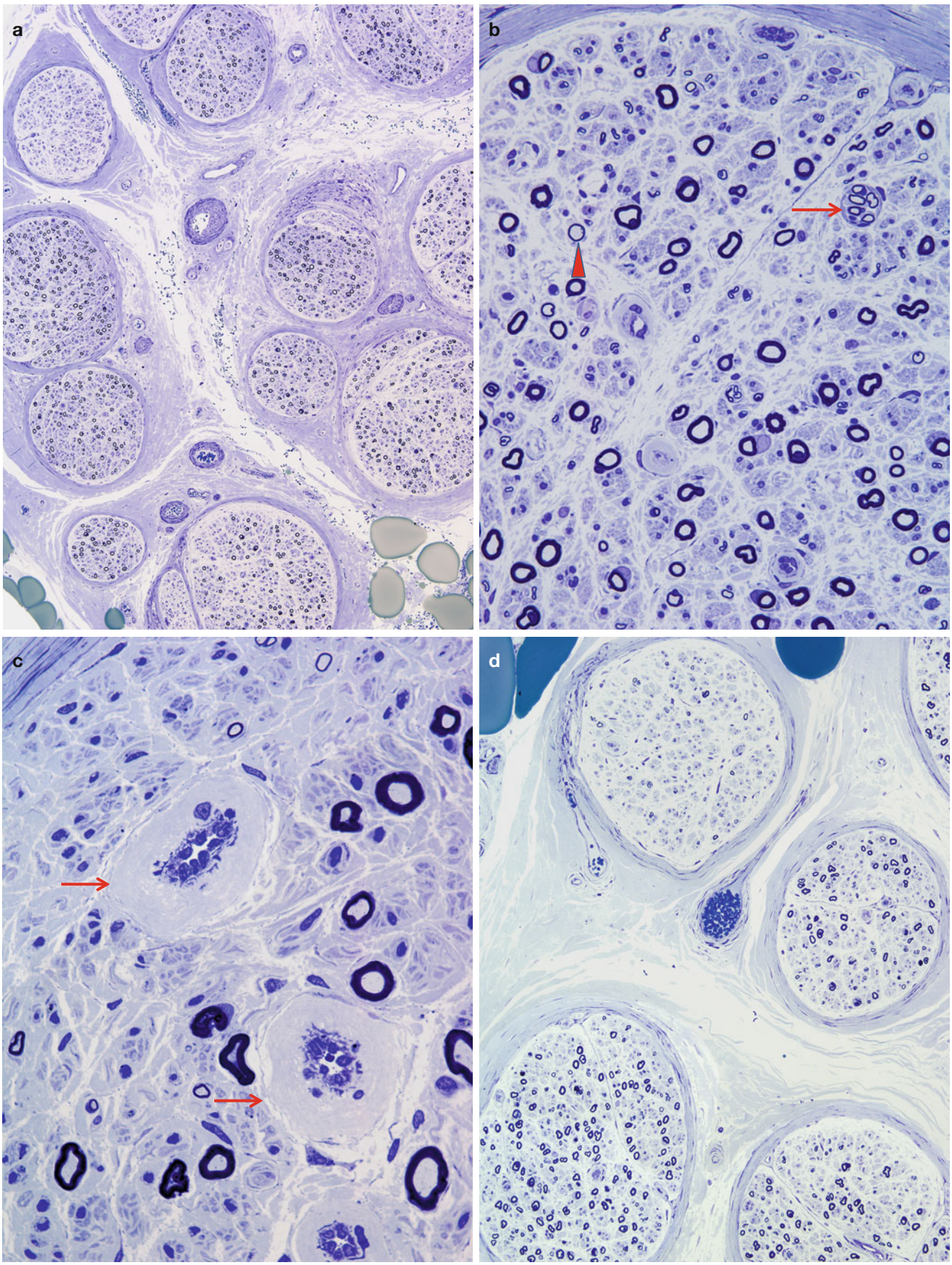

Fig. 17.1 Diabetic neuropathy: (**a**, **b**) moderate reduction in myelinated fiber density, regenerating clusters (*arrow*, **b**) disproportionately thin myelin sheaths (*arrowhead*, **b**) and marked thickening of vessel walls (*arrows*, **c**). There may be significant fascicle-to-fascicle variability in axon number (**d**) (1 **µ**m plastic sections. Magnifications: **a**, 100×; **b**, 400×; **c**, 1,000×; **d**, 200×)

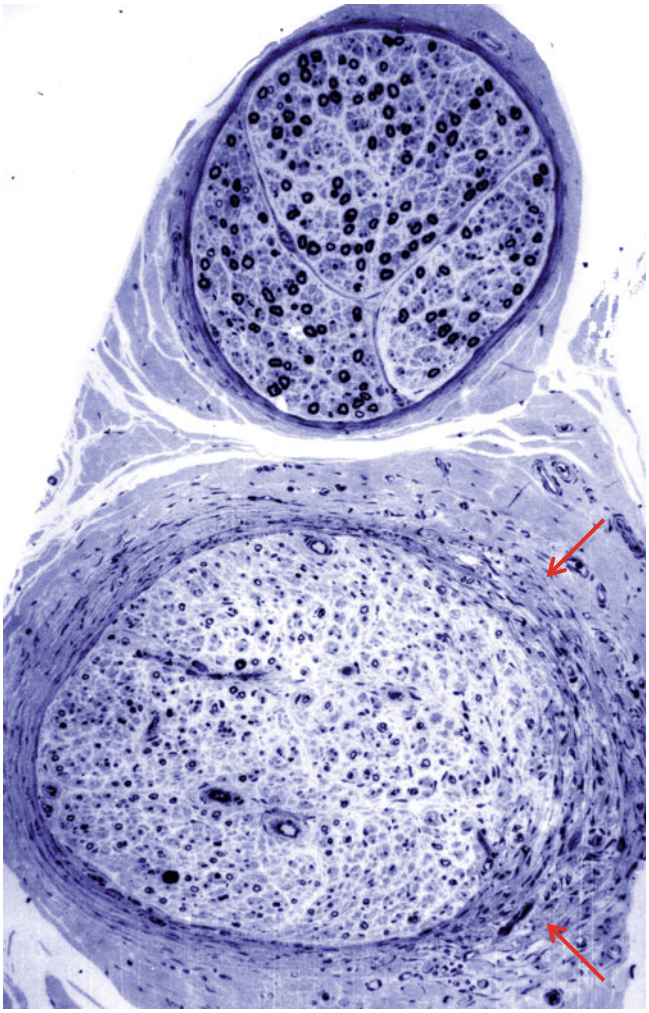

Fig. 17.2 Diabetic neuropathy, reparative phase following focal fascicular damage. Compare MF density between two adjacent fascicles. Note perineurial damage (*arrows*) (1 μm plastic sections, 200×)

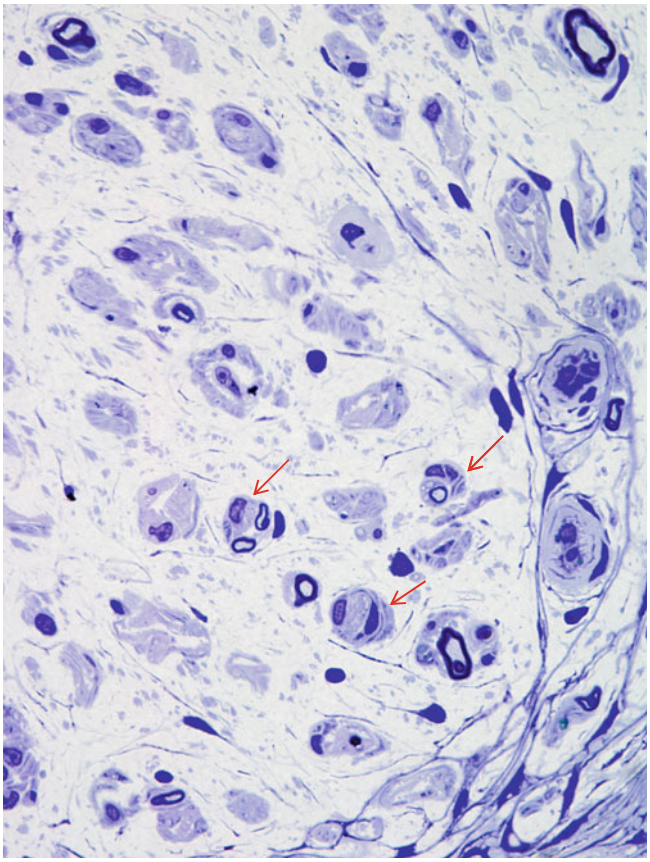

Fig. 17.3 Diabetic neuropathy: high-power view of affected fascicle in Fig. 17.2 showing pseudo-onion bulbs (*arrows*) resulting from axonal regeneration through a region of focal damage (1 μm plastic sections, magnification, 600×)

reported to develop fewer foci of patchy axonal degeneration and more evidence of myelinopathy than those with type 2 diabetes, in which axonal degeneration is most prominent.

Increased numbers of denervated Schwann cell subunits give early evidence of involvement of unmyelinated fibers, and at later stages of the disease, unmyelinated fiber counts can drop below the lower limit of normal. An apparent increase in rigidity and durability of Schwann cell basal lamina has been observed in diabetics, where after axonal degeneration and regeneration the original basement membrane outline is abnormally maintained (King et al. 1989) (Fig. 17.6a). Perineurial cells may display a thickened basal lamina (Johnson et al. 1981; King et al. 1989).

17.1.2.2 Pathology in Painful/Small Fiber Neuropathy (Sural and Skin Biopsy)

In three patients described by Brown and colleagues, nerve biopsy revealed more severe loss of small MFs than of large MFs (Brown et al. 1976). Unmyelinated axons were not severely depleted, but showed a striking shift to the left in size range, with only 4 % more than 1 μm in diameter (control 33 %). The Schwann cell–axon relationships appeared altered in the unmyelinated fibers, with the frequent occurrence of axons incompletely surrounded by Schwann cells or abutting one another. Two reports by Said and colleagues include a total of nine patients with clinical features suggesting small fiber disease (Said et al. 1983a, 1992). A dramatic depletion of unmyelinated fibers was noted, often to 10 % or less of the lower limit of normal. Myelinated fibers were also severely affected, but to a slightly lesser degree. Regenerating clusters were frequent. A proximodistal gradient of severity compatible with distal axonopathy was observed. Segmental myelin abnormalities were seen in a high proportion of teased fibers and thought to occur both primarily and secondary to axonal alterations (Said et al. 1983a, 1992). Onion-bulb and "pseudo"-onion bulbs (formed by regenerating clusters) were noted.

In contrast to the above observations, other workers (Behse and Buchthal 1977; Dyck et al. 1986b; Llewelyn et al. 1991) have failed to identify biopsies showing a

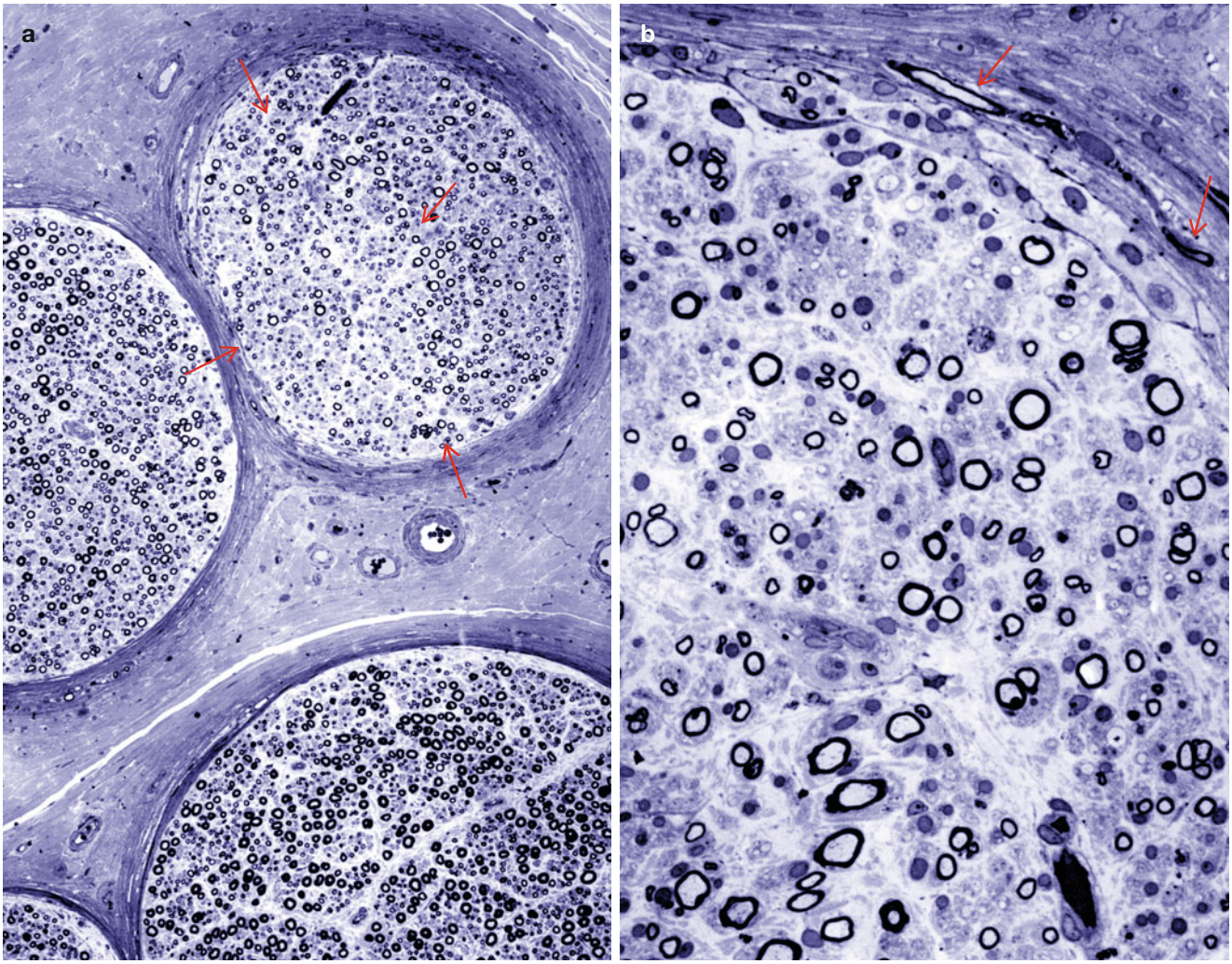

Fig. 17.4 Diabetic neuropathy, reparative phase following focal fascicular damage. Subtle fascicular damage is shown characterized by a geographic region of diminished numbers fibers with attenuated myelin (outlined by *arrows*, **a**). Note aberrant regeneration of fibers in perineurium (*arrows*, **b**) (1 **µ**m plastic sections, magnification **a**, 200×; **b**, 600×)

selective involvement of small myelinated and unmyelinated axons, even in patients with severe autonomic or painful neuropathies. Such cases may represent the extreme manifestations of a single type of symmetrical diabetic neuropathy (Dyck et al. 1986b; Llewelyn et al. 1991). Nerve biopsy in an acute painful neuropathy associated with establishment of tight glycemic control biopsy revealed only a chronic axonal neuropathy with no special features (Llewelyn et al. 1986). Another group of patients suffering from acute painful neuropathy associated with severe weight loss did not display any special features except perhaps an increased degree of active axonal degeneration (Archer et al. 1983).

Skin biopsies have been recently used in the analysis of diabetic symmetrical sensory polyneuropathy and autonomic neuropathy (illustrated in Chap. 1, Fig. 1.2a, b and Fig. 1.8) (Lauria et al. 2005; Luo et al. 2011; McCarthy et al. 1995; Periquet et al. 1999). Fiber loss demonstrable in skin biopsies has been reported in the absence of axon loss in the sural nerve in diabetic sensory neuropathy, which reflects loss of the most distal sensory complement of axons. The ability to perform multiple skin biopsies in the essential absence of neuropathic residua is one of the assets of skin biopsy and can potentially be used to follow the progress of treatment. After capsaicin-induced sensory skin denervation, diabetic patients show a slower rate of intraepidermal nerve fiber regeneration compared with healthy subjects, a result which may identify incipient neuropathy by regenerative stress at the earliest stages in which it may be most amenable to therapy. The demonstration of distal preterminal nerve fiber swellings in skin biopsies of diabetics may represent a "predegenerative" change and relate to synaptic/nerve terminal pathology in DRG and autonomic ganglia. The skin biopsy technique has been expanded to glabrous, non-hairy skin demonstrating in diabetic patients a significant reduction of

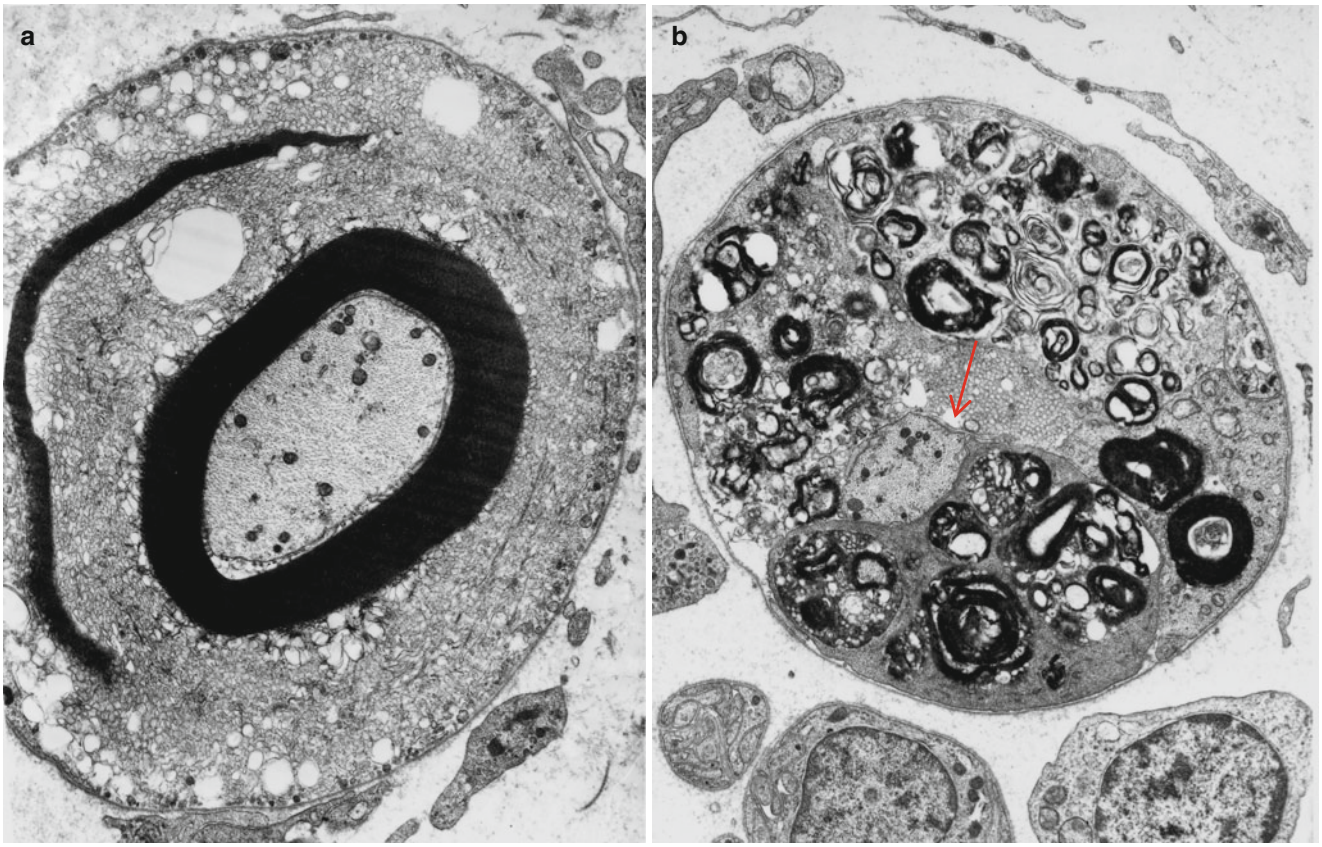

Fig. 17.5 Diabetic neuropathy: segmental demyelination. Note vesicular pattern in (**a**). The demyelinated axon (*arrow*, **b**) is surrounded by Schwann cells containing myelin debris (**a** ×8,892, **b** ×6,384)

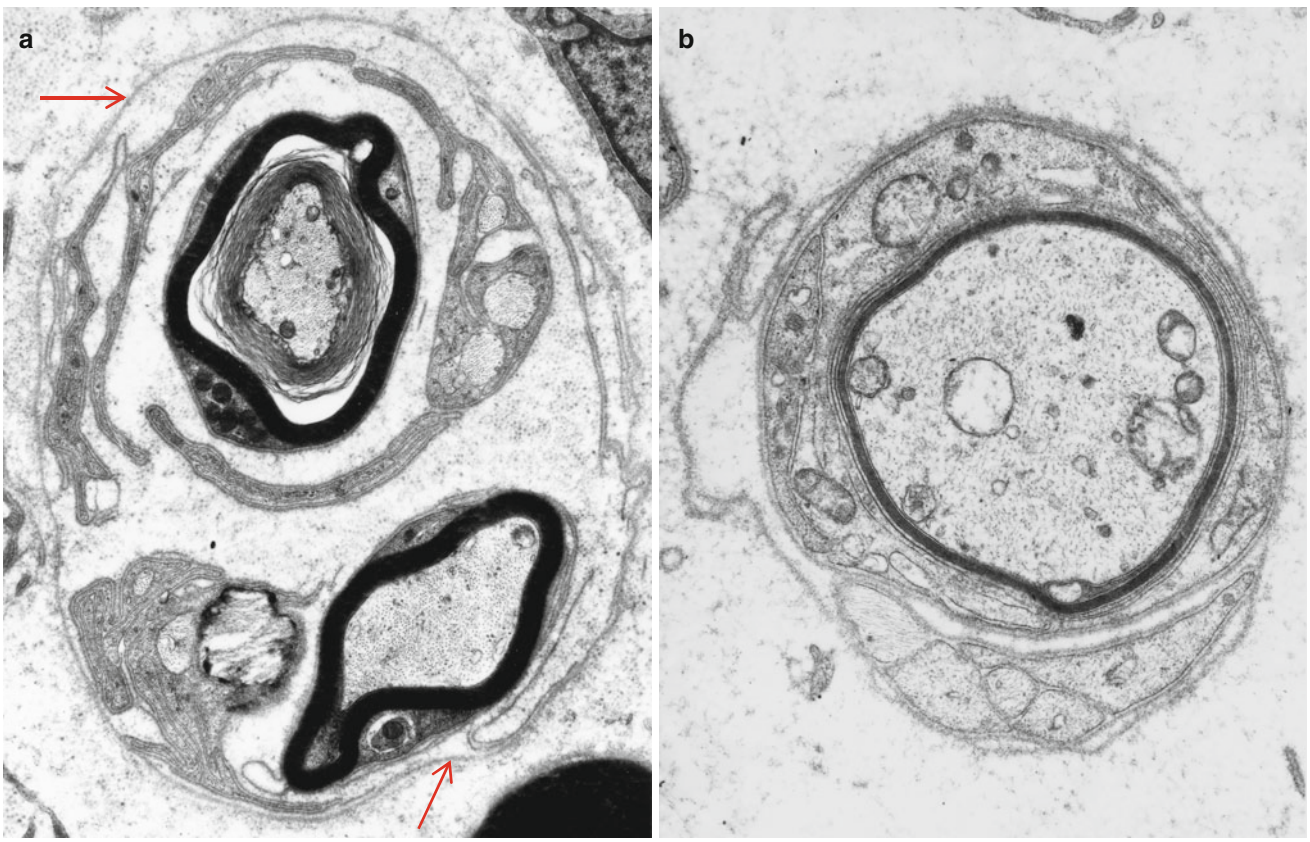

Fig. 17.6 Diabetic neuropathy, regenerative processes: in (**a**) note continuous basement membrane (*arrows*) surrounding a regenerating cluster of myelinated and unmyelinated fibers. Remyelination is illustrated in (**b**) (**a** ×9,576, **b** ×15,048)

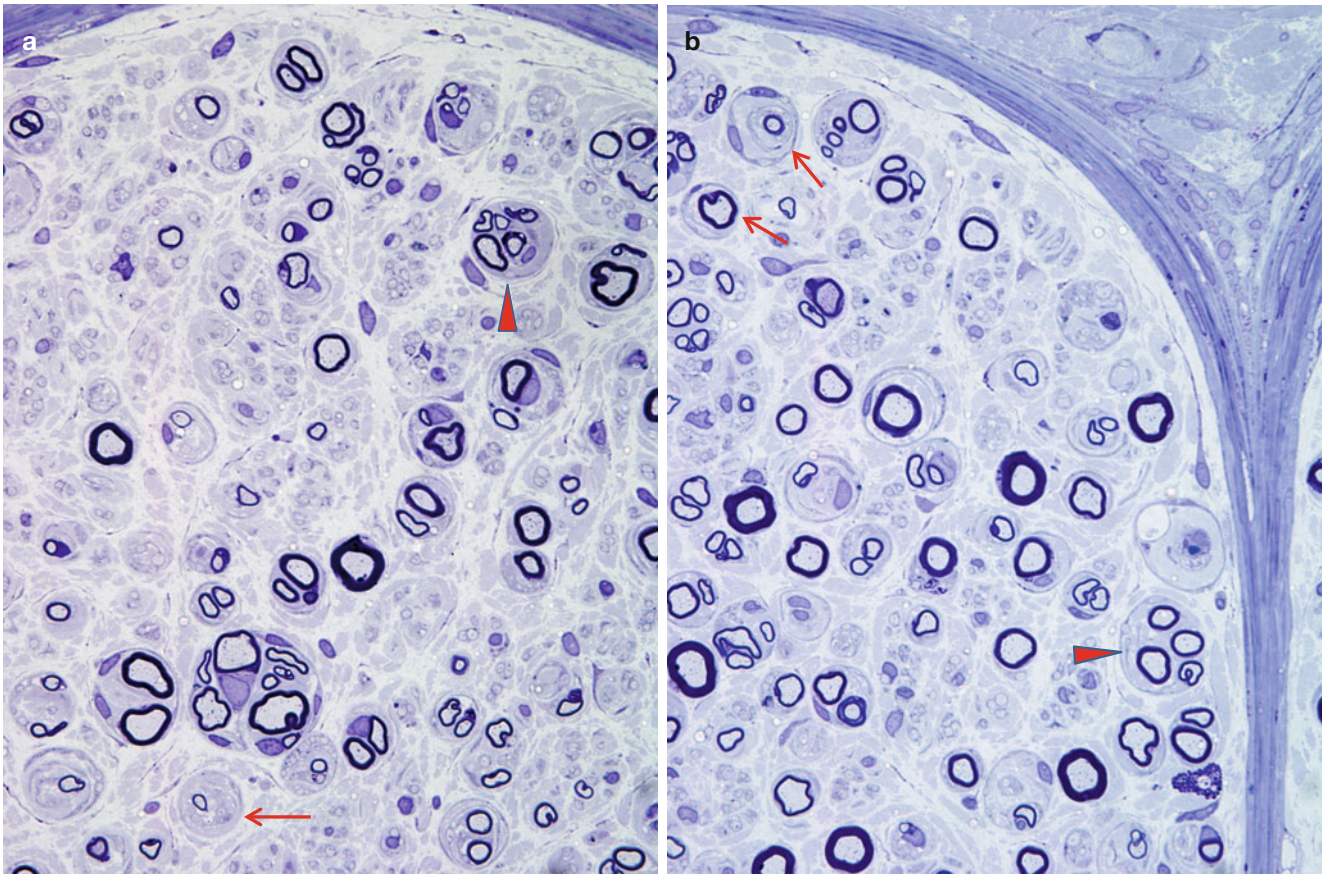

Fig. 17.7 Diabetic neuropathy: note axon loss with (pseudo)-onion bulbs (*arrows*, **a**, **b**) and regenerating cluster (*arrowheads*, **a**, **b**) (1 µm plastic sections. Magnification: **a**, **b**, 1,000×)

both mechanoreceptive Meissner corpuscles and their afferent myelinated nerve fibers (Peltier et al. 2013). Myelinated nerve fiber loss was correlated with the decreased amplitudes of sensory/motor responses in nerve conduction studies. Skin biopsies may distinguish neuropathies from radiculopathies, since dorsal roots are proximal to DRG and do not directly produce epidermal nerve changes.

Recent studies (Cheng et al. 2013) have examined skin biopsies from nondiabetic controls, diabetic patients without neuropathy, diabetics with neuropathy and neuropathic pain, and diabetics with neuropathy but without pain. They reported that patients with painful diabetic neuropathy showed a higher percentage of GAP43-immunoreactive epidermal axons compared to total numbers of PGP fibers (suggesting regenerating axons) and increased numbers of tropomyosin-receptor kinase A and substance P containing axonal swellings per PGP-immunoreactive axon, suggesting that the axons are associated with nociception. The DRG of autopsied diabetic patients contain neurofilament-laden, calcitonin gene-related peptide (CGRP)-, trkA-, and substance P-immunoreactive dystrophic swellings involving recurrent collateral axons that terminate on adjacent perikarya (Schmidt et al. 1997) but have no proven association with painful neuropathy. Other levels of the CNS (e.g., integration in the spinal cord or higher brain centers) may also contribute to painful neuropathy.

17.1.2.3 Pathology in Asymmetric Diabetic Neuropathy

The array of names given to the proximal, often asymmetric, diabetic neuropathies betrays the uncertainty that exists regarding the site of the lesion. At times this syndrome has been termed "diabetic amyotrophy," "Bruns–Garland Syndrome," "diabetic polyradiculopathy," "diabetic lumbosacral plexopathy," or "diabetic femoral neuropathy" (also see Chap. 1, Sects. 1.3, 1.4). The usual coexistence of symmetrical and asymmetric polyneuropathy confounds interpretation of nerve biopsy material. In 10 sural nerve biopsies, myelinated fiber loss was almost invariably seen, with the nerve damage distributed in both multifocal and diffuse patterns (Barohn et al. 1991). This group of neuropathies may involve an autoimmune component resulting in an inflammatory microvasculitis with T-cell infiltrates (Younger et al. 1996; Dyck et al. 1999; Prasnoor et al. 2013), although not typically angionecrosis, involving the epineurial vasculature (Fig. 17.9a, b) with endoneurial and perineurial hemosiderin

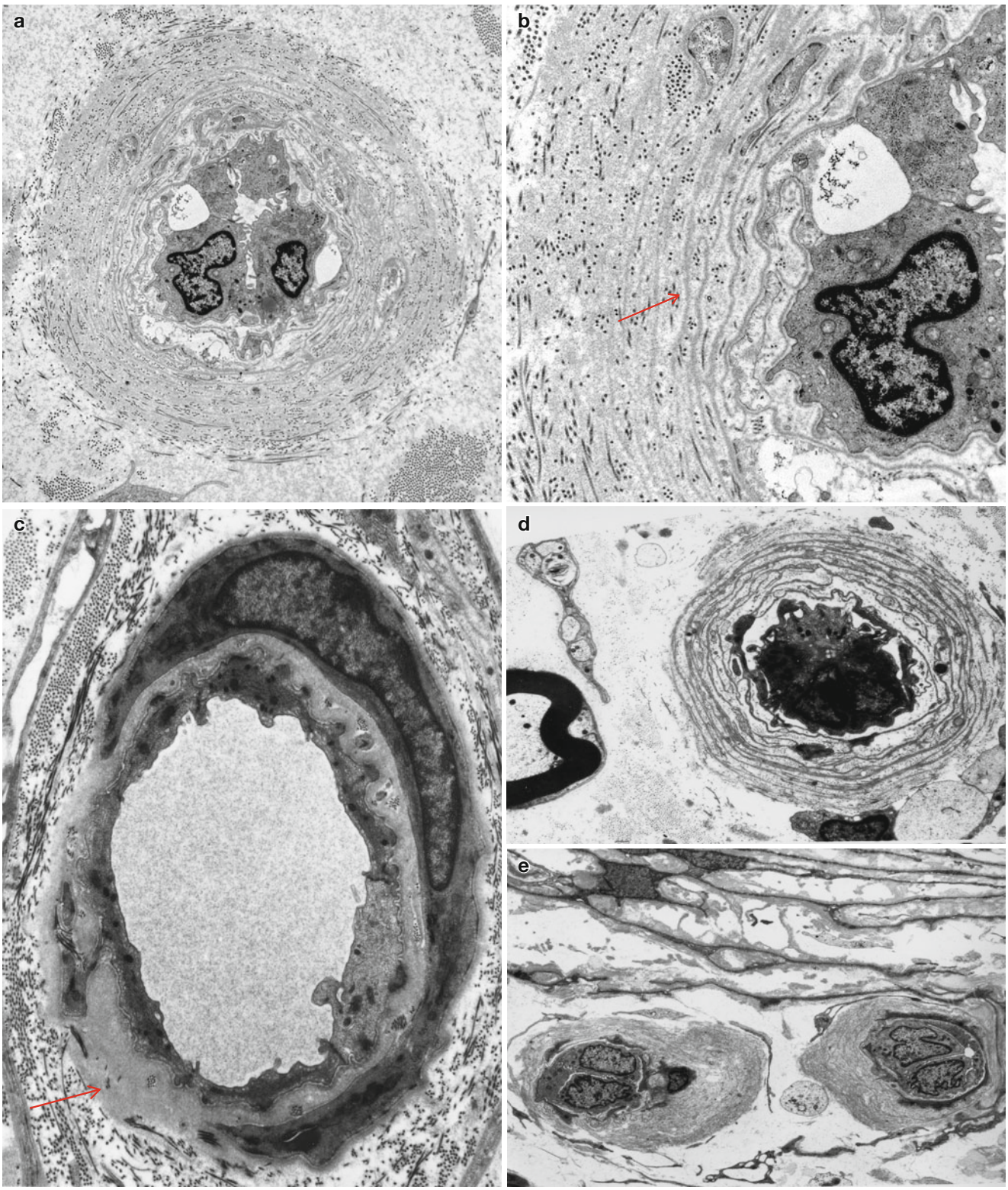

Fig. 17.8 Diabetic neuropathy: the range of endoneurial microvascular alterations is illustrated. Marked duplication of basal lamina about endothelium and pericytes (**a**, **b**). In (**c**) there is thickening of endothelial basal lamina (*arrow*, **c**). "Closed" vessels are illustrated in (**d**, **e**) (**a**, 6,000×; **b**, 15,000×; **c**, 8,379×; **d**, 5,426×; **e**, 2,880×)

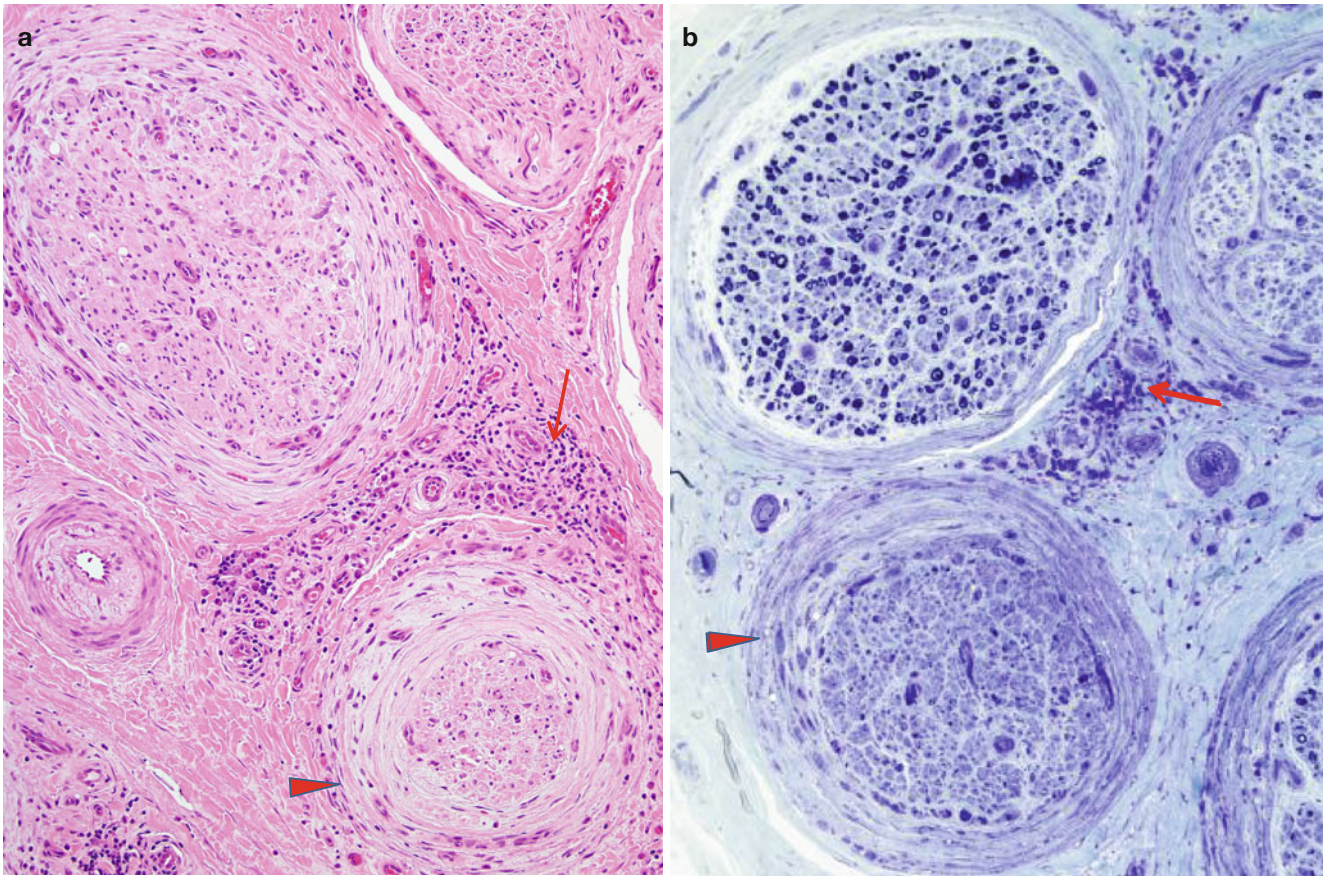

Fig. 17.9 Diabetic lumbosacral radiculoplexus neuropathy (DLPRN): characteristic pathological findings include perivascular inflammation involving the epineurium (*arrows*, **a**, **b**), perineuritis with infiltrating macrophages (*arrowheads*, **a**, **b**), and differential fascicular axon loss (**b**) (**a**, H&E paraffin, 200×; **b**, 1 **µ**m plastic section, 200×)

deposition. The possibility that immune-mediated and inflammatory lesions are important in proximal diabetic neuropathy has been suggested by workers who have observed low-grade perivascular inflammatory infiltrates, sometimes associated with vascular occlusion and focal axonal loss, in this setting (Costigan et al. 1990; Said et al. 1994). Frank necrotizing vasculitis has not been illustrated. Vasculopathy accompanies perineuritis (Fig. 17.9a, b) and an axonopathy, which ranges from scattered degenerating axons to the formation of an "injury neuroma" (Llewelyn et al. 1998; Said et al. 2003). Frequently there is fascicle-to-fascicle variability in axon loss (Fig. 17.9b) At least some "infarcts" described in early studies (Raff et al. 1968) may have represented Renaut bodies (Sugimura and Dyck 1981; Thomas and Tomlinson 1993). Other workers have shown only patchy demyelination or fibrosis in the roots or plexus (Bastron and Thomas 1981). A demyelinating picture suggestive of CIDP was seen in nerve biopsy of three patients with "proximal diabetic neuropathy," and the patients improved with immune suppressive therapy (Costigan et al. 1990). Loss of intraepidermal nerve fibres has also been described in biopsies taken from symptomatic areas. Diabetic LRPN histological findings are identical to those seen in patients with nondiabetic LRPN, although it has been suggested that "nondiabetic" patients with LRPN may also have abnormal glucose tolerance (Kelkar and Hammer-White 2005). The usual course results in spontaneous recovery over several months.

17.1.2.4 Pathology of Diabetic Autonomic Neuropathy

Investigation of the pathology of diabetic autonomic neuropathy has been neglected in comparison to symmetrical sensorimotor neuropathy (reviewed in Schmidt 2002). Systematic autopsy studies of diabetic patients have demonstrated the development of markedly swollen neurofilament-laden axons and synapses (neuroaxonal dystrophy) in the relative absence of neuron loss in diabetic prevertebral sympathetic autonomic ganglia that serve the bowel and viscera (Schmidt et al. 1993). The presence of lymphocytic infiltrates in some diabetic sympathetic ganglia has been noted, although without proof of an immune pathogenesis. Significant loss of preganglionic myelinated axons and elements of the abdominal vagus nerve has also been described.

The examination of the innervation of sweat glands, arrector pili, and arterioles extends the usefulness of skin biopsies to the analysis of autonomic nerves in diabetic autonomic neuropathy (Kennedy 2004) by concentrating on sweat gland nerve fiber density, neuropathic symptoms, neurological deficits, and sweat production in diabetic patients.

17.1.3 Pathogenesis

17.1.3.1 Site of the Lesion

The length-dependent nature of diabetic symmetrical sensorimotor polyneuropathy and the consistent finding of axonal loss in patients with established neuropathy indicate unequivocally that axonal degeneration is the predominant pathology. Whether the frequently observed myelin alterations occur secondary to axonal influences or are due to a primary schwannopathy remains unsettled. The latter possibility has been favored by the description of segmental myelin changes in patients with early or presymptomatic neuropathy (Chopra et al. 1969; Dyck et al. 1980; Thomas and Lascelles 1966; Vital et al. 1974). No evidence for axonal atrophy was found by Sugimura and Dyck (1981). However, some workers have found remyelinating internodes to cluster along certain axons, suggesting secondary demyelination (Dyck et al. 1986b; Said et al. 1983a, 1992). Said and colleagues proposed that both primary and secondary demyelination occurred in diabetic neuropathy (Said et al. 1983a, 1992).

The fiber types involved in symmetrical diabetic neuropathy may vary between different syndromes. In the most common distal symmetrical sensorimotor polyneuropathy, there is either no selectivity or a preferential involvement of large myelinated fibers. In painful sensory neuropathy with prominent autonomic dysfunction, involvement of small myelinated and unmyelinated fibers may be selective. A predominantly motor neuropathy is rarely observed. It remains unclear if these distinct syndromes are part of the spectrum of the same pathophysiological process or if different pathogenic mechanisms are at play.

17.1.3.2 Cause of the Lesion

The precise pathogenesis of diabetic neuropathy remains unknown (Harati 1992; Thomas 1992). Nonetheless, a number of pathogenetic mechanisms have been proposed and tested in experimental animal models (Table 17.3) which have been recently reviewed (Llewelyn et al. 2005; Obrosova 2009; Vincent et al. 2011). There may be no single mechanism underlying diabetic neuropathy, rather multiple mechanisms may participate and may vary between different forms of diabetic neuropathy. Tight glucose control does delay the development of somatic and autonomic neuropathy, but it does not prevent it, even intensive diabetic treatment of type 2 diabetics does not correct established neuropathy (UK Prospective Diabetes Study Group 1998). Two main schools of thought have existed regarding the cause of the neuropathy. The distally predominant and symmetrical pattern, combined with the known metabolic alterations of diabetes, has suggested that metabolic axonopathy with or without schwannopathy directly underlies nerve changes (Sima 1993). Conversely, the focal nature of some diabetic neuropathies has led to consideration of ischemic causes, and such hypotheses have gradually been extended to the entire spectrum of diabetic neuropathies (Dyck 1989).

The Metabolic Hypothesis

The metabolic hypothesis began in earnest with the analysis of the role of the polyol pathway in the pathogenesis of diabetic neuropathy (Sima et al. 1988a; Sima 1993). Intraneural concentrations of sorbitol and fructose are increased in both experimental and human diabetic neuropathy, presumably as a consequence of increased metabolic activity along the aldose reductase pathway but do not have an osmotic effect on nerve function. The increase in intracellular sorbitol was reported to influence intracellular myoinositol content and producing a subsequent defect in Na^+/K^+-ATPase activity (Sima et al. 1988a). These biochemical changes were thought to cause accumulation of Na^+ at the nodal axon where sodium channels are found, causing node swelling and detachment of the terminal myelin loops which attach to the axon, so-called axoglial dysjunction. This results in widening of the node and exposure of K^+ channels which hyperpolarize the axonal membrane and impair conduction. Sima and co-workers have found that aldose reductase inhibitors promote regeneration of peripheral nerve in diabetic neuropathy (Sima et al. 1988b).

Against this theory stands the failure of aldose reductase inhibitors (Tsai and Burnakis 1993) to have a substantial clinical effect in many studies, although controversy continues. Salutary use of aldose reductase inhibitors would reasonably be expected to interrupt the process in its earliest phases, in order to improve diabetic neuropathy.

Recent work reveals that hyperglycemia in diabetes triggers a multitude of biochemical metabolic changes (see Table 17.3) which are currently thought to underlie the development of diabetic neuropathy. Currently, Fernyhough and co-workers (Chowdhury et al. 2010, 2011, 2013; Fernyhough et al. 2003) propose that nutrient excess in neurons mediates mitochondrial damage through alteration of the AMP-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) signaling axis. This signaling pathway is thought to affect an energy-sensing metabolic pathway which modulates mitochondrial function, biogenesis, and regeneration. The bioenergetic phenotype (maximal oxygen consumption rate, coupling efficiency, respiratory control ratio, and spare respiratory capacity) of mitochondria in diabetic neurons is

Table 17.3 Diabetic neuropathy – proposed pathogenetic mechanisms

Mechanism	Synopsis	References
Oxidative stress	Increased production of oxidant stressors and/or reduction in antioxidant defenses. DRG neurons from diabetic rats show low MnSOD and increased reactive oxygen species associated with dystrophic axonal swellings and impaired axon outgrowth which may lead to axonal degeneration	Obrosova (2002), Campanucci et al. (2010), Zherebitskaya et al. (2009)
Mitochondriopathy	Insulin- and NT-3-sensitive decrease in mitochondrial membrane potential Nutrient excess interferes with activity of AMP kinase and PGC-1α in sensing neuronal metabolic demands in diabetic DRG, decreasing mitochondrial respiratory chain and Krebs cycle function which may interfere with peripheral nerve energy supply and result in distal axonopathy, a result reversible with resveratrol	Chowdhury et al. (2011, 2012, 2013), Fernyhough et al. (2003), Choi et al. (2014)
Calcium homeostasis	Axons of diabetic DRG neurons exhibit aberrant Ca2+ homeostasis resulting from suboptimal neuronal SERCA activity	Zherebitskaya et al. (2011)
Neurotrophic substances	Deficiencies in the amount or regulation of various neurotrophic substances (e.g., NGF, NT-3, IGF-I, insulin, erythropoietin, C-peptide)	Hellweg and Hartung (1990), Harati (1992), Brewster et al. (1994), Ekberg et al. (2003), Ishii (1993), Pierson et al. (2003a, b), Schmidt (1996), Dey et al. (2013)
Polyol pathway	Excess glucose is converted to sorbitol and then to fructose using aldose reductase and sorbitol dehydrogenase, diminishing glutathione and increasing oxidative stress.	Obrosova (2002)
Ischemia	(See text)	
Advanced glycation end products (AGEs)	Hyperglycemia-induced glycosylation of various intracellular and extracellular proteins converted into AGEs which cross-link a variety of proteins and subcellular organelles and increase oxidative stress by binding to AGE receptors (RAGEs)	Thornalley (2002), Juranek et al. (2013)
Mitogen-activated protein kinases	Mitogen-activated protein kinases (MAPKs) are activated by glucose, and oxidative stress may induce excessive protein (e.g., neurofilament) phosphorylation	Purves et al. (2001)
Neuronal apoptosis	A contentious mechanism, proposed from in vitro cell culture studies conflicting with largely maintained numbers of dorsal root and sympathetic ganglia in experimental rat models, even in the face of increased neuronal activated caspase 3. Mice with experimental diabetes apparently do lose sensory neurons and axons	Cheng and Zochodne (2003), Russell et al. (1999), Kennedy and Zochodne (2005)
Protein kinase C	Increased activity of protein kinase C (PKC) βII isoform may injure the microvasculature and induction of TGF-β effects on the extracellular matrix	Way et al. (2001)
Axonal transport	Axonal transport defects have been identified in diabetic animal models involving a variety of materials	Sidenius and Jakobsen (1987)
Abnormal axonal regeneration/synaptic turnover	Abnormal regeneration and synaptic turnover have been proposed to explain dystrophic nerve terminals and synapses in diabetic DRG and prevertebral sympathetic ganglia	Ekstrom et al. (1989), Longo et al. (1986), Pierson et al. (2002), Schmidt (1996)
Autoimmune pathogenesis	Autoimmune attack has been proposed to explain diabetic autonomic neuropathy in which a variety of antibodies against parasympathetic and sympathetic ganglia have been found, although unproven as a cause of clinical diabetic autonomic neuropathy	Bird and Brown (1996), Granberg et al. (2005), Rabinowe et al. (1990)
Neuronal insulin resistance	Neuronal insulin resistance is thought to alter PI3K/pAkt/GSK-3β signaling pathways, mitochondrial biogenesis and function, oxidative stress, and 12/15 lipoxygenase activation	Kim and Feldman (2012)
Dyslipidemia	Dyslipidemia is a major independent risk factor for the development of neuropathy as shown by epidemiologic studies and demonstration of dysregulated genes associated with lipid metabolism in experimental animals	Vincent et al. (2009)
Channelopathy	Dysregulation of Na+ and K+ channel activities as a consequence of posttranslational modifications	Arnold et al. (2013), Zenker et al. (2013), Hammond et al. (2013)
ER stress	Unfolded protein response (UPR) is insufficient to alleviate diabetes-induced ER stress resulting in apoptosis	O'Brien et al. (2014)

aberrant as the result of changes in expression and activity of respiratory chain components brought about as a direct consequence of abnormal AMPK/PGC-1α signaling. Other investigators have confirmed and expanded these ideas, demonstrating decreased PGC-1α and downregulation of its transcription factors including TFAM and NRF1 in mouse streptozotocin-diabetic dorsal root ganglion neurons (Choi et al. 2014). A neuropathic phenotype is exacerbated in PGC-1α (–/–) diabetic mice accompanied by a severe neuropathy with reduced mitochondrial DNA and a further increase in protein oxidation. Conversely, overexpression of PGC-1α in cultured adult mouse neurons prevents oxidative stress associated with increased glucose levels (Choi et al. 2014). The result of these changes in cellular bioenergetics is a reduced adaptability to fluctuations in ATP demand, thought to be distally accentuated. This diabetes-induced maladaptive process is hypothesized to result in exhaustion of the ATP supply in the distal nerve compartment and induction of axonal degeneration, a result which can be corrected by resveratrol treatment (Chowdhury et al. 2012, 2013).

The Ischemic Hypothesis

Proponents of the ischemic hypothesis have emphasized the primacy of axonal lesions, their multifocal distribution, alterations in endoneurial and epineurial vessels, and association with disease in other organs (eye, kidney) where microvascular injury is thought to play a central role (Dyck et al. 1986a, b; Dyck 1989; Johnson et al. 1986; Sugimura and Dyck 1982; Timperley et al. 1985). The observation that endoneurial microvascular changes differ quantitatively and qualitatively from those seen in other tissues in diabetics mitigates against the suggestion that these simply reflect nonspecific systemic microvasculopathy (Malik et al. 1989, 1993). The distally predominant symmetrical pattern of clinical involvement may result from the summation of numerous random focal insults, with the longest fibers sustaining the greatest number of injuries (Johnson et al. 1986; Waxman et al. 1976). In support of this hypothesis, reduced microvascular luminal area, increased numbers of closed endoneurial capillaries (Dyck et al. 1985; Malik et al. 1993), and plugging of vessel lumina by degenerate cellular material have been described (Timperley et al. 1985; Yasuda and Dyck 1987) and debated. The observation of increased resistance of diabetic peripheral nerve to ischemia (Thomas and Tomlinson 1993) may suggest chronic neural ischemia in these patients.

Correlation of the severity of microvasculopathy and neuropathy has supported an ischemic pathogenesis (Malik et al. 1993; Yasuda and Dyck 1987), but similar microvascular alterations are seen in chronic neuropathies in the absence of ischemia (Bradley et al. 1990). Indeed, endothelial cell hyperplasia was more prominent in CMT-1 than in diabetes and reduced vascular luminal area, and capillary "closure" has not been consistently detected (Bradley et al. 1990), or found to be no different than in age-matched controls (Sima et al. 1991). Moreover, the finding of multifocal nerve fiber loss of equal degree in CMT-1 and diabetic neuropathy weakens the assertion that this pattern suggests an ischemic neuropathy (Bradley et al. 1990; Dyck 1989; Llewelyn et al. 1988). Sima and colleagues (Sima et al. 1988a) found multifocal degeneration of axons in NIDDM (mean age 56) but not IDDM (mean age 38) patients with neuropathy, suggesting that multifocality of nerve lesions in diabetes may relate to age. Geographic regions of fibers with attenuated myelin (Fig. 17.4b) may represent foci of axonal degeneration and subsequent regeneration showing the hypomyelination typical of regenerating fibers or may indicate demyelination with remyelination. Johnson and colleagues (1986) found that the frequency of such focal lesions proximally correlated with the degree of distal nerve fiber depletion in patients with symmetrical sensorimotor neuropathy. The multifocality of the axonal lesions was verified statistically by Dyck et al. (1986b). However, multifocal loss of axons is not pathognomonic of vasculopathy or ischemia and is typical in some forms of inherited neuropathy. Endothelial cell and pericyte degeneration, endothelial cell hyperplasia/hypertrophy, decreased capillary luminal area, and altered blood–nerve barrier permeability are seen in endoneurial microvasculature in patients with diabetic symmetrical sensorimotor neuropathy, findings that correlated with electrophysiological measures of the severity of the neuropathy (Watkins and Thomas 1998; Malik et al. 1993; Giannini and Dyck 1995). Nonetheless, Theriault and colleagues (1997) measured nerve blood flow using laser Doppler flowmetry in diabetic patients with early polyneuropathy and determined that local blood flow was not decreased, even in the presence of definitive evidence of decreased sensory nerve action potential (SNAP) amplitudes and decreased numbers of axons. Finally, axon and Schwann cell nodal and paranodal changes similar to those emphasized in diabetes have been produced by chronic endoneurial ischemia alone (Sladky et al. 1991). Alterations in microvessel junctional structures, similar to "axoglial dysjunction," have been described (Sima et al. 1991). Increased sural nerve water content due to accumulation of osmotically active solutes may cause a rise in endoneurial pressure and result secondarily in endoneurial ischemia (Griffey et al. 1988; Kalichman and Myers 1991). Nonenzymatic glycosylation of intracellular and extracellular structures may directly cause axon dysfunction or cause cellular and extracellular alterations resulting in endoneurial ischemia (Harati 1992; King et al. 1989).

In summary, the role of microvascular pathology in diabetic neuropathy has been variously interpreted as evidence for an ischemic pathogenesis in diabetic neuropathy or as unrelated but parallel changes (Zochodne 2002); however, there is evidence for tissue hypoxia in the nerve and ganglia in experimental animal models and humans (Zochodne and Ho 1992). Thus, nerve ischemia is associated with nerve dysfunc-

tion but may not represent the primary or major cause; rather, it may simply accelerate the process (Llewelyn et al. 2005).

Other Mechanisms

The ischemic and metabolic hypotheses are not mutually exclusive and can be unified by suggestion that metabolic alterations in the neural milieu cause direct or indirect injury to the neural blood supply. An overview of the number of hypotheses proposed to underlie development of neuropathy in diabetes is provided in Table 17.3. It is clear that many of the hypotheses proposed may be interdependent (e.g., oxidative stress, mitochondriopathy, polyol pathway, etc.).

17.2 Neuropathy in Thyroid Disease

17.2.1 Clinical Manifestations

Peripheral neuropathy (excluding carpal tunnel syndrome) is seen in 17–60 % of hypothyroid patients (Nickel et al. 1961; Watanakunakorn et al. 1965). Typically this is a mild, predominantly sensory polyneuropathy with prominent paresthesias. Physical examination is confounded by the frequent coexistence of muscle disease. Electrophysiological studies can be suggestive of a demyelinating or an axonal process, but are usually mixed. CSF protein may be increased. Thyroid replacement is efficacious, but recovery is not always complete (Dyck and Lambert 1970; Nemni et al. 1987; Pollard et al. 1982). Nerve biopsy studies are mixed favoring axonal degeneration (Nemni et al. 1987) or demyelination (Dyck and Lambert 1970).

17.2.2 Pathology

Deposition of mucopolysaccharide materials, a characteristic systemic feature of myxedema, was described in endoneurium and subperineurium in an early nerve biopsy study (Nickel et al. 1961), but subsequent reports have not confirmed this (Dyck and Lambert 1970; Meier and Bischoff 1977; Nemni et al. 1987; Pollard et al. 1982; Shirabe et al. 1975). Renaut bodies were seen in only 2 of 17 cases presented by these latter authors. A predominance of segmental demyelination has been noted in some reports (Dyck and Lambert 1970; Shirabe et al. 1975), while, in others, the degeneration of myelinated and unmyelinated axons has been emphasized (Meier and Bischoff 1977; Nemni et al. 1987; Pollard et al. 1982). It is, however, agreed that larger myelinated fibers are most prominently involved. The neuropathy is usually histologically indolent, and regenerating clusters may be readily visualized. Reich Pi granules may be more prominent than usual (Dyck and Lambert 1970; Meier and Bischoff 1977). Onion-bulb formations have rarely been observed.

Excessive deposition of glycogen in various cell types including Schwann cells, myelinated and unmyelinated axons, endothelial cells, and perineurial cells has been emphasized as the most interesting ultrastructural feature of hypothyroid neuropathy (Dyck and Lambert 1970), but the extent to which this exceeds the amounts seen in normal nerves and other neuropathies is uncertain. The observation of "axonal atrophy" (Meier and Bischoff 1977) awaits quantitative confirmation.

17.2.3 Pathophysiology

The symptomatic and objective improvement seen in these patients after thyroid replacement leaves no doubt regarding the causal connection between thyroid deficiency and the neuropathy, but the mechanism remains obscure. Thyroid hormone is a key mediator of neuronal development, interacts with a variety of important growth factors, and may modulate axonal growth through regulation of microtubule assembly (Stein et al. 1991; Timiras and Nzekwe 1989), suggesting a possible mechanism for axonal degeneration. The observed glycogen deposition may be due to a decreased utilization of energy associated with the hypothyroid state (Nemni et al. 1987).

17.2.4 Neuropathy Associated with Hyperthyroidism

The most common neuromuscular complication of hyperthyroidism is a myopathy, but some have observed additional neurogenic features that were clinically overshadowed by muscle disease (Feibel and Campa 1976; Ludin et al. 1969). Neuropathy may be electrophysiologically detectable in as many as 30 % of patients (Berlit et al. 1992). The single biopsy report found in the literature describes active axonal degeneration with no specific features; however, the patient's clinical course was complex and other neuropathy etiologies may have been present (Szollar et al. 1988).

17.3 Neuropathy in Acromegaly

17.3.1 Clinical Manifestations

The frequency of peripheral neuropathy, excluding carpal tunnel syndrome, in acromegaly is controversial, with general reviews of the disease claiming a low incidence of symptoms (Molitch 1992; Pickett et al. 1975), but series focusing on neuropathy find an incidence of more than 50 % (Low et al. 1974; Jamal et al. 1987). Distal paresthesias and weakness predominate, with palpable enlargement of superficial

nerves in a substantial proportion of the patients (Low et al. 1974; Stewart 1966; Jamal et al. 1987). Electrophysiological studies have demonstrated mixed axonal and demyelinating features.

17.3.2 Pathology

The pathological reports available have emphasized an increase in connective tissue within the perineurium and endoneurium (Stewart 1966; Low et al. 1974; Dinn and Dinn 1985). A diminution in axon numbers with little or no active degeneration, afflicting both myelinated and unmyelinated fibers, has been the usual observation. The presence of onion-bulb formations has been noted by most observers. Segmental demyelination represented the predominant change in two teased fiber studies, one of which suggested that the demyelination was primary (Low et al. 1974; Dinn and Dinn 1985).

17.3.3 Pathogenesis

Pathogenesis of neuropathy in acromegaly remains obscure, especially as regarding the role of the frequent occurrence of glucose intolerance in acromegaly. Although a potential contributing role of diabetes cannot be excluded, many patients with a definite polyneuropathy have had no evidence of glucose intolerance (Low et al. 1974; Jamal et al. 1987; Dinn and Dinn 1985). Teased fiber preparations demonstrate a combination of axonal degeneration and segmental demyelination, although some studies show demyelination combined with hypertrophic onion bulbs thought to reflect excessive Schwann cell stimulation by increased IGF-I levels (Dinn and Dinn 1985). The fascicular area is increased by subperineurial and endoneurial connective tissue. Growth hormone elevations and associated thyroid dysfunction have also not correlated with the presence of a polyneuropathy (Low et al. 1974; Jamal et al. 1987).

17.4 Uremic Neuropathy

17.4.1 Clinical Manifestations

The majority (up to 90 %, Krishnan and Kiernan 2007) of patients with end-stage renal failure have a peripheral neuropathy, independent of underlying disease (Bolton and Young 1990), although the neuropathy is uncommon in children. The pattern of involvement is sensorimotor, distal, and symmetrical, evolving acutely in a small fraction of patients and more frequently in a subacute or chronic fashion (Said et al. 1983a; Asbury et al. 1963; Ropper 1993; Thomas et al. 1971). Paresthesias, sensory loss, weakness, and early distal areflexia are all typical features. Electrophysiological studies reveal a combination of mild to moderate conduction slowing and amplitude reductions that suggests a mixed or predominantly axonal sensorimotor polyneuropathy (Bolton and Young 1990; Thomas et al. 1971). CSF protein can be increased. Hemodialysis and peritoneal dialysis usually halt progression and may improve the neuropathy, but only kidney transplant is likely to result in a major improvement (Bolton and Young 1990).

17.4.2 Pathology

Uremic neuropathy was the subject of intense scrutiny in the early 1970s, and much of the available histological material dates from this period (Ahonen 1981; Appenzeller et al. 1971; Asbury et al. 1963; Dayan et al. 1970; Dinn and Crane 1970; Dyck et al. 1971; Said et al. 1983b; Thomas et al. 1971). Active and chronic axonal degeneration is invariably present, with severity increasing proximally to distally along the nerve fibers (Asbury et al. 1963; Dyck et al. 1971). Large myelinated fibers are most severely affected, but unmyelinated fibers are not spared. Regenerative activity is variably present.

Some contention has occurred around the frequency and importance of segmental myelin changes, which have at times been prominent enough to suggest a primary Schwann cell defect (Dinn and Crane 1970; Appenzeller et al. 1971), while other authors felt that axonal changes were the fundamental substrate of uremic neuropathy (Asbury et al. 1963; Thomas et al. 1971). Certainly, paranodal demyelination, segmental demyelination, remyelinated internodes, and even some onion-bulb formations may be seen in uremic neuropathy (Dayan et al. 1970; Appenzeller et al. 1971). Dyck and colleagues (1971) seemed to have resolved this issue by demonstrating that the segmental myelin changes were secondary to a distal axonopathy with axonal atrophy, an impression shared by others (Thomas et al. 1971; Burn and Bates 1998). Nevertheless, some authors continue to believe that a primary defect of Schwann cells is present in some cases (Said et al. 1983b). The neuropathy of uremia shows no specific ultrastructural features, but the entire spectrum of axonopathic changes may be present.

Dayan and colleagues (1970) have suggested that in acute renal failure the neuropathy is predominantly demyelinating, while in chronic renal failure it is essentially axonal, while Said et al. (1983b) found quite the opposite pattern. In the latter report, a number of surprising observations were made, including multifocal sausage-like myelin swellings reminiscent of tomaculous neuropathy, apparent macrophage-mediated demyelination, and several axons surrounded by a single myelin sheath (polyaxonal myelination) (Said et al.

1983b). These unusual findings have not been described by other authors nor have we seen them in our experience with six cases of uremic neuropathy.

17.4.3 Pathogenesis

Bolton and Young provide a comprehensive review of the current understanding of the pathogenesis of uremic neuropathy (Bolton and Young 1990). An accumulation of circulating toxins ("the middle molecule hypothesis") has been implicated in various aspects of the uremic syndrome, including neuropathy (Burn and Bates 1998). Circulating substances of intermediate molecular weight, 0.5–500 kD, are cleared better by peritoneal dialysis than hemodialysis, and this has been thought to explain the clinical observation that peritoneal dialysis, or better yet a renal transplant, is more likely to result in improvement of neuropathy than chronic hemodialysis (Ahonen 1981; Bolton and Young 1990; Baumgaertel et al. 2014).

17.5 Neuropathy Associated with Liver Disease

Neuropathy in liver disease may take several forms. Most common is a mild sensorimotor, usually subclinical, polyneuropathy. Physical examination or electrophysiological abnormalities, usually mild, are seen in 20–91 % (Seneviratne and Peiris 1970; Knill-Jones et al. 1972; Chari et al. 1977; Kardel and Nielsen 1974). Electrophysiological testing reveals mild prolongation of distal latency and conduction slowing. The histological findings vary from little or no abnormality to a 50 % loss of axons, without active signs of fiber degeneration. Teased fiber studies have consistently shown segmental demyelination and remyelination to be more prominent than axonal loss (Chari et al. 1977; Dayan and Williams 1967; Knill-Jones et al. 1972). The neuropathy does not seem to be explicable on the basis of alcohol abuse (Seneviratne and Peiris 1970) and has been thought to be due to a toxic metabolite that accumulates in hepatic failure, although conduction velocities do not appear to be lower in patients with a portacaval shunt than in those without (Chari et al. 1977).

Hepatitis B infection has been associated with both acute and chronic inflammatory demyelinating neuropathies (Niermeijer and Gips 1975; Berger et al. 1981; Inoue et al. 1987). Tsukada and colleagues (1987) have associated elevated hepatitis B titers and HBsAg–antibody complex titers with a Guillain–Barré and CIDP-like illness. On histological examination, a mixed axonal-demyelinating inflammatory neuropathy was seen, with focal electron-dense deposits in small vessels and endoneurium, perhaps representing hepatitis B antigen–antibody complexes (Tsukada et al. 1987; Inoue et al. 1987). In some instances, onion-bulb formation was said to be prominent.

A rare but distinctive association has been described between primary biliary cirrhosis (PBC) and sensory neuropathy (Thomas and Walker 1965; Charron et al. 1980; Illa et al. 1989), often with symptoms prior to the diagnosis of liver disease. In the cases presented by Thomas and Walker (1965), there was infiltration of the peripheral nerve, especially perineurium, with lipid-filled macrophages. This report is unique, and subsequent cases of sensory neuropathy in PBC demonstrated only axonal loss, perhaps length dependent, without inflammation, demyelination, or lipid deposition (Illa et al. 1989; Charron et al. 1980). Ludwig et al. (1982) described xanthomatous changes in intrahepatic unmyelinated nerves of a patient with juvenile-onset diabetes and cholestatic hepatitis of unknown cause, but other peripheral nerves were not similarly affected. We have examined a case of "sensory perineuritis" in which striking lipid deposition reminiscent of "xanthomatous" neuropathy was present in the perineurium (Chap. 10). However, perineurial inflammation was also a feature of this biopsy, and autopsy revealed only early nutritional cirrhosis.

17.6 Neuropathy Due to Vitamin Deficiency

Vitamin deficiency has long been identified as a cause of polyneuropathy. No longer common in the developed world, these nutritional disorders are nevertheless important to the clinician because of their treatability. In the underdeveloped world, nutritional deficits continue to be a major cause of peripheral neuropathy (Osuntokun 1980). Isolating the features of a particular vitamin deficiency is difficult because most often human malnutrition involves the lack of several essential nutrients which may combine to produce the clinical picture. Recently, nutritional neuropathies (deficiencies of thiamine; vitamins B12, E, and D; and copper) have been reported in up to 16 % of patients (Becker et al. 2012) following bariatric surgery presenting as either sensory predominant polyneuropathy (acute, subacute, and chronic), mononeuropathy, or less frequently radiculoplexopathy.

17.6.1 Thiamine (B_1) Deficiency

The central features of thiamine deficiency (beriberi) are cardiac failure and peripheral nerve disease (Windebank 1993; Igata 1984). The latter typically begins with painful paresthesias and progresses over weeks to months to produce sensory loss and weakness in a length-dependent fashion (Takahashi and Nakamura 1976; Ohnishi et al. 1980; Igata

1984). Study of thiamine deficiency has been hampered by the fact that nearly all cases in the developed world occur in association with alcohol abuse. Nevertheless, the available data suggests that the neuropathy is a distal axonopathy (Djoenaidi and Notemrans 1990; Collins et al. 1964).

Modern-era histological data regarding the neuropathy of pure thiamine deficiency comes almost exclusively from Japan, where intake of milled rice without thiamine supplementation precipitated an epidemic of beriberi in the 1970. In these cases, the nutritional defect was likely confined to thiamine and not confounded by issues such as malnutrition, multiple vitamin deficiency, or concurrent alcohol intake. Of interest, Wernicke encephalopathy was not seen (Igata 1984).

Axonal degeneration, both chronic and active, was present, with large MFs most severely involved (Takahashi and Nakamura 1976; Ohnishi et al. 1980; Igata 1984). Pathological findings most consistently involve the extremities, vagus nerve, and phrenic nerves, in which distal axonal degeneration is most prominent with proximal secondary demyelination (Vedder 1938). No axonal regenerative activity was seen in untreated patients, but with treatment this became prominent. Unmyelinated fibers also showed evidence of degeneration and regeneration, but their total numbers were normal. Teased fibers invariably showed axonal degeneration. Segmental myelin alterations were clustered on certain axons, and thus likely secondary to axonal pathology. Ultrastructural examination in patients who had not yet been treated revealed axonal accumulations of tubular or tubulovesicular structures of various sizes and focal aggregates of filaments, microtubules, glycogen, and mitochondria (some of which were degenerating, Takahashi and Nakamura 1976). Two patients with a relapsing course showed infrequent remyelinated internodes with the beginnings of onion-bulb formation (Takahashi and Nakamura 1976). Enlargement of the subperineurial space, so-called subperineurial edema, was emphasized in one report, where this finding was far more prominent in thiamine deficiency than in any other type of neuropathy (Watanabe and Ohnishi 1979; Ohnishi et al. 1980). The biochemical mechanism involves the effect of deficiency of thiamine pyrophosphate (TPP), a cofactor in the formation of acetyl coenzyme A from pyruvate which is catalyzed by pyruvate dehydrogenase.

17.6.2 Pyridoxine (B_6) Deficiency and Excess

Pyridoxine deficiency produces a syndrome of distal symmetrical numbness and tingling. In humans, this occurs with medicinal use of isoniazid (Ochoa 1970), hydralazine, or phenelzine (Raskin and Fishman 1965), hemodialysis, alcoholism, penicillamine, and pharmaceutical agents which inhibit phosphorylation of pyridoxine to the active coenzyme pyridoxal phosphate (Windebank 1993; Hammond et al. 2013). The most thorough study of this neuropathy in man was performed by Ochoa, who demonstrated an axonal neuropathy affecting myelinated and unmyelinated fibers, with ample regenerative activity in both populations (Ochoa 1970).

Pyridoxine excess also produces a predominantly sensory neuropathy which displays nonspecific axonal degeneration on biopsy (Schaumburg et al. 1983). Intake of excessive amounts of pyridoxine produces a diffuse distal sensory neuropathy in which sural nerves show nonspecific axon loss, which is likely to result from the degeneration of DRG neurons with high doses (Krinke et al. 1985) possibly due to blockade of fast axonal transport. Rats treated with excessive doses of pyridoxine also develop an exclusively sensory, reversible neuropathy which improves with 4-methylcatechol, a drug known to induce NGF synthesis (Callizot et al. 2001). Neurotrophin 3 has also been shown to be protective in the animal model.

17.6.3 Cobalamin (B_{12}) Deficiency

Central nervous system involvement usually overshadows peripheral nervous symptoms in vitamin B12 deficiency. The neurological manifestations include distal paresthesias, sensory ataxia, and lower limb weakness. Amyotrophy and diminished distal reflexes may give evidence of a neuropathy. Electrophysiological studies suggest the presence of axonal (McCombe and McLeod 1984) or, less often, demyelinating (Steiner et al. 1988) neuropathy. B_{12} replacement arrests disease progression, but may not result in complete improvement (McCombe and McLeod 1984). Nitrous oxide inactivates the cobalamin-dependent enzyme methionine synthase (Frasca et al. 1986), and thus intoxication with this inhaled analgesic causes a syndrome with clinical and histological features similar to those of B12 deficiency (Sahenk et al. 1978; Vishnubhakat and Beresford 1991). Proton pump inhibitors and metformin may also contribute to vitamin B12 deficiency (Hammond et al. 2013).

Nerve biopsies and postmortem studies have been reported by several authors (Abarbanel et al. 1986; McCombe and McLeod 1984; Bischoff et al. 1975; Kosik et al. 1980), and Greenfield and Carmichael (1935) series remains a major work on the subject. Histological examination has consistently shown an axonal neuropathy, with large MFs more severely depleted, and the severity of MF loss paralleling disease duration. The histological picture may be one of the very active axonal degeneration in cases with an aggressive course (Kosik et al. 1980). McCombe and McLeod (1984) found little axonal regenerative activity, but Bischoff et al. (1975) commented on the prominence of this finding. Unmyelinated fibers are also affected, although less severely.

While some reports have emphasized the absence of segmental myelin changes (McCombe and McLeod 1984; Bischoff et al. 1975), others have detected this finding occasionally, although it was in all cases felt to be most likely secondary to the axonal process.

Axonal atrophy and nonspecific ultrastructural evidence of axonopathy are readily seen (Bischoff et al. 1975). Schochet and Chesson (1977) reported a patient with malabsorption-related vitamin B12 deficiency where nerve biopsy demonstrated occasional massively swollen axons which on EM were shown to be distended with a dense accumulation of neurofilaments, a picture of "giant axonal" neuropathy. Although Coers and Woolf (1959) noted that intramuscular axonal swellings were particularly prominent in B12 deficiency, the significance of this observation is hard to interpret in light of the frequency of such swellings in intramuscular nerves of normals as well as in a wide range of neuromuscular diseases (Alderson 1992).

17.6.4 Tocopherol (Vitamin E) Deficiency

Deficiency of vitamin E results in a syndrome of cerebellar degeneration, pigmentary retinopathy, and sensorimotor neuropathy (Muller and Goss-Sampson 1990). Most often this occurs in the setting of fat malabsorption, such as chronic liver disease or abetalipoproteinemia, congenital biliary sclerosis, people with mutation in the gene for α-tocopherol-transfer protein (Gotoda et al. 1995), and, in the past, cases of cystic fibrosis without pancreatic enzyme supplementation. The scanty histological material available in vitamin E-deficient states other than abetalipoproteinemia indicates that a predominantly axonal process, involving large myelinated axons more than small myelinated fibers, is the basis of the neuropathy (Laplante et al. 1984; Rosenblum et al. 1981; Hammond et al. 2013). The few cases we have seen with demonstrated vitamin E deficiency show substantial large and small myelinated axon loss (Fig. 17.10a) and scattered dystrophic axons (Fig. 17.10b–d).

17.6.5 Folate Deficiency

A few reports exist suggesting that isolated folic acid deficiency might cause a length-dependent sensorimotor neuropathy or neurological disease similar to subacute combined degeneration (Su 1976; Botez et al. 1978; Fehling et al. 1974; Lossos et al. 1991; Parry 1990). Electrophysiological studies suggest an axonal neuropathy. Biopsy in a patient with Crohn's disease, who had not received metronidazole and had normal B12 indices but markedly reduced folate levels, showed a severe loss of large and small myelinated fibers (Lossos et al. 1991). The mechanism may relate to interference with thiamine or cobalamin metabolism, but may also result directly from the folate deficiency (Botez et al. 1978).

17.7 Alcoholic Neuropathy

17.7.1 Clinical Manifestations

Neuropathy is a well-established feature of the neurological disease that afflicts chronic alcoholics (Victor et al. 1990). Patients may complain of weakness and painful paresthesias or may be asymptomatic despite signs of neuropathy on examination. The distribution is distal and symmetrical and spares the cranial nerves unless very severe. Sensory loss to all modalities can be seen, and distal reflexes are diminished or lost. Evolution usually occurs over weeks to months, but can be more rapid, mimicking a Guillain–Barré picture, usually in association with a recent worsening in dietary intake (Tabaraud et al. 1990; Walsh and McLeod 1970). CSF examination is normal, and nerve conductions are most consistent with an axonal process. With cessation of alcohol and adequate nutritional intake, most patients improve, albeit slowly and sometimes incompletely, over several months.

17.7.2 Pathology

The central question regarding neuropathy in alcoholics is whether it is due to a toxic effect of alcohol, the nutritional deficits often seen in this population, or combinations of both. Patients with neuropathy not infrequently give a history of excessive alcohol intake, and thus it is important to recognize those histological changes that lie outside of the spectrum of pathology seen with alcoholic neuropathy. Excellent data regarding the pathology of pure thiamine deficiency is available (vide supra), and the discussion below is confined to patients with documented alcohol abuse. However, no histological features separate these groups.

Alcoholic neuropathy is an axonal degenerative process (Behse and Buchthal 1977; Tredici and Minazzi 1975; Said and Landrieu 1978; Said 1980; Walsh and McLeod 1970; Tabaraud et al. 1990). The severity of MF depletion and degree of active degeneration relate to the chronicity of the process and range from an extremely indolent axonal degenerative neuropathy with some regenerative activity to a very active picture of Wallerian degeneration. In mild lesions, large MFs are affected first, but with increasing severity, all sizes are involved. Rarely, diameter–frequency histograms have revealed selective loss of small myelinated fibers (Behse and Buchthal 1977; Said 1980).

Unmyelinated fibers also show evidence of degenerative and regenerative activity and occasionally can be

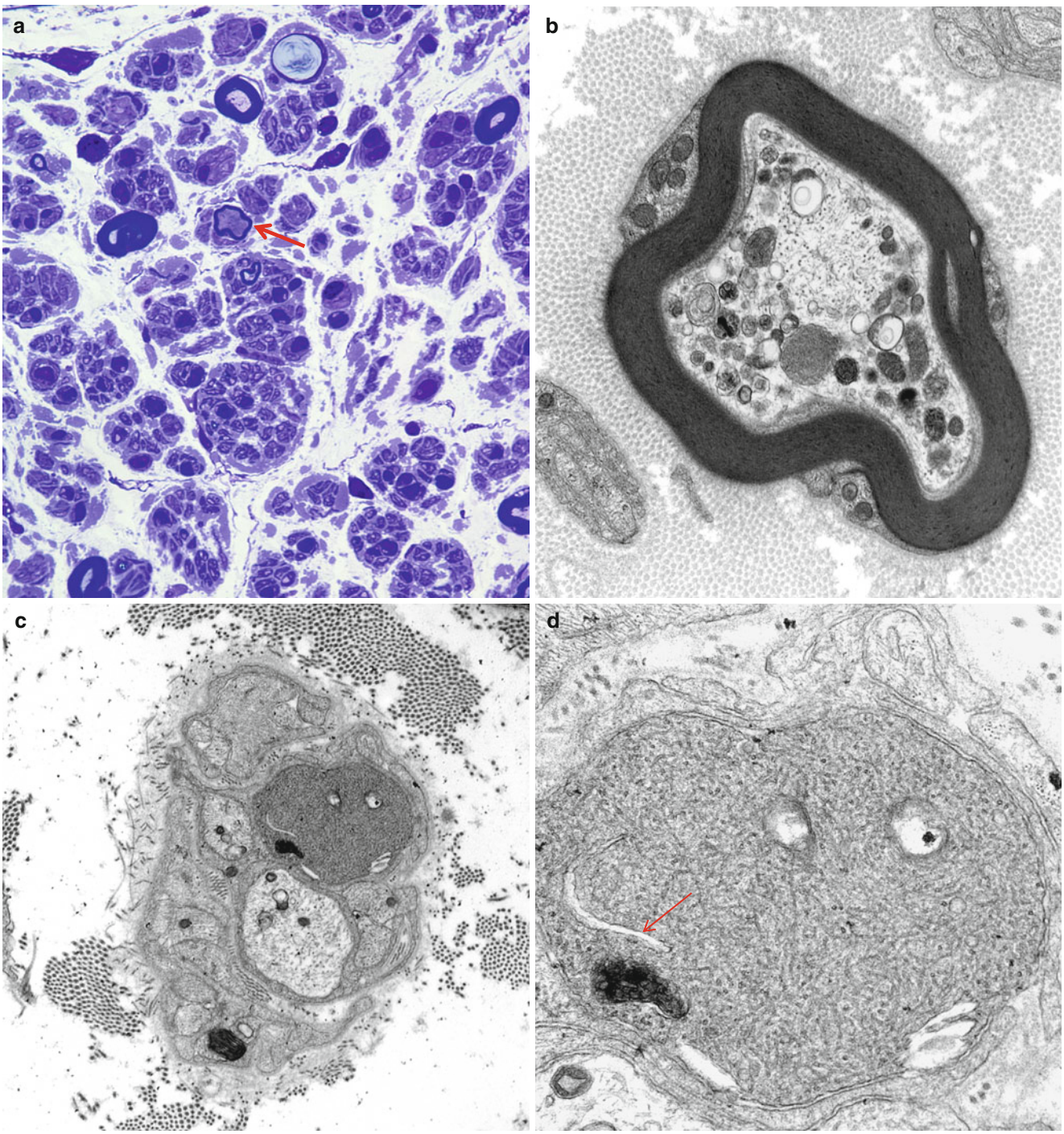

Fig. 17.10 Vitamin E deficiency: loss of large and small myelinated axons is accompanied by a single axon with dense axoplasm (*arrow*, **a**). Ultrastructure demonstrates a myelinated axon with increased subcellular organelles (**b**). Unmyelinated axons contain increased numbers of tubulovesicular elements (**c**, **d**) and often an axoplasmic cleft (*arrow*, **d**). (**a**) 1 μm thick plastic section; (**b**–**d**) electron micrographs (Magnification: **a**, 1,000×; **b**, 15,000×; **c**, 20,000×; **d**, 50,000×)

severely depleted (Said 1980). Axonal atrophy and nonspecific axoplasmic degenerative alterations are commonly seen. Segmental demyelination and remyelination and paranodal myelin alterations may be detected, but are not major features and seem to be secondary to axonal influences.

