Pathology of small vessel stroke

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Disease of small intracerebral vessels is widely assumed to be responsible for the majority of small, deep-seated (lacunar) infarcts and primary intracerebral haemorrhages. Our present, limited understanding of the pathogenesis of these stroke subtypes, which together constitute up to one-third of all strokes, is based on a limited number of detailed pathology studies, supported by clinical, risk factor and imaging data. Further progress using these traditional approaches has been prevented by a variety of largely technical obstacles. It is suggested that advances in our understanding of the genetic basis of established and new animal stroke models, in turn linked to more focused human genetic stroke surveys, may hold the key to further insights.

An impasse appears to have been reached in our attempts to understand the pathogenesis and, therefore, the prevention, of strokes due to disease of small intracerebral vessels. Current dogma has it that small vessel disease causes a significant proportion of small, deep-seated (lacunar) infarcts and primary (*i.e.* non-traumatic) intracerebral haemorrhage (PICH). This is a problem of clear socio-economic importance, for lacunar strokes constitute approximately 25% of first in-a-lifetime ischaemic stroke[1], and PICH some 10% of all stroke, at least in the West[2]. To this should be added the probable contribution of small vessel disease to global, dementing brain injury, further discussion of which lies outside the scope of this article. I shall describe the central role of pathology in achieving our current, if limited, understanding of small vessel disease related stroke, in particular lacunar stroke, outline the obstacles that have prevented further progress, and suggest how the modern molecular pathologist may offer fresh insights.

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Lacunar infarction and the lacunar hypothesis

The original pathological descriptions of lacunar infarcts were made by the beginning of this century by Durand Fardell and Marie in Paris[3]. Lacunar infarcts vary in maximal dimension from 3–20 mm, and are

most commonly found in the putamen, caudate, thalamus, pons, internal capsule and cerebral white matter, in descending order of frequency. By the end of the century, Poirier and Derousne had proposed a neuropathological classification of lacunes. They described old, small infarcts (type I lacunes), old, small haemorrhages (type II) and dilated perivascular spaces (type III)[4]. Type I lacunes are by far the most important clinically. Between times, Fisher had taken the descriptive pathological approach a stage further by his meticulous serial section autopsy reconstructions of the vascular supply of a limited number of lacunar infarcts[5]. His fundamental observation was that lacunar infarcts result from occlusion of small perforating cerebral arteries, in some cases by a destructive process he termed 'segmental arterial disorganisation' or 'lipohyalinosis', and in others by atherosclerosis. In a small minority, he found no occlusive lesion and assumed embolism. Thus arose what became known as the 'lacunar hypothesis', according to which lacunar infarcts are caused by characteristic vascular lesions involving single perforating brain arteries, often in combination with hypertension.

The nature of small vessel lesions causing lacunar infarcts – the legacy of Fisher

There are a large number of potential causes of small vessel occlusion, some autopsy-proven, others inferred (Table 1). These 'causes' may not necessarily be mutually exclusive; for example, small vessel spasm may mediate destructive lesions or exacerbate atheroma[6]. Despite this multiplicity of possible causes, Fisher's work suggested, and there has been no convincing contradictory data since, that there are two small vessel pathologies of major pathogenetic significance to stroke, the first 'lipohyalinosis', the second atherosclerosis.

Lipohyalinosis, as originally described, is a destructive vessel lesion characterised by a loss of normal arterial architecture, mural foam cells and, in acute cases, evidence of fibrinoid vessel wall necrosis. Fisher noted that such vascular lesions involved small arteries of 40–200 μm diameter, and caused correspondingly small (3–7 mm diameter), often asymptomatic, cerebral infarcts, particularly in the striatocapsule[5]. By whatever name, although perhaps less prevalent today in the era of controlled hypertension[5], such vessel lesions are still seen at post mortem in close proximity to lacunar infarcts (Fig. 1b). The important, unresolved questions concerning this lesion are what proportion of lacunar infarcts is it now responsible for and what is the underlying molecular mechanism?

