Greenfield's Neuropathology

Fourth Edition

Edited by

J. Hume Adams

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J. Hume Adams PhD, MBChB, FRCP, FRCPath
Professor of Neuropathology, University of Glasgow and Institute of Neurological Sciences, Southern General Hospital, Glasgow

J.A.N. Corsellis FRCP, FRCPath, FRCPsych
Emeritus Professor of Neuropathology, University of London
Honorary Consultant Neuropathologist, Bethlem Royal and Maudsley Hospitals, London and Runwell Hospital, Wickford, Essex

L.W. Duchen MD, PhD, DSc, FRCP, FRCPath
Professor of Neuropathology, Institute of Neurology
Honorary Consultant Neuropathologist, The National Hospital for Nervous Diseases, Queen Square, London

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J. G. Greenfield, MD, FRCP, LLD
(1884–1958)

Born in Edinburgh, Scotland. Graduated in Medicine at Edinburgh University 1908. House physician (1910) subsequently Pathologist to the National Hospital, Queen Square 1914–1949 and Dean of the Medical School 1923–1943. Visiting Professor National Institutes of Health, Bethesda 1954–1958. Founder of the Neuropathological Club (now the British Neuropathological Society) and Editor of the first edition of this book, 1958.

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Contributors

J. Hume Adams, PhD, MBChB, FRCP, FRCPath
Professor of Neuropathology, University of Glasgow and Institute of Neurological Sciences, Southern General Hospital, Glasgow

Ingrid V. Allen, MD, DSc, FRCPath
Professor of Neuropathology, Neuropathology Laboratory, Department of Pathology, Queen's University, Belfast

J.B. Brierley, MD, FRCPath, FRCPsych
Formerly Scientific Officer (Clinical), MRC Laboratories, Woodmansterne Road, Carshalton, Surrey

Betty Brownell, BA, MB, BCh, BAO, FRCPath
Consultant Neuropathologist, Department of Neuropathology, Frenchay Hospital, Bristol

J.A.N. Corsellis, FRCP, FRCPath, FRCPsych
Emeritus Professor of Neuropathology, University of London:
Honorary Consultant Neuropathologist, Bethlem Royal and Maudsley Hospitals, London and Runwell Hospital, Wickford, Essex

L.W. Duchen, MD, PhD, DSc, FRCP, FRCPath
Professor of Neuropathology, Institute of Neurology
Honorary Consultant Neuropathologist, The National Hospital for Nervous Diseases, Queen Square, London

D.I. Graham, PhD, MB, BCh, MRCP, FRCPath
Professor of Neuropathology, University of Glasgow:
Honorary Consultant in Neuropathology Institute of Neurological Sciences, Southern General Hospital, Glasgow

D.G.F. Harriman, MD, FRCP, FRCPath
Reader in Neuropathology, University of Leeds:
Honorary Consultant Neuropathologist, The General Infirmary, Leeds

J. Trevor Hughes, MA, DPhil, MD, FRCP(Lond and Ed), FRCPath
Clinical Lecturer in Neuropathology and Fellow of Green College, University of Oxford:
Consultant Neuropathologist, The Radcliffe Infirmary, Oxford

Jean M. Jacobs, BSc, PhD
Lecturer in Neuropathology, Institute of Neurology, The National Hospital for Nervous Diseases, Queen Square, London

R.H.M. King, PhD, MSc, BSc
Lecturer, Department of Neurological Science, Royal Free Hospital School of Medicine, London

Brian D. Lake, BSc, PhD, FRCPath
Reader in Histochemistry, Department of Histopathology, Hospital for Sick Children and Institute of Child Health, Great Ormond Street, London

D.N. Landon, BSc, MB, BS, MRCS, LRCP
Reader in Neurocytology, Institute of Neurology, The National Hospital for Nervous Diseases, Queen Square, London

Jeanne-Claudie Larroche, MD
Maître de Recherches au CNRS
Hôpital Port-Royal, Paris, France

Pamela M. Le Quesne, DM, BSc, FRCP
Member MRC Toxicology Unit; Honorary Consultant Neurologist, Department of Neurological Studies, The Middlesex Hospital, London

B.S. Meldrum, PhD, MB, BChir
Reader in Experimental Neurology, Department of Neurology, Institute of Psychiatry, London

J. Douglas Miller, MD, PhD, FRCS, FACS, FRCP
Professor of Surgical Neurology, University of Edinburgh at the Western General Hospital, Edinburgh

D.R. Oppenheimer, MA, DM, FRCPath
Formerly University Lecturer and Honorary Consultant in Neuropathology, The Radcliffe Infirmary, Oxford: and Fellow of Trinity College, Oxford

F. Scaravilli, MD, PhD
Senior Lecturer in Neuropathology, Institute of Neurology, The National Hospital for Nervous Diseases, Queen Square, London

P.K. Thomas, DSc, MD, FRCP
Professor of Neurology in the University of London at the Royal Free Hospital School of Medicine and the Institute of Neurology, London

A.H. Tomlinson, MA, D.Phil, FRCPath
Formerly at the Virology Laboratory, Public Health Laboratory, John Radcliffe Hospital, Oxford

B.E. Tomlinson, CBE, MD, FRCP, FRCPath
Professor of Pathology and Consultant Pathologist, Department of Pathology, Newcastle General Hospital, Newcastle upon Tyne

C.S. Treip, MA, MD, PhD, FRCPath
University Lecturer in Pathology and Fellow of Wolfson College, Cambridge.
Honorary Consultant in Neuropathology, Addenbrooke's Hospital, Cambridge

