PHARMACOLOGICAL REVIEWS
Vol. 29, No. 1

Printed in U.S.A.

Drug-Induced Alterations of Ion Distribution at the Cellular Level of the Central Nervous System*

LEIF HERTZ

Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

I. Introduction

A variety of drugs influence the central nervous system either as a major part of their therapeutic action (*e.g.*, hypnotics and antiepileptics) or as an unwanted side effect (*e.g.*, local anaesthetics). In spite of a vast amount of experimental investigation, the mechanisms of action for several of these drugs remain unknown. One reason for this is that the study of drug effects may be hampered by the complexity of the central nervous system. The adult mammalian central nervous system is extremely heterogeneous in both its gross anatomy and its histology. The gross ana-

* This study was supported by Grant DG 120 from the Medical Research Council of Canada.

tomical complexity does not impede experimental investigations to a major degree, but the histological heterogeneity represents an enormous obstacle for experimental work. In addition, evidence is accumulating that the different cell types in grey matter of the central nervous system have widely different metabolic properties (130) and also that the biophysical characteristics of their membranes show fundamental differences (296). This heterogeneity is essential for the understanding of ion distribution in the brain.

Like other excitable cells, neurons take up sodium ions and lose potassium ions by "downhill" transport during excitation. It had already been theorized that the potassium release is large enough to cause a substantial increase of the potassium concentration in the narrow extracellular clefts (24, 58, 80, 105, 116, 117, 119, 121, 137, 176, 187, 197, 241, 244, 264, 284, 306, 316) when the introduction of potassium-sensitive electrodes made this conjecture a firmly established fact (88, 140, 171–173, 193, 200–202, 229, 243, 258, 293, 296, 297, 334, 335). The effects of the increase in potassium concentration on adjacent cells will depend upon the excitability of the cells (201, 298) as well as upon active or passive homeostasis mechanisms regulating the local concentrations of potassium. These homeostasis mechanisms are probably reflected by effects of elevated potassium concentrations on membrane potentials (176, 244, 296) and on transport of ions and water (24, 27, 84, 85, 97, 119, 197, 228), as well as by the potassium-induced stimulation of Na^+-K^+-ATPase activity (58, 116) and of energy metabolism (9, 71, 122, 129, 154, 239, 281). These phenomena seem to a large extent to occur in glial cells but ultimately the released potassium ions must of necessity be reaccumulated into neurons and the accumulated sodium ions must be extruded again (for a more detailed discussion, see 128, 130).

Drugs acting upon the central nervous system may affect ion distribution either by altering the down hill movements during excitation or by influencing the subsequent homeostasis mechanisms. Thus, it has been suggested that diphenylhydantoin (phenytoin) owes its antiepileptic effect to either a reduction of ion movements during excitation (303) or a stimulation of the subsequent active transport of potassium and sodium (75, 200, 355, 356), and a multitude of drugs counteract the potassium-induced stimulation of energy metabolism, which has been observed *in vitro* (section III B 1). The purpose of this paper is, after a brief outline of the functional and histological heterogeneity of nervous tissue, to review current information and concepts related to ion distribution and transport at the cellular level of the central nervous system and to discuss effects of certain selected drugs on this distribution *in vivo* and *in vitro*. The drugs have been selected either because of their established therapeutic action on the central nervous system, combined with indications that they affect ion distribution (*e.g.*, phenytoin) or because of their known action on transport phenomena (*e.g.*, ouabain, tetrodotoxin). The latter drugs may serve as tools for understanding ion homeostasis in the nervous system during physiological and pathological conditions. No attempt will be made to review the vast amount of information about drugs interacting with the production, release, enzymatic degradation or reaccumulation of classical or putative transmitters. Several of these do appear as ions at pH 7.3 but, with the exception of glutamate, in concentrations so low that their quantitative importance as charged particles at the cellular (but maybe not at the subcellular) level probably can be neglected. Also, transport processes across the brain barriers (61, 160) will be regarded as beyond the scope of this review.

II. Complexity of Nervous Tissue

A. Neurons and Glial Cells

Tissue from the central nervous system contains an interstitial space and two

widely different types of cells, *i.e.*, neurons and glial cells, which on morphological grounds have been subdivided into different types. The rapidly increasing amount of information about function at the cellular level may, however, lead to a much more refined classification based upon neurochemical, neurophysiological and neuropharmacological characteristics. The neurons are [with few exceptions (296)] excitable cells, *i.e.*, they react to a certain depolarization of their membranes with an "all-or-none" action potential, whereas no such response is given by glial cells (64, 134, 135, 176–178, 340). Potential changes may, however, spread between individual, electrically coupled glial cells over a distance of more than 1 mm (176–178, 336). An excellent review of glial electrophysiology has recently appeared (296) and should be consulted for further, up-to-date information, including functional criteria for the distinction of glial cells from neurons; the pioneer work by the Kuffler group and others, which was initiated only about 10–15 years ago, has been reviewed by Kuffler and Nicholls (176).

No unequivocal answers are found to the fundamental questions of what fraction of the total volume of different regions is occupied by, respectively, neurons and glial cells, or to what extent each of the different cell types contributes, *e.g.*, to the total energy expenditure or the total content of potassium or other constituents in brain cortical tissue; it is also not known with certainty which features of the complex electrical activity of the central nervous system reflect phenomena occurring in each of the two cell types. These topics are discussed in more detail elsewhere (124, 130, 296). Here it will suffice to recapitulate that the cell bodies (perikarya) of the neurons in the brain cortex of larger mammals only account for about 5% of the total volume, that the volume occupied by their processes is a few times larger, that the nerve endings (synaptosomes) constitute another 15 to 25% of the brain volume and that glial cells according to several authors outnumber nerve cells by a factor of 2 to 10 (for references, see 124, 130). Nevertheless, the volume occupied by easily recognizable glial cell bodies is relatively small, and a considerable fraction of the tissue volume remains to be accounted for. In grey matter, a large part of the volume is made up by the neuropil, *i.e.*, an intricately interwoven network of minute neuronal and neuroglial processes, tapering into lamellar sheets of 200 to 1000 Å thickness (248, 350). The relative amount of the neuropil seems to increase as the brain size increases (for references, see 130), and the possible importance of neuropil for higher mental activities has been speculated upon. The ratio between neuronal and glial components in the neuropil is uncertain but it seems reasonable to suggest that up to one-half of the total brain cortical volume may be occupied by glial cells; evidence has accumulated during the last decade that these cells are not at all so functionally inert as they were previously considered to be. Glial cells may thus account for *at least* one-third of the total oxygen consumption (for references, see 124, 130), about one-half to three-fourths of the total glutamate content (14, 124, 282) and the majority of the Na^+-K^+-ATPase activity (58, 116, 222–224, 231) in brain cortex. The brain-specific S-100 protein is mainly, but maybe not exclusively, localized in glial cells (145) and the primary lesion in certain pathological conditions may be glial rather than neuronal (101). Glial cells and certain neurons (interneurons) are formed at a late ontogenetic stage, *i.e.* (in rats and humans), to a large extent after birth (3–6, 72, 76, 253, 259) and glial cell formation can be modified by learning (69). Maturation of neurons and glial cells seems to occur hand-in-hand as indicated by the identical developmental pattern of the neuronal-specific protein 14-3-2 and the glia-specific protein S-100 (45).[1]

[1] In spite of the small amount of S-100 which may be localized in neurons this protein is generally regarded as a glial marker.

Further information about glial and neuronal cell biology can be found in previously published reviews by Windle (348), Nakai (232), De Robertis and Carrea (67), Kuffler *et al.* (177), Hydén (143, 144), Lasansky (182), Johnston and Roots (156), Watson (341), Hertz and Schousboe (130) and Somjen (296).

B. Extracellular and Intracellular Spaces

Another fundamental question that has yet to be unanimously agreed upon is the magnitudes of the extra- and intracellular fluid spaces in the brain. These magnitudes are of crucial importance for exact estimates of intracellular concentrations or of fluxes across cell membranes. Certain drugs affect the magnitude of the fluid spaces (section III B 2), and unless these effects are taken into account, gross errors may be introduced in deduction of any effects on intracellular concentrations or transcellular fluxes.

One estimate (*e.g.*, 139, 178) has been that the extracellular space in the brain cortex *in vivo* accounts for only about 5% of the total volume and is restricted to interstitial clefts of about 150 Å (15 nm) width. Van Harreveld (321, 322) has, on the other hand, reported that the extracellular space is considerably larger *in vivo*, but shrinks during the period between the arrest of the circulation and conventional fixation, and recent determinations of diffusion profiles for "extracellular markers" support the concept of an extracellular space of about 15 to 20% (79, 160, 263). This space is mainly found between nonmyelinated axons, whereas other cellular elements (and thus also neurons and glial cells) are separated by the narrow slits described above (328). Accordingly, compounds (*e.g.*, potassium ions, glutamate) which are released from cells may in any case become confined to such a small volume that their concentrations become high enough to exert biochemical effects on adjacent cells. The demonstration of an increase in the extracellular potassium concentration during excitation of the brain or the spinal cord proves the correctness of this concept.

During incubation of brain slices *in vitro*, the extracellular space increases during the first ½ hr (228, 252, 311). This increase may constitute a considerable source of inaccuracy if transport studies are initiated within this period. Once the extracellular space has approached a steady magnitude, it should theoretically be accessible for determination by aid of extracellular markers. As discussed below (section III B 2), such determinations are, however, encumbered with difficulties and shortcomings.

Also the intracellular space in brain slices increases during incubation, accounting for most of the "swelling" (fluid uptake) of incubated brain cortex tissue. Electron micrographic studies have shown that the intracellular swelling during incubation in an oxygenated, physiological medium mainly occurs in glial cells (46, 93, 146, 228, 311). The glial swelling is even more pronounced after exposure to potassium-rich, sodium-deficient (146) or ouabain-containing media (section III B 2), whereas exposure to a low temperature (incubation at 0°C) causes a pronounced neuronal swelling (228) and anoxia or exposure to glutamate affects both cell types (228, 325). The differences in cellular localization of the swelling evoked by different agents should be emphasized since they probably reflect fundamental differences in the mechanisms of action for these agents.

III. Ion Contents

A. In Vivo

1. Intracellular and extracellular ion concentrations: Effects of barbiturates, metrazol, ouabain, tetrodotoxin and aminopyridines. The potassium concentration in grey matter from adult mammals is about 100 μmol/g fresh weight and the sodium concentration is about half of this value; in white matter the potassium concentration may be slightly lower, and the

sodium concentration slightly higher, but the differences between grey and white matter are relatively small (for references, see 130, 160, 322). The concentration of chloride may increase with an increasing brain volume (25).[2] In the rat cerebral cortex the chloride concentration is thus about 30 and in the rabbit about 40 μmol/g fresh weight (25, 160). It may be slightly lower in white matter (314). The deficit between the sum of the concentrations of the two major cations ($Na^+ + K^+$) and the chloride concentration is probably mainly accounted for by the content of protein, but glutamate also is found in the adult brain in such a high (10 mM) concentration (*e.g.*, 205, 312, 318) that it may be of importance (as an anion) in the ion balance.

In the cerebrospinal fluid, most ionic concentrations are relatively close to those in plasma but the potassium concentration is significantly lower, *i.e.*, approximately 3.0 mM (27, 31, 53, 160, 226, 270) and the chloride concentration is higher, *i.e.*, about 130 mM (25, 27, 61, 222). The concentration of glutamate is low, *i.e.*, at most 0.1 mM (20) and probably even lower (for references, see 131). The maintenance of constant ionic concentrations in the brain and the cerebrospinal fluid is based upon a restriction to free movements between blood on one hand, and cerebrospinal fluid and brain tissue on the other, known as the blood-liquor and the blood-brain barriers. These barriers are so effective that *e.g.*, intraperitoneally or intravenously injected ^{42}K has scarcely reached equilibrium with the potassium in cerebrospinal fluid and in brain after 18 to 24 hr (31, 61, 237). The ionic movements between the cerebrospinal fluid and the brain tissue seem, in contrast, to be relatively unlimited (31), although there is a slight hindrance to diffusion of potassium ions into the brain at the cortical pial surface (246, 254). It is in keeping with a relatively free exchange between cerebrospinal fluid and brain extracellular fluid that the "resting" level of extracellular potassium in cat or rat brain cortex and in spinal cord grey substance of the cat consistently has been found to be between 2.5 and 4.1 mM[3] by several groups of investigators in the absence of anesthesia as well as in animals anesthetized by ether or several different barbiturates (159).

Based upon Na^+, K^+ and Cl^- equilibrium potentials in spinal motoneurons of the cat, and on the assumption that the extracellular fluid of the central nervous system has the same ionic composition as an ultrafiltrate of cat blood, Eccles (73) calculated intracellular concentrations of potassium, sodium and chloride in cat motoneurons to be, respectively, 150, 15 and 9 mM. The more recent finding that the potassium concentration in the extracellular fluid corresponds to that of the cerebrospinal fluid (3.0 instead of 5.5 mM) will reduce the calculated potassium concentration to about 80 mM. Kuffler and Nicholls (176) have, in a similar manner, estimated the intracellular potassium concentration in leech glial cells to about 110 mM. This result as well as the high membrane potentials (up to -95 mV) generally observed also in other types of glial cells (for references, see 130, 296) is in contrast to the previously widespread concept that glial cells from the central nervous system should have a high sodium content and a low potassium content (68, 93, 112, 158, 166). Measurements of ion contents in preparations of one cell type have demonstrated a high content of potassium in microdissected (107) but not in bulk-prepared glial cells (105), and high potassium contents as well as low sodium contents have been found in cultured astrocytes originating from neonatal animals (97, 183, 186) or from the C-6 glioma cell line (164, 180, see also 124). A high content of sodium has,

[2] Such a species variation, which has been challenged by Katzman and Pappius (160), might indicate a relatively high chloride content in glial cells and/or terminal parts of neuronal processes since it roughly corresponds to the species variation with respect to the relative amount of neuropil (*cf.* section II A).

[3] The values seem to center around 3.0 mM, *e.g.*, 3.24 ± 0.21 mM as an average of 1260 determinations (254).

however, been reported in the Schwann cells of the squid nerve (332, 333).

Electrical or chemical excitation in intact animals has long been known to lead to a release of potassium and an uptake of sodium in the brain (43, 44, 51, 207) and circumstantial evidence was obtained in the middle 1960's by Kuffler *et al.* (176, 244) that stimulation leads to an increase of the extracellular potassium concentration in leech ganglia and in *Necturus* optic nerve. It is, however, only since 1972 that reports have appeared from all over the world stating that repetitive electrical stimulation of the mammalian cortical surface or of afferent pathways, or even physiological stimulation of the optic pathway (293), leads to a slow (*i.e.*, within 0.1–0.3 sec) rise of the potassium concentration in the extracellular fluid of the brain or the spinal cord (140, 154, 159, 171, 172, 193, 194, 200–202, 229, 297). This rise has generally been measured to 1 mM[4] or a few mM, *i.e.*, from 3 to, at the very most, 10 to 12 mM.[5] The upper level of about 12 mM potassium can be exceeded *only* by massive electrical, mechanical or chemical excitation leading to spreading depression (*cf.* below), and spreading depression in inevitably evoked whenever the extracellular potassium concentration exceeds 12 mM (193, 201).[6] The facts that spreading depression is evoked when the extracellular potassium concentration rises above 12 mM and that the lowest potassium concentration observed under spreading depression is 25 to 30 mM (*cf.* below) indicate that potassium levels between 12 and 25 to 30 mM are nonexistent, *i.e.*, not stable (193).

Also, during seizures the extracellular potassium concentration reaches a maximum of about 12 mM (*e.g.*, 193). A direct correlation between the level of the extracellular K^+ concentration and seizure activity is unlikely (265), but some connection (70, 306) may nevertheless be suggested by the well established fact that exposure of the brain (or certain parts of the brain) to excess potassium may provoke seizure activity (*e.g.*, 78, 98, 148, 155, 238, 365).

Studies of drug effects on ion distribution *in vivo* are hampered by the presence of the barrier systems. Certain drugs, *e.g.*, metrazol, do, however, penetrate the barriers fast and efficiently, and a very dramatic swelling of astrocytic processes has been observed within 5 min after intraperitoneal injection of a large dose of metrazol (150 mg/kg) into rats (see fig. 2 in ref. 66); for osmotic reasons this accumulation of fluid must have been accompanied by an uptake of ions and it is an indication of ion redistribution at the cellular level that no changes in *total* H_2O, Na^+ or K^+ were found in the cerebral cortices (66). Other drugs have been studied after direct application into the central nervous system. Intracranial application of ouabain thus leads to motor hyperactivity and convulsions followed by more or less complete recovery or by death (6, 12, 17, 188, 189, 255). The application of ouabain leads to a decreased potassium content and an increased sodium content in the brain (189, 331) as well as to a concomitant increase in the potassium concentration of the perfusion fluid or the cerebrospinal fluid (12,

[4] After physiological stimulation of the optic pathway the increase is thus from 3 to 4 mM (293).

[5] Although the diameter of the tip of the potassium-sensitive electrode is small (1–3 μm), it is still considerably larger than the width of the interstitial clefts (150 Å or 0.015 μm). The recorded changes in interstitial potassium concentrations after activity of adjacent neurons may therefore reflect alterations in a somewhat expanded extracellular "pocket" and thus represent minimum values (*cf.* also 306). In opposition to this view it has been stated (115) that measurements of membrane potentials in the inexcitable ("idle," "silent," "unresponsive") glial cells, which have been used to deduce changes in extracellular potassium concentrations on the assumption that the intracellular potassium concentration remains unaltered (176, 244, 265, 267), have confirmed the upper level of 12 mM. This argument is, however, weakened by the fact that the membrane potential of a glial cell is determined by the ratio between the intracellular potassium concentration and the extracellular potassium concentration surrounding the *entire* cell.

[6] The level of 12 mM can not be exceeded in the spinal cord, where no spreading depression can be evoked (G. Somjen, personal communication).

255). Morphological investigations have demonstrated that primarily glial cells are affected by ouabain and show a very pronounced vacuolization and swelling, whereas neurons remain largely intact (17, 56, 184). It is in agreement with a primarily glial localization of the morphological effects that the potassium content in cultured glial cells is decreased more rapidly than that in neurons after exposure to ouabain, and that a lower concentration (10^{-5} M) of this drug is required to obtain a maximum effect in glial cells (184). In the presence of a high concentration of ouabain (0.5–1 mM) both C-6 glioma cells and C1300 neuroblastoma cells do, however, show a pronounced loss of potassium (largest in the glial cells) and the neuroblastoma cells gain a corresponding amount of sodium (164, 179).