Table 1 Postulated causes of small deep cerebral infarcts

Common (autopsy proven)
      Destructive small vessel disease ('lipohyalinosis')
      Perforating/parent artery atherosclerosis

Uncommon/rare (may be autopsy proven but mechanism often assumed or inferred)
      Embolism
      Vasculitis
            Collagen vascular disease
            Infective
            Recreational drugs (e g cocaine)
            Isolated CNS angiitis
      Infection
            HIV
            Tuberculosis
            Neurosyphylis
            Cystercicosis
            Lyme disease
      Hypoperfusion
      In situ thrombosis/hypercoaguability
            Antiphospholipid antibodies
            SLE
            Disseminated malignancy
            Thrombocythaemia
            Polycythaemia
      Arterial dissection
      CADASIL
      Cerebral amyloid angiopathy

Speculative (difficult to prove)
      Vasospasm
      Oedema
      Pulsatile trauma
      Destructive ('lytic') agent

The term 'lipohyalinosis' has subsequently been misused to describe almost any cerebral small vessel pathology. It is distinct from, and should not be confused with, the concentric, hyaline wall thickening that is a feature of most aged brains (Fig. 1a), particularly those from the hypertensive and diabetic elderly. Such poorly distensible collagen-rich 'tubes', for which the term 'hyaline arteriosclerosis' is appropriate, are ill-equipped to match cerebral blood supply with demand, particularly if systemic blood pressure is abnormally high or low. They are an almost invariant feature of brains with diffuse, presumed ischaemic, white matter disease or leukoaraiosis. However, although such hyaline arteriosclerosis is often severe in brains harbouring lacunar infarcts, there is no direct evidence that they are a cause of focal brain lesions and lacunar stroke.

The other vascular lesion of pathologically-proven relevance to lacune formation is intracranial atherosclerosis. This is pathologically similar to the disease more familiar in the larger cervicocranial arteries, and affects
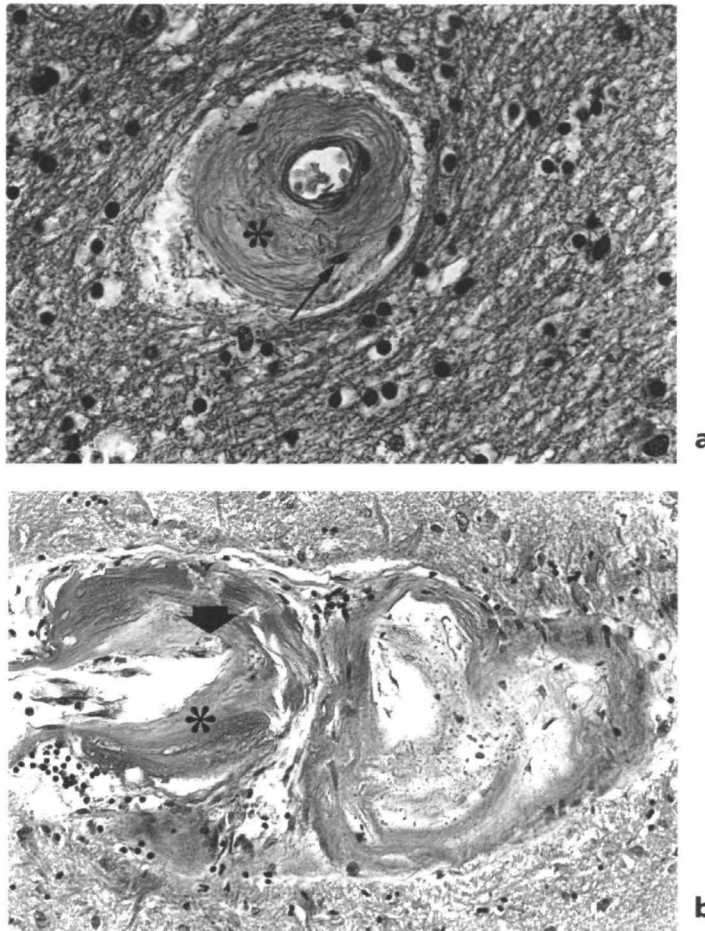
Fig. 1 Photomicrographs illustrating two distinct, but often confused, types of intrinsic cerebral small vessel pathology. (a) Hyaline arteriosclerosis ('simple' small vessel disease) in the putamen. Roughly concentric vessel wall thickening by hyaline collagenous material (asterix), with occasional surviving smooth muscle cell nuclei (arrow). (b) Healed 'lipohyalinosis' ('complex' small vessel disease) in the putamen of an elderly woman with multiple basal ganglia lacunes. An asymmetrically thickened, disorganised vessel wall with focal fibrosis (asterix) and foam cell infiltration (thick arrow). The vessel is cut in two planes. Haematoxylin and eosin: (a) x430; (b) x200.