17.7.3 Pathogenesis

Alcoholic neuropathy is regarded as an axonal process. Evidence of axonal atrophy and a proximodistal gradient of severity suggest that it is a distal axonopathy (Said 1980; Takeuchi and Saito 2005; Tredici and Minazzi 1975).

The contention that alcoholic neuropathy is due to nutritional, especially thiamine, deficiency has rested on several observations. History of a nutritional deficiency is very frequently obtained in these patients, and biochemical evidence for a thiamine defect is seen in low activity of RBC transketolase, a thiamine-dependent enzyme (Blass and Gibson 1977; Victor et al. 1990). Nonalcohol-associated thiamine deficiency, rare in the modern era, produces a neuropathy clinically and pathologically similar to that seen in alcoholics (vide supra), although neither has specific features.

A concern that alcohol has a direct toxic effect on nerve arises from the frequent observations that patients with chronic alcohol abuse but no identifiable nutritional defect develop neuropathy (Behse and Buchthal 1977; Said 1980; Walsh and McLeod 1970; Claus et al. 1985). The neuropathy of postgastrectomy patients differs from that of chronic alcoholics (Behse and Buchthal 1977). Measurements of thiamine, directly and indirectly, do not always correspond to the presence either of neuropathy or of chronic alcoholism (Poupon et al. 1990; D'Amour et al. 1991). Investigations have continued to separate alcoholic neuropathy without thiamine deficiency (ALN) from nonalcoholic thiamine deficiency neuropathy (TDN), finding that they differ significantly in sensory (ALN) vs. motor (TDN) dominance, rapidity of progression (slow ALN, rapid TDN), small fiber-predominant axonal loss (ALN) vs. large fiber loss (TDN), greater pain in ALN, presence of subperineurial edema (TDN), and segmental de-/remyelination resulting from widening of consecutive nodes of Ranvier (ALN) (Koike et al. 2003; Koike and Sobue 2006). Alcoholic toxic neuropathy may be due to the accumulation of acetaldehyde, a neurotoxic metabolite, and in one Japanese study, individuals with inactive ALDH2 (aldehyde dehydrogenase which metabolizes acetaldehyde) developed more severe alcoholic neuropathy (Masaki et al. 2004).

Early animal experiments have usually failed to produce neuropathy on the basis of chronic alcohol ingestion (Windebank 1993; Hallett et al. 1987), although mild axonal alterations have been reported (Bosch et al. 1979; Claus et al. 1985). In vitro studies of axonal transport using a cultured rat DRG preparation, however, identified an inhibitory effect of long-term alcohol exposure (McLane 1987) as well as in vivo impairment of retrograde axonal transport (Malatova and Cizkova 2002). Neurofilament protein levels in cultured hippocampal neurons treated with ethanol are reduced (Saunders et al. 1997).

At the present time, the issue is unresolved, with workers feeling that nutritional deficiency, perhaps of multiple essential elements, is the central but perhaps not entire explanation for alcoholic neuropathy. Alcohol intake may cause a defect in thiamine utilization (Paladin and Russo Perez 1987), or both alcohol toxicity and thiamine deficiency may both contribute to the pathogenesis.

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Toxic Neuropathies

18

18.1 General Aspects of Toxic Neuropathy

Exogenous substances that can cause peripheral nerve injury include pharmaceutical agents, heavy metals, industrial chemicals, and nutritional and biological toxins. If a history of toxic exposure is not obtained or an alternative etiology is under consideration, nerve biopsy might be performed.

A discussion of each of the many substances that are toxic to the peripheral nerve is beyond the scope of this book (see recent review, Manji 2013; Diezi et al. 2013). As most human toxic neuropathies show nonspecific histological changes, we will concentrate on a small number of neuropathies that have unusual pathological features or have been extensively studied and serve as paradigms for toxic nerve injury. Tables 18.1 and 18.2 provide a broader listing.

Axonal degeneration underlies nearly all toxic neuropathies, and in many instances, a distal axonopathy has been identified. The histological features of axonal degeneration are discussed in detail in Chap. 4. Several principles are helpful in understanding the clinical and histological features of toxic neuropathies:

1. *Axons are affected earlier than Schwann cells*, probably because the complexity of maintaining an axon that is as long as a meter from the cell body is greater than that of maintaining a myelinated internode. Exceptions to this rule arise when the toxin specifically upsets some aspect of myelin or Schwann cell metabolism, such as diphtheria toxin or the amphiphilic drugs amiodarone and perhexiline.
2. *Large myelinated axons are affected first and most severely*. The larger the volume of axoplasm, the more challenging the metabolic task of maintaining the axon and the more vulnerable the axon is to exogenous toxins. Examples of possible exceptions to this rule are dimethylaminopropionitrile (Pestronk et al. 1980) and pyridoxine exposure (Berger et al. 1992). It is possible that this observation has been overemphasized because of difficulties in quantitating unmyelinated fiber injury pathologically and electrophysiologically.
3. There is usually a *correlation between the dose and duration* of intoxicant exposure and rate of onset and severity of neuropathy. However, this is not always evident, perhaps due to interindividual differences in drug metabolism. Some neuropathies seem to occur only after massive exposure to the toxin (lithium, amitriptyline), while others occur only after many years of drug use (anticonvulsants).

In keeping with these tenets, most toxic neuropathies are length dependent and affect predominantly large axons. This has certain clinical consequences. The longest peripheral nerve axons in the body are sensory fibers to the toes, explaining why distal paresthesias often herald a neuropathy well before the appearance of weakness. Thus, unless permitted to progress despite early symptoms, most toxic neuropathies are predominantly sensory. Furthermore, alteration in large fiber sensation, i.e., vibration, is an early objective finding. Cranial nerves are almost never involved, but evidence of CNS dysfunction is sometimes present. The tempo of progression depends on the duration and dose of exposure, as well as interindividual variations in metabolism, and is usually not characteristic for a particular toxin. Nerve conductions confirm that a predominantly axonal process is occurring, and most ancillary tests, including CSF examination, are normal. There are, of course, exceptions to all these generalizations. Amphiphilic drugs (amiodarone, perhexiline) cause a demyelinating or mixed neuropathy, and thus motor involvement is early, and nerve conduction may be slowed. Secondary demyelination can result in prominent conduction slowing in severe hexacarbon neuropathy (vide infra). Gold, arsenic, and nitrofurantoin are particularly liable to cause an acute–subacute neuropathy.

Prognosis is usually good if the toxin is withdrawn, with axonal neuropathies recovering to a variable extent over months to years and demyelinating neuropathies more rapidly and completely.

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Table 18.1 Toxic neuropathies caused by pharmaceutical agents

Toxin	Used for	Clinical features	Pathological features and comments
Almitrine	COPD	Sensory, chronic	Axonal, large MFs > small MFs and UFs. Uncertain role of concomitant hypoxia (Bouche et al. 1989; Gherardi et al. 1987)
*Allopurinol	Gout Rx	Sensory > motor, chronic	One biopsy described: this shows axonal and demyelinating changes, but the latter may have caused by previous exposure to chloroquine (Azulay et al. 1993; Castot et al. 1991)
Amiodarone	(See text)		
*Amitriptyline	Antidepressant, suicide attempts	Acute severe sensorimotor neuropathy	Axonal. Significant neuropathy occurs mostly after acute massive intoxication (Boudouresques et al. 1977; LeWitt and Forno 1985)
Bortezomib	Chemotherapy	Acute severe mostly sensory small fiber predilection	Severe axon degeneration, endoneurial edema, and inflammation with increased endoneurial macrophages (Saifee et al. 2010; Cavaletti and Jakubowiak 2010)
*Chloramphenicol	Antibiotic	Sensory + optic	No human material (Joy et al. 1960)
Chloroquine	Antimalarial	Myopathy > neuropathy	Demyelinating and axonal, lamellar/crystalline inclusions (as in amiodarone) in all endoneurial cells except axons; may reflect increased intralysosomal pH followed by the accumulation of undegraded lysosomal phospholipids (Tegner et al. 1988; Herskovitz and Schaumburg 2005)
Cisplatin	Chemotherapy	Sensory > motor	Loss large MF, axonal degeneration, and swollen/vacuolated mitochondrial DNA damage and dysfunction. Experimental Rx results in DRG neuronopathy (Podratz et al. 2011; Zheng et al. 2011)
Colchicine	Gout, FMF, Behçet's syndrome	Myopathy > neuropathy Sensory > motor	Axonal, involves large MFs > small MFs with evidence of axonal degeneration, regeneration (Kuncl et al. 1987)
Dapsone	Leprosy, malaria, rheumatoid arthritis, pneumocystis pneumonia, dermatologic	Motor > sensory	Axonal degeneration (Navarro et al. 1989)
ddI/ddC	HIV Rx	Distal sensory > motor	Electrophysiology indicates an axonal process (Berger et al. 1993)
Disulfiram	(See text)		
Doxorubicin	Antineoplastic	Not described in humans	Sensory neuronopathy through spinal ganglion injury (animal data) (Cho 1977)
Ethambutol	Antituberculous	Sensory > motor	Axonal, sometimes with secondary segmental demyelination (Critchley 1987; Takeuchi et al. 1980)
*FK506	Immune suppressant	CIDP-like picture	Axonal depletion, demyelination, no inflammation (1 report only) (Wilson et al. 1994)
Gold	Rheumatoid arthritis	Subacute motor, sensorimotor neuropathy, myokymia	Axonal > demyelinating (Katrak et al. 1980)
*Hydralazine	Antihypertensive	Sensorimotor	No biopsy data. Possible role of hydralazine-induced pyridoxine deficiency (Raskin and Fishman 1965)
Isoniazid	Antituberculous	Sensory > motor	Axonal, involves MFs > UFs; periaxonal vacuolation, proliferation of intra-axonal agranular reticulum and vacuoles, progressing to axonal degeneration. Inactivates cofactor pyridoxal phosphate (Ochoa 1970; Schroder 2000)
*Lithium	Mood stabilizer	Acute–subacute sensorimotor	Axonal. Occurs most convincingly after acute intoxication (Vanhooren et al. 1990)
L-tryptophan	(See text)		
Metronidazole	Antibiotic, antiprotozoal, radiation sensitizers	Sensory > motor, large fiber	Axonal degeneration and demyelinating, relative sparing of UFs (Takeuchi et al. 1988)
Misonidazole	(See text)		
Nitrofurantoin	Antibiotic (urinary)	Subacute sensorimotor, potentially severe. Higher risk in renal failure	Axonal, involving large MFs most affected (Yiannikas et al. 1981)

(continued)

Toxin	Used for	Clinical features	Pathological features and comments
Nitrous oxide	Mild anesthetic, abuse	Sensory > motor polyneuropathy and myelopathy (SCD-like)	Axonal. Probably due to alteration of B12-dependent metabolic pathways (Frasca et al. 1986; Sahenk et al. 1978; Vishnubhakat and Beresford 1991)
Perhexiline	Antianginal	Sensorimotor	Demyelinating > axonal. Slow hydroxylators develop excessive blood levels of perhexiline. Typical inclusions of amphiphilic substance toxicity (see Sect. 18.2) (Fardeau et al. 1979; Said 1978)
[A]Phenytoin	Anticonvulsant	Sensorimotor, usually subclinical	Axonal, large MFs most affected, ?secondary demyelination (Ramirez et al. 1986)
Cis-platinum	Antineoplastic	Large > small fiber sensory	Axonal, involving large > small fibers, with (probably secondary) segmental myelin changes (Gastaut and Pellissier 1984; Roelofs et al. 1984)
Podophyllin	Herbal remedy, topical agent	Severe acute–subacute sensorimotor	Axonal, MF and UF involved. Likely mechanism relates to effect on microtubules, similar to vincristine (Ng et al. 1991; O'Mahoney et al. 1990)
*Procarbazine	Antineoplastic	Mild–moderate sensorimotor	Not available (Samuels et al. 1967)
Pyridoxine	Vitamin abuse	Sensory, large > small fiber	Axon loss/atrophy, DRG neuron loss, ? blockade of fast axonal transport in the proximal axon (Schaumburg et al. 1983)
Sodium cyanate	Sickle cell anemia	Sensorimotor	Axonal degeneration with secondary demyelination (Ohnishi et al. 1975; Peterson et al. 1974)
Suramin	Antineoplastic and antiparasitic	Severe sensorimotor	Mixed axonal and demyelinating; known inhibitor of lysosomal enzymes which accumulates in DRG (LaRocca et al. 1990; Chaudhry et al. 1996). The mechanism is unestablished, but suramin is a known inhibitor of lysosomal enzymes and accumulates in DRG neurons in experimental animals; in addition, suramin blocks the receptor function of IGF-I and NGF
Tacrolimus	Immune suppression	Sensorimotor	Axonal loss and demyelination, CIDP mimic
Taxanes (paclitaxel, docetaxel)	Antineoplastic	Distal sensory > motor axonopathy	MF and UF loss, axonal atrophy, membranotubular aggregates but without microtubule aggregates (occur in cultured cells, localized nerve injection) and with inconstant evidence of regeneration. Calpain inhibitors may inhibit axonal degeneration in experimental animals. NGF, IGF-I, ORG 2766, and LIF are reported to protect against the neuropathy (Lipton et al. 1989; Sahenk et al. 1994; Herskovitz and Schaumburg 2005)
Thalidomide	Behçet's disease, graft vs. host Dz, skin disease, leprosy, HIV, plexiform neurofibromas, Crohn's disease, and as an antiangiogenic antitumor, immune-modulating agent	Sensory > motor	Axonal degeneration, large MFs most affected (Chapon et al. 1985; Fullerton and O'Sullivan 1968; Chaudhry et al. 2002; Fleming et al. 2005; Giannini et al. 2003). Painful paresthesias of the hands and feet. Axonal degeneration and loss of large MFs, regenerating axons, increased numbers of regenerating axons, and loss of dorsal root ganglia neurons, a pattern consistent with dying-back neuropathy and/or with sensory ganglionitis
Thiophenicol	Antibiotic	Subacute, sensory	? demyelinating (Shinohara et al. 1977)
Vincristine	(See text)		

This table provides a brief summary of toxic substances that have been identified as causing a peripheral neuropathy. The pathological features are categorized as axonal, demyelinating, or both. "Axonal" usually indicates distal axonopathy, the most common mechanism through which toxins cause peripheral neuropathy

*Literature supportive but not entirely convincing of existence of neuropathy with this drug

[A]Although in the past only chronic phenytoin administration has associated with peripheral neuropathy (Lovelace and Horowitz 1968), more recent data suggests that any of the anticonvulsants can cause an indistinguishable minimal neuropathy with similar frequency (Bono et al. 1993; Krause and Berlit 1990; Swift et al. 1981)

18.2 Amiodarone

18.2.1 Clinical Manifestations

Initially used in the treatment of angina nearly three decades ago, amiodarone is presently employed with increasing frequency in the treatment of cardiac arrhythmia. Although not mentioned in one large review (Raeder et al. 1985), some studies indicate that 6–10 % of amiodarone-treated patients develop a peripheral neuropathy (Charness et al. 1984; Palakurthy et al. 1987). Usually, the neuropathy occurs in a patient who has been on the drug for 6 months or more, at

Table 18.2 Neuropathy caused by metals and biological/occupational/household toxins

Toxin	Source	Clinical features	Pathological features
Acrylamide	Grouting/strengthening agent in mining and paper industries	Chronic sensorimotor	Human data suggests distal axonopathy with giant axonal filamentous changes, although not to same extent as hexacarbon toxicity. May reflect interference with slow and fast axonal transport; inhibiting fast motor kinesin interaction with microtubules. Experimental data is similar (Davenport et al. 1976; Fullerton 1969; Gold et al. 1985; Schaumburg and Berger 1993; Berger and Schaumburg 2005)
Arsenic	Antimicrobial, poisoning	Acute sensorimotor	Axonal, with large MFs most involved, minimal segmental demyelination (Donofrio et al. 1987)
Buckthorn	Shrub endemic to SW North America	Acute severe sensorimotor	Demyelinating, ? toxic schwannopathy, but experimental literature suggests a primary axonopathy (Heath et al. 1982; Weller et al. 1980)
Carbon disulfide	Rayon manufacture	Chronic sensorimotor	Experimental work indicates distal axonopathy with giant axonal filamentous accumulations reflecting cross-linking of neurofilament proteins, metal chelation, enzyme inhibition, and free radicals (Gottfried et al. 1985; Seppalainen and Haltia 1980; Berger and Schaumburg 2005)
Cyanide	Cassava, sorghum, laetrile	Chronic distal sensorimotor	Axonal and demyelinating. Some cases of African tropical ataxic neuropathy probably due to cyanide intoxication from cassava intake (Kalyanaraman et al. 1983; Montgomery 1979; Williams and Osuntokun 1969)
Dimethylaminopropionitrile	Grouting agent, polyurethane foam manufacturing	Sensorimotor, small fiber	Axonal, possibly involving UFs most prominently with particulate organelle hyperplasia and neurofilament disorganization (Pestronk et al. 1980)
Diphtheria toxin	(See Sect. 11.5)		
Ethylene oxide	Cool sterilization, industrial chemical manufacture	Sensorimotor neuropathy; encephalopathy	Axonal. Vesicular myelin change (Gross et al. 1990; Schaumburg and Berger 1993; Schroder et al. 1985)
Lead	(See text)		
Mercury	Industrial pollutant, accidental exposure	Sensorimotor neuropathy, encephalopathy	Axonal, probably a neuronopathy (Windebank 1993)
Methyl methacrylate	Resin (dental, orthopedic)	Sensorimotor, chronic	Axonal, involving large MFs > small MFs (Donaghy et al. 1991)
Methyl n-butyl ketone	(See text – Sect. 18.7)		
N-Hexane	(See text – Sect. 18.7)		
Organophosphate	Insecticides, plastics, petroleum additives	Acute/subacute sensorimotor	Axonal. TOCP model studied extensively in animals. Mechanism may involve phosphorylation of neuropathy target esterase (NTE) and metabolism into a neurotoxic substance. Impairment of retrograde transport (Kaplan et al. 1993; Schaumburg and Berger 1993)
Thallium	Rat poison, depilating agent	Acute/subacute sensorimotor, often painful	Axonal with prominent mitochondrial abnormalities (Cavanagh et al. 1974); inhibition of flavin-dependent enzymes (Manzo 2000)
Vacor	Rat poison	Hyperacute sensorimotor neuropathy	No human biopsy material. Animals show distal axonopathy with active axonal degeneration. ? Impairment of fast axonal transport (LeWitt 1980; Watson and Griffin 1987)

This table provides a brief summary of toxic substances that have been identified as causing a peripheral neuropathy. The pathological features are categorized as axonal, demyelinating, or both. "Axonal" usually indicates distal axonopathy, the most common mechanism through which toxins cause peripheral neuropathy

doses of 200–600 mg/day. However, onset after 1 month with high doses (1,400 mg/day) and many months with very low doses (50 mg/day for 18 months) have been described (Pellissier et al. 1984). Progression is typically over weeks to months, with symmetrical distal paresthesias, followed by diminished motor and sensory function. Reflexes are diminished or absent. Myopathy often accompanies the neuropathy. CSF protein can be elevated, occasionally markedly (Pellissier et al. 1984). Conduction studies may show elements of both axonal loss and demyelination, but either can predominate, and denervation is commonly seen on EMG. If the clinician is not aware of the patient's use of this medication, an incorrect diagnosis of Guillain–Barre syndrome or CIDP is possible. Most workers report improvement within

1–6 months of stopping the drug. This has also been our experience with three cases.

It is noteworthy that an examination of the incidence of polyneuropathy in patients treated with a variety of antiarrhythmic drugs suggested that this occurred no more frequently with amiodarone than with other antiarrhythmics and that clinically significant neuropathy was rare (Collaborative 1994). However, the average dose (200 mg) of amiodarone used was small.

18.2.2 Pathology

The characteristic peripheral nerve finding in amiodarone neuropathy is an accumulation of lysosomal inclusions. These are found in great numbers in endothelial cells, vascular smooth muscle cells, pericytes, perineurial cells, fibroblasts, and Schwann cells, especially nonmyelinating Schwann cells (Figs. 18.1, 18.2, 18.3, 18.4, and 18.5). We have rarely observed them within axons (Fig. 18.5). These inclusions are well visualized in semithin toluidine blue-stained sections (Figs. 18.1 and 18.2) but are not retained in paraffin-embedded material.

The pathological process is usually active axonal degeneration (Fig. 18.1a and 18.3) with variable amounts of demyelination (Figs. 18.1b and 18.3a) (Pellissier et al. 1984). Axonal loss ranges from mild to subtotal and can affect large myelinated fibers preferentially, with relative sparing of unmyelinated fibers (Meier et al. 1979; Jacobs and Costa-Jussa 1985; Pellissier et al. 1984). Regenerating clusters may be readily seen (Fig. 18.3a). It is the occasionally prominently demyelinating nature of this neuropathy that sets it apart from most other toxic exposures. In our experience, the numbers of thinly myelinated fibers, debris-filled macrophages, large naked axons, and even onion-bulb formations have varied considerably between biopsies. Some authors have reported very prominent segmental demyelination with little or no axon loss (Jacobs and Costa-Jussa 1985). In one instance, we observed focal perineurial disruption with axonal degeneration in the adjacent endoneurium (Fig. 18.2).

Electron microscopy reveals that the cytoplasmic inclusions are 0.5–1.5 μm in size and consist of membrane-bound osmiophilic material (Figs. 18.3b and 18.4a, b). The inclusions may take a lamellar or granuloreticular/crystalline appearance. The former may demonstrate a circular or linear, tightly or loosely packed lamellar structure (Fig. 18.4b).

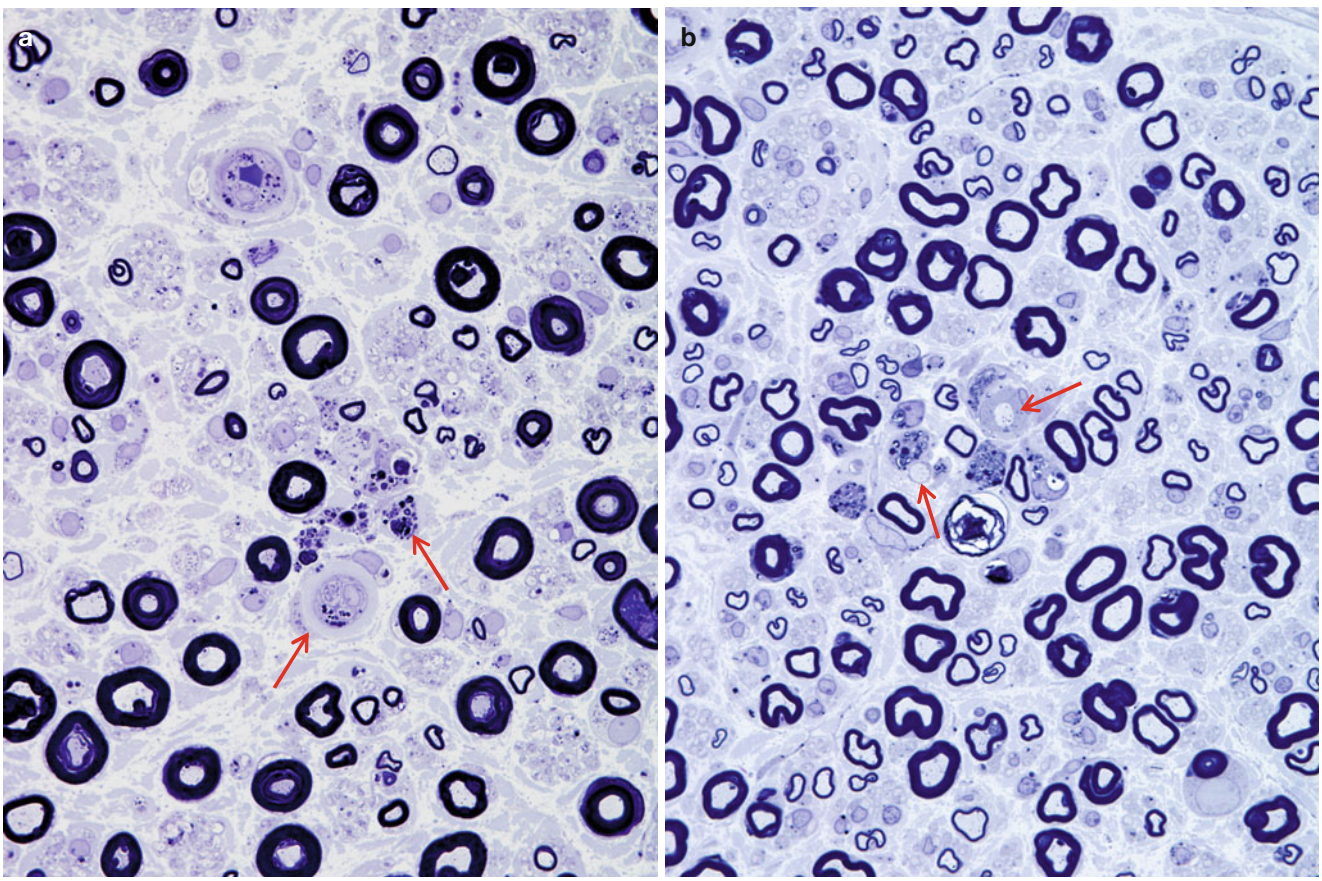

Fig. 18.1 Amiodarone: note loss of myelinated fibers and small lipid inclusions in vessels and endoneurial cells (*arrows*, **a**). Several denuded axons are shown (*arrows*, **b**) (1 μm thick plastic sections; magnification: **a**, **b** 1,000×)

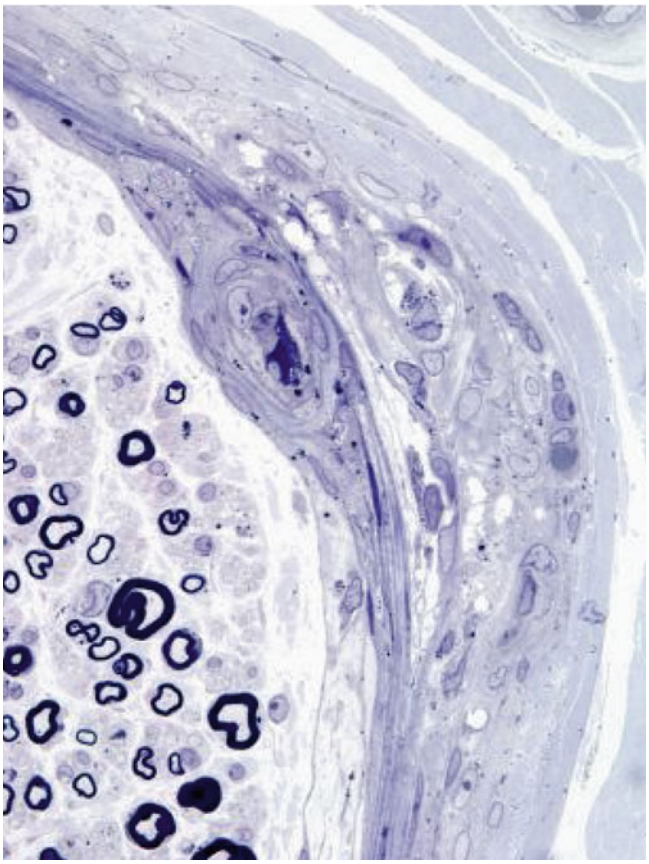

Fig. 18.2 Amiodarone: focal damage to fascicle including perineurium is seen. Note scattered osmiophilic bodies in perineurium and blood vessels (1 μm thick plastic section; magnification 1,000×)

In our experience, there is no association between cell and inclusion types, although some authors have claimed otherwise (Pellissier et al. 1984). Moreover, it is not difficult to find inclusions which show features of both ultrastructural patterns (Fig. 18.4b), suggesting that the two inclusion types are related, and the different morphology may reflect different stages of evolution.

Jacobs and Costa-Jussa (1985) observed increased amounts of a finely granular Schwann cell cytoplasm with diminished numbers of organelles, even in the absence of inclusions. It was reported that similar alterations were seen in endothelial and perineurial cells and postulated that these changes may precede the formation of inclusions. The axoplasm itself often shows nonspecific changes (filamentous hyperplasia, crystalloid osmiophilic filamentous aggregates, and glycogen deposits), although typical inclusions are uncommon (Fig. 18.5).

18.2.3 Pathogenesis

Amiodarone belongs to the class of amphiphilic substances, which include chloroquine (Figs. 18.6, 18.7a, b, and 18.8a, b) and perhexiline, and are well known to cause systemic formation of myelinoid bodies (lamellar, granuloreticular or crystalline) having all the characteristics discussed above for amiodarone (Hruban et al. 1972; Hruban 1984; Lullmann et al. 1978). The inclusions appear to reflect lysosomal accumulation of membranes whose degradation is impaired by the toxin (Hruban et al. 1972; Hruban 1984). Amiodarone accumulates within affected tissue (Meier et al. 1979; Fraser et al. 1985) and likely forms a drug–phospholipid complex which resists degradation; amiodarone appears to block the action of phospholipase A (Hruban 1984; Kannan et al. 1991).

The mechanism by which axons or Schwann cells are damaged remains unknown. Jacobs and Costa-Jussa (1985) postulated a toxic schwannopathy based on case material that showed dominant demyelination with early Schwann cell cytoplasmic changes. Most authors, however, have shown a significant axonal loss which remains unexplained.

18.2.4 Differential Diagnosis

The inclusions of amiodarone neuropathy, whether lamellated or paracrystalline, are nonspecific, simply reflecting a disturbance of lysosomal function. Several storage diseases should be considered.

Any of the causes of systemic phospholipidosis may demonstrate similar inclusions (Hruban 1984), but in the setting of neuropathy, perhexiline and chloroquine toxicity should be considered. If the history of exposure to amiodarone is missed, the clinician will usually be considering the diagnosis of inflammatory demyelinating neuropathy, and this issue is easily resolved once the characteristic inclusions are observed. Neuropathy in patients taking amiodarone has been described in the absence of inclusions (Dubois et al. 1984; Vital and Vallat 1987a, p185). It remains unclear whether or not these patients suffered from another cause of peripheral neuropathy (e.g., hypothyroidism due to amiodarone or an unrelated cause) or whether it is possible to have significant neuropathy without characteristic inclusions. In our experience, the inclusions are so abundant that we would be reluctant to consider the possibility of an amiodarone neuropathy in their absence.

Fabry and Niemann-Pick diseases also demonstrate intracellular storage in a variety of endoneurial cells and under light microscopy may be superficially similar to amiodarone neuropathy (see Chap. 20). One helpful distinguishing point is the rarity of Fabry inclusions in Schwann cells. The inclusions of Fabry disease demonstrate the "Maltese cross" pattern on frozen sections under polarized light and are larger than those of amiodarone even on light microscopy. Paracrystalline inclusions are not a significant feature of Fabry or Niemann-Pick disease but are abundant in amiodarone neuropathy. These are

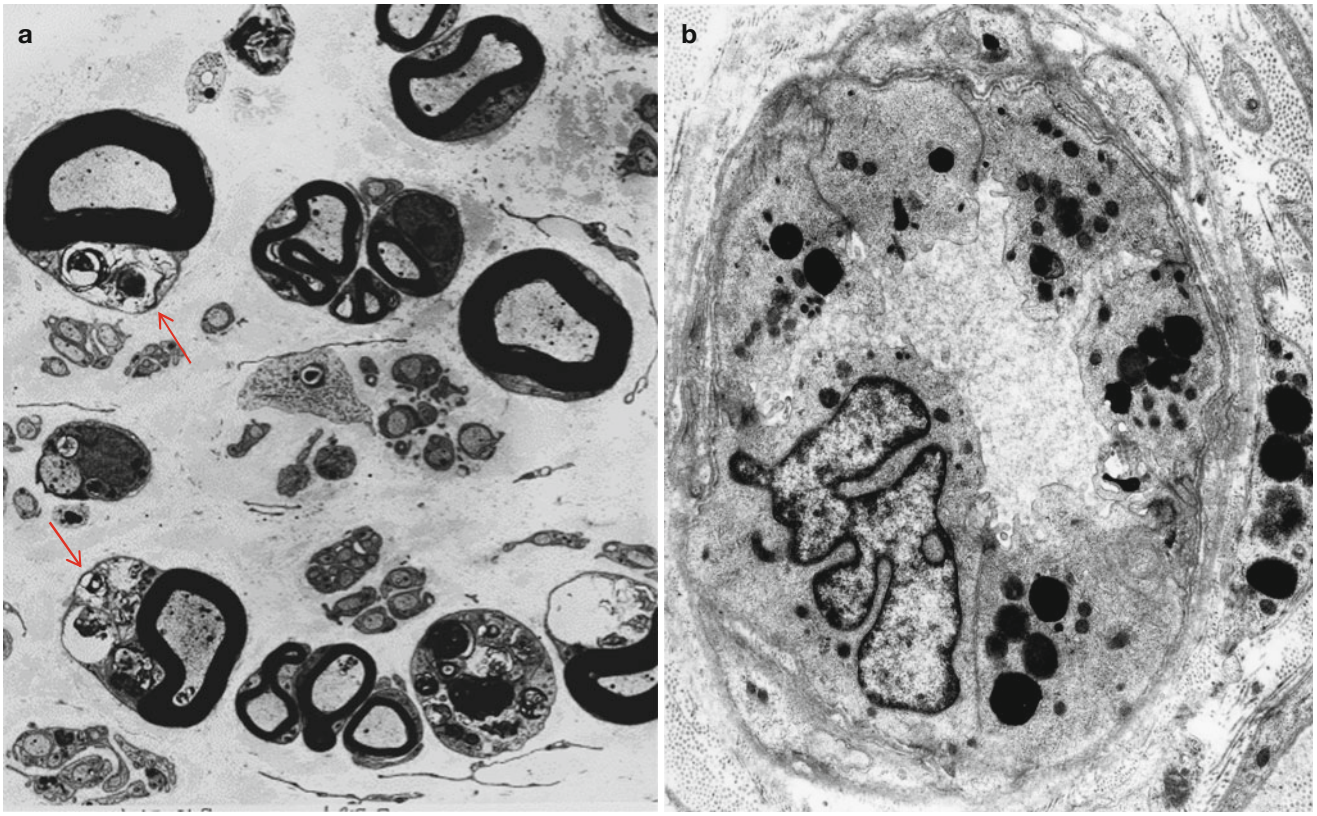

Fig. 18.3 Amiodarone: numerous cytoplasmic osmiophilic inclusions are seen in Schwann cells (*arrows*, **a**) of degenerating and demyelinated axons. (**b**) Inclusions are prominent in endothelium and pericytes (**a**, 3,000×; **b**, 14,910×)

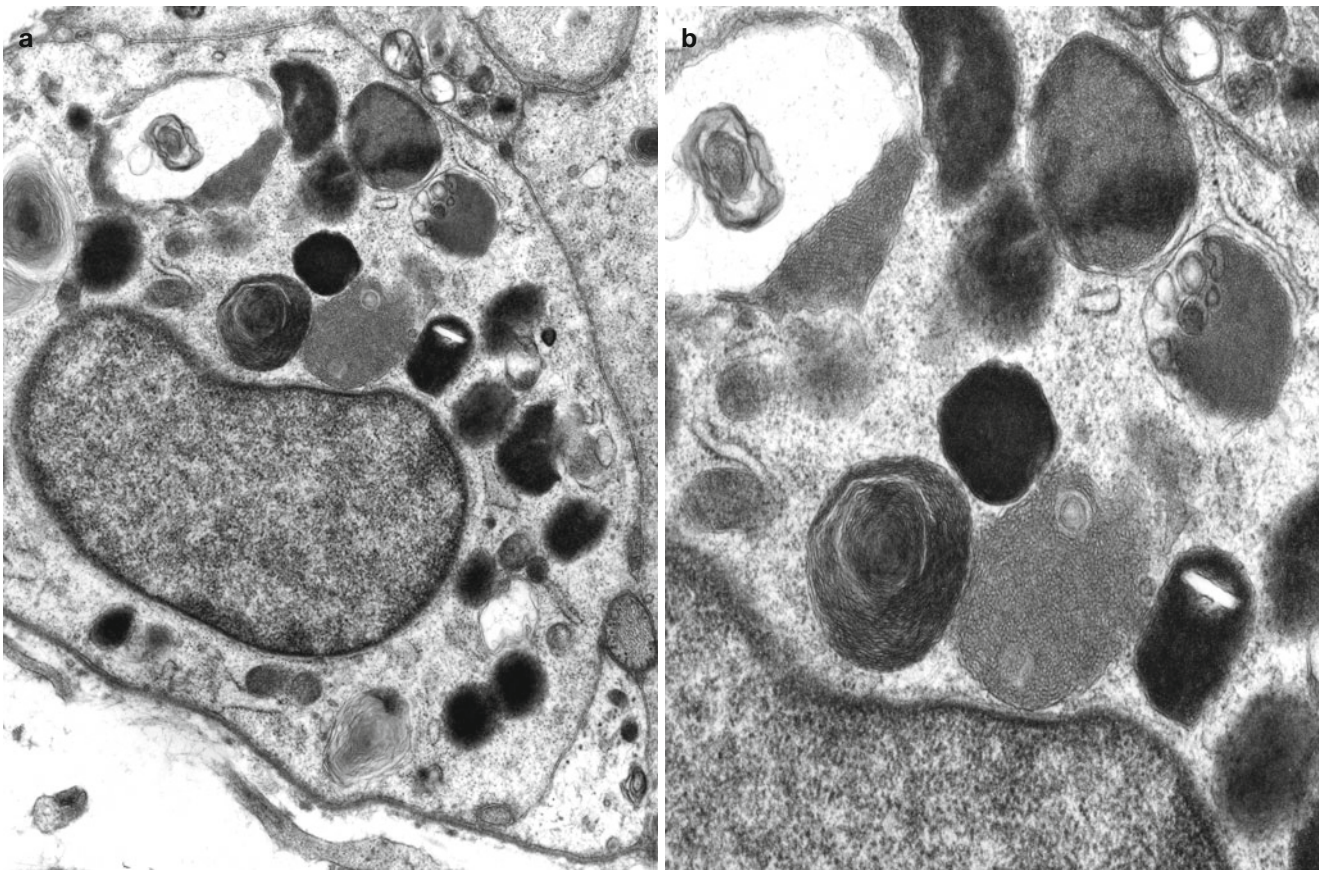

Fig. 18.4 Amiodarone: lysosomal inclusions of varying morphologies are shown in Schwann cells of denervated band (**a**) and at higher magnification (**b**) (**a**, 14,560×; **b**, 25,000×)

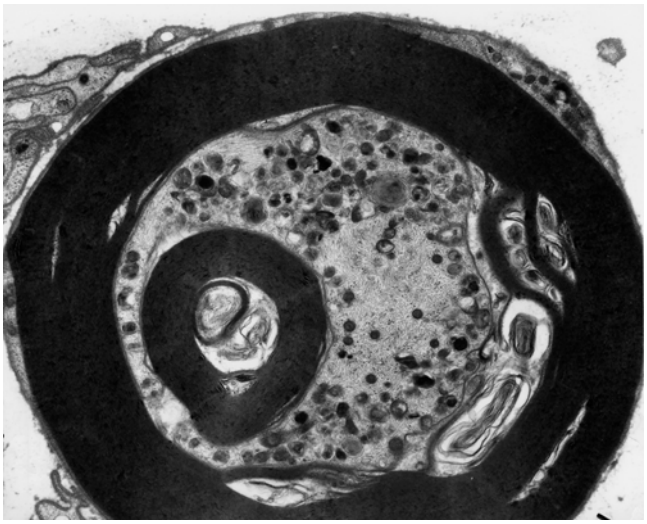

Fig. 18.5 Amiodarone: note accumulation of lamellar and dense osmiophilic vesicles in axon, with appearance of axonal atrophy and myelin irregularities (12,070×)

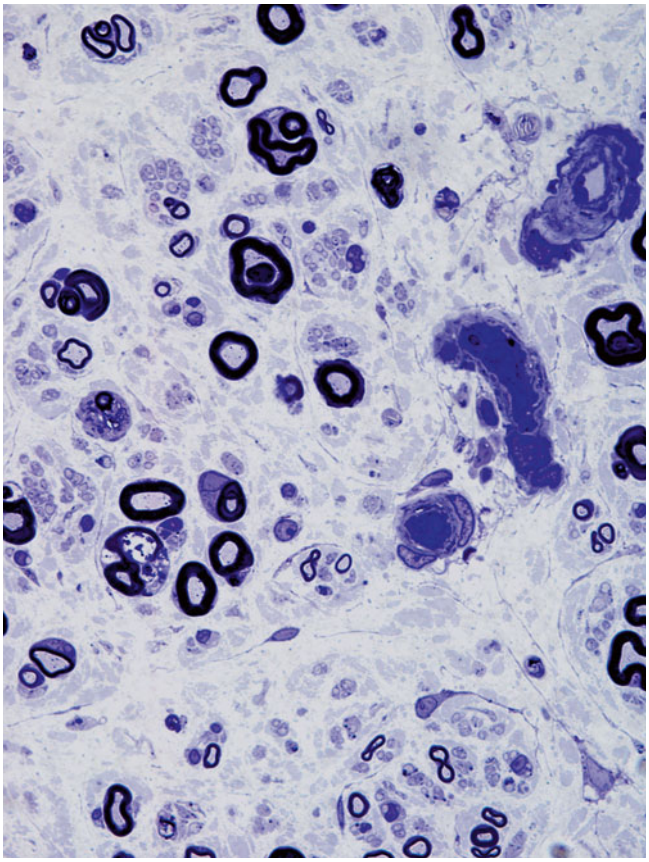

Fig. 18.6 Chloroquine neuropathy: characterized by significant axon loss and active axonal degeneration (1 μm thick plastic section, 1,000×)

also seen in small numbers in a variety of neuropathies. Zebra bodies are not seen in amiodarone neuropathy. The osmiophilic storage material of Fabry disease usually contains regions with regular 4–6 nm periodicity. In Niemann-Pick disease, peripheral nerve inclusions may take several forms, most often amorphous and lipopigment-like. Other storage diseases including metachromatic leukodystrophy, globoid cell leukodystrophy, and adrenoleukodystrophy have very characteristic inclusion ultrastructure and will not pose a diagnostic problem (Chap. 20).

Case 18.1

Four weeks prior to presentation, a 50-year-old male noted numbness and tingling in his feet followed by progressive weakness in the lower extremities to the point where he became bed-bound. Mild weakness in the arms was also present. Medical history was notable for several myocardial infarctions, congestive failure, and cardiac arrhythmia. Medications included amiodarone, which had been started at 600 mg/day 3 months previously. Examination disclosed an alert man with normal cranial nerve function. There was diffuse grade 4+/5 (MRC) weakness in the upper extremities and severe lower extremity weakness, worse in the right leg, involving both proximal and distal muscles. All reflexes were absent, and mild distal sensory loss was present to all modalities in the lower extremities.

Investigations on admission to hospital included CSF examination which demonstrated a normal CSF protein and cell count. Other serological and hematologic tests were unremarkable. Nerve conduction studies showed a moderate to severe mixed axonal and demyelinating polyneuropathy. Because of the rapid progression and asymmetric features, the differential diagnostic possibilities were felt to include Guillain–Barre syndrome and vasculitic neuropathy. Sural nerve biopsy was performed.

Following a diagnosis of amiodarone neuromyopathy, the dosage was reduced to 200 mg/day, with subsequent complete resolution of the patient's symptoms over a period of 3–6 months.

18.3 Disulfiram Neuropathy

18.3.1 Clinical Manifestations

Disulfiram (Antabuse) is used in the treatment of alcoholism, at a dose of 250–500 mg daily. Peripheral neuropathy occurs in a small fraction of patients and is probably dose dependent. Onset is usually within months after initiating therapy but can occur as soon as 10 days with high-dose treatment (van Rossum et al. 1984; Watson et al. 1980; Nukada and Pollock 1981). Distal symmetrical sensory and motor deficits are present. Some patients have also demonstrated evidence of central nervous system dysfunction (Kane 1970). Electrophysiological tests reveal an axonal neuropathy.

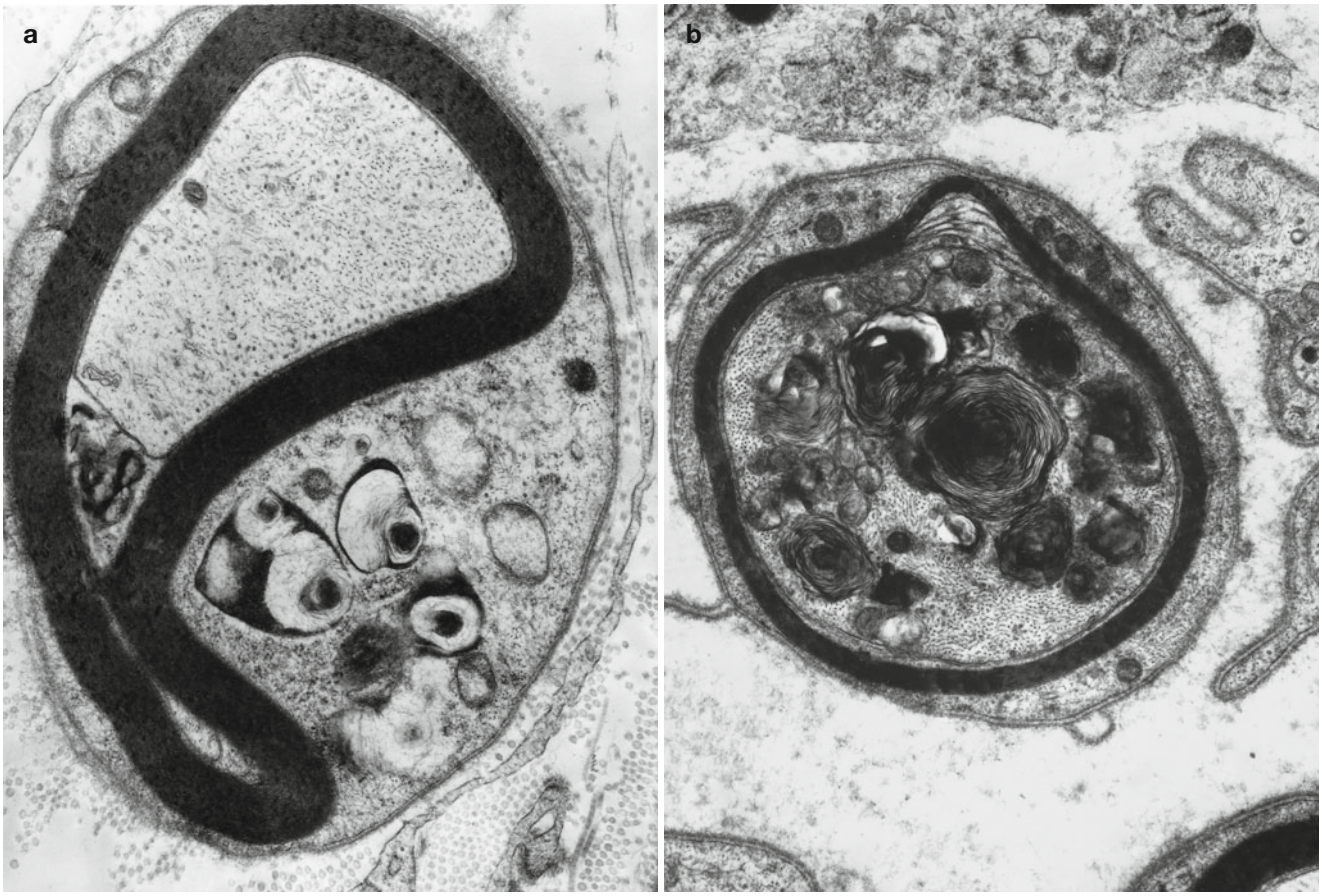

Fig. 18.7 Chloroquine neuropathy: compact and loose osmiophilic lamellar inclusions in Schwann cell cytoplasm (**a**) and within myelinated axon (**b**) (Magnification: **a**, 16,800×; **b**, 22,880×)

Withdrawal of the drug usually results in substantial improvement over several months, although mild residual symptoms and electrophysiological abnormalities tend to persist.

18.3.2 Pathology

We have examined nerve biopsies from five patients with disulfiram neuropathy, and a substantial quantity of pathological material is available in the literature (Bilbao et al. 1984; Watson et al. 1980; van Rossum et al. 1984; Mokri et al. 1981; Moddel et al. 1978; Ansbacher et al. 1982; Nukada and Pollock 1981; Bergouignan et al. 1988; Bouldin et al. 1980). Active axonal degeneration is usually visible, with little or no segmental demyelination (Fig. 18.9a–d). Larger myelinated fibers may be preferentially involved, and unmyelinated fibers tend to be spared. In cases biopsied after cessation of the drug, regenerating clusters have been prominent (Moddel et al. 1978). One reported case has demonstrated a predominance of segmental demyelination which seemed to be independent of axonal disease (Nukada and Pollock 1981), and a rare onion-bulb formation was seen. We have never observed any changes suggesting a primary demyelinating component to this toxic neuropathy.

Ultrastructural examination usually reveals a nonspecific pattern of active axonal degeneration with relative or absolute sparing of unmyelinated fibers. A minority of the reported cases (1 of 5 in our material) have demonstrated axoplasmic filamentous accumulations (see Chap. 5), producing a giant axonal change (Fig. 18.10a, b), which may be accompanied by a variety of accumulated organelles (Fig. 18.10c) (Bilbao et al. 1984; Bergouignan et al. 1988; Ansbacher et al. 1982).

18.3.3 Pathogenesis

The clinical picture of disulfiram neuropathy suggests a distal axonopathy. A significant minority of the reported cases have demonstrated axonal swelling and filamentous hyperplasia of the type seen and extensively investigated in hexacarbon experimental and human neuropathies (vide infra). Disulfiram is catabolized to carbon disulfide (Prickett and Johnson 1953), and indeed, experimental CS_2 neuropathy

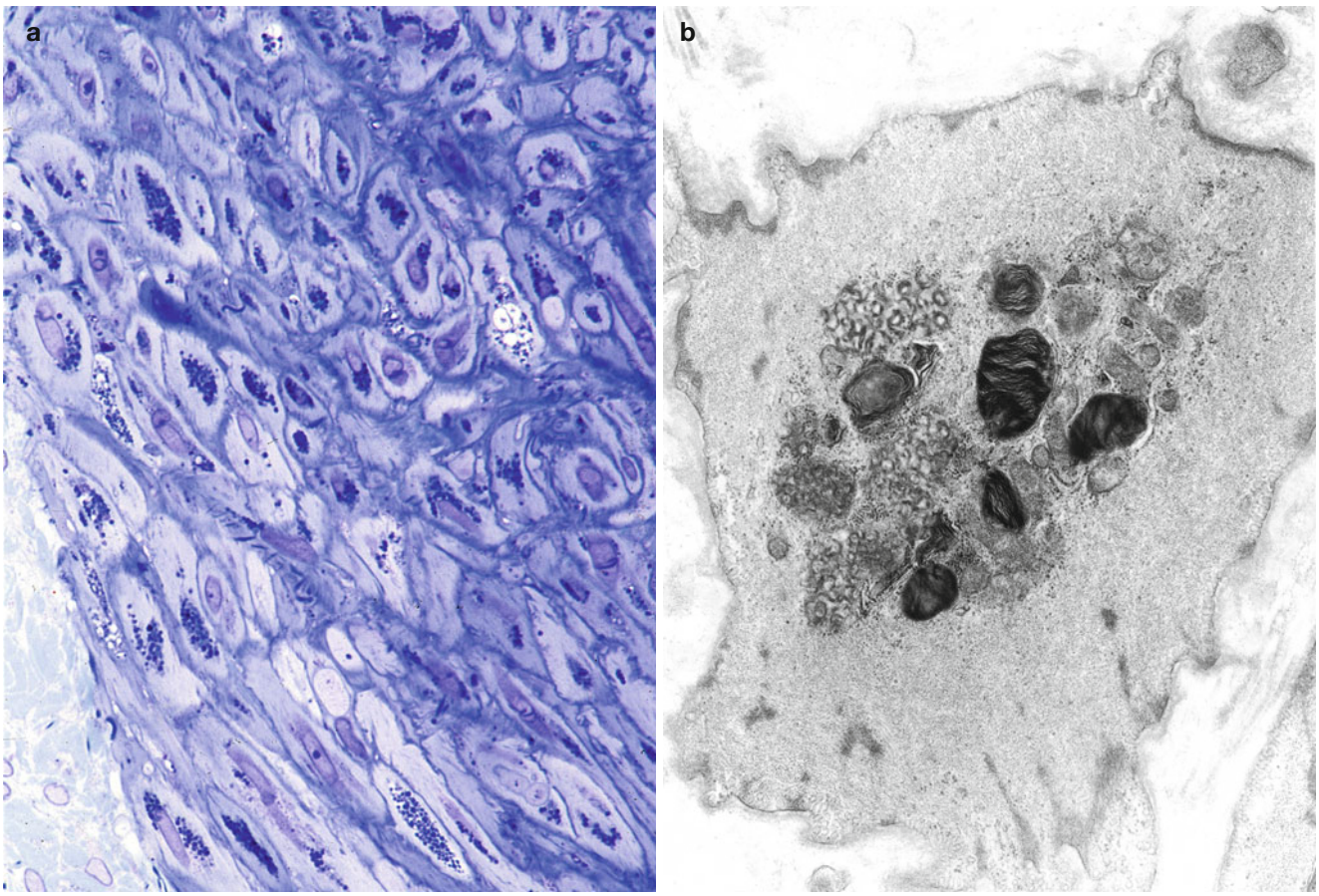

Fig. 18.8 Chloroquine epineurial vessel shows accumulation of osmiophilic inclusions in smooth muscle cells (**a**, 1 μm thick plastic section, 400×; **b**, 15,000×)

also demonstrates focal axonal swelling and filamentous hyperplasia (Seppalainen and Haltia 1980; Pappolla et al. 1987; Gottfried et al. 1985). As discussed previously, alterations in axonal transport mechanisms have been implicated in this group of toxic neuropathies. Evidence exists for an increased rate of neurofilament transport in carbon disulfide neurotoxicity (Pappolla et al. 1987), similar to that observed by some in the hexacarbon neuropathies (Monaco et al. 1989). One hypothesis is that carbon disulfide impairs energy metabolism (Seppalainen and Haltia 1980), and another possibility is that CS_2 disrupts microtubule and neurofilament interactions (Pappolla et al. 1987). The variable finding of filamentous axonopathy in nerve biopsy specimens may relate to the pace of the intoxication, as seems to be the case in experimental acrylamide toxicity (Gold et al. 1985).

18.4 Chemotherapy-Induced Peripheral Neuropathies (CIPN)

CIPNs represent the most rapidly developing class of toxins whose side effects limited in dose and duration interferes with their clinical use (Diezi et al. 2013; Cavaletti et al. 2013; Grisold et al. 2012). Most result in the development of a subacute or chronic, length-dependent distal symmetrical, mostly sensory neuropathy. The number of drugs is extensive ranging from well-known microtubular agents (vinca alkaloids, taxanes such as paclitaxel and docetaxel, epothilones) to ion channels, including voltage-gated and the transient receptor potential (TRP) family of ion channels (paclitaxel, oxaliplatin, cisplatin), proteasome inhibitors (bortezomib), DRG cytotoxicity, and mitotoxicity (thalidomide, lenalidomide, bortezomib, nucleoside analog reverse-transcriptase inhibitors). Newly developed targeted pathway chemotherapy may produce demyelination (etanercept, infliximab) or interfere with normal VEGF function by targeting VEGF receptors (sorafenib, sunitinib) or as a taxane (paclitaxel), neutralizing antibody (bevacizumab). With a few exceptions, the peripheral nerve pathology is not unique in each neuropathy and most are summarized in Table 18.1.

18.5 Misonidazole Neuropathy

Misonidazole is used as a cell sensitizer in radiotherapy of solid tumors; typical doses range from 1 to 2 g/m^2, 1–3 times a week. Peripheral neuropathy, seen in about a third of patients, is dose (more accurately: serum level) dependent, appears

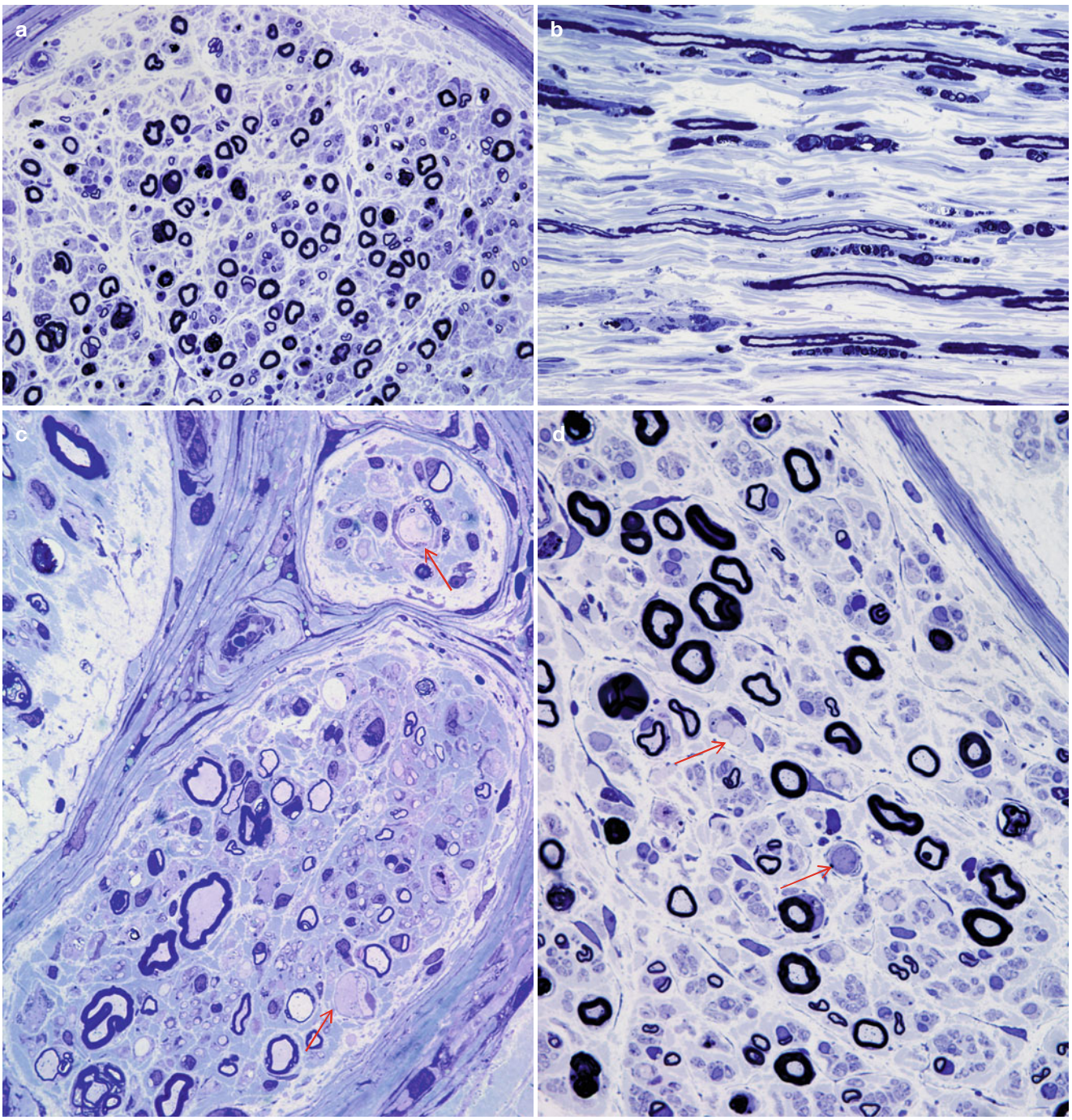

Fig. 18.9 Disulfiram: active axonal degeneration is seen against a backdrop of moderate axonal depletion (**a**, **b**). Several swollen axons are shown (*arrows*, **c**, **d**) (1 μm thick plastic sections; magnification: **a**, **b** 400×; **c**, **d** 1,000×)

within weeks of initiation of the medication, and may develop after completion of the drug regimen (Urtasun et al. 1978; Melgaard et al. 1982, 1988). Sensory manifestations, including paraesthesias and multimodality sensory loss in a stocking-glove distribution, predominate. Electrophysiological studies reveal a sensory more than motor axonal neuropathy. Symptoms improve over weeks to months after the medication is stopped, but some residua may persist (Melgaard et al. 1982).

Sural nerve histological studies (Urtasun et al. 1978; Melgaard et al. 1982) have shown active axonal degeneration, more severe in large myelinated fibers, and frequent regenerating clusters. One biopsy demonstrated a degree of neurofilament accumulation with axonal swelling (Urtasun et al. 1978). We have observed very active axonal degeneration with no specific light or electron microscopic features (Fig. 18.11).

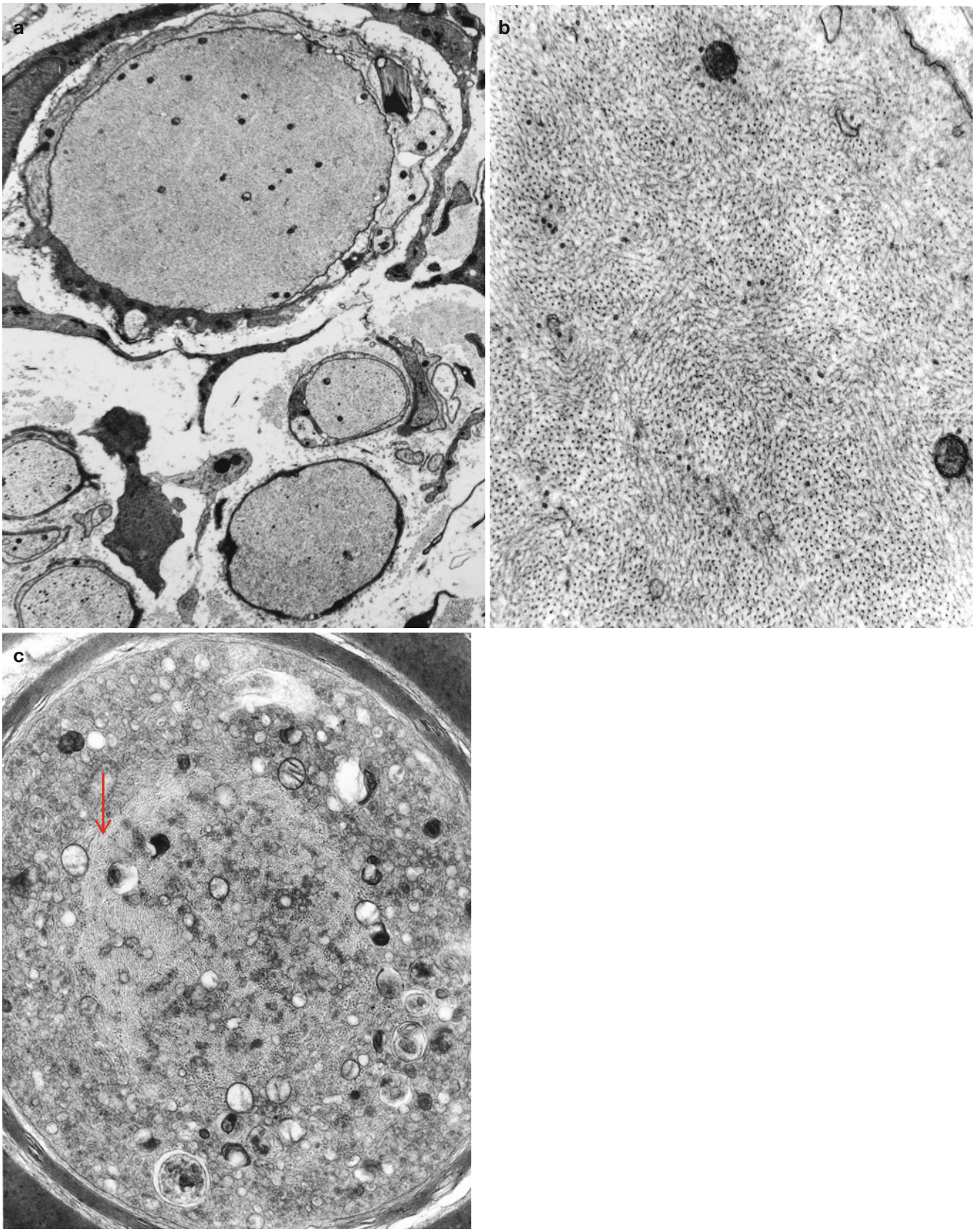

Fig. 18.10 Disulfiram: (**a**) filamentous axonopathy. (**b**) Note paucity of microtubules except adjacent to membranous organelles. (**c**) Swollen myelinated axon shows accumulation of subcellular organelles including mitochondria, neurofilaments (*arrow*), multivesicular organelles (**a**, 15,000×; **b**, 55,404×; electron micrograph ~10,000×)

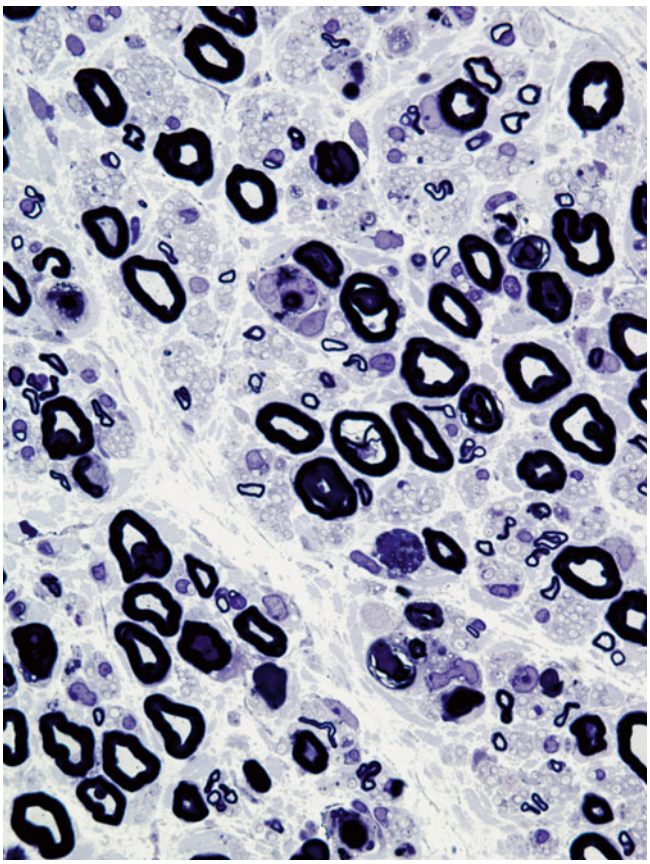

Fig. 18.11 Misonidazole: axonal loss and axonal degeneration largely involve large myelinated axons (1 μm thick plastic section, 1,000×)

The mechanism of neuropathy is unknown. Metronidazole, closely related structurally to misonidazole, also produces a predominantly sensory axonal neuropathy (Table 18.1).

18.6 Vincristine

18.6.1 Clinical Manifestations

Vincristine is used extensively as an antineoplastic agent. It belongs to the class of vinca alkaloids, related to vinblastine and vindesine. The discussion below should apply to all three substances, but the vast majority of clinical and experimental data is based on vincristine (Bradley et al. 1970; Gottschalk et al. 1968; McLeod and Penny 1969; Casey et al. 1973). Typical doses are 1–2 mg/week, and the neuropathy occurs in a highly predictable dose-related fashion in all patients maintained on the drug for a sufficient period. The typical clinical manifestation is a symmetrical distal sensorimotor polyneuropathy, which can progress over weeks to months to severe debilitation. Electrophysiological tests suggest that axonal degeneration is the predominant process. Symptomatic recovery occurs over weeks to months, but residual hyporeflexia and electrophysiological abnormalities are often present.

18.6.2 Pathology

Given the frequency of this condition, there is a relative paucity of human pathological material in vincristine neuropathy (Wulfhekel and Dullmann 1972; McLeod and Penny 1969; Bradley et al. 1970; Gottschalk et al. 1968; Vital and Vallat 1987a, b). Although examination of neuronal cell bodies at autopsy has shown neurofilamentous accumulations (Shelanski and Wisniewski 1969), sural nerve biopsy generally shows nonspecific axonal degeneration, with little or no segmental demyelination (McLeod and Penny 1969). Myelinated and unmyelinated axons are involved, and a variable amount of regenerative activity may be seen. A variety of axonal ultrastructural findings have been commented upon, including clustering or disorganization of neurotubules, marked diminution in tubule numbers, vesicular or filamentous accumulations, unusual filamentous aggregates, and increased glycogen, but these findings are all nonspecific.

18.6.3 Pathogenesis

Vincristine neuropathy is important in the understanding of axonal degeneration because the mechanism of disease is relatively well understood. Vincristine binds to tubulin, the microtubule subunit protein, and this may result in impairment of the normal assembly and maintenance of microtubules from their soluble subunits (Paulson and McClure 1975; Sahenk et al. 1987). Microtubules are thought to play a fundamental role in fast axonal transport. Disruption of their normal structure and function results in misalignment of cytoskeletal components and impaired axonal transport, manifesting as a visible disorganization of the axoplasmic architecture (Shelanski and Wisniewski 1969; Sahenk et al. 1987; Green et al. 1977). Experiments showing an accumulation of axon vesicles proximal to a region of focal vincristine exposure further support the hypothesis that fast axonal transport, normally responsible for anterograde transport of vesicular organelles, is impaired as a consequence of the observed alterations in neurotubule organization (Sahenk et al. 1987). Similar considerations probably apply to the neuropathy caused by colchicine and podophyllin. The microtubule-targeting drugs eribulin, vincristine, paclitaxel, and ixabepilone differ significantly in the incidence of induced neuropathy. Recent studies, using a cell-free microtubule-gliding assay, demonstrate that all 4 drugs inhibited kinesin-dependent anterograde transport but different substantially in dynein-dependent retrograde transport (LaPointe et al. 2013).

18.7 Hexacarbons

18.7.1 Clinical Manifestations

The hexacarbon neuropathies include those due to n-hexane and methyl n-butyl ketone, both metabolized to the neurotoxic product 2,5-hexanedione (Spencer et al. 1980). These toxic substances are used in glues, lacquers, and cleaning solvents (Allen et al. 1975; Chang et al. 1993; Korobkin et al. 1975; Herskowitz et al. 1981; Rizzuto et al. 1977; Davenport et al. 1976). Exposure is thus occupational or due to intentional inhalant abuse (Altenkirch et al. 1977; Shirabe et al. 1974; Goto et al. 1974; Towfighi et al. 1976; Oh and Kim 1976). Symptoms are symmetrical and progress slowly or subacutely, paresthesias and sensory loss being followed by motor defects. The "coasting" phenomenon, a tendency of the neuropathy to get worse for several weeks to month after removal of the intoxicant, is commonly seen in the hexacarbon neuropathies. Electrophysiological tests demonstrate axonal features in mild disease, but as the symptoms worsen, conduction velocities become progressively slower. Other clinical features and evoked potential testing may give evidence of central nervous system dysfunction.

18.7.2 Pathology

The characteristic pathology of the hexacarbon neuropathies, as seen in humans (Korobkin et al. 1975; Chang et al. 1993; Towfighi et al. 1976; Rizzuto et al. 1977; Oh and Kim 1976; Davenport et al. 1976; Goto et al. 1974; Altenkirch et al. 1977) and in experimental animals (Spencer and Schaumburg 1977), is a distal axonopathy with some axons demonstrating fusiform swelling to several times their normal diameter. Ultrastructural examination demonstrates that the focal swelling is filled with neurofilaments, arrayed in swirling masses in various planes of orientation. Other organelles, especially microtubules, may be diminished in numbers. The focal swellings involve only part of an internode and are most often seen just proximal to the node of Ranvier, their frequency increasing with a proximal to distal gradient along the nerve fiber (Spencer and Schaumburg 1977; Oh and Kim 1976). Myelin overlying the axonal swellings may be thinned, and paranodal myelin retraction with focal demyelination can occur, seen best with teased fibers. A few human cases have not demonstrated these alterations, showing no change (Gonzalez and Downey 1972; Herskowitz et al. 1981) or nonspecific axonal degeneration and segmental demyelination (Shirabe et al. 1974), but some of these biopsies were performed 1–3 months after cessation of the toxic exposure.

Overall, the neuropathic process is predominantly axonal, of mild to moderate severity, with preferential loss of larger diameter myelinated fibers. Varying amounts of segmental demyelination have been observed and are probably secondary to axonal alterations (Griffin and Price 1981). The differential diagnosis of giant axonal change and filamentous hyperplasia is reviewed elsewhere. Similar changes can be seen in intramuscular nerves, which may be involved earlier in the disease (Spencer and Schaumburg 1978; Herskowitz et al. 1981), but this is nonspecific and frequently seen even in normal patients (Alderson 1992).

18.7.3 Pathogenesis

The accumulation of neurofilaments typical of these neuropathies suggests that a defect of slow axonal transport is involved in this intoxication, but only fast axonal transport has been shown to be significantly impaired in the animal models (Ochs and Brimijoin 1993). One hypothesis suggests that a toxic diketone hexacarbon metabolite causes cross-linking of axonal neurofilament proteins, forming inflexible aggregates which become blocked at sites of narrowing such as the node of Ranvier, thus explaining the tendency for the axonal swellings to be found proximal to this location (Graham et al. 1984). Axonal degeneration might then follow as a consequence of the "plugging up" of the normal transport pathways (Mendell et al. 1977). However, this hypothesis is not consistent with the increase in neurofilament transport speed and ultrastructural changes observed by some workers (Monaco et al. 1989).

18.8 Lead

18.8.1 Clinical Manifestations

Lead is found in paints, fuel additives, metal food containers, and batteries, to name a few commonly used products. Inorganic lead is the offending form of the metal and can be absorbed by inhalation or ingestion. Lead neuropathy (reviewed in Windebank and Dyck 1984; Windebank 2005) is now rare. Manifestations include abdominal colic, nephropathy, encephalopathy, anemia, and neuropathy. Lead neuropathy has a notable predilection toward involvement of upper extremities, especially the radial nerve. Motor symptoms and signs usually predominate. The CSF is typically normal or shows mild protein elevation. Electrophysiological testing demonstrates mild conduction slowing in subclinical toxicity but mixed or predominantly axonal changes in patients with overt neuropathy (Buchthal and Behse 1979; Ehle 1986). The diagnosis is confirmed by finding evidence of excessive lead in tissue, usually blood, urine, bone, or teeth. Treatment is with chelating agents such as penicillamine or EDTA.

18.8.2 Pathology

There is remarkably little modern human pathological material on lead neuropathy (Behse et al. 1972; Oh 1975; Buchthal and Behse 1979; Dupuy et al. 1984; Schlenska and Spalke 1975). The older literature, based on data from 50 to 100 years ago, does not permit a meaningful analysis of whether axonal degeneration or primary demyelination predominates (Goldstein et al. 1975). Two patients reported by Schlenska and Spalke (1975) demonstrated a mild mixed axonal and demyelinating neuropathy. The patient with severe neuropathy described by Oh (1975) had axonal degeneration, but concurrent alcoholism was a confounding factor. A patient studied by Buchthal and Behse (1979) showed a loss of large myelinated axons, regenerating clusters, and no evidence of demyelination. In the same study, seven patients with subclinical neuropathy showed trivial paranodal myelin changes without frank segmental demyelination and remyelination. Dupuy et al. (1984) observed a loss of large myelinated axons, some regenerating clusters, as well as a mild degree of segmental demyelination and remyelination.

Ultrastructurally, Dupuy et al. (1984) drew attention to nonspecific features such as reactive Schwann cell changes (well-developed Golgi apparatus and endoplasmic reticulum) and reduplication of basal laminae around unmyelinated fibers' Schwann cells and endothelial cells. Schlenska and Spalke (1975) described prominent axon–Schwann cell networks, a nonspecific indicator of axonopathy. No inclusions or specific ultrastructural features have been observed. Overall, our reading of the limited modern literature suggests that a nondescript axonal degeneration is the predominant process in clinical human lead neuropathy, and while demyelination is sometimes present, it is likely secondary.

18.8.3 Pathophysiology

The experimental literature on lead neuropathy, historically one of the classic experimental models of peripheral nerve demyelination, is substantial (Lampert and Schochet 1968; Fullerton 1966). However, the data is contradictory as to whether axonal or myelin changes are predominant, perhaps depending on the species studied and dose schedule used (Ohnishi et al. 1977; Fullerton 1966; Windebank and Dyck 1984). Rats fed 4 % lead carbonate for several months develop a primary demyelinating neuropathy, perhaps because of interference with Schwann cell mitochondrial metabolism, and secondary alterations in the blood–nerve barrier (Windebank and Dyck 1984).

In humans it is not possible to reconcile the mild conduction slowing, more severe in sensory nerves, seen in subclinical lead toxicity, with the predominantly motor axonal degeneration seen in clinical lead neuropathy (Ehle 1986). In light of the unusual clinical manifestations (preferential involvement of upper extremities and radial nerve) and the contradictions within and between the human and animal pathological data, it is not yet possible to formulate a satisfying pathophysiological mechanism for lead neuropathy.

18.9 Epidemic Toxic Inflammatory Neuropathy

In recent years, two toxin-caused epidemics in which peripheral nerve disease was a prominent manifestation have been recognized. No new cases are now occurring, but these epidemics continue to be an active field of study, and a brief review of clinical and histological features is worthwhile. Despite differing epidemiology, these two syndromes seem to have much in common clinically and histologically.

18.9.1 Eosinophilia Myalgia Syndrome

18.9.1.1 Clinical Manifestations

In 1989 and 1990, over 1,500 cases of a syndrome most consistently characterized by severe debilitating myalgia and peripheral blood eosinophilia were described. Many were initially misdiagnosed as "eosinophilic fasciitis" until the association with L-tryptophan ingestion was recognized (Kilbourne 1992). Scleroderma-like skin changes were often seen in chronic disease (Kilbourne 1992). Ultimately, nearly all identified cases were traced back to a contaminated L-tryptophan product originating in one manufacturing site in Japan (Kilbourne 1992). Peripheral nerve involvement occurred in about a third of patients and could be part of a multisystem syndrome, or present in isolation. Painful predominantly sensory neuropathy and distal sensorimotor neuropathy were the typical manifestations (Smith and Dyck 1990; Smith et al. 1990; Kaufman et al. 1990a). Electrophysiological studies most often showed a predominantly axonal process, but demyelinating or mixed pictures could be seen (Smith and Dyck 1990; Freimer et al. 1992; Donofrio et al. 1992). Although treatment with steroids often resulted in rapid improvement of fever, skin lesions, and eosinophilia, neuromuscular manifestations did not respond so readily, and many patients became chronically disabled, sometimes progressing to death despite cessation of exposure of the offending substance (Freimer et al. 1992; Martin et al. 1990; Smith and Dyck 1990; Kaufman et al. 1990a; Donofrio et al. 1992).

18.9.1.2 Pathology

Nerve biopsies have been reported in eosinophilia myalgia syndrome (Smith and Dyck 1990; Kaufman et al. 1990a, b; Freimer et al. 1992; Heiman-Patterson et al. 1990; Burns

et al. 1994). Most often these describe an inflammatory axonal neuropathy. The inflammation predominates in the epineurium, and sometimes evidence of vascular injury can be seen by way of luminal narrowing and angioneogenesis. Kaufman et al. (1990b) thought that epineurial venules were more often involved than arterioles. Lymphocytes predominate in the inflammatory infiltrate, but eosinophils can be prominent in patients not treated with steroids prior to biopsy.

Active axonal degeneration of varying severity is usually seen, and some nerves show regenerative cluster formation. The pattern of active axonal degeneration may be multifocal, or there can be panfascicular acute axonal degeneration, both suggestive of acute ischemic insults (Heiman-Patterson et al. 1990). Segmental demyelination has also been seen, with occasional onion bulbs, thinly myelinated axons, and appropriate teased fiber findings (Smith and Dyck 1990). Although this observation was generally much less prominent than the axonal changes, in two cases described as presenting with a subacute demyelinating neuropathy, active segmental demyelination and numerous thinly myelinated axons were seen (Freimer et al. 1992). Perivascular inflammation was not as prominent in these two cases, and a tendency toward perineurial infiltration with inflammatory cells was noted (Freimer et al. 1992).

We know of only one report of peripheral neuropathy in idiopathic eosinophilic fasciitis, and this showed features similar to those above (Satsangi and Donaghy 1992).

18.9.2 Toxic Oil Syndrome

Ingestion of adulterated rapeseed oil has been associated with an epidemic illness that struck 20,000 people in Spain in 1981 and 1982 (Kilbourne et al. 1983; Martinez-Tello and Tellez 1991). Pneumonia, GI upset, rash, and peripheral eosinophilia were often followed by progressive peripheral nerve and muscle involvement and scleroderma-like skin lesions. A remarkable 73 sural nerve biopsies were reported in one study (Ricoy et al. 1983). Perivascular inflammation involving epineurium, perineurium, and endoneurial vessels was uniformly seen, consisting mostly of lymphocytes, although occasional PMNs, including eosinophils, were present. A particularly striking feature was a tendency toward perineurial inflammation, progressing from focal involvement to confluent circumferential collections of inflammatory cells. True necrotizing vasculitis was not described, although subendothelial fibrosis was sometimes seen. Immunofluorescence studies were unremarkable. Axonal degeneration was the predominant pathological process and often occurred in a multifocal distribution. A marked perineurial and endoneurial fibrosis could be seen in chronic lesions.

