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I. INTRODUCTION

The function of the mammalian brain depends on a continuous supply of O₂ and glucose. When the brain no longer receives either of these substances, loss of function occurs quickly (83, 254), and viability is endangered when the lack of substrates persists for more than a few minutes (51, 83). These special conditions, which are not found in any other organ, present an important pathophysiological and clinical problem.
This review deals with ion movements in the brain under conditions in which the energy metabolism of the brain has been compromised, with special emphasis on ion concentration changes in the interstitial space. The dysfunction of the brain and of ion movements may be linked, because normal brain function requires an intact ion distribution. During action potentials and synaptic activity there is a net gain of Na\(^+\) and Ca\(^{2+}\) in nerve cells. Albeit passive in nature, these processes can only take place if the ions are maintained in electrochemical disequilibrium across the nerve membrane at the expense of ATP hydrolysis. Brain energy metabolism regenerates ATP, which is the main substrate fueling active ion transport. Accordingly the effects of anoxia, hypoglycemia, and related conditions on brain function and ion distribution are believed to be linked to insufficient ATP regeneration. The link between brain function and energy metabolism is the key problem in brain anoxia, and this relationship is still poorly understood.

Because of the importance of the ionic environment in synaptic transmission and in the function of neurons, the mechanisms that regulate ion content in the brain interstitial fluid and in cellular compartments are considered first. The two central problems in brain anoxia—the rapid loss of function and the irreversible damage or death of brain cells—are addressed with reference to ion movements. The loss of consciousness is not specifically related to the maintenance of ion gradients across the nerve membrane but rather is due to activation of specific ion conductances, whereas the damage may be related to the gross disturbances in ion distribution observed in prolonged anoxia. A description of the spreading depression of Leão (170) is included because this phenomenon exhibits ion changes resembling those observed during cerebral anoxia.

II. ION CONCENTRATIONS IN THE BRAIN

The ion distribution in water spaces of the brain must be known before the ion movements observed during the conditions described below can be understood. The interstitial space in the brain is confined to narrow clefts 20–50 nm wide between the densely packed nerve and glial cells (291, 296). The enormous surface area of brain cells constitutes the basis for an interstitial space volume of ~20% (176, 221, 235, 245, 296). The interstitial space and the vascular volume together constitute the extracellular space. Because the vascular space has a volume of only 1–3% (180), extracellular and interstitial spaces are for all practical purposes identical. The intracellular space has a volume of 80% (146). The cerebrospinal fluid (CSF) volume represents ~10–20% of brain weight (31).

A. Methodology: Ion-Sensitive Microelectrode

Much of the data described in this review stems from experiments with ion-sensitive microelectrodes (ISM). The ISM mechanism and the results of in vivo measurements in brain tissue are briefly described next.
The ISM consists of two glass micropipettes sintered together and possessing a common tip diameter of \(\sim 1-5 \mu m\). One pipette serves as a common potential-recording electrode; the other contains the ion-selective material. The potential difference between the pipettes represents the ion activity at the tip. The ion-sensitive material is usually a liquid ion exchanger. Ion exchangers are now available in microelectrodes for the detection of \([K^+]\), \([Na^+]\), \([Ca^{2+}]\), \([H^+]\), and \([Cl^-]\) (10, 202). The microdimensions of the electrodes allow them to be positioned in tissue without disturbing the local microcirculation. In measurements of interstitial space, special considerations must be taken: the width of the electrode tip is a few micrometers, or \(\sim 100\) times the width of the intercellular cleft (291, 296), and therefore the intrusion of the electrode is likely to disturb local conditions in the interstitial space. Positioning of the electrode in the tissue may create a dead space around the tip (123, 192). However, the region around the electrode tip is in functional equilibrium with the surrounding undisturbed tissue. Lothman and Somjen (187) determined changes in interstitial \(K^+\) concentration (\([K^+]_i\)) by two methods: direct measurement in the interstitial space with an ISM (disturbed space) or measurement of changes in the membrane potential of glial cells (undisturbed space) (233). They found that the two methods recorded induced changes in \([K^+]_i\) similarly. Interstitial space volume, as determined from concentration changes recorded with an ISM in response to iontophoretic injection of ions, compares with noninvasive techniques (221). Furthermore the results are independent of the distance between the two electrodes within a range of 40–200 \(\mu m\) (221), indicating that the brain cell microenvironment is not markedly disturbed by the presence of microelectrodes. Accordingly the ion concentrations recorded with an ISM in the interstitium are supposed to reflect the events in the undisturbed brain.

Ion-sensitive microelectrodes determine ion activities rather than ion concentrations. However, most papers dealing with ISM measurements state the values in concentrations, probably because activity coefficients are difficult to determine in mixed solutions. No significant error is introduced if the activity coefficients in the electrode-calibration solutions and in the biological fluid are the same. Because this is usually not known, values from ISM recordings should be given in terms of ion activity. In this review, however, the values are expressed in concentrations, in accordance with common usage.

**B. Brain Interstitial Ion Concentrations**

The brain possesses homeostatic mechanisms that maintain constant ion concentrations in CSF despite variations in plasma composition (31). The homeostatic mechanisms are especially efficient for ions such as \(K^+\), \(Ca^{2+}\), and \(Mg^{2+}\) (31). They operate in conjunction with a low ion permeability in the brain capillaries (65, 111) and active ion-transport mechanisms in the choroid plexus (61, 315) and brain endothelial cells (21, 34). The interstitial fluid (ISF) and the CSF are thus protected against changes in plasma ion composition.
Before the advent of ISMs, virtually no techniques were available for direct measurement of ion concentrations in the brain interstitial space (146). Variations in ion concentrations could be estimated indirectly by electrophysiological techniques. For example, the \([K^+]\) was determined by glial cell membrane potential (233) or by the magnitude of the undershoot after the action potential (57). Estimation of the \([Na^+]\) was obtained from the height of the action potential (125), and the \([Cl^-]\) was determined from the magnitude of the inhibitory postsynaptic potential (IPSP) (184, 191). For the older techniques to be used, the intracellular concentration of the measured ion must remain constant and the entire surface of the cell must be exposed to the same ion concentration, which may not always be the case.

The ion composition of brain ISF has been inferred from measurements of CSF (32, 60, 145, 234). That solutes of high molecular weight readily pass between CSF and brain ISF supports the supposition that no significant concentration gradients exist between the two compartments in the steady state (176, 245). Hence the ionic composition of the interstitial space is reflected by the CSF. The exchange of substances between ISF and CSF involves diffusion processes and bulk flow (62). The rather long time course of these processes creates a disequilibrium between the two spaces during acute changes in ISF composition. Moreover dilution in the large CSF cavities lessens the reliability of the CSF composition as a representative ISF composition.

Many ISM measurements of brain interstitial ion concentrations have been reported. Some results are shown in Table 1 along with values of CSF composition. The table shows that the ion compositions in the two fluids are similar.

C. Intracellular Ion Concentrations

Several methods can be employed to determine intracellular ion concentrations.

With early techniques the total ion content of a brain volume was determined and the extracellular ion content subtracted (146). More recent techniques employ the permeability characteristics of cell membranes. As mentioned in section II B, the glial cell membrane is selectively permeable to \(K^+\), whereas the nerve cell membrane is mainly permeable to \(Na^+\) and \(K^+\) during various phases of the action potential and to \(Cl^-\) during the IPSP. On the basis of this knowledge the intracellular concentrations are calculated according to the Nernst equation; the interstitial ion concentrations and the respective equilibrium potentials are used in these determinations. Obviously the most direct method is determining ion concentrations by ISM. This technique has been used in nerve and glial cells from various invertebrates (40, 52, 79, 307) and in frog motoneurons (44) but not yet in mammalian nerve cells. The use of ISM for intracellular measurements is somewhat hampered by the limited selectivities of the available liquid ion exchangers (44, 202).
### Table 1. Ion concentrations in brain interstitial and cerebrospinal fluid

<table>
<thead>
<tr>
<th></th>
<th>[K⁺] (mM)</th>
<th>[Na⁺] (mM)</th>
<th>[Cl⁻] (mM)</th>
<th>[HCO₃⁻]</th>
<th>[Ca²⁺]</th>
<th>[Mg²⁺]</th>
<th>pH</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interstitial fluid of cerebral cortex</strong></td>
<td></td>
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<tr>
<td>Rat</td>
<td>3.1</td>
<td>154</td>
<td>129</td>
<td>1.3</td>
<td>23</td>
<td>7.33</td>
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<td></td>
<td>2.8</td>
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<td></td>
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<td>18</td>
<td>7.22</td>
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<tr>
<td></td>
<td>3.0</td>
<td>143</td>
<td>127</td>
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<td>1.2</td>
<td>7.39</td>
<td>264</td>
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<tr>
<td></td>
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<td>148</td>
<td>128</td>
<td></td>
<td></td>
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<td>318</td>
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</tr>
<tr>
<td>Cat</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td>146</td>
<td>1.2</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>140</td>
<td>1.2</td>
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<td>3.5</td>
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<td>Cerebrospinal fluid from cisterna magna</td>
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<tr>
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<td>155</td>
<td>125</td>
<td>1.4</td>
<td>27</td>
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<td>214</td>
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<tr>
<td></td>
<td>3.2</td>
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<td>1.3</td>
<td>1.3</td>
<td>7.38</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
<td>0.9</td>
<td>1.5</td>
<td>8</td>
</tr>
</tbody>
</table>

Interstitial concentrations (mM) measured by ion-sensitive microelectrodes. Calculations made with measured pH values and a PCO₂ of 46 mmHg for rats (242) and 32 mmHg for cats (282). Cerebrospinal samples analyzed by conventional methods. * Determined from Henderson-Hasselbalch equation: pH = pK' + log([HCO₃⁻]/PCO₂ X S), where solubility factor (S) for CO₂ is 0.0318 mM/mmHg and pK' is 6.133 (204). † Determined from surface electrode.

The ISM results compare with the data obtained with electrophysiological techniques and confirm the well-known unequal distribution of ions across cell membranes. The data from tissue sampling express the ionic content, not the cytoplasmic activity, of the ions and therefore give higher values than the electrode techniques. The high values for [K⁺], [Ca²⁺], and [Mg²⁺] obtained with this technique are due to sequestration into specialized intracellular organelles (131, 174, 257).

Table 2 presents values of intracellular ion concentrations determined by the different methods. The volume of glial cells in smaller animals like the rat and the cat constitutes only 10–20% of the brain volume (81, 313), implying that these results refer mainly to the ion content in nerve cells. With the results in Table 1, the following intracellular-to-extracellular ratios of ion concentrations across a rat nerve cell membrane are likely (and are shown in millimoles per liter): K⁺, 100/3; Na⁺, 30/150; Cl⁻, 5/130; Ca²⁺, 0.0001/1.3; Mg²⁺, 0.8/1; HCO₃⁻, 12/24; the pH ratio is 7.00/7.30. The corresponding
<table>
<thead>
<tr>
<th>Method</th>
<th>Site</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>pH</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
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<td>Cat cortical neuron</td>
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<td></td>
<td>147</td>
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<tr>
<td>(-98 mV, [Cl⁻], = 149 mM)</td>
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<tr>
<td>Undershoot of action potential</td>
<td>Cat motoneuron</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76</td>
</tr>
<tr>
<td>(-90 mV, [K⁺], = 3.0 mM)</td>
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<tr>
<td>Overshoot of spike potential</td>
<td>Motoneuron</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>146</td>
</tr>
<tr>
<td>(+25 mV, [Na⁺], = 146 mM)</td>
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<tr>
<td>Glia membrane potential</td>
<td>Cat cortical glia</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>94</td>
</tr>
<tr>
<td>(-93 mV, [K⁺], = 3.1 mM)</td>
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<tr>
<td>Ion-sensitive microelectrode</td>
<td>Frog motoneurons</td>
<td>118</td>
<td>36</td>
<td>31</td>
<td>2.3 × 10⁻²</td>
<td></td>
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<tr>
<td>Frog spinal glia</td>
<td></td>
<td>-100</td>
<td>22</td>
<td>11</td>
<td>8.8 × 10⁻²</td>
<td></td>
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<td>44</td>
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<tr>
<td>Giant neuron</td>
<td>(Helix aspersa)</td>
<td>1.7 × 10⁻⁴</td>
<td></td>
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<td>Giant neuron</td>
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<td>Tissue sampling</td>
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<tr>
<td></td>
<td>Cat brain cortex</td>
<td>163</td>
<td>59</td>
<td>24</td>
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<td>10.5</td>
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<tr>
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<td>Primary cultures of</td>
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<td></td>
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<td>103</td>
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<tr>
<td></td>
<td>rat astroglia</td>
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<td></td>
<td>Distribution of DMO</td>
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<td></td>
<td>Rat brain cortex</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td>253</td>
</tr>
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</table>

Intracellular ion concentrations (mM) determined by different techniques. **Upper section:** ion equilibrium potentials determined by electrophysiological techniques, and the intracellular ion concentration determined from interstitial concentrations via the Nernst equation. **Second section:** measurements from ion-sensitive microelectrodes studies. **Third section:** values calculated from total tissue concentrations (mmol/kg wet wt) assuming an interstitial space volume of 20% with an ion composition as indicated in Table 1 and assuming that all dry matter (~20%) is located inside the cell. **Lower section:** values determined from [³⁵Cl⁻] exchange and from distribution of 5,5-dimethyloxazolidine-2,4-dione (DMO) in the brain. IPSP, inhibitory postsynaptic potential; [Cl⁻], [K⁺], and [Na⁺], interstitial concentrations.
equilibrium potential ($E$) values (in millivolts) are $E_{K^+}$, -93; $E_{Na^+}$, +43; $E_{Cl^-}$, -86; $E_{Ca^{2+}}$, +125; $E_{Mg^{2+}}$, -3; and $E_{HCO_3^-}$ and $E_{H^+}$, 19. With a neuronal membrane potential of -70 mV, these values imply that active processes are required to maintain the ion gradients. Any increase in membrane ionic permeability not counteracted by active transport leads to an increase in $K^+$ and $HCO_3^-$ in the interstitium and to a decrease in all of the remaining interstitial ion concentrations.

