Blood brain barrier, fluid flow, oedema, immune responses

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3 anatomical compartments

- Cerebrospinal fluid
- Meninges
- Parenchyma of brain and spinal cord

3 blood-brain barriers

- Endothelial BBB in CNS parenchymal blood vessels
- Blood-leptomeningeal barrier in microvasculature on surface of brain and spinal cord
- Epithelial Blood-CSF-brain barrier in choroid plexus

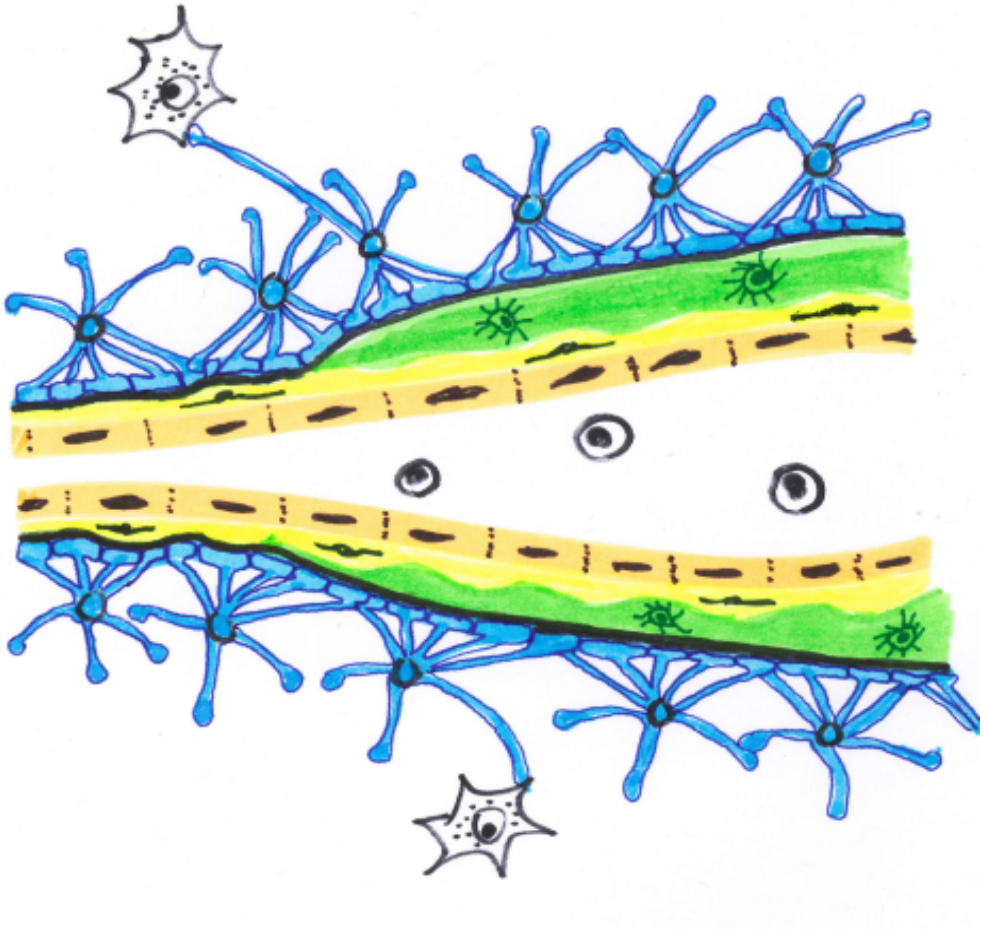
- BBB **lacking** in circumventricular organs

Blood-brain barrier – why?

Protection of CNS

- CNS homeostasis necessary to maintain precisely regulated EC fluid milieu
  - required to provide an optimal chemical environment for electrical transmission
- Inflammation potential to cause injury
  - Neurones (almost all) are end-stage cells
  - Significant adverse effects of brain swelling as encased in bony calvarium

Blood-brain barrier



astrocyte foot processes

parenchymal basement membrane (BM)

perivascular space (post capillary venule) between parenchymal and endothelial BMs draining into CSF containing antigen presenting cells

vascular lumen

endothelium

endothelial BM containing embedded pericytes (merges with parenchymal BM at level of capillaries)

Blood-brain barrier – characteristics

- Endothelium
  - low pinocytotic activity, lack of fenestrations and tight and adherens junctions tightly restrict paracellular and transcellular passage of hydrophilic molecules into the CNS
  - sophisticated transport mechanisms ensure transport of nutrients from blood into CNS and rapid extrusion of toxic metabolites out of CNS
- Endothelial basement membrane
  - laminins α4 and α5, contains pericytes, fused with glial BM at level of capillaries
- Glial basement membrane
  - laminins α1 and α2, fibronectin and type IV collagen
- Astrocyte foot processes
  - linked to BM by transmembrane receptor dystroglycan

Blood-CSF barrier – characteristics

- Choroid plexus
  - Secretes CSF
  - Villous structures composed of fenestrated capillaries – free diffusion of solutes between blood and CP parenchyma
  - Overlain by cuboidal epithelium with unique parallel tight junctions
  - Polarised expression of specific transmembrane transport systems
  - Kolmer (epiplexus) cells – tight association with apical aspect, probably APCs

- Leptomeninges
  - meningeal bvs – differences *cf* parenchymal BBB *eg* contain adhesion molecules such as P-selectin in Weibel Palade bodies
  - APCs – macrophages
  - glia limitans superficialis – surrounds entire surface of brain and spinal cord

CNS – 'immunologically priviledged'

- Blood / CSF – brain barrier
  - limits access of soluble factors and immune cells to CNS
- Low constitutive activation of MHC class I and II molecules
  - microglia and lesser extent astrocytes
- Local production of suppressive factors
  - *eg* interaction between normal physiologically active neurones expressing CD200 receptor exerts quiescent effect on microglia expressing its ligand
- Limited numbers of APCs
  - Dendritic cells appear to be absent from brain parenchyma
- Lacks a substantial lymphocyte population
- However, the CNS can mount effective innate and adaptive immune responses

CNS – innate immune capability

- Pattern recognition receptors for pathogen-associated molecular patterns (PAMPs) in response to CNS infection and for danger-associated molecular patterns (DAMPs) in response to CNS injury and release of altered tissue constituents
  - on microglia and lesser extent on astrocytes
- Activate members of *eg* Toll-like receptor (TLR) family *eg*
  - TLR1 ligands include Mycobacteria
  - TLR2 ligands include peptidoglycans eg Streptococci, *Listeria monocytogenes* ; HSP70 (host)
  - TLR4 ligands include LPS – gram negative bacteria eg *E coli;* fibronectin, fibrinogen (host)
  - Evidence that neurones also express TLR4 and can mediate response to LPS
- Leads to rapid recruitment of neutrophils, macrophages and lymphocytes (natural killer cells)

CNS – immune surveillance

- Activated T cells are able to cross the B-B/CSF barrier to perform immune surveillance in the CSF spaces (perivascular, leptomeningeal and ventricular spaces)
- Occurs more quickly (and predominantly?) in leptomeninges (entering CSF space) and in choroid plexus (entering CP parenchyma, i.e. not in CSF space), compared with microvasculature
- Extravasation into CSF spaces involves 3 main stages
  - Upregulation of adhesion molecules on leptomeningeal endothelium and T cell arrest
  - T cell crawling preferentially against direction of blood flow to find a site permissive for extravasation, may be long distance
  - Diapedesis across endothelial barrier preferentially through endothelial cytoplasm rather than paracellular route
- Leptomeningeal macrophages produce matrix metalloproteinases (MMP2, MMP9) cleave dystroglycan from astrocyte endfeet allowing T cells to penetrate glia limitans into CNS parenchyma

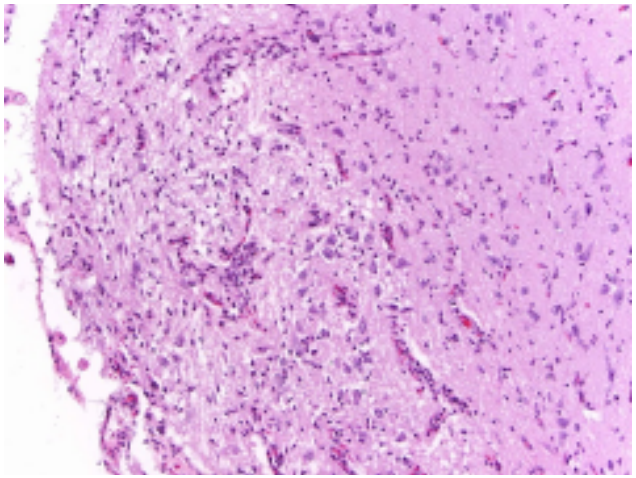
Immune response – morphology

- Accumulation of leucocytes in perivascular spaces ('cuffing') is the hallmark of CNS inflammatory conditions
- Leptomeningeal infiltrate is common (vasculature more permissive, at least in early stages)
- However, induction of clinical signs (EAE model) occurs only once the immune cells penetrate into CNS parenchyma
- Nature of cellular infiltration depends on nature of pathogen / insult, host factors etc. B cell infiltration produces intrathecal antibody response
- Parenchymal (gliovascular) responses
  - Diffuse and nodular proliferation of glia
  - 'Glial' nodules consist of mixture of microglia / macrophages, astrocytes and infiltrating leucocytes, occurring particularly in protozoal and viral infections
  - Vascular activation – largely endothelial hypertrophy resulting in increased prominence. Unclear how much proliferation / sprouting contributes – it is worth noting that the inter-capillary distance in brain is about 40 μm, which is space for 2 neurons: in effect, in most areas of the brain, each neuron is perfused by its own blood vessel.