Roy O. Weller, BSc, PhD, MD, FRCPath
Professor of Neuropathology in the University of Southampton at the Department of Pathology (Neuropathology), Southampton General Hospital, Southampton

Preface to the fourth edition

More than 25 years have passed since the publication of the first edition of Neuropathology, which, since the second edition in 1963, has been known as Greenfield's Neuropathology in recognition of the pioneering work of Dr Greenfield at the National Hospital for Nervous Diseases, Queen Square. The fundamental approach and the format of the book remain true to the Greenfield tradition which was established in the first edition but the subject has inevitably enlarged and changed reflecting the rapid developments which are taking place in all branches of the neurosciences. Many of the chapters have been completely rewritten and there is now a greater emphasis on ultrastructure and biochemistry. A new generation of neuropathologist contributors has been joined by specialists in clinical fields and in other laboratory disciplines to provide what we think are up-to-date and authoritative surveys of their topics. To bring in the new does not necessitate discarding the old and much of the irreplaceable expertise expressed by the original authorship has been retained. Many of the recent discoveries in neurobiology have yet to be applied to the clinical and neuropathological study of man and the subject inevitably continues to grow. This is reflected in the size of the book which has grown with each edition but it is still contained within a single volume and this has unfortunately led to the omission of some of the historical aspects of the subject. Although the personal links with J.G. Greenfield himself are now not strong, many of the contributors were students of his fellow authors Blackwood, Meyer, McMenemey, Norman and Dorothy Russell who together with Greenfield laid the foundations of Neuropathology in Great Britain.

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Contents

1

General pathology of neurons and neuroglia

L. W. Duchen

Neuropathology, as surveyed in this volume, deals mainly with morphological aspects of the study of diseases of the nervous system. The foundations of this science were established in the nineteenth century by the pioneer neurohistologists in France, Germany and Spain, many whom combined clinical neuropsychiatric practice with laboratory investigation. Greenfield (1958) and subsequently Greenfield and Meyer (1963) and Blackwood (1976) briefly reviewed the historical development of neuropathology.

The complexity of structure and composition of the nervous system necessitates a wide range of technical methods for its microscopic study. Each component has some method of staining most appropriate for its visualization, and the importance of each method varies in different pathological conditions. The methods applied to the study of cases with complex neurological disease which may have required many hours of clinical investigation should be the most appropriate for each individual case. Obviously, therefore, the details of the clinical history of a case under study need to be known. As complete a post-mortem examination of the nervous system as is possible should be the aim in every case. Widespread sampling of tissues should be a routine procedure, so that the examination will include not only different areas of the brain and spinal cord but also nerve roots, sensory and autonomic ganglia, peripheral nerves, muscles, visceral innervation and sensory receptor organs. The brain should normally be fixed intact. Indiscriminate slicing of the fresh unfixed brain may preclude further useful study but it is possible to obtain fresh brain tissue without compromising later histological study. For example, a cut through one frontal lobe anterior to the lateral ventricle will provide a good sample of cortex and white matter. In diseases affecting the brain symmetrically, a mid-sagittal division will provide one hemisphere for histological examination while the other can be studied by techniques requiring unfixed tissue. This type of combined approach enables advances in neurobiology to become applicable to the study of human neurological disease. The application of electron microscopy to human clinical neuropathology remains a considerable, perhaps intractable, problem. The rapid development of post-mortem artefact due to autolytic changes, the disruption of membranes and the consequent impairment of adequate fixation and staining make it difficult to apply criteria for significant pathological change even when the period between death and fixation is reduced to 3 to 4 hours. Only the most stable, and metabolically therefore the most inactive, constituents can be identified with certainty in post-mortem electron micrographs. Nevertheless, electron microscopy of post-mortem tissue has made important contributions to our understanding of the pathogenesis of neurological disease and could be used more widely and routinely when the material is suitable.

Brain weight

The weight of the brain is a useful measure of its pathological state. Fixation in 10 per cent formalin usually causes a small but variable increase in weight while fixation in 30 per cent formalin leads to a small decrease. Brain weight is related to age, height and sex, and many tables have been published giving the normal range. A recent extensive survey (Dekaban and Sadowsky, 1978) was based on recorded fresh unfixed weights from more than 2700 male and nearly 2000

female patients dying with histologically normal brains. Brain weight was correlated with height, weight, age and sex, and it was found that in males brain weight is almost 10 per cent greater than in females of similar age. The largest rate of increase occurs between birth and 3 years, the maximum weight is in young adults around the 19th year and there is a progressive decline after 45–50 years of age. Lowest values occur after 86 years, when brain weight is about 11 per cent lower than the maximum. Average brain weights for males are 380 g at birth; 970 g at 1 year; 1120 g at 2 years; 1300 g at 5 years; 1400 g at 10; 1450 g at 19–21; 1430 g at 50; 1370 g at 60 and 1330 g at 80. In females the maximum is 1340 g at age 18, which declines to 1140 g at 80.