18.9.3 Pathogenesis

The precise pathogenesis of these syndromes remains obscure, although the association with the particular toxin is unequivocal (Kilbourne et al. 1983; Kilbourne 1992; Ortega-Benito 1992). Given the clinical and histological similarity between the two entities, unifying hypotheses have been offered by a number of authors, focusing on aniline-derived contaminants that have been identified in both syndromes (Aldridge 1992; Mayeno et al. 1992; Philen and Hill 1993).

18.10 Differential Diagnosis of Toxic Neuropathies

Peripheral nerve biopsy has little to offer in the investigation of toxic neuropathy, because the diagnosis is made on history and the findings are almost always nonspecific. Nevertheless, a few points regarding differential diagnosis can be made.

Almost all toxic neuropathies are axonal, so the finding of a significant demyelinating component does serve to restrict the list of possible offenders to a handful, including amiodarone, perhexiline, chloroquine, diphtheria, and perhaps to lead, gold, buckthorn, cyanide, or allopurinol. The first three of these have characteristic inclusions. Inflammation does not ordinarily suggest a toxic neuropathy, but the remarkable toxic oil and L-tryptophan epidemics of recent years should be kept in mind.

The finding of giant axonal changes with filamentous accumulation should lead to consideration of the hexacarbon neuropathies, acrylamide, disulfiram toxicity, and nontoxic (familial) giant axonal neuropathy. A subtle difference between nontoxic (genetically based) giant axonal neuropathy and the others is that the former has focal condensations of filaments scattered among the filament accumulations, while the toxic giant axonal neuropathies do not (Asbury and Brown 1980; King et al. 1993). There are reports about giant axons with filament accumulations in amyloid neuropathy, misonidazole toxicity, and B12 neuropathy, but these do not represent typical examples of the neuropathy seen in those diseases. The significance of finding occasional axons with filamentous accumulations but without massive axonal swelling is unclear. This has been described in vincristine and cisplatin toxicity, and we have seen such changes in several cases including CIDP and a granulomatous neuropathy of uncertain etiology (Figs. 4.4 and 4.5). Focal filament masses which only occupy part of the axonal cross section are an entirely nonspecific finding.

18.11 Drug Interactions

Increasingly, it is possible for one treatment to couple with another to exaggerate the neuropathic effect of each other (Manji 2013) as has been considered for VEGF-neutralizing

antibodies (bevacizumab) or VEGF receptor inhibitors (sorafenib, sunitinib) on the neuroprotective action of VEGF on paclitaxel-induced neuropathies (Verheyen et al. 2012; Diezi et al. 2013). Similarly, increased susceptibility to peripheral neurotoxicity has been reported in polymorphisms in genes involved in a variety of pathways (Diezi et al. 2013).

The mechanisms which underlie drug-induced toxicity have been recently reviewed and run the gamut from altered ion channels including the transient receptor potential (TRP) family (ankyrin-1, vanilloid-1,vanilloid-4), voltage-gated ion channels (Na^+, K^+, Ca^{+2}), reduction of VEGF neuroprotective effect, microtubular function, mitotoxicity and oxidative stress, DRG cytotoxic inflammation, and apoptosis.

Other changes of axonal degeneration, including abnormalities of mitochondrial number and morphology, vesicular and dense body hyperplasia, axonal "atrophy", formation of Schwann cell-axon networks, accumulations of glycogen, etc., are discussed in Chap. 4. These are all nonspecific findings of degenerating axons and are most typically seen in toxic neuropathies, but can be seen in ischemia, metabolic disturbances, nutritional deficiencies, and any other situation where axonal metabolism is disturbed.

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Genetically Determined Neuropathies

19

Peripheral neuropathy caused by a genetic defect is common in clinical practice as the manifestation of a variety of inherited disorders. The polyneuropathy may occur as the sole expression of the genetic defect or may be seen in association with the involvement of the CNS or as part of a complex multisystem disorder. Many of these diseases have a relatively benign and slowly progressive course, with most patients leading a nearly normal life. Recent advances in molecular genetics have reduced the utility of nerve biopsy in the diagnosis of genetically determined neuropathies and allow for the beginnings of a rational classification. The full gamut of genes responsible for inherited neuropathies is now so extensive that it has spawned its own database "The Inherited Peripheral Neuropathies Mutation Database" (http://www.molgen.ua.ac.be/CMTMutations/). We will not attempt to catalogue the pathology of each of these mutations; rather, we will illustrate distinctive neuropathy phenotypes which characterize multiple different genotypes. For additional information the reader is directed to a number of excellent recent reviews (Reilly et al. 2011; Klein et al. 2013; Rossor et al. 2013; Saporta and Shy 2013; Sagnelli et al. 2013). Inherited neuropathies with accumulation of storage material are discussed in Chap. 20. With the exception of clear-cut familial occurrence, often not demonstrable, the single most important clue to the presence of a genetically determined neuropathy is duration of illness. While some neuropathies can manifest episodically or acutely (e.g., porphyria), the vast majority have an insidious onset and progress over many years, except for hereditary neuralgic amyotrophy.

A heterogeneous group of neuropathies is classified under the rubric of Charcot–Marie–Tooth (CMT), previously known as Hereditary Motor and Sensory Neuropathy (HMSN), and in practice these account for the majority of genetically determined neuropathies. Peripheral nerve dysfunction associated with cerebellar or spinocerebellar disease is next in frequency.

19.1 Charcot–Marie–Tooth and Cognate Disorders

The prevalence of CMT has been estimated at 5–41 per 100,000 (Dyck et al. 1993; Berciano and Combarros 1990). This wide range is largely due to the fact that many cases are mild and patients never require medical attention. Dyck has estimated that only 10 % of individuals with CMT-1 seek a physician for symptoms relating to the disease (Dyck et al. 1993). The importance of full assessment of patients' families cannot be overemphasized, whether in adults or in children (Dyck et al. 1981a; Hagberg and Lyon 1981).

The syndrome of hereditary CMT or peroneal muscular dystrophy covers a heterogeneous group of inherited polyneuropathies (Rautenstrauss 2011) that have a similar clinical phenotype. Charcot–Marie–Tooth disease historically has been divided into the demyelinating or hypertrophic forms (CMT1) in which nerve conduction velocity is markedly and diffusely reduced) and neuronal forms (CMT-2) characterized by normal or slightly slowed nerve conduction velocity) (Shy and Patzko 2011). A third form, intermediate CMT (X-linked CMT), comprises patients in whom the MCV lies in the range of 25 m/s and 45 m/s. Early-onset genetic neuropathies (vide infra), including those of prenatal development, are incorporated into the forms of CMT-1 and CMT-2.

Two other less frequently inherited groups of polyneuropathies are closely related to CMT: the hereditary sensory neuropathies (HSN), which affects predominantly sensory fibers, and the distal hereditary motor neuropathies (HMN), which display only motor involvement (Rossor et al. 2013). These three disorders are collectively included into the family of CMT by some authors.

Traditional classifications of CMT diseases combine elements of clinicopathological phenotypes, inheritance patterns, motor nerve conduction velocities, and causative genes. Up to the end of 2013, 70 disease genes have been described for CMT (of which 33 were discovered after

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2009!) and related disorders. Nonetheless, more than 70 % of patients with CMT, in Western countries, have genetic abnormalities affecting only four genes: *PMP22*, *MPZ*, *MFN2*, and *GJB1* (Tazir et al. 2013).

The rapid evolution in gene discovery is attributed to the development and affordability of high-throughput sequencing technologies, has affected diagnosis and management of patients with CMT, and has enabled the design of classifications where genetic data plays a significant role (Rossor et al. 2013).

Clinical and pathological similarities exist between CMT and some hereditary spastic paraplegias with a subset of patients having an overlap condition termed Silver syndrome (Timmerman et al. 2013).

CMT may be the presenting phenotype of mitochondrial disorders. MFN2 and GDAP1 are involved in the fusion and fission of mitochondria, fundamental in regulating mitochondrial transport along the cell. CMT can result from mutations of *MFN2* (autosomal dominant CMT-2A, autosomal recessive CMT-2, CMT-5, and CMT-6) and *GDAP1* (CMT-2 K, CMT-4A, recessive intermediate CMT, and autosomal dominant CMT CMT-2 K) (Pareyson et al. 2013) (vide infra).

19.2 CMT-1

Traditionally referred to as hypertrophic Charcot–Marie–Tooth disease or hereditary sensory motor neuropathy (HSMN-I), the central features of CMT-1 are marked slowing of nerve conduction velocity with demyelination, remyelination, and onion bulb formation. Nerve conduction studies play a critical role in separating the hypertrophic and the neuronal forms of CMT. Conduction velocities of less than 38 m/s have been proposed to separate CMT-1 from CMT-2 (Harding and Thomas 1980a). Slowing of conduction is seen in asymptomatic or presymptomatic carriers (Nicholson 1991). CSF examination is normal or shows slight elevation of protein.

Different inheritance and molecular-genetic studies within this group of patients indicate that CMT-1 is a genetically heterogeneous syndrome. The most common variant, accounting for 70–80 % of all patients, is now known to be due to a defect in expression of PMP22 protein that is caused by a point mutation or tandem duplication of the *PMP22* gene (on chromosome 17p11.2–p12) (Hoogendijk et al. 1993). PMP 22 is an integral membrane protein produced by Schwann cells, where it plays a crucial role in the development and maintenance of compact myelin. A smaller group linked to chromosome 1, classified as CMT-1B (Lebo et al. 1991; Ionasescu et al. 1993), is characterized by defects in the *MPZ* gene which codes for the peripheral myelin protein P_0 (Hayasaka et al. 1993c). Patients with CMT-1B are clinically indistinguishable from patients with CMT-1A. Other cases of autosomal dominant CMT-1 (CMT-1C, CMT-ID, CMT-IF, and CMT1 plus retinal degeneration) are caused by *LITAF*, *EGR2* (which influences the transcription of other myelin protein genes), *NEFL*, and *FBLN5* gene defects (Rossor et al. 2013). The CMT-1A and CMT-1B groups demonstrate autosomal dominant inheritance with age-dependent (eventually 100 %) but variably severe penetrance. In Western Europe, North America, and Japan, the dominant forms of CMT-1 are by far the most frequent (Tazir et al. 2013). About 20 % of cases are sporadic, and although segregation analysis had previously suggested that most were recessive (Harding and Thomas 1980c), recent genetic studies indicate that most sporadic cases are caused by new mutation of the CMT-1A type (Hoogendijk et al. 1992; Gabreels-Festen et al. 1993). Autosomal recessive forms are classified as CMT-4 (see Charcot–Marie–Tooth disease type 4).

19.2.1 Clinical Manifestations

While individual cases of CMT-1A and CMT-1B are not clinically distinguishable, CMT-1B is typically more severe (Dyck et al. 1993). Symptoms usually begin in the first or second decade although nerve conduction is abnormal in early life. Onset in the 3rd–5th and rarely up to seventh decade is well described (Harding and Thomas 1980a). The usual presenting manifestations are skeletal deformity, distal leg muscle atrophy, weakness, or indirectly through findings detected while undergoing evaluation for non-neurological symptoms (Dyck et al. 1993). Pes cavus, hammertoes, and scoliosis may be present as early manifestations in childhood and serve as important clues to the diagnosis of a familial neuropathy. Symptoms can be so mild and insidious that some patients never present to a physician for disease-related complaints. Visible and palpable hypertrophy of peripheral nerves, especially the greater auricular nerve, can be found in approximately a quarter of patients. Weakness and atrophy are often restricted to the distal legs, even after many years of illness, with about half of patients progressing to distal hand muscle weakness. Reflexes are typically diminished or absent in a distal to proximal gradient. Sensory loss involves all modalities but is usually less prominent than motor symptoms, joint destruction, or sensory ataxia being uncommon. Essential tremor is seen in a third of CMT-1 patients and its presence defines the "Roussy–Lévy" variant, but these patients are not genetically distinct from those with other CMT-1 variants. The disease progresses very slowly over a patient's lifetime and does not influence most individuals' longevity; only a minority eventually become wheelchair dependent. A small subset of patients with dominantly inherited CMT-1 present in early childhood or even at birth, some

progressing very slowly, but others reaching a debilitated state (Ouvrier et al. 1987; Vanasse and Dubowitz 1981; Hagberg and Lyon 1981).

Medication-induced (vincristine, metronidazole, nitrous oxide, nitrofurantoin, etc.) exacerbation of neuropathy in CMT is well known (Weimer and Podwall 2006). In CMT patients, acute initiation of weakness, including a Guillain–Barré-like pattern, may occur. In ten previously unsuspected and undiagnosed CMT patients, nerve deficit developed following the administration of pharmaceuticals as the initial manifestation of the genetic disease.

19.2.2 Pathology

19.2.2.1 Role of Biopsy

Given that a familial neuropathy is often clinically suspected, that examination of even "normal" family members in CMT-1 will often disclose affected individuals, and that nerve conduction studies can distinguish between hypertrophic and neuronal variants, there is little reason to biopsy patients in this setting. Some of the cases referred for biopsy in our institutions had been suspected to represent CIDP because of atypical features. For the most part, however, the diagnosis of CMT-1 was strongly suspected clinically, but perhaps because there is no specific therapy to offer, the referring clinicians felt the need to prove their suspicion and make every effort to rule out a treatable neuropathy (especially CIDP) before offering genetic counseling. Even so, recent advances in molecular biology permit noninvasive diagnosis of CMT-1A with greater specificity than biopsy (Hoogendijk et al. 1992; Gabreels-Festen et al. 1993), and rapid progress has been made in the genetic characterization of the other forms of CMT (Rossor et al. 2013).

In the setting of a long history consistent with CMT, but a recent rapid worsening, the possibility of a superimposed diagnosis, such as CIDP or paraproteinemic neuropathy, should be considered. Authors observing such a co-occurrence have suggested an increased risk for CIDP or paraproteinemic neuropathy with CMT-1 (Gregory et al. 1993; Dyck et al. 1982). Although clinical acumen is more important than biopsy for the identification of such patients, histological examination may provide useful confirmatory evidence. Sural nerve biopsy may be diagnostic of familial neuropathy even if a patient is clinically and electrophysiologically normal (Dyck et al. 1983).

In infants and young children, where the spectrum of clinical and histological findings is wide, biopsy may provide useful prognostic and inheritance information. Infantile or childhood-onset CIDP may be clinically indistinguishable from inherited neuropathies, but is treatable and should not be overlooked (Sladky et al. 1986). Although there is disagreement (Hagberg and Lyon 1981), the literature suggests that biopsy can reliably separate CMT-1 and CMT-3, a distinction with prognostic and genetic implications (vide infra). The light-microscopic features are also indistinguishable, but an ultrastructural finding that suggests the diagnosis of the myelin P_0 (*MPZ*) mutation (CMT-1B) is the presence of uncompacted myelin.

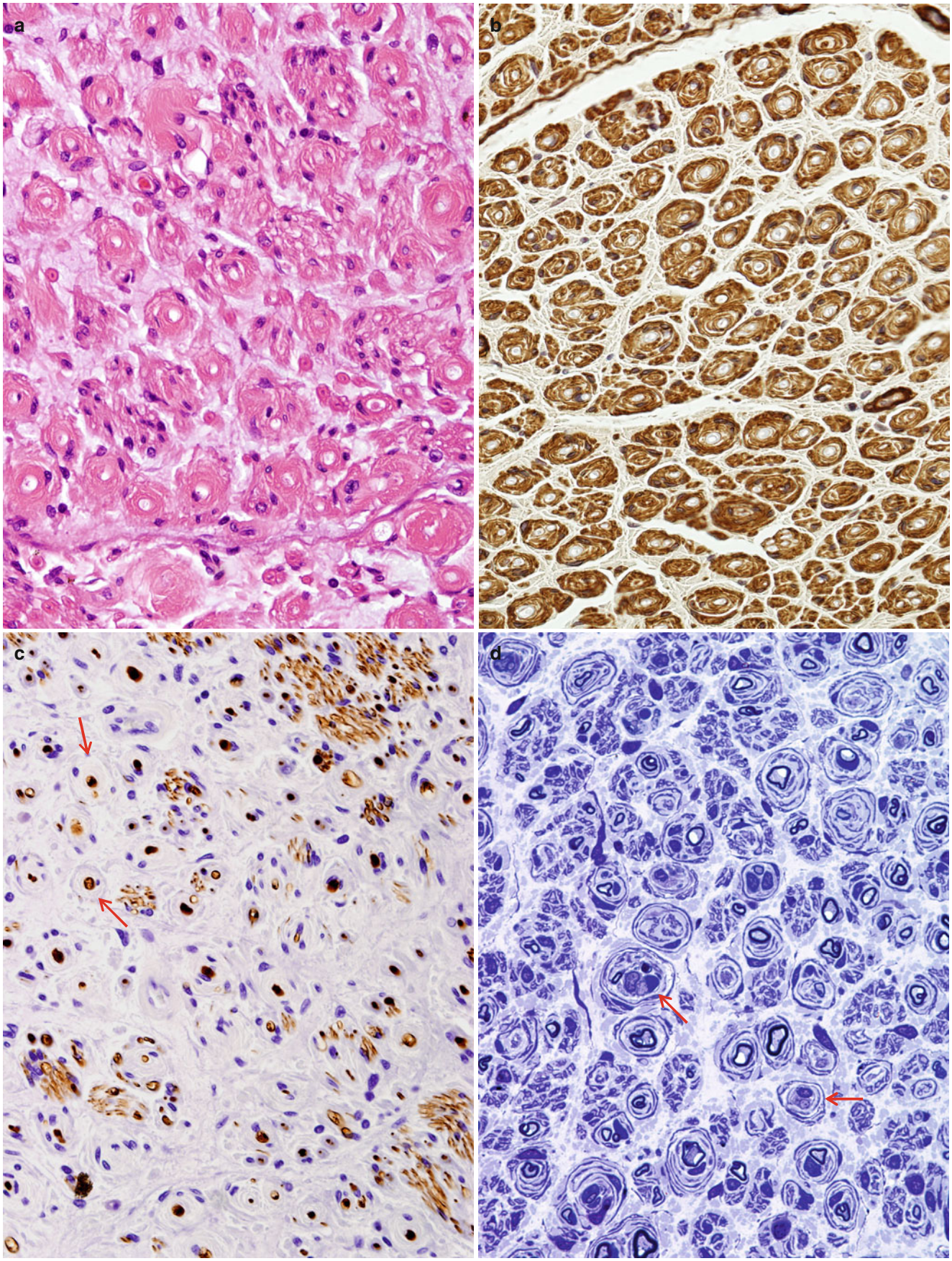
19.2.2.2 Light Microscopy

The literature contains several large biopsy series, and our experience is entirely congruent with the information these present (Behse and Buchthal 1977; Gabreels-Festen et al. 1992b; Madrid et al. 1977; Gherardi et al. 1983; Ouvrier et al. 1987; Low et al. 1978).

An increase in the size of nerve fascicles, sometimes severalfold, is often readily apparent in advanced CMT-1, reflecting the additive accumulation of diffuse onion bulb formations, edema, extracellular matrix, and collagen in the endoneurium (Behse et al. 1974). Histochemical stains often indicate that the endoneurial interstitial "edema" accumulation has a mucoid component. Proliferation of Schwann cells and fibroblasts results in increased endoneurial cellularity. The fascicle-to-fascicle distribution of pathology is relatively homogenous. Variable amounts of chronic inflammation can be found in some cases (Thomas et al. 1997). Many thinly myelinated fibers are seen. Increased myelin spiral length in relation to axon diameter, which has been interpreted as evidence of axonal atrophy, possibly reflects overexpression of PMP22, leading to myelin thickening before demyelination occurs.

A reduction in myelinated fiber (MF) numbers is evident and varies in severity from moderate to subtotal, correlating with duration and severity of disease. Both large and small MFs are depleted (Fig. 3.1c), and a unimodal size-frequency histogram is characteristic (Gabreels-Festen and Gabreels 1993). Unmyelinated fiber (UF) densities are usually normal, and sometimes even increased (Tome et al. 1979). Regenerating clusters are ordinarily found in numbers no greater than controls, but we have seen them feature prominently in some cases which were otherwise typical of CMT-1 (see "Intermediate CMT" below).

Repeated episodes of primary segmental demyelination and remyelination lead to the formation of onion bulbs, a term that refers to imbricated layers of supernumerary Schwann cell processes forming rings around individual axons, an appearance which can be confidently detected only in cross sections. Although hypertrophic neuropathy is a prominent feature of several of the genetically determined neuropathies, onion bulbs can also occur in CIDP and, to a lesser extent, in diabetic neuropathy and other acquired polyneuropathies. The hallmark of familial hypertrophic neuropathy is the presence of onion bulbs (OBs) involving a substantial fraction (30–100 %) (Gherardi et al. 1983; Behse et al. 1974; Low et al. 1978) of the visible myelinated and demyelinated axons,


a
b
c
d

distributed diffusely among the nerve fascicles (Figs. 19.1 and 19.2). Onion bulbs can be visualized in paraffin-embedded material using H&E sections (Fig. 19.1a), an appearance confirmed by collagen IV staining of Schwann cell basal laminae (Fig. 19.1b). Onion bulbs may have a central myelinated or demyelinated axon (arrows, Fig. 19.1c), no central axon, or several axons as shown by neurofilament immunoreactivity. Paraffin-embedded thick sections do not reveal these structures as well as as semithin toluidine blue (Figs. 19.1d and 19.2a–d) and especially ultrathin EM sections (Fig. 19.3). Naked axons may be present, more frequently in children (arrow, Fig. 19.2d). Myelin debris is rarely seen, especially in adults (Fig. 19.2d). While myelin sheaths can be inappropriately thin indicating remyelination, they may also appear normal or even hypermyelinated relative to axon diameter (arrow, Fig. 19.2b; arrowhead, Fig. 19.2c) and may show focal redundant loops (Dyck et al. 1970; Gabreels-Festen et al. 1992b). Small myelinated fibers in particular may demonstrate inappropriately thick myelin for axonal diameter, perhaps due to axonal atrophy (arrowhead, Figs. 19.2c and 19.3).

Evolution of the histological picture from childhood into adult life has been studied by several authors (Gabreels-Festen et al. 1992b; Ouvrier et al. 1987; Meier et al. 1976). Children have fewer and smaller OBs, sometimes almost none, while naked or actively demyelinating axons are more prominent. In one study 2.7–9.2 % of MFs were demyelinated and 0–1.7 % were actively demyelinating in patients below the age of 10, whereas 0–2 % and 0–0.2 % respectively showed these changes in patients above the age of 10 (Gabreels-Festen et al. 1992b). In children debris-filled cells remain unusual but are more common than in adults. The finding of a fair number of naked axons with only a few cells containing myelin debris suggests delayed remyelination. With increasing age the active segmental myelin changes become less frequent, and OBs increase in number and size. By the age of 6, onion bulbs are usually well developed (Gabreels-Festen et al. 1992b). Loss of MFs is also age related, although even at early stages there is a significant diminution in their numbers. As with adults, degenerating axons are generally not seen. However, nonspecific axonal alterations, particularly atrophy, can be present. Denervated Schwann cell subunits and collagen pockets may be seen more often than in normals, indicating unmyelinated fiber disease, but UF density is usually maintained or even increased (Behse and Buchthal 1977; Low et al. 1978).

Denervated OBs become more frequent with time, as may the endoneurial edema frequently present in hypertrophic neuropathy (Meier et al. 1976). Even so, a correlation is not always seen between endoneurial area and age (Gabreels-Festen et al. 1992b).

19.2.2.3 Electron Microscopy

Onion Bulb Formations

Onion bulb formations are a nonspecific but nevertheless significant finding on nerve biopsy. They are discussed here in detail because they comprise a central part of the histological picture in CMT-1. However, there are no morphologic features of a single onion bulb which allow specific diagnosis. Helpful clues may be obtained from the pattern of distribution of OBs within the nerve fascicle and from other findings in the nerve biopsy.

Onion bulbs are easily detected by light microscopy on semithin sections, particularly under oil immersion, but they are best defined with the electron microscope. A typical formation consists of a myelinated axon surrounded by concentric layers of Schwann cell processes; these can be plump, thin and elongated, or tightly packed stacks (Figs. 19.3 and 19.4). The identity of the cellular profiles is established by the finding of a surrounding basal lamina. Collagen fibers run longitudinally and separate the individual OB layers. Numerous variations on this theme can be seen. An individual axon may be denuded, thinly myelinated, or hypermyelinated. An OB forming around what appears to be a large unmyelinated axon (Fig. 19.4c) likely represents a previously myelinated fiber that has become atrophic with the loss of its myelin sheath. Rarely, more than one myelinated axon is seen in the center, representing a regenerating cluster within an onion bulb.

Unmyelinated fibers are frequently incorporated into the circumferential lamellae of an OB (arrows, Fig. 19.4c). These UFs may be tiny sprouts arising from the demyelinated axon as part of the regenerative response after segmental demyelination or neighboring UFs which have gravitated into the onion bulb. Ochoa (1978) has observed that the outermost lamellae of OB formations often have the configuration of denervated Schwann cell bands and believes that neighboring UFs can make a significant contribution to OB formation. A "pseudo"-onion bulb is formed when a myelinated axon is surrounded by several unmyelinated axons and their accompanying Schwann cells as part of a regenerating

Fig. 19.1 Onion-bulb neuropathy: characteristic histopathology. (**a**, **b**) Onion bulbs consist of prominent concentric rings of Schwann cell processes surrounding a central pale axon by H&E (**a**) and collagen IV immunohistochemistry (**b**). (**c**) Single axons form the center of most onion bulbs (*arrows*), although some have several axons or lack axons altogether (neurofilament immunohistochemistry). (**d**) Plastic sections show onion bulbs with thinned or absent (*arrows*) myelin sheaths (Magnification: paraffin, **a**, H&E, 400×; **b**, collagen IV, 400×; **c**, neurofilament, 400×; **d**, 1 μm thick plastic section 1,000×)

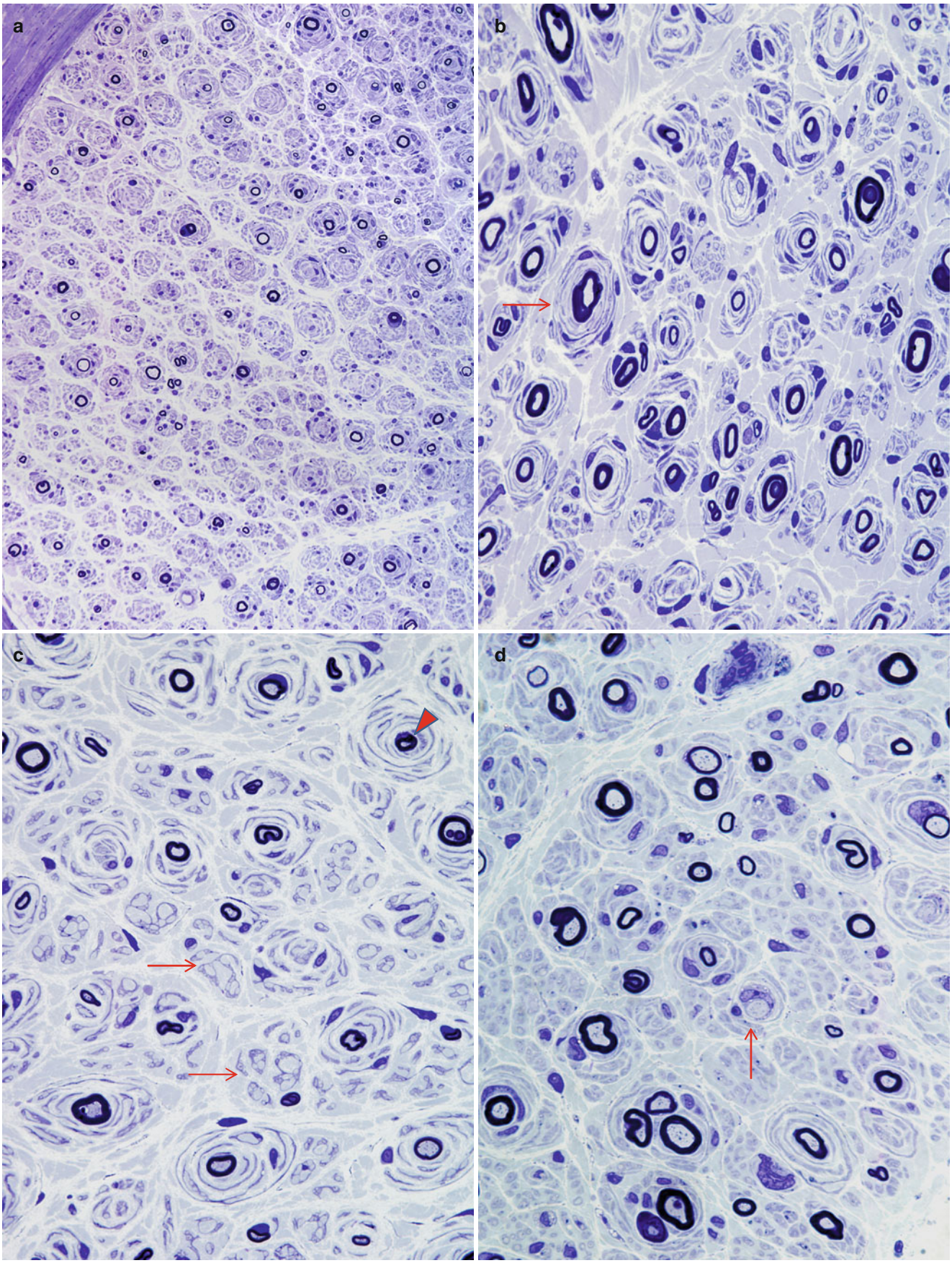


Fig. 19.2 CMT-1: spectrum of onion bulb morphology from different cases is shown. Most axons have attenuated myelin (**a**–**d**), while some appear relatively hypermyelinated (*arrow*, **b**; *arrowhead*, **c**). (**c**) The delicate processes between onion bulbs (*arrows*) represent denervated Remak bundles. (**d**) Naked axons are also noted (*arrow*) (Magnification: 1 μm thick plastic section **a**, 400×; **b**–**d**, 1,000×)

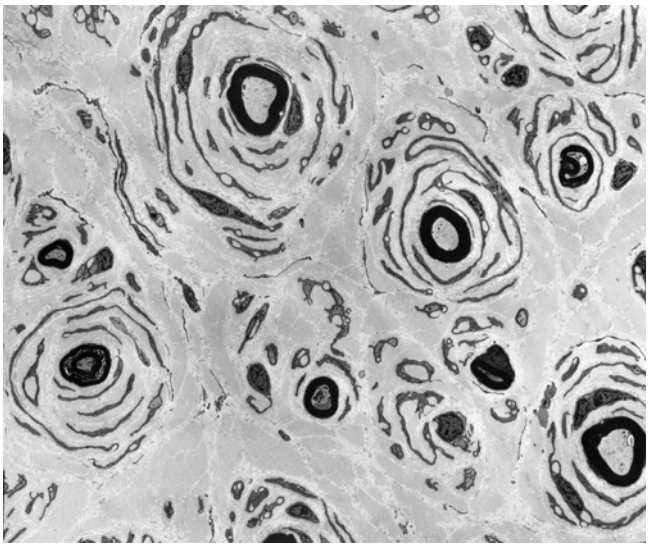


Fig. 19.3 CMT-1: classic ultrastructural appearance of onion bulbs (Magnification 2,250×)

cluster (Fig. 7.13). Misinterpretation of these formations as true onion bulbs may lead to the false impression that a hypertrophic neuropathy is present.

Typically there are 2–5 concentric layers of Schwann cell processes (Gherardi et al. 1983), but we have observed giant OBs with 20 such lamellae (Fig. 9.31). The Schwann cell processes may disappear, leaving behind closely apposed basement membranes. The latter can only be recognized by electron microscopic examination and are infrequently seen in typical cases of CMT-1 (vide infra). Ultimately the axon at the center may disappear, replaced by collagen or Schwann cells, creating a denervated or obsolete onion bulb (Fig. 19.5). The frequency of these structures parallels disease duration.

Other Ultrastructural Changes in CMT-1

Active demyelination is rarely seen in CMT-1, but when present appears as a focal myelin degeneration or an accumulation of debris in Schwann cells. Debris-filled macrophages are uncommon, but may be more frequently detected in children, where the demyelination is most active (Gabreels-Festen et al. 1992b; Ouvrier et al. 1987; Meier et al. 1976). Madrid et al. (1977) and Vital et al. (1992) have commented on the rare finding of macrophage-mediated myelin stripping in familial hypertrophic neuropathy but we have never seen this, and the illustrative figures may simply represent a scavenger macrophage that has entered the Schwann tube to clear away damaged myelin. Alternatively, this may represent inflammatory demyelination superimposed on familial neuropathy, as described by some authors (Sect. 19.2.4 below). In addition, in mouse models of CMT-1A, CMT-1B, and CMT-1X (mice homo/hemizygously deficient for Cx32), peripheral nerve macrophages and $CD8^+$ T cells have been shown to contribute significantly to the pathogenesis of these genetic disorders (Kobsar et al. 2005; Ip et al. 2006).

Schwann cells in the process of remyelinating recently demyelinated axons are characterized by an increased amount of electron dense cytoplasm and evidence of anabolic activity. A few lamellae of myelin may be deposited, and in the earliest stages of remyelination, these appear uncompacted (Figs. 5.13 and 14.7). Naked or demyelinating axons often seem atrophic and show an increased axonal filament density and a wrinkled axolemma, similar to that described in other demyelinating conditions (Fig. 9.24) (Gabreels-Festen et al. 1992b).

19.2.2.4 Fiber Teasing

In CMT-1 segmental myelin changes, including entirely demyelinated segments, are seen in 30–100 % of teased fibers (Dyck 1966; Dyck et al. 1968, 1993; Behse and Buchthal 1977; Low et al. 1978). Frequent segmental demyelination and remyelination results in shortened (<0.60 mm) internodes. Two thirds of 6 mm segments of teased nerve fibers will show an abnormal internode, rising to 95 % of 8 mm segments, in comparison with 0.5 % of 5 mm segments in normals. Segmental myelin changes cluster on certain fibers, suggestive of secondary demyelination (Dyck et al. 1993). Axonal degeneration is not present significantly more often than in normals.

19.2.2.5 CMT-1 Variants

The description of histological findings provided above applies to the large majority of CMT-1 patients who demonstrate autosomal dominant inheritance. It is not thought that a significant difference exists between the histology of CMT-1A and CMT-1B; however, the large biopsy series of the past were performed before the genetic delineation of the 1A and 1B groups and demonstrated homogenous findings, with the exception of the controversial "intermediate" form (Madrid et al. 1977). CMT-1A is a less severe phenotype (on average) than CMT-1B (Dyck et al. 1989, 1993; Bird et al. 1983), and very scanty evidence suggests that nerve biopsy might parallel this (Bird et al. 1983). However, the dramatic variation of biopsy findings demonstrated in one dominantly inherited kinship makes comparison on the basis of single cases meaningless (van Weerden et al. 1982). In another report two related patients (father and son) with CMT-1B showed very frequent myelin tomaculae and a mutation of myelin P_0 protein (Thomas et al. 1994). Such meager evidence makes it difficult to distinguish CMT-1A from CMT-1B histologically.

Some reports of recessive CMT-1 have shown histology similar to that described for dominant CMT, but the available material is scanty (Harding and Thomas 1980b). Early-onset dysmyelinating neuropathies with focally folded myelin or with basal lamina onion bulbs (discussed below with the

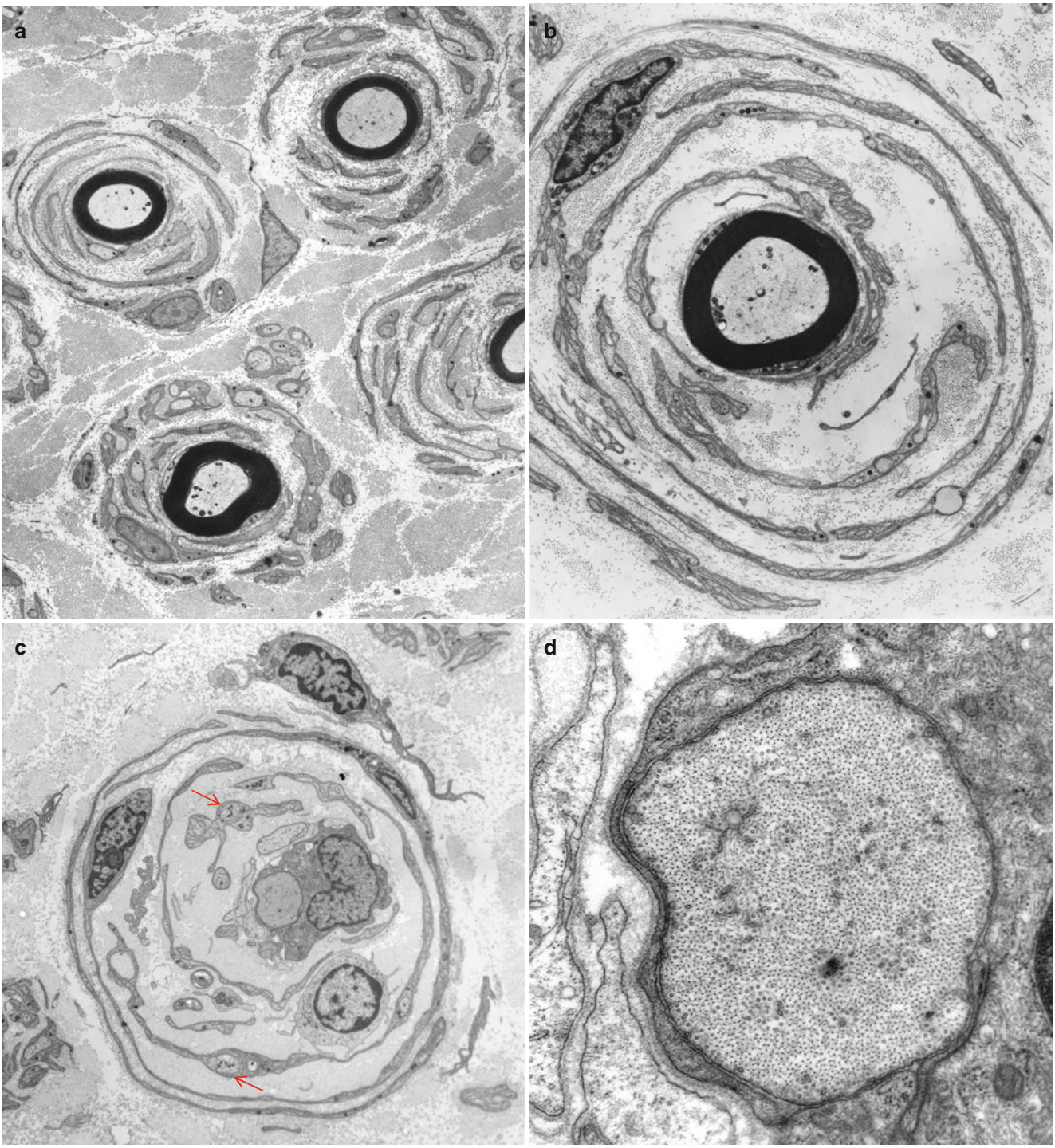


Fig. 19.4 CMT-1: (**a**, **b**) classic onion bulbs consist of concentric imbricated Schwann cell processes alternating with collagen fibers. (**c**, **d**) Note central naked axon in (c) which is also shown at higher magnification in (d). The demyelinated axon contains increased numbers and density of neurofilaments and microtubules, reminiscent of concentrated intra-axonal organelles seen at nodes of Ranvier. A few small unmyelinated axons are seen in surrounding Schwann cell processes (*arrows*, **c**) (Magnification: **a**, 2,240×; **b**, 3,300×; **c**, 5,000; **d**, 40,000×)

congenital dysmyelinating neuropathies) are also recessively inherited and may not be distinguishable from the cases described by Harding and Thomas (1980b).

For X-linked CMT there has been a controversy regarding whether the changes are primarily hypertrophic or neuronal. While Rozear et al. (1987) and Nicholson and Nash (1993) describe features suggestive of a hypertrophic neuropathy in a total of 3 biopsies in X-linked kindreds, Hahn argues that the "onion bulb-like" structures seen in two cases in her laboratory were "pseudo"-onion bulbs formed by regenerating

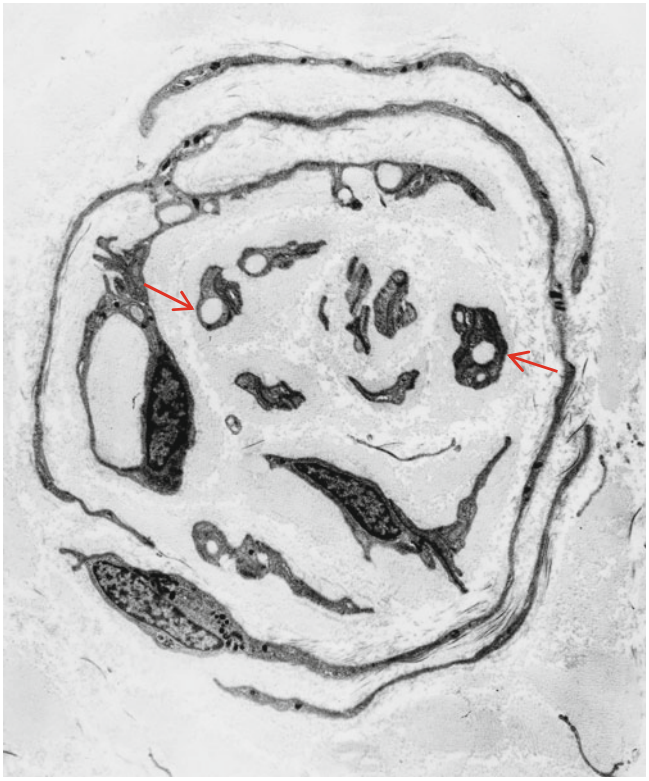


Fig. 19.5 CMT-1, obsolete onion bulb: concentric layers of Schwann cell processes enclose collagen which is arranged longitudinally or circumferentially. Many Schwann cells display collagen pockets (*arrows*) (Magnification: 3,120×)

clusters and that true onion bulbs are not present (Hahn 1993). X-linked CMT is also genetically heterogeneous (Rossor et al. 2013).

The pathological features seen in a case of homozygous AD CMT-1 were not particularly marked, despite the more severe clinical phenotype (Killian and Kloepfer 1979). In a pedigree demonstrating severe dominantly inherited CMT-1, biopsy showed massive onion bulb formation and nerve enlargement, with hypomyelination, more suggestive of Dejerine–Sottas disease than typical CMT-1. Genetic analysis demonstrated a point mutation in the PMP gene on chromosome 22 rather than the more common PMP-22 duplication (vide infra) (Hoogendijk et al. 1993).

A small number of axons with redundant myelin loops or outfoldings may be infrequently seen in classic CMT-1. However, a few cases are found in the literature, in patients with the childhood or adult onset of a peripheral neuropathy syndrome similar to ordinary CMT-1, which emphasize focal myelin abnormalities, described variously as "unstable myelin" "globules," "outfoldings," or "tomaculae." In some patients biopsy showed numerous focal myelin masses, measuring 10–30 μm in thickness and 15–100 μm in length, found in a paranodal and less often internodal location, typical of those seen in hereditary neuropathy with pressure palsies (Barbieri et al. 1990; Malandrini et al. 1992); these may represent phenotypic variants of HNPP. One interesting case combines a clinical phenotype of CMT-1, a histological picture of typical tomaculous neuropathy, and a documented mutation in the myelin P_0 protein (Thomas et al. 1994). A second group of patients combines a clinical picture of CMT-1 with histology revealing frequent focal myelin degeneration into "blebs" or "globules," quite distinct from the tomaculae of HNPP; this group is discussed further below. Possibly a third group is identified by Gabreels-Festen and Gabreels (1993) as "CMT-1 with focally folded myelin," discussed with the congenital dysmyelinating neuropathies (Sect. 19.6.5).

19.2.3 Pathogenesis

19.2.3.1 CMT-1A

CMT-1A is defined as an autosomal dominant disease with duplication or point mutation of the *PMP22* gene on chromosome 17 (Lupski 1993; Dyck et al. 1993; Rossor et al. 2013), a defect which accounts for the largest group of CMT-1 patients. Furthermore, most sporadic CMT-1 patients have tested positive for this mutation (Hoogendijk et al. 1992; Gabreels-Festen et al. 1993; Mancardi et al. 1994). It is suggested that new mutations arise from errors during gametogenesis due to misalignment of a 17 KB tandem repeat segment which may normally serve to line up the chromosome for recombination events (Lupski 1993). A point mutation in *PMP22* is the cause of the mouse Trembler phenotype, long considered a model for CMT-1 (Suter et al. 1992). This notion has been strongly supported by the detection of pedigrees of CMT-1A with a point mutation in the PMP-22 gene, in one case identical to that found in the Trembler mouse (Roa et al. 1993a; Valentijn et al. 1992).

The mechanism by which the genetic defect causes the neuropathy is unknown. A gene dosage effect might be postulated for the majority of patients with CMT-1 who have a duplication. However, a similar phenotype is present in a small number in patients with a point mutation in the *PMP22* gene (Roa et al. 1993a; Valentijn et al. 1992). Deletion of a 1.5 MB segment at 17p11.2, the same region duplicated in CMT-1A, has been consistently associated with hereditary neuropathy with pressure palsies (vide infra Sect. 19.3). In one remarkable pedigree, a patient with a point mutation in one PMP-22 gene and a 1.5 MB deletion in the 17p11.2 region of the other chromosome had a CMT-1 phenotype, while two children with only 1.5 MB deletion had the HNPP phenotype, and another child with the point mutation only was clinically unaffected (Roa et al. 1993b). In this family the point mutation appeared to act in a recessive manner, providing a genetic basis for the

clinical observation of recessively inherited CMT-1 (Roa et al. 1993b; Harding and Thomas 1980a).

PMP22 has the structure of an integral membrane protein, with 4 highly conserved transmembrane domains, mutations in 3 of which have been shown to cause a dysmyelinating neuropathy in humans or animals (Lupski 1993; Suter et al. 1993). PMP22 shows some structural similarities to proteolipid protein, found only in the CNS, and both are seen only in compact myelin. A possible role in intercellular communication has been postulated; however, the normal function of PMP22, and details of precisely how a defect results in the disease phenotype are unclear (Lupski 1993; Suter et al. 1993).

Prior to the discovery of the CMT-1A mutation, a large body of evidence pointed to a primary axonal cause of the disease, with secondary myelin change (Nukada et al. 1983; Dyck et al. 1974, 1993; Smith et al. 1980). Similar evidence had been used as the basis for the conclusion that secondary demyelination is an important mechanism in a variety of neuropathies, including uremia (Dyck et al. 1971a), Friedreich's ataxia (Dyck and Lais 1973), paraproteinemia (Ohi et al. 1985), and the permanent axotomy model (Dyck et al. 1981b). Given that overwhelming evidence now suggests a myelin defect, the question of why there was the appearance of a primarily axonal defect is still relevant. Presumably the role of Schwann cells in regulating the axon's metabolism is more profound than was previously realized, and defects in the first must cause metabolic and physiologic disturbances in the second (Suter et al. 1993) to account for the axonal loss which is a common feature of CMT-1.

19.2.3.2 CMT-1B

The dramatic genetic advances made in sorting out CMT-1A have been applied to the investigation of CMT-1B. The CMT-1B mutation was previously linked to chromosome 1, q21.2-q25, and this coincides with the locus of P_0 protein, the major peripheral nerve myelin protein (Hayasaka et al. 1993a). Point mutations in the P_0 protein have been found in the large pedigrees of CMT-1B identified a decade earlier by linkage analysis (Hayasaka et al. 1993b, d; Kulkens et al. 1993; Himoro et al. 1993; Su et al. 1993). The 9 kb P_0 gene includes 6 exons, of which 2 and 3 are extracellular components, exon 4 is the transmembrane domain, and exons 5 and 6 represent the cytoplasmic domain. The extracellular domain has homologies with the immunoglobulin variable region and has been thought to play a role in the compaction of myelin at the intraperiod line. Disease-associated mutations in the protein's structure are all located at the extracellular segment of the molecule. Interestingly, a point mutation in P_0 protein has been associated with a CMT clinical phenotype and histological features of tomaculous neuropathy (Thomas et al. 1994).

19.2.4 X-Linked CMT

Seven different defects in the gene encoding the gap junction protein connexin-32 have been detected in 8 families with X-linked CMT (Bergoffen et al. 1993b), while unaffected family members and control subjects did not possess the mutations. This type of CMT disease, which is probably the second most common form of the disease (accounting for around 10 % of all CMT patients), is a genetically separate, X-linked condition. More than 250 mutations of the CX32 (connexin 32, gap junction protein β1, GJB1) gene have been found to be the cause of this syndrome (Bergoffen et al. 1993a, b). Males with CMT-X display a more severe clinical, electrophysiological and histopathological disease than heterozygous females, befitting the notion that having two X chromosomes confers some protection. Connexin-32 is a member of a family of transmembrane proteins, the connexins, which assemble to form gap junctions. Immunohistochemistry reveals that these proteins are found in myelinated peripheral nerve fibers at the nodes of Ranvier and at Schmidt–Lanterman incisura (Bergoffen et al. 1993b). The workers identifying this mutation hypothesized that dysfunction of putative intracellular gap junctions at these sites might impair flow of ions and nutrients to the innermost layers of compact myelin and perhaps the axon, resulting in myelin disruption and axonal degeneration (Bergoffen et al. 1993a, b).

The pathological changes are somewhat nonspecific, but just as the condition is often considered "intermediate" between CMT-1 and CMT-2, so too are its nerve conduction velocities and histopathological findings (Sander et al. 1998; Vital et al. 2001). Classic onion bulbs are not very frequent, but "pseudo-onion bulbs" are common. In these formations, a regenerating cluster is centered by a myelinated fiber and surrounded by several unmyelinated axons and their associated Schwann cells. Axon numbers are often relatively preserved in CMT-X, as large myelinated fibers degenerate or atrophy, but regenerative clustering activity provides numerical compensation. As is typical for genetic processes, the histopathological picture appears chronic, with no active axonal loss (i.e., Wallerian degeneration) or active demyelination visible on cross sections, although teased fibers may show some ongoing axonal degeneration.

19.2.5 Differential Diagnosis

When a nerve biopsy shows prominent hypertrophic changes, the most important diagnostic considerations are CIDP and CMT-1. These can usually be distinguished by the pattern of involvement: multifocal in CIDP and diffuse in CMT-1 and definitively by genetic analysis. However, minimal pathological expression of CMT-1 may demonstrate multifocal regions containing onion bulbs interspersed

with fibers of normal appearance (Dyck et al. 1983). Prominent inflammatory infiltrates are not typical of classic CMT-1; however, a few epineurial lymphocytes should not be over interpreted as CIDP. Because of the chronicity of the process, macrophages filled with myelin debris are rare in adult CMT-1, and when several are seen in the same section, CIDP should be considered. We do not accept macrophage-mediated myelin stripping as a feature of CMT-1. If evidence is conflicting, consideration should be given to the possibility of CMT with a superimposed inflammatory neuropathy, which may be steroid responsive (Dyck et al. 1982; Bird and Sladky 1991; Vital et al. 1990; Crawford and Griffin 1991). Gabreels-Festen et al. (1993) have considered this issue, and a detailed discussion is provided in Chap. 9. PMP-22 expression in nerve biopsy material can distinguish between CMT-1A and other demyelinating neuropathies, but there is an overlap between the two groups (Yoshikawa et al. 1994).

In an infant with a dysmyelinating neuropathy, the differential diagnostic possibilities include CMT-1, Dejerine–Sottas syndrome, the other congenital dysmyelinating neuropathies (vide infra), and CIDP. An infantile or early childhood neuropathy progressing for many years can be due to CIDP, and nerve biopsy may be the only means of making the diagnosis (Sladky et al. 1986), although nerve conduction studies are very helpful. In the absence of a family history of dominant inheritance, sporadic infantile CMT-1 is distinguished from Dejerine–Sottas in that the latter shows very prominent hypomyelination and demyelination with a g-ratio >0.7, few or no axons over 6–8 μm in diameter on the myelinated fiber histogram, and prominent (>50 % of myelinated fibers) onion bulb formations at a young age (Ouvrier et al. 1987). The distinction is important because prognosis is better for infantile onset CMT-1 than for Dejerine–Sottas syndrome (Vanasse and Dubowitz 1981; Ouvrier et al. 1987). Moreover, nerve biopsy might suggest the mode of transmission, i.e., dominant (CMT-1) or recessive (Dejerine–Sottas). The available literature suggests that biopsies revealing numerous basement membrane onion bulbs or focally folded myelin are more likely to be recessive (vide infra). Uniformly thin myelination is not specific for Dejerine–Sottas syndrome. We have examined CIDP biopsies in adults with a picture of uniformly distributed, strikingly thinly myelinated axons in an adult, and a similar appearance may be present in metachromatic leukodystrophy (Bardosi et al. 1987). Nerve biopsy features typical of Dejerine–Sottas syndrome were seen in a patient with a point mutation in the PMP gene who had dominant inheritance of a particularly severe CMT-1 phenotype (Hoogendijk et al. 1993) and in a patient with homozygous inheritance of dominant CMT-2 (Sghirlanzoni et al. 1992).

CMT-1 can show dramatic histological variability. In one CMT-1 kinship in which 5 biopsies were performed, one showed classic OBs, 2 showed thinly myelinated axons, and 2 showed only axonal loss (van Weerden et al. 1982).

19.3 Hereditary Neuropathy with Pressure Palsies

19.3.1 Clinical Manifestations

Hereditary neuropathy with pressure palsies (HNPP) is an increasingly recognized entity that has emerged in recent decades. Patients experience recurrent bouts of mononeuropathy, sometimes precipitated by trivial injury to the affected nerve and usually resolving over hours to weeks. Painless peroneal, radial, or ulnar palsies are most common (Meier and Moll 1982; Verhagen et al. 1993; Earl et al. 1964), and a mononeuritis multiplex-like picture may occur (Behse et al. 1972). Brachial plexus involvement is not infrequent, accounting for 8 % of episodes in one review (Meier and Moll 1982), while cranial nerves are only rarely affected. A mild polyneuropathy may be superimposed. Uncommonly, weakness in the territory of a single nerve can be slowly progressive but may remit even after many years if close attention is paid to avoidance of pressure or traction (Earl et al. 1964, case 19-1). Although most patients identify the second or third decades as the time of disease onset, focal paralysis may be present at birth. Alternatively, the diagnosis can be delayed as long as the seventh decade if the recurrent nature and family history of such symptoms are not appreciated (Meier and Moll 1982). The inheritance pattern is autosomal dominant, with complete or nearly complete penetrance but variable disease severity (Verhagen et al. 1993). Genetic studies have shown point mutation in *PMP22* or deletions affecting the region of chromosome 17 harboring the *PMP22* gene (Chance et al. 1992, 1993) in 85 % of patients. Therefore, HNPP can be caused by deletion of the gene *PMP22* while duplication of *PMP22* causes Charcot–Marie–Tooth disease type 1A, and point mutation in *PMP22* may result in either HNPP or CMT1A.

Rarely, patients with the typical histological findings of HNPP seem to suffer a slowly progressive, distally predominant sensorimotor polyneuropathy (Madrid and Bradley 1975; Malandrini et al. 1992; Barbieri et al. 1990; Pellissier et al. 1987) similar to the Charcot–Marie–Tooth syndrome. It is unclear if this is a phenotypic variant, a spurious consequence of insufficient detailed electrophysiological testing (Felice et al. 1994), or an entirely different disease, perhaps CMT-1B (Thomas et al. 1994). The pediatric cases of HNPP reported by Gabreels-Festen and colleagues (1992a) often had a chronic progressive neuropathy, while a history of recurrent focal palsies could be found in some of the affected older relatives, suggesting that HNPP can manifest as a chronic progressive neuropathy in early life, with the typical focal palsies coming

later. Other pedigrees exist in which some affected individuals showed recurrent focal palsies while others had only a chronic neuropathy (Leblhuber et al. 1991).

Electrophysiological testing reveals a diffuse abnormality of peripheral nerves, with mild-moderate slowing of conduction, worse distally, even in clinically unaffected nerves. Sites of nerve compression are particularly likely to be affected. EMG reveals neurogenic changes, usually chronic, indicating that axonal degeneration does occur (Behse et al. 1972). CSF examination is unremarkable.

19.3.2 Pathology

19.3.2.1 General Considerations

Although Behse and colleagues (1972) were the first to describe the classic pathology, it was Madrid and Bradley (1975) who coined the term "Tomaculous Neuropathy." At least two of the four patients described in this report suffered from recurrent pressure palsies, and the term "tomaculous neuropathy" has often been used synonymously with HNPP. However, myelin "tomaculae" are seen in other settings such as the dysmyelinating neuropathies and some patients with a Charcot–Marie–Tooth syndrome (vide supra). Thus, the term "tomaculous neuropathy" should be used in a descriptive sense only. The histological features of HNPP are well described (Madrid and Bradley 1975; Behse et al. 1972; Verhagen et al. 1993; Pellissier et al. 1987; Yoshikawa and Dyck 1991; Drac 1989), and the pathology does not appear to differ significantly in children (Gabreels-Festen et al. 1992a). We have examined six cases.

19.3.2.2 Light Microscopy

Light microscopic examination usually reveals a normal myelinated fiber density and mild nonspecific changes such as endoneurial fibrosis or vascular hyalinization, although occasionally individual markedly enlarged axons can be seen in H&E-stained paraffin sections (Fig. 19.6a, b). The striking abnormality is the presence of many fibers, large and small myelinated axons alike, with a myelin sheath which may be thickened circumferentially or eccentrically or may show prominent focal outfoldings and infoldings (Fig. 19.6c–f). Such changes are seen in <1 % to 10 % of axons in cross sections. The most dramatic circumferentially hypermyelinated fibers often show a "jelly roll" appearance (Figs. 19.6c, e, f and 19.7). Axons associated with such configurations often seem to be constricted, with an abnormally dense axoplasm. In the same specimen, some axons have inappropriately thin sheaths, suggesting demyelination and remyelination.

In HNPP, intramuscular nerves may show focal myelin swellings (Oda et al. 1990), but these should be distinguished from axonal swellings, which are a common and nonspecific abnormality (Alderson 1992).

Most often myelinated fiber counts are normal, and active axonal degeneration is rare. In a total of 29 cases from three large series (Pellissier et al. 1987; Verhagen et al. 1993; Behse et al. 1972), only 3 showed a mild-moderate reduction in axon numbers. However, the rare observation of degenerating axons and regenerating clusters suggests that axons are not entirely spared in HNPP (Verhagen et al. 1993). The largest diameter fibers are reduced in number relative to controls (Behse et al. 1972; Pellissier et al. 1987; Verhagen et al. 1993). Unmyelinated fibers seemed to be entirely spared in one study (Behse et al. 1972) but showed a shift in diameter towards the small end of the spectrum in another (Pellissier et al. 1987). These changes in the myelinated and unmyelinated axon diameter-frequency histograms might be interpreted as due to axonal atrophy (which may be secondary to myelin abnormalities in myelinated fibers) or as a consequence of degeneration and regeneration.

19.3.2.3 Electron Microscopy

The true nature of myelin tomaculae is revealed best with electron microscopy (Fig. 19.7). All sizes of axons are involved, even individual fibers in a regenerating cluster. Madrid and Bradley (1975) provide a detailed exposition of the various alterations. The most common formation is a double-folded redundant myelin loop that wraps around the axon to varying degrees, forming up to 3 or 4 complete tightly packed turns (Fig. 19.8a) but can be mimicked by compaction of a spiraled redundant myelin loop (Fig. 19.8b). Most often the redundant loops wrap around the outside of the fiber, but they may turn inwards and wrap around the axon internally (Madrid and Bradley 1975). Infolded or outfolded redundant myelin loops that are not adherent to the contour of the axon are common (Fig. 19.9). The existence of true circumferential hypermyelination, defined as a sheath that is normally constructed except for a pathological increase in the number of myelin lamellae, remains to be proven (Fig. 19.8c) (Meier and Moll 1982; Madrid and Bradley 1975; Behse et al. 1972). Whereas normally there are less than 200 myelin lamellae around even the largest of sural nerve axons, up to 480 such lamellae were described in one report (Meier and Moll 1982). However, Yoshikawa and Dyck (1991) feel that close examination of "hypermyelinated" fibers (such as Fig. 19.8b) will show that the increase in myelin lamellae is due to either folding of internodal myelin in a circumferential or longitudinal direction or a result of relative axonal atrophy. In the cases we have examined the fragility of massive myelin tomaculae made it impossible to find a grossly hypermyelinated axon (Fig. 19.8c) whose lamellae were sufficiently well preserved to resolve this issue.

Other myelin alterations may be seen. In hypermyelinated fibers, the sheath often appears somewhat ragged, and there may be frank myelin degeneration with debris and denuded axons (blue arrow, Fig. 19.7). Onion bulb formations may be seen but are not usually a dominant feature (arrowhead, Fig. 19.7). At nodes of Ranvier, myelin from one side may seem to overgrow the node and bridge over into the adjacent internode, so-called transnodal myelination. Some workers

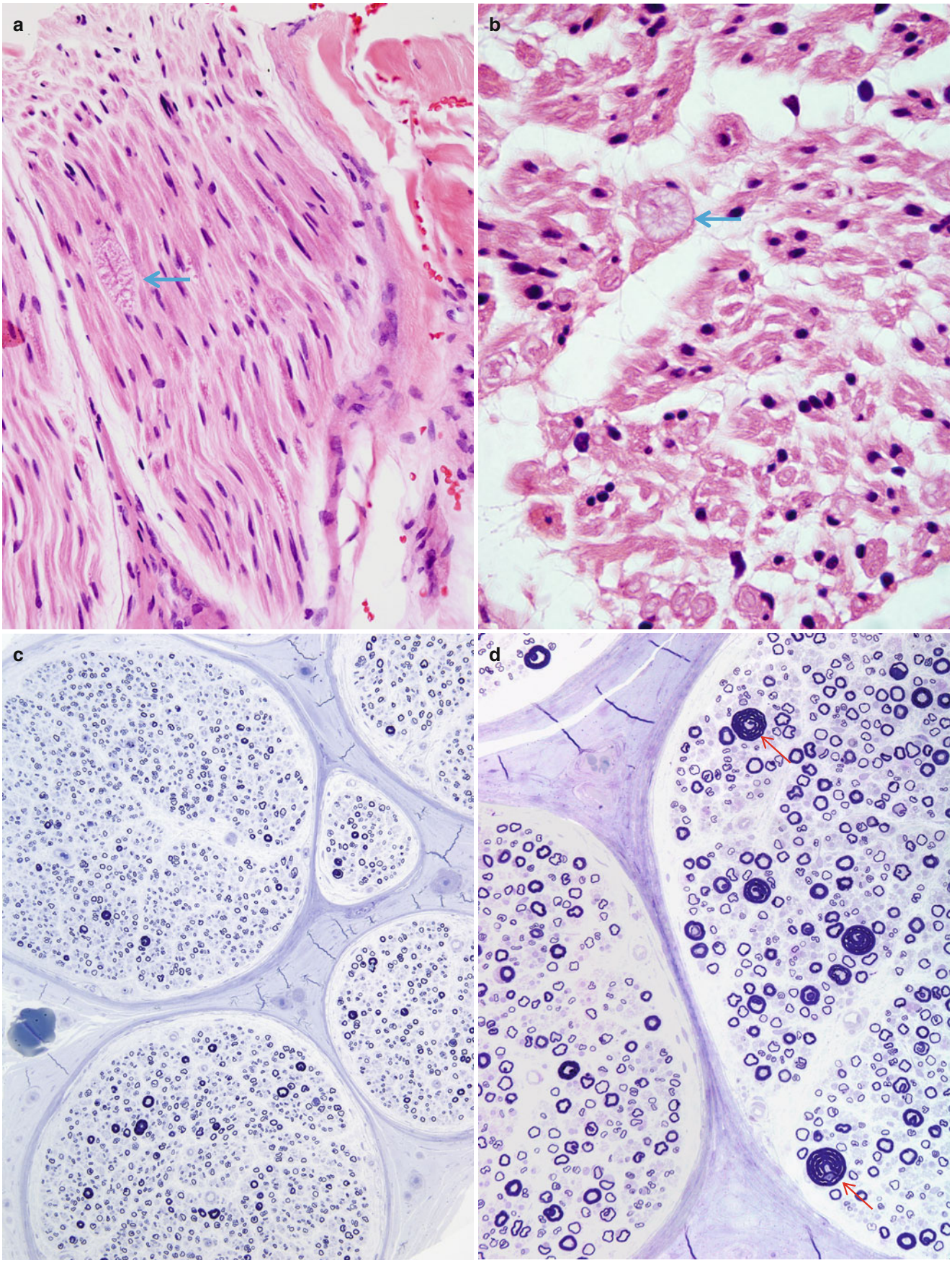


Fig. 19.6 HNPP: (**a**, **b**) H&E-stained nerve shows a single tomaculum visible in this fascicle in longitudinal (*arrow*, **a**) and cross section (*arrow*, **b**). (**c**–**f**) Note the relative preservation of axons (**c**), wide variation of myelin thickness relative to axon diameter and scattered "jelly rolls" (*arrows*, **d**–**f**) (Magnification: **a**, **b**, 600×, H&E paraffin; **c**, 200×,1 μm thick plastic section; **d**, 400×,1 μm thick plastic section; **e**, **f**, 1,000×)

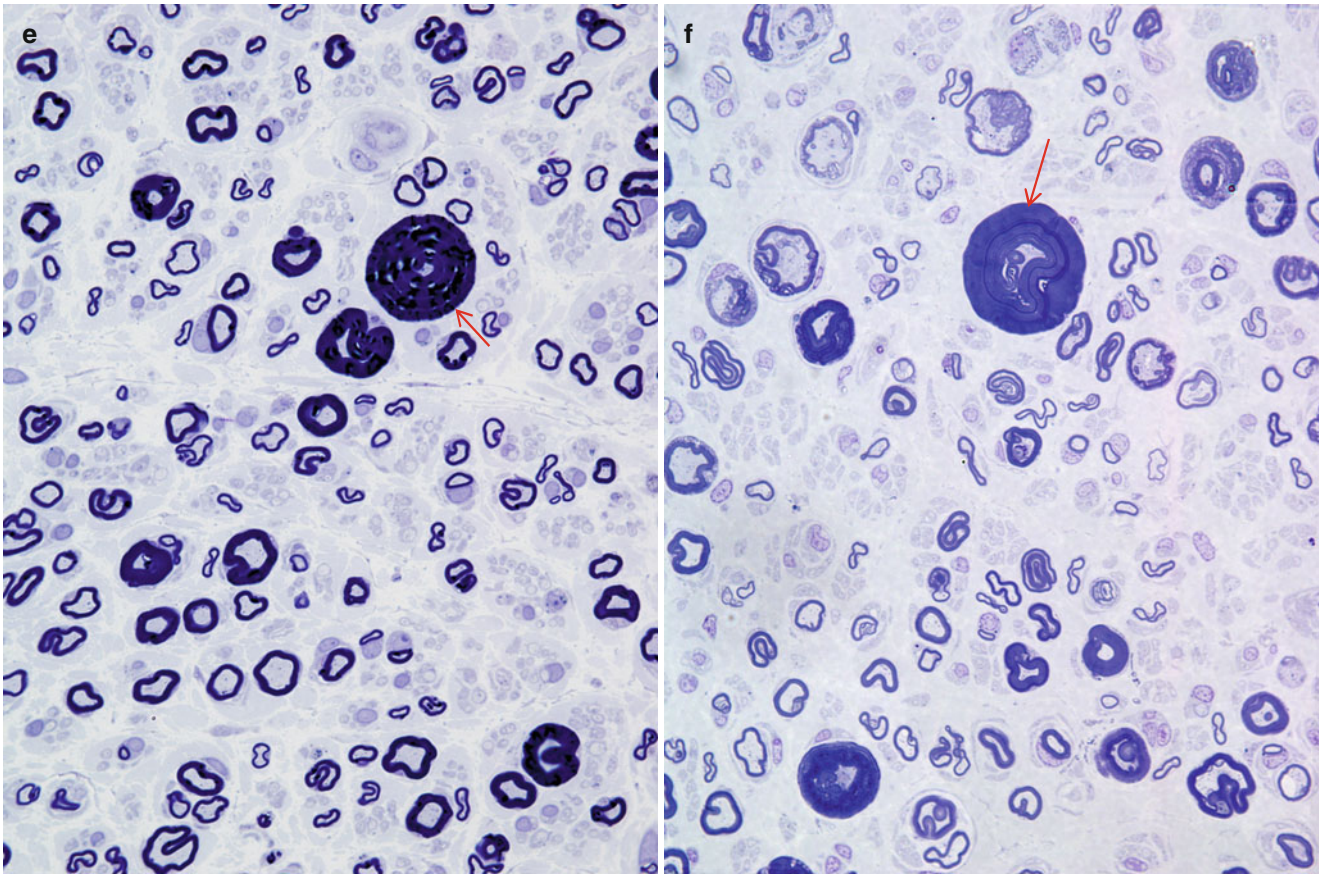


Fig. 19.6 (continued)

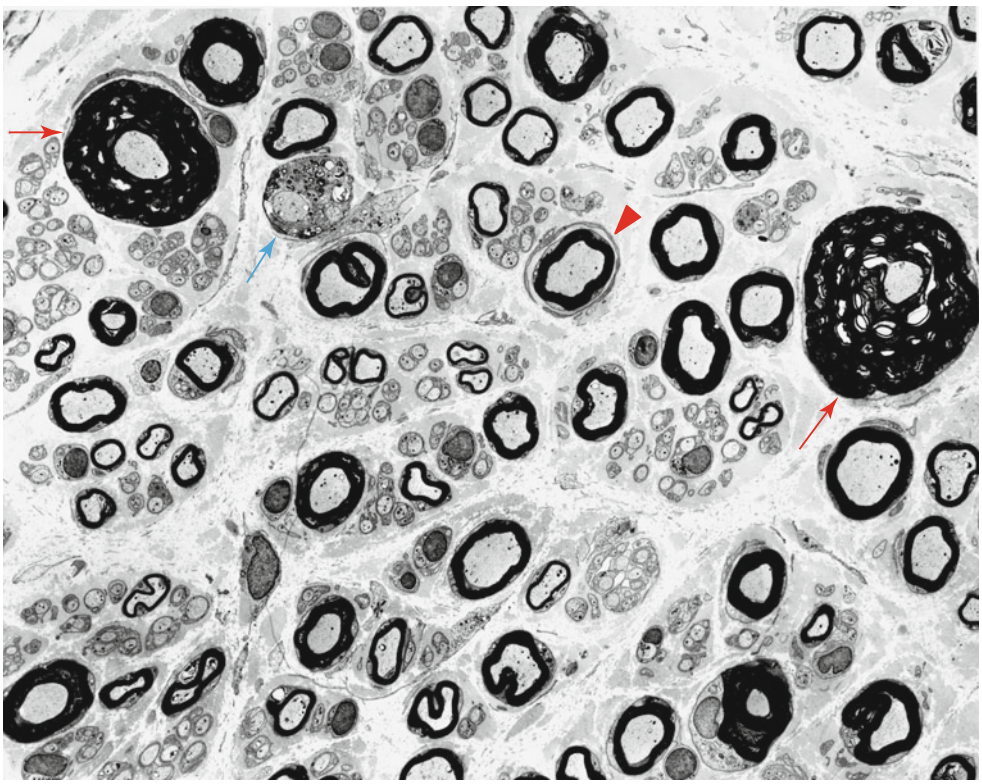


Fig. 19.7 HNPP: low-power view showing the spectrum of findings including "jelly rolls" (*red arrow*), active demyelination (*blue arrow*), and rudimentary onion-bulb formation (*arrowhead*) (1,740×)

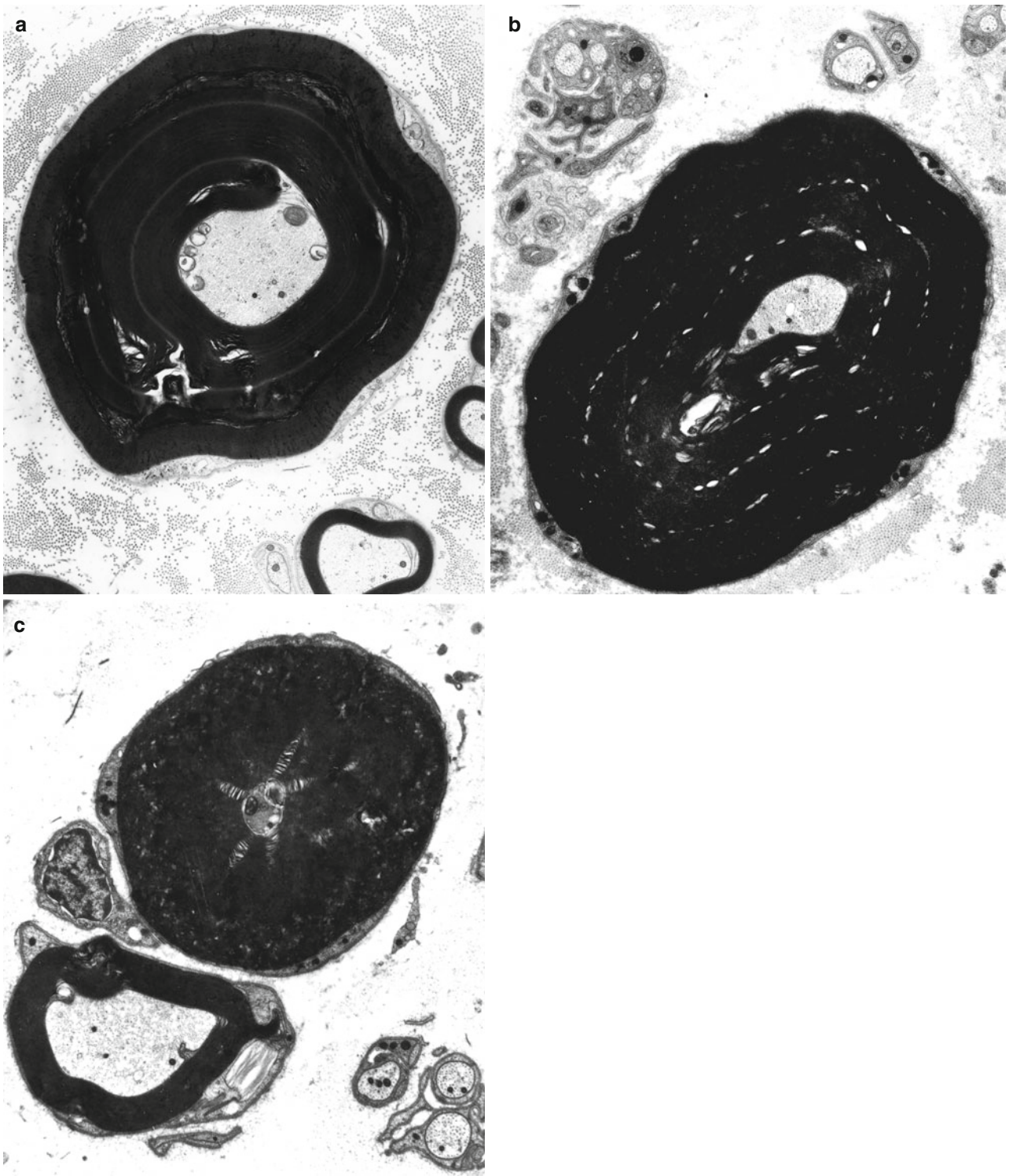


Fig. 19.8 HNPP: "hypermyelination," spurious "hypermyelinated" fiber formed by compaction of spiraled redundant myelin loop is shown in (**a**, **b**). Ultrastructural examination failed to resolve the nature of increased myelin thickness in (**c**) (**a**, 5,000×; **b**, 6,160×; **c**, 6,840×)

were able to demonstrate two Schwann cell nuclei per internode (Madrid and Bradley 1975). Uncompacted myelin has been described in HNPP (Yoshikawa and Dyck 1991; Jacobs and Gregory 1991; Hall 1994) and may appear as enlarged Schmidt–Lanterman incisura (Debruyne et al. 1980). This seems to be an uncommon finding that requires an extensive search, for the majority of ultrastructural descriptions of HNPP do not comment on uncompacted myelin, even when

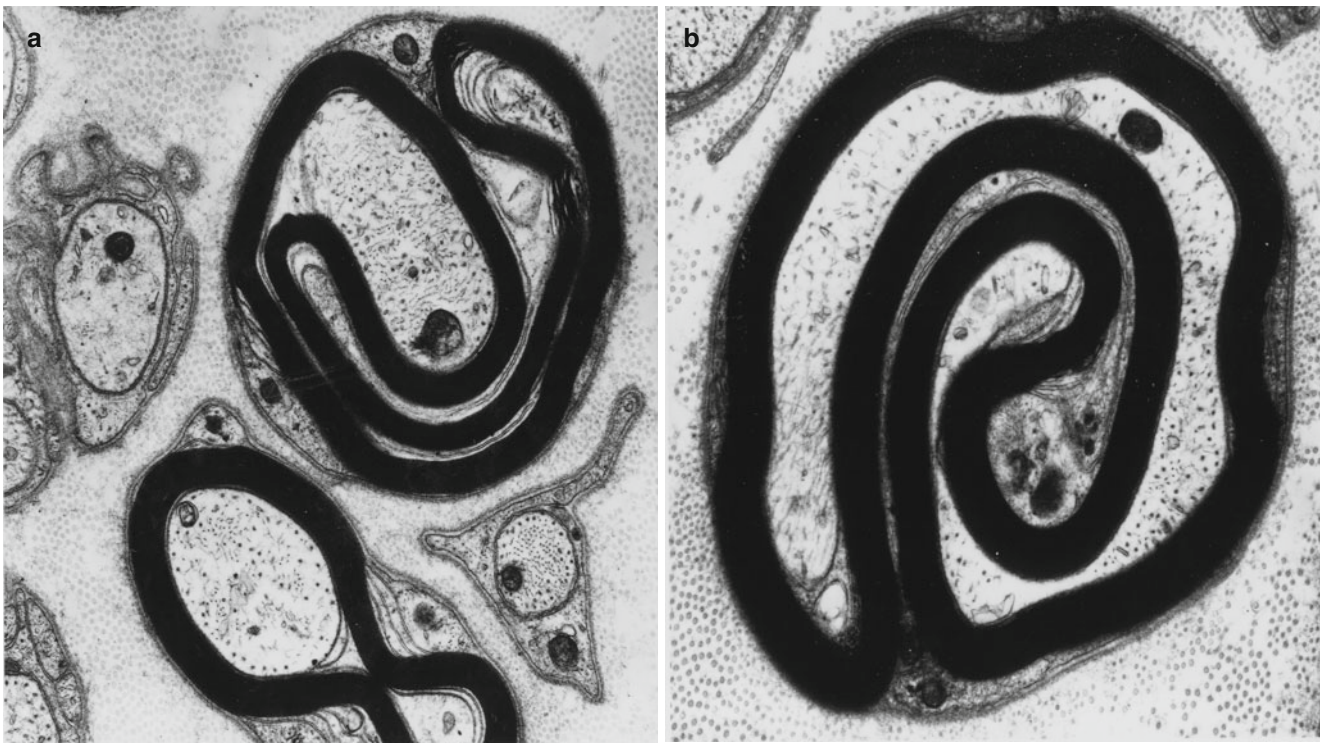


Fig. 19.9 HNPP: redundant outer and inner loops of attenuated myelin. In (**b**) note the unusual axonal configuration, almost divided by the myelin infolding (**a** ×15,600, **b** ×18,000)

specifically looking for it (Verhagen et al. 1993). We have not observed convincing examples of this alteration in our material. The uncompacted lamellae are said to consistently be found on the axonal aspect of the myelin sheath and were seen on normal and on demyelinated/remyelinated internodes alike (Yoshikawa and Dyck 1991; Jacobs and Gregory 1991).

The hyperplastic myelin sheath seems to be unduly fragile and is often seen to be disrupted in a variety of ways, including focal intramyelinic vacuoles, myelin splitting, granular or vesicular myelin disintegration, and at times complete degeneration. This does not occur to the extent seen in the "Neuropathies with Unstable Myelin " (Sect. 19.2.5, above). Focal collections of degenerating myelin are often located between an intact outer myelin layer and the axon.

Some authors have used the size of myelin tomaculae as a criterion for distinguishing the myelin swellings in HNPP from those of other tomaculous neuropathies. In HNPP tomaculae can be up to 40 µm in width (axon + thickened myelin), with a longitudinal extension of 30–300 µm (Meier and Moll 1982; Behse et al. 1972).

At times, an infolded myelin loop or circumferentially thickened myelin may appear to constrict, even obliterate, of the axon within. The axon may show increased filament and tubule density. Serial cross sections of a single fiber indicate that proximal and distal to the area of constriction the axon can regain its normal diameter (Behse et al. 1972). This may be an example of the postulated "demyelinative internal strangulation" mechanism of axonal degeneration discussed by Dyck et al. (1993).

