Section 1 Etiology, Pathophysiology, and Imaging

Chapter 1

Neuropathology and Pathophysiology of Stroke

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Neuropathology

Vascular Origin of Cerebrovascular Disease

In the latest edition of the International Classification of Diseases and Related Health Problems (ICD-11) cerebrovascular diseases (CVD) are listed in the section of diseases of the nervous system [1]. However, they have their origin in the vessels supplying or draining the brain, and the knowledge of pathological changes occurring in the vessels and in the blood are essential for understanding the pathophysiology and therapy of the various types of CVD. Changes in the vessel wall lead to obstruction of blood flow; by interacting with blood constituents they may cause thrombosis and blockade of blood flow in this vessel. In addition to vascular stenosis or occlusion at the site of vascular changes, disruption of blood supply and consecutive infarcts can also be produced by emboli arising from vascular lesions situated proximally to otherwise healthy branches located more distal in the arterial tree or from a source located in the heart. At the site of occlusion, opportunity exists for thrombus to develop in anterograde fashion throughout the length of the vessel, but this event seems to occur only rarely.

Changes in large arteries supplying the brain, including the aorta, are mainly caused by atherosclerosis. Middle-sized and intracerebral arteries can also be affected by acute or chronic vascular diseases of inflammatory origin due to subacute to chronic infections, e.g. tuberculosis and lues or due to collagen disorders, e.g. giant cell arteritis, granulomatous angiitis of the CNS, panarteritis nodosa, and even more rarely systemic lupus erythematosus, Takayasu's arteritis, Wegener granulomatosis, rheumatoid arteritis, Sjögren's syndrome, Sneddon and Behçet's disease. In some diseases affecting the vessels of the brain, the etiology and pathogenesis are still unclear, e.g. Moyamoya disease and fibromuscular dysplasia, but these disorders are characterized by typical locations of the vascular changes. Some arteriopathies are hereditary, like CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), in some like cerebral amyloid angiopathy a degenerative cause is discussed. All these vascular disorders can cause obstruction, and lead to thrombosis and embolizations. Small vessels of the brain are affected by hyalinosis and fibrosis; this "small-vessel disease" can cause lacunes and, if widespread, is the substrate for vascular cognitive impairment and vascular dementia.

Atherosclerosis is the most widespread disorder leading to death and serious morbidity including stroke [2]. The basic pathologic lesion is the atheromatous plaque, the most commonly affected sites are the aorta, the coronary arteries, the carotid artery at its bifurcation, and the basilar artery. Arteriosclerosis, a more generic term describing hardening and thickening of the arteries, includes as additional types Mönkeberg's sclerosis and is characterized by calcification in the tunica media and arteriolosclerosis with proliferative and hyaline changes affecting the arterioles. Atherosclerosis starts at young age, lesions accumulate and grow throughout life and become symptomatic and clinically evident when end organs are affected [3].

Atherosclerosis: atheromatous plaques, most commonly in the aorta, the coronary arteries, the bifurcation of the carotid artery and the basilar artery.

The initial lesion of atherosclerosis has been attributed to "fatty streaks" and the "intimal cell mass." Those changes already occur in childhood and adolescence and do not necessarily correspond to the future sites of atherosclerotic plaques. Fatty streaks are focal areas of intracellular lipid collection in both macrophages and smooth muscle cells. Various concepts have been proposed to explain the progression of such precursor lesions to definite atherosclerosis [3, 4], most remarkable of which is the response-to-injury hypothesis postulating a cellular and molecular response to various atherogenic stimuli in the form of an inflammatory repair process [5]. This inflammation develops

concurrently with the accumulation of minimally oxidized low density lipoproteins [6, 7], stimulates vascular smooth muscle cells (VSMCs), endothelial cells and macrophages [8], and as a result foam cells aggregate with an accumulation of oxidized LDL. In the further stages of artherosclerotic plaque development VSMCs migrate, proliferate, and synthesize extracellular matrix components on the luminal side of the vessel wall, forming the fibrous cap of the atherosclerotic lesion [9]. In this complex process of growth, progression, and finally rupture of an atherosclerotic plaque, a large number of matrix modulators, inflammatory mediators, growth factors, and vasoactive substances are involved. The complex interactions of these many factors are discussed in the special literature [6–10].

The fibrous cap of the atherosclerotic lesion covers the deep lipid core with a massive accumulation of extracellular lipids (*atheromatous plaque*), or fibroblasts and extracellular calcifications may contribute to a *fibrocalcific lesion*. Mediators from inflammatory cells at the thinnest portion of the cap surface of a *vulnerable plaque* – which is characterized by a larger lipid core and a thin fibrous cap – can lead to plaque disruption with formation of a thrombus or hematoma or even to total occlusion of the vessel. During the development of artherosclerosis the entire vessel can enlarge or constrict in size [11]. However, once the plaque covers >40% of the vessel wall, the artery no longer enlarges, and the lumen narrows as the plaque grows. In vulnerable plaques thrombosis forming on the disrupted lesion further narrows the vessel lumen and can lead to occlusion or be the origin of emboli. Less commonly, plaques have reduced collagen and elastin with a thin and weakened arterial wall, resulting in aneurysm formation which when ruptured may be the source of intracerebral hemorrhage (Figure 1.1).

> Injury hypothesis of progression to atherosclerosis: fatty streaks (focal areas of intracellular lipid collection) → inflammatory repair process with stimulation of vascular smooth muscle cells → atheromatous plaque.

