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Chapter 14

QUANTITATIVE NEUROCHEMICAL HISTOLOGY

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I. NEUROHISTOLOGICAL COMPLEXITY: BIOCHEMICAL CONSTITUENTS AS QUANTITATIVE INDICES OF HISTOLOGY

The uniquely complex histological structure of the vertebrate CNS poses serious problems for interpretation of data on the biochemical composition and metabolism of normal and pathological tissue. Although nervous tissue is comprised of only three principal types of cells, its complexity differs from that of other tissues by several orders of magnitude. First, the neuron population shows enormous variety in morphology. Neuronal processes have unique elaborative tendencies and may extend for long distances from the cell body. In particular, the wealth of neuronal interrelationships through synaptic contacts, which are the basis of the highly organized function of the nervous system, represents a structural and metabolic variant of a complexity without parallel in nonnervous tissues. Second, the glial population, both oligodendroglial and astroglial, also shows some variety in morphology and function. Finally, the absolute numbers and proportions of these three cell types and their varieties differ greatly in regional distribution in the CNS.

One approach to the solution of the problem of histological complexity has been to isolate the different cells and parts of cells and to analyze them separately. This attack has succeeded in revealing some of the biochemical properties and behavior of neurons and glia individually, and extensive reference will be made to this approach in this article.

An ultimate goal of neurochemistry and other neurosciences, however, is the analysis of higher nervous functions, and this would seem to require, in addition, study of *in situ* organized populations of neurons and glia. A second

approach, therefore, has been to try to quantitate the cytology by anatomical methods of counting and measuring. Many attempts have been made to determine the absolute and relative numbers and types of cells, as well as their volumes, masses, surface areas, and organelles in various regions of brain, but except for counting of total cells and neurons, such anatomical quantitation has been excessively laborious and generally of low accuracy. The problem has not been simplified by the high resolution of the electron microscope. In fact, the latter has only illustrated the intricacy of the problem, and the limitations of such methods of quantitation by revealing the profusion of membranes and organelles of both neurons and glia and the involved interrelationships of the surface membranes of the two types of cells.

Although quantitative anatomical measurements of cytology are tedious and fraught with methodological difficulties, biochemical measurements can be performed rapidly and accurately on large numbers of samples. Consequently, a third approach has been to find chemoanatomical indices or biochemical marker substances that can clearly be established as related to specific types of cells or cell parts, with the aim of using the indices to quantitate the anatomical elements. Indices of this kind when used as bases of reference for expression of other biochemical data on the same tissue can greatly increase the yield of information from analysis of any component. Such more or less specific chemoanatomical indices supplement rather than replace more general referents, which include wet weight or volume, total dry weight, total lipid, total protein, and subclasses of lipids or proteins.

When chemoanatomical indices are used in conjunction with sampling methods that provide anatomically or pathologically discrete divisions of the CNS for biochemical analysis, the resulting discipline may be termed quantitative neurochemical histology or pathology.

In quantitative neurochemical histology, chemical assays are thus substituted for morphological measurements of histological and cytological components in nervous tissue. At the cellular level, the attempt is to enumerate the cells present as well as to identify and describe a cell species as a morphological unit differing qualitatively and quantitatively from other cell species in chemical and enzymatic constitution. Cell enumeration *in situ* can be accomplished by combining DNA assays as an estimate of total cells per unit weight with differential cell counts on analogous fixed stained tissue to determine both relative and absolute numbers of neuronal and nonneuronal cells.[1] At the subcellular level the attempt is to identify and evaluate chemical components or ratios of components as indices of organelles, extensive membranes, and other structures of cells. In combination with micromethods of serial frozen section sampling and analysis, cellular and subcellular quantitation can be extended over a significant spatial interval in a region of brain. A composite picture can thus be constructed of the normal histology and metabolism of a region for comparison with analogous samples of functionally or pathologically affected brain tissue studied similarly.

This article will first consider the establishing and testing of chemoanatomical indices by the overlapping methods of (a) light and electron microscopic histochemistry, (b) biochemical analysis of segregated cells and parts of cells isolated in various ways, and (c) biochemical analysis of selected cell populations obtained by frozen section sampling. The principal established indices for subcellular structures or for specific cell types as well as the possibilities for developing new indices will be evaluated. The application of chemoanatomical indices in quantitative neurochemical histology will be illustrated by two examples. The first will show how the distributions of certain indices in selected tracts and nuclei of human CNS relate to the histological picture. The second will involve the more specialized use of chemoanatomical indices to study the architectonics of human and rat cerebral cortex. The results on cortex will illustrate how the techniques of quantitative neurochemical histology are designed to divide a region into small parts for analysis by serial section sampling and to reconstruct after analysis a holistic picture of the region as an integral part of the nervous system. The holistic view is expected to make possible eventual correlations with neuroanatomy and electrophysiology at gross, microscopic, and ultramicroscopic levels. Finally, the usefulness of chemoanatomical indices in pathological studies will be considered.

II. METHODOLOGY FOR ESTABLISHING CHEMOANATOMICAL INDICES*

The effort to correlate biochemical composition with histological and cytological structure is as old as the study of microscopic anatomy itself. During the past three decades, however, several fruitful methodological approaches to the problem were developed and widely employed for the histochemical analysis of brain and nerve. These may be grouped under the following headings.

A. Light and Electron Microscopic Histochemistry: *In Situ* Demonstration of Biochemical Constituents

From a certain point of view, all histology is histochemistry, but a subdiscipline has developed for visualization of known compounds or enzymatic reactions in tissues and cells at the level of light microscopy.[2–5] Specific chemical properties of certain structural compounds and enzymes are utilized for demonstrating their histological and cytological localization. Essentially the same principles are employed for refinement of such techniques to the ultrastructural level visualized by electron microscopy.[6,7] Closely allied in aim are physical techniques, such as ultraviolet microscopy and microspectrography, interference microscopy, absorption cytophotometry, microspectrophotometry, microdensitometry, X-ray absorption spectroscopy and microradiography,[8,9] fluorescence microscopy, including immunofluorescence techniques,[10] quantitative electron microscopy,[11] and elemental analyses by the laser microprobe[12] or the electron microprobe.[13,14]

* Abbreviations used: NANA, *N*-acetylneuraminic acid; PLP, proteolipid protein; C, cerebrosides; ChE, nonspecific cholinesterase; CN, cyclic nucleotide; AChE, acetylcholinesterase.

The particular advantage of microscopic histochemistry in general is that when carefully carried out, it results in maximal anatomical precision. However, the danger of erroneous localization due to shifts in loci of chemical constituents during the complex preparative steps, the necessity of using often artificial conditions for *in situ* demonstrations, especially in the case of enzymes, and the difficulties of obtaining quantitative information by such means are limiting factors in the value of these techniques. Nevertheless, an impressive array of widely ranging information upon the structural and enzymatic histochemistry of the nervous system has been and continues to be provided by these means.[2,15,16] The number of available reviews is such that further consideration in this article is unnecessary.[2–5,7,15,16]

B. Isolation and Analysis of Cells or Parts of Cells

In the study of a tissue as complex as the CNS, an obvious first goal is to determine the specific composition and metabolic characteristics of neurons, oligodendrocytes, and astrocytes and the cytological substituents thereof. Two prerequisites are (a) the isolation of identifiable cells and cell parts and (b) the availability of microchemical analytical techniques for microdissected material of the degree of refinement required for precise quantitative analyses upon such minute biological objects.

Biochemical analyses have been performed on neuronal and glial cells isolated by a number of procedures, as outlined below. Microdissection and centrifugation methods are briefly summarized because they have been covered in previous volumes of this Handbook and in the general literature. Furthermore, many of the results are discussed in Section III of this article. Tissue culture and electrophoresis are treated in somewhat greater detail because they are new in their application to the CNS.

1. *Microdissection and Sieving*

Direct isolation of brain cells has been achieved by dissection of both dried and fresh tissue. Dried, frozen microtome-prepared brain slices have been microdissected under direct microscopic observation. At first, groups of cells and later single nerve cell bodies were sampled from sensory ganglia, spinal cord, and cerebellum. Many biochemical determinations have been performed on cells and isolated cell nuclei prepared in this way.[17–19]

Cells have been isolated by direct microdissection from fresh brain tissue. The lateral vestibular nucleus contains large neurons with satellite glial cells which are mainly oligodendroglia. The neuron and its group of glia can be isolated separately and compared with respect to their macromolecular constituents and metabolic and enzymatic properties[20,21]; similar studies have been made of neurons and glia from cerebral cortex.[20,22]

Neurons have been isolated from ox brain nuclei by passing fresh sliced tissue through sequential nylon sieves of decreasing apertures. The resulting suspension was examined microscopically and the neurons were recovered

by means of a nylon loop.[23] About 300–600 cells could be collected in 3 hr. Amounts sufficient for certain chemical analyses were obtainable.

2. *Centrifugation (Differential, Density Gradient, and Zonal)*

a. Cells. Neuron and glial cell-enriched fractions of brain have been prepared by sucrose and Ficoll-density gradient centrifugation,[24–30] though reproducibility and homogeneity appear to be a problem.[31] A glial cell fraction from white matter (presumably oligodendroglia) has been prepared by sucrose density gradient centrifugation.[32] Zonal centrifugation in combined Ficoll-sucrose media has been used to prepare a neuron fraction, an oligodendroglia fraction also containing cells with the appearance of microglia, and an astrocyte fraction containing capillaries.[33]

While it appears feasible to obtain nerve cell bodies in relative purity, the mechanical nature of the preparative methods and the cytological characteristics of the preparations obtained show that the nerve cells have been severely damaged by severance of their axonal and protoplasmic processes. Nevertheless, isolation techniques of this kind for nerve cell body preparations have potential promise and value.

b. Subcellular Fractions. Attempts to prepare in pure form some of the structures peculiar to the CNS have led to more efficient centrifugation methods for subcellular particles. Myelin and synaptic endings have been subfractionated from the crude mitochondrial fraction and synaptic vesicles and nerve end membranes from the synaptic ending fractions, using sucrose density gradients.[34–39] Zonal centrifugation is also being applied and has yielded a fraction enriched in nerve end membranes.[40] Chemical studies support rather specific localization of some of the components in particular subcellular fractions (Table I).

3. *Tissue and Cell Culture*

Another means for segregation of cytological constituents of the nervous system is selective cultivation *in vitro*, either in organ culture or in dispersed cell culture. A number of observations have been made in the morphology and biochemistry, as well as physiology, of organ cultures of nervous tissues.[41–49] Harvesting of specific constituents is accomplished by microdissection under direct visualization. In principle, however, the use of cell cultures affords the best opportunity for obtaining the different types of cells of the nervous system in a purified form in amounts suitable for biochemical analysis. Procedures or conditions which select for specific cell types *in vitro*, including selective cloning, are employed. The propagation of human fetal spongioblasts and astrocytes in dispersed cell cultures as well as techniques for preparation of pure cultures of astrocytes have been described.[50]

Recently, from trypsinized whole newborn hamster brains, 7- or 14-day suspension or dispersed cultures of astroglial cells have been produced. By subcutaneous injection of these cells in newborn hamsters, astroglial nodules

TABLE I

Established or Potential Indices of Subcellular Components of CNS

Anatomical component	Biochemical constituent
Nucleus	DNA; histones (?)
Soluble cytoplasm	K^+; water-soluble proteins
Mitochondria	Cytochrome oxidase; succinic dehydrogenase
Ribosomes and endoplasmic reticulum (rough)	RNA
Smooth endoplasmic reticulum	Sialyl transferase (?)
Golgi apparatus	An enzyme synthesizing glycoprotein (?) Thiamine pyrophosphatase
Lysosomes	Hydrolases (active on proteins; RNA;DNA; many carbohydrate-containing substances; lipids, etc.)
Neurotubules and neurofilaments	Actomyosinlike protein (Mg–Ca–ATPase)
Astroglial fibrils	α-keratin type protein
Neuron plasma membranes	Gangliosides; Na-K ATPase; AChE; ? specific glycoprotein
Astroglial plasma membranes	?
Oligodendroglial free plasma membranes	Cerebrosides (high sulfated/neutral ratio)?
Myelin	Cerebrosides (low-sulfated/neutral ratio) Proteolipid protein Basic encephalitogenic protein 2′,3′-Cyclic nucleotide 3′-phosphohydrolase
Synapses	Gangliosides; Na-K ATPase, K-nitrophenyl phosphatase; AChE; choline acetylase. Specific protein of vesicles? Specific glycoprotein?

(3–20 mg wet wt) have been produced and shown to be suitable for biochemical study.[51,52] Similar cultures of newborn and fetal ($\frac{2}{3}$ gestation) hamster glial cells have been transformed with SV40 and polyoma virus, respectively.[53–55] The resulting neoplastic cells produce tumors when injected subcutaneously into newborn hamsters. The cultures and tumors both can be grown in amounts suitable for biochemical analysis. Two culture lines of adult rat brain neuroglia transformed *in vivo* by *N*-nitrosomethylurea have also been developed,[56] and some biochemical studies have been done.[51,57,58]

Neoplastic brain cell cultures offer opportunities to sort out normal and malignant characteristics of neuroglia, both by conventional biochemical techniques and by the technique of cell fusion.[59] Of course, data on the biochemistry of *in vitro* preparations of any sort must be viewed critically, and extrapolations to the structure and metabolism of organized tissue *in vivo* made with reservations.

4. *Electrophoresis*

Cells or other microparticles suspended in a liquid carry a charge, which may arise from the intrinsic ionic character of their surface or from adsorbed ions. They may therefore be fractionated by electrophoresis, on the basis of the density of such surface charges.

As yet, few chemical analyses have been done on cells and subcellular particles separated electrophoretically. Methods of dissociating cells and cell parts rapidly without serious damage and the availability of efficient commercial equipment allowing quantitative separations are two requirements for advancement in this field.

