Cerebral biopsy and assessment of brain damage in hydrocephalus

R. O. WELLER and BERNARD N. WILLIAMS

From the Department of Pathology (Neuropathology), Southampton General Hospital, and Midland Centre for Neurosurgery and Neurology, Warley, Worcestershire

Weller, R. O., and Williams, B. N. (1975). Archives of Disease in Childhood, 50, 763. Cerebral biopsy and assessment of brain damage in hydrocephalus. Needle biopsies of the cerebral mantle were taken from 12 hydrocephalic children aged between 14 days and 3 years. 5 children were biopsied twice or more often during subsequent shunting operations. Histological studies of biopsies embedded in epoxy resin for light and electron microscope examination revealed more useful information than those embedded in paraffin wax. There was evidence of axonal degeneration in the white matter of patients with acute hydrocephalus. Progressive gliosis was seen in more chronic hydrocephalus together with signs of cerebral atrophy. No measurable effect of hydrocephalus on myelination was detected.

This histological study of needle biopsies taken at shunt operations could be useful in assessing brain damage and thus in predicting future intellectual development in hydrocephalic children.

Recent clinical surveys have shown that hydrocephalic children are more likely to develop normal intelligence if there is prompt recognition and adequate treatment of the hydrocephalus (Young et al., 1973; Raimondi and Soare, 1974). Furthermore, children shunted before the age of 6 months appear to be significantly brighter than those treated after 6 months. Lorber (1968) has also emphasized the correlation with age, but he found no correlation between the preoperative thickness of the cerebral mantle and ultimate intellectual development. These findings suggest that brain tissue damage occurs in the early stages of hydrocephalus and that the young infant’s brain is in some way more resilient than that of the older child.

Many data on patterns of histological damage in hydrocephalus have been obtained from experimental studies on animals (De, 1950; Hochwald et al., 1969; Clark and Milhorat, 1970). Oedema and nerve fibre damage were seen in the periventricular tissues during the early, acute, high pressure phase of experimental hydrocephalus in puppies (Weller et al., 1971); there was gliotic scarring of this region in the more chronic stages of ventricular enlargement.

There are a number of important differences between the brains of most young mammals and of human infants. Not least of these is the pattern of myelination. Much of the white matter around the child’s ventricles is poorly myelinated until about 3 months of age (Flechsig, 1920), whereas in non-primate mammals, myelination begins earlier and progresses more rapidly after birth. Nevertheless, biopsies from a small number of human hydrocephalic children (Weller and Shulman, 1972) have shown similar histological changes to those seen in experimental animals.

We have studied the histological changes in needle biopsies of the cerebral mantle from hydrocephalic children in an effort to correlate the brain tissue damage with the previous course of the hydrocephalus.

Material and Methods

Clinical material. Biopsies of the cerebral mantle were taken from 12 patients at the time of ventricular shunt insertion (see Table). 5 patients were biopsied twice or more often.

Method of biopsy. A 14 gauge needle with a sharpened circular rim was attached to a 1 ml syringe and all the air was expelled with saline. The needle was
carefully rotated to give a circular cut in the pia-arachnoid and then inserted along the line that the ventricular drainage cannula would eventually adopt. By continuing the rotary cutting motion and applying strong suction throughout, a core of cortex and white matter was obtained.

**Preparation of histological material.** 18 cerebral biopsies were taken from the 12 patients. 9 biopsies were fixed in 3% glutaraldehyde in 0·2 mol/l cacodylate buffer at pH 7·4 for 4 hours and then washed in cacodylate-sucrose buffer for at least 12 hours. Blocks were taken and postfixed in Millonig's osmium tetroxide, dehydrated, and embedded in araldite. 1 µm sections were cut and stained with 1% toluidine blue; ultrathin sections of selected areas were cut from these blocks with diamond knives and examined in a Philips EM 300 electron microscope. The other 9 biopsies were fixed in buffered formalin and embedded in paraffin wax; 5–7 µm longitudinal sections of the needle cores were stained by H & E, Luxol fast blue, PTAH, Palmgren, Nissl, and haematoxylin-van Gieson techniques.