somewhat larger perforating arteries than lipohyalinosis (200–800 μm diameter), causing correspondingly larger infarcts, 5 mm or more in diameter, which are more often symptomatic. According to Fisher, the culprit atheromatous plaques were seen in the proximal portion of the perforating artery (microatheroma), at its origin (junctional atheroma) or in the parent artery itself (mural atheroma)[5]. Infarcts were related to stenotic or occlusive plaques, some but not all of which were complicated by overlying thrombus.

Progress in lacune research since Fisher

Since Fisher's landmark pathological observations, most lacune research has been in the clinical arena. Consistent with the lacunar hypothesis, lacunar stroke patients have been found to have a relatively low frequency of cardiac and large vessel atheromatous embolic sources compared to those with cortical infarcts[7]. That lacunar infarcts tend not to have a risk of early recurrence would also seem to mitigate against their being caused by an active embolic source. The risk factor profile for lacunar infarcts continues to be refined, but appears similar to that for ischaemic stroke in general, with the possible exception of hypertension. Hypertension was a more severe disease when Fisher first studied lacunes and he perhaps overestimated its importance in lacune pathogenesis, but it may still be more prevalent in this stroke subtype today[8]. Thus, although not accepted by everyone[9], the lacunar hypothesis has gained general support, not least because the distinct clinical lacunar syndromes have proved useful in patient management[10].

However, it could be argued that this large body of clinical and epidemiological work has not actually advanced our understanding of what actually causes lacunar infarcts. As one eminent stroke physician wrote of lacune research[11], 'attempts to infer the underlying disease by the analysis of clinical risk factors...is at best an approximation of what would be learned by microscopy'. So what of recent pathology research? Perhaps unsurprisingly, there have been no further attempts at serial section analysis, widely held to be the gold standard technique for visualising small vessel anatomy, and there have been few novel technical approaches to visualising the small vessels supplying lacunes[12]. A few large-scale autopsy surveys of lacune brains have been undertaken[13,14], but these have merely confirmed the epidemiological and risk factor trends. A new pathological variant of lacune has been described which is characterised by partially or 'incompletely' infarcted brain tissue, the potential significance of which lies in the fact that it suggests subtotal or temporary stenosis/occlusion as likely causes of lacunar infarcts, for example temporary embolic small vessel occlusion or vessel spasm[15]. Clearly, pathology has contributed little to the debate in recent years, overshadowed perhaps by the promise of the new genetics.