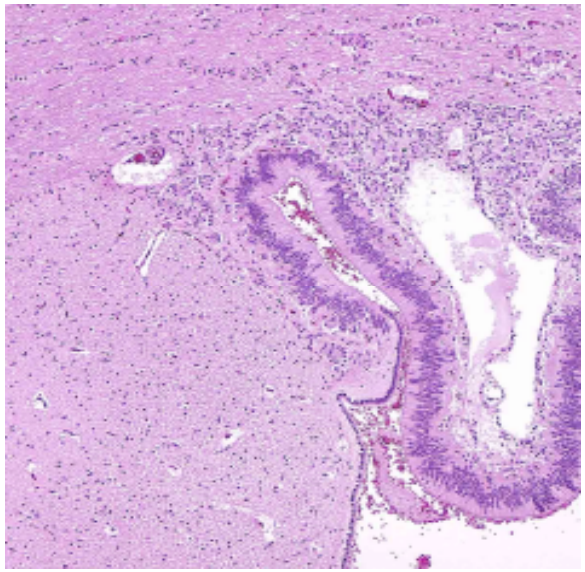
Circumventricular organs

Brain regions usually without BBB characteristics that are responsible for sensing the internal milieu of the body

- serum osmolarity
  - subfornical organ, area postrema of the brain stem
- involved in either sensing hormone levels or releasing hormonal factors into the circulation
  - pineal gland, median eminence, neurohypophysis
- subcommisural organ
  - does not have fenestrated capillaries therefore not a CVO?



Area postrema on lateral aspect fourth ventricle at level of obex. Note highly vascularised and contains neuronal cell bodies

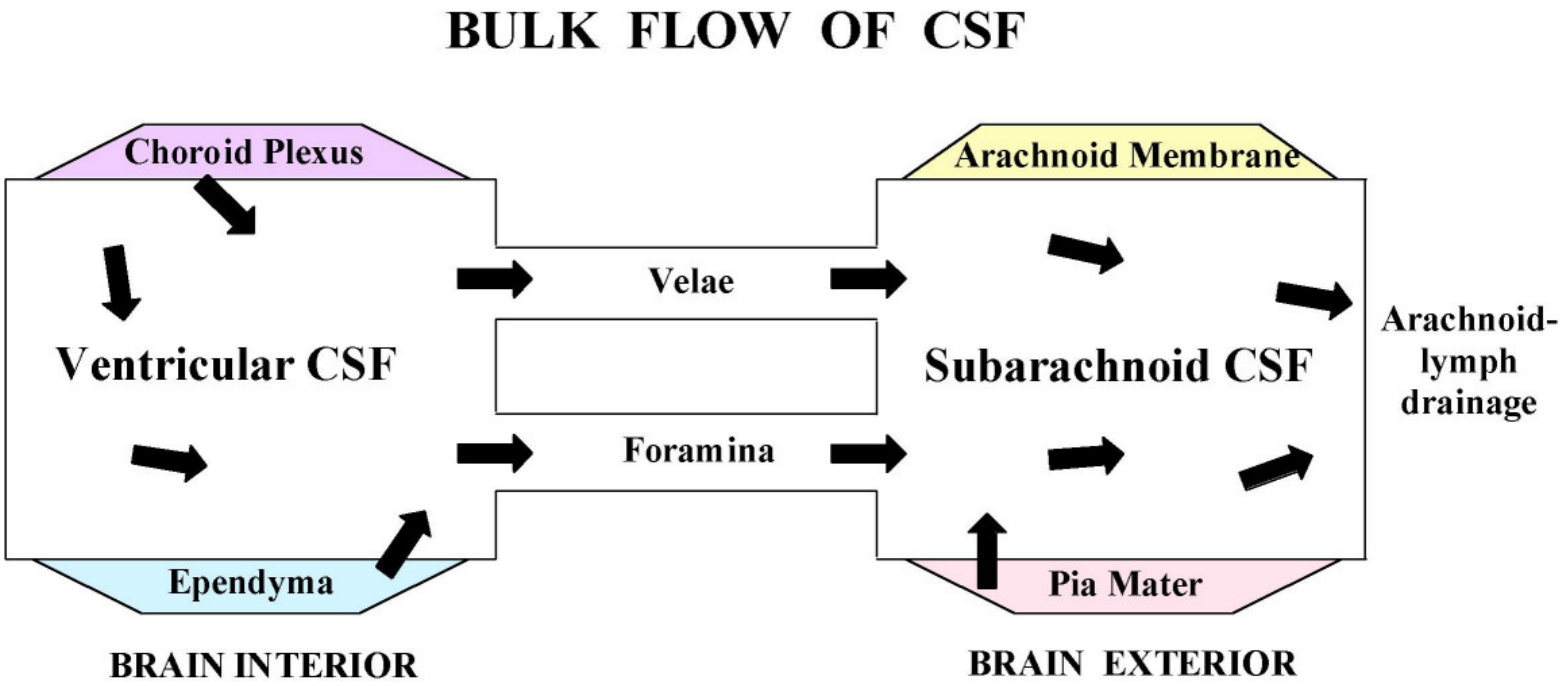


Subcommisural organ on dorsal aspect third ventricle

Fluid flow in the CNS

CNS fluid flow

- Most (~66%) cerebrospinal fluid produced in choroid plexus, rest by ependymal lining and capillary-astrocyte complex
- Ependyma cilia function important for CSF flow
- CSF acts as a sink for brain extracellular proteins: *in vivo* two photon imaging reveals that a substantial portion of CSF cycles through the interstitial space
- CSF enters the parenchyma along paravascular spaces that surround penetrating arteries and brain interstitial fluid is cleared along paravenous drainage pathways; there is evidence that the bulk fluid flow between these influx and efflux sites is supported by astrocytic water transport (animals lacking AQP4 have reduced efflux)
- Drainage / reabsorption of CSF via the olfactory and optic nerves, cribriform plate, nasal submucosa and cervical lymphatics now regarded as the primary bulk flow routes of CSF egress from the brains of pigs and sheep
- Prevailing view is that CSF flow into nasal lymph is the major route of CSF outflow, rather than via arachnoid granulations into venous system, in sheep and other species

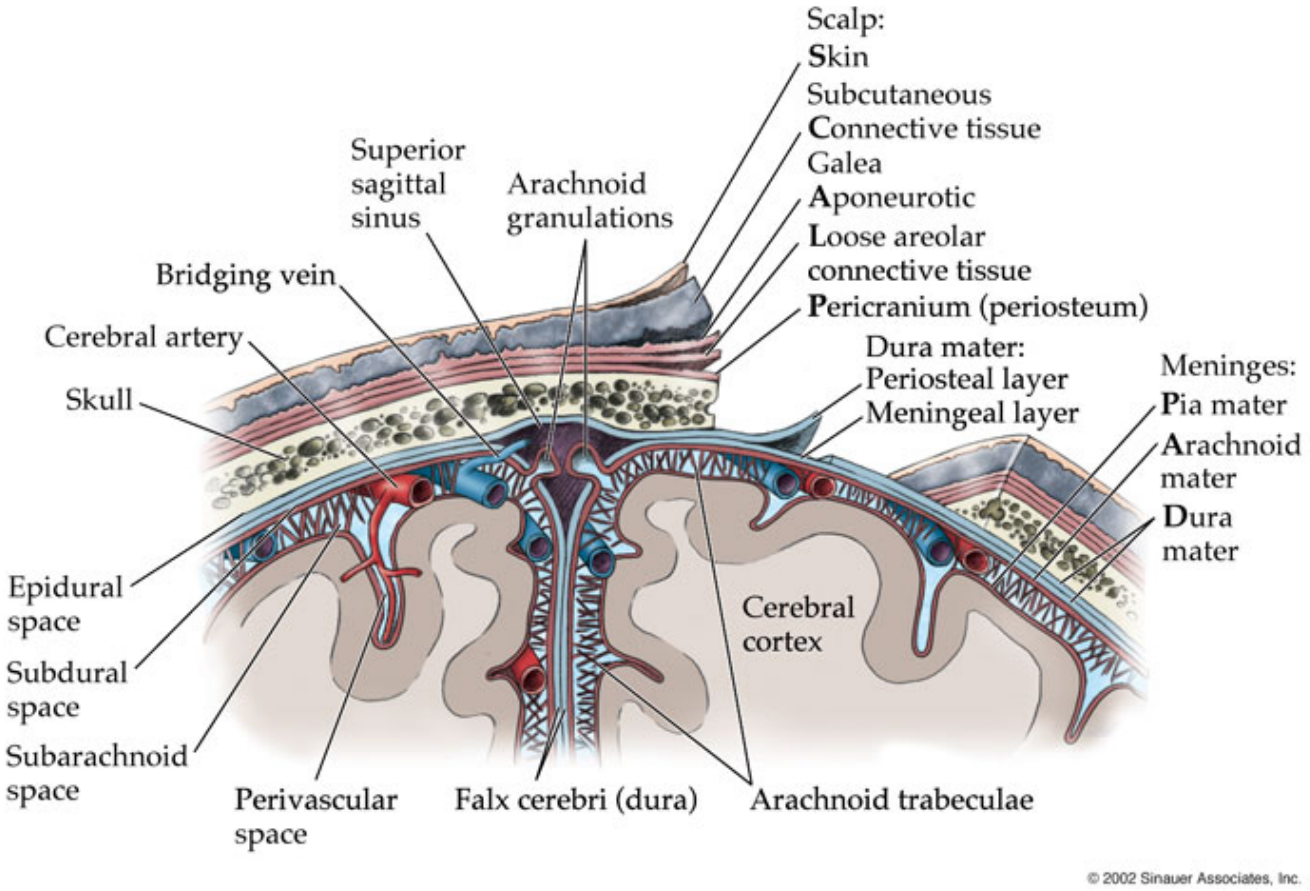


Large cavity CSF compartments and fluid flow:

Extracellular fluid enables volume transmission (convection) of fluid from ventricles to subarachnoid space (SAS), CSF formed by lateral, 3rd and 4th ventricles choroid plexus flows from the lateral to 3rd ventricle via the cerebral aqueduct and 4th ventricle to SAS in cisterna magna. Then CSF is transmitted by bulk flow through cisternal foramina into basal cisterns. CSF is also convected from ventricles through velae channels to the quadrigeminal and ambient cisterns, into subarachnoid extensions of the velum interpositum (from dorsal 3rd ventricle) and superior medullary velum (rostral 4th ventricle). Thereafter fluid is convected to the SAS of the spinal cord and brain convexities. As CSF flows through the ventriculo-subarachnoid system, there are diffusional and bulk flow exchanges between CSF and brain, depending on region-specific gradients for concentration and hydrostatic pressure hat promote widespread distribution of CSF-borne materials. Normally CSF is readily distributed from the ventricles to arachnoidal drainage sites.