The neuron

The great variety of shapes and sizes of nerve cells makes it difficult, if not impossible, to define the morphological characteristics of a typical neuron. The identification of alterations in the morphology of neurons is one of the cornerstones of neuropathology and it is useful to compare suspected alterations in pathological states with control material of the same type of cell. Some features are common to all nerve cells, large or small. Nuclei tend to be rounded in section—i.e. they are spherical (Fig. 1.1)—and on electron microscopy can be seen to be bounded by a double membrane which in places is fused to form nuclear pores or diaphragms. Often, and usually in the large neurons such as large pyramidal (Betz) cells of motor cortex and Purkinje* cells of cerebellum, there is a large prominent, nearly central, nucleolus. Typically, neuronal nuclei lie in the centre of the cell body, and displacement to the periphery may be evidence of pathology. However, the nucleus may be normally eccentric in some neurons such as in the cells of Clarke's column of the spinal cord and in neurosecretory cells of the hypothalamus. Nuclei may also seem to be displaced by perikaryal accumulation of lipofuscin pigment and

*In view of its common usage the spelling Purkinje has been retained instead of the more correct form, Purkyně.

a b

Fig. 1.1 Normal neurons. (**a**) Anterior horn cell from lumbar spinal cord. Note the central round nucleus, prominent nucleolus, and Nissl bodies in the perikaryon extending into dendrites but absent from the axon hillock and axon. Thionin (Nissl's method) × 550. (**b**) Cerebellar cortex showing two Purkinje cells with their dendrites extending up into the molecular layer. Axons from basket cells surround the cells and pass up the dendrites. Bielschowsky silver impregnation × 400.

in normal cells of the substantia nigra or locus ceruleus where there is abundant perikaryal neuromelanin. In small neurons such as cerebellar granule cells the nucleus forms the major constituent of the cell body. The sex chromatin, characteristically larger in females, was described in nerve cells of the cat where it forms a juxtanucleolar mass (Barr and Bertram, 1949). In other species, including man, it may be seen as attached to the internal aspect of the nuclear membrane.

Perikaryal constituents vary in amount and in proportion among neurons but most elements are common to all nerve cells. As in other cell types actively synthesizing protein, neuronal perikarya are rich in ribosomes (composed of ribonucleic acid) which may be grouped in polysomes or arranged along stacks of membrane in parallel array (the rough endoplasmic reticulum) forming Nissl bodies or granules (Fig. 1.1). These granules are readily visible with light microscopy and are stainable with haematoxylin and with basic aniline dyes such as cresyl fast violet or thionin which also stain the nucleolus. The presence and distribution of Nissl granules throughout the perikaryon or the more diffuse basophilia produced by free ribosomes is of importance because abnormalities in these components of the cell occur under pathological conditions. Normally ribosomes are absent from the axon but they are found in dendrites.

In addition to the stacks of organized rough endoplasmic reticulum there are other perikaryal organelles which include arrays of agranular (smooth) endoplasmic reticulum, often lying close to the nucleus and forming the Golgi apparatus, abundant mitochondria, smooth as well as coated vesicles, multivesicular bodies, neurofilaments and neurotubules (Fig. 1.2). Lipofuscin granules and, in specific sites, neuromelanin granules are other normal constituents. The normal electron microscopic structure of nerve cells has been reviewed by Peters, Palay and Webster (1976). With light microscopy the use of silver impregnation techniques in either paraffin or frozen sections adds very greatly to the extent of neuronal visualization. Neuronal processes and perikarya rich in neurofilaments are readily stainable in paraffin sections by methods such as those of Palmgren (1948) and Marsland, Glees and Erikson (1954), while the Golgi silver technique (as used and modified by Valverde, 1970, and Ramon-Moliner, 1970) in thick sections gives the most extensive picture of the neuron and is particularly valuable in the assessment of the size of the dendritic arborization and the density of dendritic spines.

The density of the perikaryon varies considerably among neurons, with both light and electron microscopy, giving rise to the terms 'large light' and 'small dark' cells. This is particularly apparent in sensory ganglia where the large light cells have prominent well-ordered stacks of rough endoplasmic reticulum forming Nissl granules widely separated from each other by cytoplasm containing abundant neurofilaments and microtubules and dense organelles (see Lieberman, 1976). Small dark cells have more closely packed ribosomes, mostly free or in packets, and few well-organized Nissl granules. The cells of autonomic ganglia differ in many respects from neurons of the CNS. A comprehensive account of structure and function of adrenergic neurons has been given by Burnstock and Costa (1975).

Synaptic morphology was reviewed by D.G. Jones (1975) and by Peters, Palay and Webster (1976) and can, for convenience, be divided into symmetrical types in which densities are present on both pre- and postsynaptic membranes and asymmetrical where the postsynaptic density is the more prominent. The characteristic organelles of the axonal terminal are synaptic vesicles (Fig. 1.3) which are agranular (clear) or dense-cored in type. Agranular vesicles may be spherical with a diameter of 40–50 nm or somewhat flattened or ellipsoidal, depending to some extent on the type of fixative used. The association of flattened vesicles with symmetrical synapses and their neurophysiological correlates points towards an inhibitory function in these synapses while the spherical vesicles are the preponderant type in excitatory synapses as at the neuromuscular junction. Dense-cored vesicles of 40–60 nm diameter are considered to be sites of storage of noradrenaline. Larger dense-cored vesicles of 80–90 nm diameter are found throughout the central nervous system and in peripheral autonomic fibres but their functional correlate is uncertain. Other constituents associated with the transmitter function of neurons include neurosecretory vesicles which are much larger than other synaptic vesicles, up to 300 nm in diameter, and are found in the neurons of the supraoptic and paraventricular nuclei and their terminals in the neurohypophysis. At a light microscopic level these vesicles correspond to the stainable