Little is known about the ionic composition of mammalian glial cells, but there is no indication that it differs significantly from the ionic composition of nerve cells. A possible exception may be that the $[Cl^-]$ is larger (149).

III. CHANGES IN INTERSTITIAL ION COMPOSITION

Ionic gradients across cell membranes are maintained by active processes that move the ions against an electrochemical gradient. The active processes are executed by membrane-bound ATPases (73, 269). The dissipative processes do not take place to any significant extent via leak pathways in the lipid membrane but via transport systems composed of integral membrane proteins (178). These channels permit selective movements of cations and anions and are activated by voltage changes, transmitters, or other chemical means. Other dissipative systems include $HCO_3^-\text{Cl}^-$ exchange and transport systems in which the downhill movement of $Na^+$ provides for the uphill movements of other ions. Accordingly the net movement of ions across the membrane can result from a reduction of the ATPase activity or an activation of passive-transport systems, in essence a decreased pump-to-leak ratio. Because the interstitial space represents one-fourth of the cellular volume, its ion composition is particularly sensitive to net ionic movements across the cell membrane.

A. Changes During Nervous Activity

The ion composition of brain interstitial space undergoes changes related to the electrical activity within the particular area, because the rate of passive ion movements through the nerve membrane increases severalfold more than the rate of active ion transport (128, 129). Application of ISM has corroborated the activity-dependent increase of $[K^+]_o$ (117, 159, 243) and has shown that $[Ca^{2+}]_o$ decreases during activity (119); the interstitial pH ($pH_o$) also decreases, often after an alkaline shift (153, 293).

The changes in brain interstitial ion composition during physiological stimulation are modest (273). Touching a skin area increases the $[K^+]_o$ in the corresponding cortical receiving area by 0.1–0.5 mM, whereas $[Ca^{2+}]_o$ decreases by 0.1 mM (119). Spontaneous activity in the respiratory centers induces rhythmical changes in the $[K^+]_o$ of 0.3 mM (250). However, in the vicinity of a spontaneously firing Purkinje cell, the $[K^+]_o$ may rise as much as 1–3 mM
(138). The interstitial concentration changes are transmitted to the surrounding cells and may affect their function. The interstitial space thus serves as a communication channel and is therefore important for the integrative function of the central nervous system (CNS) (219).

During intense stimulation of afferent pathways and during direct stimulation of the nervous tissue or epileptiform activity, the [K⁺]₀ may increase to 10–12 mM and the [Ca²⁺]₀ may decrease to 0.5 mM (94, 117, 162, 186, 223); the pH₀ falls ~0.2 units (153), whereas the [Na⁺]₀ and the interstitial [Cl⁻] ([Cl⁻]₀) only show small changes (71). It is generally not possible to increase the [K⁺]₀ above 10–12 mM (117). This ceiling has been ascribed to the inhibitory effect of the increased [K⁺]₀ on synaptic transmission in combination with a stimulation of reuptake mechanisms (117, 160), but it also probably involves uptake by the glial cells (52, 96, 122). The ceiling is only exceeded under conditions in which failure of energy metabolism prevails or during spreading depression (SD). The low permeability of the brain capillaries (111, 210) prevents overflow from brain to blood; the ion changes can thus be regarded as an exchange between the cellular constituents and the interstitial space. In this regard, brain tissue is different from all other tissues, and a detailed knowledge of the kinetics of ion transport in the blood-brain barrier is required for an understanding of prolonged disturbances.

B. Interstitial Ion Concentrations During Anoxia

The dependence of brain function on a continuous blood supply coupled with the fact that the brain contains virtually no reserve supply of O₂ and only small stores of glucose or energy-rich compounds (266) suggests that function ceases in instances of substrate deficiency because active ion transport is inadequate or arrested. The cells lose K⁺, gain Na⁺, depolarize, and stop functioning. Determination of ion movements in brain during anoxia shows that the cells do indeed lose K⁺ and take up Na⁺. Surprisingly, however, the time course is not directly related to the functional deficits. This interesting dissociation of function and a fundamental prerequisite for electrical activity are discussed in this section.

The energy metabolism of the brain can be compromised by ischemia, hypoxia, anoxia, or intoxication. Ischemia is defined as a state of insufficient blood flow; hypoxia signifies a lowered O₂ tension (P⁰₂) of the tissue, and anoxia refers to lack of O₂ in blood. In the next section, anoxia is used as a common denominator for all of these conditions unless otherwise stated.

1. Response of brain to anoxia

The substrate sensitivity of the brain is most strikingly revealed in experiments that completely arrest the blood supply to the brain. Brain ischemia was produced in humans by inflation of a neck cuff to a pressure
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of 600 mmHg and led to unconsciousness within 6–7 s (254). At this time the electroencephalogram (EEG) exhibited slow-wave activity, and the corneal reflexes were lost in <10 s. The subjects experienced paresthesia and narrowing of the visual field followed by loss of vision. Complete ischemia for up to 100 s was tolerated without neurological sequelae. Similar findings have been made in animals (68, 126). The EEG in ischemia initially shows increases in activity, followed by cessation of activity within 10–20 s. Reflexes (e.g., corneal reflexes) and spontaneous respiration disappear 10–30 s later, whereas evoked responses are more resistant (53, 126, 136). The response evoked by electrical stimulation of the cerebral cortex and recorded in the pyramidal tract reveals that synaptic transmission is abolished after 2 min and electrical activation after 4 min of ischemia (136). Functions that rely on integration of cortical processes are more susceptible than functions in lower brain structures (35, 241). On the whole, spontaneous activity is less resistant to anoxia than evoked activity. The relationship between interstitial ion concentration and loss of function is discussed in further detail below.

2. Changes in interstitial \([K^+]\)

Variations in the \([K^+]\) have several effects on nerve function; some effects are excitatory and some inhibitory. Increasing the \([K^+]\) moderately causes increased excitability (161), but at the same time, synaptic transmission is hampered by a presynaptic action (160). Studies indicate that the \([K^+]\) in brain increases during anoxia. The outflux of \(K^+\) from the cerebral cortex a few minutes after initiation of anoxia increases severalfold (24, 38). In rats the \([K^+]\) in cisterna magna fluid increases from 3 to 20 mM after 15 min of sustained anoxia (106, 306), whereas in larger animals (including humans) the increase in the CSF \([K^+]\) occurs more slowly because of the longer diffusion distances (20, 215). The temporal and spatial resolution of the CSF changes, however, provide inadequate information about brain ISF changes.

Studies with a \(K^+\)-sensitive microelectrode have amply confirmed and extended the knowledge about the \([K^+]\) increases during anoxia and have further established that the rise in the \([K^+]\), occurs in several distinct phases. Most studies have been undertaken in the brains of small animals such as the rat. Figure 1 illustrates the changes in the \([K^+]\) in rat brain cortex during anoxia. Immediately after induction of cardiac arrest, there is a short-lived enhancement of the activity of the EEG, causing a small transient increase in the \([K^+]\) before complete arrest of activity. This is followed by a slow increase in the \([K^+]\), with the rate of rise becoming progressively faster. After ~2 min the \([K^+]\) suddenly rises steeply from ~10 mM to ~60 mM within a few seconds. A subsequent slower rate of rise during the next few minutes augments the \([K^+]\) to almost 80 mM. The rise in the \([K^+]\) can apparently be resolved into three phases: an initial slow rate of rise (phase 1), a rapid rate of rise (phase 2), and a final slow increase (phase 3). This pattern has been observed in brain gray matter during ischemia, hypoxia,
and anoxia (14, 107, 108, 305). It seems to be most pronounced in the brains of small animals and less distinct in larger animals such as cats and baboons (25, 115, 279). In this section, emphasis is placed on events in the rat brain cortex.

The increase in the [K⁺]₀ during phase 1 is most likely explained by a net efflux from neurons that is caused by insufficient inward pumping due to the reduced Na⁺-K⁺-ATPase activity. The K⁺ permeability probably is also increased. The equilibrium potential for K⁺ explains the K⁺ movements and the concomitant hyperpolarization (sect. II.C; 110). Only a small part of the K⁺ efflux, however, mediates the hyperpolarization. If one assumes a membrane capacity of 1 μF/cm² and a membrane area of 25 × 10⁴ cm²/cm³ ISF (cf. sect. IV), a change in the membrane potential of 10 mV gives a capacitive transfer of K⁺, which raises [K⁺], by 25 μM. Therefore any excess [K⁺], increase must take place by exchange with cations or be accompanied by anions. The mechanism by which K⁺ enters the ISF is unknown, as is the role played by the glial cells.

No study has demonstrated whether the K⁺ released from neurons during anoxia is taken up by glial cells, as has been found during nervous activity (52) and during SD (207). However, conditions that preserve a high level of ATP in brain during ischemia prolong the duration of phase 1, i.e., decrease the rate of increase in the [K⁺]₀. The rapid and dramatic change during phase 2 represents a sudden increase in overall ionic permeability of the cell membrane and is described in more detail in section IV.D. The level attained in the interstitial space after several minutes of anoxia, equivalent to phase 3 (Table 3), is significantly lower than expected if K⁺ is distributed freely.
TABLE 3. Interstitial ion concentrations in cerebral cortex in anoxia and spreading depression

<table>
<thead>
<tr>
<th></th>
<th>[K⁺]</th>
<th>[Na⁺]</th>
<th>[Cl⁻]</th>
<th>[HCO₃⁻]</th>
<th>pH</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>56</td>
<td>75</td>
<td>48</td>
<td>72</td>
<td>0.06</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>48</td>
<td>72</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cuff</td>
<td>80</td>
<td>49</td>
<td>48</td>
<td>45</td>
<td>0.13</td>
<td>263</td>
</tr>
<tr>
<td>N₂ inhalation†</td>
<td>78</td>
<td>49</td>
<td>48</td>
<td>45</td>
<td>0.13</td>
<td>263</td>
</tr>
<tr>
<td>Respiratory arrest</td>
<td>100</td>
<td>25</td>
<td>55</td>
<td>50</td>
<td></td>
<td>318</td>
</tr>
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<td>Cat</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N₂ inhalation†</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Clamping of main arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Baboon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clamping of middle cerebral artery†</td>
<td>60-70</td>
<td></td>
<td></td>
<td></td>
<td>0.28</td>
<td>115</td>
</tr>
<tr>
<td>Spreading depression</td>
<td>Rat</td>
<td>55</td>
<td>59</td>
<td>74</td>
<td>0.07</td>
<td>114</td>
</tr>
</tbody>
</table>

Ion concentrations (mM) determined by ion-sensitive microelectrodes. Duration of anoxia, which was generally >5 min, was sufficient to elicit anoxic depolarization * Calculated from the Henderson-Hasselbalch equation (cf. Table 1 legend) with measured pH values and PCO₂ of 120 mmHg (148). † Values represent maximum deviations.

in brain. Because the water content of gray matter is 80%, the [K⁺] of 100 mM in brain matter (Table 4) corresponds to a concentration of 130 mM in brain water. The lower final level of 80 mM may be explained by K⁺ binding to cellular constituents such as mitochondria (131, 257).