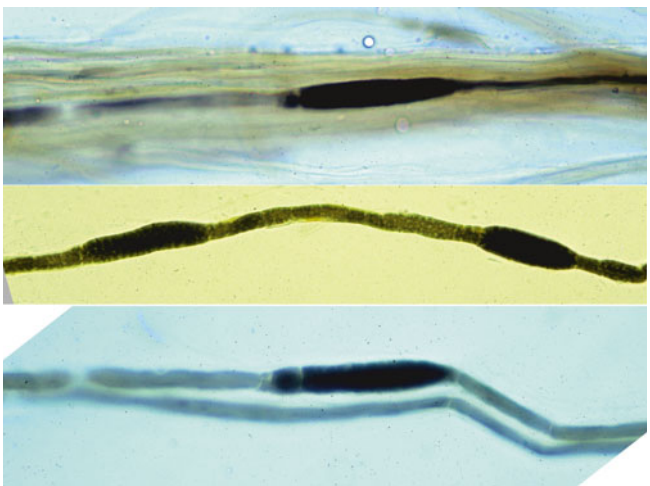


Fig. 19.10 HNPP: teased nerve fiber preparation. This shows characteristic finding of tomaculae and remyelinated internodes

19.3.2.4 Teased Fiber Studies

Tomaculous neuropathies are dramatically demonstrated upon examination of teased fibers (Fig. 19.10). The focal nature of the myelin swellings is best appreciated, as is the distribution, paranodal or internodal (Drac 1989). Typically 25–50 % but anywhere from 5 to 82 % of internodes show

focal myelin swellings (Madrid and Bradley 1975; Behse et al. 1972; Verhagen et al. 1993; Pellissier et al. 1987; Drac 1989). Several tomaculae can be seen in one internode, up to 6 in one study (Madrid and Bradley 1975). Teased fibers will also reveal evidence of segmental demyelination and remyelination.

19.3.2.5 HNPP without Myelin Tomaculae

Not all patients with HNPP actually show myelin tomaculae (Castaigne et al. 1976; Earl et al. 1964; Mayer and Garcia-Mullin 1968; Roos and Thygesen 1972; Pellissier et al. 1987; Grossiord et al. 1973; Lhermitte et al. 1973; Attal et al. 1975), although some of the cases and pedigrees described are less than convincing, and electron microscopy, fiber teasing, and semithin toluidine blue-stained sections were often not performed in these reports. The findings include entirely normal biopsies, diffuse loss of myelinated fibers, or nonspecific segmental myelin changes. Absence of the tomaculous change and emergence of fiber loss may indicate a late stage in the evolution of HNPP (Windebank 1993), but many biopsied patients showing tomaculous change have had symptoms for decades (case 19.1).

19.3.3 Pathogenesis

Madrid and Bradley (1975) have discussed the mechanisms that might form the myelin swellings of HNPP, including infoldings, outfoldings, and splitting of the mesaxon. These authors pointed out that similar findings are seen during myelinogenesis but that ordinarily myelin remodeling produces the normal smoothly contoured sheath. The morphologic findings of HNPP are not disease specific, but can be seen, albeit less dramatically, in other demyelinating diseases. A reasonable postulate is that there is a defect in either the ability of the axon to send regulating signals to the Schwann cell or a defect in the Schwann cell's ability to interpret signals from the axon.

Yoshikawa and Dyck (1991) observed uncompacted myelin in HNPP and found evidence that this change could precede segmental demyelination. The suggestion was made that the neuropathy might arise due to a defect in membrane proteins or lipids involved in myelin compaction, P_0 protein and myelin-associated glycoprotein being identified as leading candidates. Gross chemical and immunohistochemical analysis of the myelin produced in HNPP reveals no abnormalities of composition, and Schwann cell cultures from patients with HNPP showed no special features when compared to controls (Federico et al. 1989).

In 1993 a chromosome 17 deletion was found to be strongly associated with HNPP in 3 pedigrees (Chance et al. 1993), and this observation has subsequently been confirmed in both inherited and sporadic cases of HNPP (Verhalle et al. 1994; Roa et al. 1993b; Reisecker et al. 1994). Remarkably, the deleted region is seemingly identical to that normally duplicated in CMT-1A (vide supra), such that only one copy of the PMP-22 gene is present in patients with HNPP. Although attention has focused on *PMP22*, other genes in this deleted region or genes disrupted by the deletion could contribute to the problem. Mutation in the myelin P_0 protein has recently been reported to cause a neuropathy with clinical features of CMT-1 and histological features of HNPP (Thomas et al. 1994).

19.3.4 Differential Diagnosis

Focal myelin swelling, excessive myelin infolding and outfolding, and circumferential hypermyelination are in and of themselves nonspecific, occurring to varying degrees in classic CMT-1, CMT-3, congenital dysmyelinating neuropathy, IgM paraprotein-associated neuropathy, and neuropathy with unstable myelin. Such alterations can also be present to a limited degree in patients with nonspecific neuropathy or even without neuropathy (Drac 1989). However, in the large HNPP series reviewed, above no less than 5 %, and usually a greater proportion, of internodes contained tomaculae (Drac 1989). This is beyond the frequency of such changes in patients with classic CMT-1 or Dejerine–Sottas syndrome (vide infra) or in controls (Drac 1989). No diagnostic significance can be attached to a nerve biopsy with myelin swellings or foldings on less than 1 % of internodes (Drac 1989).

A growing number of patients and pedigrees have been described where histological findings typical of tomaculous neuropathy were found in association with an atypical history: suggestive of either CMT (neuronal or hypertrophic) (Malandrini et al. 1992; Madrid and Bradley 1975; Barbieri et al. 1990; Gabreels-Festen et al. 1992b; Thomas et al. 1994) or even GBS (Joy and Oh 1989). It is unclear if these represent phenotypic variants of the same disease or if they are altogether different entities with tomaculum formation as a nonspecific response. The severity of axonal loss described in some of these reports is more typical of CMT-1 than of HNPP, and a mutation in the myelin P_0 protein has been identified in one instance (Thomas et al. 1994), suggesting that at least some of these cases represent HSMN-1B rather than HNPP.

The patients designated by Gabreels-Festen and Gabreels (1993) as "CMT-1 with focally folded myelin" have myelin tomaculae that resemble those of HNPP in size, ultrastructural appearance, and frequency (vide supra). However, hypomyelination, onion bulb formation, and especially axon loss are much more prominent in this group than in HNPP. Clinically there should be little trouble distinguishing the two, as in "CMT-1 with focally folded myelin" focal palsies are not a feature, the phenotype and electrophysiological findings are more severe, and autosomal dominant inheritance is not usually seen.

In IgM paraproteinemic neuropathy, typically associated with antibody activity against myelin-associated glycoprotein, redundant myelin folds and hypermyelination very similar to that seen in HNPP are sometimes seen (Vital et al. 1989). Widely spaced, not uncompacted, myelin is present in IgM paraproteinemic neuropathy, allowing a distinction to be made purely on morphologic grounds, although clinically there is no difficulty separating the two.

Frequent "sausage-like" myelin swellings apparently typical of HNPP have been described by Said and colleagues in alcohol-associated "acrodystrophic" neuropathy (Said 1980), uremic neuropathy (Said et al. 1983), and in cytomegalovirus neuritis in a patient with AIDS (Said et al. 1991). However, the ultrastructural correlate of these swellings was not provided, other workers have not reported such data, and we have never made this observation in a number of nerves taken from patients with these diseases.

We wish to emphasize that "tomaculous neuropathy" is not synonymous with HNPP. If typical histological features of the tomaculae are present, HNPP can be diagnosed with a high degree of confidence, although a small group of patients with a chronic progressive neuropathy will remain whose classification is uncertain at present. Molecular genetics should help resolve this issue. However, detection of rare myelin tomaculae or redundant myelin loops is of little diagnostic utility.

19.3.5 Inherited Recurrent Brachial Plexus Neuropathy (Hereditary Neuralgic Amyotrophy)

Autosomal dominant inherited tendency to recurrent brachial plexopathy (inherited brachial plexus neuropathy = IBPN) has been described (Windebank 1993). Symptoms of the individual attack are identical to those of idiopathic brachial neuritis. Pain, which can be very severe, heralds the onset of paralysis by hours to days. Weakness occurs predominantly in a proximal upper limb distribution but can be present in the distal arm, cranial nerves, and lower limbs. Recovery is the rule, although this may take many months. Nosologically, this entity poses a problem, for it shares many features with HNPP. Patients with HNPP may have episodic weakness involving brachial plexus muscles, and patients with IBPN can involve the lower limbs (Dunn et al. 1978). Compression or traction on the brachial plexus may precipitate an attack in both entities. Inheritance is autosomal dominant and typical age of onset is in the second or third decade for both. Nevertheless, most neurologists separate the two based on clinical and electrophysiological criteria (Windebank 1993; Verhagen et al. 1993), although there are dissenters (Martinelli et al. 1989). Pain with paralysis is an almost invariable feature of IBPN, and preceding infection and pregnancy are common precipitating factors. Neither of these is true for HNPP. In HNPP electrophysiological tests reveal evidence of a diffuse sensorimotor polyneuropathy (Behse et al. 1972). In IBPN most studies have pointed to isolated involvement of the plexus (Windebank 1993), but not all have found this (Dunn et al. 1978).

There is little histological material on IBPN, presumably because it does not seem rational to biopsy the sural nerve in a disease of the brachial plexus. In one case studied at the Mayo clinic, no abnormality was found on exploration and fascicular biopsy of the brachial plexus (Windebank 1993). Verhagen et al. (1993) make reference to two patients in which sural nerve biopsy similarly did not reveal myelin tomaculae. Arts et al. (1983) found no myelin tomaculae in nerve biopsy and autopsy of 2 patients with IBPN. This data would suggest that IBPN is a different entity with different histological findings than HNPP.

Several pedigrees have been described with familial recurrent brachial plexopathy and myelin tomaculae on nerve biopsy (Martinelli et al. 1989; Pou Serradell et al. 1992). These patients had a painless disease, showed diffuse abnormalities on electrophysiological testing, and are thus likely to represent examples of HNPP with selective involvement of the brachial plexus. However, case 1 reported by Madrid and Bradley (1975) had prominent pain with her illness and tomaculae on nerve biopsy, as did the case we report below. The IBPN locus had been mapped to chromosome 17q24-25, and recently, genetic analysis has shown that IBPN is caused by mutation in the *SEPT9* gene (Hannibal et al. 2009) which encodes a septin family member, a family of genes most involved in cytokinesis and cell cycle control. However, there remains a proportion of families with IBPN without the genetic abnormalities described above, indicating the existence of other genes involved.

Case 19.1

At the age of 12, this patient developed weakness and pain involving the right shoulder. He was unable to lift the arm due to weakness, and some muscle atrophy was subsequently noted. A diagnosis of polio was made. The weakness gradually improved over several months. A right wrist drop developed at the age of 17 and recovered over 6–12 months. The patient was fit enough to join the army subsequently. Intermittent paraesthesias, particularly involving the ulnar nerve territory and more prominent on awakening, plagued the patient over during his third decade. A few moments of pressure on any nerve resulted in numbness and tingling lasting for minutes to hours. After crossing his legs, he often found that he experienced difficulty walking for a few minutes. At the age of 38, the patient experienced sudden onset of pain and weakness in the left shoulder girdle, and a diagnosis of brachial neuritis was ultimately made. Despite initially complete paralysis of arm abduction and definite atrophy, full recovery was eventually attained. At the age of 43 the patient presented for neurological consultation with a complaint of bilateral symmetrical hand and foot weakness and clumsiness.

The patient had no siblings but two sons were said to be normal. His father was said to have "numb hands" and wasting of small hand muscles. Neurological examination documented normal muscle bulk, normal power, normal reflexes, and a slight multimodality decrease of sensation in a right ulnar nerve distribution.

Nerve conduction studies showed focal conduction slowing across the right ulnar nerve at the elbow, the right median nerve at the wrist, and the right peroneal nerve across the fibula. All F-wave responses were slowed. Motor conduction velocities of the ulnar and median nerves in the forearms were on the lower limit of normal, and the peroneal nerve below the fibula was definitely slowed at 33 m/s. Sensory potentials were moderately delayed and diminished in amplitude. Subsequent examination of the patient's sons showed no clinical abnormalities, but conduction slowing was found across some sites of compression.

Biopsy confirmed a clinical diagnosis of HNPP. Subsequently the patient underwent bilateral carpal tunnel release and transposition of the right ulnar nerve, resulting in considerable improvement in his hand symptomatology and function (clinical material courtesy of Dr. P. Ashby).

19.4 CMT-2

19.4.1 Clinical Manifestations

Neuronal (type 2) CMT (CMT-2) is the typically autosomal dominant (50–70 %) neuronal form of the disease showing motor nerve conduction velocities of 38 m/s or greater, with 25–50 % sporadic and 5 % recessive cases in the largest series (Harding and Thomas 1980c; Bouche et al. 1983; Berciano and Combarros 1990). Segregation analysis suggests that 25 % of sporadic cases are recessive and the rest new dominant mutations (Harding and Thomas 1980c). Genetic analysis of autosomal dominant CMT-2 now has identified 19 gene defects and 5 abnormal genes in autosomal recessive CMT-2. Its prevalence is about one-third that of CMT-1 and CMT-2A; its most frequent form (20 % of CMT-2 cases) is associated with a missense mutation of *Mfn2* (mitochondrial fusion protein 2 gene). Mitofusin (Mfn2) is a mitochondrial membrane protein that participates in mitochondrial fusion in mammalian cells and in pyruvate, glucose, and fatty acid oxidation, defects of which culminate in reduced mitochondrial membrane potential. Experimental data support the notion that Mfn2 loss of function causes axonopathy by impairing energy production along the axon which may reflect abnormal tethering of mitochondria to ER (Pich et al. 2005). Type 2A CMT is clinically similar to CMT-1, although on average the phenotype is milder (peripheral nerves are not enlarged) and the disease develops later (mean age of onset 20 years), there is less prominent involvement of upper limbs (particularly the hands) and less ataxia, tendon areflexia, and sensory loss. Mutations in the *RAB7* (small GTPase late endosomal protein) gene cause CMT-2B, a predominantly sensory neuropathy complicated by chronic foot ulcers and amputations (Lawson et al. 2005). Type CMT-2D is associated with mutations of the *GARS* (glycil tRNA synthetase) gene and consists of upper extremity wasting and weakness, with corresponding sensory impairment; lower extremities are involved to a lesser degree (Lawson et al. 2005). Mutations in the *NEFL* gene are associated with axonal neuropathy and designated as CMT-2E. This condition is characterized by variable severity and sensory ataxia. NCS may show preservation of normal values or slight slowing. Pathological findings are of interest in that they reveal the presence of giant axons with attenuated myelin and containing aggregates of disorganized neurofilaments (Pareyson et al. 2013).

Autosomal recessive forms of CMT-2 are called autosomal recessive CMT (AR-CMT). Recessive mutations of the *LMNA* (laminin A/C) gene are the cause of the axonal neuropathy in AR-CMT-2 (formerly known as CMT-4C and CMT-2B1). Whether a neuronal X-linked form exists is unclear (Hahn 1993; Dyck et al. 1993). Clinical manifestations are much like those of CMT-1, summarized above. No clinical features definitively distinguish the two, although onset in the first decade and palpable nerves suggest the hypertrophic form, and variations in distribution of atrophy, weakness, and reflex changes may be helpful (Hahn 1993). As with CMT-1, examination of "normal" family members is essential because the disease may be asymptomatic.

Patients with childhood onset are more severely affected, often resulting in debilitation by adulthood (Ouvrier et al. 1981; Gabreels-Festen et al. 1991). Most of these are probably genetically distinct from dominantly inherited CMT-2. From a total of 29 cases with childhood onset and a severe clinical course (Gabreels-Festen et al. 1991; Ouvrier et al. 1981), only 3 had a dominant inheritance pattern, 8 had similarly affected siblings and normal parents suggesting recessive inheritance, and most were sporadic.

Nerve conduction studies are usually the basis for a definitive distinction of CMT-2 from CMT-1, but rarely there are situations where the conduction velocities are slowed, presumably due to the selective and sometimes very severe loss of large (rapidly conducting) myelinated fibers (vide infra). Conduction velocity is usually normal or near normal, with reduced amplitudes suggesting loss of axons. EMG shows changes of very chronic denervation. CMT-2 has been reviewed by Hahn (1993), Dyck et al. (1993), and genetic analysis (Rossor et al. 2013; Saporta and Shy 2013; Sagnelli et al. 2013; Tazir et al. 2013; Vallat et al. 2013).

19.4.2 Pathology

There are several large CMT-2 biopsy series (Behse and Buchthal 1977; Gherardi et al. 1983; Ouvrier et al. 1981; Berciano et al. 1986; Gabreels-Festen et al. 1991).

19.4.2.1 Light Microscopy

Histologically, this disorder displays some neuron loss in ventral horn and spinal ganglia of the lumbosacral region and tract degeneration in the gracilis fasciculi. In peripheral nerves there is mostly chronic axonal loss affecting large myelinated fibers, axonal atrophy as evidenced by an increase in the axon caliber/myelin thickness ratio, abundant regenerating clusters, and no hypertrophy. Endoneurial edema and fascicular enlargement are not features of CMT-2 biopsies, but atrophy of the nerve fascicle is occasionally seen. In longstanding cases fibrosis is prominent. Myelinated fiber numbers are usually less dramatically reduced than in CMT-1 of comparable duration, with a more selective loss of large MFs. Unmyelinated fibers are spared or only slightly reduced in number with autosomal dominant adult cases, but may be significantly reduced in recessive/sporadic childhood cases. Interpretation of axon numbers is difficult due to the high frequency of regenerating clusters. In the recessive and infantile onset forms, MF loss can be very severe, often with no axons greater than 6 μm in diameter remaining (Gabreels-Festen et al. 1991). In one extreme case all remaining MFs were within the 0.6–1.8 μm diameter size range! Despite the severity of axonal loss, Wallerian degeneration is a rarity, even in the more severe recessive/childhood cases.

In contrast to CMT-1, regenerating clusters are quite frequent in dominant CMT-2, quantified at 20–400/mm^2 (normal being <20/mm^2) (Gherardi et al. 1983; Behse and Buchthal 1977) (Fig. 19.11a–d, arrows, d). This has also been expressed as the "cluster ratio," measuring the number of regenerating clusters per 1,000 MFs, and values in dominant CMT-2 range from 2.4 to 38 (Ouvrier et al. 1981; Gherardi et al. 1983; Gabreels-Festen et al. 1991), with normal being less than 2, often 0, in adults (extrapolated from data of Gherardi et al. (1983) Behse and Buchthal (1977)). In the severe recessive or sporadic childhood cases described by Gabreels-Festen et al. (1991) and Ouvrier et al. (1981), regenerating clusters were usually not seen. One exception was an autosomal dominant case (Gabreels-Festen et al. 1991), which had a cluster ratio of 8.1. An interesting observation is that cluster numbers diminish more distally in a nerve, and with increased disease duration, presumably because the distal axon is "sicker" and less able to regenerate (Berciano et al. 1986; Gherardi et al. 1983).

The presence of MFs with inappropriately thin myelin sheaths is variable, but can be surprisingly prominent for a neuropathy which is not regarded as having a demyelinating component. Fiber teasing may demonstrate an increase in segmental myelin alterations above normals, and it is presumed that these are secondary to axonal atrophy (Berciano et al. 1986; Dyck et al. 1993).

19.4.2.2 Electron Microscopy

Well-formed onion bulbs are absent or rare in CMT-2, even by ultrastructural criteria. Those cases where their presence has been noted may represent secondary demyelination or "pseudo"-onion bulbs (Tome et al. 1979; Hahn 1993). The latter explanation also probably accounts for the presence of inappropriately thin myelin sheaths. Nonspecific axonal alterations may be seen in myelinated and unmyelinated fibers (Vital et al. 1979; Julien et al. 1988; Yasuda et al. 1990; Hahn et al. 1990), but are not a dominant feature.

One pedigree with neurological features typical of CMT-2, some patients having evidence of cardiomyopathy, has been reported to show giant axonal swellings due to massive focal accumulations of neurofilaments, similar to that seen in hexacarbon toxicity (Chap. 18) and recessive/sporadic giant axonal neuropathy (Vogel et al. 1985). The X-linked family described by Hahn et al. (1990) had features typical of CMT-2, supporting the existence of X-linked neuronal CMT-2.

19.4.3 Pathogenesis

The genetics of the various forms of CMT-2 (Rossor et al. 2013) suggest a variety of pathogenetic mechanisms ranging from altered mitochondria and mitochondria-based metabolism of axons/Schwann cells to axonal cytoskeleton, endosomal functions, and others. Autopsy and teased fiber studies support the hypothesis that a distal axonopathy with axonal atrophy is the fundamental pathogenic mechanism (Berciano et al. 1986; Yasuda et al. 1990; Dyck et al. 1993).

19.4.4 Differential Diagnosis

Individually, the histological findings of CMT-2 are nonspecific. However, a biopsy showing diffuse loss of myelinated fibers, with relatively selective large fiber involvement, no active degeneration, and very prominent regenerating cluster formation, is highly suggestive of the diagnosis. In theory, a similar picture may be seen in other very longstanding axonal neuropathies such as diabetes, chronic renal failure, or long duration toxic exposures, but in our experience these always show a degree of active (Wallerian) degeneration that is out of keeping with the diagnosis of CMT-2.

19.5 Autosomal Dominant Intermediate CMT

Validity of the classification of CMT into neuronal and hypertrophic types is strongly supported by the bimodal nerve conduction velocity distribution (Dyck et al. 1993; Buchthal and Behse 1977). As discussed above, this usually correlates well with histological findings: onion bulbs and no regenerating clusters in CMT-1 and the reverse in CMT-2.

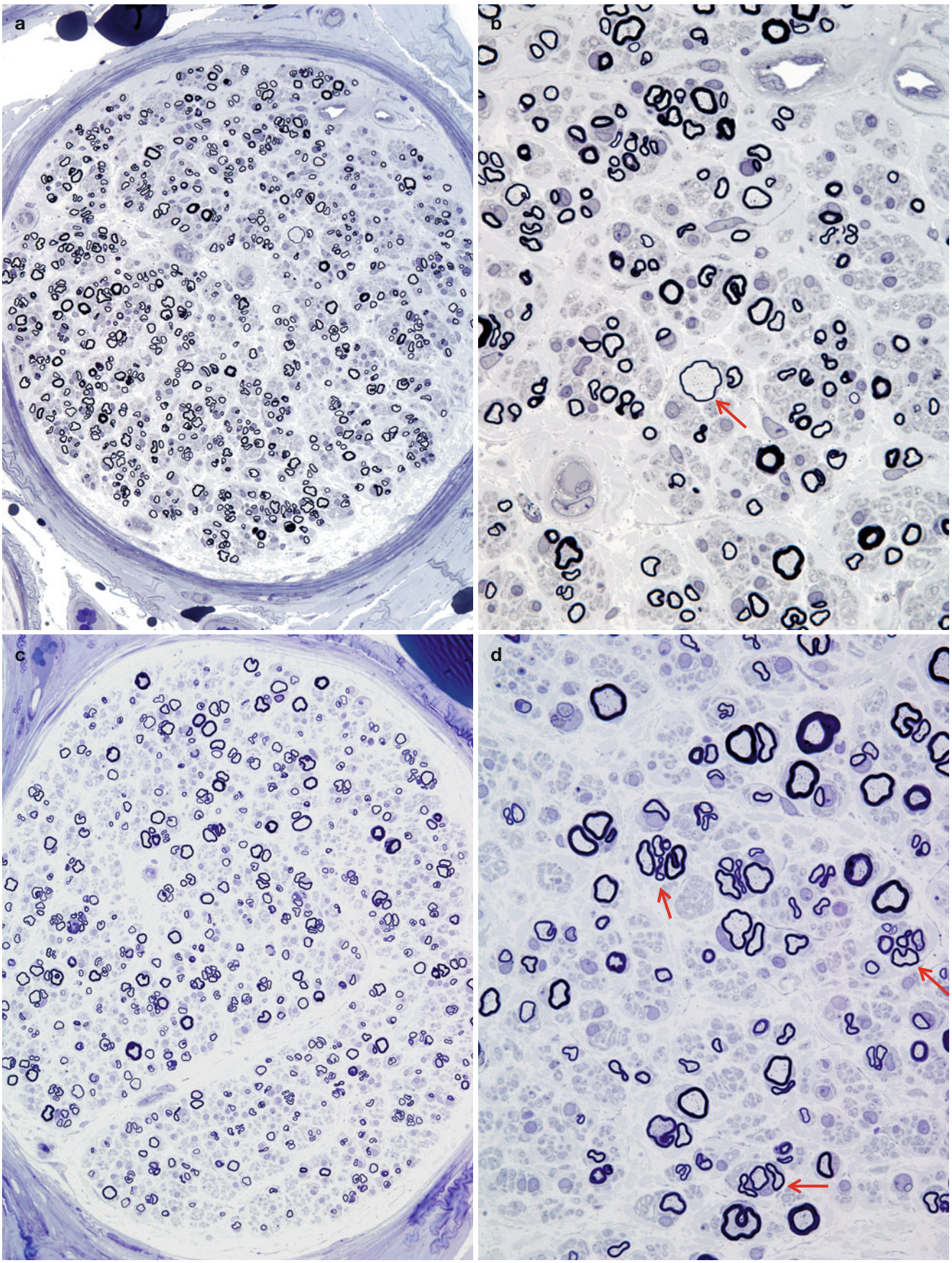
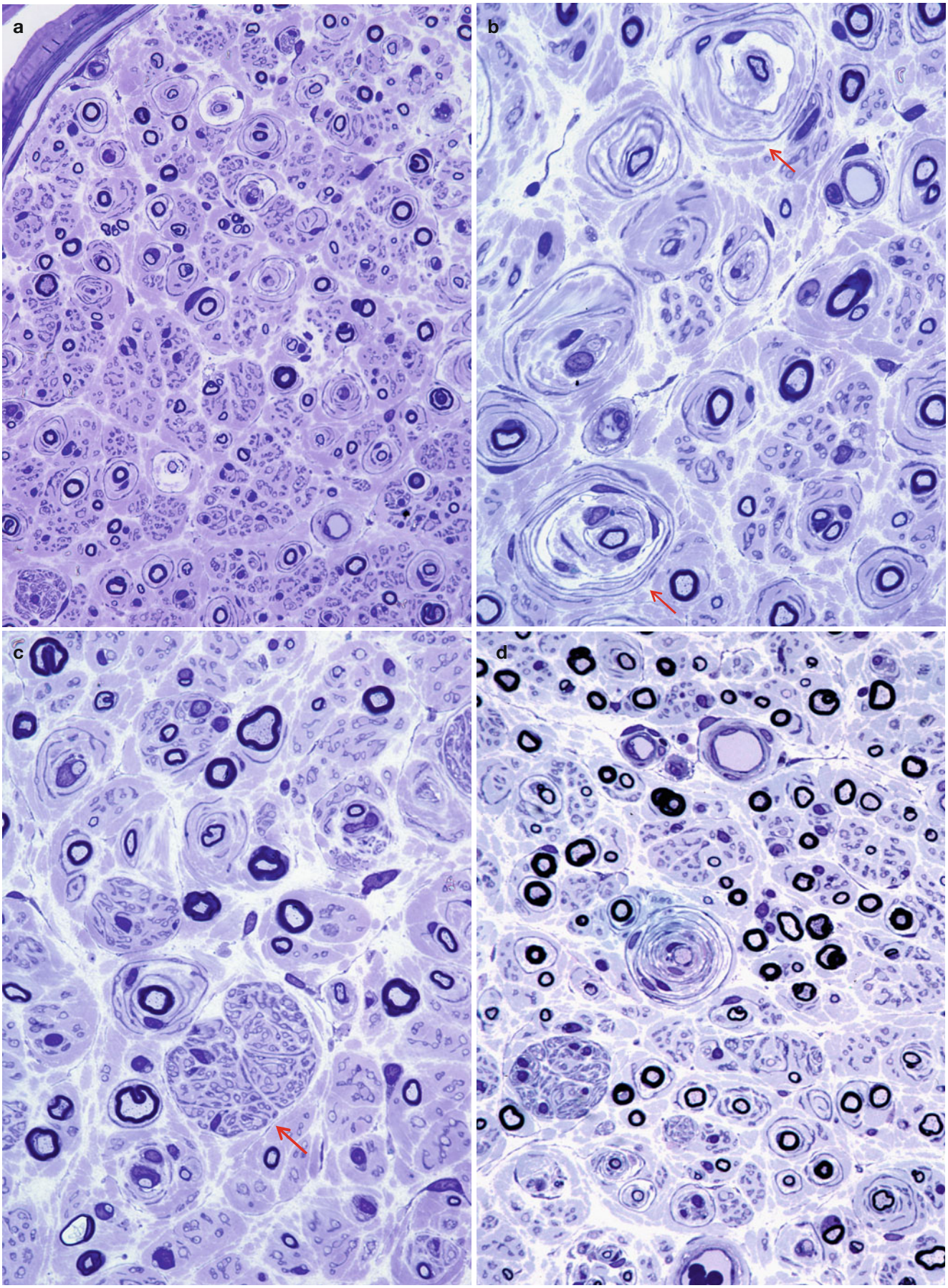


Fig. 19.11 Two cases of hereditary axonal neuropathy show moderate reduction in number of myelinated fibers and regenerating clusters. Rare thinly myelinated swollen axons are a nonspecific finding (*arrow*, **b**). Biopsy from a 28-year-old woman with dominantly inherited CMT-2 of 18 years duration is depicted in figs (**a**, **b**). Biopsy from a 58-year-old woman with dominantly inherited axonal neuropathy and spastic paraparesis, likely CMT-5, is illustrated in figs (**c**, **d**). Note the regenerative clusters (*arrows*, **d**) (1 μm thick plastic section. Magnification: **a**, **c** 400×; **b**, **d** 1,000×)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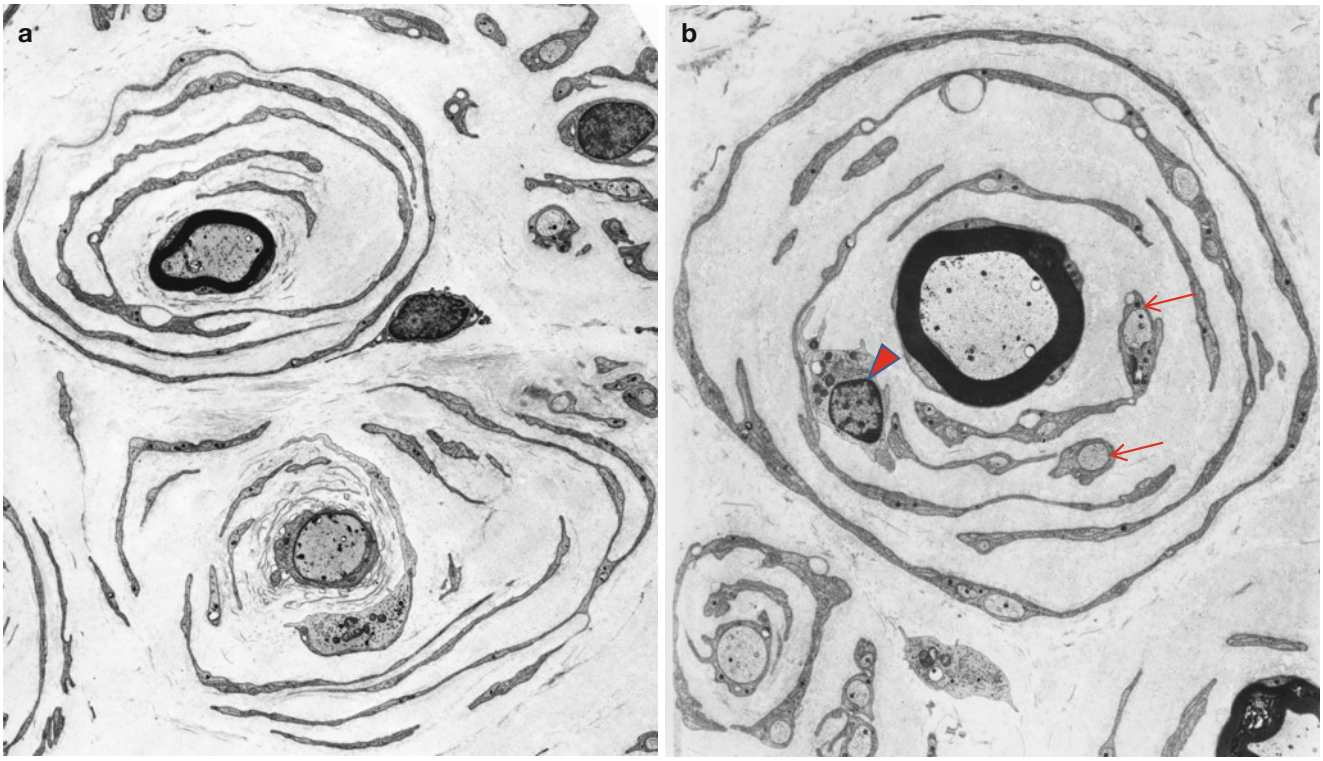


Fig. 19.13 CMT-"intermediate": variation in morphology of onion bulbs. Central axons are surrounded by short segments of redundant basal lamina, one denuded and one thinly myelinated (**a**). In (**b**) the central fiber is fully myelinated and miniature axons can be seen enclosed within Schwann cells of the onion-bulb rings (*arrows*). The significance of a lymphocyte (*arrowhead*) within the OB is undetermined – inflammation was absent otherwise. Same case as Fig. 19.12 (Magnification: **a**, 3,360×; **b**, 4,080×)

However, Madrid, Davis, and Bradley (1977; Bradley et al. 1977) found clinical, electrophysiological, and histological evidence for an "intermediate group" of CMT patients. The most distinctive features were motor conduction velocities in the 25–45 m/s range, biopsies revealing a combination of onion bulb and regenerating cluster formations, and ultrastructural evidence of active axonal degeneration. Other authors have found that nerve conduction velocities (Brust et al. 1978) and histological observations (Rossi et al. 1985; Gherardi et al. 1983) do not invariably support easy division into neuronal and hypertrophic forms. We have occasionally examined biopsies in patients with genetically determined neuropathy showing both OBs and denervated bands (Figs. 19.12 and 19.13). However, the biopsies reported as "intermediate" by Madrid and colleagues (1977) were said to show "not infrequent" active axonal degeneration and axonal ultrastructural abnormalities. This is a finding that we have never observed in patients with any type of CMT and which we would not consider compatible with the diagnosis, unless an additional disease was superimposed. None of the other large nerve biopsy series in CMT-1 or CMT-2 report significant active axonal degeneration, even in severe infantile and childhood-onset neuronal cases (Ouvrier et al. 1981; Gabreels-Festen et al. 1991).

Genetic analysis of such patients has identified dominant gene defects (*DNM2*, *YARS*, *MPZ*, *INF2*, *GNB4*) and recessive (*GDAP1*, *KARS*, *PLEKHG5*) forms, different gene defects involving a wide variety of targeted genes (Rossor et al. 2013).

An excellent correlation exists between electrophysiological and histological subdivision of autosomal dominant CMT in types 1 and 2 (Buchthal and Behse 1977), but there are rare exceptions (Harriman and Currie 1979; Gherardi et al. 1983). A biopsy showing prominent onion bulbs may come from a patient with conduction velocities greater than 38 m/s, and conversely a patient with markedly showed conduction may show the histological picture of neuronal CMT. The latter may be ascribed to a severe loss of axons, especially large MFs, resulting in a surviving population that conducts slowly. Some of the former might fall under the "intermediate" group or may represent a disjunction between the pathology of motor and sensory nerves.

Fig. 19.12 CMT-"intermediate": a 30-year-old woman with familial neuropathy and mildly reduced conduction velocity, inheritance uncertain. Nonuniform pattern of pathological alterations is shown (**a**). While some fibers are normal, others demonstrate either typical or giant onion bulbs (*arrows*, **b**) or large denervated band (*arrow*, **c**) or both (**d**) (1 μm thick plastic section: **a**, 400×; **b**–**d**, 1,000×)

19.6 CMT-Associated Demyelinating Neuropathies of Early Onset

The majority of perinatal early-onset hereditary neuropathies represent demyelinating phenotypes (58 %) with slowed motor nerve conduction velocities, indicative of demyelination or amyelination (Baets et al. 2011). Early-onset hereditary polyneuropathies are not a single entity but rather represent a broad and heterogeneous clinical and genetic spectrum of disorders, which include both dominant and recessive mutations in multiple genes (Baets et al. 2011). Several factors militate against the formulation of a heuristic classification of congenital neuropathies; these include as follows: a single gene abnormality can cause multiple phenotypes of variable severity, a single phenotype can be caused by mutations in different genes, the same phenotype may result from a de novo dominant mutation or from a recessive mutation, and that many of the children with early-onset congenital neuropathy remain without a specific diagnosis after multiple testing. (Yiu and Ryan 2012; Baets et al. 2011). For current classifications of demyelinating prenatal and infantile developmental neuropathies, the reader is directed to Yiu and Ryan (2012), Landrieu and Baets (2013), and Baets et al. (2011).

Congenital hypomyelinating neuropathy (*CHN*), *Dejerine–Sottas Disease* (*DSD*), also termed CMT-3, and CMT-4 (also designated as *autosomal recessive CMT-1*) are forms of demyelinating CMT with prenatal or infantile development. Most infants with early-onset neuropathies may display one of two phenotypes. The first group is genuinely congenital and is characterized by perinatal onset with hypotonia, muscle weakness at birth, arthrogryposis, respiratory insufficiency, and marked conduction slowing to 6–12 m/s or less (Ouvrier et al. 1987; Benstead et al. 1990). These patients experience delayed development. The second and most common group, after an uneventful neonatal period, manifests in infancy with motor delay and foot deformities and show slow progression into adult life (Yiu and Ryan 2012).

Dejerine–Sottas syndrome, formerly designated CMT-III (CMT-3 and DSD), is the first and most clearly defined subgroup of CDN; however, it shows substantial clinical and pathological overlap with CHN. Molecular genetics has shown that both are in fact at the severe end of the CMT-1 disease spectrum (Landrieu and Baets 2013). Noteworthy is the fact that of the original two siblings reported by Dejerine and Sottas, one showed disease onset in infancy and the other at the age of 14 years (Dyck et al. 1993).

Dejerine–Sottas and CHN are genetically complex syndromes (Gabreels-Festen 2002) that have been reported as sporadic or autosomal recessive. They are associated with de novo dominant mutations involving at least 3 different genes (*EGR2*, *PMP22*, *MPZ*) and recessive mutations in *EGF2* and *periaxin* genes. In its classic form, CMT-3 is a severe hypertrophic polyneuropathy of early onset (first 2 years of life) with delayed milestones, progressive and debilitating clinical course, with half of patients becoming wheelchair bound by the age of 10 years (Dyck et al. 1993; Anderson et al. 1973; Ouvrier et al. 1987; Guzzetta et al. 1982; Weller 1967). Nerves are palpable in most patients, CSF protein is elevated, and motor nerve conduction velocity is below 6–12 m/s. There is clinical and electrophysiological overlap between DSS and CMT-1 given that they share mutations of the same group of genes. Although debility in DSD is more severe than for CMT, DSD is now placed by many among the CMT-1 group.

19.6.1 Light Microscopy

Nerve biopsy shows large numbers of onion bulbs, endoneurial fibrosis, loss of axons, and, specifically, hypomyelination: Many fibers that, on the basis of axon diameter, should be myelinated lack myelin completely or show disproportionately thin myelin sheaths (Fig. 19.14a, b) (Guzzetta et al. 1982). Histological features are reminiscent of dominant CMT-1, but more severe as regards nerve enlargement, onion bulb formation, and axonal loss (Fig. 19.14). As many as 50 % of internodes may be demyelinated on cross sections and even more dramatically on teased fibers (Dyck and Gomez 1968; Dyck et al. 1970, 1971b; Ouvrier et al. 1987). Despite the large number of denuded axons, macrophages containing myelin debris are not prominent. The most salient observation, however, is that when axons do have a myelin sheath, it is always inordinately thin, regardless of axon size (Figs. 19.14 and 19.15). The myelinated axon population is reduced, sometimes very severely, with a marked shift towards small diameters. There can be overlap between the degree of axon loss seen in autosomal dominant CMT-1 and DSS in patients of roughly the same age (Ouvrier et al. 1987). Unmyelinated axon counts seem normal, but with the shift of the myelinated axon population towards small fiber size and the florid demyelination, it may be difficult to classify small nonmyelinated axons.

19.6.2 Electron Microscopy

Onion bulbs are a prominent feature and may reach large proportions. The lamellae are made up of concentric imbricated layers of Schwann cell processes, as well as redundant basement membranes (Figs. 19.15 and 19.16a). Hypomyelination is the rule, with only 10–20 myelin lamellae per myelinated axon, regardless of its diameter (Dyck et al. 1971a). Ouvrier et al. (1987) reported that this represents the most consistent means of distinguishing morphologically between autosomal dominant CMT-1 and CMT-3: The g-ratio (see Chap. 3) was never above 0.69 in 10 cases of

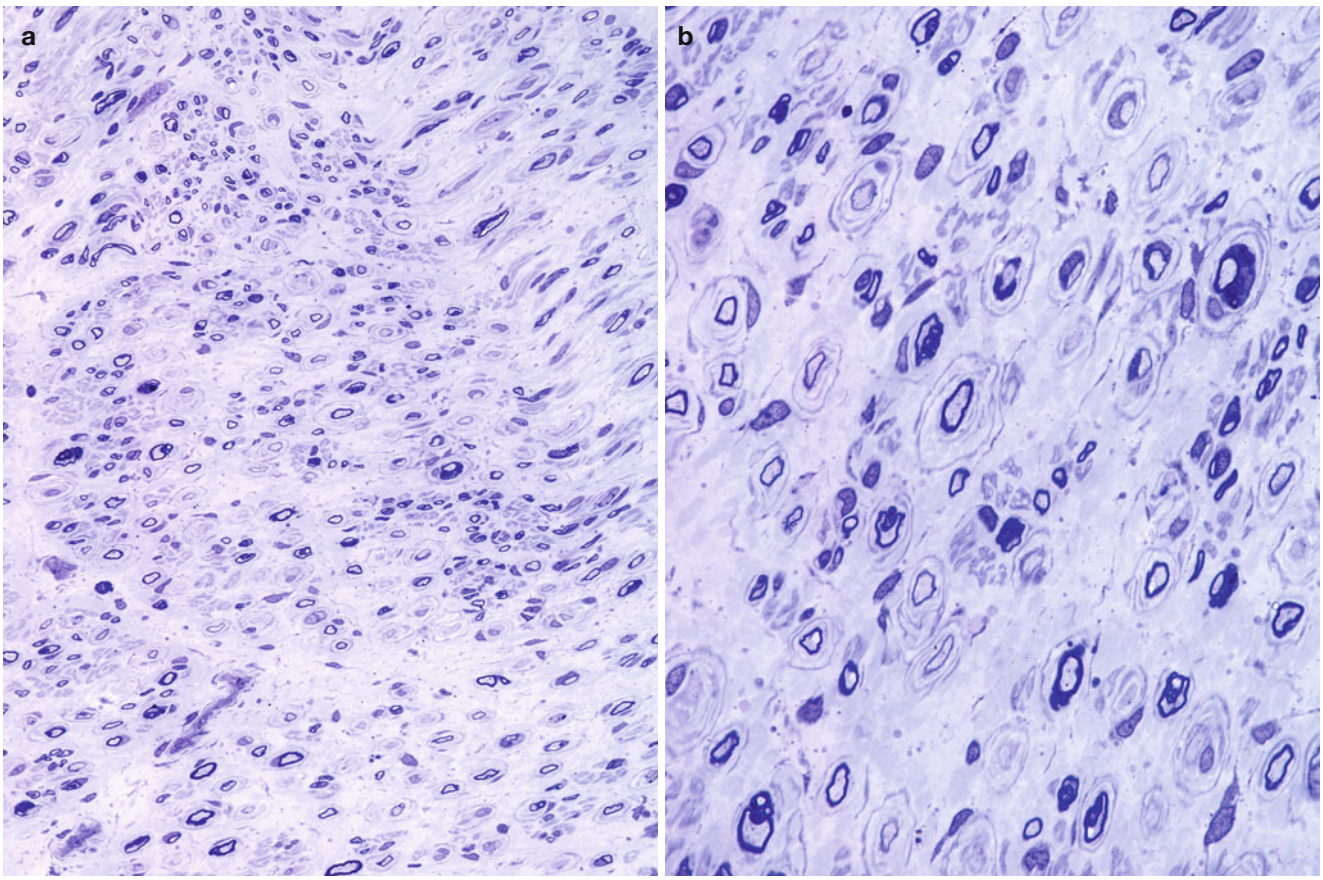


Fig. 19.14 CDN-Classic Dejerine–Sottas (CMT-3): note that none of the fibers are fully myelinated and many are amyelinic, yet no lipid debris is seen. Onion bulbs with thin leaves are prominent (**b**) (1 μm thick plastic section, magnification: **a**, 200×; **b**, 1,000×) (Tissue courtesy of Dr. V. Jay, Toronto)

childhood-onset CMT-1 and never below 0.81 for 6 cases of Dejerine–Sottas. Myelin debris may be seen within Schwann cells. A slight increase in collagen pockets and denervated Schwann cell bands, subtle indicators of mild unmyelinated axon loss, has been reported (Ouvrier et al. 1987).

19.6.3 Pathogenesis

Since PMP duplications and point mutations are also associated with CMT-1A (vide supra), it is evident that the clinical and histological phenotype is dependent on additional factors, perhaps the site of mutation in the gene. Mutations in myelin P_0 protein have been found in patients satisfying clinical or histological criteria for DSS (Hayasaka et al. 1993d; Himoro et al. 1993). One of the three mutations occurred in the transmembrane domain of the P_0 protein and the others in the extracellular variable immunoglobulin-like region. In one instance these workers showed that P_0 was expressed in normal quantities in peripheral myelin (Tachi et al. 1994). As with the PMP-22 mutations, mutations in P_0 are also associated with the CMT-1 phenotype (vide supra: CMT-1B).

19.6.4 CMT Type III with Basal Lamina Onion Bulbs (CMT-3 BLOB)

Of historical interest was the proposed grouping, by Gabreels-Festen and Gabreels (1993), of patients under the description of CMT type III with basal lamina onion bulbs (CMT-3-BLOB). Their review of the literature identified about 30 such cases (Harati and Butler 1985; Joosten et al. 1974; Lutschg et al. 1985; Balestrini et al. 1991; Ono et al. 1982; Moss et al. 1979; Vital et al. 1987; Vallat et al. 1987; Lyon 1969; Boylan et al. 1992; Guzzetta et al. 1982; Kennedy et al. 1977). Onset is most often at birth or infancy, with a variable disease severity ranging from arthrogryposis to survival to adult life with moderate disability. Conduction velocity is often below 6 m/s. Thus, these patients are clinically indistinguishable from the classic DSS syndrome discussed above.

19.6.4.1 Pathology

Light microscopic examination may show enlarged nerve fascicles, with an increase in endoneurial matrix (Joosten et al. 1974). Only a few thinly myelinated fibers remain, but the axonal loss is not as severe as a quick glance would indicate, for the majority of axons in the nerve fascicle are

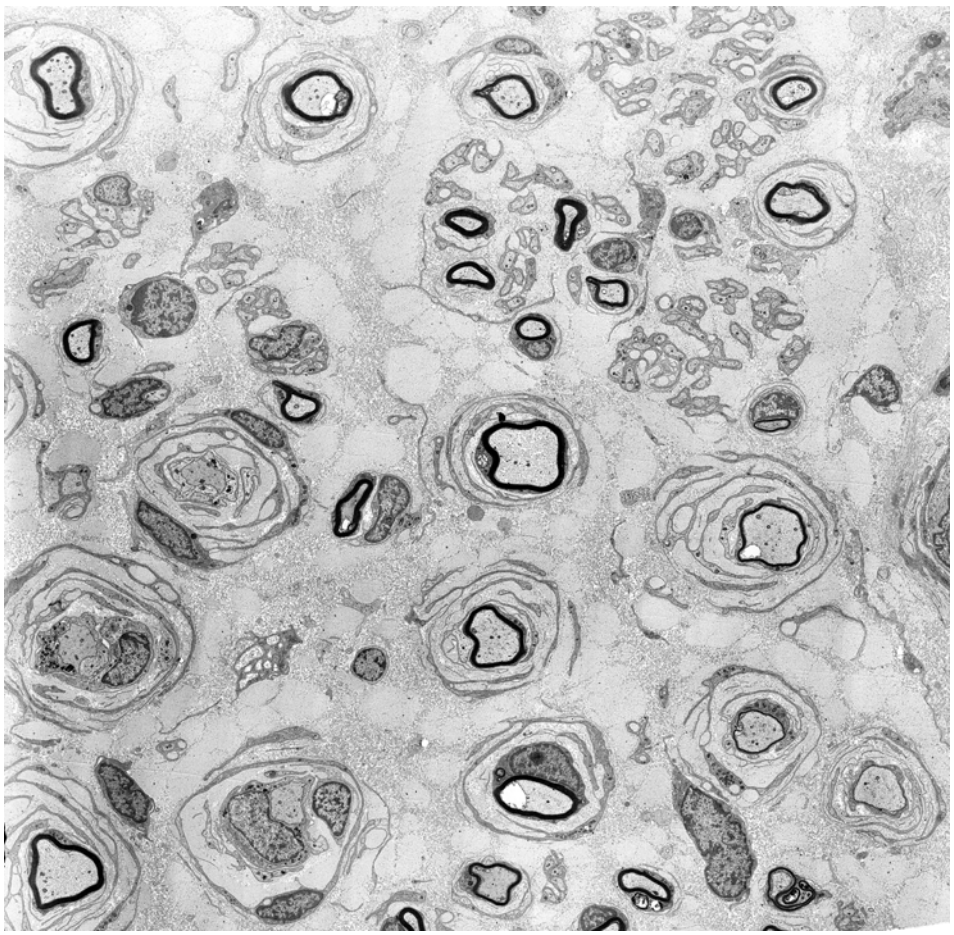


Fig. 19.15 CDN-Classic Dejerine–Sottas: low-power view showing lack of full myelination and many onion bulbs containing demyelinating and remyelinating fibers (1,800×)

demyelinated. In the various case reports, axon numbers ranged from severely reduced (Gabreels-Festen and Gabreels 1993) to relatively preserved (Harati and Butler 1985; Boylan et al. 1992). Large axons seem to be more severely affected.

Ultrastructural examination reveals attenuated myelin, which occasionally may be uncompacted. The characteristic finding is that many axons, regardless of whether they are myelinated or not, are surrounded by basal lamina rings (arrow, Fig. 19.16b), with few or no classic onion bulb structures. Very thin Schwann cell processes may be found at the periphery. Sometimes the Schwann cell does not fully surround the naked axon, and the axon is adjacent to basement membrane without intervening Schwann cell cytoplasm. The rare regenerating cluster may be seen, as well as infrequent redundant myelin folds. Myelin debris and active demyelination in the presence of an intact axon has been described in some of these cases (Joosten et al. 1974; Balestrini et al. 1991; Moss et al. 1979; Lyon 1969; Kennedy et al. 1977).

19.6.4.2 Discussion

CMT-3-BLOB and classic DSS share many features including typical age of onset, variation in severity of disease, similar inheritance patterns, very slow conduction velocities, frequent demyelination with myelin debris, and striking hypomyelination. The main distinction appears to be the presence of numerous basement membrane onion bulbs. However, these are also seen in "classic" DSS (Dyck and Gomez 1968; Ouvrier et al. 1987), and the difference seems to be only a matter of degree. BLOBs are also part of the CMT-4A and CMT-4C groups, which have *GDAP1* (ganglioside-induced differentiation-associated protein 1) and *SH3TC2* gene defects identified. Many workers (Dyck et al. 1993; Ouvrier et al. 1987) do not identify two distinct groups of patients with Dejerine–Sottas syndrome.

19.6.5 CMT-1 with Basal Lamina Onion Bulbs

Gabreels-Festen and colleagues suggested the delineation of a group of patients in which BLOBs are prominent, but which are distinguished histologically from the two groups previously mentioned by virtue of milder myelin abnormalities (Gabreels-Festen et al. 1992a; Gabreels-Festen and

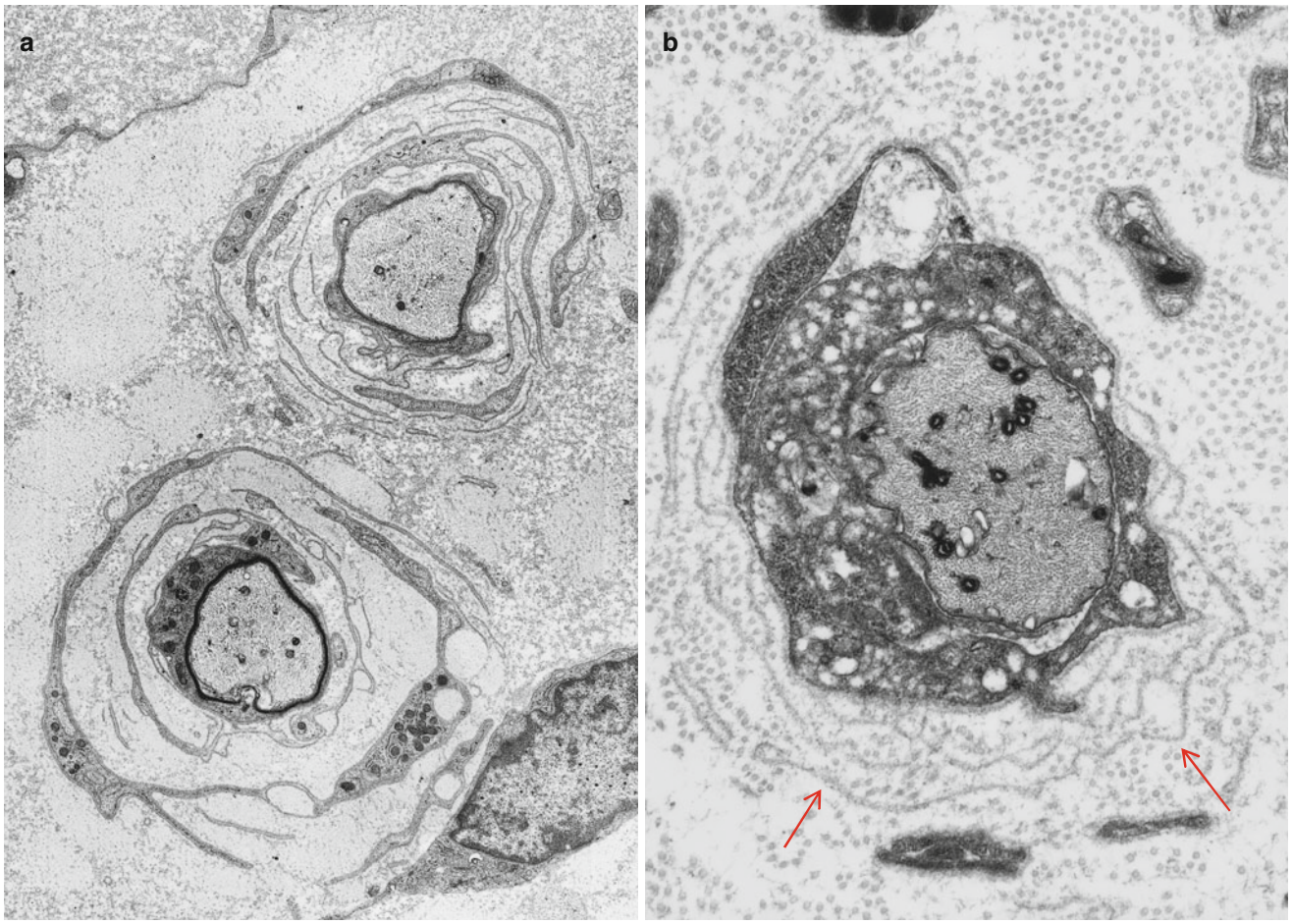


Fig. 19.16 CDN-Classic Dejerine–Sottas: (**a**, **b**) basal lamina rings form a prominent part of these onion bulbs (Magnification **a**, 7,500×; **b**, 10,000×)

Gabreels 1993). These authors identified several similar cases in the literature (Meier et al. 1976; Nordborg et al. 1984; Smith et al. 1980). Clinically the patients suffer from a disease intermediate in severity between typical Dejerine–Sottas syndrome and CMT-1. Conduction velocities are usually in the 10–30 m/s range. All reported cases have been sporadic or autosomal recessive. Testing for the CMT-1A mutation has been negative (Gabreels-Festen and Gabreels 1993), but there may be overlap with CMT-4A and CMT-4C groups which have *GDAP1* (ganglioside-induced differentiation-associated protein 1) and *SH3TC2* gene defects identified.

Biopsy may show an increased endoneurial area with interstitial edema. There is reduction in myelinated fiber density, but unmyelinated fibers are relatively spared. Classic onion bulb formations are small and less frequent than BLOBs. Occasional myelin tomaculae are present. Teased fibers show segmental myelin changes without signs of active axonal degeneration. The major distinguishing point from the Dejerine–Sottas picture is that far fewer demyelinated axons are seen, and there is no evidence of the severe hypomyelination that characterized DSS. When measured, the g-ratio has been in the range 0.64–0.77.

The patients reported by Nordborg et al. (1984) showed intra-axonal lamellated inclusions in unmyelinated fibers and less often in myelinated fibers, as well as frequent redundant myelin loops.

The existence of yet another morphologically unusual variant of CDN, designated "autosomal recessive CMT with focally folded myelin (FFM)" was postulated by Gabreels-Festen and colleagues (Gabreels-Festen et al. 1990), who identified similar cases from the literature (Barbieri et al. 1994; Lutschg et al. 1985; Routon et al. 1991; Vital et al. 1987; Ouvrier et al. 1990 case 14.3; Nordborg et al. 1984). Umehara et al. 1993 described a dominantly inherited case of motor and sensory neuropathy with excessive myelin folding complex. Symptoms are present at birth or appear within the first year, with slow progression. Some are wheelchair bound in childhood, while others retain the ability to walk well into adulthood. Conduction velocities have been above and below the cutoff of 10–12 m/s usually used to delineated Dejerine–Sottas syndrome. CSF protein is usually normal.

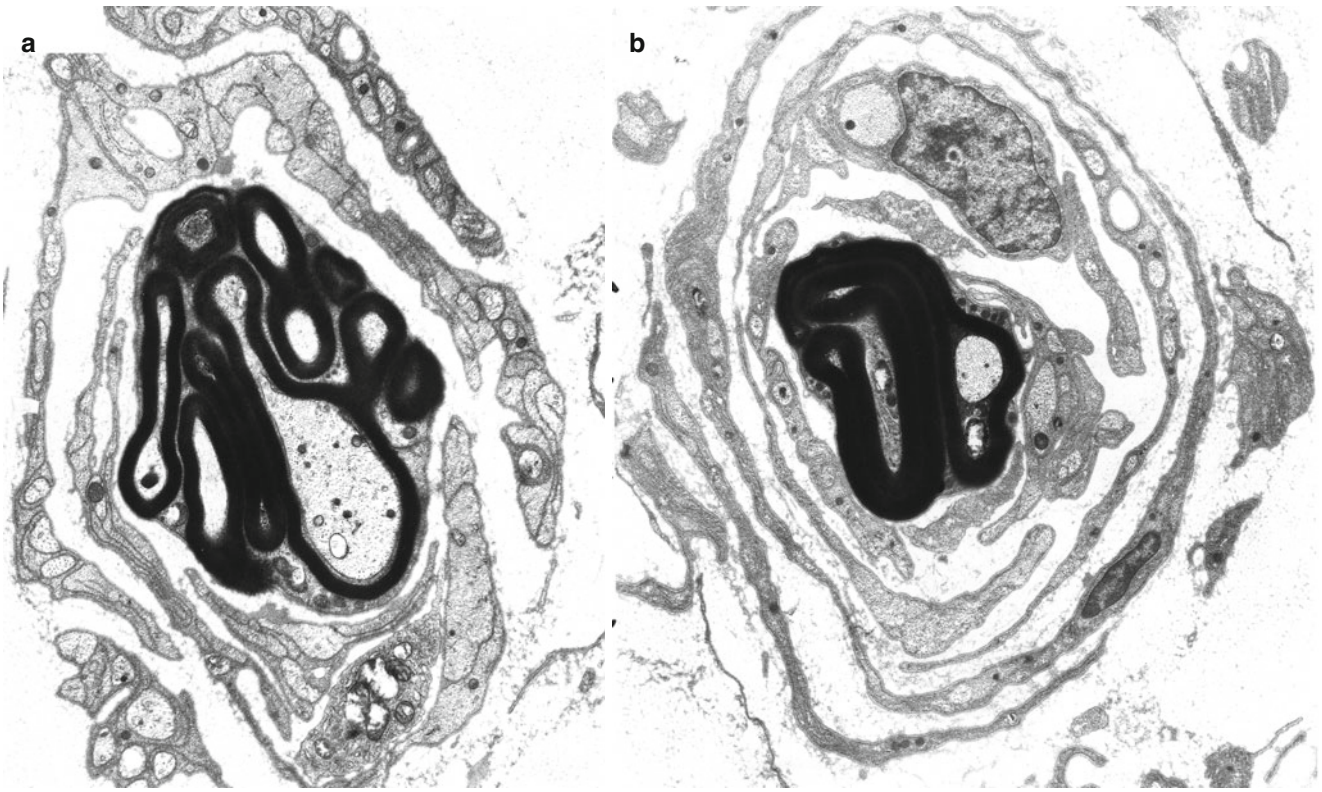


Fig. 19.17 CDN-Focally folded myelin: (**a**, **b**) two onion bulbs are centered by axons that display elaborate outer redundant loops of myelin (Magnification a, b, 7,500)

19.6.5.1 Pathology

Microscopic examination reveals two populations of axons. Many are thinly myelinated or amyelinic, and the g-ratio is frequently in the range associated with DSS. A second group of axons demonstrate folded redundant myelin loops or circumferential hypermyelination with occasional signs of degeneration. Onion bulb formations are present, in numbers seemingly proportional to the patient's age. In patients reaching adult life, onion bulbs are more frequent, may attain a substantial size, and are often denervated. Classic OBs and BLOBs can be seen. The myelinated axon population is normal in very young patients and declines with age, with large myelinated axons being more severely depleted. Myelin debris may be seen. Unmyelinated axons are usually unscathed. Teased fiber studies show that myelin "tomaculae" are present in many as 50–75 % of internodes.

19.6.5.2 Discussion

The finding of redundant myelin loops and outfoldings without onion bulb formation in even the youngest of patients might suggest a primary defect of myelinogenesis (Gabreels-Festen et al. 1990). Myelin tomaculae seen in this situation are usually smaller than those typically described in HNPP, but there is overlap. However, focally folded myelin is also part of the CMT-4 syndromes with identified mutations in *MTMR2*, *PRX*, *FGD4*, and *MPZ*-related CHN/DSD (Yiu and Ryan 2012; Tazir et al. 2013). The 9 cases reported by Nordborg et al. (1984) seemed to show both very prominent basement membrane onion bulbs and redundant myelin loops.

19.6.6 CMT-3 with Amyelination

Several cases with near or total amyelination of the peripheral nerves have been described (Hakamada et al. 1983; Seitz et al. 1986; Routon et al. 1991; Charnas et al. 1988; Kasman et al. 1976); this represents the most severe form of peripheral myelinopathies. These patients often have arthrogryposis multiplex, cranial nerve abnormalities, and a short lifespan. Biopsy has usually shown a diminution of axon numbers, but normal axonal morphology. The histological hallmark is a striking absence of myelin around nearly all axons, some perhaps showing a few myelin lamellae. Onion bulb formations and myelin debris are not seen. Biopsies in

very young patients aged 4 days and 1 month (Seitz et al. 1986; Hakamada et al. 1983) have shown the absence of myelin with no debris. These cases probably represent a failure of axons to become myelinated (Charnas et al. 1988). Whether this is due to Schwann cell or axon defects is not known, although some evidence exists for the latter (Sahenk et al. 1991). Some patients with congenital hypomyelinating syndrome with amyelination have defects in MPZ and EGR2 (Warner et al. 1996, 1998). In 2012 Funalot et al. reported a homozygous deletion of an *EGR2* enhancer (the myelinating Schwann cell element) in a child with congenital amyelinating neuropathy.

19.6.7 Reconsideration of the Classification

It cannot be overemphasized that the complexity and overlap of pathology in CHN is imperfectly aligned with gene defects involving at least 4 different genes (EGR2, PMP22, MPZ, and PRX). In some cases separation is based on sural nerve pathology with the absence of active myelin breakdown and infrequent OBs in CHN and the presence of demyelination/remyelination and OBs in DSS with the implication that CHN represents a congenital impairment in myelin formation and DSS aberrant demyelination and subsequent remyelination (Balestrini et al. 1991).

We have discussed 5 groups (A–E above) from a histologically based classification proposed by Gabreels-Festen and Gabreels (1993). There is overlap in terms of age of onset, inheritance pattern, and degree of conduction slowing. There is much in common between groups A and B, and prominent hypomyelination and basement membrane onion bulbs are also present in group D. One reported patient's nerve biopsies showed near amyelination at 4 months (group E) and basal lamina onion bulbs with myelin formation and degeneration 1 year later (group B) (Ulrich et al. 1981). Such evolution from one histopathological group to another is a strong argument against the ability of this histopathologic classification to identify and group together specific genetic defects. The histological picture may simply vary depending on the degree of impairment of ability of Schwann cells to make or maintain myelin and on maturation factors. The significance of focal myelin folds is also unclear. Overlap between groups C and D is demonstrated in the patients reported by Nordborg et al. (1984). Finally, typical CMT-1 may show focally redundant myelin and basement membrane onion bulbs, although these are infrequent features.

Overall, we are unconvinced that the subgroups discussed above are sufficiently distinct clinically or histologically to merit consideration as separate entities. The most important conclusion that can be drawn upon review of this group of patients is that the presence of very prominent basal lamina onion bulbs, severe hypomyelination, or frequent myelin infoldings or outfoldings are suggestive of nondominant inheritance, valuable for genetic counseling in situations where the neuropathy is sporadic.

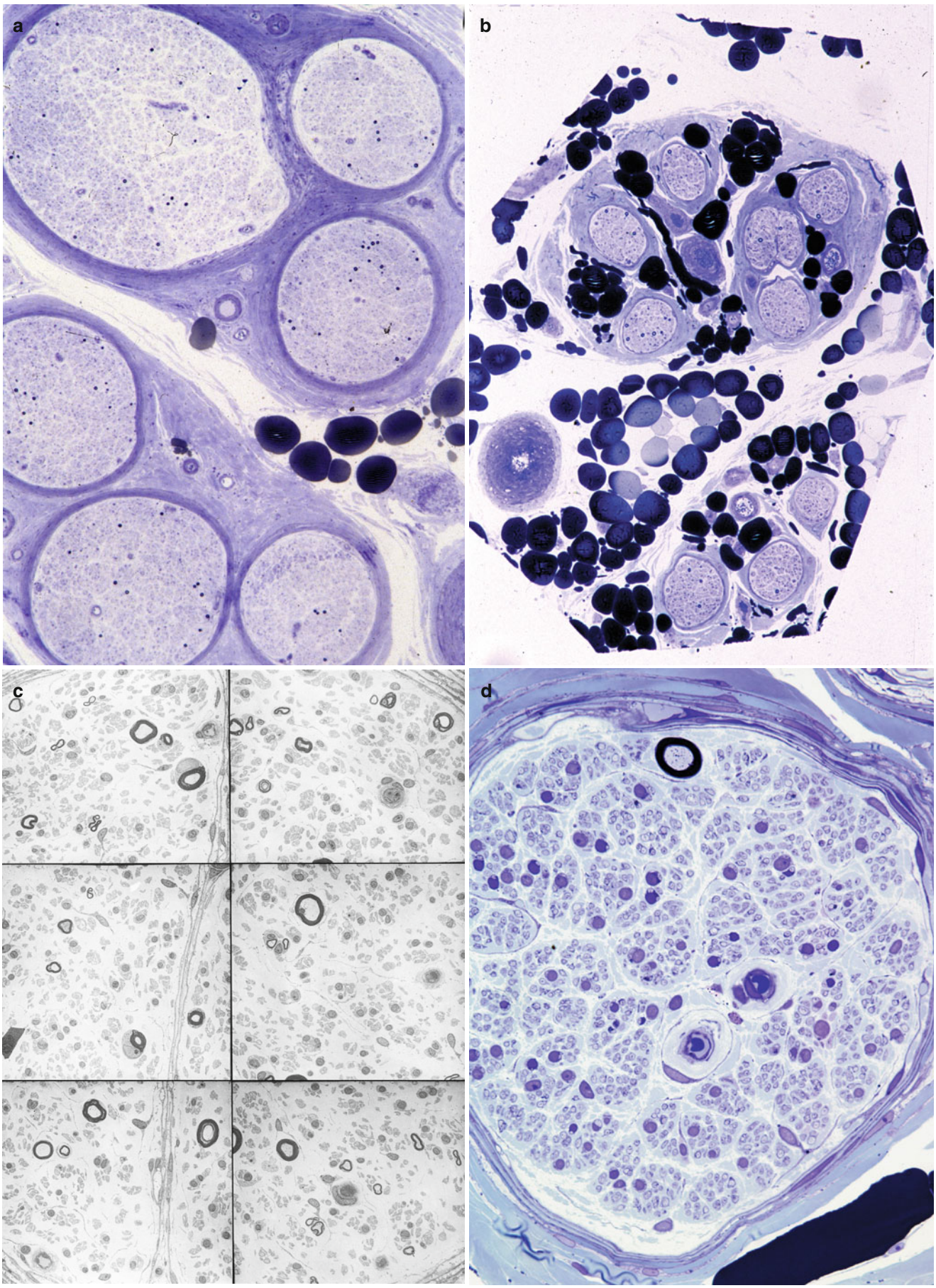
19.7 CMT-4 (Charcot–Marie–Tooth Type 4)

Autosomal recessive CMT-1 is commonly termed CMT-4. This includes rare neuropathies exclusively inherited as autosomal recessive disorders, some of which are more prevalent in North Africa (Parman et al. 2004).

CMT-4A begins with delayed motor milestones before the age of 2 years, with delayed motor milestones and weakness, culminating in wheelchair dependence. CMT-4A is caused by a mutation in the *GDAP1* (ganglioside-induced differentiation-associated protein 1) gene which codes for a cell membrane protein. Nerve biopsy shows features of severe reduction of myelinated fiber density and hypomyelination and, in some reports, onion bulbs with redundant layers of basal lamina referred to as "basal lamina onion bulbs" (vide supra).

CMT-4B constitutes a heterogeneous patient group with autosomal recessive hereditary motor and sensory neuropathy with focally folded myelin. Symptoms develop in the second or third year of life, with a severe neuropathy, both distal and proximal, followed by relentless progression culminating in wheelchair dependency and early death. A prominent and consistent pathological finding consists of irregular redundant loops and folding of the myelin sheath. The *MTMR2* (myotubularin-related protein 2) gene is mutated in patients with CMT-4B1 with marked depression of MNCV usually less than 12 m/s (Gabreels-Festen et al. 1992b). Mutation in the *MTMR13/SBF2* (myotubularin-related protein-13/set binding factor 2) gene leads to disease in CMT-4B2 (Young and Suter 2003).

Patients with disabling scoliosis and childhood onset of a demyelinating sensorimotor neuropathy is the presenting manifestation of CMT-4C which is linked to mutations in an uncharacterized transcript *K1AA1985* (chromosome 5q23–q33). This is a relatively frequent cause of CMT-4, accounting for approximately 17 % of all cases (Yiu and Ryan 2012). Nerve biopsy shows loss of large myelinated fibers, few of which exceed 8 μm. Ultrastructural findings include basal lamina onion bulbs and extended Schwann cell processes (Gabreels-Festen et al. 1999).


a
b
c
d

Mutation in the *NDRG1* (N-Myc-downstream-regulated gene 1) gene has been found to be responsible for CMT-4D (also known as HMSN-Lom from its involvement of Romany population in Lom, Bulgaria). This rare recessive demyelinating syndrome also includes hearing loss and dysmorphism. Sural nerve histological features include demyelination, remyelination, onion bulbs, and axonal regenerative clusters (King et al. 1999).

CMT-4E is caused by mutations in the *EGR2* gene and is allelic to CMT-1D (Warner et al. 1999). Patients present with floppy infant syndrome at birth but, although delayed, may reach motor milestones. Motor nerve conduction velocities are extremely reduced to 3–8 m/s, and sural nerve biopsies show virtual absence of myelin.

CMT-4 F have severe phenotype with marked sensory impairment, respiratory failure shortly after birth, and a mutation in the *PRX* (periaxin, chromosome 19q13) gene (Takashima et al. 2002). Periaxin is a selective myelinated Schwann cell membrane-associated protein. Sural nerve biopsies reveal demyelination, onion bulbs, and occasional tomacula formation, with focal myelin thickening, abnormalities of the paranodal myelin loops, and focal absence of paranodal septate-like junctions between the terminal loops and axon (Takashima et al. 2002).

CMT-V is a syndrome combining peroneal muscular atrophy and spastic paraplegia (Serratrice et al. 1990; Behse and Buchthal 1977; Harding and Thomas 1984; Brust et al. 1978). Onset is usually in the second decade, with slow progression sometimes to a wheelchair-dependent state. Inheritance is autosomal dominant. Electrophysiological studies and nerve biopsy demonstrate features typical of the neuronal form of CMT. Some biopsies have been normal (Behse and Buchthal 1977). The affected genes are *MFN2*, *GJB1*, and *BSCL2*. (Pareyson et al. 2013). BSCL2 mutations may generate a wide spectrum of diseases, including spastic paraplegia type 17 with upper limb atrophy, also termed Silver syndrome (Pareyson et al. 2013).

CMT-VI is dominant and combines features of peroneal muscular atrophy (PMA) with Leber hereditary optic neuropathy. It is caused by mutations in the *MFN2* gene. In 3 cases described by Sommer and Schroder (1989), a mild depletion of myelinated fibers, especially those of large diameter, was noted. Unmyelinated fibers were also affected, with appearance of a bimodal diameter-frequency histogram and mild-moderately reduced numbers. Onion bulb formations were few and, judging by the pictures provided, quite rudimentary. The authors commented on a number of ultrastructural findings, including an increased number of paracrystalline inclusions of the nonspecific type seen in various neuropathies, as well as some enlarged mitochondria containing tightly packed parallel cristae or amorphous matrix. Axonal dense core vesicles were also noted to be increased in number (Sommer and Schroder 1989). These findings are of great interest in light of the fact that Leber Hereditary Optic Atrophy is caused by a defect in mitochondrial DNA and that MFN2 protein is involved in mitochondrial dynamics.

19.8 Hereditary Sensory Neuropathies

As suggested by Dyck and colleagues, the familial sensory (and sometimes autonomic) neuropathies (HSAN) are divided into five groups (Dyck 1993; Thomas 1993a) with some features in common including that the primary sensory neurons of spinal ganglia and the autonomic system either fail to develop or undergo degeneration with a hereditary basis.

Type I is the most frequent clinically, presenting in the second decade or later with slowly progressive distal sensory loss and little or no weakness. Autonomic features are actually not prominent in type I HSAN. The presenting feature is often a complication of acrodistal sensory loss (especially pain and temperature), such as foot ulcers, atrophy and distal anhidrosis, or arthropathy. Inheritance is autosomal dominant. One form of this disease is caused by a point mutation in the *SPLTLC1* (serine-palmitoyltransferase 1) gene (Kuhlenbaumer et al. 2002). Nerve biopsy shows preservation of fascicle size, notwithstanding severe loss of small myelinated and unmyelinated fibers (Fig. 19.18a, c) and smoldering axonal disintegration with occasional myelin ovoids. Large myelinated fibers are less affected. Spinal ganglia neurons are lost, and there is significant reduction in the number of fibers entering the dorsal columns of the spinal cord.

Types II-V HSAN are congenital or infantile onset illnesses in which autonomic abnormalities play a more prominent role and progression is absent or extremely slow (Dyck 1993; Thomas 1993a). Genetic defects have been identified as follows:

HSAN II (mutations in *FAM134B* and *HSN2* exon of *WNK1* as well as *KIF1A*, with defects in off-loading axonal cargos at nerve terminals) and prominent atrophy (Fig. 19.18b, d)

HSAN III (Riley–Day syndrome, familial dysautonomia), mutations in the *IKBKAP* (inhibitor of kappa light polypeptide) gene

HSAN IV (Congenital insensitivity to pain with anhidrosis, mutation in NTRK1 (neurotrophin receptor tyrosine kinase 1) gene

Fig. 19.18 Hereditary sensory and autonomic neuropathy. (**a**, **c**) HSAN type 1 shows marked axon loss with preservation of fascicular area (**a**) which is seen at higher magnification in (**c**). (**b**, **d**) HSAN type 2 shows marked fascicular atrophy (**b**) and marked axon loss (**d**) (EM and 1 μm thick plastic sections, magnification: (**a**, **b**) 100×; **c** ~1,500×, **d** 600×)

HSAN V (similar symptoms to HSAN IV), caused by *NGF-B* gene defect

This group of rare diseases typically shows sporadic or autosomal recessive inheritance. A hereditary sensory neuropathy with spastic paraplegia has been described by Cavanagh et al. (1979), and its existence and autosomal recessive pattern of inheritance are confirmed by Thomas et al. (1994).

The small amount of pathological material available (Dyck 1993) suggests that HSAN I is a distal axonopathy. Loss of unmyelinated fibers is greater proportionally than the loss of myelinated fibers, with large myelinated fibers least involved. Given the slow rate of progression, active axonal degeneration is unlikely to be captured on a nerve biopsy. The segmental myelin changes observed on with teased fiber preparations are probably secondary to axonal atrophy (Dyck 1993). No specific findings are present, but the observation of selective unmyelinated and small myelinated fiber loss has a limited differential diagnosis (Table 7.7).

The "congenital" sensory neuropathies are a heterogeneous group with very little pathological material available for analysis. Reviews are provided by Dyck (1993) and Thomas (1993a). The most important aspect of histological examination is quantitative morphometry, which demonstrates selective loss of various subpopulations of fibers: myelinated fibers in type II (Schoene et al. 1970; Nukada et al. 1982; Ohta et al. 1973), unmyelinated fibers in types III (Aguayo et al. 1971) (Riley-Day) and IV (Goebel et al. 1980), and small myelinated with or without unmyelinated fibers in type V HSAN (Donaghy et al. 1987). In some cases these may be caused by a congenital hypoplasia of nerve fibers, while in others the disease may be a slowly progressive degenerative process beginning in utero (Nukada et al. 1982; Ohta et al. 1973). In the cases of hereditary sensory neuropathy with spastic paraplegia described by Cavanagh et al. (1979), ultrastructural study demonstrated severe loss of myelinated and unmyelinated fibers.

The "vacuolated fibroblasts" first noted and emphasized by Schoene et al. (1970) in a case of HSAN II have subsequently been described in other patients with this disorder (Nukada et al. 1982; Ohta et al. 1973), in patients with hypertrophic neuropathy (Asbury et al. 1971), with CMT-1II-BLOB histology (Joosten et al. 1974), and a case of CMT-1 showing "focal mucoid degeneration" (Meier and Bischoff 1977). The common element to all these situations is the deposition of focal or diffuse endoneurial amorphous mucoid (on LM) and fibrillar (on EM) material (Asbury et al. 1971). Fibroblasts with a similar appearance are seen in Renaut bodies, and the deposited "mucoid" material contains large amounts of oxytalan. We do not feel that this finding has any diagnostic significance.

19.9 Peripheral Neuropathy Associated with Hereditary Ataxias

Inherited ataxia syndromes are frequently associated with peripheral nerve alterations, typically a predominantly sensory axonal neuropathy. Most common of these is Friedreich's ataxia, but cerebellar and peripheral nerve dysfunction are combined in other neurodegenerative diseases. The classification of hereditary ataxia is beyond the scope of this book (de Jong et al. 1991; Subramony and Currier 1991). In this section we review only Friedreich's ataxia, abetalipoproteinemia, and Chediak–Higashi syndrome and briefly mention other cerebellar degenerations where peripheral nerve has been examined. Several additional disorders combining cerebellar and peripheral nerve syndromes are considered elsewhere in this chapter including Sects. (19.10, 19.11, 19.12.1, 19.12.2, and 19.12.3). A spinocerebellar/neuropathy syndrome develops in some storage diseases including the sphingolipidoses (metachromatic leukodystrophy, Niemann–Pick disease), adrenoleukodystrophy, and cerebrotendinous xanthomatosis.

Nerve biopsy is not useful in a chronic cerebellar ataxia – axonal peripheral neuropathy syndrome – as findings are invariably nonspecific. A demyelinating neuropathy in this setting might suggest one of the storage diseases (Chap. 20), but given the data available from modern imaging and biochemical and molecular biologic techniques, biopsy is rarely necessary. Although not of diagnostic value, morphometric analysis of peripheral nerve in Friedreich's ataxia has yielded valuable insights into mechanisms of peripheral nerve disease, especially the concepts of distal axonopathy and secondary demyelination (Dyck and Lais 1973).

19.9.1 Friedreich's Ataxia

19.9.1.1 Clinical Manifestations

Friedreich's ataxia (FA) is an autosomal recessive disease with onset in the first two decades of life, characterized by progressive gait ataxia and dysarthria, loss of lower limb tendon jerks, skeletal and cardiac abnormalities, and large fiber sensory neuropathy. The loss of large fiber sensation in FA is due to a combination of dorsal column and distal axonal degeneration. FA patients with a severe peripheral neuropathy may form a distinct subgroup (Ben Hamida et al. 1991). Electrophysiological studies demonstrate a distally predominant axonal neuropathy affecting sensory more than motor fibers (Caruso et al. 1983; McLeod 1971). FA is distinguished from "Friedreich's-like" early-onset cerebellar

ataxia in that tendon jerks in the latter are relatively preserved and optic nerve, severe skeletal deformity, and cardiac disease are not present (Harding 1993).