Thromboembolism: Immediately after plaque rupture or erosion, subendothelial collagen, the lipid core, and procoagulants such as tissue factor and von Willebrand factor are exposed to circulating blood. Platelets rapidly adhere to the vessel wall through the platelet glycoproteins (GP) Ia/IIa and GP Ib/IX [12] with subsequent aggregation to this initial monolayer through linkage with fibrinogen and the exposed GP IIb/IIIa on activated platelets. As platelets are a source of nitrous oxide (NO), the resulting deficiency of bioactive NO, which is an effective vasodilator, contributes to the progression of thrombosis by augmenting platelet activation, enhancing VSMC proliferation and migration, and participating in neovascularization [13]. The activated platelets also release adenosine diphosphate (ADP) and thromboxane A2 with subsequent activation of the clotting cascade. The growing thrombus obstructs or even blocks the blood flow in

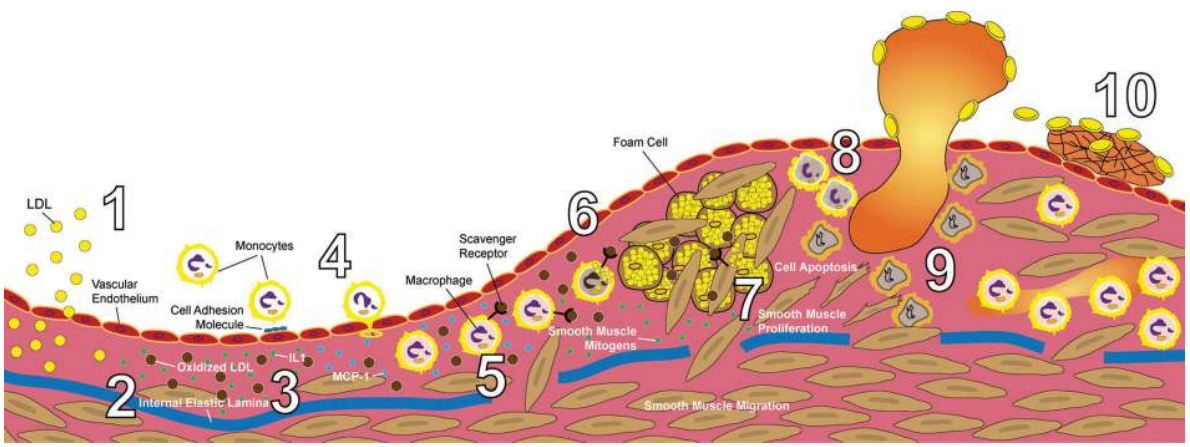

Figure 1.1 The stages of development of an atherosclerotic plaque. (1) LDL moves into the subendothelium and (2) is oxidized by macrophages and smooth muscle cells (SMC). (3) Release of growth factors and cytokines (4) attracts additional monocytes. (5) Macrophages and (6) foam cell accumulation and additional (7) SMC proliferation result in (8) growth of the plaque. (9) Fibrous cap degradation and plaque rupture (collagenases, elastases). (10) Thrombus formation.
(Modified from Faxon *et al.* 2004 [6].)

the vessel. Atherosclerotic thrombi are also the source for embolisms, which are the primary pathophysiologic mechanism of ischemic strokes, especially from carotid artery disease or of cardiac origin.

> Rupture or erosion of atheromatous plaques → adhesion of platelets → thrombus → obstruction of blood flow and source of emboli.

Small-vessel disease usually affects the arterioles and is associated with hypertension. It is caused by subendothelial accumulation of a pathological protein, the hyaline, formed from mucopolysaccharides and matrix proteins. It leads to narrowing of the lumen or even occlusion of these small vessels. Often it is associated with fibrosis, which affects not only arterioles, but also other small vessels and capillaries and venules. Lipohyalinosis also weakens the vessel wall predisposing for the formation of "miliary aneurysms." Small-vessel disease results in two pathological conditions: status lacunaris (lacunar state) and status cribrosus (état criblé). Status lacunaris is characterized by small irregularly shaped infarcts due to occlusion of small vessels; it is the pathological substrate of lacunar strokes and vascular cognitive impairment and dementia. In status cribrosus small, round cavities develop around affected arteries due to disturbed supply of oxygen and metabolic substrate. These "criblures" together with miliary aneurysms are the sites of vessel rupture causing typical hypertonic intracerebral hemorrhages [14–17]. A second type of small-vessel disease is characterized by the progressive accumulation of congophilic, βA4 immuno-reactive, amyloid protein in the walls of small to medium-sized arteries and arterioles. Cerebral amyloid angiopathy is a pathological hallmark of Alzheimer's disease and also occurs in rare genetically transmitted diseases, e.g. CADASIL and Fabry disease [18]. For a more detailed discussion of the etiology and pathophysiology of the various specific vascular disorders see [19–21].

> Small-vessel disease: subendothelial accumulation of hyaline in arterioles.

Types of Cerebrovascular Disease

Numbers relating to the frequency of the different types of acute CVD are highly variable depending on the source of data. The most reliable numbers come from the in-hospital assessment of stroke in the Framingham study determining the frequency of completed stroke: 60% were caused by atherothrombotic brain infarction, 25.1% by cerebral embolism, 5.4% by subarachnoid hemorrhage, 8.3% by intracerebral hemorrhage, and 1.2% by undefined diseases. In addition, transient ischemic attacks accounted for 14.8% of the total cerebrovascular events [22]. Since the first Framingham reports, the rate of stroke death has declined by more than one-third, but the relative frequency distribution of completed stroke is essentially the same [23].

