1999

Circulation and energy metabolism in the brain / Donald D. Clarke and Louis Sokoloff

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The biochemical pathways of energy metabolism in the brain are in most respects like those of other tissues, but special conditions peculiar to the central nervous system in vivo limit full expression of its biochemical potentialities. In no tissue are the discrepancies between in vivo and in vitro properties greater or the extrapolations from in vitro data to conclusions about in vivo metabolic functions more hazardous. Valid identification of normally used substrates and products of cerebral energy metabolism, as well as reliable estimations of their rates of utilization and production, can be obtained only in the intact animal; in vitro studies identify pathways of intermediary metabolism, mechanisms and potential rather than actual performance.

Although the brain is said to be unique among tissues in its high rate of oxidative metabolism, the overall cerebral metabolic rate for O₂ (CMRO₂) is of the same order as the un-stressed heart and renal cortex [1]. Regional fluxes in the brain may greatly exceed CMRO₂, however, and these are closely coupled to changes in metabolic demand.

INTERMEDIARY METABOLISM

ATP production in brain is highly regulated

Oxidative steps of carbohydrate metabolism normally contribute 36 of the 38 high-energy phosphate bonds (~P) generated during aerobic metabolism of a single glucose molecule. About 15% of brain glucose is converted to lactate and does not enter the Krebs cycle, also termed the citric acid cycle. However, this might be matched by a corresponding uptake of ketone bodies. The total net gain of ~P is 33 equivalents per mole of glucose utilized. The steady-state concentration of ATP is high and represents the sum of very rapid synthesis and utilization. On average, half of the terminal phosphate groups turn over in about 3 sec; this is probably much faster in certain regions [2]. The level of ~P is kept constant by regulation of ADP phosphorylation in relation to ATP hydrolysis. The active adenyllyl kinase reaction, which forms equivalent amounts of ATP and AMP from ADP, prevents any great accumulation of ADP. Only a small amount of AMP is present under steady-state conditions; thus, a relatively small decrease in ATP may lead to a relatively large increase in AMP, which is a positive modulator of many reactions that lead to increased ATP synthesis. Such an amplification factor provides a sensitive control for maintenance of ATP levels [3]. Between 37 and 42°C, the brain metabolic rate increases about 5% per degree.

The concentration of creatine phosphate (CRP) in brain is even higher than that of ATP, and creatine phosphokinase (CPK) is extremely active. The CRP level is exquisitely sensitive to changes in oxygenation, providing ~P for ADP...
phosphorylation and, thus, maintaining ATP levels. The CPK system also may function in regulating mitochondrial activity. In neurons with a very heterogeneous mitochondrial distribution, the CRP shuttle may play a critical role in energy transport [4]. The BB isoenzyme of CPK is characteristic of, but not confined to, brain. Thus, its presence in body fluids does not necessarily indicate disruption of neural tissue.

**Glycogen is a dynamic but limited energy store in brain**

Although present in relatively low concentration in brain (3.3 mmol/kg brain in rat), glycogen is a unique energy reserve that requires no energy (ATP) for initiation of its metabolism. As with glucose, glycogen levels in brain appear to vary with plasma glucose concentrations. Biopsies have shown that human brain contains much more glycogen than rodent brain, but the effects of anesthesia and pathological changes in the biopsied tissue may have contributed. Glycogen granules are seen in electron micrographs of glia and neurons of immature animals but only in astrocytes of adults. Barbiturates decrease brain metabolism and increase the number of granules seen, particularly in astrocytes of synaptic regions; however, biochemical studies show that neurons do contain glycogen and that enzymes for its synthesis and metabolism are present in synaptosomes. Astrocyte glycogen may form a store of carbohydrate made available to neurons by still undefined mechanisms. Associated with the granules are enzymes involved in glycogen synthesis and, perhaps, degradation. The increased glycogen found in areas of brain injury may be due to glial changes or to decreased utilization during tissue preparation.

The accepted role of glycogen is that of a carbohydrate reserve utilized when glucose falls below need. However, rapid, continual breakdown and synthesis of glycogen occur at a rate of 19 μmol/kg/min. This is about 2% of the normal glycolytic flux in brain and is subject to elaborate control mechanisms. This suggests that, even under steady-state conditions, local carbohydrate reserves are important for brain function. However, if glycogen were the sole supply, normal glycolytic flux in brain would be maintained for less than 5 min.

The enzymes which synthesize and catabolize glycogen in other tissues are found in brain also, but their kinetic and regulatory properties do differ [5]. Glycogen metabolism in brain, unlike in other tissues, is controlled locally. It is isolated from the tumult of systemic activity, evidently by the blood–brain barrier (BBB). Although glucocorticoid hormones that penetrate the BBB increase glycogen turnover, circulating protein hormones and biogenic amines have no effect. Beyond the BBB, cells are sensitive to local amine concentrations; drugs that cross the BBB and modify local amine concentrations or membrane receptors thus cause metabolic changes (see Chap. 32).

Separate systems for the synthesis and degradation of glycogen provide a greater degree of control than if glycogen were degraded by simply reversing its synthesis (Fig. 31-1). The amount of glucose-6-phosphate (G6P), the initial synthetic substrate, usually varies inversely with the rate of brain glycolysis because of greater facilitation of the phosphofructokinase step relative to transport and phosphorylation of glucose. Thus, a decline in G6P during energy need slows glycogen formation.

The glucosyl group of uridine diphospho-glucose (UDP-glucose) is transferred to the terminal glucose of the nonreducing end of an amylose chain in an α-1,4-glycosidic linkage (Fig. 31-1). This reaction, catalyzed by glycogen synthetase (GS), is rate-controlling for glycogen synthesis [5]. In brain, as in other tissues, GS occurs in both a phosphorylated (D) form, which depends on G6P as a positive modulator, and a dephosphorylated, independent (I) form sensitive to, but not dependent on, the modulator. Although in brain the I form of GS requires no stimulator, it has a relatively low affinity for UDP-glucose. At times of increased energy demand, not only is there a change from the D to the I form but also an I form with even lower affinity for substrate develops. Inhibition of glycogen synthesis is enhanced, and this increases the availability of G6P for energy needs. Goldberg and O'Toole [5] hypothesize that the I form in brain is associated with inhibition of glycogen synthesis under conditions of energy demand, whereas the D form causes a relatively
small regulated synthesis under resting conditions. Regulation of the D form may reduce glycogen formation in brain to approximately 5% of its potential rate. In liver, where large amounts of glycogen are synthesized and degraded, the I form of GS is associated with glycogen formation. At present, it appears that the two tissues use the same biochemical apparatus in different ways to bring about their different overall metabolic patterns.

Under steady-state conditions, it is probable that less than 10% of phosphorylase in brain (Fig. 31-1) is in the unphosphorylated b form (requiring AMP), which is inactive at the very low AMP concentrations present normally. When a steady state is disturbed, there may be an extremely rapid conversion of enzyme to the a form, which is active at low AMP. Brain phosphorylase b kinase is activated indirectly by cAMP and by the molar concentrations of Ca$^{2+}$ released during neuronal excitation (see Chap. 22). The endoplasmic reticulum of brain, like muscle, is capable of taking up Ca$^{2+}$ to terminate its stimulatory effect. These reactions provide energy from glycogen during excitation and when cAMP-forming systems are activated. It has not been possible to confirm directly that the conversion from phosphorylase b to a is a control point of glycogenolysis in vivo. Norepinephrine and, probably, dopamine activate glycogenolysis through cAMP; but epinephrine, vasopressin and angiotensin II do so by another mechanism, possibly involving Ca$^{2+}$ or a Ca$^{2+}$-mediated proteolysis of phosphorylase kinase.

Hydrolysis of the α-1,4-glycoside linkages leaves a limit dextrin that turns over at only half the rate of the outer chains (see also Chap. 42). The debrancher enzyme that hydrolyzes the α-1,6-glycoside linkages may be rate-limiting if the entire glycogen granule is utilized. Because one product of this enzyme is free glucose, approximately one glucose molecule for every 11 of G6P is released if an entire glycogen molecule is degraded (Fig. 31-1). α-Glucosidase, also termed acid maltase, is a lysosomal enzyme whose precise function in glycogen metabolism is not known. In Pompe's disease, which is the hereditary absence of α-glucosidase, glycogen accumulates in brain as well as elsewhere (see Chap. 42). The steady-state concentration of glycogen is regulated precisely by the coordination of synthetic and degradative processes through enzymatic regulation at several metabolic steps [6].
Brain glycolysis is regulated mainly by hexokinase and phosphofructokinase

Aerobic and anaerobic glycolysis have been defined historically as the amount of lactate produced under conditions of "adequate" oxygen and no oxygen, respectively. More recently, glycolysis refers to the Embden-Meyerhoff glycolytic sequence from glucose, or glycogen glucosyl, to pyruvate. Glycolytic flux is defined indirectly: it is the rate at which glucose must be utilized to produce the observed rate of ADP phosphorylation.

Figure 31-2 outlines the flow of glycolytic substrates in brain. Glycolysis first involves phosphorylation by hexokinase. The reaction is essentially irreversible and is a key point in the regulation of carbohydrate metabolism in brain. The electrophoretically slow-moving (type I) isoenzyme of hexokinase is characteristic of brain. In most tissues, hexokinase may be soluble and may exist in the cytosol or be attached firmly to mitochondria. Under conditions in which no special effort is made to stop metabolism while isolating mitochondria, 80 to 90% of brain hexokinase is bound. In the live steady state, however, when availability of substrate keeps up with metabolic demand and end products are removed, an equilibrium exists be-

**Figure 31-2.** Glycolysis in brain. Enzyme and metabolic data expressed as in Figure 31-1. *Ten-day-old mouse brain [43]. (Data from [44].)
between the soluble and the bound enzyme. Binding changes the kinetic properties of hexokinase and its inhibition by G6P so that the bound enzyme on mitochondria is more active. The extent of binding is inversely related to the ATP:ADP ratio, so conditions in which energy utilization exceeds supply shift the solubilization equilibrium to the bound form and produce a greater potential capacity for initiating glycolysis to meet the energy demand. This mechanism allows ATP to function both as the substrate of the enzyme and, at another site, as a regulator to decrease ATP production through its influence on enzyme binding. It also confers preference on glucose in the competition for the MgATP$^{2-}$ generated by mitochondrial oxidative phosphorylation. Thus, a process that will sustain ATP production continues at the expense of other uses of energy. Because energy reserves are exhausted rapidly postmortem, it is not surprising that brain hexokinase is bound almost entirely.

The significance of reversible binding of other enzymes to mitochondria is not clear. The measured glycolytic flux, when compared with the maximal velocity of hexokinase, indicates that in the steady state the hexokinase reaction is inhibited 97%. Brain hexokinase is inhibited by its product G6P, to a lesser extent by ADP and allosterically by 3-phosphoglycerate and several nucleoside phosphates, including cAMP and free ATP$^4^-$. The ratio of ATP to Mg$^{2+}$ also may have a regulatory action. In addition to acting on enzyme kinetics, G6P solubilizes hexokinase, thus reducing the efficiency of the enzyme when the reaction product accumulates. The sum total of these mechanisms is a fine tuning of the activity of the initial enzyme in glycolysis in response to changes in the cellular environment. Gluconeokinase, also termed low-affinity hexokinase, a major component of liver hexokinase, has not been found in brain.