Application of a concentrated solution of KCl or of ouabain, depolarizing amino acids, metabolic inhibitors or "labilizing" (289, 290) drugs, *e.g.*, veratrine, to a localized point of the exposed cortex as well as massive electrical stimulation lead to the so-called spreading depression originally described by Leão (185) and recently reviewed by Bureš and co-workers (38). This is a peculiar neurophysiological phenomenon, during which a wave of suppression of the normal electrical activity spreads from an intensely stimulated point of the exposed brain over almost its entire surface at a rate of 2 to 6 mm/min. The propagation of the depression is accompanied by a release of radioactive potassium ions from brain tissue which has been preloaded with ^{42}K (34, 36, 170, 335), but no concomitant decline occurs in the total content of potassium in the brain after a prolonged period of spreading depression (38). A key role in the process has been attributed to the potassium ion (103) and to glutamate (322, 324, 326). Spreading depression has been observed in the cerebral cortex, cerebellar cortex, basal ganglia and retina (38), whereas the spinal cord seems to be immune (296). An essential role of glial cells (Müller cells) has been suggested for propagation of spreading depression in the retina (109), and it is in keeping with the concept of a major role for glial cells in spreading depression that neurophysiological characteristics of spreading depression have been observed in glial cell cultures devoid of neurons (337).

During spreading depression, the extracellular concentration of chloride is drastically reduced (235), whereas the extracellular potassium concentration rises to at least 30 mM and often to the high level of 60 to 80 mM (88, 159, 193, 208, 258, 335).In order to maintain electroneutrality, the extracellular sodium concentration must be greatly decreased [as has recently been demonstrated (168)] and if the extracellular fluid remains isotonic, the concentration of an anion is probably increased. The latter could conceivably be glutamate which is released from retina during spreading depression (323) and from brain cortex slices by exposure to electrical pulses or high concentrations of potassium (section IV C 2). By aid of histochemical techniques it has been shown that chloride ions enter the apical dendrites together with water (leading to a swelling) and sodium ions (320, 322, 327, 329). The cell bodies of the astrocytes have been observed to shrink, but the volumes occupied by the astrocytic processes could reportedly not be measured (52), and it is thus not known whether chloride enters both neurons and glial cells or only the former cell type. In this context it should be remembered that after exposure to potassium-rich media, in which the increase in potassium concentration is compensated for by a reduction in sodium concentration (*i.e.*, the situation in brain during spreading depression), swelling and ion uptake can both qualitatively and quantitatively be explained as a result of osmotic, electrical and Donnan equilibria (30, 271, 288).

Typical spreading depression is obtained under moderate barbiturate anaesthesia, whereas the electroencephalogram amplitude is so low under very deep anaesthesia that it shows little further alteration; local treatment with cocaine or procaine or with high concentrations of $CaCl_2$ or $MgCl_2$

counteracts spreading depression (38). Treatment of the cortex with tetrodotoxin, which inhibits the sodium flux of the neuronal action potential, prevents the initiation of spreading depression by electrical stimulation but not that by application of concentrated solutions of KCl, indicating that the stimulus is an increased extracellular potassium concentration rather than neuronal excitation *per se* (133, 300–302). It seems most noteworthy that the propagation of spreading depression can occur through an area treated with tetrodotoxin (167, 300–302) because this supports the concept that neuronal excitation may not be indispensable for the propagation. In the presence of drugs inhibiting the potassium flux of the action potential (aminopyridines, Ba^{2+}), a large extracellular potassium concentration (about 25 mM) as well as certain neurophysiological characteristics of spreading depression can be evoked by a single local surface stimulus (236).

2. Metabolic manifestations of alterations in ion distribution: Effects of ouabain and barbiturates. Fluorometric measurements of NAD^+/NADH concentrations[7] have shown that the increased extracellular potassium concentration during excitation and spreading depression (181, 208–210, 276) is accompanied by an increase in metabolism. During "normal" excitation, there is a very good correlation between the external potassium concentration and the magnitude of the metabolic increase (191, 193) suggesting that the increased potassium concentration *per se* might trigger a process requiring energy. The rate at which NADH oxidation occurs is decreased by ouabain, whereas that at which the subsequent reduction takes place is drastically decreased by phenobarbital or amobarbital (54, 181, 210, 275).[8] The maintained oxidation observed in the presence of barbiturates is not due to a blockade of electron transport at a later stage of the chain of oxidative coenzymes since both NADH and cytochrome *a* oxidize and re-reduce with approximately the same time course (54, 298). The good correlation between extracellular potassium concentration and metabolism breaks down during spreading depression, when the metabolic activity becomes greater than could be deduced from extrapolation of the normal correlation between metabolic activity and extracellular potassium concentration (154, 193). For this reason, Somjen and co-workers (193) suggested the existence of a secondary source of ADP, *i.e.*, another energy-requiring process leading to hydrolysis of ATP, which becomes evident under pathological stress when the extracellular potassium concentration rises above 12 mM. Also during seizures (provoked, *e.g.*, by intense electrical stimulation or by metrazol), the increase in metabolic activity (190, 193) is larger than that corresponding to the elevation of the potassium concentration although the latter remains below 12 mM, at least in the extracellular pocket where it is measured. There is an increase in oxygen uptake (*e.g.*, 225) and in CO_2 production (256) during the seizures, and in spite of a sufficient oxygen supply to the brain cortex (at least as long as the respiration is maintained), the lactate production is also raised (21, 256). In the case of metrazol-

[7] Oxidative metabolism *in vivo* has been estimated by fluorometric monitoring of the concentration of NADH and other coenzymes of the respiratory chain (41, 42, 154). An increased metabolic demand (*e.g.*, during seizures) is in general accompanied by a shift toward a more oxidized state. The fascinating advantage of this method is that it allows determinations *in vivo*. It does not distinguish between events occurring in neurons and in glial cells, and quantitative estimates are uncertain, since alterations in mitochondrial coenzymes affect the measurements much more than alterations in extramitochondrial coenzymes (193).

[8] Since many data about ion distribution and energy metabolism *in vivo* originate from experiments on anaesthetized animals, it seems of major importance from a pharmacological point of view that some authors have remarked that different results were obtained in awake and in anaesthetized animals (200, 201, 209, 210).

induced seizures, an increase (up to 20%) of Na^+-K^+-ATPase activity has been observed (18).

It is an obvious possibility that the increased metabolism during excitation, seizures and spreading depression may serve the purpose of redistributing potassium ions lost from neurons and/or sodium ions taken up. Evidence to be discussed later (*e.g.*, a potassium-induced stimulation of energy metabolism in glial cells) suggests that the *immediate* uptake may occur into glial cells. This would represent an analogy to the current-carried redistribution of potassium ions through glial cells suggested by Kuffler and his co-workers on the basis of their classical experiments in leech ganglia and *Necturus* optic nerve, in which the contained *glial cells* behave as perfect K^+ electrodes[9] and are electrically coupled over long distances (>1 mm) (176–178, 244, 337). Subsequently, the potassium ions must of necessity be reaccumulated into neurons, and there is at present no method to estimate whether the increase of oxidative metabolism observed *in vivo* reflects neuronal or glial uptake of potassium.

B. In Vitro

1. Stimulation of metabolism: Effects of "labilizers," ouabain, local anaesthetics, tetrodotoxin, chlorpromazine, phenytoin, magnesium, ethanol and barbiturates. Much of the currently available information about energy metabolism and ion contents in the central nervous system in the presence or absence of drugs originates from *in vitro* studies of brain tissue.[10] Such data supplement those obtained *in vivo*, since quantitative determinations are easily obtained and since different cell types can be studied separately under more or less physiological conditions. In view of the increased extracellular potassium concentration during excitation, seizures and spreading depression, the fact is important that exposure of brain slices to high concentrations of potassium, depolarizing electrical pulses or labilizing drugs (289, 290), *e.g.*, protoveratrine (261, 353, 362) leads to pronounced increases in the rates of oxygen uptake, aerobic glycolysis and CO_2 production (9, 65, 71, 102, 121, 129, 211, 214, 217, 220, 221, 242, 307, 308). Simultaneously, there is an accelerated turnover of the terminal phosphate in ATP (37, 338) and the concentration of energy-rich posphates is decreased (352–354, 362). The substrate requirement for the drug-induced and the electrical stimulation is the same as that for the potassium-induced increase of oxygen uptake (130, 352) and the stimulation is in both cases dependent upon the presence of a certain concentration of potassium in the medium (19, 59,

[9] Glial cells in the mammalian cerebral cortex, in contrast to those in leech ganglia and *Necturus* optic nerve, may not behave as perfect potassium electrodes (180, 205, 247, 266, *cf.*, 89), whereas those in spinal grey matter do (194, 296).

[10] The "*in vitro*" term is used here to comprise both studies using surviving brain tissue prepared directly from the animal (*e.g.*, brain slices) and those using cultures, *i.e.*, neural tissue grown and maintained *in vitro* for at least 24 hr. The latter may be either primary cultures (*i.e.*, cultures started from tissue taken directly from the living organism) or established cell lines (*i.e.*, cultures obtained from parent cultures by subculturing, and demonstrating the ability to be subcultured and maintained indefinitely in culture). The use of cultures in neurobiological work is rapidly increasing (*e.g.*, 77, 279), and cultured tissues and cells may become of great value in pharmacological and toxicological research, since the preparations, which functionally are remarkably intact (*e.g.*, 77), can be exposed to an accurately known concentration of a drug or toxin for a prolonged period (weeks to months) without interference from other tissues or organs. The development of cultures consisting predominantly or exclusively of one cell type, *e.g.*, either glial cells (22, 76, 292) or neurons (100, 149, 287) is becoming of major importance for studies of the characteristics of the different cell types in the nervous system, although attempts to grow pure populations of neurons in primary cultures has until now not been too successful (*e.g.*, 287). Also brain slices are in several respects metabolically and morphologically well maintained. Their rate of oxygen uptake and content of ATP compare reasonably well with the corresponding *in vivo* values (130, 262), and the content of potassium may under suitable conditions correspond to more than two-thirds of the *in vivo* value (87, 130, 197).

352). However, the potassium-induced stimulation of oxygen consumption is accompanied by an increased oxidation of NAD(P)H, whereas the electrically induced increase in metabolic rate is accompanied by an increase in the reduced component (57). The anaerobic glycolysis is decreased by excess potassium (9, 71); this potassium-induced decrease may be counteracted by morphine, ethanol or barbiturates (309).

A threshold potassium concentration of about 20 mM is required to exert the potassium-induced respiratory stimulation (123, 129, 142) as well as the increase in aerobic glycolysis (307, 308) and the decrease in the level of ATP (142, 308). The maximum effect on oxygen consumption (*i.e.*, almost a doubling) occurs at about 50 mM K^+ (129). Approximately identical potassium concentrations are required to obtain threshold and maximum effects on swelling (section III B 2) in brain cortex slices (122, 123, 197), whereas the activity of the Na^+-K^+-ATPase reaches its maximum at a distinctly lower concentration, *i.e.*, 10 to 20 mM (116, 278), corresponding to the upper limit for the extracellular potassium concentration in the absence of spreading depression (section III A 1) and to the most distinct potassium accumulation and sodium extrusion in brain slices (section III B 2).

The potassium-induced stimulation of oxygen uptake is present in grey matter from the rostral parts of the central nervous system but absent in typical white matter and in grey matter from the spinal cord (125, 126, 130, 272). An increasing amount of solid evidence is accumulating that the potassium-induced stimulation of Na^+-K^+-ATPase activity, the decrease in ATP concentration and the increase in oxygen consumption are pronounced in glial cells (for references, see 130).[11] This conclusion is corroborated by the distinct morphological effect of ouabain on glial morphology in cellularly mixed preparations (17, 56, 184, 299). The question of whether a potassium-induced stimulation of the oxygen uptake also is found in neurons has been more ambiguous. Little or no stimulation was observed in the experiments by Hertz (118), Aleksidze and Blomstrand (1), Haljamäe and Hamberger (105) and Hertz *et al.* (127) using, respectively, microdissected, bulk-prepared and cultured neurons. Bradford and Rose (33) reported, however, approximately the same reaction to addition of potassium in their bulk-prepared neuronal and glial cell fractions and, using a microspectrophotometric technique, Hultborn and Hydén (141) observed a potassium-induced stimulation of oxygen uptake in microdissected neurons. The rate of oxygen uptake by synaptosomal preparations[12] is increased by electrical stimulation (63) or excess potassium (345) and nerve fibers have been shown histochemically to be enriched in Na^+-K^+-ATPase activity (298a).

Drugs which affect the distribution of cations in nervous tissue exert pronounced effects on the metabolism of brain slices. A similarity between the effects of excess potassium, electrical stimulation and the labilizing drugs has already been mentioned; application of ouabain, which affects the active transport of potassium and sodium by an inhibition of the Na^+-K^+-ATPase (257, 286, 293, 294, 304), may, in concentrations above 10^{-6} M, lead to a transient increase in the rate of oxygen uptake (151, 304, 351). The presence of calcium seems to be essential for this stimulation (285, 305, 313) and in the absence of this ion, a decreased oxygen consumption is evoked (346). The aerobic glycolysis is increased during incubation in media holding 10^{-4} M ouabain (274) and, in contrast to the effects exerted by high concentrations of potassium, 10^{-4} to 10^{-5} M oua-

[11] The initial stimulation of oxygen uptake in glial cells (and in brain slices (126, 129)) is followed by an increased rate of respiratory decline (1, 118, 127), which can be counteracted by phenobarbital (125).

[12] It has recently been demonstrated that such preparations may be heavily contaminated (up to 40%) by glial cells (for references, see 131).

bain leads also to an increase in the rate of anaerobic glycolysis (L. Hertz and T. Arnfred, unpublished experiments). Some "stabilizing" (289, 290) drugs (*e.g.*, the local anaesthetics procaine and cocaine) counteract the increase in membrane permeability resulting from excitation. Related to these is tetrodotoxin which in peripheral nerve (234) and in brain slices (39, 241) has been found specifically to inhibit the sodium conductance mechanism. Tetrodotoxin (39, 216, 233, 241, 291) and to some extent 0.1 mM chlorpromazine (215, 261), 1.0 mM procaine or 0.2 mM cocaine (40, 92, 212, 291) inhibit the electrical stimulation of oxygen uptake with little or no effect on that exerted by high concentrations of potassium or by dinitrophenol (104, 212, 218, 241),[13] and certain anticonvulsant hydantoin derivatives, including phenytoin (0.1 mM), inhibit the oxidative response evoked by electrical stimulation in a frequency-dependent manner (82, 104). Other drugs, *e.g.*, magnesium in high concentrations (10 mM) (129), 0.4% ethanol (94, 339) or barbiturates (*e.g.*, 0.1 mM pentothal) (94, 213) seem to exert an almost instantaneous (213, 344) inhibition of the stimulation regardless of whether it is evoked by application of electrical pulses (*e.g.*, 213), high concentrations of potassium (*e.g.*, 94, 213), dinitrophenol (94, 213) or labilizers (260). At least some of these drugs do, however, also have some inhibitory effect on the "nonstimulated" oxygen consumption (94, 213, 342, 344). Ouabain has both been reported to inhibit (65, 227) and to enhance (29) the respiratory stimulation, and Gonda and Quastel (102) found the same percent inhibition of the resting and the stimulated oxygen uptake after addition of 1×10^{-4} M ouabain.

Some of the drugs which counteract the stimulation of oxygen uptake by electrical pulses or excess potassium also prevent the concomitant fall of energy-rich phosphates. This has been demonstrated with *e.g.*, 0.4% ethanol (338), 5×10^{-4} M ouabain (227) or 0.3 mM phenytoin (303). The latter drug has in accordance with this been found to inhibit the brain Na^+-K^+-ATPase (96, 269); the absence of any effect has, however, also been reported (83), and with a certain K^+/Na^+ ratio in the medium a stimulation has been observed (81).

2. Contents of ions and water: Effects of ouabain, cocaine, procaine, phenytoin, acetazolamide, barbiturates, lithium, ethanol, tetrodotoxin, protoveratrine and chlorpromazine. Studies on brain slices to elucidate drug effects on ion contents are faster and technically less demanding than measurements of extracellular ion concentrations in the central nervous system *in vivo* and may thus be well suited for a screening of drug effects. As in the intact brain, reciprocal alterations in different cell types or between cells and the interstitial space may, however, be obscured when ion contents are measured in this composite structure. A deeper insight into the mechanisms of action for a drug may therefore either require an attempt to identify the cellular localization of the alterations induced [*e.g.*, by electron micrographic determination of swelling (section II B); kinetic analysis of different ion compartments (section IV C 1 and 2); simultaneous use of other drugs specifically affecting certain cell types (15); or work on isolated cells]. Such investigations are in their beginnings.

Brain slices generally take up fluid ("swell") even during incubation in a physiological medium. High concentrations (>20 mM) of potassium (24, 26–28, 74, 138, 192, 197, 250, 283), electrical stimulation (10, 240, 310) and ouabain in a concentration of at least 10^{-5} M (29) lead to an increase in swelling,[14] which in the case of

[13] High concentrations of at least some of these drugs do, however, also affect the potassium-induced stimulation (215, 344).

[14] The swelling is generally expressed as the percent increase of the fresh wet weight resulting from exposure to the medium and incubation and amounts to at least 10 to 15% (84, 87, 197). A correction has often been made for the swelling when the contents of potassium, sodium or chloride have been expressed. To this end, the tissue was considered as consisting of two compartments, *i.e.*, one corresponding to the increase in weight during the incubation (swelling) and the other to the fresh wet weight; the concentrations of

potassium and ouabain has been shown to occur in glial cells (17, 26, 46, 56, 184, 228, 299, 364; *cf.* section II B). The potassium-induced swelling is prevented by replacement of all chloride with the indiffusible isethionate ion (24, 27, 28, 192, 197). This reflects the fact that the swelling fluid appears to be a rapidly exchanging, isotonic fluid expansion composed predominantly of potassium and chloride ions (24, 283). The observations that the potassium-induced swelling is diminished by a lowering of the temperature (24) and that the concomitant uptake of ^{36}Cl follows Michaelis-Menten kinetics [with respect to both chloride and potassium (section IV C 2)] seem to indicate participation of an active transport mechanism. The presence of the potassium-induced swelling in media with unaltered sodium concentration (197) demonstrates also that it is not a simple, passive consequence of replacing sodium in the medium with potassium (30, 271, 288). Therefore, it has been suggested that the water uptake during the potassium-induced swelling is secondary to an accumulation of potassium and chloride (possibly with chloride as the ionic species actively transported) which probably occurs into glial cells (24, 27, 28, 121, 130, 197, 241, 249). Such an uptake of potassium without a concomitant extrusion of sodium would provide an explanation for the view that brain tissue is capable of concentrating potassium to a larger extent than of excluding sodium (121, 138). The potassium-induced swelling is efficiently counteracted by such drugs as cocaine (291) and procaine but to a much smaller extent by phenytoin (C. S. Kjeldsen and L. Hertz, unpublished experiments).[15] It is also counteracted by acetylcholine (15) and inhibited by acetazolamide, an inhibitor of the enzyme carbonic anhydrase (26). This enzyme has much higher activity in glial cells than in neurons (95) and its possible importance for ion transport in the brain has been discussed by Bourke and Nelson (26). Barbiturates (*e.g.*, 0.25 mM pentymal or pentobarbital) lead to a, probably intracellular, swelling (C. S. Kjeldsen and L. Hertz, unpublished experiments), the cellular localization and mechanism of which are unknown.