Ischemic strokes result from a critical reduction of regional cerebral blood flow lasting beyond a critical duration, and are caused by atherothrombotic changes of the arteries supplying the brain or by emboli from sources in the heart, the aorta, or the large arteries. The pathological substrate of ischemic stroke is ischemic infarction of brain tissue, the location, extension, and shape of which depend on the size of the occluded vessel, the mechanism of arterial obstruction, and the compensatory capacity of the vascular bed (Figure 1.2). Occlusion of arteries supplying defined brain territories by atherothrombosis or embolizations lead to *territorial infarcts* of variable size: they may be large – e.g. the whole territory supplied by the middle cerebral artery – or small, if branches of large arteries are occluded or if compensatory collateral perfusion – e.g. via the circle of Willis or leptomeningeal anastomoses – is efficient in reducing the area of critically reduced flow [15, 17]. In a smaller number of cases, *infarcts* can also develop *at the borderzones* between vascular territories, when several large arteries are stenotic and the perfusion in these "last meadows" cannot be constantly maintained above the critical threshold of morphological integrity [24]. *Borderzone infarctions* are a subtype of the *low-flow* or hemodynamically induced *infarctions*, which are the result of critically reduced cerebral perfusion pressure in far-downstream brain arteries. The more common low-flow infarctions affect subcortical structures within a vascular bed with preserved but marginal irrigation [25]. *Lacunar infarcts* reflect disease of the vessels penetrating the brain to supply the capsule, the basal ganglia, thalamus, and paramedian regions of the brainstem [26]. Most often they are caused by lipohyalinosis of deep arteries (small-vessel disease), less frequent causes are stenosis of the MCA stem and microembolization to penetrant arterial territories. Pathologically these lacunes are defined as small cystic trabeculated scars about 5 mm in diameter, but they are more often observed on magnetic resonance images where they are accepted as lacunes up to 1.5 cm diameter. The classic lacunar syndromes include pure motor, pure sensory, and sensorimotor syndromes,

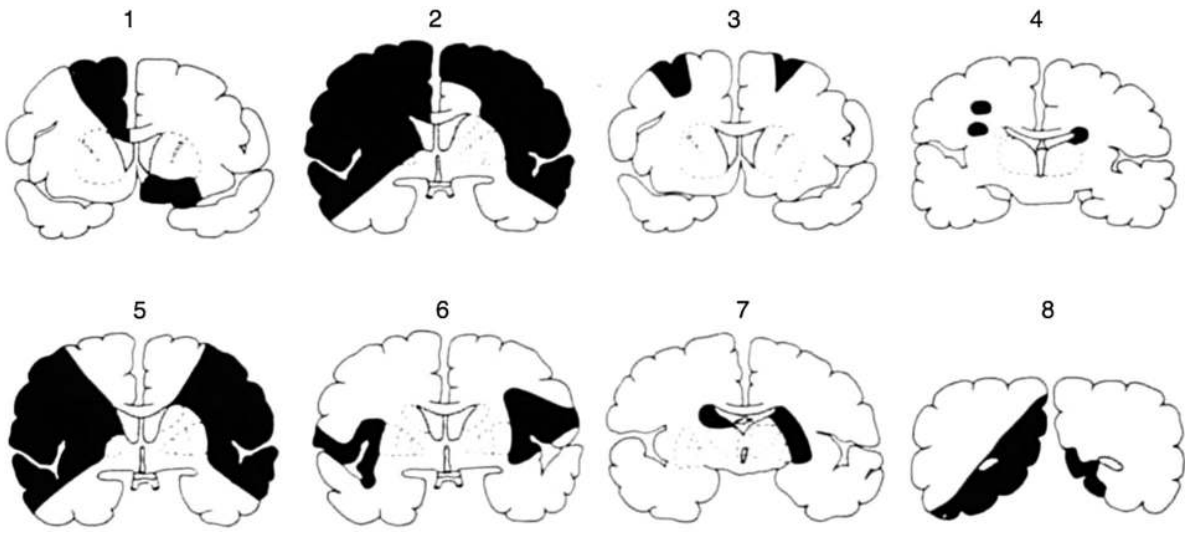

Figure 1.2 Topography of the most common types of cerebral infarcts: (1) anterior cerebral artery (left: total infarction, right: infarct of recurrent artery of Heubner); (2) anterior and middle cerebral arteries (left: with, right: without lenticulostriate arteries); (3) borderzone infarcts between anterior and middle cerebral arteries; (4) cystic infarcts (left: centrum ovale, right: caudate); (5) and (6) middle cerebral artery (5 left: total, right: cortical, 6 left: minimal, right: wedge-shaped); (7) end-artery and borderzone infarcts of the perforating branches of middle cerebral artery; (8) posterior cerebral artery (left: total, right: subtotal).

(With permission, Zülch 1985 [15].)

sometimes ataxic hemiparesis, clumsy hand, dysarthria, and hemichorea/hemiballism, but higher cerebral functions are not involved. A new classification of stroke subtypes is mainly oriented on the most likely cause of stroke: atherosclerosis, small-vessel disease, cardiac source, or other cause [27].

