Review Article

Cerebral circulation and metabolism

Bo K. Siesjö, M.D.

Laboratory for Experimental Brain Research, University of Lund, Lund, Sweden

Recent developments in the field of cerebral circulation and metabolism are reviewed, with emphasis on circulatory and metabolic events that have a bearing on brain damage incurred in ischemia. The first part of the treatise reviews aspects of cerebral metabolism that provide a link to the coupling of metabolism and blood flow, notably those that lead to a perturbation of cellular energy state, ionic homeostasis, and phospholipid metabolism. In the second part, attention is focused on the derangement of energy metabolism and its effects on ion fluxes, acid-base homeostasis, and lipid metabolism. It is emphasized that gross brain damage, involving edema formation and infarction, is enhanced by tissue acidosis, and that neuronal damage, often showing a pronounced selectivity in localization, appears related to a disturbed Ca2+ homeostasis, and to Ca2+-triggered events such as lipolysis and proteolysis.

KEY WORDS: cerebral circulation · cerebral metabolism · coupling factors · ischemia · edema · energy state · ion flux · phospholipid degradation · Ca2+ homeostasis · acidosis

During recent decades, the subject of cerebral circulation and metabolism has evolved as an area of ever-growing scope and complexity, amalgamating knowledge from the basic fields of physiology, chemistry, anatomy, pharmacology, and pathology. The explosive development within the field has been made possible by the advent of new methodologies. It is an exacting task to grasp the complexities of the technological advances, and the wealth of biological information amassed. This task becomes even more demanding with the need to relate information on the coupling of metabolic rate and blood flow to corresponding developments in the field of neurochemistry, even if the scope is restricted to a few areas of immediate and obvious concern.

From the very outset, the subject of cerebral circulation and metabolism has attracted the interest of clinicians; in fact, some of the most significant advances can be attributed to the research efforts of neurologists and neurosurgeons. Their interest is understandable, since some of the most important conditions encountered by these workers are disorders of cerebral circulation, and the outcome of such disorders is largely determined by the neurochemical response to a failing circulation.

The present review article is an attempt to summarize some key developments within the field of cerebral circulation and metabolism. Of necessity, any treatise on this vast research field must be selective, and burdened with personal prejudice. It has been my intention, though, to emphasize aspects that seem of obvious interest to neurosurgeons. I have chosen to begin by reviewing some aspects of cerebral metabolism that provide a link to the coupling of metabolism and blood flow, to proceed by briefly discussing mechanisms that may mediate this coupling, and to end by discussing the relationship between circulatory and metabolic events in ischemia, with emphasis on events that may underlie any brain cell damage incurred. I have deemed it necessary to restrict quotations to original works, and have tried to select those of recent origin which provide references to the older literature. The reader can also consult the review articles quoted for further references.
Definitions of Abbreviations

acyl-CoA = acylcoenzyme A
ADP = adenosine diphosphate
AMP = adenosine monophosphate
ATP = adenosine triphosphate
CBF = cerebral blood flow
CDP = cytidine diphosphate
CMP = cytidine monophosphate
CoQ10 = coenzyme Q
CPP = cerebral perfusion pressure
Cr = creatine
CTP = cytidine triphosphate
Cyt = cytochrome
DG = diglyceride
EC = energy charge
FFA = free fatty acid
FP = flavoprotein
GABA = gamma aminobutyric acid
GPI = inosine phosphoglyceride
IMP = inosine monophosphate
αKG = α-ketoglutarate
MCA = middle cerebral artery
NADH = nicotinamide adenine dinucleotide
NE = norepinephrine
PA = phosphatidic acid
PCr = phosphocreatine
PG = prostaglandin
Pi = inorganic phosphate
PLA = phospholipase A
PLC = phospholipase C

COUPLING OF CELL ACTIVITY, METABOLISM, AND BLOOD FLOW IN NORMAL BRAIN TISSUE

Coupling of Neuronal Activity and Brain Metabolism

In this section, we will ask the question how cell activity, exemplified by nerve cell depolarization, perturbs cerebral metabolism (that is, how it triggers an increased metabolic rate and what "error" signals may arise). For details of metabolic events the reader is referred to available textbooks. It seems justified to begin by reviewing three features of the subcellular organization and metabolism of neurons in general. First, in relation to its volume the cell has a large membrane mass. Second, most of the axonal and some of the dendritic ramifications of the cell are widely separated from the perikaryon. Third, although ionic fluxes and membrane perturbations occur at central as well as peripheral locations, protein synthesis and macromolecular assembly are restricted to the cell body. It follows from these features that energy requirements for restoration of ionic gradients are high, and that additional energy must be spent both for maintaining membrane structure and for propelling material between central and peripheral sites. We also recall that communication between cells involves the release of excitatory or inhibitory transmitters, and that information transmission at synaptic clefts is associated with intense metabolic activities in axonal and dendritic domains. At first sight, it is tempting to assume that the structural and metabolic features discussed contribute to the reputed vulnerability of neurons to an interruption of oxygen and/or substrate supply. As the subsequent discussion will bear out, though, the term "vulnerability" is ambiguous. Thus, the existence of a selective vulnerability (that is, of marked differences in sensitivity between neurons) suggests that additional structural and/or metabolic features which determine neuronal vulnerability must exist.

Cell energy metabolism is simply the balance between utilization of adenosine triphosphate (ATP) during the performance of work, and its resynthesis in anabolic sequences which provide the energy required to repolish adenosine diphosphate (ADP, Fig. 1). During intense activity, or when production is impeded by oxygen lack, some ATP can be produced by shifts in the creatine kinase and adenylate kinase equilibria, or by glycolysis. Since the adenylate pool (ATP + ADP + AMP) can vary in size, some advantage is gained if the adequacy of energy metabolism is assessed from the adenylate energy charge. On the basis of this simplified scheme, we proceed to discuss production and utilization of ATP in brain tissues.

In the absence of ketosis (such as occurs in starvation, diabetes, and ethanol ingestion), the brain uses glucose as its sole substrate. As in other tissues, glucose can either be anaerobically degraded to lactic acid, or oxidized to CO2 and water. Since the energy yield of glycolysis is small compared to oxidation, the brain relies for its continuing function on oxidative metabolism. We may thus envisage cerebral energy metabolism as the coupling of the glycolytic production of pyruvate to its aerobic oxidation. Pyruvate is metabolized by the mitochondria in a cyclic series of reactions, yielding various citric acid cycle intermediates. When some of
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![Diagram of electron flow and coupled ATP production in the respiratory chain.](image)

These intermediates are oxidized, their electrons enter the series of reactions constituting the respiratory chain, to be finally accepted by oxygen at the cytochrome oxidase (a-a3) step. The key to aerobic energy production is the fact that the energy released during electron transport can be tapped off in packages, three of which allow ATP to be synthesized from ADP and inorganic phosphate (Pi) (Fig. 2). In the figure, the ATP-generating steps have been written as equilibrium reactions. What this means is that the rate of electron transport and, thereby, of oxygen utilization is determined by the rate of consumption of ATP (and rate of accumulation of ADP and Pi).

It is conventionally taught that mitochondrial oxidations involve the acceptance by oxygen of a package of four electrons (with formation of water), and that electron flow is coupled to ATP production in an obligatory fashion. Clearly this is an oversimplification. Thus, it is now realized that reactions exist in which univalent reduction of oxygen occurs, with the subsequent formation of superoxide radicals (•O2), hydrogen peroxide (H2O2), and hydroxyl radicals (•OH), one site of such radical formation being the cytochrome Q10 step.

When pathologically enhanced, dislocation of free radicals may induce untoward reactions that threaten to damage cells. Furthermore, it should be emphasized that electron transport can be dissociated from ATP formation (so-called “uncoupling” or “loose coupling”). As we will discuss below, this occurs under conditions in which ion transport across mitochondrial membranes takes preference over ATP phosphorylation, and it is conceivable that pathological uncoupling contributes to energy failure in disease.

It is customary to divide the work tasks of the cell (that is, the cellular events that require expenditure of energy) into “osmotic” and “biosynthetic.” Osmotic work includes all forms of transport, and thus both transmembrane transport of ions and intracellular (“axonal”) transport of macromolecules and macromolecular assemblies. In order to exemplify how cell activity causes consumption of energy we focus on events associated with ionic fluxes during cell activity before dealing with biosynthesis.

