GREENFIELD'S
NEUROPATHOLOGY

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PREFACE

Neuropathology was founded at the end of the last and the beginning of the present century by scientists of distinction among whom Franz Nissl and Santiago Ramon-y-Cajal were pre-eminent. The body of knowledge which they and many others, especially Charcot and his colleagues and pupils, have handed down is a precious heritage. Most of it has stood the test of the intervening years and, although modern technical advances have added many details, few fundamental errors have been discovered in the older work. Many of these early studies were remarkably complete and little has been added to some of them in more than half a century.

The pioneers of neuropathology were led on by the hope that the study of the diseased brain would lead to the interpretation of all disorders of action and conduct. Some, who found this quest unrewarding, were diverted to the more hopeful approach offered by psychopathology. But those who have followed the older discipline have been encouraged by seeing the return of a more organic conception of many disorders of behaviour. Each lobe of the brain has now its own symptomatology and the relation of hypothalamic, thalamic and rhinencephalic centres to emotion and emotional expression is becoming better understood. The pathology of the basal ganglia and brain stem has also been explored during the past fifty years, but physiological research has not yet clarified the relation between symptoms and lesions in that area of the brain.

Since many of the classical monographs of neuropathology are little known and few are available in the English language, more attention than usual is given in this book to the history of the subject and some sections are largely based on the original descriptions. To recall the names of the founders of neuropathology and the steps by which the science was built up has more than a pious interest since the principles of scientific advance remained unchanged.

The rapid progress of scientific technique is now challenging the older disciplines and not least histopathology. The advent of the electron microscope and of cytochemical and enzyme stains have called for a complete review of the older cytology which was based on the light microscope and traditional staining methods. Already knowledge of the structure of the neurone has been greatly extended and many of its changes under experimental conditions explored by these new techniques. Their application to human pathology has scarcely begun but offers an attractive field in which many advances are likely to be made in the near future. These new explorations must start from a base of existing knowledge and the provision of this is one of the objects of this book. In preserving and presenting in the English language what has been discovered by the established methods the authors hope not only to assist research but also to give the neurologist and psychiatrist that basis for diagnosis and logical treatment which only pathology can supply.

In a book compiled by several authors some overlapping and restatement have been unavoidable. In some places there may be difference of emphasis, but the editor considers this preferable to a dogmatic presentation of one view-point.

As the large subject of cerebral and spinal tumours is treated in a companion volume edited by Professor Dorothy S. Russell, it is omitted from this book.

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PREFACE TO THE SECOND EDITION

Dr. Greenfield did not live to see the publication of the first edition of this book, which crystallises so much of his unrivalled experience at Queen Square. The loss of his guiding hand has been acutely felt in the preparation of this second edition but it has been the purpose of his original colleagues, now joined by Professor Dorothy Russell, to try to maintain the standards that he would have demanded.

Our chapters have been brought up to date, bearing in mind that this textbook is intended as a balanced account of the subject for senior students of neuropathology. The considerable amount of new information has been offset to some extent by the elimination of less important references and by some rearrangement of the subject matter to obviate undue repetition. Many new photographs have been added and a more useful index provided. Mrs. Greenfield's help in preparing the latter is greatly appreciated.

The difficult task of revising Dr. Greenfield's own chapters has had to be faced, but so much of his fundamental teaching remains valid that in most instances only some amplification has been needed. In the section on demyelinating diseases, however, so many new observations have accumulated during the last five years that parts have had to be substantially rewritten in the interest of clarity and continuity of style.

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R. M. NORMAN

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CHAPTER 1

GENERAL PATHOLOGY OF THE NERVE CELL AND NEUROGLIA

By J. G. GREENFIELD and A. MEYER

Introduction

The nervous system consists almost entirely of the cell bodies and processes of neurones. The science of neuropathology is therefore primarily concerned with the various methods by which neurones undergo degeneration and death. But since the neurones are fed by blood-vessels and are bound together by neuroglial and collagenous connective tissues, neuropathology must take account of changes in these tissues as also in the meningeal coverings of the brain and spinal cord. These may be more obvious than the changes in the neurones. For example, in inflammatory diseases, cellular proliferation may hide or mask the changes in the neurones which can only react by degeneration or death. It is true that in certain virus diseases the degenerative changes which the neurones undergo may be quite unusual and almost pathognomonic. But these changes are usually less evident than the 'inflammatory' and cellular proliferation and may need special staining methods for their demonstration. In many degenerative diseases also the overgrowth of astrocytes and proliferation of their fibres may be more striking than the disappearance of nerve cells or their axons. For this reason the term 'sclerosis' with various descriptive adjectives such as 'primary lateral', 'cortical' or 'lobar' has been given to processes which consist essentially in degeneration of certain neurones as a whole, and with the prefixes 'multiple', 'disseminated' or 'diffuse' to degenerations in which the myelin sheath is most affected. It was for a time thought that the neuroglial sclerosis in some of these diseases was primary and that the axons or myelin sheaths were strangled by the contraction of newly formed glial fibres. Similarly, before the virus etiology of poliomyelitis was discovered it was thought by some that the motor nerve cells were compressed and destroyed by the cellular exudate which invaded the ventral horns of the spinal cord. At the present time it is considered that the proliferation of neuroglial cells is almost always either a reaction to the degeneration of neurones or a concomitant reaction to the noxa to which this is due.