The content of potassium in brain slices is lower than that in the brain *in vivo*, whereas the sodium content is higher. One reason for the lower content of potassium during incubation *in vitro* is a pronounced loss of this ion during and immediately after the preparation of the tissue, which is followed by a more or less complete reaccumulation (10, 29, 84, 147, 169, 197, 251, *cf.* 138); it should, however, also be recalled that the extracellular space[16] is increased in brain slices (section II B). A

potassium, sodium and chloride in the compartment formed during the incubation were regarded as identical to those in the medium (*i.e.*, about 5 mM K^+ and 120–135 mM Na^+ and Cl^-) and subtracted from the total amounts of these ions. The remaining amounts (*i.e.*, virtually all the potassium content but only a fraction of the sodium content) were subsequently calculated as contents per gram *remaining* (after subtraction of the swelling) weight. The more recent demonstrations that the swelling is not exclusively, and not even predominantly, an extracellular phenomenon and that the swelling fluid does not have the same composition as the medium imply that this correction can no longer be regarded as permissible.

[15] The effect of procaine and cocaine on a phenomenon localized in glial cells may either suggest that these two local anaesthetics exert other effects on the central nervous system than those related to a "stabilizing" of the membranes of excitable cells or that the potassium-induced swelling of glial cells is secondary to a potassium effect on neurons.

[16] Reliable calculation of intracellular ion concentrations in brain slices would be the most meaningful way of expressing ion contents. Several substances, including sucrose (62), inulin (113) and chloride (317) have functioned as indicator substances to measure the size of the presumably extracellular and thus, by subtraction from the total, the intracellular fluid space in brain tissue incubated *in vitro*. The spaces accessible to inulin, sucrose and chloride are, however, of unequal magnitude, the "inulin space" being the smallest and the "chloride space" the largest (28, 132, 249, 250, 283, 330). All of these spaces can accordingly not delineate the true extracellular space, and studies of temperature effects on the marker spaces and of the washout of radioactively labelled markers have shown that all extracellular markers, including inulin, have access to one or more intracellular compartments (2, 47–50, 283). However, kinetic analysis of the

concomitant initial gain in sodium content seems more difficult to revert (*cf.* above), but a sodium extrusion has been observed when the tissue is prepared with special care (10, 87). Massive oxygenation seems of major importance to obtain as "physiological" contents of both potassium and sodium as possible (8, 84, 87).

Electrical "stimulation" of neocortical brain slices[17] as developed by McIlwain (211, 220) leads to a depolarization and is accompanied by a considerable sodium gain and potassium loss (10, 59, 152, 153, 330). The decline in the potassium content begins immediately, is independent of the substrate used and reaches a maximum of about 20 μmol/g wet weight after 5 to 10 min (59, 153). Subsequently, the potassium content remains stable at a level which depends upon the sodium concentration of the medium (10), and 15 years ago it was calculated that the potassium concentration in the extracellular clefts may increase as much as 20 to 30 mM (137). During the first 2 to 3 min after the termination of the stimulation an almost quantitative, substrate-dependent, reaccumulation of potassium occurs and also the previous content of sodium is more or less completely regained (136, 163).

Also the presence of increased potassium concentrations in the incubation medium affects the content of sodium and potassium in brain slices. An increase of the external potassium concentration to 15 to 20 mM leads to an enhanced accumulation of potassium and extrusion of sodium (29, 122, 197), probably reflecting a stimulation of the Na^+-K^+-ATPase which has its maximum activity at that potassium concentration (section III B1). It is in keeping with the concept of a 1:1 ion exchange that the swelling is unaffected below 20 mM potassium (*cf.* above). A further increase of the external potassium concentration causes a pronounced rise in the sodium content. This rise is attenuated, but not abolished, in chloride-free media (where no increased swelling occurs) and is accordingly not a simple consequence of the potassium-induced swelling (197). It may be comparable to the sodium gain during electrical stimulation but is more difficult to explain since the increased sodium conductance resulting from the potassium-induced depolarization of excitable tissue in general is a transient phenomenon. At the same time the potassium content in the tissue, expressed per gram wet weight, is less increased than could be expected from the increase in the medium concentration. The potassium content is affected in exactly the same manner by the external potassium concentration after incubation in chloride-free (or glutamate-containing) media, in which the swelling is not influenced by the external potassium concentration (197). Accordingly, the content of potassium per gram dry weight is relatively constant between 20 and 50 mM external potassium in the chloride-free and the glutamate-containing media, whereas it shows a continuous rise in the potassium-rich, otherwise "normal" medium (197, *cf.* 251). These findings led Lund-Andersen and Hertz (197) to suggest the hypothesis that the depolarization evoked by the increase of the external potassium concentration in any case leads to a potassium loss and a sodium gain, and that the

---

washout of labelled inulin has indicated the presence of one rapidly exchanging compartment, which does follow diffusion kinetics and is washed out from brain slices of 0.5 mm thickness with a half-time of about 5 min (196, 198). This component seems to represent the extracellular compartment and may be used to determine its magnitude; the practical importance of such a determination is, however, probably small since the procedure is time-consuming and since several compounds, *e.g.*, glucose analogues, penetrate into a larger "functional extracellular space" in accordance with diffusion kinetics (199).

[17] Most studies of the effect of electrical stimulation on metabolism and ion distribution in brain slices have been performed using slices from the neocortex, which give little or no electrical response to the stimulation; during recent years such responses have, however, been obtained in slices prepared from piriform cortex (361), hippocampus (359) or the superior colliculus (161); conceivably metabolic and ionic characteristics as well as responses to stimulation and drugs may be different in these preparations.

potassium loss in the normal medium may be balanced by a compensatory accumulation of potassium and chloride (*cf.* 23, 24) which for osmotical reasons leads to an increased swelling (*cf.* above) and thus becomes obscured when the potassium content is expressed in relation to the wet weight.

The effects of ouabain on ion contents have been studied extensively and there is no doubt that both the potassium accumulation and the extrusion of sodium and chloride are reduced in brain slices exposed to at least a 10^{-6} to 10^{-5} M concentration of this drug (29, 91, 151, 285, 304, 305, 347). It is in keeping with the concept that the increased potassium accumulation and sodium extrusion at about 15 to 20 mM external potassium are brought about by a stimulation of the Na^+-K^+-ATPase that these phenomena are completely abolished by 10^{-6} M ouabain (C. S. Kjeldsen and L. Hertz, unpublished experiments). In addition, 10^{-5} M ouabain has been found to reduce the membrane potential in cultured glioma cells exposed to about 10 mM potassium in a manner consistent with inhibition of an ion pump (205). Barbiturates (0.25 mM amobarbital or 0.25–1.00 mM pentobarbital) cause a dose-dependent inhibition of both potassium accumulation and sodium extrusion but have little or no effect upon the increased potassium content and decreased sodium content at 20 mM potassium (C. S. Kjeldsen and L. Hertz, unpublished experiments). At a concentration of 1 to 2 mM, lithium ion seems to cause a lowering of both potassium and sodium contents (147, 165, 358) and, if anything, enhances the increased potassium accumulation and sodium extrusion at 20 mM potassium (165). Ethanol has, in pharmocological concentrations, an inhibitory effect on both the Na^+-K^+-ATPase and the accumulation of potassium (147).

Tetrodotoxin (40, 108, 216, 219), 50 μM chlorpromazine (136) and certain local anaesthetics, *e.g.*, procaine (40), almost completely prevent both the potassium loss and the sodium gain during electrical stimulation; tetrodotoxin also inhibits a sodium uptake evoked by protoveratrine (15) but has no effect on the sodium uptake evoked by 100 mM potassium (15, 241).[18] However, 5 mM procaine does lead to a substantial reduction (about 50%) of the sodium uptake evoked by 40 to 50 mM potassium (C. S. Kjeldsen and L. Hertz, unpublished experiments). In addition, a concentration of chlorpromazine as low as 10 μM may enhance the extrusion of sodium from brain slices after pretreatment in the cold (19). In the presence of phenobarbital (0.3 mM) [or other barbiturates (40)], electrical stimulation causes the usual release of potassium and gain of sodium. The reaccumulation of potassium takes place as in the absence of any drug, but the cells in the slice show a faster repolarization after termination of the stimulation (136).[19] Phenytoin (0.3 mM) seems to inhibit the potassium loss and sodium gain during electrical stimulation (303), probably due to a stabilizing action on the cell membrane and/or increased rates of the restitution processes. The latter mechanism of action may be suggested by the observation that 0.5 mM phenytoin seems to act somewhat differently from

[18] This uptake of sodium is counteracted by acetylcholine in the presence of eserine (15). Since, on the other hand, acetylcholine has no effect on the protoveratrine-induced sodium uptake, it has been suggested that the sodium uptake during exposure to 100 mM potassium occurs via a channel which is different from the neuronal channel activated by protoveratrine and that it possibly occurs into glial cells (15). This hypothesis is at some variance with the concept (24, 197) that excess extracellular potassium leads to an uptake of KCl into glial cells. If the chloride ions are accumulated actively and potassium and/or sodium ions follow as the counterions, relatively minor alterations in experimental conditions might, however, affect the accumulation of, respectively, sodium and potassium by alterations of membrane permeabilities toward the two ions.

[19] Originally, the membrane potentials observed in neocortical brain slices were presumed to occur in neurons (137). The large amount of recent information about glial potential measurements *in vivo* may conceivably challenge this concept.

procaine. At an external K^+ concentration of 5 mM phenytoin leads to a slightly decreased potassium content, but a rise of the external potassium concentration to 10 to 20 mM leads to a larger increase in potassium content than that observed in the absence of any drug or in the presence of procaine (5 mM). This may support the hypothesis that phenytoin, in addition to possibly evoking a reduction of ion movements during excitation, may also act directly upon potassium accumulation (*cf.* section I) and may do so only at certain external potassium concentrations (section IV C 2), *e.g.*, 10 to 20 mM where the Na^+-K^+-ATPase has its maximum activity (section III B 1).

IV. Ion Transport

A. In Vivo: Effects of Ouabain, Barbiturates and Phenytoin

Both membrane potentials and the unequal distribution of sodium and potassium ions across cell membranes depend upon active transport of one or more ionic species. *In vivo* studies of penetration of radioactive substances from the blood into the brain often reflect penetration through the blood-brain barrier and will not be dealt with here. Very useful information has, however, been obtained after intracranial application of radioactive potassium (246, 247) or by superfusion of the cortex after previous loading with a radioisotope (36, 80, 170).[20] Also continuous monitoring of the extracellular K^+ concentration after an increase evoked by stimulation or electrophoretic application of potassium to the brain parenchyma (202) has yielded important information about the rate at which excess potassium is removed. Some of the findings have recently been reviewed by Katzman and Grossman (159), who discussed the relative importance of diffusion, cellular reuptake and potassium clearance by brain capillaries. The uptake into brain capillaries was calculated to lead to an exchange of the total extracellular potassium (3.0 mM in an extracellular space of 15%) about every 10 min. It was concluded that this value is far too small to account for the clearance of extracellular K^+ after rapid local alterations, and the crucial question seems to be the relative contribution by active, energy-requiring processes and by diffusion. Since diffusion per time unit is related to the square of the distance, Katzman and Grossman (159) reached the conclusion that it is only in the case of a point source (rather than a distributed source) that diffusion will contribute significantly to the potassium clearance during the time period involved. A redistribution of potassium via the current-carried transport mechanism (section III A 2) suggested for the leech brain does not seem to contribute to the extracellular clearance of potassium in mammalian brain cortex to any greater extent (99, 202), which is in agreement with the failure of the contained glial cells to behave as perfect potassium electrodes (180, 247, 266, *cf.*, however, 89).

The rate of clearance of extracellular potassium, *i.e.*, the kinetics for the reduction of an increased extracellular potassium concentration, might conceivably help to distinguish between an unsaturable diffusion mechanism and a saturable active component. There is fairly good agreement in the literature that the increased potassium concentration declines more (191) or less exponentially with a half-time of 1 to 10 sec (193, 201, 202, 268), but the deviation from a strictly exponential course has been large enough to suggest a saturable component (298) and G. Cordingley and G. Somjen (personal communication) observed a considerable faster removal of extracellular potassium than can be explained by diffusion. The rate constant for the decline in potassium concentration can be modified, since applica-

[20] The *in vitro* studies by Bourke (23, 24) on chloride uptake and swelling in brain cortex slices have also been repeated in the superfused monkey cortex where essentially similar results were obtained (27) and where acetazolamide was found to inhibit both the swelling and the increased influx of Cl^- evoked by a high concentration of potassium (26).

tion of several stimulus trains in close succession results in a more rapid potassium removal (and a more pronounced metabolic increase, *i.e.*, NADH oxidation, *cf.* section III A 2) after the later trains (191). Often, the decline continues below the baseline level. This "undershoot" has been described in both the brain (191, 202, 268) and the spinal cord (295), and it is specially pronounced after more intense stimulation (191, 193, 268). Electrophoretic injection of potassium during the undershoot results in a significantly smaller than usual elevation of the potassium concentration (201).

The undershoot is more distinct in the absence of anaesthesia (114, 201) and there seems to be no doubt that barbiturates (*e.g.*, 60 mg of phenobarbital per kg body weight) decrease the rate constant for clearance of excess extracellular potassium (54, 210, 298) and also that the metabolic response persists for a longer time in the presence of these drugs (section III A 2). Phenytoin may exert a similar, although less consistent, effect on potassium removal (298), and Krnjevic and Morris (174) observed a pronounced inhibition (50%) of potassium clearance in the cuneate nucleus after superfusion with 10^{-4} M strophantidin. Cordingley and Somjen (55) found a somewhat smaller (13–25%) effect in spinal cord grey matter after digitoxigenin doses of 100 μg/kg.

Transport kinetics have also been studied in gliotic scars evoked by freeze lesions and accompanied by seizure susceptibility (254); no differences from normal brains were reported. On the other hand, Glötzner (99) and Ward (339a) have described membrane properties of glial cells in epileptogenic gliosis (evoked by aluminum hydroxide injection) of the cat motor cortex that would facilitate the passive, ion-carried redistribution of potassium.

In addition to causing a release of intracellular potassium, afferent stimulation also enhances the glutamate release (150). No details are known about the kinetics for the subsequent removal *in vivo*.

B. Cultures: Effect of Ouabain

Cultures of neurons and/or glial cells are well suited for transport studies since the kinetics often are relatively simple, the barrier systems are bypassed and neurons and glial cells may be studied separately or in cultures containing both cell types. Latzkovits *et al.* (183) compared accumulation of ^{42}K (or the potassium analog ^{86}Rb) into pure glial and mixed neuronal/glial chick embryo cultures and observed that the uptake followed a simple exponential course in the glial culture, whereas the uptake curves for the mixed cultures indicated a complex interaction between different compartments. A kinetic model analysis suggested a direct uptake of potassium from the medium into both cell types *plus* a transport from one cell type into the other. In a continuation of this work (184), ouabain ($1\text{-}100 \times 10^{-5}$ M) was found to inhibit the glial uptake of potassium to a larger extent than the neuronal uptake. The latter was concluded to consist of two components: 1) a main uptake regulated by intact glial function and thus sensitive to ouabain, and 2) a secondary neuronal uptake directly from the medium and much more resistant to ouabain.

Conceivably, the uptake of potassium into glial cells and neurons could either occur in exchange with sodium or together with an anion, *i.e.*, probably chloride. A potassium-activated uptake of chloride following Michaelis-Menten kinetics with a K_m (for K^+) of about 30 mM has been observed in the NN astroglial line (97), and active transport of potassium in exchange with sodium has been reported in the C-6 glioma line (179). In the C-6 cells, potassium influx and efflux are roughly identical and can be calculated to amount to about 7 μmol/min/g wet weight (180); the uptake rate for potassium is approximately the same in the NN glial cells (164) and in chick glial cells in primary cultures (183). The sodium fluxes in the C-6 cells seem to be of about the same magnitude or slightly higher and from the uptake curve

for ^{36}Cl the chloride influx can be calculated to about 3 μmol/min/g (180). These fluxes are all rather high compared to the corresponding values in brain slices (*cf.* below) and the potassium uptake per gram wet weight is about 50% of that observed *in vivo* after excitation (*cf.* section V). A 20 times higher uptake rate, *i.e.*, a potassium uptake of about 150 μmol/min/g wet weight has, however, recently been demonstrated into mouse glial cells in primary cultures (124). This impressive uptake rate which increases further with a rise of the extracellular potassium concentration is more than intense enough to account for the *in vivo* observation that the raised extracellular potassium concentration resulting from excitation decreases with a half-time of 1 to 10 sec,[21] and supports the concept that glial cells may be of major importance for clearance of extracellular potassium *in vivo*.

In the C-6 cells, a decrease of temperature was observed to inhibit the potassium uptake slightly and, remarkably enough, to lead to a very pronounced decrease of both sodium influx and potassium efflux (179). Ouabain inhibits the Na^+-K^+ exchange, but at a concentration of 5×10^{-4} M the potassium influx is only decreased by about one-third (179).

Also uptake and release of glutamate have been studied in cultured cells. A very efficient uptake has been found in both glial cell lines and glial cells in primary cultures (for references, see 131).