Johanson *et al. Cerebrospinal Fluid Research* 2008 **5**:10

Meninges



http://missinglink.ucsf.edu/lm/ids_104_cns_injury/Response%20_to_Injury/Meninges.htm

CNS oedema

Cerebral (CNS) oedema

- Definition of cerebral oedema: increase in brain tissue volume due to an increase in its water content (Greenfield's neuropathology, 6th ed)

- Classification of CNS oedema
  - **Cytotoxic** : impairment of osmoregulation
  - **Vasogenic** : blood-brain barrier breakdown
  - Hydrostatic : rapid decompression in severe intracranial hypertension
  - Interstitial (hydrocephalus) : acutely elevated CSF pressure
  - Hypo-osmotic

Cytotoxic oedema

- Intracellular accumulation of fluid
- Impaired ionic homeostasis (Na-K ATPase membrane pump, ATP)
    - Ischaemia
    - Thiamine deficiency
- Usually more conspicuous in grey matter
    - Astrocytes
        - Grey matter: cytoplasmic and nuclear swelling, may progress to astrogliosis
        - White matter: nucleus swollen and pale, cytoplasm visible and swollen

Vasogenic oedema

- Extracellular accumulation of fluid
- Consequence of injury to vascular endothelium to allow leakage of plasma constituents
  - Inflammation
  - Trauma
  - Haemorrhagic lesions
  - Intoxications eg *Clostridium perfringens* epsilon
- Usually more conspicuous in white matter [depending on cause] – less resistance to fluid flow?
  - Astrocytes
    - Grey matter: cytoplasmic and nuclear swelling, may progress to astrogliosis
    - White matter: nucleus swollen and pale, cytoplasm visible and swollen
  - White matter: rarefaction of myelin
    - spaces empty / proteinaceous fluid / hyaline droplets

Cerebral (CNS) oedema

Diagnosis

- MRI
- Macroscopic evidence of brain swelling
- Microscopic

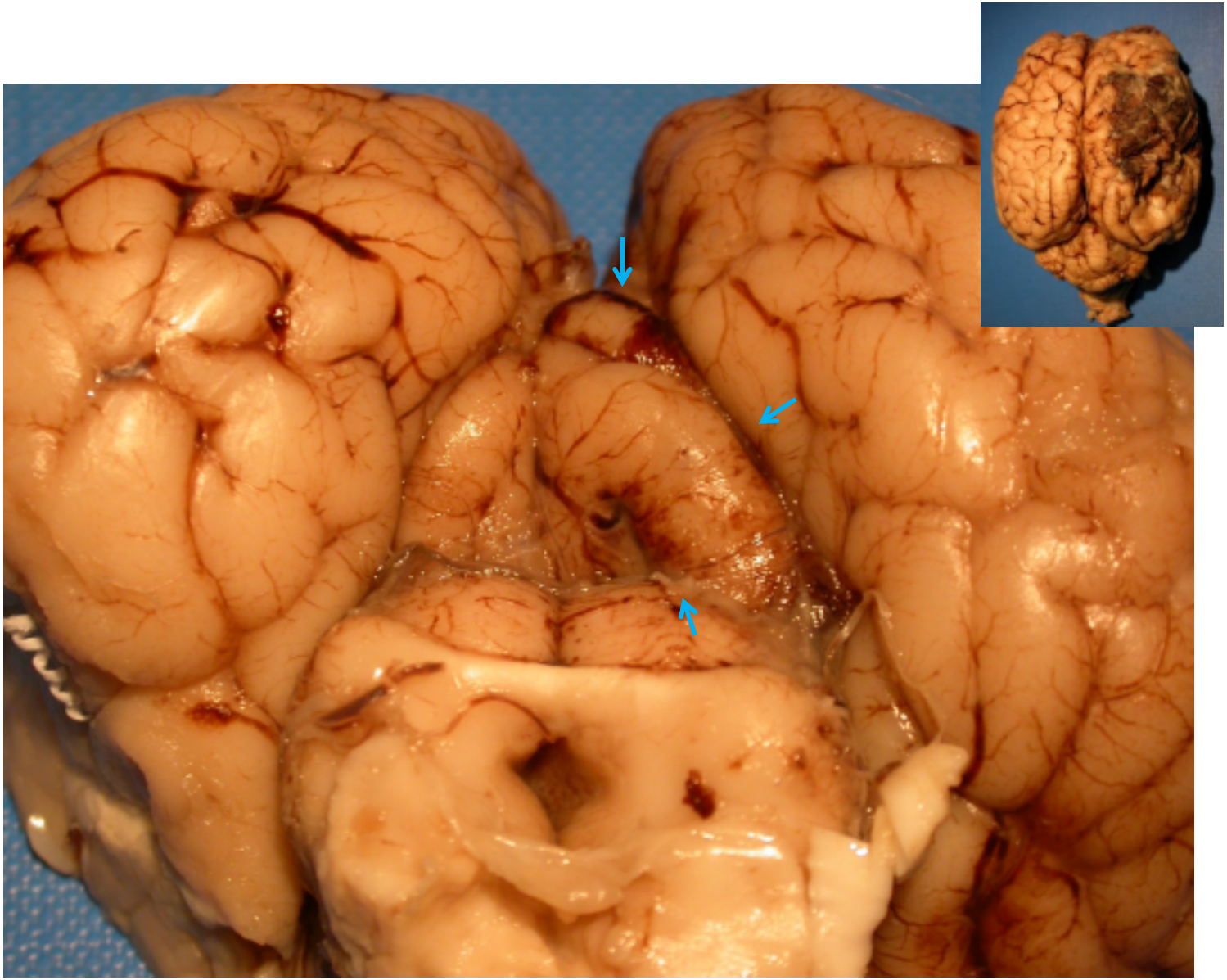
Cerebral (CNS) oedema

Macroscopic evidence of brain swelling

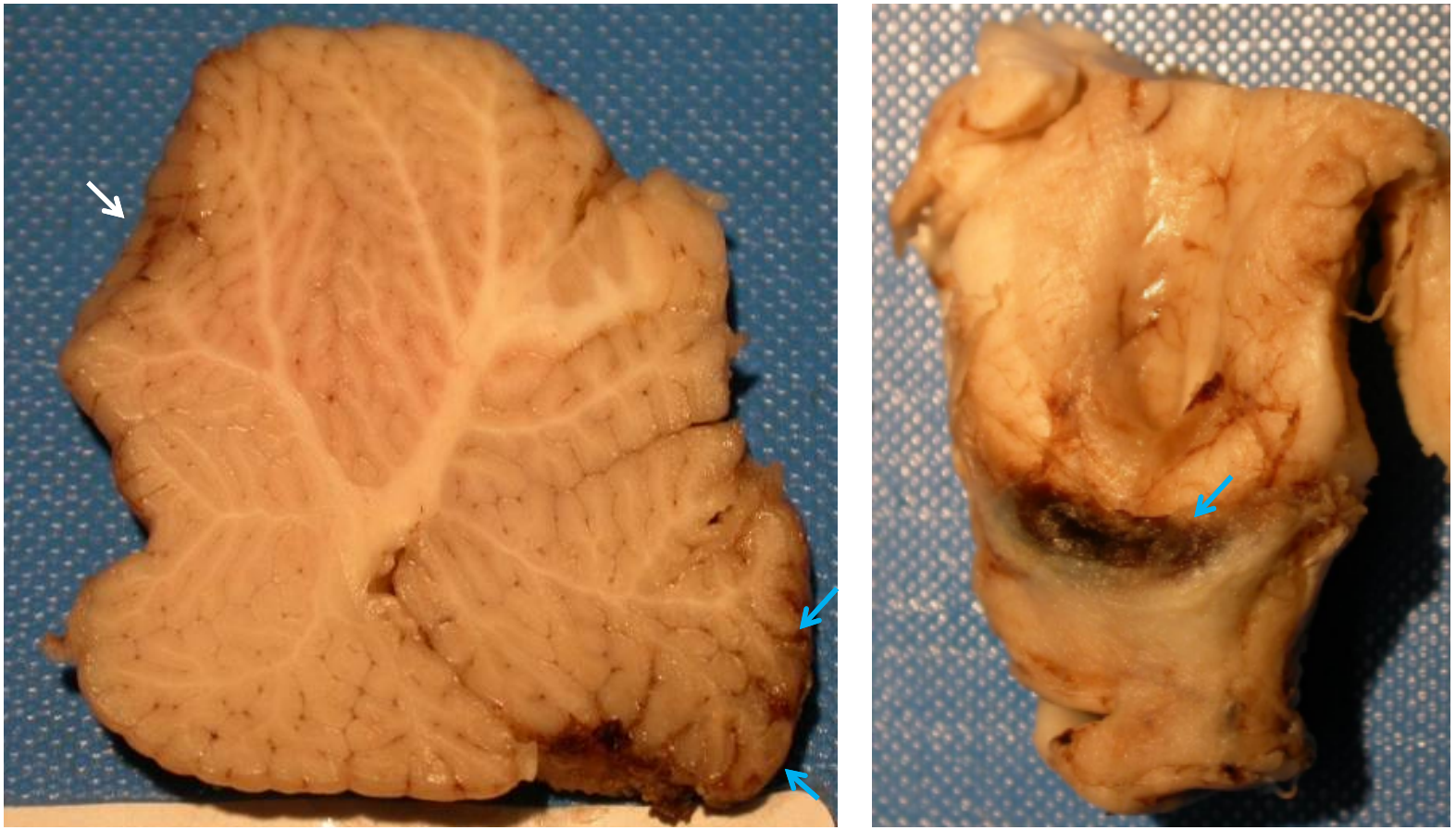
- Cerebellar coning
- Tentorial herniation of occipital cerebrum
- Flattening of cerebral gyri