G6P represents a branch point in metabolism because it is a common substrate for enzymes involved in glycolytic, pentose phosphate shunt and glycogen-forming pathways. There is also slight but detectable G6Pase activity in brain, the significance of which is not clear. The liver requires this enzyme to convert glycogen to glucose. The differences between the hexokinases and the modes of glycogen metabolism of liver and brain can be related to the function of liver as a carbohydrate storehouse for the body, whereas brain metabolism is adapted for rapid carbohydrate utilization for energy needs. In glycolysis, G6P is the substrate of phosphohexose isomerase. This is a reversible reaction, with a small free energy change and a 5:1 equilibrium ratio in brain that favors G6P.

Fructose-6-phosphate is the substrate of phosphofructokinase, a key regulatory enzyme controlling glycolysis [3]. The other substrate is MgATP$^{2-}$. Like other regulatory reactions, it is essentially irreversible. It is modulated by a large number of metabolites and cofactors, whose concentrations under different metabolic conditions have a great effect on glycolytic flux. Prominent among these are availability of $\sim$P and citrate concentrations. Brain phosphofructokinase is inhibited by ATP, Mg$^{2+}$ and citrate and stimulated by NH$_4^+$, K$^+$, PO$_4^{2-}$, 5'-AMP, 3',5'-cAMP, ADP and fructose-1,6-bisphosphate.

When oxygen is admitted to cells metabolizing anaerobically, utilization of O$_2$ increases, whereas utilization of glucose and production of lactate drop; this is known as the Pasteur effect. Modulation of the phosphofructokinase reaction can account directly for the Pasteur effect. In the steady state, ATP and citrate concentrations in brain apparently are sufficient to keep phosphofructokinase relatively inhibited as long as the concentration of positive modulators, or inhibitors, is low. When the steady state is disturbed, activation of this enzyme produces an increase in glycolytic flux, which takes place almost as fast as events changing the internal milieu.

Fructose-1,6-bisphosphate is split by brain aldolase to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Dihydroxyacetone phosphate is the common substrate for both glycerophosphate dehydrogenase, an enzyme active in reduced nicotinamide adenine dinucleotide (NADH) oxidation and lipid pathways (see Chap. 3), and triose phosphate isomerase, which maintains an equilibrium between dihydroxyacetone phosphate and glyceraldehyde-3-phosphae; the equilibrium strongly favors accumulation of dihydroxyacetone phosphate.

After the reaction with glyceraldehyde-3-phosphate dehydrogenase, glycolysis in brain proceeds through the usual steps. Brain enolase,
also termed D-2-phosphoglycerate hydrolyase, which catalyzes dehydration of 2-phosphoglycerate to phosphoenolpyruvate, is present as two related dimers, one of which (γ) is associated specifically with neurons and the other (α) with glia. The neuronal subunit is identical to the neuron-specific protein 14-3-2. Immunocytochemical determination of the enolases makes them useful in determining neuron:glia ratios in tissue samples, but neuron-specific enolase is not confined to neural tissue. Brain phosphoenolpyruvate kinase controls an essentially irreversible reaction that requires not only Mg\(^{2+}\), as do several other glycolytic enzymes, but also K\(^+\) or Na\(^+\). This step also may be regulatory.

Brain tissue, even when at rest and well oxygenated, produces a small amount of lactate, which is removed in the venous blood, accounting for 13% of the pyruvate produced by glycolysis. The measured lactate concentration in brain depends on success in rapidly arresting brain metabolism prior to tissue processing. Five lactate dehydrogenase isoenzymes are present in adult brain; the one that electrophoretically moves most rapidly toward the anode, termed band 1, predominates. This isoenzyme is generally higher in tissues that are more dependent on aerobic processes for energy; the slower moving isoenzymes are relatively higher in tissues such as white skeletal muscle, which is better adapted to function at suboptimal oxygen levels. The distribution of lactate dehydrogenase isoenzymes in various brain regions, layers of the retina, brain neoplasms and brain tissue cultures and during development indicates that their synthesis might be controlled by tissue oxygen concentrations. Lactate dehydrogenase functions in the cytoplasm to oxidize NADH, which accumulates as a result of the activity of glyceraldehyde-3-phosphate dehydrogenase in glycolysis. This permits glycolytic ATP production to continue under anaerobic conditions. Lactate dehydrogenase also functions under aerobic conditions because NADH cannot easily penetrate mitochondrial membranes. Oxidation of NADH in the cytoplasm depends on this reaction and on the activity of shuttle mechanisms that transfer reducing equivalents to mitochondria.

Glycerol phosphate dehydrogenase is another enzyme indirectly associated with glycolysis that participates in cytoplasmic oxidation of NADH. This enzyme reduces dihydroxyacetone phosphate to glycerol-3-phosphate, oxidizing NADH in the process. Under hypoxic conditions, α-glycerophosphate and lactate increase initially at comparable rates, although the amount of lactate produced greatly exceeds that of α-glycerophosphate. The relative concentrations of the oxidized and reduced substrates of these reactions indicate much higher local concentrations of NADH in brain than are found by gross measurements. In fact, the relative proportions of oxidized and reduced substrates of the reactions that are linked to the pyridine nucleotides may be a better indicator of local redox states (NAD\(^+/\)NADH) in brain than the direct measurement of pyridine nucleotides themselves [3,7].

An aspect of glucose metabolism that has led to much confusion is the observation that labeled glucose appears in carbon dioxide much more slowly than might be suggested from an examination of the glycolytic pathway plus the citric acid cycle [8]. Glucose flux is 0.5 to 1.0 μmol/min/g wet weight of brain in a variety of species. The concentration of glycolytic plus Krebs cycle intermediates is 2 μmol/g. Hence, the intermediates might turn over every 2 to 4 min and \(^{14}\)CO\(_2\) production might reach a steady state in 5 to 10 min. This is not seen experimentally. Also, large amounts of radioactivity are trapped in amino acids related to the Krebs cycle (70 to 80%) from 10 to 30 min after a glucose injection. This is due to high transaminase activity in comparison with flux through the Krebs cycle, and amino acids made by transamination of cytoplasmic intermediates behave as if they are part of the cycle. When pools of these amino acids (~20 μmol/g) are added to the Krebs cycle components plus glycolytic intermediates, the calculated time for \(^{14}\)CO\(_2\) evolution is increased by a factor of 10, in agreement with experimental results.

In contrast, in liver, amino acids related to the Krebs cycle are present at much lower steady-state values, and approximately 20% of the radioactivity from administered glucose is trapped in these amino acids shortly after injection. Thus, ignoring the radioactivity trapped in amino acids has a relatively small effect on esti-
mates of glycolytic fluxes in liver but makes an enormous difference in brain. Immature brain resembles liver more nearly in this respect. The relationship of the Krebs cycle to glycolysis undergoes a sharp change during development, coincident with the metabolic compartmentation of amino acid metabolism characteristic of adult brain.

**The pyruvate dehydrogenase complex plays a key role in regulating oxidation**

Pyruvate dehydrogenase has an activity of 14 nmol/min/mg protein in rat brain and controls the rate of pyruvate entry into the Krebs cycle as acetyl coenzyme A (acetyl-CoA). Pyruvate dehydrogenase, or decarboxylase, is part of a mitochondrial multienzyme complex that also includes the enzymes lipoate acetyltransferase and lipoamide dehydrogenase; the coenzymes thiamine pyrophosphate, lipoic acid, CoA and flavine; and nicotinamide adenine dinucleotides. It is inactivated by being phosphorylated at the decarboxylase moiety by a tightly bound Mg\(^{2+}\)/ATP\(^2-\)-dependent protein kinase and activated by being dephosphorylated by a loosely bound Mg\(^{2+}\)- and Ca\(^{2+}\)-dependent phosphatase. About half the brain enzyme is usually active. Pyruvate protects the complex against inactivation by inhibiting the kinase. ADP is a competitive inhibitor of Mg\(^{2+}\) for the inactivating kinase. Under conditions of greater metabolic demand, increases in pyruvate and ADP and decreases in acetyl-CoA and ATP make the complex more active. Pyruvate dehydrogenase is inhibited by NADH, decreasing formation of acetyl-CoA during hypoxia and allowing more pyruvate to be reduced by lactate dehydrogenase, thus forming the NAD\(^+\) necessary to sustain glycolysis. Pyruvate dehydrogenase defects do occur in several mitochondrial enzyme-deficiency states (see below and Morgan-Hughes [9], also Chap. 42).

Although acetylcholine synthesis normally is controlled by the rate of choline uptake and choline acetyltransferase activity (see Chap. 11), the supply of acetyl-CoA can be limiting under adverse conditions. Choline uptake is, however, independent of acetyl-CoA concentration. The mitochondrial membrane is not permeable to the acetyl-CoA produced within it, but there is efflux of its condensation product, citrate. Acetyl-CoA can then be formed from citrate by ATP citrate lyase. The acetyl moiety of acetylcholine is formed in a compartment, presumably the synaptosome, with rapid glucose turnover. The cytosol of cholinergic endings is rich in citrate lyase, and it is possible that citrate shuttles the acetyl-CoA from the mitochondrial compartment to the cytosol. During hypoxia or hypoglycemia, acetylcholine synthesis can be inhibited by failure of the acetyl-CoA supply.

**Energy output and oxygen consumption are associated with high rates of enzyme activity in the Krebs cycle**

The actual flux through the Krebs cycle depends on glycolysis and acetyl-CoA production, which can “push” the cycle, the possible control at several enzymatic steps of the cycle and the local ADP concentration, which is a prime activator of the mitochondrial respiration to which the Krebs cycle is linked. The steady-state concentration of citrate in brain is about one-fifth that of glucose. This is relatively high compared with glycolytic intermediates or isocitrate.

As in other tissues, there are two isocitrate dehydrogenases in brain. One is active primarily in the cytoplasm and requires nicotinamide adenine dinucleotide phosphate (NADP\(^+\)) as cofactor; the other, bound to mitochondria and requiring NAD\(^+\), is the enzyme that participates in the citric acid cycle. The NAD\(^+\)-linked enzyme catalyzes an essentially irreversible reaction, has allosteric properties, is inhibited by ATP and NADH and may be stimulated by ADP. The function of cytoplasmic NADP\(^+\) isocitrate dehydrogenase is uncertain, but it has been postulated that it supplies the NADPH necessary for many reductive synthetic reactions. The relatively high activity of this enzyme in immature brain and white matter is consistent with such a role. α-Ketoglutarate (α-KG) dehydrogenase, which oxidatively decarboxylates α-KG, requires the same cofactors as does the pyruvate decarboxylation step.

Succinate dehydrogenase, the enzyme that catalyzes the oxidation of succinate to fumarate, is bound tightly to the mitochondrial mem-
brane. In brain, succinate dehydrogenase also may have a regulatory role when the steady state is disturbed. Isocitrate and succinate concentrations in brain are affected little by changes in the flux of the citric acid cycle as long as an adequate glucose supply is available. The highly unfavorable free energy change of the malate dehydrogenase reaction is overcome by the rapid removal of oxaloacetate, which is maintained at low concentrations under steady-state conditions by the condensation reaction with acetyl-CoA [6].