> Territorial infarcts are caused by an occlusion of arteries supplying defined brain territories by atherothrombosis or embolizations.
>
> Borderzone infarcts develop at the borderzone between vascular territories and are the result of a critically reduced cerebral perfusion pressure (low-flow infarctions).
>
> Lacunar infarcts are mainly caused by small-vessel disease.

Hemorrhagic infarctions, i.e. "red infarcts" in contrast to the usual "pale infarcts," are defined as ischemic infarcts in which varying amounts of blood cells are found within the necrotic tissue. The amount can range from a few petechial bleeds in the gray matter of cortex and basal ganglia to large hemorrhages involving the cortical and deep hemispheric regions. Hemorrhagic transformation frequently appears during the second and third phases of infarct evolution, when macrophages appear and new blood vessels are formed in tissue consisting of neuronal ghosts and proliferating astrocytes. However, the only significant difference between "pale" and "red infarcts" is the intensity and extension of the hemorrhagic component, since in at least two-thirds of all infarcts petechial hemorrhages are microscopically detectable. Macroscopically, red infarcts contain multifocal bleedings which are more or less confluent and predominate in cerebral cortex and basal ganglia which are richer in capillaries than the white matter [28]. If the hemorrhages become confluent intrainfarct hematomas might develop, and extensive edema may contribute to mass effects and lead to malignant infarction. The frequency of hemorrhagic infarctions (HIs) in anatomic studies ranged from 18% to 42% [29], with a high incidence (up to 85% of HIs) in cardioembolic stroke [30].

Mechanisms for hemorrhagic transformation are manifold and vary with regard to the intensity of bleeding. Petechial bleeding results from diapedesis rather than vascular rupture. In severe ischemic tissue vascular permeability is increased and endothelial tight junctions are ruptured. When blood circulation is spontaneously or therapeutically restored, blood can leak out of these damaged vessels. This can also happen with fragmentation and distal migration of an

embolus (usually of cardiac origin) in the damaged vascular bed, explaining delayed clinical worsening in some cases. For the hemorrhagic transformation also the collateral circulation might have an impact: in some instances reperfusion via pial networks may develop with the diminution of peri-ischemic edema at borderzones of cortical infarcts. Risk of hemorrhage is significantly increased in large infarcts with mass effect supporting the importance of edema for tissue damage and the deleterious effect of late reperfusion. In some instances also the rupture of the vascular wall secondary to ischemia-induced endothelial necrosis might cause an intrainfarct hematoma. Vascular rupture can explain very early hemorrhagic infarcts and early intrainfarct hematoma (between 6 and 18 hours after stroke), whereas hemorrhagic transformation usually develops within 48 hours to 2 weeks.

> Hemorrhagic infarctions (HI) are defined as ischemic infarcts in which varying amounts of blood cells are found within the necrotic tissue. They are caused by leakage from damaged vessels, due to increased vascular permeability in ischemic tissue or vascular rupture secondary to ischemia.

Intracerebral hemorrhage (ICH) occurs as a result of bleeding from an arterial source directly into the brain parenchyma and accounts for 5–15% of all strokes [31, 32]. Hypertension is the leading risk factor, but in addition advanced age, race, and also cigarette smoking, alcohol consumption, and high serum cholesterol levels have been identified. In a number of instances ICH occurs in the absence of hypertension usually in atypical locations. These causes include small vascular malformations, vasculitis, brain tumors, and sympathomimetic drugs (e.g. cocaine). ICH may also be caused by cerebral amyloid angiopathy and rarely is elicited by acute changes in blood pressure, e.g. due to exposure to cold. The occurrence of ICH is also influenced by the increasing use of anti-thrombotic and thrombolytic treatment of ischemic diseases of the brain, heart, and other organs [33, 34].

Spontaneous ICH occurs predominantly in the deep portions of the cerebral hemispheres ("typical ICH") [35]. Its most common location is the putamen (35–50% of cases). The subcortical white matter is the second most frequent location (approximately 30%). Hemorrhages in the thalamus are found in 10–15%, in the pons in 5–12%, and in the cerebellum in 7% of cases [36]. Most ICHs originate from the rupture of small, deep arteries with diameters of 50–200 μm, which are affected by lipohyalinosis due to chronic hypertension. These small vessel changes lead to weakening of the vessel wall and miliary micro-aneurysm and consecutive small local bleedings, which might be followed by secondary ruptures of the enlarging hematoma in a cascade or avalanche fashion [37]. After active bleeding started it can continue for a number of hours with enlargement of hematoma that is frequently associated with clinical deterioration [38].

Putaminal hemorrhages originate from a lateral branch of the striate arteries at the posterior angle resulting in an ovoid mass pushing the insular cortex laterally and displacing or involving the internal capsule. From this initial putaminal-claustral location a large hematoma may extend to the internal capsule and lateral ventricle, into the corona radiata, and into the temporal white matter. Putaminal ICHs are considered the typical hypertensive hemorrhages.

Caudate hemorrhage, a less common form of bleeding from distal branches of lateral striate arteries, occurs in the head of the caudate nucleus. This bleeding early connects to the ventricle and usually involves the anterior limb of the internal capsule.

Thalamic hemorrhages can involve most of this nucleus and extend into the third ventricle medially and the posterior limb of the internal capsule laterally. The hematoma may press on or even extend into the midbrain. Larger hematomas often reach the corona radiata and the parietal white matter.