The increased [K⁺] in anoxia does not seem to be responsible for the

TABLE 4. Brain cortical ion concentrations

<table>
<thead>
<tr>
<th></th>
<th>[K⁺]</th>
<th>[Na⁺]</th>
<th>[Cl⁻]</th>
<th>[Ca²⁺]</th>
<th>[Mg²⁺]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>105</td>
<td>50</td>
<td>30</td>
<td></td>
<td></td>
<td>214</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.1*</td>
<td>6.5*</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>101</td>
<td>62</td>
<td>42</td>
<td></td>
<td></td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.2</td>
<td>5.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are in millimoles per kilogram wet weight. * Whole brain.
initial functional changes, because the EEG becomes isoelectric when the [K\(^+\)]\(_{\text{int}}\) is near the normal value (108, 135). Impulse propagation of cortical nerves only disappears when the [K\(^+\)]\(_{\text{int}}\) exceeds 16 mM and returns when the [K\(^+\)]\(_{\text{int}}\) falls below this value during reoxygenation (135). This important dissociation of the drastic interference with EEG activity and the [K\(^+\)]\(_{\text{int}}\) changes is one of the surprising observations made possible with the ISM technique. A satisfactory explanation of this puzzling observation has not yet been provided. This finding sheds doubt on the often-repeated statement that one of the functions of the blood-brain barrier is to stabilize the [K\(^+\)]\(_{\text{int}}\) at a lower level than that present in plasma.

a) Changes in DC potential. The local electrical potential, also called the DC potential, as measured between an extracellularly placed electrode in the brain and a remote electrode, displays distinct changes related to changes in the [K\(^+\)]\(_{\text{int}}\). The most notable change is a fast negative deflection with an amplitude of ~20 mV at the time of the steep increase in the [K\(^+\)]\(_{\text{int}}\) (cf. Fig. 1). The DC potential obviously reflects the rise in the [K\(^+\)]\(_{\text{int}}\). The potential change during the steep increase in the [K\(^+\)]\(_{\text{int}}\) is called anoxic depolarization (41, 294) and is likely to be accompanied by depolarization of nerve and glial cells, although this has apparently not been experimentally substantiated (55). Observations of SD, in which a similar fast rise in the [K\(^+\)]\(_{\text{int}}\) and a negative deflection of the DC potential take place, show that glial and nerve cells depolarize concomitantly with the negative change in the DC potential (280).

The origin of the DC-potential shifts are not firmly established. The observation that anoxic depolarization is detected only when the recording electrode is placed in brain gray matter and not in the CSF of the ventricles rules out the blood-brain barrier as the potential generator (114). Part of the potential is probably due to a current induced by cell depolarization, as seems to be the case in SD (220). However, because the negative potential persists for several hours (292), a diffusion potential arising between the local brain ISF and the surrounding normal extracellular fluid in the brain is a more likely possibility (114). (For more detailed descriptions of this unresolved problem, see refs. 46, 47, 185, 232, 272.) Because the potential is closely related to the steep increase in the [K\(^+\)]\(_{\text{int}}\), it can be used to compare the time course of concentration changes in other ions with that in the [K\(^+\)]\(_{\text{int}}\).

3. Changes in interstitial [Ca\(^{2+}\)]

The level of the [Ca\(^{2+}\)]\(_{\text{int}}\) influences membrane excitability and synaptic transmission and could be involved in the arrest of nervous activity in anoxia. The intracellular [Ca\(^{2+}\)] is extremely low (0.0001 mM; 5, 48); therefore the interstitial concentration is inclined to decrease when active extrusion is hampered. However, no decline in the [Ca\(^{2+}\)]\(_{\text{int}}\) is initially observed during anoxia. During phase 1 there is a small but significant increase of 0.1 mM in the [Ca\(^{2+}\)]\(_{\text{int}}\), (114, 264). The maintained level in [Na\(^+\)]\(_{\text{int}}\) ensures a continuous
action of the $\text{Na}^+:\text{Ca}^{2+}$ exchanger (26), and shrinkage in the interstitial space is probably responsible for the $[\text{Ca}^{2+}]_o$ level in this phase. An increase in the $[\text{Ca}^{2+}]_o$ has a charge-screening effect on the neurons and tends to depress neuronal activity (90). The small increase observed initially in ischemia, however, insufficiently explains the observed changes in excitability.

The pronounced and rapid fall in the $[\text{Ca}^{2+}]_o$ to ~10% of the resting level observed in phase 2 can seriously impair synaptic transmission (72, 144). A similar decrease has been observed in the cerebral cortex of the baboon during ischemia (115) and in the rat cerebellum in anoxia (222). The influence that $\text{Ca}^{2+}$ has on transmitter release (144) and on $K^+$ conductance (200) makes it especially important to explore whether the $[K^+]_o$ changes or the other ion movements described below are related to the movements of $\text{Ca}^{2+}$. A decline in the $[\text{Ca}^{2+}]_o$ preceding the $[K^+]_o$ increase provoked by anoxia and SD has not been reported (114, 115, 154, 222). This may rule out the possibility that a net $\text{Ca}^{2+}$ influx is involved in eliciting the $K^+$ movements by changing the $K^+$ conductance, but the ISM technique is probably too crude to detect the small $\text{Ca}^{2+}$ influxes that induce transmitter release and the increase in $K^+$ conductance.

Information on $[\text{Mg}^{2+}]_o$ is not available. Magnesium is an important ion that is required in many processes in the cell, and therefore its movements during anoxia must be determined. The lack of a suitable ion exchanger is the main impediment to the measurement of $\text{Mg}^{2+}$ movements (202). An increase in the CSF $[\text{Mg}^{2+}]$ during anoxia probably reflects augmentation of the $[\text{Mg}^{2+}]_o$ (215).

4. Changes in interstitial $[\text{Na}^+]$ and $[\text{Cl}^-]$

The dominant ions in the interstitial fluid are $\text{Na}^+$ and $\text{Cl}^-$ (Table 1). Their significance in the action potential, in synaptic inhibition, and in cell volume regulation makes them important ions in connection with brain anoxia. In contrast to the characteristic $[K^+]_o$ change induced by anoxia, earlier studies of CSF concentrations did not reveal marked $[\text{Na}^+]$ or $[\text{Cl}^-]$ changes (306). However, studies with ISM have demonstrated marked alterations. The changes in the $[\text{Na}^+]$, and the $[\text{Cl}^-]$, and in the $[K^+]$, and the $[\text{Ca}^{2+}]$, are closely related to the DC-potential changes. Initially in ischemia there is no decrease in the concentrations of these ions despite the gradual depletion of energy-rich compounds in the brain cortex (266); rather, there is a small increase (114, 318). This might signify that the $\text{Na}^+-\text{K}^+$-ATPase can still maintain the transmembrane gradients. However, this does not seem likely. Because the interstitial space gradually shrinks during phase 1 to ~80% of the control value (113), whereas $[\text{Na}^+]_o$ and $[\text{Cl}^-]_o$ only rise ~5% (cf. Table 5), these ions must enter the cells.

The fact that the interstitial concentrations of these ions are maintained at near-normal levels may be explained as follows. The lowered ATPase activity cannot counteract the passive $\text{Na}^+$ influx, which is accompanied partly
TABLE 5. Ion contents of brain cortex in anoxia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial space, liter/liter</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>brain cortex</td>
<td>20%</td>
<td>20–16%</td>
<td>16–9%</td>
<td>113</td>
</tr>
<tr>
<td>Interstitial ion concentrations, mM</td>
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<tr>
<td>Na⁺</td>
<td>154</td>
<td>157</td>
<td>63</td>
<td></td>
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<tr>
<td>Cl⁻</td>
<td>129</td>
<td>136</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>3.1</td>
<td>9.1</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Content of ions in interstitial space, mM brain cortex</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>30.8</td>
<td>25.1</td>
<td>5.7</td>
<td>114</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>25.8</td>
<td>21.8</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>0.62</td>
<td>1.5</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Intracellular concentrations, mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>30</td>
<td>27</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>10</td>
<td>17</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>100</td>
<td>93</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

Ionic changes in rat cerebral cortex during complete ischemia. Phase 1 starts with onset of ischemia and lasts a few min, whereas phase 2 only lasts a few seconds and is characterized by a steep, negative deflection of interstitial electrical potential. Ion content in interstitial space calculated from upper sections. Control values for intracellular concentrations taken from suggested values in section 16'. It is assumed that dry matter of cerebral cortex (~20%) is located inside cells given ~60% intracellular control volume.

by Cl⁻ influx and partly by K⁺ efflux to maintain electroneutrality. Because of osmosis, the combined influx of Na⁺ and Cl⁻ is accompanied by water and constitutes an isosmotic influx of NaCl, as suggested by Van Harreveld (296). Because the interstitial and intracellular spaces are tightly coupled in a closed system such as the ischemic brain, the swelling of the cells is accompanied by an equivalent shrinkage of the interstitial space. Thus the coupling of ATP generation, Na⁺ net flux, and cell volume expansion is responsible for the initial maintenance of the [Na⁺], the [Cl⁻], and the [Ca²⁺], in ischemia. The intracellular production of osmotically active solutes, including lactic acid from glycolysis (187, 266), is responsible for the elevation of the osmolarity in the interstitial fluid (cf. Table 5).

Accompanying the anoxic depolarization (phase 2) there is a marked decrease in both the [Na⁺] and the [Cl⁻], which is followed by a somewhat slower rate of decrease in the [Na⁺] and the [Cl⁻], to ~50 mM (114, 318). The rapid and dramatic changes during phase 2 reflect a sudden increase in overall ionic permeability of the cell membrane (cf. sect. vi). The results of Cl⁻ movements recorded with ISMs corroborate the histochemical finding by Van Harreveld and Schade (304) of an accumulation of Cl⁻ within brain cortical cells during anoxia—an accumulation assumed to occur mainly in apical dendrites.
5. Changes in interstitial pH

The pH in brain ischemia begins within 15 s after the onset of ischemia and continues during the next few minutes. The fact that the decrease is due mainly to increased CO₂ liberated from intracellular HCO₃⁻ and not to net transport of H⁺ and HCO₃⁻ across the cell membrane is supported by the observation that the inhibition of HCO₃⁻-Cl⁻ exchange with 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS) (30) or the inhibition of the Na⁺-H⁺ exchange with amiloride (3) does not affect the time course or the magnitude of pH changes (209).

At the beginning of anoxic depolarization there is an unexpected alkaline shift of 0.1 units (153, 209). This finding was only made possible by the development of fast-responding ISM with liquid ion exchangers (202). This early alkaline shift was overlooked in the past or considered to be an electrode artifact due to a spatial separation of the pH and reference electrodes in earlier electrode designs. The explanation of the alkaline shift is somewhat obscure. The brain ISF is devoid of weak acids or bases, and the pH is solely determined by the PCO₂ and the value of buffer base or base excess (265). The base excess is equal to the difference between the sum of all nonbuffer cations (K⁺, Na⁺, Ca²⁺, etc.) and the sum of nonbuffer anions (Cl⁻, lactate, etc.). In brain ISF the value of the base excess is equivalent to the [HCO₃⁻]. Determination of interstitial ion concentrations in the brain during ischemia indicates an increased base excess during anoxic depolarization (114, 318), in accordance with the observed alkaline shift at that moment. There is no clear explanation of the increased base excess. The fact that the magnitude of the alkaline shift is increased by pretreatment with DIDS or amiloride (209) suggests that it is due to an inhibition of the Cl⁻-HCO₃⁻ and Na⁺-H⁺ exchange mechanisms (30, 150).

During the alkaline shift, the [HCO₃⁻] increases, whereas the [Cl⁻] decreases. This process stimulates the Cl⁻-HCO₃⁻ exchange, resulting in cellular uptake of HCO₃⁻ and efflux of Cl⁻, which attenuates the alkaline shift and probably also the decrease in the [Cl⁻] (45). The importance of the exchange mechanisms for the HCO₃⁻ level during anoxic depolarization can be calculated from the Henderson-Hasselbalch equation (see Table 2). If one assumes a local PCO₂ of 120 mmHg and uses a solubility factor(s) for CO₂ of 0.0318 mM/
mmHg and a pK' of 6.133, one finds that the \([\text{HCO}_3^-]\) increases by 6 mM during the alkaline shift. After application of DIDS and amiloride, however, the values are 16 and 10 mM, respectively (209). The anoxic alkaline shift could be abolished by pretreatment with Mn²⁺ in the rat cerebellum (153), which points toward involvement of Ca²⁺ channels. In rat cerebral cortex, Mn²⁺ was without effect (209).

The alkaline shift is followed by an acidic shift, which further lowers the pH. The maximal pH value in ischemia depends on the preischemic glucose content in brain (183, 264). If a trickling blood flow is maintained during anoxia, the continuous delivery of glucose causes further lactic acid accumulation (247).

C. Interstitial Ion Concentrations During Spreading Depression of Leão

Because the mechanisms responsible for the ionic shifts in anoxia are still unresolved, it may be profitable to examine other situations with large ion shifts. The phenomenon of SD has been thoroughly described (43, 170–172, 196, 230). The striking similarities between the events that occur in SD and in brain anoxia suggest that the mechanisms controlling the ionic shifts in the two events are similar.