CF post mortem change

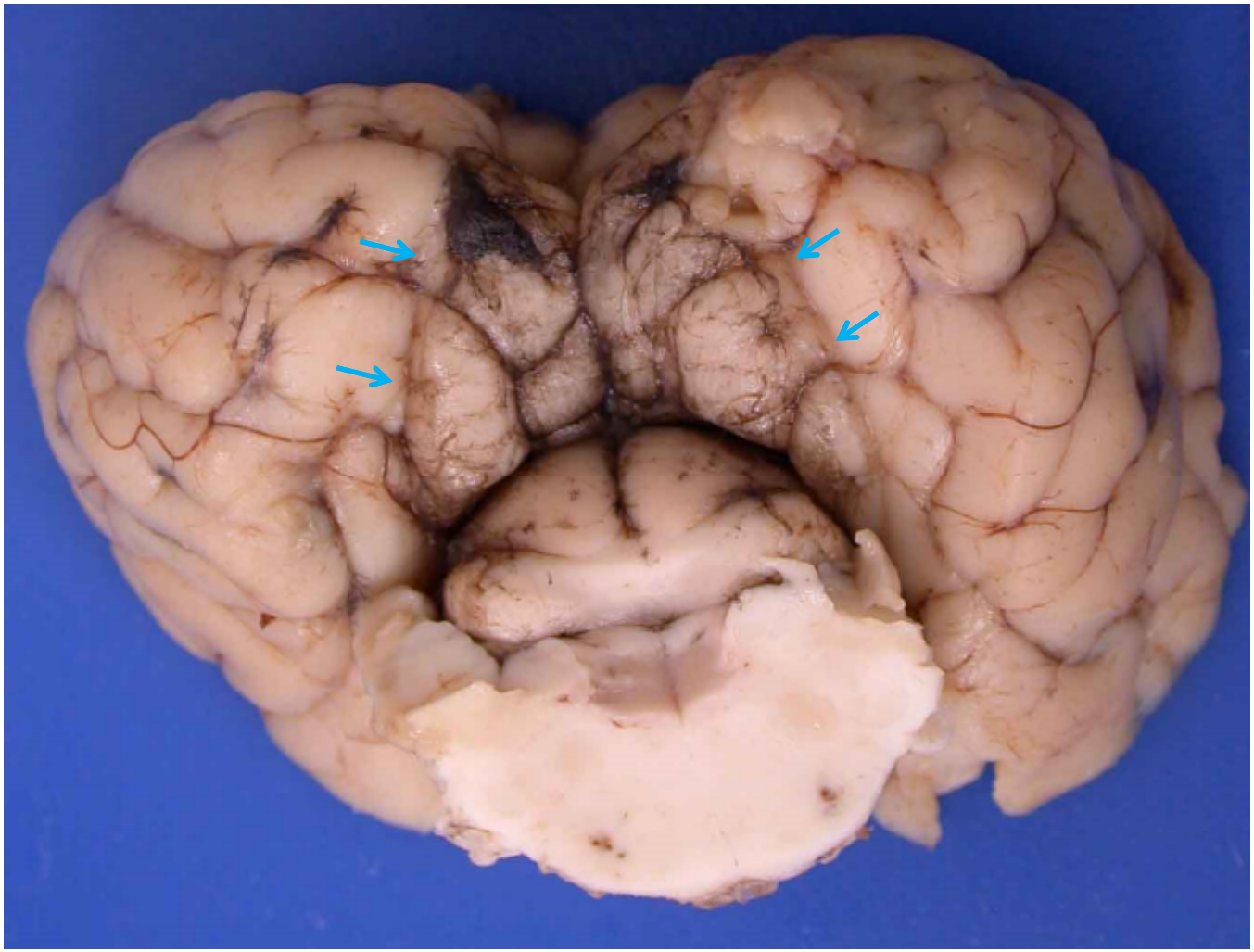
- Tentorial imprint on occipital cerebrum
- ‘Flattening’ of cerebral gyri



Disbudding injury, calf, right cerebral hemisphere. Note congestion and haemorrhage in herniated portion of cortex (arrows)

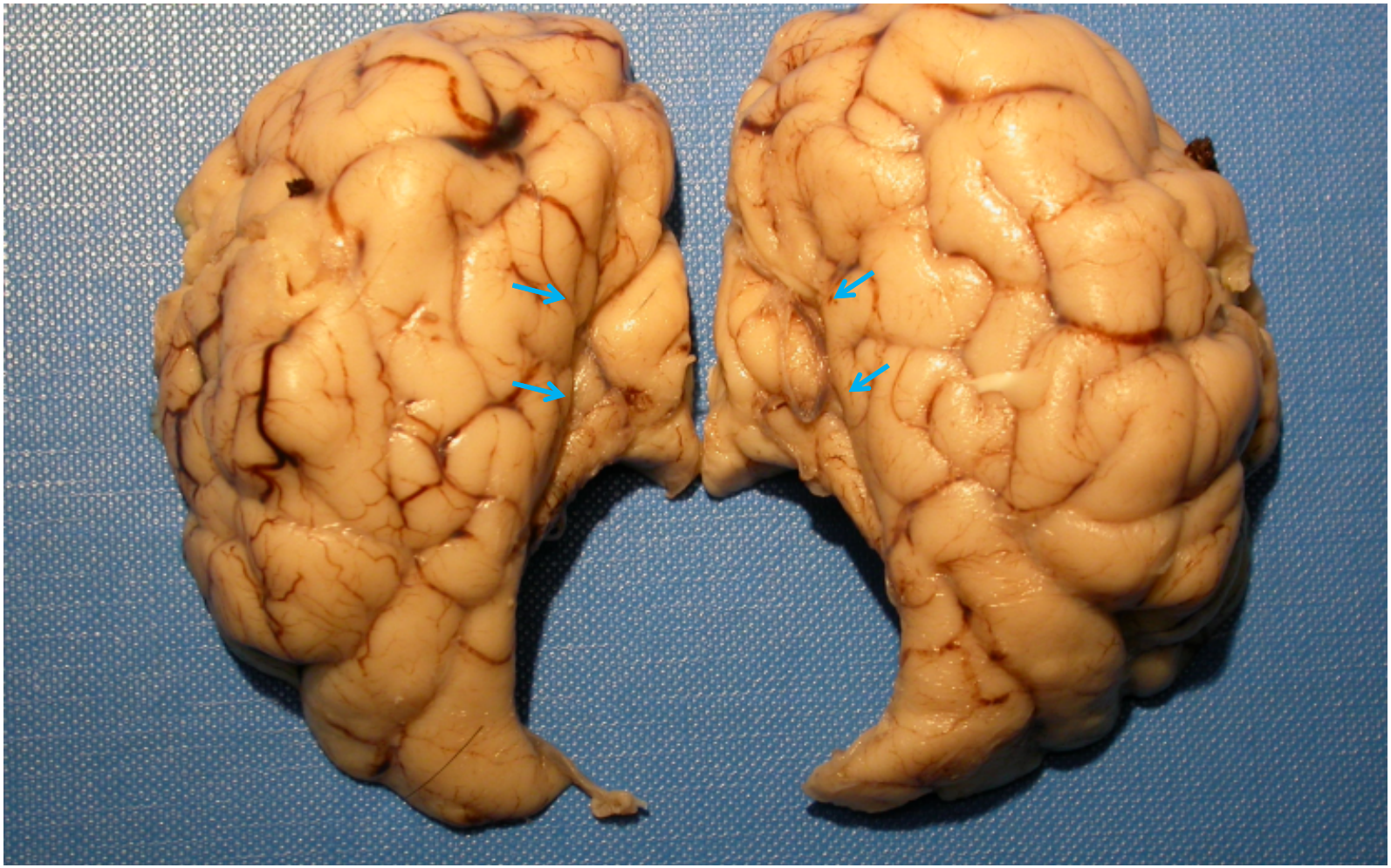


Disbudding injury, calf. Note congestion in herniated portion of cerebellum and immediately caudal to obex (blue arrows) and distortion of rostral cerebellar vermis by herniated cerebral cortex (white arrow)



