Outline Studies in Biology

Brain Biochemistry

Second Edition

H.S. Bachelard

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Editor's Foreword

The student of biological science in his final years as an undergraduate and his first years as a graduate is expected to gain some familiarity with current research at the frontiers of his discipline. New research work is published in a perplexing diversity of publications and is inevitably concerned with the minutiae of the subject. The sheer number of research journals and papers also causes confusion and difficulties of assimilation. Review articles usually presuppose a background knowledge of the field and are inevitably rather restricted in scope. There is thus a need for short but authoritative introductions to those areas of modern biological research which are either not dealt with in standard introductory textbooks or are not dealt with in sufficient detail to enable the student to go on from them to read scholarly reviews with profit. This series of books is designed to satisfy this need. The authors have been asked to produce a brief outline of their subject assuming that their readers will have read and remembered much of a standard introductory textbook on biology. This outline then sets out to provide by building on this basis, the conceptual framework within which modern research work is progressing and aims to give the reader an indication of the problems, both conceptual and practical, which must be overcome if progress is to be maintained. We hope that students will go on to read the more detailed reviews and articles to which reference is made with a greater insight and understanding of how they fit into the overall scheme of modern research effort and may thus be helped to choose where to make their own contribution to this effort. These books are guidebooks, not textbooks. Modern research pays scant regard for the academic divisions into which biological teaching and introductory textbooks must, to a certain extent, be divided. We have thus concentrated in this series on providing guides to those areas which fall between, or which involve, several different academic disciplines. It is here that the gap between the textbook and the research paper is widest and where the need for guidance is greatest. In so doing we hope to have extended or supplemented but not supplanted main texts, and to have given students assistance in seeing how modern biological research is progressing, while at the same time providing a foundation for self help in the achievement of successful examination results

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Contents

1 Introduction

The brain is the most complex and highly specialised of all mammalian organs. Understanding the complexity of its function remains man's greatest challenge. The functional unit is the neurone, or excitable nerve cell, making anatomical and chemical connections with other units in the system. Many of the essential biochemical connections of the nerve cell are dependent upon special morphological features: synaptic contact is mediated by chemical molecules, 'neuro-transmitters' which ensure the continued propagation of electrical impulses through sequential units of the system. Also closely related to the morphology of the nervous system is the chemical energy expended in maintaining distribution gradients of cations across cellular membranes. Chemical neurotransmission results in an alteration in cation distribution and while the energy-utilising mechanisms which underly their redistribution are not peculiar to the nervous system, they are of particular importance to neural function. The mechanisms of chemical transmission, in contrast, are peculiar to the nervous system.

Nerve cells are unique in their ability to trigger off and maintain conduction of electrical impulses over long distances, which may be measured in metres, without significant loss of strength of the conducted impulse. Remarkable also is the specificity of their connections, not only with other nerve cells, but also with non-neural target cells in sites such as the endocrine glands or muscles.

These unique features rest in the possession of semi-permeable excitable membranes which can be caused, rapidly and transiently, to undergo changes in permeability to small chemical molecules and to cations. The highly specialised nature of the constituent cells, with their unique function and specificity, is closely related to the structure of the whole tissue. The underlying chemical processes cannot be discussed or seen in perspective without constant awareness of related aspects of physiology and morphology. The brain is structurally extraordinarily complex in its distinct anatomical regions, each of which is heterogeneous in the types and structures of the constituent cells.

One aspect of the biochemical function of the brain can be seen in its efficient production of the energy required to support the unique processes referred to above. This energy, essentially stored as ATP, is produced from the oxidation of glucose by mechanisms common to all biological cells. The importance in the brain of these processes is quantitative, rather than qualitative. The brain depends absolutely for its ability to function normally on a constant supply of glucose and oxygen from the blood stream. It has virtually no reserves of