19.9.1.2 Pathology

A large volume of nerve biopsy and autopsy material is available for FA (Caruso et al. 1983; Santoro et al. 1990; McLeod 1971; Ouvrier et al. 1982; Rizzuto et al. 1981; Said et al. 1986; Lamarche et al. 1984). An indolent axonal degeneration which affects large myelinated fibers most severely is found almost universally and may result in almost total depletion of myelinated fibers (Hughes et al. 1968). Some authors have commented on the occasional presence of increased numbers of regenerating clusters (Caruso et al. 1983), but more often these are surprisingly absent given the prominent clustering seen in other chronic axonal neuropathies (McLeod 1971; Said et al. 1986). Wallerian degeneration is almost never seen. There is usually little or no evidence of a significant demyelinating component, although the occasional thinly myelinated axon may be seen. Onion bulb formations are rare, rudimentary if present, and may sometimes represent "pseudo"-onion bulbs formed by regenerating clusters (Rizzuto et al. 1981). Said et al. did describe "… a few large onion bulb formations" in one case (Said et al. 1986). Unmyelinated fiber number and frequency distribution are normal.

Teased fiber abnormalities are minimal. Segmental demyelination and remyelination may be seen in a few percent of fibers, and rare images of Wallerian degeneration can be present (Said et al. 1986; Dyck and Lais 1973; McLeod 1971; Caruso et al. 1983). Dyck and Lais (1973) reported that segmental myelin changes were clustered on certain axons and likely secondary to axonal changes.

19.9.1.3 Pathogenesis

The CNS pathology of Friedreich's ataxia is felt to represent a distal axonopathy with length-dependent degeneration of the spinocerebellar, corticospinal, and dorsal column long tracts. In addition, a marked depletion of spinal ganglion cells occurs (Hughes et al. 1968). Some controversy exists regarding whether the peripheral nerve disease is a progressive distal axonopathy with axonal atrophy (Dyck and Lais 1973) or a maturational abnormality with relatively little progression (Said et al. 1986; Santoro et al. 1990). Some authors feel that poor correlation between duration of disease and severity of axon loss (Caruso et al. 1983), a surprising paucity of regenerating clusters, and a lack of visible histological progression (Santoro et al. 1990) favors the latter. However, Ouvrier and coworkers (1982) did find that severity of axon loss correlated with patient age. The genetic defect is in *FXN* which codes for frataxin, a nuclear-encoded mitochondrial iron chaperone and a pathogenetic mechanism involving oxidative stress (Schmucker et al. 2008).

19.9.2 Non-Friedreich's Inherited Spinocerebellar/Cerebellar Degenerations

Non-Friedreich's cerebellar and spinocerebellar degenerations are sometimes distinguished on the basis of a predominance of corticospinal tract findings or cerebellar features, in comparison to the dorsal column deficits very prominent in Friedreich's ataxia. Separation from FA is also possible in the presence of autosomal dominant inheritance or an age of onset out of the range normally seen in FA. Classification is complex (Soubramony and Currier 1991). The importance of peripheral neuropathy in these entities is less well studied than in FA.

Some cases of "Friedreich's-like" early-onset cerebellar ataxia with retained tendon reflexes (Harding 1993) studied by Santoro et al. (1992) showed a nonspecific axonal neuropathy with variably severe loss of myelinated fibers, sometimes affecting large MFs more than small MFs.

McLeod and Evans (1981) reviewed 19 patients with familial olivopontocerebellar (OPCA-Menzel type) or cerebello-olivary (Holmes-type) degeneration. Neuropathy was significant in only 2, but axonopathic nerve conduction abnormalities were seen in 9 of 19. Five nerve biopsies were examined. Loss of myelinated fibers was demonstrated in 3, without the predominance of large MF depletion typical of FA. Unmyelinated fibers were normal. Biopsies reported by Rossi et al. (1986) in dominantly inherited OPCA showed a mild axonal dropout affecting large MFs only in one case and only a slight increase in regenerating clusters in a second (Rossi et al. 1986).

In 12 patients with autosomal dominant olivopontocerebellar degeneration commonly seen in India, 8 had clinical evidence and 11 had electrophysiological evidence of an axonal neuropathy. Quantitative sural nerve examination was performed in 3 cases and showed chronic axonal degeneration affecting large myelinated fibers more severely (Wadia et al. 1978). Nerve biopsies in 13 Finnish patients with recessively inherited infantile onset cerebellar ataxia revealed an axonal sensory neuropathy which worsened with age (Koskinen et al. 1994). Large MFs were more severely depleted, and onion bulbs and regenerative clusters were infrequently observed.

A Japanese report of nerve biopsy in 9 hereditary and 12 sporadic non-Friedreich's spinocerebellar degenerations noted mild axonal dropout without a selectivity for large myelinated fibers was described (Matsuoka et al. 1984). Segmental myelin changes were frequent on teased fibers

and felt to most likely be secondary to axonal alterations. No significant difference was present between the inherited and sporadic groups.

19.9.3 Abetalipoproteinemia (Bassen-Kornzweig Disease)

19.9.3.1 Clinical Manifestations

Abetalipoproteinemia is a rare autosomal recessive multisystem disease in which serum betalipoproteins are absent. Symptom onset is in the first or second decade, and peripheral neuropathy manifests with diminished reflexes and a mild predominantly sensory neuropathy (Yao and Herbert 1993; Wichman et al. 1985). Ataxia, intention tremor, gastrointestinal symptoms, retinal pigmentary degeneration, loss of vibration sense and proprioception, and the typical musculoskeletal stigmata of weakness and inherited neuropathy are also seen. Electrophysiological studies indicate a mild axonal neuropathy affecting sensory more than motor fibers (Miller et al. 1980; Wichman et al. 1985). Peripheral red cells demonstrate acanthocytosis, and serum cholesterol and triglyceride are decreased. Deficiency of vitamin E secondary to intestinal fat malabsorption has been implicated in the pathogenesis of the neurological disease (Yao and Herbert 1993).

Abetalipoproteinemia is believed to result from a defect in the *MTP* gene encoding the microsomal triglyceride transfer protein, involved in the transfer of triglycerides, cholesteryl esters, and phospholipids across phospholipid surfaces (Sharp et al. 1993). A genetically distinct but clinically similar condition, hypobetalipoproteinemia, is caused by a defect in the apolipoprotein B (*APOB*) gene (Pessah et al. 1993; Kane and Havel 1989).

19.9.3.2 Pathology

Peripheral nerve histological material is rare. Three early reports had suggested that the neuropathy of abetalipoproteinemia or hypobetalipoproteinemia was demyelinating in nature, but the histological studies performed in these reports were inadequate (Asbury and Johnson 1978). More recent data derives from four cases reported by Wichman et al. (1985) and Miller et al. (1980). In these reports the MF density did not fall outside the range of normal, but a mild axonopathy was evidenced by the depletion of large MFs and a somewhat increased number of regenerating clusters (30–64/mm^2). The distal sural nerve was somewhat more severely affected than the proximal part (Wichman et al. 1985). Although a slight shift to the left of the UF histogram was noted, the UF density and the density of denervated Schwann cell subunits were within normal limits, indicating little or no involvement of unmyelinated fibers (Wichman et al. 1985). Studies of Rhesus monkeys with vitamin E deficiency resulted in loss of axons in the dorsal columns and in peripheral nerve as well as development of dystrophic axons (Nelson et al. 1981), a result seen in the sural nerve of patients with vitamin E deficiency caused by abetalipoproteinemia and cystic fibrosis (Fig. 17.10). Teased fibers revealed frequent widening of the Node of Ranvier, but no increase in the incidence of segmental demyelination or remyelination. No onion bulbs were noted, and the mild myelin alterations were felt to most likely be secondary (Wichman et al. 1985).

In patients with vitamin E deficiency of other etiologies, usually malabsorption, peripheral nerve pathology is similar (Sect. 17.6.4).

19.9.4 Chediak–Higashi Syndrome

Chediak–Higashi syndrome is an autosomal recessive disorder characterized by immunodeficiency, lymphoreticular malignancies, oculocutaneous albinism, and a neurological syndrome that may include cerebellar ataxia and peripheral neuropathy (Blume and Wolff 1972). Mutations in the *CHS1* or *LYST* gene on chromosome 1q are thought responsible. The pathognomonic abnormality is the finding of giant lysosomes in leukocytes.

A small number of nerve biopsies have been reported (Lockman et al. 1967; Misra et al. 1991; Pezeshkpour et al. 1986; Blume and Wolff 1972; Myers et al. 1963). These have shown myelinated fiber degeneration with relative sparing of small myelinated axons. Unmyelinated fibers have also been decreased in number, with the histogram showing a bimodal peak indicating regenerative activity. No significant segmental myelin change is present. Giant lysosomes, appreciated best with electron microscopy, have been described in Schwann cells, endothelial cells, and fibroblasts, and endoneurial mast cells were said to have granules 10 times larger than normal (1–2 μm vs. 0.1–0.2 μm), with loss of the normal scrolled appearance (Misra et al. 1991; Blume and Wolff 1972; Lockman et al. 1967). Given the increased incidence of lymphoreticular malignancies, the possibility of neoplastic infiltrative neuropathy must also be considered in these patients (Myers et al. 1963).

19.10 Giant Axonal Neuropathy

19.10.1 Clinical Manifestations

The first case of the typically autosomal recessive disorder giant axonal neuropathy (GAN) was reported in 1972 (Asbury et al. 1972), with about thirty cases documented by the mid-1990s. With the realization that CNS involvement is common and that histological changes can be seen outside the nervous system, a more accurate name for this entity might be giant axonal disease (Maia et al. 1988; Richen and Tandan 1992). A generalized disorder of intermediate filament organization has been postulated (Guazzi et al. 1991) as the cause of the disease. Clinical onset is before the age of 7 years. Tandan

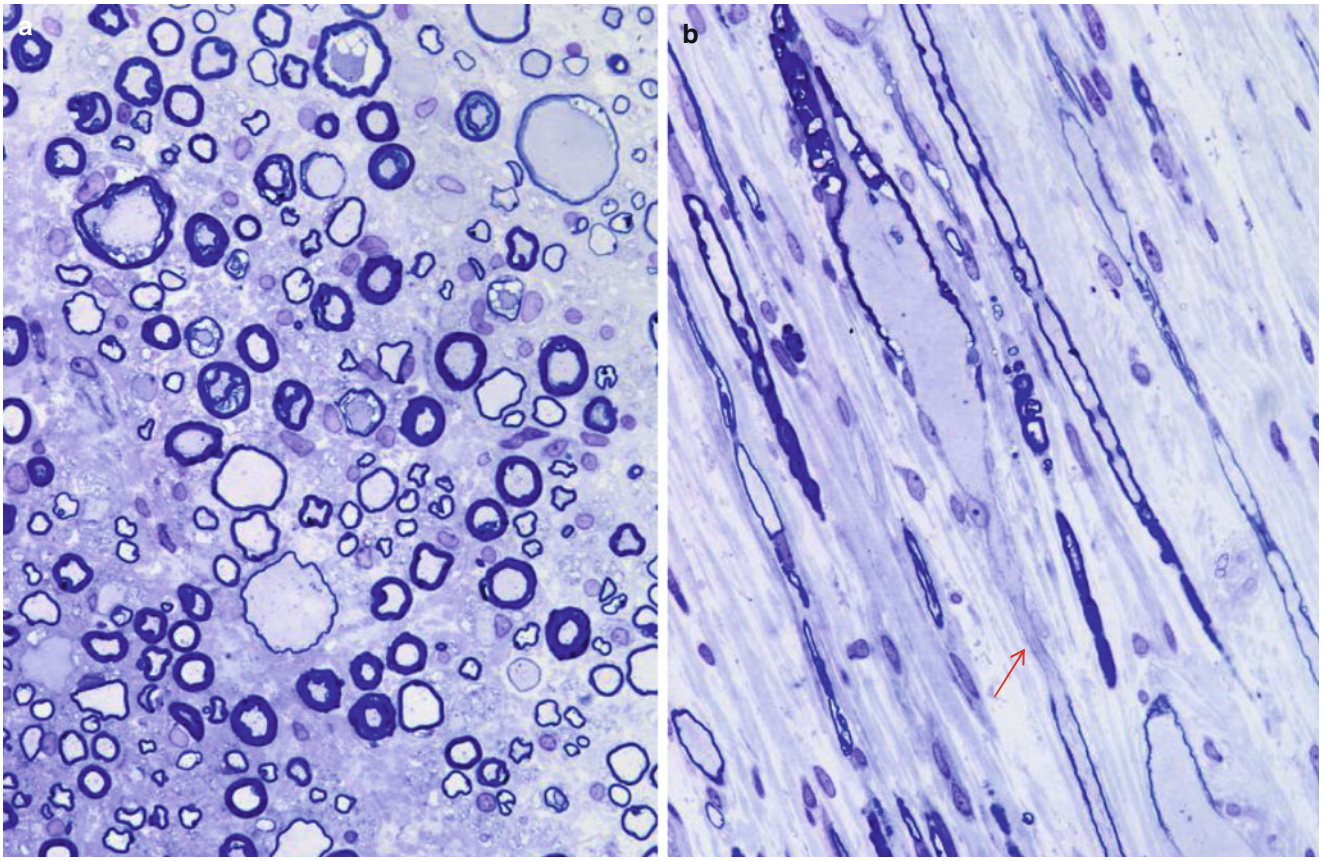


Fig. 19.19 GAN: (**a**, **b**) Many myelinated fibers are distended and display attenuated myelin sheaths. Longitudinal sections illustrate the segmental nature of axonal swellings and the origin of a swelling from an axon of normal diameter (*arrow*, **b**) (1 μm thick plastic section; **a**, **b** 1,000×) (Tissue courtesy of Dr. J. Lamarche, Sherbrooke)

et al. (1987) provide a comprehensive clinical review (Tandan et al. 1987). Most cases reported have been sporadic, but enough have been associated with parental consanguinity or shown sibling involvement (Kumar et al. 1990; Takebe et al. 1981; Maia et al. 1988) to suggest autosomal recessive inheritance. Bomont et al. (2000) identified the GAN gene defect on chromosome16q23.2 which codes for gigaxonin, which is thought to be a substrate adaptor for an E3 ubiquitin ligase, which affects proteasome-dependent degradation of microtubule-related proteins (e.g., MAP1B, MAP8, and the tubulin folding chaperone) (Hentati et al. 2013).

GAN is largely a pediatric disease, typically presenting in the first 3 years of life with clumsy gait difficulty progressing to a bed-bound state by adolescence. Ataxia and cognitive impairment may be seen, and skeletal abnormalities are common. Strikingly curly hair is almost invariably present. Neurological examination documents a symmetrical distally predominant sensorimotor neuropathy, with variably prominent central nervous system findings suggesting involvement of cerebellum, cranial nerves, corticospinal tracts, and cerebral cortex. MRI studies may show a picture reminiscent of leukodystrophy (Richen and Tandan 1992; Donaghy et al. 1988b). Electrophysiological tests suggest a length-dependent axonal neuropathy with variably prominent demyelinating features. The CSF is normal. Following a report of an adult with B12 deficiency and giant axonal change (Schochet and Chesson 1977), B12 supplementation was given to patients with no such deficiency, with unimpressive results (Fois et al. 1985; Takebe et al. 1981), and usually the disease progresses relentlessly to death in the second or third decade. Dogs suffer from a similar condition (King et al. 1993).

19.10.2 Pathology

The diagnosis of GAN does not require nerve biopsy. If the clinical picture is suspicious the finding of filamentous accumulations in various cell types seen on skin biopsy should be sufficient to confirm the diagnosis (vide infra).

19.10.2.1 Light Microscopy

The characteristic change of GAN in sural nerves is the presence of massively dilated axons usually surrounded by thinned or no myelin (Fig. 19.19a, b). Longitudinal sections show that axonal swellings represent focal enlargement of axons (arrow, Fig. 19.19b). Some of the demyelinated

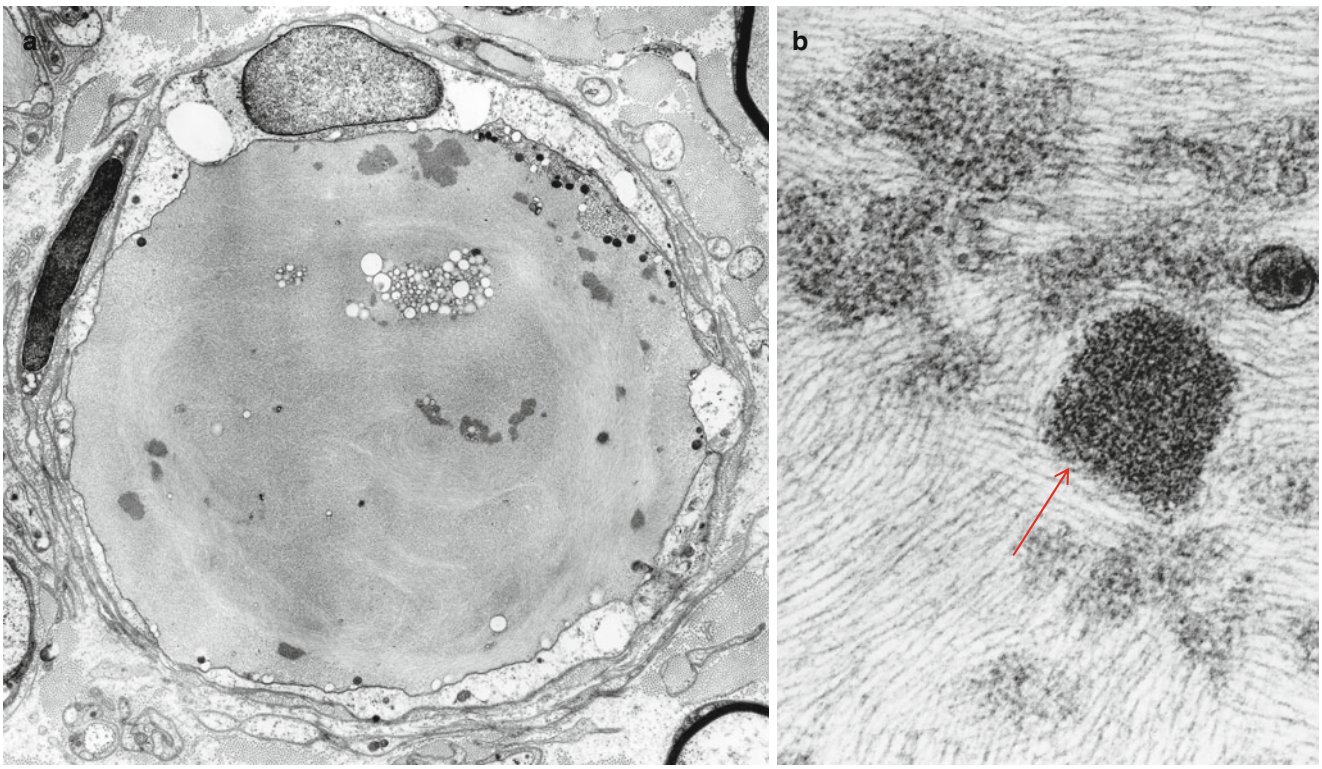


Fig. 19.20 GAN: (**a**) discloses a massively enlarged axon, which still possesses a thin myelin sheath. Higher magnification shows patchy osmiophilic condensations (*arrow*, **b**) merging with filaments (**a**, 3,000×; **b**, 52,700×)

swollen axons are associated with several surrounding Schwann cells. The distended axons may reach a size of 50 µm in diameter, but are typically in the 20–30 µm range. There are usually several such giant axons per fascicle, and as many as 1–5 % of axons in cross section may show the change (Fig. 19.19a). A few onion bulb formations and regenerating clusters can be seen, but these do not dominate the picture.

Quantitation of nerve fibers reveals a variable severity of axon loss that correlates with the duration and severity of the disease and involves all fiber types and sizes. However, actively degenerating axons are a rare finding. Teased fiber studies show that the axonal swellings are fusiform, 100–200 µm in length, and probably do not show any relation to the node of Ranvier (Asbury et al. 1972; Koch et al. 1977; Prineas et al. 1976; King et al. 1993). As many as 50 % of teased fibers may show axonal swellings (Tandan et al. 1987). Segmental demyelination and remyelination can be seen, and demyelinated segments may be present in internodes without any focal swellings (Koch et al. 1977).

19.10.2.2 Electron Microscopy

Ultrastructural examination reveals enlarged axons distended with neurofilaments which randomly swirl and stream in various configurations (Figs. 19.20a, b, 19.21a, b, and 19.22a, b) through the axoplasm, segregating mitochondria, microtubules, and other organelles to a subaxolemmal location. In some myelinated and unmyelinated fibers, there may be an increase in filament density without swelling. Although accumulation of filaments is the essential characteristic of GAN, one can also see non-dilated axon profiles filled with mitochondria, tubules, or membranous organelles, but few or no filaments, or only a small central core of filaments. Longitudinal sections reveal that these regions can be explained as a consequence of a relatively constant microtubule and other organelle content throughout the axon, with segmental accumulation of filaments associated with focal swelling in some regions, and the total or near total absence of filaments associated with axonal atrophy in adjacent segments of the axon (Asbury et al. 1972).

An important characteristic of the filamentous accumulations in GAN are focal irregular condensations in the midst of a sea of neurofilaments (arrow, Fig. 19.20a, b). These are typically 0.1–1 µm in diameter, are not surrounded by any membrane, and most have a tangled irregular amorphous/granular shape, while others appear as paracrystalline osmiophilic bodies (Gambarelli et al. 1977; Donaghy et al. 1988a). The condensations do not display a filamentous substructure, but the observation that filaments seem to enter these focal densities suggests that they are composed of tightly packed neurofilaments (Fig. 19.20b) (Donaghy et al. 1988a). Their similarity to glial Rosenthal fibers has been commented upon (Koch et al. 1977). A distinctive feature of the filaments encountered in GAN is that they lack the side arms that extend laterally and that are characteristic of the filaments in

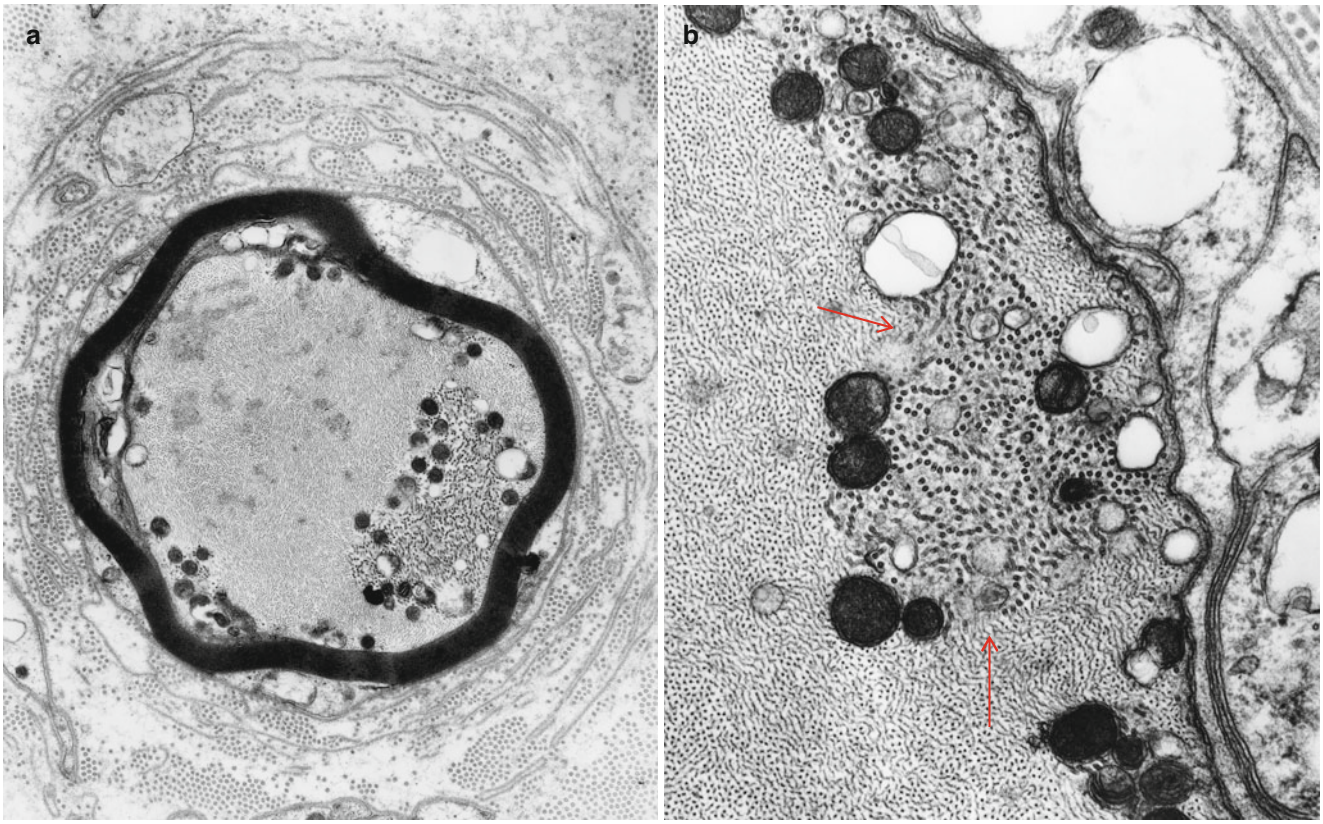


Fig. 19.21 GAN: Myelinated fiber is surrounded by reduplicated basal laminae (**a**). Note segregation and margination of microtubules and mitochondria towards the axolemma (**b**: *arrows*) (Magnification: **a**, 11,360×; **b**, 33,600×)

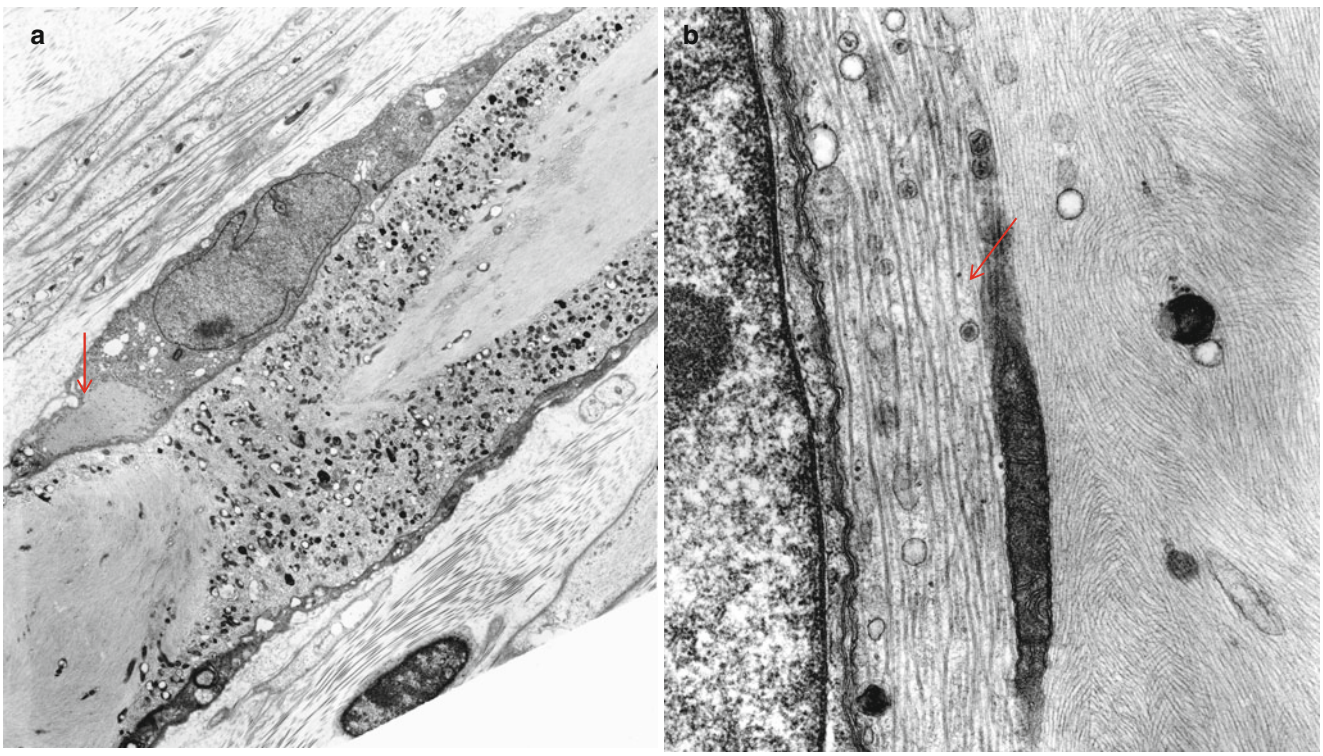


Fig. 19.22 GAN: Longitudinal view illustrates that because of the focal nature of filament masses, a transverse section might have shown filament accumulation alone, membranous organelle only, or combinations of both (**a**). Note filament accumulation in Schwann cell (*arrow*, **a**). The whorled appearance of neurofilament accumulations is contrasted with the longitudinal orientation of microtubules in the subaxolemmal area (*arrow*, **b**) (Magnification: **a**, 3,840×; **b**, 22,400×)

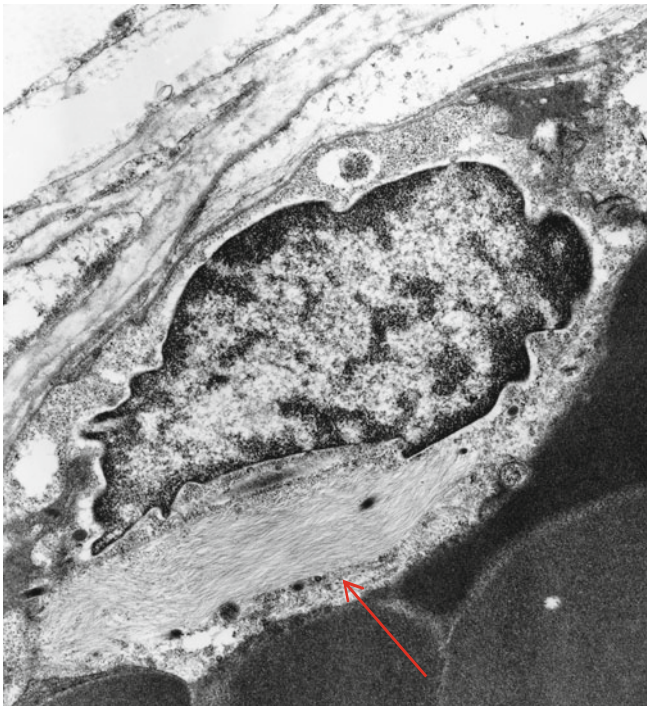


Fig. 19.23 GAN: abnormal filament aggregates in endoneurial endothelial cell (*arrow*) (Magnification: 9,940×)

swollen axons featured in diabetes and in patients suffering from the toxic effects of solvents and disulfiram.

Most reports of GAN have noted the presence of filament accumulations in Schwann cells (Fig. 19.22a) and less often in fibroblasts and perineurial and endothelial cells (Fig. 19.23) (Bolthauser et al. 1977; Gambarelli et al. 1977; Koch et al. 1977; Prineas et al. 1976). Similar accumulations were seen in fibroblasts, Langerhans cells, mast cells, and melanocytes from skin biopsy, thus permitting a diagnosis without nerve biopsy (Prineas et al. 1976; Takebe et al. 1981; Fois et al. 1985).

Occasional onion bulb formations are usually seen with EM, but are not a very prominent feature. The thinned myelin sheaths around some of the giant axons can be seen to be in various stages of disintegration, but there is no suggestion of a primary myelin change around normal-sized axons. Several adjacent Schwann cells may be spread over the surface of a giant demyelinated axon.

Serial nerve biopsies (Gambarelli et al. 1977; Donaghy et al. 1988a) clearly show a progression of the severity of the giant axonal change parallel with progression of disease. Indeed, the giant axonal change may be entirely absent in the first biopsy, but very prominent subsequently.

19.10.3 Pathogenesis

Axonal disease underlies the peripheral neuropathy of GAN. Donaghy and colleagues (1988a) and King et al. (1993) have studied the ultrastructure of GAN axons in detail. At very high magnifications it was not possible to detect the sidearms normally seen to separate longitudinally arrayed neurofilaments in normal axons, yet immunostaining for NF-H, the polypeptide forming the sidearms, was normal. The minimum spacing between neurofilaments in GAN was 12–30 nm, less than the normal value of 24–60 nm. These authors reported that the neurofilaments of GAN were visibly thicker than normal (12.5 nm vs. 10.1 nm in normals). They thus hypothesized that the basis of the axonal filament accumulation was a failure of the neurofilament sidearms to project perpendicular to the long axis of the filament, instead falling to the side, thus explaining both the presence of normal neurofilament immunostaining and the increased filament thickness and decreased separation. Giant axonal changes seen in hexacarbon-induced toxic neuropathies differ from those of GAN in that the tight packing and increased neurofilament thickness with absent sidearms are not seen, and axonal swelling is clearly related to the paranodal area (Spencer and Schaumburg 1977). These observations suggest that the mechanisms of these two giant axonal neuropathies are quite different.

Clinical observation (kinky hair), biopsy, autopsy (Peiffer et al. 1977), and radiological data indicate that not only peripheral nerve axons are affected in GAN. The pattern of CNS long tract disease suggests a central–peripheral distal axonopathy (Thomas et al. 1987). Involvement of the central nervous system is widespread, including the cerebellum, cortical white matter, and ascending and descending tracts, with neuronal loss, prominent giant axonal swellings, gliosis, and Rosenthal fiber formation (Dubeau et al. 1985; Peiffer et al. 1977; Thomas et al. 1987; Kretzschmar et al. 1987). In cultured fibroblasts of patients with GAN, filamentous accumulations have been reported to immunostain positively for vimentin (Pena 1982). Neurofilaments, glial filaments, keratin filaments, and vimentin all fall into the class of intermediate filaments, but are unique proteins coded for by different genes (Klymkowsky and Plummer 1985). Similar filamentous accumulations occur in Langerhans cells, mast cells, and melanocytes from skin biopsy, thus permitting a diagnosis without nerve biopsy. Studies on the structure, synthesis, and biochemical properties of filament accumulations seen in cultured fibroblasts from patients with GAN have demonstrated no defect in synthesis or structure of the actual intermediate filament subunit proteins. These observations suggested that in giant axonal neuropathy, the abnormality lies in the control of assembly and structural organization of intermediate filaments (Pena 1982; Klymkowsky and Plummer 1985; Donaghy et al. 1988a). Recent analysis, however, identifies gigaxonin as a major factor in the degradation of cytoskeletal intermediate filaments using the proteasomal degradation pathway (Mahammad et al. 2013).

19.10.4 Differential Diagnosis

Giant axonal swelling with filamentous accumulations is a striking histological finding, but seeing a single swollen axon or finding some normal-sized axons with filament accumulations is not sufficient to make the diagnosis. When swollen axons with filament accumulations are common (1 or more per fascicle) in peripheral nerve, the differential diagnosis is narrowed to a group of toxic neuropathies including hexacarbons, acrylamide, and carbon disulfide and related products (Chap. 18) and the genetically based giant axonal neuropathy discussed above. These two groups are only superficially similar. Axonal swelling is more dramatic in GAN than in the toxic neuropathies, and the osmiophilic condensations seen in GAN are not a feature of hexacarbon toxic neuropathy (King et al. 1993; Asbury and Brown 1980). Filaments are larger in cross section and packed more tightly in GAN (King et al. 1993).

A small number of neuropathies have been described with giant axonal swelling but atypical clinical features which do not share the same genetic defect. These include an autosomal dominant CMT-2 phenotype with cardiac disease (Vogel et al. 1985), neuromyopathy and cardiomyopathy with desmin filament storage (Sabatelli et al. 1994), and sporadic adult-onset neuropathy with B12 deficiency (Schochet and Chesson 1977). The description of the pathology in these cases suggests that the swollen axons are not as numerous and not as large as in typical GAN. The highly consanguineous family reported by Ben Hamida et al. (1990) had typical histological findings, but showed much slower progression, with survival to late adult life, and none of the 6 patients had kinky hair.

Axoplasmic osmiophilic paracrystalline inclusions without giant axonal swelling can be seen in a variety of neuropathies and even in normals and are in and of themselves nonspecific. The axonal swellings of neuroaxonal dystrophy are packed with membranous organelles, not filaments (vide infra 19.14.1), but one recorded case had some filamentous axonal swellings and accumulations of filaments in various nonneural cell types similar to GAN and the authors speculated on a relation between the two (Begeer et al. 1979). The focal axonal swellings of polyglucosan body disease are obviously not due to filamentous accumulations (Chap. 21). Although neurofilament filled focal axonal swellings can be detected in intramuscular nerves in GAN, such a finding is nonspecific and may be observed in normals (Alderson 1992).

19.11 Refsum Disease (CMT-4)

19.11.1 Clinical Manifestations

Refsum disease is a rare disorder of lipid metabolism inherited in autosomal recessive fashion (Skjeldal et al. 1993). In most patients suffering from Refsum disease (RD) phytanoyl-CoA hydroxylase is deficient, due to mutations in the structural gene *PHYH*/*PAHX* (Wanders and Komen 2007), which results in the accumulation of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid: a 3-methyl branched-chain fatty acid) in tissues and bodily fluids (Poll-The and Gaerner 2012). Most of the cases reported to date originate from Scandinavia, northern France, and Britain. The characteristic, and nearly invariable, clinical features are retinitis pigmentosa, peripheral neuropathy, cerebellar degeneration, and elevated CSF protein. Other common but not invariable features are ichthyosis and hearing loss. Onset is usually in the first or second decade with night blindness due to retinal degeneration. Neuropathy and ataxia usually follow, with relentless progression if untreated. Peripheral neuropathy may dominate the clinical picture and presents as a peroneal muscular atrophy-type syndrome, with motor involvement more prominent than sensory in most cases. Peripheral nerves may be palpably enlarged. Electrophysiological tests usually reveal a peripheral neuropathy with dramatic conduction slowing, sometimes to 10 m/s or less (Staunton et al. 1989), and CSF protein is invariably elevated.

Refsum disease is associated with failure of phytanic acid α-oxidation (Steinberg 1989). Most patients have clear-cut manifestations of the disorder before the age of 20 years. Making the diagnosis is critical because the treatment, long-term restriction of dietary phytanic acid, can arrest and partially reverse the manifestations (Skjeldal et al. 1993; Gibberd et al. 1985). Plasma exchange is useful for rapid reduction of serum phytanic acid levels (Harari et al. 1991). An elevation in serum phytanic acid is probably invariably seen in untreated patients and does not occur in normal patients. However, it is not completely specific to Refsum disease, occurring in other peroxisomal disorders such as Zellweger syndrome (Steinberg 1989; Skjeldal et al. 1993). Phytanic acid levels may be normal in treated or dietary restricted patients, but assay of α-oxidation of fatty acids by cultured fibroblasts will be abnormal (Skjeldal et al. 1993). The clinical picture may be mimicked by spinocerebellar degenerations or familial neuropathies. Symptoms can worsen dramatically with febrile illness, surgical procedures, pregnancy, or weight loss. If the peripheral nerves are involved in such an exacerbation, a diagnosis of GBS or CIDP might even be entertained and would be supported by the CSF and electrophysiological findings (Veltema and Verjaal 1961; Staunton et al. 1989; Kuntzer et al. 1993; Hungerbuhler et al. 1985).

19.11.2 Pathology

Peripheral nerve histological studies were published before (Veltema and Verjaal 1961; Cammermeyer 1956; Nevin et al. 1967; Allen et al. 1978; Schott et al. 1968) and after (Barbieri et al. 1981; Dereux and Gruner 1963; Dotti et al.

1985; Fardeau and Engel 1969; Fardeau et al. 1970; Lenz et al. 1979; Lapresle et al. 1974; Hungerbuhler et al. 1985; Flament-Durand et al. 1971; Savettieri et al. 1982) the availability of electron microscopy.

19.11.2.1 Light Microscopy

The neuropathy of Refsum disease is commonly regarded as hypertrophic, but the most prominent changes occur proximally, in the plexuses and cauda equina (Lapresle et al. 1974). Although the hypertrophic character of the neuropathy had been appreciated prior to the advent of EM for the examination of peripheral nerves (Cammermeyer 1956), biopsies with only nonspecific changes and no onion bulb formations were described (Schott et al. 1968). EM reveals onion bulbs in some cases where they cannot confidently identified with light microscopy alone, but the structures seen may be quite unimpressive (Lapresle et al. 1974; Fardeau et al. 1970; Barbieri et al. 1982). Moreover, even in the modern era some authors have reported the absence or near absence of onion bulb formations (Dotti et al. 1985; Hungerbuhler et al. 1985; Staunton et al. 1989). Thus, the prominence of onion bulb formations can vary widely in Refsum disease, and the diagnosis is not excluded even if onion bulb formations are not seen.

The total number of myelinated fibers is reduced, often dramatically, with large myelinated fibers showing more severe depletion. A diffuse thinning of myelin gives evidence of segmental demyelination and remyelination, and active segmental demyelination is only infrequently observed, with debris-filled cells a rarity. Endoneurial vessel hyalinization and nerve fibrosis are common nonspecific changes.

Some nerve biopsies have been reported as normal, but it is unclear if electron microscopy was employed in those cases (Petit et al. 1986). Even with electron microscopy the abnormalities can be quite mild (Barbieri et al. 1981).

19.11.2.2 Electron Microscopy

In early reports of the ultrastructural morphology of Refsum disease (Fardeau and Engel 1969; Fardeau et al. 1970; Flament-Durand et al. 1971), the presence of paracrystalline inclusions within nonmyelinating Schwann cells, about 1 μm in diameter and perhaps intramitochondrial, was emphasized (Fig. 5.9a, b). However, it is now clear that these inclusions can be seen in a variety of other neuropathies, and that they are not necessarily present in Refsum disease. Other nonspecific dense bodies may also be prominently present within Schwann cells in Refsum disease (Fardeau and Engel 1969). Atypical axonal mitochondria have also been described (Savettieri et al. 1982). Special note has been made of the observation of lipofuscin in myelinating Schwann cells (Thomas 1993b) as this pigment is usually confined to nonmyelinated Schwann cells. However, we have also made this observation in a case of Dejerine–Sottas syndrome. Involvement of unmyelinated fibers has not been studied to our knowledge.

19.11.3 Pathogenesis

Refsum disease patients examined are homozygous for inactivating mutations in the *PHYH* or *PAHX* gene which encodes phytanoyl-CoA hydroxylase on chromosome 10 (Mihalik et al. 1997). The inability to properly metabolize phytanic acid is correlated with serum and tissue phytanic acid elevation and disease severity is strong evidence that the accumulation of phytanic acid itself causes the cell and organ damage. Incorporation of phytanic acid into membrane lipids, especially myelin, may interfere with membrane stability (Steinberg 1989), although some measurements of peripheral nerve endoneurial phytanic acid have not agreed (Yao and Dyck 1987). The possibility remains that other consequences of an α-oxidation defect contribute to the disease spectrum (Steinberg 1989).

19.12 Peripheral Neuropathy in Diseases with Defective DNA Repair Mechanisms

An elaborate repair system has evolved to cope with the continuous damage to cellular DNA that occurs as a result of environment influences. Genetic defects in this repair mechanism are implicated in the pathogenesis of several human neurodegenerative diseases (Wood 1991; Friedberg 1992), some of which have peripheral nerve manifestations.

19.12.1 Cockayne Syndrome

Cockayne syndrome (CS) is an autosomal recessive leukodystrophy-like disease characterized by growth failure and microcephaly, developmental delay and mental retardation, microcephaly, progeria (premature senility), and cachexia (Nance and Berry 1992). Cockayne syndromes A & B are caused by homozygous or compound heterozygous mutations in the gene encoding the group 8 or 6, respectively, excision-repair cross-complementing protein (*ERCC8* or *ERCC6*) genes (Mallery et al. 1998). In addition to DNA repair deficiency, transcription dysregulation, altered redox balance, and mitochondrial dysfunction have been implicated in the pathogenesis of this disorder (Cleaver et al. 2013). Neuropathy can be an early and important manifestation (Campistol Plana et al. 1991), but more often it is present only on nerve conduction studies, which show diffuse moderate to marked slowing.

A number of reports on peripheral nerve pathology have conclusively shown that the neuropathy is due to a primary

demyelinating process, often with onion bulb formation (Ohnishi et al. 1987; Grunnet et al. 1983; Roy et al. 1973; Vos et al. 1983; Moosa and Dubowitz 1970; Sasaki et al. 1992; Weidenheim et al. 2009). Not all biopsies have shown abnormalities (Gamstorp 1972), but this may result from use of relatively insensitive histological techniques (Vos et al. 1983). In a review of 6 patients, 3 with a severe infantile onset, 3 with a milder childhood onset, all showed evidence of segmental demyelination and remyelination, with many hypomyelinated axons (Vos et al. 1983). In the infantile cases the disease seems to be more aggressive with occasional naked axons. In the childhood-onset group, denuded axons were not seen, but onion bulb formations were frequent. Although little evidence of active axonal disease is present, a variable degree of chronic axonal loss is seen, correlating with duration of disease. Large myelinated, small myelinated, and unmyelinated fibers may be affected. Teased fiber studies suggest that the demyelination is primary (Ohnishi et al. 1987; Sasaki et al. 1992).

In some instances, electron microscopy revealed inclusion material in both myelinating and nonmyelinating Schwann cells and in perineurial cells (Vos et al. 1983; Grunnet et al. 1983; Roy et al. 1973). This has been described as membrane-bound vacuoles containing an electron dense granular ground substance within which small empty spaces can be seen, some of which have a granular or membranous content.

19.12.2 Xeroderma Pigmentosum (XP)

This is an autosomal recessive syndrome characterized by a high incidence of sunlight-induced eye and skin lesions and cerebral and cerebellar degeneration of variable severity. Several genetic defects (in *ERCC2*, *ERCC5*, and *XPA*) have been identified on several different chromosomes, but all relate to a family of genes involved in excision repair of DNA (Wood 1991). Some patients with defects associated with XP have features of Cockayne syndrome (Wood 1991; Vermeulen et al. 1993). Involvement of peripheral nerves is usually a minor feature and progresses very slowly with increasing age. Kanda and colleagues (1990) provide a comprehensive review.

There is a surprising wealth of peripheral nerve biopsy and autopsy material (Kanda et al. 1990; Thrush et al. 1974; Tachi et al. 1988; Fukuhara et al. 1982; Hentati et al. 1992), and this has shown chronic depletion of myelinated, and, to a much lesser extent, unmyelinated fibers that correlates with the duration and severity of the disease. Large MFs are most severely affected. Active axonal degeneration is rarely observed. Some fibers may be too thinly myelinated, and small onion bulb formations with only 1 or 2 circumferential layers of Schwann cells are frequently seen. Teased fiber studies suggest an axonal or mixed axonal/demyelinating process. No specific ultrastructural features are present. The pattern of fiber loss has variably been interpreted as suggestive of a distal axonopathy or a neuronopathy (Kanda et al. 1990).

19.12.3 Ataxia Telangiectasia

Ataxia telangiectasia is an autosomal recessive spinocerebellar degeneration with onset in early childhood of progressive ataxia, dementia, and extrapyramidal movement disorders (Woods and Taylor 1992). Cutaneous and ocular telangiectasias are a nearly constant feature, and there is a high incidence of lymphoreticular disorders, with recurrent infections, lymphoid hypoplasia, and absent IgA and IgE. An axonal sensorimotor neuropathy is a late but common manifestation (Woods and Taylor 1992). Lymphoid cells show an increased sensitivity to radiation, and lymphoreticular malignancies, especially Non-Hodgkin's lymphoma and lymphocytic leukemia, are common. The genetic defect has been linked to chromosome 11q (Gatti 1993). To date more than 500 biallelic mutations in the ataxia-telangiectasia gene (*ATM*) have been identified (Buzin et al. 2003; Huang et al. 2013). The ATM protein, which is located in the nucleus and cytosol associated with peroxisomes, is multifunctional and plays a critical role regulation of cell cycle control, DNA damage repair, and cell survival and death by orchestrating the phosphorylation of multiple substrates (Huang et al. 2013).

A small number of nerve biopsy reports are available (Asbury and Johnson 1978; Gardner and Goodman 1969; Malandrini et al. 1990; Cruz-Martinez et al. 1977; Jerusalem and Bischoff 1972; Barbieri et al. 1986; Gressner et al. 1972). These have shown a picture of chronic myelinated axon loss, but usually most pronounced in the large MF population. Some segmental myelin changes and rare small onion bulbs may be seen. Where performed, unmyelinated axon counts have been normal. A highly characteristic but not always detected feature is the finding of Schwann cells with large bizarre hyperchromatic nuclei with irregular outlines and multiple infoldings. The presence of polymorphous membrane-bound debris in Schwann cells, macrophages, and other endoneurial cells has been emphasized by some authors, but many of the provided illustrations show Pi granules or lipofuscin (Gardner and Goodman 1969; Jerusalem and Bischoff 1972). Mitochondrial morphology may be altered (Gardner and Goodman 1969).

19.13 Neuropathy with Defective Porphyrin Metabolism

19.13.1 Clinical Manifestations

Defects in the porphyrin metabolic pathways underlie the porphyrias (Moore 1993). The enzymes involved in heme synthesis in hepatic and erythroid cells differ, and only the

hepatic porphyrias, caused by defects in hepatic heme synthesis, are associated with peripheral neuropathy. These include acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria. The enzymatic defects and their chromosomal localization are well known, and all are inherited in autosomal dominant fashion (Moore 1993). The neuropathic manifestations of these three diseases are indistinguishable (Windebank and Bonkovsky 1993). Patients often present with crises characterized by abdominal pain, encephalopathy, and the acute onset of a peripheral neuropathy, but the latter may occur in isolation (Eales and Linder 1962). A slowly evolving polyneuropathy with infrequent exacerbations is rarely seen (di Trapani et al. 1984). Motor defects predominate, proximal limb and facial weakness are not uncommon, and autonomic disturbances are usually prominent. Electrophysiological studies reveal an axonal neuropathy, and the CSF examination is usually unremarkable (Albers et al. 1978; Eales and Linder 1962).

Porphyric crises are often precipitated by drugs which induce microsomal P450 enzyme activity; stress and starvation have the same effect (Windebank and Bonkovsky 1993). Hereditary tyrosinemia, an inborn error of the final step in tyrosine metabolism (fumarylacetoacetate hydrolase), can manifest with acute neurological crises similar to those of porphyria, possibly resulting from an indirect impairment of porphyrin metabolism (Mitchell et al. 1990).

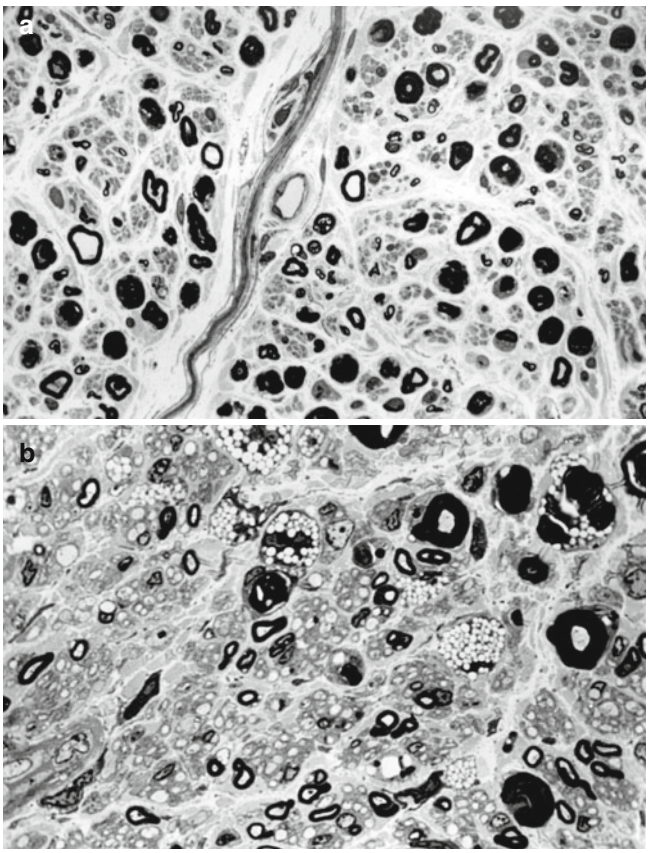


Fig. 19.24 Porphyria: initial sural nerve biopsy (**a**) shows acute massive axonal degeneration. At autopsy 2 months later many foamy macrophages are dispersed in the endoneurium against a background of MF depletion (**b**) (Plastic, magnification: **a**, **b** ~600×)

19.13.2 Pathology

Acute porphyric neuropathy (see Lin et al. 2013 for review) is now recognized as an axonal disease (Thorner et al. 1981; Anzil and Dozic 1978; Cavanagh and Mellick 1965; di Trapani et al. 1984; Barohn et al. 1994), despite earlier emphasis on patchy demyelinating lesions (Campbell 1963; Denny-Brown and Sciarra 1945; Gibson and Goldberg 1956). We have examined one case at nerve biopsy and at autopsy 2 months later (case 19.2) (Thorner et al. 1981). Light microscopic examination during the acute phase revealed active axonal degeneration with no significant segmental myelin change (Fig. 19.24a). Axonal atrophy, axon-Schwann cell networks, and other nonspecific axonal alterations were frequently noted. Large myelinated fibers were most severely involved. Unmyelinated axons were normal in total number, but demonstrated a leftward shift in the fiber diameter-frequency histogram. A variety of nonspecific degenerative alterations were present, and particularly notable was a small population of unmyelinated fibers that displayed axonal swelling with dense accumulations of neurofilaments (Thorner et al. 1981). At autopsy, performed 2 months after biopsy, a more chronic axonal neuropathy was seen in the tibial nerve, with large numbers of foamy macrophages present in the endoneurium (Fig. 19.24b). Frequent regenerating clusters were present. Examination of the dorsal and ventral spinal roots revealed a much milder axonal neuropathy. Quantitative analysis of fiber counts and the ratio of axonal to myelin lamellar numbers demonstrated that the axonal atrophy and fiber dropout was worse distally than proximally. Cell body dropout in the spinal ganglia and anterior horns was mild to moderate.

Only a small number of modern era reports on the pathology of porphyric neuropathy exist, and these are largely congruent with our findings (Barohn et al. 1994; di Trapani et al. 1984; Anzil and Dozic 1978; Cavanagh and Mellick 1965; Sweeney et al. 1970). Interestingly, in an autopsy study reported by Sweeney et al. (1970), the severity of axonal degeneration was greater in proximal nerves than in distal nerves, the reverse of that seen the case we examined and the patient reported by Cavanagh and Mellick (1965). In hereditary coproporphyria of long duration, biopsy revealed acute and chronic axonal changes, but onion bulb formations were also seen (di Trapani et al. 1984). The authors interpreted these myelin changes as likely secondary to axonal disease (di Trapani et al. 1984). In hereditary tyrosinemia an active

axonal degeneration was documented in three nerve biopsies taken during acute neurological crises (Mitchell et al. 1990).

19.13.3 Pathogenesis

Our analysis of the histological material discussed above indicates that acute porphyric neuropathy is a distal axonopathy, although it is unclear why clinically motor fibers are more severely affected than sensory fibers. This is in agreement with the conclusions of Cavanagh and Mellick (1965). The focal filament accumulations seen in unmyelinated fibers are suggestive of an alteration in axonal transport. A deficiency of neural heme might explain disturbance of axonal function (Moore 1993). Conversely, the occurrence of neuropathy in only the hepatic porphyrias might be explained on the basis of accumulation of porphobilinogen or delta-aminolevulinic acid (δ-ALA), not seen in the erythropoietic porphyrias. The presence of neurological manifestations similar to those of porphyria, correlating with elevation of urine δ-ALA excretion, in hereditary tyrosinemia (Mitchell et al. 1990) and in hereditary δ-ALA dehydratase deficiency (Mercelis et al. 1990), suggests that elevation of δ-ALA is the proximate cause. Possible mechanisms involve competition for GABA binding sites mimicking physiologic effects GABA binding, free radical generation with oxidative stress, and both nuclear and mitochondrial DNA damage (see Lin et al. 2013 for review).

Case 19.2
A 46-year-old Caucasian woman with a previous history of depression and one episode of severe abdominal pain with diarrhea was admitted to a hospital because of protracted vomiting. Cholecystitis was diagnosed, but despite surgical treatment the vomiting continued over the subsequent 3 weeks. During this interval the patient's affect was observed to become flattened, and impairment of recent memory was noted, although she remained alert and oriented. Over a period of several days she developed four-limb weakness, legs more affected than arms. Constipation and overflow urinary incontinence were noted. Examination revealed grade 4/5 power (MRC) in the upper extremities diffusely and grade 3/5 power in the lower extremities. Tendon reflexes were reduced in the biceps and absent elsewhere. Decreased sensation to light touch was present in both hands and feet. Nerve conduction studies and EMG revealed an axonal neuropathy. CSF examination was unremarkable. The diagnosis of variegate porphyria was confirmed by analysis of blood, urine, and stool porphyrins. Nerve biopsy was performed 1 month into the acute illness. Over the subsequent month the patient's encephalopathy and neuropathy worsened, and the patient died of systemic candidiasis 3 months after admission.

19.14 Other Genetically Determined Neuropathies

19.14.1 Infantile Neuroaxonal Dystrophy

19.14.1.1 Clinical Manifestations
Neuroaxonal dystrophy (INAD) is an autosomal recessive degenerative disease manifesting progressive encephalopathy with motor and cognitive regression in the first years of life usually with death before the end of the first decade of life (Seitelberger 1986). There is cerebellar atrophy and accumulation of iron in the globus pallidus. Involvement of the peripheral nervous system is usually overshadowed by CNS manifestations but may reveal itself through hypotonia, diminished reflexes, and muscle atrophy. The most common variant demonstrates early infantile onset, but disease manifestations may begin later (Seitelberger 1986). Onset with neuropathy in childhood has been described (Shimono et al. 1977). Thus nerve, skin, and conjunctival biopsy have been important avenues to diagnosis, although any tissue containing axons may be sufficient (Ceuterick and Martin 1990; Duncan et al. 1970; Lehmann and Goebel 1992; Martin et al. 1979; Wisniewski and Wisniewski 1980). There is some disagreement about the sensitivity of conjunctival biopsy (Ceuterick and Martin 1990; Crisci et al. 1989), but given its low morbidity it may be used before nerve biopsy.

19.14.1.2 Light Microscopy
The major site of pathology in NAD is the CNS where axonal spheroidal swelling to twice or more the normal diameter is seen throughout the neuraxis (Seitelberger 1986). However, similar swellings are seen in peripheral nerve and intramuscular nerve fibers (Duncan et al. 1970; Berard-Badier et al. 1971; Lehmann and Goebel 1992; Shimono et al. 1976, 1977; Martin and Martin 1972; Sengel and Stoebner 1972; Yagishita et al. 1978). Frank Wallerian degeneration is uncommon, and myelinated fiber density is normal or mildly reduced (Fig. 19.25a). The dystrophic axons may be infrequent, only one or two per fascicle (Shimono et al. 1976, 1977); Martin and Martin (1972) found only three such axons in six nerve fascicles. Dystrophic axons may also be atrophic rather than swollen, and their axoplasm may be irregular in contour and granular or inhomogenous in texture (Fig. 19.25b). The overlying myelin is often thinned or absent altogether. The axonal spheroids are PAS positive and variably argyrophilic.

19.14.1.3 Electron Microscopy
Ultrastructural examination reveals that the axon swellings mostly consist of accumulations of randomly disposed tubulovesicular profiles (presumably the axoplasmic reticulum) with associated focal congregations of mitochondria, filaments, glycogen and various dense bodies, granules, and vacuoles (Figs. 19.26a, b, and 19.27a, b). Loosely separated

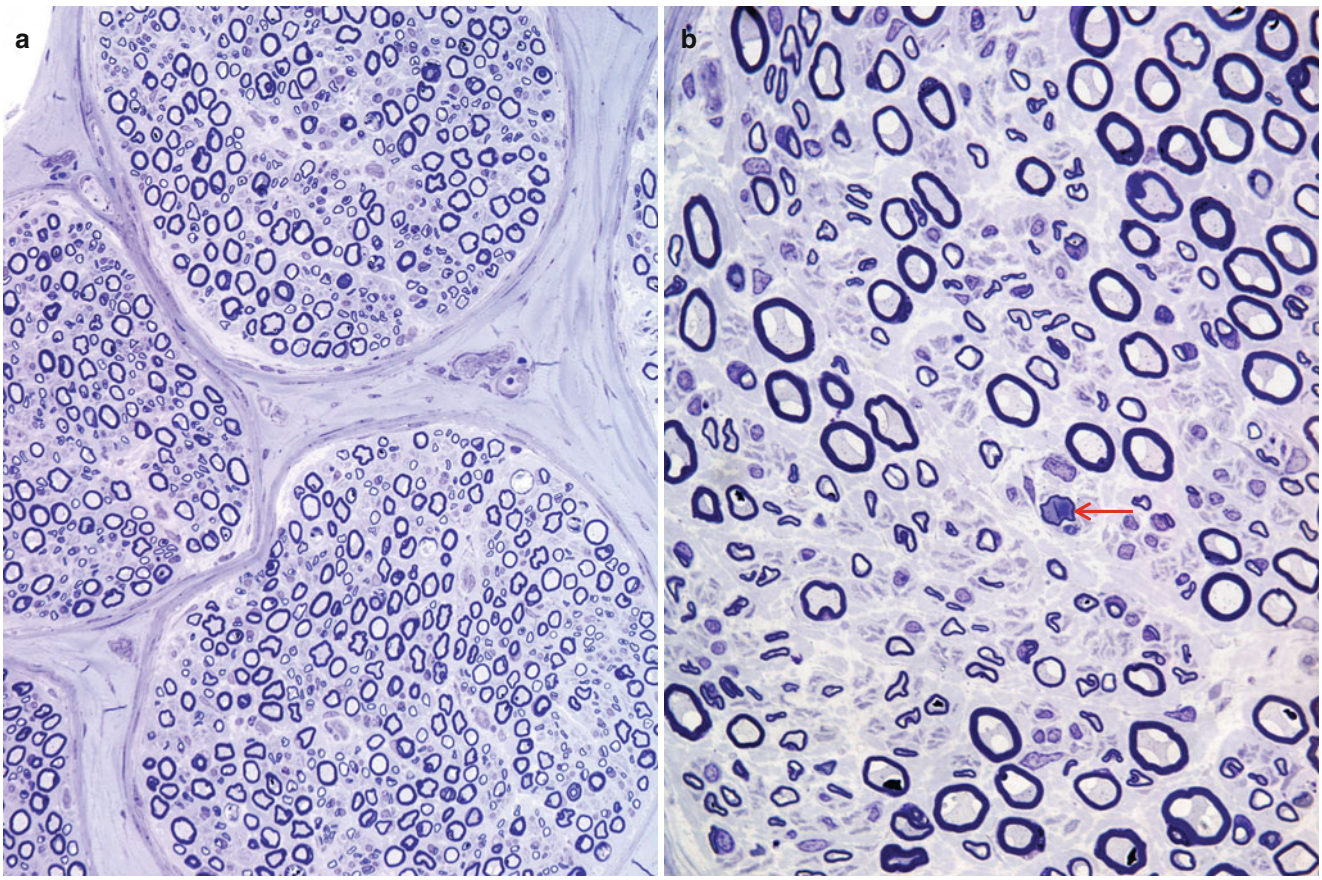


Fig. 19.25 NAD: (**a**) note normal density of MFs, many of which have an attenuated myelin sheath. In (**b**) a single irregular fiber displays heterogeneously dense axoplasm (*arrow*) (1 μm thick plastic sections, magnification: **a**, 200×; **b**, 1,000×) (Tissue courtesy of Dr. J. Michaud, Montreal)

parallel linear or circular concentric membrane arrays can be seen. Dysmorphic mitochondria may be prominent, and vacuoles containing glycogen-like granules are frequently seen. Microtubules and neurofilaments seem to be diminished in density (Berard-Badier et al. 1971). Unmyelinated axons show the dystrophic changes most prominently (Barlow et al. 1989; Sengel and Stoebner 1972), but they are also readily seen in myelinated axons and in nerve terminals. Nonneural cells, particularly Schwann cells, may also demonstrate the characteristic accumulations (Wisniewski and Wisniewski 1980; Yagishita et al. 1978).

Accumulations of tubulovesicular organelles are one of the nonspecific signs of axonal disease (compare Figs. 19.26 and 19.27 with Fig. 4.6a, b). However, the detection of dystrophic axons per nerve fascicle on light microscopy combined with the observation of tubulovesicular and dystrophic mitochondrial aggregates on microscopy is very suggestive of neuroaxonal dystrophy.

19.14.1.4 Pathogenesis

INAD (Seitelberger disease, also called neurodegeneration with brain iron accumulation – 2A, NBIA2A) largely results from mutations in *PLA2G6* gene which were found (Gregory et al. 2008; Landrieu and Baets 2013) in 45 (79 %) of 56 patients with INAD1. Seitelberger regards this as part of a spectrum of diseases in which unspecified defects in axonal transport or neuronal metabolism result in the formation of the characteristic swellings (Seitelberger 1986). Lehmann and Goebel (1992) have suggested a defect in fast axonal transport mechanisms. However, the finding of abnormal cytoplasmic organization in non-neuronal cells (Yagishita et al. 1978) suggests a more systemic defect which may be more pronounced in axons due to their unique metabolic requirements.

19.14.2 The Neuropathy of Oxalosis

Primary hyperoxaluria is an autosomal recessive defect in glyoxylate metabolism which results in renal and extrarenal deposition of calcium oxalate crystals. There are at least three forms of primary hyperoxaluria caused by several genes: type I (*AGXT*, alanine-glyoxylate aminotransferase), type 2 (*GRHPR*, glyoxylate reductase/hydroxypyruvate reductase), and type 3 (*DHDPSL*). Renal failure invariably

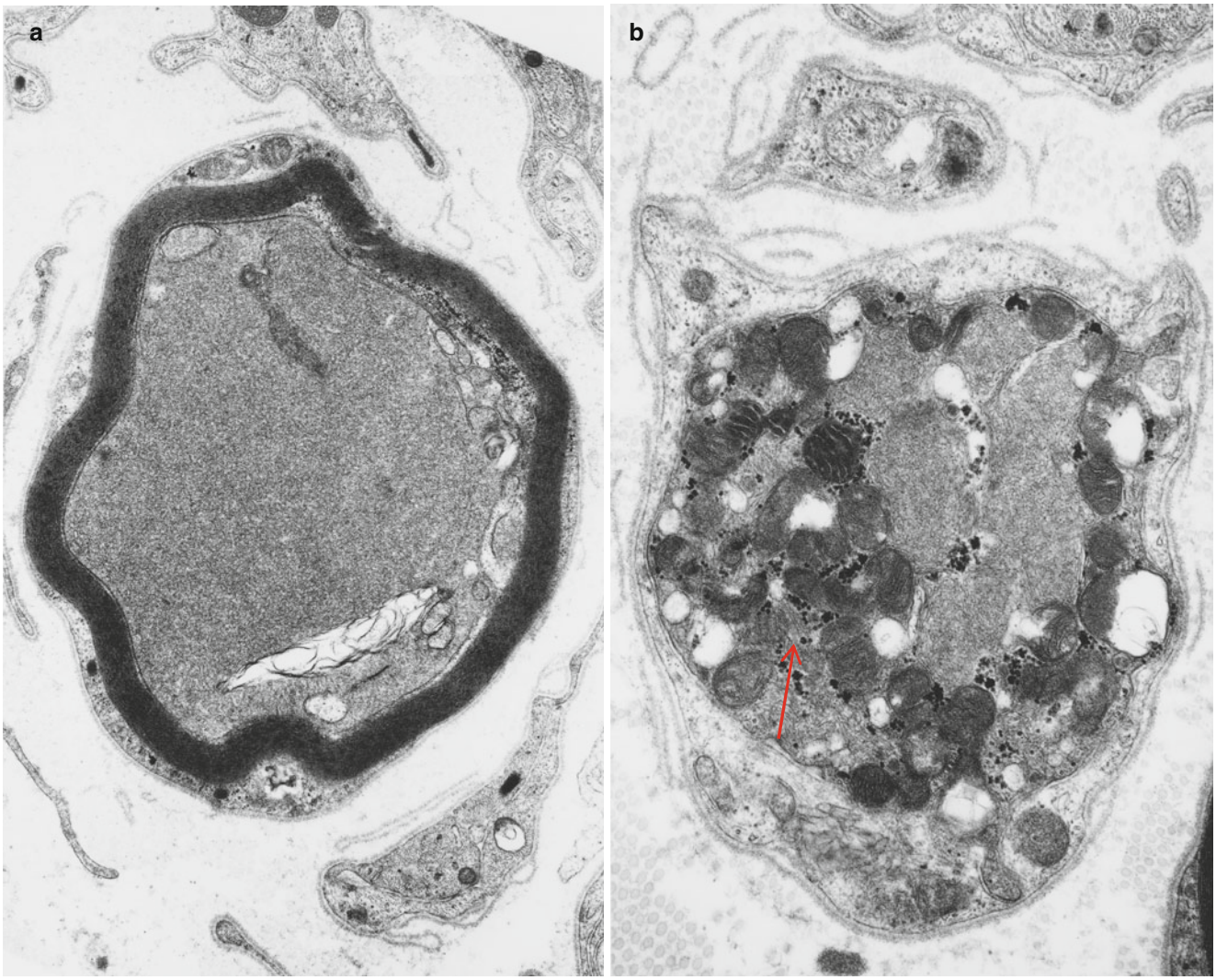


Fig. 19.26 NAD: the typical intra-axonal tubulovesicular profiles are illustrated in myelinated and unmyelinated axons. Note mitochondrial aggregates at the periphery of an unmyelinated fiber (*arrow*, **b**) (Magnification: **a**, 15,620×; **b**, 25,200×)

occurs. Peripheral neuropathy in this setting may be due to renal failure, but severe conduction slowing seen in the cases summarized below is not typical of uremic neuropathy and fits with the prominent segmental demyelination seen on histological examination of nerves in oxalosis. We have had experience with one such case in which the presence of a severe neuropathy, progressing despite aggressive hemodialysis and stable BUN and creatinine, suggested that calcium oxalate deposition in nerve might play a contributory role (Bilbao et al. 1976). At autopsy, specimens taken from radial and sciatic nerves revealed a mixed picture of segmental demyelination with chronic and active axonal degeneration. The most striking observation was the presence of numerous refractile crystals which distended otherwise intact myelin sheaths (Fig. 19.28a, b). A few endoneurial crystals were seen, enclosed by giant cell formations. There was no evidence of microvascular injury. Electron microscopy confirmed the presence of both segmental demyelination and axonal degeneration and revealed that the crystals were found in the Schwann cells. Other authors have documented similar findings (Moorhead et al. 1975; Hall et al. 1976), although Hall et al. (1976) also found crystal deposition in vessel walls and favored the possibility that the segmental demyelination was secondary to axonal disease.

19.14.3 Peripheral Nerve Changes in Myotonic Dystrophy

Myotonic dystrophy (MyD) is a multisystem disease in which muscle involvement has traditionally been emphasized. Several forms occur as the result of heterozygous

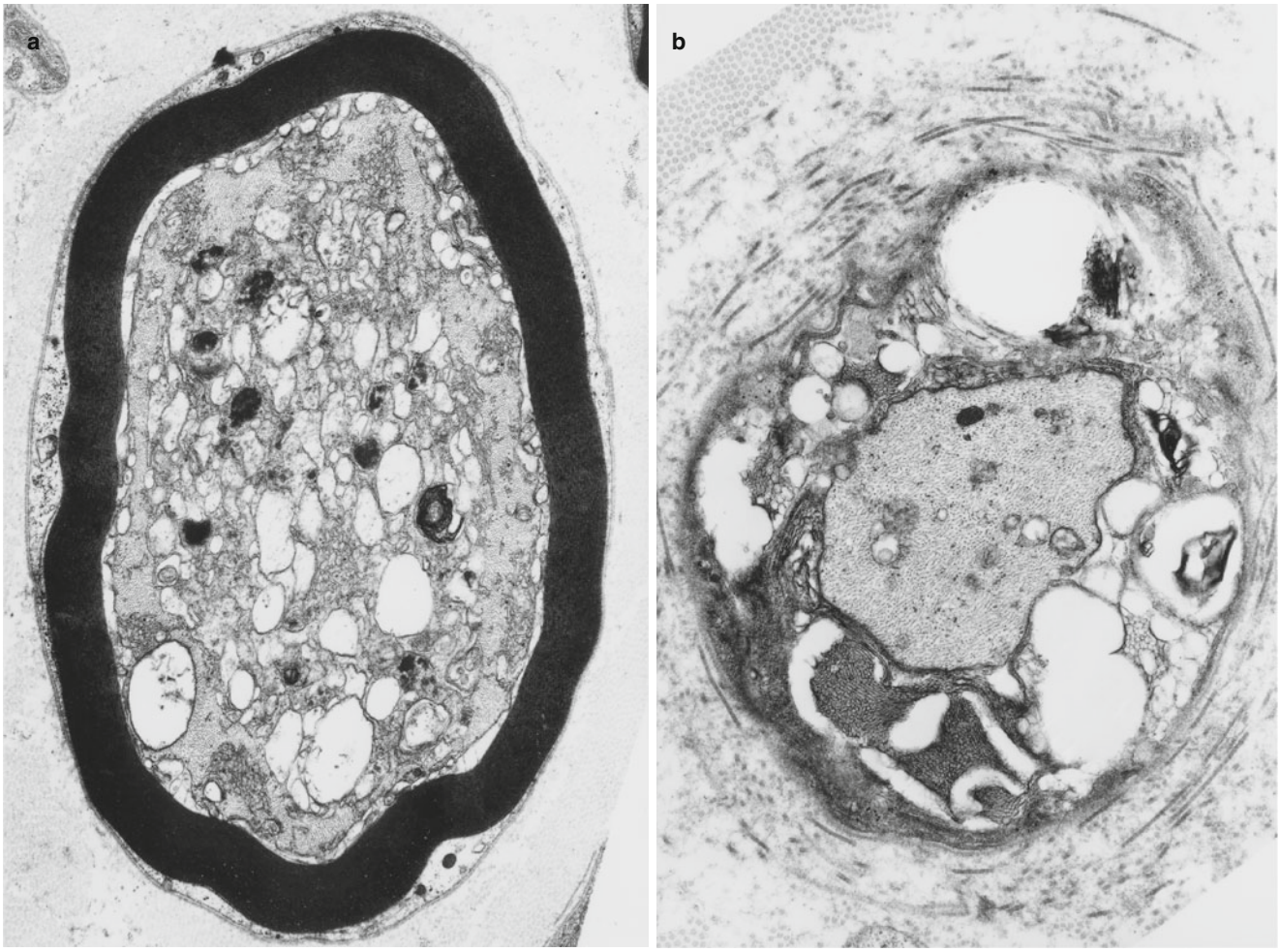


Fig. 19.27 NAD: (**a**) axoplasm is filled with large vacuoles, dense bodies, and myeloid bodies. In (**b**) segmental demyelination is present (Magnification: **a**, 9,680×; **b**, 12,780×)

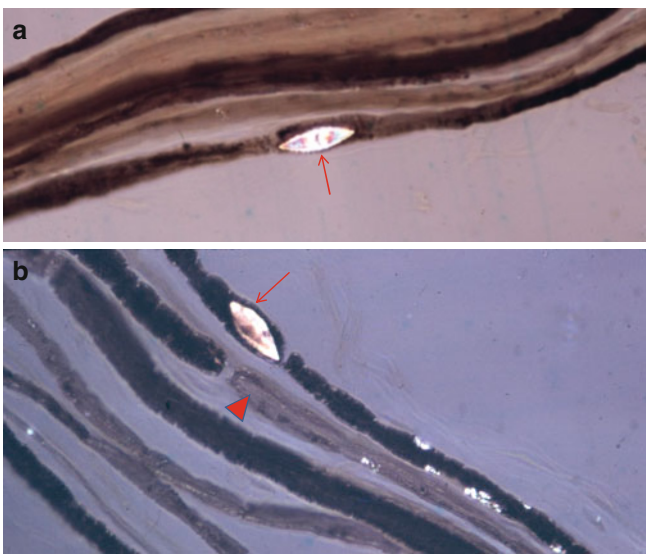


Fig. 19.28 Oxalosis: teased fiber preparation shows crystals (**a** and **b** *arrows*) with MFs. Ultrastructural examination revealed that these were found within Schwann cells. Note segmental demyelination (**b**: *arrowhead*)

expansions of trinucleotide repeats in the *DMPK* gene or *ZNF9*. However, neuropathic features (not related to the occurrence of glucose intolerance in MyD) have also been described (Panayiotopoulos and Scarpalezos 1976; Mondelli et al. 1993). Electrophysiological studies have shown a mild sensorimotor axonal neuropathy (Mondelli et al. 1993). This has been supported by the results of histological examination, which have at times shown a mild loss of myelinated fibers, especially those of large diameter, with infrequent occurrence of Wallerian degeneration (Cros et al. 1988). Axonal atrophy with probable secondary demyelination has also been described (Mondelli et al. 1993; Cros et al. 1988). Some morphometric analyses of peripheral nerve in MyD have been entirely normal (Pollock and Dyck 1976). Endoneurial fibrosis has at times been described as very prominent (Kito et al. 1973) and in one report resulted in palpable nerves (Borenstein et al. 1977). A few rudimentary onion bulb formations have been described (Borenstein et al. 1977). There may be two groups of patients with MyD

and a neuropathy: those with mild axonal changes as part of the myotonic dystrophy disease and those with a co-occurrence of MyD and a more severe inherited familial neuropathy.

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Storage Diseases

20

Inborn errors of metabolism can manifest as multisystem diseases characterized by abnormal intracellular accumulation of storage material, the best known involving lysosomal enzymes. In a recent review (Boustany 2013), a less restrictive classification of lysosomal storage disorders has been proposed which includes diseases that display defects in cellular storage, synthetic enzymes, lysosome membrane or other membrane proteins, and trafficking. In the past, tissue examination for identification and characterization of storage material, including nerve biopsy, was an important means of diagnosis. However, advances in understanding of the biochemical and genetic basis of disease have revealed specific enzymatic and genetic defects in many of these conditions (Table 20.1), and noninvasive diagnosis using genetic techniques, assays of enzyme activity in fluids or cultured cells, or detection of abnormal storage products has increasingly become the diagnostic method of choice. Nevertheless, atypical clinical manifestations or inaccessibility of advanced biochemical and genetic techniques occasionally make nerve biopsy useful in some patients, and for a small number of storage diseases, histologic examination remains essential.

20.1 The Sphingolipidoses

Sphingolipids are lipids in which sphingosine, a complex amino alcohol, replaces glycerol in the lipid backbone. This class of molecules includes gangliosides, sulfatides, and cerebrosides and plays a role in biological membrane structure and function. Specific enzymatic defects result in pathological accumulation of lipid within lysosomes in various tissues, although how this leads to cell injury is often not known. The diseases included under this aegis include metachromatic leukodystrophy, globoid cell leukodystrophy (Krabbe disease), Fabry disease, Niemann–Pick disease, Farber disease, GM1 and GM2 gangliosidosis, and Gaucher disease.

20.1.1 The Metachromatic Leukodystrophies

The metachromatic leukodystrophies (MLD) are caused by defective activity of the arylsulfatase enzyme system, with a consequent accumulation of sulfatides. The most common variant (Phenotype MIM#250100) is caused by a defect in the *arylsulfatase A* gene (*ARSA*, protein product cerebroside sulfatase). The AB variant of MLD (MIM#249900) is produced by a mutation in the prosaposin gene (*PSAP*) on chromosome 10q22.1 which produces a deficiency of sphingolipid activator protein saposin B resulting in diminished ARSA activity. Multiple sulfatase deficiency (MIM#272200) is produced by a defect of arylsulfatases A, B, and C due to mutation in the *sulfatase-modifying factor-1 gene* (*SUMF1*). This form is clinically similar to the others but includes the presence of urinary mucopolysaccharides in addition to sulfatides (Hahn et al. 1981; Thomas 1993; Fluharty 2006). All are transmitted in autosomal recessive fashion. Finally, ARSA pseudodeficiency (MIM#250100), in which ARSA enzyme activity is 5–20 % that of normal controls, has been reported in otherwise healthy individuals. The clinical picture of MLD has been divided into infantile (50–60 % cases, juvenile (20–30 %), and adult-onset variants (15–20 %, Fluharty 2006), with the age of onset being consistent within a pedigree (Alves et al. 1986; Haltia et al. 1980; Percy et al. 1977). Advances in molecular biology have validated these subdivisions (Polten et al. 1991).

20.1.1.1 Clinical Manifestations

Infantile cases present before the third year of life with motor delay or regression and cognitive difficulties. As the disease progresses, ataxia and hypotonia appear and mental function worsens, with death resulting within the first decade. The adult form of ARSA deficiency, defined as age of onset after 15–21 years of life, is much less common than the infantile variety and usually presents as a disturbance of mental function with subsequent evidence of diffuse CNS disease, including visual loss, ataxia, and movement

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Table 20.1 Storage diseases causing polyneuropathy

Sphingolipidoses	Protein/enzyme defect
Metachromatic leukodystrophies	Arylsulfatase A (cerebroside sulfatase)
	Saposin B arylsulfatase A activator
	Arylsulfatase A, B, and C (multiple sulfatase deficiency)
Globoid cell leukodystrophy (Krabbe disease)	Galactosylceramide-β-galactosidase
Fabry disease (angiokeratoma corporis diffusum)	α-Galactosidase A
Niemann–Pick disease (type A)	Acid sphingomyelinase
Farber disease (lipogranulomatosis)	Acid ceramidase
GM1 gangliosidosis	β-Galactosidase
GM2 gangliosidosis (various syndromes)	Hexosaminidase A
	GM2-activator protein
Others	
Adrenoleukodystrophy (X-linked form)	ABCD1 peroxisomal transporter
Tangier disease	Alpha lipoprotein
Cerebrotendinous xanthomatosis	Mitochondrial sterol-27-hydroxylase
Glycogenosis type III (Cori–Forbes)	Debrancher enzyme (amylo-1,6-glucosidase)
Sialidosis type I	Glycoprotein specific α-neuraminidase
Wolman disease	Lysosomal acid lipase/cholesteryl esterase
Niemann-Pick disease (type C)	*NPC1* gene related product

Some storage diseases discussed in this chapter, such as NCL, MPS, and Pompe, do not cause polyneuropathy and are not tabulated

disorders. Severe debilitation and death are inevitable, but may take 15 or more years to occur. Manifestations of the juvenile form are intermediate between those of the infantile and adult variants. Multiple sulfatase deficiency is clinically more severe than ARSA deficiency, with organomegaly and ichthyosis being unique to this variant (Thomas 1993).

