PROGRESS IN BRAIN RESEARCH

VOLUME 29

BRAIN BARRIER SYSTEMS

EDITED BY

A. LAJTHA

AND

D. H. FORD

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Preface

In recent years, many important developments have occurred in many research disciplines that are directly related to brain permeability. In morphological fields, the fine resolution provided by electron microscopy has resulted in new concepts of membrane structure. Electron microscopy, combined with autoradiography, have contributed significant information concerning dynamic events occurring in and around such membranes. Permeability measurements with drugs have led to new theories of the physical and chemical requirements which determine the penetration of drugs into the central nervous system. Measurements of permeability during development and in later more mature stages of life have resulted in theories that relate brain permeability and function and, as in senescence, brain permeability and pathology. Recent work with normal metabolites in the brain has shown complex active transport mechanisms to be present in the walls of the various cell types making up the nervous system. Cerebral transport phenomena have been shown to have a considerable degree of selectivity and specificity and seem highly significant in controlling the mechanisms of brain metabolism. Inasmuch as these membranes and transport systems sometimes appear to restrict entry of materials into the brain, it is apparent that they represent, in part at least, what has been termed the "Blood-Brain Barrier". Thus, the brain-barrier system may determine which metabolites can gain access to the brain, may determine the level of these metabolites available to various brain parts and brain cells, and may determine the rate of supply and the rate of elimination. It has also been suggested that specific transport processes may be interfered with in pathological states (*i.e.* amino acidurias, and phenylketonuria), and therefore that alterations of permeability can be involved in altered mental function.

It is obvious from this very brief survey that great advances have been made recently on a number of fronts, in such areas as anatomy, physiology, neurochemistry, and pharmacology.

Despite these advances, however, it is not uncommon to attend meetings and learn that the failure of almost any compound to enter the brain is due to the Blood-Brain Barrier, or that the only amino acid capable of penetrating the brain is glutamine. Most textbooks of neuroanatomy treat the concept of "Brain-Barrier" as being too complicated for discussion or provide some very structural rigid concept for restricting entry of most compounds.

Although a number of conferences have been planned to consider these important advances in cerebral permeability, barriers or transport, none has taken place in recent years. Thus it was the purpose of the conference held in Amsterdam from September 26 to 30, entitled "Brain Barrier Systems" to gather together leading investigators from America and Europe who have been working on the various anatomico-bio-

chemical-physiologic aspects of the complex membrane systems in the brain, and attempt to define as concisely as possible our state of knowledge today about these "barriers".

It is most fortunate that so many of the outstanding contributors to this field could come and participate at the conference. Many more investigators with important contributions are missing from the volume, because many of the authors had previous commitments and so were, to our regret, unable to participate. While their absence is certainly felt, it was fortunate that it was possible to have contributions on most of the important aspects of the problem. The conference, therefore, could show the relationship and interdependence of the disciplines concerned with barrier phenomena, and it was highly successful in clarifying the nature of the investigations still required to permit us to fully understand how these "membranes" serve to maintain, or influence, normal brain function.

In planning this conference, we were delighted to obtain the cooperation of the Netherlands Central Institute for Brain Research, which has taken care of all the local arrangements. We were indeed fortunate to be able to gather in the stimulating atmosphere provided by this castle (De Hooge Vuursche), which by its nature led us to many fruitful hours of discussion after the close of the formal meetings. Unfortunately, it was not feasible to hide microphones along the paths in the beautiful garden and in the woods surrounding the conference to record all the free discussions that went on till late in the evening hours. We are also indebted to the Office of Naval Research for their interest, both intellectual and financial, in the support of this conference. Additional financial support was provided by the drug houses of E. R. Squibb and Sons, Organon, Abbott Laboratories, and the Warner Lambert Research Laboratories. Without the assistance of the Brain Institute, the Office of Naval Research, and the research directors of the above drug companies, all the best intentions and hope for having a conference dealing with the importance of the various Brain-Barriers in the neurobiologic system would have long ago foundered. Thus, it is proper that we should express our thanks for their interest.

Finally, thanks are due to all the participants for their enthusiastic participation, for the excellent contributions, and for the stimulating discussions, all of which made the conference such a success and makes the present book an excellent summary of the problems.

DONALD H. FORD
ABEL LAJTHA

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ION MOVEMENTS

FACTORS INFLUENCING BARRIER FUNCTION

SPACES IN THE CENTRAL NERVOUS SYSTEM

The Composition of Nervous Membranes

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The literature on the chemistry of membranes is much too vast to allow a meaningful review within the space and time available for this presentation. Therefore, instead of attempting such an impossible task, the subject matter of this presentation will be limited to the discussion of four groups of compounds which occur mainly, if not exclusively, in the nervous system and all of which are clearly identifiable as membrane constituents. These compounds are gangliosides, proteolipids, polyphosphoinositides and neurokeratin. Gangliosides are neuronal components; proteolipids, polyphosphoinositides and neurokeratin are myelin components. Since even our information on these compounds — and specially the sum total of our uncertainties about them — is much too vast for adequate presentation in the time available, the following comments will bear mainly on those aspects of their chemistry that are specially pertinent to their function as membrane constituents.