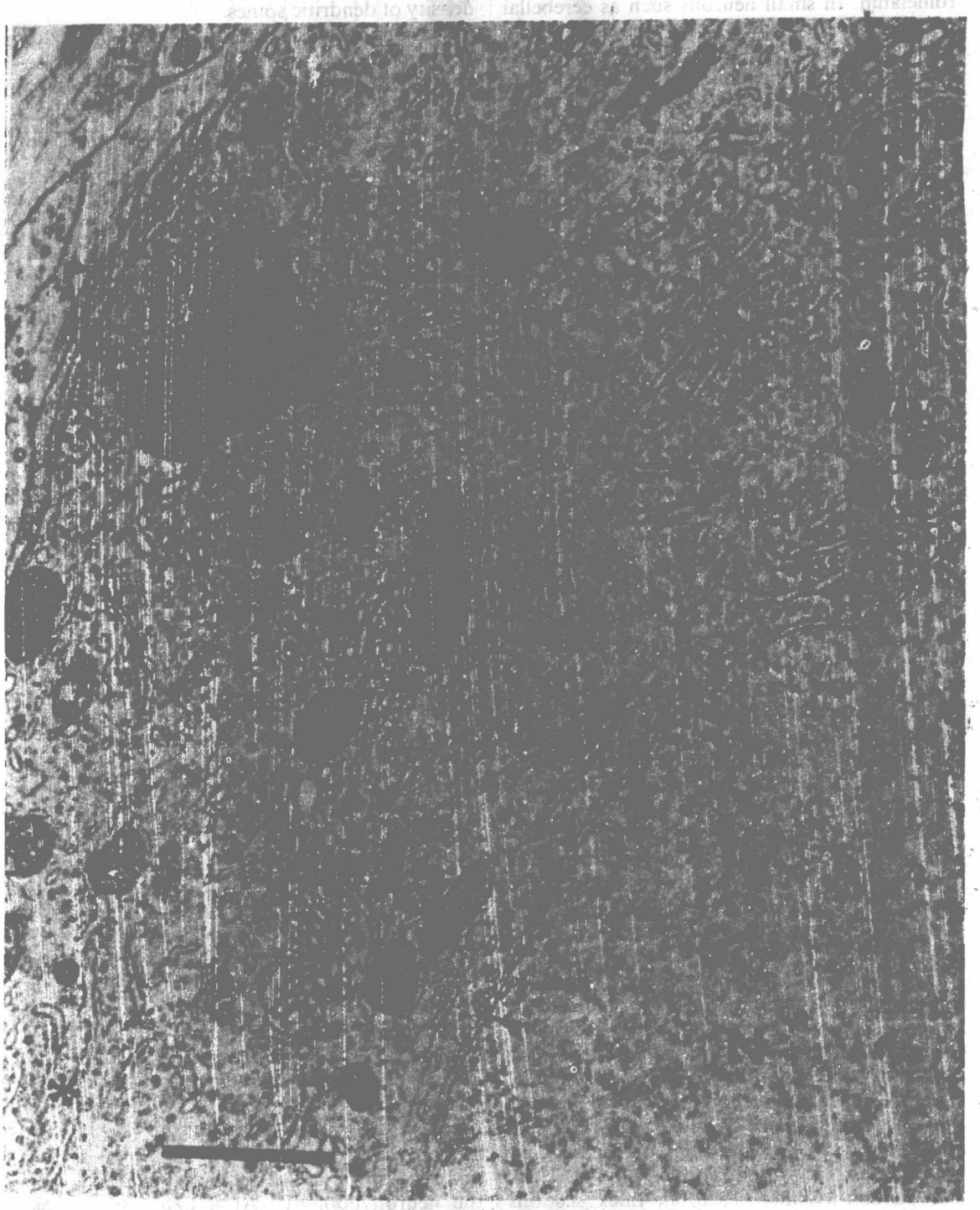

Fig. 1.2 Electron micrograph of part of an anterior horn cell of the spinal cord of a normal rat. The nucleus (N) is bounded by a double membrane which is fused at the nuclear pores (arrowheads). In the perikaryon the parallel arrays of rough endoplasmic reticulum (✱) correspond to the Nissl bodies seen by light microscopy. There are abundant polysomes (small groups of ribosomes), mitochondria (m) and cisternae of smooth endoplasmic reticulum forming the Golgi apparatus (G). Occasional microtubules and one lipofuscin inclusion (L) are also shown. Bar = 1 μm. (Preparation by Dr Jean M. Jacobs).