While not ordinarily the dominant manifestation of MLD in any of its variant forms, peripheral neuropathy is usually present, albeit sometimes only electrophysiologically (Percy et al. 1977; Vos et al. 1982). Rare reports document a chronic progressive demyelinating neuropathy as the presenting manifestation of MLD in adults or children (Fressinaud et al. 1992; Vos et al. 1982; Yudell et al. 1967). The demyelinating nature of the neuropathy is evidenced by a marked slowing of conduction velocities (Vos et al. 1982; Martin et al. 1982a).

Diagnosis of MLD is usually easily made by demonstration of decreased cerebroside sulfatase activity in serum samples or cultured cells or by analysis of a gene defect. However, enzyme assays are normal in the rare cases of defective arylsulfatase A activator protein (Hahn et al. 1981; Shapiro et al. 1979; Stevens et al. 1981). Conversely, a relatively common pseudodeficiency of ARSA has been identified which can make assays of enzyme activity misleading (Hohenschutz et al. 1989; Kappler et al. 1991; Penzien et al. 1993). Without biochemical testing of family members, interpretation of low ASA activity can be difficult (Vos et al. 1982). Accumulation of sulfatides separates MLD from ARSA pseudodeficiency, is seen in all MLD variants, and is traditionally demonstrated by the finding of metachromasia in urinary sediment cells or a quantitative increase in urinary sulfatide excretion (Moser 1985). Identification of defects in *arylsulfatase A ARSA*, *prosaposin* (*PSAP*), or *sulfatase-modifying factor-1 gene* (*SUMF1*) now obviates some of the confusion.

20.1.1.2 Pathology

The central nervous system shows diffuse demyelination sparing subcortical "U" fibers, with some loss of neurons, and numerous metachromatic-debris-filled macrophages (Gregoire et al. 1966; Haberland et al. 1973; Alves et al. 1986; Satoh et al. 1988). The ultrastructure of the inclusions seen in the CNS is similar to that of the PNS as described below (Gregoire et al. 1966). Metachromatic storage material is also detected in non-nervous tissues (Haberland et al. 1973; Gregoire et al. 1966; Satoh et al. 1988).

Role of Biopsy

The literature on nerve biopsy in MLD is considerable (Vos et al. 1982; Martin et al. 1982a; Thomas et al. 1977; Percy et al. 1977; Bardosi et al. 1987; Bischoff 1979). Characteristic storage material can probably be seen in all nerve biopsies postnatally, even well before the development of clinical and electrophysiological abnormalities (Argyrakis et al. 1977). Storage substance was reported at 23 and 26 weeks of gestation, even before the appearance of demyelinating changes (Martin et al. 1982a; Meier and Bischoff 1976). In one report of two siblings who had no characteristic inclusions in nerve despite clinical and electrophysiological evidence of a neuropathy, the diagnosis of MLD is questionable because of

very atypical biochemical features (Iannaccone et al. 1993). Skin biopsy is less likely to be helpful (Dolman et al. 1975).

Nerve biopsy is usually not required for the diagnosis of MLD. The diagnosis of MLD must be confirmed by one or more of the following additional tests: molecular genetic testing of *ARSA* (the only gene in which mutation is known to cause arylsulfatase A deficiency), urinary excretion of sulfatides, and/or finding of metachromatic lipid deposits in nervous system tissue (Fluharty 2006). However, when biochemical tests are falsely negative, biopsy may be of value. MLD may not be suspected in those rare patients who have a neuropathy without detectable CNS disease, and nerve biopsy will lead to a surprise diagnosis (Fressinaud et al. 1992; Vos et al. 1982, case 20). In a report by Vos and colleagues (1982) the authors found nerve biopsy exposed misleading biochemical information in 2 of 22 patients, resulted in an unexpected diagnosis of MLD in 1 patient, and helped distinguish between MLD and a competing diagnosis in 2 further patients.

Light Microscopy

Histochemical properties of the storage material of MLD are reliably assessed only on frozen material. A search for brown or brown-yellow metachromasia with acidified cresyl violet (Hirsch–Peiffer technique) or toluidine blue on frozen sections appears to be the method of choice, although other aniline stains such as acridine or toluidine blue will also reveal metachromasia (Olsson and Sourander 1969). The storage material of MLD is also PAS positive, and with cresyl-violet staining, polarized light demonstrates yellow-green dichroism (Dayan 1967; Takahashi and Naito 1985). Sections stained with acriflavine may demonstrate a yellow-orange secondary fluorescence (Olsson and Sourander 1969).

The staining properties of Reich Pi granules on frozen sections are similar to those of MLD inclusions (Olsson and Sourander 1969), although Dayan (1967) indicated that Pi granules stained red, not brown, with the Hirsch–Peiffer technique. The perinuclear and paranodal "storage material" observed by Dayan (1967) in teased fibers of a 15-year-old with MLD might have represented Pi granules, but, if so, the quantity of this material would have been most remarkable for this age. Prior to the availability of electron microscopy, the diagnosis of MLD could be made when the metachromatic material was present in large quantities and especially when it was seen in endoneurial phagocytes; subtle Schwannian accumulation of metachromatic material could not be reliably interpreted (Olsson and Sourander 1969). Electron microscopy is highly sensitive in detecting inclusion material and demonstrates a variety of pathognomonic ultrastructural appearances.

In Schwann cells, storage material is concentrated most densely in the perinuclear region and takes the form of 0.5–2.0 μm granules. In addition, myelinating Schwann cells often demonstrate, external to the myelin sheath, concentric lamellar osmiophilic bodies surrounded by a halo which do not represent metachromatic storage material (vide infra). Endoneurial macrophages, usually arrayed in a perivascular distribution, can contain inclusion material.

In MLD, the myelinated axon population is often reduced, and axons of all sizes are lost such that the diameter–frequency histogram may retain its bimodal appearance (Martin et al. 1982a; Bardosi et al. 1987). Actively degenerating axons and regenerating clusters are uncommon. Unmyelinated fibers have been less rigorously studied, but seem to be spared (Case Records NEJM 1984). A picture of diffuse hypomyelination is usually present (Fig. 20.1a, b), and small onion bulb formations may be seen, in both infantile and adult cases (Bardosi et al. 1987; Martin et al. 1982a; Thomas et al. 1977; Vos et al. 1982). The onion bulbs are usually rudimentary, but were said to be quite prominent in the five cases of juvenile MLD reported by Haltia et al. (1980) and in a report of MLD due to activator protein deficiency (Hahn et al. 1981). The myelin sheath is often irregular, with evidence of remodeling and occasional formation of redundant myelin loops. Active demyelination, with naked axons and debris-filled macrophages (Fig. 20.2), is seen most prominently in the infantile cases, the macrophages often congregating in a perivascular or subperineurial distribution. In the adult cases the process is usually more indolent and active demyelination and debris-filled cells (Fig. 20.2) are uncommon (Martin et al. 1982a; Thomas et al. 1977; Haltia et al. 1980). However, Percy and colleagues (1977) found that the pace of demyelination correlated more with the disease stage than the age of onset, i.e., a biopsy taken during the rapidly progressive early stage of adult-onset disease showed the same active demyelination as that of an infantile-onset case. Fibrotic changes may be prominent.

Studies of teased fibers have shown that segmental demyelination is the predominant pathological process and that this does not correlate with the local prominence of inclusions (Dayan 1967).

Electron Microscopy

The storage material of MLD is single membrane bound and can take a variety of ultrastructural appearances. "Tuffstone" bodies are readily identified as sharply demarcated inclusions containing an intermingling of granular material, electron-lucent vacuoles, and regions with periodicity (Figs. 20.3a, b and 20.4a, b). A second type of inclusion ultrastructure (Fig. 20.5a, b) has been described under a confusing plethora of names including "stacked disc," "prismatic," or "herringbone" inclusions (Gregoire et al. 1966; Luijten et al. 1978; Martin et al. 1982a; Rutsaert et al. 1973). Depending on the plane of section, the appearance may

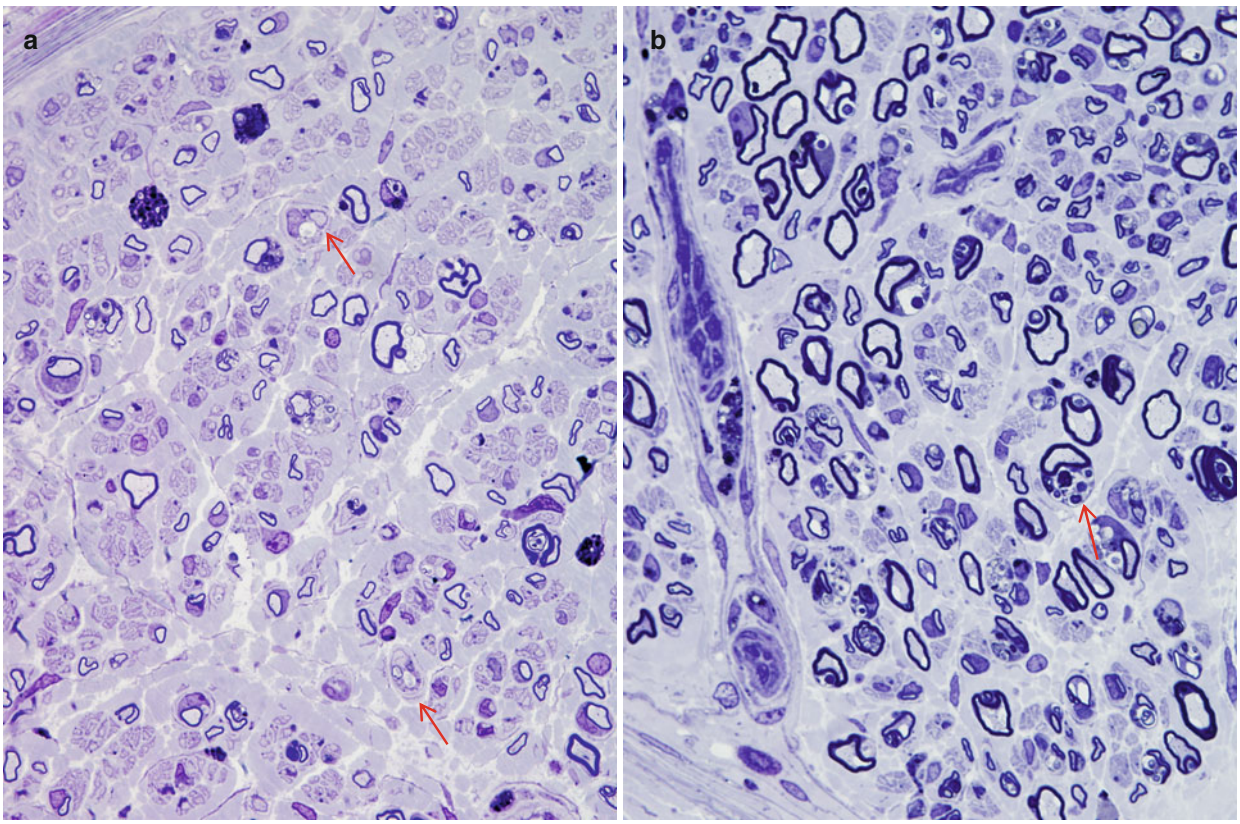

Fig. 20.1 MLD: most fibers are thinly myelinated (**a**, **b**). Segmental demyelination is seen in (**a**) (*arrows*), and many Schwann cells contain concentric lamellar osmiophilic bodies (*arrow*, **b**) (1 μm thick plastic sections: **a**, 600×; **b**, 1,000×) (Tissue courtesy of Dr. D. Agamanolis)

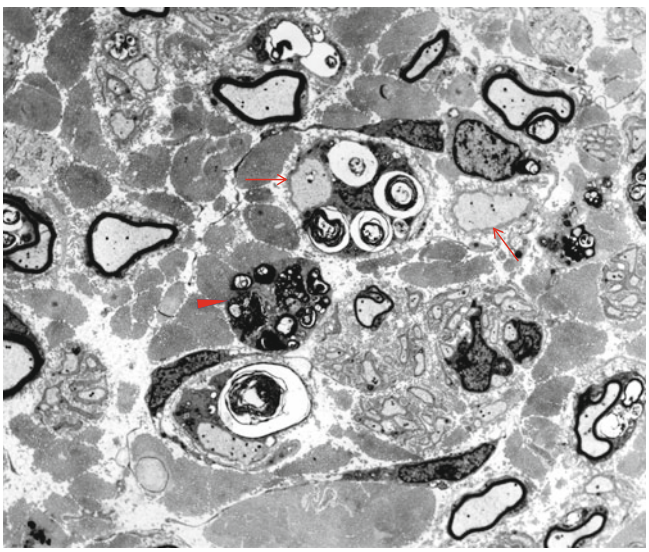

Fig. 20.2 MLD: low magnification view of the sural nerve shows constituent elements ranging from naked axons (*arrows*) to intact, thinly myelinated elements and macrophages containing phagocytosed sulfatide (*arrowhead*) (2,500×)

range from maze-like or honeycombed to parallel arrays of lamellated material, and we will use the term "prismatic" to describe these various morphologies, as it seems to have been applied most consistently in the literature. Inclusions best categorized as "tuffstone bodies" often show regions of prismatic structure which blend into less structured material, suggesting that there is an evolution from one appearance of the stored material into the other. A third type of storage cytosome is the "zebra body," an oval or round membrane bound structure traversed by alternating light and dark bands, the latter made up of several closely packed lamellae. When seen in myelinating Schwann cells, these may be confused with Reich Pi granules, but this is less of an issue when detected in nonmyelinating Schwann cells and macrophages (Bischoff 1979). The various inclusion morphologies blend into each other and thus probably reflect different orientations and packing of the same storage material. Regardless of inclusion type, periodicity of storage material, when present, is 5.6–6.0 nm, visibly less than that of myelin, and typical of the appearance of stored intracellular sulfatide in experimental work (Rutsaert et al. 1973).

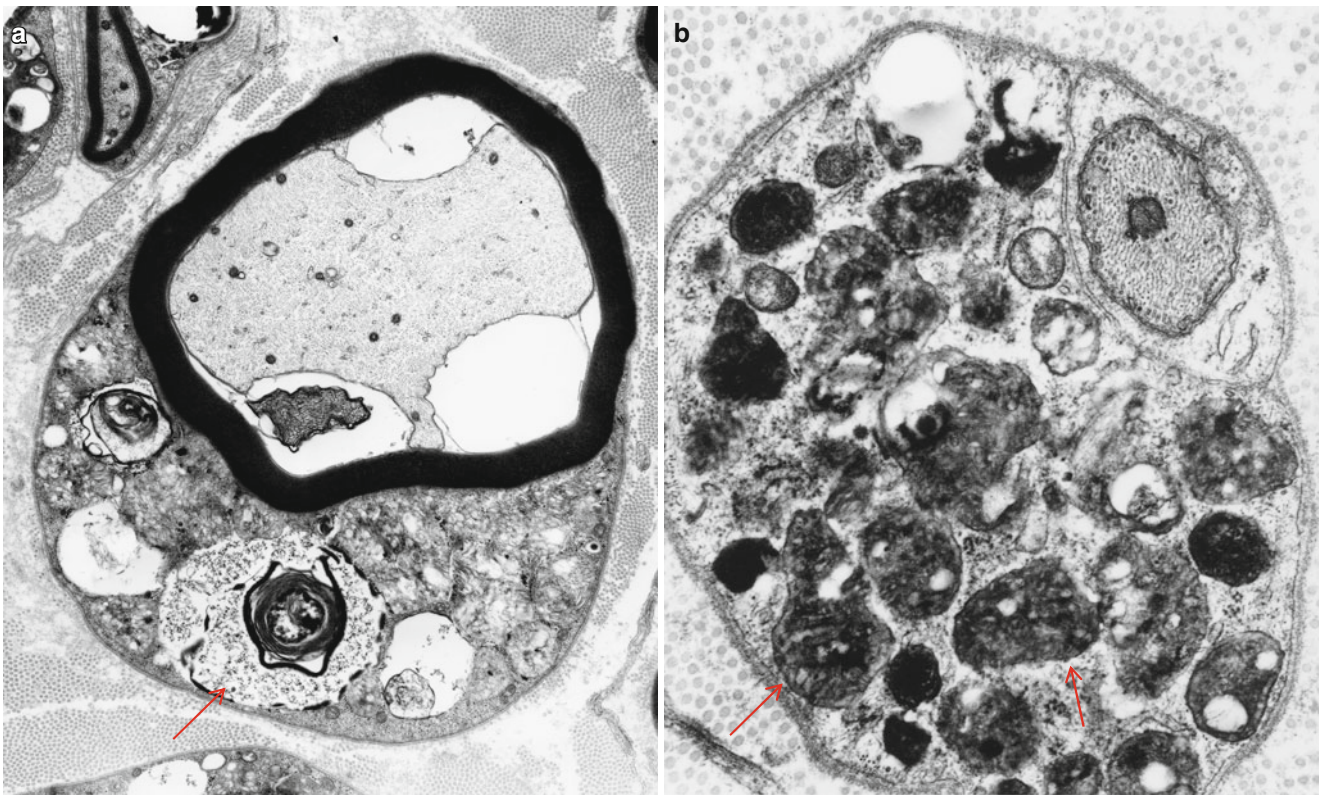

Fig. 20.3 MLD: (**a**) EM reveals that the concentric lamellar structures seen on LM are secondary lysosomes containing a myeloid whorl surrounded by glycogen (*arrow*, **a**). (**b**) In comparison, the characteristic inclusion of MLD, "Tuffstone" bodies (*arrows*) are seen (**a**, 11,000×; **b**, 23,660×)

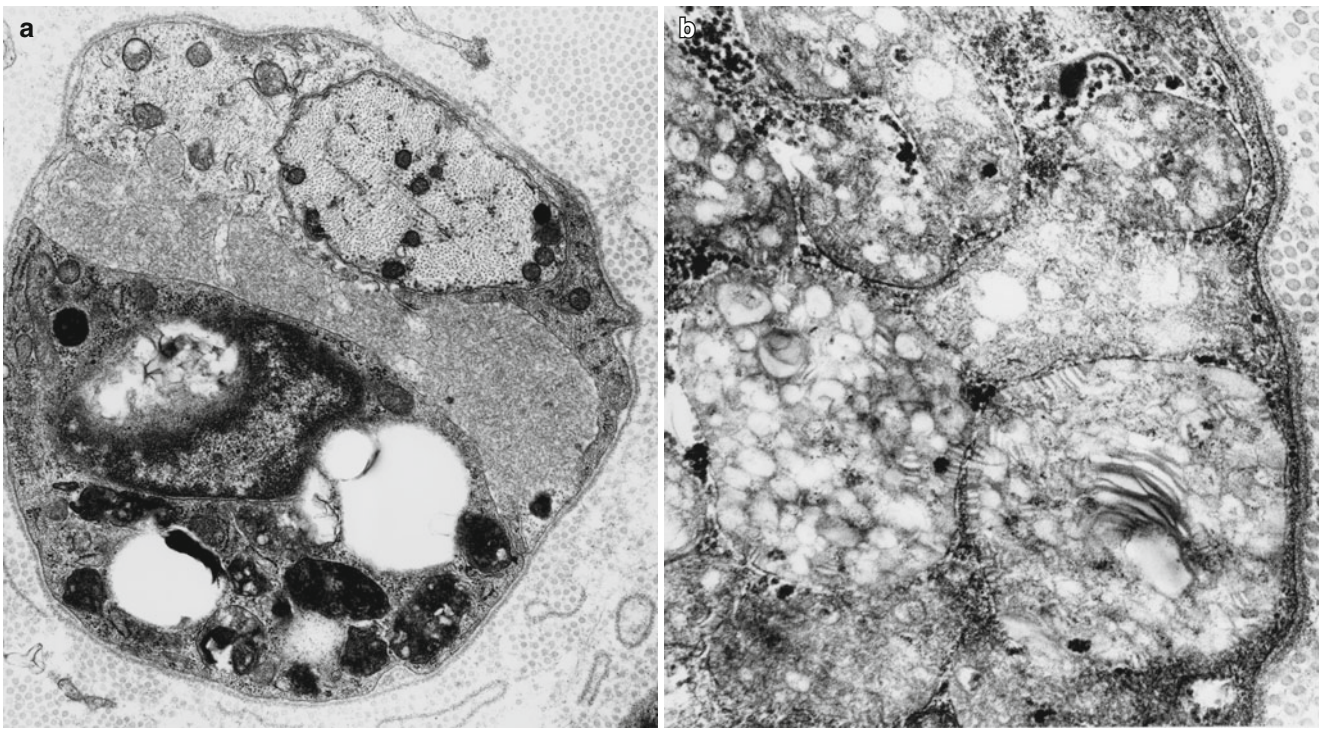

Fig. 20.4 "Tuffstone" inclusions are seen within the Schwann cell cytoplasm surrounding a demyelinated axon and at higher magnification (**b**) (**a**, 13,520×; **b**, 49,010×)

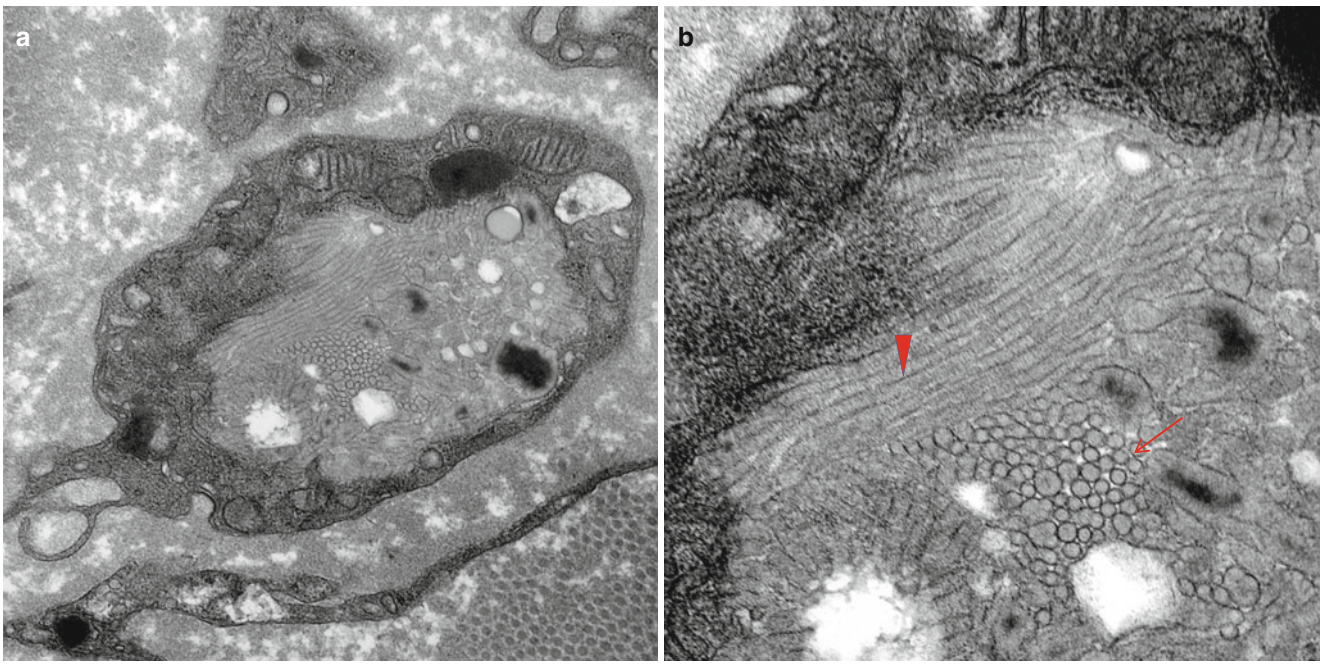

Fig. 20.5 (**a**, **b**) Large prismatic inclusion in macrophage seen in stacked (*arrow*, **b**) and longitudinal orientation (*arrowhead*, **b**) (**a**, 25,000×; **b**, 60,000×)

The storage material is seen in myelinating Schwann cells, nonmyelinating Schwann cells (and, thus, are not derived from myelin breakdown), and in macrophages. Myelinating Schwann cells are most likely to contain tuffstone bodies, while macrophages most often show "prismatic" inclusions (Martin et al. 1982a), both likely derived from lysosomes. The single detailed histological report of MLD with activator deficiency demonstrated typical findings, but also revealed lamellated material in endothelial cells (Hahn et al. 1981). Although not mentioned in most reports, a few authors have stated that inclusions were observed in axons, and one example has been illustrated (Thomas et al. 1977).

Inclusions are seen regardless of the age of onset, although they are more numerous in the infantile cases (20–45 % of myelinated fibers) than in adult- or juvenile-onset cases (10 % of myelinated fibers) (Martin et al. 1982a). Luijten et al. (1978) felt that ultrastructure of the inclusions varied with age, infantile cases characterized by tuffstone bodies, juvenile cases showing prismatic inclusions, and adult patients mostly displaying zebra bodies, but this was based on the study of only one case in each group. The ten patients studied by Martin et al. (1982a) showed a trend towards this pattern, but overall, the full spectrum of ultrastructural pathology has been seen regardless of age of onset and genetic subtype (Hahn et al. 1981; Martin et al. 1982a; Thomas et al. 1977).

In MLD, structures composed of concentric lamellar osmiophilic substance are often seen in myelinated fibers, usually external to an otherwise normally compacted sheath; these correspond to the ovoids seen on LM (Cravioto et al. 1966). The difference in periodicity between these structures and the inclusions of MLD, the observation that the periodicity is similar to myelin (Argyrakis et al. 1977), and the occasional detection of continuity between the myeloid whorl and intact compact myelin (Cravioto et al. 1966) suggest that this structure is a stage in the remodeling of the myelin sheath and not directly related to the storage material. Indeed, similar myelin configurations are seen in a wide variety of neuropathies, albeit not usually so prominently. However, Thomas et al. (1977) found the lamellated bodies to have a periodicity of 8 nm and failed to detect sites of continuity with intact myelin. Thus, an alternative possibility is that these bodies represent appearance of lipid storage material being degraded within secondary lysosomes. Cellular organelles may show nonspecific changes such as increases in glycogen (Argyrakis et al. 1977), abnormally formed mitochondria (Argyrakis et al. 1977; Cravioto et al. 1966), or dilation of the endoplasmic reticulum (Argyrakis et al. 1977).

A peculiar "loosened" myelin appearance has been illustrated in MLD on several occasions (Cravioto et al. 1966; Percy et al. 1977; Hahn et al. 1981; Webster 1962; Bischoff 1979). This differs from the widely spaced myelin seen in some paraprotein-associated neuropathies (Chap. 14) in that "loose" myelin is often wavy and the separation between major dense lines is less uniform. Similar "loosened" myelin sheaths are illustrated by Bischoff (1979) in diabetic and uremic neuropathies (Figs. 4.38, 4.39 reference Bischoff 1979)

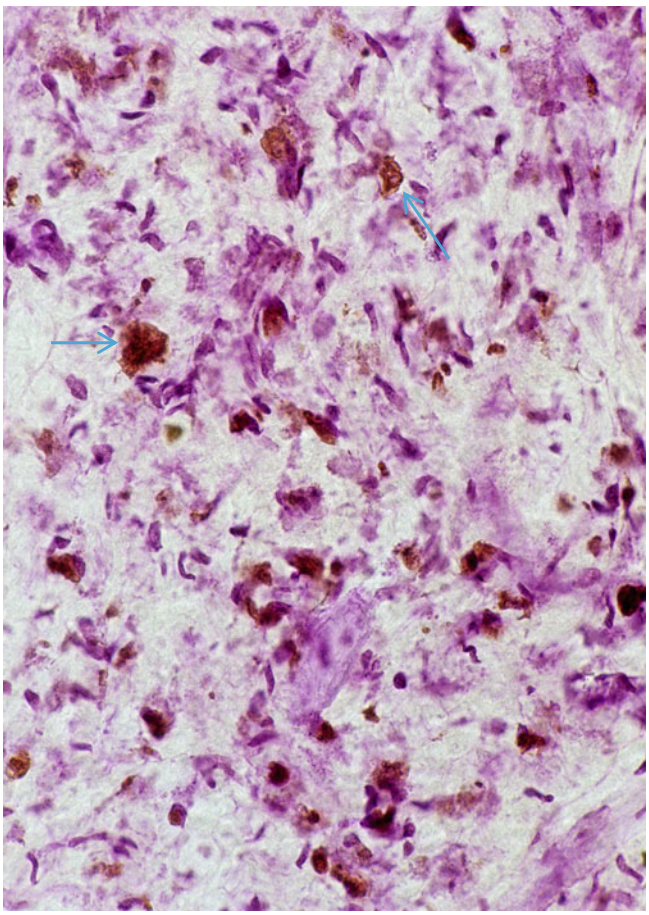

Fig. 20.6 Metachromatic leukodystrophy (MLD) inclusions (*arrows*) stained brown with acidified cresyl violet (Hirsh Pfeiffer stain) (400×)

and by Vital and Vallat in idiopathic and leprous neuropathies (Figs. 90, 91, 168, 169 in Vital and Vallat 1987). We have never observed this alteration and wonder whether it represents an artifact of fixation or tissue trauma to which nerves in MLD patients are more susceptible.

20.1.1.3 Pathogenesis and Therapy

The common denominator of all the MLD is the accumulation of sulfatide, and this has been shown to be the major component of the material accumulated in the characteristic metachromatic inclusions (Fig. 20.6) (Suzuki et al. 1967). The storage material is seen prior to the onset of myelination and in nonmyelinating Schwann cells and is thus unlikely to reflect uncleared myelin debris (Meier and Bischoff 1976). The lack of correlation between sulfatide accumulation and the presence of segmental myelin damage (Martin et al. 1982a; Dayan 1967) suggests that Schwann cell dysfunction is not necessarily due to toxic accumulations of storage material. An alteration in the fatty acid composition and possibly the physical properties of myelin may be present in MLD, and this may somehow result in myelin damage (Thomas 1993).

The biochemical errors that cause MLD are dysfunction of arylsulfatase A, its activator protein (AB variant), or of multiple sulfatases. The ARSA gene has been fully characterized (Gieselmann et al. 1991). The fundamental difference between the various age-defined MLD subsets seems to be related to the amount of residual ARSA activity (Polten et al. 1991). In cell culture, fibroblasts from patients with adult-onset MLD are better able to clear sulfatide than those taken from infantile-onset patients (Percy et al. 1977). Polten and colleagues (1991) identified two important mutations of the ARSA gene: the "I" allele and the "A" allele. The former results in an unstable RNA that does not permit synthesis of a functional ASA, while the latter leads to synthesis of a rapidly degraded ASA protein (Polten et al. 1991). Patients with an I/I genotype have no functional ASA activity and present in infancy. Patients with the A/A genotype have sufficient enzyme activity to prevent presentation until early adult life, and patients with the I/A genotype are intermediate (Polten et al. 1991). The MLD pseudodeficiency gene is yet another ARSA allele which leaves sufficient enzyme activity to permit a normal phenotype, even if paired with an MLD allele (Penzien et al. 1993). Although combination of such alleles seem to give a satisfactory account for phenotypic variability, there are some pedigrees with phenotype variability which would be hard to explain on this basis (Clarke et al. 1989).

Pathogenetic mechanisms in MLD have been further examined in transgenic mice deficient in cerebroside sulfotransferase (CST). CST mice show impaired maintenance of Nav 1.6 sodium channels in mature nodes and migration of Kv1.2 channels from the paranodal to the juxtaparanodal region, resulting in the disappearance of Caspr and neurofascin 155 (NF155) clusters, essential components of the axo-glial junction (Eckhardt 2008; Dupree et al. 2005). In the peripheral nervous system, CST-deficient nerves extend axonal protrusions at the node of Ranvier, abnormal enlarged vesicles, and unusually short or absent Caspr and NF155 clusters (Hoshi et al. 2007). Additionally, the number of Schmidt–Lanterman incisures are increased and elevated levels of annexin II suggest structural changes at the Schmidt–Lanterman incisures (Eckhardt 2008; Hayashi et al. 2007).

A variety of treatment schemes have been developed for a number of lysosomal storage disorders (Miranda et al. 2013; Patil and Maegawa1 2013; Pierret et al. 2008). Enzyme replacement therapy (ERT) is undergoing clinical trials. Small molecules capable of crossing the blood–brain or blood–nerve barriers can be used as enzyme enhancers of diverse ASA mutants, either as pharmacological chaperones or as proteostasis regulators, to reduce the biosynthesis of sulfatides or target different affected pathways downstream of the primary ARSA deficiency. Hematopoietic stem cell transplantation using bone marrow-derived or umbilical

cord blood-derived sources represents another therapeutic possibility, especially using cells which overexpress the ARSA gene. Bone marrow-derived mesenchymal stromal cells (BM-MSCs) have also been investigated as therapeutic agents (Miranda et al. 2013; reviewed in Patil and Maegawal 2013). BM-MSCs can provide the defective protein, secrete a number of neurotrophic factors and chemokines, have low immunogenicity, lack tumorigenic potential, and have a strong ability to home to injured tissues after being systemically injected. Some small molecule pharmacological chaperones may assist misfolded-prone mutant enzymes by assisting their ER folding and others may work on the lysosomal environment normalizing calcium levels (Patil and Maegawal 2013).

20.1.2 Globoid Cell Leukodystrophy (Krabbe Disease)

20.1.2.1 Clinical Manifestations

Globoid cell leukodystrophy (GLD, MIM#245200) is an autosomal recessively inherited disease caused by mutation in the *galactosylceramidase gene* (*GALC*) resulting in defective galactosylceramide-β-galactosidase activity causing accumulation of galactosylceramide in neural and nonneural tissues. Similar to MLD, an atypical form of GLD (MIM#611722) is produced by saposin A activator deficiency. Onset is usually in the first year of life with retarded development and irritability, rapid progression to spasticity, blindness, deafness, and decerebration, and death following within a few years of onset (Hagberg 1984). Some patients, usually those with onset later in childhood, have a more indolent course characterized by visual failure, ataxia, spasticity, and dementia and may survive into the second or third decade (Kolodny et al. 1991). Onset in adulthood is very rare (Hagberg 1984; Hedley-Whyte et al. 1988). Peripheral neuropathy is a usually a minor part of the infantile disease, but may be evidenced by diminished distal reflexes or hypotonia, and becomes more prominent with increasing age (Hogan et al. 1969; Kolodny et al. 1991; Dunn et al. 1969). Cases with neuropathy as the dominant manifestation have been described (Hedley-Whyte et al. 1988; Lyon et al. 1991). More often there is only electrophysiological evidence of neuropathy, marked conduction slowing being typical (Dunn et al. 1969; Suzuki and Grover 1970; Kolodny et al. 1991; Lyon et al. 1991). Diagnosis may be made by assay of β-galactosidase activity in cultured fibroblasts, leukocytes, or serum (Kolodny et al. 1991) or, more recently, from gene analysis.

20.1.2.2 Pathology

Globoid cell leukodystrophy derives its name in part from the prominent demyelination that afflicts the CNS and in part from the presence of "globoid" cells, multinucleated giant cells up to 120 μm in diameter containing PAS-positive material (Allen and de Veyra 1967; Suzuki and Grover 1970). Globoid cells are seen predominantly in the brain, often around vessels, and may be seen in nonneural tissues, but are not present in peripheral nerves. However, ultrastructural examination reveals that the material stored in globoid cells is the same as the storage product detected in the peripheral nervous system.

Light Microscopy

Light microscopy does not always give evidence of peripheral nerve disease. In a review of GLD, Lyon et al. (1991) noted that seven of ten biopsies showed segmental demyelination but that three were normal, and typical inclusions were said to be present in only three of the ten. However, it is unclear how closely the nerves were studied. Review of the case reports and small series described in the literature lead us to suspect that electron microscopy would reveal typical inclusions in most nerve biopsies (Bischoff and Ulrich 1969; Hedley-Whyte et al. 1988; Hogan et al. 1969; Lake 1968; Dunn et al. 1969; Martin et al. 1974; Lyon et al. 1991; Schlaepfer and Prensky 1972; Schochet et al. 1976; Sourander and Olsson 1968; Suzuki and Grover 1970; Thomas et al. 1984; Vital and Vallat 1987; Yunis and Lee 1972). Storage material was detected in nerve biopsy from a 23 week fetus, but not from a 20 week fetus (Martin et al. 1981).

The multinucleate PAS-positive cells seen in the CNS are not found in peripheral nerve. Some authors have described cells containing fine PAS-positive granules or sudanophilic material in the endoneurium, but this does not seem prominent (Suzuki and Grover 1970; Schochet et al. 1976; Schlaepfer and Prensky 1972; Allen and de Veyra 1967) and may not be distinguishable from control material (Bischoff and Ulrich 1969). No metachromatic material is detected, and Oil Red O staining is also negative (Dunn et al. 1969; Hogan et al. 1969). The substantial effort that went into characterizing the histochemical properties of the storage product is now of historical interest only (Sourander and Olsson 1968; Allen and de Veyra 1967).

Nerve biopsy in GLD reveals a demyelinating neuropathy, with some thinly myelinated fibers and a variable prominence of onion-bulb formations and ongoing demyelination. In some reports, active segmental demyelination with naked axons and prominent debris-filled macrophages has been emphasized (Dunn et al. 1969; Suzuki and Grover 1970), while in others the picture has been that of diffuse hypomyelination and an indolent pathology (Thomas et al. 1984; Vos et al. 1983). Axon numbers are mildly to moderately reduced in most cases, but Wallerian degeneration is typically not seen. Large myelinated fibers may at times be more severely depleted, and unmyelinated fibers seem to be spared (Martin et al. 1974; Schlaepfer and Prensky 1972). Debris-filled macrophages may be seen in the endoneurium, often aggregated around capillaries (Fig. 20.7). Under oil immersion, needlelike inclusions may be identified

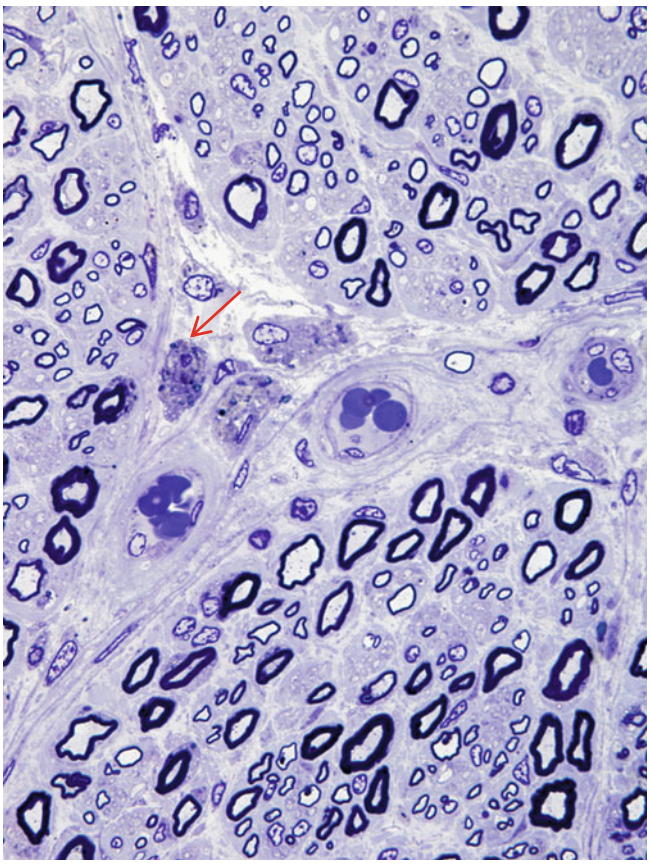

Fig. 20.7 Krabbe disease (globoid cell leukodystrophy, GLD). Perivascular macrophages (*arrow*) are accompanied by relatively large number of thinly myelinated axons (1 μm thick plastic section, 1,000×)

within these cells. Fiber teasing indicates that segmental demyelination with remyelination is the predominant pathological process.

Electron Microscopy

Ultrastructural examination reveals the characteristic appearance of GLD inclusions: tubular or prismatic, straight or slightly curved, and elongated membrane-bound structures (Fig. 20.8a–c). Depending on the plane of section they range in appearance from polygonal or irregular optically empty clefts to hollow spicules as little as 10 nm in width. These optically empty clefts may contain a small amount of granular osmiophilic material or a few parallel lamellae of storage substance (Martin et al. 1974). Presumably most of the stored substance has been removed during tissue preparation. The inclusions seem to be rigid and very resistant to degradation, with needlelike clefts sometimes appearing to protrude into and even disrupt the plasmalemma of the cells containing them, becoming extruded into the extracellular space (Bischoff and Ulrich 1969). Inclusion material may be seen lying freely in the endoneurium (Thomas 1993). Typical GLD inclusions are seen in macrophages and in as many as half of myelinating and nonmyelinating Schwann cells (Bischoff and Ulrich 1969).

Some reports have described storage material within macrophages taking the form of cytosomes containing straight and twisted tubules, 30 nm in diameter (Schochet et al. 1976; Yunis and Lee 1972; Vos et al. 1983; Goebel et al. 1990). A similar substance has at times been found in CNS globoid cells and resembles the ultrastructural appearance of purified bovine cerebroside (Yunis and Lee 1972; Schochet et al. 1976).

20.1.2.3 Pathogenesis and Therapy

The mechanism of myelin damage and cell death in GLD remains uncertain (Suzuki and Suzuki 1989). Defective function of the enzyme galactosylceramide-β-galactosidase (also called galactosylceramidase) is always associated with GLD. Dogs and rats with the same defect have an identical disease (Suzuki and Suzuki 1989), and GLD affects only those tissues where galactosylceramide accumulates. Injection of cerebroside, but not other sphingolipids, into animal tissue produces typical globoid cells (Olsson et al. 1966). The "indigestibility" of the storage substance of GLD (as described above) may relate to the formation of multinucleate giant cells. A related sphingolipid, psychosine, also broken down by galactosylceramide-β-galactosidase, is consistently found to be increased in GLD tissue and is highly cytotoxic (Suzuki and Suzuki 1989). Various neuropathic mechanisms have been proposed in studies of the twitcher mouse model of GLD including activation of the pro-apoptotic protease caspase 3 in sciatic nerves (which occurs before demyelination), axonal instability, defective axonal transport, and deregulated ion channels including loss of Na^+ channel concentration at the nodes of Ranvier (Siddiqi et al. 2006; Sakai 2009; Kagitani-Shimono et al. 2008; Smith et al. 2011). Psychosine rapidly accumulates in lipid rafts (White et al. 2009) and may interfere in multiple signaling pathways (Suzuki 1998). Thus, it is likely that the accumulation of galactosylceramide and its toxic metabolite psychosine causes the histologic features and tissue injury seen in GLD (Suzuki and Suzuki 1989).

As in other lysosomal disorders, experimental treatment methods have been investigated in human or animal models including hematopoietic stem cell transplantation, chaperone therapy, enzyme replacement therapy, deprivation of the substrate, gene therapy, and cytokine therapy (Sakai 2009). Siddiqi et al. (2006) found that nerve conduction studies improved in 7 (60 %) of 12 patients after hematopoietic stem cell transplantation followed for an average of 18 months.

20.1.3 Fabry Disease

20.1.3.1 Clinical Manifestations

Fabry disease (MIM#301500), also called angiokeratoma corporis diffusum in reference to the frequent presence of cutaneous angiokeratomas, is an X-linked disease resulting

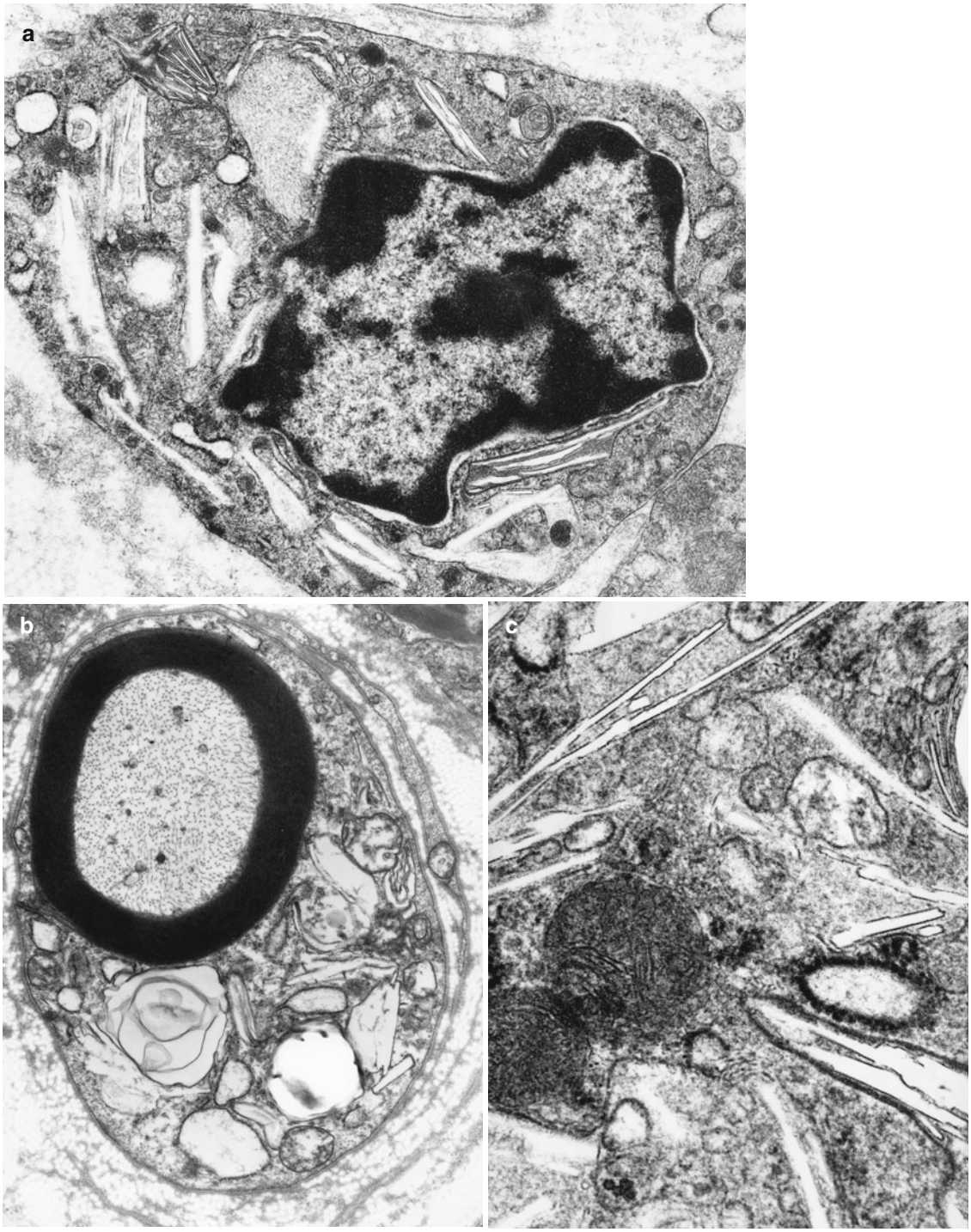

Fig. 20.8 Krabbe disease (GLD). Characteristic inclusions in macrophage (**a**) and Schwann cell (**b**) and at higher magnification in (**c**) (Magnification; **a**, 27,600×; **b**, 12,780×; **c**, 46,200×)

from a mutation in *alpha-galactosidase A* (GLA), has been localized to the long arm of the X chromosome, and codes for α-galactosidase A which in its full blown form affects only males, although heterozygote females may be mildly symptomatic (Morgan et al. 1990; Brady 1993; Biegstraaten et al. 2012). Age of symptom onset is usually childhood or adolescence, with diagnosis often delayed into adult life, the significance of skin lesions and corneal

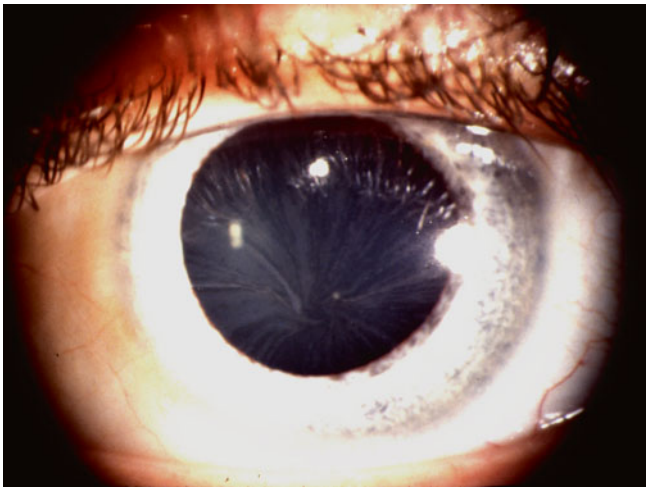

Fig. 20.9 Fabry disease cornea verticillata ("vortex keratopathy") is characteristic of Fabry disease

"whorl" dystrophy (Fig. 20.9) going unappreciated for many years (Morgan et al. 1990). Presentation with renal dysfunction or episodic painful paresthesia is typical, with cardiac and cerebral vascular disease representing important causes of morbidity and renal failure in mid-adult life being the usual cause of death prior to the availability of dialysis and renal transplantation. Autonomic features include hypo- or anhidrosis, reduced salivation and lacrimation, and gastrointestinal tract disordered motility; however, decreased sweating has been attributed both to autonomic neuropathy and to direct sweat gland involvement (Bersano et al. 2012). Routine clinical and electrophysiological testing for evidence of a peripheral neuropathy is often unrewarding, but specific assessment of small fiber function (Ohnishi and Dyck 1974; Morgan et al. 1990) and quantitative determination of numbers of axons within the epidermis (IENFD, Biegstraaten et al. 2012) will reveal impairment. Accumulation of glycolipid in DRG neuron cell bodies and their eventual loss may also play a role (Schiffmann 2006). Diagnosis is often suspected on the basis of the clinical picture and is confirmed by assay of the activity of the affected enzyme, α-galactosidase A (lysosomal hydrolase a-galactosidase A), in plasma or leukocytes. This is typically 10 % or less of the normal value in affected individuals and 50 % of normal value in heterozygote females. Molecular techniques may be used to demonstrate *GLA*, *alpha-galactosidase A*, gene defects.

20.1.3.2 Pathology

Nerve biopsy has minimal diagnostic value in Fabry disease because of the characteristic clinical manifestations and the presence of reliable noninvasive tests. If tissue is taken, the storage material of Fabry disease will be found predominantly in vascular cells: endothelial cells, smooth muscle cells, and pericytes; skin biopsy will readily demonstrate

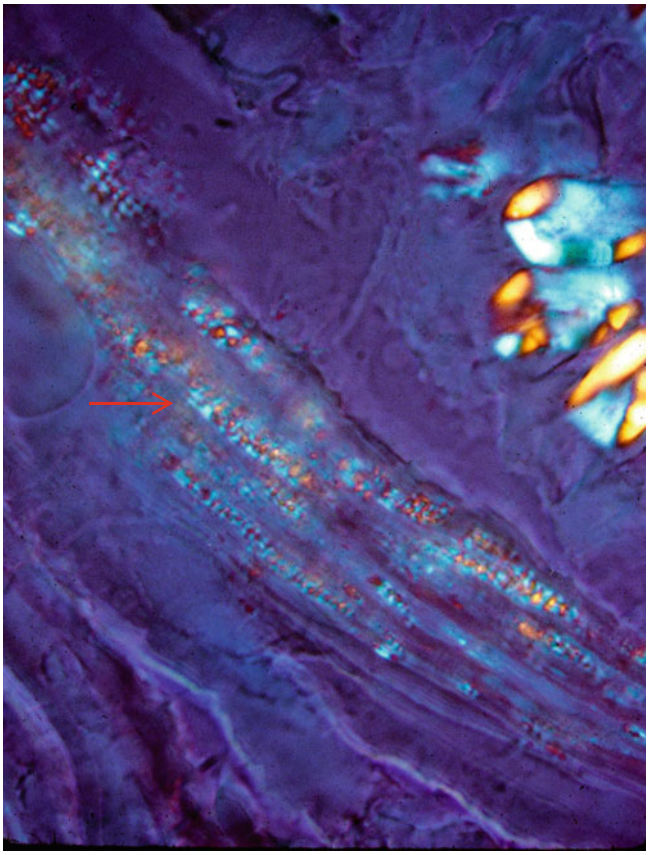

Fig. 20.10 Fabry disease: polarization microscopy discloses "Maltese cross" birefringence in perineurium (*arrows*). Unstained fresh frozen tissue (400×)

characteristic inclusions. However, when the diagnosis is unsuspected, nerve biopsy reveals pathognomonic histology, as has occurred once in our experience. Other detailed reports of nerve histology are available (Ohnishi and Dyck 1974; Kocen and Thomas 1970; Bischoff et al. 1968; Gemignami et al. 1984; Sima and Robertson 1978; Tabira et al. 1974; Fukuhara et al. 1975; Vital et al. 1984; Tome et al. 1977; Pellissier et al. 1981; Schiffmann 2006).

Light Microscopy

Light microscopy invariably reveals an accumulation of storage material in various cell types. The inclusions are birefringent, and a "Maltese cross" pattern can be readily demonstrated on polarized light examination of fresh frozen sections (Fig. 20.10). Frozen sections, and less reliably paraffin-embedded material, show Sudan Black B, Oil Red O, LFB, and PAS positivity, with the latter reduced by digestion with diastase (Fukuhara et al. 1975; Kocen and Thomas 1970; Hashimoto et al. 1965). The storage material is partially dissolved in the process of paraffin embedding, and H&E staining of processed material often shows little pathology (Fig. 20.11a), but exposure of formalin-fixed tissue to 3 % potassium chromate for 1 week reduces this problem (Desnick and Bishop 1989). Osmicated toluidine

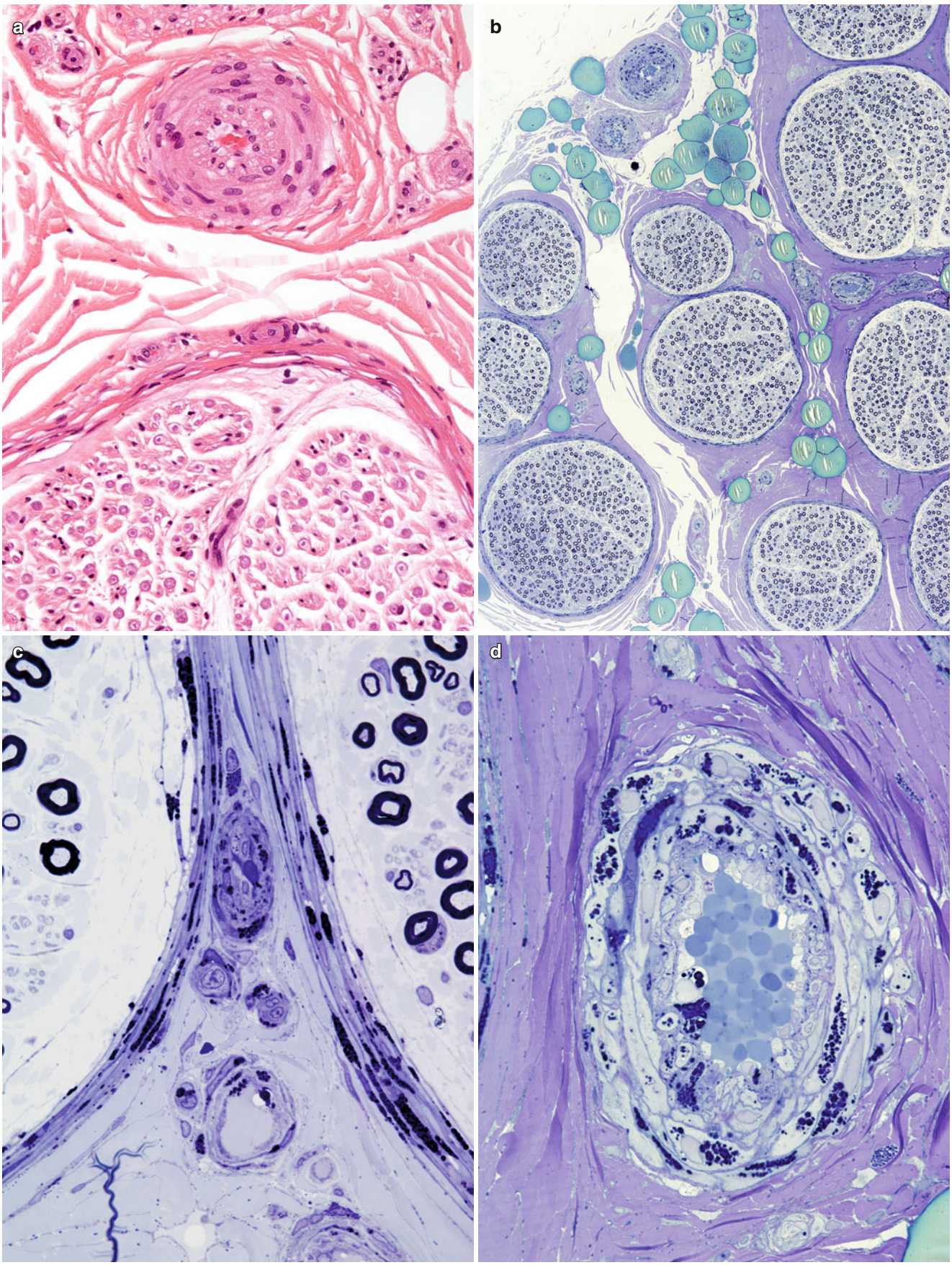

Fig. 20.11 Fabry disease: (**a**) H&E-stained section of small epineurial artery shows only subtle involvement. (**b**) All fascicles are equally affected and show relatively small loss of axons. (**c**–**e**) Note the large quantity of osmiophilic lipid within the cytoplasm of perineurial, endothelial, and endoneurial macrophages (1 μm thick plastic sections; magnifications: **a**, 400×; **b**, 10×; **c**–**e**, 1,000×)

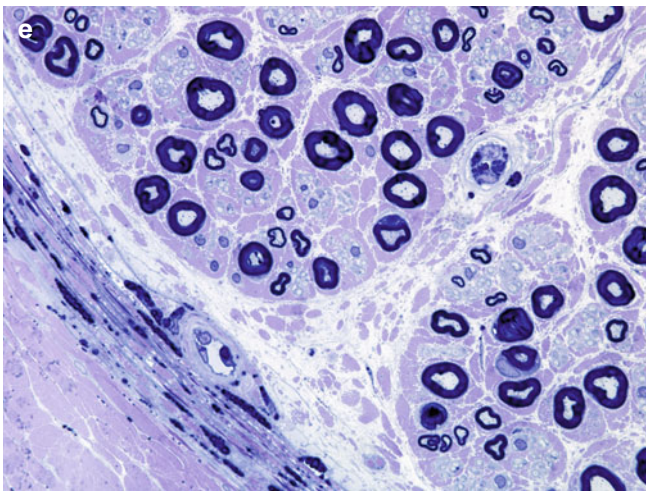

Fig. 20.11 (continued)

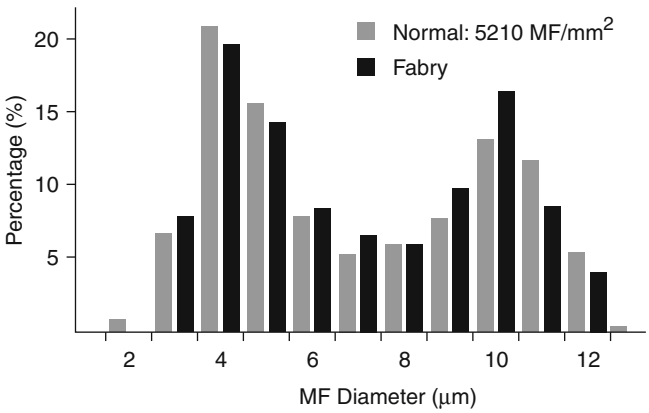

Fig. 20.12 Fabry disease: slight decrease in fiber density for age without loss of small myelinated axons

blue-stained plastic-embedded sections showing relatively well-preserved axonal population (Fig. 20.11b) circumvent this problem, and the inclusions, typically 1–2 µm in diameter, can be readily seen in endothelial cells, pericytes, smooth muscle cells, perineurial cells, and fibroblasts, but usually not in Schwann cells or axons (Fig. 20.11c–e). Endothelial cells seem to be particularly distended with the storage material.

The classic peripheral nerve alteration in Fabry disease is said to be a relatively selective loss of small myelinated and perhaps unmyelinated fibers (Ohnishi and Dyck 1974; Kocen and Thomas 1970; Gemignami et al. 1984). However, this was not seen in our material (Case 20.1) (Figs. 20.11 and 20.12), and a number of literature reports describe large myelinated or pan-myelinated fiber depletion (Fukuhara et al. 1975; Pellissier et al. 1981; Vital et al. 1984; Tabira et al. 1974). Active degeneration is rarely seen due to the indolent nature of the process, and regenerating clusters can even be prominent (Gemignami et al. 1984). It is possible that concomitant renal failure accounts for depletion of large myelinated fibers, but a predominantly large fiber neuropathy has been seen in Fabry patients with minimally abnormal or normal renal function (Fukuhara et al. 1975; Pellissier et al. 1981, Case 20.1). Demyelination is not a significant element of the neuropathy of Fabry disease, although teased fibers may show some demyelinated–remyelinated internodes, presumably of a secondary nature (Gemignami et al. 1984; Ohnishi and Dyck 1974).

Electron Microscopy

Ultrastructural examination reveals the characteristic appearance of the osmiophilic inclusions. Although acid phosphatase staining indicates that the storage material is contained within lysosomes (Hashimoto et al. 1965), a limiting membrane is sometimes not seen. The electron-dense inclusions may be rounded (particularly in perineurial cells, Fig. 20.13a, b), have a comma-like shape (Fig. 20.14), or take the form of "zebra" bodies. At high magnification a 4–7 nm periodicity is commonly seen, and other patterns include regular arrays of concentric rings or hexagons, or amorphous electron-dense material with no visible periodicity (Fig. 20.14). Most studies have not described inclusions in Schwann cells or axons, but a few authors have illustrated examples of inclusions in these cells (Sima and Robertson 1978; Perrelet et al. 1969; Vital and Vallat 1987, Fig. 251).

Deposition of inclusion material in endothelial cells can be very impressive, but rarely occurs to such an extent that the lumen is occluded (Fig. 20.15a, b). Unmyelinated fibers may show active degenerative changes more frequently than in controls, and the number of denervated nonmyelinating Schwann cell subunits may be increased (Sima and Robertson 1978; Ohnishi and Dyck 1974). A histogram of unmyelinated fiber counts demonstrates a shift in size towards small UF diameters (Pellissier et al. 1981).

20.1.3.3 Pathogenesis and Therapy

The defective gene (*GLA*, *alpha-galactosidase A*, MIM#300644) has been localized to the long arm of the X chromosome and codes for α-galactosidase A. A number of different deletions and point mutations causing an abnormally formed or an abnormally processed protein have been found in patients with Fabry disease (Bernstein et al. 1989; Lemansky et al. 1987). Precisely how the genetic defect relates to disease manifestations is unclear. The peripheral nerve disease is thought to be a neuronopathy preferentially involving small myelinated and unmyelinated axon cell bodies (Ohnishi and Dyck 1974; Gemignami et al. 1984; Morgan et al. 1990). Alternatively, the prominent vascular involvement seen in Fabry disease has been suggested as a cause of peripheral nerve dysfunction (Fukuhara et al. 1975).

Enzyme replacement therapy and small molecule chaperones have increased residual enzyme activity in animal models and cultured cells from Fabry disease patients (Bersano et al. 2012; Toyooka 2011, 2013). Nonetheless, the IENFD was unchanged after 12–18 months

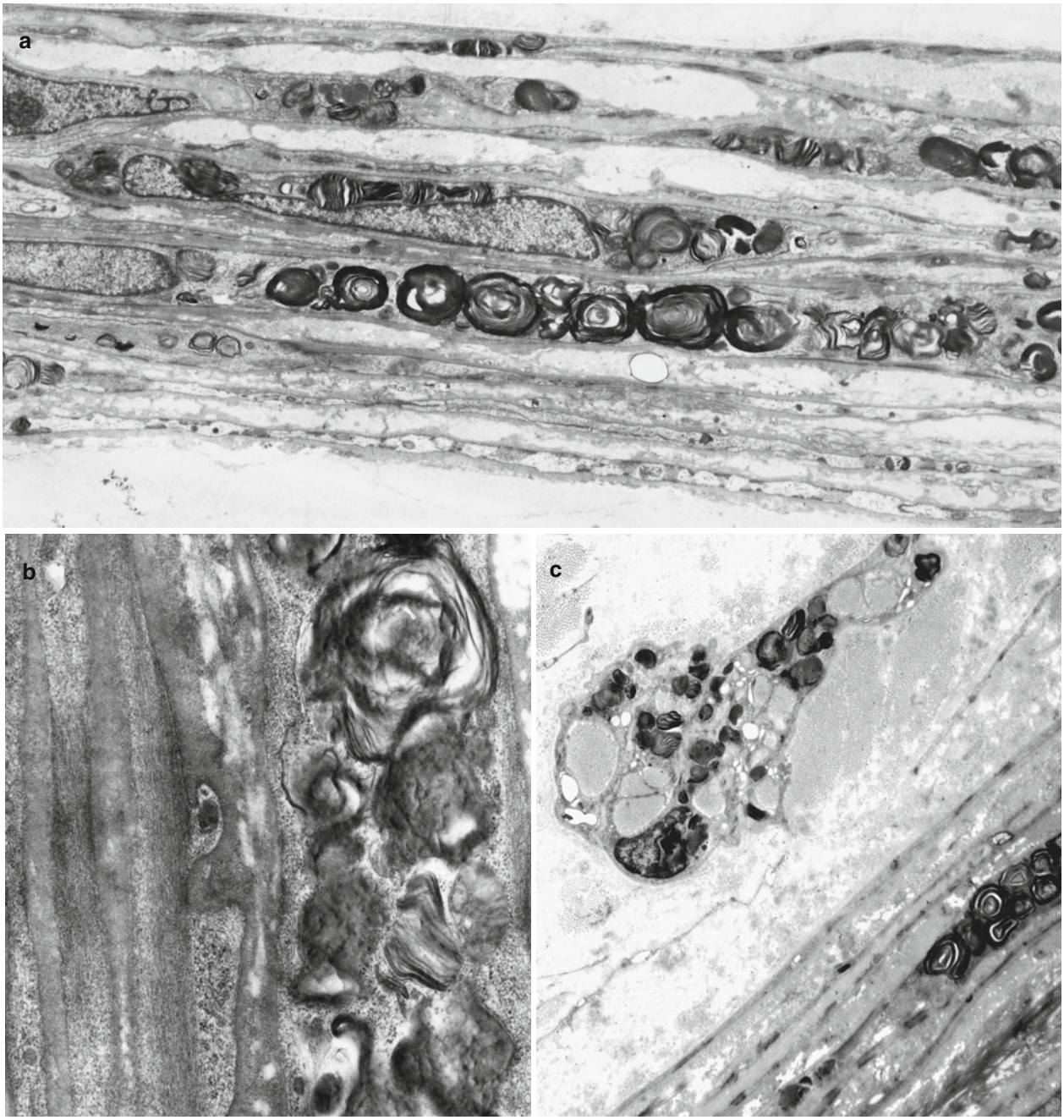

Fig. 20.13 Fabry disease: lipid storage in perineurium (**a**, **b**) and endoneurial macrophage (**c**) (**a**, 3,840×; **b**, 19,760×; **c**, 6,000×)

of enzyme replacement treatment (Schiffmann et al. 2006), although there was evidence of improved small nerve fiber function (Hilz et al. 2004).

Case 20.1

A 34-year-old male of Northern Irish descent was referred because of headache and transient visual obscurations. One year previously he had experienced transient weakness of the left arm and leg lasting several days. Additional questioning revealed a history dating to the teen years of undiagnosed sharp shooting pains in the legs and arms, and a childhood appendectomy for abdominal pain which revealed a normal appendix. The patient's family included four brothers (one nonidentical twin) and one sister, all of whom were reportedly well. The parents were also apparently unaffected. Neurological examination was essentially normal,

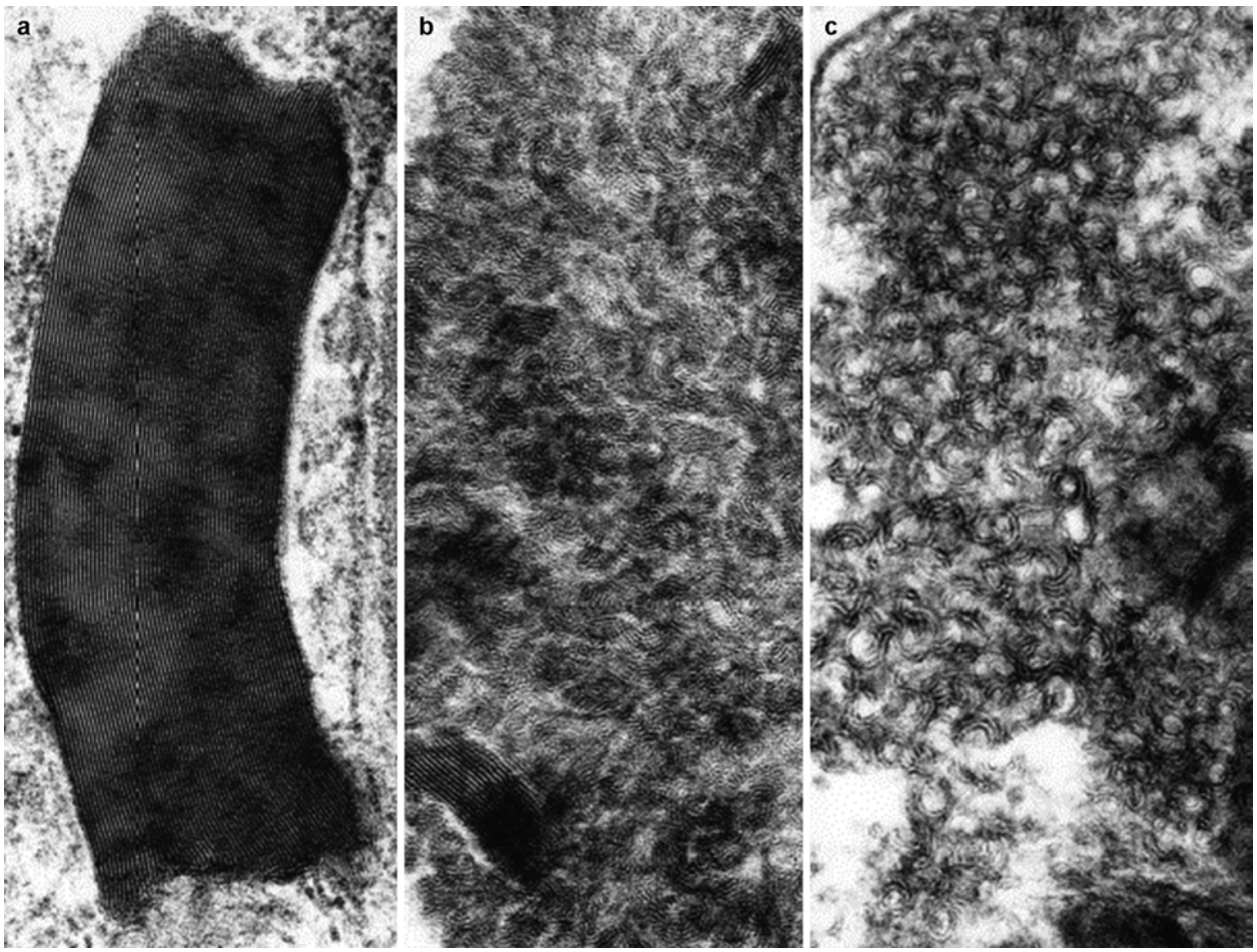

Fig. 20.14 Fabry disease: the variable morphology of stored lipid is illustrated (Magnification: **a**, 165,600×; **b**, **c**, 164,800×)

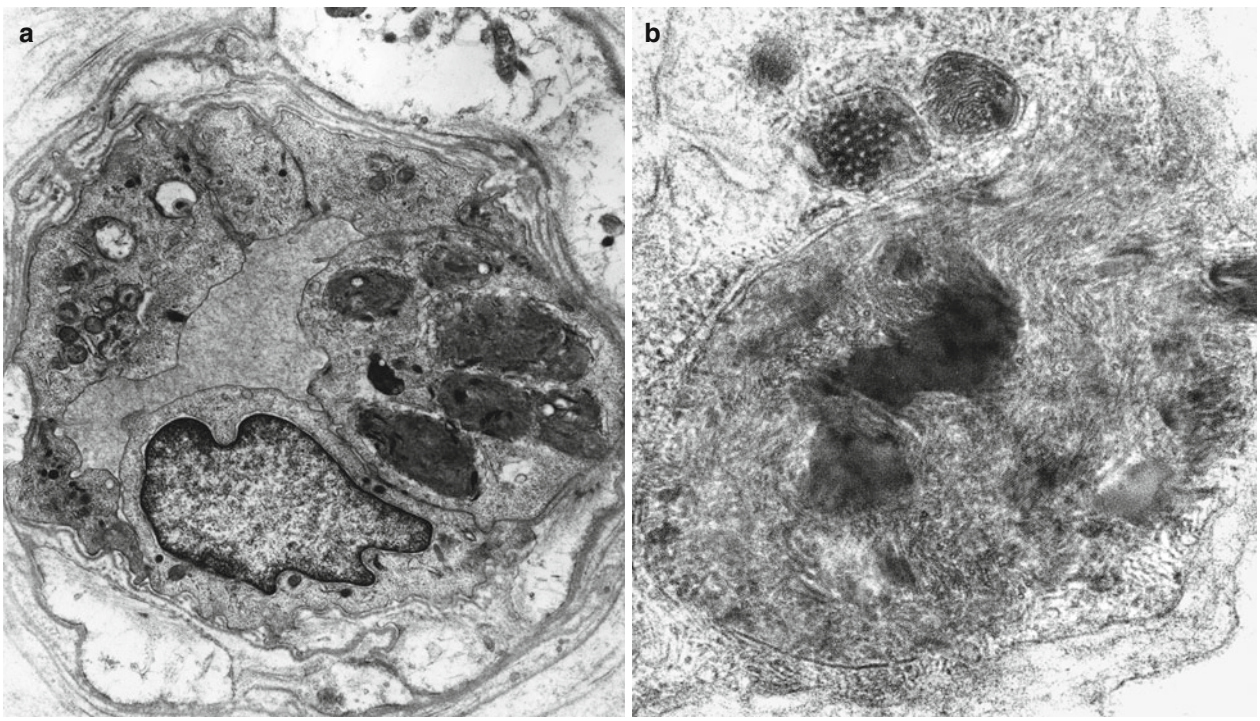

Fig. 20.15 Fabry disease: epineurial vessels showing lipid accumulation in endothelial and smooth muscle cells (Magnification: **a**, 6,250×; **b**, 20,000×)

but ophthalmological opinion was requested because of suspected mild papilledema of the left optic disk. The consultant ophthalmologist did not feel that papilledema was present, but noted corneal opacification and raised the possibility of Fabry disease at that time. Fluorescein angiography was normal.

The patient was admitted to hospital 5 months later with sudden onset of near-total vision loss in the left eye. A diagnosis of optic neuropathy was made, but the question of an inflammatory or vascular basis remained unclear; the complete lack of improvement favored the latter. Note was made of "whorl keratopathy" of the cornea. Erythematous papules were observed on the backs of the shoulders and in the flanks and groin area. Buccal and labial telangiectasias were also noted. A neurological examination at this time revealed mild right arm and leg weakness, diffuse hyperreflexia, and bilaterally upgoing toes. His course over the next 2 years was notable for urinary difficulties ultimately requiring intermittent catheterization. Although the diagnosis of Fabry disease was established biochemically by this time, nerve biopsy was prompted by suspicion of a diagnosis of polyglucosan body disease.

Laboratory testing included normal renal function and normal 2-D echocardiogram. CSF examination revealed a slight pleocytosis on two occasions 6 months apart (9, 17 cells, respectively, predominantly lymphocytes), and normal protein, glucose, and opening pressure. Oligoclonal banding was negative. CT and MRI of the head were normal on three separate occasions. Serum α-galactosidase was 22.5 ng/mg/h (normal >100), with increased urinary ceramide trihexoside excretion. Nerve conduction and EMG studies were normal. Small fiber function and sweating were not specifically tested.

20.1.4 Niemann–Pick Disease

20.1.4.1 Clinical Manifestations

The Niemann–Pick (NP) group of diseases is characterized by tissue storage of sphingomyelin and/or cholesterol (Spence and Callahan 1989). All subtypes are inherited as autosomal recessive traits. Niemann–Pick disease is currently subdivided on the basis of biochemical and molecular criteria into several separate classes: types A (MIM#257200) and B (MIM#607616) (both type I in older classification) are deficient in acid sphingomyelinase and are sphingomyelin storage diseases (Takahashi et al. 1992), and types C and D (MIM#257220) (both previously type II) are deficient in the NPC1 protein and represent lysosomal disease of intracellular cholesterol trafficking. Foam cell infiltration and visceromegaly are common to all, but severe neurological involvement occurs only in types A (a rapidly progressive neurodegenerative course leading to death by 2–3 years of age) and C and not in type B (McGovern and Desnick 2011).

Hepatosplenomegaly is a central element of the NP clinical picture, and biopsy reveals "foam cells," macrophages distended with storage material ultrastructurally similar to that described below. Peripheral neuropathy is usually not a clinically significant part of the disease (Gumbinas et al. 1975). Nearly all the NP patients with neuropathy discussed below had conduction slowing suggestive of a demyelinating neuropathy.

Diagnosis traditionally has been made through clinical suspicion and the histologic demonstration of "foam cells" on bone marrow or liver biopsy. More recently assays for sphingomyelinase in tissue culture or fluids have been replaced by genetic studies. Niemann–Pick type A is caused by mutation in the sphingomyelin phosphodiesterase-1 gene (*SMPD1*), which encodes acid sphingomyelinase (ASM; Schuchman et al. 1992) and Niemann–Pick disease types C and D are caused by mutation in the *NPC1* gene. Recently a number of small molecule agents including cyclodextrin have been used to improve visceral and neurodegeneration (Perez-Poyato and Pineda 2011; Liu et al. 2010).

20.1.4.2 Pathology

Clinicopathological studies of peripheral nerve in NP are rare (Landrieu and Said 1984; Da Silva et al. 1975; Gumbinas et al. 1975; Hahn et al. 1994; Elleder 1989; Elleder et al. 1986; Babel et al. 1970 p 130; Bischoff 1979; Dubois et al. 1990). Most reports have considered NP type A except those of Elleder (1989) and Hahn et al. (1994). Our experience consists of three nerve biopsies in a brother and sister with NP type A who survived well into adult life (Case 20.2). Two biopsies in the sister were taken 15 years apart.

Light Microscopy

Sural nerves from patients with Niemann–Pick type A show generalized attenuation of myelin sheaths (Fig. 20.16a, b). Classic "foam cells" are sometimes observed in the endoneurium (Gumbinas et al. 1975). These large cells are often found in a perivascular distribution and demonstrate a finely multivacuolated cytoplasm (Fig. 20.16c). Light microscopy provides sufficient resolution to detect some Schwann cell inclusions, about 1 μm in size. With cryostat sections, the storage material is said to display "Maltese cross" birefringence similar to that of Fabry disease (Elleder 1989), although we have not detected this in our material. Oil Red O-positive material is seen on both frozen and paraffin-embedded sections, and the storage substance is particularly well highlighted with Giemsa staining. Some of the lipid inclusions are autofluorescent.