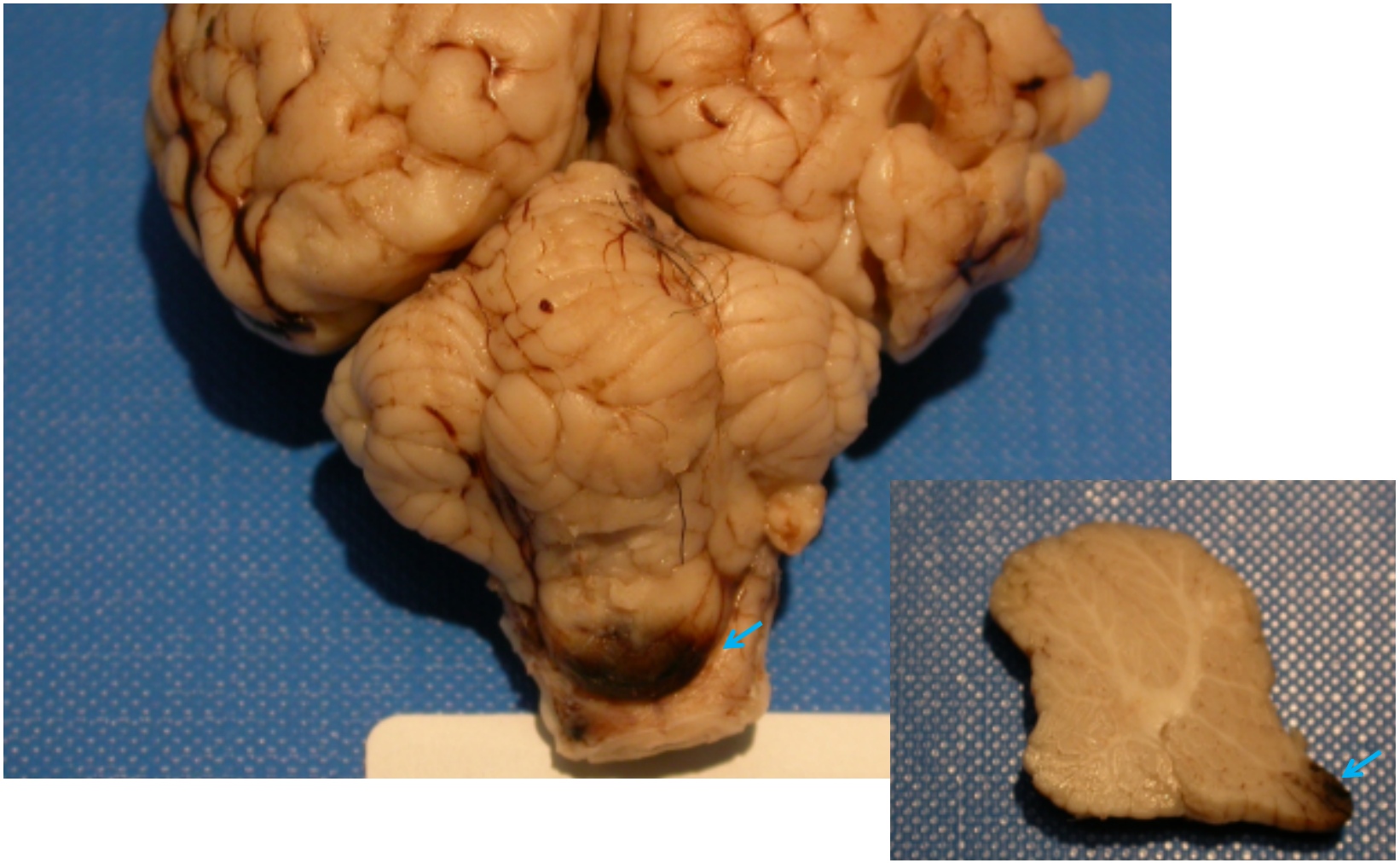
Brain swelling, 18 months male alpaca.

Note tentorial notch (arrows) on occipital cortex and leptomeningeal haemorrhages and congestion of herniated part of cerebral cortex



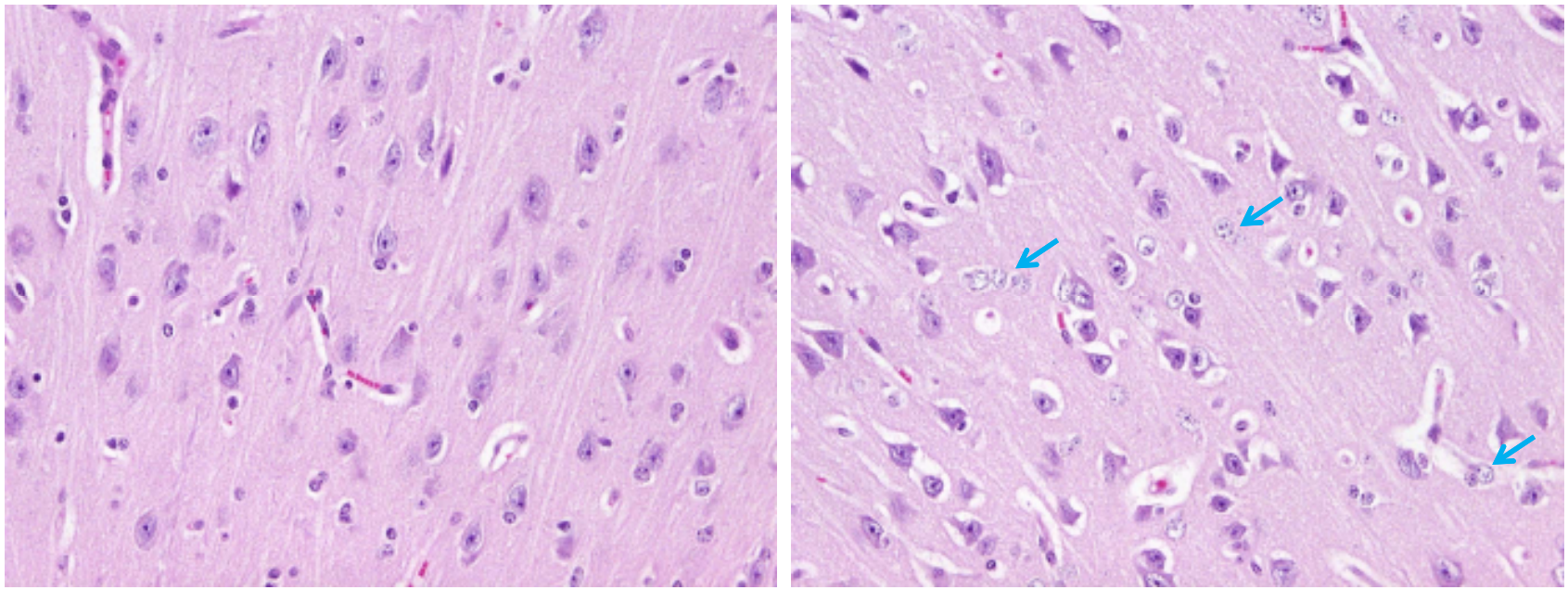
Tentorial herniation cerebral cortex (arrows) bovine