Spreading depression of Leão represents a chain of events that can be elicited in most parts of the CNS (including the retina) by chemical, electrical, or mechanical stimulation (43). Nicholson and Kraig (220) have specified several characteristics of SD. As the term implies, the chief feature of SD is a transient reduction of EEG activity and of evoked neuronal activity, spreading like a wave from the site of origin. A concomitant transient increase in \([\text{K}^+]\), to at least 20 mM occurs and is accompanied by a transient negative deflection in the DC potential (when recorded against a remote electrode) of at least 10 mV. The propagation rate is ~3 mm/min, and the event is followed by a refractory period of 1–2 min. If the changes are not transient, it is likely that anoxia or hypoglycemia prevails.

During SD in the rat brain cortex there is a characteristic pattern of interstitial ion concentrations that resembles that seen during anoxia (114). The negative deflection in the DC potential is accompanied by a rapid increase in the \([\text{K}^+]\), from 3 to ~60 mM within a few seconds and by a decrease in the \([\text{Na}^+]\), the \([\text{Cl}^-]\), and the \([\text{Ca}^{2+}]\), to levels similar to those observed during anoxia (Fig. 2; Table 3; 114). After ~0.5–1 min there is a spontaneous normalization of the ion concentrations (114, 154).

It is evident that the process is spontaneously self-limiting. The similarities between the ionic changes in SD and in anoxia (phase 2) suggest that both events have common mechanisms. In SD (as in anoxia) there is an early increase in the \([\text{K}^+]\), to ~10 mM before the concentrations of the other ions change. In SD, however, the early small \([\text{K}^+]\), increase lasts only a few seconds before it changes to a rapid rise (114, 220). The lack of recovery in interstitial ion concentration in persisting anoxia, in contrast to the tran-
sitory nature of SD, is clearly due to the lack of an energy supply. However, the cause of the initial functional derangement and of the later spontaneous restitution remains a mystery. Consequently, although the two abnormalities resemble each other and may have common explanations, the common denominator is not evident.

D. Brain Interstitial Space Volume During Anoxia

To understand the quantitative ionic changes in the interstitial space, the respective distribution volumes of the ions, both extracellular and intracellular, must be known. Under normal conditions the cell volume is precisely regulated and in part determined by a "double Donnan system" (193), in which the extra osmotic pressure exerted by the intracellular macromolecules is counteracted by the excess osmotic pressure from $[\text{Na}^+]$ and $[\text{Cl}^-]$; hence the maintenance of cell volume requires the presence of an intact energy metabolism fueling the Na$^+$ pump.

Two mechanisms contribute to cell swelling during anoxia. First, the depletion of energy stores and the consequent arrest of active ion transport lead to dissipative ion movements, resulting in colloid-osmotic swelling due to the presence of intracellular, nonpermeant macromolecules (169, 288). Second, there is an intracellular production of osmotically active particles by anaerobic metabolism, in the form of lactic acid and other metabolites (289).
Fluid displacements occur rapidly between extra- and intracellular compartments. Therefore traditional tracer methods for determination of extracellular space volume are useless because of the long equilibration times they require (11, 176). A rapid resolution has been obtained with extracellular markers, which can be sensed by ISM (70, 113, 221). These markers are charged and include choline \([(\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2\text{OH}]\) and tetramethylammonium \((\text{CH}_3)_4\text{N}^+\) (70, 113, 221). They enter cells slowly because they are polar in nature (113, 221), and accordingly they have a low permeability through the blood-brain barrier. They must therefore be introduced into brain ISF by either superfusion of the brain or iontophoresis (70, 113, 221). The level of the extracellular marker concentration during steady-state conditions is a relative measure of the interstitial volume. Any subsequent change in the marker concentration is inversely related to the volume of the space. Figure 3 shows the extracellular choline concentration during complete cerebral ischemia in the rat. During phase 2 there is a small increase, elevating the concentration by ~20%. During anoxic depolarization the concentration rapidly doubles and then decreases more slowly. Thus the interstitial space shrinks significantly during anoxia (initially only modest shrinkage occurs, but later a fast, pronounced decrease nearly halves the size of the space). The method, however, does not permit an unequivocal quantitative assessment of the volume changes. An increase in cellular marker permeability may allow the transport of marker into cells, as illustrated by the decrease in concentration after anoxic depolarization (Fig. 3). Although the method may have some limitations, it nevertheless represents the first in vivo determi-

**FIG. 3.** Interstitial concentration of choline ([choline]) in rat cerebral cortex after cardiac arrest (arrow). Because choline is an extracellular marker, its concentration is a relative measure of interstitial volume. Before cardiac arrest, brain was superfused with isotonic mock cerebrospinal fluid in which 50 mM NaCl was replaced by 50 mM choline chloride. Concentration was measured at depth of 500 μm by ion-sensitive microelectrode with liquid exchanger (Corning 477317). Lower curve shows change in interstitial electrical potential (DC potential). Note rapid rise in marker concentration at time of anoxic depolarization (phase 2), indicating 50% reduction in interstitial space. Ensuing concentration fall probably reflects increase in cellular permeability to choline. [From Hansen and Olsen (113).]
nation of extracellular space shrinkage. In anoxia there is first a slow increase in tissue impedance, then a fast rise at the time of anoxic depolarization (135, 173, 296, 303). With this technique and mathematical models the size of the interstitial space could be estimated (50). The equations employed assume that 1) suspension of cellular elements of a given shape is uniform; 2) the shape of the cellular elements in question remains the same; 3) the membrane ion permeability is unaltered, so that current passes exclusively through the extracellular space; and 4) the total ion concentration, i.e., the ISF conductivity, remains constant. None of these criteria is necessarily fulfilled during either normal conditions or anoxia. Probably the impedance method has served its purpose and should now be replaced by ISM techniques.

The shrinkage of interstitial space and the cellular swelling during anoxia are now firmly established. It is not known, however, whether neurons, glial cells, or both are involved. Earlier studies in which the brain was prepared for electromicroscopic analysis showed virtually no interstitial space (133), because the above-mentioned fluid distribution had already taken place. Fixation of the tissue in vivo cannot prevent this process (302). The elaborate technique used by Van Harreveld and associates (295, 296, 299), consisting of rapid in situ freezing followed by freeze-substitution or freeze-drying, is probably the best way to preserve the in vivo size of the cellular elements. In agreement with the results obtained with extracellularly distributed marker molecules, morphological studies reveal an interstitial space of ~20% (mouse brain cortex). After anoxic depolarization the size of the space decreases to <6% (299). The swollen cellular elements are preferentially apical dendrites; only minor swelling of glial elements is observed (295).

The changes in the cortical interstitial space during SD are similar to those in anoxia. The marker space decreases (113) and the impedance increases (130) simultaneously with the occurrence of the negative change in the DC potential. In addition, morphological studies demonstrate a swelling of apical dendrites (301). Thus the changes in the interstitial space in anoxia and in SD are related to ion movements, mainly through the membranes of apical dendrites.

The movements of ions between interstitial space and cellular compartments during ischemia can be calculated from the time course of the concentration and volume changes described above (see Table 5). Currently it is not possible to distinguish between fluxes across glial membranes and those across nerve membranes. In one study, however, uptake of K⁺ in Müller's cells (glia) during SD was demonstrated in the frog retina by measurement of the intracellular [K⁺] with a K⁺-sensitive microelectrode (207). From a resting [K⁺] of ~80 mM, the initiation of SD caused a concentration rise of 50–100% accompanied by depolarization. The cells, however, deteriorated rapidly, precluding a thorough study of the sequence of events. This interesting report supplements the observation of K⁺ uptake into glial cells during neuronal activity (52), but it must be regarded as preliminary and should be repeated.
E. Interstitial Ion Inventory

The interstitial ion concentrations as measured with ISM (Table 1) indicate an anion deficit of ~25 mM in the normal state. Calculation via the Henderson-Hasselbalch equation of the $[\text{HCO}_3^-]$ from the pH, and arterial $\text{PCO}_2$ verifies that $\text{HCO}_3^-$ covers the major part of the anion gap. Thus Na$^+$, K$^+$, Cl$^-$, and $\text{HCO}_3^-$ form the bulk of the ions in the interstitial space. The osmolarity calculated from the concentrations of these ions, with an osmotic coefficient of 0.9, agrees with the reported CSF and brain tissue values of ~300 mosM (137, 214). Because the membranes of mammalian cells are highly permeable to water generally (139), the intracellular osmolarity is also ~300 mosM, but the ion composition is not well characterized. Most of the cations are K$^+$ and Na$^+$. The anions remain largely unidentified, although they are considered to be rather large polyvalent molecules.

The ion movements that take place in phase 2 induce changes in the ion composition of the ISF that seem to violate the principles of osmotic balance and electroneutrality (Table 3).

1. Osmotic balance

The values in Table 3 indicate that large reductions in interstitial osmotic pressure and in ionic strength occur in anoxia. The sum of the $[\text{Na}^+]$, the $[\text{K}^+]$, the $[\text{Cl}^-]$, and the calculated $[\text{HCO}_3^-]$ is 200-250 mM, implying that 50 mosM are missing from the interstitial space to match the number of osmoles in the control brain. Because brain osmolality increases by 50 mosM after 1 h of complete cerebral ischemia (137), the number of osmoles required is apparently higher. The high osmotic water permeability of mammalian cells (139) ensures that osmotic disequilibrium between intra- and extracellular compartments rapidly dissipates. In addition the cell membrane cannot sustain any significant hydrostatic pressure difference. Consequently the cells undergo colloid-osmotic swelling, which continues until either the cells burst or the ISF is engulfed. The latter is more likely because of the limited ISF volume. Therefore the apparent osmotic imbalance across the cell membranes could in part be maintained by constraints in the interstitial space. The presence of an extracellular matrix (cell surface coat) composed of negatively charged glycoproteins and glycolipids may hamper the shrinkage of the interstitial space and preserve a distribution volume for solutes outside the cells (190, 219).

2. Electroneutrality

The values in Table 3 indicate that there is an anion deficiency of ~50 mM, 20 mM can be accounted for by the $[\text{HCO}_3^-]$. The lacking solutes may be amino acids (glutamate and aspartate), which are negatively charged at
the prevailing pH and together comprise \( \sim 15 \) mM in the cerebral cortex \((227)\), and lactate, which accounts for 10–15 mM in complete cerebral ischemia \((266)\). This argument presupposes that these substances can be transferred quickly to the interstitial space. Supporting evidence indicates that glutamate diffuses out of the cells during cerebral ischemia \((69)\).

The problems encountered with the interstitial ion inventory in anoxia are similar to those in SD \((114, 154, 220)\). Spreading depression is also accompanied by an efflux of glutamate from the cells \((300)\). Table 3 shows the interstitial ion concentrations at the peak of SD.

IV. BRAIN ENERGY METABOLISM AND ION TRANSPORT

A. Energy Requirement for Ion Transport

Glucose is the main metabolic substrate for the adult brain; oxidation of 1 mol of glucose supplies \( \sim 35 \) mol of ATP \((266)\). The relation between ion transport and energy metabolism is complicated and far from clear. This is partly because cellular ion fluxes in the intact CNS cannot yet be measured. It is assumed that the high rate of energy metabolism in the brain is largely used in active ion transport, counteracting dissipative ion fluxes across the cell membranes. The intracellular sequestration of \( \text{Ca}^{2+} \), which provides an extremely low cytoplasmic \([\text{Ca}^{2+}]_c\), is an important energy-requiring process.

To evaluate the steady-state situation it is instructive to look at the factors determining the passive transmembrane fluxes of ions. The following equation gives the passive net flux \((J)\) of \( \text{Na}^+ \)

\[
J_{\text{Na}^+} = A \times P_{\text{Na}^+} \times \frac{[C]}{[C]} \frac{\exp(-VF/RT)}{1 - \exp(-VF/RT)}
\]

where \(J_{\text{Na}^+}\) is the net flux of \( \text{Na}^+ \); \(A\) is the area of cell membranes; \(P_{\text{Na}^+}\) is the membrane permeability; \(V\) is the transmembrane potential; \([C]\) and \([C]\) are the concentrations in interstitial and intracellular compartments, respectively; \(R\) is the gas constant; \(T\) is absolute temperature; and \(F\) is Faraday constant.