**Ion Transport**

We can now ask how neuronal activity is coupled to metabolic rate. Let us first consider the events that occur when an excitatory transmitter leads to transient depolarization of the entire cell membrane by allowing a coupled flux of Na+ (in) and K+ (out). These fluxes activate the membrane-bound Na+ – K+ dependent ATPase (Fig. 3). As a result, the ionic gradients are restored at the expense of ATP which is hydrolyzed to ADP and Pi. Inspection of Fig. 2 reveals that ATP is then resynthesized during consumption of glucose (the source of pyruvate and citric acid cycle intermediates) and oxygen (the terminal electron acceptor), and with production of CO2. Normally, this coupling of neuronal activity and metabolic rate is remarkably tight, and any “error” signal is hard to detect unless the neuronal activity is intense, as during seizures.

Nonetheless, there is reason to believe that the metabolic perturbation involves other reactions as well. For example, the compensatory stimulation of glycolysis may overshoot and allow some lactic acid to accumulate. Furthermore, accumulation of ADP will shift the adenylate kinase equilibrium (ADP + ADP ⇔ ATP + AMP) and the ensuing accumulation of AMP will, among other things, lead to adenosine production via the 5′-nucleotidase reaction. Thus, neuronal activity is apt to yield both H+ ions (from CO2 and lactic acid) and adenosine (via the 5′-nucleotidase reaction). Since CO2, undissociated lactic acid, and adenosine are diffusible, one must assume that acidosis and adenosine accumulation occur in extracellular fluid as well. Two additional features of nucleotide metabolism should be mentioned. First, the dephosphorylation and deamination of AMP decreases the adenine nucleotide pool (ATP + ADP + AMP), mechanisms of resynthesis must exist. Since de novo synthesis from amino acids and other precursors...
FIG. 3. Passive and energy-dependent ion fluxes across plasma membranes. Neuronal depolarization leads to cellular influx of Na⁺ and Ca²⁺, and efflux of K⁺. Re-pumping of Na⁺ and K⁺ occurs at the expense of ATP. It is hypothesized that Ca²⁺ efflux occurs by Na⁺/Ca²⁺ exchange (that is, it utilizes the energy stored in the Na⁺ gradient created by ATPase activity). This gradient is also believed to transport H⁺ from cells to extracellular fluid. See Definitions of Abbreviations.

is slow, recovery mainly occurs by ATP-dependent kinase reactions and by salvage pathways.33,109,208,258 These utilize adenosine, adenine, and hypoxanthine molecules which have been retained within the tissue; however, if some of these have been lost to the circulation it can take many hours before the pool has been reconstituted by de novo synthesis.109,125,131 Second, although adenosine may diffuse from cells to extracellular fluid, other mechanisms exist for extracellular adenosine accumulation. It has been documented that electrical stimulation (and depolarization) leads to cellular release of nucleotides, including ATP.24,25,138,177,248,258 At least in part, this release must occur in conjunction with transmitters with which ATP is packed in the synaptic vesicles. Obviously, ectoenzymes must exist that can convert nucleotides to adenosine. It is of clear interest that 5'-nucleotidase has been found attached to glial cell membranes, notably to the external surface.4,117,218 The importance of this extracellular adenosine formation is twofold: adenosine has a transmitter action in that it modulates signal transmission in the brain,118,171,198 and, as we will discuss below, the nucleoside is a potent vasodilator.

We must now return to Fig. 3 and consider activity-coupled fluxes of Ca²⁺. It is common knowledge that influx of Ca²⁺ into presynaptic terminals is a prerequisite for transmitter release; however, such influx occurs in some postsynaptic elements as well and seems to constitute an important mechanism of cell excitation (for instance, in pyramidal cells in the hippocampus and the cerebral cortex, as well as in cerebellar Purkinje cells).75,177,201 Restoration of normal Ca²⁺ gradients requires energy. This follows from the fact that external concentrations (about 10⁻³ mol-liter⁻¹) are very much higher than the internal (about 10⁻⁷ mol-liter⁻¹), and that the negative membrane potential tends to drag Ca²⁺ into the cell.13,28 Probably, export of Ca²⁺ mainly occurs by Na⁺/Ca²⁺ exchange (that is, the energy for Ca²⁺ transport comes from the large Na⁺ gradient created by the Na⁺-K⁺-dependent ATPase). However, several other mechanisms exist for long- and short-term transport/sequestration of Ca²⁺. Thus, Ca²⁺ export may occur by the operation of a Ca²⁺-ATPase, and sequestration includes the binding of Ca²⁺ to Ca²⁺-regulatory proteins and to endoplasmic reticulum (Fig. 5).

In all probability, an important role in the precise regulation of intracellular Ca²⁺ activity is played by the mitochondria.3,141 According to the chemiosmotic theory of Mitchell,24 the energy released during electron transfer through the respiratory chain is used for the synthesis of ATP. This ATP is then used to pump Na⁺ from the cell into the extracellular fluid, thereby creating a gradient of Na⁺ that can drive the entry of Ca²⁺ into the cell. Ca²⁺ is then removed from the cell by the operation of the Na⁺/Ca²⁺ exchange process, which is driven by the Na⁺ gradient. The net result is that the cell actively pumps Ca²⁺ into the extracellular fluid, thereby maintaining a low concentration of Ca²⁺ in the cell. This process is known as the Ca²⁺-ATPase pump, and it is a key mechanism for the regulation of intracellular Ca²⁺ concentration.

FIG. 4. Pathways of degradation of adenine nucleotides, slightly modified from the scheme of Deuticke, et al.43
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transport is utilized to split water in a vectorial way so that H⁺ is released on the outside and OH⁻ on the inside of the inner mitochondrial membrane. This separation of H⁺ and OH⁻ stores the respiratory energy in the form of a large electrochemical gradient for H⁺. When this is dissipated, it can reverse a mitochondrial ATPase reaction (ADP + Pᵢ + H⁺ → ATP + H₂O) and thus constitute the mechanism whereby ATP is produced. However, another way of utilizing the electrochemical H⁺ gradient (that is, its electrical component) is to drag Ca²⁺ into the mitochondria by electrophoretic action. We note, therefore, that ATP production and Ca²⁺ sequestration are alternative ways of utilizing metabolic energy. In the long run, though, Ca²⁺ must be released from the mitochondria and be exported from the cell by translocation across the plasma membrane. Release of Ca²⁺ from mitochondria, which occurs via a channel separate from that allowing Ca²⁺ uptake, is activated by Na⁺/Ca²⁺ exchange (c) and by ATP-driven translocation (d), while sequestration/release occurs between cytosol and endoplasmic reticulum (e,f) or mitochondria (g,h). (Reproduced with permission from Berridge MJ: Modulation of nervous activity by cyclic nucleotides and calcium, in Schmitt FO, Worden FG (eds): The Neurosciences: Fourth Study Program. Cambridge, MA: MIT Press, 1979, pp 873-889.) See Definitions of Abbreviations.

Membrane Degradation and Resynthesis

Macromolecules and macromolecular assemblies are constantly being synthesized in the cell body, and transported to peripheral domains to replace what has been lost or degraded. The rate at which this “wear and tear” occurs is not accurately known, but it has been estimated that a cell body may reproduce up to 2000 mitochondria, and renew its whole population of macromolecules in 1 day. It has also been assumed that synaptic vesicles are used only a couple of times before being degraded. Clearly, such estimates are uncertain but the figures emphasize that synthetic tasks must consume a large share of the energy expended. It also seems likely that various structural components turn over at markedly varying rates, and that turnover rates are related to intensity of impulse traffic. We will exemplify this by considering the perturbation of membrane structure during activity.

Membrane structure is now viewed as consisting of a bimolecular leaflet of phospholipid molecules of which the polar heads face the outside solutions and the fatty acid tails form the inner, hydrophobic zone. Intercalated into this fluid matrix are cholesterol molecules and proteins, some of which span the distance between the inside and outside solutions. The activity of such proteins, of which ATPases, adenylate cyclases, and cytochrome oxidases form examples, depends on their phospholipid environment. This, in turn, is in-
The second cycle (Fig. 6C) involves the degradation of other phospholipid classes by a phospholipase A, activated by Ca\(^{2+}\), to lysophospholipids and FFA's, a cycle that is completed by an energy-dependent reacylation of the lysocompound.\(^{222}\) Again, we must ask why this cycle operates. One clue to the problem is provided by the scheme of Hirata and Axelrod,\(^{81}\) who consider that interaction of agonists with membrane receptors alters membrane fluidity (by phospholipid interconversions, see above), the latter allowing activation of membrane-bound enzymes (such as adenylate cyclases) and/or opening of membrane gates for Ca\(^{2+}\) entry (see also other references\(^{309,24}\)). In the scheme of Hirata and Axelrod,\(^{81}\) Ca\(^{2+}\) entry is envisaged to trigger the decacylation of phospholipids. These authors also emphasized that accumulation of arachidonic acid, an important component of membrane-bound phospholipids, must trigger the production of both prostaglandins and leukotrienes, catalyzed by cyclo-oxygenase and lipoxygenase, respectively (see further below). We may tentatively conclude, therefore, that interaction of agonist and receptor leads to an energy-requiring turnover of membrane structure and that such interactions lead to the formation of compounds (such as FFA's and prostaglandins) with established or putative effects on metabolism and circulation. It also appears that, since agonist-receptor interaction enhances both ion fluxes and turnover of cell structure, any attempt to partition the energy expenditure into osmotic and biosynthetic work is fraught with difficulties.