Migrations of synaptosomes, synaptic vesicles, and mitochondria have been found to be similar by sucrose density gradient electrophoresis[60] and unaffected by neuraminidase.

Migrations of brain cells dissociated by trypsin and hyaluronidase were studied in an apparatus of special design and the types of cells isolated were reported briefly[61] to show differences in nonspecific cholinesterase (ChE), lactic, and malic dehydrogenase activity. Neurons of bovine brain stem obtained by dissecting and sieving[23] were studied by electrophoresis, but were not separated from extraneous material. The external (neuronal) plasma membrane of frog retinal rod outer segments has been found to carry a charge causing it to migrate at a rate similar to that of frog erythrocytes in a continuous particle electrophoresis apparatus.[62]

The more highly developed technique of microelectrophoresis[63] for studying the surface charge on single cells has not been exploited for cells of brain origin.

C. Frozen Section Sampling and Microanalysis of Selected Cell Populations

1. *Normal CNS Tissue*

An approach for establishing correlation between chemical composition and histological structure, differing significantly from those considered in the foregoing paragraphs, is one that takes advantage of the partial segregation of microanatomical components provided by nature. This is widely obvious in the segregation of neuron cell bodies, dendrites, and synapses in gray nuclei and of glia and axons in white tracts. In addition, in the mammalian nervous system those portions of gray matter that represent late migrations of neuroblasts into the marginal layer, thus producing a gray outer cortex, are organized in a laminated fashion. Thus, adult cerebral and cerebellar cortices and quadrigeminal bodies display characteristic alternations of nerve cell body layers and of feltworks of axonal and dendritic expansions that partially separate these components in a stratified way.

Procedures have been devised for sampling the relatively discrete cell body layers and the axonal and dendritic plexuses of such structures as hippocampus,[64] cerebellum,[65] and retina[66,67] directly by microdissection of vertical frozen dried sections. It is also possible to prepare serial horizontal

samples through successive layers that can be used alternately for microchemical and histological analyses in accordance with the principles developed by Linderstrøm–Lang for other laminated tissues.[68] In the CNS the latter techniques have been particularly adapted for retina[69] and mammalian cerebral isocortex.[70,71] Both of these forms of quantitative histochemical analyses provide a means for comparing and correlating the varying distributions of biochemical constituents with the nonuniform distribution of histological components. This, in turn, makes possible correlative histochemical inferences as well as establishing the microchemical fine structure of the brain subdivision under scrutiny.

In these procedures, the sample size is of the order of 10–100 μg fresh weight. An extensive array of analytical methods is available for assay of both biochemical structural components and enzymes in samples of this size. These include procedures for tissue fractionation and for ultramicrochemical analyses by means of microrespirometry as well as microvolumetric, spectrophotometric, fluorimetric, and gravimetric techniques.[72–74] The methods have operational simplicity, precision, and reliability equal to those of standard tissue biochemistry and yield similarly exact quantitative information. Thus, the degree of anatomical precision is considerably less, but the chemical precision far greater than that provided by classical microscopic histochemistry.

2. *Pathological Tissue*

An extension of the principles just considered to a further type of experiment provided by nature makes use of selective changes in histological composition encountered in the course of disease. Thus, correlative inferences may often be made from comparative observations on regions of the central and peripheral nervous system which have undergone specific forms of parenchymal degeneration and substitution by interstitial elements. In the CNS, areas of replacement gliosis provide an occasional example of a relatively pure culture of astroglia suitable for biochemical analysis, preferably done at the microchemical level so as to maximize histological control. Intracerebral tumors of the glioma group furnish another at least tentative means for ascertaining the biochemical properties of glia, though as with tissue culture studies, extrapolations to the normal nonneoplastic cell types may be hazardous. Nevertheless, analysis of the chemical composition and enzymatic properties of well-differentiated astrocytomas and oligodendrogliomas by means of histologically supported microchemical techniques can sometimes provide suggestive information concerning the biological attributes of the cell types of origin of such neoplasms.[75–80]

Interpretation of results on spontaneously occurring brain gliomas must confront the question of whether only tumor cells or tumor cells plus remnants of invaded brain gray or white matter were analyzed. Such remnants, although inconspicuous microscopically, may influence the results in the case of some biochemical constituents. Sequential microanalyses decrease but do not eliminate the danger that such contaminations will be misleading.

The problem of contamination of tumors by brain tissue can be circumvented by study of experimental gliomas induced by viral and chemical agents and grown subcutaneously in young animals.[57,81]

III. ASSESSMENT OF BIOCHEMICAL INDICES OF SUBCELLULAR COMPONENTS IN NEURAL CELLS

In this section, a selective review of existing knowledge of the cytological localization of biochemical constituents in brain cells will be attempted, based on the contributions of all the previously mentioned approaches, but without detailed references to methods, since information from all sources should form a homogeneous matrix. The two main aims will be to consider the specificity of established indices which to date have proved to be most useful and to select others which have potentialities and merit future investigation. Additional anatomical structures and physiological or metabolic functions for which quantitative indices would be desirable will be mentioned and the likelihood discussed that indices may be developed for them.

A. Definition and Requirements for a Good Index

The pioneers of quantitative chemical histology thought principally in terms of correlating a chemical component or enzyme activity with a particular type of cell present, and this continues to be an aim of the field. However, with study of more components and progress in electron microscopy as well as tissue fractionation by centrifugation, it became clear that in most cases a component could more readily be correlated with a subcellular element than with a particular type of cell. Two types of chemoanatomical index related to subcellular elements came to be recognized: (1) an index of general significance denoting the presence of a kind of structure, organelle, or membrane common to all the cell types, although perhaps more abundant in one cell type; and (2) an index of special significance denoting a structure, organelle, or membrane largely or totally confined to a particular type of cell. Each type of chemoanatomical index has particular advantages.

The first requirement of a good index for a subcellular component or cell type is that it be present in a readily measurable concentration; the permissible concentration thus may depend on the sensitivity of existing methods of analysis. The second requirement is that the component be absent from or occur in only low concentration in other structures or cells present in the sample to be analyzed. The specificity and usefulness of an index, therefore, may vary with the species or age of animal and the region of brain studied.

As considered here, subcellular components will be anatomical structures as revealed by microscopy, not as operationally defined by physical fractionation procedures. Subcellular components for which chemical indices have been or might be sought are listed (Table I), together with the established or suggested indices. Some biochemical characteristics of the three types of cells of the nervous system, a number well-established, others inferred, are also briefly summarized (Table II).

TABLE II

Some Biochemical Characteristics of Nervous Tissue Cells

Constituents	Neurons[a]	Oligodendroglia[b]	Astrocytes[c]
Lipids			
Gangliosides	× × × ×	×	×
Cerebrosides (neutral and sulfated)		× × × ×	
Cholesterol	×	× × × ×	×
Proteins			
Proteolipid proteins	×	× × × ×	
Myelin basic protein		× × × ×	
S-100		×	×
14-3-2	×		
Histone specific to brain and spermatogonia	× × × ×		
Enzymes			
Oxidative enzymes	× × × ×	× ×	×
Hexose monophosphate shunt enzymes	×	× × ×	×
Na-K ATPase	× × × ×	× ×	×
Neurohumor-related enzymes	× × × ×		
Carbonic anhydrase		×	
2′,3′-CN 3′-phosphohydrolase		× × × ×	
Nonspecific cholinesterase	×	× ×	× ×
Glycogen granules	×	×	× × × ×
RNA/cell	× × × ×	×	×

[a] Neurons include dendrites, axis cylinders, and synapses.
[b] Oligodendrocytes include processes and myelin.
[c] Astrocytes include processes.

Much of our knowledge of subcellular constituents has come from studies of centrifugally prepared fractions. However, even when a fraction is pure in terms of the type of anatomical component it contains, it is likely to be heterogeneous in that it is derived from not one but several of the cell types present, and/or from different special regions of the same cell (i.e., the cell body versus the synaptic ending of a neuron, as in the case of mitochondria). This often imposes a serious inherent limitation on interpretation of the results of chemical analysis of such fractions and, when such analyses are not in agreement with metabolic studies by isotope incorporation or with staining histochemistry, such heterogeneity may be an explanation.

B. Subcellular Anatomical Components: Present and Potential Indices

1. *Nucleus*

The prototype and prime example of a type 1 chemoanatomical index is DNA, which assumed this status when it was established that diploid cell

nuclei of a given animal species contain a constant amount of DNA. The specificity of this index is complicated only by the occurrence of small amounts of DNA in extranuclear sites, such as mitochondria,[82] and by tetraploidy of large neurons.[83,84] Moreover, a chemical count of the total number of nuclei of all cells falls short of the desired goal of revealing the numbers of each type of cell. At present this can be derived from the DNA only if the percentages of the different cells are determined by counting them relative to one another in fixed stained tissue from the identical region of brain. Unfortunately, even in stained tissue, oligodendroglia and astrocytes cannot always be distinguished reliably from each other or from small neurons. Therefore, if total DNA as an index could be supplanted by type 2 indices such as special histones used as nuclear markers for neurons, oligodendrogli, and astroglia, respectively, a great refinement in brain cell enumeration would result. A suggestion that such special histones may exist is that fluorescent antibody to a basic protein, of the histone type, from pig brain reacts specifically with neuronal nuclei but not with Schwann cell nuclei or myelin.[85] The authors seem to imply that in the CNS astrocytes and oligodendroglia fail to react, and photomicrographs included suggest they do not; spermatogonia react, but kidney, liver, ovary, and spleen cells do not.

2. *Soluble Cytoplasm*

K^+ as an intracellular ion is probably a better indicator of total cytoplasmic volume of all cells than are the so-called soluble glycolytic enzymes, which not only exist in multiple forms differing in activity (isoenzymes) but also may form a part of a plasma membrane.[86] Other water-soluble enzymes and proteins include hexose monophosphate shunt enzymes,[87,88] ChE,[89] carbonic anhydrase,[90] and S-100 protein.[91–95] These constituents are thought to be localized mainly in glia,[21,88,95] and thus might offer an estimate of glial cytoplasmic volume. The concentration of the brain-specific S-100 protein per unit wet weight of human brain is highest in the molecular layer of cerebellum; cerebral cortical gray matter, however, contains only one fourth as much as cerebral or cerebellar white matter.[93] This protein has been demonstrated in spontaneous human astrogliomas[95] and in a well-differentiated cloned line of astroglial cells obtained by culture of adult rat brain after *in vivo* transformation by *N*-nitrosomethylurea.[56] These findings suggest S-100 as an index of cytoplasmic volume in astrocytes. Another water-soluble protein, 14-3-2, appears to be localized mainly in nerve cells, as indicated by immunofluorescence and nerve cell degeneration studies.[94,96]

3. *Mitochondria*

Mitochondria differ in size and shape and to some extent in biochemical composition. Cytochrome oxidase and succinic dehydrogenase are examples of enzymes exclusively localized in them and ideally suited to be markers; they have been used widely for this purpose. Moreover, succinic dehydrogenase is a marker for the inner membrane of mitochondria,[97] while

monoamine oxidase is present only in the outer membrane.[98] Brain mitochondria have intrinsic proteolytic activity commensurate with their rate of protein turnover; outer membranes contain slightly higher activities of aminopeptidase (Leu-Gly-Gly) and acid proteinase (Hb); the incorporation of radioactive amino acids into inner membranes exceeds that into outer membranes.[99] That not all mitochondrial enzymes make good markers is illustrated first by the fact that the citric acid cycle enzyme malic dehydrogenase also exists in a soluble form.[100] Second, certain enzymes (acetyl-CoA synthetase, glutamic dehydrogenase, glutamine synthetase, and glutaminase I) appear to vary greatly in activity in mitochondrial populations subfractionated on continuous sucrose gradients.[101] This may be related to postulated multiple citric acid cycle intermediate pools suggested by isotopic labeling experiments, and to special groups of mitochondria within brain cells.

Neurons typically have an abundance of mitochondria in perikarya, large proximal dendrites, and synaptic endings. The scanty cytoplasm of oligodendroglia also contains many of these organelles, but normal astrocytes have a significantly lower concentration, as shown by electron microscopy.[4] Consequently, in normal mammalian gray matter, neurons contain the bulk of the mitochondria—that is, a mitochondrial enzyme marker will largely indicate the oxidative capacity of the neurons present. In normal mammalian white matter, it is clear that the abundant oligodendroglia, despite their relatively small volume of cytoplasm, account for the bulk of glial respiration. However, the question of the relative abundance of mitochondria in axons and whether the increased respiration during axonal activity occurs mainly in axons have not been answered.

4. *Ribosomes (Rough Endoplasmic Reticulum)*

Whole brain contains 80% ribosomal RNA, 15% soluble or transfer RNA, and only 5% remains for messenger and preribosomal varieties.[102] The amount of RNA in brain cells varies from 1500 *pg* in large neurons to 45 *pg* or less in small neurons and about 3.5–5.7 *pg* in glial cells.[103] Because of these wide differences, variations in RNA levels in normal mammalian gray matter regions are largely due to neurons; in white matter, RNA is mostly due to glia.[103] Total RNA thus largely indicates the presence of ribosomes (wherever localized in the cell) and may be considered a physiological or metabolic index of the protein synthesizing capacity of the cell or tissue.

5. *Smooth Endoplasmic Reticulum*

Many synthetic functions are performed by smooth endoplasmic reticulum, some involved with glycoproteins, but in brain no enzyme has been shown to be specifically localized there. In liver, however, smooth endoplasmic reticulum contains the enzyme sialyl transferase, which attaches *N*-acetylneuraminic acid (NANA) as a terminal group to the carbohydrate

of glycoproteins.[104] This is an example of a smooth endoplasmic reticulum component that is not also thought to be in Golgi apparatus and/or plasma membranes.