With a membrane potential of \( -70 \) mV, the value of \(VF/RT\) at \(37^\circ \text{C}\) is 2.62 and the value of \(\exp(-VF/RT)\) is 0.073. The \([\text{Na}^+]_o\) is 155 mM, and the \([\text{Na}^+]_i\) is 30 mM (cf. Table 5). With a \(P_{\text{Na}^+}\) of \(3 \times 10^{-6}\) cm/s (see ref. 140 for squid axon) and a membrane area per cubic centimeter of brain of \(\sim 50,000\) cm\(^2\) \((87, 133)\) the passive \(J_{\text{Na}^+}\) per gram of tissue per minute is calculated to be \(37\) \(\mu\)mol. It is commonly accepted that three \( \text{Na}^+ \) are transported per molecule of ATP split. A net dissipative \( \text{Na}^+ \) transport of \(37\) \(\mu\)mol \(\cdot \text{g}^{-1} \cdot \text{min}^{-1}\) requiring an active \( \text{Na}^+ \) transport of a similar magnitude would need an ATP hydrolysis at a rate of \(12\) \(\mu\)mol \(\cdot \text{g}^{-1} \cdot \text{min}^{-1}\). With a P:O ratio of 3 \((266)\) the \( \text{O}_2 \) consumption is \(2\) \(\mu\)mol \(\text{O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}\) and \(22.4 \times 2 \times 100\) \(\mu\)l \(100\) g\(^{-1} \cdot \text{min}^{-1} = 4.5\) ml \(\text{O}_2 \cdot 100\) g\(^{-1} \cdot \text{min}^{-1}\), assuming 100% efficiency in the energy
conversion. The O$_2$ consumption in the rat brain is 10 ml O$_2$·100 g$^{-1}$·min$^{-1}$ (80), and therefore Na$^+$ transport requires about one-half of the total O$_2$ consumption. Although the calculation rests on a somewhat uncertain basis (due to the ambiguous values of the participating factors), the calculation nevertheless confirms the supposition that a large percentage of brain energy metabolism is required for ion transport.

Because neuronal activity is associated with increased ion fluxes, energy metabolism is expected to be augmented in these conditions. The discovery of a close coupling between the functional activity in brain and glucose consumption (271) and of the relationship between the [K$^+$], and O$_2$ consumption (179) supports this notion but does not allow quantitative assessment of the coupling.

The subject may be clarified by separating the total energy brain metabolism into basal metabolism (maintenance processes in an electrically silent brain) and activation metabolism (supporting processes related to functional activity) (203). Inhibition of the membrane-bound ATPases carrying out transport of Na$^+$ and K$^+$ has been used to assess the percentage of energy metabolism expended by ion transport (296). In cortical slices from the rat brain, Whittam (310) measured a 40% reduction in oxygen uptake when the slices were either treated with ouabain or incubated in sodium-free solutions. A similar reduction has been obtained by Ritchie and Straub (252) in unmyelinated nerve fibers exposed to ouabain and by Astrup et al. (13) in the EEG-silent canine brain. Nerve tissue in vitro and the EEG-silent brain require only one-half of the normal energy metabolic rate (266). These experiments illustrate the impact of ouabain on basal metabolism and may not be particularly relevant in the brain in vivo.

It is important to notice that there is a linear relationship between spike frequency and glucose utilization in cervical ganglions (316) and that the poststimulation increase in O$_2$ uptake is abolished by ouabain treatment (252). These findings support the concept that the increased metabolic rate in response to stimulation results from the extra load on the Na$^+$-K$^+$ pump and that ion transport demands a substantial part of brain metabolism. In contrast, Na$^+$-K$^+$ transport in liver (88) and in quiescent striated muscle (58) requires only $\sim$5% of the O$_2$ consumption.

B. Ion Movements and Energy Metabolism in Anoxia

In the ischemic brain, ATP regeneration occurs via anaerobic conversion of endogenous substrates. The brain contains only small O$_2$ stores. The blood in the microvessels in the brain contains $\sim$150 ml O$_2$/liter, corresponding to 0.15 ml O$_2$/100 g brain, because the blood volume in brain is $\sim$1%. With a mean P$_{O_2}$ in brain of 20 mmHg (189), a solubility coefficient of 0.022 ml O$_2$/ml H$_2$O atm (284), and a water content of 80%, the amount of dissolved O$_2$ is 0.05 ml/100 g. Therefore the stores of O$_2$ are 0.2 ml/100 g and can support normal O$_2$ consumption for only a few seconds. Consequently the
value of \( P_0 \) in the brain falls to zero after a few seconds of ischemia (248). Anaerobic glycolysis only yields 2 mol of ATP per mol of glucose (3 ATP starting from glycogen). The rate of anaerobic glycolysis would have to increase \( \sim 15 \) times to maintain the preanoxic rate of ATP regeneration—obviously an impossibility. The endogenous stores of ATP, ADP, and phosphocreatine (PCr) are soon drawn on. The rate of cleavage of high-energy phosphate bonds (\( \Delta \sim P/\Delta t \)) can be assessed in a closed system by measuring the change in the sum of the concentration changes in the following metabolites (188)

\[
\Delta \sim P/\Delta t = (2\Delta [ATP] + \Delta [ADP] + \Delta [PCr] + 2.9\Delta [glucose] + 2.9\Delta [glycogen])/\Delta t
\]

The equation takes into account that ATP contains two high-energy phosphate groups, PCr and ADP (one of each), and that anaerobic conversion of glucose and glycogen yields two and nearly three high-energy phosphate groups, respectively (188). The method involves determination of the metabolites from several brains at various times after induction of ischemia. Apart from determination of the heat-accumulation rate (86), this method is the only way to assess the metabolic rate during ischemia.

In the initial period of ischemia the \( \Delta \sim P/\Delta t \) in rat brain cortex is \( \sim 30 \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \), which is comparable with the preanoxic value (266). The cortical concentrations of energy-rich metabolites (\( \mu \text{mol/g} \)) are as follows: ATP, 3; PCr, 5; ADP, 0.3; glucose, 3; and glycogen, 2. In anaerobiosis this allows a maximal yield of high-energy phosphate groups of \( \sim 25 \mu \text{mol/g} \), sufficient to support energy metabolism for almost 1 min in ischemia. The rate of cleavage of high-energy phosphate groups, however, declines rapidly after induction of brain ischemia and is only one-third of normal after 10 s. In the second minute it is one-sixth of normal, and after 2 min it is nearly zero (188, 228).

Studies in cats, baboons, and humans with reduced cerebral blood flow indicate one flow threshold for maintenance of EEG and evoked responses and another threshold for \( K^+ \) homeostasis (18, 36, 120, 290). When cortical blood flow is reduced to 15–20 ml \cdot 100 g^{-1} \cdot \text{min}^{-1} (30% of normal), EEG activity and evoked cortical responses disappear, but the \( [K^+]_0 \) level is still normal or slightly elevated (18, 36), whereas a reduction to \( \sim 10\% \) induces a pronounced increase in \( [K^+]_0 \) (18, 36).

The total \( O_2 \) supply at the two flow-threshold values is \( \sim 3 \) and \( \sim 1.5 \) ml \cdot 100 g^{-1} \cdot \text{min}^{-1}, respectively, which corresponds to an \( O_2 \) consumption of approximately two-thirds and one-third of the human cortex if the extraction fraction of \( O_2 \) were 1 (165). This is not the case, and the values are accordingly lower, but precise values are not available. The metabolic rate for brain cortex in the ischemic rat brain is reduced to 10–20% at the end of the initial period (phase 1) (112). At this time the ATP level is reduced to 10–20% of its normal value (12, 112). Thus the rapid increase in the \( [K^+]_0 \) takes place only when the rates of ATP regeneration and \( O_2 \) consumption have fallen
to extremely low values, but other mechanisms responsible for neuronal transmission (e.g., early cessation of EEG and synaptic transmission) are much more sensitive.

The quantitative link between energy metabolism and ion homeostasis in brain during ischemia can be evaluated from other studies. If the content of glucose in rat brain is increased by augmenting the plasma concentration of glucose (183), the duration of phase 1 is doubled, whereas the opposite occurs in hypoglycemic animals (Fig. 4; 108, 264). The $[K^+]_i$ at the end of phase 1 remains within 7-10 mM, despite a variation in the duration of this phase. These results are explained by the changed substrate stores available for immediate anaerobic glycolysis. The rate of decline in ATP during ischemia is significantly affected by the glucose level in the brain, but the level of ATP at the end of phase 1 is the same in normo- and hyperglycemic animals (112). The $[Ca^{2+}]_i$ remains at the normal level during a prolonged phase 1 in hyperglycemic rats (264). The fact that phase 1 can be prolonged by increasing the glucose stores shows that it is not the metabolic apparatus but the stores that are the limiting factor. As discussed in section vB, the large capacity in cold-blooded animals for maintaining interstitial ion concentrations in the brain during anoxia is due to the large stores of glycogen.

The ability to keep a certain level of ATP in the brain may be important for ion homeostasis in two ways: to provide sufficient substrate for transport ATPases and to keep the membrane constituents in a phosphorylated state to maintain membrane permeability properties. It is not yet possible to distinguish between the two possibilities, but studies in heart muscle have shown that the reduction in the height and duration of an action potential during anoxia can be abolished by increasing the glucose concentration in the medium (198, 283), implying an effect of anaerobically generated ATP on membrane permeability properties (198).

![Graph](http://physrev.physiology.org/)

**FIG. 4** Importance of glucose content in brain to rate of increase of interstitial $K^+$ concentration ($[K^+]_i$) in rat brain cortex after cardiac arrest (here induced by intravenous injection of saturated MgCl₂ solution). Brain glucose content was changed by intraperitoneal administration of either glucose or insulin. [From Hansen (108).]
The importance of ATP during anoxia is also demonstrable in brain slices, where the ability to maintain evoked responses in anoxia is prolonged by pretreatment of the slices with creatine (182). This exposure leads to a fivefold increase in PCr, and the ability to regenerate ATP during anoxia is enhanced. Surprisingly ion homeostasis in brain slices has not been investigated during anoxia.

The above experiments illustrate that augmented capacity for ATP regeneration preserves the brain interstitial ion concentration during ischemia. It is not known whether there is a causal relationship or just a covariation with some unknown factor that is more directly involved. It is certain that other mechanisms do operate. In rats, where the preischemic cerebral metabolic rate is changed by analeptics, sedatives, cooling, and heating, the duration of phase 1 is inversely correlated with the preischemic O2 uptake (15). Because the stores of carbohydrates and high-energy phosphates in brain were almost the same in the different groups, the authors ascribed the varying rate of rise in the [K+]o and the different duration of phase 1 to changes in general membrane ion permeability. Thus the preischemic O2 uptake of the brain may have an impact on the membrane ion permeability. Accordingly it has been reported that the rate of decline of ATP in brain cortex is faster in animals with high preischemic O2 uptake (225).

The fast rise (phase 2) is consistently found in lissencephalic brains of small animals but not invariably in gyrencephalic brains (sect. vB). In a tissue like skeletal muscle, the rate of rise in the [K+]o during anoxia is much slower than in the brain. After 10 min of circulatory arrest, no significant increase is seen (268). The increase in impedance (sect. III D) also occurs much more slowly in other organs (298). The initiation of phase 2 was formerly ascribed to the severe reduction in ATP levels in the brain and to the ensuing insufficient ion-pumping activity. A simple calculation shows that an increase in the ionic permeability is necessary. Suppose that 90% of the ATP hydrolysis in the brain cortex (30 μmol·g⁻¹·min⁻¹) is related to maintenance of ion gradients across cell membranes. The Na⁺-K⁺-ATPase uses 1 mol of ATP to transport 3 mol of Na⁺ out of and 2 mol of K⁺ into the cell. The pump counteracts equivalent leaks that, in an interstitial space of 20%, would increase the [K⁺]o, at a rate of 5 mM/s and decrease the [Na⁺]o, at a rate of 7 mM/s. These calculated values are much lower than the maximal rates determined experimentally [Δ[K⁺]o, 80 mM/s (108); Δ[Na⁺]o, 100 mM/s (A. J. Hansen, unpublished observation)]. The reduction in the interstitial volume during phase 2 augments the rate of rise but can only explain part of the discrepancy between observed and calculated values (113). Other than the mechanisms mentioned, the most likely explanation for the fast ionic changes during phase 2 is an increase in the ion permeability of the cell membrane. No hard data are available on this important point, but studies

1 Lissencephalic brains have a smooth surface; gyrencephalic brains have gyri and sulci.
during a similar rapid change in ion concentrations during SD support this suggestion (see sect. VII).

Most studies dealing with the $[K^+]_o$ changes during anoxia show that the $[K^+]_o$ remains below 10 mM during phase 1, irrespective of the duration of this phase; i.e., above this ion concentration, the pathophysiological disturbance becomes regenerative and self-amplifying (15, 107, 108, 305). This concentration is similar to the one described as the ceiling level of the $[K^+]_o$ during functional activity (see sect. III). Any acute elevation above this ceiling leads to an SD response of the $[K^+]_o$ (114, 186, 220).