Malic dehydrogenase is one of several enzymes in the citric acid cycle present in both the cytoplasm and mitochondria. The function of the cytoplasmic components of these enzyme activities is not known, but they may assist in the transfer of hydrogen equivalents from the cytoplasm into mitochondria.

The Krebs cycle functions as an oxidative process for energy production and as a source of various amino acids, for example, glutamate, glutamine, γ-aminobutyrate (GABA), aspartate and asparagine. To export net amounts of α-KG or oxaloacetate from the Krebs cycle, the supply of dicarboxylic acids must be replenished. The major route for this seems to be the fixation of CO₂ to pyruvate or other substrates at the three-carbon level. Thus, the CO₂ fixation rate sets an upper limit at which biosynthetic reactions can occur. In studies of acute ammonia toxicity in cats, this has been estimated as 0.15 μmol/g wet weight brain/min, or approximately 10% of the flux through the citric acid cycle (see below). Liver seems to have ten times the capacity of brain for CO₂ fixation, as is appropriate for an organ geared to making large quantities of protein for export [10]. In brain, pyruvate carboxylase, which catalyzes CO₂ fixation, appears to be largely an astrocytic enzyme. Pyruvate dehydrogenase seems to be the rate-limiting step for the entry of pyruvate into the Krebs cycle from glycolysis.

The pentose shunt, also termed the hexose monophosphate pathway, is active in brain

Under basal conditions 5 to 8% of brain glucose is likely to be metabolized via the pentose shunt in the adult monkey and 2.3% in the rat [8]. Both shunt enzymes and metabolic flux have been found in isolated nerve endings. The pentose pathway has relatively high activity in developing brain, reaching a peak during myelination. Its main contribution is probably to produce the NADPH needed for reductive reactions necessary for lipid synthesis (see Chap. 3). Shunt enzymes and metabolic flux are found in synaptosomes. Although the capacity of the pathway, as determined using nonphysiological electron acceptors, remains constant throughout the rat life span, activity with physiological acceptors could not be detected in middle-aged (18 months) and older animals. It seems that the shunt serves as a reserve pathway for use under such stresses as the need for increased lipid synthesis or repair or reduction of oxidative toxins. The shunt pathway also provides pentose for nucleotide synthesis; however, only a small fraction of the activity of this pathway would be required. As with glycogen synthesis, turnover in the pentose phosphate pathway decreases under conditions of increased energy need, for example, during and after high rates of stimulation. Pentose phosphate flux apparently is regulated by the concentrations of G6P, NADP⁺, glyceraldehyde-3-phosphate and fructose-6-phosphate. Since transketolase, an enzyme in this pathway, requires thiamine pyrophosphate as a cofactor, poor myelin maintenance in thiamine deficiency may reflect failure of this pathway to provide sufficient NADPH for lipid synthesis [6].

Glutamate in brain is compartmented into separate pools

The pools that subserve different metabolic pathways for glutamate equilibrate with each other only slowly. This compartmentation is a vital factor in the separate regulation of special functions of glutamate (see Chap. 15) and GABA (see Chap. 16), such as neurotransmission, and of general functions, such as protein biosynthesis. Glutamate metabolism in brain shows at least two distinct pools; in addition, the Krebs cycle intermediates associated with these pools are distinctly compartmented. Mathematical models to fit data from radiotracer experiments that require separate Krebs cycles to satisfy the hypotheses of compartmentation have been developed. A key assumption of current models is that GABA
is metabolized at a site different from its synthesis. The best fit of kinetic data is obtained when glutamate from a small pool actively converted to glutamine flows back to a larger pool (8 μmol/g), which is converted to GABA. Of possible relevance to this is the finding that glutamate decarboxylase (GAD) is localized at or near nerve terminals, whereas GABA transaminase, the major degradative enzyme, is mitochondrial.

Evidence points to an inferred small pool of glutamate (2 μmol/g) as probably glial. Glutamate released from nerve endings appears to be taken up by glia or neurons, converted to glutamine and recycled to glutamate and GABA (see Chaps. 5 and 15). Various estimates of the proportion of glucose carbon that flows through the GABA shunt have been made, but the most definitive experiments show this to be about 10% of the total glycolytic flux. While this may seem small, that portion of the Krebs cycle flux used for energy production, including ATP synthesis and maintenance of ionic gradients, does not require CO₂ fixation, while the portion used for biosynthesis of amino acids does. Recycling the carbon skeleton of some of the glutamate released in neurotransmission through glutamine and GABA to succinate diminishes the need for dicarboxylic acids to replenish intermediates of the Krebs cycle when export of α-KG to make amino acids takes place.

It is difficult to get good estimates of the extent of CO₂ fixation in brain; the maximum capability measured during ammonia stress, when glutamine increases rapidly, suggests that CO₂ fixation occurs at 0.15 μmol/g/min in cat and 0.33 μmol/g/min in rat; this is about the same rate as for the GABA shunt.

For comparison, only about 2% of the glucose flux in whole brain goes toward lipid synthesis and approximately 0.3% is used for protein synthesis. Thus, turnover of neurotransmitter amino acids is a major biosynthetic activity in brain.

Metabolic compartmentation of glutamate usually is observed when labeled ketogenic substrates are administered to animals. It is interesting that acetoacetate and β-hydroxybutyrate do not show this effect, apparently because ketone bodies are a normal substrate for brain and are taken up in all kinds of cells. Acetate and similar substrates, which are not taken up into brain efficiently, appear to be more readily taken up or activated, or both, in glia. This is believed to lead to the observed abnormal glutamine/glutamate ratio. Similarly, metabolic inhibitors, like fluoroacetate, appear to act selectively in glia and to produce their neurotoxic action without marked inhibition of the overall Krebs cycle flux in brain. This difference in behavior has led to suggestions that acetate and fluoroacetate may be useful markers for the study of glial metabolism by autoradiography [11].

A nonuniform distribution of metabolites in living systems is a widespread occurrence. Steady-state concentrations of GABA vary from 2 to 10 mM in discrete brain regions, and it has been estimated that GABA may be as high as 50 mM in nerve terminals. Observations in brain indicate the existence of pools of metabolites with half-lives of many hours for mixing, which is most unusual. The discovery of subcellular, morphological compartmentation, that is, different populations of mitochondria in cerebral cortex that have distinctive enzyme complements, may provide a somewhat better perspective by which to visualize such a separation of metabolic function [12].

In addition to the phasic release of both excitatory and inhibitory transmitters, there may be a continuous tonic release of GABA, dependent only on the activity of the enzyme responsible for its synthesis and independent of the depolarization of the presynaptic membrane. Such inhibitory neurons could act tonically by constantly maintaining an elevated threshold in the excitatory neurons so that the latter would start firing when a decrease occurred in the continuous release of GABA acting on them. This is consistent with a known correlation between the inhibition of GAD and the appearance of convulsions after certain drug treatments. GABA is depleted by some convulsant drugs and elevated by others.

DIFFERENCES BETWEEN IN VITRO AND IN VIVO BRAIN METABOLISM

In addition to the usual differences between in vitro and in vivo studies that pertain to all tissues, there are two unique conditions that pertain only to the central nervous system.
In contrast to cells of other tissues, individual nerve cells do not function autonomously.

They are generally so incorporated into a complex neural network that their functional activity is integrated with that of various other parts of the central nervous system and with somatic tissues. In addition, neurons and adjacent glia are linked in their metabolism. Any procedure that interrupts the structural and functional integrity of the network inevitably would alter quantitatively and, perhaps, qualitatively its normal metabolic behavior.

The blood-brain barrier selectively limits the rates of transfer of soluble substances between blood and brain

This barrier discriminates among various potential substrates for cerebral metabolism (see Chap. 32). The substrate function is confined to those compounds in the blood that are not only suitable substrates for cerebral enzymes but also can penetrate from blood to brain at rates adequate to support the considerable energy demands of brain. Substances that can be readily oxidized by brain slices, minces or homogenates in vitro and that are utilized effectively in vivo when formed endogenously within the brain often are incapable of supporting cerebral energy metabolism and function when present in the blood because of restricted passage through the BBB. The in vitro techniques establish only the existence and potential capacity of the enzyme systems required for the use of a given substrate; they do not define the extent to which such a pathway actually is used in vivo. This can be done only in intact animals, and it is this aspect of cerebral metabolism with which this part of the chapter is concerned.

CEREBRAL ENERGY METABOLISM IN VIVO

Numerous methods have been used to study the metabolism of the brain in vivo; these vary in complexity and in the degree to which they yield quantitative results. Some require such minimal operative procedures on the laboratory animal that no anesthesia is required, and there is no interference with the tissue except for the effects of the particular experimental condition being studied. Some of these techniques are applicable to normal, conscious human subjects, and consecutive and comparative studies can be made repeatedly in the same subject. Other methods are more traumatic and either require the animal to be killed or involve such extensive surgical intervention and tissue damage that the experiments approach an in vitro experiment carried out in situ. All, however, are capable of providing specific and useful information.

Behavioral and central nervous system physiology are correlated with blood and cerebrospinal fluid chemical changes

The simplest way to study the metabolism of the central nervous system in vivo is to correlate spontaneous or experimentally produced alterations in the chemical composition of the blood, spinal fluid or both with changes in cerebral physiological functions or gross central nervous system-mediated behavior. The level of consciousness, the reflex behavior or the electroencephalogram (EEG) generally is used to monitor the effects of chemical changes on functional and metabolic activities of brain. Such methods first demonstrated the need for glucose as a substrate for cerebral energy metabolism; hypoglycemia produced by insulin or other means altered various parameters of cerebral function that could not be restored to normal by administering substances other than glucose.

The chief virtue of these methods is their simplicity, but they are gross and nonspecific and do not distinguish between direct effects of the agent on cerebral metabolism and those secondary to changes produced initially in somatic tissues. Also, negative results are often inconclusive, for there always remain questions of insufficient dosage, inadequate cerebral circulation and delivery to the tissues or impermeability of the BBB.
Brain samples are removed for biochemical analyses

The availability of analytical chemical techniques makes it possible to measure specific metabolites and enzyme activities in brain tissue at selected times during or after exposure of the animal to an experimental condition. This approach has been very useful in studies of the intermediary metabolism of brain. It has permitted estimation of the rates of flux through the various steps of established metabolic pathways and the identification of control points in the pathways where regulation may be exerted. Such studies have helped to define more precisely changes in energy metabolism associated with altered cerebral functions, for example, those produced by anesthesia, convulsions or hypoglycemia. While such methods require killing animals and analyzing tissues, in effect, they are in vivo methods since they attempt to describe the state of the tissue while it is still in the animal at the moment of killing. They have encountered their most serious problems with regard to this point. Postmortem changes in brain are extremely rapid and not always retarded completely even by the most rapid freezing techniques available. These methods have proved to be very valuable, nevertheless, particularly in the area of energy metabolism.

Radioisotope incorporation can identify and measure routes of metabolism

The technique of administering radioactive precursors followed by the chemical separation and assay of products in the tissue has added greatly to the armamentarium for studying cerebral metabolism in vivo. Labeled precursors are administered by any of a variety of routes; at selected later times the brain is removed, the precursor and the various products of interest are isolated and the radioactivity and quantity of the compounds in question are assayed. Such techniques facilitate identification of metabolic routes and rates of flux through various steps of a pathway. In some cases, comparison of specific activities of the products and precursors has led to the surprising finding of higher specific activities in products than in precursors. This is conclusive evidence of the presence of compartmentation. These methods have been used effectively in studies of amine and neurotransmitter synthesis and metabolism, lipid metabolism, protein synthesis, amino acid metabolism and the distribution of glucose carbon through the various biochemical pathways present in brain.