The genetics of lacunar stroke

It is now clear from twin, family and population studies that there is a significant familial or genetic component underlying ischaemic stroke, and there is now increasing effort to identify and characterise the susceptibility genes[16,17]. It is attractive, for example, to speculate that 'cortical' and

'lacunar' stroke patients, who have similar risk factor profiles, suffer different types of stroke because of different genetic predispositions. Of the rare inherited Mendelian disorders with an increased stroke risk, CADASIL and familial variants of cerebral amyloid angiopathy are both associated with specific cerebral small vessel pathologies and cortical/subcortical microinfarcts[18], but neither is likely to contribute significantly to the overall prevalence of lacunar strokes. Gene polymorphism association studies have, however, shown a weak but significant association of the DD genotype of the angiotensin converting enzyme (ACE) gene with ischaemic stroke in general[19]. Two studies have suggested an association between I/D polymorphism status and lacunar stroke in particular[20,21], in one of which there was also suggested to be an association of the GG genotype of the Glu298Asp endothelial nitric oxide synthase gene polymorphism with lacunes[17]. Such genetic studies promise much, but thus far have yielded marginally significant, negative or conflicting results, for reasons which are discussed below.

Obstacles to progress in lacune research

50 years after Fisher began his studies of lacunes, the bulk of clinical, radiological and epidemiological data would appear to support his conclusion that lacunes are caused predominantly by some form of intrinsic small vessel disease. However, we remain largely ignorant as to the underlying cause of the small vessel lesions he described, and so have been powerless to prevent or treat them, apart from managing their associated risk factors. The reasons for this lack of progress are several, and most are intractable.

For the pathologist, the low case fatality[1] means that only rarely does the pathogenetically informative acute small vessel lesion come to autopsy; when it does, it is usually late after the onset of stroke and so is organised and at least partially healed, its appearance perhaps reflecting as much a response to injury as its cause. Even when the fresh lesion does present itself, the technical difficulties inherent in tracing the lesion's vascular supply are formidable. The current climate of research funding would seem to preclude further serial section analysis in a sufficiently large number of informative cases. As discussed, risk factor analysis is unlikely to provide further insight and current methods of imaging small intracerebral vessels during life, lack the necessary resolution. To these problems should be added the limitations inherent in a traditional descriptive pathology approach – the classical descriptions of lipohyalinosis, important though they were, have not of themselves shed light on the underlying cellular or molecular mechanisms. Further, our understanding of the potential contribution of intracranial atheroma is limited, is largely derived from

somewhat crude radiological data, and lacks the detailed pathology study which has proved so informative in coronary artery and more recently carotid artery research[22]. A further problem is the clinical and pathogenetic heterogeneity of subcortical, as well as cortical, brain infarction[23]. For example, small infarcts in the centrum ovale may be manifestations of cardiac or large artery disease more commonly than striatocapsular lesions[24]

Faced with these problems, there has not been a satisfactory animal model of lacunar infarction to which the experimental pathologist can turn[25]; most animal stroke models have been used to study the pathophysiology and salvage of ischaemic brain tissue. The stroke-prone spontaneously hypertensive rat (SHRSP) does suffer microinfarcts and haemorrhages, as well as fibrinoid small vessel lesions, and may yet prove to be a useful model of small vessel stroke. However, the SHRSP suffers these lesions only in association with blood pressure levels not commonly encountered in modern clinical practice[25], and in this context may be regarded as a model of malignant hypertension.

Finally, the early genetic studies of human lacunar and other types of stroke have had major limitations which are becoming familiar in other complex or multifactorial diseases[26]. Many studies have had extremely small sample sizes, a lack of adequate numbers of genetically matched controls, an absence of relevant biological data or even of a convincing biological rationale ('fishing'). They too of course have suffered from the difficulties inherent in accurate clinical identification of pathological stroke subtypes.

Primary intracerebral haemorrhage – a related problem

Problems have also beset researchers studying the pathogenesis of PICH. Some of these reflect the same inaccessibility of the culprit intracerebral vessels which has frustrated lacune research, although others are unique.