C. Brain Slices

1. Resting conditions: Effect of ouabain. The lack of complete information about intracellular potentials and ion concentrations in brain slices impedes calculation of electrochemical potentials on either side of the membranes and makes it difficult, if possible at all, to establish whether a single ion species, *e.g.*, chloride or potassium, is in electrochemical equilibrium. Also the thickness of ordinary brain-cortex slices (0.25 to more than 0.5 mm) may complicate the determination of membrane-limited unidirectional fluxes by aid of isotope tracers (110, 199). If information is sought about transport phenomena in central nervous tissue, which may turn out to be very specific (*cf.* section V), they *must* be studied in this tissue and not in simpler preparations such as frog skin or peripheral nerve which may display quite different transport phenomena. Measurements of influx (uptake) and efflux (washout) by aid of radioactively labelled ions have been performed not only in brain slices (28, 59, 84, 86, 119, 162, 169, *cf.* also 34), but also in isolated sympathetic ganglia (35, 111, 357) and in the total frog brain (32, 363). The influx is measured from the *rapidity* with which the tissue becomes radioactive after exposure to a radioisotope, and the efflux from the speed with which the radioactivity subsequently is washed out into a non-radioactive medium (349). Influx and efflux will be identical, if no net changes in ion content [and no production (*e.g.*, in the case of an amino acid)] occur, and one of these can be calculated from the other when the magnitude of a possible net change is known.

Uptake studies have shown that practically all potassium in brain slices from adult [but not from newborn (130)] animals is exchangeable within about 1 hr (84), and desaturation curves (349) describing the washout of ^{42}K from slices previously loaded with the radioisotope have indicated the presence of at least two, kinetically different compartments (86, 119). By refinement of the kinetic analysis, Franck (84) distinguished between one extracellular and two kinetically different, rapidly exchanging, cellular compartments. These rapidly exchanging cellular compartments (half-times, respectively, 5

[21] With the potassium-sensitive electrode in an "extracellular pocket" of, *e.g.*, 3 μm, a glial cell width of, *e.g.*, 15 μm, and a glial content of 50%, a potassium uptake of 150 μmol/min/g cell weight would correspond to a potassium removal of about 750 μmol/min (12.5 μmol/sec) per ml of extracellular fluid. An extracellular potassium concentration of, *e.g.*, 10 mM would thus decline with a half-time of less than 1 sec.

and 15 min) comprise by far the largest amount of radioactive potassium in the tissue and were envisaged by Franck (84) to represent, respectively, glial cells and nerve cells plus dendrites (for further details, see 84).[22]

From the potassium concentrations and the rate constants, fluxes between the rapidly exchanging cellular compartment(s) and the medium have been estimated to be 1 to 6 μmol/min/g wet weight during incubation in oxygenated physiological media (59, 84, 86, 119, 169). Under anoxia the rate constants of the efflux from the rapidly exchanging compartment(s) are slightly increased (84, 119), but the concentration of potassium is lower and the fluxes thus decreased. Ouabain (10^{-4} M) leads almost to a tripling of the rate constant, but at the same time the potassium concentration is reduced to about one-third (84).

The desaturation curve observed after loading with radioactive sodium resembles in principle that obtained with potassium, since again two or more compartments are observed. In this case, it is the fastest of the two rapidly exchanging cellular fractions described by Franck (84) and suggested to represent glial cells, which accounts for most of the intracellularly located radioactivity, and the fluxes between the rapidly exchanging fraction(s) and the medium can be calculated to about 5 μmol/min/g final wet weight (84, 119, 162, 303, 363). Anoxia or metabolic inhibitors have relatively little effect on this part of the sodium efflux (84, 119, 363), but ouabain (10^{-4} M) causes a distinct decrease of the rate constant (84). The sodium exchange with the slowly exchanging fraction occurs at a rate of about 0.1 to 0.3 μmol/min/g final wet weight (162, 363), and is decreased by metabolic inhibitors or by a lowering of the temperature (363), but not by ouabain (84).

Also the chloride fluxes in brain-cortex slices amount to a few micromoles per minute per gram wet weight (23, 283). Both influx and efflux depend upon the chloride concentration of the medium, and the influx into the cellular compartment has been shown to conform to Michaelis-Menten kinetics with a K_m of 245 mM and a V_{max} of 7.7 μmol/min/g wet weight (23).

Glutamate is accumulated fast and with a relatively high affinity into brain cortex slices; the combination of a fast uptake *and* a high affinity probably leads to an underestimate of the uptake rate in brain slice experiments, since diffusion will be rate-limiting, when the low concentrations in the range of the K_m value are used (131).[23] The intense uptake of glutamate into glial cells in primary cultures has already been mentioned (section IV B), and Benjamin and Quastel (14) have used tetrodotoxin sensitivity of a release of glutamate induced by protoveratrine together with ouabain as a tool to distinguish between uptake into neurons and glial cells in brain slices. They concluded that the major part of exogeneous glutamate is accumulated into glial cells and have suggested that also glutamate released from neurons during excitation may be taken up by glial cells, converted to glutamine and returned to the neurons (13). Studies of "metabolic compartmentation" in the brain had previously suggested a connection between a smaller and a bigger metabolic "pool" by a flow of glutamine in one direction and of γ-aminobutyric acid

[22] These half-times are so short that it has been argued that these compartments represent extracellular and not cellular phases. In that case the efflux should follow diffusion kinetics, *i.e.*, become 4 times slower when the thickness of the slice is doubled and 4 times faster when it is halved. The half-time of the larger, more slowly exchanging of these compartments is, however, only slightly changed (from 12 to 15 min) when the thickness of the slice is increased from 0.25 to 1.0 mm indicating that diffusion is not the rate-limiting process (121). These compartments do accordingly not represent an extracellular space and are also far larger (*i.e.*, contain far more potassium) than the extracellular space in brain tissue (84, 119, 363).

[23] This qualification does not seem to apply for studies of potassium or sodium fluxes where higher medium concentrations are used. This can be seen from the previously mentioned observation of a virtually unaltered potassium efflux when the slice thickness if halved or doubled (121).

(formed from glutamate) in the other (for details, see 11, 16, 106, 124, 319).

2. Stimulation: Effects of phenytoin and ouabain. An increase of the potassium concentration of the medium from 5 to 55 mM causes a considerable increase in both efflux and influx of potassium in the rapidly exchanging cellular fraction(s) (84, 119). At the same time, the potassium concentration in the tissue increases and the magnitude of the fluxes increases from 1 to 2 μmol/min/g wet weight in the medium with 5 mM potassium to about 10 μmol/min/g wet weight after the potassium addition. This increase is inhibited by 10^{-4} M ouabain (L. Hertz, unpublished experiments).

Also, during application of electrical pulses, both influx and efflux of potassium increase to about 10 μmol/min/g wet weight (59, 84, 86). The raised influx continues during the first minutes after the termination of the electrical stimulation [as does the metabolic response (214)] and leads to a considerable (4–10 μmol/min/g wet weight) net gain of potassium (10, 59, 163).

Phenytoin has been reported to enhance the potassium uptake into synaptosomes[24] from epileptic foci in the cat brain if the incubation medium contains 10 mM potassium [*cf.* the maximum Na^+-K^+-ATPase activity at 10–20 mM potassium (section III B 1) and the effect of 15 to 20 mM K^+ on potassium accumulation and sodium extrusion (section III B 2)], but not at lower potassium concentrations; this stimulation may be prevented by ouabain, but only at low sodium concentrations (75).

The curve describing the influx of sodium into brain cortex slices seems to be independent of an increase in the external potassium concentration (84, 119), but the tissue is not in a steady state because the sodium content and the weight increase. The rate constant of the efflux from the rapidly exchanging cellular fraction, or at least from its quantitatively dominant, possibly glial compartment is also not affected by excess potassium (84, 119). Franck (84) found analogously that electrical stimulation has little or no effect on the efflux of ^{24}Na, but it has been claimed by Keesey and Wallgren (162) that the efflux of sodium is increased by application of electrical pulses. The identical slopes of the washout curves with and without stimulation (fig. 4 in their paper) are, however, not in agreement with this conclusion.

The initial velocity of the chloride influx into the cellular compartment of brain cortex slices is significantly increased by excess potassium and follows Michaelis-Menten kinetics with a K_m (for potassium) of about 30 mM (23). This effect is localized in glial cells (97). Addition of excess potassium during the washout of ^{36}Cl from brain cortex slices has no effect on the rate constant (119, 283). The chloride content is, however, increased and the high potassium concentrations lead to a pronounced increase in a rapidly exchanging, cellular chloride fraction in brain tissue from adult, but not from neonatal, rats (283). The volume of the cells with which chloride ions rapidly exchange is thus greatly expanded after exposure to excess potassium. This is in accordance with the observed rapid exchange of the chloride-rich fluid of the potassium-induced intracellular swelling (23, 24) and also with the concept that the expansion occurs into glial cells, which develop at a late stage of ontogenesis (for references, see 124).

The decreased content of glutamate after incubation in potassium-rich media

[24] Detailed studies have been reported on fluxes of sodium, potassium and chloride in synaptosomes and on the effect of certain drugs on these fluxes (206). From fluxes given per cm^2, the fluxes in the absence of any drugs can be recalculated to about 65 μmol/min (sodium), 20 μmol/min (chloride) and 2 to 3 μmol/min (potassium) per g wet weight. The sodium and chloride fluxes are high compared to those in brain slices, but the much larger surface/volume ratio should be kept in mind. In view of the possible contamination of synaptosomal preparations with up to 40% glial elements (for references, see 131), and the risk of membrane impairment during the preparation of the synaptosomes, these findings will not be described in further detail in the present review.

(with glucose as the substrate) is correlated with an increased efflux of this amino acid from preparations of brain cortex, spinal cord or retina exposed to excess potassium (or to electrical stimulation) after previous loading with ^{14}C-glutamate (7, 119, 120, 157, 230, 323). A similar potassium-induced release of endogeneously formed glutamate has been observed in synaptosomes (63) but may be less distinct in brain slices (203, 204). By aid of chromatography, it has been shown that the increased amount of ^{14}C, which is released after labelling with glutamate, is due largely to an efflux of unmetabolized glutamate (7, 323).

Both the spontaneous and the potassium-induced releases of glutamate from brain slices are largely unaffected by pentobarbital (60, 343) but a high concentration (4 mM) of this drug inhibits the enhanced release evoked by electrical stimulation (360). Tetrodotoxin, lidocaine and procaine have no effect on the stimulated efflux of glutamate (108, 343) indicating that the response is no direct result of an excitation. It is in agreement with this concept that the potassium concentrations required for a maximum response are much higher than those needed for a depolarization (123, 343). In synaptosomes, a maximum release of glutamate is, in contrast, evoked by 10 mM potassium (245) which might suggest that the response in brain slices occurs from other sources than synaptosomes. A potassium-induced release of ^{14}C-glutamate from isolated rat dorsal ganglia, in which exogeneous glutamate seems to be accumulated into glial cells (280), suggests a glial localization of the response (273).[25]

V. Concluding Remarks

The whole field of ion distribution at the cellular level of central nervous tissue and especially of drug effects on this distribution is still so much in its beginnings that the concluding remarks of this review to a great extent will be in the form of questions and vague hypotheses rather than actual conclusions. One fact that recently has been firmly established is the physiological (excitation, stimulation of visual pathways) and pathological (seizures, spreading depression) increase in extracellular potassium concentration to a level that would hardly have been believed 5 years ago. These extracellular potassium concentrations of 10, 30, 60 and even 80 mM attribute a very physiological significance to the *in vitro* studies of brain biochemistry, physiology and pharmacology under exposure to potassium concentrations of a similar magnitude, which have been performed since the demonstration by Ashford and Dixon (9) and Dickens and Greville (71) in 1935 that incubation in potassium-rich (about 50 mM) media leads to a very considerable increase in the oxygen uptake of brain slices. The similarity between the recent *in vivo* and the older *in vitro* observations is remarkably good. A reasonably close correlation between the intensity of energy metabolism *in vivo* and *in vitro* has previously been pointed out (*e.g.*, 130, 262); during exposure to high K^+ concentrations, there seems in both cases to be an increase in oxidative metabolism as well as in aerobic lactate formation, and it has been estimated by several authors (159, 195, 315, 347) that ion transport may account for a major part of the energy consumption by the brain both *in vivo* and *in vitro*. The potassium fluxes seem to be comparable *in vivo* and in brain slices since the "stimulated" *in vitro* value of 10 μmol of K^+ per min per g wet weight is approximately similar to a removal of 10 mM potassium from 15% of the tissue (the extracellular space) with a half-time of 1 to 10 sec.[26] Compared to this value, the potassium uptake of up to 150 μmol/min/g wet weight in cultured glial cells is remarkably high. Conceivably the *in vivo* uptake

[25] Attempts to demonstrate a potassium-induced release of glutamate from mouse astrocytes in primary cultures have, however, failed (L. Hertz, unpublished experiments).

[26] With an extracellular potassium concentration of 10 mM and a half-time of 5 sec, 1.39 μmol of potassium is removed from the clefts per sec per ml of extracellular space or 83.4 μmol/min/ml. Since the

rates may, however, have been grossly underestimated since it is likely that the uptake will occur considerably faster from a cleft of 150 Å width (due to the large surface/volume ratio) than from the extracellular "pocket" of 3 μm diameter surrounding the potassium-sensitive electrode.

Both during incubation *in vitro* and under *in vivo* conditions it has more or less reluctantly been accepted that there may be two distinctly different levels of raised potassium concentrations, *i.e.*, 1) one level with a maximum of about 10 to 12 mM which *in vivo* occurs during excitation and seizures[27] and is accompanied by an increase of energy metabolism, and which *in vitro* leads to a stimulation of Na^+-K^+-ATPase activity but not of energy metabolism or swelling, and 2) another level, potassium concentrations above 20 mM, which *in vivo* only have been observed in connection with spreading depression and *in vitro* lead to immense increases of oxygen uptake, swelling and aerobic glycolysis. The *in vivo* experiments have not been able to distinguish between phenomena occurring in neurons and in glial cells, but there is a remarkable agreement between most authors that the stimulation of energy metabolism and ion transport *in vitro* to a large extent occurs in glial cells. If it is assumed that the stimulated metabolism mainly serves the purpose of regulating potassium homeostasis in the extracellular space, an analogy becomes obvious with the role of glial cells in the current-carried potassium redistribution suggested for the leech and amphibian nervous system (176, 316). One may thus wonder whether an active mechanism for potassium removal from the extracellular clefts in the mammalian brain cortex represents a further phylogenetic development from an analogous, passive (current-carried) system in simpler central nervous systems (128), and one may also ask what advantage for brain function is worth the extra price of an energy-requiring uptake of potassium ions into glial cells before an eventual reaccumulation into neurons. These questions cannot be answered today but the ultimate possibility is that glial cells may play a crucial role in higher mental activity by conveying and regulating impulses over short distances as originally suggested by Galambos (90) and Hertz (117). The studies by Latzkovits *et al.* (183, 184) on potassium uptake in the presence and absence of ouabain strongly suggest a neuronal/glial interaction in ion metabolism, and the finding by Sugaya and co-workers (300–302) that spreading depression can propagate through a tetrodotoxin-treated area may suggest a major role of glial cells in this *in vivo* phenomenon in analogy with the previously established importance of these cells for the potassium-induced stimulation of energy metabolism *in vitro*. The absence of both these phenomena in mammalian spinal cord, which on the other hand seems to fulfill the requirements for a current-carried redistribution of potassium ions (through glial cells reacting as perfect potassium electrodes), is thought-provoking and might give a hint of different mechanisms working at different levels of the central nervous system.

The role of glutamate in connection with both excitation and spreading depression is difficult to evaluate. This compound is found in much higher concentrations than other classical or putative transmitters and might, in addition to its likely action as a transmitter (175), also play a role in a possible metabolic interaction between neurons and glial cells. The lack of effect by procaine on the potassium-induced glutamate release is in keeping with the concept that this amino acid has other functions in the brain in addition to its possible role as a transmitter.

---

extracellular clefts account for about 15% of the volume, this corresponds to about 12.5 μmol of potassium per min per g of brain.

[27] It should, however, be remembered that 10 to 12 mM is the upper level observed experimentally in the "pocket" of extracellular fluid surrounding the electrode and that the concentrations conceivably could be higher in the narrow extracellular clefts (*cf.* 306).

Many drugs other than those mentioned here do affect ion contents in brain tissue, but it was felt appropriate to discuss only drugs of which the effects on ion distribution at the cellular level *in vivo* and *in vitro* have been studied in some detail. Even with this approach, no full understanding was obtained of the *exact* mechanism of action for any of the drugs discussed. The results are probably most clear-cut with respect to ouabain which *in vitro* inhibits the Na^+-K^+-ATPase and may counteract potassium effects on metabolism and ion distribution, and *in vivo* delays the response to excitation. It seems likely that phenytoin may exert *both* a stabilizing action counteracting downhill movements of potassium and sodium during excitation (303) *and* a specific stimulation of potassium uptake in the range 5 to 10 (20) mM potassium (75, 81). The former of these mechanisms may be reflected by the stimulus-dependent inhibition of the electrical stimulation (83). The large delay of potassium removal and the maintenance of the concomitant metabolic increase *in vivo* under the influence of barbiturates is remarkable; evidence was given that barbiturates do not affect passive ion movements during excitation *in vitro* (40, 136) or *in vivo* (*cf.* the unaltered "on" response in 54, 275, 298), and that they also do not inhibit the increased potassium uptake after electrical stimulation (136) *in vitro* or exert a general blockade of energy metabolism *in vivo* (54, 298). The latter finding is in accordance with the concept that it is a decreased function of neural tissue that affects energy metabolism during anaesthesia and not a reduction in metabolism that affects function (195). The exact manner(s) in which barbiturates affect the nervous system remain(s) unknown[28] but it might conceivably be by interference with more or less unknown mechanisms directly involved in the regulation of ion distribution between individual cells or between cells and their surroundings. Such mechanisms might include transport processes which are specific for central nervous tissue. The importance of a primary anion(chloride) transport in the brain was pointed out by Bourke and Nelson (26) and this concept is supported by the inhibitory effect of acetazolamide on the potassium-induced increase in swelling and chloride uptake.

If the knowledge about ion distribution at the cellular level of the central nervous system continues to progress as it has done during the past 10 to 15 years, one may foresee an answer to many questions about physiological and pharmacological characteristics of the brain cortex and other central nerve tissue structures within another decade. In the view of the present author, such progress will to a major extent originate from a further development within the domains which have contributed extensively to our present understanding of ion metabolism at the cellular level of the central nervous system, *i.e.*, electrophysiological monitoring of local ion concentrations and of changes in energy metabolism *in vivo* and studies of ion turnover and energy metabolism in individual cell types *in vitro*. Only integration of results obtained within each of these fields will ultimately lead to an understanding of ion distribution at the cellular level of the nervous system, of the physiological importance of ion homeostasis and of the mechanism of action for neurotropic drugs affecting ion distribution.