Lobar (white matter) hemorrhages originate at the cortico-subcortical junction between gray and white matter and spread along the fiber bundles most commonly in the parietal and occipital lobes. The hematomas are close to the cortical surface and usually not in direct contact with deep hemisphere structures or the ventricular system. As atypical ICHs they are not necessarily correlated with hypertension.

Cerebellar hemorrhages usually originate in the area of the dentate nucleus from rupture of distal branches of the superior cerebellar artery and extend into the hemispheric white matter and into the fourth ventricle. The pontine tegmentum is often compressed. A variant, the midline hematoma, originates from the cerebellar vermis, always communicates with the fourth ventricle, and frequently extends bilaterally into the pontine tegmentum.

Pontine hemorrhages from bleeding of small paramedian basilar perforating branches cause medially placed hematomas involving the basis of the pons. A unilateral variety results from rupture of distal, long circumferential branches of the basilar artery.

These hematomas usually communicate with the fourth ventricle, and extend laterally and ventrally into the pons.

The frequency of recurrent ICHs in hypertensive patients is rather low (6%) [39]. Recurrence rate is higher with poor control of hypertension and also in hemorrhages due to other causes. In some instances multiple simultaneous ICHs may occur, but also in these cases the cause is another than hypertension.

In ICHs, the local accumulation of blood destroys the parenchyma, displaces nervous structures, and dissects the tissue. At the bleeding sites fibrin globes are formed around accumulated platelets. After hours or days extracellular edema develops at the periphery of the hematoma. After 4–10 days the red blood cells begin to lyse, granulocytes and thereafter microglial cells arrive, and foamy macrophages are formed, which ingest debris and hemosiderin. Finally, the astrocytes at the periphery of the hematoma proliferate and turn into gemistocytes with eosinophylic cytoplasma. When the hematoma is removed, the astrocytes are replaced by glial fibrils. After that period – extending to months – the residue of the hematoma is a flat cavity with a reddish lining resulting from hemosiderin-laden macrophages [36].

> Intracerebral hemorrhage (ICH) occurs as a result of bleeding from an arterial source directly into the brain parenchyma, predominantly in the deep portions of the cerebral hemispheres (typical ICH). Hypertension is the leading risk factor, and the most common location is the putamen.

Cerebral venous thrombosis can develop from many causes and due to predisposing conditions. Cerebral venous thrombosis (CVT) is often multifactorial, when various risk factors and causes contribute to the development of this disorder [40]. The incidence of septic CVT has been reduced to less than 10% of cases, but septic cavernous sinus thrombosis is still a severe, however rare problem. Aseptic CVT occurs during puerperium and less frequently during pregnancy, but may also be related to use of oral contraceptives. Among the non-infectious causes of CVT congenital thrombophilia, particularly prothrombin and factor V Leiden gene mutations, as well as anti-thrombin, protein C, and protein S deficiencies must be considered. Other conditions with risk for CVT are malignancies, inflammatory diseases, and systemic lupus erythematosus. However, in 20–35% of CVT the etiology remains unknown. The fresh venous thrombus is rich in red blood cells and fibrin and poor in platelets. Later on, it is replaced by fibrous tissue, occasionally with recanalization. The most common location of CVT is the superior sagittal sinus and the tributary veins.

Whereas some thromboses, particularly of the lateral sinus, may have no pathological consequences for the brain tissue, occlusion of large cerebral veins usually leads to a venous infarct. These infarcts are located in the cortex and adjacent white matter and often are hemorrhagic. Thrombosis of the superior sagittal sinus may lead only to brain edema, but usually causes bilateral hemorrhagic infarcts in both hemispheres. These venous infarcts are different from arterial infarcts: cytotoxic edema is absent or mild, vasogenic edema is prominent, and hemorrhagic transformation or bleeding is usual. Despite this hemorrhagic component heparin is the treatment of choice.

> Cerebral venous thrombosis can lead to a venous infarct. Venous infarcts are different from arterial infarcts: cytotoxic edema is absent or mild, vasogenic edema is prominent, and hemorrhagic transformation or bleeding is usual.

Cellular Pathology of Ischemic Injury

Acute interruption of cerebral blood flow causes a stereotyped sequel of cellular alterations which evolve over a protracted period of time and which depend on the topography, severity, and duration of ischemia [41]. Traditionally, these alterations have been studied by classical histological techniques, but recent developments in high resolution in vivo optical imaging such as multi-photon laser scanning microscopy (MPM), optical coherence tomography (OCT), or photoacoustic imaging (PAI) have opened the way to correlate morphological alterations with functional disturbances [42].

The most sensitive brain cells are neurons, followed – in this order – by oligodendrocytes, astrocytes, and vascular cells. The most vulnerable brain regions are hippocampal subfield CA_1, neocortical layers 3, 5, and 6, the outer segment of striate nucleus, and the Purkinje and basket cell layers of cerebellar cortex. If blood flow decreases below the threshold of energy metabolism, the primary pathology is necrosis of all cell elements, resulting in ischemic brain infarct. If ischemia is not severe enough to cause primary energy failure, or if it is of so short duration that energy metabolism recovers after reperfusion, a delayed type of cell injury may evolve which exhibits the morphological characteristics of necrosis, apoptosis, necroptosis, or other forms of programmed cell

death [43]. In the following, primary and delayed cell death will be described separately.