GANGLIOSIDES

In 1941, Klenk isolated from brain a new amino acid to which he gave the name of *neuraminic acid*. In 1942, he described a group of new brain glycolipids that were characterized by the presence of neuraminic acid and which were otherwise constituted by a lipid moiety, presumably a ceramide, and a carbohydrate moiety presumably consisting of one or more monosaccharides. He named them *gangliosides* because their distribution in the tissue suggested that they were components of ganglion cells. Subsequent work by Klenk and other workers established that neuraminic acid was present in gangliosides usually as an N-acetyl derivative, and that it was identical with *sialic acid* which had been isolated by Blix from submaxillary mucin a few years previously (Klenk, 1936); that the carbohydrate moiety of gangliosides contained hexosamine(s) in addition to neutral sugars; and, finally, especially after the introduction of thin layer chromatography, that gangliosides comprised a large number of closely related chemical compounds.

The chemistry of gangliosides has been the object of recent authoritative reviews, to which the reader is referred for a detailed discussion of the subject (Svennerholm, 1964; Ledeen, 1966). In summary, gangliosides are complex glycolipids consisting,

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according to Kuhn and Wiegant (1963), of a lipid moiety in the form of a ceramide, and of a carbohydrate moiety in the form of a tetrasaccharide, as follows:

galactose (1–3)N-acetylgalactosamine(1–4)galactose(1–4)glucose(1–1)ceramide

To this backbone are attached, 1, 2 or 3 sialic acid residues constituting respectively mono-, di- or trisialogangliosides. Variations in the carbohydrate moiety have been reported and at present upward to 12 different gangliosides have been recognized. In addition, the ceramide moiety, although consisting mainly of sphingosine and stearic acid, contains also higher and lower homologs of sphingosine, and a host of different fatty acids, thus multiplying several times the number of individual gangliosides that occur in nature.

In parallel with this chemical work, it was observed that, although gangliosides were extracted from brain tissue with conventional lipid solvents, they were easily soluble in water as undialyzable solutes. In aqueous solutions they appeared to be monodisperse (Folch *et al.*, 1951), with an apparent molecular weight which was first computed, from ultracentifuge data, as being 250 000 and which by other methods of measurement employing different parameters has been given values ranging from 180 000 to 400 000. This observation led to the assumption that a physically homogeneous high molecular weight compound was being dealt with, and to it was given the name of *strandin* (Folch *et al.*, 1951). With advances in the chemistry of gangliosides it became apparent that strandin was a polymeric form of gangliosides, and even a critical micellar concentration of 0.015 % was suggested (Howard and Burton, 1964). Since it had been observed that preparations of gangliosides contained small amounts of polypeptides (Folch *et al.*, 1951; Folch and Lees, 1959) or proteins, it has been also suggested that these presumed protein contaminants might play a part in determining the remarkable homogeneity of the micellar solutions of strandin (Rosenberg and Chargaff, 1956).

That gangliosides are, at least in part, membrane components appears to be a reasonable assumption on the basis of their distribution in the nervous system, of their rate of accumulation during brain development (Folch, 1955), of histochemical evidence (Diezel, 1955), and of their distribution among subcellular fractions of brain tissue (Wolfe, 1961; Wherrett and McIlwain, 1962; Seminario *et al.*, 1964; Burton *et al.*, 1964; Eichberg *et al.*, 1964; Spence *et al.*, 1964). In addition, a consideration of some of the properties of gangliosides clearly points to them as being exceptionally well designed as membrane constituents: the presence of the carboxyl group of sialic acid which permits binding with organic and inorganic cations, the presence of the lipophilic groups of the ceramide and of the hydrophilic groups of the carbohydrate moiety which permit interaction with many different substances including proteins, lipids, and many small molecule substances, and the ability to form micelles of fairly uniform size. Indeed, it is not surprising that gangliosides have been implicated by many workers in different membrane functions: cation transport (McIlwain, 1962), acetylcholine release at the presynaptic membrane, synaptic inhibition, receptor function for serotonin (Burton *et al.*, 1964), for tetanus toxin (Van Heyningen, 1963), which parallel the well established function of sialic acid as a viral receptor in red

blood cells, just to mention a few highlights in a considerable literature dealing with possible functions of gangliosides.

We will close this brief survey by discussing the interaction of gangliosides with sodium, potassium, calcium and magnesium, and an apparent effect of the presence or absence of polypeptide on the behavior of the resulting complexes. As expected, all four cations combine with gangliosides, presumably by simple electrostatic bonds. These combinations are reversible, and each cation can displace the others from combination with the ganglioside, the divalent cations being more effective than the monovalent. However, the calcium-ganglioside complex is much less polar than either the free ganglioside or the complexes of ganglioside with the other cations (Quarles and Folch-Pi, 1965). Thus, when these salts of gangliosides are dissolved in the biphasic system chloroform : methanol + water 8 : 4 : 3, v/v/v, free ganglioside, and its sodium, potassium or magnesium salts remain in the upper (polar) phase. On the other hand, the calcium complex will remain in the upper phase at low and at high concentrations of calcium ions, but at intermediate concentrations of these ions, it will partition into the lower, least polar phase.