Type D enterotoxaemia in sheep – cerebellar coning



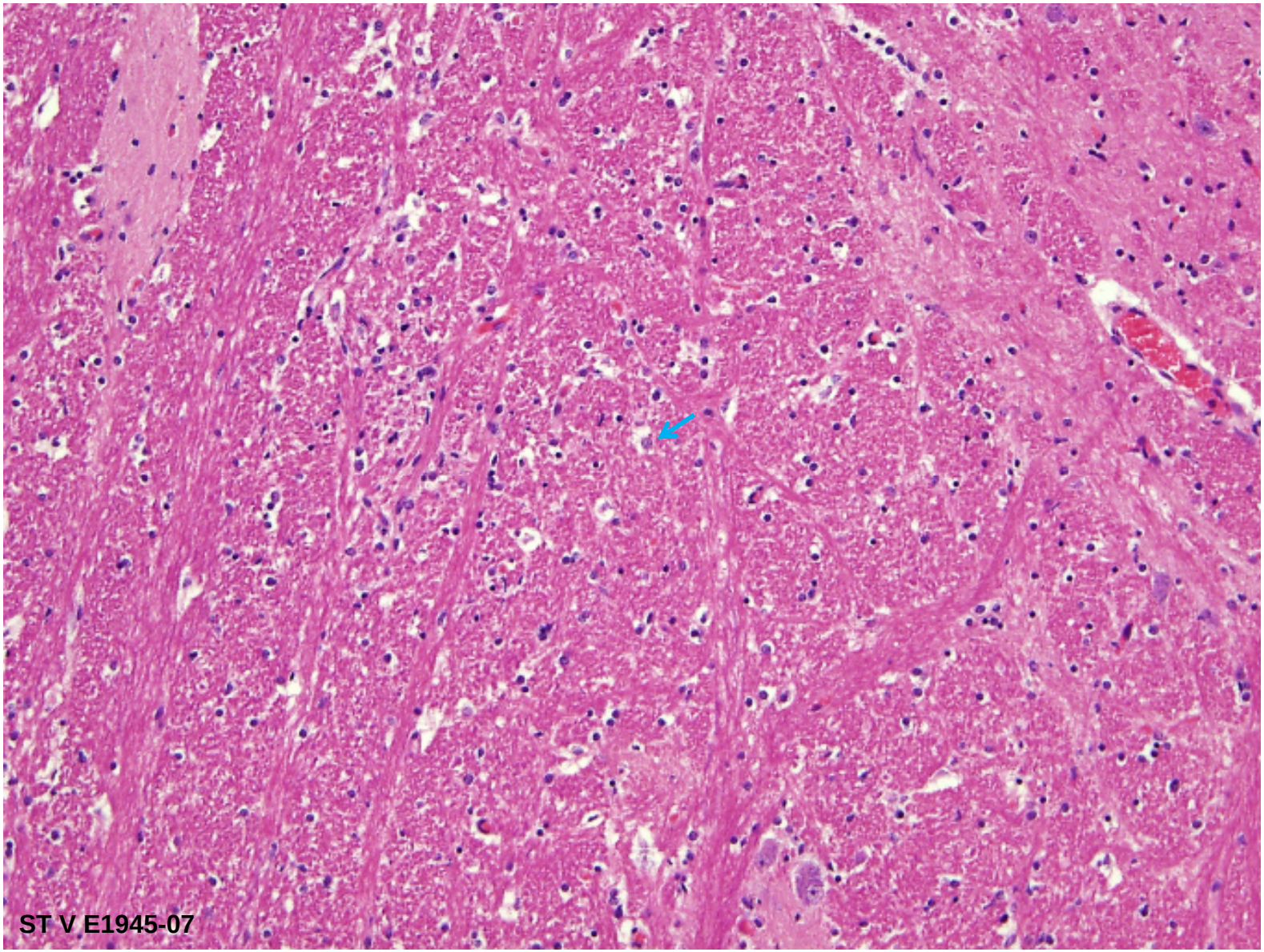
Cerebral (CNS) oedema

- Microscopic



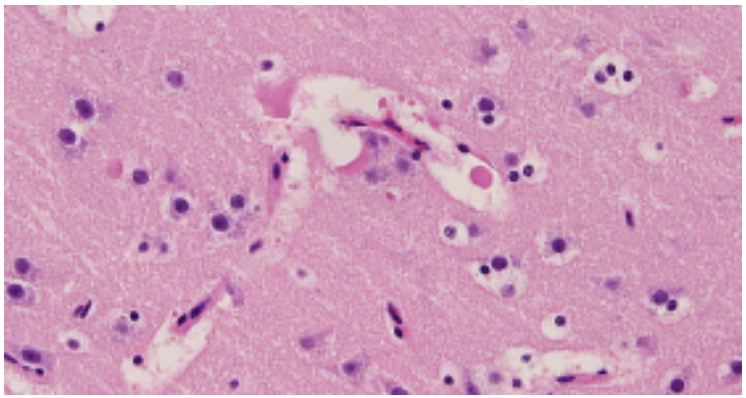
Thiamine dependent encephalopathy, sheep: note marked astrocyte nuclear swelling (arrows) and neuropil porosity in area immediately adjacent to area of cortical necrosis (right) compared with unaffected cortex from the same region, same animal (left).

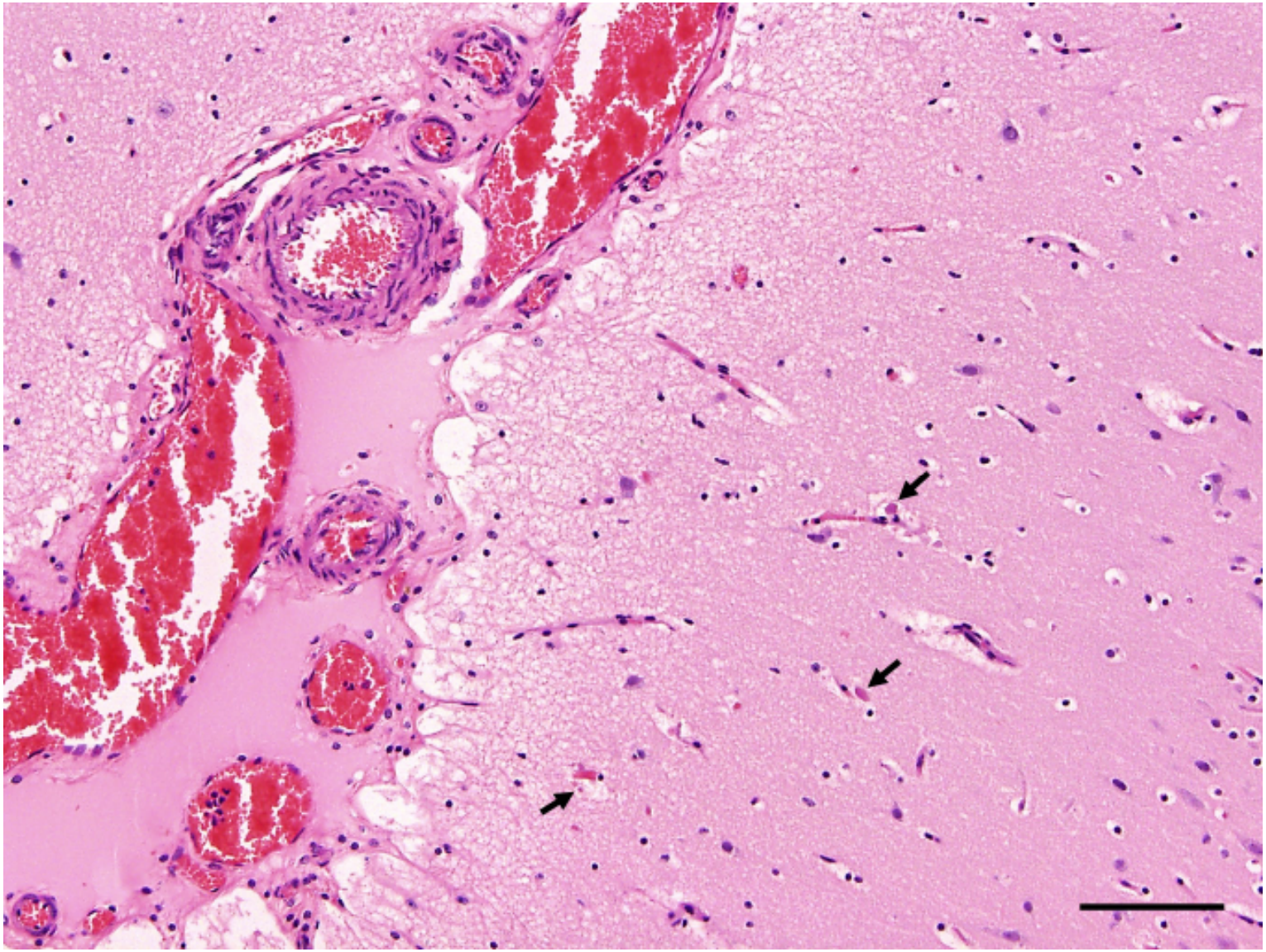
Interpret with care: postmortem astrocyte cytoplasmic swelling (arrows)



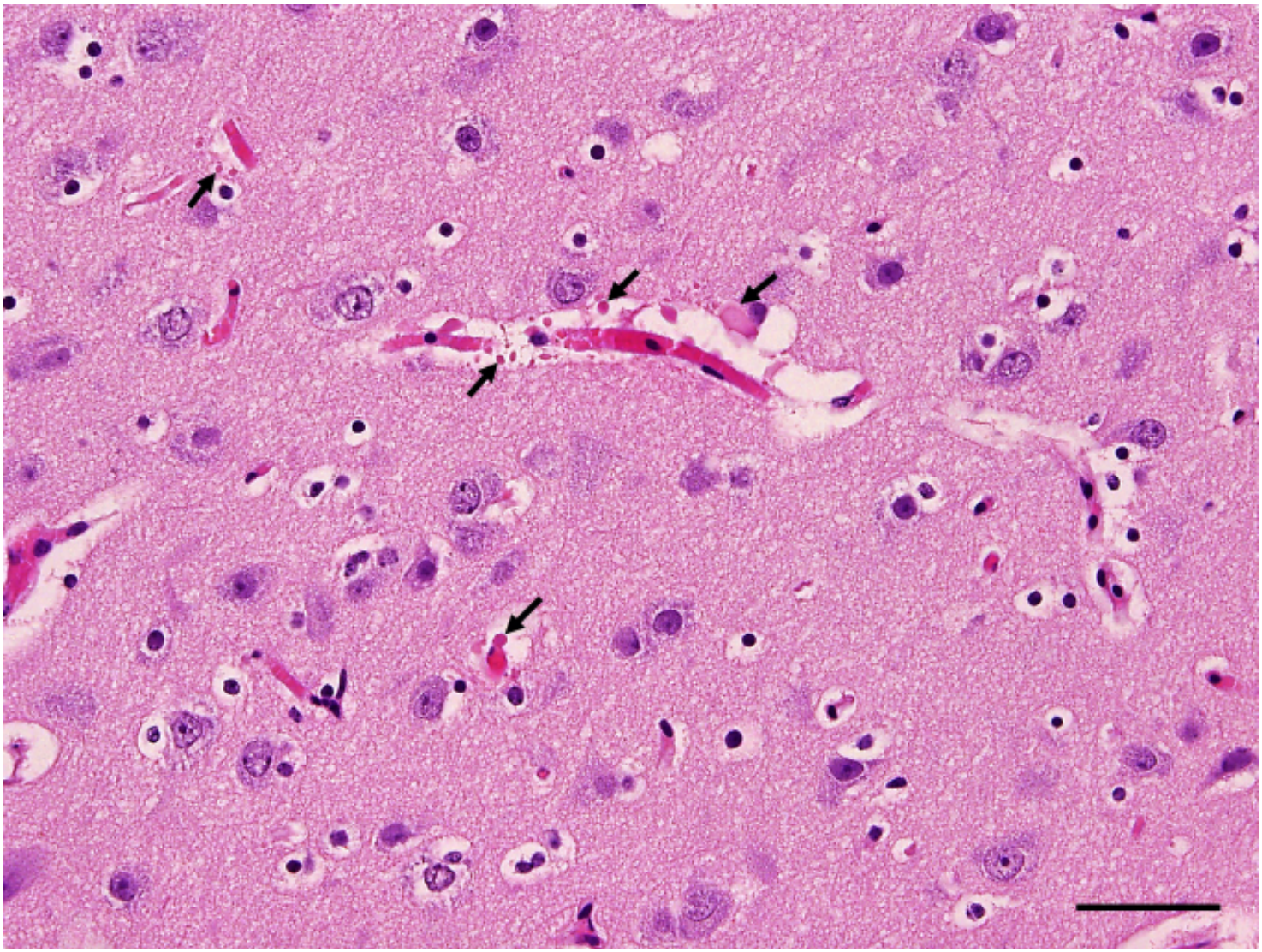
Clostridium perfringens epsilon intoxication