The nature of the couple between the $[K^+]_o$ and the ATP level in brain is still only speculative. Elevation in the $[K^+]_o$ stimulates the Na$^+$-K$^+$-ATPase, increasing the decline in the ATP level. Because the ATP level of the cell in some unknown way is important for the K$^+$ permeability and for general membrane permeability (sect. viD), $K^+$ is lost from the cells. The relation among the levels of the $[K^+]_o$, ATP, and permeability may explain the progressively increasing rate of increase in the $[K^+]_o$ in anoxia.

During hypoglycemia the series of events is similar: 1) an arrest of EEG, 2) a slow increase in the $[K^+]_o$ (14), and 3) a steep increase in the $[K^+]_o$ (14) and an increase in cortical impedance (237). These changes occur without interfering with the supply of $O_2$ and are readily reversed by supply of glucose (14, 237).

C. Energy Metabolism During Spreading Depression

The similarities between ionic changes in anoxia and in SD suggest a common genesis. This is surprising because it is difficult to envisage similar drastic changes in the supply of ATP and other energy-rich compounds during the rather brief transient condition present in SD. Determinations of cortical metabolites in the rat brain show only small decreases in the level of energy-rich phosphates before or at the moment of the steep ionic changes, similar to the anoxic phase 2 (155, 244). These observations strongly suggest that marked interstitial ion changes can occur without a significant decrease in substrates for ion pumping—a surprising finding that is difficult to reconcile with conventional concepts of cause and effect in ionic derangements. It is as if some other effective mechanism or intermediary compound controls membrane permeability. Despite the continued blood supply during SD, the rate of glycolysis rises before the marked ionic events occur (100), and in both conditions an accumulation of cyclic AMP is observed (156, 259, 278); the significance of this finding is obscure.

V. COMPARATIVE ASPECTS OF ION CHANGES DURING ANOXIA

A. Young Animals

The immature brain tolerates anoxia longer than the adult brain (66, 82); therefore it is interesting to see whether young and newborn animals
have better ion homeostatic capabilities during ischemia. A much slower rate of increase in the [K⁺], in the newborn cerebral cortex during anoxia than in the adult amply supports this possibility (107, 195). The slow rise in the [K⁺], lasted 25 min in a 4-day-old rat during nitrogen inhalation, compared with 2 min in an adult (107), and was followed by a faster rate of increase accompanied by a negative deflection in the DC potential. The fast rate of the [K⁺]ᵢ rise was slower than in adults and started at the higher level of 30 mM. This high concentration parallels the finding of an increased ceiling level of the [K⁺]ᵢ during activity in the immature nervous system (56, 208). The long duration of phase 1 is in part explained by the more anoxia-resistant cardiovascular system in the immature animals (66), which ensures continuous substrate supply for brain energy metabolism during anoxia. This is illustrated by the observation that when ischemia is established, phase 1 is shortened to 10 min in the 4-day-old rat.

The better K⁺ homeostasis in the immature brain during ischemia is not well understood but may be explained by smaller demands for ion pumping in the immature brain. The neurons are smaller and less highly branched (75) and there are fewer synapses (2), indicating less functional surface for ion dissipation than in the adult. In addition the immature brain has a larger interstitial space (28). The stores of energy-rich substrates in the immature brain are not larger than those in the adult, but the rate of energy metabolism in the immature brain is only 5–20%, depending on age (74, 112, 132). The fact that the glycolytic capacity in the immature brain is sufficient to regenerate ATP at ~50% of the normal rate (74, 112), compared with only 25% in the adult brain (228), may be important in this connection.

B. Cold-Blooded Animals

The ability of cold-blooded animals to withstand long periods of anoxia is remarkable (127). Their ability to maintain the [K⁺]ᵢ near the normal value during anoxia is equally impressive. During nitrogen inhalation the [K⁺]ᵢ of the telencephalon in the turtle (Pseudemys scripta) never exhibits a steep increase and increases only by a few millimoles per liter even when the anoxic period is extended to 4 h (260). The explanation for this exceptional ability to keep the brain [K⁺]ᵢ low during anoxia touches on several aspects of turtle brain energy metabolism. In turtles the rate of energy metabolism is only ~2% of that in rats (199). The brain is equipped with unusually large stores of glycogen (eight times the amount in rats), which is the main reason for the potential of high-energy phosphate group generation of 70 µmol/g—about three times that of the rat (sect. IVB). Because of the high anaerobic glycolytic capacity, it is possible to maintain ~50% of the normal rate of energy metabolism via this route during anoxia (199). Accordingly the ATP content after decapitation in the turtle declines very slowly and attains ~10% of the initial level after only 4 h (199). The importance of glycolysis for the impressive ion homeostasis during anoxia becomes apparent with inhibition of the glycolytic pathway. When the turtle brain is superfused
with iodoacetate, the effect of anoxia on the brain \([K^+]_o\) becomes qualitatively similar to that on the rat brain \([K^+]_o\) (260).

C. Gyrencephalic Animals

The changes observed in gyrencephalic animals are comparable with those obtained in rats, but there are quantitative differences. Occlusion of the middle cerebral artery in cats and baboons (18, 115, 279) or nitrogen inhalation in cats is followed by a slow initial rate of rise succeeded by a faster rate of rise in the \([K^+]_o\) (25). The \([K^+]_o\) threshold at which the fast rate of rise starts corresponds to that in rats; it is 6–10 mM in rats (25) and 13 mM in baboons (115). The different phases are considerably longer than in the rat, but direct comparison is difficult because of differences in the cardiovascular tolerance to anoxia and to the uncharacterized collateral blood supply that occurs with middle cerebral artery occlusion.

Some workers were apparently unable to demonstrate a biphasic increase in the \([K^+]_o\) during anoxia or ischemia in cats and dogs (17, 135). Even though the \([K^+]_o\) increases beyond the ceiling level, an SD type of response cannot be elicited. Thus the larger brain in gyrencephalic animals does not invariably show the characteristically rapid increase in the \([K^+]_o\) during anoxia. The fact that eliciting SD in gyrencephalic animals requires more extensive noxious stimuli than in lissencephalic animals like the rat might thereby be important (43, 196). Spreading depression has, however, been demonstrated in most animals and possibly in humans (276).

D. Regional Differences in the Brain

For practical purposes, most studies dealing with interstitial ion concentration in the brain have been conducted on the cerebral cortex. Bureš and Burešová (42) investigated regional differences in the \([K^+]_o\) increase in response to respiratory arrest. They observed the well-known response of a slow initial rise, followed by an abrupt increase in gray matter structures such as the neocortex, hippocampus, amygdala, caudate nucleus, and thalamus, whereas a considerably lower rate of rise during the second phase was demonstrated in the reticular formation, zona incerta, and hypothalamus. Again it seems likely that the same mechanisms controlling elicitation of SD operate because these latter structures have a low susceptibility to SD (48). Apart from this investigation, regional differences in the anoxic pattern have not been reported, although such studies might shed light on possible relationships between structure and function. Moreover it might be expected that different ratios between nerve and glial elements in individual regions might be reflected in the \(K^+\) response.

VI. CHANGES IN NEURONS AND GLIAL CELLS DURING ANOXIA

Most of the described ionic changes in the interstitial space are probably a consequence of events occurring primarily in nerve cell membranes and
only to a lesser degree in glial cell membranes. As noted in section IIIA, the severe ionic changes during anoxia occur somewhat later than the drastic changes in brain function, e.g., loss of consciousness and cessation of EEG activity. Subsequent sections review the possible cause of this surprising dissociation of events. First, the nerve cell and glial cell membrane potential changes are discussed, then the conditions for synaptic transmission, and finally the mechanisms for possible membrane changes.

A. Nerve Cell Membrane Potential

There are few detailed reports on this topic because of substantial technical difficulties [e.g., tissue movements and cellular swelling (sect. III D)], particularly with in vivo studies. The results of available studies differ somewhat. Initially a slight depolarization occurs in spinal and cortical neurons after the anoxic challenge (54, 55, 78, 152, 217). Later investigations found a hyperpolarization preceded by a depolarization, which was seen in spinal motoneurons (275), cortical neurons (101, 103, 104), and hippocampal neurons (110). Possibly the discrepancy is due to technical problems, because depolarization during anoxia is diminished when precautions are taken to reduce tissue movements (217). Because only a few investigations recorded the membrane potential with a reference electrode placed close to the recording electrode, which reduces the effect of variations of the interstitial DC potential, the studies are difficult to compare. All investigators agree, however, that the membrane potential of nerve cells in anoxia is quite stable for a few minutes before a fast depolarization occurs that coincides with the extracellular anoxic depolarization (55). These findings alone cannot explain the abrupt arrest of nervous activity; other mechanisms must be invoked.

B. Glial Cell Membrane Potential

Only one report is available on the response of glial cell membrane potentials to anoxia (102). It was found (not unexpectedly) that glial cells depolarized during the anoxic period, most likely because of the well-known sensitivity of the glial cell membrane potential to external K+ concentration (164). The glial membrane resistance fell by 30–40% (102).

C. Synaptic Transmission

At the onset of anoxia there is a transient increase in spike activity, which then ceases while the synaptic potentials abate (47, 104). The fact that spike generation fails at the moment when postsynaptic potentials can still be elicited (110, 274) suggests a particular sensitivity of the action-potential mechanism (274) but could equally be caused by inadequate synaptic transmission. Indeed, synaptic transmission is arrested in anoxia (54, 78, 104, 110, 143, 274). The mechanism behind this blockage is not firmly established.

Transmission failure has been ascribed to impairment in transmitter
synthesis, and the synthesis of several transmitters depends on O₂ availability. When glucose oxidation is reduced, even in mild hypoxia, the synthesis rate of acetylcholine (97, 98) and of other glucose-derived neurotransmitters is also reduced (99).

The rate-limiting enzymes (tyrosine and tryptophan hydroxylase) for the synthesis of dopamine, norepinephrine, and serotonin have a Michaelis constant for O₂ ($K_m$) of $P_{O_2} = 5-10$ mmHg (84, 92), which is quite near the $P_{O_2}$ level normally encountered in the brain (189); therefore the rate of synthesis declines during hypoxia (63, 64). Despite a dependence on O₂ availability, the reduced rate of synthesis has not been shown to have any impact on the transmission process. It may be that the reduced rate reflects, rather than causes, a lowered synaptic activity. Consequently the rate of synthesis can be increased by depolarization of brain tissue (205). The release of amino acid neurotransmitters in cerebellar slices is unaffected by 30 min of anoxia (29); in fact, the release of glutamate was augmented in anoxia (29, 69).

The transmission failure may instead be related to depolarization due to pump failure (78, 181), most probably occurring in the thin presynaptic terminals (54, 78). However, no direct evidence is available to support this hypothesis. The early neuronal response is a hyperpolarization (101, 104, 110, 274, 275), accompanied by a reduction of membrane resistance (110, 274), due to a selective increase in $K^+$ conductance (110), all of which are important factors in this connection. Hyperpolarization lowers the excitability of nerve cells, whereas increased membrane conductance to $K^+$ clamps the membrane potential and short-circuits the membrane, thereby attenuating potentials induced by presynaptic activity. This theory favors the postsynaptic site as the source of anoxic transmission failure. The observation that transmitter-evoked activity is blocked in anoxic nerve cells points in the same direction (102), as does the observation that presynaptic potentials persist when postsynaptic responses are abolished in anoxia (143).

The two key phenomena, hyperpolarization and lowered membrane resistance, have been reported after exposure of cortical neurons to metabolic inhibitors such as 2,4-dinitrophenol (102, 157, 163). Thus the loss of activity in nerve cells can currently be explained by hyperpolarization and by a reduction of membrane resistance. The site of action for this effect is the neuronal soma. The axon is much less susceptible to anoxia: an anoxic period of ~20 min elapses before action potentials and axoplasmic transport are blocked (1, 89, 175, 231), and the conduction block only occurs when the marked changes in interstitial ion concentrations take place (135).

D. Mechanisms of Membrane Changes

1. Initial changes

The central role of the $K^+$-permeability change in the reduced excitability makes it important to elucidate the underlying mechanisms. Several $K^+$
conductances have been demonstrated in nerve membranes activated by either voltage changes or chemical means (105, 121, 168, 200, 285). The suggestive hypothesis formulated by Krnjević (157) gives a central role to the Ca\(^{2+}\)-activated K\(^+\) conductance. The low intracellular activity of Ca\(^{2+}\) is the result of several processes: 1) ATP-dependent Ca\(^{2+}\) extrusion (73), 2) Na\(^+\)-Ca\(^{2+}\) exchange driven by the Na\(^+\) gradient (26), and 3) Ca\(^{2+}\)-uptake systems located in the mitochondria and in other cell organelles (27, 39, 174). The dependence of Ca\(^{2+}\) sequestration on ATP regeneration makes its intracellular level sensitive to the rate of energy metabolism (27, 39, 174). When it is compromised, Ca\(^{2+}\) is released from the organelles (19, 39), activating the K\(^+\) conductance in the cell membrane and thus decreasing excitability. In this situation the Ca\(^{2+}\) acts as a messenger between the metabolic apparatus and the excitable cell membrane, adjusting cell activity to the metabolic capacity. Intraneuronal injection of Ca\(^{2+}\) mimics the effect of metabolic poisoning (158, 201), and intracellular injection of the Ca\(^{2+}\) chelator ethylene glycol-bis(β-amino-ethylether)-N,N'-tetraacedic acid (EGTA) attenuates the 2,4-dinitrophenol effect (163), both in accordance with the Ca\(^{2+}\) hypothesis. The acidification of cells during anoxia may also contribute to the increase of intracellular [Ca\(^{2+}\)] via H\(^+\)-Ca\(^{2+}\) exchange in mitochondria (201).