Radioisotope incorporation methods are particularly valuable for studies of intermediary metabolism, which generally are not feasible by most other in vivo techniques. They are without equal for the qualitative identification of the pathways and routes of metabolism. They suffer, however, from a disadvantage: only one set of measurements per animal is possible because the animal must be killed. Quantitative interpretations often are confounded by the problems of compartmentation. Also, all too frequently, they are misused; unfortunately, quantitative conclusions are drawn incorrectly based on radioactivity data without appropriate consideration of the specific activities of precursor pools.

Oxygen utilization in the cortex is measured by polarographic techniques

The O₂ electrode has been used for measuring the amount of O₂ consumed locally in the cerebral cortex in vivo [13]. The electrode is applied to the surface of the exposed cortex, and the local partial pressure for O₂ (P₀₂) is measured continuously before and during occlusion of the blood flow to the local area. During occlusion, P₀₂ falls linearly as O₂ is consumed by tissue metabolism, and the rate of fall is a measure of the rate of O₂ consumption locally in the cortex. Repeated measurements can be made successively in the animal, and the technique has been used to demonstrate the increased O₂ consumption of the cerebral cortex and the relation between changes in the EEG and the metabolic rate during convulsions [13]. The technique is limited to measurements in the cortex and, of course, to O₂ utilization.

Arteriovenous differences identify substances consumed or produced by brain

The primary functions of the circulation are to replenish the nutrients consumed by the tissues and to remove the products of their metabolism.
These functions are reflected in the composition of the blood traversing the tissue. Substances taken up by a tissue from the blood are higher in concentration in the arterial inflow than in the venous outflow, and the converse is true for substances released by the tissue. The convention is to subtract the venous concentration from the arterial concentration so that a positive arteriovenous difference represents net uptake and a negative difference, net release. In nonsteady states, as after a perturbation, there may be transient arteriovenous differences that reflect changes in tissue concentrations and re-equilibration of the tissue with the blood. In steady states, in which it is presumed that the tissue concentration remains constant, positive and negative arteriovenous differences mean net consumption or production of the substance by the tissue, respectively. Zero arteriovenous differences indicate neither consumption nor production.

This method is useful for all substances in blood that can be assayed with enough accuracy, precision and sensitivity to enable the detection of arteriovenous differences. The method is useful only for tissues from which mixed representative venous blood can be sampled. Arterial blood has essentially the same composition throughout and can be sampled from any artery. In contrast, venous blood is specific for each tissue, and to establish valid arteriovenous differences the venous blood must represent the total outflow or the flow-weighted average of all of the venous outflows from the tissue under study, uncontaminated by blood from any other tissue. It is not possible to fulfill this condition for many tissues.

The method is fully applicable to the brain, particularly in humans, in whom the anatomy of venous drainage is favorable for such studies. Representative cerebral venous blood, with no more than approximately 3% contamination with extracerebral blood, is readily obtained from the superior bulb of the internal jugular vein in humans. The venipuncture can be made percutaneously under local anesthesia; therefore, measurements can be made during conscious states undistorted by the effects of general anesthesia. Using this method with monkeys is similar, although the vein must be exposed surgically before puncture. Other common laboratory animals are less suitable because extensive communication between cerebral and extracerebral venous beds is present and uncontaminated representative venous blood is difficult to obtain from the cerebrum without major surgical intervention. In these cases, one can sample blood from the confluence of the sinuses, also termed the torcular herophili, even though it does not contain fully representative blood from the brainstem and some of the lower portions of the brain.

The chief advantages of these methods are their simplicity and applicability to unanesthetized humans. They permit the qualitative identification of the ultimate substrates and products of cerebral metabolism. They have no applicability, however, to those intermediates that are formed and consumed entirely within brain without being exchanged with blood or to those substances that are exchanged between brain and blood with no net flux in either direction. Furthermore, they provide no quantification of the rates of utilization or production because arteriovenous differences depend not only on the rates of consumption or production by the tissue but also on blood flow (see below). Blood flow affects all of the arteriovenous differences proportionately, however, and comparison of the arteriovenous differences of various substances obtained from the same samples of blood reflects their relative rates of utilization or production.

Combining cerebral blood flow and arteriovenous differences permits measurement of rates of consumption or production of substances by brain

In a steady state, the tissue concentration of any substance utilized or produced by brain is presumed to remain constant. When a substance is exchanged between brain and blood, the difference in its steady state of delivery to brain in the arterial blood and removal in the venous blood must be equal to the net rate of its utilization or production by brain. This relation can be expressed as follows:

\[ CMR = CBF(A - V) \]
where \((A - V)\) is the difference in concentration in arterial and cerebral venous blood, \(CBF\) is the rate of cerebral blood flow in volume of blood per unit time and cerebral metabolic rate (CMR) is the steady-state rate of utilization or production of the substance by brain.

If both the rate of cerebral blood flow and the arteriovenous difference are known, then the net rate of utilization or production of the substance by brain can be calculated. This has been the basis of most quantitative studies of cerebral metabolism in vivo.

The most reliable method for determining cerebral blood flow is the inert gas method of Kety and Schmidt [14]. Originally, it was designed for use in studies of conscious, unanesthetized humans, and it has been employed most widely for this purpose; but it also has been adapted for use in animals. The method is based on the Fick principle, which is an equivalent of the law of conservation of matter; and it utilizes low concentrations of a freely diffusible, chemically inert gas as a tracer substance. The original gas was nitrous oxide, but subsequent modifications have substituted other gases, such as \(^{85}\text{Kr},^{79}\text{Kr}\) or hydrogen, which can be measured more conveniently in blood. During a period of inhalation of 15% \(\text{N}_2\text{O}\) in air, for example, timed arterial and cerebral venous blood samples are withdrawn and analyzed for their \(\text{N}_2\text{O}\) contents. The cerebral blood flow in milliliters per 100 g of brain tissue per minute can be calculated from the following equation:

\[
\text{CBF} = 100 \frac{\lambda}{X} \frac{V(T)}{T} \left[ A(t) - V(t) \right] dt
\]

where \(A(t)\) and \(V(t)\) are the arterial and cerebral venous blood concentrations of \(\text{N}_2\text{O}\), respectively, at any time \(t\); \(V(T)\) is concentration of \(\text{N}_2\text{O}\) in venous blood at the end of the period of inhalation, that is, time \(T\); \(\lambda\) is the partition coefficient for \(\text{N}_2\text{O}\) between brain tissue and blood; \(t\) is variable time in minutes; \(T\) is total period of inhalation of \(\text{N}_2\text{O}\), usually 10 min or more; and \(\int_0^T [A(t) - V(t)] dt\) is the integrated arteriovenous difference in \(\text{N}_2\text{O}\) concentrations over the total period of inhalation.

The partition coefficient for \(\text{N}_2\text{O}\) is approximately 1 when equilibrium has been achieved between blood and brain tissue; at least 10 min of inhalation is required to approach equilibrium. At the end of this interval, the \(\text{N}_2\text{O}\) concentration in brain tissue is about equal to the cerebral venous blood concentration. Because the method requires sampling of both arterial and cerebral venous blood, it lends itself readily to the simultaneous measurement of arteriovenous differences of substances involved in cerebral metabolism. This method and its modifications have provided most of our knowledge of the rates of substrate utilization or product formation by brain in vivo.

**REGULATION OF CEREBRAL METABOLIC RATE**

The brain consumes about one-fifth of total body oxygen utilization

The brain is metabolically one of the most active of all organs in the body. This consumption of \(\text{O}_2\) provides the energy required for its intense physicochemical activity. The most reliable data on cerebral metabolic rate have been obtained in humans. Cerebral \(\text{O}_2\) consumption in normal, conscious, young men is approximately 3.5 ml/100 g brain/min (Table 31-1); the rate is similar in young women. The rate of \(\text{O}_2\) consumption by an entire brain of average weight (1,400 g) is then about 49 ml \(\text{O}_2\)/min. The magnitude of this rate can be appreciated more fully when it is compared with the metabolic rate of the whole body. An average man weighs 70 kg and con-

<table>
<thead>
<tr>
<th>Function</th>
<th>Per 100 g of brain tissue</th>
<th>Per whole brain (1,400 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow</td>
<td>57</td>
<td>798</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral (\text{O}_2) consumption</td>
<td>3.5</td>
<td>49</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral glucose utilization</td>
<td>5.5</td>
<td>77</td>
</tr>
<tr>
<td>(mg/min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on data derived from the literature, in Sokoloff [16].
sumes about 250 ml O₂/min in the basal state. Therefore, the brain, which represents only about 2% of total body weight, accounts for 20% of the resting total body O₂ consumption. In children, the brain takes up an even larger fraction, as much as 50% in the middle of the first decade of life [15].

O₂ is utilized in the brain almost entirely for the oxidation of carbohydrate [16]. The energy equivalent of the total cerebral metabolic rate is, therefore, approximately 20 W, or 0.25 kcal/min. If it is assumed that this energy is utilized mainly for the synthesis of high-energy phosphate bonds, that the efficiency of the energy conservation is approximately 20% and that the free energy of hydrolysis of the terminal phosphate of ATP is approximately 7 kcal/mol, then this energy expenditure can be estimated to support the steady turnover of close to 7 mmol, or approximately $4 \times 10^{21}$ molecules, of ATP per minute in the entire human brain. The brain normally has no respite from this enormous energy demand. Cerebral O₂ consumption continues unabated day and night. Even during sleep there is only a relatively small decrease in cerebral metabolic rate; indeed, it may even be increased in rapid eye movement (REM) sleep (see below).

The main energy-demanding functions of the brain are those of ion flux related to excitation and conduction

The brain does not do mechanical work, like that of cardiac and skeletal muscle, or osmotic work, as the kidney does in concentrating urine. It does not have the complex energy-consuming metabolic functions of the liver nor, despite the synthesis of some hormones and neurotransmitters, is it noted for its biosynthetic activities. Considerable emphasis has been placed on the extent of macromolecular synthesis in the central nervous system, an interest stimulated by the recognition that there are some proteins with short half-lives in brain. However, these represent relatively small numbers of molecules, and in fact, the average protein turnover and the rate of protein synthesis in mature brain are slower than in most other tissues, except perhaps muscle. Clearly, the functions of nervous tissues are mainly excitation and conduction, and these are reflected in the unceasing electrical activity of the brain. The electrical energy ultimately is derived from chemical processes, and it is likely that most of the energy consumption of the brain is used for active transport of ions to sustain and restore the membrane potentials discharged during the processes of excitation and conduction (see Chaps. 5 and 6).