REFERENCES

1. Aleksidze, N. G. and Blomstrand, C.: Influence of potassium ions on the respiration of the neuron and the neuroglia of the lateral vestibular nucleus of the rabbit. Dokl. Akad. Nauk SSSR 186: 1429–1430, 1969. (Proc. Acad. Sci. USSR, Biochem. Series 186: 140–141, 1969).
2. Allen, J. N.: Extracellular space in the central nervous system. Arch. Neurol. Psychiatr. 73: 241–248, 1955.
3. Altman, J.: Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. *In* Neurosciences, ed. by G. C. Quarton, T. Melnechuk and F. O. Schmitt, pp. 723–743, The Rockefeller University Press, New York, 1967.
4. Altman, J.: DNA metabolism and cell proliferation. *In* Handbook of Neurochemistry, ed. by A. Lajtha, vol. 2, pp. 137–182, Plenum Press, New York, 1969.

[28] Evidence has been obtained that barbiturates have a marked effect on glial cells (277).

5. ALTMAN, J.: Postnatal development of the cerebellar cortex in the rat. The external germinal layer and the transitional molecular layer. J. Comp. Neurol. 145: 353–398, 1972.
6. AQUINO-CÍAS, J., HÁRMONY, T., GUMÁ, E., TORO, A., ANEIROS, R., HERNÁNDEZ, N. and FERNÁNDEZ, G.: Effect of strophantine and ouabain on cortical afterdischarges. Arch. Int. Pharmacodyn. Ther. 168: 352–365, 1967.
7. ARNFRED, T. AND HERTZ, L.: Effects of potassium and glutamate on brain cortex slices: Uptake and release of glutamic and other amino acids. J. Neurochem. 18: 259–265, 1971.
8. ARNFRED, T., HERTZ, L., LOLLE, L. AND LUND-ANDERSEN, H.: An improved holder for transfer of brain slices during *in vitro* incubation. Exp. Brain Res. 11: 373–375, 1970.
9. ASHFORD, C. A. AND DIXON, K. C.: The effect of potassium on the glycolysis of brain tissue with reference to the Pasteur effect. Biochem. J. 29: 157–168, 1935.
10. BACHELARD, H. S., CAMPBELL, W. J. AND MCILWAIN, H.: The sodium and other ions of mammalian cerebral tissues, maintained and electrically stimulated *in vitro*. Biochem. J. 84: 225–232, 1962.
11. BALAZS, R. AND CREMER, J. E.: Metabolic Compartmentation in the Brain, The MacMillan Press, London, 1972.
12. BALDY-MOULINIER, M., ARIAS, L. P. AND PASSOUANT, P.: Hippocampal epilepsy produced by ouabain: Studies of cerebral circulation and ionic metabolism. Eur. Neurol. 9: 333–348, 1973.
13. BENJAMIN, A. M. AND QUASTEL, J. H.: Metabolism of amino acids and ammonia in rat brain cortex slices *in vitro*: A possible role of ammonia in brain function. J. Neurochem. 25: 197–206, 1975.
14. BENJAMIN, A. M. AND QUASTEL, J. H.: Cerebral uptakes and exchange diffusion *in vitro* of L- and D-glutamates. J. Neurochem. 26: 431–441, 1976.
15. BENJAMIN, A. M. AND QUASTEL, J. H.: Effects of acetylcholine and tetrodotoxin on transport processes of high [K^+] and protoveratrine-stimulated rat brain cortex slices. Proc. Can. Fed. Biol. Soc. 20: 9, 1977.
16. BERL, S., NICKLAS, W. J. AND CLARKE, D. D.: Compartmentation of glutamic acid metabolism in brain slices. J. Neurochem. 15: 131–140, 1968.
17. BIGNAMI, A. AND PALLADINI, G.: Experimentally produced cerebral status spongiosus and continuous pseudorhythmic electroencephalographic discharges with a membrane-ATPase inhibitor in the rat. Nature (London) 209: 413–414, 1966.
18. BIGNAMI, A., PALLADINI, G. AND VENTURINI, G.: Effect of cardiazol on sodium-potassium-activated adenosine triphosphatase of the rat brain *in vivo*. Brain Res. 1: 413–414, 1966.
19. BILODEAU, F. AND ELLIOTT, K. A. C.: The influence of drugs and potassium on respiration and potassium accumulation by brain tissue. Can. J. Biochem. Physiol. 41: 779–792, 1963.
20. BITO, L., DAVSON, H., LEVIN, E., MURRAY, M. AND SNIDER, N.: The concentration of free amino acids and other electrolytes in cerebrospinal fluid, *in vivo* dialysate of brain, and blood plasma of the dog. J. Neurochem. 13: 1057–1067, 1966.
21. BOLWIG, T. G. AND QUISTORFF, B.: *In vivo* concentration of lactate in the brain of conscious rats before and during seizures: A new ultra-rapid technique for the freeze-sampling of brain tissue. J. Neurochem. 21: 1345–1348, 1973.
22. BOOHER, J. AND SENSENBRENNER, M.: Growth and cultivation of dissociated neurons and glial cells from embryonic chick, rat and human brain in flask cultures. Neurobiology 2: 97–105, 1972.
23. BOURKE, R. S.: Evidence for mediated transport of chloride in cat cerebral cortex *in vitro*. Exp. Brain Res. 8: 219–231, 1969.
24. BOURKE, R. S.: Studies of the development and subsequent reduction of swelling of mammalian cerebral cortex under isosmotic conditions *in vitro*. Exp. Brain Res. 8: 232–248, 1969.
25. BOURKE, R. S., GREENBERG, E. S. AND TOWER, D. B.: Variation of cerebral cortex fluid spaces *in vivo* as a function of species brain size. Amer. J. Physiol. 208: 682–692, 1965.
26. BOURKE, R. S. AND NELSON, K. M.: Further studies on the K^+-dependent swelling of primate cerebral cortex *in vivo*: The enzymatic basis of the K^+-dependent transport of chloride. J. Neurochem. 19: 663–685, 1972.
27. BOURKE, R. S., NELSON, K. M., NAUMANN, R. A. and YOUNG, O. M.: Studies of the production and subsequent reduction of swelling in primate cerebral cortex under isosmotic conditions *in vivo*. Exp. Brain Res. 10: 427–466, 1970.
28. BOURKE, R. S. AND TOWER, D. B.: Fluid compartmentation and electrolytes of cat cerebral cortex *in vitro*. I. Swelling and solute distribution in mature cerebral cortex. J. Neurochem. 13: 1071–1097, 1966.
29. BOURKE, R. S. AND TOWER, D. B.: Fluid compartmentation and electrolytes of cat cerebral cortex *in vitro*. II. Sodium, potassium and chloride of mature cerebral cortex. J. Neurochem. 13: 1099–1117, 1966.
30. BOYLE, P. J. AND CONWAY, E. J.: Potassium accumulation in muscle and associated changes. J. Physiol. (London) 100: 1–63, 1941.
31. BRADBURY, M. W. B. AND DAVSON, H.: The transport of potassium between blood, cerebrospinal fluid and brain. J. Physiol. (London) 181: 151–174, 1965.
32. BRADBURY, M. W. B., VILLAMIL, M. AND KLEEMAN, C. R.: Extracellular fluid, ionic distribution and exchange in isolated frog brain. Amer. J. Physiol. 214: 643–651, 1968.
33. BRADFORD, H. F. AND ROSE, S. P. R.: Ionic accumulation and membrane properties of enriched preparation of neurons and glia from mammalian cerebral cortex. J. Neurochem. 14: 373–375, 1967.
34. BRINLEY, F. J., JR.: Ion fluxes in the central nervous system. Int. Rev. Neurobiol. 5: 183–242, 1963.
35. BRINLEY, F. J.: Potassium accumulation and transport in the rat sympathetic ganglion. J. Neurophysiol. 30: 1531–1560, 1967.
36. BRINLEY, F. J., KANDEL, E. R. AND MARSHALL, W. H.: Potassium outflux from rabbit cortex during spreading depression. J. Neurophysiol. 23: 246–256, 1960.
37. BROSSARD, M. AND QUASTEL, J. H.: Studies of the cationic, and acetylcholine, stimulation of phosphate incorporation into phospholipids in rat brain cortex *in vitro*. Can. J. Biochem. Physiol. 41: 1243–1256, 1963.
38. BUREŠ, J. BUREŠOVÁ, O. AND KŘIVÁNEK, J.: The Mechanism and Applications of Leão's Spreading Depression of Electroencephalographic Activity, Academia, Prague, 1974.
39. CHAN, S. L. AND QUASTEL, J. H.: Tetrodotoxin: Effects on brain metabolism *in vitro*. Science (Washington) 156: 1752–1753, 1967.
40. CHAN, S. L. AND QUASTEL, J. H.: Effects of neurotropic drugs on sodium influx into rat brain cortex *in vitro*. Biochem. Pharmacol. 19: 1071–1085, 1970.
41. CHANCE, B., COHEN, P., JÖBSIS, F. AND SCHOENER, B.: Intracellular oxidation-reduction states *in vivo*. Science (Washington) 137: 499–508, 1962.
42. CHANCE, B., OSHINO, N., SUGANO, T. AND MAYEVSKY, A.: Basic principles of tissue oxygen determination from mitochondria signals. Advan. Exp. Med. Biol. 37A: 277–292, 1973.
43. CICARDO, V. H.: Physiochemical mechanisms in experimental epilepsy. J. Nerv. Ment. Dis. 101: 527–536, 1945.
44. CICARDO, V. H. AND TORINO, A: Release of potassium by the brain of the dog during electrical stimulation. Science (Washington) 95: 625, 1942.
45. CICERO, T. J., FERRENDELLI, J. A., SUNTZEFF, V. AND

MOORE, B. W.: Regional changes in CNS levels of the S-100 and 14-3-2 proteins during development and aging of the mouse. J. Neurochem. 19: 2119–2125, 1972.

46. COHEN, M. M. AND HARTMANN, F. J.: Biochemical and ultrastructural correlates of cerebral cortex slices metabolizing *in vitro*. *In* Morphological and Biochemical Correlates of Neural Activity, ed. by M. M. Cohen and R. S. Snider, pp. 57–74, Harper and Row, New York, 1964.
47. COHEN, S. R.: The estimation of extracellular space of brain tissue *in vitro*. *In* Research Methods in Neurochemistry, ed. by N. Marks and R. Rodnight, vol. 1, pp. 179–219, Plenum Press, New York, 1972.
48. COHEN, S. R., BLASBERG, R., LEVI, G. AND LAJTHA, A.: Compartmentation of the inulin space in mouse brain slices. J. Neurochem. 15: 707–720, 1968.
49. COHEN, S. R. AND LAJTHA, A.: The effect of tonicity, D-mannitol, D-sorbitol and choline chloride on the water content of mouse brain slices, and on the compartmentation of the space indicated by extracellular space markers. Brain Res. 23: 77–93, 1970.
50. COHEN, S. R. AND LAJTHA, A.: The effect of loading and extraction temperatures, composition of medium, and molecular size on retention of extracellular space markers by mouse cerebrum slices. Intern. J. Neurosci. 1: 251–258, 1971.
51. COLFER, H. F. AND ESSEX, H. E.: The distribution of total electrolyte, potassium and sodium in the cerebral cortex in relation to experimental convulsions. Amer. J. Physiol. 150: 27–36, 1947.
52. COLLEWIJN, H. AND SCHADÉ, J. P.: Changes in the size of astrocytes and oligodendrocytes during anoxia, hypothermia and spreading depression. Prog. Brain Res. 15: 184–195, 1965.
53. COOPER, E. S., LECHNER, E. AND BELLET, H.: Relation between serum and cerebrospinal fluid electrolytes under normal and abnormal conditions. Amer. J. Med. 18: 613–621, 1955.
54. CORDINGLEY, G., LAMANNA, J., ROSENTHAL, M. AND SOMJEN, G.: Phenobarbital effects on extracellular potassium activity and oxidative metabolism in cat cerebral cortex. Fed. Proc. 35: 1245, 1976.
55. CORDINGLEY, G. AND SOMJEN, G.: Clearance of locally accumulated extracellular potassium in the spinal cord of cats. Neuroscience Abstracts, Sixth Annual Meeting of the Society for Neuroscience, p. 962, 1976.
56. CORNOG, J. L., JR., GONATAS, N. K. AND FEIERMAN, J. R.: Effects of intracerebral injection of ouabain on the fine structure of rat cerebral cortex. Amer. J. Pathol. 51: 573–590, 1967.
57. CUMMINS, J. T. AND BULL, R.: Spectrophotometric measurements of metabolic responses in isolated rat brain cortex. Biochim. Biophys. Acta 253: 29–38, 1971.
58. CUMMINS, J. AND HYDÉN, H.: Adenosine triphosphate levels and adenosine triphosphatases in neurons, glia and neuronal membranes of the vestibular nucleus. Biochim. Biophys. Acta 60: 271–283, 1962.
59. CUMMINS, J. T. AND MCILWAIN, H.: Electrical pulses and the potassium and other ions of isolated cerebral tissues. Biochem. J. 79: 330–341, 1961.
60. CUTLER, R. W. P., MARKOWITZ, D. AND DUDZINSKI, D. S.: The effect of barbiturates on (^{3}H)GABA transport in rat cerebral cortex slices. Brain Res. 81: 189–197, 1974.
61. DAVSON, H.: Physiology of the Cerebrospinal Fluid, J. & A. Churchill, London, 1967.
62. DAVSON, H. AND SPAZIANI, E.: The blood-brain barrier and the extra-cellular space of brain. J. Physiol. (London) 149: 135–143, 1959.
63. DEBELLEROCHE, J. S. AND BRADFORD, H. F.: The synaptosome: An isolated, working, neuronal compartment. Progr. Neurobiol. 1: 275–602, 1973.
64. DENNIS, M. J. AND GERSCHENFELD, H. M.: Some physiological properties of identified mammalian neuroglial cells. J. Physiol. (London) 203: 211–222, 1969.
65. DE PIRAS, M. AND ZADUNAISKY, J. A.: Effect of potassium and ouabain on glucose metabolism by frog brain. J. Neurochem. 12: 657–661, 1965.
66. DE ROBERTIS, E., ALBERICI, M. AND DE LORES ARNAIZ, R. G.: Astroglial swelling and phosphohydrolases in cerebral cortex of metrazol convulsant rats. Brain Res. 12: 461–466, 1969.
67. DE ROBERTIS, E. AND CARREA, R.: Biology of neuroglia. Prog. Brain Res. 15: 296–297, 1965.
68. DE ROBERTIS, E. AND GERSCHENFELD, H. M.: Submicroscopic morphology and function of glial cells. Int. Rev. Neurobiol. 3: 1–65, 1961.
69. DIAMOND, M. C., LAW, F., RHODES, H., LINDNER, B., ROZENZWEIG, M. R., KRECH, D. AND BENNETT, E. L.: Increases in cortical depth and glia numbers in rats subjected to enriched environment. J. Comp. Neurol. 128: 117–126, 1966.
70. DICHTER, M. A., HERMAN, C. J. AND SELZER, M.: Silent cells during interictal discharges and seizures in hippocampal penicillin foci. Evidence for the role of extracellular K^+ in the transition from the interictal state to seizures. Brain Res. 48: 173–183, 1972.
71. DICKENS, F. AND GREVILLE, G. D.: The metabolism of normal and tumour tissue. (XIII). Neutral salt effects. Biochem. J. 29: 1468–1483, 1935.
72. DOBBING, J.: Undernutrition and the developing brain: The use of animal models to elucidate the human problem. Advan. Exp. Med. Biol. 13: 399–412, 1971.
73. ECCLES, J. C.: The Physiology of Nerve Cells, Johns Hopkins Press, Baltimore, 1957.
74. ELLIOTT, K. A. C.: The relation of ions to metabolism in brain. Can. J. Physiol. 33: 466–480, 1955.
75. ESCUETA, A. V., DAVIDSON, D., HARTWIG, G. AND REILLY, E.: The freezing lesion. III. The effects of diphenylhydantoin on potassium transport within nerve terminals from the primary foci. Brain Res. 86: 85–96, 1974.
76. FEDOROFF, S.: The development of glial cells in primary cultures. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press, 1977.
77. FEDOROFF, S. AND HERTZ, L.: Cell, Tissue and Organ Cultures in Neurobiology, Academic Press, New York, in press, 1977.
78. FELDBERG, W. AND SHERWOOD, S. L.: Effects of calcium and potassium injected into the cerebral ventricles of the cat. J. Physiol. (London) 139: 408–416, 1957.
79. FENSTERMACHER, J. D., LI, C. L. AND LEVIN, V. A.: Extracellular space of the cerebral cortex of normothermic and hypothermic cats. Exp. Neurol. 27: 101–114, 1970.
80. FERTZIGER, A. P. AND RANCK, J. B., JR.: Potassium accumulation in interstitial space during epileptiform seizures. Exp. Neurol. 26: 571–585, 1970.
81. FESTOFF, B. W. AND APPEL, S. H.: Effect of diphenylhydantoin on synaptosome sodium-potassium-ATPase. J. Clin. Invest. 47: 2752–2758, 1968.
82. FORDA, O. AND MCIWAIN, H.: Anticonvulsants on electrically stimulated metabolism of separated mammalian cerebral cortex. Brit. J. Pharmacol. 8: 225–229, 1953.
83. FORMBY, B.: The *in vivo* and *in vitro* effect of diphenylhydantoin and phenobarbitone on K^+-activated phosphohydrolase and (Na^+, K^+)-activated ATPase in particulate membrane fractions from rat brain. J. Pharm. Pharmacol. 22: 81–85, 1970.
84. FRANCK, G.: Sur la Composition Ionique des Tranches de Cerveau de Rat. Thesis, Université de Liege, 1970.
85. FRANCK, G.: Ion contents and fluxes in brain slices. *In* Abstracts, Fourth International Meeting of the In-