However much the study of the reactions of the vascular and conjunctival tissues may help towards our understanding of the pathogenesis of nervous diseases, it should never be allowed to supplant the study of the changes in the neurones themselves since it is to these that the symptoms of disease are due. This study has two chief objectives, first the *topography* of the lesion and secondly its *nature*. The relative importance of these two objectives varies in different conditions; when the nature of the process is evident the study of its topography may be more important and vice versa. But in most diseases the two objectives are complementary and both are essential.

In the early days of neuropathology the great French and German schools were distinguished by the emphasis laid on one or the other of these objectives. The French genius for anatomy was reflected in the School of Charcot for which the distribution of the lesions in relation to nuclei and tracts had special importance. When the lesion was focal, or confined to a special system or related group of systems, it could be correlated with the symptoms observed during

life and thus provide evidence of the function of tracts or nuclei in the human brain. Such clinicopathological studies formed an important part of French neurological literature during the latter part of the nineteenth and the earlier part of the twentieth centuries. Many observations contributed in this way, for example on the sensory tracts and nuclei and on various aspects of mental activity, especially aphasia and apraxia, cannot, by their nature, be confirmed by animal experiment. Some have been confirmed by neurosurgery; others still await full confirmation; but the influence which studies of this kind have had on clinical neurology has been far reaching and profound.

On the other hand the German school of Weigert, Nissl and Alzheimer was specially interested in the alterations which the neurones and neuroglial cells underwent in disease and in the interpretation of these changes in relation to etiology and pathogenesis. The experimental studies of these scientists and of their pupils, especially Jakob and Spielmeyer, many of which were published in the Nissl-Alzheimer Arbeiten during the years 1904–20, form a wide and for the most part solid foundation for the structure of neuropathology. It was unfortunate that Alzheimer's work on the neuroglia was considerably invalidated by his failure to distinguish the different forms of cell included in this tissue. This distinction was later made in Madrid by Ramon-y-Cajal and his pupil del Rio Hortega, whose description and nomenclature is now universally accepted, although all do not entirely agree with their interpretations of the functions of these cells. This method of study has a less immediate relation to clinical neurology, but it is fundamental to the understanding of diseases of the nervous system. It is the object of present-day neuropathology to integrate these two disciplines, and while retaining the careful study of the topography of diseases, to seek to advance ever further knowledge of the pathology of the individual cells which constitute the nervous system.

This twofold quest of neuropathology imposes a heavy task on those who undertake it, since they must be not only pathologists but also anatomists and to some extent clinical neurologists and psychiatrists. A neuropathologist must know the history and details of the patient's illness before he makes his examination, otherwise he will often fail to examine some part of the nervous system. This applies most obviously to such outlying parts as the optic nerves and retina, the internal ear, and the dorsal root ganglia and peripheral nerves, but it happens too often that no examination is made of the spinal cord even in cases with signs of paraplegia or ataxia.

This combined aim of modern neuropathology has resulted in a technique which is far removed from the simpler methods in every day use for the other tissues of the body. In general, paraffin embedding is not so suitable for nervous tissues as either celloidin or frozen sections, owing to the greater shrinkage of the tissues and more complete removal of lipids during the process of embedding. Post-mordanting with mercury or chrome salts may reduce these disadvantages, but restricts the choice of staining methods. Very large paraffin sections are also less easily handled than celloidin sections of similar size. In some laboratories skill in dealing with large frozen sections had made their use the method of choice for many techniques. More general is the use of smaller frozen sections both as a preliminary to further examination and for special staining methods such as fat stains and silver impregnation. But celloidin embedding remains, in many laboratories, the most popular routine method. Its relative freedom from shrinkage and other artefacts, the greater retention of lipids, even without post-mordanting, and the wide range of staining processes available for celloidin sections offset the disadvantage of slowness and expense.

Not only does neuropathology demand a technique different from that of general pathology but it also uses a different language. This is largely inevitable in so far as it deals with quite different tissues. The nerve cell, nerve fibre, myelin sheath and neuroglial cell have characters