- swelling and rupture of perivascular astrocyte processes
- extravasation of fluid - vasogenic oedema (hyaline protein droplet formation considered pathognomonic)
- brain swelling
- neuroparenchymal necrosis





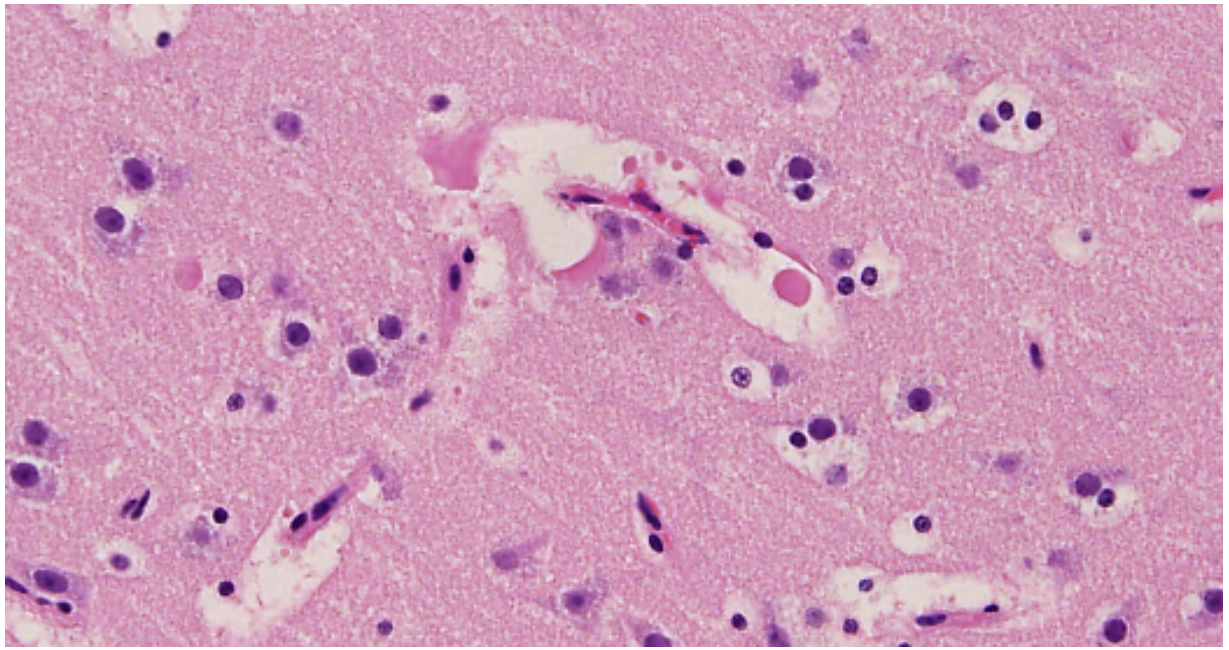
Clostridium perfringens epsilon intoxication, neonatal calf; protein rich fluid in meninges and hyaline protein droplets (arrows). Bar = 100 µm



Clostridium perfringens epsilon intoxication, neonatal calf; hyaline protein droplets (arrows). Bar = 50 µm

Astrocytes

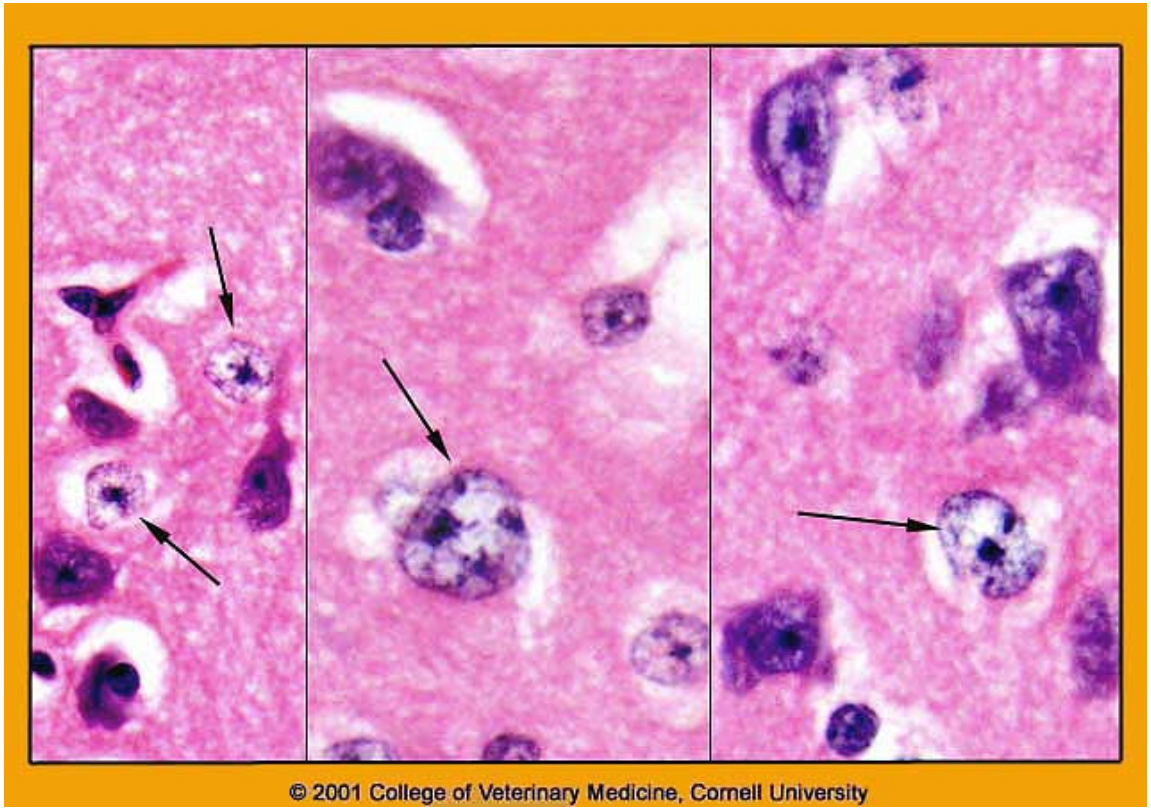
- Fluid accumulation in processes (‘hyaline protein droplets’)



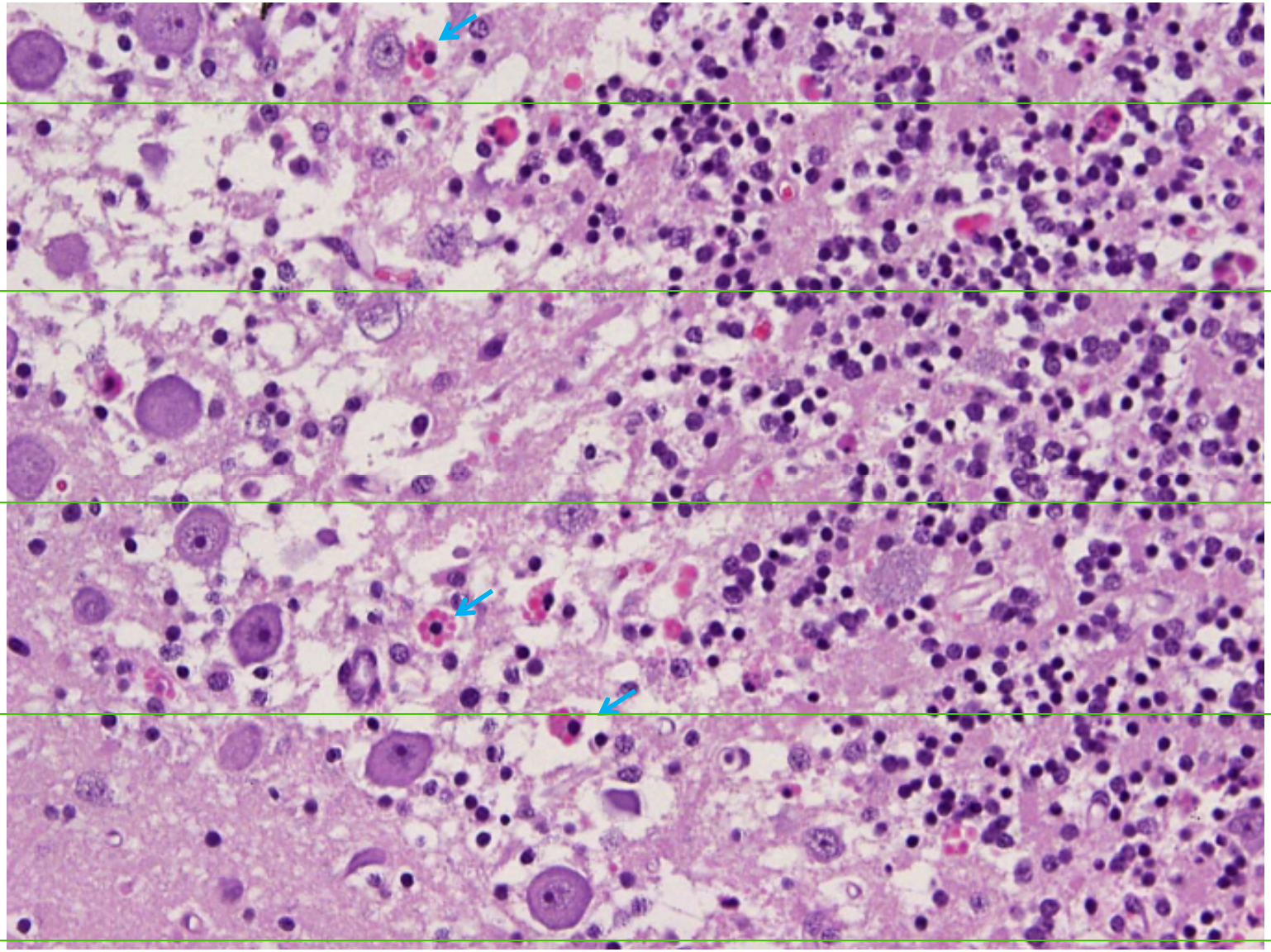
Clostridium perfringens type D intoxication, 3 day old lamb