Primary Neuronal Cell Death

In the core of the territory of an occluded brain artery the earliest sign of cellular injury is neuronal swelling or shrinkage, the cytoplasm exhibiting microvacuolation (MV), which ultrastructurally has been associated with mitochondrial swelling [44]. These changes are potentially reversible if blood flow is restored before mitochondrial membranes begin to rupture. One to two hours after the onset of ischemia, neurons undergo irreversible necrotic alterations (red neuron or ischemic cell change [ICC]). In conventional hematoxilin-eosin stained brain sections such neurons are characterized by intensively stained eosinophilic cytoplasma, formation of triangular nuclear pyknosis, and direct contact with swollen astrocytes (Figure 1.3). Electron microscopically mitochondria exhibit flocculent densities, which represent denaturated mitochondrial proteins. Ischemic cell change must be distinguished from artifactual dark neurons, which stain with all (acid or basic) dyes and are not surrounded by swollen astrocytes [45].

With ongoing ischemia, neurons gradually lose their stainability with hematoxilin, they become mildly eosinophilic, and, after 2–4 days, transform to ghost cells with hardly detectable pale outline. Interestingly, neurons with ischemic cell change are mainly located in the periphery and ghost cells in the center of the ischemic territory, which suggests that manifestation of ischemic cell change requires some residual or restored blood flow, whereas ghost cells may evolve in the absence of flow [41].

Primary ischemic cell death induced by focal ischemia is associated with reactive and secondary changes. The most prominent alteration during the initial 1–2 hours is perivascular and perineuronal astrocytic swelling, after 4–6 hours the blood–brain barrier

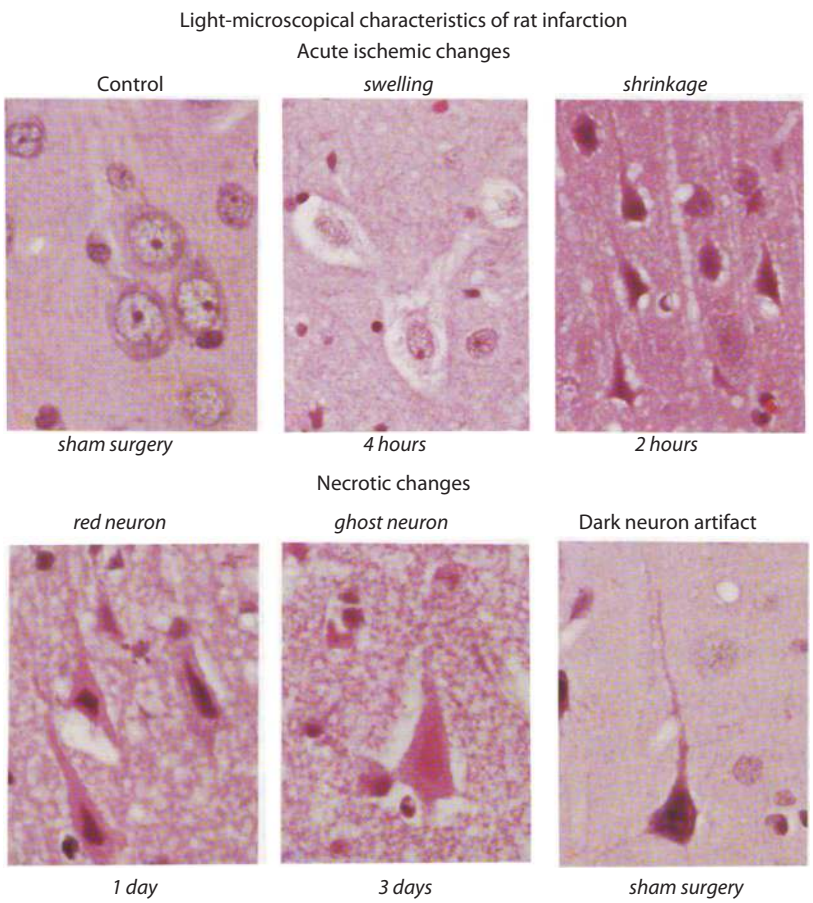

Figure 1.3 Light-microscopical evolution of neuronal changes after experimental middle cerebral occlusion.
(Modified with permission from Garcia *et al.* 1995 [168].)

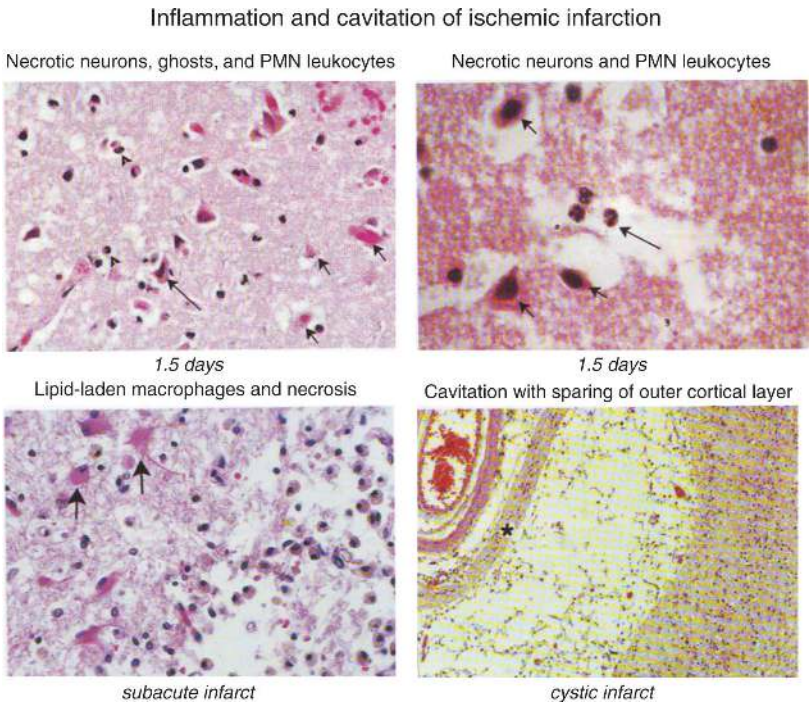

Figure 1.4 Transformation of acute ischemic alterations into cystic infarct. Note pronounced inflammatory reaction prior to tissue cavitation.