6. *Golgi Apparatus*

In the Golgi apparatus, for the most part, complex carbohydrates are (1) synthesized, (2) joined to protein to form glycoproteins and (3) prepared for secretion, which for most neural cells means secretion to become part of the cell membrane.[105,106] It contains, therefore, enzymes necessary for these functions, but none has been shown to be a specific marker. The presence of thiamine pyrophosphatase is significant, but this enzyme also occurs to a lesser extent in smooth endoplasmic reticulum.[105]

7. *Lysosomes*

Lysosomes are evident histologically in both neurons and neuroglia,[101] and are probably involved in normal growth and maintenance processes, as well as in pathological reactions.[108] Hence their estimation is of obvious importance. In nervous tissues, the enzymes used most satisfactorily for their detection have been: β-galactosidase, β-glucuronidase, arylsulfatase, and acid phosphatase (with glycerophosphate or α-naphthyl acid phosphate as substrates, but not *p*-nitrophenyl acid phosphate). These enzymes are more active in neuron cell bodies than in dendrites, axons, and glial cells.[109–111] Acid phosphatase and β-galactosidase are abundant in lipofuscin granules of aging neurons, suggesting that these arise from lysosomes.[112] The lysosomal hydrolases are not completely specific for lysosomes, since they occur to some extent in the Golgi apparatus, which may be the precursor of lysosomes, as well as in endoplasmic reticulum. Lysosomes of other tissues contain lipolytic enzymes[113] as well as acid RNase, acid DNase, β-glucosidase, β-*N*-acetylglucosaminidase, and phosphoprotein phosphatase.

8. *Glycogen Granules*

Glycogen granules are a characteristic feature of astrocytes, as shown by electron microscopy,[114] and are greatly increased in reactive astrocytes, such as those multiplying after brain trauma.[115] The percentage of total brain glycogen that is present in astrocytes and the part these cells may play in overall brain glycogen metabolism deserves study. Such granules are far less abundant in other types of brain cells.

9. *Microtubules, Neurotubules, and Neurofilaments*

Neurotubules are analogous to microtubules, which are comprised of an actomyosinlike protein that binds GTP and colchicine.[116] A GTP- and colchicine-binding protein has been isolated from pig brain[117] and a colchicine-binding actomyosinlike protein has been prepared from rat, cat,

and ox brains.[118,119] The latter protein is dissociable into actinlike and myosinlike proteins which react with muscle myosin and actin, respectively, with the expected increase in relative viscosity and in Mg^{2+} and Ca^{2+} activated ATPase activity. Since a microtubule fraction, confirmed by electron microscopy, has been prepared from rat brain homogenate by sucrose density gradient centrifugation,[120] biochemical study of this fraction may provide evidence for the localization of mammalian actomysinlike protein in micro- and neurotubules.

Neurotubules and neurofilaments have been suggested as agents in axonal transport processes.[121] Quantitative estimation of neurotubular and neurofibrillar material would be useful in the study of brain during development and in pathological conditions such as Alzheimer's dementia, in which these proteinaceous organelles are severely affected. The usefulness of the actomyosinlike Mg-ATPase as an index of neurotubular protein will depend upon what proportion of the total actomyosin Mg-ATPase the neurotubules represent and whether the enzyme can be distinguished by chemical means, such as inhibitors, from other Mg-ATPases in mitochondria and perhaps in plasma membranes and endoplasmic reticulum.

10. *Astroglial Fibrils*

The protein of astroglial fibrils is of the α-keratin type.[122] It would be worthwhile to determine whether this particular keratin is specific for astrocytes and could be used to quantitate glial fibrils or for identifying astrocytes for counting purposes by immunofluorescence techniques.

11. *Plasma Membranes*

The plasma membrane of a particular cell type may offer the possibility of yielding a highly specific index which could be useful both in estimating the cell surface area by quantitative chemistry or in identifying the cell membranes *in situ* by immunofluorescence. For example, carbohydrate components linked to lipid (gangliosides, cerebrosides, sulfatides, ceramide di- and trihexosides, aminoglycolipids, etc.) and to protein (glycoproteins) are known plasma membrane constituents, and have immunological significance. Of the two, glycoproteins have much broader and greater potentialities for specificity reactions because of the almost infinite variety in macromolecular composition and configuration conferred by a combination of protein and polysaccharide chains. Glycoproteins are detectable by electron microscopic techniques in CNS surface membranes.[123] Progress in methods of separating glycoproteins from brain have been reviewed (Vol. 1 of this *Handbook*, Chapters 5 and 11). The study of glycoproteins in brain is not sufficiently advanced to allow definite assignment of any particular pure glycoprotein to a given cell type, but it seems likely that this will eventually be possible.

Present biochemical indices for plasma membranes do not have such a complete specificity for cell type, but have been useful, nevertheless. As discussed below, the enzyme Na–K ATPase and complex gangliosides are the principal indices of neuronal plasma membranes; neutral and sulfated cerebrosides may be indicative of oligodendrocyte plasma membranes as well as of myelin; but no index has been proposed for astrocyte surface membranes. In liver tissue, the enzyme 5′-nucleotidase is a plasma membrane marker[124]; possibly the much higher level of this enzyme activity in white than in gray matter[87] may indicate its presence in the free membranes of oligodendrocytes. In fat cell ghosts[125] the enzyme adenyl cyclase, which synthesizes cyclic 3′,5′-AMP, is tightly bound to the plasma membranes. This enzyme occurs in brain in concentrations 2–10 times as great as in heart, adrenal, epididymal fat pad, kidney, and liver, per unit wet weight.[126] However, whether it may be localized in neuronal or glial plasma membranes has not been established.

a. Neuronal Plasma Membranes. The plasma membranes of the neuron cell body, dendrites, axon, and synapses will be discussed together. More complex aspects of synapses will be considered separately (Section III, B.12).

Support for Na–K ATPase activity and complex gangliosides as indices of neuron plasma membranes is compelling. Localization of Na–K ATPase mainly in neuron plasma membranes is indicated by studies of synaptic ending fractions[35,37,39] and single neurons,[127] and is consistent with its intralaminar distribution in rat somatosensory cortex.[128] Possibly, Na–K ATPase is a better index of neuron surfaces and synapses than gangliosides are, since the latter are comprised of up to 12 compounds. Ganglioside NANA and Na–K ATPase activity do not parallel one another perfectly in distribution in tissues and subcellular fractions, but the significance of the differences is not clear.

Localization of gangliosides in neuron plasma membranes is supported by findings of high concentrations of lipid NANA in synaptic endings though not in synaptic vesicles,[129] and higher concentrations in surrounding neuropil than in wet-dissected neurons.[130] Distributions of gangliosides in the layers of human frontal and rat somatosensory cortex[131] and bovine hippocampal and cerebellar cortex[132] are consistent with such a localization. The variations in the relative amounts of the separate species of ganglioside in a number of histologically different regions of human brain[133] suggest either discrete localization or discrete turnover (or both) of certain ganglioside molecules in anatomical structures in those regions. The anatomical structures, however, have not been identified. No single species of complex neural ganglioside has been shown to be uniquely localized in a specific type of neuronal membrane, such as that of cell body, dendrite, axon, or synapse.

The second requirement for a good index is also satisfied for Na–K ATPase and gangliosides, namely, that they occur in relatively low concentration in other cells or subcellular elements. Several types of preparations derived from astrocytes and oligodendrocytes have been examined for these neuronal indices. "Normal" astroglial nodules grown subcutaneously in

newborn hamsters by injection of cells of a 7- or 14-day suspension or dispersed culture of trypsinized whole newborn hamster brain have low but detectable levels of Na–K ATPase activity,[134] as do astrocytoma tissues derived from virally or chemically transformed astrocytes of hamster or rat origin.[81] These results suggest that astrocytes may resemble nonnervous tissue cells (such as erythrocytes or liver cells), which function adequately with a much lower capacity for Na^+ pumping than neurons require. Both the normal glial nodules and the experimental astrocytomas contain low but detectable levels of ganglioside NANA and ceramide hexosides.[51,52] Astrocytes separated by a centrifugation procedure from adult rat brain also contain little ganglioside and ceramide hexoside.[135] In glial cells (probably mainly oligodendroglia) separated from bovine white matter by centrifugation procedures,[136] gangliosides have not been observed; however, small amounts occur in myelin fractions,[137] mainly the monosialoganglioside G_{M1} which presumably derives either from the oligodendrocyte or from the axon. Na–K ATPase activity is very low in myelin fractions, but has not been studied in the oligodendroglia fraction.

Glycoprotein NANA does not perfectly parallel ganglioside NANA in distribution in subcellular fractions, although both are enriched in microsomal and synaptosomal fractions.[106] Results of further subfractionations suggest that the dense membranes of the synaptosome fraction contain a higher ratio of glycoprotein : ganglioside than do lighter fractions presumed to be mainly from the other neuron cell surfaces (cell body, dendrites, and axons).

Acetylcholinesterase is an established constituent of neuron plasma membranes (and of endoplasmic reticulum and synapses), but varies widely in concentration in different types of neurons and in different parts of the same type of neuron.[138] Since it is restricted to neurons, it is a useful indicator of the presence of neuronal membranes, especially those of cholinergic neurons, in which it is most highly concentrated.

b. Astroglial Plasma Membranes. At present, no established or potential biochemical index of astrocyte plasma membranes exists. Subcellular fractions identifiable as astroglial plasma membranes have not been prepared. However, several types of astroglial preparations are now available, as discussed in the previous section, which should eventually yield such a fraction. The results of the studies on the whole cells of these preparations may indicate something of the trend to be expected for astrocyte plasma membranes. In most regions of mammalian gray and white matter, astrocytes are greatly outnumbered by neurons and oligodendrocytes, respectively. Whatever distinctive biochemical properties their plasma membranes possess would not be predominantly displayed in such a setting, but studies of the cells isolated by culture or tissue fractionation methods should eventually reveal them.

c. Oligodendroglial Plasma Membranes. These membranes may be considered to occur in two forms: (1) the free membranes and (2) their derivative compact myelin. Compact myelin is built up by an imperfectly understood developmental process from windings of the free membranes. Myelin may represent a simplified model of the oligodendrocyte plasma membrane. The chemical composition of myelin has been studied in detail, as reviewed in Vol. 1, Chapter 9 of this *Handbook*.

For compact myelin the simplest and best biochemical structural index is total cerebroside (neutral plus sulfated), which surpasses cholesterol and proteolipid protein in specificity.[139] Neutral cerebrosides and their sulfate esters comprise 28–31% of the lipids of purified CNS myelin.[140] Furthermore, they appear to comprise 14–16% of the lipids of glial cells (probably oligodendroglia) isolated from bovine white matter; interestingly, the proportion of sulfate esters is significantly greater in the cell fraction than in myelin.[136] Neutral cerebroside may therefore prove to be a slightly more specific index of myelin itself, while sulfatide may be more indicative of oligodendrocyte free plasma membranes.

The second requirement for a good index is also satisfied by total cerebroside, namely, that it should occur only in low concentration in other cells or structures. Cerebrosides show high relative enrichment in myelin fractions as compared with synaptosomal, mitochondrial, microsomal, and nuclear subcellular fractions.[141] The relative enrichment in myelin is much greater for cerebroside than for cholesterol, sphingomyelin, or any other lipid studied. That cerebrosides may comprise only a small fraction of the lipids of neuron membranes is indicated by the low values in gray matter. Furthermore, the low concentrations of lipid hexose in astroglial nodules[52] and tumors[51] probably reflect little cerebroside in astrocytic cells. Astrocytes are as abundant in gray as in white matter.

Total cerebrosides are therefore a good index of myelinated fibers because of their high concentration in oligodendroglial free membranes and their derivative compact myelin, as well as their low concentrations in mitochondria, synapses, and neuronal and astrocytic membranes.

Proteolipid proteins, which are extractable from fresh tissue by 2:1 chloroform–methanol, constitute one of the major types of protein in myelin. As defined and isolated in this way, they are less satisfactory than cerebrosides as an index of myelin because some nonmyelin "gray matter" proteolipid proteins occur in synapses and mitochondria.[142–144]

A basic protein responsible for experimental allergic encephalitogenic activity of myelin is the second most abundant protein of myelin, but its possibilities as a biochemical index of myelin have not been explored. It is extractable from purified myelin by 2:1 chloroform–methanol,[145] but this solvent does not remove it from fresh tissue, aqueous acid being necessary for its solubilization.[146–149] It differs from proteolipid protein in being digestible by trypsin and this property is of assistance in its histochemical demonstration.[150] Immunofluorescence localization also has been studied.[151]

Myelin may also contain a third major protein, with a content of 23% dicarboxylic acids.[152,153]

For both developing and adult myelin an enzymatic marker with a specificity like that of cerebrosides is 2′,3′-cyclic nucleotide 3′-phosphohydrolase.[154] The function of this enzyme in myelin is unknown. Myelin is for the most part low in enzyme activity.

Free plasma membranes of oligodendrocytes may reasonably be expected to have some properties like myelin, such as high cerebrosides, especially sulfated cerebrosides, but probably have an active complement of enzymes and other surface components lacking in myelin.

12. *Synapses*

Synapses as functional units include not only the specialized plasma membrane junctional regions of the axonal ending and the receptor cell involved, but also the usual plasma membranes (discussed under neuron plasma membranes) adjacent to the junction on both sides, the mitochondria, synaptic vesicles or storage granules, cytoplasm and soluble enzymes.

A chemical index which would quantitate the total number of synapses or those of a certain variety in a tissue sample would be a great aid in detecting changes in connectivity during growth and in functional or pathological states of the CNS. The specificity required is to be looked for in the two unique aspects of the synapse, namely, the synaptic vesicles and the synaptic junctional regions themselves. First, for example, each type of storage vesicle might contain a specific protein which could serve as an index. No evidence for this has been obtained for brain, but 50% of the solubilizable protein of adrenal catecholamine storage vesicles consists of a single protein.[155] Second, the postsynaptic (receptor) membrane, the interjunctional gap, or the presynaptic membrane may contain a special component characteristic of the type of synapse.