of their own and undergo processes of degeneration which have no counterpart in other tissues. Therefore some terms used in general pathology such as 'cloudy swelling' and 'hyaline change' are inapplicable to changes in neurones. The term 'cloudy swelling' is often applied to the very characteristic changes in the cell body which follow section of the axon, but it would be equally applicable to Nissl's *acute swelling*, to the swollen cells of Pick's disease and of Lissauer's paresis, and to the distension of the nerve cell with lipid which occurs in the various cerebrospinal lipidoses. Such attempts to bring neuropathological terminology into line with the much less exact terminology of general pathology can only result in confusion. The old names 'central chromatolysis' and 'Wallerian degeneration' have, through long study, acquired a very precise meaning, and indicate a method of reaction to injury which is fairly well understood. The meaning of other terms used in neuropathology is less definite. For example the terms 'demyelination' and 'demyelinating disease' are often used for conditions in which the nerve fibre is damaged on its course through the white matter of the brain, although in some of these conditions the damage to the myelin may not be much more severe than that to the axons. It is often also convenient or even necessary to postulate, from what is seen by a general cell stain, changes which can only be fully proved by the use of special techniques. For example a phagocytic cell laden with the products of myelin katabolism may be recognised in a section stained by haematoxylin and eosin and the name 'fat granule cell', instead of the more descriptive term 'foamy cell', may be given to it, although the constitution of the granules can only be decided by the use of special stains for lipids. Similarly some forms of neuroglial cell can only be recognised by their nuclei when general cell stains are used, but the terms 'astrocytic proliferation', 'Alzheimer type of astrocyte' and 'hyperplasia' or 'multiplication of microglia' may be quite justified by such an examination alone.

Thus the value of special techniques in neuropathology is, for the most part, rather to make more evident and more definite what can be faintly discerned by more ordinary staining methods, than to discover structures or abnormalities which are otherwise invisible. In many cases, however, their use is obligatory. The extent and degree of demyelination, of damage to axons or neurofibrils, of nerve cells and of fibrous gliosis can only be judged by the use of special techniques, and such changes as neurofibrillary tangles in nerve cells and senile plaques are difficult to see without the use of silver impregnations. In this way some techniques come into routine use while others are reserved for special cases.

In the past neuropathology has suffered through being tied to too few special technical methods. For example, Nissl's stain has, in many laboratories, been the only cytological method in routine use, and details which are more easily visible by haematoxylin and eosin or other general stains have thereby been missed. In recent years there has been a trend in the opposite direction, and polychrome stains, and some special histochemical methods, such as those for amyloid and polysaccharides have proved to have a special value when applied to the nervous system.

The rapid growth of neurochemistry and the development of ultramicroscopes imposes a heavy task upon the neuropathologist who will be responsible for integrating these developments with established techniques. Since a single worker cannot hope to acquire expert knowledge in all these new fields, arrangements must be made to include trained specialists in the neuropathological laboratory. Many neuropathologists now appoint biochemists in their departments, where also the electron microscope is no longer an unfamiliar sight. Such teams will succeed, however, only to the extent that the specialists have a basic understanding of each other's provinces.

In a sense, the old-established techniques of neuropathology have always been histochemical, although often based more on empiricism than on scientific principles. Thus

Nissl's method is founded on the well-known affinity of acid proteins for basic dyes. Weigert's use of haematoxylin to stain myelin is not very different from Baker's stain for phosphatides, and the osmic acid techniques are based upon the reducing power of unsaturated fatty acids on osmium tetroxide. On the other hand, the various staining methods for glial fibres have been developed almost entirely from empirical observations by such masters as Carl Weigert and Frank B. Mallory. A similar empiricism appears to have guided the Spanish school as well as Golgi and Bielschowsky in their use of silver stains.

Modern histochemical techniques aim less at staining structural elements than at localising and making biochemical activities visible within the cells and their organelles. Numerous methods have been developed to visualise the activities of enzymes such as the cytochrome oxidases, dehydrogenases, specific and unspecific phosphatases, and cholinesterases, to quote only a few. Most of these techniques are still in their experimental stage, and their present limitations as well as their potentialities have been emphasised by workers experienced in this field (Roizin 1955; Potanos, Wolf and Cowen 1959; Feigin and Wolf 1955; Koelle 1955). Moreover, they usually require tissue near the living state, sectioning before fixation, freezing–drying techniques etc.: conditions which, with the exception of biopsies, are not obtainable in ordinary neuropathological work. This applies also to certain procedures which are not histochemical in a strict sense, but are, nevertheless, of considerable interest to neuropathology: such as, for example, differential centrifugation (introduced by Bensley and Hoerr, 1934) and the bold microchemical analyses on microdissected single neurones and glial cells which have been carried out by Lowry (1957) and Hydén and Pigon (1960).

Among the ultramicroscopes, Caspersson's ultraviolet histospectroscope, small angle X-ray diffraction and the electron microscope appear to be the most promising. Microscopy in ultraviolet light increases magnification to the extent, for example, which includes wavelengths of 2,600 Å, at which absorption of ribonucleic acids of the Nissl bodies takes place. X-ray diffraction gives valuable information of the atomic structure of molecules; although its data are not 'histological' in a narrow sense, it has nevertheless proved of considerable value in comparison with electron microscopic findings. On the other hand, the electron microscope is very much in the histological tradition: fixation, often by perfusion, is carried out in osmium tetroxide or potassium permanganate. 'Staining' with phosphotungstic acid increases the contrasts. After dehydration, the tissue is embedded in substances such as methacrylate or araldite and eventually cut by specially constructed microtomes provided with glass knives which allow sectioning to the required thinness of approximately 0.1 μ. The resolving power is so high that useful magnification is possible up to $\times$ 1,000,000, though the most frequent magnification for nervous tissue is well below $\times$ 200,000. Even so, the prospect offered is stupendous and brings into the range of observation most of the larger and medium-sized molecules and viruses. As Roizin (1960) has pointed out, we are moving fast from the era of cellular pathology to that of molecular pathology. The very magnitude of this advance naturally evokes many initial difficulties and divergencies of interpretation and, although certain facts have been established (as will be described presently), it will be some time before the new ultramicroscopic as well as cytochemical developments become an assured province of neurohistology.