Not all of the O₂ consumption of the brain is used for energy metabolism. The brain contains a variety of oxidases and hydroxylases that function in the synthesis and metabolism of a number of neurotransmitters. For example, tyrosine hydroxylase is a mixed-function oxidase that hydroxylates tyrosine to 3,4-dihydroxyphenylalanine (DOPA), and dopamine β-hydroxylase hydroxylates dopamine to form noradrenaline. Similarly, tryptophan hydroxylase hydroxylates tryptophan to form 5-hydroxytryptophan in the pathway of serotonin synthesis. The enzymes are oxygenases, which utilize molecular O₂ and incorporate it into the hydroxyl group of the hydroxylated products. O₂ also is consumed in the metabolism of these monoamine neurotransmitters, which are deaminated oxidatively to their respective aldehydes by monoamine oxidases. All of these enzymes are present in brain, and the reactions catalyzed by them use O₂. However, the total turnover rates of these neurotransmitters and the sum total of the maximal velocities of all oxidases involved in their synthesis and degradation can account for only a very small, possibly immeasurable, fraction of the total O₂ consumption of brain.

Continuous cerebral circulation is absolutely required to provide sufficient oxygen

Not only does the brain utilize O₂ at a very rapid rate, but it is absolutely dependent on uninterrupted oxidative metabolism for maintenance of its functional and structural integrity. There is a large Pasteur effect in brain tissue, but even at its maximal rate anaerobic glycolysis is unable to provide sufficient energy. Since the O₂ stored in brain is extremely small compared with its rate of utilization, the brain requires continuous re-
plenishment of its O\textsubscript{2} by the circulation. If cerebral blood flow is interrupted completely, consciousness is lost within less than 10 sec, or the amount of time required to consume the O\textsubscript{2} contained within the brain and its blood content. Loss of consciousness as a result of anoxemia, caused by anoxia or asphyxia, takes only a little longer because of the additional O\textsubscript{2} present in the lungs and the still-circulating blood. The average critical level of O\textsubscript{2} tension in brain tissues, below which consciousness and the normal EEG pattern are invariably lost, lies between 15 and 20 mm Hg. This seems to be so whether the tissue anoxia is achieved by lowering the cerebral blood flow or the arterial oxygen content. Cessation of cerebral blood flow is followed within a few minutes by irreversible pathological changes within the brain, readily demonstrated by microscopic anatomical techniques. In medical crises, such as cardiac arrest, damage to the brain occurs earliest and is most decisive in determining the degree of recovery.

Cerebral blood flow must be able to maintain the avaricious appetite of the brain for O\textsubscript{2}. The average rate of blood flow in the human brain as a whole is approximately 57 ml/100 g tissue/min (see Table 31-1). For a whole brain this amounts to almost 800 ml/min, or approximately 15% of the total basal cardiac output. This must be maintained within relatively narrow limits, for the brain cannot tolerate any major drop in its perfusion. A fall in cerebral blood flow to half its normal rate is sufficient to cause loss of consciousness in normal, healthy, young men. There are, fortunately, numerous reflexes and other physiological mechanisms to sustain adequate levels of arterial blood pressure at the head level, such as the baroreceptor reflexes, and to maintain cerebral blood flow, even when arterial pressure falls in times of stress for example, autoregulation. There are also mechanisms to adjust cerebral blood flow to changes in cerebral metabolic demand.

Regulation of cerebral blood flow is achieved mainly by control of the tone or the degree of constriction, or dilation, of the cerebral vessels. This in turn is controlled mainly by local chemical factors, such as PaCO\textsubscript{2}, PaO\textsubscript{2}, pH and others still unrecognized. High PaCO\textsubscript{2}, low PaO\textsubscript{2} and low pH, which are products of metabolic activity, tend to dilate the blood vessels and increase cerebral blood flow; changes in the opposite direction constrict the vessels and decrease blood flow [17]. Cerebral blood flow is regulated through such mechanisms to maintain homeostasis of these chemical factors in the local tissue. The rates of production of these chemical factors depend on the rates of energy metabolism; therefore, cerebral blood flow is adjusted to the cerebral metabolic rate [17].

**Local rates of cerebral blood flow and metabolism can be measured by autoradiography and are coupled to local brain function**

The rates of blood flow and metabolism presented in Table 31-1 and discussed above represent the average values in the brain as a whole. The brain is not homogeneous, however; it is composed of a variety of tissues and discrete structures that often function independently or even inversely with respect to one another. There is little reason to expect that their perfusion and metabolic rates would be similar. Indeed, experiments clearly indicate that they are not. Local cerebral blood flow in laboratory animals has been determined from the local tissue concentrations, measured by a quantitative autoradiographic technique, and from the total history of the arterial concentration of a freely diffusible, chemically inert, radioactive tracer introduced into the circulation [18]. The results reveal that blood-flow rates vary widely throughout the brain, with average values in gray matter approximately four to five times those in white matter [18].

A method has been devised to measure glucose consumption in discrete functional and structural components of the brain in intact, conscious laboratory animals [19]. This method also employs quantitative autoradiography to measure local tissue concentrations but utilizes 2-deoxy-D-\textsuperscript{14}C]glucose as the tracer. The local tissue accumulation of \textsuperscript{14}C]deoxyglucose as \textsuperscript{14}C]deoxy-G6P in a given interval of time is related to the amount of glucose that has been phosphorylated by
hexokinase over the same interval, and the rate of glucose consumption can be determined from the $[^{14}\text{C}]$deoxy-G6P concentration by appropriate consideration of (i) the relative concentrations of $[^{14}\text{C}]$deoxyglucose and glucose in the plasma, (ii) their rate constants for transport between plasma and brain tissue and (iii) the kinetic constants of hexokinase for deoxyglucose and glucose. The method is based on a kinetic model of the biochemical behavior of 2-deoxyglucose and glucose in brain. The model (diagrammed in Fig. 31-3) has been analyzed mathematically to derive an operational equation that presents the variables to be measured and the procedure to be followed to determine local cerebral glucose utilization.
General equation for measurement of reaction rates with tracers:

\[
\text{Rate of reaction} = \frac{\text{Labeled Product Formed in Interval of Time, 0 to } T}{\left[ \frac{\text{Isotope effect correction factor}}{\int \text{Integrated specific activity of precursor}} \right]}
\]

Operational equation of \([^{14}\text{C}]\text{deoxyglucose method:}\)

\[
R_i = \frac{\left( \frac{\lambda \cdot V_m \cdot K_m}{\Phi \cdot V_m \cdot K_m} \right) \left( \frac{\int_0^T \left( \frac{C_p}{C_r} \right) dt}{\Phi} \right) - e^{-\left( k_i^* + k_i^* \right)T} \int_0^T \left( \frac{C_p}{C_r} \right) e^{\left( k_i^* + k_i^* \right)t} dt}{\left( \int_0^T \left( \frac{C_p}{C_r} \right) dt - e^{-\left( k_i^* + k_i^* \right)T} \int_0^T \left( \frac{C_p}{C_r} \right) e^{\left( k_i^* + k_i^* \right)t} dt \right)}
\]

Isotope effect correction factor

Integrated plasma specific activity

Correction for lag in tissue equilibration with plasma

**FIGURE 31-3.** (Continued.) B: Functional anatomy of the operational equation of the radioactive deoxyglucose method. \(T\) represents the time at the termination of the experimental period; \(\lambda\) equals the ratio of the distribution space of deoxyglucose in the tissue to that of glucose; \(\Phi\) equals the fraction of glucose that, once phosphorylated, continues down the glycolytic pathway; \(K_m, V_m, K_m, V_m\) represent the familiar Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose, respectively. Other symbols are the same as those defined in A. (From [19].)

To measure local glucose utilization, a pulse of \([^{14}\text{C}]\text{deoxyglucose}\) is administered intravenously at time zero and timed arterial blood samples are drawn for determination of the plasma \([^{14}\text{C}]\text{deoxyglucose}\) and glucose concentrations. At the end of the experimental period, usually about 45 min, the animal is decapitated, the brain is removed and frozen and 20-\(\mu\)m-thick brain sections are autoradiographed on X-ray film along with calibrated \([^{14}\text{C}]\text{methyl-methacrylate}\) standards. Local tissue concentrations of \(^{14}\text{C}\) are determined by quantitative densitometric analysis of the autoradiographs. From the time courses of the arterial plasma \([^{14}\text{C}]\text{deoxyglucose}\) and glucose concentrations and the final tissue \(^{14}\text{C}\) concentrations, determined by quantitative autoradiography, local glucose utilization can be calculated by means of the operational equation for all components of the brain identifiable in the autoradiographs. The procedure is designed so that the autoradiographs reflect mainly the relative local concentrations of \([^{14}\text{C}]\text{deoxy-G6P}\). The autoradiographs, therefore, are pictorial representations of the relative rates of glucose utilization in all of the structural components of the brain.

Autoradiographs of the striate cortex in monkey in various functional states are illustrated in Figure 31-4. This method has demonstrated that local cerebral consumption of glucose varies as widely as blood flow throughout the brain (Table 31-2). Indeed, in normal animals, there is remarkably close correlation between local cerebral blood flow and glucose consumption \([20]\). Changes in functional activity produced by physiological stimulation, anesthesia or deafferentation result in corresponding changes in blood flow and glucose consumption \([21]\) in the structures involved in the functional change. The \([^{14}\text{C}]\text{deoxyglucose method}\) for the measurement of local glucose utilization has been used to map the functional visual pathways and to identify the locus of the visual cortical
FIGURE 31-4. Autoradiograms of coronal brain sections from rhesus monkeys at the level of the striate cortex. A: Animal with normal binocular vision. Note the laminar distribution of the density; the dark band corresponds to layer IV. B: Animal with bilateral visual deprivation. Note the almost uniform and reduced relative density, especially the virtual disappearance of the dark band corresponding to layer IV. C: Animal with right eye occluded. The half-brain on the left represents the left hemisphere contralateral to the occluded eye. Note the alternate dark and light striations, each approximately 0.3 to 0.4 mm in width, representing the ocular dominance columns. These columns are most apparent in the dark lamina corresponding to layer IV but extend through the entire thickness of the cortex. Arrows point to regions of bilateral asymmetry, where the ocular dominance columns are absent. These are presumably areas that normally have only monocular input. The one on the left, contralateral to the occluded eye, has a continuous dark lamina corresponding to layer IV that is completely absent on the side ipsilateral to the occluded eye. These regions are believed to be the loci of the cortical representations of the blind spots. (From [21].)
representation of the retinal "blind spot" in the brain of rhesus monkey [21] (Fig. 31-4). These results establish that local energy metabolism in the brain is coupled to local functional activity and confirm the long-held belief that local cerebral blood flow is adjusted to metabolic demand in local tissue. The method has been applied to humans by use of 2-[18F]-fluoro-2-deoxy-D-glucose and positron emission tomography (PET), with similar results (see Chap. 54).