chemical energy, compared with other tissues and organs. Stored concentrations of glucose and glycogen (each of the order of 1–2 μmoles/g) and of ATP (3 μmoles/g) are sufficient to maintain function in isolation for minutes only, if permanent damage is not to ensue and under normal circumstances, the brain cannot utilise alternative sources for its energy requirements [1]. The importance of the constant blood supply of essential nutrients can be readily appreciated if we remember that this organ, only some 3% of the total adult body weight, consumes some 20% of the glucose required by the whole body. This supply is in fact supported by the blood: one-fifth of the output of the heart passes through the brain. The brain is therefore the most sensitive part of the body to failure in oxygen or glucose. In the absence of either of these, fainting occurs within seconds, and if not corrected, coma and death follow rapidly. It is usually the first organ to suffer. Its peculiar sensitivity to abnormalities in energy metabolism can also be seen in the features of vitamin deficiency, especially of those vitamins such as the B group which function as coenzymes in intermediary energy metabolism. Although any deficiency affects the same metabolic pathways in the same way throughout the body, one of the most profound consequences is impaired mental function and in children, often mental retardation. It must be stressed that this is due, not to specialised qualitative metabolism by the brain, but to its very high sensitivity to any impairment in the normal processes of energy production.

This is of particular importance in the nutrition of the underdeveloped 'third world', where deficiency or dietary imbalance may cause irreparable mental damage to the developing child, and which has been the concern of special symposia [2, 3]. Not only is an inadequate environment increasingly suspected of leading to impaired intelligence in the poorer parts of the world, but evidence is also to hand that this can be seen in countries normally regarded as rich and developed. Although current discussions on the relative influences of heredity and of environment on the development of intelligence are heated and controversial, studies such as those on Scottish children over a 15 year period indicate that a consistent if small increase in intelligence can result from progressive improvement in their environment [4]. Further indications of the sensitivity of the brain to general metabolic impairment arise from the high proportion of inherited metabolic disorders which result in mental disturbance or retardation so important in Neurology and Psychiatry [5];

1.1 Regional cerebral metabolism

The great dependence of the brain on its supplies of glucose and oxygen, noted above, was originally assumed with good reason to be associated solely with energy production, yet early pioneering studies on the rates at which the normal adult brain uses these nutrients showed no difference with function. That is, the brain seemed to require the same energy whether the subject was responding to sensory stimuli, was

thinking intensively or resting [6]. These studies were based on arteriovenous differences over the whole brain, and regional variations could not be assessed. Since then, measurements of regional variations in rates of cerebral blood flow have shown changes in specific regions in response to sensory stimuli or mental effort [7]. However this does not tell us if metabolic rates are changing in a similar manner with function: in epilepsy or induced fits in animals, overall rates of cerebral consumption of glucose and oxygen increase to a considerably greater extent than does the blood flow rate.

Some evidence for regional rates of glucose utilization in experimental animals is emerging from use of the autoradiographic 2-deoxyglucose technique [8], based on knowledge of the transport and metabolism of deoxyglucose in the brain [9]. While this provides much information about regional variations in metabolism with function in animals, it is subject to limitations in the types of function that can be studied. Furthermore it cannot be applied directly to studies in man, requiring as it does removal of the brain for autoradiography. The method has now been adapted for use in man. The ^{18}F-isotope emits positrons which penetrate the skull, so its localization within the brain can be monitored externally. The attachment of ^{18}F to the No. 2 position of the deoxyglucose molecule is thought not to affect its metabolism and it is being used, by positron emission computed tomography, to study regional cerebral glucose metabolism in conscious normal man [10]. It too has one essential limitation in that the isotope has a very short half-life, and a cyclotron is required to produce it in the vicinity of the research or clinical laboratory. Its expense renders it unlikely to come into general use, but so much promise in research and diagnosis is offered that one can foresee extensive use in selected specialist centres.

1.2 Cerebral requirements for glucose and oxygen

A direct relation between function and the requirements of glucose and oxygen for energy production is also being questioned in view of recent observations in man and experimental animals that mild hypoglycaemia and hypoxia show changes in the EEG and in behaviour without any perceptible energy deficit. This poses two possibilities: 1) that small regional variations in energy production are escaping detection; 2) that the brain is responding to changed availability of these nutrients in a protective homeostatic manner. The first possibility is regarded as an unlikely answer in itself (though it may contribute) because of the extraordinary rapidity with which an experimental animal can be aroused from deep hypoglycaemic coma by giving glucose: the metabolic machinery is thought not to be able to respond so fast. The second possibility is receiving much attention. This puzzling apparent anomaly is seen in a general sense if various aspects of energy metabolism and synaptic function are compared. We know that maintenance of synaptic function depends to a great extent on the cation pumping processes which require energy (Chapter 2).