At present, less is known about turnover rates of various proteins, whether bound to membranes or not. However, we will discuss below experimental results that bear on protein degradation and protein synthesis in ischemic and postischemic states.

**Metabolism of Glial Cells**

Clearly, it would be a gross oversimplification to disregard the fact that metabolic perturbations associated with nerve impulse traffic involve glial cells as well.\(^{196,221}\) These cells (astrocytes, oligodendrocytes, and microglia), have been estimated to occupy about half the volume of the brain. One established function of oligodendroglia (and Schwann's cells) is the formation of myelin. Since astrocytes separate neurons from each other and from blood vessels, they form part of the diffusion (or transport) pathway for neuronal nutrients and waste products. The close structural association between neurons and glia has inspired proposals that glial cells do not only provide structural support and electrical insulation to neuronal elements but that they are also instrumental in regulating the composition of the perineuronal fluid environment. As we will see, though, opinions differ on the quantitative aspects of this matter.

There is now extensive information on the electrophysiology of glial cells in brains of both invertebrates and vertebrates.\(^{65,66,130,164,174}\) Three important features have emerged. First, glial-cell membranes behave as

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**Figure 6.** Reactions involved in turnover of neuronal phospholipids, showing the interaction between inositol phosphoglyceride (GPI) turnover (A) and general phosphoglyceride turnover (C). (Reproduced with permission from Wieloch T, Siesjö BK: Ischemic brain injury: the importance of calcium, lipolytic activities, and free fatty acids. *Pathol Biol* 30: 269-277, 1982.) See Definitions of Abbreviations.

flavoured by the properties of the phospholipid classes involved and thus by their fatty acid composition. One interesting feature of membrane composition is that phospholipids may be asymmetrically distributed in the two leaflets, and that enzymes exist (phosphatidyl methyltransferases) that can convert one phospholipid into another.\(^{81}\) Such interconversions may alter membrane fluidity, this in turn having an influence on ion gates and enzymatic activities (see below).

Figure 6 illustrates two cyclic reaction sequences for turnover of membrane phospholipids, and a bidirectional pathway for base exchange between the two cycles. The first cycle (Fig. 6A), involves the degradation of inosine phosphoglyceride (GPI) to diglyceride, with the subsequent resynthesis of GPI at the expense of two ATP molecules (one of which is used to re-form cytidine triphosphate (CTP) from cytidine diphosphate (CDP)).\(^{73,142}\) Noting that the degradation of GPI is dependent on Ca\(^{2+}\), we must ask why an energy-dependent GPI cycling occurs. Since the cycle is stimulated by transmitters and receptor agonists,\(^{73,142}\) one can envisage that agonist-dependent alterations in ion conductances requires that membrane gates are remodelled by changes in their phospholipid environment.
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nearly perfect potassium electrodes, in that their membrane potentials (−70 to −90 mV) vary with extracellular K⁺ concentrations as predicted by the Nernst equation. This feature suggests that, normally, the permeability of the membranes to all ions other than K⁺ is small. Second, although glial cells show neither synaptic nor spike potentials, they depolarize when extracellular K⁺ concentration rises. Third, since low resistance pathways exist between neighboring glial cells (at least partly in the form of gap junctions) they form a syncytium, allowing spread of electrical potentials and diffusion of material over relatively large distances. One consequence of this is that local depolarization of glial membranes due to leakage of K⁺ from active neurons will lead to an inward current, carried by K⁺ ions, and to distribution of the K⁺ taken up within the glial syncytium. In other words, glial cells may function as K⁺ buffers, aiding in the removal of K⁺ from the site of the neuronal membranes.77–79

Another facet of the ability of glial cells to act as spatial buffers is the uptake of neurotransmitters released from neurons during impulse traffic.80,221 Since some of these neurotransmitters, notably excitatory and inhibitory amino acids, are not degraded by ectoenzymes following their release, transmitter inactivation depends on reuptake, either into presynaptic nerve terminals or into glial cells. It is a general consensus that at least some of this reuptake occurs into glial cells, and it seems likely that compartmentation of amino acid metabolism of the brain involves glial uptake of amino acids such as gamma aminobutyric acid (GABA), their conversion by glia to glutamine, and the subsequent reexport of the glutamate to neurons for subsequent deamination and resynthesis of the original amino acid.

Glial cells are purported to aid in the delivery of substrate to neurons, and in the removal of waste products. It seems likely that a major share of the glycogen content of the brain (about 2 μmol. gm⁻¹) is stored in glial cells: one may envisage that this provides for rapid delivery of glucose to neighboring neuronal elements in emergency situations.40,78,79 It has also been established that the accumulation of glycogen that occurs after trauma and other adverse conditions is largely confined to glial cells, notably the astrocytic cytoplasm. It is less clear if glucose delivery to neurons occurs by diffusion through the narrow intercellular clefts or by transport across glia. However, even if the latter is the case, rapid delivery of glucose seems secured by a high-capacity glucose transport system across cell membranes.126 Although glial cells are not known to facilitate transport of oxygen between capillaries and neurons, the possibility exists that they enhance transport of CO₂. It is thus recognized that CO₂ transport is faster in bicarbonate-containing media, suggesting that HCO₃⁻ transport aids in the mass transfer of CO₂.191 This facilitation requires that CO₂ is rapidly hydrated to H₂CO₃, a reaction catalyzed by the enzyme, carbonic anhydrase.29,133 Since glial cells contain high concentra-

tions of carbonic anhydrase they are equipped to fulfill this transport function.58,195,218 However, in view of the fact that unfacilitated diffusion of CO₂ is rapid, the importance of the enzyme in CO₂ transport is unclear, and the enrichment of oligodendroglial cells in carbonic anhydrase195,218 suggests that the major function of the enzymes may be something else (see also below).

Opinions differ as to the contribution of glial cells to the overall cerebral metabolic rate. Cerebral tissues respond to increased K⁺ concentrations with an increased metabolic rate, both in vitro69,79 and in vivo.71 The question has arisen whether this increase is due to neuronal or glial events. Based on experiments showing that dissected, disaggregated, or cultured glial cells seem to contain higher activities of Na⁺-K⁺-ATPase than neurons,64,77 and that they respire in vitro at rates purported to correspond to overall cerebral metabolic rate in vivo, Hertz78,79 has suggested that the metabolic rate is at least as high in glial cells as in neurons, and that uptake of K⁺ released from neurons predominantly occurs in glial cells. This contention is not supported by electrophysiological results, which indicate that a major share of the K⁺ released is taken up by neurons. Thus, since the Na⁺-K⁺ pump seems to be electrogenic in the sense that it carries more Na⁺ (out) than K⁺ (in), it should be stimulated by internal Na⁺.230 As glial cells do not show action potentials and normally have a low permeability to Na⁺, their uptake of K⁺ may be ancillary rather than dominating. One may also argue that, since glial cells have a lower mitochondrial density than neurons, their metabolic capacity would not allow them to be as metabolically active. This assertion receives support from data on cellular protein synthesis, which show intense synthesis in neuron-dense areas of the brain.104 One must conclude, therefore, that although glial cells normally play a role in the spatial buffering of K⁺ and transmitters released during neuronal activity, they should normally have a lower metabolic rate. This conclusion is in keeping with the well established fact that neurons succumb to hypoxia (or hypoglycemia) when glial cells do not only survive but also multiply.