**TABLE 31-2. REPRESENTATIVE VALUES* FOR LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE NORMAL CONSCIOUS ALBINO RAT AND MONKEY (μmol/100g/min)**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Albino rat*</th>
<th>Monkey*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual cortex</td>
<td>107 ± 6</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>162 ± 5</td>
<td>79 ± 4</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>112 ± 5</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>120 ± 5</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>Thalamus: lateral nucleus</td>
<td>116 ± 5</td>
<td>54 ± 2</td>
</tr>
<tr>
<td>Thalamus: ventral nucleus</td>
<td>109 ± 5</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>131 ± 5</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>Lateral geniculate body</td>
<td>96 ± 5</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>54 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>Mammillary body</td>
<td>121 ± 5</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>79 ± 3</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Amygdala</td>
<td>52 ± 2</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Caudate putamen</td>
<td>110 ± 4</td>
<td>52 ± 3</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>82 ± 3</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>58 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>58 ± 3</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Vestibular nucleus</td>
<td>128 ± 5</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>Cochlear nucleus</td>
<td>113 ± 7</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Superior olivary nucleus</td>
<td>133 ± 7</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>197 ± 10</td>
<td>103 ± 6</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>95 ± 5</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>Pontine gray matter</td>
<td>62 ± 3</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>57 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Cerebellar nuclei</td>
<td>100 ± 4</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>40 ± 2</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>33 ± 2</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Cerebellar white matter</td>
<td>37 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Weighted average for whole brain</td>
<td>68 ± 3</td>
<td>36 ± 1</td>
</tr>
</tbody>
</table>

* Values are the means plus or minus standard errors from measurements made in ten rats or seven monkeys.
  * From Sokoloff and co-workers [19].
  * From Kennedy and co-workers [22].

**SUBSTRATES OF CEREBRAL METABOLISM**

Normally, the substrates are glucose and oxygen and the products are carbon dioxide and water.

In contrast to most other tissues, which exhibit considerable flexibility with respect to the nature of the foodstuffs extracted and consumed from the blood, the normal brain is restricted almost exclusively to glucose as the substrate for its energy metabolism. Despite long and intensive efforts, the only incontrovertible and consistently positive arteriovenous differences demonstrated for the human brain under normal conditions have been for glucose and oxygen [16]. Negative arteriovenous differences, significantly different from zero, have been found consistently only for CO₂, although water, which has never been measured, also is produced. Pyruvate and lactate production have been observed occasionally, certainly in aged subjects with cerebral vascular insufficiency but also irregularly in subjects with normal oxygenation of the brain.

In the normal in vivo state, glucose is the only significant substrate for energy metabolism in the brain. Under normal circumstances, no other potential energy-yielding substance has been found to be extracted from the blood in more than trivial amounts. The stoichiometry of glucose utilization and O₂ consumption is summarized in Table 31-3. The normal, conscious human brain consumes oxygen at a rate of 156 μmol/100 g tissue/min. CO₂ production is the same, leading to a respiratory quotient (RQ) of 1.0, further evidence that carbohydrate is the ultimate substrate for oxidative metabolism. O₂ consumption and CO₂ production are equivalent to a rate of glucose utilization of 26 μmol/100 g tissue/min, assuming 6 μmol of O₂ consumed and of CO₂ produced for each micromole of glucose completely oxidized to CO₂ and H₂O. The actual glucose utilization measured is, however, 31 μmol/100 g/min, which indicates that glucose consumption not only is sufficient to account for total O₂ consumption but is in excess by 5 μmol/100 g/min. For complete oxidation of glucose, the theoretical ratio of O₂:glucose utilization is 6.0; the excess glucose utilization is re-
Chapter 31  Brain Circulation and Metabolism

RELATIONSHIP BETWEEN Cerebral Oxygen Consumption and glucose utilization IN A NORMAL Young Adult Man

<table>
<thead>
<tr>
<th>Function</th>
<th>Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ consumption (µmol/100 g brain tissue/min)</td>
<td>156</td>
</tr>
<tr>
<td>Glucose utilization (µmol/100 g brain tissue/min)</td>
<td>31</td>
</tr>
<tr>
<td>O₂/glucose ratio (mol/mol)</td>
<td>5</td>
</tr>
<tr>
<td>Glucose equivalent of O₂ consumption (µmol glucose/100 g brain tissue/min)</td>
<td>26b</td>
</tr>
<tr>
<td>CO₂ production (µmol/100 g brain tissue/min)</td>
<td>156</td>
</tr>
<tr>
<td>Cerebral respiratory quotient (RQ)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

From Sokoloff [16].

a Values are the median of values reported in the literature.
b Calculated on the basis of 6 mol of O₂ required for complete oxidation of 1 mol of glucose.

This does not imply that the pathways of glucose metabolism in the brain lead, like combustion, directly and exclusively to production of CO₂ and H₂O. Various chemical and energy transformations occur between uptake of the primary substrates glucose and O₂ and liberation of the end products CO₂ and H₂O. Compounds derived from glucose or produced through the energy made available from glucose catabolism are intermediates in the process. Glucose carbon is incorporated, for example, into amino acids, protein, lipids and glycogen. These are turned over and act as intermediates in the overall pathway from glucose to CO₂ and H₂O. There is clear evidence from studies with [¹⁴C]glucose that not all of the glucose is oxidized directly and that at any given moment some of the CO₂ being produced is derived from sources other than glucose that enter the brain at the same moment or just prior to that moment. That O₂ and glucose consumption and CO₂ production are essentially in stoichiometric balance and that no other energy-laden substrate is taken from the blood means, however, that the net energy made available to the brain ultimately must be derived from the oxidation of glucose. It should be noted that this is the situation in the normal state; as discussed later, other substrates may be used in special circumstances or in abnormal states.

In brain, glucose utilization is obligatory

The brain normally derives almost all of its energy from the aerobic oxidation of glucose, but this does not distinguish between preferential and obligatory utilization of glucose. Most tissues are largely facultative in their choice of substrates and can use them interchangeably more or less in proportion to their availability. This does not appear to be so in the brain. Except in some unusual and very special circumstances, only the aerobic utilization of glucose is capable of providing the brain with sufficient energy to maintain normal function and structure. The brain appears to have almost no flexibility in its choice of substrates in vivo. This conclusion is derived from the following evidence.

Glucose deprivation is followed rapidly by aberrations of cerebral function. Hypoglycemia, pro-
duced by excessive insulin or occurring spontaneously in hepatic insufficiency, is associated with changes in mental state ranging from mild, subjective sensory disturbances to coma, the severity depending on both the degree and the duration of the hypoglycemia. The behavioral effects are paralleled by abnormalities in EEG patterns and cerebral metabolic rate. The EEG pattern exhibits increased prominence of slow, high-voltage δ rhythms, and the rate of cerebral oxygen consumption falls. In studies of the effects of insulin hypoglycemia in humans [24], it was observed that when arterial glucose fell from a normal concentration of 70 to 100 mg/100 ml to an average of 19 mg/100 ml, subjects became confused and cerebral O₂ consumption fell to 2.6 ml/100 g/min, or 79% of normal. When arterial glucose fell to 8 mg/100 ml, a deep coma ensued and cerebral O₂ consumption decreased even further to 1.9 ml/100 g/min (Table 31-4).

These changes are not caused by insufficient cerebral blood flow, which actually increases slightly during coma. In the depths of coma, when the blood glucose content is very low, there is almost no measurable cerebral uptake of glucose from the blood. Cerebral O₂ consumption, although reduced, is still far from negligible; and there is no longer any stoichiometric relationship between glucose and O₂ uptakes by the brain, evidence that the O₂ is utilized for the oxidation of other substances. The cerebral RQ remains approximately 1, however, indicating that these other substrates are still carbohydrate, presumably derived from the endogenous carbohydrate stores of the brain. The effects are clearly the result of hypoglycemia and not of insulin in the brain. In all cases, the behavioral, functional and cerebral metabolic abnormalities associated with insulin hypoglycemia are reversed rapidly and completely by the administration of glucose. The severity of the effects is correlated with the degree of hypoglycemia and not the insulin dosage, and the effects of insulin can be prevented completely by the simultaneous administration of glucose with the insulin.

Similar effects are observed in hypoglycemia produced by other means, such as hepatectomy. Inhibition of glucose utilization at the phosphohexose isomerase step with pharmacological doses of 2-deoxyglucose also produces all of the cerebral effects of hypoglycemia despite an associated elevation in blood glucose content.

In hypoglycemia, substrates other than glucose may be utilized. The hypoglycemic state provides convenient test conditions to determine whether a substance is capable of substituting for glucose as a substrate of cerebral energy metabolism. If it can, its administration during hypoglycemic shock should restore consciousness and normal cerebral electrical activity without raising the blood glucose concentration. Numerous potential substrates have been tested in humans and animals. Very few can restore normal cerebral function in hypoglycemia, and of

<table>
<thead>
<tr>
<th>TABLE 31-4 EFFECTS OF INSULIN HYPOGLYCEMIA ON CEREBRAL CIRCULATION AND METABOLISM IN HUMANS a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial blood</strong></td>
</tr>
<tr>
<td>Glucose concentration (mg%)</td>
</tr>
<tr>
<td>O₂ content (vol%)</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
</tr>
<tr>
<td><strong>Cerebral circulation</strong></td>
</tr>
<tr>
<td>Blood flow (ml/100 g/min)</td>
</tr>
<tr>
<td>O₂ consumption (ml/100 g/min)</td>
</tr>
<tr>
<td>Glucose consumption (mg/100 g/min)</td>
</tr>
<tr>
<td>Respiratory quotient</td>
</tr>
</tbody>
</table>

aFrom Kety et al. [24].
these all but one appear to operate through a variety of mechanisms to raise the blood glucose concentration rather than by serving directly as a substrate (Table 31-5).

Mannose appears to be the only substance that can be utilized by the brain directly and rapidly enough to restore or maintain normal function in the absence of glucose [25]. It traverses the BBB and is converted to mannose-6-phosphate. This reaction is catalyzed by hexokinase as effectively as the phosphorylation of glucose. The mannose-6-phosphate is then converted to fructose-6-phosphate by phosphomannose isomerase, which is active in brain tissue. Through these reactions mannose can enter directly into the glycolytic pathway and replace glucose.

Maltose also is effective occasionally in restoring normal behavior and EEG activity in hypoglycemia but only by raising the blood glucose concentration through its conversion to glucose by maltase activity in blood and other tissues [16]. Epinephrine is effective at producing arousal from insulin coma, but this is achieved through its well-known stimulation of glycogenolysis and the elevation of blood glucose concentration. Glutamate, arginine, glycine, GABA and succinate also act through adrenergic effects that raise glucose concentrations of the blood [16].

It should be noted, however, that failure to restore normal cerebral function in hypoglycemia is not synonymous with an inability of the brain to utilize the substance. Many of the substances that have been tested and found ineffective are compounds normally formed and utilized within the brain and are normal intermediates in its intermediary metabolism. Lactate; pyruvate; fructose-1,6-bisphosphate; acetate; β-hydroxybutyrate; and acetoacetate can be utilized by brain slices, homogenates or cell-free fractions; and the enzymes for their metabolism are present in brain. Enzymes for the metabolism of glycerol or ethanol, for example, may not be present in sufficient amounts. For other substrates, for example, D-β-hydroxybutyrate and acetoacetate, the enzymes are adequate but the substrate is not available to the brain because of inadequate blood levels or restricted transport through the BBB.