Axon numbers are minimally or not at all reduced in most literature reports, and in our experience, even with the passage of many years (Dubois et al. 1990; Hahn et al. 1994; Landrieu and Said 1984). In two nerve biopsies from the same patient taken at the ages of 35 and 50, there was no evidence of any histological progression of the disease

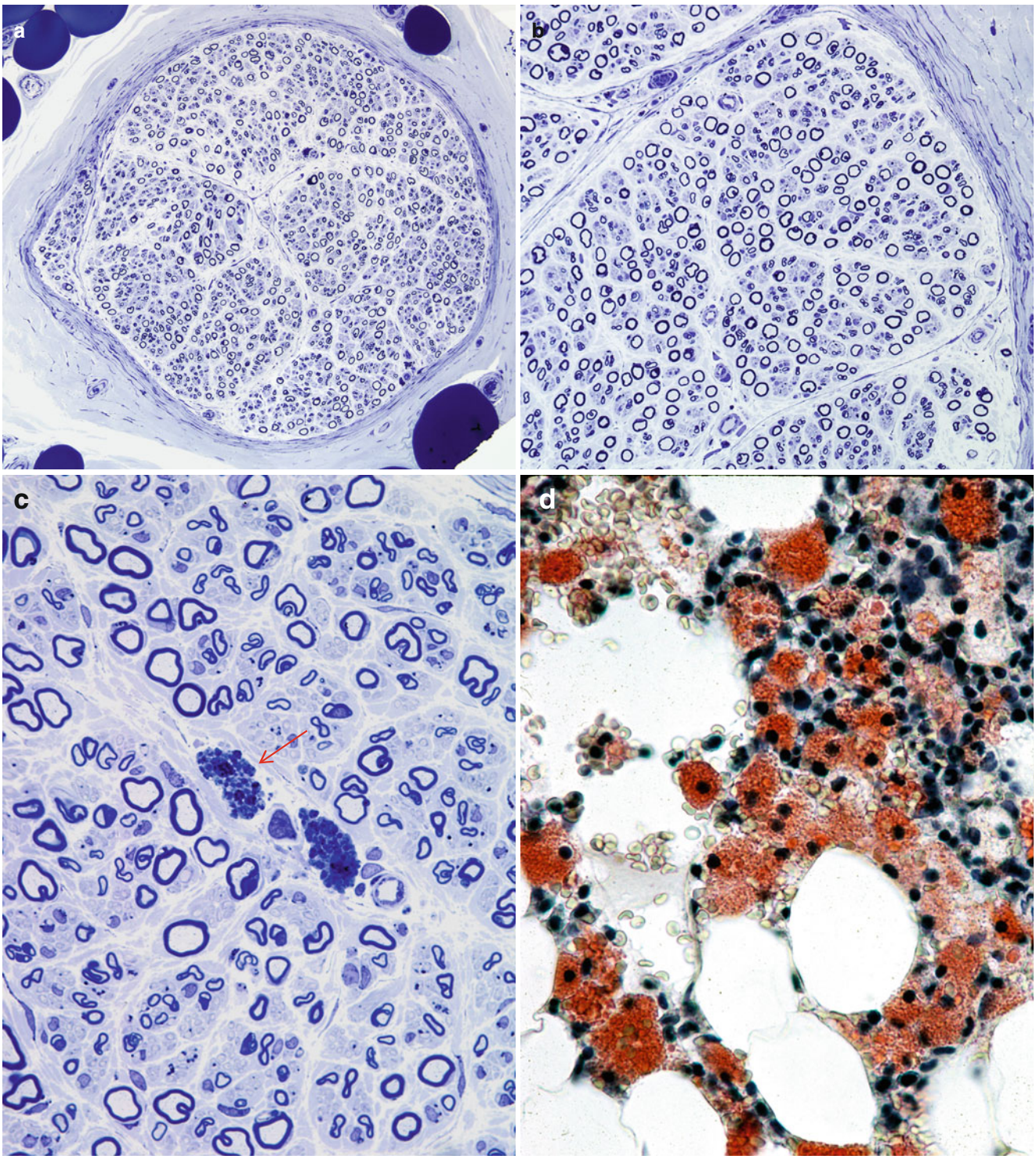

Fig. 20.16 Niemann–Pick disease, type A: note universal attenuation of myelin sheaths (**a**, **b**), lipidized perivascular endoneurial macrophages (*arrow*, **c**) and foamy bone marrow histiocytes (**d**). (**a**–**c**, 1 μm thick plastic section; **d**, frozen section, Oil Red O stain) (Magnifications: **a**, 400×; **b**, 600×; **c**, 1,000×; **d**, 1,000×)

(Fig. 20.17a–c). A picture of excessively thin myelin sheaths is most common (Fig. 20.16a–c), with naked axons and active demyelination being quite rare (Gumbinas et al. 1975; Hahn et al. 1994; Landrieu and Said 1984; Da Silva et al. 1975). Onion-bulb formations were present in our material, but have not been reported otherwise. The two patients reported by Dubois et al. (1990) had no light microscopic evidence of neuropathy, but ultrastructural examination revealed characteristic storage material in Schwann cells.

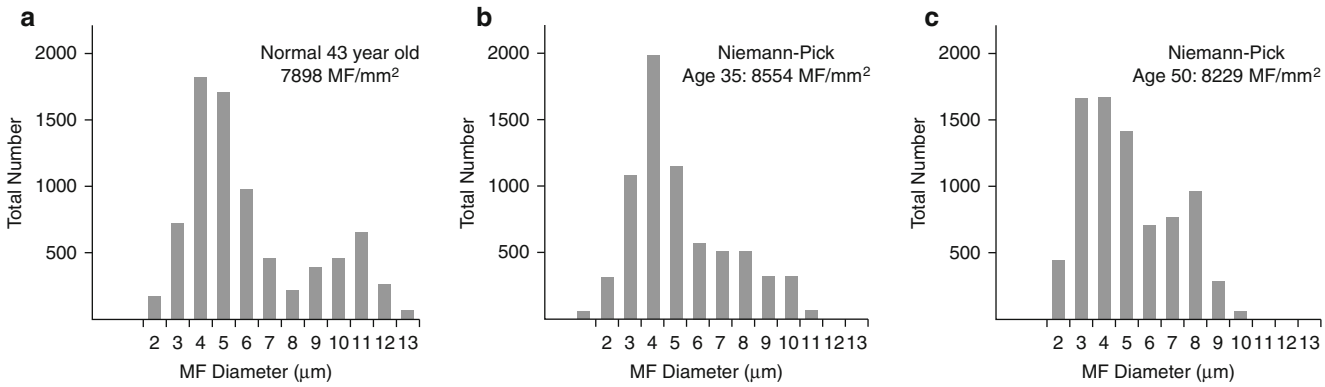

Fig. 20.17 Two biopsies spanning 15 years show a normal fiber density (**b**, **c**) as compared to an age-matched control (**a**)

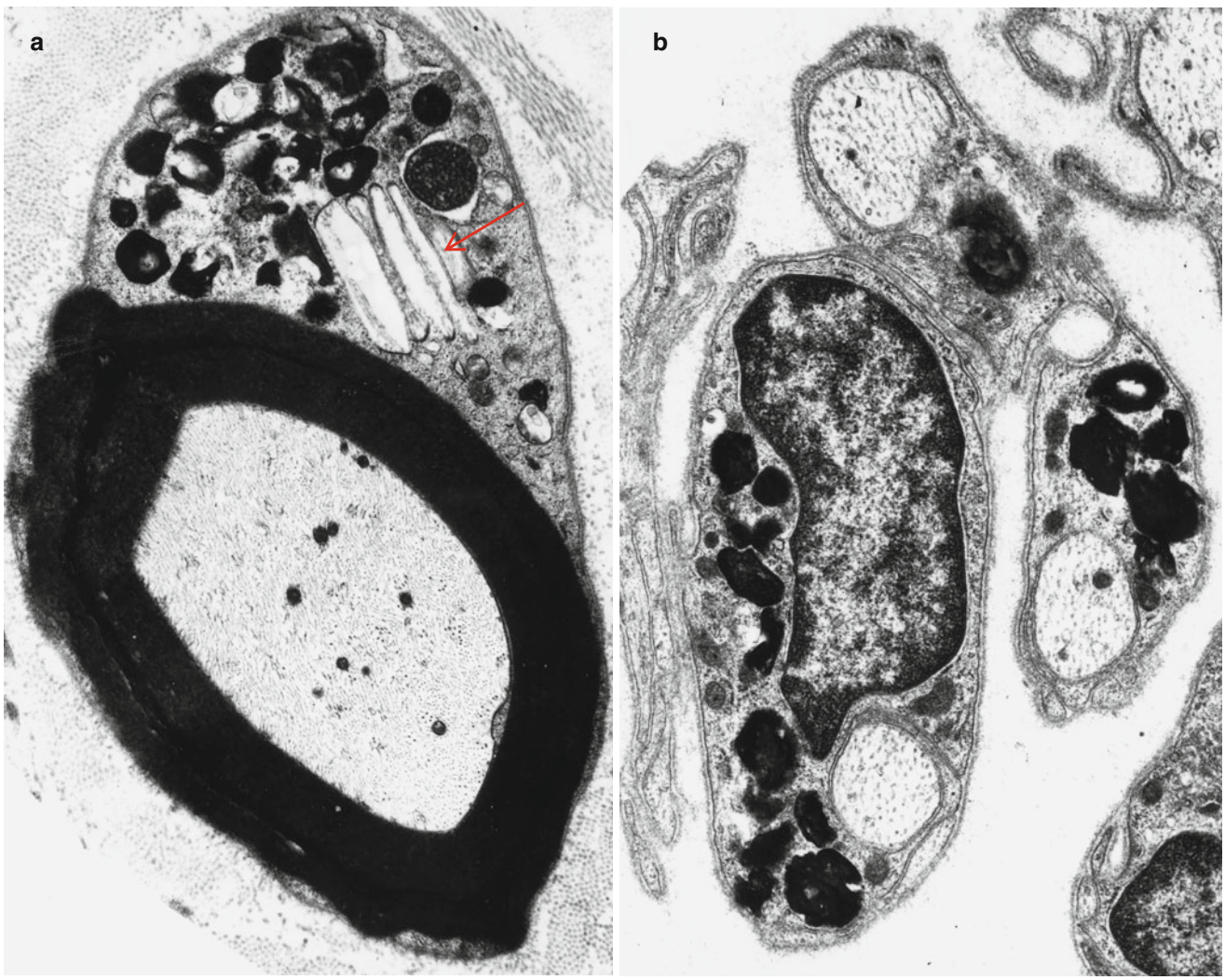

Fig. 20.18 Niemann–Pick type A: lipid inclusions, most of which are indistinguishable from lipofuscin, are seen in the cytoplasm of myelinating and nonmyelinating Schwann cells. Note Pi-body (*arrow*, **a**) (Magnification: **a**, 11,172×; **b**, 16,188×)

Electron Microscopy

The typical ultrastructural finding in NP type A is an accumulation of membrane-bound inclusions, most prominently in Schwann cells of myelinated and unmyelinated fibers (Fig. 20.18) and in histiocytes (Fig. 20.19a, b). In reported cases the storage material has taken a variety of appearances,

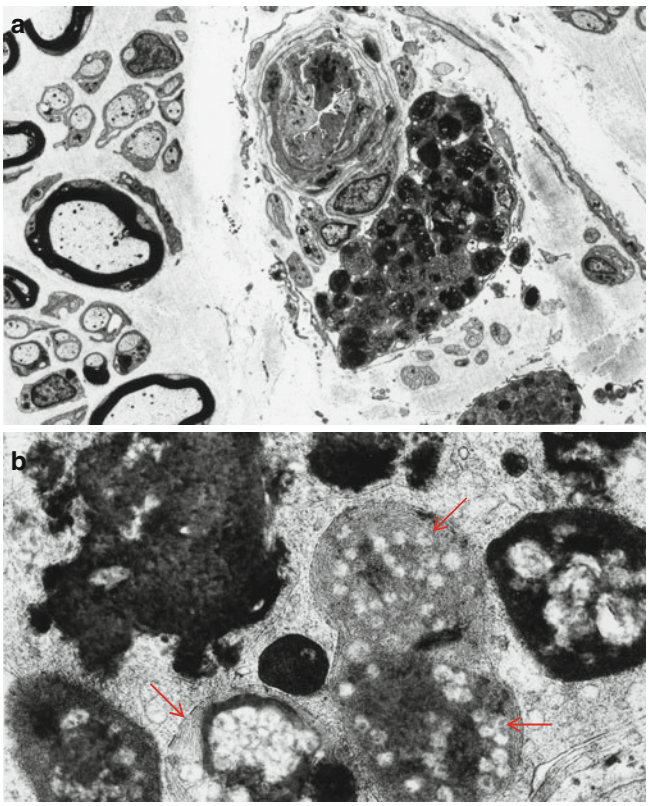

Fig. 20.19 Niemann–Pick type A: perivascular endoneurial macrophage laden with lipid debris. In (**b**) regions of concentric periodicity (*arrows*) are intermingled with amorphous material of variable osmiophilia (**a**, 3,040×; **b**, 45,144×)

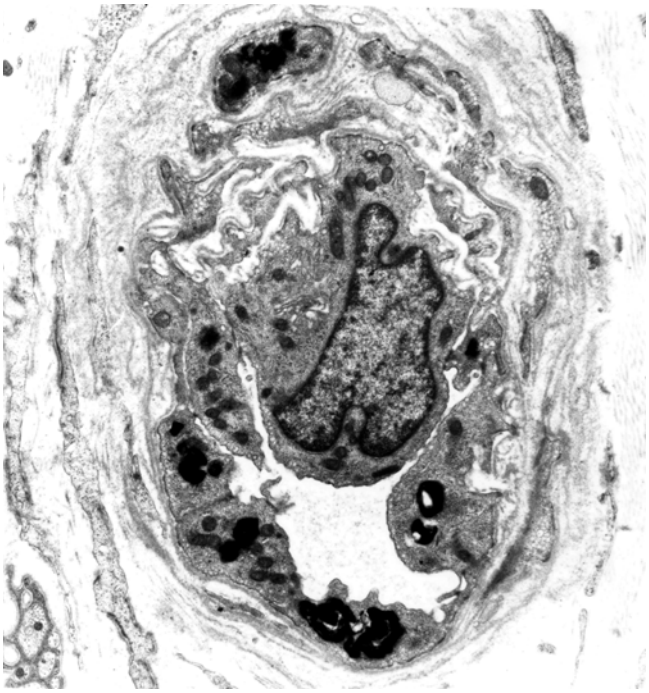

Fig. 20.20 Niemann–Pick type A: lipid inclusions in endothelial cells and pericytes (Magnification: 7,040×)

including concentric whorls of loosely packed membranous material, amorphous or granular electron-dense material in which a subtle periodicity may be seen, single or multiple lucent vacuoles, or "zebra" body-like inclusions. Some of the stored material is indistinguishable from lipofuscin and ceroid (Figs. 20.18 and 20.19a, b), and as Elleder (1989) points out, a major part of the storage material found in the reticuloendothelial system is indeed lipopigment, especially in the late-onset disease variants (Spence and Callahan 1989). In our patients the stored substance was most often amorphous and lipofuscin-like, and "zebra bodies" were not seen. However, at times osmiophilic regions showing a periodicity of 4–6 nm emerged from the amorphous background (Fig. 20.19b). The foamy cells seen on LM are revealed as macrophages containing storage material similar to that seen in the Schwann cells (Fig. 20.19a). Inclusions can also be seen in endothelial cells, pericytes, myocytes, perineurial cells, bone marrow, and fibroblasts (Fig. 20.20a, b). Axonal changes are less obvious and nonspecific and include loss of filaments, mitochondrial accumulations, increases in axoplasmic vesicles, and numerous dense bodies (Babel et al. 1970; Landrieu and Said 1984). Babel et al. (1970, Fig. 62) illustrate a membrane-bound axonal inclusion composed of lamellated material with a periodicity of 5.6–6 nm, but lamellated intra-axonal cytosomes are a nonspecific finding and might not relate to the storage process occurring in Schwann cells.

In NP type C, axonal alterations seem to be a central part of the histological picture (Hahn et al. 1994; Elleder 1989), but the paucity of available material precludes generalization. An accumulation of whorled and multi-lobulated flocculent material in most endoneurial cellular components is described by Hahn et al. (1994). However, these authors emphasize the presence of axonal alterations, with occasional "… accumulation and condensation of neurofilaments, vesicular profiles, dense bodies, and abnormal mitochondria resembling changes of neuroaxonal dystrophy" (Hahn et al. 1994). Elleder (1989) has also commented on "… neuroaxonal dystrophy of a nonspecific type …" in cutaneous nerve axons. Skin biopsy in NP type C reveals lysosomes containing dense material or loosely packed lamellated material in a clear background in axons and Schwann cells, as well as fibroblasts, epithelial cells, and pericytes (Boustany et al. 1990). Curvilinear inclusions in endothelial cells described by Hahn et al. (1994) appear similar to those seen in neuronal ceroid lipofuscinosis.

Case 20.2

A 37-year-old man was referred with a history of dementia and ataxia. At the age of 3 he was found to have an enlarged spleen. A psychiatric disorder with paranoid features emerged during his teen years, and in his 20s, he became frankly demented. Progressive gait unsteadiness followed by

limb ataxia also appeared in the second decade. Examination at the age of 37 revealed severe dementia, no significant weakness, preservation of reflexes, grossly intact sensation, and ataxia of all limbs. Nerve conduction studies showed normal amplitudes, with slightly prolonged distal latencies and conduction velocity of 30 m/s in both motor and sensory nerves, suggesting a mild demyelinating neuropathy. CT scan showed severe cerebellar atrophy affecting the vermis more than the hemispheres.

The patient's sister presented with gait difficulty in her teens, progressing very slowly to walker dependence by the age of 40. She was also thought to be cognitively impaired and demonstrated impulse control difficulties requiring treatment with neuroleptics. Examination of at the age of 50 years revealed mild mental impairment, normal fundoscopy, normal extraocular movements, and a slight dysarthria. The limbs displayed normal tone and strength throughout, with all reflexes reduced but obtainable, and no significant sensory loss to pin or vibration. Mild ataxia was present on heel–shin testing, but the gait was wide based and grossly ataxic. Hepatosplenomegaly was noted. Nerve conduction studies performed at the age of 35 showed a very mild neuropathy with amplitudes and conduction velocities just below the lower limit of normal.

Liver biopsy was performed on the brother and demonstrated numerous foamy histiocytes. Lipid analysis of the liver biopsy material revealed an accumulation of sphingomyelin. Cultured fibroblast sphingomyelinase activity in the sister was 1.1 nmol/mg/h (10 % interdecile range 8.6–289 nmol/mg/h). The brother's cultured fibroblast and serum sphingomyelinase activities were also less than 10 % of the lower limit of normal. Nerve biopsies were performed on the brother at the age of 38, and on the sister at the ages of 35 and 50 (Figs. 20.16, 20.17, 20.18, 20.19, and 20.20).

20.1.5 Farber Disease (FD, Lipogranulomatosis)

20.1.5.1 Clinical Manifestations

Farber disease is a rare autosomal recessive infantile disease (MIM#228000) caused by mutation in the *ASAH1* gene encoding acid ceramidase resulting in the deficiency of acid ceramidase, necessary for metabolism of the sphingolipid ceramide (Moser et al. 1989; Zappatini-Tommasi et al. 1992; Alayoubi et al. 2013). The characteristic clinical features are disseminated nodular subcutaneous, periarticular swellings and vocal cord thickening. The central nervous system is affected in about half of the patients. Peripheral nerve involvement is common, manifesting with hypotonia and atrophy, and nerve conduction studies have shown significant slowing (Fujiwaki et al. 1992; Pellissier et al. 1986). The usual outcome is rapid progression to death in the first few years of life, although some patients survive into the third decade (Moser et al. 1989).

20.1.5.2 Pathology

Histologic examination of the nodules of Farber disease reveals a granulomatous infiltrate, sometimes with mature granuloma formation. Storage material may take on a variety of ultrastructural appearances including Farber bodies, "banana" bodies, and "zebra" bodies (Zappatini-Tommasi et al. 1992; Burck et al. 1985). Farber bodies are curved tubular structures 14–17 nm in diameter found in great numbers within membrane-bound vacuoles or free within the cytoplasm and believed to correspond to stored ceramide (Rauch and Aubock 1983; Takahashi and Naito 1985; Pellissier et al. 1986). They bear a slight resemblance to the curvilinear profiles of Batten–Kufs disease, but are smaller and tubular, not lamellated. Farber bodies are seen in fibroblasts, histiocytes, and endothelial cells in extraneural tissues. Zebra bodies have been described in endothelial cells (Rauch and Aubock 1983).

A few nerve biopsy specimens have been closely studied (Vital and Vallat 1987; Vital et al. 1976; Pellissier et al. 1986; Rauch and Aubock 1983; Burck et al. 1985). Light microscopic examination of nerves has shown widespread hypomyelination and may reveal mild loss of large axons not sufficient to reduce the total MF density below normal (Fig. 20.21a) (Pellissier et al. 1986). Vital et al. (1976) indicated that unmyelinated fibers were spared in their specimen, but this was not quantitated. High-power LM can reveal pathognomonic vacuoles in myelinating Schwann cells (Fig. 20.21b). Frozen sections did not reveal any sudanophilic or metachromatic material in the case of Vital et al. (1976).

Ultrastructural examination is diagnostic (Fig. 20.22). Myelinating Schwann cells contain electron-lucent membrane-bound clefts, which take a variety of shapes ranging from needlelike to elongated rectangular, angulated, polygonal, and even annular profiles (Fig. 20.23a, b). These may be up to 5 μm in diameter, may indent the axon, and are almost exclusively seen in myelinating Schwann cells (Rauch and Aubock 1983; Schmoeckel and Hohlfed 1979). Presumably the content of these structures is lipid, washed out in preparation. At times there is a residual content which Pellissier et al. (1986) described as "… either homogenous and osmiophilic, or less dark with an electron-dense lamellar component." We have also observed, in nonmyelinating Schwann cells only, novel membrane-bound elongated bodies containing a central longitudinal striated region surrounded by amorphous electron-dense material (Fig. 20.24).

20.1.5.3 Pathogenesis and Therapy

A mouse model has been developed (Alayoubi et al. 2013) via "knock-in" of a known human ASAH1 mutation into a

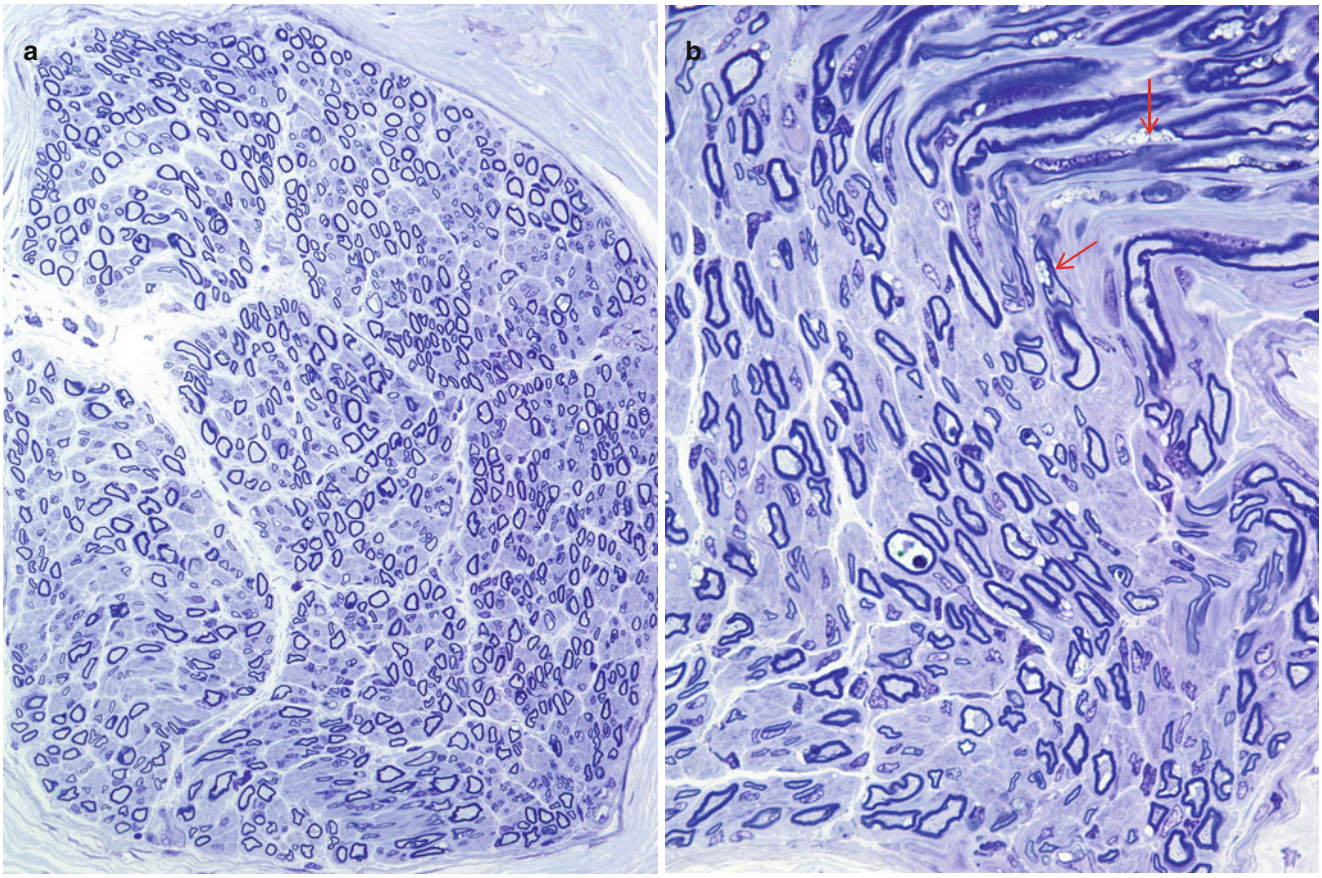

Fig. 20.21 Farber disease: note normal MF density (**a**), attenuated myelin sheaths, and accumulation of optically empty inclusions in Schwann cell cytoplasm (*arrows*, **b**) (1 μm thick plastic sections; magnification **a**, 400×; **b**, 1,000×) (Tissue courtesy of Dr. V. Jay)

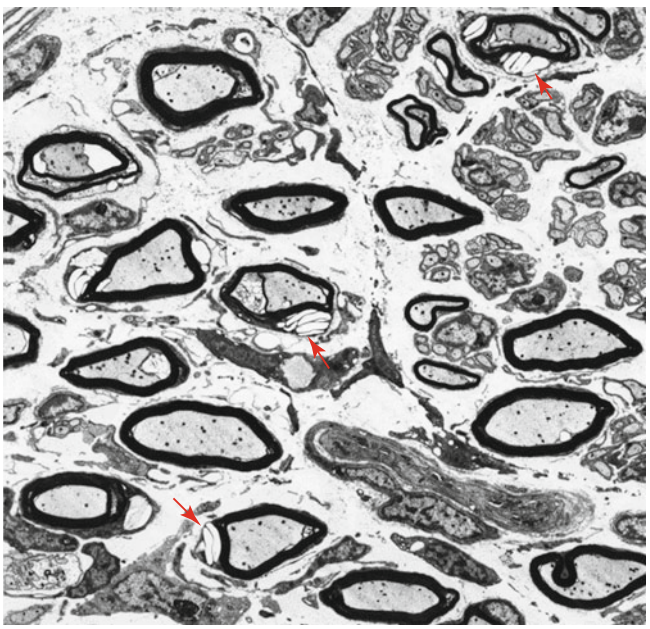

Fig. 20.22 Farber disease: numerous "banana" bodies are shown in myelinating Schwann cells (*arrows*) (4,000×)

conserved murine Asah1 gene locus. In that model it has been determined that the accumulation of ceramide prompts the release of MCP-1 which recruits circulating monocytes to tissues where they accumulate and fail to degrade the sphingolipids and ceramide they engulf, amplifying the foamy appearance which is characteristic of FD. Neonatal gene therapy using recombinant lentivectors encoding the human acid ceramidase gene has shown a salutary effect in this experimental model. In patients allogeneic bone marrow transplantation has shown success in reducing the peripheral symptoms in Farber disease but failed to improve neurological symptoms and patient lifespan (Ehlert et al. 2007).

20.1.6 Other Sphingolipidoses

Examination of nerve biopsy has been carried out in a number of other sphingolipidoses, but the available peripheral nerve material is too scanty for detailed discussion. For completeness, these are discussed briefly.

GM2 gangliosidosis (MIM#272800) is caused by defects in hexosaminidase activity (Table 20.1) and manifests with a

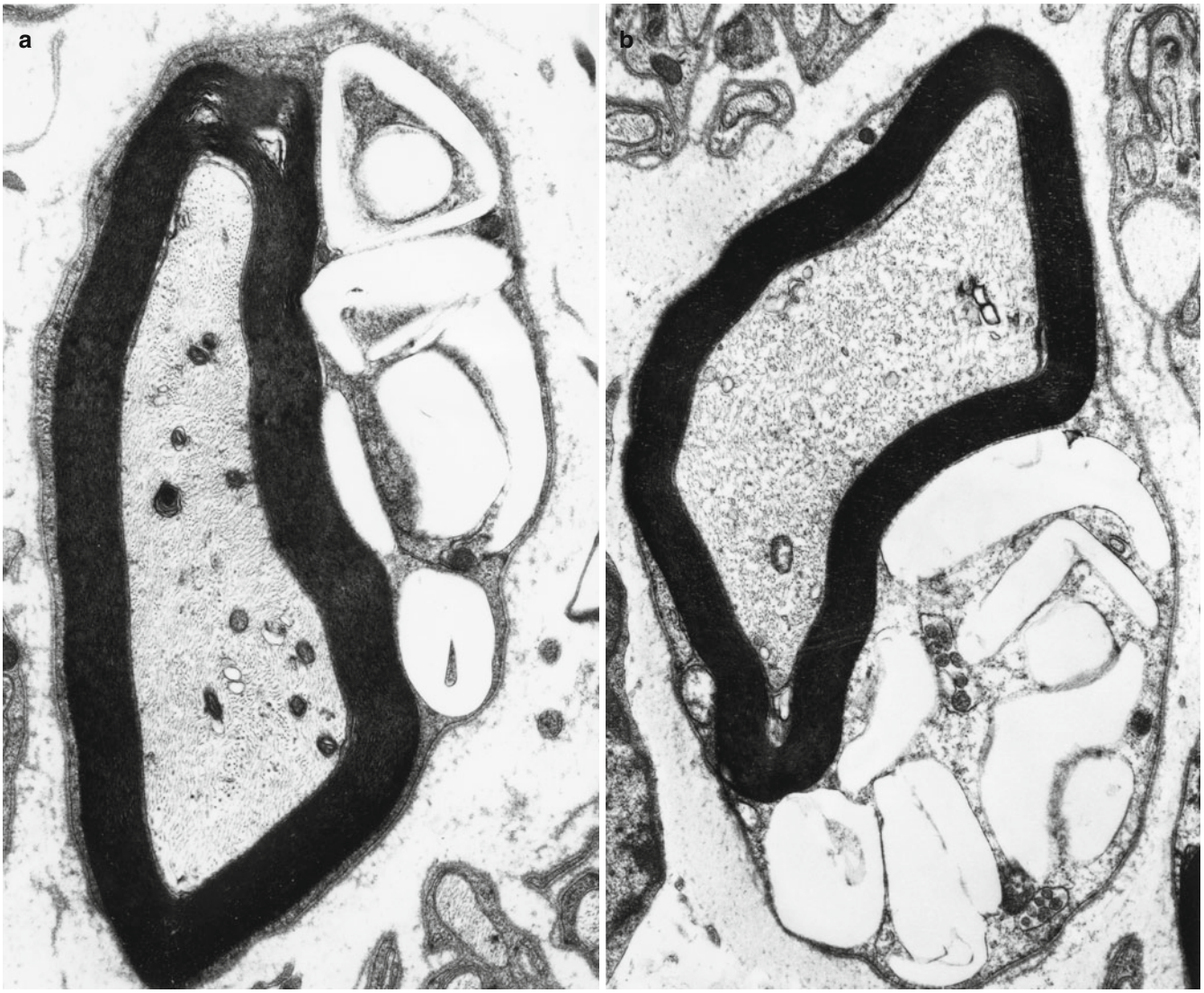

Fig. 20.23 Farber disease: typical morphologies of "banana" bodies (**a**, 14,910×; **b**, 12,320×)

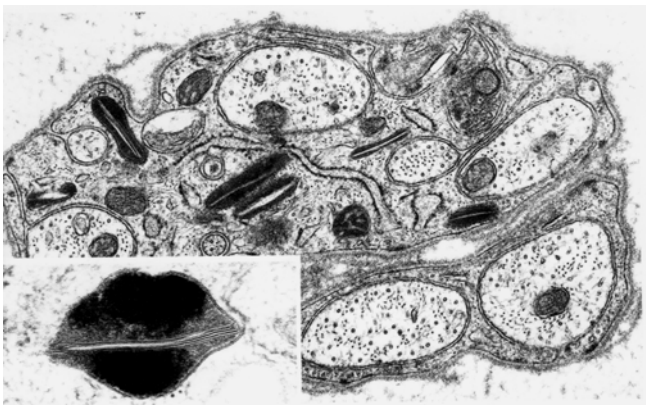

Fig. 20.24 Farber disease: nonmyelinating Schwann cells contain membrane-bound elongated striated bodies (20,080×, *inset* 91,200×)

wide spectrum of infantile- and adult-onset diseases, the most common of which is Tay–Sachs disease (Federico et al. 1991a). Peripheral neuropathy is not a significant part of the disease, but motor neuronopathy is common in late-onset cases. The characteristic storage inclusions, seen best in neuronal perikarya, are membranous cytoplasmic bodies. Sural nerve biopsy in some of the adult-onset cases has shown a reduction in myelinated fiber density and evidence of axonal regenerative activity (Mitsuo et al. 1990; Mitsumoto et al. 1985; Federico et al. 1986, 1991a). Unfortunately, these case reports do not mention ultrastructural findings, and there are no well-documented reports of inclusions in sural or other peripheral sensory nerve biopsies. Severe destruction of myelin sheaths and axonal swellings were seen in a light microscopic study of sciatic nerve in Tay–Sachs disease, but

no EM examination was performed (Kristensson et al. 1967). In the infantile (Tay–Sachs) and juvenile (Sandhoff) variants of GM2 gangliosidosis, intra-axonal membranous cytoplasmic bodies, "zebra" bodies, and dense amorphous inclusions have been observed in terminal myelinated and unmyelinated nerve fibers present in skin and muscle specimens (Schmitt et al. 1979; Abe et al. 1985; Dolman et al. 1977; Thomas et al. 1989; Burck et al. 1979). Bischoff (Fig. 4.61) illustrates unmyelinated fibers containing osmiophilic multilamellated inclusions in a patients with Tay–Sachs disease, but the tissue source is not specified (Bischoff 1979). Recent work with pharmacologic chaperones results in the protection of the mutated protein from degradation, increasing its lysosomal quantity and metabolic function (Chiricozzi et al. 2013). Chaperone therapy has been attempted in GM1 gangliosidosis, Fabry disease, Gaucher disease, and Morquio B disease (Chiricozzi et al. 2013).

GM1 gangliosidosis is an autosomal recessive disease (multiple types include M230500, 230600, 230650) caused by a deficiency of ganglioside β-galactosidase (distinct from the galactosylcerebroside β-galactosidase defect of Krabbe disease). Onset is usually at birth or infancy, with hypotonia, organomegaly, and a cherry red macula. Bischoff (1979) illustrates membrane-bound Schwann cell inclusions with a slightly electron-dense granular and amorphous content. In studies of muscle and skin biopsy specimens (Tome and Fardeau 1976; Vissian et al. 1970), Schwann cells and axons demonstrated similar inclusions which sometimes contained parallel arrays of lamellae. Pericytes, perineurial, endothelial, and smooth muscle cells contained electron-lucent vacuoles similar to those seen in this disease in other tissues (O'Brien et al. 1975; Dolman et al. 1977). Correction of the enzyme deficiency and reduction in glycosphingolipid accumulation have been shown in β-gal −/− mice treated with intravenous injection of an adenoviral vector or intracerebroventricular injection of an adeno-associated virus expressing the mouse b-galactosidase (reviewed in Brunetti-Pierri and Scaglia 2008). A second approach with a chemical chaperone (*N*-octyl-4-epi-b-valienamine, NOEV) has been used to stabilize the mutant β-galactosidase protein for restoration of enzyme activity (Matsuda et al. 2003).

20.2 Adrenoleukodystrophy

20.2.1 Clinical Manifestations

Adrenoleukodystrophy (ALD, MIM#300100) is an X-linked disorder caused by a defect in very long-chain fatty acid (VLCFA) metabolism (recently reviewed, Faust et al. 2010). Several disease variants are identified, and more than one phenotype can occur in the same family (Moser et al. 1992). The childhood ALD form of the disease is most common, with onset between the ages of 3 and 12, and neurological manifestations usually preceding clinical signs of adrenal insufficiency. Patients present with behavior disturbances, followed by impaired hearing and vision, and motor abnormalities. Death due to progressive disease occurs within 1–10 years after onset. The adrenomyeloneuropathy (AMN) variant is next in frequency, age of onset typically in the second to fourth decade, with a progressive spastic paraparesis, urinary disturbance, and sensory dysfunction (Griffin et al. 1977). Adrenal insufficiency is overt in many patients with either variant and can usually be demonstrated biochemically in the rest. Addison disease without neurological symptoms can occur, but the latter usually appear with time. Given that the disease is X-linked recessive, all severely affected patients are male, but significantly symptomatic female heterozygotes are seen, usually with AMN. Neuropathy is not usually a feature of the childhood disease (Schaumburg et al. 1975). In AMN a neuropathy is usually present, with conduction slowing found frequently although not invariably (Griffin et al. 1977). Neonatal adrenoleukodystrophy is an entirely different autosomal recessive disease (Moser et al. 1984; Mito et al. 1989).

Sural nerve biopsy does not have a significant role to play in this disease, but some biopsy material has been studied (Martin et al. 1982b; Pages and Pages 1985; Chazot et al. 1979; Cohadon et al. 1975; Gastaut et al. 1988; Mito et al. 1989; Julien et al. 1981; Vital and Vallat 1987, Figs. 253; 254; Kumamoto et al. 1985; Case Records NEJM 1982; Schaumburg et al. 1975,1977).

20.2.2 Pathology

The histological picture of adrenoleukodystrophy varies with the tissue examined. In the CNS there is diffuse loss of myelin most severe in the posterior brain regions and perivascular inflammatory infiltration with lymphocytes and plasma cells. Lipid-laden macrophages are found in regions of active demyelination, and ultrastructural examination reveals characteristic inclusion material. A similar storage substance can be identified in adrenal cortical cells and testicular Leydig cells, as well as in peripheral nerve, but inflammation is not an element of the disease in these tissues.

20.2.2.1 Light Microscopy

Light microscopic studies of peripheral nerve in both AMN and ALD have at times shown no abnormalities or revealed a nonspecific combination of chronic axonal loss

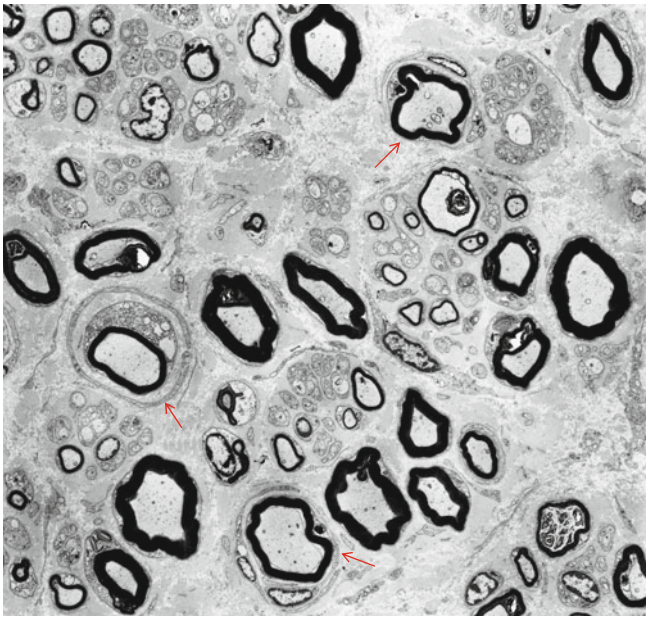

Fig. 20.25 Adrenomyeloneuropathy: 21-year-old male. Low-power EM view of endoneurium shows attenuation of myelin sheaths and onion-bulb formations (*arrows*) (2,340×) (Tissue courtesy of Dr. J. Michaud)

and demyelinating changes. Large myelinated fibers can be selectively lost, and some regenerating clusters may be seen. Unmyelinated axons may be normal or reduced in number (Griffin et al. 1977; Case Records of the Massachusetts General Hospital 1982; Pages and Pages 1985). Onion bulbs are readily detected in most cases, and thinly myelinated fibers indicative of demyelination and remyelination are common (Fig. 20.25). The demyelinating process appears indolent, as naked axons and debris-filled endoneurial macrophages are rarely seen. The lymphocytic infiltrate seen in CNS tissues is not a feature of the peripheral nerve involvement in this disease.

20.2.2.2 Electron Microscopy

ALD and AMN may reveal characteristic ultrastructural inclusions, but these are much more difficult to detect in peripheral nerve than in the CNS, even in the AMN variant (Schaumburg et al. 1975, 1977). Indeed, in only a minority of reported cases has the typical inclusions of the disease been demonstrated (Martin et al. 1982b; Gastaut et al. 1988; Julien et al. 1981; Pages and Pages 1985; Schaumburg et al. 1975). The storage material is seen in Schwann cells and macrophages, may be single membrane bound, and is usually composed of randomly disposed or regularly arrayed aggregates of linear profiles. The typical individual profile is elongated and can be bilaminar or trilaminar, with the two outer lamellae each 2.5 nm thick and separated by a space of 1–7 nm. The underlying structure of the storage material is trilaminar, but the bilaminar cross-sectional appearance can result depending on the plane of section (Ghatak et al. 1983). When the third (inner) lamella is identifiable, it is seen to be thinner than the two which flank it (Julien et al. 1981; Gastaut et al. 1988). Glycogen particles and electron-lucent regions often demarcate the storage material. On occasion, perhaps in relation to tissue preparation methods, the lamellar material is not seen, and only curvilinear clear spaces and glycogen particles may be identifiable (Martin et al. 1982b). Large regions of the Schwann cell or macrophage cytoplasm can be filled with randomly arrayed inclusions intermingled with vacuolated spaces and glycogen granules (Gastaut et al. 1988). Martin and colleagues (1982b) found such inclusions in about 5 % of Schwann cells associated with myelinated fibers.

Some workers have commented on an increase in Reich Pi granules or Pi granule-like inclusions (Askanas et al. 1979; Chazot et al. 1979; Kumamoto et al. 1985; Schaumburg et al. 1977). In a case of AMN in a 21-year-old man generously sent to us by Dr. J. Michaud of Montreal, this was also the main finding on ultrastructural examination (Fig. 20.26a, b). When these Pi granules are examined closely, there is often a bilamellar substructure that suggests that these inclusions are made up of stacks of the individual bilamellar structures regarded as typical of ALD storage substance (Figs. 20.26b and 20.27) (Gastaut et al. 1988). Abnormal accumulation of lipofuscin in myelinating Schwann cells has also been noted (Askanas et al. 1979; Chazot et al. 1979) and was present in the nerve we examined.

Ghatak et al. (1983) have suggested that inclusions seen in macrophages are membrane bound, while those seen in parenchymal cells (i.e., Schwann cells) are not (Ghatak et al. 1983).

One reported case of neonatal adrenoleukodystrophy has shown a combination of axonal degeneration and segmental myelin change with typical inclusions on ultrastructural examination (Mito et al. 1989).

20.2.3 Pathogenesis

An accumulation of very long-chain fatty acids (VLCFA) had been considered the essential diagnostic characteristic of ALD. This disease has been called a peroxisomal disorder because VLCFA are metabolized in peroxisomes, not in mitochondria as with shorter-chain fatty acids. However, linkage analysis localized the genetic defect of ALD to the q28 region of the X chromosome (Moser et al. 1992) and not to peroxisomal lignoceroyl-CoA ligase activity which had been thought previously (Singh et al. 1992). Rather, the gene defect involves adenosine triphosphate [ATP]-binding cassette transporter superfamily D member 1 (ABCD1, MIM 300371) encoding a peroxisomal ATP-binding cassette transporter (Mosser et al. 1993). It is likely

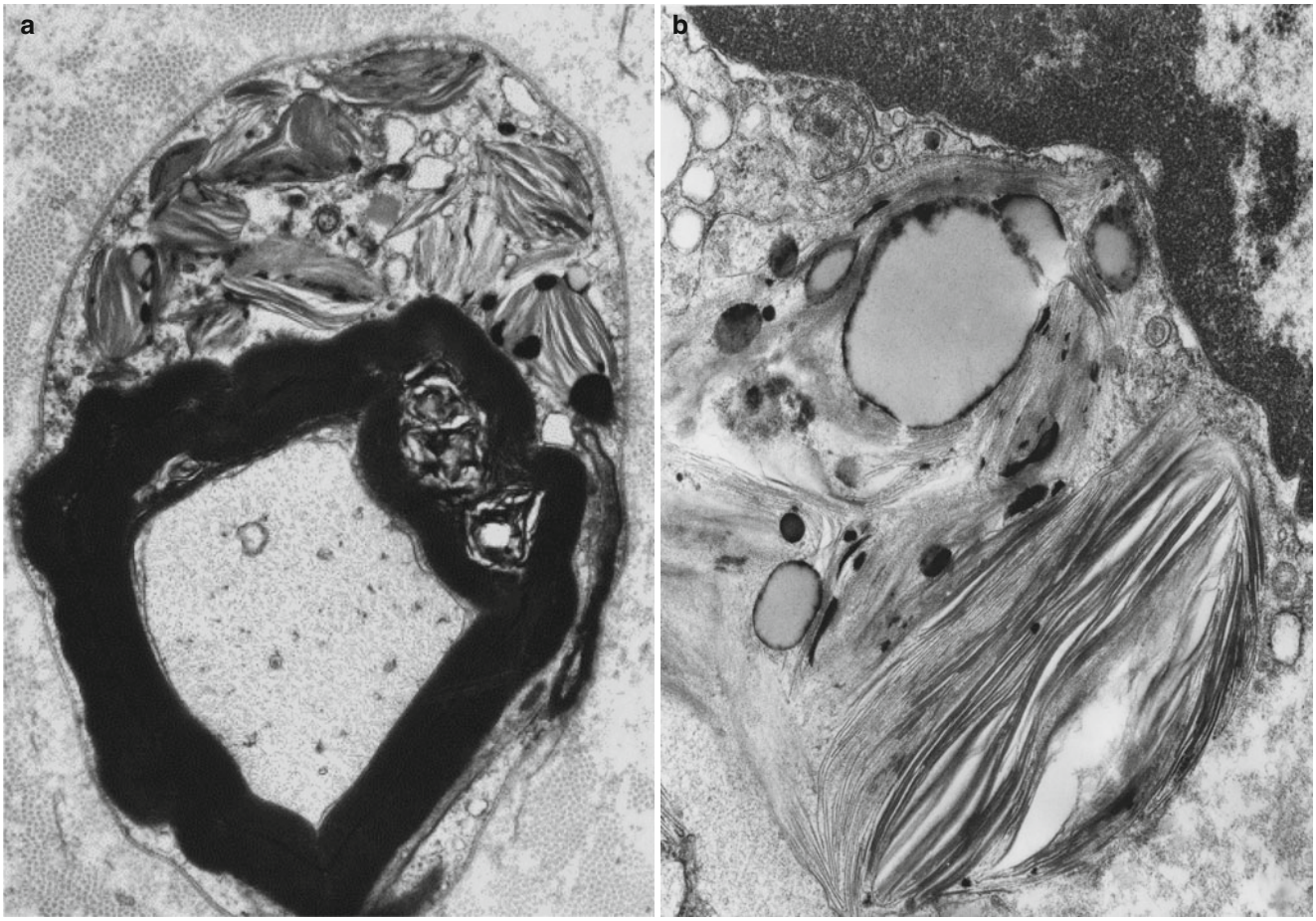

Fig. 20.26 Adrenomyeloneuropathy: Pi-body-like cytosomes distending the cytoplasm of a myelinating Schwann cell (**a**). These bodies are composed of fusiform aggregates of bilaminar subunits (**b**) and may contain lipid droplets at the poles (**a**, 11,440×; **b**, 35,000×)

that the genetic defect is responsible for entry of very long-chain fatty acids into peroxisomes for degradation (Jang et al. 2011). Diagnosis now can be performed by genetic analysis rather than measurement of long-chain fatty acid content or biopsy. The phenotypic variability of the disease within families remains unexplained, although the presence of an autosomal modifier gene locus has been suggested (Moser et al. 1992).

It is unclear how the disease manifestations are caused. Direct toxicity of the storage material has been postulated and seems to be particularly relevant to adrenal cell damage (Moser and Moser 1989). Therapeutic trials which lower serum VLCFA show an improvement in nerve conductions, but even this was not impressive (Moser et al. 1992). There is no correlation between plasma VLCFA levels and disease severity either before or after treatment (Moser and Moser 1989; Aubourg et al. 1993). However, part of the issue may be the age at which treatment is begun. Young asymptomatic boys (normal MRI, < 6 years old) may respond to a degree to dietary treatment with "Lorenzo's oil" by decreasing serum long-chain fatty acids. Bone marrow transplantation results in reversal of early neurologic and neuroradiologic features of ALD (Aubourg et al. 1990). Recently, autologous $CD34^+$ cells were removed from two ALD patients lacking donor matches, genetically corrected ex vivo with a lentiviral vector encoding wild-type ABCD1, and then reinfused into the patients after they had received myeloablative treatment (Cartier et al. 2009). Over 24–30 months of follow-up, a population of granulocytes, monocytes, and T and B lymphocytes expressed the normal ALD protein, suggesting transduction of hematopoietic stem cells, and the patients maintained a stable MRI. Peripheral nerve structure or function was not examined in this study.

Myelin from patients with ALD may contain an increased proportion of cholesterol esters (Brown et al. 1983). The prominent inflammatory infiltrate seen in the CNS of patients with ALD has suggested that immune mechanisms play a role, but immunosuppressive therapy has been unhelpful (Griffin et al. 1985; Moser and Moser 1989) for ALD. Inflammation does not seem to be important in the peripheral nerve lesion of ALD. It is currently thought that

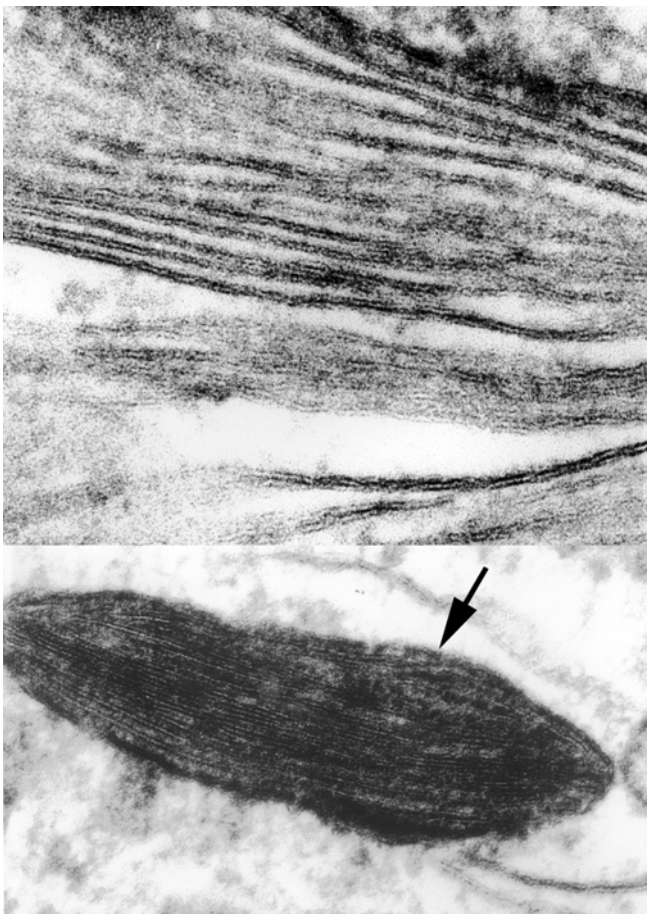

Fig. 20.27 Adrenomyeloneuropathy: isolated elongated bilaminar profiles are associated with a fusiform striated cytoplasmic body (*arrow*) in macrophage cytoplasm (48,300×)

abnormal oxidative homeostasis, perhaps induced by VLCFA, may initiate axonal degeneration in ALD/AMN (Faust et al. 2010). The atrophy of DRG neurons and posterior column degeneration in AMN patients correlates with the strong expression of ABCD1 in these neurons (Faust et al. 2010).

20.3 Neuronal Ceroid Lipofuscinoses (Batten–Kufs Disease)

20.3.1 Clinical Manifestations

Commonly referred to as the neuronal ceroid lipofuscinoses (NCL; see Table 20.2 for MIM gene and phenotype numbers), the Batten–Kufs group of diseases are clinicopathological entities whose common denominator is the intralysosomal accumulation of autofluorescent material reminiscent of lipofuscin storage in the aging brain (Carpenter et al. 1977). The incidence of NCLs varies depending on geographical location, with prevalence estimates ranging from 1 per 1,000,000 in some regions to 1 in 100,000 in Scandinavian countries (Mole et al. 2011; Boustany 2013). The various NCLs, now including 10–14 distinct genetic NCL variants (Table 20.2, see Boustany 2013; Haltia and Goebel 2013 for a comprehensive review), are characterized by electron-dense ultrastructural features that are unique to each disorder, result from mutations in a group of genes, represent storage of a variety of substances, and present in a number of age-related patterns (Haltia and Goebel 2013). The NCLs are caused by defects in "ceroid lipofuscinosis, neuronal" (CLN) genes (Table 20.2, Boustany 2013; Cotman et al. 2013), the first identified in 1995 with characterization of defects in the endosomal–Golgi membrane protein CLN3 in the juvenile-onset pattern. NCLs are among the most common pediatric neurodegenerative diseases and are inherited in an autosomal recessive manner with few exceptions, although adult patients may have an autosomal dominant pattern of inheritance (Jarvela et al. 1992; Wisniewski et al. 1992). The clinical picture depends on the age of onset and is reasonably constant within a family (Wisniewski et al. 1992). Patients with symptom onset before the age of 10, previously subdivided into infantile (Haltia–Santavuori), late infantile (Jansky–Bielschowsky), and juvenile (Spielmeyer–Vogt) groups, were characterized by epilepsy, myoclonus, and progressive cerebral and retinal degeneration. Patients with later onset, i.e., Kufs disease, do not demonstrate retinal degeneration and may present with progressive myoclonic epilepsy or a dementing disease with extrapyramidal or cerebellar motor manifestations (Berkovic et al. 1988).

Peripheral neuropathy is not a significant part of the disease spectrum, and nerve conduction studies are normal or minimally abnormal, such changes being difficult to divorce from the effects of anticonvulsants or chronic illness (Joosten et al. 1973; Dom et al. 1979; Blatt-Lyon 1975).

20.3.2 Pathology

20.3.2.1 General Considerations

The pathology of Batten–Kufs group of diseases has been studied extensively (Carpenter et al. 1972, 1977; Berkovic et al. 1988; Wisniewski et al. 1992; Boustany 2013; Haltia and Goebel 2013). Tissue examination remains an important diagnostic tool, although molecular genetic analysis has become the most critical separation of subtypes. Electron microscopic examinations of skin, conjunctiva, and peripheral blood buffy coat are the least invasive and are most informative diagnostic procedures (Carpenter et al. 1977;

Table 20.2 Human neuronal ceroid lipofuscinoses variants

Disease	Eponym	OMIM	Clinical phenotype	Gene	Gene product
CLN1	Haltia–Santavuori	256730	Classic infantile, late infantile, juvenile, adult[a]	*CLN1* (*PPT1*)	PPT-1
CLN2	Janský–Bielschowsky	204500	Classic late infantile, juvenile[a]	*CLN2* (*TPP1*)	TPP-1
CLN3	Spielmeyer–Sjögren	204200	Juvenile[a]	*CLN3*	CLN3 protein (battenin)
CLN4	Parry	162350	Adult autosomal dominant[a]	*CLN4* (*DNAJC5*)	DnaJ homologue subfamily C member 5193
CLN5	Finnish variant late infantile, variant juvenile (previously CLN9)	256731	Late infantile variant, juvenile, adult[a]	*CLN5*	Protein CLN5
CLN6	Early juvenile (Lake Cavanaugh), late infantile Costa Rican–Indian variant, adult Kufs type A	601780	Late infantile variant, adult[a] (Kufs, type A)[a]	*CLN6*	Protein CLN6
CLN7	Turkish variant late infantile	610951	Late infantile variant[a], juvenile[a], adult[a]	*CLN7* (*MFSD8*)	Major facilitator superfamily domain-containing protein 8
CLN8	Northern epilepsy, progressive EPMR	610003	Late infantile variant EPMR[a]	*CLN8*	Protein CLN8
CLN10	Congenital	610127	Congenital classic[a], late infantile[a], adult[a]	*CLN10* (*CTSD*)	Cathepsin D
CLN11	Adult variant	–	Adult[a]	*CLN11* (*GRN*)	Progranulin194
CLN12	Juvenile variant	–	Juvenile, Kufor–Rakeb syndrome[a]	*CLN12* (*ATP13A2*)	–
CLN13	Adult Kufs type B	–	Adult Kufs type[a]	*CLN13* (*CTSF*)	Cathepsin F
CLN14	Infantile	–	Infantile, progressive myoclonus epilepsy 3[a]	*CLN14* (*KCTD7*)	Potassium channel tetramerization domain-containing protein 7195

Credit: Reprinted with permission from Boustany (2013)
EPMR epilepsy with mental retardation
[a]Diseases with neurological involvement

Goebel et al. 1976; Wisniewski et al. 1992; Dolman et al. 1975). Neuron-containing tissues, i.e., rectal or brain biopsy, are also likely to have a high yield (Martin 1991; Goebel et al. 1976) but may be complicated by age changes in adults. The storage material demonstrates autofluorescence and strong staining with lipid and carbohydrate stains, characteristics shared by lipofuscin and ceroid (age-related pigment accumulation). However, ultrastructural examination reveals a variety of inclusion morphologies not at all like the granular and amorphous appearance of lipofuscin, although variable amounts of the latter may also be seen.

The inclusions containing cytosomes of Batten–Kufs disease are typically 1–2 μm in size, and a number of ultrastructural varieties have been delineated (Figs. 20.28 and 20.29). "Curvilinear profiles" have been defined as "curved stacks of lamellae showing alternate dark and pale lines, with the dark lines being 35–40 A and the pale lines 40–45 A in width. The stacks of lamellae generally form arcs or semicircles. The number of pale lines in a stack varies between 2 and 6" (Carpenter et al. 1977). Accumulations of these profiles are contained within a single limiting membrane (Fig. 20.28). When the profiles are straight but otherwise have same properties, they have been designated "rectilinear profiles" (Carpenter et al. 1977). "Fingerprint profiles" (Fig. 20.29) are defined as structures "… formed from groups of parallel paired dense lines, each pair of lines being separated by a thin lucent space about 15 A wide. The dense lines each measure about 23 A, and each pair of dense lines is separated from its neighboring pair by a space of 25 A. The lines may be straight or curved. In some projections the pairs of lines intersect to produce a honeycomb-like pattern or a hexagonal lattice" (Carpenter et al. 1977). As with curvilinear profiles, groups of fingerprint bodies are seen within membrane-bound cytosomes, and indeed the two types of inclusion may be intermingled in a single cytosome (Fig. 20.28). Another type of inclusion shows osmiophilic granular nonperiodic contents (Carpenter et al. 1977).

The various inclusions are seen in numerous cell types including neurons, Schwann cells, fibroblasts, endothelial cells, skeletal muscles cells, and eccrine sweat glands. The nervous and visceral organ distribution of the variety of inclusion types is complex and beyond the scope of this book (Berkovic et al. 1988; Carpenter et al. 1977; Boustany 2013). There is a strong but not absolute correlation between the type of inclusion and the disease subset (Carpenter et al.

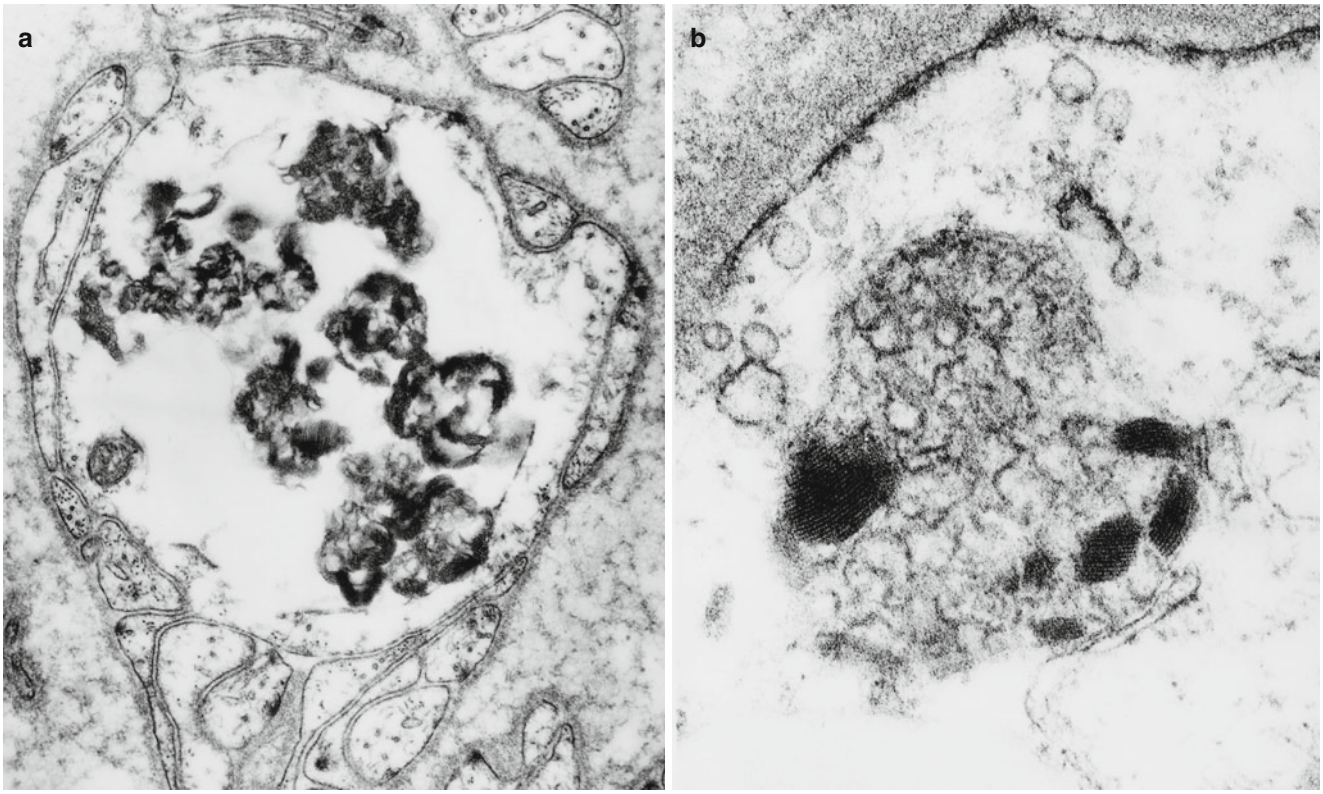

Fig. 20.28 Batten disease, onset age 7, death age 35. Nonmyelinating Schwann cells contain curvilinear and fingerprint profiles (**a**, 27,300×; **b**, 64,220×) (Tissue courtesy of Dr. J. Maguire)

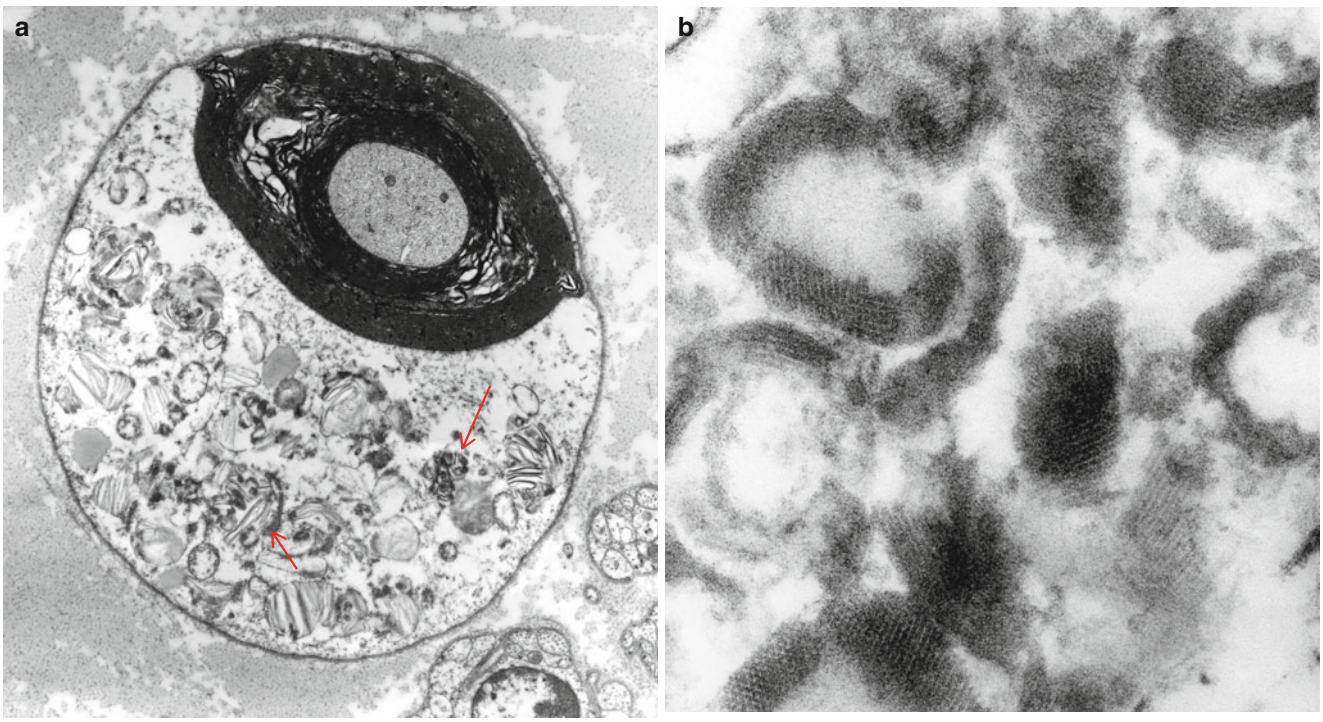

Fig. 20.29 Batten disease, same case as Fig. 20.28. Typical fingerprint profiles (*arrows*, **a**) are mingled with Pi bodies in Schwann cell cytoplasm. The pathognomonic appearance of fingerprint profiles is shown in high magnification (Magnification: **a**, 16,640×; **b**, 12,6040×)

1977; Wisniewski et al. 1992; Haltia et al. 1973). More recently, Goebel and Müller (2013) describe 3 patterns of NCL lipopigments: mixed or separate granular (infantile NCL or CLN1), curvilinear (late infantile NCL or CLN2), fingerprint (classic juvenile NCL or CLN3 type), and mixed patterns (CLN5–CLN8). Granular osmiophilic deposits are strongly associated with the infantile variety of the disease, curvilinear profiles with the late infantile group, and fingerprint bodies with the juvenile-onset group. In the adult-onset group (Kufs disease) fingerprint and curvilinear inclusions are usually present, but some may represent a more complex pattern (Josephson et al. 2001).

20.3.2.2 Role of Nerve Biopsy

The sensitivity of nerve biopsy relative to muscle or skin biopsy has not been formally assessed, but the substantial literature on the latter two compared with the paucity of reports on the former suggests that nerve biopsy is an inferior approach to diagnosis. However, if investigating a neurodegenerative process of unknown etiology, nerve biopsy may be more useful as it is abnormal in a wide range of diseases that may share the clinical picture of Batten–Kufs disease, as discussed elsewhere in this chapter.

Autopsy or biopsy examination of peripheral nerve material has been reported in Kufs disease (Berkovic et al. 1988; Dom et al. 1979; Josephson et al. 2001) and in the early infantile (Anzil et al. 1975; Haltia et al. 1973), late infantile, and juvenile variants of Batten disease (Goebel et al. 1976; Joosten et al. 1973; Carpenter et al. 1972; Towfighi et al. 1973). A few other cases have been reported as Batten–Kufs disease, but had atypical features or were incompletely studied, leading to uncertainty about the diagnosis (Vallat et al. 1985; Kristensson et al. 1967). In some subtypes there is not enough material to allow generalization regarding the type and localization of inclusions in peripheral nerve.

20.3.2.3 Peripheral Nerve Pathology

Most often there is no alteration in the nerve fiber population (Goebel et al. 1976; Joosten et al. 1973). Mild Wallerian degeneration was noted in one case of Kufs disease, but the significance of this is uncertain in a debilitated patient (Berkovic et al. 1988). There are reports of peripheral nerve biopsies with no abnormalities, even with thorough ultrastructural examination (Berkovic et al. 1988; Towfighi et al. 1973; Dom et al. 1979), but the proportion of nerve biopsies showing diagnostic changes in Batten–Kufs disease is unknown. Autofluorescent material was not observed in most cases.

Ultrastructurally, curvilinear profiles and fingerprint bodies were observed in peripheral nerve biopsies and autopsy material from the juvenile and late infantile cases (Carpenter et al. 1972; Joosten et al. 1973; Goebel et al. 1976; Towfighi et al. 1973) and granular osmiophilic deposits in early infantile cases (Anzil et al. 1975; Haltia et al. 1973). Myelinating and nonmyelinating Schwann cells appear to show the most prominent changes. Endothelial cells, macrophages, perineurial cells, and smooth muscle cells also show the inclusions, but axons do not (Carpenter et al. 1972). In Kufs disease nerve biopsy is unlikely to reveal characteristic inclusions (Dom et al. 1979; Berkovic et al. 1988); the report of Vallat et al. (1985) which showed atypical inclusions in Schwann cells was not accepted as Kufs disease in a subsequent literature review (Berkovic et al. 1988). Pi granules (or Pi granule-like inclusions) were thought to be numerous in some reports (Goebel et al. 1976).

20.3.2.4 Pathogenesis

The subtypes of NCL are genetically distinct (Yan et al. 1992; Boustany 2013), with the gene defects causing different forms located on a variety of chromosomes and with different gene products (Table 20.2) (Yan et al. 1992; Hall et al. 1991; Goebel 1995; Boustany 2013; Haltia and Goebel 2013). The storage material consists mostly of insoluble protein and 20–40 % lipid (Hall et al. 1991; Martin 1991). Defects in endosomal–Golgi membrane proteins, the soluble lysosomal proteins palmitoyl protein thioesterase 1 (PPT1) and tripeptidyl peptidase 1 (TPP1), cathepsins D and F, and ER-resident proteins have been established (Jalanko and Braulke 2009; Boustany 2013). Mouse models of NCL have been developed (Shacka 2012) and development of therapeutic methods are following the multifaceted approach of lysosomal storage disorders generally (Pierret et al. 2008).

20.4 Tangier Disease (Analphalipoproteinemia)

20.4.1 Clinical Manifestations

Tangier disease is a very rare autosomal recessive genetic disorder (MIM#205400) characterized by a severe high-density lipoprotein (HDL) deficiency, sterol deposition in tissue macrophages, and atherosclerosis. Mutations in the ATP-binding cassette transporter ABCA1 cause Tangier disease and other familial HDL deficiencies (Rust et al. 1999; Oram 2000). Peripheral nerve disease is the principal cause of morbidity and is clinically evident in at least half of patients, onset occurring in the second to seventh decade (Pollock et al. 1983). The neuropathy is protean, with sensory and motor findings of acute or chronic onset, relapsing or progressive course, proximal or distal predominance, and a symmetrical or asymmetric pattern (Fazio et al. 1993). In some cases there is relative sparing of large fiber sensation presenting in upper limbs resulting in a

pseudosyringomyelic picture and in others a Lewis–Sumner-like type of CIDP (Zyss et al. 2012). Facial palsy is often present. The pathognomonic orange-gray tonsils are a result of cholesterol ester accumulation in reticuloendothelial cells of this lymphoid tissue. Electrophysiological studies usually show axonal features, but some reports document a reduction in conduction velocities. The diagnosis of Tangier disease is suggested by the finding of hypocholesterolemia and normal or elevated serum lipids and confirmed when further study of the serum lipid profiles indicates absence of HDL and lipoprotein electrophoresis reveals absent or extremely low apo-A1 levels and an increase in the pro-apo A-1 fraction or ABCA1 mutation (Assmann et al. 1989; Rust et al. 1999).

20.4.2 Pathology

Tangier disease is rare and with its highly variable clinical picture may not be suspected clinically. Nerve biopsy can be highly suggestive of the diagnosis. A number of detailed morphologic and morphometric studies have been performed (Antoine et al. 1991; Fazio et al. 1993; Kocen et al. 1973; Marbini et al. 1985; Dyck et al. 1978; Pollock et al. 1983; Pietrini et al. 1985; Engel et al. 1967; Gibbels et al. 1985; Zyss et al. 2012). Skin biopsy may be an adequate and less invasive means of morphologic diagnosis (Gibbels et al. 1985).

20.4.2.1 Light Microscopy

It is worthy of mention that no lipid accumulation in peripheral nerve was seen in the initial report emphasizing neuropathy in Tangier disease (Engel et al. 1967). However, vacuolation of Schwann cells, endothelial cells, and pericytes can usually be observed light microscopically, even in cases where there is little or no abnormality of axons or myelin (Dyck et al. 1978; Pietrini et al. 1985; Zyss et al. 2012). Routine preparation dissolves the lipid storage material, but with fresh frozen sections the storage material stains bright red with Oil Red O.

Axonal loss of variable severity is usually observed and may be devastating in the most severely affected cases. The changes are chronic, and the prominence of regenerative activity has varied between reports (Marbini et al. 1985). Active and focal axonal dropout was noted in one description of nerve biopsy in a rapidly evolving neuropathy (Fazio et al. 1993). Unmyelinated fibers are also diminished in number. Evidence of a demyelinating component to the disease is less frequently seen, but has been commented upon by several authors, with some thinly myelinated or even naked axons and rudimentary onion-bulb formations and hypermyelinated fibers (Pollock et al. 1983; Fazio et al. 1993; Marbini et al. 1985; Gibbels et al. 1985).

There is a good correlation between the clinical disease and the nerve biopsy findings. Thus, in cases where the hands have been severely involved but the legs spared, sural nerve biopsy has shown little or no abnormality of axons and myelin (Dyck et al. 1978; Pietrini et al. 1985). Patients with a pseudosyringomyelic neuropathy show an axonal process, with variable regenerative activity, and unmyelinated and small myelinated fibers are affected relatively more severely than large myelinated fibers (Antoine et al. 1991; Dyck et al. 1978; Kocen et al. 1973; Schmalbruch et al. 1987). However, Gibbels et al. (1985) studied a case of pseudosyringomyelic neuropathy where large MFs, small MFs, and UFs were proportionately depleted. Patients with a multifocal or relapsing neuropathy show a mild nonselective axonal loss with prominent segmental myelin change, but the nonrandom pattern of demyelination/remyelination suggests that it might be secondary to an axonal influence (Pollock et al. 1983). In one biopsied case of a chronic progressive neuropathy, a mixed axonal/demyelinating picture with regenerating clusters and onion-bulb formations was seen. There was no attempt made to determine whether the myelin changes were primary or secondary (Marbini et al. 1985).

20.4.2.2 Electron Microscopy

Ultrastructural examination reveals cytoplasmic round or elliptic storage vacuoles 0.5–3 μm in diameter, appearing as an electron-lucent space presumably due to removal of lipid content during tissue preparation. Authors have variably described the inclusions as either membrane bound (Dyck et al. 1978), not membrane bound (Gibbels et al. 1985), or both (Pollock et al. 1983). The vacuoles have frequently been described as most numerous in denervated Schwann cells (Antoine et al. 1991; Dyck et al. 1978). Indeed, Dyck and colleagues (1978) noted that in their case the number of lipid vacuoles in Schwann cells with an intact myelinated axon did not differ from control. However, other workers have described lipid vacuoles in intact axon–Schwann cell structures, especially nonmyelinating Schwann cells (Pollock et al. 1983), less often in myelinating Schwann cells, and also in pericytes, perineurial, and endothelial cells (Fazio et al. 1993; Gibbels et al. 1985; Marbini et al. 1985). Up to 25–50 % of nonmyelinating Schwann cells may show one or more cytoplasmic vacuoles (Pollock et al. 1983). Gibbels et al. (1985) also commented on the prominence of lipofuscin-like material in nonmyelinating Schwann cells and on the presence of electron-dense amorphous or lamellated material in

myelinating Schwann cells (Gibbels et al. 1985). Rarely, vacuolated macrophages may be observed, often in a perivascular distribution.

In some reports a very severe depletion of unmyelinated fibers has been observed, these instances corresponding with the pseudosyringomyelic clinical pattern (Antoine et al. 1991; Kocen et al. 1973; Dyck et al. 1978; Gibbels et al. 1985). Nonspecific changes of axonal degeneration such as mitochondrial enlargement or filament and membranous organelle accumulations may be seen.

20.4.3 Pathogenesis

Dyck et al. (1978) noted a correlation between loss of axons and presence of lipid globules, suggesting that the storage material was a consequence, not the cause, of the neuropathy. A failure of the normal mechanisms for debris removal following Wallerian degeneration was postulated (Dyck et al. 1978). However, other authors have failed to confirm this observation (Marbini et al. 1985; Gibbels et al. 1985). Some workers have commented on the multifocal nature of the disease, microvascular involvement, and evidence of focal axonal damage, postulating that ischemia somehow underlies the neuropathy (Fazio et al. 1993; Marbini et al. 1985). Autopsy studies in two cases with the pseudosyringomyelic variant have demonstrated inclusion material in anterior horn and DRG neurons, suggesting that at least in these cases the disease may be a neuronopathy, although the precise nature of the lipid accumulations did not appear to be the same in these two reports (Antoine et al. 1991; Schmalbruch et al. 1987).

More recent investigation has proposed that the basis of Tangier disease and the associated neuropathy arises from a mutation in the ATP-binding cassette 1 gene (ABCA1). ABCA1 controls a cellular pathway that secretes cholesterol and phospholipids to lipid-poor apolipoproteins. The defect in Tangier disease supports the hypothesis that newly synthesized apolipoproteins do not acquire cellular lipids by the ABCA1 pathway, resulting in their rapid degradation and formation of an excess of macrophage cholesterol. ABCA1 modulates cholesterol and phospholipid flux into the reverse cholesterol transport pathway, which is critical for the clearance of excess cholesterol from macrophages and preventing atherosclerosis (Oram 2000; Iatan et al. 2012). Recent evidence suggests paranodal malfunction due to cholesterol ester accumulation in Schwann cells and lipid accumulation in dorsal roots and sensory nerves (Cai et al. 2006) supporting a mechanism of primary neuronopathy and secondary axonal degeneration (Zyss et al. 2012).

20.5 Cerebrotendinous Xanthomatosis (Cholestanosis)

20.5.1 Clinical Manifestations

Cerebrotendinous xanthomatosis (CTX) is a rare autosomal recessive disease (MIM#213700) characterized by a progressive spinocerebellar syndrome, dementia, and tendon xanthomata, with onset usually in childhood and slow progression to adult life (Bjorkhem and Skrede 1989). Xanthomata may be absent (van Hellenberg-Hubar et al. 1992). Peripheral neuropathy varies from minor changes to serious polyneuropathy (Wang et al. 2007; Pilo et al. 2011). Conduction velocities have been slowed, suggesting a significant demyelinating component (Argov et al. 1986; Kuritsky et al. 1979; van Hellenberg-Hubar et al. 1992). The diagnosis is usually clinically obvious, and confirmation is provided by detection of high cholestanol levels in plasma, bile, feces, and urine or identification of the gene defect. Treatment with chenodeoxycholic acid or with HMG-CoA reductase inhibitors lowers blood cholestanol levels, stops progression, and may substantially reverse the disease (Salen et al. 1991; Keren and Falik-Zaccai 2009).

20.5.2 Pathology

Nerve biopsies (Ohnishi et al. 1979; Argov et al. 1986; Donaghy et al. 1990; Pop et al. 1984; Pilo et al. 2011) have shown a variably severe and chronic reduction in fiber density, large MFs being most affected. Unmyelinated fibers have been spared or only slightly reduced in number. Axonal regenerative activity may be seen. Most biopsy reports have emphasized segmental demyelination with remyelination, as evidenced both on teased fiber studies and through the observation of onion-bulb formations, ranging from very prominent (Argov et al. 1986) to minimal (Donaghy et al. 1990; Pop et al. 1984). Wang et al. (2007) have classified the polyneuropathy in CTX into three pathological types: (1) axonal polyneuropathy, in which axonal degeneration and regenerative clusters of myelinated fibers are the main pathological changes; (2) demyelinating polyneuropathy, characterized by profuse demyelination, thin myelin sheaths, and onion bulbs of myelinated fibers; and (3) mixed polyneuropathy, in which both axon and myelin are equally involved. There are no characteristic inclusions and lipid deposition is usually not seen in nerve, although one report describes small membrane-bound clear spaces in Schwann cell cytoplasm of myelinated and unmyelinated fibers (Donaghy et al. 1990).

20.5.3 Pathogenesis

CTX is caused by a defect in bile acid synthesis and resultant accumulation of substrates which are diverted into the formation of cholestanol, with deposition of this and cholesterol in various tissues throughout the body. Absence of activity of mitochondrial sterol 27-hydroxylase, encoded on human chromosome 2, correlates with the presence of the disease, and mutations in *CYP27A1* gene, which encodes sterol 27-hydroxylase, have been identified (Leitersdorf et al. 1993; Meiner et al. 1994; Gallus et al. 2006).

20.6 Other Storage Diseases

20.6.1 The Mucopolysaccharidoses

20.6.1.1 Clinical Manifestations

Disorders resulting from identifiable defects in degradation of glycosaminoglycans are categorized as mucopolysaccharidoses (MPS). There are many clinical subtypes and a review is provided by Neufeld and Meunzer (1989). Characteristic features are short stature, skeletal deformities, coarse features, and variably severe CNS manifestations. Peripheral polyneuropathy generally does not occur, with the exception of focal entrapment neuropathies due to connective tissue proliferation or inanition (Neufeld and Meunzer 1989; Karpati et al. 1974; Swift and McDonald 1976). A few peripheral nerve biopsy and autopsy studies have been reported in MPS: type I Hurler (Jellinger et al. 1990); type II, Hunter (Swift and McDonald 1976; Schmitt 1981; Karpati et al. 1974); and type III Sanfilipo (Bischoff 1979; Martin et al. 1979) MPS. In one instance a patient presented with multiple entrapment neuropathies; and the diagnosis of MPS was not suspected until characteristic inclusions were seen on nerve biopsy (Karpati et al. 1974).