Yet in addition to the conditions of hypoglycaemia and hypoxia noted above, treatment with various centrally active drugs (anaesthetics, depressants and excitants) may also cause changes in the EEG and in behaviour, and in synaptic function, without detectable change in the energy state [11, 12]. This has now been confirmed *in vitro*: electrical activity evoked from slices of hippocampus incubated *in vitro* can be affected markedly by slightly lower concentrations of glucose (2m*M*) which seem to have no effect on the intermediary metabolism of the tissue [13]. No clear pointers on the basis for this have emerged so far, but it may have important clinical significance in our understanding of such conditions as coma, stroke, epilepsy and also perhaps, dementia.

To be able to understand what he is trying to achieve, the biochemist who studies brain function must acquaint himself with related aspects of morphology, physiology and pharmacology; the chemical function of the brain cannot be separated from the architectural integrity of the cellular relationships. For a small book of this type the topics selected concentrate on the chemical events related to excitability and transmission and to the adaptability of the brain to react to various stimuli both from within and without the body. To this end, a brief description of the associated morphology and physiology seems an essential requirement and is treated first. Then follows a description of membrane permeability phenomena and neurotransmission. The final section of the book is concerned with aspects of the chemical response of the brain to its immediate environment and as a result of some of the hormonal signals reaching the brain.

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2 Appearance of the brain

2.1 Gross appearance

Biochemists tend to study the brains of small mammals and consciously or subconsciously extrapolate to what might occur in the human brain, itself subject to obvious limitations in opportunities for chemical exploration. Yet what do we mean by the 'mammalian' brain, since the brain of a rat or guinea pig is obviously far different in appearance and many functions from that of Man? The brain has evolved and specialised within mammalian species more than any other organ of the body: Fig. 2.1 shows a comparison of the brains of a selected group of mammals and it should be remembered that increasing size is not necessarily associated with increased intelligence or sophistication of function. The main discernible change that has occurred during evolution of the mammalian brain is in the size and complexity of the cerebral cortex. The increase in surface area per unit of volume of the cortex has been effected by increased folding so that the convolutions of the human cerebral cortex are considerably more extensive than of the rat or rabbit. The function of the cortex has altered also: the 'primary cortex' concerned with sensorimotor function (Fig. 2.1) has remained proportionately much the same, but the areas devoted to 'association', i.e. areas concerned with higher functions of learning and decision-taking, have increased considerably [1].

Other areas, such as the limbic system (Fig. 2.1), concerned with more primitive functions of homeostasis, motivation and especially emotion [2], are phylogenetically older, and have changed little in relative size, as the shaded areas show [3, 4]. For the non-anatomically trained, the nomenclature of the regions and specialised parts of an organ as complex as the brain is daunting. In fact the brain should