ternational Society for Neurochemistry, Tokyo, pp. 117–118, 1973.
86. FRANCK, G. AND CORNETTE, M.: Composition cationique et flux du K au niveau de tranches de cerveau de rat incubées *in vitro*. Rev. Neurol. 115: 312–314, 1966.
87. FRANCK, G., CORNETTE, M. AND SCHOFFENIELS, E.: The cationic composition of incubated cerebral cortex slices. J. Neurochem. 15: 843–857, 1968.
88. FUTAMACHI, K., MUTANI, R. AND PRINCE, D. A.: Potassium activity in rabbit cortex. Brain Res. 75: 5–25, 1974.
89. FUTAMACHI, K. J. AND PEDLEY, T. A.: Glial cells and extracellular potassium: Their relationship in mammalian cortex. Brain Res. 109: 311–322, 1976.
90. GALAMBOS, R.: A glia-neuronal theory for brain function. Proc. Nat. Acad. Sci. U.S.A. 47: 129–136, 1961.
91. GÁRDOS, G.: Potassium accumulation in guinea pig brain cortex slices. J. Neurochem. 5: 199–201, 1960.
92. GEDDES, J. C. AND QUASTEL, J. H.: Effects of local anaesthetics on respiration of rat brain cortex *in vitro*. Anesthesiology 17: 666–671, 1956.
93. GERSCHENFELD, H. M., WALD, F., ZADUNAISKY, J. A. AND DE ROBERTIS, E. D. P.: Function of astroglia in the water-ion metabolism of the central nervous system. Neurology 9: 412–425, 1959.
94. GHOSH, J. AND QUASTEL, J. H.: Narcotics and brain respiration. Nature (London) 174: 28–31, 1954.
95. GIACOBINI, E.: A cytochemical study of the localization of carbonic anhydrase in the nervous system. J. Neurochem. 9: 169–177, 1962.
96. GILBERT, J. C. AND WYLLIE, M. G.: The effects of phenytoin on adenosine triphosphatase activities of synaptosomes and their components. Brit. J. Pharmacol. 52: 445P, 1974.
97. GILL, T. H., YOUNG, O. M. M. AND TOWER, D. B.: The uptake of ^{36}Cl into astrocytes in tissue culture by a potassium-dependent, saturable process. J. Neurochem. 23: 1011–1018, 1974.
98. GLASER, G. H.: Experimental derangements of extracellular ionic environment. *In* Experimental Models of Epilepsy, ed. by D. P. Purpura, J. K. Penry, D. B. Tower, D. M. Woodbury and R. D. Walter, pp. 315–345, Raven Press, New York, 1972.
99. GLÖTZNER, F. L.: Membrane properties of neuroglia in epileptogenic gliosis. Brain Res. 55: 159–171, 1973.
100. GODFREY, E. W., NELSON, P. G., SCHRIER, B. K., BREUER, A. C. AND RANSOM, B. R.: Neurons from fetal rat brain in a new cell culture system: A multidisciplinary analysis. Brain Res. 90: 1–21, 1975.
101. GOMIRATO, G. AND HYDÉN, H.: A biochemical glia error in the Parkinson disease. Brain 86: 773–780, 1968.
102. GONDA, O. AND QUASTEL, J. H.: Effects of ouabain on cerebral metabolism and transport mechanisms *in vitro*. Biochem. J. 84: 394–406, 1962.
103. GRAFSTEIN, B.: Mechanism of spreading cortical depression. J. Neurophysiol. 19: 154–171, 1956.
104. GREENGARD, O. AND MCILWAIN, H.: Anticonvulsants and the metabolism of separated mammalian cerebral tissues. Biochem. J. 61: 61–68, 1955.
105. HALJAMÄE, H. AND HAMBERGER, A.: Potassium accumulation by bulk-prepared neuronal and glial cells. J. Neurochem. 18: 1903–1912, 1971.
106. HAMBERGER, A., NYSTRÖM, B., SELLSTRÖM, Å, AND WOILER, C. T.: Amino acid transport in isolated neurons and glia cells. Advan. Exp. Med. Biol. 69: 221–236, 1976.
107. HAMBERGER, A. AND RÖCKERT, H.: Intracellular potassium in isolated nerve cells and glial cells. J. Neurochem. 11: 757–760, 1964.
108. HAMMERSTAD, J. P. AND CUTLER, W. P.: Sodium ion movements and the spontaneous and electrically stimulated release of [^3H]GABA and [^{14}C]glutamic acid from rat cortical slices. Brain Res. 47: 401–413, 1972.
109. HANAWA, I., KUGE, K. AND MATSUMURA, K.: Mechanism of the slow depressive potential production in the isolated frog retina. Jap. J. Physiol. 18: 59–70, 1968.
110. HARRIS, E. J. AND BURN G. P.: The transfer of sodium and potassium ions between muscle and the surrounding medium. Trans. Faraday Soc. 45: 508–528, 1949.
111. HARRIS, E. J. AND MCLENNAN, H.: Cation exchanges in sympathetic ganglia. J. Physiol. (London) 121: 629–637, 1953.
112. HARTMANN, J. F.: High sodium content of cortical astrocytes: Electron microscopic evidence. Arch. Neurol. 15: 633–643, 1966.
113. Harvey, J. A. AND MCILWAIN, H.: Excitatory acidic amino acids and the cation content and sodium ion flux of isolated tissues from the brain. Biochem. J. 108: 269–274, 1968.
114. HEINEMANN, U. AND LUX, H. D.: Effects of diphenylhydantoin on extracellular (K^+) in cat cortex. Electroencephalogr. Clin. Neurophysiol. 34: 735, 1973.
115. HEINEMANN, U. AND LUX, H. D.: Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. Brain Res. 120: 231–249, 1977.
116. HENN, F. A., HALJAMÄE, H. AND HAMBERGER, A.: Glial cell function: Active control of extracellular K^+ concentration. Brain Res. 43: 437–443, 1972.
117. HERTZ, L.: Possible role of neuroglia: A potassium-mediated neuronal-neuroglial-neuronal impulse transmission system. Nature (London) 206: 1091–1094, 1965.
118. HERTZ, L.: Neuroglial localization of potassium and sodium effects on respiration in brain. J. Neurochem. 13: 1373–1387, 1966.
119. HERTZ, L.: Potassium effects on ion transport in brain slices. J. Neurochem. 15: 1–16, 1968.
120. HERTZ, L.: The biochemistry of brain tissue. *In* The Biological Basis of Medicine, ed. by E. E. Bittar, vol. 5, pp. 3–37, Academic Press, New York, 1969.
121. HERTZ, L.: Ion Effects on Metabolism in the Adult Mammalian Brain *in Vitro*, FADL's Forlag, Copenhagen, 1973.
122. HERTZ, L.: Potassium ion homoeostasis in the cerebral cortex. Biochem. Soc. Trans. 1: 115–118, 1973.
123. HERTZ, L: Potassium effects on transport of amino acids, inorganic ions and water: Ontogenetic and quantitative differences. Adv. Exp. Med. Biol. 69: 371–383, 1976.
124. HERTZ, L.: Biochemistry of glial cells. *In* Cell, Tissue and Organ Cultures in Neurobiology, ed. by S. Fedoroff and L. Hertz, Academic Press, New York, in press, 1977.
125. HERTZ, L.: Energy metabolism. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press, 1977.
126. HERTZ, L. AND CLAUSEN, T.: Effects of potassium and sodium on respiration: Their specificity to slices from certain brain regions. Biochem. J. 89: 526–533, 1963.
127. HERTZ, L. DITTMANN, L. AND MANDEL, P.: K^+ induced stimulation of oxygen uptake in cultured cerebral glial cells. Brain Res. 60: 517–520, 1973.
128. HERTZ, L. AND NISSEN, C.: Differences between leech and mammalian nervous systems in metabolic reaction to K^+ as an indication of differences in potassium homeostasis mechanisms. Brain Res. 110: 182–188, 1976.
129. HERTZ, L. AND SCHOU, M.: Univalent cations and the respiration of brain-cortex slices. Biochem. J. 85: 93–104, 1962.
130. HERTZ, L. AND SCHOUSBOE, A.: Ion and energy metabolism of the brain at the cellular level. Int. Rev. Neurobiol. 18: 141–211, 1975.

131. Hertz, L., Schousboe, A., Boechler, N., Mukerji, S. and Fedoroff, S.: Kinetic characteristics for the glutamate uptake by normal astrocytes in cultures. Neurochem. Res. in press, 1977.
132. Hertz, L., Schousboe, A. and Weiss, G.: Estimation of ionic concentrations and intracellular pH in slices from different areas of rat brain. Acta Physiol. Scand. 79: 506–515, 1970.
133. Higashida, H., Mitarai, G. and Watanabe, S.: A comparative study of membrane potential changes in neurons and neuroglial cells during spreading depression in the rabbit. Brain Res. 65: 411–425, 1974.
134. Hild, W., Chang, J. J. and Tasaki, I.: Electrical responses of astrocytic glia from the mammalian central nervous system cultivated *in vitro*. Experientia (Basel) 14: 220–221, 1958.
135. Hild, W. and Tasaki, I.: Morphological and physiological properties of neurons and glial cells in tissue culture. J. Neurophysiol. 25: 277–304, 1962.
136. Hillman, H. H., Campbell, W. J. and McIlwain, H.: Membrane potentials in isolated and electrically stimulated mammalian cerebral cortex. Effects of chlorpromazine, cocaine, phenobarbitone and protamine on the tissue's electrical and chemical responses to stimulation. J. Neurochem. 10: 325–339, 1963.
137. Hillman, H. H. and McIwain, H: Membrane potentials in mammalian cerebral tissues in vitro: Dependence on ionic environment. J. Physiol. (London) 157: 263–278, 1961.
138. Hillman, H., Stollery, S. and Joanny, P.: The mechanism of the exclusion of sodium ions and the concentration of potassium ions by guinea-pig cerebral cortex slices. J. Neurochem. 22: 573–579, 1974.
139. Horstmann, E. and Meves, H.: Die Feinstruktur des molekularen Rindengraues und ihre physiologische Bedeutung. Z. Zellforsch. Mikrosk. Anat. 49: 569–604, 1959.
140. Hotson, J. R., Sypert, G. W. and Ward, A. A.: Extracellular potassium concentration changes during propagated seizures. Exp. Neurol. 38: 20–26, 1973.
141. Hultborn, R. and Hydén, H.: Microspectrophotometric determination of nerve cell respiration at high potassium concentration. Exp. Cell Res. 87: 346–350, 1974.
142. Huttunen, M. O.: Protein and Ribonucleic Acid Metabolism in Rat Brain Cortex Slices. Thesis, Helsinki, 1969.
143. Hydén, H.: The neuron. *In* The Cell, ed. by J. Brachet and A. E. Mirsky, pp. 215–323, Academic Press, New York, 1960.
144. Hydén, H.: Dynamic aspects on the neuron-glia relationship. *In* The Neuron, ed. by H. Hydén, pp. 179–219, Elsevier, Amsterdam, 1967.
145. Hydén, H. and McEwen, B.: A glial protein specific for the nervous system. Proc. Nat. Acad. Sci. U.S.A. 55: 354–358, 1966.
146. Ibata, Y., Piccoli, F., Pappas, G. D. and Lajtha, A.: An electron microscopic and biochemical study on the effect of cyanide and low Na^+ on rat brain slices. Brain Res. 30: 137–158, 1971.
147. Israel, Y., Kalant, H. and Le Blanc, A. E.: Effects of lower alcohols on potassium transport and microsomal adenosine-triphosphatase activity of rat cerebral cortex. Biochem. J. 100: 27–33, 1966.
148. Izquierdo, I., Nasello, A. G. and Marichich, E. S.: Effects of potassium on rat hippocampus: The dependence of hippocampal evoked and seizure activity on extracellular potassium levels. Arch. Int. Pharmacodyn. Ther. 187: 318–328, 1970.
149. Jaros, G. G., Moonen, G., Sensenbrenner, M. and Mandel, P.: Cultures de cellules dissociées d'hémisphères cérébraux à composante essentiellement neuronale. C. R. Acad. Sci.Paris 280: 327–330, 1975.
150. Jasper, H. H. and Koyama, I.: Rate of release of amino acids from the cerebral cortex in the cat as affected by brain stem and thalamic stimulation. Can. J. Physiol. Pharmacol. 47: 889–905, 1969.
151. Joanny, P. and Corriol, J.: Influence de l'ouabaïne sur les mouvements ioniques, la respiration et la glycolyse aérobie du cortex cérébral isolé de mammifère. Arch. Sci. Physiol. 18: 325–337, 1964.
152. Joanny, P. and Hillman, H. H.: Substrates and the potassium and sodium levels of guinea pig: Cerebral cortex slices *in vitro*: Effects of application of electrical pulses, of inhibitors and of anoxia. J. Neurochem. 10: 655–664, 1963.
153. Joanny, P. and Hillman, H.: Further studies on the potassium and sodium concentrations of mammalian cerebral slices *in vitro*. J. Neurochem. 11: 413–422, 1964.
154. Jöbsis, F., Rosenthal, M., LaManna, J., Lothman, E., Cordingley, G. and Somjen, G.: Metabolic activity in epileptic seizures. *In* Brain Work, Alfred Benzon Symposium, ed. by D. H. Ingvar and N. A. Lassen vol. 8, pp. 185–196, Munksgaard, Copenhagen, 1975.
155. John, E. R., Tschirgi, R. D. and Wenzel, B. M.: Effects of injections of cations into the cerebral ventricles on conditioned responses in the cat. J. Physiol. (London) 146: 550–562, 1959.
156. Johnston, P. V. and Roots, B. I.: Nerve Membranes, Pergamon Press, Oxford, 1972.
157. Katz, R. I., Chase, T. N. and Kopin, I. J.: Effects of ions on stimulus induced release of amino acids from mammalian brain slices. J. Neurochem. 16: 961–967, 1969.
158. Katzman, R.: Electrolyte distribution in mammalian central nervous system. Are glia high sodium cells? Neurology 11: 27–36, 1961.
159. Katzman, R. and Grossman, R.: Neuronal activity and potassium movement. *In* Brain Work, Alfred Benzon Symposium, ed. by D. H. Ingvar and N. A. Lassen, vol. 8, pp. 149–166, Munksgaard, Copenhagen, 1975.
160. Katzman, R. and Pappius, H. M.: Brain Electrolytes and Fluid Metabolism, The Williams & Wilkins Company, Baltimore, 1973.
161. Kawai, N. and Yamamoto, C.: Effects of serotonin, LSD and related compounds on potentials evoked *in vitro* in thin sections from the superior colliculus. *In* Abstracts, Second International Meeting of the International Society for Neurochemistry, ed. by R. Paoletti, R. R. Fumagalli and C. Galli, pp 238–239, Tamburini, Milano, 1969.
162. Keesey, J. C. and Wallgren, H.: Movements of radioactive sodium in cerebral-cortex slices in response to electrical stimulation. Biochem. J. 95: 301–310, 1965.
163. Keesey, J. C., Wallgren, H. and McIlwain, H.: The sodium, potassium and chloride of cerebral tissues: Maintenance, change on stimulation and subsequent recovery. Biochem. J. 95: 289–300, 1965.
164. Kimelberg, H. K.: Active potassium transport and (Na^+ + K^+)ATPase activity in cultured glioma and neuroblastoma cells. J. Neurochem. 22: 971–976, 1974.
165. Kjeldsen, C. S., Lund-Andersen, H. and Hertz, L.: Effects of lithium in a pharmacological concentration on potassium and sodium in rat brain-cortex slices. Trans. Biochem. Soc., 1: 111–114, 1973.
166. Koch, A., Ranck, J. B. and Newman, B. L.: Ionic content of the neuroglia. Exp. Neurol. 6: 186–200, 1962.
167. Kow, L. M. and Van Harreveld, A. Ion and water movements in isolated chicken retinas during spreading depression. Neurobiology 2: 61–69, 1972.
168. Kraig, R. P. and Nicholson, C.: Sodium changes: Account for electrolyte shifts of spreading depression. Neuroscience Abstracts, Sixth Annual Meet-