This effect of calcium appears to require the presence of small amounts of other lipids, sulfatides being especially effective in this action. In addition, the presence of polypeptide will tend to produce an accumulation of calcium gangliosidate at the interphase. All these interactions illustrate the dramatic changes that may occur in the physical properties of gangliosides, hence on their possible behavior as membrane constituents, and they also point to a possible crucial influence of the presence of small amounts of polypeptides on ganglioside properties. The effect of polypeptides does not appear to be a general protein property. Since the concentrations of calcium that effect the change in polarity of the ganglioside fall in part within the physiological range of concentrations of calcium, it is clear that the observations on the model employed may have implications for the behavior of gangliosides *in vivo*.

Proteolipids, polyphosphoinositides and neurokeratin

These three groups of substances are closely related biochemically and anatomically. As will be detailed below, these are myelin constituents and, since myelin itself is formed by the infolding of the plasma membrane of the satellite glial cells around the axons, it is obvious that myelin components are membrane components by definition. In addition, polyphosphoinositides are constituents of both proteolipids and neurokeratin and proteolipids and neurokeratin appear to be very closely related.

Since polyphosphoinositides are components of both proteolipid and neurokeratin, it might be pertinent to review highly their history. In 1941 Folch and Woolley (1942) reported the occurrence of inositol as a constituent of brain lipids. Subsequent work resulted in the isolation of an inositol-rich lipid fraction (Folch, 1949) which appeared to have as constituents, inositol diphosphate, glycerol and fatty acids in integral molar ratios and to which the name diphosphoinositide was given (DPI). Later work, using chromatographic techniques, showed, that besides diphosphoinositide, there was a *triphosphoinositide* (Dittmer and Dawson, 1961; Brockerhoff and Ballou, 1961),

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and that, in fact, the latter might well be the most abundant of the two, DPI possibly being derived by partial dephosphorylation of TPI.

Proteolipids — The name proteolipid was introduced in 1951 by Folch and Lees to designate substances consisting of a protein moiety and a lipid moiety and characterized by a complete insolubility in water and solubility in some organic solvents, especially in chloroform : methanol mixtures. The name is intended to emphasize that proteolipids are lipoproteins which behave like lipids.

The original observation that led to the discovery of proteolipids was that chloroform : methanol extracts of brain, presumably freed of nonlipid material by water washing, contained protein material (Folch and Lees, 1951). This protein material remained in chloroform through successive water washings, i.e., it was not only soluble in chloroform but insoluble in water. The protein material could be obtained by simply taking to dryness the extract, and extracting the residue with chloroform : methanol. Apparently, in the course of drying the protein underwent some rearrangement that resulted in the loss of its original solubility in chloroform : methanol. As a consequence, the protein remained as an insoluble residue. It contained 14 % N, 1.75 % S and, after acid hydrolysis, 91 % of its nitrogen could be recovered as free amino acids. Its amino acid composition revealed a preponderance of monoamino-mono-carboxylic acids, a high concentration of methionine and cysteine (or cystine) and a relatively small concentration of acidic and of basic amino acids. The material was resistant to the action of trypsin, pepsin, papain and erepsin. Later, it was found to be hydrolyzable by pronase.

Distribution of proteolipids. — Although especially abundant in nervous tissue, proteolipids are also found in a wide variety of animal and vegetable tissues. Bovine tissues contain the following amounts of proteolipid protein (mg/g tissue weight): heart, 3.5; kidney 2.0; liver, 1.6; lung, 0.95; uterus, 0.6; biceps, 0.4. In spinach chloroplasts they represent 2–4 % of dry weight (Zill and Harmon, 1961). These values are only indicative because the yields obtained may have been incomplete.

In the nervous system, proteolipids are found at highest concentration in white matter (20–25 mg/g wet tissue) and at about 1/5 this concentration in gray matter. They are present in peripheral nerve at only 1/20 to 1/80 the concentration in white matter (Folch *et al.*, 1958), which may well indicate a qualitative difference between peripheral and central myelin. They are absent from fetal brain and their appearance and progressive accumulation is concurrent with myelination (Folch, 1955).

In a study of 28 different anatomical areas of the human nervous system, Amaducci (1962) has observed marked and consistent differences from one anatomical area to another. He has shown that the highest concentration of proteolipids occurs in central white matter, with 1/5 to 1/10 as much in gray matter, and only 1/20 to 1/80 as much in peripheral nerve. Within this general pattern the concentration of proteolipids decreases progressively from cerebral white matter to spinal cord white matter, with cerebellar white matter showing an intermediate value. In spinal cord itself, the concentration of proteolipids appears to decrease in the anterolateral columns