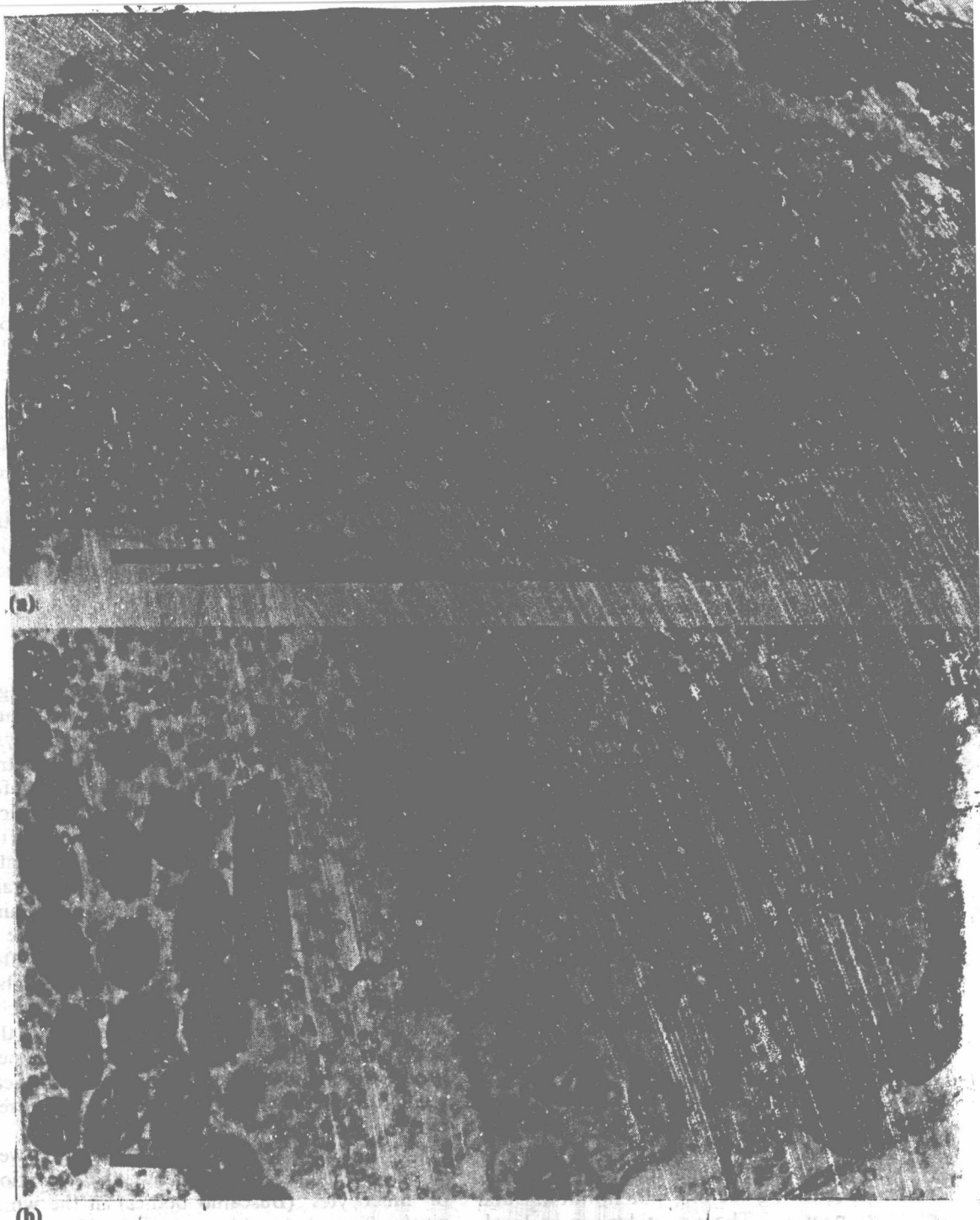

Fig. 1.3 Electron micrographs showing nerve terminals and synapses. (**a**) Several axonal terminals are seen containing synaptic vesicles which have a varied morphology. Some are round and clear, others are round and have a dense core. Clear but elliptical vesicles are also shown. One synapse (arrow) on the perikaryal membrane of an anterior horn cell has an associated flattened cisterna lying close to the postsynaptic membrane. Bar = 0.5 μm. (**b**) In the cerebellar granule cell layer a large axonal terminal (A) of a mossy fibre contains abundant mitochondria and clear rounded synaptic vesicles. Several dendritic profiles (D) and synapses (arrowheads) are shown. In some there is a prominent postsynaptic density but in others pre- and postsynaptic densities are of similar thickness. Bar = 0.5 μm. (Preparations by Dr Jean M. Jacobs)

neurosecretory material demonstrable by the Gomori chrome alum haematoxylin stain and contain the hormones oxytocin and vasopressin and their carrier proteins.

A great deal of interest attaches to the functional relationships between vesicles, neurotransmitters and the mechanism of transmitter release from nerve terminals. Much discussion has centred on the problems as they present at the neuromuscular junction, where the physiological studies of Katz and his collaborators established the quantal nature of transmitter release (see Katz, 1969). The question as to whether this release is by exocytosis from vesicles or by some other mechanism controlling acetylcholine release from a cytoplasmic pool has been discussed by Israel, Dunant and Manaranche (1979) and by Ceccarelli and Hurlbut (1980). It seems that although a proportion (estimated at about 40 per cent) of transmitter is present within vesicles and that an exocytotic process does occur, it is by no means certain that quantal transmitter release is by exocytosis or is derived from vesicles.

The correlation between neuronal structure, the identification of neurotransmitter function of identified neurons and their pathology is still a new field (see Iversen, 1982). The putative transmitters include gamma-aminobutyric acid (GABA), glycine, glutamic acid, acetylcholine, dopamine, noradrenaline, adrenaline, 5-hydroxytryptamine and histamine. Methods for the localization of these transmitters, particularly in nerve terminals, may rely more on identification of synthesizing enzymes such as glutamic acid decarboxylase, choline acetyltransferase or tyrosine hydroxylase than of the transmitter substance itself. Other hormone-like, rather than transmitter, substances include oxytocin and vasopressin, while recently identified neuropeptides seem likely to act as modulators of neuronal activity rather than as neurotransmitters and include the opioid peptides, substance P, hypothalamic releasing factors, gut hormones and pituitary peptides. These peptides can be localized by immunocytochemical methods (see Chan-Palay and Palay, 1982; Gaspar et al., 1983; Pearson, 1983) which can also be applied to receptor localization. Clearly, methods for the identification of transmitters are set to make great contributions to the understanding of neurological disease.