Volume Regulation in the Brain

It has been recognized for a long time that regulation of cellular volume in various tissues is intimately related to the energy metabolism of those tissues.100,131 The “double-Donnan pump-leak” hypothesis can be explained as follows. Since cells contain impermeant, negatively-charged macromolecules, the Gibb-Donnan theory predicts that they will accumulate permeant ions from the extracellular fluid, and that the associated water flux will lead to swelling of the cells. This swelling tendency can be counteracted if one or more of the permeant ions is prevented from entering. Cells in general seem to achieve this by actively extruding Na⁺ which is, therefore, effectively prevented from entering the cell (“double-Donnan equilibrium”). However, since the nonequilibrium distribution of Na⁺ is due to
metabolic energy it follows that energy failure will lead to influx of Na⁺ and Cl⁻ in excess of any K⁺ lost and, therefore, to cell swelling. At any given moment, the distribution of Na⁺ will depend on the balance between active extrusion and passive influx ("pump-leak" hypothesis). It follows from this that at any given pump activity the swelling tendency will be related to the magnitude of the leak (that is, to ionic permeability). One can envisage that cells with manifest ATP shortage ("pump failure") will swell if membrane permeability to Na⁺ and Cl⁻ is increased; if it is not (that is, if K⁺ permeability is much higher), pump failure could conceivably even cause cell shrinkage. Since glial cells have permeability much higher), pump failure could contribute to Na⁺ and Cl⁻ influx of the cell at the expense of K⁺, and water.

FIG. 7. Ion and water fluxes of importance for cell volume regulation. Upper Panel: Coupled Na⁺/H⁺ and Cl⁻/HCO₃⁻ fluxes, sustained by metabolic production of CO₂ and H₂O, can lead to influx of water. Lower Panel: Reexport of Na⁺ by an Na⁺/K⁺-ATPase. It is suggested that an electrogenic pump which is short-circuited by Cl⁻ can balance passive influx of Na⁺, Cl⁻, and water.

Two questions arise. First, how is this normal swelling tendency counteracted? Second, what is the function of the coupled ion fluxes?

Since glial cell membranes normally have a low permeability to ions other than K⁺, the tendency to swelling due to the coupled Na⁺/H⁺ and Cl⁻/HCO₃⁻ fluxes could be small. Furthermore, it seems that balance could be upheld by the electrogenic nature of the Na⁺ pump. An electrogenic pump carries charge across the membrane, increasing the negativity of the membrane potential. It is recognized that the electrogenic part of the potential is short-circuited by negative ions, chiefly Cl⁻, which are translocated from intra- to extracellular fluids. Thus, since the transport of Na⁺ and Cl⁻ (out) will exceed that of K⁺ (in), water will leave the cell. Assuming that this transport normally balances influx of Na⁺, Cl⁻, and water, we will later return to conditions in which glial cells swell due to pump failure and/or increased membrane permeability for Na⁺ and Cl⁻.

There is no straightforward answer to the question as to the function of the coupled ion fluxes. No doubt, Na⁺/H⁺ exchange represents a general mechanism for intracellular pH regulation (see above), but the role of the Cl⁻/HCO₃⁻ exchange is less clear. In erythrocytes, this anion antiport system allows the cells to transport CO₂ as HCO₃⁻ but, in the brain, a similar function seems less likely.

Coupling of Metabolism and Blood Flow

Since regulation of cerebral blood flow (CBF) has been discussed in several recent review articles, I will confine the present account to some key features, emphasizing relationships of putative pathophysiologial importance. The influence of the extrinsic and intrinsic innervation of cerebral vessels will not be reviewed, nor will I consider the effects of exogenous or endogenous monoamines or cholinergic agonists-antagonists. It seems justified to begin by recalling some general aspects.

Regulation of CBF, mainly subserved by precapillary resistance vessels, must occur in response both to endogenous metabolic alterations and to exogenous influences that threaten to upset the coupling of metabolism and blood flow. A wealth of information now exists showing that alterations in neuronal activity and metabolic rate can be quite localized. Clearly, this calls for equally localized regulation of CBF. On the other hand, conditions exist in which global changes in cerebrovascular regulation seem purposeful, if not mandatory. We recognize that this is the case when, for example, body (and brain) temperature is altered. However, of even greater importance are those conditions in which either blood flow or oxygen and substrate delivery are reduced, such as occurs with reductions in cerebral perfusion pressure, or in arterial oxygen tension and glucose concentration.

In seeking a mechanistic explanation for cerebrovascular control we must consider the point where regula-
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Adenosine

Adenosine is a potent dilator of vessels in many tissues, including the brain. It is tempting to conclude, therefore, that adenosine may enter as an important vasodilator in epileptic seizures and hypoxia and that it is instrumental in preventing further vasoconstriction at low CO\textsubscript{2} tensions. It seems somewhat less clear that the nucleoside is responsible for vascular autoregulation. Most workers in the field favor the view that autoregulation represents an intrinsic myogenic property of the vasculature, and it has previously been shown that changes in perfusion pressure within the autoregulatory range do not cause detectable alterations in metabolites reflecting the cerebral energy state, nor are they accompanied by changes in extracellular pH or K\textsuperscript{+} activity. At present, though, there are no reasons to dismiss the possibility that adenosine mediates, at least in part, vascular autoregulation.

In all probability, adenosine induces its circulatory effects by reducing Ca\textsuperscript{2+} availability at the site of the actin-myosin complex. Its site of action is unknown, but it may act either by reducing influx of Ca\textsuperscript{2+} through membrane channels or by interacting with intracellular sequestration sites.

METABOLIC EVENTS IN ISCHEMIA

With this background information on the coupling of function, metabolism, and blood flow in the normal brain we proceed to discuss how ischemia perturbs cell metabolism and consider how this perturbation sets in motion a series of reactions which, if sustained for a certain minimal period, lead to irreversible cell damage.

As we will see, metabolic changes during ischemia constitute grossly exaggerated responses to physiological stimuli, responses that become distorted by the avalanche character of the metabolic cascades elicited. In a discussion of metabolic events that take place in ischemia, it is immaterial whether or not the models are clinically relevant; what matters is the impact of the density and the duration of the ischemia on metabolism. For this reason, I will use data derived from several different models. However, it seems profitable to begin by considering metabolic events in transient ischemia of sufficient severity to cause what appears to be a complete disruption of cerebral energy metabolism before discussing ischemic states in which variable amounts of flow persist.

Metabolic perturbations due to transient ischemia should be considered in relation to changes in CBF and in oxygen supply. Figure 9 synthesizes current views of these variables. The principal difference between complete and near-complete ischemia is that the latter
allows some continuing oxygen supply and oxygen utilization. The graph does not show the fact that some substrate is also carried to the tissue, and that the trickle of flow provides a source of ions and water as well. However, whether the ischemia is complete or incomplete, restoration of an adequate perfusion pressure normally leads to an initial hyperemia, later to be followed by a secondary ("delayed") hypoperfusion.95-96 Since the postischemic metabolic rate is initially low, the tissue must pass through a stage of transient hyperoxia. Later, when the oxygen utilization rate increases, relative hypoxia may develop. In some experimental settings an increase in metabolic rate above normal has been recorded, possibly reflecting uncoupling of oxidative phosphorylation. However, this is not a regular finding. Whatever is the change in metabolic rate, it seems that, for some time during recirculation, the delayed hypoperfusion gives rise to a mismatch between metabolic rate and blood flow of possible pathogenetic importance.

Although it is not my intention to discuss the evolution of the no-reflow concept, as originally described by Ames, et al., and considerably modified in later publications from the same laboratory, it should be emphasized that subsequent work has shown that the phenomenon does indeed exist, notably as an initial hindrance to recirculation following complete ischemia. However, since this type of ischemia is of rather limited clinical relevance, I will not discuss its effect on circulation but use it merely to illustrate neurochemical differences between complete and near-complete cessation of tissue blood flow. I also wish to emphasize that the circulatory and metabolic events discussed pertain to reversible ischemia of moderate duration. With more prolonged ischemia recovery may be incomplete and involve failure of circulation, often with patchy distribution, and gross metabolic derangement.

Complete and Near-Complete Ischemia

Major Metabolic Changes. We begin by considering transient ischemia of 15 minutes' duration and concentrate on three major alterations, namely, those affecting cellular energy state, acid-base metabolism, and FFA's (Fig. 10). Following complete interruption of oxygen supply, tissue ATP content decreases to zero within 5 to 7 minutes, as exemplified by the reduction in adenylate energy charge to minimal values within that time. Tissue concentration of phosphocreatine (PCr), the storage form of ATP, is reduced to nil within about 5 min. As observed, the lactic acid concentration reaches a maximum value within 2 to 3 minutes. This is the expected result in complete ischemia during which lactic acid production is limited by the preischemic stores of glucose and glycogen. With this amount of lactic acid accumulated, pH has been estimated to fall to

Fig. 9. Changes in cerebral blood flow (CBF), tissue pO₂ (PtO₂), and cerebral rate of oxygen utilization (CMRO₂) during and following complete (closed circles) and incomplete (open circles) ischemia. (Reproduced with permission from Siesjo BK: Cell damage in the brain: a speculative synthesis. J Cereb Blood Flow Metab 1981.)