Apart from those rare cases in which PICH is presumed to follow rupture of structurally normal vessels, usually in association with acute rises in blood pressure or blood flow[27], it is reasonable to assume that rupture of an intracerebral vessel is a consequence of focal vessel wall pathology. As Caplan has pointed out, the so-called Charcot-Bouchard microaneurysm has never been clearly identified as the definitive cause of even a single haematoma[27], and its very existence has now been questioned by elegant histochemical studies suggesting it was an illusion of injection studies produced by complex arteriolar coils and perivascular clots[28]. The consensus view at present is perhaps that the same small vessel lesion observed by Fisher in relation to lacunar infarcts is responsible for the majority of PICH[29,30], a lesion in its acute form characterised by

fibrinoid vessel wall necrosis[31]. PICH and lacunar infarction appear to co-localise, often co-exist in the same brain at autopsy, and show broad similarities in risk factor profile[29]. However, why the same or a similar vessel lesion should in some cases lead to a small infarct and in others to an intracerebral haemorrhage is unclear. There is clearly potential for a complex interplay of acute haemodynamic and acquired structural vessel wall changes in the pathogenesis of these stroke subtypes.

Study into the cause of PICH by traditional, observational clinical and autopsy studies have been beset by now familiar difficulties. Intracerebral haemorrhages are a heterogenous stroke type, and it is often difficult to distinguish clinically those cases due, for example, to haemorrhagic transformation of infarcts, haemorrhagic venous infarction, cryptic vascular malformations and cerebral amyloid angiopathy[32]. Clinical series of PICH are, therefore, likely to have included a diversity of patho-genetically unrelated entities. Although a relatively high proportion of severe PICH cases have come to autopsy shortly after the ictus, the problem of causal lesion localisation is the same as for lacunes, and is further complicated by the fact that the vessel lesion is destroyed or at least modified by the rupture itself and the consequent haemorrhage. Yet again, *in vitro* and *in vivo* models of PICH have been sought, but are used mainly to examine the clinical properties of clots and their response to drugs, or the effects of haemorrhage on surrounding brain[33]. There has, to date, been no satisfactory *in vivo* model that sheds light on the mechanism of human PICH.

Future prospects

It is clear from the above that traditional pathological lines of investigation have been pivotal in formulating our current ideas as to how small vessel-strokes occur. There is still much that such an approach can offer, in particular regarding the nature and consequences of cervicocranial atherosclerosis. Stroke researchers have been slow to apply the paradigm of plaque instability to the arteries supplying the brain. Thus, whilst it seems that thrombotic occlusion of the carotid artery usually occurs in a manner directly analogous to coronary artery thrombosis[22], very little is known of the relations between plaque stability, stenosis, plaque pro-gression, thrombosis and embolism. This is particularly true of intracranial vessels, despite the obvious implications for lacunar infarct pathogenesis.

The prospects of such traditional approaches clarifying the role of intracerebral small vessel disease in stroke are by comparison bleak. However, there should be some attempt to standardise pathological terminology in this field, particularly in relation to small vessel lesions. Thereafter, it is perhaps the emerging breed of experimental molecular

pathologist who has most to offer[34], ideally in the context of a multidisciplinary stroke research team. Questions which logically pose themselves are 'is there a specific intracerebral vessel lesion associated with lacunar infarction and PICH?' and then, 'is this lesion merely a stochastic event caused by the unfortunate concurrence of multiple risk factors or is it, to an extent at least, (genetically) predictable and hence maybe preventable?' Fisher's work, and the debate it provoked, have provided a provisional answer to the first question – a characteristic destructive cerebral vessel lesion characterised in its acute form by fibrinoid necrosis, does appear to be specifically associated with lacunar infarction, and albeit less convincingly, PICH. The answer to the second question is at one level straightforward – small vessel stroke will, like the vast majority of multi-factorial diseases, prove to be a combination of genetic and environmental risk factors. Advances in our understanding of the genetics of established stroke risk factors, such as hypertension, will complement the search for novel genes predisposing to stroke independent of such factors. Also, small vessel stroke researchers may now begin to take advantage of an improved understanding of the genetic basis of established animal models, such as the SHRSP, in which chromosomal regions have recently been identified containing blood pressure-independent genetic factors predisposing to stroke[16]. In addition, a variety of newer transgenic and gene knock-out animal models[35] have the potential now to model the specific small vessel lesions implicated by human autopsy research. Both approaches will be essential in focusing on biologically plausible candidate genes for association studies in human stroke, an increasingly important concern given the impending availability of the entire human genome sequence and its variability, as well as the development of comprehensive sets of single nucleotide polymorphisms spanning the human genome[36]. These human genetic studies will, of course, continue to require valid stroke subtyping. Finally, more, and more informative, stroke intermediate phenotypes need to be developed. This is partly because such phenotypes are likely to be influenced by a smaller number of genes than stroke itself, thereby improving the power of linkage and association studies, but also as they may provide insights into the mechanisms underlying postulated genetic associations[16]. A well-defined small vessel lesion, modelled in a genetically defined animal, would be an obvious intermediate phenotype which would integrate the fields of Virchowian pathology and molecular genetics in small vessel-related stroke research.