The use of monoclonal antibody techniques, developed by Köhler and Milstein (1975), has greatly facilitated the exact localization of antigens in the nervous system and these methods are now being applied to the identification and characterization of cells, both neuronal and glial. Neuronal cell markers, localizable by immunocytochemical techniques (fluorescence or immunoperoxidase) include neurofilament polypeptide (Anderton et al., 1980; Yen and Fields, 1981), and the antibodies A2B5 (Eisenbarth, Walsh and Nirenberg, 1979), A4 and 38D7. Other methods for the identification of neurons are tetanus toxin binding and the visualization of neuron-specific enolase. These cell markers are already making great contributions to neurobiological research, particularly for identification of cells in tissue culture, and their applicability to problems of human neurological disease is apparent (see Kennedy, 1982).

Artefact

Artefact occurs all too readily at various stages of neurohistological preparation and may cause errors of interpretation. *Post mortem* autolysis seems to be less of a problem in the central nervous system than in other viscera, at least for light microscopy, and reasonable histological staining can be obtained even several days after death, provided refrigeration has been adequate. The autolytic changes which develop after brain death has occurred but with the patient maintained with life-supporting systems may be a major obstacle to useful histological study. After only 24 hours on a respirator, changes resemble hypoxic cell damage. Longer periods on artificial ventilation after brain death lead to failure of the brain to become adequately fixed and hardened in formalin and renders detailed histological study useless. The 'respirator brain' is considered in more detail in Chapter 4.

Other artefactual changes have been reviewed by Blackwood (1976) and include the formation of 'mucocytes' (Buscaino bodies) in the white matter. These 'metachromatic bodies' were studied by Smith (1949). They occur only in some cases, and are seen in frozen sections as well as paraffin- and celloidin-embedded tissue. Separation of cell layers from each other is also artefactual and affects particularly the granule cell and molecular layers of cerebellum and the hippocampus in paraffin-embedded blocks. These are probably akin to the perineuronal and

perivascular spaces which appear in paraffin sections and are shrinkage artefacts occurring during tissue processing. They can be partially avoided by prolonging the duration of each stage of dehydration, clearing and impregnation with paraffin wax and by celloidin embedding. Fixation artefact is also probably the cause of 'selective granule cell necrosis' in which the granule cell layer of cerebellar cortex appears pale grey or whitish macroscopically and the cells fail to stain adequately. The exact cause is not known, perhaps being autolytic, but it is a very rare occurrence when brains are fixed in a stronger formalin solution (20–30%) than in general use and the volume of formalin relative to brain is adequate (i.e. 6 litres or more of formalin).

Shrunken dark neurons have been clearly demonstrated to be artefactual (Cammermeyer, 1960, 1961; Brown, 1977) and are caused by pressure on unfixed brain tissue. They are irregular in outline, the apical dendrite has a twisted, corkscrew-like appearance, the nucleus and cytoplasm stain darkly and Nissl bodies cannot be identified (Fig. 1.4). There may also be a perinuclear halo. The presence of dark neurons is often a problem, particularly in biopsy specimens of cortex and in experimental animal material in which brain tissue is handled in the fresh state and subjected to pressure. The artefact can be prevented or at least reduced by minimizing handling of the brain when fresh and unfixed. In experimental animals the use of perfusion-fixation and leaving the brain untouched in the skull for several hours before removal helps to prevent artefact.

Artefactual changes are easily induced during removal and handling of the spinal cord, which, if bent or kinked sharply before fixation, may develop swellings which may resemble intrinsic tumours. The cord should therefore not be subjected to pressure and should be kept as straight

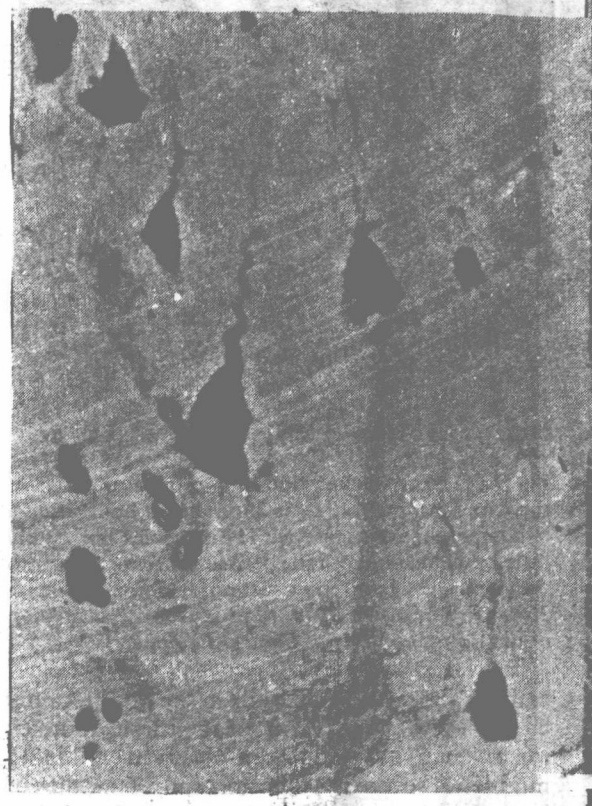

Fig. 1.4 Artefactually shrunken neurons near the cut edge of a cortical biopsy. These cells are darkly stained and the apical dendrite has a twisted corkscrew-like appearance. Haematoxylin and van Gieson × 400.