ing of the Society for Neuroscience, p. 994, 1976.
169. KREBS., H. A., EGGLESTON, L. V. AND TERNER, C.: *In vitro* measurements of the turnover rate of potassium in brain and retina. Biochem. J. 48: 530–537, 1951.
170. KŘIVÁNEK, J. AND BUREŠ, J.: Ion shifts during Leão's spreading cortical depression. Physiol. Bohemoslov. 9: 494–503, 1960.
171. KŘÍŽ, N., SYKOVÁ, E., UJEC, E. AND VYKLICKÝ, L.: Changes of extracellular potassium concentration induced by neuronal activity in the spinal cord of the cat. J. Physiol. (London) 238: 1–15, 1974.
172. KRNJEVIC, K. AND MORRIS, M. E.: Extracellular K^+ activity and slow potential changes in spinal cord and medulla. Can. J. Physiol. Pharmacol. 50: 1214–1217, 1972.
173. KRNJEVIC, K. AND MORRIS, M. E.: Extracellular accumulation of K^+ evoked by activity of primary afferent fibers in the cuneate nucleus and dorsal horn of cats. Can. J. Physiol. Pharmacol. 52: 852–871, 1974.
174. KRNJEVIC, K. AND MORRIS, M. E.: Strophantidin effects on extracellular K^+ and electrogenic pumping in the cuneate nucleus. J. Physiol. (London) 250: 36P–37P, 1975.
175. KRNJEVIC, K. AND PHILLIS, J. W.: Iontophoretic studies of neurones in the mammalian cerebral cortex. J. Physiol. (London) 165: 274–304, 1963.
176. KUFFLER, S. W. AND NICHOLLS, J. G.: The physiology of neuroglial cells. Ergeb. Physiol. 57: 1–90, 1966.
177. KUFFLER, S. W., NICHOLLS, J. G. AND ORKAND, R. K.: Physiological properties of glial cells in the central nervous system of amphibia. J. Neurophysiol. 29: 768–787, 1966.
178. KUFFLER, S. W. AND POTTER, D. D.: Glia in the leech central nervous system: Physiological properties and neuron-glia relationship. J. Neurophysiol. 27: 290–320, 1964.
179. KUKES, G., DE VELLIS, J. AND ELUL, R.: A linked active transport system for Na^+ and K^+ in a glial cell line. Brain Res. 104: 93–105, 1976.
180. KUKES, G., ELUL, R. AND DE VELLIS, J.: The ionic basis of the membrane potential in a rat glial cell line. Brain Res. 104: 71–92, 1976.
181. LAMANNA, J. C. AND ROSENTHAL, M: Effect of ouabain and phenobarbital on oxidative metabolic activity associated with spreading cortical depression in cats. Brain Res. 88: 145–149, 1975.
182. LASANSKY, A.: Nervous function at the cellular level: Glia. Annu. Rev. Physiol. 33: 241–256, 1971.
183. LATZKOVITS, L., SENSENBRENNER, M. AND MANDEL, P.: Tracer kinetic model analysis of potassium uptake by dissociated nerve cell cultures: Glial-neuronal interrelationship. J. Neurochem. 23: 193–200, 1974.
184. LATZKOVITS, L., SENSENBRENNER, M. AND MANDEL, P.: Tracer kinetic model analysis of potassium uptake by dissociated nerve cell cultures. II. Effects of ouabain on ^{86}Rb and ^{42}K incorporation. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press 1977.
185. LEÃO, A. A. P.: Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 7: 359–390, 1944.
186. LEES, M. B. AND SHEIN, H. M.: Sodium and potassium content of normal and neoplastic rodent astrocytes in cell culture. Brain Res. 23: 280–283, 1970.
187. LEBOVITZ, R. M.: A theoretical examination of ionic interactions between neural and non-neural elements. Biophys. J. 10: 423–444, 1970.
188. LEWIN, E.: Epileptogenic foci induced with ouabain. Electroencephalogr. Clin. Neurophysiol. 29: 402–403, 1970.
189. LEWIN, E.: Epileptogenic cortical foci induced with ouabain: Sodium, potassium, water content and sodium–potassium-activated ATPase activity. Exp. Neurol. 30: 172–177, 1971.
190. LEWIS, D. V., O'CONNOR, M. J. AND SCHUETTE, W. H.: Oxidative metabolism during recurrent seizures in the penicillin-treated hippocampus. Electroencephalogr. Clin. Neurophysiol. 36: 347–356, 1974.
191. LEWIS, D. V. AND SCHUETTE, W. H.: NADH fluorescence and $[K^+]_o$ changes during hippocampal electrical stimulation. J. Neurophysiol. 38: 405–417, 1975.
192. LIPTON, P.: Effects of membrane depolarization on light scattering by cerebral cortical slices. J. Physiol. (London). 231: 365–383, 1973.
193. LOTHMAN, E., LAMANNA, J., CORDINGLEY, G., ROSENTHAL, M. AND SOMJEN, G.: Responses of electrical potential, potassium levels and oxidative metabolic activity of the cerebral neocortex of cats. Brain Res. 88: 15–36, 1975.
194. LOTHMAN, E. W. AND SOMJEN, G. G.: Extracellular potassium activity, intracellular and extracellular potential responses in the spinal cord. J. Physiol. (London) 252: 115–136, 1975.
195. LOWRY, O. H.: Energy metabolism in brain and its control. *In* Brain Work, Alfred Benzon Symposium, ed. by D. H. Ingvar and N. A. Lassen, vol. 8, pp. 48–63, Munksgaard, Copenhagen, 1975.
196. LUND-ANDERSEN, H.: Extracellular and intracellular distribution of inulin in rat brain cortex slices. Brain Res. 65: 239–254, 1974.
197. LUND-ANDERSEN, H. AND HERTZ, L.: Effects of potassium and glutamate on swelling and on sodium and potassium content in brain-cortex slices from adult rats. Exp. Brain Res. 11: 199–212, 1970.
198. LUND-ANDERSEN, H. AND HERTZ, L.: Duffusion-limited (extracellular) and non-diffusion-limited (intracellular) movements of inulin in brain-cortex slices. Biochem. Soc. Trans. 1: 123–126, 1973.
199. LUND-ANDERSEN, H., KJELDSEN, C. S., HERTZ, L. AND BRØNDSTED, H. E.: Uptake of glucose analogues by rat brain cortex slices: Na^+-independent membrane transport. J. Neurochem, 27: 369–373, 1976.
200. LUX, H. D.: Fast recording ion specific microelectrodes: Their use in pharmacological studies in the CNS. Neuropharmacology 13: 509–517, 1974.
201. LUX, H. D.: Extracellular potassium in the CNS: Relation to excitability changes. *In* Brain Work, Alfred Benzon Symposium, ed. by D. H. Ingvar and N. A. Lassen, vol. 8, pp. 172–181, Munksgaard, Copenhagen, 1975.
202. LUX, H. D. AND NEHER, E.: The equilibration time course of $[K^+]_o$ in cat cortex. Exp. Brain Res. 17: 190–205, 1973.
203. MACHIYAMA, Y., BALAZS, R., HAMMOND, B. J., JULIAN, T. AND RICHTER, D.: The metabolism of γ-aminobutyrate and glucose in potassium ion-stimulated brain tissue *in vitro*. Biochem. J. 116: 469–481, 1970.
204. MACHIYAMA, Y., BALAZS, R. AND RICHTER, D.: Effect of K^+-stimulation on GABA metabolism in brain slices *in vitro*. J. Neurochem. 14: 591–594, 1967.
205. MANUELIDIS, L., MANUELIDIS, E. E. AND PRICHARD, J.: Relationship between membrane potential and external potassium in human glioblastoma cells in tissue culture. J. Cell. Physiol. 87: 179–188, 1976.
206. MARCHBANKS, R. M. AND CAMPBELL, C. W. B.: Sodium and chloride fluxes in synaptosomes *in vitro*. J. Neurochem. 26: 973–980, 1976.
207. MARICHICH, E. S. AND IZQUIERDO, I.: Potassium loss from rat hippocampus during electrical activity. Arch. Int. Pharmacodyn. Ther. 196: 353–356, 1972.
208. MAYEVSKY, A. AND CHANCE, B.: Repetitive patterns of metabolic changes during cortical spreading depression of the awake rat. Brain Res. 65: 529–533, 1974.
209. MAYEVSKY, A. AND CHANCE, B.: Metabolic responses of the awake cerebral cortex to anoxia, hypoxia, spreading depression and epileptiform activity. Brain Res. 98: 149–165, 1975.
210. MAYEVSKY, A., ZEUTHEN, T. AND CHANCE, B.: Mea-

surement of extracellular potassium, ECoG and pyridine nucleotide levels during cortical spreading depression in rats. Brain Res. 76: 347–349, 1974.
211. McIlwain, H.: Metabolic response *in vitro* to electrical stimulation of sections of mammalian brain. Biochem. J. 49: 382–393, 1951.
212. McIlwain, H.: Atropine and related compounds on the metabolism of electrically stimulated sections of mammalian cerebral cortex. Brit. J. Pharmacol. Chemother. 6: 531–539, 1951
213. McIlwain, H.: The effect of depressants on metabolism of stimulated cerebral tissues. Biochem. J. 53: 403–412, 1953.
214. McIlwain, H.: Characteristics required in electrical pulses for stimulation of the respiration of separated mammalian cerebral tissues. J. Physiol. (London) 124: 117–129, 1954.
215. McIlwain, H.: Appraising enzymic actions of central depressants by examining cerebral tissues. *In* Enzymes and Drug Action, CIBA Foundation Symposium, ed. by. J. L. Mongar and A. V. S. De Reuck, pp. 170–205, J. & A. Churchill, London, 1962.
216. McIlwain, H.: Tetrodotoxin and the cation content, excitability and metabolism of isolated mammalian cerebral tissues. Biochem. Pharmacol. 16: 1389–1396, 1967.
217. McIlwain, H., Anguiano, G. and Cheshire, J. D.: Electrical stimulation *in vitro* of the metabolism of glucose by mammalian cerebral cortex. Biochem. J. 50: 12–18, 1952.
218. McIlwain, H. and Greengard, O.: Excitants and depressants of the central nervous system, on isolated electrically stimulated cerebral tissues. J. Neurochem. 1: 348–357, 1957.
219. McIlwain, H., Harvey, J. A. and Rodriquez, G.: Tetrodotoxin on the sodium and other ions of cerebral tissues, excited electrically and with glutamate. J. Neurochem. 16: 363–370, 1969.
220. McIlwain, H. and Joanny, P.: Characteristics required in electrical pulses of rectangular time-voltage relationships for metabolic change and ion movements in mammalian cerebral tissues. J. Neurochem. 10: 313–323, 1963.
221. McIlwain, H. and Tresize, M. A.: The glucose, glycogen and aerobic glycolysis of isolated cerebral tissues. Biochem. J. 63: 250–257, 1956.
222. Medzihradsky, F., Nandhasri, P. S., Idoyaga-Vargas, V. and Sellinger, O. Z.: A comparison of the ATPase activity of the glial cell fraction and the neuronal perikaryal fraction isolated in bulk from rat cerebral cortex. J. Neurochem. 18: 1599–1603, 1971.
223. Medzihradsky, F., Sellinger, O. Z., Nandhasri, P. S. and Santiago, J. C.: ATPase activity in glial cells and in neuronal perikarya of rat cerebral cortex during early postnatal development. J. Neurochem. 19: 543–545, 1972.
224. Medzihradsky, F., Sellinger, O. Z., Nandhasri, P. S. and Santiago, J. C.: Adenosine triphosphatase activity in glial cells and in neuronal perikarya of edematous rat brain. Brain. Res. 67: 133–139, 1974.
225. Meldrum, B. S. and Nilsson, B.: Cerebral blood flow and metabolic rate early and late in prolonged epileptic seizures induced in rats by bicuculline. Brain 99: 523–542, 1976.
226. Merritt, H. H. and Fremont-Smith, F.: The Cerebrospinal Fluid, p. 28, W. B. Saunders Company, Philadelphia, 1938.
227. Minakami, S., Kakinuma, K. and Yoshikawa, M: The control of respiration in brain slices. Biochim. Biophys. Acta 78: 808–811, 1963.
228. Møller, M., Lund-Andersen, H., Møllgård, K. and Hertz, L.: Concordance between morphological and biochemical estimates of fluid spaces in rat brain cortex slices. Exp. Brain Res. 22: 299–314, 1974.
229. Moody, W. J. Futamachi, K. J. and Prince, D. A.: Extracellular potassium activity during epileptogenesis. Exp. Neurol. 42: 248–263, 1974.
230. Mulder, A. H. and Snyder, S. H.: Potassium-induced release of amino acids from cerebral cortex and spinal cord slices of the rat. Brain Res. 76: 297–308, 1976.
231. Nagata, Y., Mikoshiba, K. and Tsukada, Y.: Neuronal cell body enriched and glial cell enriched fractions from young and adult rat brains: Preparation and morphological and biochemical properties. J. Neurochem. 22: 493–503, 1974.
232. Nakai, J.: Morphology of Neuroglia, Igaku Shoin Ltd., Tokyo, 1963.
233. Nakasawa, T., and Ogura, Y.: Ann. Rep. Inst. Food Microbiol. Chiba Univ. 13: 59–61, 1961 (Quoted from Kao, C. Y.: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmacol. Rev. 18: 997–1049, 1966).
234. Narahashi, T., Moore, J. W. and Scott, W. R.: Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J. Gen. Physiol. 47: 965–974, 1964.
235. Nicholson, C. and Kraig, R. P.: Chloride and potassium changes measured during spreading depression in catfish cerebellum. Brain Res. 96: 384–389, 1975.
236. Nicholson, C., Ten Bruggencate, G. and Senekowitsch, R.: Large potassium signals and slow potentials evoked during aminopyridine or barium superfusion in cat cerebellum. Brain Res. 113: 606–610, 1976.
237. Noonan, T. R., Fenn, W. O. and Haege, L.: The distribution of injected radioactive potassium in rats. Amer. J. Physiol. 132: 474–488, 1941.
238. O'Connor, M. J. and Lewis, D. V.: Recurrent seizures induced by potassium in the penicillin treated hippocampus. Electroencephalogr. Clin. Neurophysiol. 36: 337–345, 1974.
239. O'Connor, M. J., Lewis, D. V. and Herman, C. J.: Effects of potassium on oxidative metabolism and seizures. Electroencephalogr. Clin. Neurophysiol. 35: 205–208, 1973.
240. Okamoto, K. and Quastel, J. H.: Water uptake and energy metabolism in brain slices from rat. Biochem. J. 120: 25–36, 1970.
241. Okamoto, K. and Quastel, J. H.: Tetrodotoxin-sensitive uptake of ions and water by slices of rat brain *in vitro*. Biochem. J. 120: 37–47, 1970.
242. O'Neill, J., Simon, S. H. and Shreeve, W. W.: Alternate glycolytic pathways in brain. A comparison between the action of artificial electron acceptors and electrical stimulation. J. Neurochem. 12: 797–802, 1965.
243. Ookawa, T. and Burĕs, J.: Extracellular potassium shifts accompanying epileptic discharge induced in chicken hyperstriatum by systematic injection of Metrazol. Brain Res. 97: 171–176, 1975.
244. Orkand, R. K., Nicholls, J. G. and Kuffler, S. W.: Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J. Neurophysiol. 29: 788–806, 1966.
245. Osborne, R. H. and Bradford, H. F.: The influence of sodium, potassium and lanthanum on amino acid release from spinal-medullary synaptosomes, J. Neurochem. 25: 35–41, 1975.
246. Pape, L. G. and Katzman, R.: K^{42} distribution in brain during simultaneous ventriculocisternal and subarachnoid perfusion. Brain Res. 38: 49–69, 1972.
247. Pape, L. G. and Katzman, R.: Response of glia in cat sensori-motor cortex to increased extracellular potassium. Brain Res. 38: 71–92, 1972.
248. Pappas, G. D. and Purpura, D. P.: Fine structure of dendrites in the superficial neocortical neuropil. Exp. Neurol. 4: 507–530, 1961.
249. Pappius, H.: The distribution of water in brain tissues

swollen *in vitro* and *in vivo*. Prog. Brain Res. 15: 135–154, 1965.

250. PAPPIUS, H. AND ELLIOTT, K. A. C.: Water distribution in incubated slices of brain and other tissues. Can. J. Biochem. Physiol. 34: 1007–1022, 1956.
251. PAPPIUS, H. M. AND ELLIOTT, K. A. C.: Factors affecting the potassium content of incubated brain slices. Can. J. Biochem. Physiol. 34: 1053–1067, 1956.
252. PATEL, K. K., HARTMANN, J. F. AND COHEN, M. M.: Ultrastructural estimation of relative volume of extracellular space in brain slices. J. Neurol. Sci. 12: 275–288, 1971.
253. PATERSON, J., PRIVAT, A. A., LING, E. A. AND LEBLOND, C. P.: Investigation of glial cells in semithin sections. III. Transformation of subependymal cells into glial cells, as shown by radioautography after ^{3}H-thymidine injection into the lateral ventricle of the brain of young rats. J. Comp. Neurol. 149: 83–102, 1973.
254. PEDLEY, A. T., FISHER, R. S. AND PRINCE, D. A.: Focal gliosis and potassium movement in mammalian cortex. Exp. Neurol. 50: 346–361, 1976.
255. PEDLEY, T. A., ZUCKERMANN, E. C. AND GLASER, H. G.: Epileptogenic effects of localized ventricular perfusion of ouabain on dorsal hippocampus. Exp. Neurol. 25: 207–219, 1969.
256. PLUM, F. AND DUFFY T. E.: The couple between cerebral metabolism and blood flow druing seizures. *In* Brain Work, Alfred Benzon Symposium, ed. by D. H. Ingvar and N. A. Lassen, vol. 8, pp. 197–214, Munksgaard, Copenhagen, 1975.
257. POST, R. L., MERRITT, C. R., KINSOLVING, C. R. AND ALBRIGHT, C. D.: Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. Biol. Chem. 235: 1796–1802, 1960.
258. PRINCE, D. A., LUX, H. D. AND NEHER, E.: Measurement of extracellular potassium activity in cat cortex. Brain Res. 50: 489–495, 1973.
259. Privat, A.: Postnatal gliogenesis in the mammalian brain. Int. Rev. Cytol. 40: 281–323, 1975.
260. QUASTEL, J. H.: Carbohydrate-aminoacid interrelations in brain cortex *in vitro*. The effects, thereon, of drugs affecting the nervous system. *In* Structure and Function of the Cerebral Cortex. Proceedings of the 2nd International Meeting of Neurobiology, Amsterdam, ed. by D. B. Tower and J. P. Schade, pp. 374–385, Elsevier, Amsterdam 1960.
261. QUASTEL, J. H.: Effects of drugs on metabolism of the brain *in vitro*. Brit. Med. Bull. 21: 49–56, 1965.
262. QUASTEL, J. H. AND QUASTEL, D. M. J.: The Chemistry of Brain Metabolism in Health and Disease, p. 11, Charles C Thomas, Publisher, Springfield, Ill., 1961.
263. RALL, D. P., OPPELT, W. W. AND PATLAK, C. S.: Extracellular space of brain as determined by diffusion of inulin from the ventricular system. Life Sci. 2: 43–48, 1962.
264. RANCK, J. B., JR.: Specific impedance of cerebral cortex during spreading depression, and analysis of neuronal, neuroglial, and interstitial contributions. Exp. Neurol. 9: 1–16, 1964.
265. RANSOM, B. R.: The behavior of presumed glial cells during seizure discharge in cat cerebral cortex. Brain Res. 69: 83–99, 1974.
266. RANSOM, B. R. AND GOLDRING, S.: Ionic determinants of membrane potential of cells presumed to be glia in cerebral cortex of cat. J. Neurophysiol. 36: 855–868, 1973.
267. RANSOM, B. R. AND GOLDRING, S.: Slow depolarization in cells presumed to be glia in cerebral cortex of cat. J. Neurophysiol. 36: 869–78, 1973.
268. RANSOM, B. R. AND GOLDRING, S.: Slow hyperpolarization in cells presumed to be glia in cerebral cortex of cat. J. Neurophysiol. 36: 879–892, 1973.
269. RAWSON, M. D. AND PINCUS, J. H.: The effect of diphenylhydantoin on sodium, potassium, magnesium-activated adenosine triphosphatase in microsomal fractions of rat and guinea pig brain and on whole homogenates of human brain. Biochem. Pharmacol. 17: 573–579, 1968.
270. REED, D. J., WITHROW, C. D. AND WOODBURY, D. M.: Electrolyte and acid-base parameters of rat cerebrospinal fluid. Exp. Brain Res. 3: 212–219, 1967.
271. REUBEN, I. P., LOPEZ, E., BRANDT, P. W. AND GRUNDFEST, H.: Muscle: Volume changes in isolated single fibres. Science (Washington) 142: 246–248, 1963.
272. RIDGE, J. W.: Resting and stimulated respiration *in vitro* in the central nervous system. Biochem. J. 105: 831–835, 1967.
273. ROBERTS, P. J.: Amino acid release from isolated rat dorsal root ganglia. Brain Res. 74: 327–332, 1974.
274. ROLLESTON, F. S. AND NEWSHOLME, E. A.: Control of glycolysis in cerebral cortex slices. Biochem. J. 104: 524–533, 1967.
275. ROSENTHAL, M. AND LAMANNA, J. C.: Effect of ouabain and phenobarbital on the kinetics of cortical metabolic transients associated with evoked potentials. J. Neurochem. 24: 111–116, 1975.
276. ROSENTHAL, M. AND SOMJEN, G.: Spreading depression, sustained potential shifts and metabolic activity of cerebral cortex of cats. J. Neurophysiol. 36: 739–749, 1973.
277. ROTH-SCHECHTER, B. F. AND MANDEL, P.: Glial cell reactions and aberrations: Drug effects. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press, 1977.
278. SAMSON, F. E. AND QUINN, D. J.: Na^+-K^+-activated ATPase in rat brain development. J. Neurochem. 14: 421–427, 1967.
279. SATO, G.: Tissue Culture of the Nervous system, Plenum Press, New York, 1973.
280. SCHON, F. AND KELLY, J. S.: Autoradiographic localization of [^{3}H] GABA and [^{3}H] glutamate over satellite glial cells. Brain Res. 66: 275–288, 1974.
281. SCHOUSBOE, A., BOOHER, J. AND HERTZ, L.: Content of ATP in cultivated neurons and astrocytes exposed to balanced and potassium-rich media. J. Neurochem. 17: 1501–1504, 1970.
282. SCHOUSBOE, A., FOSMARK, H. AND HERTZ, L.: High content of glutamate and of ATP in astrocytes cultured from rat brain hemispheres: Effect of serum withdrawal and of cyclic AMP. J. Neurochem. 25: 909–911, 1975.
283. SCHOUSBOE, A. AND HERTZ, L.: Effects of potassium on indicator spaces and fluxes in slices of brain cortex from adult and new-born rats. J. Neurochem. 18: 67–77, 1971.
284. SCHOUSBOE, A. AND HERTZ, L.: Effects of potassium on concentrations of ions and proteins and on pH in brain-cortex slices from new-born and adult rats. Int. J. Neurosci. 1: 235–242, 1971.
285. SCHWARTZ, A.: The effect of ouabain on potassium content, phosphoprotein metabolism and oxygen consumption of guinea pig cerebral tissue. Biochem. Pharmacol. 11: 389–391, 1962.
286. SCHWARTZ, A., LINDENMAYER, G. E. AND ALLEN, J. C.: The sodium-potassium adenosine triphosphatase: Pharmacological, physiological and biochemical aspects. Pharmacol. Rev. 27: 3–134, 1975.
287. SENSENBRENNER, M.: Dissociated cells in primary cultures. *In* Cell, Tissue and Organ Cultures in Neurobiology, ed. by S. Fedoroff and L. Hertz, Academic Press, New York, in press, 1977.
288. SHANES, A. M.: A neglected factor in studies of potassium distribution in relation to the resting potential of nerve. J. Cell. Comp. Physiol. 27: 115–118, 1946.
289. SHANES, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. I. The resting cell and its alteration by extrinsic factors. Pharmacol. Rev. 10: 59–164, 1958.