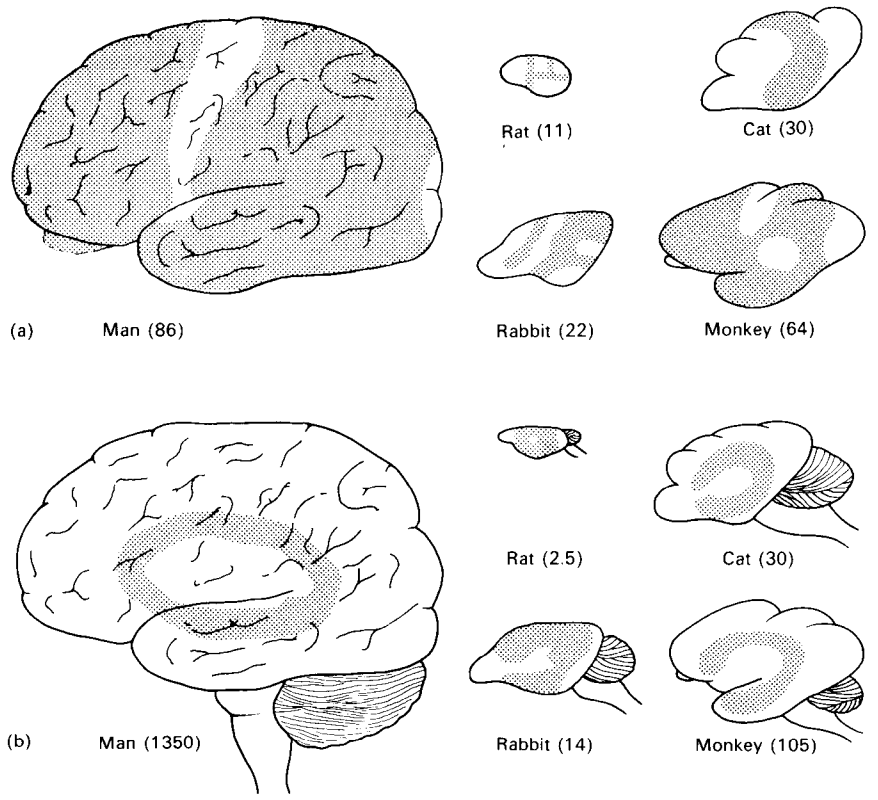

Fig. 2.1 Comparison of some functional areas of the brains of various mammals. **(a)** Proportions of sensorimotor and association (shaded) areas. Values in parenthesis are percentages of association cortex. **(b)** Schematic representation of the relative areas of the limbic system. Values in parenthesis are the weights of the brains in grams. The drawings are approximately half-size (linear).

really be regarded as a collection of highly specialised organs rather than as a single organ. Often various areas of the brain are cited in biochemical articles as experimental material and the untutored reader may be uncertain of the site and significance of the part named. The major areas of the human brain which are likely to be referred to are shown in Fig. 2.2 (see also Table 3.2 of Chapter 3). The first part of the illustration (a) shows the lateral aspect of the whole brain, viewed from the left hand side: the next drawing (b) shows some of the internal parts which are seen if the brain is divided into the two hemispheres. This is the right hemisphere viewed from the left hand side. The whole brain has been divided into four main parts for convenience: the cerebrum, the cerebellum, the mid-brain and the brain stem, and it is the latter which contains a large number of specialised parts indicated in Fig. 2.2. The third illustration (c) shows the interior of the brain cut horizontally and viewed from above.

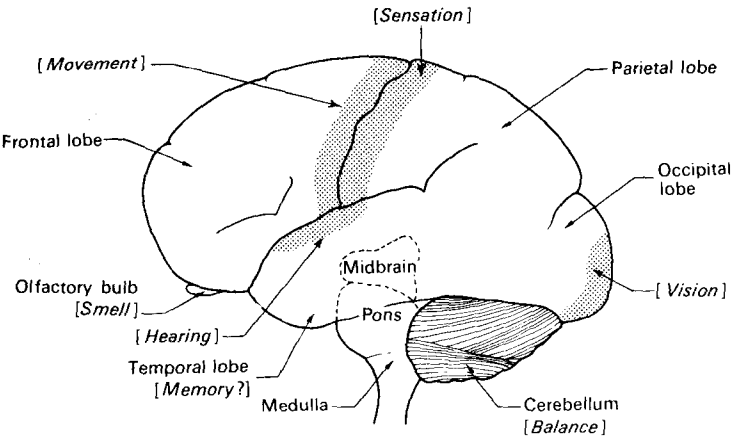

Fig. 2.2 Drawings of the human brain. **(a)** View from the left side, showing major areas with some indication of function.

2.2 Fluid compartments

One prominent feature of the gross anatomy of the brain is its extensive blood supply–perhaps not surprisingly, since it uses about one-fifth of the total blood used in the body. The blood volume of the brain is only about 3% of the total brain volume and the efficiency of the supply is ensured by the extensive ramified system of capillaries. Exchange of solutes between the various fluid compartments (blood, cerebrospinal fluid and the extracellular tissue space) and the cells themselves, exhibits features not always found in other parts of the body. The most studied of these features is the 'blood-brain barrier'. Originally this concept arose from the limited penetration of injected dyestuffs from the bloodstream to the brain substance, which was also found to occur with a variety of small highly water-soluble chemicals of a wide range of classes: sugars such as fructose and sucrose, and charged molecules such as thiocyanate and most amino acids. Microscopic examination of the endothelial cells of the walls of the blood capillaries of the brain indicated that they were packed more tightly together than in capillaries outside the brain, so there seemed to be a sound basis for a physical permeability barrier at the capillary wall. This suffices for relatively large molecules like proteins but is inadequate to explain the apparent limited permeability of substances such as glutamate. Indeed if radioactive glutamate is present in the blood-stream it equilibrates rapidly with the glutamate within the brain, to judge from the extent of its labelling, but a massive increase in concentration of the external glutamate does not materially change its internal concentration. For substances like glutamate, and many others (including precursors of amines with specific neural function, Chapter 3), the 'blood-brain barrier' can be regarded as a homeostatic mechanism whereby the internal concentration is maintained by active processes of extrusion [5].