At present, a certain degree of quantitation of synapses is afforded by assay of gangliosides, Na–K ATPase, K-nitrophenyl phosphatase, AChE and choline acetylase—all of which occur in characteristically high concentration in synaptosomes isolated by centrifugation procedures. The broader localizations of these components in neuronal plasma membranes and in some cases in endoplasmic reticulum (AChE, for example) are well known, so that a greatly increased concentration in the junction itself would be required to give the results significant specificity.

Assays of enzymes involved in the metabolism of 5-hydroxytryptamine, norepinephrine, and dopamine, as well as γ-aminobutyric acid can provide information complementing that on ACh metabolizing enzymes. Quantitative assay of neurohumors directly is not ideal, even when accurate, since these substances exist in several compartments with different physiological significance. Qualitative topographical studies of bioamines by histofluorescence have been useful in revealing serotonin-containing neurons in the central raphe system of mesencephalon and myelencephalon, norepinephrine-containing neurons in the lateral part of the bulbopontine tegmentum (mainly locus coeruleus) and dopamine neurons in ventral mesencephalon.[156–159]

TABLE III

Biochemical Indices Useful in Quantitative Neurochemical Histology

Biochemical index	Cytological component	Architectonic picture
DNA	Nuclei	Total cytoarchitecture
DNA combined with percent neurons and glia by count		Neuron cytoarchitecture Glia cytoarchitecture
Cerebrosides (neutral + sulfated)	Myelin, nerve fibers	Myeloarchitecture
Gangliosides, Na–K ATPase	Neuropil (dendrites, fine axons, synapses and glial membranes)	Piloarchitecture
Cytochrome oxidase	Mitochondria	Mitochondrial membranes (sites of oxidative metabolism)
RNA	Ribosomes	Rough endoplasmic reticulum (principal sites of protein synthesis)

13. *Summary*

A brief list is given (Table III) of some well-established and useful chemoanatomical indices, with the subcellular elements they represent and the architectonic picture that results when they are employed in quantitative neurochemical histology. The terms cyto- and myelo-architecture are familiar from long usage, but the term piloarchitecture has been coined by analogy to describe the distribution of the material complementary to cells and myelin, namely, the neuropil.

The listed group of biochemical indices, measured per unit dry weight, can be combined with the histological procedure of counting neurons as a percent of total cells, to provide interrelated data on: (1) the total numbers of cells, and (2) the total numbers of neurons and nonneuronal cells (referred to as glia in the table, but also including vascular cells) separately.

From the preceding review of possible new indices, it is difficult to choose others that are likely to prove as useful. Obviously most constituents can be expected not to be good indices. It is far easier to point out specific cellular or subcellular anatomical components which it would be desirable to quantitate chemically. In most instances, however, either a highly specific constituent of the anatomical component has not yet been identified, or the constituent occurs in such low concentration that no suitable method exists to allow its assay in a small sample. Hence, in the one case an index must be sought and in the other a more sensitive method of assay devised. Nuclear or cell surface constituents specific for neurons, oligodendroglia, and astrocytes, respectively, would be exceedingly useful, even if they could be applied only

qualitatively to identify a particular cell in histological sections. This would allow, for the first time, truly accurate counting of the relative numbers of the three types of neural cells (and endothelial cells) in CNS tissues. Quantitative chemical assays for nuclear (histones?) or surface membrane (mucoprotein?) indices of the three types of cells would be even more useful. Equally desirable would be indices for the number of total or special synapses and for neurotubular and neurofibrillar material.

Future refinements in knowledge of the molecular constitution of membranes, as well as nuclei and chromatin of nervous tissue cells may provide such precise chemical indices for subcellular constituents of specific cell types.

IV. ILLUSTRATIVE USE OF INDICES

Two illustrations will be given of the use of biochemical indices to construct a quantitative picture of the histology of brain regions. The first will consist of a group of human CNS regions, chosen for marked differences in their microscopic anatomy in order to survey the resulting range of variation in concentrations of the constituents. The second will represent a further extension of the methods of quantitative neurochemical histology to the depiction of detailed architectonics within the stratified tissue of human and rat cerebral cortex. The results on cortical tissue demonstrate some architectonic features related to the basic plan of intracortical microanatomy in mammals and other features related to the area of cortex and the species of animal.

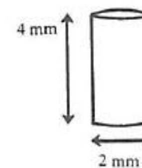
The indices used include most of those listed in Table III, as well as proteolipid protein and the chloroform–methanol insoluble protein of the residue. The residue protein is a reference substance that is relatively uniform per unit volume, thus substituting for wet weight on the microscale. The scheme of analysis[103,160–163] is designed for simultaneous assay of constituents whose concentrations are to be compared accurately (Fig. 1).

A. Selected Tracts and Nuclei of Human CNS

Analyses were performed in 14 regions of normal human brain and spinal cord obtained at autopsy.[103,163] Included are eight regions of gray matter, four of white matter, and two special regions which lie outside the blood–brain barrier and have secretory functions. The data (Figs. 2–4) illustrate quantitatively the characteristic histological features of each region.

1. *Cerebral Cortex and Caudate Nucleus*

Frontal cortex (Brodmann area 9) is one of the secondary association areas which are late in myelinating and never attain as high a density of myelinated fibers as other cortical regions do. Only caudate nucleus (chosen for its paucity of myelinated fibers) has a lower concentration of cerebroside



Frozen cylinder of tissue, sectioned serially into 20 or 40 μ thick slices, dry weight 10–35 μg (av.)

Sections are grouped in consecutive sets of 4 slices for analysis:

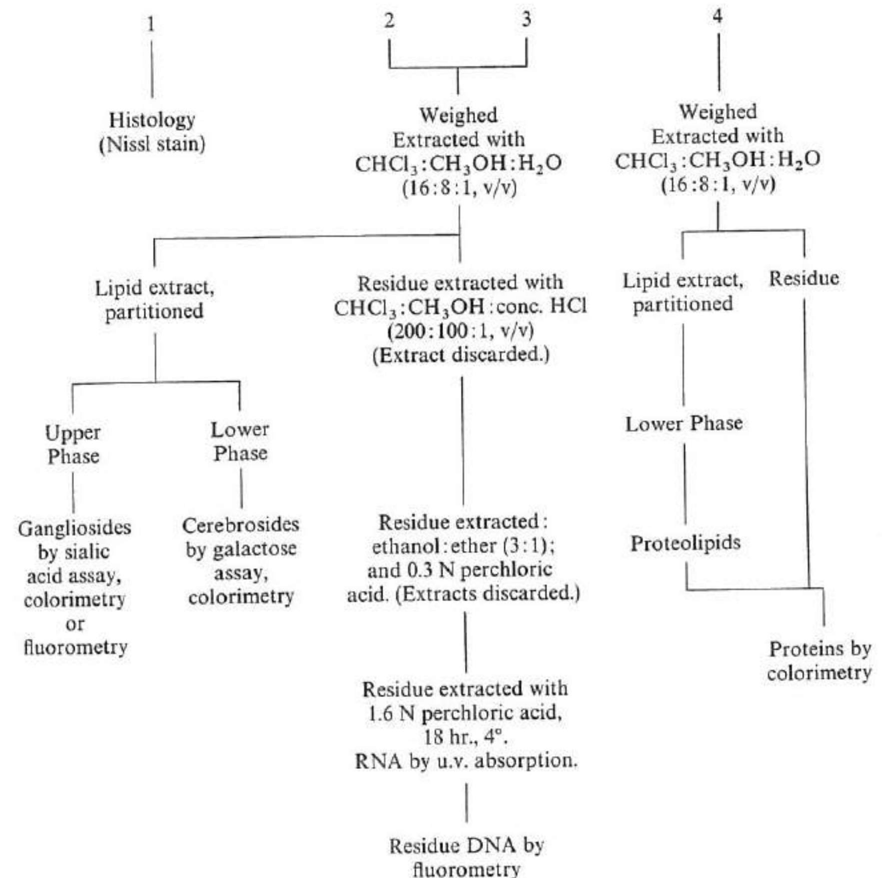


Fig. 1. Scheme of analysis for biochemical structural components in CNS.

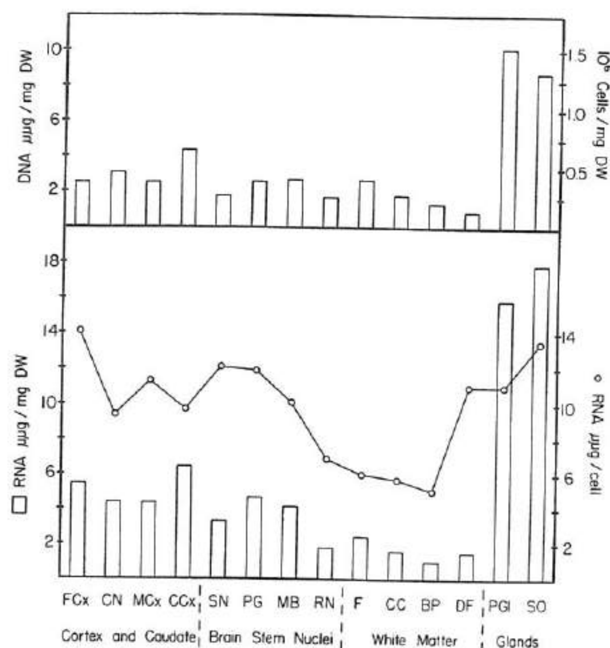


Fig. 2. Regional distribution of DNA and RNA in human CNS. Dried sections (20–110 μg dry weight) were analyzed as noted in Fig. 1. DNA was converted to numbers of cells based on a human diploid nuclear value of 6.6 pg. In this figure and in Figs. 3 and 4 the following abbreviations are used: FCx, frontal cortex; CN, caudate nucleus; MCx, motor cortex; CCx, calcarine cortex; SN, substantia nigra; PG, periaqueductal gray matter; MB, mammillary body; RN, red nucleus; F, fornix; CC, corpus callosum; BP, basis pedunculi; DF, dorsal funiculi; PGl, pineal gland; and SO, subfornical body.

(Fig. 3). Compared with caudate nucleus, frontal cortex has a lower cell content and a higher RNA concentration, based on dry weight; hence, the RNA per cell is appreciably greater (Fig. 2). The RNA per cell is greater in frontal cortex than in any other region, including the two secretory regions. The ganglioside NANA (calculated both per unit dry weight and per cell) is likewise greater than in any other region. The NANA-to-RNA ratio (Figs. 3, 4) is equally high in frontal cortex, caudate nucleus, and substantia nigra. These results on frontal cortex point to a population of neurons with high protein synthetic capacity in the cell body (RNA per cell) and a high ratio of ambient dendritic and axonal surface area (including synapses) in relation to the cell body surface area. The percentages of the cells that are neurons in the three cortical areas vary from 46% in calcarine to 25% in frontal and 16% in the

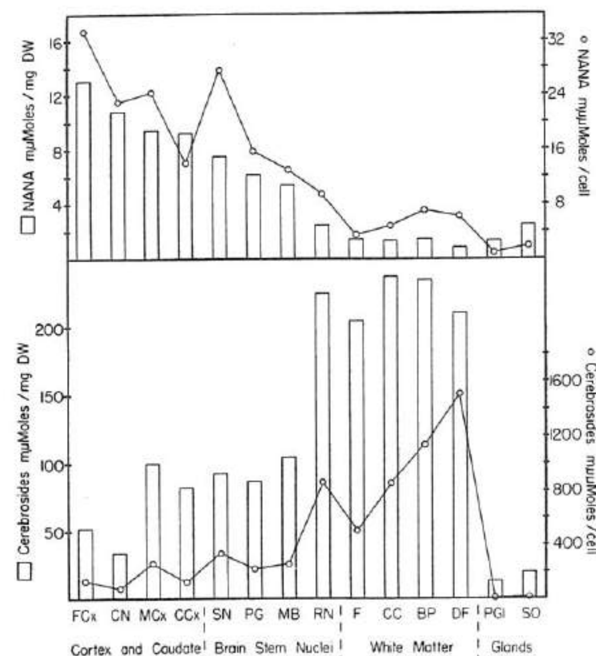


Fig. 3. Ganglioside-NANA and cerebrosides; regional distribution in human CNS. NANA was assayed by a microversion of the Aminoff method after 2 hr hydrolysis in 0.1 N H_2SO_4.

motor region; hence, if ganglioside NANA were calculated per neuron present, the respective values would be 31, 134, and 152 moles NANA in calcarine, frontal, and motor cortices, respectively; the fact that motor cortex has the highest value can be accounted for by the higher percentage of large neurons present.

Calcarine cortex has a cell density greater than that of any other region except the two secretory regions (Fig. 2). Although the percentage of the cells that are neurons is greater than in the other two cortical regions, the neurons are smaller on the average and the RNA per cell is lower. The cerebroside per milligram dry weight is much greater than in caudate nucleus and frontal cortex and like that in motor cortex and the nuclei of the diencephalon and mesencephalon (Fig. 3). The cerebroside per cell is similar to that of frontal cortex and appreciably less than that in motor cortex.