Acknowledgements

References

1   Bamford J, Sandercock P, Jones L, Warlow CP. The natural history of lacunar infarction: the Oxfordshire Community Stroke Project. *Stroke* 1987; 18: 545–51

2   Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. A prospective study of acute cerebrovascular disease in the community The Oxfordshire Community Stroke Project 1981–1986; II: incidence, case fatality rates and overall outcome at one year of cerebral infarction, primary intracerebral and subarachnoid haemorrhage. *J Neurol Neurosurg Psychiatry* 1990; 53: 16–22

3   Poirier J, Derouesre C. Le concept de lacune cerebrale de 1838 a nos jours. *Rev Neurol (Paris)* 1985; 141: 3–17

4   Poirier J, Derouesne C Cerebral lacunae. A proposed new classification *Clin Neuropathol* 1989; 3: 266

5   Fisher CM. Lacunar infarcts – a review. *Cerebrovasc Dis* 1991; 1: 311–20

6   Gutstein WH. Vasospasm, vascular injury and atherogenesis: a perspective. *Hum Pathol* 1999; 30 365–71

7   Lodder J, Bamford JM, Sandercock PAG, Jones LN, Warlow CP. Are hypertension or cardiac embolism likely causes of lacunar infarction? *Stroke* 1990; 21: 375–81

8   Donnan GA Lacunes and lacunar syndromes In: Ginsberg MD, Bogousslavsky J. (eds) *Cerebrovascular Disease Pathophysiology, Diagnosis and Treatment*. Malden, MA Blackwell Science, 1998, 1090–102

9   Millikan C, Futrell N. The fallacy of the lacune hypothesis. *Stroke* 1990; 21: 1251–7

10 Bamford JM, Warlow CP. Evolution and testing of the lacunar hypothesis. *Stroke* 1988; 19: 1074–82

11 Mohr JP. The pathogenesis of single penetrator infarcts. In. Donnan G, Norrving B, Bamford J, Bogousslavsky J. (eds) *Lacunar and other Subcortical Infarctions*. Oxford, OUP, 1995; 29–31

12 Challa VR, Bell MA, Moody DM. A combined haematoxylin-eosin, alkaline phosphatase and high resolution microradiographic study of lacunes. *Clin Neuropathol* 1990; 9: 196–204

13 Tuszynski MH, Petito CK, Levy DE. Risk factors and clinical manifestations of pathologically verified lacunar infarctions *Stroke* 1989, 20. 990–9

14 Dozono K, Ishii N, Nishihara Y, Horie A An autopsy study of the incidence of lacunes in relation to age, hypertension and arteriosclerosis. *Stroke* 1991; 22: 993–6

15 Lammie GA, Brannan F, Wardlaw JM. Incomplete lacunar infarction (type 1b lacunes). *Acta Neuropathol* 1998; 96: 163–71