### TABLE 31-5. EFFECTIVENESS OF VARIOUS SUBSTANCES IN PREVENTING OR REVERSING THE EFFECTS OF HYPOGLYCEMIA OR GLUCOSE DEPRIVATION ON CEREBRAL FUNCTION AND METABOLISM

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>Substance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective</td>
<td>Epinephrine</td>
<td>Raises blood glucose concentration</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>Converted to glucose and raises blood glucose concentration</td>
</tr>
<tr>
<td>Partially or occasionally effective</td>
<td>Mannose</td>
<td>Directly metabolized and enters glycolytic pathway</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>Occasionally effective by raising blood glucose concentration</td>
</tr>
<tr>
<td></td>
<td>Arginine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Aminobenzoate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Succinate</td>
<td></td>
</tr>
<tr>
<td>Ineffective</td>
<td>Glycerol</td>
<td>Some of these substances can be metabolized to various extents by brain tissue and conceivably could be effective if it were not for the blood–brain barrier</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glyceraldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hexose diphosphates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fumarate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β-Hydroxybutyrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td></td>
</tr>
</tbody>
</table>

*Summarized from the literature, in Sokoloff [16].
Nevertheless, nervous system function in the intact animal depends on substrates supplied by the blood, and no satisfactory, normal, endogenous substitute for glucose has been found. Glucose, therefore, must be considered essential for normal physiological behavior of the central nervous system.

The brain utilizes ketones in states of ketosis

In special circumstances, the brain may fulfill its nutritional needs partly, although not completely, with substrates other than glucose. Normally, there are no significant cerebral arteriovenous differences for D-β-hydroxybutyrate and acetoacetate, which are "ketone bodies" formed in the course of the catabolism of fatty acids by liver. Owen and coworkers [26] observed, however, that when human patients were treated for severe obesity by complete fasting for several weeks, there was considerable uptake of both substances by the brain. If one assumed that the substances were oxidized completely, their rates of utilization would have accounted for more than 50% of the total cerebral oxygen consumption, more than that accounted for by the glucose uptake. D-β-hydroxybutyrate uptake was several times greater than that of acetoacetate, a reflection of its higher concentration in the blood. The enzymes responsible for their metabolism, D-β-hydroxybutyrate dehydrogenase and acetoacetate-succinyl-CoA transferase and acetoacetyl-CoA-thiolase, are present in brain tissue in sufficient amounts to convert them into acyl-CoA and to feed them into the tricarboxylic acid cycle at a sufficient rate to satisfy the metabolic demands of the brain [27].

Under normal circumstances, that is, ample glucose and few ketone bodies in the blood, the brain apparently does not oxidize ketones in any significant amounts. In prolonged starvation, the carbohydrate stores of the body are exhausted and the rate of gluconeogenesis is insufficient to provide glucose fast enough to meet the requirements of the brain; blood ketone concentrations rise as a result of the rapid fat catabolism. The brain then apparently turns to the ketone bodies as the source of its energy supply.

Cerebral utilization of ketone bodies appears to follow passively their concentrations in arterial blood [27]. In normal adults, ketone concentrations are very low in blood and cerebral utilization of ketones is negligible. In ketogenic states resulting from starvation; fat-feeding or ketogenic diets; diabetes; or any other condition that accelerates the mobilization and catabolism of fat, cerebral utilization of ketones is increased more or less in direct proportion to the degree of ketosis [27]. Significant utilization of ketone bodies by the brain is, however, normal in the neonatal period. The newborn infant tends to be hypoglycemic but becomes ketogenic when it begins to nurse because of the high fat content of the mother's milk. When weaned onto the normal, relatively high-carbohydrate diet, the ketosis and cerebral ketone utilization disappear. Studies have been carried out mainly in the infant rat, but there is evidence that the situation is similar in the human infant.

The first two enzymes in the pathway of ketone utilization are D-β-hydroxybutyrate dehydrogenase and acetoacetyl-succinyl-CoA transferase. These exhibit a postnatal pattern of development that is well adapted to the nutritional demands of the brain. At birth, the activity of these enzymes in the brain is low; activity rises rapidly with the ketosis that develops with the onset of suckling, reaches its peak just before weaning and then gradually declines after weaning to normal adult rates of approximately one-third to one-fourth the maximal rates attained [27,28].

It should be noted that D-β-hydroxybutyrate is incapable of maintaining or restoring normal cerebral function in the absence of glucose in the blood. This suggests that, although it can partially replace glucose, it cannot fully satisfy the cerebral energy needs in the absence of some glucose consumption. One explanation may be that the first product of D-β-hydroxybutyrate oxidation, acetoacetate, is metabolized further by its displacement of the succinyl moiety of succinyl-CoA to form acetoacetyl-CoA. A certain rate of glucose utilization may be essential to drive the tricarboxylic cycle, to provide enough succinyl-CoA to permit the further oxidation of acetoacetate and, hence, to pull along the oxidation of D-β-hydroxybutyrate.
Chapter 31  Brain Circulation and Metabolism

AGE AND DEVELOPMENT INFLUENCE CEREBRAL ENERGY METABOLISM

Metabolic rate increases during early development

The energy metabolism of the brain and the blood flow that sustains it vary considerably from birth to old age. Data on the cerebral metabolic rate obtained directly in vivo are lacking for the early postnatal period, but the results of in vitro measurements in animal brain preparations and inferences drawn from cerebral blood flow measurements in intact animals [29] suggest that cerebral oxygen consumption is low at birth, rises rapidly during the period of cerebral growth and development and reaches a maximal level at about the time maturation is completed. This rise is consistent with the progressive increase in the levels of a number of enzymes of oxidative metabolism in the brain. The rate of blood flow in different structures of the brain reaches peak levels at different times, depending on the maturation rate of the particular structure. In structures that consist predominantly of white matter, the peaks coincide roughly with maximal rates of myelination. From these peaks, blood flow and, probably, cerebral metabolic rate decline to the levels characteristic of adulthood.

Metabolic rate declines and plateaus after maturation

Reliable quantitative data on the changes in cerebral circulation and metabolism in humans from the middle of the first decade of life to old age are summarized in Table 31-6. By 6 years of age, cerebral blood flow and oxygen consumption already have attained high rates, and they decline thereafter to the rates of normal young adulthood [15]. Cerebral oxygen consumption of 5.2 ml/100 g brain tissue/min in a 5- to 6-year-old child corresponds to total oxygen consumption by the brain of approximately 60 ml/min, or more than 50% of the total body basal oxygen consumption, a proportion markedly greater than that occurring in adulthood. The reasons for the extraordinarily high cerebral metabolic rates in children are unknown, but presumably they reflect the extra energy requirements for the biosynthetic processes associated with growth and development.

Tissue pathology, but not aging, produces secondary changes in metabolic rate

Whole-brain cerebral blood flow and oxygen consumption normally remain essentially unchanged between young adulthood and old age. In a population of normal elderly men in their eighth decade of life, who were selected carefully for good health and freedom from all disease including vascular disease, both blood flow and oxygen consumption were not significantly different from rates of normal young men 50 years younger (see Table 31-6) [30]. In a comparable group of elderly subjects, who differed only by the presence of objective evidence of minimal arteriosclerosis, cerebral blood flow was signifi-

<table>
<thead>
<tr>
<th>Life period and condition</th>
<th>Age (years)</th>
<th>Cerebral blood flow (ml/100 g/min)</th>
<th>Cerebral O2 consumption (ml/100 g/min)</th>
<th>Cerebral venous O2 tension (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood</td>
<td>6b</td>
<td>106b</td>
<td>5.2b</td>
<td>—</td>
</tr>
<tr>
<td>Normal young adulthood</td>
<td>21</td>
<td>62</td>
<td>3.5</td>
<td>38</td>
</tr>
<tr>
<td>Aged</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal elderly</td>
<td>71b</td>
<td>58</td>
<td>3.3</td>
<td>36</td>
</tr>
<tr>
<td>Elderly with minimal arteriosclerosis</td>
<td>73b</td>
<td>48b</td>
<td>3.2</td>
<td>33b,c</td>
</tr>
<tr>
<td>Elderly with senile psychosis</td>
<td>72b</td>
<td>48b,c</td>
<td>2.7b,c</td>
<td>33b,c</td>
</tr>
</tbody>
</table>

*From Kennedy and Sokoloff [15] and Sokoloff [30].
*Statistically significant difference from normal young adult (p < 0.05).
*Statistically significant difference from normal elderly subjects (p < 0.05).
Part Five Metabolism

cantly lower. It had reached a point at which the oxygen tension of the cerebral venous blood declined, an indication of relative cerebral hypoxia. However, normal cerebral oxygen consumption was maintained through extraction of larger than normal proportions of the arterial blood oxygen. In senile, psychotic patients with arteriosclerosis, cerebral blood flow was no lower but cerebral oxygen consumption had declined. These data suggest that aging per se need not lower cerebral oxygen consumption and blood flow but that, when blood flow is reduced, it is probably secondary to vascular disease, which produces cerebral vascular insufficiency and chronic relative hypoxia in the brain, or to another tissue pathology that decreases function, such as dementia (see Chap. 46). Because arteriosclerosis and Alzheimer's disease are so prevalent in the aged population, most individuals probably follow the latter pattern. However, age-related changes in local regulation are possible and are the subject of research (see Chap. 30).

CEREBRAL METABOLIC RATE IN VARIOUS PHYSIOLOGICAL STATES

Cerebral metabolic rate is determined locally by functional activity in discrete regions

In organs such as heart or skeletal muscle that perform mechanical work, increased functional activity clearly is associated with increased metabolic rate. In nervous tissues outside the central nervous system, electrical activity is an almost quantitative indicator of the degree of functional activity; and in structures such as sympathetic ganglia and postganglionic axons, increased electrical activity produced by electrical stimulation definitely is associated with increased utilization of oxygen. Within the central nervous system, local energy metabolism also is correlated closely with the level of local functional activity. Studies using the [14C]deoxyglucose method have demonstrated pronounced changes in glucose utilization associated with altered functional activity in discrete regions of the central nervous system specifically related to that function [21]. For example, diminished visual or auditory input depresses glucose utilization in all components of the central visual or auditory pathways, respectively (Fig. 31-4). Focal seizures increase glucose utilization in discrete components of the motor pathways, such as the motor cortex and the basal ganglia (Fig. 31-5).

Convulsive activity, induced or spontaneous, often has been employed as a method of increasing electrical activity of the brain (see Chap. 37). Davies and Remand [13] used the oxygen electrode technique in the cerebral cortex of cat and found increases in oxygen consumption during electrically induced or drug-induced convulsions. Because the increased oxygen consumption either coincided with or followed the onset of convulsions, it was concluded that the elevation in metabolic rate was the consequence of the increased functional activity produced by the convulsive state (see Chaps. 37 and 54).

Metabolic rate and nerve conduction are related directly

The [14C]deoxyglucose method has defined the nature and mechanisms of the relationship between energy metabolism and functional activity in nervous tissues. Studies in the superior cervical ganglion of the rat have shown almost a direct relationship between glucose utilization in the ganglion and spike frequency in the afferent fibers from the cervical sympathetic trunk [31]. A spike results from the passage of a finite current of Na⁺ into the cell and of K⁺ out of the cell, ion currents that degrade the ionic gradients responsible for the resting membrane potential. Such degradation can be expected to stimulate Na,K-ATPase activity to restore the ionic gradients to normal, and such ATPase activity in turn would stimulate energy metabolism. Indeed, Mata et al. [32] have found that, in the posterior pituitary in vitro, stimulation of glucose utilization due to either electrical stimulation or opening of Na⁺ channels in the excitable membrane by veratridine is blocked by ouabain, a specific inhibitor of Na,K-ATPase activity (see Chap. 5). Most, if not all, of the stimulated energy metabolism associated with increased functional activity is confined to the axonal terminals.
FIGURE 31-5. Local glucose utilization during penicillin-induced focal seizures. Penicillin was applied to the hand and face area of the left motor cortex of a rhesus monkey. The left side of the brain is on the left in each of the autoradiograms in the figure. Numbers are rates of local cerebral glucose utilization in micromoles per 100 g tissue per minute. Note the following: upper left, motor cortex in region of penicillin application and corresponding region of contralateral motor cortex; lower left, ipsilateral and contralateral motor cortical regions remote from area of penicillin applications; upper right, ipsilateral and contralateral putamen and globus pallidus; lower right, ipsilateral and contralateral thalamic nuclei and substantia nigra. (From [21].)
rather than to the cell bodies in a functionally active pathway (Fig. 31-6) [33]. Astrocytes also contribute to the increased metabolism [34].