THE NEURONE

Normal histology

The neurone is the most complex cell in the body and the alterations which it may undergo in disease are similarly complex. These are manifested histologically by a number of alterations, in shape, staining reactions and even in structure, which cannot be interpreted without

a more intimate knowledge of the composition of the neurone than we possess at present. Certain typical histological alterations have, however, been recognised for many years to be the results of certain forms of lesion or disease, and the metabolic changes which lead to some of these histological changes are beginning to be understood. These are essentially problems in histochemistry which may soon be clarified by the rapid advances which are taking place in this subject.

In the central nervous system the cell bodies or perikarya of the neurones are for the most part grouped either in layers, as in the cerebral and cerebellar cortex, or in irregular collections of similar cells termed 'nuclei'. In the brain and cerebellum there is a 'cortex' containing several layers of the bodies of nerve cells and a dense mass of their dendrites, and a 'white matter' which contains only nerve fibres. The cerebrum also contains large central nuclei of grey matter or basal ganglia. The cerebellum has basal nuclei in which its efferent fibres are relayed. In the spinal cord the 'grey matter' containing the nerve cells forms a central H-shaped column which is surrounded by white matter. In the brain stem grey and white matter are less separable, but various nuclei, such as the substantia gelatinosa of the medulla, the substantia nigra and the nuclei in the floor of the 4th centricle, consist chiefly of grey matter. Other nuclei, such as the nucleus subthalamicus, nucleus ruber and nuclei pontis, contain a larger admixture of myelinated fibres, whereas in others, especially the reticular formation, the nerve cells are rather loosely scattered in a heavy network of myelinated fibres. Therefore in sections of the brain stem stained for myelin some nuclei are clearly outlined areas containing very little myelin, others are less clearly defined areas with some myelin staining, while yet others can scarcely be outlined at all.

It has been calculated that there are in the brain more than 20,000 million nerve cells. These are of varied form and size. The larger cells in the cerebral cortex and corpus striatum, and those which give rise to the motor nerves in the brain stem and spinal cord, are pyramidal in shape with their axons arising from the base of the cell. This shape is characteristic of efferent cells. The cells of the dorsal root ganglia and those of the mesencephalic root of the trigeminal nerve are spherical in shape; and in the secondary sensory nuclei of the brain stem and spinal cord and the thalamus a more or less oval shape is the rule. Other nerve cells vary between these two extremes, some being more multipolar, others having more rounded outlines.

Bipolar cells are found in the cochlear and vestibular ganglia, the olfactory organ and the retina. The fusiform cells described by Cajal in the auditory cortex are a special form. The shape of the Purkinje cells of the cerebellar cortex is also unusual. They may be described as flask-shaped. The spherical cell body gives off at one pole an axon and at the other a thicker process which breaks up into a frond of dendrites which spreads out across the lamella at right angles to its length.

The size of nerve cells varies with all gradations from very large to very small cells. The largest cells, the Betz cells in the 5th layer of the motor cortex, the large anterior horn cells in the cervical and lumbosacral enlargements of the spinal cord and the cells in the dorsal root ganglia, may measure 80μ in their greatest diameter. The smallest neurones, the granule cells in the cerebellar cortex, and in the retina and olfactory bulb, consist of little more than a rounded darkly staining nucleus, about 5μ in diameter, and an axon which runs for a comparatively short distance among neighbouring cells. Golgi divided neurones into two types. Neurones of his Type 1 have a long axon which passes into the white matter and extends for a considerable distance from the perikaryon. In Type 2 the axon does not leave the grey matter in which the nutrient cell body lies. These terms are only applicable to the largest and smallest cells, and there are many of intermediate type. In the brain stem particularly there are cells of all sizes, the axons of which run for very varied distances from the perikarya.

According to Hydén (1947) in the larger neurones the axon may have 1,000 times the bulk of the cell body and the nutrition of this large process must make great demands on the metabolism of the cell.

The neurone theory

According to the neurone theory which arose from the pioneer work of Cajal, His and Forel in 1889–91, each neurone is a separate cellular entity consisting of a perikaryon or cell body, dendrites or short processes which ramify in the neighbourhood of the cell body, and a longer or shorter axon which passes from the cell body to make contact by means of nerve endings of various kinds either with the dendrites or cell body of other neurones, or with muscle or other effector cells. The primary afferent neurones ot the peripheral nerves differ from this general scheme in having no dendrites; they are connected, by nerve endings at one end of the axon, to the skin or a sensory end organ, and at the other end of the axon make synaptic connection with other neurones; their nutrient cell body lies at one side of the axon, on some part of its course, being attached to it by a short side process. Cajal postulated that the neurone is 'polarised' in the sense that the nervous impulse normally passes from the dendrites to the axon. In this sense he considered that in such receptor neurones as those of the olfactory organ, the retina and the internal ear, the peripheral processes represented the dendrites.