A number of compounds induce hyperpolarization and the reduction of membrane resistance in nerve cells. Among these are general anesthetics such as fluorotane and ether (224) and opiates (238). It has been suggested that anesthetics and anoxia both activate K\(^+\) channels in the nerve cell membrane by releasing Ca\(^{2+}\) from intracellular stores (157).

Direct evidence for the [Ca\(^{2+}\)] change in nerve cells during anoxia is, however, still lacking. Simultaneous measurement of membrane resistance and intracellular Ca\(^{2+}\) activity during anoxia would be helpful. Also, nerve membrane resistance should be measured in anoxia during application of inhibitors of the Ca\(^{2+}\)-activated K\(^+\) conductance (e.g., quinidine; see ref. 85).

The fact that hyperpolarization and the reduction of membrane resistance in nerve cells of hippocampal slices were attenuated by aminopyridines but were left unchanged by blockers of synaptic transmission (Mg\(^{2+}\) and Mn\(^{2+}\)) and by tetrodotoxin precludes transmitter involvement (110). It also supports the hypothesis that K\(^+\) channels are involved in anoxic events. Aminopyridines are known blocking agents of a voltage-dependent K\(^+\) channel in molluscan (121, 285) and mammalian (105) neurons, but they do not interfere with the Ca\(^{2+}\)-controlled K\(^+\) channel (110, 121, 285). The blockage of the anoxically activated K\(^+\) conductance via aminopyridine conflicts with the theory that Ca\(^{2+}\) acts as the direct controller of K\(^+\) channels in anoxia, and thus other explanations are necessary.

Recently it was shown that 4-aminopyridine reduced the level of \(^{32}\)P incorporation in a 25,000-dalton phosphoprotein in nerve tissue (216). This effect of 4-aminopyridine and the previously described effect on K\(^+\) conductance in nerve cells during anoxia are in line with the hypothesis that part of the effect of anoxia on nervous tissue may result from an effect on protein
phosphorylation/dephosphorylation. The fact that the permeability of excitable membranes may be related to protein phosphorylation is in line with this hypothesis (218, 249). It is now well established that extracellular signals such as hormones and transmitters produce specific physiological responses in nerve cells involving phosphorylation processes (213, 309). The first messengers act by changing the intracellular levels of cyclic AMP, cyclic GMP, and Ca\(^{2+}\). The second messengers in turn produce their responses through activation of protein kinases, resulting in phosphorylation of substrate proteins, again followed by a specific biological response.

It is highly likely, although not proven, that anoxia can manifest its action in the nervous system via the system described above, because the start of anoxia immediately increases the level of cyclic AMP (259) and of intracellular Ca\(^{2+}\) (39). The ensuing fall in the ATP level probably influences the synthesis of cyclic AMP and protein phosphorylation (278).

Recently, a patch-clamp technique demonstrated ATP-dependent K\(^{+}\) channels in cardiac muscle cells (226). Treatment with cyanide or exposure of the inside of the membrane to ATP-free solutions activates the channels, whereas high ATP levels cause inactivation. This observation suggests that the action of anoxia on nervous function may be mediated through an activation of K\(^{+}\) channels, probably by a change in the state of phosphorylation of some membrane protein. Whether these channels also are present in the nerve membrane is not yet established.

Perhaps \(O_2\) in itself affects nerve cell excitability. This suggestion is partly based on the time relationship between the very fast decline in \(\Phi_{O_2}\) in brain and the arrest of the EEG activity (248). Because axons conduct impulses for several minutes in total anoxia and because the arrest of EEG activity in brain anoxia can be delayed by either increasing the stores of energy-rich substances in the brain or by lowering the metabolic rate (15, 108), \(O_2\) per se probably does not play a direct role. Evoked responses in brain slices maintained in vitro persist four times longer during anoxia when the stores of PCr are artificially increased before the anoxia (311). This result emphasizes that nervous tissue is able to maintain a high rate of ATP regeneration, and that it is the ATP level that is critical for the excitability of the mammalian brain during anoxia and not the \(P_{O_2}\) itself.

2. Late changes

When the anoxic period is sufficiently long, the early hyperpolarization or slow depolarization is followed by a much faster rate of depolarization (54, 55, 104, 110, 274). Possibly neurons become critically depolarized by the elevated [K\(^{+}\)], during phase 1, and the rapid and gross ion shifts are related to those occurring during an action potential, caused by abnormal opening and closing of voltage-dependent channels. However, the routes for the ionic movements are not firmly established. The simultaneous movements of all interstitial ions during phase 2 suggest an unselective permeability increase.
different from the activation of voltage-dependent channels (see sect. III.B). This specific problem, however, has not been addressed. Unselective cation channels activated by an increase in intracellular [Ca\(^{2+}\)] have been demonstrated in nerve cells (317), but their significance in this respect remains unknown. Transmitter-operated channels activated by a synchronous release of excitatory and inhibitory transmitters is a possible explanation and may account for both cation and anion movements. Iontophoresis of glutamate in the interstitial space of the cat cerebral cortex causes a decline in the [Ca\(^{2+}\)], similar to that observed in ischemia, and a less-pronounced fall in the [Na\(^+\)] (118). Studies of nerve membrane resistance in anoxia have not been particularly helpful. The early reduction in resistance is followed by an almost complete loss of the resistance in the initial phase (110), which precludes detection of any additional decrease in membrane resistance by this technique.

There are, however, signs of an increased membrane permeability in brain during anoxia. Under these conditions, retinas kept in vitro display an increased distribution volume for mannitol in absolute terms and also relative to the inulin space (7, 236). In fact the cell membrane permeability to ions and nonelectrolytes (e.g., uridine and deoxyglucose) increases when the intracellular level of ATP is lowered (194, 256). Again it is tempting to invoke the involvement of protein phosphorylation for the permeability changes observed in anoxia.

The cellular elements responsible for the ionic fluxes in anoxia are thought to be the neurons because (unlike astrocytes) they are able to vary their ionic conductances (164). In small animals the astrocytes comprise only 10–20% of the brain volume, in agreement with the supposition that these cells are of minor importance with respect to the marked ionic fluxes in anoxia. Changes in the membrane structure of astrocytes have, however, been disclosed by the freeze-fracture technique after a few minutes of anoxia (166)—an interesting finding that calls for renewed study of glial cell function in anoxia.

Rigorous data on membrane permeability in brain anoxia are still scanty. Therefore studies of SD may be useful if similar mechanisms operate in the two conditions.

VII. MEMBRANE CHANGES DURING SPREADING DEPRESSION

The fast ionic changes in SD are accompanied by a depolarization of nerve and glial cells (280). The membrane resistance of a nerve cell is reduced and nearly completely lost during depolarization (270), whereas the membrane resistance of the glial cell is only slightly decreased (207, 281).

Studies with foreign probe ions have given the most significant results. Phillips and Nicholson (239, 240) and Nicholson and Kraig (220) measured the interstitial concentration of different-sized ions in the rat cerebellum during SD. The low cell permeability to these ions prevents them from entering
the cells during normal conditions. To the extent that the cells remain impermeable during SD, the ions are concentrated because of the shrinkage of the interstitial space (sect. III D), whereas a permeability increase would cause their concentration to fall. The largest anion found to leave the interstitial space during SD was hexafluoroarsenate (0.635 nm diam), and the smallest anion to be concentrated was hexafluoroantimonate (0.73 nm diam). No probe cations entered the cells; the smallest to be concentrated was tetramethylammonium (0.70 nm diam; 239, 240).

These results are important because they imply that the ionic shifts during SD are not the result of a total breakdown of membrane integrity but rather are due to activation of a specific permeability mechanism, excluding ions above a certain size, thus preserving some selectivity.

The molecular mechanisms of the permeability changes are far from established. The involvement of the classic ion channels is not proven but cannot be crucial, as evidenced by two facts: the diameters of the K⁺ channels (0.3 nm) and the Na⁺ channels (0.3 × 0.5 nm) (124) are obviously too small to allow passage of the probe anions mentioned above. Also tetrodotoxin can prevent neither the occurrence of SD (280) nor the decline in the [Na⁺] during SD (287). This does not preclude activation of these channels during SD but rather suggests that they are of minor importance. Instead of invoking an unknown permeability mechanism, Nicholson and Kraig (220) suggested that the channels activated during SD are transmitter dependent, in line with the glutamate theory by Van Harreveld (297). However, they implicated both excitatory and inhibitory transmitter-operated channels. The initiating mechanism for transmitter release might be the initial slow increase in the [K⁺]₀ in SD (114, 154). This induced depolarization of presynaptic terminals might activate Ca²⁺ channels, and the Ca²⁺ influx would in turn cause the release of both excitatory and inhibitory transmitters from the nerve endings. The excitatory transmitters acting on Na⁺ and Ca²⁺ channels in the postsynaptic membrane would lead to depolarization and to the exit of K⁺ down the electrochemical gradient. The increase in the [K⁺]₀ leads to further depolarization of the presynaptic terminals and to sustained transmitter release evoked by the influx of Ca²⁺. A positive-feedback loop between the [K⁺]₀ and the transmitters is thus established and could explain the cascade character of the fast [K⁺]₀ increase. Likewise the inhibitory transmitters activate anion channels on the postsynaptic site. Release of glutamate has in fact been demonstrated during SD (300).

Observations that reduced [Ca²⁺]₀ (197) and high [Mg²⁺]₀ (297) antagonize SD elicitation seem to corroborate the transmitter hypothesis. More important is the observation that the current induced by glutamate in the vertebrate neuron increases when the cell is depolarized. Thus the current has properties resembling that of a regenerative system, e.g., the Na⁺ conductance during the action potential (229). Furthermore, Nowak et al. (229) demonstrated that the presence of Mg²⁺ reduced the probability of opening the glutamate-activated channels. However, the presence of high concentrations of divalent cations cannot totally block SD. Recently it was shown in hippocampal brain
slices that a high [Mn^{2+}] and a low [Ca^{2+}] sufficient to block synaptic transmission cannot antagonize SD. The reason for these contrasting findings may be that part of the release of glutamate is Ca^{2+} independent (212, 308).

Unequivocal information on the size of transmitter-dependent channels is lacking. Reports on the size of anion channels activated during the IPSP are not consistent. In cortical neurons the channel sizes have been estimated to allow passage of anions with a diameter twice that of hydrated Cl^− (147), which is appreciably greater than the estimated channel size in hippocampal pyramidal cells and spinal motoneurons (77, 142). No precise information is available on the size of transmitter-activated cation channels. The patch-clamp technique is ideally suited to characterize neuronal channels activated by various transmitters, but so far the technique has not been used for this purpose.

Whether the findings in SD also apply to the ionic movements in anoxia awaits more definitive studies. Choline [(CH$_3$)$_3$N$^+$CH$_2$CH$_2$OH] and “N-TRIS” [(CH$_3$)$_3$N$^+$C(CI$_3$OII)$_3$] do not enter brain cells during anoxic depolarization (113), indicating that cations with a diameter of ~0.8 nm, as determined from stereo models, are excluded from the cells. This also supports the notion that the permeability increase in anoxia is not due to unspecific breakdown of membrane structure; rather, it is caused by an activation of a well-defined permeability mechanism. Glutamate release has been demonstrated in the anoxic brain (69), supporting the concept that transmitter release may be important in anoxia, thus leading to depletion of vesicles in the synaptic terminals and to subsequent transmission failure (312).

Exaggerated and uncontrolled transmitter release, although a likely event, may nevertheless not be at the heart of the matter. The uncertainty exists because primary intracellular events (e.g., increases in intracellular [Ca^{2+}] and in cyclic nucleotides and an abnormal function of protein kinases) may act on ion channels from the inside. These and other hypothetical intracellular events can be consequences of the fundamental disturbance: a damaged energy supply. Further studies are evidently needed to gain more solid evidence on these inferences.