**It is difficult to define metabolic equivalents of consciousness, mental work and sleep**

**Mental work.** Convincing correlations between cerebral metabolic rate and mental activity have been obtained in humans in a variety of pathological states of altered consciousness [35]. Regardless of the cause of the disorder, graded reductions in cerebral oxygen consumption are accompanied by parallel graded reductions in the degree of mental alertness, all the way to profound coma (Table 31-7). It is difficult to define or even to conceive of the physical equivalent of mental work. A common view equates concentrated mental effort with mental work, and it is fashionable to attribute a high demand for mental effort to the process of problem solving in mathematics. Nevertheless, there appears to be no increased energy utilization by the brain during such processes. From resting levels, total cerebral blood flow and oxygen consumption remain unchanged during the exertion of the mental effort required to solve complex arithmetical problems [35]. It may be that the assumptions that relate mathematical reasoning to mental work are erroneous, but it seems more likely that the areas that participate in the processes of such reasoning represent too small a fraction of the brain for changes in their functional and metabolic activities to be reflected in the energy metabolism of the brain as a whole.

**Sleep.** Physiological sleep is a naturally occurring, periodic, reversible state of unconsciousness; and the EEG pattern in deep, slow-wave
Chapter 31  Brain Circulation and Metabolism

### RELATIONSHIP BETWEEN LEVEL OF CONSCIOUSNESS AND CEREBRAL METABOLIC RATE

<table>
<thead>
<tr>
<th>Level of consciousness</th>
<th>Cerebral blood flow (ml/100 g/min)</th>
<th>Cerebral O₂ consumption (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentally alert</td>
<td>54</td>
<td>3.3</td>
</tr>
<tr>
<td>Normal young men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mentally confused</td>
<td>48</td>
<td>2.8</td>
</tr>
<tr>
<td>Brain tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic acidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin hypoglycemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral arteriosclerosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comatose</td>
<td>57</td>
<td>2.0</td>
</tr>
<tr>
<td>Brain tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic coma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin coma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anesthesia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From Sokoloff [35].

### CEREBRAL ENERGY METABOLISM IN PATHOLOGICAL STATES

The cerebral metabolic rate of the brain as a whole is normally fairly stable and varies over a relatively narrow range under physiological conditions. There are, however, a number of pathological states of the nervous system and other organs that affect the functions of the brain either directly or indirectly, and some of these have profound effects on cerebral metabolism.

#### Psychiatric disorders may produce effects related to anxiety

In general, disorders that alter the quality of mentation but not the level of consciousness (described in Chaps. 51 and 52), such as the functional neuroses, psychoses and psychotomimetic states, have no apparent effect on the average blood flow and oxygen consumption of the brain as a whole. Thus, no gross changes in either function are observed in schizophrenia [35] or LSD intoxication (Table 31-8) [35]. There is still uncertainty about the effects of anxiety, mainly because of the difficulties in evaluating quantitatively the intensity. It is generally believed that ordinary degrees of anxiety, or "nervousness," do not affect the cerebral metabolic rate but that severe anxiety, or "panic," may increase cerebral oxygen consumption [35]. This may be related to the level of epinephrine circulating in the blood. Small doses of epinephrine that raise heart rate and cause some anxiety do not alter cerebral blood flow and metabolism, but large doses that are sufficient to raise the arterial blood pressure cause significant increases in both.

### CEREBRAL BLOOD FLOW AND METABOLIC RATE IN SCHIZOPHRENIA AND IN NORMAL YOUNG MEN DURING LSD-INDUCED PSYCHOTOMIMETIC STATE

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cerebral blood flow (ml/100 g/min)</th>
<th>Cerebral O₂ consumption (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>67</td>
<td>3.9</td>
</tr>
<tr>
<td>LSD intoxication</td>
<td>68</td>
<td>3.9</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>72</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* From Sokoloff [35].
Coma and systemic metabolic diseases depress brain metabolism

Coma is correlated with depression of cerebral oxygen consumption; progressive reductions in the level of consciousness are paralleled by corresponding graded decreases in cerebral metabolic rate (Table 31-7). There are almost innumerable derangements that can lead to depression of consciousness. Table 31-9 includes only a few typical examples that have been studied by the same methods and by the same or related groups of investigators. Metabolic encephalopathy is discussed in detail in Chapter 38.

Inadequate cerebral nutrient supply leads to decreases in the level of consciousness, ranging from confusional states to coma. The nutrition of the brain can be limited by lowering the oxygen or glucose concentrations of arterial blood, as in anoxia or hypoglycemia, or by impairment of their distribution to the brain through lowering cerebral blood flow, as in brain tumors. Consciousness is then depressed, presumably because of inadequate supplies of substrate to support the energy metabolism necessary to sustain function of brain.

In a number of conditions, the causes of depression of both consciousness and the cerebral metabolic rate are unknown and must, by exclusion, be attributed to intracellular defects in the brain. Anesthesia is one example. Cerebral oxygen consumption always is reduced in the anesthetized state regardless of the anesthetic agent used, whereas blood flow may or may not be decreased and may even be increased. This reduction is the result of decreased energy demand and not insufficient nutrient supply or a block of intracellular energy metabolism. There is evidence that general anesthetics interfere with the balance of excitatory and inhibitory synaptic transmission, particularly by an agonist action at GABA-ergic inhibitory synapses, thus reducing neuronal interaction, functional activity and, consequently, metabolic demands (see Chap. 16).

Several metabolic diseases with broad systemic manifestations also are associated with disturbances of cerebral function. Diabetes mellitus, when allowed to progress to states of acidosis and ketosis, leads to mental confusion and, ultimately, deep coma, with parallel proportionate decreases in cerebral oxygen consumption (see Table 31-9) [39]. The abnormalities usually are reversed com-

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**TABLE 31-9.** CEREBRAL BLOOD FLOW AND METABOLIC RATE IN HUMANS WITH VARIOUS DISORDERS AFFECTING MENTAL STATE*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mental state</th>
<th>Cerebral blood flow (ml/100 g/min)</th>
<th>Cerebral O₂ consumption (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Alert</td>
<td>54</td>
<td>3.3</td>
</tr>
<tr>
<td>Increased intracranial pressure (brain tumor)</td>
<td>Coma</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin hypoglycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial glucose level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>74 mg/100 ml</td>
<td>Alert</td>
<td>58</td>
<td>3.4</td>
</tr>
<tr>
<td>19 mg/100 ml</td>
<td>Confused</td>
<td>61</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8 mg/100 ml</td>
<td>Coma</td>
<td>63</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thiopental anesthesia</td>
<td>Coma</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Convulsive state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before convulsion</td>
<td>Alert</td>
<td>58</td>
<td>3.7</td>
</tr>
<tr>
<td>After convulsion</td>
<td>Confused</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidosis</td>
<td>Confused</td>
<td>45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coma</td>
<td>Coma</td>
<td>65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hepatic insufficiency</td>
<td>Coma</td>
<td>33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* All studies listed were carried out by Kety and/or his associates, employing the same methods. For references, see Kennedy and Sokoloff [15] and Sokoloff [35].

<sup>b</sup> Denotes statistically significant difference from normal level (p < 0.05).
pletely by adequate insulin therapy. The cause of the coma or depressed cerebral metabolic rate is not known. Deficiency of cerebral nutrition cannot be implicated because the blood glucose concentration is elevated and cerebral blood flow and oxygen supply are more than adequate. Neither is insulin deficiency, presumably the basis of the systemic manifestations of the disease, a likely cause of the cerebral abnormalities since no absolute requirement of insulin for cerebral glucose utilization or metabolism has been demonstrated. Ketosis may be severe in this disease, and there is evidence that a rise in the blood concentration of at least one of the ketone bodies, acetoacetate, can cause coma in animals. In studies of human diabetic acidosis and coma, a significant correlation between the depression of cerebral metabolic rate and the degree of ketosis has been observed, but there is an equally good correlation with the degree of acidosis [39]. Hyperosmolarity itself may cause coma. Ketosis, acidosis, hyperosmolarity or a combination may be responsible for the disturbances in cerebral function and metabolism.

Coma occasionally is associated with severe impairment of liver function, or hepatic insufficiency (see Chap. 38). In human patients in hepatic coma, cerebral metabolic rate is depressed markedly (see Table 31-9). Cerebral blood flow also is depressed moderately but not sufficiently to lead to limiting supplies of glucose and oxygen. Blood ammonia usually is elevated in hepatic coma, and significant cerebral uptake of ammonia from the blood is observed. Ammonia toxicity, therefore, has been suspected as the basis for cerebral dysfunction in hepatic coma. Because ammonia can, through glutamic dehydrogenase activity, convert $\alpha$-KG to glutamate by reductive amination, it has been suggested that ammonia might thereby deplete $\alpha$-KG and, thus, slow the Krebs cycle (see Chaps. 15 and 38). The correlation between the degree of coma and blood ammonia is far from convincing, however, and coma has been observed in the absence of an increase in blood ammonia concentration. Although ammonia may be involved in the mechanism of hepatic coma, the mechanism remains unclear, and other causal factors probably are involved.

Depression of mental functions and of the cerebral metabolic rate has been observed in association with kidney failure and uremic coma (see Chap. 38). The chemical basis of the functional and metabolic disturbances in the brain in this condition also remains undetermined.

In the comatose states associated with these systemic metabolic diseases, there is depression of both conscious mental activity and cerebral energy metabolism. From the available evidence, it is impossible to distinguish which, if either, is the primary change. It is more likely that the depressions of both functions, although well correlated with each other, are independent reflections of a more general impairment of neuronal processes by some unknown factors incident to the disease.

Measurement of local cerebral energy metabolism in humans

Most of the in vivo measurements of cerebral energy metabolism described above, and all of those in humans, were made in the brain as a whole and represent the mass-weighted average of the metabolic activities in all of the component structures of the brain. The average, however, often obscures transient and local events in the individual components, and it is not surprising that many of the studies of altered cerebral function, both normal and abnormal, have failed to demonstrate corresponding changes in energy metabolism (see Table 31-8). The $[14C]$-deoxyglucose method [19] has made it possible to measure glucose utilization simultaneously in all of the components of the central nervous system, and it has been used to identify regions with altered functional and metabolic activities in a variety of physiological, pharmacological and pathological states [21]. As originally designed, the method utilized autoradiography of brain sections for localization, which precluded its use in humans. However, later developments with PET [40] made it possible to adapt it for human use, which is described fully in Chapter 54.

REFERENCES

Part Five Metabolism