20.6.1.2 Pathology

Nerve biopsies usually reveal a normal MF and UF population, mirroring the fact that peripheral neuropathy is not a significant aspect of the disease spectrum (Karpati et al. 1974; Swift and McDonald 1976; Schmitt 1981). Ultrastructurally, the characteristic inclusion of the MPS group is the "zebra body," a single membrane-bound cytosome with alternating electron-lucent and electron-dense areas, the latter made up of a few lamellae with a regular periodicity. These are most prominently seen in Schwann cells of myelinated fibers, but can also be seen in fibroblasts and perineurial cells. Dense granular inclusions were described by Swift and McDonald (1976) in paranodal Schwann cell cytoplasm. Vacuolated inclusions which may contain small amounts of dispersed granular or fibrillar material can be seen in fibroblasts and perineurial cells. The scanty information available does not suggest that there is any means of distinguishing types I, II, and III MPS on nerve biopsy. Skin biopsy shows similar inclusions (O'Brien et al. 1975).

Electron-lucent vacuoles have been described in nonmyelinating Schwann cells, fibroblasts, and pericytes of peripheral nerve in I-cell disease (mucolipidosis II) (Martin et al. 1975).

20.6.2 Glycogen Storage Diseases

Peripheral neuropathy is not a significant manifestation of the glycogen storage diseases. *Pompe disease* is an autosomal recessive disorder caused by a defect in gene encoding acid alpha-1,4-glucosidase (GAA; MIM#606800), also known as acid maltase, which maps to chromosome 17. Nerve biopsy in Pompe disease has not revealed any morphometric or morphologic evidence of fiber loss, although mild-nonspecific axoplasmic alterations were emphasized by Goebel et al. (1977). Ultrastructural examination has shown accumulations of glycogen in all endoneurial cells, especially Schwann cells (Araoz et al. 1974; Goebel et al. 1977; Vital and Vallat 1987, Fig. 266; de Martin et al. 1976). Axonal glycogen accumulations are also seen, but less prominently than in Schwann cells, and are nonspecific. The stored glycogen may be membrane bound or free in the cytoplasm. Goebel et al. (1977) suggested that the co-occurrence of glycogen and lipofuscin in a single lysosome may be a finding specific to Pompe disease.

Peripheral neuropathy may be seen in *type III glycogenosis* (*Cori–Forbes disease*, *MIM#232400*) (Ugawa et al. 1986) caused by homozygous or compound heterozygous mutation in the gene (*AGL*) on chromosome 1p21 encoding the glycogen debrancher enzyme, inherited in an autosomal recessive pattern. In a sural nerve biopsy Ugawa et al. (1986) observed moderate–severe axonal dropout and rare massively swollen axons filled with glycogen. Glycogen accumulation was present in all endoneurial cell types (Ugawa et al. 1986). Muscle biopsy has revealed prominent glycogen accumulation in Schwann cells of unmyelinated fibers in intramuscular nerves (Powell et al. 1985; Pellissier et al. 1979), although dermal nerve fibers were said to be unremarkable in another report (Sancho et al. 1990).

Type IV glycogenosis (*glycogen brancher deficiency*, *Andersen disease*, *MIM#232500*) is caused by mutation in the gene encoding the glycogen branching enzyme (*GBE1*; MIM#607839). Polyglucosan bodies accumulate in various cells, including axons (Schroder et al. 1993). The histologic differential diagnosis includes adult polyglucosan body disease and Lafora disease (Chap. 21).

20.6.3 Sialidosis

Sialidosis I (MIM#256550) and II are autosomal recessive multisystem storage diseases caused by mutation in the gene encoding neuraminidase (NEU1) resulting in a defect in glycoprotein specific α-neuraminidase (Beaudet and Thomas 1989; Federico et al. 1991b). Type I sialidosis is characterized by nonpigmentary retinal degeneration with a cherry red macula and a progressive myoclonic epilepsy syndrome. In type II sialidosis coarse facies and bony abnormalities are present, and impaired β-galactosidase activity is found along with α-neuraminidase deficiency (Beaudet and Thomas 1989). Peripheral neuropathy is not usually part of the picture, but has been documented in both type I (Steinman et al. 1980) and type II (Miyatake et al. 1979) sialidosis. The diagnosis is confirmed by demonstration of the gene defect or measurement of neuraminidase activity in fresh fibroblasts or leukocytes (Beaudet and Thomas 1989).

Storage material is found in a wide variety of tissues including circulating lymphocytes (Beaudet and Thomas 1989; Miyatake et al. 1979). Examination of the sural nerve in type I sialidosis has shown a mixed picture of axonal dropout and segmental demyelination and remyelination (Steinman et al. 1980). Light microcopy on toluidine blue semi-thin sections revealed Schwann cell vacuoles, which on EM were seen as vacuolated membrane-bound spaces occasionally containing membranous and globular electron-dense material (Steinman et al. 1980) The storage material in this report was said to, at times, show a resemblance to "tuffstone bodies" of metachromatic leukodystrophy. In type II sialidosis no sural nerve abnormalities were seen on light microscopy, but the ultrastructural examination revealed vacuoles containing a fine granular material in Schwann cells and fibroblasts (Miyatake et al. 1979).

20.6.4 Wolman Disease

This is a rare (<1/100,000 live births) autosomal recessive disorder (MIM#278000) of neutral lipid metabolism where lipids and cholesterol accumulate in a variety of tissues (Byrd and Power 1979; Fasano et al. 2012). The defect is produced by mutation in the *lysosomal acid lipase A* (*LIPA*) *gene* on chromosome 10q24-q25 and produces two distinct disease phenotypes: Wolman disease and cholesteryl ester storage disease (CESD), the latter caused by the complete or partial deficiency of lysosomal acidic lipase (LAL), respectively. LAL is required for the lysosomal hydrolysis of cholesteryl esters and triglycerides that cells acquire through the receptor-mediated endocytosis of low-density plasma lipoproteins resulting in a failure of hydrolysis and buildup of undigested triglycerides and cholesteryl esters in the cell (Anderson et al. 1993). Lysosomal acid lipase deficiency impairs regulation of the ABCA1 gene and formation of high-density lipoproteins in cholesteryl ester storage disease (Bowden et al. 2011). If enzyme activity is very low or absent, clinical presentation is in infancy with failure to thrive, intestinal malabsorption, hepatosplenomegaly, and early death (Wolman disease), but with higher but still low enzyme activity, presentation is later in life with hepatic fibrosis, dyslipidemia, and early atherosclerosis (CESD, Reynolds 2013).

Targeted disruption of the mouse lysosomal acid lipase gene (Du et al. 1998) has produced a mouse model of human CESD and Wolman disease. Enzyme supplementation of LAL by repeated injection LAL protein (Du et al. 2001) and adenovirus-mediated *LIPA* gene transfer in mice (Du et al. 2002) has been shown to diminish stored triglycerides and cholesteryl esters.

Sudanophilic lipid droplets accumulate in neurons, glia, endothelial cells, and histiocytes. Peripheral nerve has shown similar lipid inclusions most prominently in the perinuclear region of myelinating Schwann cells, but also in endothelial cells, nonmyelinating Schwann cells, and perineurial cells (Byrd and Power 1979). In an earlier report no abnormalities were observed on light microscopic examination (Guazzi et al. 1968).

20.7 Aspects of Differential Diagnosis in the Storage Diseases

Reading of this chapter reveals a bewildering array of inclusions that can be seen in various storage diseases. Notwithstanding the fact that clinical manifestations, biochemical tests, and genetic analysis can resolve differential diagnostic issues in nearly all instances, a brief tabulation is provided below of the storage diseases and their histologic features (Tables 20.3 and 20.4). Certain inclusions have ultrastructural features that are pathognomonic of their respective disease, such as the Tuffstone bodies or prismatic inclusions of MLD, the curvilinear profiles of Batten–Kufs disease, or the trilaminar linear bodies of ALD. Fingerprint bodies are strongly associated with neuronal ceroid lipofuscinosis, but have been described in GM1 and GM2 gangliosidosis and mucopolysaccharidosis (Goebel et al. 1981; Goebel and Braak 1989).

Lipid storage may also take an entirely nonspecific appearance such as lamellated inclusions, empty vacuoles, or cytosomes with osmiophilic granular/amorphous contents. Identification of the cell types involved in the storage process is helpful in differential diagnosis. It is also important to be familiar with normal structures such as Reich Pi granules and lipofuscin accumulation, so as not to diagnose a storage

Table 20.3 Polyneuropathies in storage diseases

Neuropathies with demyelination
Metachromatic leukodystrophy
Globoid cell leukodystrophy
Adrenomyeloneuropathy
Niemann–Pick disease (type 1)
Cerebrotendinous xanthomatosis
Tangier disease (some cases)
Type 1 sialidosis
Axonal neuropathies
Fabry disease
Tangier disease (some cases)
GM1 and GM2 gangliosidosis
Niemann–Pick disease (type 2)
Type 3 glycogenosis

Table 20.4 Differential diagnosis of storage inclusions

Osmiophilic inclusions with periodicity
Metachromatic leukodystrophy (msc, nmsc, mp≫en, ax)
Fabry disease (en, pc, sm, pn>ax, nmsc)
Niemann–Pick disease type A (all except ax)
Batten–Kufs disease (msc, nmsc, en, pc, fb)
Zebra bodies
Metachromatic leukodystrophy (msc, nmsc, mp)
Mucopolysaccharidosis (msc, fb)
Fabry disease (en, pc, sm, pn)
Niemann–Pick disease (msc, nmsc, en, mp)
Pi granules (msc, nmsc)
? GM1 gangliosidosis (ax)
? Farber disease (en)
Membrane-bound clefts or polygonal spaces
Krabbe disease (msc, nmsc, mp)
Adrenoleukodystrophy (msc, umsc, mp)
Farber disease (msc > nmsc, mp)
Empty vacuoles
Tangier disease (nmsc≫other cell type, not in axon)
GM1 gangliosidosis (en, pc, pn, fb))
I-cell disease (nmsc, fb, pn)
Mucopolysaccharidoses (fb, pn, sc, en, pc)
Cerebrotendinous xanthomatosis (msc, nmsc)
Wolman disease (? All except ax)
Sialidosis type 1 (sc)

msc myelinating Schwann cell, *nmsc* nonmyelinating Schwann cell, *ax* axon, *en* endothelial cell, *fb* fibroblast, *mp* macrophage, *pn* perineurial cell, *pc* pericyte, *sm* smooth muscle cell
[?] inclusions may be very rare and/or ambiguous

disease where one does not exist. Since myelinating Schwann cells do not ordinarily accumulate significant amounts of lipopigment, such an observation suggests the possibility of a storage disease including Niemann–Pick, NCL, adrenoleukodystrophy, and Tangier disease. We have also detected lipofuscin in myelinating Schwann cells in a congenital dysmyelinating neuropathy.

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Unclassified Neuropathies and Mimics

21

Some polyneuropathies which have not been discussed elsewhere in this monograph deserve specific mention. Some of these are parts of clinical syndromes, while others are specific entities. Although mitochondrial diseases might well be considered to be "genetically determined," they are also considered here. Peripheral neuropathy resulting from a mitochondrial defect associated with MFN2 and GDAP1 mutations has been addressed as part of the CMT group of peripheral nerve disorders and will not be readdressed here.

21.1 Neuropathy in Mitochondrial Diseases

21.1.1 Clinical Manifestations

Mitochondrial diseases are diagnosed when a disturbance of number, structure, or function of mitochondria is identified in association with an appropriate clinical picture. The spectrum of these diseases is growing rapidly. Mitochondria contain 2–10 copies of a 16,569 base-pair ring which encodes 13 proteins involved in oxidative phosphorylation and the special transfer RNA and ribosomal RNA required for intramitochondrial protein synthesis. Mitochondrial diseases usually arise as a consequence of mitochondrial DNA (mtDNA) mutations, but may also result from defects in nuclear DNA related to mitochondrial function. Although mitochondrial diseases may directly result in neuropathy, it is important to recognize that polyneuropathy may reflect a secondary associated condition (e.g., diabetes, renal failure, hypothyroidism) which may be part of some mitochondrial disorders (Finsterer 2005). Finsterer's study (2005) of 108 patients with mitochondrial disease and polyneuropathy attributed 35 % directly to a primary mitochondrial pathogenesis.

Mitochondrial DNA defects are divided into deletions and point mutations (Zeviani and Antozzi 1992). Deletions are usually sporadic and typically manifest as a progressive external ophthalmoplegia (PEO) syndrome, either in isolation, with myopathy, or as the full Kearns–Sayre syndrome (KSS). Point mutations are usually inherited (typically maternal transmission) and are associated with a variety of clinical phenotypes including MELAS (myopathy, encephalopathy, lactic acidosis, stroke-like episodes), MERRF (myoclonus epilepsy with ragged red fibers), and Leber hereditary optic neuropathy. Peripheral neuropathy defines several mitochondrial syndromes: (1) NARP (neuropathy, ataxia, and retinitis pigmentosa) caused by a missense mutation in the mitochondrial ATPase gene (*MTATP6*, Holt et al. 1990), (2) MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) due to a thymidine phosphorylase (*TYMP*) gene defect, and (3) SANDO (sensory ataxic neuropathy, dysarthria, and ophthalmoparesis) thought, in some patients, to be caused by mutations in the mitochondrial polymerase γ (*POLG*) gene (Van Goethem et al. 2003). MNGIE is a particularly interesting neuropathy in which a nuclear-encoded defect in endothelial cell growth factor 1 (ECGF1) results in secondary mutations, deletions, and defects in mtDNA. Neuropathy has also been described in a variety of other mitochondrial syndromes, including MELAS, MERFF, PEO, and KSS (Nardin and Johns 2001). Leigh subacute necrotizing encephalopathy has been associated with several different mtDNA and nuclear DNA defects, illustrating the potential genetic heterogeneity of mitochondrial disease syndromes.

Neuropathy is present in 25–50 % of patients with most mitochondrial diseases (Eymard et al. 1991; Mizusawa et al. 1991; Yiannikas et al. 1986) and is usually mild, length dependent, with sensory alterations often predominating over motor deficits. Nerve conduction studies show axonal neuropathy, but demyelinating features have also been reported (Finsterer 2005). Peripheral neuropathy may occur (1) in mitochondrial disorders associated with defects in mitochondrial DNA maintenance or replication which may be caused by nuclear gene mutations (*POLG1*, *C10ORF2*, *TYMP*, and *MPV17*), (2) in the respiratory chain complexes particularly complex V associated with *MTATP6* mutations, and (3) in mitochondrial dynamics, i.e., the distribution of mitochondria by fission and fusion (*MFN2*, *GDAP1*

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mutations) (Pareyson et al. 2013). A sense of the current complexity of mitochondriopathy in association with peripheral neuropathy is provided by the recent review by Pareyson and colleagues (2013).

Recent studies using mutant mice lacking Schwann cell Tfam, which controls the transcription, replication, and stability of mtDNA, show neuropathy characterized by the depletion of mtDNA, mitochondrial morphologic abnormalities, failure to create a normal electron transport chain, and respiration deficiency (Viader et al. 2013). In this mouse model, neuropathy was characterized by a preferential loss of small unmyelinated C fibers, followed by that of large myelinated axons and, ultimately, extensive demyelination of residual axons (Viader et al. 2013).

21.1.2 Pathology

Many single cases and small series of nerve biopsies and autopsies have been recorded in mitochondrial diseases including PEO/myopathy (Drachman 1968; Gemignami et al. 1982; Gonatas 1967; Markesbery 1979; Mizusawa et al. 1991; Peyronnard et al. 1980; Pezeshkpour et al. 1987; Schroder and Sommer 1991; Yiannikas et al. 1986), Leigh disease (Goebel et al. 1986; Jacobs et al. 1990; Schroder and Sommer 1991), MERRF (Mizusawa et al. 1991; Sasaki et al. 1983), MELAS (Mizusawa et al. 1991; Nicoll et al. 1993; Schroder and Sommer 1991), and MNGIE (Bardosi et al. 1987; Hirano et al. 1994, 2004; Li et al. 1991; Simon et al. 1990; Steiner et al. 1987; Threkeld et al. 1992; Uncini et al. 1993). As discussed below, the histological picture is nonspecific and does not permit differentiation between the various mitochondrial diseases.

21.1.2.1 Light Microscopy

Nerve biopsies and autopsy reports in patients with PEO/myopathy syndrome have almost invariably revealed chronic depletion of myelinated fibers, the larger MFs usually being more severely affected. In one report, the study of myelin lamellar numbers vs. axonal diameter suggested the presence of axonal atrophy (Pezeshkpour et al. 1987). Regenerating clusters may be increased (Mizusawa et al. 1991; Peyronnard et al. 1980). Unmyelinated fibers are relatively spared, although observations such as increased numbers of denervated bands or collagen pockets suggest a mild involvement (Pezeshkpour et al. 1987; Yiannikas et al. 1986). Myelin alterations such as hypomyelination (Peyronnard et al. 1980), and segmental demyelination and remyelination with small onion-bulb (OB) formations (Mizusawa et al. 1991), have been frequently noted, but whether these are primary or secondary to axonal alterations has not been explored. The dramatic hypertrophic neuropathy described in Drachman's "Ophthalmoplegia Plus" paper (Drachman 1968) has not been described by other workers, is clinically and histologically atypical for a mitochondrial disease, and most likely represents a superimposed peripheral nerve condition, perhaps multifocal neuropathy with persistent conduction block or CIDP.

Study of peripheral nerve in patients with Leigh disease has indicated preservation of myelinated and unmyelinated fiber densities, with thinly myelinated axons resulting from demyelination and remyelination (Goebel et al. 1986), or intrinsic hypomyelination (Jacobs et al. 1990; Schroder 1993). "Incipient" onion bulbs in Leigh disease were described by Goebel et al. (1986). Wallerian degeneration, active segmental demyelination, and dominant onion-bulb formation have not been noted.

In the point mutation syndromes, MELAS and MERRF, biopsies and autopsy studies have revealed chronic axonal depletion with some thinly myelinated fibers and occasional onion bulbs (Mizusawa et al. 1991; Nicoll et al. 1993; Robitaille et al. 1980). Sural nerve biopsies in patients with SANDO demonstrate severe loss of both large and small myelinated fibers with endoneurial fibrosis (Fadic et al. 1997).

Light microscopy in the MNGIE syndrome has also revealed chronic axonal depletion with variable regenerative activity and evidence of demyelination and remyelination. Dystrophic mitochondria with loss of cristae and paracrystalline inclusions have been described (Pareyson et al. 2013). The symptoms of MNGIE are an example of a mitochondrial syndrome caused by an mtDNA replication dysfunction that results from an imbalance in the mitochondrial nucleotide pool. Most often some small onion bulbs are seen, with individual reports describing a spectrum ranging from none to numerous onion bulbs. Whether the myelin alterations are primary or secondary remains unclear. Various workers have commented on nonspecific findings such as endoneurial vascular hyalinization and perineurial or endoneurial fibrosis.

21.1.2.2 Electron Microscopy

Of interest as to pathogenesis of the neuropathy is whether abnormal mitochondrial morphology can be detected in peripheral nerve as readily as it is in the muscle. Increased numbers or focal accumulations of mitochondria have occasionally been reported in Schwann cells (Peyronnard et al. 1980; Threkeld et al. 1992), and we have encountered an example in a case of neuropathy with genetically unestablished mitochondrial encephalomyopathy (Fig. 21.1a–c). Similar features were noted in neural endothelial and vascular smooth muscle cells in KSS and MELAS (Schroder and Sommer 1991), as previously described in other tissues (Ohama et al. 1987). Enlarged mitochondria have also been described in these mitochondrial neuropathies (Goebel et al. 1986; Hirano et al. 1994). Schroder and Sommer (1991) have

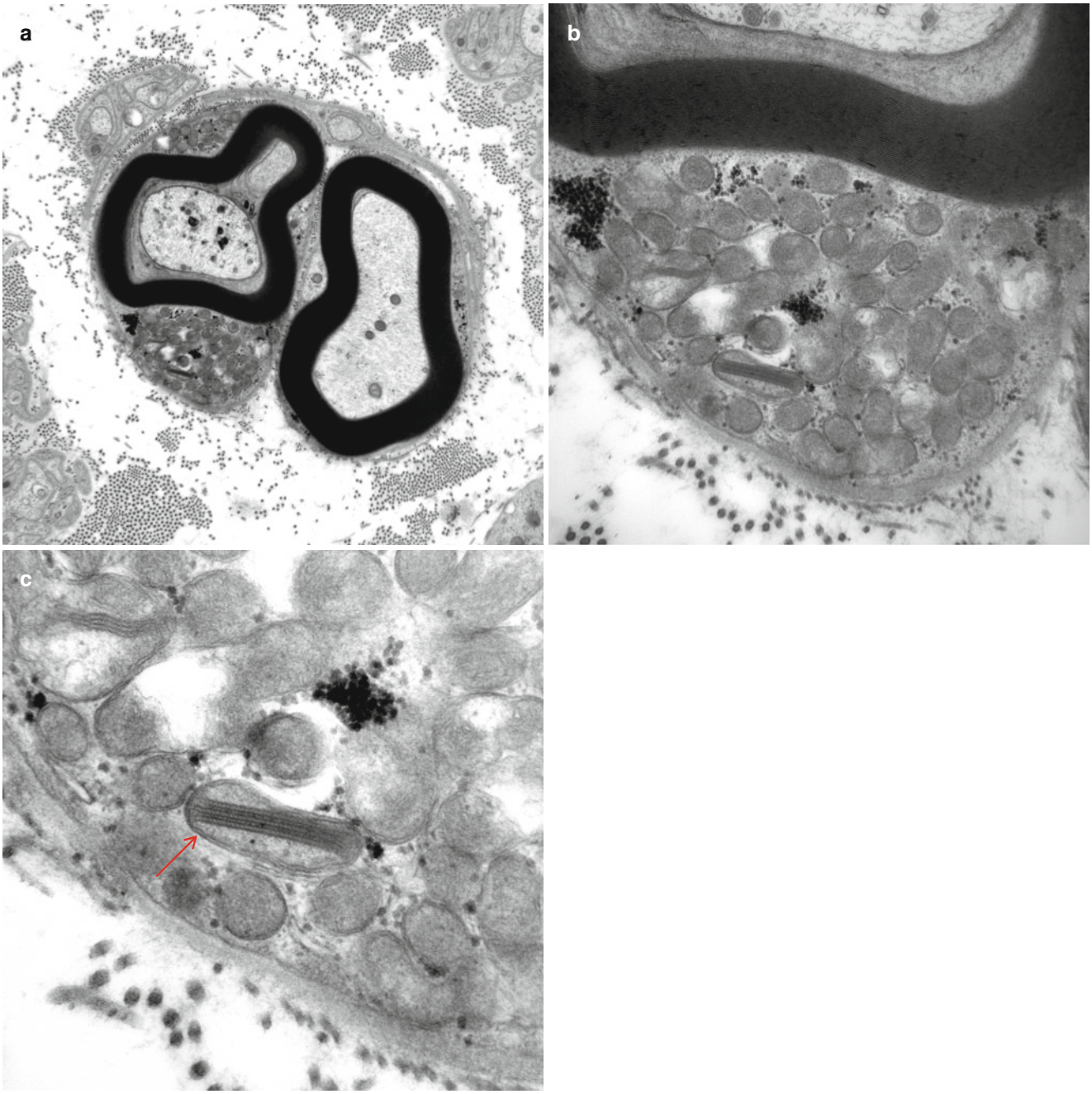

Fig. 21.1 Sural nerve in a patient with unestablished mitochondrial disease. Note that the Schwann cell collections of mitochondria (**a**, **b**) demonstrate one with a crystalline inclusion (*arrow*, **c**) (magnification: (**a**) 12,000×; (**b**), 32,000×; (**c**), 72,000×)

studied this issue most thoroughly and have concluded that enlarged mitochondria, some containing distorted cristae, are more common in mitochondrial cytopathies than in other neuropathies, but are not specific. Recent studies of a variety of a large number of mitochondrial peripheral neuropathies (Vital and Vital 2012) confirm the presence of ultrastructural mitochondrial changes including enlarged mitochondria with intertwined, or amputated cristae in Schwann cell cytoplasm. Increased numbers of Reich Pi granules have been emphasized in some reports (Gemignami et al. 1982; Gonatas 1967).

A number of authors have described and illustrated nonmyelinating Schwann cells containing paracrystalline material surrounded by a double membrane, likely of mitochondrial origin (Schroder 1993; Yiannikas et al. 1986). However, such inclusions are not infrequently seen in a variety of non-mitochondrial peripheral nerve diseases (Schroder 1993).

Classic inclusions of the paracrystalline stacked bar variety have been illustrated in the nerve in only one instance (Li et al. 1991). Other, less characteristic but unusual intramitochondrial structures have been infrequently noted (Goebel et al. 1986; Pezeshkpour et al. 1987). In two patients with CMT type 6 and another with an idiopathic sensorimotor neuropathy, Sommer and Schroder (1989) illustrated enlarged mitochondria in Schwann cells of small MFs containing several linearly arrayed and closely apposed cristae with a paracrystalline material filling the intercristal space. However, the "mitochondrial" nature of some CMT-6 patients remains speculative pending further evidence of mitochondrial dysfunction in this rare syndrome.

In one report of a patient with PEO/myopathy, examination of intramuscular nerve fibers revealed intra-axonal structures identical in ultrastructural appearance to Hirano bodies (Atsumi et al. 1980).

21.2 Adult Polyglucosan Body Disease

21.2.1 Clinical Manifestations

A number of entities are associated with tissue polyglucosan bodies, including type IV glycogenosis, phosphofructokinase deficiency, Lafora body disease, and normal aging (Cavanagh 1999). In our experience, polyglucosan bodies are found in about 3 % of sural nerve biopsies. In recent years, adult polyglucosan body disease (APGBD) has emerged as another distinct entity associated with characteristic inclusions (Cafferty et al. 1991; Gray et al. 1988; Karpati and Carpenter 1983; McDonald et al. 1993; Robitaille et al. 1980; Mochel et al. 2012). The APBGD syndrome includes adult-onset progressive upper motor neuron disease, urinary incontinence, dementia, and sensorimotor or pure motor peripheral neuropathy. Family history is positive in about one-third of cases, in a pattern suggestive of autosomal recessive inheritance. APGBD (Phenotype MIM#263570) is caused by mutation in the glycogen branching enzyme gene (*GBE1*; Gene/Locus MIM#607839). However, mutation in the same gene (*GBE1*) causes type IV glycogen storage disease (GSD IV; Phenotype MIM#232500), an early childhood disorder with systemic manifestations. Polyglucosan bodies are found throughout the central and peripheral nervous systems, as well as in myocardium and other nonneural tissues (Gray et al. 1988; Robitaille et al. 1980). Electrophysiological testing demonstrates an axonal neuropathy.

21.2.2 Pathology

A nerve biopsy showing polyglucosan bodies (PGBs) is commonly employed for the diagnosis of APGBD in life, although axillary skin biopsy may be a reliable and less invasive procedure, showing large numbers of PGBs in the myoepithelial cells of apocrine glands (Busard et al. 1991; Milde et al. 2001).

Nerve biopsies reported in APGBD have shown from one to many polyglucosan bodies (Fig. 21.2a–c) per nerve fascicle cross section. Typically the bodies are intra-axonal, round, and range from 5 to 70 μm in diameter (Fig. 21.2a–c), may result in minimal change in the axon/myelin (Fig. 21.2d), or induce expansion of the axon dimensions with myelin thinning (Fig. 21.2e). Polyglucosan bodies are composed of aggregates of 6–8 nm diameter branched filaments (Fig. 21.2f) which lack a limiting membrane. The inclusions are seen in myelinated axons and, less frequently, in unmyelinated axons or Schwann cells (Cafferty et al. 1991; Vos et al. 1983). Longitudinal sections may indicate that the inclusions are elongated, and several may be seen within one axon (Cafferty et al. 1991; Robitaille et al. 1980). Deposits of similarly staining, but less distinctively organized, material can be seen in axons or other peripheral nerve cells including endothelial and perineurial cells, fibroblasts, and macrophages (Vos et al. 1983). Extracellular PGBs, surrounded by inflammatory cells, may result from the recent death of the axon previously containing that inclusion (Robitaille et al. 1980).

In most reported cases of APGBD axonal dropout ranging from mild to severe has been the dominant finding, the infrequency of debris-filled macrophages indicating a slow degenerative process. Regenerating clusters may be present. Prominent segmental demyelination is less commonly seen (Matsumuro et al. 1993; Vos et al. 1983). Axons with large inclusion bodies can show attenuation of surrounding myelin, but this may be due to axonal dilation, not demyelination and remyelination (Yoshikawa et al. 1990).

21.2.3 Pathogenesis

Polyglucosan bodies (including Lafora bodies and corpora amylacea) are made up almost entirely by glucose polymers, with only a small amount of protein, and some sulfate and phosphate groups (Stam and Roukema 1973; Steyaert et al. 1990; Milde et al. 2001). A study in alloxan diabetic rats demonstrated an intimate relationship between glycogen accumulation and formation of polyglucosan bodies (Powell et al. 1979). Seemingly identical inclusions are seen in type IV (brancher deficiency) glycogenosis (McMaster et al. 1979; Schroder et al. 1993). Thus, it seems reasonable to suppose that a disturbance of carbohydrate metabolism might result in the production of PGBs. Indeed, several Ashkenazi Jewish patients with APGBD have been shown to have a tissue-specific defect of brancher enzyme (Bruno et al. 1992; Lossos et al. 1991).

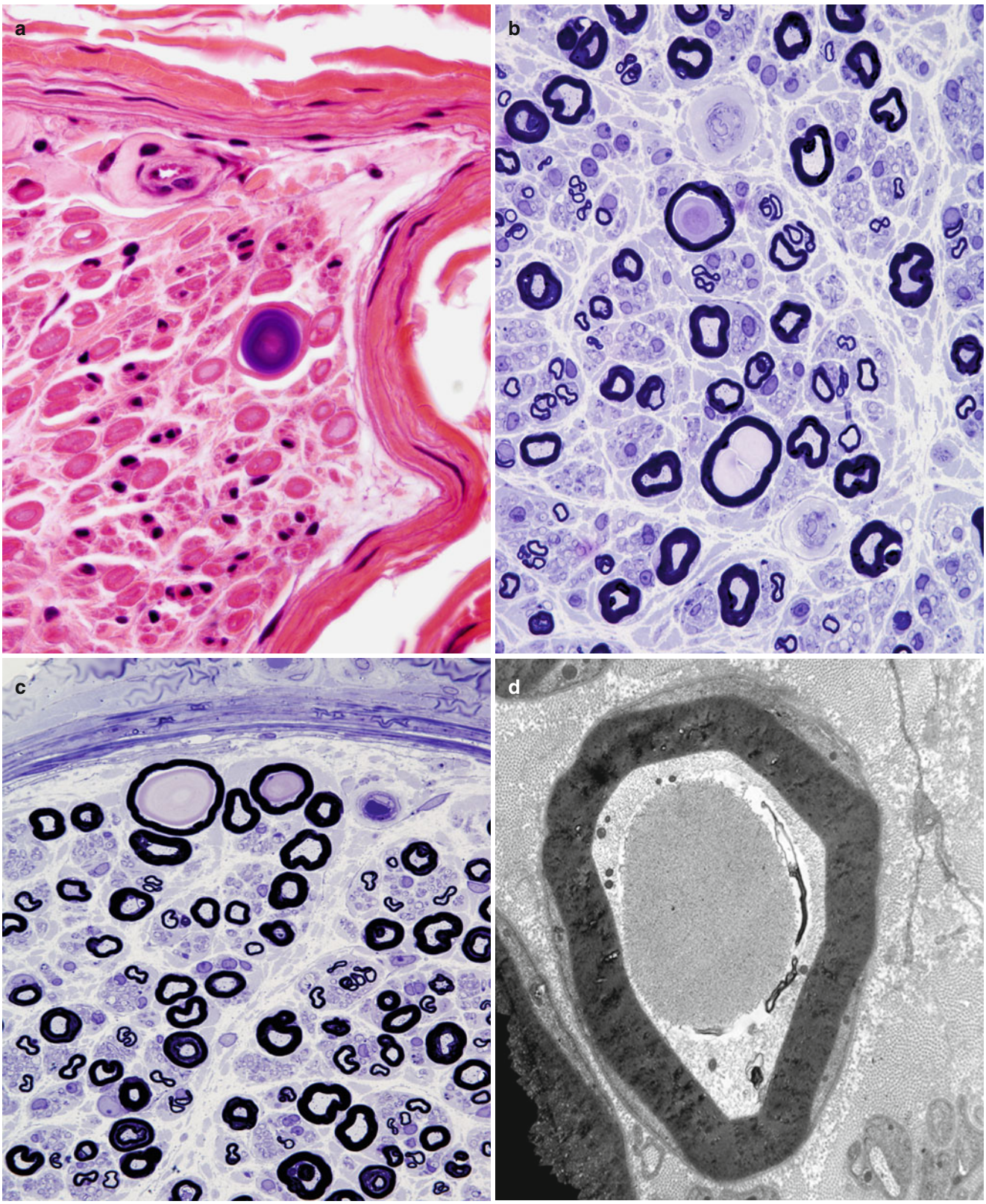

Fig. 21.2 Sural nerve in a 65-year-old patient with vasculitis. In the older age group, rare polyglucosan bodies (**a**–**c**) are of undetermined significance in the absence of an appropriate clinical context. Ultrastructural analysis demonstrates intra-axonal polyglucosan bodies (**d**) which may dilate the axon and thin the myelin sheath (**e**). Ultrastructural analysis shows filamentous aggregates which are not membrane delimited (**f**) (**a**, H&E; **b**, **c** 1 μm thick plastic sections; **d**–**f**)

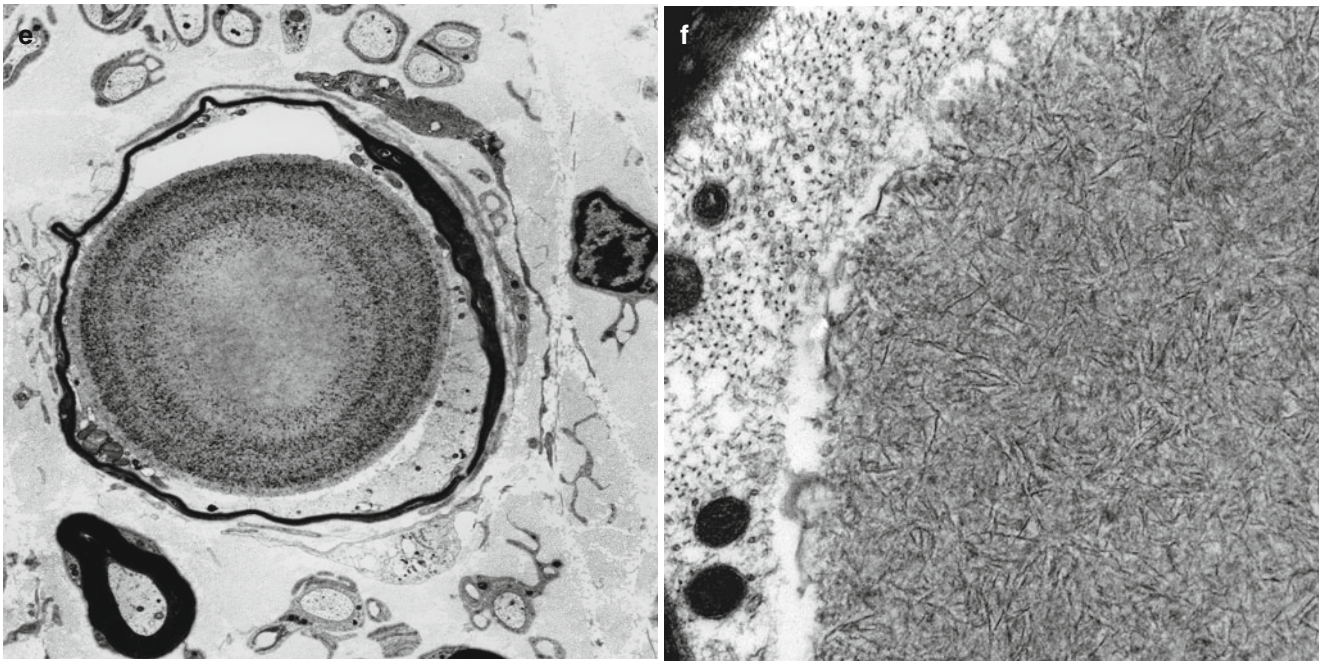

Fig. 21.2 (continued)

The mechanism of production of the neuropathy is unclear. Polyglucosan bodies are most commonly seen in the presence of an axonopathy (Busard et al. 1990); however, the axon is not necessarily disrupted by the inclusions, and in fact seems to be able to adapt to their presence (Yoshikawa et al. 1990). Whether the polyglucosan bodies, when massive in size or present in large numbers, cause the neuropathy or are an epiphenomenon of the metabolic defect that underlies the neuropathy remains unknown.

21.2.4 Differential Diagnosis

Our experience suggests that one or two polyglucosan bodies seen in a nerve biopsy specimen are a nonspecific finding with no diagnostic implications. We have noted polyglucosan bodies in 2–3 % of sural nerve biopsies, and others have reported that 8–15 % of unselected sural nerve biopsies in adults may show this finding (Averback and Langevin 1978; Busard et al. 1990). Intramuscular nerve twigs show polyglucosan bodies even more frequently (Averback and Langevin 1978; Bernsen et al. 1989; Fukuhara 1977). The incidence of PGBs in the nerve clearly rises with age, as with corpora amylacea in the central nervous system (Averback and Langevin 1978; Bernsen et al. 1989; Busard et al. 1990; Thomas et al. 1980), and there may be an association between the presence of nonspecific CNS disease and the finding of peripheral nerve PGBs, even if a syndrome suggestive of APGBD is absent (Busard et al. 1990).

Observation of more than one PGB per nerve fascicle, of a PGB outside an axon, or of unusually large PGBs (>30 μm) should, however, lead to consideration of a number of disease entities, including APGBD, type IV glycogenosis (McMaster et al. 1979; Schroder and Sommer 1991), and Lafora disease (Bernsen et al. 1989; Busard et al. 1990). Similar suspicions should be raised by the finding of even a single PGB in a patient under the age of 5 (Busard et al. 1990) and perhaps under the age of 20 (Bernsen et al. 1989). PGBs have also been described in the setting of CIDP (Busard et al. 1990; Yoshikawa et al. 1990) and neuropathy in a diabetic (Mancardi et al. 1985). One patient with numerous PGBs in peripheral nerve and clinical features suggestive of APGBD who came to autopsy failed to reveal any unusual pathological features suggestive of a systemic accumulation of polyglucosan bodies (Gray et al. 1988).

21.3 Sensory Neuropathy Syndrome

21.3.1 Clinical Manifestations

The differential diagnosis of sensory neuropathy is considerable (Table 21.1). Once systemic malignancy and identifiable neuropathies are excluded, there remain a considerable number of patients in whom no explanation can be given and who have a relatively characteristic syndrome (Dalakas 1986; Griffin et al. 1990; Sobue et al. 1993; Windebank et al. 1990). These patients develop paresthesias, sensory loss, and

Table 21.1 Miscellaneous neuropathies

Neuropathy associated with mitochondrial disease
Adult polyglucosan body disease
Idiopathic sensory neuropathy/ganglionopathy
Hypereosinophilic syndrome
"Motor neuron" diseases
Critical illness polyneuropathy
Multiple symmetrical lipomatosis (Madelung disease)

sensory ataxia, but show no significant weakness. Sensory abnormalities may be distal and symmetrical, asymmetric, or segmental and can include the cranial nerves. As suggested by the sensory ataxia, large fiber damage often predominates, and small fiber function may be strikingly spared. Rate of progression varies widely, from days to years, and the morbidity ranges from insignificant to debilitation. Despite an extensive search, investigations reveal no evidence of malignancy, as confirmed with long-term follow-up (Windebank et al. 1990). There is an association with collagen disease, especially Sjögren syndrome (Griffin et al. 1990), but this accounts for a minority of patients (Dalakas 1986; Sobue et al. 1993; Windebank et al. 1990). CSF protein is normal or minimally elevated, and cell count is normal. Nerve conduction abnormalities suggest sensory axon damage with and minimal or no involvement of motor axons. No therapy has been of benefit, but a small proportion of patients show some spontaneous improvement (Dalakas 1986; Windebank et al. 1990).

21.3.2 Pathology

Sural nerve biopsy has been performed in many of these patients (Dalakas 1986; Griffin et al. 1990; Sobue et al. 1993; Windebank et al. 1990). Inflammation is not a feature, except in patients with Sjögren syndrome who may demonstrate perivascular mononuclear cell collections. Axon loss ranges from minimal to severe, and status ranging from very active to indolent. Regenerating clusters are infrequent. Large myelinated fibers may be most involved, but small myelinated and unmyelinated axons can also be lost (Sobue et al. 1993). Evidence of segmental demyelination and remyelination on teased fiber preparations has been interpreted as secondary to axonal atrophy (Sobue et al. 1993; Windebank et al. 1990).

The sural nerve findings in idiopathic sensory ganglionopathy are entirely nonspecific and cannot be distinguished from those in the paraneoplastic variant, although prominent inflammatory infiltration should suggest the presence of the Sjögren syndrome. Other potential causes of a sensory neuropathy (Table 21.1), especially vasculitis and CIDP, should be looked for.

21.3.3 Pathogenesis

A small number of autopsies and dorsal root ganglion biopsies have demonstrated inflammation and cell body loss in the spinal ganglia (Griffin et al. 1990). However, spinal ganglionitis would not explain the occasional evidence of mild motor fiber involvement (Windebank et al. 1990). Despite the association with autoimmune diseases, especially Sjögren syndrome, circulating antibodies reactive against spinal ganglion neurons have not been found (Griffin et al. 1990), in contrast to paraneoplastic polyganglionopathy. Inflammation may be seen in proximal nerve trunks, as contrasted with the absence of inflammation in nerve biopsy specimens (Sobue et al. 1993). The sural nerve histological findings described above suggest that a "sensory variant" of CIDP is not an important cause of this syndrome (Windebank et al. 1990).

21.4 Neuropathy in the Hypereosinophilic Syndrome

21.4.1 Clinical Manifestations

The hypereosinophilic syndrome (HES) is characterized by peripheral blood eosinophilia in the absence of known associated disease such as parasitic or fungal infection, granulomatous angiitis, recognized allergic reaction, or malignancy (Moore et al. 1985). The clinical picture is of a multisystem disease with fevers, skin rash, pulmonary infiltrates, and cardiac disease (Chusid et al. 1975). Neuropathy may occur in as many as 50 % of patients, most often a mild symmetrical sensory polyneuropathy (Mondelli et al. 1993). However, a severe rapidly evolving axonal neuropathy, with distal predominance and some asymmetry, has been described as well (Dorfman et al. 1983; Grisold and Jellinger 1985; Sunohara et al. 1989). Response to steroids is usually gratifying.

21.4.2 Pathology

Nerve biopsy has been reported by several authors (Dorfman et al. 1983; Grisold and Jellinger 1985; Lupo et al. 1989; Monaco et al. 1988; Sunohara et al. 1989; Werner and Wolf 1990; Wichman et al. 1985). Most often a severe acute or chronic axonal neuropathy with little or no regenerative activity has been demonstrated. Large myelinated fibers may be more severely depleted. Biopsies in mildly affected or asymptomatic patients have shown only mild axonal loss or even a normal nerve (Vos et al. 1983; Wichman et al. 1985). Despite the usual presence of a marked peripheral eosinophilia, only one report describes an inflammatory neuropathy with significant vascular injury, and this case may have been an example of allergic eosinophilia, not an idiopathic HES

(Grisold and Jellinger 1985). Endoneurial edema was prominent in the two cases described by Monaco et al. (1988). In some reports, the authors have been impressed by the prominence of degranulated mast cells or thickening of capillary walls and endothelial cells (Lupo et al. 1989; Monaco et al. 1988).

21.4.3 Pathogenesis

Many of the manifestations of the HES are reminiscent of Churg–Strauss angiitis, and the possibility that this is a clinical variant must be considered. The neuropathy in HES is often asymmetric, suggestive of ischemic origin. However, most nerve biopsy material in the HES has shown a noninflammatory axonal neuropathy, which differs from the necrotizing angiitis of Churg–Strauss. Ischemia may be due to eosinophil-induced endothelial cell injury (Dorfman et al. 1983). A possible role of axonotoxic eosinophil products has been postulated and receives some support from experimental data (Lupo et al. 1989; Sunohara et al. 1989).

21.5 Motor Neuron Diseases

The motor neuron diseases, including ALS and spinal muscular atrophy, are generally said to spare sensory fibers, and as such one would expect sensory nerve biopsy to be normal. However, the literature suggests that this is an erroneous assumption and that although motor abnormalities completely overshadow the sensory changes, the latter do occur (Dyck et al. 1975; Mondelli et al. 1993).

21.5.1 Amyotrophic Lateral Sclerosis

Studies of sural or other sensory nerve biopsy in patients with ALS (Ben Hamida et al. 1987; Bradley et al. 1983; Dayan et al. 1969; di Trapani et al. 1986; Dyck et al. 1975; Heads et al. 1991; Tohgi et al. 1977) have not infrequently revealed slight but definite axonal depletion, sometimes with mild ongoing Wallerian degeneration, usually affecting large more than small MFs. Unmyelinated fibers have not been studied as closely, but these too seem to be affected (Ben Hamida et al. 1987; Heads et al. 1991). Regenerative activity, as evidenced by the presence of increased numbers of regenerating clusters, has at times been observed. Teased fiber studies have ranged from normal (Ben Hamida et al. 1987) to showing a marked increase in the incidence of segmental demyelination and remyelination, with abnormal internodes tending to be clustered along certain axons, suggesting a secondary demyelinating process (Heads et al. 1991). Neural inflammatory infiltrates are no more prominent in ALS than in normal nerves (Kerkoff et al. 1993). No specific ultrastructural findings have been described, although nonspecific axonal degenerative changes have been commented upon (Dalakas 1986).

In early ALS, a morphometric comparison of eight patients and eight age-matched controls showed no significant reduction in MF density in sural/sensory nerves, but demonstrated a shift to the left in myelinated fiber diameter, i.e., axonal atrophy, and a bimodal unmyelinated fiber histogram, suggesting a degree of UF degeneration and regeneration (Heads et al. 1991). In contrast, with relatively late-stage ALS, a 30 % loss of myelinated fibers relative to controls was noted in sural nerves and an increase in the number of denervated Schwann cell subunits indicating unmyelinated fiber pathology (Bradley et al. 1983).

21.5.2 Other Motor Neuron Diseases

Distally accentuated axonal loss with axonal atrophy and secondary demyelination and remyelination has been found in X-linked bulbospinal neuronopathy (Kennedy syndrome), and these patients clearly demonstrate a loss of primary sensory neurons (Sobue et al. 1989). In distal spinal muscular atrophy, some studies have shown a chronic axonal process with prominent regenerating clusters (Gherardi et al. 1983), while in others the sensory nerves were entirely normal (Frequin et al. 1991; McLeod and Prineas 1971). The available information on proximal spinal muscular atrophies (Werdnig Hoffman, Kugelberg-Welander) indicates that axonal loss can occur in sensory nerves (Carpenter et al. 1978; Probst et al. 1981; Winder and Auer 1989).

The practical significance of these observations lies in two considerations. First, it is obviously inappropriate to use ALS or spinal muscular atrophy patients as "normal" sural nerve controls. Second, some patients with these diseases have atypical features, and nerve biopsy is performed in the search for a treatable illness. The finding of mild axonal degeneration and regeneration or slight segmental demyelination and remyelination does not exclude the diagnosis of motor neuron disease.

The possibility that a few motor axons run in the sural nerve, thus accounting for the observed axonal degeneration, seems unlikely (Carpenter et al. 1978), and loss of spinal ganglion neurons has been demonstrated in both ALS and spinal muscular atrophy (Carpenter et al. 1978; Kawamura et al. 1991; Marshall and Duchen 1975).

21.6 Critical Illness Polyneuropathy

The "critical illness" syndrome of systemic sepsis and multiple organ failure is characterized (Stevens et al. 2009) by prolonged periods of bacteremia and evidence of pulmonary, central nervous system, hepatic, cardiac, and renal

dysfunction. Neuropathy is being increasingly recognized as a part of this syndrome (recently reviewed by Koshy and Zochodne 2013), which is present in up to 70 % of these patients. Most often this emerges after periods ranging from days to weeks on a ventilator, when patients are unable to be weaned (Garcia Garcia et al. 1991; Gorson and Ropper 1993; Spitzer et al. 1992; Witt et al. 1991; Zochodne et al. 1987). Because of the difficulties associated with examining a critically ill patient, this neuropathy is often not suspected clinically, and the most reliable means of making the diagnosis is electrical testing, which reveals near normal nerve conduction velocities and decreased sensory nerve action potentials consistent with an axonal neuropathy of mild to marked severity. If systemic disease is controlled, patients can make an excellent recovery (Witt et al. 1991), although long-term studies have shown significant functional residua in some patients (Fletcher et al. 2003).

In critically ill patients, depending on the underlying illness, this syndrome may be clinically indistinguishable from a diffuse vasculitic neuropathy or severe Guillain–Barré syndrome. Biopsy might be performed so that a treatable diagnosis is not missed.

Pathological examination of autopsy and nerve biopsy material has demonstrated active axonal degeneration, distally enhanced, without a significant demyelinating component, nor any evidence of inflammation, serving to distinguish this neuropathy from the aforementioned alternatives (Zochodne et al. 1987). The absence of inflammation is a key element of the differential diagnosis in this setting, and its presence should suggest alternative diagnoses including vasculitis, GBS, and even endocarditis-associated neuropathy (Pamphlett and Walsh 1989).

No specific pathogenic mechanism has been identified, although nutritional and iatrogenic factors seem unlikely to be important (Zochodne et al. 1987). Most likely the metabolic changes associated with sepsis, especially high concentrations of various cytokines and increased levels of nitric oxide during sepsis (Angel et al. 2007), compounded by organ damage which promotes accumulation of toxic circulating substances and diminishes nutrition to stressed tissue, act together to cause axonal damage (Bolton et al. 1993).

21.7 Multiple Symmetrical Lipomatosis (Madelung Disease)

The essential feature of multiple symmetrical lipomatosis (MSL) is the presence of multiple axial and proximal limb non-encapsulated fat masses, most commonly around the neck, appearing during adult life (Enzi 1984). No consistent defects in lipid or glucose metabolism have been identified, but excessive alcohol intake has frequently been noted (Enzi 1984; Pollock et al. 1988). Sensorimotor polyneuropathy is often motor predominant, and autonomic features may be prominent. Clinical and electrophysiological features suggest axonal neuropathy (Chalk et al. 1990; Enzi 1984). The polyneuropathy of MSL affects the majority of patients with MSL and often progresses to incapacitation (Saiz Hervas et al. 2000). A minority of patients demonstrate a multisystem disease with myopathy, cerebellar degeneration, and pyramidal signs, and a growing body of evidence suggests that at least some patients with multiple symmetrical lipomatosis suffer from a mitochondrial cytopathy (Berkovic et al. 1991; Holme et al. 1993; Klopstock et al. 1994) which may be exaggerated by alcohol (Saiz Hervas et al. 2000).

A small number of nerve biopsies of MSL have been reported (Chalk et al. 1990; Enzi et al. 1986; Pollock et al. 1988), and we have examined a case. Biopsy reveals a very indolent process causing depletion of myelinated axons, especially large myelinated fibers, with some regenerative cluster formation and no Wallerian degeneration (Fig. 21.3a). Unmyelinated fibers may be mildly depleted. In our experience, and in most other reports, no significant myelin alterations were noted, even with teased fibers (Chalk et al. 1990), although features of demyelination/remyelination have been noted in some cases, seemingly clustered along certain axons, suggestive of a primary axonal influence (Pollock et al. 1988).

Electron microscopic examination has not revealed any specific features. We have noted rare axons containing focal accumulations of normal appearing mitochondria (Fig. 21.3b), an intriguing finding given the evidence of mitochondrial dysfunction in these patients (vide supra). However, this was an uncommon finding and probably within the spectrum of nonspecific axonopathic alterations. The case we examined demonstrated numerous paracrystalline inclusions in muscle mitochondria, but these were completely absent in the nerve biopsy material. Pollock and colleagues (1988) noted a frequent honeycomb-like degeneration of inner myelin lamellae.

21.8 Traumatic Injuries

Traumatic injury to the nerve takes a number of forms. Chronic nerve compression which occurs in entrapment neuropathy is characterized by focal loss of myelin and, often, increased numbers of Renaut bodies (collections of fibroblast-like cells surrounded by mucoid extracellular matrix). Chronic compression may produce fibrosis of the underlying nerve accompanied by endoneurial edema, demyelination, remyelination, and axon loss. Morton neuroma, resulting from chronic compression of interdigital nerves of the foot, is a fusiform enlargement or thickening of the intact but chronically compressed nerve. Similarly, constriction of the lateral femoral cutaneous nerve by fascia/inguinal ligament is thought to produce pain, numbness, and paresthesia in the territory of the outer and anterior thigh, a syndrome

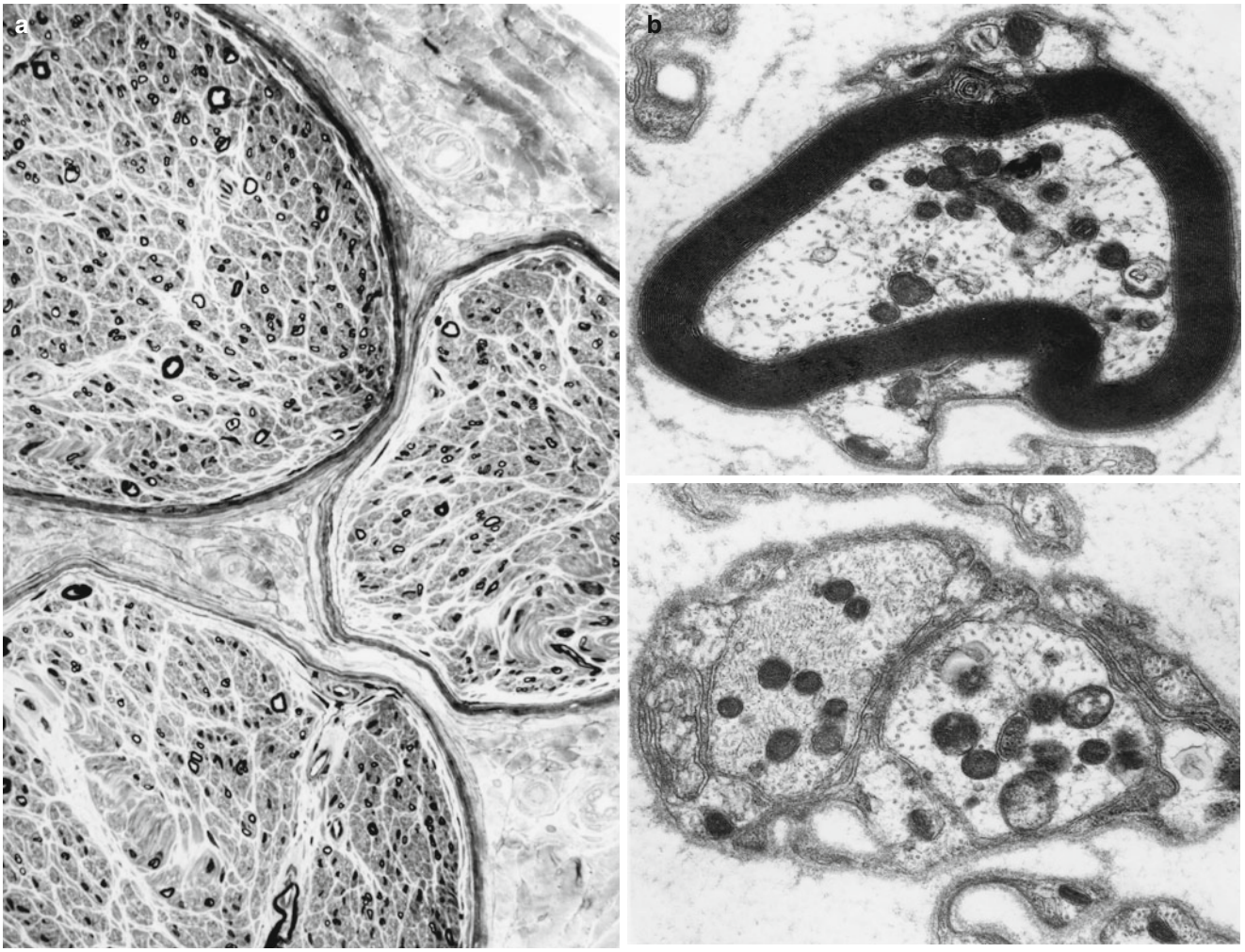

Fig. 21.3 Madelung disease. Diffuse dropout of MFs of all sizes is seen (**a**). The finding of rare intra-axonal accumulations of normal mitochondria is of undetermined significance (**b**). The muscle biopsy in this patient showed numerous abnormal mitochondria with paracrystalline inclusions (**a**, **b** 23,660×) (plastic, tissue courtesy of Dr. Y. Yucel)

designated meralgia paresthetica. This syndrome is often seen in obese patients of either gender between the fourth and sixth decades of life and usually improves spontaneously (reviewed in Arnold and Elsheikh 2013).

Irrespective of the suggestion of neoplasm by the name "neuroma," this is a nonneoplastic reactive proliferation of axons, fibroblasts, and Schwann and perineurial cells which may develop in somatic or visceral nerves, spontaneously or as the result of prior surgery or injury. Traumatic damage to peripheral nerve results in efficient regeneration in some cases but, in others, particularly those with injury in which there is loss of nerve continuity, regeneration is interrupted and frustrated. These swollen nerve segments represent the neuroma (i.e., traumatic or amputation neuroma). Damage to the nerve trunk may reflect focal proximal interruption by neuropathy, particularly vasculitis, vertebral collapse, or the results of prior surgery in which nerves were intentionally or inadvertently sectioned.

The resultant pathological response represents a combination of degenerative and regenerative responses resulting in disorganized aggregates of collagen and minifascicles (arrows, Fig. 21.4a, b) of regenerating and sprouted axons (Fig. 21.4c) searching for their distal connections (Fig. 21.4d). Sprouted axons are typically ensheathed by Schwann cells shown by S100 immunohistochemistry (Fig. 21.4e) which may myelinate axons or surround them as unmyelinated axons. Minifascicles contain thinly myelinated and unmyelinated axons (Fig. 21.4f) and are surrounded by perineurial cells which stain with EMA (arrows, Fig. 21.4g).

Neuroma tissue has been found to contain increased levels of the chemorepulsive protein semaphorin 3A, which may increase fasciculation and inhibit organized neurite outgrowth (Tannemaat et al. 2007). Large clusters of frustrated regenerating axons suggest the failure to eliminate supernumerary axons in regenerating nerve. Neuromas may be painful to touch or movement which may reflect alterations in sodium channels in constituent axons. This discovery holds the promise for targeted blockade of NaV1.7 or ERK1/2 as a therapeutic strategy for amelioration of chronic pain that

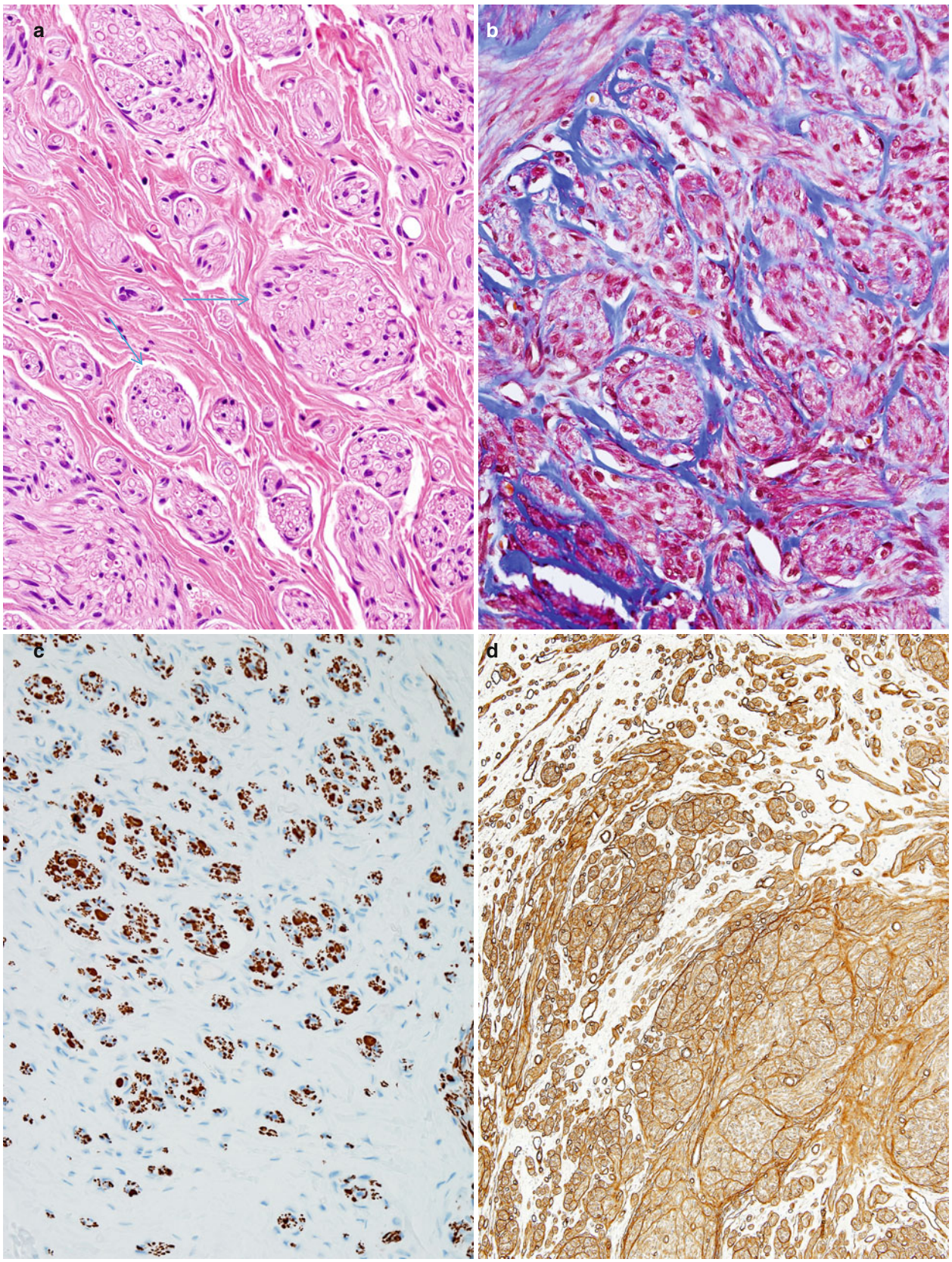

Fig. 21.4 Neuroma, traumatic sections of neuroma composed of minifascicles (*arrows*, **a**) surrounded by collagenous processes (**b**, trichrome) which contain clusters of axons (**c**, neurofilament immunohistochemistry). The distal end of injured nerve stump shows minifascicles arising from nerve trunk (**d**, collagen IV stain). S100 immunohistochemistry highlights Schwann cells within minifascicles (**e**). Minifascicles contain small and large myelinated axons with a thin perineurial rim (*arrow*, **f**) which is highlighted by EMA immunohistochemistry (**g**)

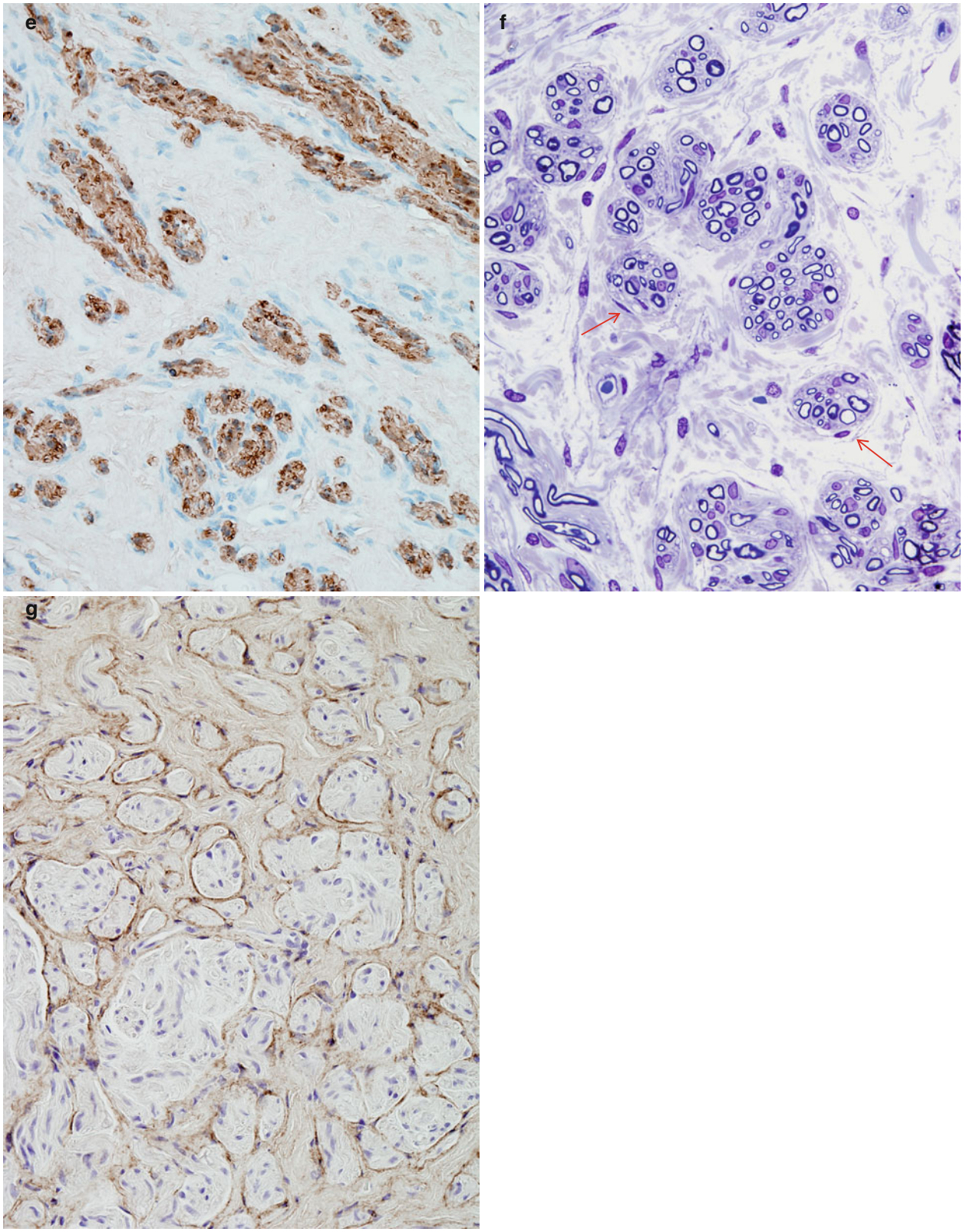

Fig. 21.4 (continued)

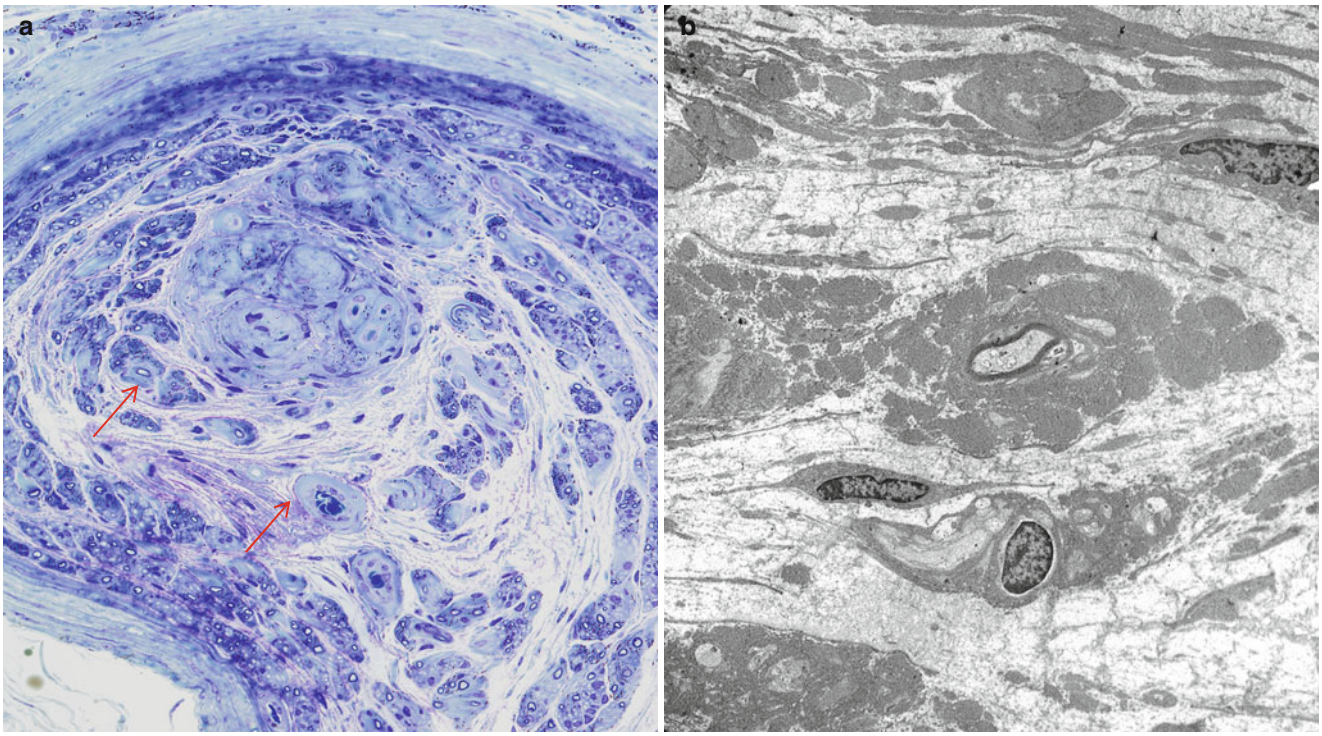

Fig. 21.5 Meralgia paresthetica, sections of lateral femoral cutaneous nerve show marked axon loss and prominently hyalinized vasculature (*arrow*, **a**). Ultrastructural examination confirms the wholesale loss of axons and increased endoneurial collagen (**a**, 1 μm thick plastic section, 600×; **b**, 3,000×)

often follows nerve injury and formation of neuromas and may be extrapolated to other conditions with intractable pain (Persson et al. 2011). Resection of the distal portion of the proximal stump and apposition of proximal and distal stumps or transplantation of a nerve graft may be curative.

The lateral femoral cutaneous (LFC) nerve is a pure sensory nerve which passes under the inguinal ligament and supplies sensation to the skin over the anterior and lateral portion of the thigh. Meralgia paresthetica is a syndrome of numbness, paresthesia, and pain associated with compression of the LFC which is increased in incidence in diabetes, obesity, pregnancy, external compression by clothes, or prolonged positioning during surgery (Parisi et al. 2011; Arnold and Elsheikh 2013). We have seen one example of LFC nerve resected from a patient with meralgia paresthetica which was characterized by marked axon loss accompanied by hyalinized endoneurial blood vessels (Fig. 21.5a, b).

21.9 Perineurioma

Intraneural perineurioma is a benign intraneural neoplasm consisting of aggregates of perineurial cells which superficially resemble onion-bulb neuropathy. Although chromosome 22 alterations are seen in perineurioma, it is not associated with neurofibromatosis NF1 or NF2 (Emory et al. 1995). There is no history of prior traumatic injury associated with perineurioma which typically involves somatic nerves in the extremities rather than cranial nerves. Perineurioma may involve individual or multiple fascicles of nerve, with plexiform growth in some cases (Fig. 21.6a–c). The tumors are composed of multiple concentric layers of perineurial processes (Fig. 21.6b) forming coarse structures superficially resembling onion bulbs. Neurofilament immunohistochemistry illustrates that concentric processes variably surround axons (Fig. 21.6d), which are often found in clusters, forming larger, coarser structures than true onion bulbs. The processes are immunoreactive for epithelial membrane antigen (EMA, arrows, Fig. 21.6e). Mitoses are uncommon and Ki67 immunostaining shows a modest frequency (5–15 %, Scheithauer et al. 2010). Plastic sections (Fig. 21.6f) confirm the broad coarse composition of the concentric processes. Ultrastructurally, processes with patchy basal lamina, unbranched processes, and numerous pinocytotic vesicles are seen (Fig. 21.6g), as expected in perineurium-derived cells (Emory et al. 1995; Scheithauer et al. 2010).

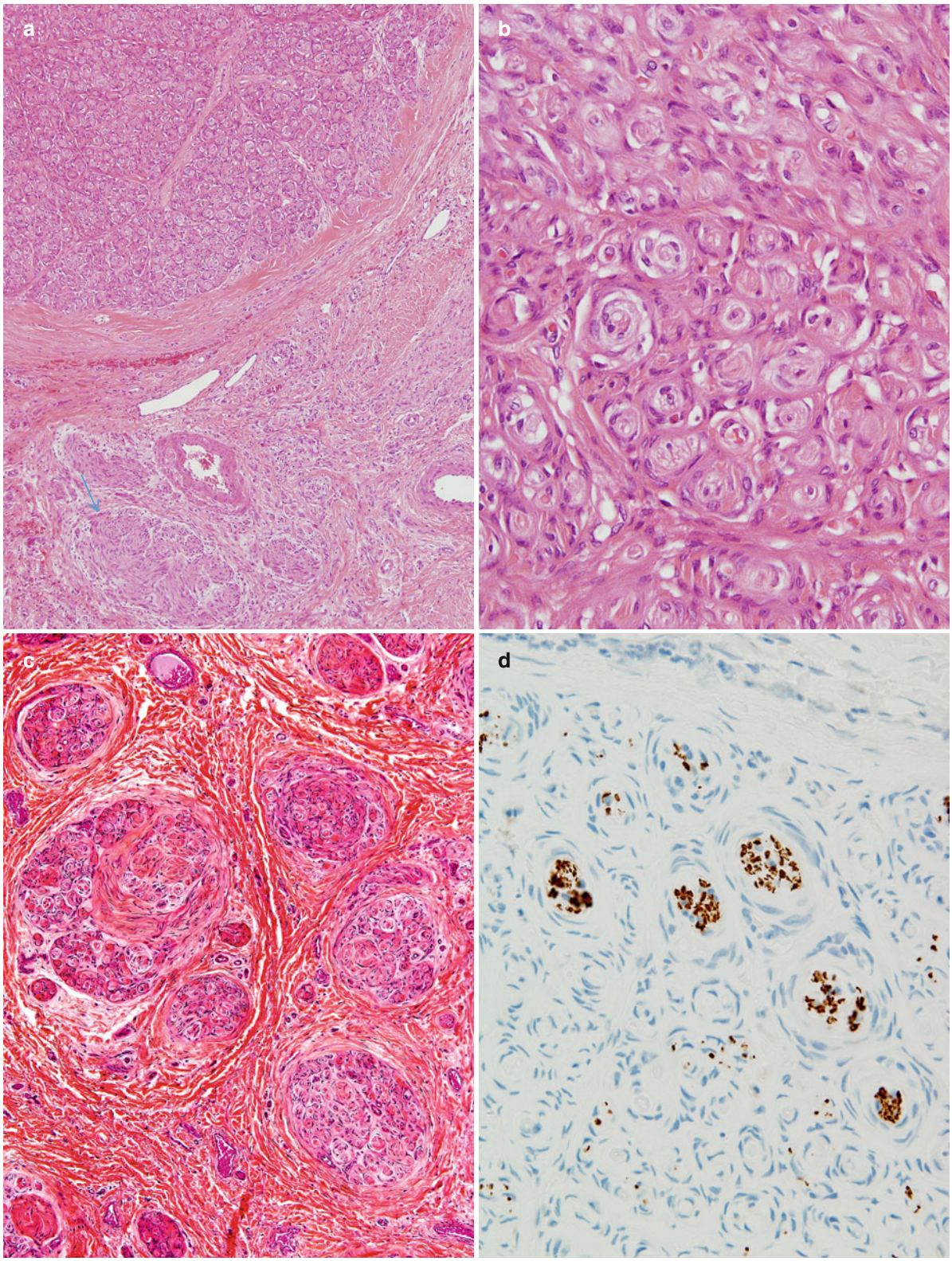

Fig. 21.6 Perineurioma. This intraneural tumor is composed of coarse concentric processes involving some fascicles and sparing others (*arrow*, **a**). The coarse concentric processes seen at higher magnification in (**b**) are reminiscent of onion bulbs. Plexiform growth is frequent (**c**). Immunolocalization of neurofilament immunoreactivity shows clumps of axons within perineurial processes and others without axonal cores (**d**). The perineurial origin of the tumor is confirmed by EMA histochemistry (**e**). A 1 μm thick plastic section further characterizes the coarse nature and arrangement of the tumor cells (**f**). Ultrastructure illustrates the unbranched nature of the perineurial processes, focal basement membranes, and pinocytotic vesicles (**g**) (**a**, **b**, H&E; **c**, HPS trichrome; **d**, **e,** neurofilament and EMA immunohistochemistry, respectively; **f**, 1 μm thick plastic section; **g**, electron micrograph, 2,500×)

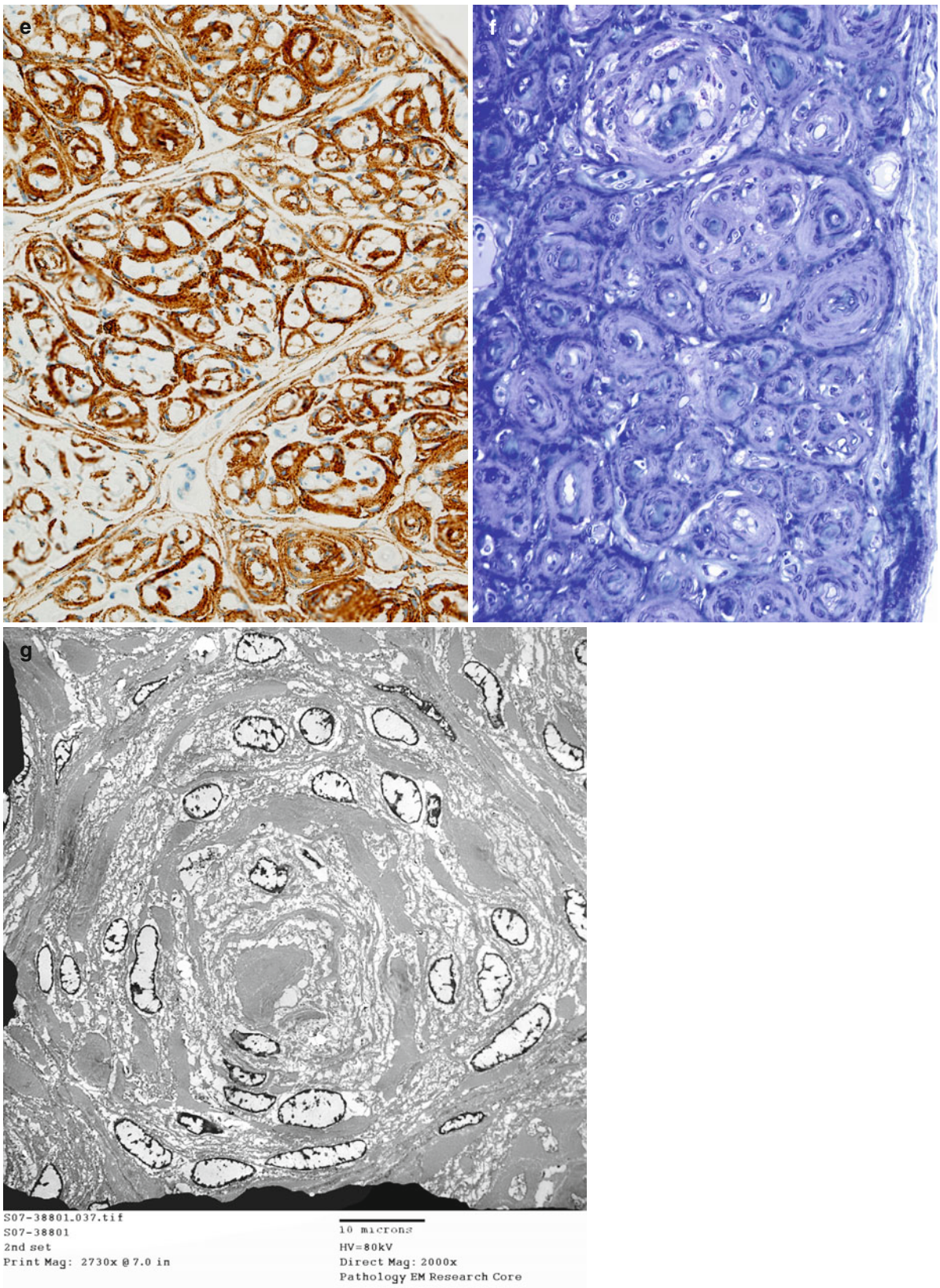

Fig. 21.6 (continued)

Separation of intraneural perineuriomas from true onion-bulb neuropathies is usually not difficult since true onion-bulb neuropathies are often hereditary, consist of delicate processes which are S100 immunoreactive but are not immunoreactive for EMA, and typically do not involve only portions of nerves sparing some fascicles and not others.

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