2. *Nuclei of Diencephalon and Mesencephalon*

The mammillary body of the hypothalamus has a cell content per milligram dry weight like that in frontal and motor cortex, periaqueductal

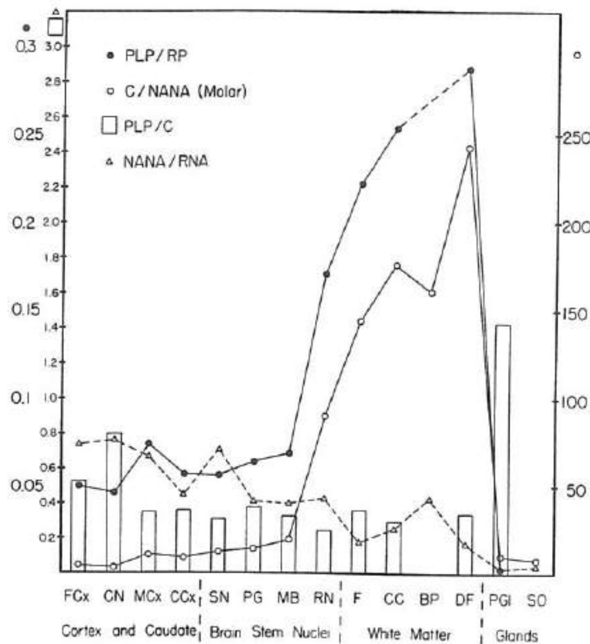


Fig. 4. Ratios of biochemical constituents in human CNS regions. The C/NANA ratio was calculated on a molar basis; the other ratios on a weight basis. ●——●, PLP/residue protein. Bars, PLP/C. ⊙——⊙, C/NANA. △----△, NANA/RNA.

gray matter and fornix (Fig. 2). The ganglioside NANA per milligram dry weight and per cell is lower than in any other gray region except red nucleus (Fig. 3). The cerebroside value in mammillary body is slightly greater than that in motor cortex, but only half that in red nucleus, which has a high density of myelinated fibers (Fig. 3). Of the three mesencephalic nuclei studied, the periaqueductal gray matter contains the highest density of cells and the highest concentration of RNA/mg dry weight (Fig. 2). The RNA per cell, however, is equally high in the substantia nigra. The NANA/mg dry weight decreases in the order substantia nigra, periaqueductal gray, and red nucleus (Fig. 3). The substantia nigra is outstanding in its level of NANA per cell.

3. *White Matter*

Of the white matter regions studied, fornix was chosen for its small diameter fibers and high cell density. The DNA is appreciably greater than in the other fiber tracts, as is the RNA per milligram dry weight (Fig. 2). The RNA per cell is greater than in the other two brain tracts, but in the spinal cord the dorsal funiculus (chosen for its large diameter fibers) has twice as

high a value of RNA per cell. It may be concluded that the value of RNA in white matter of mammals is almost entirely glial, since axonal RNA concentrations are very low.[103,164] The results suggest that oligodendroglial cells associated with large diameter heavily myelinated fibers may contain more RNA than those associated with small fibers. Greater protein synthetic potential may be required of a cell responsible for maintenance of myelin around such a large fiber, with a large internode.

Ganglioside NANA per milligram dry weight is lowest in dorsal funiculus (Fig. 3), but the amount per cell is the greatest; the amount per unit fiber length, if estimated, would surely have been by far the greatest. Cerebroside per milligram dry weight is similar in the four tracts (Fig. 3); consequently, since the cell density decreases in the order fornix, corpus callosum, basis pedunculus, and dorsal funiculus, the cerebroside per cell increases in that order.

4. *Secretory Organs*

Pineal gland contains the highest population of cells found in any region, but subfornical organ has the highest RNA (Fig. 2). Ganglioside NANA is greater in subfornical organ, probably reflecting the fact that it contains some neurons and synapses (Fig. 3).

5. *Ratios of Constituents*

The cerebroside-to-NANA molar ratio is far greater in the white tracts than elsewhere (Fig. 4); this ratio gives a clear distinction between the regions that contain neuron cell bodies, dendrites, and synapses from those that contain only axons (and glial cells). It is lowest in frontal cortex and caudate nucleus. The proteolipid protein-to-residue protein ratio gives a similar but less marked distinction between gray and white regions (Fig. 4).

The proteolipid protein-to-cerebroside ratio (Fig. 4) is relatively constant in all regions except for frontal cortex, caudate nucleus, and pineal gland (subfornical organ was not analyzed). These regions contain less cerebroside; in other words, proteolipid protein is present in structures with little cerebroside, in contrast with its usual occurrence in myelin, which contains high cerebroside concentrations. Subsequent studies on the intralaminar distributions of cerebrosides and proteolipid proteins in human frontal cortex and rat somatosensory cortex have cast further light on this high ratio, as discussed in the next section.

B. Human and Rat Cerebral Cortex

Studies in the authors' laboratories on the chemoarchitectonics of human and rat isocortex[165] and the bearing they have on the issues under consideration in this article, may be illustrated by three examples, two dealing with structural myeloarchitecture, the third with the enzymatic architecture. In these investigations the quantitative distributions of biochemical constituents are determined in microgram-sized tissue samples obtained at 20–25

evenly spaced intracortical intervals. The resulting profile of the intracortical distribution of a given constituent can then be related to the characteristic stratification of nerve cell bodies, myelinated axonal plexuses, and other histological elements.

1. *Cerebrosides*

The intralaminar distributions of total cerebrosides (neutral and sulfated) in the prefrontal cortex of man[166] and the somatosensory cortex of rat[139] give a quantitative dimension to previous findings with Weigert stains of these regions (Fig. 5). This human secondary association area (Brodmann area 9) is thinly myelinated. In contrast, the primary sensory area is one of the first to myelinate and is the most heavily myelinated region of rat cerebral cortex. The intracortical patterns in the two species have obvious similarities, despite the areal differences. In human cortex the lowest values were seen in the upper half of layer III; the next lowest value was in layer II. In rat cortex, layer II values were lower than the lowest value in upper layer III. In both human and rat cortex the highest values were in white matter and represented five- and fourfold increases, respectively, over the lowest values in the cortex. The cerebroside as a percent of dry weight was only slightly greater in rat than in human white matter. Elevations were seen

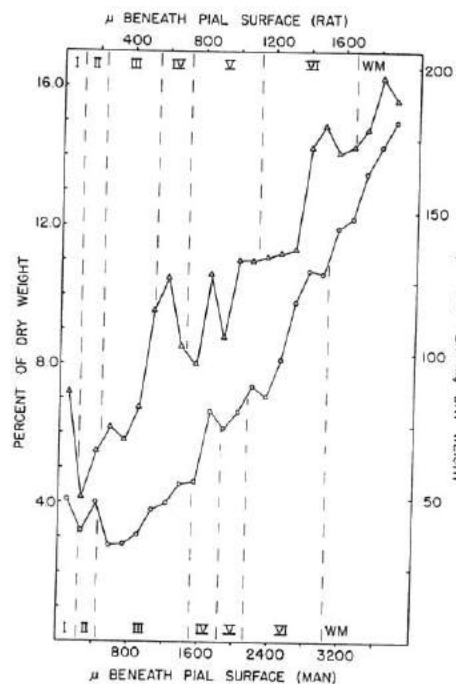


Fig. 5. Cerebrosides as an index of the myeloarchitecture in human frontal and rat somatosensory cortex, expressed on percentage and molar bases. ○——○, human cortex; △——△, rat cortex.

in layer I (tangential plexus), the II–III border (stripe of Kaes and Bechterew), layer IV (outer line of Baillarger), the V–VI junction (inner line of Baillarger), and in the lower half of layer VI (the inner tangential plexus in rat, analogous to subcortical U fibers in man). The greater prominence of the peak of layer IV in the rat is consistent with its reception of well-myelinated specific sensory afferent fibers from the posterior ventral nucleus of the thalamus. On the other hand, it has been suggested that human area 9 may be athalamic and the origin of the myelinated fibers in layer IV is uncertain.[167]

This work illustrates that within a subdivision of the nervous system having an internal structure describable only at the microscopic and submicroscopic levels, the distribution pattern of cerebrosides both in terms of their gray matter-to-white matter ratio and details of their intralaminar profiles are consistent with principal localization in the myelin sheath in conformity with other extensive evidence to which reference has already been made. Furthermore, it seems a reasonable assumption that within such a tissue as cerebral cortex as a whole, and even within the most discrete substituent strata of medullated fibers, cerebroside concentrations can be used as quantitative measurements for total and relative amounts of myelin and as sensitive indices of its integrity.

2. *Proteolipid Proteins*

In contrast with cerebrosides, proteolipid proteins have a quite different distribution in human frontal[168] as compared with rat somatosensory[139] cortex (Fig. 6). Although this type of protein comprises about 45% of the proteins of myelin,[153] it is not confined to this structure. In rat cortex the distribution of the proteolipid protein-to-cerebroside ratio demonstrates that these substances parallel each other throughout except for an elevation at the junction of layers I and II and another one in layer IV. In human cortex, however, the characteristic ratio of myelinated fibers (0.3–0.4) is seen only in the lower half of the cortex; in the upper layers, proteolipid protein is present in cerebroside-poor structures, as has been found also in caudate nucleus and pineal gland. The gray matter nonmyelin proteolipid protein may be related to the abundance of fine plexuses of dendrites, axons, and synapses in the upper layers of human prefrontal cortex, which has a high ratio of neuropil in relation to neuron somata. Two histological elements shown to contain proteolipid protein and little cerebroside are synapses and mitochondria, as discussed previously.[139] Synaptosomes appear to contain 5–14% of their protein as proteolipid protein[142–144] and their cerebroside content is low.[141] It is interesting that in human cortex, where gray matter proteolipid protein may comprise up to 60% of the total in upper layer III, the pattern of the proteolipid protein/cerebroside ratio is similar to that of the mitochondrial marker cytochrome oxidase (Fig. 7). This would be consistent with a largely parallel intracortical distribution of mitochondria and synapses (or other components with a similar ratio) in cortex having especially abundant fine plexuses of dendrites and axons. A suggested function of the

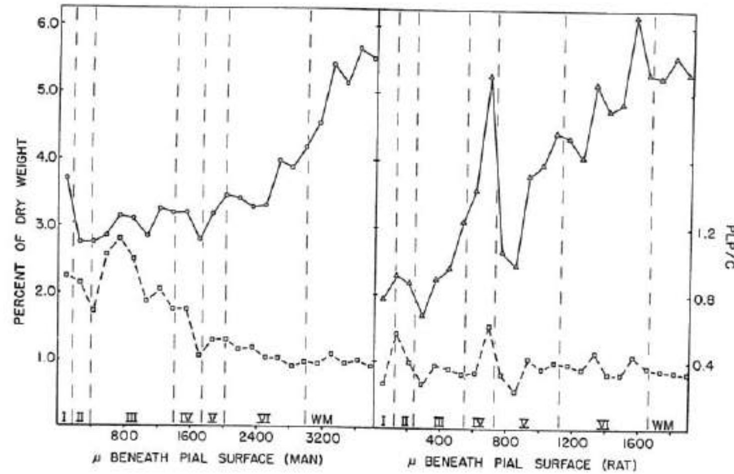


Fig. 6. Proteolipid proteins (PLP) as an index of the myeloarchitecture in human frontal and rat somatosensory cortex, expressed as a percentage of dry weight. ○——○, human cortex; △——△, rat cortex. □——□, PLP/C ratio in human and rat cortex (left and right lower curves, respectively).

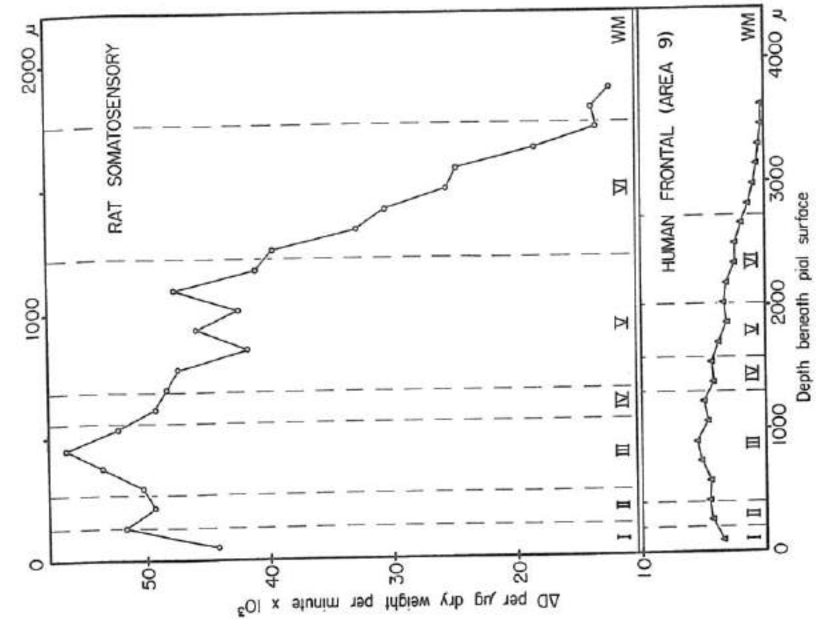
proteolipid protein of synapses is as a high affinity binder of certain neurohumors.[169]

3. *Cytochrome Oxidase*

The intralaminar distributions of cytochrome oxidase activity in the same two areas of human and rat cortex (Fig. 7) show equally interesting similarities and differences.

In a given tissue sample, the activity of this enzyme can be considered indicative of the potential (and perhaps also steady state) rate of biological oxidations and hence of energy metabolism. Mediating, as it does, a critical step in electron transport, the enzyme is part of the complex for oxidative phosphorylation localized in mitochondria. It serves as a quantitative histochemical marker for these organelles whatever their cytological localization, and in this sense Fig. 7 depicts the distribution of mitochondria within the two cortices. It is clear that in both species mitochondria are abundant and energy metabolism high throughout layers I–IV, especially so in the midzone of layer III, and that there is a considerable and progressive decline in the potential for biological oxidations through the infragranular layers and into the subjacent white matter; the decline is more pronounced in human than in rat cortex.