At the present time the neurone theory is accepted by most histologists. A few, however, consider that axons at their terminations blend with the cytoplasm of the cell with which they make contact (Boeke 1950). Others admit that in the sympathetic nervous system there are nerve plexuses in which the processes of different cells blend with one another. The old catenary theory, according to which the peripheral nerve fibre consists of a chain of internodal segments, joined together at the nodes of Ranvier and each nourished by a cell of Schwann, is no longer accepted for the axon, although it is probably true in a more limited sense for the myelin sheath.

Constitution and structure

The nerve cell is one of the very few types of cell in the human body which cannot be replaced if it is destroyed and which does not undergo cell division after the first few weeks of infancy. At the same time it has great metabolic activity, requiring a constant large supply of oxygen and glucose. No nerve cell in the central nervous system can tolerate absence of oxygen for much more than 15 minutes at the normal temperature of the body, and the more susceptible cells in the cerebral cortex cannot survive 5 minutes, or perhaps less, of complete arrest of the circulation. When the metabolic needs of cortical neurones are reduced by lowering the body temperature these times can be considerably exceeded, but the relation between body temperature and the time for which it is safe to stop the heart beating has not yet been fully worked out. Similarly if the level of blood sugar falls to 20 mg. per 100 ml. consciousness is lost and hypoglycaemia at this level for several hours usually entails the death of many neurones in the cerebral cortex. (Lawrence, Meyer and Nevin (1942) point out that low blood sugar estimations, if made by the usual laboratory techniques, may mean much lower true blood sugar values or even complete absence of blood sugar as other substances in the blood may have a reducing power equivalent to that of 20 mg. glucose per 100 ml. It is certain that when the blood sugar is estimated at 20 mg. per 100 ml. no trace of glucose may be found in the cerebrospinal fluid.)

These two characteristics, ability to survive for 100 years or more and great susceptibility to environmental and metabolic disturbances, are associated with an unusual toughness and density of cytoplasm and tenuity of cell membrane. De Renyi (1931) showed by micro-

dissection that if a fresh unfixed frog's nerve cell were stretched until it tore into two halves, there was no escape of cytoplasm from the cell membrane; the torn edges remained irregular and when the two halves were again brought into apposition, the irregularities of one surface fitted more or less exactly into those of the other. The high density of nerve cells has enabled Chu (1954) to study isolated neurones, since they can be differentially sedimented by centrifugalising an emulsion of grey matter from the ventral horns of a rabbit's spinal cord in isotonic fluid with a specific gravity of 1,060. He found that nerve cell cytoplasm behaved like jelly in a bag. The consistency of this jelly was so viscid that no Brownian movement could be seen in it. This toughness may be associated with a high lipid content which is reckoned by Brante (1949) to be about 40 per cent. The lipids are chiefly ganglioside and lecithin. They are present not only in the mitochrondria and Golgi apparatus, but also more widely throughout the cytoplasm of nerve cells, especially in the neighbourhood of the axon hillock where they may become apparent as granules of 'lipochrome' or 'lipofuscin'.

The neurone contains in its cell body and processes various structures. About some of these, we know little. Others have been thoroughly studied and the changes which they may undergo in disease form the basis of neuropathology. As these changes can only be understood by comparison with the normal the various constituents of the nerve cell must first be shortly described.

Nucleus and nucleolus

The nucleus varies considerably in different types of nerve cell and in the same cell type in different mammalian species. The vesicular nucleus with large central nucleolus seen in larger nerve cells appears distinctive, but it contains the same chemical elements as other nuclei, although in different proportions. These are (1) *nucleoplasm* composed of weakly acidophilic protein. (2) *Chromatin* or desoxyribose nucleic acid (DNA) in granules of varying size and fine threads. These stain with basic aniline dyes and are Feulgen positive. (3) *True nucleolus*, which is spherical, more or less central and is composed of a mixture of ribosenucleic acid (RNA) and a basic (acidophilic) protein. It is amphiphilic and Feulgen negative. Its ribosenucleic acid content gives it the same staining and optical properties as Nissl granules, but in virtue of its basic protein it takes the red acid dye in mixtures of eosin, phloxin or acid fuchsin with dyes of more neutral character (Mann, Alzheimer's fuchsin light green, Mallory's and Masson's trichrome stains and their variants). With Unna-Pappenheim's methyl green-pyronin also it stains red while the chromatin stains green. (If the methyl green is impure, it may stain chromatin violet.) The nucleolus often contains one or more 'vacuoles'.