290. SHANES, A. M.: Electrochemical aspects of physiological and pharmacological action in excitable cells. II. The action potential and excitation. Pharmacol. Rev. 10: 165–273, 1958.
291. SHANKARAN, R. AND QUASTEL, J. H.: Effects of anaesthetics on sodium uptake into rat brain cortex *in vitro*. Biochem. Pharmacol. 21: 1763–1773, 1972.
292. SHEIN, H. M., BRITVA, A., HESS, H. H. AND SELHOB, D. J.: Isolation of hamster brain astro-glia by *in vitro* cultivation and subcutaneous growth and content of cerebroside, ganglioside, RNA and DNA. Brain Res. 19: 497–501, 1970.
293. SINGER, W. AND LUX, H. D.: Extracellular potassium gradients and visual receptive fields in the cat striate cortex. Brain Res. 96: 378–383, 1975.
294. SKOU, J. C.: Further investigations on a Mg^{++} + Na^{+}-activated adenosinetriphosphatase, possibly related to the active, linked transport of Na^{+} and K^{+} across the nerve membrane. Biochim. Biophys. Acta 42: 6–23, 1960.
295. SOMJEN, G. G.: Evoked sustained focal potentials and membrane potential of neurons and of unresponsive cells of the spinal cord. J. Neurophysiol. 33: 562–582, 1970.
296. SOMJEN, G. G.: Electrophysiology of neuroglia. Annu. Rev. Physiol. 37: 163–190, 1975.
297. SOMJEN, G. G. AND LOTHMAN, E. W.: Potassium, sustained focal potential shifts, and dorsal root potentials of the mammalian spinal cord. Brain Res. 69: 153–157, 1974.
298. SOMJEN, G. G., ROSENTHAL, M., CORDINGLEY, G., LAMANNA, J. AND LOTHMAN, E.: Potassium, neuroglia, and oxidative metabolism in central gray matter. Fed. Proc. 35: 1266–1271, 1976.
298a. STAHL, W. L., SPENCE, A. M., COATES, P. W. AND BRODERSON, S. H.: Studies on cellular localization of the Na^{+}, K^{+}-ATPase activity in nervous tissue. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press, 1977.
299. STEFANELLI, A., PALLADINI, G. AND IEARADI, L.: Effetto della ouabaina sul tessuto nervoso in coltura *in vitro*. Experientia (Basel) 21: 717–719, 1965.
300. SUGAYA, E., TAKATO, M. AND NODA, Y.: Spreading depression under the effect of tetrodotoxine. J. Physiol. Soc. Jap. 33: 591–592, 1971.
301. SUGAYA, E., TAKATO, M. AND NODA, Y.: Glial membrane potential during spreading depression under the effect of tetrodotoxine. J. Physiol. Soc. Jap. 33: 654–655, 1971.
302. SUGAYA, E., TAKATO, M. AND NODA, Y.: Neuronal and glial activity during spreading depression in cerebral cortex of cat. J. Neurophysiol. 38: 822–841, 1975.
303. SWANSON, P. D. AND CRANE, P. O.: Diphenylhydantoin and movement of radioactive sodium into electrically stimulated cerebral slices. Biochem. Pharmacol. 21: 2899–2905, 1972.
304. SWANSON, P. D. AND MCILWAIN, H.: Inhibition of the sodium-ion-stimulated adenosine triphosphatase after treatment of isolated guinea pig cerebral cortex with ouabain and other agents. J. Neurochem. 12: 877–891, 1965.
305. SWANSON, P. D. AND ULLIS, K.: Ouabain-induced changes in sodium and potassium content and respiration of cerebral cortex slices: Dependence on medium calcium concentration and effects of protamine. J. Pharmacol. Exp. Ther. 153: 321–328, 1966.
306. SYPERT, G. W. AND WARD, A. A., JR.: Unidentified neuroglia potentials during propagated seizures in neocortex. Exp. Neurol. 33: 239–255, 1971.
307. TAKAGAKI, G.: Control of aerobic glycolysis and pyruvate kinase activity in cerebral cortex slices. J. Neurochem. 15: 903–916, 1968.
308. TAKAGAKI, G.: Control of aerobic glycolysis in guinea-pig cerebral cortex slices. J. Neurochem. 19: 1737–1751, 1972.
309. TAKEMORI, A. E.: The influence of morphine on glucose utilization in cerebral preparations of rats. J. Pharmacol. Exp. Ther. 145: 20–26, 1964.
310. THOMSON, C. G. AND MCILWAIN, H.: An attachment of protamines to cerebral tissues, studied in relation to gangliosides, suramin and tissue permeability. Biochem. J. 79: 342–347, 1961.
311. TORACK, R. M., DUFFY, M. L. AND HAYNES, J. M.: The effect of anisotonic media upon cellular ultrastructure in fresh and fixed rat brain. Z. Zellforsch. Mikroskop. Anat. 66: 690–700, 1965.
312. TOWER, D. B.: The neurochemistry of asparagine and glutamine. *In* Neurochemistry of Nucleotides and Amino Acids, ed. by R. O. Brady and D. B. Tower, pp. 173–204, John Wiley & Sons, New York, 1960.
313. TOWER, D. B.: Ouabain and the distribution of calcium and magnesium in cerebral tissues *in vitro*. Exp. Brain Res. 6: 273–283, 1968.
314. TOWER, D. B.: Inorganic constituents. *In* Handbook of Neurochemistry, ed. by A. Lajtha, vol. 1, pp. 1–24, Plenum Press, New York, 1969.
315. TOWER, D. B.: Neurochemical mechanisms. *In* Basic Mechanisms of the Epilepsies, ed. by H. H. Jasper, A. A. Ward and A. Pope, pp. 611–638, Little, Brown and Co., Boston, 1969.
316. TRACHTENBERG, M. C. AND POLLEN, D. A.: Neuroglia: Biophysical properties and physiologic function. Science (Washington) 167: 1248–1251, 1970.
317. TSUKADA, Y., HIRANO, S., NAGATA, Y. AND MATSUTANI, T.: Metabolic studies of gamma-aminobutyric acid in mammalian tissues. *In* Inhibition in the Nervous System and GABA, ed. by E. Roberts, C. F. Baxter, A. Van Harreveld, C. A. G. Wiersma, W. R. Adey and K. Killam, pp. 163–168, Pergamon Press, Oxford, 1960.
318. VAN DEN BERG, C. J.: Glutamate and glutamine. *In* Handbook of Neurochemistry, ed. by A. Lajtha, vol. 3, pp. 355–379, Plenum Press, New York, 1970.
319. VAN DEN BERG, C. J. AND GARFINKEL, D.: A simulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. Biochem. J. 123: 211–218, 1971.
320. VAN HARREVELD, A.: Changes in the diameter of apical dendrites during spreading depression. Amer. J. Physiol. 192: 457–463, 1958.
321. VAN HARREVELD, A.: Water and electrolyte distribution in central nervous tissue. Fed. Proc. 21: 659–664, 1962.
322. VAN HARREVELD, A.: Brain Tissue Electrolytes, Butterworths, London, 1966.
323. VAN HARREVELD, A. AND FIFKOVA, E.: Glutamate release from the retina during spreading depression. J. Neurobiol. 2: 13–29, 1970.
324. VAN HARREVELD, A. AND FIFKOVA, E.: Effects of glutamate and other amino acids on the retina. J. Neurochem. 18: 2145–2154, 1971.
325. VAN HARREVELD, A. AND FIFKOVA, E.: Light- and electronmicroscopic changes in central nervous tissue after electrophoretic injection of glutamate. Exp. Mol. Pathol. 15: 61–81, 1971.
326. VAN HARREVELD, A. AND FIFKOVA, E.: Mechanisms involved in spreading depression. J. Neurobiol. 4: 375–387, 1973.
327. VAN HARREVELD, A. AND KHATTAB, F. I.: Changes in cortical extracellular space during spreading depression investigated with the electron microscope. J. Neurophysiol. 30: 911–929, 1967.
328. VAN HARREVELD, A. AND MALHOTRA, S. K.: Extracellular space in the cerebral cortex of the mouse. J. Anat. 101: 197–207, 1967.
329. VAN HARREVELD, A. AND SCHADÉ, J. P.: Chloride movements in cerebral cortex after circulatory arrest and during spreading depression. J. Cell. Comp. Physiol. 54: 65–77, 1959.
330. VARON, S. AND MCILWAIN, H.: Fluid contents and compartments in isolated cerebral tissues. J. Neuro-

chem. 8: 262–275, 1961.

331. VENTURINI, G. AND PALLADINI, G.: ATPase activity, sodium and potassium content in guinea pig cortex after ouabain treatment *in vivo*. J. Neurochem. 20: 237–239, 1973.

332. VILLEGAS, J.: Transport of electrolytes in the Schwann cell and location of sodium by electron microscopy. J. Gen. Physiol. 51: 61–71, 1968.

333. VILLEGAS, J., VILLEGAS, L. AND VILLEGAS, R.: Sodium, potassium and chloride concentrations in the Schwann cell and axon of the squid nerve fiber. J. Gen. Physiol. 49: 1–7, 1965.

334. VYKLICKY, L., SYKOVÁ, E., KŘÍŽ, N. AND UJEC, E.: Post-stimulation changes of extracellular potassium concentration in the spinal cord of the rat. Brain Res. 45: 608–611, 1972.

335. VYSKOČIL, F., KŘÍŽ, N. AND BUREŠ, J.: Potassium-selective micro-electrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. Brain Res. 39: 255–259, 1972.

336. WALKER, F. D. AND HILD, W. J.: Neuroglia electrically coupled to neurons. Science (Washington) 165: 602–603, 1969.

337. WALKER, F. D. AND HILD, W. J.: Spreading depression in tissue culture. J. Neurobiol. 3: 223–235, 1972.

338. WALLGREN, H.: Rapid changes in creatine and adenosine phosphates of cerebral cortex slices on electrical stimulation with special references to the effect of ethanol. J. Neurochem. 10: 349–362, 1963.

339. WALLGREN, H. AND KULONEN, E.: Effect of ethanol on respiration of rat-brain-cortex slices. Biochem. J. 75: 150–158, 1960.

339a. WARD, A.: Epilepsy. *In* Dynamic Properties of Glial Cells, ed. by G. Franck, L. Hertz, E. Schoffeniels and D. B. Tower, Pergamon Press, Oxford, in press, 1977.

340. WARDELL, W. M.: Electrical and pharmacological properties of mammalian neuroglial cells in tissue-culture. Proc. Roy. Soc. London Ser. B Biol. Sci. 165: 326–361, 1966.

341. WATSON, W. E.: Physiology of neuroglia. Physiol. Rev. 54: 245–271, 1974.

342. WEBB, J. L. AND ELLIOTT, K. A. C.: Effects of narcotics and convulsants on tissue glycolysis and respiration. J. Pharmacol. Exp. Ther. 103: 24–34, 1951.

343. WEISS, G. B. AND HERTZ, L.: Effects of different potassium ion concentrations and of procaine and pentobarbital on [^{14}C] glutamate fluxes in rat brain-cortex slices. Biochem. Soc. Trans. 2: 274–277, 1974.

344. WEISS, G. B., HERTZ, L. AND GOODMAN, F. R.: Drug-induced alterations in respiration of rat brain cortex and striatum slices in a CO_2/bicarbonate buffered medium. Biochem. Pharmacol. 21: 625–634, 1972.

345. WHITTAKER, V. P.: The synaptosome. *In* Handbook of Neurochemistry, ed. by A. Lajtha, vol. 2, pp. 327–364, Plenum Press, New York, 1969.

346. WHITTAM, R.: Active cation transport as a pacemaker of respiration: Nature (London) 191: 603–604, 1961.

347. WHITTAM, R.: The dependence of the respiration of brain cortex on active cation transport. Biochem. J. 82: 205–212, 1962.

348. WINDLE, W. F.: Biology of Neuroglia, Charles C Thomas, Publisher, Springfield, Ill., 1958.

349. WINEGRAD, S. AND SHANES, A. M.: Calcium flux and contractility in guinea pig atria. J. Gen. Physiol. 45: 371–394, 1962.

350. WOLFF, J.: Elektronmikroskopische Untersuchungen über Struktur und Gestalt von Astrozytenfortsätzen. Z. Zellforsch. Mikrosk. Anat. 66: 811–828, 1965.

351. WOLLENBERGER, A.: Metabolic action of the cardiac glycosides. I. Influence on respiration of heart muscle and brain cortex. J. Pharmacol. Exp. Ther. 91: 39–51, 1947.

352. WOLLENBERGER, A.: Action of protoveratrine on the metabolism of cerebral cortex. I. Unstimulated cerebral-cortex tissue. Biochem. J. 61: 68–77, 1955.

353. WOLLENBERGER, A.: Action of protoveratrine on the metabolism of cerebral cortex. II. Electrically stimulated cerebral-cortex tissue. Biochem. J. 61: 77–80, 1955.

354. WOLLENBERGER, A. AND WAHLER, B.: Einfluss von Protoveratrin und Strophanthin auf den Kationen- und Phosphatgehalt von Gehirnrindegewebe. Naunyn-Schmiedebergs. Arch. Exp. Pathol. Pharmakol. 228: 134–136, 1956.

355. WOODBURY, D. M.: Effect of diphenylhydantoin on electrolytes and radiosodium turnover in brain and other tissues of normal, hyponatremic and postictal rats. J. Pharmacol. Exp. Ther. 115: 74–95, 1955.

356. WOODBURY, D. M. AND KEMP, J. W.: Pharmacology and mechanisms of action of diphenylhydantoin. Psychiat. Neurol. Neurochir. 74: 91–115, 1971.

357. WOODWARD, J. K., BIANCHI, C. P. AND ERULKAR, S. D.: Electrolyte distribution in rabbit superior cervical ganglion. J. Neurochem. 16: 289–299, 1969.

358. WRAAE, O., HILLMAN, H. AND ROUND, E.: The uptake of low concentrations of lithium ions into rat cerebral cortex slices and its dependence on cations. J. Neurochem. 26: 835–843, 1976.

359. YAMAMOTO, C. AND KAWAI, N.: Seizure discharges evoked *in vitro* in thin section from guinea pig hippocampus. Science (Washington) 155: 341–342, 1967.

360. YAMAMOTO, C. AND MATSUI, S.: Effect of stimulation of excitatory nerve tract on release of glutamic acid from olfactory cortex slices *in vitro*. J. Neurochem. 26: 487–491, 1976.

361. YAMAMOTO, C. AND MCILWAIN, H.: Potentials evoked *in vitro* in preparations from the mammalian brain. Nature (London) 210: 1055–1056, 1966.

362. YOSHIDA, H., FUJISAWA, H. AND KAJIKAWA, W.: Breakdown of high energy phosphate compounds in brain slices in relation with an increase in permeability to cations. Jap. J. Pharmacol. 13: 297–304, 1963.

363. ZADUNAISKY, J. A. AND CURRAN, P. F.: Sodium fluxes in isolated frog brain. Amer. J. Physiol. 205: 949–956, 1963.

364. ZADUNAISKY, J. A., WALD, F. AND DE ROBERTIS, E.: Osmotic behaviour and ultrastructural modifications in isolated frog brains. Exp. Neurol. 8: 290–309, 1963.

365. ZUCKERMANN, E. C. AND GLASER, G. H.: Activation of experimental epileptogenic foci. Action of increased K^+ in extracellular spaces of brain. Arch. Neurol. 23: 358–364, 1970.