A careful analysis of the details of the intralaminar pattern of cytochrome oxidase in human isocortex,[170] and of the essentially similar pattern found in the somatosensory cortex of the rat[171] indicates that in both species it parallels the probable total mass of nerve cell bodies, the large proximal



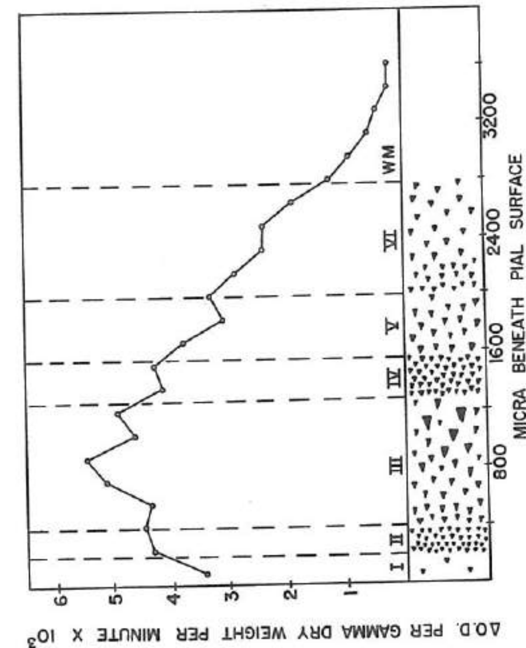


Fig. 7. Cytochrome oxidase as an index of the architectonic distribution of mitochondrial membranes in human frontal and rat somatosensory cortex. Human cortex: left curve and right lower curve. Rat cortex: right upper curve.

portions of dendrites and the axon terminals which represent the major sites of mitochondria observed microscopically.[4] The intralaminar pattern of cytochrome oxidase also parallels that of the total capillary bed, both being indicative of relative rates of oxidative metabolism. Thus, the conclusion seems warranted that cytochrome oxidase activity furnishes an effective quantitative means for estimating the abundance of mitochondrial membranes and the relative potential rates of energy metabolism within the microscopic infrastructure of nervous tissues. Correlation would exist with mitochondrial membranes rather than with numbers of mitochondria, since the latter vary markedly in size.

Slice respiration studies have suggested that rat brain does not share in the six to ten times higher metabolic rate of the smaller mammal.[172] However, if this is the case, a lack of terminal oxidase cannot be the determining factor, since it is about 10 times as active in rat as in human cortex (see lower curve of right side of figure for proportional plot of human data).

V. GENERAL CONCLUSIONS AND USEFULNESS OF BIOCHEMICAL INDICES IN NEUROPATHOLOGY

The use of biochemical indices in conjunction with serial frozen section sampling and microchemical analysis offers an approach to interpreting neurochemical data on histologically complex regions of the CNS. The combined technique may be called quantitative neurochemical histology because the indices chosen give a quantitative picture of certain histological elements, such as nuclei (DNA), myelin or myelinated fibers (cerebrosides, neutral plus sulfated), neuron plasma membranes and synapses (ganglioside sialic acid and Na–K ATPase), mitochondria (cytochrome oxidase), and ribosomes or rough endoplasmic reticulum (RNA). Possibilities exist for extending this list with progress in knowledge of the molecular architecture of membranes, as well as nuclei and chromatin of nervous tissue cells, and for achieving greater specificity.

The distributions of the indices in various tracts and nuclei of human CNS agree well with the histological pictures. Results for the distribution of cerebrosides, proteolipid proteins, and cytochrome oxidase in human and rat cerebral cortex show by their general resemblances the similarity in the underlying pattern of the anatomical fine structure of the two cortices, and by their differences the quantitative variations corresponding partly to regional and functional specialization (secondary association area 9 of man and specific somatosensory region of rat) and partly to the size of animal and to phylogeny.

Extension of the methods of quantitative neurochemical histology to pathology has necessarily lagged behind studies of normal tissues. Interpreting neurochemical data on pathological specimens is a challenging problem.

Chemoanatomical indices are used as referents supplementing wet and dry weight, total protein and total lipid. They can objectively quantify such qualitative subjective expressions as "cell loss," "cell multiplication," "status spongiosis," etc. Valid comparisons can thus be made between different tissue specimens, normal and abnormal.

A number of investigations suggest that appropriate microchemical observations can demonstrate functional or pathological change prior to the appearance of unequivocal histopathological alterations, at least at the level of light microscopy. For example, under certain circumstances homologous cortex contralateral to an established epileptogenic lesion may develop autonomous, independently generated focal spike discharges. Elevation of AChE activity[173] may occur in the secondary or mirror focus as it does in the primary in spite of the absence of unmistakable histologic changes in light microscopic preparations. Similarly, decreases in cerebroside and proteolipid protein concentrations indicative of intracortical demyelination are demonstrable in Alzheimer's disease before there are detectable changes in standard histological preparations for myelin sheaths.[174] In certain other cases of Alzheimer's disease showing definite but relatively mild histopathology, Mg-ATPase activity has been found to be considerably decreased at a time when Na–K ATPase activity is still maintained at normal levels[175]; in cases with severe pathological changes in the cortex, however, Na–K ATPase activity decreased markedly. An early loss of Mg-ATPase activity may be related to the well-known alterations in neurotubules and neurofibrils in the disease, since actomyosin Mg-ATPase activity has been shown to be present in a brain protein fraction[118,119] probably derived, at least in part, from these structures.

In neuropathological conditions in which histological changes are far advanced (Creutzfeldt–Jacob disease and experimental cyanide encephalopathy) the use of microchemical indices can provide a quantitative reflection of the extent of progress of the disease processes.[176,177]

Quantitative neurochemical histology is not limited to a static depiction of the histology of a brain region, but may also be used to detect and evaluate subtle metabolic and morphological changes accompanying growth, function, and aging. Similarly, quantitative neurochemical pathology is not limited to the construction of a static description of the end stage of a disease process. Biochemical indices or markers serve both as measures of the concentrations of particular histological elements and as reference components. They are useful as modes of expression of other substances or enzymes that lack specific morphological localizations or that may change rapidly in functional or pathological states. Therefore, quantitative chemical histology and pathology, as defined and considered in this article, can be developed as a precise technique to detect metabolic and structural biochemical changes in CNS tissues and to give a picture of the time course of a disease process when favorable components are chosen and suitable samples are available for study.

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VI. REFERENCES

1. N. H. Bass, H. H. Hess, and A. Pope, Quantitative comparison of the intralaminar distribution of neurons and glia in the cerebral cortex of rat and man, *Anat. Rec.* **157**:311 (1968).
2. A. G. E. Pearse, *Histochemistry: Theoretical and Applied*, Little, Brown & Co., Boston (1960).
3. T. Barka and P. J. Anderson, *Histochemistry: Theory, Practice and Bibliography*, Hoeber-Harper, New York (1963).
4. C. W. M. Adams (ed.), *Neurohistochemistry*, Elsevier Publ. Co., Amsterdam (1965).
5. D. Glick, *Techniques of Histo- and Cyto-chemistry*, Interscience Publishers, New York (1949).
6. R. M. Torack, *in Neurohistochemistry* (C. W. M. Adams, ed.) pp. 161–188, Elsevier, Amsterdam (1965).
7. D. C. Pease, *Histological Techniques for Electron Microscopy*, Academic Press, New York (1964).
8. H. Hydén, *in Neurochemistry* (K. A. C. Elliott, I. H. Page, and J. H. Quastel, eds.) pp. 331–375, Charles C Thomas, Springfield, Ill. (1962).
9. G. L. Wied (ed.), *Introduction to Quantitative Cytochemistry*, Academic Press, New York (1966).
10. F. Haurowitz, *Immunochemistry and the Biosynthesis of Antibodies*, Interscience Publishers, New York (1968).
11. G. F. Bahr, Dry mass determinations with the electron microscope as an adjunct to cell biological studies, Third Intern. Congr. Histochem. Cytochem., Summary Reports, p. 10, Springer-Verlag, New York (1968).
12. D. Glick, Elemental analysis by the laser microprobe, Third Intern. Congr. Histochem. Cytochem., Summary Reports, pp. 77–78, Springer-Verlag, New York (1968).
13. A. J. Hale, Elemental analysis by the electron microprobe, Third Intern. Congr. Histochem. Cytochem., Summary Reports, pp. 90–91, Springer-Verlag, New York (1968).
14. T. A. Hall, A. J. Hale, and V. R. Switsur, *in The Electron Microprobe* (McKinley *et al.*, eds.) pp. 805–833, Wiley, New York (1966).
15. R. L. Friede, *Topographic Brain Chemistry*, Academic Press, New York (1966).
16. A. N. Davison and N. A. Gregson, *in Neurohistochemistry* (C. W. M. Adams, ed.) Elsevier, Amsterdam (1965).
17. O. H. Lowry, The chemical study of single neurons, *Harvey Lecture Ser.* **58** (1962–1963) pp. 1–19, Academic Press, New York (1964).
18. O. H. Lowry, N. R. Roberts, and M. -L. W. Chang, The analysis of single cells, *J. Biol. Chem.* **222**:97–107 (1956).
19. A. Hamberger and J. Sjöstrand, Respiratory enzymes in neurons and glial cells of the hypoglossal nucleus during nerve regeneration, *Acta Physiol. Scand.* **67**:76–88 (1966).
20. H. Hydén, *in The Neuron* (H. Hydén, ed.) pp. 179–219, Elsevier, Amsterdam (1967).
21. E. Giacobini, *in Morphological and Biochemical Correlates of Neural Activity* (M. M. Cohen and R. S. Snider, eds.) pp. 15–38, Hoeber-Harper, New York (1964).

22. M. H. Epstein and J. S. O'Connor, Respiration of single cortical neurons and of the surrounding neuropile, *J. Neurochem.* **12**:389–395 (1965).
23. B. I. Roots and P. V. Johnston, Neurons of ox brain nuclei: their isolation and appearance by light and electron microscopy, *J. Ultrastruct. Res.* **10**:350–361 (1964).
24. S. P. R. Rose, Preparation of enriched fractions from cerebral cortex containing isolated, metabolically active neuronal and glial cells, *Biochem. J.* **102**:33–43 (1967).
25. S. P. R. Rose, *in Handbook of Neurochemistry* (A. Lajtha, ed.) Vol. 2, pp. 183–193, Plenum Press, New York (1969).
26. M. Satake and S. Abe, Preparation and characterization of nerve cell perikaryon from rat cerebral cortex, *J. Biochem.* **59**:72–75 (1966).
27. M. Satake, S. Hasegawa, S. Abe, and R. Tanaka, Preparation and characterization of nerve cell perikarya from pig brain stem, *Brain Res.* **11**:246–250 (1968).
28. L. Freysl, R. Bieth, C. Judes, M. Sensenbrenner, M. Jacob, and P. Mandel, Distribution quantitative des divers phospholipides dans les neurones et les cellules gliales isoles du cortex cerebral de rat adulte, *J. Neurochem.* **15**:307–313 (1968).
29. S. Varon and C. W. Raiborn, Jr., Dissociation, fractionation and culture of embryonic brain cells, *Brain Res.* **12**:180–199 (1969).
30. G. M. McKhann, W. Ho, C. Raiborn, and S. Varon, The isolation of neurons from normal and abnormal human cerebral cortex, *Arch. Neurol.* **20**:542–547 (1969).
31. J. E. Cremer, P. V. Johnston, B. I. Roots, and A. J. Trevor, Heterogeneity of brain fractions containing neuronal and glial cells, *J. Neurochem.* **15**:1361–1370 (1968).
32. M. E. Fewster, A. B. Schiebel, and J. F. Mead, The preparation of isolated glial cells from rat and bovine white matter, *Brain Res.* **6**:401–408 (1967).
33. A. L. Flangas and R. E. Bowman, Neuronal perikarya of rat brain isolated by zonal centrifugation, *Science* **161**:1025–1027 (1968).
34. L. A. Autilio, W. T. Norton, and R. D. Terry, The preparation and some properties of purified myelin from the CNS, *J. Neurochem.* **11**:17–27 (1964).
35. V. P. Whittaker, *in Progress in Biophysics and Molecular Biology* (J. A. V. Butler and H. E. Huxley, eds.) Vol. 15, pp. 39–96, Pergamon Press, London (1965).
36. V. P. Whittaker, Storage of transmitters in CNS, *Biochem. J.* **109**:20P (1968).
37. E. De Robertis, Ultrastructure and cytochemistry of the synaptic region, *Science* **156**: 907–914 (1967).
38. G. Rodrigues de Lores Arnaiz. M. Alberici, and E. De Robertis, Ultrastructural and enzymic studies of cholinergic and non-cholinergic synaptic membranes isolated from brain cortex, *J. Neurochem.* **14**:215–225 (1967).
39. R. J. Hosie, The localization of ATPases in morphologically characterized subcellular fractions of guinea-pig brain, *Biochem. J.* **96**:404–412 (1965).
40. C. Cotman, H. R. Mahler, and N. G. Anderson, Isolation of a membrane fraction enriched in nerve-end membranes from rat brain by zonal centrifugation, *Biochim. Biophys. Acta* **163**:272–275 (1968).
41. M. D. Bornstein and M. R. Murray, Serial investigations on patterns of growth, myelin formation, maintenance and degeneration in cultures of newborn rat and kitten cerebellum, *J. Biophys. Biochem. Cytol.* **4**:499–504 (1958).
42. S. M. Crain, Resting and action potentials of cultured chick embryo spinal ganglion cells, *J. Comp. Neurol.* **104**:285–330 (1955).
43. R. Levi-Montalcini and P. U. Angeletti, Essential role of the nerve growth factor in the survival and maintenance of dissociated sensory and sympathetic embryonic nerve cells *in vitro*, *Develop. Biol.* **7**:653–659 (1963).
44. R. P. Bunge, M. B. Bunge, and E. R. Peterson, An electron microscope study of cultured rat spinal cord, *J. Cell Biol.* **24**:163–191 (1965).