Fig. 10. Changes in cerebral cortical concentrations of phosphocreatine (PCr), lactate, and free fatty acids (FFA), as well as in the adenylate energy charge and intracellular pH (pHᵢ), during and following transient complete ischemia. For discussion see text.
about 6.5, a value close to that recently measured with an ingenious histochemical technique. It should also be recalled that, since flow is nil, the CO₂ arising from HCO₃⁻ is trapped in the tissue; as a result, tissue CO₂ tension must rise to excessive values.

By contrast, the FFA content does not attain a plateau value; in fact, FFA concentrations continue to rise even if the ischemia is prolonged beyond the 15-minute point. As originally observed by Bazán, the FFA showing the largest relative rise is arachidonic acid.

Provided that perfusion pressure is restored at the end of the ischemia, all variables return to control values. Not shown in Fig. 10 is the fact that the rise in AMP concentration triggers its deamination and dephosphorylation, leading (among other things) to gross adenosine accumulation. Since some of the nucleosides and bases are lost during recirculation, the ATP concentration and the sum of adenine nucleotides will remain depressed for some time.

Two of the recovery events warrant comment. First, we note that normalization of the FFA content and (not shown) the arachidonic acid concentration occurs gradually over 30 minutes or longer. We will discuss the implication of this later. Second, we note that post-ischemic PCr concentrations rise above control levels and that pH overshoots the control value (that is, a transient alkalosis develops). The rise in PCr concentration follows from the fact that the concentration of H⁺ influences the equilibrium of the creatine kinase equilibrium (see above), while the transient alkalosis probably reflects the simultaneous occurrence of two events: oxidation of the lactate accumulated, with removal of a stoichiometrical amount of H⁺, and extrusion of H⁺ from the cells, for instance, via the Na⁺/H⁺ antiporter. I wish to point out, though, that intracellular alkalosis may indicate an ongoing pathological process rather than recovery events. Using their microembolization procedure, Kogure, et al., noted that tissue areas with low ATP values and high lactate concentrations had pH values in the range of 7.2 to 7.5, a finding which they considered to reflect formation of sodium lactate. The findings may be interpreted differently, though, and taken to indicate passive equilibration of H⁺/HCO₃⁻ across depolarized and leaky membranes. This interpretation receives support from results obtained in hypoglycemia. Conceivably, if badly injured cells exist in an extracellular environment with normal or increased pH, they may become markedly alkaline in spite of high lactate contents. The importance of such alkalosis is not known, but, interestingly, it has been claimed that ischemic cell changes of the Spielmeyer type can be induced in vitro if the incubating fluid is alkaline.

Ion and Water Fluxes. Figure 11 illustrates associated changes in extracellular concentrations of K⁺, Ca²⁺, Cl⁻, and Na⁺ as well as of extracellular space volume (ECS) and pH (pHₑ), during and following ischemia. These data are reported in the literature (see text). Ion concentrations are given in μmol·ml⁻¹ and ECS in percent of tissue volume.

The K⁺ concentration rises only slowly during the first 1 to 2 minutes, and then suddenly floods the extracellular fluid. Possibly, the slow release is due to relative energy failure (that is, shortage of ATP at membrane sites which is replenished by diffusion from cytoplasmic stores), while the fast release may reflect overt ATP depletion; however, it is also possible that the fast release requires opening of ionic gates. What seems to be established is that Ca²⁺ influx occurs when extracellular K⁺ approaches 15 μmol·ml⁻¹, probably because the concomitant depolarization then opens voltage-dependent Ca²⁺ gates. There is evidence that Ca²⁺ influx is normally stimulated by excitatory amino acids, and that it then predominantly occurs at postsynaptic sites, notably in apical dendrites of neocortical and limbic pyramidal cells. It is not known where Ca²⁺ accumulates during ischemia; however, it seems likely that cells with high Ca²⁺ conductances also have a large Ca²⁺ influx. This assumption receives support from experiments showing that induced seizures lead to accumulation of Ca²⁺ in the mitochondria of hippocampal pyramidal cells.

The changes in Na⁺ and Cl⁻ concentrations and in extracellular fluid volume deserve comment. Van Harreveld and Ochs originally showed that tissue anoxia was associated with an increase in tissue im-
pedance, with an electron microscopically discernible reduction in extracellular fluid space, and with uptake of Cl⁻ into glial elements. Subsequent work by Bourke and Kimelberg and their associates, who defined a HCO₃⁻/Cl⁻ antiporter in glial cells, showed in vitro as well as in vivo that increases in extracellular K⁺ to values exceeding 10 μmol·ml⁻¹ stimulated uptake of Na⁺, Cl⁻, and water into glial cells with an ensuing astrocytic swelling. Probably this K⁺-stimulated uptake of ions and water is what causes the perivascular and perineuronal astrocytic edema which accompanies not only hypoxia/ischemia but also severe hypoglycemia and status epilepticus. The question arises as to the mechanism involved, especially since status epilepticus does not cause K⁺ accumulation in excess of 10 μmol·ml⁻¹. We will return to this issue later.

Provided that the ischemia is of limited duration, extracellular ion concentrations and fluid spaces normalize during recirculation. Extracellular acidosis seems to persist for at least 40 minutes, though, indicating that changes in intra- and extracellular pH become dissociated. Possibly, expulsion of H⁺ from cells retards the normalization of extracellular pH.

So far, we have tacitly assumed that water and ion fluxes are limited to an exchange between extra- and intracellular spaces (that is, that no net increase in tissue fluid content occurs). Although this may be true for the period of ischemia, conditions existing in the immediate recirculation period favor net ion and water fluxes between blood and brain. Net water flux seems partly triggered by an increase in tissue osmolality during the ischemia; however, changes in extracellular ion concentrations may lead to loss of K⁺ to the blood, and gain of Na⁺, Cl⁻, and water from the blood. With adequate recirculation, such changes are eventually reversed, but if circulation is deficient they are not, and progressive deterioration of ion and water homeostasis may involve massive accumulation of Ca²⁺ as well. We return to the problem of edema when discussing regional ischemia.

**Metabolic Cascades.** In all probability, the series of metabolic reactions described is initiated by ATP shortage, which allows release of K⁺ from cells: this in turn leads to both influx of Ca²⁺ into neurons and uptake of Na⁺ and Cl⁻ by glial cells, with an ensuing cellular edema. The metabolic cascades elicited also encompass reactions other than those considered. We will discuss some of these, emphasizing events secondary to K⁺ release, Ca²⁺ uptake, and adenosine accumulation.

Energy failure is known to cause not only adenosine accumulation but also release from cells of monoamine and amino acid transmitters, as well as in vitro that increases in extracellular K⁺ concentration, which leads to influx of Ca²⁺ and accelerated exocytosis at a time when reuptake or metabolic inactivation of transmitters is inhibited. Since noradrenaline and adenosine are known activators of membrane-bound adenylate cyclases, the expected result is accumulation of cyclic AMP (cAMP). This has indeed been documented, as a transient event both during ischemia and, to an exaggerated degree, during recirculation. Probably, the burst of cAMP production during recirculation is due to the fact that the agonists abound at receptor sites when ATP, the substrate of adenylate cyclase, is being resynthesized. Although cAMP must trigger a multitude of reactions, we consider only its presumed influence on ionic gates. It has been established that both adenosine and noradrenaline enhance uptake of Na⁺, Cl⁻, and water into glial cells. We can envisage, therefore, that the effect of K⁺ accumulation is to cause massive transmitter release, which in turn augments the normally low permeability of glial cell membranes to other ions. This assertion is supported by findings showing that exposure of brain tissues to adenosine or noradrenaline can induce glial swelling even in the absence of a rise in K⁺ concentration, and could explain why seizures are accompanied by such swelling. Thus, even though the extracellular K⁺ concentration does not rise above 10 μmol·ml⁻¹, seizures lead to accumulation of adenosine and elevated noradrenaline turnover. Augmented transmitter release may also explain why seizures are accompanied by cellular uptake of Ca²⁺ even though ATP concentrations are close to normal, and extracellular K⁺ does not reach the threshold value of close to 15 μmol·gm⁻¹. Thus, if excitatory transmitters like glutamate abound at postsynaptic Ca²⁺ gates, an increase in Ca²⁺ conductance may overload the transport capacity of membranes.

Two of the primary events, ATP shortage and Ca²⁺ influx, probably act in conjunction to initiate and sustain release of FFA's from phospholipids (Fig. 12). Thus, depletion of ATP arrests resynthesis of GPI and reacylation of lysophospholipids, while Ca²⁺ influx must accelerate phospholipid breakdown. A contributory cause to the rapid accumulation of FFA's is the presence in the tissue of diglyceride lipases and lysophospholipases. It should be emphasized that enhanced lipolysis during ischemia and other adverse conditions is a threshold event that occurs first when overt energy failure is at hand. The evidence for this has been obtained in experiments on gradual hypoxia and hypoglycemia (T. Wieloch, et al., unpublished data).