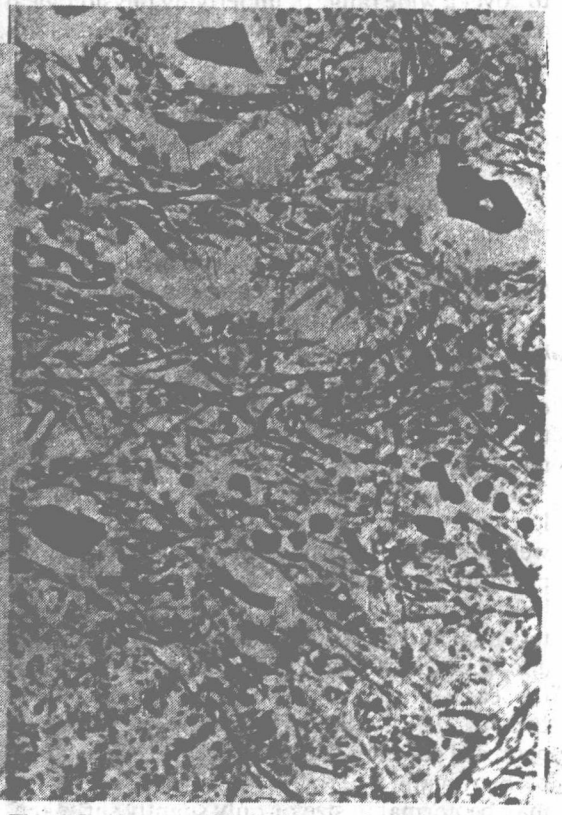

Fig. 1.5 Argyrophilic bodies in the spinal cord are shown in grey matter close to several anterior horn cells. Paraffin section of lumbar spinal cord of a patient without neurological disease. Glees silver impregnation × 160.

as possible until placed in fixative in a suitable container.

Argyrophilic bodies in the spinal cord

Argyrophilic bodies (Fig. 1.5) up to 30 μm in diameter are a constant finding in the anterior horns of the grey matter of the spinal cord in adults (Smith, 1955). Their nature is uncertain but because of their ubiquity they are not considered to be indicative of any specific pathology in spite of their resemblance to axonal retraction balls.

General pathology of the neuron

Hypoxic neuronal damage

One of the commonest forms of injury to neurons is that caused by hypoxia, this term being used to cover a wide range of underlying physiological disturbances such as arrested or impaired blood flow, reduced oxygen tension in circulating blood or toxic factors. Furthermore, because of the dependence of the neuron on glucose as its main metabolic substrate, hypoglycaemia of whatever cause can induce morphological changes similar to those caused by hypoxia due to the neuron's inability to utilize oxygen (Lawrence, Meyer and Nevin, 1942; Meyer, 1963). The mechanisms of these disturbances are discussed in Chapter 4.

The early neuropathological literature referred to Nissl's acute cell or severe cell disease but these terms have been superseded. For details of the older terminology the reader may refer to the previous editions of this book or to Spielmeyer (1922).

The sequence and time-course of events following hypoxic injury to the neuron have been well defined in experimental animals. The use of perfusion-fixation and delayed removal of the brain has allowed the differentiation between artefactual and significant pathological alterations and in particular has facilitated the definition of the early stages of hypoxic cell damage (see Brown, 1977). The earliest stage of ischaemic neuronal pathology was named *microvacuolation* by Brown and Brierley (1966). The cell (Fig. 1.6) may be normal in size or only slightly shrunken, and the nucleus also only slightly shrunken while the perikaryon is vacuolated. The majority of vacuoles (which are up to about 2 μm in diameter) are swollen mitochondria, while some may be due to dilatations of endoplasmic reticulum or of other intracytoplasmic organelles (Fig. 1.7). Microvacuolation has been identified in hippocampal and cortical neurons in the rat after only 5–15 minutes of hypoxia. In the rhesus monkey microvacuolation has been observed within 15 minutes of severe hypotension and 30 minutes of hypoglycaemia, and Brierley (unpublished) has identified the changes in the brain of a human case dying 1 hour after cardiac arrest. Microvacuolation can, however, only rarely be identified in man because of early autolytic changes. Microvacuolation occurs earlier and seems to persist for a shorter time in small neurons than in

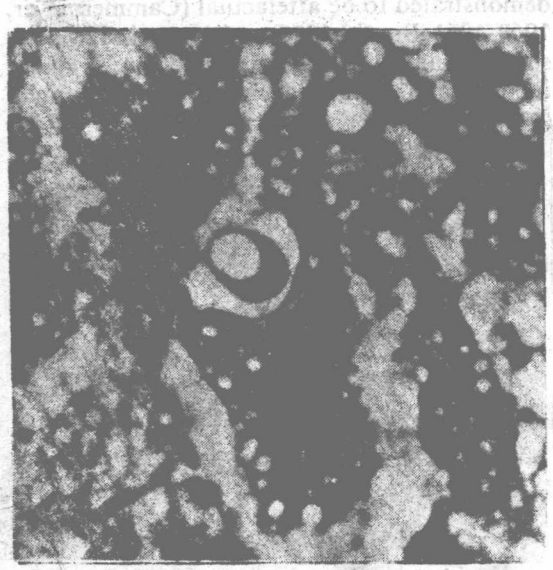

Fig. 1.6 Microvacuolation in pyramidal neurons of the hippocampus of a rat surviving 1 hour after a hypoxic-ischaemic episode. The vacuoles are evenly distributed throughout the perikaryon, extending into the dendrites. 1 μm epon section. Toluidine blue × 2000. (From Brown, 1977. Reproduced by permission of the Editor, *Journal of Clinical Pathology*).