Astrocytes

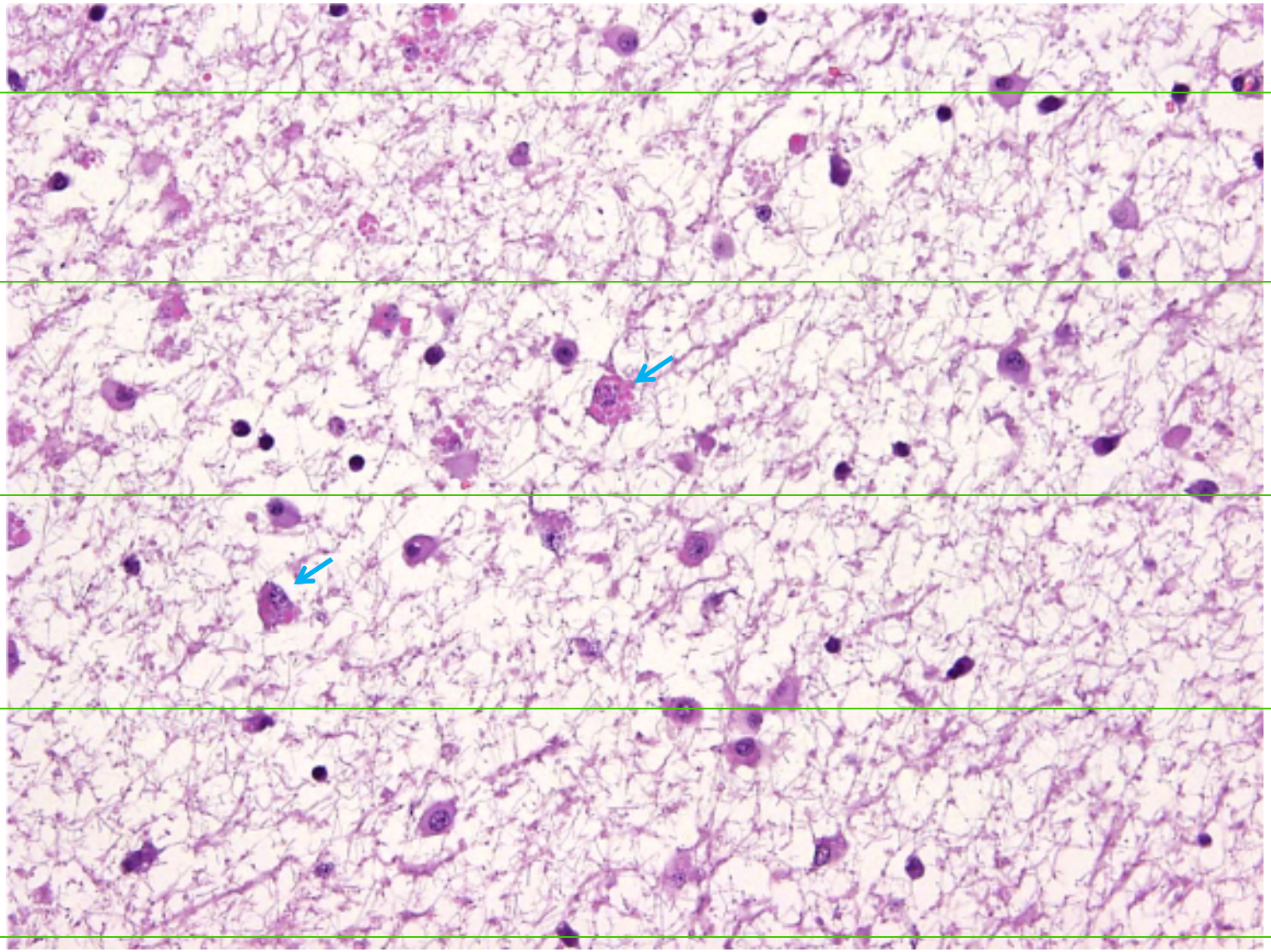
- Alzheimer type II cell formation
  - Clusters of ACs with large 'clear' nuclei;
  - Label for S100 but poorly for GFAP



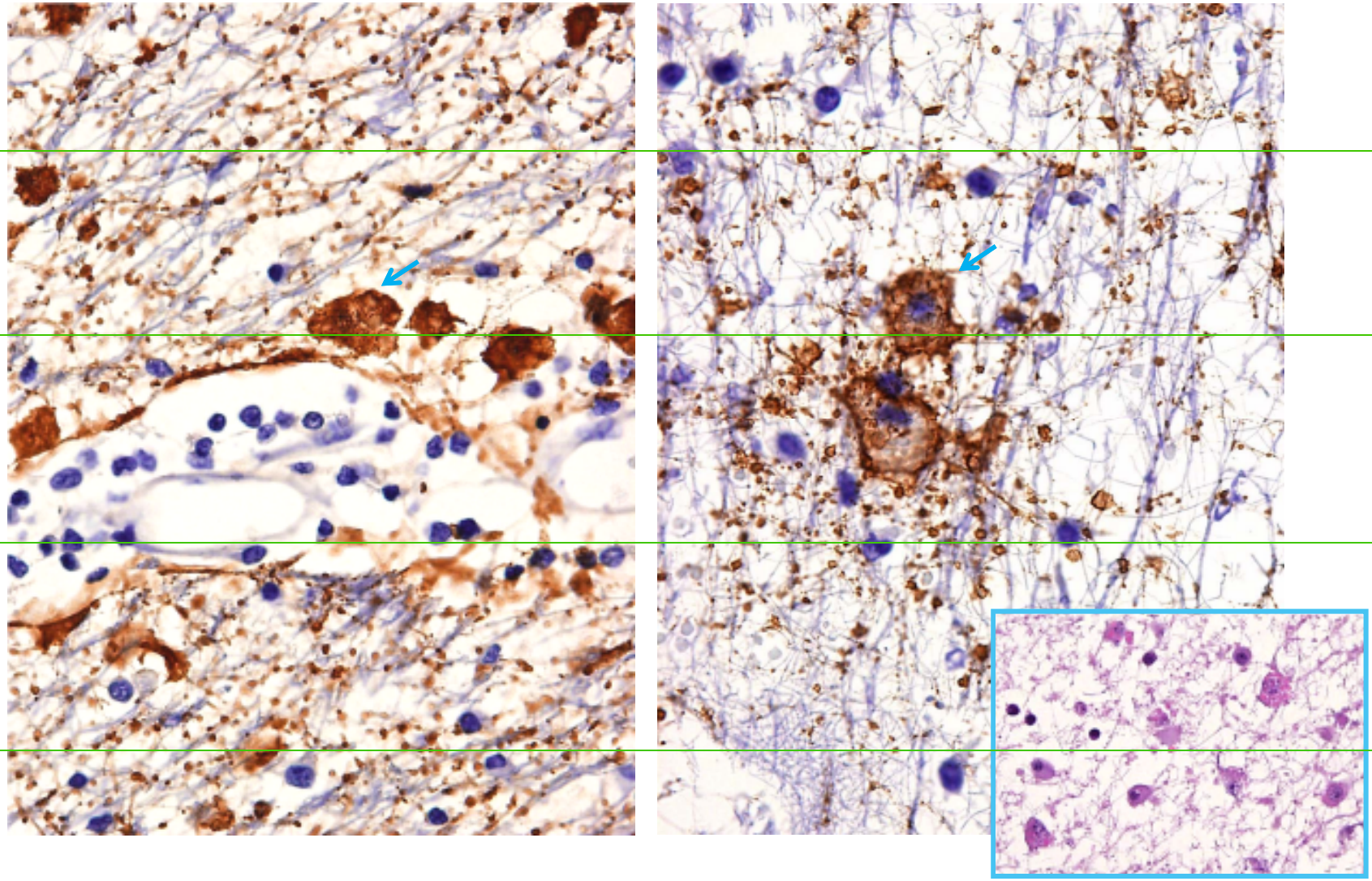
Bovine Hepatic Encephalopathy - High magnification views of cortical gray matter showing significant enlargement of astrocyte nuclei (arrows), to equal or exceed the size of neuronal nuclei.



PCV2-associated cerebellar oedema and vasculopathy; hyaline protein inclusions in astrocytes (arrows) and neuroparenchymal rarefaction (oedema)



PCV2-associated cerebellar oedema and vasculopathy; hyaline protein inclusions in astrocytes (arrows) and neuroparenchymal rarefaction (oedema) white matter



PCV2-associated cerebellar oedema and vasculopathy; hyaline protein inclusions in astrocytes (arrows) and neuroparenchymal rarefaction (oedema); GFAP (left) and Aquaporin 4 (right) IHC confirm identity of cells with inclusions as astrocytes