As remarked, increased levels of FFA's including arachidonic acid persist for some time during recirculation. Since resumption of perfusion leads to a phase of cellular hyperoxia (see above), conditions are at hand for an arachidonic acid cascade along the cyclo-oxygenase and lipoxygenase pathways. As Fig. 8 shows, this cascade leads to the production not only of prostaglandins, thromboxane A₂, and 12-lipoxygenase products, but also of free radicals (denoted by Q-). Additional free radicals may be formed during reoxidation of the members of the respiratory chain, by autoxidation of catecholamines, and when xanthine and hypoxanthine are metabolized by xanthine oxidase.
A burst of postischemic production of prostaglandins has been unequivocally documented; however, it has been more difficult to prove that free radicals are formed. It was originally proposed by Demopoulos, Flamm, and their collaborators that free-radical damage occurs in regional ischemia due to occlusion of the middle cerebral artery (MCA). However, conditions during recirculation following transient ischemia could favor radical formation as well. Free-radical formation is potentially harmful since it can lead to peroxidation and subsequent fragmentation of polyenic, phospholipid-bound fatty acids, as well as to cross-linking of proteins with inactivation of enzymes.

The Demopoulos-Flamm hypothesis considers that free radicals are dislocated during incomplete ischemia due to lack of electron acceptor (oxygen) at the cytochrome oxidase step. Suggestive evidence was the reduction in tissue concentrations of ascorbic acid (a natural radical scavenger) and polyenoic phospholipid-bound fatty acids (the main site of radical attack); in addition, it was proposed that barbiturates protect against incomplete ischemia by acting as free-radical scavengers.

It can readily be shown in vitro that initiation of free-radical formation by suitable pro-oxidants (such as Fe$^{2+}$ and ascorbic acid) leads to the production of malondialdehyde and fluorescent products, to diene conjugation, and to reduction in tissue concentrations of polyenoic fatty acids as well as of scavengers such as glutathione (GSH). It has been more difficult to prove that such changes occur in vivo during transient ischemia, and it seems less likely that barbiturates in general act as free-radical scavengers. In view of these results, it is tempting to conclude that radical generation during regional ischemia due to MCA occlusion represents a late event, reflecting dissolution of cells that have died from reasons other than the cause of death.

In spite of these negative results, though, free-radical hypothesis has probably come to stay. Thus, failure to assess unequivocal radical generation may be due to the fact that, with transient ischemia, such generation is limited to part of the cell population, and to discrete cellular domains. It now seems likely that polyenic fatty acids induce cellular edema in the brain by free-radical mechanisms. Furthermore, free-radical damage to cortical vessels appears to occur in response to an arachidonic acid-triggered burst of PG formation, and may well contribute to vasospasm following subarachnoid hemorrhage. Finally, some evidence exists that transient ischemia does reduce tissue concentrations of lipid-soluble radical scavengers. The key to the problem may be that radical damage occurs only when lipolysis due to energy failure and Ca$^{2+}$ influx/release is pronounced, when suitable pro-oxidants are generated, or when the antioxidant capacity is overwhelmed. Such conditions may be at hand in certain cell groups (for instance, in cells with high Ca$^{2+}$ conductances and/or limited antioxidative capacity), making whole-tissue analysis a blunt tool.

One can hypothesize that the adverse effects of Ca$^{2+}$ influx into cells also encompass cascades other than those considered. For example, since intracellular concentrations of free Ca$^{2+}$, Na$, and FFA's are raised during transient ischemia, such generation could favor radical formation as well. Free-radical formation is potentially harmful since it can lead to peroxidation and subsequent fragmentation of polyenic, phospholipid-bound fatty acids, as well as to cross-linking of proteins with inactivation of enzymes.
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remarkable finding that ischemia, hypoglycemia, and status epileptics are accompanied by shrinkage of many neurons but by swelling of glial cells.23,53

It is clear that, since ischemia deprives cells of their energy supply, all ATP-requiring synthetic reactions must come to a halt. This also applies to protein synthesis.36,44,107,108,259-261 Three features of protein metabolism are especially noteworthy. First, although complete ischemia arrests peptide chain initiation and other energy-requiring steps in protein synthesis, it also seems to inhibit protein breakdown. As a result, polyribosomes remain aggregated until recirculation is initiated.36,107,108 Second, recovery of protein synthesis is slow and lags considerably behind restoration of cerebral energy state.34,108 Third, in severely ischemic tissue both neuronal and glial fractions show marked reduction in protein synthesis.259

At present, it is not possible to identify those mechanisms that are responsible for protein breakdown and inhibition of protein synthesis during and following ischemia. One problem is that inhibition of protein synthesis is exquisitely sensitive to disturbances of cerebral energy metabolism.260 For example, although seizures may cause only minimal perturbation of cerebral energy state, they nevertheless markedly inhibit protein synthesis.101,240 It seems clear, though, that influx/release of Ca2+ can accelerate proteolysis and degrade structural proteins, including those constituting the cytoskeletons. Although difficult to prove by analyses on tissue in bulk,132,260 it also seems likely that accelerated lipolysis can cause damage to membrane-bound enzymes. Thus, aberrations of protein metabolism and inactivation of enzymes may well constitute yet another cascade triggered by ATP shortage and Ca2+ influx/release.

Complete Versus Near-Complete Ischemia

It has often been assumed that complete ischemia carries a far greater risk than incomplete ischemia, even if the latter only leaves a trickle of flow.62 However, summarizing their experience with electrophysiological and metabolic recovery following complete ischemia, Hossmann and Kleihues remarked that many animals failed to show such recovery if the procedure for arresting CBF failed, and some residual flow persisted. Subsequent studies on rats showed that 30 minutes of complete ischemia allowed extensive short-term recovery of cerebral metabolism, while severe incomplete ischemia (with CBF less than 5% of control) was accompanied by signs of persisting energy failure.161-163 These apparently paradoxical results were corroborated by data demonstrating recovery of respiratory functions of isolated mitochondria following complete but not after incomplete ischemia.184

The results were not only paradoxical but also controversial. Thus, previous134-138 as well as more recent220 results clearly showed that some residual perfusion allowed better recovery, or prevented the development of histopathologically verifiable cell damage, even though the ischemia was dense enough to cause extensive deterioration of the cerebral energy state.

The controversy has now been resolved. Although it had been suspected for a long time that excessive tissue acidosis may induce cellular edema and hasten the development of cell damage, it remained for Myers150-152 to provide evidence that recovery following ischemia was influenced by the nutritional state. The author found that if animals were fed or infused with glucose prior to induction of ischemia, recovery was adversely affected, a result which they attributed to the association between raised plasma glucose concentrations and enhanced tissue lactic acidosis. These basic observations have been confirmed by several groups,51,97,181,185,186,207,345 and strong evidence has been presented that recovery correlates inversely with tissue lactic acidosis. A similarly adverse effect of glucose loading has been noted in severe hypoxia as well.55 The relationship between nutritional state and lactic acidosis in ischemia is illustrated in Fig. 13. We observe that in fasted animals exposed to incomplete ischemia, in which hyperglycemia does not occur, tissue lactic acid concentrations do not rise above 15 to 17 μmol.gm-1. In contrast, in fed animals, which respond to ischemia with marked hyperglycemia, tissue lactic acidosis becomes pronounced. A comparison between these data and those shown in Fig. 10 shows that fed animals exposed to complete ischemia show a similar accumulation of lactate as fasted animals exposed to incomplete ischemia.

Rehncrona, et al.185,186 conducted experiments in which blood (and tissue) glucose concentrations were varied prior to induction of complete or incomplete
ischemia, and tissue lactic acid concentrations during ischemia were correlated to short-term metabolic and neurophysiological recovery. They obtained the following interesting results. First, fasted animals exposed to incomplete ischemia showed better recovery than fed animals with complete ischemia, results similar to those reported by Marshall, et al.,134-136 and Steen, et al.220 Second, fed, or fasted and glucose-infused, animals exposed to incomplete ischemia showed poor recovery compared to fed animals exposed to complete ischemia, corroborating the paradoxical results reported previously.161-163,184 Third, progressive deterioration of metabolic recovery was observed at tissue lactic acid concentrations exceeding about 20 µmol·gm⁻¹, arbitrarily establishing a critical degree of acidosis.