The proportions of chromatin and nucleolus vary considerably in neurones of different types, especially in relation to their size. In the large nerve cells the nucleolus is large and the chromatin is present in small amount being confined to very fine granules under the nuclear membrane and one or two larger paranucleolar granules. At the other extreme the cerebellar granule cells have a small central nucleolus which is covered and partly obscured by abundant chromatin granules. In general, as Olszewski (1947) points out, there is a positive correlation in man between the amount of Nissl substance and the size of nucleolus, and a negative correlation between the amounts of Nissl substance and nuclear chromatin. Most nerve cells have only one nucleolus, although two or more have been described in developing neurones.

In some of the larger nerve cells the chromatin, in addition to fine granules, may form a rounded or oval mass, 0·5–2μ in diameter, which usually lies close to, or in contact with, the nucleolus, but sometimes free in the nucleoplasm or in contact with the nuclear membrane. This *nucleolar satellite* has been shown by Barr and Bertram (1949) to be related to the sex

chromosome and to be larger in the female than in the male in many nerve cells. In the human this sex difference can be well seen in the sympathetic ganglia and cerebral cortex but is not so distinct in Purkinje cells. They call this body the *sex chromatin*.

In the cat, which was chiefly studied by Barr and his associates, the sex chromatin most often lies against the nucleus (61 per cent) but may lie free in the nucleoplasm (15 per cent) or adjacent to the nuclear membrane (6 per cent). In the monkey the sex chromatin granule is most often seen in contact with the nuclear membrane. Lindsay and Barr (1955) distinguish the sex chromatin from the basophil clots described by Levi (1897) in rodents. These consist of two, three or more small reniform masses of chromatin which lie closely applied to the spherical nucleolus. These are well seen in Purkinje cells.

Cajal (1909) described an argyrophilic dot in the nucleoplasm of large nerve cells under the name *accessory body*. This has no relation to chromatin and its nature is not known (Lindsay and Barr 1955). Hydén (1950) also describes a less sharply defined, strongly acidophilic area in the neighbourhood of the nucleolus. He found it to contain basic protein along with small quantities of pentose nucleic acids. It can be seen in the larger nerve cells of the human nervous system when well stained with haematoxylin and eosin.

As Marinesco (1909) pointed out, the cells of the substantia nigra and locus coeruleus may normally contain, in the nucleoplasm, acidophilic bodies which become larger, more sharply defined and more numerous in old people. These bodies may be mistaken for intranuclear inclusions, the earlier stages of which they closely resemble, but they do not displace the nucleolus. Although usually single, two or three of such bodies may be seen lying on either side of, or around, the nucleolus.

The *electron microscope* shows the nucleus surrounded by a double membrane which exhibits deep folds, indentations and pores which apparently allow passage between nuclear and cytoplasmic material. The chromatin has a rosette-like arrangement, quite different from the basophilic material in the cytoplasm, while the nucleolus contains aggregates of extremely fine granules measuring 10–30 Å (Palay and Palade 1955; Luse 1956, Fernandez-Moran 1957, and others).

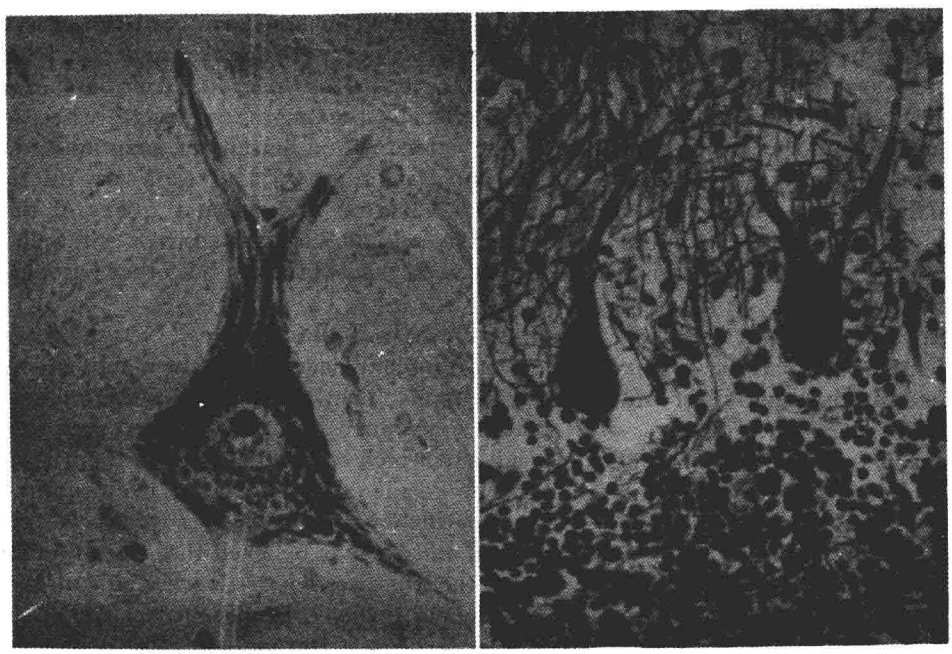
Nissl granules

This name is now in general use for the flakes of basophilic material in the cytoplasm of nerve cells and dendrites which were described by Nissl in 1894 (a). It is preferable to the term 'chromophilic substance' since in other cells such as those of the pituitary the term 'chromophilic' denotes acidophilic cells. The name 'tigroid substance' is now seldom used. It is convenient to use the terms hyperchromic and hypochromic for nerve cells which, although normal in all other respects, stain more or less intensely than normal with basic aniline dyes.