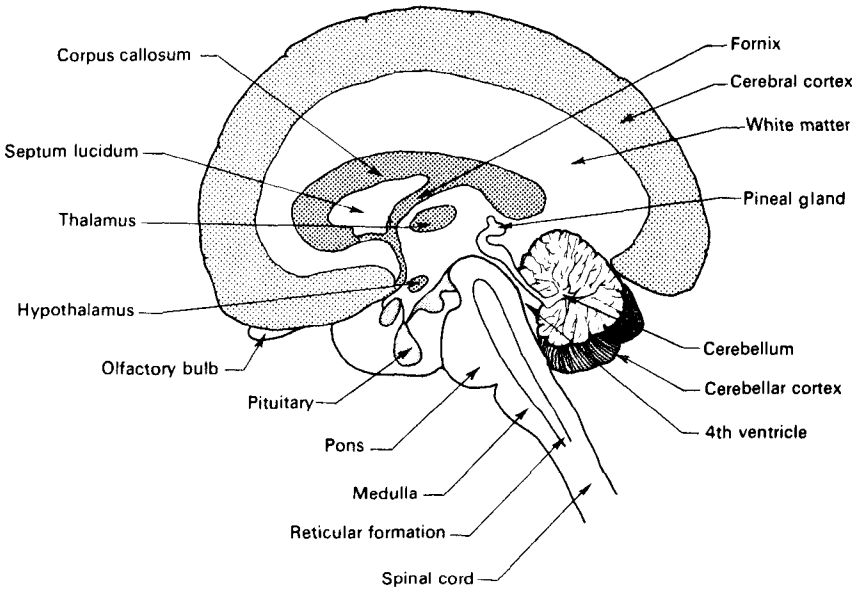

Fig. 2.2 Drawings of the human brain. **(b)** Cut-away view as in **(a)** showing some of the internal features.

The peculiarities of the 'blood-brain barrier', which is believed to fulfil a protective role for the highly sensitive brain, can therefore impede potential treatment of brain disorders by rendering difficult the internal accumulation of drugs or metabolites. Such a difficulty might be overcome by a knowledge of the biochemistry of the system at fault. Thus in Parkinson's disease, a degenerative and progressive disorder associated with muscular tremor and akinesia, anatomical observation showed degeneration of certain nerve tracts and histochemical analysis revealed a parallel loss of dopamine (see Chapter 3). Dopamine (3, 4-dihydroxyphenylethylamine) is one of those substances which are not easily transported into the brain, but its immediate metabolic precursor, Dopa (dihydroxyphenylalanine), is readily taken up. Marked improvement in many patients has been achieved by treatment with Dopa.

2.3 Microscopic appearance

The first appreciation of the morphology and cytology of nervous tissues, the network of individual cells and their processes, came towards the end of the nineteenth century with the application of the improved light microscope and the development of new staining methods. One of these, based on silver salts, causes selective staining of neuronal cell bodies and their processes in thin sections with such clarity that the structures seem to stand out in almost a three-dimensional picture. This is the Golgi stain. An alternative method, the