Recalling that the animals studied by Marshall, et al.134-136 and Steen, et al.220 accumulated 15 to 17 µmol·gm⁻¹ of lactic acid during both complete and incomplete ischemia, all results discussed became internally consistent. Two important conclusions can be drawn. The first is that, unless blood glucose and tissue lactic acid concentrations do not rise unduly, a small residual perfusion during ischemia improves recovery, even if the tissue energy state is seemingly completely deranged. The second conclusion is that, if hyperglycemia develops, a trickle of flow may be detrimental.

Critical CBF Thresholds

So far, we have considered complete or nearly complete ischemia, that is, flow reductions of sufficient severity to cause extensive deterioration of cerebral energy state as well as of ion and water homeostasis. We must now address the important problem of critical CBF thresholds for loss of electrical and metabolic functions. Experiments with global or forebrain ischemia have defined critical cerebral perfusion pressures (CPP's) of 30 to 40 mm Hg for metabolic functions,134-136,214,215 and critical CBF thresholds at flow rates of 40% to 50% of control.134-136,213 However, it was early recognized that critical reductions of CPP and CBF could lead to grossly inhomogeneous metabolic alterations,213 an assumption that was amply corroborated by workers using an elegant nicotinamide adenine dinucleotide (NADH) fluorescence technique for localizing such inhomogeneities.244,246,247 We will turn our attention, therefore, to models that provide more comprehensive data on this issue.

There is no doubt that the most useful data on CBF thresholds have been obtained in experiments with MCA occlusion in cats and subhuman primates; in fact, apart from providing a closer approximation to vascular disease in man than any other model,239 such experiments have provided some challenging new concepts. It will facilitate our discussion of these concepts if we review current knowledge of the distribution and density of the resulting ischemia and the severity of the metabolic perturbation arising from it.

The advent of methods for measuring local blood flow at multiple sites with electrodes recording hydrogen clearance has allowed repeated assessment of flow rates at focal and perifocal sites. Figure 14, illustrating results obtained in anesthetized baboons, shows that MCA occlusion reduced CBF in the infarction-prone opercular region (A) to a mean of 26% of control. Neighboring areas (B and D) showed less dense ischemia, and the parasagittal region (C) exhibited CBF values close to control.226 The standard deviation values given reflect considerable inter-animal variation; for example, flow rates varied between 6% and 41% of control levels in Zone A, and between 17% and 92% of control rates in Zone B. Similar techniques have
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been used to measure local CBF in chronic stroke, in closed-skull preparations, and also in unanesthetized animals. Although such studies, and those devised to assess infarct size, have shown that the density and distribution of ischemia varies between baboons, monkeys, and cats, the basic concept of a CBF gradient between focal, perifocal, and distant zones remains the same. It is also of clear interest that loss of autoregulation renders ischemia flow rates pressure-passive; as a result, CBF can be varied within relatively wide limits by altering CPP.

In the squirrel monkey, MCA occlusion reduced CBF in the focal area to 20% to 50% of control levels. Using an elegant method for tissue sampling, Michenfelder and Sundt showed that MCA occlusion led to a gradual decrease in ATP and a gradual rise in lactate content of the cerebral cortex; in fact, a reduction in ATP concentration to below 50% of control and a rise in lactate concentration above 15 μmol·gm⁻¹ were observed first after about 2 hours. We recognize that this time course is quite different from that observed in complete or near-complete ischemia (see Fig. 10). Clearly, the data reported reflect considerable differences in metabolic response, not only between animals but also between regions. This was amply demonstrated in experiments on cats, in which the tissue was frozen by the funnel technique and multiregional measurements of metabolites were performed. On the whole, though, the combined results favor the view that regional ischemia due to MCA occlusion allows sufficient flow to considerably retard the development of gross energy failure.

Threshold for Electrical Failure. One of the most exciting results emerging from studies of MCA occlusion is the demonstration of distinct CBF thresholds for various cellular functions. Clinical observations, based on CBF and electroencephalographic (EEG) recordings during carotid endarterectomy showed that EEG flattening occurred when CBF fell below 0.16 to 0.18 ml·gm⁻¹·min⁻¹. Subsequent experiments on baboons established the critical CBF value for loss of sensory evoked potentials as 0.15 ml·gm⁻¹·min⁻¹, and a similar threshold (0.18 ml·gm⁻¹·min⁻¹) was established for loss of neuronal spike activity in the cat.

Threshold for Ion Pump Failure. Once the threshold for electrical failure was established, the question arose as to the mechanisms involved. Studies found that cessation of spontaneous or evoked electrical activity was not related to membrane failure with release of K⁺ from ATP-derived cells, since such release required a further reduction of CBF to about 0.10 ml·gm⁻¹·min⁻¹. These studies demonstrated both a second critical threshold (for membrane pump failure) and a narrow CBF range of about 0.05 ml·gm⁻¹·min⁻¹ over which electrically quiescent nerve cells retain the metabolic capacity to transport ions. However, it should be recalled that, although cessation of evoked responses was not correlated to changes in extracellular pH, some degree of acidosis was present in the prepolarization phase.

Threshold for Energy Failure. Since it has been established that an increase in extracellular K⁺ concentrations to values exceeding 10 μmol·ml⁻¹ is accompanied both by cellular uptake of Ca²⁺ and by (glial) uptake of Na⁺, Cl⁻, and water (see above), it appears that crossing the electrical threshold elicits extensive ion and water fluxes. The triggering event has not been clearly established, but a critical lowering of cellular energy stores seems likely. Thus, experiments with gradual reduction of cerebral oxygen availability during seizures induced by bicuculline, a GABA receptor blocker, have shown that electrical failure precedes K⁺ release, and that the latter is associated with clear deterioration of cerebral energy state (G Blennow, et al., unpublished data). Furthermore, during insulin-induced hypoglycemia, release of K⁺, cellular uptake of Ca²⁺, and reduction in extracellular fluid volume occur pari passu with depletion of cellular energy stores (T Wieloch, et al., unpublished data). It is of interest that the ensuing cascade of events encompasses marked accumulation of FFA’s as well. There is reason to believe, therefore, that the thresholds for ion pump failure and energy failure are similar, if not identical.

Threshold for Infarction. In monkeys, restoration of flow within 1 to 2 hours after MCA occlusion can cause full recovery of neurological function and prevent histopathological damage; in the cat, this period is even longer. Experiments on monkeys suggest that infarct size correlates to the reduction in local CBF, and that infarcts develop only if flow is reduced below 0.12 ml·gm⁻¹·min⁻¹ for 2 hours, or longer. Similar results were obtained in the cat, but the critical CBF (about 0.20 ml·gm⁻¹·min⁻¹) was somewhat higher. In view of the fact that CBF thresholds for electrical and ion pump failure differ by only about 0.05 ml·gm⁻¹·min⁻¹ (see above), one cannot conclude that the thresholds for metabolic failure and infarction are the same. However, it seems reasonable to conclude that infarction does not develop unless cellular energy metabolism and ion homeostasis fail.

Threshold for Edema Formation. At any given moment after MCA clipping, a gradient of ischemia density extends from focal to perifocal to essentially normal tissue. Along this gradient the focus may be the site of energy and membrane failure and of infarct development, while the perifocal area (the penumbra) contains viable but electrically silent cells. However, this condition is not static since deterioration of flow may progress and involve penumbral and even more peripheral areas. It is now recognized that brain edema constitutes a major factor in this progression, often being the principal cause of death of patients due to tentorial herniation. The literature on the subject is large, and the reader should consult recent articles for references.
genic, the latter being associated with blood-brain barrier dysfunction and extravasation of proteins. The general consensus is that the edema (“ischemia edema”) is of a predominantly cytotoxic type during the first few hours of MCA occlusion. Vasogenic edema, which occurs later, will therefore be disregarded in the present discussion. We will ask the questions: what characterizes ischemic edema, when does it occur, and what are the mechanisms?

Whereas the glial swelling associated with complete ischemia does not lead to net water uptake, edema associated with regional ischemia does: that is, it leads to an increase in tissue volume due to an increase in its fluid content. The nature of the fluid is revealed by Fig. 15 which shows that water accumulates with Na⁺ (and, not shown, with Cl⁻), while the tissue K⁺ concentration is reduced. Essentially, therefore, edema formation involves the uptake by tissue of fluid with a composition similar to that of extracellular fluid or blood plasma. We recognize that such changes are those expected to occur in any tissue when the Na⁺ extrusion mechanisms of cells fail.

