Claire Roberts NP 03/12
Addendum 22.08.2012

Preamble:
It may assist in understanding this supplementary report if I explain a little about the process of routine brain sampling. After an autopsy the brain is placed in formalin to preserve it. After a period of some 2-3 weeks to allow fixation to occur, the brain is examined with the naked eye and photographed. Small postage-stamp sized pieces (blocks) are selected to provide material to look at under the microscope. The blocks are labelled with a specific number or other label to indicate which part of the brain they were taken from. They are embedded in wax to make the tissue rigid enough to cut. It is then possible to cut many hundreds of very thin slivers (sections) which are placed on glass slides.

The sections on the glass slides are stained with a number of different dyes so that different tissue components can be visualised under the microscope. Routine dyes such as haematoxylin and eosin (H&E) are virtually universal. These allow an initial assessment of the state of the tissue and visualization of basic pathological processes.

However, there are many ways to improve the diagnostic process. By using methods that are capable of identifying specific cell types, we can obtain a much clearer picture of the nature and timing of pathological processes. These include the identification of specific inflammatory cells, newly growing reactive blood vessels, and damaged nerve fibres.

In this case one complication is that the tissue blocks were not individually labeled. It appears that one set of sections was used for reporting the case originally and most of these sections were then sent to me for review. However among the sections received on 2.8.12 were some I do not think I have seen before. There is some uncertainty as they are not individually labelled.
There were also a number of sections stained with special stains that I had not seen before.

Review of additional sections:
I have received further sections which apparently include those reviewed by Dr Harding. These slides include 43 stained with H & E, 17 with immunostains (including negative controls) and 4 semi thins.

Most of the H & E stained sections are from the same blocks as those I have already seen. 4 do not appear to correspond exactly to previously seen sections. These are described in part 1, where I comment on any new observations which add to those already made. In part 2 I comment on the new sections from blocks reviewed for my first report.

Part 1: New sections:

<u>Semi thin Sections:</u>
Four sections of cerebral cortex: These sections do not add any further information.

Further H & E stained sections:

2x H & E stained sections of cerebral cortex and underlying white matter. These show swollen brain and congested surface blood vessels. In one the lateral ventricular wall is included and shows the ependyma to be intact and a few groups of small dark cells are seen in the subependymal zone.

1x section of midbrain - no further information.

1x section of very distorted medulla in which a single olivary nucleus is seen. Neurones are hyperchromatic with shrunken nuclei. There is fresh haemorrhage into the root of a cranial nerve (?the vagal nerve).

Part 2 New sections from previously reviewed blocks:

OX 1, 2, 3: 1 or 2 x new H & E stained sections received: No change in pathological observations.
OX 4, 5: 2 x new H & E stained sections each: no change.
New immunocytochemistry:-
CD68 Meningeal and perivascular macrophages with normal limits.
LCA, L26, CD3 No abnormality seen.
OX6 – 1 x new H & E : no change.
OX7 – 2 x new H & E : congested vessel in cortex with small fresh perivascular bleed.
OX8 – 2 x new H & E : no change.
OX9 – 1 x new H & E : no change.
OX10, 11 and 12: Two x new H & E. These are all unlabelled sections of hippocampus from different levels and presumably some are right and some left. There is no change to the previous observations. Gliosis and cell loss are not seen in the H & E stained sections. No further immunostained sections have been submitted. Reference to GFAP staining done in Oxford shows prominent gliosis in the dentate fascia and hilum of the hippocampus. The discrepancy between the H & E sections and the GFAP is striking.
OX13, 14- One x H & E each: no change.
OX15 – 1 x new H & E section and 13 new levels: no change.
New immunocytochemistry: -
LCA: Many small dendritic cells in parenchyma.
CD3: no positive cells seen.
OX16 – 2 x H & E sections: Very swollen, no myelin loss seen.

Comment
The findings in the new material closely resemble those in my original report. There are no significant new observations.
I have closely reviewed all the sections of hippocampus as this is pertinent to Claire's illness and death. Two new H & E sections are reviewed. These H & E sections, as those first seen, show little evidence of pathology, the dentate fascia is generally well preserved but there is perhaps a little "dispersion". Dispersion of the dentate gyral cells is seen in some forms of epilepsy. As previously noted, there is no obvious cell loss and it is unlikely that significant pathology would be diagnosed on the basis of the H & E stains alone. When the GFAP stains

are examined there is clearly an increase in glial cells (scarring) in the hilum or end-folium and in the dentate fascia. No special stains were submitted on the hippocampus; so I assume that none of the other pathologists who have examined the case have seen any special stains of the hippocampus, unless they have not been disclosed.
I assume that Dr Harding did not request or review any special stains which may explain his lack of comment on the hippocampal pathology.

Conclusion

My opinion is unchanged. The hippocampal pathology is very subtle and not readily appreciated in the routine H & E stains. There is, however, scarring demonstrated in GFAP stains. This is mild pathology, most neuropathologists do not examine the hippocampus in early epilepsy. Post mortem examinations on patients with epilepsy are not particularly common as life is not necessarily shortened by the disease. Most pathologists see samples taken from patients who undergo surgery for severe, intractable temporal lobe epilepsy many years after it has been symptomatic. In these cases the pathology would be expected to be severe. For these reasons the earliest and mildest forms are not usually encountered by pathologists.

Waney Squier

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