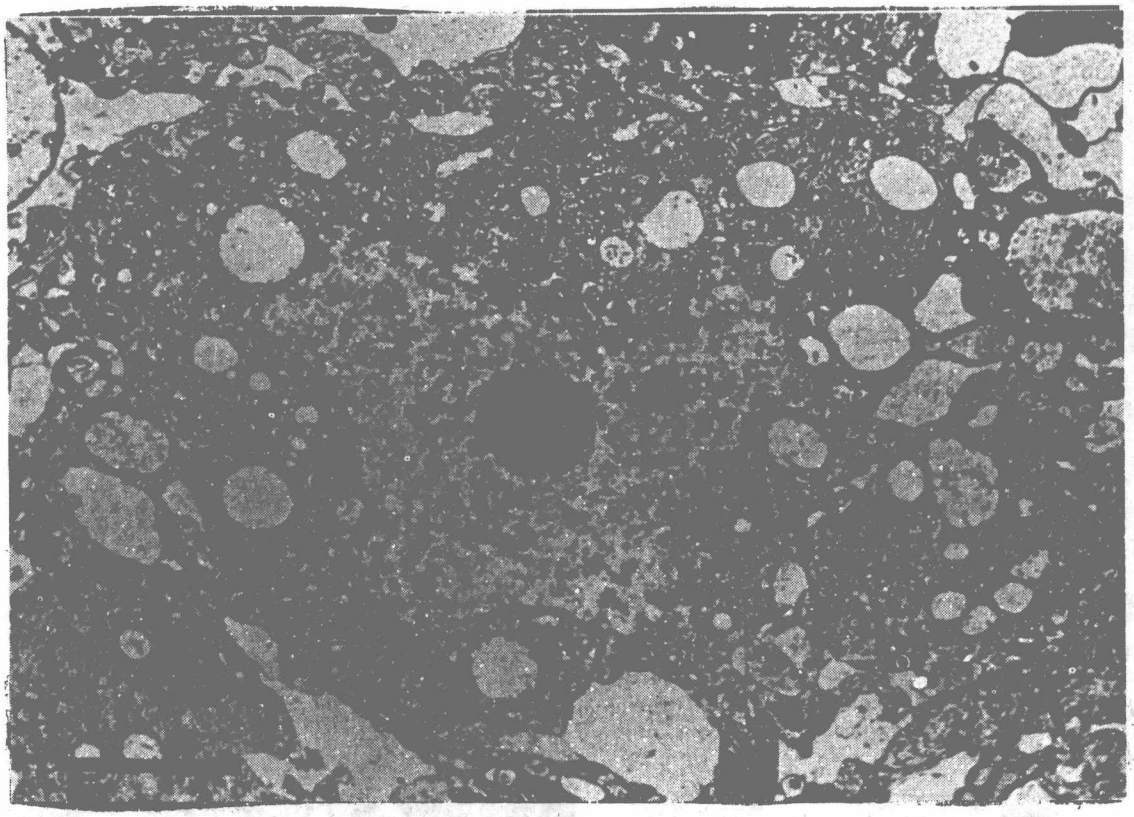

Fig. 1.7 Electron micrograph showing microvacuolation of a pyramidal cell of the hippocampus of a rat as in Fig. 1.6. Swollen mitochondria with disorganized cristae lie within a cytoplasm of increased electron density. The nucleus is irregular in shape. Bar = 5 μm. (Preparation by A. W. Brown).

large in which it has been seen for up to 4 hours after the ischaemic-hypoxic insult.

The stage termed *ischaemic cell change* (Fig. 1.8) develops as a gradual transition from the stage of microvacuolation to one in which the neuron is shrunken, staining darkly with cresyl violet, its cytoplasm becoming markedly eosinophilic and containing finely granular dispersed Nissl substance. The nucleus is also shrunken and often triangular and darkly staining, and the nucleolus may be eosinophilic. Luxol fast blue stains nucleus and cytoplasm bright blue to dark mauve. Electron microscopy has shown increased electron density of the cytoplasm which contains degenerating but usually identifiable organelles and remnants of microvacuoles. A later stage in the ischaemic cell process is the appearance of *incrustations* which stain darkly in most preparations (Fig. 1.9), and are seen on the perikaryon and dendrites. Electron microscopy has shown incrustations to be electron-dense profiles of neuronal cytoplasm, including some derived from the dendrites, formed into projections from the cell surface which is indented and distorted by clear swollen astrocytic processes (Fig. 1.10). Ischaemic cells with incrustations are found as early as 30 minutes in the rat and 90 minutes in the monkey after the hypoxic episode, and they may persist for 48 hours. As Greenfield pointed out (1958) the cells with incrustations are haphazardly distributed, in some areas almost every cell being affected, while in other cases or in other areas in the same case none is seen, although there may be other evidence of hypoxic damage.

A later stage of neuronal morphology observed after a hypoxic ischaemic insult is *homogenizing cell change* (Fig. 1.11). This was first described in Purkinje cells by Spielmeyer (1922) who considered that it was not necessarily due to ischaemia since he observed it in association with infectious and toxic disorders, and Greenfield (1958) believed it to be due to a less severe or less