45. S. M. Crain and E. R. Peterson, Complex bioelectric activity in organized tissue cultures of spinal cord (human, rat and chick), *J. Cell. Comp. Physiol.* **64**:1–13 (1964).
46. M. K. Wolf, Differentiation of neuronal types and synapses in myelinating cultures of mouse cerebellum, *J. Cell Biol.* **22**:259–279 (1964).
47. S. M. Crain and M. R. Murray, Differentiation and prolonged maintenance of bioelectrically active spinal cord cultures (rat, chick and human), *Z. Zellforsch. Mikroskop. Anat.* **66**:130–154 (1965).
48. R. Levi-Montalcini, The nerve growth factor: its mode of action on sensory and sympathetic nerve cells, *Harvey Lecture Ser.* **60** (1964–1965), pp. 217–259, Academic Press, New York (1966).
49. P. U. Angeletti, R. Levi-Montalcini, and P. Calissane, The nerve growth factor (NGF): chemical properties and metabolic effects, *Adv. in Enzymol.* **30**:51–57 (1968).
50. H. M. Shein, Propagation of human fetal spongioblasts and astrocytes in dispersed cell cultures, *Exp. Cell Res.* **40**:554–569 (1965).
51. H. H. Hess, L. J. Embree, and H. M. Shein, Biochemistry of normal astroglia and of virally and chemically induced astrocytomas grown subcutaneously, *Second Meeting, Intern. Soc. for Neurochem.*, pp. 42–43, Tamburini Editore, Milano (1969).
52. H. M. Shein, A. Britva, H. H. Hess, and D. J. Selkoe, Isolation of hamster brain astroglia by *in vitro* cultivation and subcutaneous growth, and content of cerebroside, ganglioside, RNA and DNA, *Brain Res.* **19**:497–501 (1970).
53. H. M. Shein, Neoplastic transformation of hamster astrocytes *in vitro* by simian virus 40 and polyoma virus, *Science* **159**:1476–1477 (1968).
54. H. M. Shein, Neoplastic transformation of hamster astrocytes and choroid plexus cells in culture by polyoma virus, *J. Neuropath. Exp. Neurol.* **29**:70–88 (1970).
55. H. M. Shein, Gliobastoma multiforme produced by the progeny of a single cloned polyoma virus-induced hamster astrocytoma cell, *J. Neuropath. Exp. Neurol.* **28**:156 (1969).
56. P. Benda, J. Lightbody, G. Sato, L. Levine, and W. Sweet, Differentiated rat glial cell strain in tissue culture, *Science* **161**:370–371 (1968).
57. L. J. Embree, H. H. Hess, and H. M. Shein, Biochemical structural components of cloned N-nitrosomethylurea induced astrocytomas grown subcutaneously, *Neurology* **19**:299 (1969).
58. J. De Vellis and D. Inglish, Effect of cortisol and epinephrine on the biochemical differentiation of cloned glial cells in culture and of the developing rat brain, *Second Meeting, Intern. Soc. for Neurochem.*, pp. 151–152, Tamburini Editore, Milano (1969).
59. H. Harris, "Cell Fusion," Dunham Lectures, Harvard Medical School, Harvard Univ. Press, Cambridge, Mass. (1969).
60. J. Vos, K. Kuriyama, and E. Roberts, Electrophoretic mobilities of brain subcellular particles and binding of GABA, ACh, nor-epinephrine and 5-HT, *Brain Res.* **9**:224–230 (1968).
61. G. L. Campbell, Jr., Biochemical characterization of rat cerebral cortex cells, *Fed. Proc.* **22**:633 (1963).
62. H. H. Hess, Negative surface charge of retinal rod outer segments, *Fed. Proc.* **27**:463 (1968).
63. E. J. Ambrose (ed.), *Cell Electrophoresis*, Little, Brown and Co., Boston (1965).
64. O. H. Lowry, N. R. Roberts, K. Y. Leiner, M. L. Wu, A. L. Farr, and R. W. Albers, Quantitative histochemistry of brain: III, Ammon's horn, *J. Biol. Chem.* **207**:39–49 (1954).
65. E. Robins and D. E. Smith, A quantitative histochemical study of eight enzymes of the cerebellar cortex and subjacent white matter in the monkey, *Res. Publ. Assoc. Nervous and Mental Dis.* **32**:305–327 (1953).
66. O. H. Lowry, N. R. Roberts, and C. Lewis, The quantitative histochemistry of the retina, *J. Biol. Chem.* **220**:879–892 (1956).

67. O. H. Lowry, N. R. Roberts, D. W. Schulz, J. E. Clow, and J. R. Clark, Quantitative histochemistry of retina. II. Enzymes of glucose metabolism, *J. Biol. Chem.* **236**:2813–2820 (1961).
68. K. Linderstrøm-Lang, Distribution of enzymes in tissues and cells, *Harvey Lecture Ser.* **34** (1937–1938), pp. 214–245, Academic Press, New York (1939).
69. C. B. Anfinsen, The distribution of cholinesterase in the bovine retina, *J. Biol. Chem.* **152**: 267–278 (1944).
70. A. Pope, Quantitative distribution of dipeptidase and acetylcholinesterase in architectonic layers of rat cerebral cortex, *J. Neurophysiol.* **15**:115–130 (1952).
71. A. Pope, W. Caveness, and K. E. Livingston, Architectonic distribution of acetylcholinesterase in the frontal isocortex of psychotic and nonsychotic patients, *AMA Arch. Neurol. Psychiat.* **68**:425–443 (1952).
72. P. L. Kirk, *Quantitative Ultramicroanalysis*, Wiley, New York (1950).
73. D. Glick, *Quantitative Chemical Techniques of Histo- and Cyto-chemistry*, Vols. I and II, Interscience Publishers, New York (1961).
74. S. Udenfriend, *Fluorescence Assay in Biology and Medicine*, Vol. I (1962) and Vol. II (1969), Academic Press, New York.
75. N. Allen, Cytochrome oxidase in human brain tumours, *J. Neurochem.* **2**:37–44 (1957).
76. N. Allen, Beta-glucuronidase activities in tumors of the nervous system, *Neurology* **11**:578–596 (1961).
77. G. M. Lehrer, H. S. Maker, D. J. Silides, C. Weiss, and L. C. Scheinberg, The quantitative histochemistry of a chemically induced ependymoblastoma—I. Enzymes, *J. Neurochem.* **13**:1197–1206 (1966).
78. H. S. Maker, G. M. Lehrer, C. Weiss, D. J. Silides, and L. C. Scheinberg, The quantitative histochemistry of a chemically induced ependymoblastoma—II. The effect of ischaemia on substrates of carbohydrate metabolism, *J. Neurochem.* **13**:1207–1212 (1966).
79. H. S. Maker, G. M. Lehrer, D. J. Silides, and C. Weiss, Circulatory factors in the carbohydrate metabolism of an experimental glial neoplasm, *Ann. N.Y. Acad. Sci.* **159** (2):461–471 (1969).
80. S. N. Nayyar, A study of phosphate, DNA and phospholipid fractions in neural tumors, *Neurology* **13**:287–291 (1963).
81. L. J. Embree, H. M. Shein, P. Benda, and H. H. Hess, ATPase activity in viral and chemically induced astrocytomas, *J. Neuropathol. Exp. Neurol.* **28**:115 (1969).
82. M. M. K. Nass and S. Nass, Intramitochondrial fibers with DNA characteristics, I. Fixation and electron staining reactions, *J. Cell. Biol.* **19**:593–611 (1963).
83. L. W. Lapham, Tetraploid DNA content of Purkinje neurons of human cerebellar cortex, *Science* **159**:310–311 (1968).
84. R. D. Lentz and L. W. Lapham, A quantitative cytochemical study of the DNA content of neurons of rat cerebellar cortex, *J. Neurochem.* **16**:379–384 (1969).
85. L. G. Tomasi and S. E. Kornguth, Characterization and immunochemical localization of a basic protein from pig brain. II. Peptide maps and tissue-specific nuclear localization, *J. Biol. Chem.* **243**:2507–2513 (1968).
86. D. E. Green, E. Murer, H. O. Hultin, S. H. Richardson, B. Salmon, G. P. Brierley, and H. Baum, Association of integrated metabolic pathways with membranes. I. Glycolytic enzymes of red cells and yeast, *Arch. Biochem. Biophys.* **112**:635–647 (1965).
87. O. H. Lowry, *in Biochemistry of the Developing Nervous System* (H. Waelsch, ed.) pp. 350–357, Pergamon Press, London (1955).
88. D. B. McDougal, Jr., D. W. Schulz, J. V. Passonneau, J. R. Clark, M. A. Reynolds, and O. H. Lowry, Quantitative studies of white matter. I. Enzymes involved in glucose-6-phosphate metabolism, *J. Gen. Physiol.* **44**:487–498 (1961).

89. J. B. Cavanagh, R. H. S. Thompson, and G. R. Webster, Localization of pseudo-cholinesterase activity in nervous tissue, *Quart. J. Exp. Physiol.* **39**:185–197 (1954).
90. P. K. Datta and T. H. Shepard, Intracellular localization of carbonic anhydrase in rat liver and kidney tissues, *Arch. Biochem. Biophys.* **81**:124–129 (1959).
91. B. W. Moore, A soluble protein characteristic of the nervous system, *Biochim. Biophys. Res. Commun.* **19**:739–744 (1965).
92. H. Hydén and B. McEwen, A glial protein specific for the nervous system, *Proc. Nat. Acad. Sci.* **55**:354–358 (1966).
93. B. W. Moore, *in Handbook of Neurochemistry* (A. Lajtha, ed.) Vol. 1, pp. 93–99, Plenum Press, New York (1969).
94. T. J. Cicero, W. M. Cowan, B. W. Moore, and V. Suntzef, The cellular localization of the two brain specific proteins, S-100 and 14-3-2, *Brain Res.* **18**:25–34 (1970).
95. D. E. Slagel, C. E. Wilson, and P. B. Simmons, Polyacrylamide electrophoresis and immunodiffusion studies of brain tumor proteins, *Ann. N.Y. Acad. Sci.* **159**(2):491–496 (1969).
96. A. Grasso, T. Cicero, and B. W. Moore, Purification and characterization of an acidic protein from mammalian brain, *Second Meeting, Intern. Soc. for Neurochem.*, pp. 201–202, Tamburini Editore, Milano (1969).
97. E. Bachmann, G. Lenaz, J. F. Perdue, N. Orme-Johnson, and D. E. Green, The membrane systems of the mitochondrion. V. The inner membrane of beef heart mitochondria, *Arch. Biochem. Biophys.* **121**:73–87 (1967).
98. C. Schnaitman, G. V. Erwin, and J. W. Greenwalt, The submitochondrial localization of monoamine oxidase. An enzymatic marker for the outer membrane of rat liver mitochondria, *J. Cell Biol.* **32**:719–735 (1967).
99. N. Marks, B. D'Monte, C. Bellman, and A. Lajtha, Protein metabolism in cerebral mitochondria. I. Hydrolytic enzymes and amino acid incorporation into mitochondrial membranes, *Brain Res.* **18**:309–324 (1970).
100. G. B. Kitto and N. O. Kaplan, Purification and properties of chicken heart mitochondrial and supernatant malic dehydrogenase, *Biochem.* **5**:3966–3980 (1966).
101. A. Neidle, C. J. van Den Berg, and A. Grynbaum, The heterogeneity of rat brain mitochondria isolated on continuous sucrose gradient, *J. Neurochem.* **16**:225–234 (1969).
102. Z. S. Tencheva and A. A. Hadjiolov, Characterization of rat brain RNA's by agar gel electrophoresis, *J. Neurochem.* **16**:769–776 (1969).
103. R. Landolt, H. H. Hess, and C. Thalheimer, Regional distribution of some chemical structural components of the human nervous system. I. DNA, RNA and ganglioside sialic acid, *J. Neurochem.* **13**:1441–1452 (1966).
104. G. R. Lawford and H. Schachter, Biosynthesis of glycoproteins, *J. Biol. Chem.* **241**:5408–5418 (1966).
105. H. W. Beams and R. G. Kessel, The Golgi apparatus: structure and function, *Intern. Rev. Cytol.* **23**:209–276 (1968).
106. E. G. Brunngraber, *in* Handbook of Neurochemistry (A. Lajtha, ed.) Vol. 1, pp. 223–234, Plenum Press, New York (1969).
107. A. B. Novikoff, *in The Neuron* (H. Hydén, ed.), pp. 319–377, Elsevier, Amsterdam (1967).
108. C. DeDuve, *in Ciba Foundation Symposium on Lysosomes* (A. V. S. de Reuck and M. P. Cameron, eds.) pp. 1–31, Little, Brown, Boston (1963).
109. E. Robins and H. E. Hirsch, Glycosidases in the nervous system. II. Localization of β-galactosidase, β-glucuronidase and β-glucosidase in individual nerve cell bodies, *J. Biol. Chem.* **243**:4253–4257 (1968).
110. H. E. Hirsch, Aryl sulfatase activity in individual neurons and neuropil and in layers of cerebellum, Third Intern. Congr. of Histochem. Cytochem., Summary Reports, p. 102, Springer-Verlag, New York (1968).
111. H. E. Hirsch, Acid phosphatase localization in individual neurons by a quantitative hitochemical method, *J. Neurochem.* **15**:123–130 (1968).