(Modified with permission from Petito 2005 [41].)

breaks down resulting in the formation of vasogenic edema, after 1–2 days inflammatory cells accumulate throughout the ischemic infarct, and within 1.5–3 months cystic transformation of the necrotic tissue occurs together with the development of a peri-infarct astroglial scar (Figure 1.4).

Delayed Neuronal Death

The prototype of delayed cell death is the slowly progressing injury of pyramidal neurons in CA_1 sector of hippocampus after a brief episode of global ischemia [46]. In focal ischemia delayed neuronal death may occur in the periphery of cortical infarcts or in regions which have been reperfused before ischemic energy failure becomes irreversible. Cell death is also observed in distant brain regions, notably in substantia nigra and thalamus.

The morphological appearance of neurons during the interval between ischemia and the manifestation of delayed cell death exhibits a continuum that ranges from necrosis to apoptosis with all possible combinations of cytoplasmic and nuclear morphology that are characteristic for the two types of cell death [47]. In its pure form, necrosis combines karyorhexis with massive swelling of endoplasmic reticulum and mitochondria, whereas in apoptosis mitochondria remain intact and nuclear fragmentation with condensation of nuclear chromatin gives way to the development of apoptotic bodies. In hemorrhagic stroke lysed blood may induce ferroptosis, a particular form of iron-dependent cell death, which is characterized by lethal accumulation of lipid reactive oxygen species (ROS) [48].

A widely used histochemical method for the visualization of apoptosis is terminal deoxyribonucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling (TUNEL assay), which detects DNA strand breaks. However, as this method may also stain necrotic neurons, a clear differentiation is not possible [49].

A consistent ultrastructural finding in neurons undergoing delayed cell death is disaggregation of ribosomes, which reflects the inhibition of protein synthesis at the initiation step of translation [50]. Light microscopically, this change is equivalent to tigrolysis, visible in Nissl-stained material that corresponds to the dissociation of ribosomes from the rough endoplasmic reticulum. Disturbances of protein synthesis and the associated endoplasmic reticulum stress are also responsible for cytosolic protein aggregation and the formation of stress granules [51]. In the hippocampus, stacks of accumulated endoplasmic reticulum may become visible, but in other areas this is not a prominent finding.

Pathology of Neurovascular Unit

The classical pathology of ischemic injury differentiates between the sensitivity of the various cell types of brain parenchyma with the neurons as the most vulnerable elements. The molecular analysis of injury evolution, however, suggests that ischemia initiates a coordinated multi-compartmental response of brain cells and vessels, also referred to as the neurovascular unit [52]. This unit includes microvessels (endothelial cells, basal lamina matrix, astrocytic endfeet, pericytes, and circulating blood elements), the cell body and main processes of astrocytes, the nearby neurons together with their axons, and supporting cells, notably microglia and oligodendrocytes. It provides the framework for the bi-directional communication between neuron and supplying microvessel. Under physiological condition, the most prominent function is the neurovascular coupling for maintaining adequate supply of brain nutrients and clearance of waste products. Pathophysiological disturbances of microcirculation provoke bi-directional responses, possibly mediated by alterations in the matrix of the vascular and non-vascular compartments of the ischemic territory. Pericytes positioned between endothelial cells, astrocytes, and neurons assume a central role in this process and are critically involved in mechanisms of both injury and repair of the central nervous system [53].

Severe ischemia induces primary cell death due to necrosis of all cell elements. Not so severe or short-term ischemia induces delayed cell death with necrosis, apoptosis, or a combination of both. The neurovascular unit provides the conceptual framework for the propagation of injury from microvessels to neurons.

Repair

Brain infarcts produced by focal ischemia are seemingly irresolvable in agreement with Cajal's classical statement that in the adult brain "everything may die, nothing may be regenerated." This dogma was reversed by the discovery of three permanently neurogenic regions, i.e. the subventricular zone (SVZ), the subgranular zone (SGZ), and the posterior perireticular (PPr) area, which provide lifelong supply of newly generated neurons to the hippocampus and olfactory bulb. After stroke, neurogenesis increases in these areas, and some of the newly formed cells migrate into the infarct penumbra, differentiate into oligodendrocytes and mature neurons, and survive for at least several weeks [54]. Neurogenesis may also occur through the neurovascular unit. After ischemia pericytes strongly migrate into the peri-infarct surrounding and contribute to tissue repair by controlling neurogenesis, angiogenesis, and blood–brain barrier function [55].