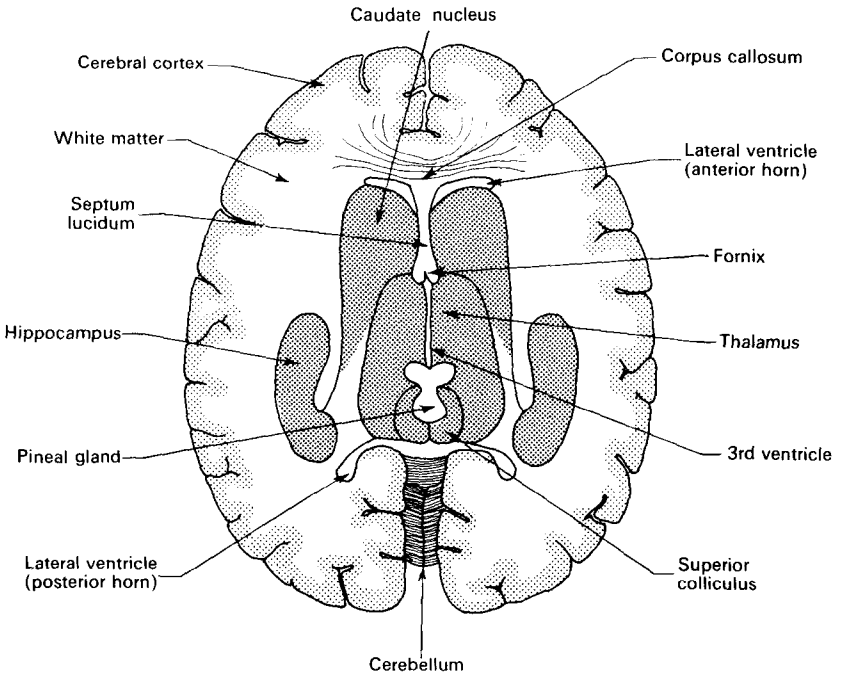

Fig. 2.2 Drawings of the human brain. **(c)** Cut-away view from above, with the front of the brain to the top of the drawing.

Nissl stain, shows the cell bodies of neurones and glia but not their processes. The observations, made using the light microscope, of cell bodies, axons, dendrites and dendritic spines (see below), were sufficient to lead to the 'neuronal hypothesis' with the concept of synaptic junctions some seventy-five years ago [6]. Further insight into fine structure, especially of the synapses, and confirmation of the neuronal hypothesis, had to wait until the advent of the electron microscope some forty years ago.

Light microscopy had clearly demonstrated the occurrence of a variety of cell types, classified into two main groups, neurones (the excitable nerve cells) and glial cells (non-excitable). Within each group, different types have been discerned.

2.3.1 Neurones

These (Fig. 2.3) may have large or small cell bodies (*perikarya*) but all are characterised in possessing a large *nucleus* containing a prominent *nucleolus*, a high content of *ribosomes* in the cytoplasm (either free or attached to an extensive *endoplasmic reticulum*) and a high content of *mitochondria*. Such features are compatible with active synthetic and secretory activities and the large capacity for energy production referred to in Chapter 1. Essential characteristics are the prominent processes which form extensions of the outer cell membrane:

axons and dendrites. Axons are usually long, relatively thin, and emerge from a swelling in the cell body – the axon hillock. The axons are sometimes branched and usually, but not always, covered by an insulating sheath, the *myelin* sheath, consisting of a spiral (giving the impression of concentric rings in cross section) of membranes. Myelinated axons form the main routes for the efficient rapid conduction of the electric impulse from the neurone (efferent) to another part of the system and the connections are made through synapses (below). Dendrites are usually thicker, shorter and highly branched, do not have a myelin sheath and carry the impulse from synapses to the nerve cell (afferent). These processes contain *neurotubules*, apparently identical with the microtubules of the mitotic apparatus and of contractile tissues, and are thought to be associated with axonal transport of materials from the perikaryon through the axon (Chapter 3).

Three main types of nerve cell can be identified by means of their processes. '*Unipolar*' cells contain only one axon and examples of these are sensory cells of ganglia. '*Bipolar*' cells have two processes, an axon and a dendrite, and are found as sensory receptor cells concerned with sight, smell, and hearing. The majority of the neurones are *multipolar*, having one axon and many dendrites. Multipolar cells fall into two main classes, named according to their shapes: the pyramidal cells of Fig. 2.4 and stellate cells.

2.3.2 Glial cells

Glial cells (Fig. 2.5) do not possess the excitable characteristics of the nerve cell, are generally smaller, but also have processes emanating from their cell bodies. These processes are relatively short and often highly branched. There are three main types. *Astrocytes* often occur close to blood vessels: their processes terminate in 'end-feet' which make contact with the blood capillary wall. These are thought to be concerned with nutrition, possibly acting as mediators in the transport of materials from the blood stream to the neurones. Indeed a highly specific means of causing degeneration of glial cells without direct and immediate damage to the neurones, is by promoting hyperammonaemia. This can occur naturally, as a result of severe liver damage, or experimentally by the portocaval shunt technique [7]; the astrocytes become swollen and vacuolated. The *oligodendroglia* are also satellite cells and are intimately concerned in the central nervous system with the myelin sheath of the axon, which they produce. The third group, the *Schwann* cells, perform the same function in myelination of peripheral nerves outside the brain. Fig. 2.6 is a diagrammatic representation of the process of myelination. The myelinating cell wraps itself around the axon so that its plasma membrane forms a spiral. The nucleus of the cell can be seen lying close to the axon. Each of the myelinating cells (oligodendroglia or Schwann cells) forms a unit of myelin along part of the length of the nerve and many such may be required for the