112. H. E. Hirsch, Enzyme measurements in lipofuscin-rich portions of individual neurons, *Second Meeting, Intern. Soc. for Neurochem.*, p. 218, Tamburini Editore, Milano (1969).
113. S. Fowler and C. De Duve, Digestive activity of lysosomes. III. The digestion of lipids by extracts of rat liver lysosomes, *J. Biol. Chem.* **244**:471–481 (1969).
114. D. S. Maxwell and L. Kruger, The fine structure of astrocytes in the cerebral cortex and their response to focal injury produced by heavy ionizing particles, *J. Cell. Biol.* **25**:141–157 (1965).
115. H. Hager, S. Luh, D. Ruščáková, and M. Ruščák, Histochemische, elektronen mikroskopische und biochemische Untersuchungen uber Glykogenanhaufung in reaktive veranderten Astrozyten der traumatische ladierten Saugergrosshirnrinde, *Z. Zellforsch. Mikroskop. Anat.* **83**:295–320 (1967).
116. M. R. Adelman, G. G. Borisy, M. L. Shelanski, R. C. Weisenberg, and E. W. Taylor, Cytoplasmic filaments and tubules, *Fed. Proc.* **27**(5):1186–1193 (1968).
117. R. C. Weisenberg and E. W. Taylor, The binding of guanosine nucleotide to microtubule subunit protein purified from porcine brain, *Fed. Proc.* **27** (2):299 (1968).
118. S. Puszkin, S. Berl, E. Puszkin, and D. D. Clarke, Actomyosin-like protein isolated from mammalian brain, *Science* **161**:170–171 (1968).
119. S. Berl and S. Puszkin, Neurostenin, an actomysin-like protein isolated from mammalian brain, *Second Meeting, Intern. Soc. for Neurochem.* pp. 87–88, Tamburini Editore, Milano (1969).
120. J. B. Kirkpatrick, Microtubules in brain homogenates, *Science* **163**:187–188 (1969).
121. F. O. Schmitt, Fibrous proteins—neuronal organelles, *Neurosciences Res. Prog. Bull.* **6**, Suppl., pp. 38–47 (1968).
122. A. Bairati, *in The Biology of Neuroglia* (W. F. Windle, ed.) pp. 66–72, Charles C Thomas, Springfield, Ill. (1958).
123. A. Rambourg and C. P. Leblond, Electron microscopic observations on the carbohydrate-rich coat present at the surfaces of cells in the rat, *J. Cell Biol.* **32**:27–53 (1967).
124. R. A. Weaver and W. Boyle, Purification of plasma membranes of rat liver. Application of zonal centrifugation to isolation of cell membranes, *Biochim. Biophys. Acta* **173**:377–388 (1969).
125. M. Rodbell, Metabolism of isolated fat cells. V. Preparation of "ghosts" and their properties: adenyl cyclase and other enzymes, *J. Biol. Chem.* **242**:5744–5750 (1967).
126. J. M. Streeto and W. J. Reddy, An assay for adenyl cyclase, *Anal. Biochem.* **21**:416–426 (1967).
127. J. Cummins and H. Hydén, ATP levels and ATPases in neurons, glia and neuronal membranes of the vestibular nucleus, *Biochim. Biophys. Acta* **60**:271–283 (1962).
128. E. Lewin and H. H. Hess, Intralaminar distribution of Na-K ATPase in rat cortex, *J. Neurochem.* **11**:473–481 (1964).
129. H. Wiegandt, The subcellular localization of gangliosides in the brain, *J. Neurochem.* **14**:671–674 (1967).
130. D. M. Derry and L. S. Wolfe, Gangliosides in isolated neurons and glial cells, *Science* **158**:1450–1452 (1967).
131. H. H. Hess, N. H. Bass, and C. N. Still, Gangliosides in relation to histological structure of cerebral cortex and of photoreceptor organelles, *First Meeting, Intern. Soc. for Neurochem.*, p. 96 (1967).
132. D. M. Derry and L. S. Wolfe, Ganglioside analyses of serial cryostat sections through Ammon's horn and cerebellar folia, *Exp. Brain Res.* **5**:32–44 (1968).
133. K. Suzuki, The pattern of mammalian brain gangliosides. III. Regional and developmental difference, *J. Neurochem.* **12**:969–979 (1965).

134. H. M. Shein, H. H. Hess, and L. J. Embree, Sodium-potassium ATPase activity and content of glycolipids and nucleic acids of normal hamster brain astrocytes grown subcutaneously, *J. Neuropath. Exp. Neurol.* **30**:138 (1971).
135. W. T. Norton and S. E. Poduslo, Isolation and some properties of whole neuroglia and neuronal perikarya from rat brain, *Second Meeting, Intern. Soc. for Neurochem.*, pp. 44–45, Tamburini Editore, Milano (1969).
136. M. E. Fewster and J. F. Mead, Lipid composition of glial cells isolated from bovine white matter, *J. Neurochem.* **15**:1041–1052 (1968).
137. K. Suzuki, S. E. Poduslo, and W. T. Norton, Gangliosides in the myelin fraction of developing rats, *Biochim. Biophys. Acta* **144**:375–381 (1967).
138. E. Giacobini, Value and limitations of quantitative chemical studies in individual cells, *J. Histochem. Cytochem.* **17**:139–155 (1969).
139. N. H. Bass and H. H. Hess, A comparison of cerebrosides, proteolipid proteins and cholesterol as indices of myelin in the architecture of rat cerebrum, *J. Neurochem.* **16**:731–750 (1969).
140. W. T. Norton and L. A. Autilio, The lipid composition of purified bovine brain myelin, *J. Neurochem.* **13**:213–222 (1966).
141. J. Eichberg, Jr., V. P. Whittaker, and R. M. C. Dawson, Distribution of lipids in subcellular particles of guinea-pig brain, *Biochem. J.* **92**:91–100 (1964).
142. M. B. Lees, Influence of sucrose on the extraction of proteolipids from brain and other tissues, *J. Neurochem.* **13**:1407–1420 (1966).
143. N. S. Radin, Y. Kishimoto, B. W. Agranoff, and R. M. Burton, Lipids and fatty acids of brain subcellular fractions, *Fed. Proc.* **26**:676 (1967).
144. E. G. Lapetina, E. F. Soto, and E. De Robertis, Lipids and proteolipids in isolated subcellular membranes of rat brain cortex, *J. Neurochem.* **15**:437–445 (1968).
145. L. Autilio, Fractionation of myelin proteins, *Fed. Proc.* **25**:764 (1966).
146. M. W. Kies, E. C. Alvord, R. E. Martenson, and F. N. LeBaron, Encephalitogenic activity of bovine basic proteins, *Science* **151**:821–822 (1966).
147. A. Na Kao, W. J. Davis, and E. R. Einstein, Basic proteins from the acidic extract of bovine spinal cord. I. Isolation and characterization, *Biochim. Biophys. Acta* **130**:163–170 (1966).
148. C. E. Lumsden, D. M. Robertson, and R. Blight, Chemical studies on experimental allergic encephalitis. Peptide as the common denominator in all encephalitogenic antigens, *J. Neurochem.* **13**:127–162 (1966).
149. R. F. Kibler and R. Shapira, Isolation and properties of an encephalitogenic protein from bovine, rabbit, and human CNS tissue, *J. Biol. Chem.* **243**:281–286 (1968).
150. C. W. M. Adams and O. B. Bayliss, Histochemistry of myelin: V. Trypsin-digestible and trypsin-resistant proteins, *J. Histochem. Cytochem.* **16**:110–114 (1968).
151. S. E. Kornguth and J. W. Anderson, Localization of a basic protein in the myelin of various species with the aid of fluorescence and electron microscopy, *J. Cell. Biol.* **26**:157–166 (1965).
152. F. Wolfgram, A new proteolipid fraction of the nervous system. I. Isolation and amino acid analyses, *J. Neurochem.* **13**:461–470 (1966).
153. F. Wolfgram and K. Kotorii, The composition of the myelin proteins of the central nervous system, *J. Neurochem.* **15**:1281–1290 (1968).
154. T. Kurihara and Y. Tsukada, 2',3'-cyclic nucleotide 3'-phosphohydrolase in the developing chick brain and spinal cord, *J. Neurochem.* **15**:827–832 (1968).
155. A. G. Kirshner and N. Kirshner, A specific soluble protein from the catechol amine storage vesicles of bovine adrenal medulla. II. Physical characterization, *Biochim. Biophys. Acta* **181**:219–225 (1969).
156. B. Flack, *in Progress in Brain Research* (H. G. Himwich, ed.) pp. 28–44, Elsevier, Amsterdam (1964).

157. A. Dahlstrom and K. Fuxe, Evidence for existence of monoamine-containing neurons in CNS. I. Demonstration of monoamines in the cell bodies of brain stem neurons, *Acta Physiol. Scand. Suppl.* **232**:1–55 (1964).
158. A. Dahlstrom and K. Fuxe, Evidence for existence of monoamine-containing neurons in CNS. II. Experimentally induced changes in intraneuronal amine levels of bulbospinal neuron systems, *Acta Physiol. Scand. Suppl.* **247**:7–36 (1965).
159. K. Fuxe, Evidence for the existence of monoamine neurons in the CNS. IV. The distribution of monoamine terminals in the CNS, *Acta Physiol. Scand. Suppl.* **247**:39–84 (1965).
160. H. H. Hess and C. Thalheimer, Microassay of biochemical structural components in nervous tissues. I. Extraction and partition of lipids and assay of nucleic acids, J. *Neurochem.* **12**:193–204 (1965).
161. H. H. Hess and E. Lewin, Microassay of biochemical structural components in nervous tissues. II. Methods for cerebrosides, proteolipid proteins and residue proteins, *J. Neurochem.* **12**:205–211 (1965).
162. H. H. Hess and E. Rolde, Fluorometric assay of sialic acid in brain gangliosides, *J. Biol. Chem.* **239**:3215–3220 (1964).
163. R. Landolt and H. H. Hess, Regional distribution of some chemical structural components of the human nervous system. II. Cerebrosides, proteolipid proteins and residue proteins, *J. Neurochem.* **13**:1453–1459 (1966).
164. E. Koenig, Synthetic mechanisms in the axon—II. RNA in myelin-free axons of the cat, *J. Neurochem.* **12**:357–361 (1965).
165. A. Pope, *in The Central Nervous System*, Intern. Acad. Pathol. Monograph No. 9, pp. 42–51, Williams and Wilkins, Baltimore (1968).
166. E. Lewin and H. H. Hess, Intralaminar distribution of cerebrosides in human frontal cortex, *J. Neurochem.* **12**:213–220 (1965).
167. J. B. Angevine, Jr., S. Locke, and P. I. Yakovev, Limbic nuclei of thalamus and connections of limbic cortex. V. Thalamocortical projection of the magnocellular medial dorsal nucleus in man, *Arch. Neurol.* **10**:165–180 (1964).
168. E. Lewin and H. H. Hess, Intralaminar distribution of proteolipid protein and residue protein in human frontal cortex, *J. Neurochem.* **14**:71–80 (1967).
169. S. Fiszer and E. De Robertis, Subcellular distribution and chemical nature of receptor for 5-hydroxytryptamine in the central nervous system, *J. Neurochem.* **16**:1201–1210 (1969).
170. H. H. Hess and A. Pope, Intralaminar distribution of cytochrome oxidase activity in human frontal isocortex, *J. Neurochem.* **5**:207–217 (1960).
171. A. Pope, H. H. Hess, J. R. Ware, and R. H. Thompson, Intralaminar distribution of cytochrome oxidase and diphosphopyridine nucleotide in rat cerebral cortex, *J. Neurophysiol.* **19**:259–270 (1956).
172. W. S. Spector (ed.), *Handbook of Biological Data*, pp. 260–261, W. B. Saunders, Philadelphia (1956).
173. A. Pope, A. A. Morris, H. Jasper, K. A. C. Elliott, and W. Penfield, Histochemical and action potential studies on epileptogenic areas of cerebral cortex in man and monkey, *Res. Publ. Assoc. Res. Nervous & Mental Dis.* **26**:218–233 (1946).
174. A. Pope, H. H. Hess, and E. Lewin, *in Morphological and Biochemical Correlates of Neural Activity* (R. S. Snider and M. M. Cohen, eds.) pp. 98–110, Hoeber-Harper, New York (1964).
175. L. J. Embree and H. H. Hess, Chemoarchitectonics of ATPases in cerebral cortex: Normal human and Alzheimer's Disease, *Second Meeting. Intern. Soc. for Neurochem.* pp. 162–163, Tamburini Editore, Milano (1969).
176. N. H. Bass, H. H. Hess, and A. Pope, Microchemical pathology of cerebral cortex in Creutzfeldt-Jakob disease, *Trans. Am. Neurol. Assoc.* **93**:108–111 (1968).
177. N. H. Bass, Pathogenesis of myelin lesions in experimental cyanide encephalopathy: A microchemical study, *Neurology* **18**:167–177 (1968).

Chapter 15

BIOCHEMISTRY OF MIDDLE AND LATE LIFE DEMENTIAS

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I. INTRODUCTION

The disease entities to be considered in this review are characterized clinically by a slow but relentless deterioration of intellectual faculties resulting in profound dementia and eventually death during middle and late life. Alzheimer's disease, Pick's disease, Huntington's chorea, Creutzfeldt–Jakob disease, and senile dementia are included in this group of "primary dementias" where the unifying pathologic feature is a degeneration of the cerebral cortex with varying degrees of involvement of related structures. These conditions, which as yet have no established cause (the special status of Creutzfeldt–Jakob disease is discussed below) and as far as can be determined at present are not related to any known disorder, stand in contrast to the "secondary dementias" that occur in association with syphilitic infection, cerebrovascular disease, brain tumor, myxedema, alcoholism, nutritional deficiencies, encephalitis, trauma, and chronic neurological diseases such as disseminated sclerosis, subacute combined degeneration, Wilson's disease, and the cerebral lipidoses. These secondary dementias are considered elsewhere in this *Handbook*.

In order to view the primary dementias in proper perspective, their incidence must be considered. Pick's disease, Huntington's chorea, and Creutzfeldt–Jakob disease are quite rare in occurrence while Alzheimer's