It was considered for many years that the appearance of Nissl granules, in fixed and stained sections, was largely an artefact of fixation and depended on the fixative used. The position was well stated by Einarson (1933) who considered Nissl material as 'truly existing during life, being present in a fluid or semi-fluid state, possibly not of a completely diffuse nature, but in localised concentrations in the cell body, finally being coagulated *post mortem* or by the fixatives employed and giving rise to the well-known histological "cell pictures"'. Nissl accepted no fixative, except alcohol, for his staining method, and warned workers not to accept the histological appearances which they found as more than 'equivalent pictures' of what was present during life. Thus for each change which occurred during life there was an 'equivalent' or corresponding change in the nerve cells after fixation. We now know that such warnings were scarcely necessary. Fixation may increase the sharpness of outline of Nissl granules, but it

does not greatly alter either their form or distribution. Further, any good fixative suitable for nuclear stains may be used. With some fixatives there is more tendency to shrinkage than with others, and with some there may even be some swelling of the cell. With these exceptions the appearance of Nissl granules in well-stained sections of tissue may therefore be accepted with little reserve as that which was present at the time of death.

It is, however, necessary to distinguish the changes which may have resulted from the mode of death, such as toxaemia or hyperpyrexia, and those due to post-mortem autolysis, from those which are related to a chronic or subacute nervous disease. Certain well-defined changes in the nerve cell such as central or peripheral chromatolysis fortunately require some little time to develop and are never the result of agonal or post-mortem changes.



(*a*) (*b*)

FIG. 1.1. Normal nerve cells.

(*a*) Cell from ventral horn of lumbar cord. (Nissl × 550.) Note absence of Nissl granules in axon hillock. (*b*) Purkinje cells. Cerebellar cortex. (Bielschowsky × 400.) Basket fibres surround the cells and pass up the dendrites.

The amount of Nissl substance and the shape and position of the condensations or 'granules' varies in different types of nerve cell. Nissl (1894 b) distinguished three main types of nerve cells: *cytochrome* or granule cells; *karyochrome*, in which the nucleus is more deeply stained than the cytoplasm, and *somatochrome* in which the cytoplasm stained more darkly than the nucleus. These special terms are now rarely used, but it is important to realise that in the cells of some centres the basophilic granules have a special arrangement. For example the larger cells of the formatio reticularis have an onion-skin arrangement of Nissl granules in concentric layers round the nucleus which is usually eccentric (Olszewski and Baxter 1954). In the cells of Clarke's column and the nucleus of Clarke-Monakow (lateral cuneate nucleus) the Nissl granules are small and lie chiefly in the periphery of the cytoplasm, and the nucleus is eccentric, an appearance which resembles the early stages of 'central chromatolysis'.

The composition of Nissl granules has been studied in many ways. The early staining method of Nissl, who used basic aniline dyes, indicated that they contained an acid protein probably allied to nucleoprotein. Weimann (1925) photographed unfixed cells by ultra-violet light and obtained appearances very similar to those in fixed and stained cells, thus showing that Nissl granules exist in the living cell. The work of Caspersson (1940), who showed that ultra-violet light with a wavelength of about 2,570 Å was absorbed by purine and pyrimidine bases present in nucleic acid, was carried further by Hydén, who photographed Nissl granules with this light band and proved also, by the use of a pure ribonuclease, that they consisted of ribose-nucleoprotein. Gersh and Bodian (1943) and Hydén and Hartelius (1948) later adapted this technique to the quantitative study of Nissl substance in nerve cells.

Nicholson (1923–4) using the Prussian blue method and Macallum's haematoxylin, after treatment by acid, showed that Nissl granules contained masked iron, and this was further demonstrated by the technique of ashing sections by Scott (1933), Alexander and Myerson (1938) and Alexander (1938) who also revealed in them by this method a considerable amount of calcium. Their composition was further studied by Einarson (1933, 1935) who used gallamin blue and gallocyanin chrome alum. The latter method permits of quantitative studies, as does also the technique of Windle, Rhines and Rankin (1943) who use a very dilute, acid-buffered thionin after fixation in formol saline. Einarson found that the area of the Nissl bodies might under certain conditions be stained by acid dyes such as eosin or acid fuchsin and considered that there were in the Nissl body not only nucleoproteins but other basophilic and acidophilic proteins.

In the *electronmicroscopic picture*, the site of the Nissl bodies is formed by a delicate laminated system of cisternae (Fig. 1.17) to which, according to Palade (1956), the name 'endoplasmic reticulum' was given by Porter and Kallman in 1952. They are sometimes also referred to as α-cytomembranes (Mercer 1961). The cisternae are lined by deeply stained granules (ribosomes) chiefly compounded of ribonucleic acid and which are responsible for the basophilia of the Nissl bodies. These were first described by Palade (1955), and have been confirmed.