16 Boerwinkle E, Doris PA, Fornage M. Field of needs. The genetics of stroke. *Circulation* 1999, 99: 331–3

17 Elbaz A, Amarenco P Genetic susceptibility and ischaemic stroke *Curr Opin Neurol* 1999; 12: 47–55

18 Pullicino P, Greenberg S, Trevisan M. Genetic stroke risk factors *Curr Opin Neurol* 1997; 10: 58–63

19 Sharma P. Meta-analysis of the ACE gene in ischaemic stroke *J Neurol Neurosurg Psychiatry* 1998; 64: 227–30

20 Elbaz A, Mallet C, Cambien F, Amarenco P. Association between the ACE 4656 (CT) 2/3 polymorphism and plasma ACE level with lacunar stroke in the GENIC study. *Cerebrovasc Dis* 1998, 8 (Suppl 4). 44

21 Markus HS, Barley J, Lunt R *et al*. Angiotensin converting enzyme gene deletion polymorphism: a new risk factor for lacunar stroke but not carotid atheroma. *Stroke* 1995; 26: 1329–33

22 Lammie GA, Sandercock PAG, Dennis MS Recently occluded intracranial and extracranial carotid arteries. The relevance of the unstable atherosclerotic plaque. *Stroke* 1999; 30. 1319–25

23 Bogousslavsky J. The plurality of subcortical infarction. *Stroke* 1992; 23: 629–31

24 Lammie GA, Wardlaw JM Small centrum ovale infarcts – a pathological study. *Cerebrovasc Dis* 1999; 9: 82–90

25 Ginsberg MD, Busto R Small-animal models of global and focal cerebral ischaemia. In: Ginsberg MD, Bogousslavsky J. (eds) *Cerebrovascular Disease Pathophysiology, Diagnosis and Management*. Malden, MA: Blackwell Science, 1998; 14–35

26 Todd JA Interpretation of results from genetic studies of multifactorial diseases. *Lancet* 1999; 354 (suppl 1): 15–6

27 Caplan LR Hypertensive intracerebral haemorrhage. In: Kase CS, Caplan LR. (eds) *Intracerebral Hemorrhage.* Boston, MA Butterworth-Heinemann, 1994; 99–116

28 Challa VL, Moody DM, Bell MA. The Charcot-Bouchard aneurysm controversy impact of a new histologic technique. *J Neuropathol Exp Neurol* 1992; 51: 264–71

29 Norrving B. Cerebral hemorrhage. In: Ginsberg MD, Bogousslavsky J (eds) *Cerebrovascular Disease. Pathophysiology, Diagnosis and Management.* Malden, MA: Blackwell Science, 1998; 1447–73

30 Fisher CM. Pathological observations in hypertensive cerebral hemorrhage. *J Neuropathol Exp Neurol* 1971; 30: 536–50

31 Rosenblum WI The importance of fibrinoid necrosis as the cause of cerebral hemorrhage in hypertension *J Neuropathol Exp Neurol* 1993; 52: 11–3

32 Garcia JH, Ho K-L, Caccamo DV. Intracerebral hemorrhage: pathology of selected topics. In: Kase CS, Caplan LR (eds) *Intracerebral Hemorrhage.* Boston, MA: Butterworth-Heinemann, 1994; 45–72

33 Kaufman HH, Schochet SS. Pathology, pathophysiology and modeling. In· Kaufman HH. (ed) *Intracerebral Haematomas.* New York: Raven, 1992; 16–21

34 Quirke P, Mapstone N. The new biology: histopathology. *Lancet* 1999, 354 (suppl 1)· 26–31

35 Chan PH. The role of transgenic animals in cerebral ischemia. In: Ginsberg MD, Bogousslavsky J. (eds) *Cerebrovascular Disease. Pathophysiology, Diagnosis and Management.* Malden, MA: Blackwell Science, 1998; 481–8

36 Collins FS, Guyer MS, Chakravarti A Variations on a theme: cataloging human DNA sequence variation. *Science* 1997, 278: 1580–1