Ischemia-induced neurogenesis is enhanced by growth factors, nitric oxide, inflammation, non-coding RNA, and various hormones and neurotransmitters, notably estradiol and dopamine, but it is repressed by activation of NMDA subtype of glutamate receptors. The functional consequences of spontaneous or drug-enhanced neurogenesis are modest, but optimism is building up for targeted interventions. Similarly, considerable expectations are placed on local or systemic transplantation of exogenous neural progenitor cells and on cerebral endothelial and bone marrow cells, particularly in combination with growth factors and/or strategies that permit recruitment of transplanted cells to the site of injury [56]. However, cell therapy carries the risk of tumorigenesis, and as major breakthroughs have not yet been achieved, further research is necessary to explore the actual potentials of stroke regenerative medicine [57].

Several brain regions may provide lifelong supply of newly generated neurons.

Pathophysiology

The evolution of stroke is a highly intricate process which can be differentiated into two consecutive phases: a rather straightforward early "plumbing" problem that arises from the interruption of blood flow and brain energy supply, and a much more complex cascade of secondary events which depends on the interaction between metabolic and functional disturbances, on the one hand, and between hemodynamic and molecular alterations, on the other. As this complexity is differently depicted in different experimental stroke models, the technical particularities of these models must be understood before attempts are made to translate experimental insights to the clinical setting.

Animal Models of Stroke

According to the Framingham study, 65% of strokes that result from vascular occlusion present lesions in the territory of the middle cerebral artery, 2% in the anterior, and 9% in the posterior cerebral artery territories. The remaining lesions are located in brainstem, cerebellum, or in watershed or multiple regions. For each of these stroke types specific experimental models have been developed [58], but in accordance with

the dominant clinical incidence middle cerebral artery occlusion models are preferentially used in experimental stroke research.

Transorbital middle cerebral artery occlusion: This model was introduced for the production of stroke in monkeys [59], and later modified for use in cats, dogs, rabbits, and even rats. The procedure is technically demanding and requires microsurgical skills. The advantage of this approach is the possibility to expose the middle cerebral artery at its origin from the internal carotid artery without retracting parts of the brain. Vascular occlusion can thus be performed without the risk of brain trauma. On the other hand, removal of the eyeball is invasive and may evoke functional disturbances, which should not be ignored. Surgery may also cause generalized vasospasm, which may interfere with the collateral circulation and, hence, induce variations in infarct size. The procedure therefore requires extensive training before reproducible results can be expected.

The occlusion of the middle cerebral artery at its origin interrupts blood flow to the total vascular territory, including the basal ganglia, which are supplied by the lenticulo-striate arteries. These MCA branches are end-arteries, which in contrast to the cortical branches do not form collaterals with the adjacent vascular territories. As a consequence, the basal ganglia are consistently part of the infarct core, whereas the cerebral cortex exhibits a gradient of blood flow, which decreases from the peripheral towards the central parts of the vascular territory. Depending on the steepness of this gradient, a cortical core region with the lowest flow values in the lower temporal cortex is surrounded by a variably sized penumbra, which may extend up to the parasagittal cortex.

Transcranial occlusion of the middle cerebral artery: Post- or retro-orbital transcranial approaches for middle cerebral artery occlusion are mainly used in rats and mice because in these species the main stem of the artery appears on the cortical surface rather close to its origin from the internal carotid artery [60]. Permanent occlusion is usually carried out by ligation or coagulation, and transient occlusion by clipping or lifting the vessel with a hook [61]. In contrast to transorbital middle cerebral artery occlusion, transcranial models do not produce ischemic injury in the basal ganglia because the lenticulo-striate branches originate proximal to the occlusion site. Infarcts, therefore, are mainly located in the temporo-parietal cortex with a gradient of declining flow values from the peripheral to the central parts of the vascular territory.

Filament occlusion of the middle cerebral artery: The presently most widely used procedure for middle cerebral artery occlusion in rats and mice is the intraluminal filament occlusion technique, first described by Koizumi [62]. A nylon suture with an acryl-thickened tip is inserted into the common carotid artery and orthogradely advanced, until the tip is located at the origin of the middle cerebral artery. Modifications of the original technique include different thread types for isolated or combined vascular occlusion, adjustments of the tip size to the weight of the animal, poly-L-lysine coating of the tip to prevent incomplete middle cerebral artery occlusion, or the use of guide-sheaths to allow remote manipulation of the thread for occlusion during polygraphic recordings or magnetic resonance imaging.

The placement of the suture at the origin of the middle cerebral artery obstructs blood supply to the total MCA-supplying territory, including the basal ganglia. It may also reduce blood flow in the anterior and posterior cerebral arteries, particularly when the common carotid artery is ligated to facilitate the insertion of the thread. As this minimizes collateral blood supply from these territories, infarcts are very large and produce massive ischemic brain edema with a high mortality when experiments last for more than a few hours. For this reason, threads are frequently withdrawn after 1–2 hours following insertion. The resulting reperfusion salvages the peripheral parts of the MCA territory, and infarcts become smaller [63]. However, as the pathophysiology of transient middle cerebral artery occlusion differs from that of the clinically more relevant permanent occlusion models, the mechanisms of infarct evolution or the pharmacological responsiveness of the resulting lesions do not properly replicate that of clinical stroke [64].

Transient filament occlusion is also an inappropriate model for the investigation of spontaneous or thrombolysis-induced reperfusion. Withdrawal of the intraluminal thread induces instantaneous reperfusion, whereas spontaneous or thrombolysis-induced recanalization results in slowly progressing recirculation. As post-ischemic recovery is greatly influenced by the dynamics of reperfusion, outcome and pharmacological responsiveness of experimental transient filament occlusion is also distinct from most clinical situations of reversible ischemia, where the onset of reperfusion is much less abrupt.