As noted in section vC, gyrencephalic animals display a slower increase in the [K^+] and may have a lower susceptibility to SD. Neurons are not as densely packed in these animals as in small rodents, from which most of the present knowledge on ion movements arises. This may be more than coincidental, because the fast changes in the interstitial space may require a great deal of synchrony and a relatively small ratio of interstitial volume to neuronal cell volume. Both conditions are more likely to exist in the lissencephalic brain, which has densely packed neurons.

VIII. REVERSIBILITY OF ION DERANGEMENT

The dramatic ionic shifts do not represent a point of no return in either anoxia or SD. Because of the obvious clinical interest in reversibility of anoxic brain damage, this theme is discussed in some detail.
A. Blood Flow and Energy Metabolism

The resumption of cerebral blood flow after a period of ischemia is clearly essential for ionic normalization. Earlier it was proposed that the remaining functional defects in the brain after anoxia could be ascribed to vascular obstruction, a so-called no-reflow phenomenon (9). The theory could explain why nervous tissue (e.g., the retina) maintained in vitro could sustain much longer periods of anoxia than the brain in vivo (6); however, it has not received much experimental support (177). Although the cerebral vessels are contracted during the anoxic period (261), perhaps because of the high [K+]o (22, 306), there is an increase of short duration in cerebral blood flow (reactive hyperemia) within minutes after the anoxia, which forms the basis for normalization of the interstitial ion concentration (109, 135, 277). Accordingly the concentrations of high-energy phosphates in brain cortex recover within minutes after anoxia (183, 266).

The process of ion normalization may be accomplished by 1) net transport across the cell membrane or 2) removal of excess ions by capillary transport. Experiments show that normalization of the [K+]o, after a 10-min period of complete cerebral ischemia occurs in two phases: an initial slow decrease, in which the [K+]o declines from 75 to 50 mM in 3-4 min, and a fast decrease, which restores the [K+]o to preischemic values within 1 min (109). The cerebral capillaries are practically impermeable to ions (65, 111), and there is no indication of increased permeability after a short period of ischemia (142, 262). At a [K+]o of 75 mM, however, the capillary transport rate calculated from a capillary K+ permeability of 3 × 10^-7 cm/s (111), and a capillary area of 200 cm²/g is sufficient to explain the initial slow decline of the [K+]o (109), but there is little reason to doubt that cellular reuptake is the important mechanism in normalization of the [K+]o. The precondition for net uptake of K+ is sufficient availability of ATP for conformation changes of the Na+-K+-ATPase. This enzyme must be strongly activated during ischemia by the high [K+]o and intracellular [Na+]o.

The inverse correlation between the vascular resistance and the [K+]o after ischemia may reflect the presence of a positive-feedback mechanism (109): the start of reflow allows for regeneration of ATP, which activates the transport enzymes lowering the [K+]o, which in turn somewhat relaxes the contracted arterioles. The diminished vascular resistance permits a more effective supply of substrates for ATP regeneration and thus a further lowering of the [K+]o. In addition the input resistance of neuronal cell membranes is gradually increased (110), counteracting the passive flux of ions and augmenting the efficiency of ion pumping.

The minimum blood flow necessary to preserve a normal [K+]o is ~20% of the normal flow (18, 36, 206), but there seems to be a connection between the magnitude of blood flow and the rate of the [K+]o normalization after ischemia. Thus the rapid decline in the [K+]o occurs when blood flow is high or even supernormal (109).
The DC potential returns to its normal small size with normalization of the \([K^+]_{\text{r}}\), indicating that the \([Na^+]_{\text{r}}\) and \([Cl^-]_{\text{r}}\) are normalized at the same time (135). The normalization of the \([Ca^{2+}]_{\text{r}}\) and \(pH_{\text{r}}\) takes considerably more time, with \([Ca^{2+}]_{\text{r}}\) attaining normal values after 15 min and \(pH_{\text{r}}\) after 30 min following 10 min of complete cerebral ischemia (264). This may be rationalized in terms of a more subtle nature of the \(Ca^{2+}\)-transport system and of the relatively slow rate of net removal of lactic acid in conditions with an efficient aerobic supply of glucose.

The period after brain anoxia is characterized by a disparity between the normalization of the interstitial ion concentrations and the electrical activity. The different disturbances are restored to normal in the following order: 1) interstitial ion concentrations, 2) evoked impulse propagation, 3) evoked synaptic activity, and 4) EEG and higher functions such as consciousness and memory (134). After 10 min of complete cerebral ischemia in rats, EEG activity only starts 15–20 min after recirculation, and it requires hours to normalize, although the \([K^+]_{\text{r}}\), the \([Ca^{2+}]_{\text{r}}\), and the \(pH_{\text{r}}\) have attained preischemic values long before (264).

**B. Role of Ion Homeostasis in Anoxic Brain Damage**

The nature and causes of the irreversible damage occurring after anoxia have recently been reviewed (134, 267), but no clear conclusion or explanation has been found. Although the origin of the damage is not known, there is little doubt that this is a multifaceted problem. Moreover certain regions (hippocampus, cerebral, and cerebellar cortices) and certain cells are particularly susceptible to anoxic damage (37, 151); this difference has not been adequately explained. The suggestions so far include reduced ATP levels (251), reduced intracellular pH (91, 247), accumulation of free fatty acids (95), formation of free radicals with subsequent lipid peroxidation, degradation of membranes (67), and intracellular calcium accumulation. However, no satisfactory and convincing explanation of this serious condition has been given. From Siesjö's (267) comprehensive review, it is clear that the many researchers have left the question open. Several attempts have been made to link ion movements with anoxic brain damage. It was originally suggested that the length of time required to attain the anoxic depolarization, i.e., the moment when rapid ion movements occur, was related to the anoxic vulnerability of the brain cortex, which was smaller when the anoxic depolarization started late (41). This is corroborated by the delayed steep increase in the \([K^+]_{\text{r}}\) in hypothermia (15) and in immature animals (107, 195). Both groups are known to possess increased tolerance to anoxia (66, 126). On the other hand, the delayed anoxic depolarization resulting from hyperglycemia (108, 264) is accompanied by more serious aftereffects (211, 263).

Others have observed the connection between anoxic depolarization (membrane failure) and the development of infarction in the brain secondary to occlusion of the middle cerebral artery (16). The important event in anoxic
Depolarization may be the influx of Ca$^{2+}$ (sect. III B). The viability of hepatocytes when treated with membrane toxins is reduced if Ca$^{2+}$ is present in the medium (258). This suggests that the cells succumb in two steps: a primary disruption of the cell membrane integrity, followed by an influx of Ca$^{2+}$, which initiates a final chain of events leading to serious damage and ultimately to cell death. This crude theory has been discussed in relation to cell damage in the anoxic brain (267). The influx of Ca$^{2+}$ activates phospholipases with subsequent generation of arachidonic acid and other free fatty acids. Restitution of the blood supply oxygenates arachidonic acid via the cyclooxygenase and lipoxygenase pathways to prostaglandins and leukotrienes, which are supposed to be the potentially harmful substances in this connection. However, compelling evidence for this notion is lacking.

Recently, Rothman (255) reported that blockage of synaptic transmission directly by a high [Mg$^{2+}$], by a low [Ca$^{2+}$], or indirectly by tetrodotoxin could prevent anoxic damage in cultured hippocampal neurons. The effect was assumed to result from the lack of an activity-dependent Ca$^{2+}$ influx.

Some observations, however, contradict the pivotal role of Ca$^{2+}$ in producing cell damage in the brain. In SD the influx of Ca$^{2+}$ is apparently similar to that in anoxia (sect. III C), but no serious cellular damage is observed. In fact the disturbance is reversed within a few minutes, but the time of Ca$^{2+}$ influx is much shorter, so the possibility remains that an effect related to the mass of Ca$^{2+}$ entering the cells may play a role. One speculation is that a synergistic damaging effect of ATP depletion plus intracellular Ca$^{2+}$ accumulation might occur. Studies on hippocampal slice preparations point toward a combined effect of ATP depletion and Ca$^{2+}$ influx for the development of anoxic damage (143). In the more mild disturbance occurring in SD, there is no depletion of ATP (155, 244). Recent studies on heart mitochondria have focused on the increased intracellular levels of phosphate and calcium during ischemia as possible causes of the damage induced by subsequent reoxygenation (167).

Despite these factors, the return of brain function requires the reestablishment of normal ion gradients. The lack of correspondence between the degree of ion derangement and the ability of the brain to recover is not surprising in view of the complexity of the system, but it emphasizes the need to search for other subtle derangements, perhaps in the synaptic transmission system. The study of Kass and Lipton (143) was focused mainly on synapses, inasmuch as they showed that anoxia and subsequent reoxygenation did not affect nerve impulse propagation but abolished the postsynaptic part of the evoked response. Thus their study also emphasized a combined role of ATP depletion and Ca$^{2+}$ influx in the genesis of anoxic brain damage.

IX. CONCLUSION

The ion concentrations in brain ISF are strongly affected when the blood or O$_2$ supply is arrested. Functionally, the condition is characterized by the loss of consciousness and of electrical activity within seconds.
An important technological innovation behind the knowledge gained in this area during the past decade has been the ion-sensitive microelectrode. Although its tip diameter is larger than the intercellular clefts, ionic changes in the brain interstitial space can be reliably and rapidly monitored.

The pattern of change in the interstitial ion concentrations during anoxia is usually divided into three phases. In the adult rat brain cortex, the first phase is characterized by only small changes in the interstitial composition. The [Na$^+$], the [Cl$^-$], and the [Ca$^{2+}$] are affected only slightly, but the [K$^+$] exhibits a slow increase from 3 to 9 mM during the first 2 min of anoxia. In the next phase the [K$^+$] suddenly rises very steeply to 60 mM, and the [Na$^+$], the [Cl$^-$], and the [Ca$^{2+}$] display a pronounced and rapid fall within few seconds; these changes continue to occur but more slowly during phase 3.

Phase 2 is accompanied by a fast negative deflection of the interstitial electrical potential with an amplitude of $\sim$20 mV (the interstitium being negative with respect to the blood).

The ionic changes correspond to features of the hypothesis that ionic equilibrium is attained between intra- and extracellular space in phase 2. These pronounced changes obviously suppress normal nervous activity, but early cessation of the EEG takes place within 20 s of anoxia, when the interstitial ion concentrations are hardly affected. Other explanations must therefore be sought for the early loss of consciousness, which may be caused by changes occurring at the postsynaptic site. Two key phenomena, early hyperpolarization and lowered membrane resistance, reduce excitability and may lead to a termination of nervous transmission. The postsynaptic membrane is presumably short-circuited, leading to diminished transmitter-evoked potentials. An important event is an increase in nerve cell K$^+$ conductance, possibly elicited by an increase in intracellular [Ca$^{2+}$].

The disruptive ionic changes in anoxia arise from the inability of anaerobic glycolysis to provide sufficient ATP, but the loss of brain electrical activity occurs before the ATP level is lowered. The opening of unselective channels in nerve cells that are critically dependent on a sufficient rate of ATP regeneration produces the extracellular-intracellular ionic equilibration during phase 2. When the brain ATP level is kept high during anoxia, either by prolonging the ability to regenerate ATP by increasing the glucose stores in brain or by lowering the metabolic rate (hypothermia), the process of ionic equilibration is significantly delayed.

The ionic changes are believed to be mediated via changes in nerve cell permeability, but this has not been proven. The possibility remains that glial cells may also change their permeability.

The spreading depression of Leão exhibits similar but much more rapid interstitial ionic changes that nevertheless normalize quickly. The ATP content is not lowered in this condition, indicating that abrupt and marked ionic changes are possible without perturbation of the ATP level. In SD the fast ionic changes are definitely not due to activation of the Hodgkin-Huxley voltage-dependent channels but are mediated by activation of pores with
diameters smaller than 0.7 nm; these pores may be transmitter-operated channels.

It is now evident that phosphorylation of membrane proteins is important for neuronal functions such as the preservation of normal membrane permeability; it may be significant that many of the compounds operating in this system (e.g., cyclic AMP, Ca$^{2+}$, and ATP) are influenced by anoxia. The role of protein phosphorylation in anoxia is still not clearly understood. The patch-clamp technique seems particularly well suited to the study of early and late membrane changes in anoxia and to the characterization of the channels involved in the abrupt and dramatic disturbances in ion concentrations.

The abnormal interstitial ion concentrations are readily reversible with reoxygenation. There is no correspondence between the time for normalization of ion concentrations and the return of electrical activity, a discordance comparable to that at the onset of anoxia. Thus the ionic changes are a sign rather than a cause of the key disturbance, which still remains enigmatic. Possibly a marked influx of Ca$^{2+}$ triggers lethal processes within the cell, which after delayed reoxygenation leads to irreversible cell damage and definitive loss of function, but there is still a substantial lack of knowledge about the nature of the intracellular derangements.

This research was supported by Købmand i Odense Johann og Hanne Weimann født Seedorfs legat.

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