The granular endoplasmic reticulum is not specific for nerve cells; it is even more developed in the so-called ergoplasm of exocrine glandular cells. It is believed that the ribosomes and their equivalents subserve protein synthesis and that the cisternae are concerned with the transport of protein products and of the various enzymes involved in this process. The granular reticulum continues into dendrites and—to a lesser degree—throughout most of the length of axons. It is observed also in small nerve cells (for example, the cerebellar granules) which in light microscopy do not appear to have cytoplasmic chromatin, and there is an agranular reticulum (Palay and Palade 1955) believed to correspond to the Golgi apparatus of the cell.

Hyperchromic and hypochromic nerve cells

In experimental work on the lower mammals it is common to find that some nerve cells appear rather pale and rounded (hypochromic) and others darker and more polygonal (hyperchromic). These appearances may sometimes be seen also in human material. Considerable confusion has arisen from interpreting the dark 'hyperchromic' cells as diseased or abnormal. Cajal (1897) considered the hyperchromic cells of healthy animals as an histological expression of a state of inactivity in those cells, an opinion with which Einarson (1933) agreed. Miller (1949) who studied the mitochondria and Golgi apparatus in hyperchromic cells as well as the Nissl substance and nucleus, found the Golgi apparatus hypertrophied and the mitochondria more numerous than in normally staining cells. Such cells also gave a strong alkaline phosphatase reaction. He interpreted these results as indicating that hyperchromic cells were in a state of increased activity. In his material, which consisted of normal animals, shrinkage of

nerve cells and a corkscrew appearance of dendrites was seen with certain fixatives and must therefore be considered as an artefact. Koenig, Groat and Windle (1945) found that swelling or shrinkage of nerve cells depended on the composition of the fixative and the method of fixation. They advise the use of intra-arterial injections with formol saline to which 2·4–4·6 per cent gum acacia is added according to the pressure employed in the injection.

Einarson has recently (1957, 1960) summarised his views on the functional significance of Nissl granules as follows: Chromoneutrality (after gallocyanin staining) indicates a resting state of the nerve cell; moderate chromophilia may point to initial activity, while extreme chromophilia suggests suppression of activity. Moderate chromophobia follows prolonged and increased activity; in extreme degree, it expresses severe stress and exhaustion. Extreme chromophobia may proceed to liquefaction, while the outcome of extreme chromophilia may be sclerosis and atrophy of the cell.

Relationship of Nissl substance to nucleus

The similarity in chemical composition of Nissl granules to most of the nucleoproteins of the nucleus led Einarson (1933) to consider that they might be derived from the nucleus, either during ontogenesis only, or both in the developing and adult nerve cell. Studying the response of motor nerve cells to prolonged electrical stimulation or to section of the axon, he found that, after the loss of Nissl granules which resulted from these procedures, there was first an increase in the amount of nucleoprotein round the nucleolus; this migrated to the periphery of the nucleus, especially in the region of the nuclear cap which became more prominent and was in these early stages partly intra- and partly extranuclear. Later it became entirely extranuclear and there was an accumulation of fine Nissl granules in its neighbourhood. As the Nissl substance increased the nuclear cap became less evident. These observations suggested that the Nissl substance was reformed after chromatolysis from the ribose nucleoprotein of tne nucleus. Hydén (1950) accepted this derivation, but it is denied by Gersh and Bodian (1943).

Mitochondrial rodlets and granules have been studied in nerve cells by Cowdry (1912, 1914) by the supravital Janus green technique, by Bertrand and Bertrand and Hadzigeorgiou (1928) by infra-red photomicrography and by numerous workers with the phase contrast microscope. In normal cells they occupy the same position as Nissl granules, but Beams *et al.* (1952) by phase contrast microscopy found them also abundant in the axon hillock. They considered that highly refractile rodlets which they saw in the axon itself were probably also of this nature.

Mitochondria are easily identified in the *electron microscope* (Fig. 1.17–18) and have been clearly illustrated by Palay and Palade (1955) and many others. On the electronic screen they show a diffuse ground substance, surrounded by a double membrane the inner lamella of which forms the characteristic 'cristae'. Belt and Pease (1956), however, described 'tubules' instead of 'cristae', but the difference seems to be of functional degree, the cristae being formations at rest (Wilke 1959). Mitochondria occur throughout the cytoplasm, even between the cisternal lamellae of the endoplasmic reticulum, and extend into dendrites and—to a lesser degree—throughout the axon. They are particularly frequent near presynaptic membranes.

Mitochondria are known to be rapidly altered in many inflammatory, viral and toxic conditions (Choja 1936; Hurst 1929). In the early stages of neurone reaction to axon section, mitochondria have been reported to increase in number and size by Hartmann (1948) and Hudson and Hartmann (1961), while Barton and Causey (1958) found them degenerating in the superior cervical ganglion after section of its preganglionic fibres. Scholz and Hager (1959), in electron-microscopic studies on various types of hypoxia, observed swelling and mild vacuolation of the mitochondria at a stage when chromatolysis was already advanced. In later stages the mitochondria became vacuolic.