To try to answer the question of when edema occurs is to probe into the problem of CBF thresholds for edema formation. Results obtained in gerbils, cats, and baboons demonstrate that edema formation begins within the first 30 minutes of vascular occlusion but that it attains significant magnitudes only after 60 to 90 minutes. Somewhat surprisingly, edema was formed in tissue with a reduction in CBF to just below 0.20 ml·gm⁻¹·min⁻¹, suggesting that the threshold for edema formation was similar to that for electrical failure (that is, higher than that for membrane failure). If true, this would imply that energy failure and changes in extracellular fluid ion concentrations need not be present. Somewhat different results were obtained by another group of investigators, who reported that ischemia of sufficient density to induce edema reduced high-energy phosphates and markedly increased lactate concentrations; what complicates the interpretation is that in “non-critical ischemia” (ischemia not leading to edema), metabolic perturbation was similarly pronounced.

Clearly, the relationship between energy failure and edema formation is equivocal. On theoretical grounds, though, it seems likely that ATP shortage and disruption of ionic homeostasis is the main cause of ischemic brain edema. The only conceivable alternative is the release of membrane-active agents which profoundly alter ion permeability, increasing membrane leak to such a degree that the membrane pumps cannot cope with ion fluxes. However, it remains to be shown that such agents exist, and that they can be released in the absence of overt energy failure.

**Brain-Cell Damage: Circulatory and Metabolic Aspects**

In this concluding section, I will briefly review the current knowledge of revival times. Revival times are defined as the maximum periods of ischemia that will allow complete recovery and preservation of cell structure. I will also discuss localization of cell damage in the brain, before attempting to briefly synthesize information on metabolic reactions that bear on the problem of irreversible cell damage.

**Revival Times**

Prior to 1963, it was generally assumed that the maximum revival time for complete ischemia was less than 10 minutes; with longer ischemic periods gross neurological deficits and widespread histopathologic damage were reported. In 1963, Neely and Youmans reported that dogs exposed to CSF compression ischemia of up to 25 minutes' duration showed unexpected neurological recovery, and Miller and Myers reported revival times for neurological function and cell structure of 14 to 15 minutes in monkeys. The remarkable series of experiments by Hossmann, et al., then demonstrated that extensive short-term recovery of neurophysiological and metabolic functions could be achieved even after 60 minutes of ischemia in cats and monkeys. An even more extensive recovery of cerebral energy metabolism was demonstrated after 15 and 30 minutes ischemia in rats.

At that time, it was suspected that the short revival times reported in the earlier literature were due to unrecognized complicating events such as the presence of no-reflow zones and of postischemic hypotension, both events tending to prolong the ischemic period. However, this conclusion did not receive support from the carefully controlled series of experiments by Marshall, et al., who noted failure of neurological

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**Fig. 15.** Changes in cerebral cortical blood flow, water content, and K⁺-Na⁺ concentrations following middle cerebral artery occlusion in cats. d.w. = dry weight; w.w. = wet weight. (Reproduced with permission from Hossmann KA: Treatment of experimental cerebral ischemia. J Cereb Blood Flow Metab 2:275–297, 1982.)
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recovery and the appearance of histopathological cell damage following 15 minutes of ischemia in five of seven rabbits. It also became clear from experiments on monkeys that the maximum revival time following complete ischemia was around 15 minutes. Then came the demonstration by Ito, et al., Kirino, and Pulsnelli, et al., that even shorter ischemic periods (5 to 10 minutes) were followed by definite cell damage that “matured” over hours and days. We have recently confirmed this, using a model of severe forebrain ischemia allowing recovery for days or weeks (Auer, et al., unpublished data). In fact, although this model allows rapid reperfusion and is not complicated by postischemic hypotension, the animals show relatively extensive neuronal damage after 10 minutes of ischemia.

It seems likely that the perplexing differences in outcome between these various series can be explained by the fact that the pre-1963 workers, and those documenting maturation of cell damage, all employed long postischemic recovery periods (usually days or weeks), whereas the remarkable neurophysiological and metabolic restoration noted by others after 30 to 60 minutes of ischemia was obtained in short-term recovery studies. It is admittedly of considerable interest that such recovery is possible after prolonged ischemia, and that many nerve cells seem to survive the insult. However, the hypothesis that the true revival times exceed 15 minutes under optimal conditions seems untenable.

Clearly, revival times correlate inversely with the density of ischemia. Thus, provided that hyperglycemia does not develop, even a trickle of flow in the ischemic tissue seems to prolong revival times. It is perplexing that this seems to be the case even though complete energy failure is at hand. One may speculate that the maintenance of ATP production, albeit too slow to measurably raise tissue ATP concentrations, significantly retards degradation of cell structure.

Selective Vulnerability

It has been known for more than 50 years that brain cells differ in their vulnerability to ischemia and other insults. Maximum damage is often inflicted upon pyramidal cells in the hippocampus and certain neocortical layers, Purkinje cells in the cerebellum, and small neurons in the caudoputamen. Recent examples of such selectivity in response are provided by the results obtained in monkeys, cats, gerbils, and rats. In one respect, therefore, our statement regarding revival times requires modification. However, we are left with the intriguing question: what determines the short revival times of vulnerable cells, and the long revival times for resistant cells?

Mechanisms of Cell Damage

Although we cannot identify the mechanisms that lead to the death of a cell, it seems obvious that irreversible damage is incurred when autolytic processes caused by lysosomal and other enzymes prevent the maintenance or reassembly of orderly membrane structures. In all probability, this action results from irreversible loss of energy production. The question then arises how such a loss comes about. It is clear that, at normothermia, periods of complete ischemia exceeding 1 hour lead to brain death. However, this does not necessarily mean that all cells die in that time. Brain death could result from the fact that this period of ischemia does not allow adequate recirculation, or that it gives rise to a secondary deterioration of circulation, such as due to massive edema. If this is the case, cell structure will be degraded, even if hours are required to reach the point of no recovery. The difficult question, though, is to identify the mechanisms that lead to the death of selectively vulnerable cells which have suffered only 5 to 10 minutes of ischemia, and which share the environment of those that survive.

Obviously, our task is to define two series of events: those that lead to brain death, and those that only affect individual cells in a large population. In all probability, the common denominator (the initiating event) is energy failure with an ensuing derangement of membrane function. As already discussed, efflux of K⁺ will allow Ca²⁺ to enter cells, and enhance transmitter release. These events, and an accelerated lipolysis, promote both swelling of glial cells and tissue edema due to shifts of water and electrolytes between blood and tissue. At this stage, reversibility depends on the availability of ATP for maintenance or resumption of Na⁺ extrusion, K⁺ uptake, and cellular export of Ca²⁺. One can envisage that when sufficient ATP is no longer available, progressive edema impedes nutritional blood flow to such an extent that the process of infarction is initiated; alternatively, resumption of circulation to a tissue with impaired mitochondrial function quickly leads to such extensive edema that secondary and fatal ischemia ensues.

Although opinions differ, there is strong evidence that tissue acidosis, especially if excessive, accelerates edema formation. For example, edema has been a conspicuous feature in experimental settings in which feeding, or glucose-loading, proved to shorten revival times in complete or near-complete ischemia. The mechanisms have not been defined, but may be related to glial cell function, such as pH-dependent ion exchanges across glial membranes.

Although infarction or brain death may depend heavily on the association of shortage of ATP, membrane failure, and acidosis, and on an irreversible impediment of CBF, the selective death of vulnerable cells requires another explanation. The inescapable conclusion is that the cells affected share special metabolic characteristics which make them succumb to only 5 to 10 minutes of ischemia. An intriguing factor of this vulnerability is that cell death, as defined by morphological techniques, may occur days after the insult. As reviewed elsewhere, there is now circumstantial evidence that Ca²⁺ influx into cells set in motion a series of reactions that lead to the death of cells. It is a fascinating possibility that the differences between cells in their suscep-
tibility to ischemia and other insults reside in corres-
ponding differences in membrane permeability to 
Ca$^{2+}$. It also seems possible that one of the con-
sequences of such influx is initiation of free-radical re-
actions, and that maturation of cell damage reflects the slow time course of such reactions, whether leading to 
degradation of membrane structure or inactivation of enzymes. Although no direct proof is available that defines Ca$^{2+}$ influx and Ca$^{2+}$-triggered metabolic cascades as the basis for selective neuronal vulnerability, the problem is of such magnitude that the hypothesis deserves intense exploration. Obviously, the task of unravelling mechanisms of cell death due to ischemia is demanding, and brings us into an era of neurochem-
ical pathology at the cellular and molecular level.

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Address reprint requests to: Bo K. Siesjö, M.D., Laboratory for Experimental Brain Research, E-Blocket, Lund Hospital, S-221 85 Lund, Sweden.

**B. K. Siesjö**

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