**Results**

**Tissue preservation.** Small cores of brain tissue were immersed in formalin or glutaraldehyde immediately after removal and this produced good fixation. Some artefact, in the form of dark, shrunken neurones which possibly reflects the trauma of the biopsy was detected in the cortical layers of most biopsies. A small area of distortion of the white matter was usually seen at the deep end of the biopsy where the tissue was severed from the surrounding brain, but despite the small diameter (1–1·5 mm) of the cores the main part of the biopsy showed little distortion. One limitation

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**TABLE**

**Histology of white matter**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (d)</th>
<th>Prepara-</th>
<th>Biopsy</th>
<th>Clinical history</th>
<th>Myelin</th>
<th>Oedema</th>
<th>Dilated Ventricles</th>
<th>Basal Cistern</th>
<th>Reactive astocytes</th>
<th>Microglia &amp; macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>A</td>
<td>Fragments</td>
<td>Myelomeningocele</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>P</td>
<td>10 x 1</td>
<td>Staph. albus infection; acute hydrocephalus</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>P</td>
<td>8 x 1</td>
<td>Shunt revision</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>P</td>
<td>6 x 1</td>
<td>Myelomeningocele</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>P</td>
<td>Fragments</td>
<td>Meningocele</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>A</td>
<td>12 x 1</td>
<td>Spina bifida; meningocoele</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>A</td>
<td>10 x 1</td>
<td>Shunt revision</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>A</td>
<td>7 x 1</td>
<td>Staph. albus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>P</td>
<td>6 x 1</td>
<td>Ventriculitis; shunt revision</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>P</td>
<td>20 x 1</td>
<td>?birth trauma; basal cistern block</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>110</td>
<td>A</td>
<td>Multiple pieces</td>
<td>Spina bifida</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>12</td>
<td>175</td>
<td>A</td>
<td>15 x 1</td>
<td>Infection; shunt revision</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>140</td>
<td>A</td>
<td>10 x 1</td>
<td>Spina bifida; Acute hydrocephalus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>196</td>
<td>A</td>
<td>12 x 1</td>
<td>Revision of shunt</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>192</td>
<td>P</td>
<td>15 x 1</td>
<td>Spina bifida</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>1 yr</td>
<td>A</td>
<td>12 x 1</td>
<td>Spina bifida</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>2 m</td>
<td>A</td>
<td>12 x 1</td>
<td>Acute hydrocephalus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>11</td>
<td>A</td>
<td>19 x 1</td>
<td>(1st shunt aged 25 d)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>9 m</td>
<td>P</td>
<td>10 x 1</td>
<td>Aqueduct stenosis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>2 yr</td>
<td>P</td>
<td>8 x 1</td>
<td>Several reasons</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Axonal degeneration detected.
A, araldite;
P, paraffin.
Cerebral biopsy and assessment of brain damage in hydrocephalus

of the technique, however, is the failure to include the gliotic subependymal region in the biopsy.

Paraffin-embedded material can be embedded as one block and sectioned in a routine laboratory, but, as with all small pieces of tissue, only a restricted number of useful sections can be obtained from each block. Embedding and sectioning of the araldite-prepared material is more arduous and the cores are necessarily divided into short lengths. Nevertheless, this technique permits better tissue preservation and gives better histological detail. In addition, selected areas may be subsequently examined by electron microscopy.

Histological changes in biopsies. The major histological changes are given in the Table together with clinical diagnoses. Little pathological change could be detected in the cortex in any of the cases, though some damage may have been masked by shrinkage of the neurones. No focal loss of neurones or cortical scarring was observed. Histological changes were, however, seen in the white matter of most of the biopsies. In the very young children with acute hydrocephalus, the non-myelinated white matter was often oedematous, especially in Case 3 where there was a marked spongy appearance. Some increase in the extracellular space was detected by electron microscope in the white matter of an older child (Case 8) but the oedema had largely subsided in the subsequent biopsy.

Direct evidence of axonal degeneration was seen only in Cases 7 and 11 where biopsies had been taken during an acute stage of the hydrocephalus. In both cases ballooned axons were observed in araldite sections either by light or electron microscopy.

Astrocyte hypertrophy and proliferation were the main evidence of tissue damage in the white matter. This could be detected in paraffin-embedded material and in light and electron microscope preparations of araldite-embedded material. Comparison of astrocyte size and density in the biopsies and control post-mortem brains was difficult due to the different degrees of histological preservation. Therefore, the most useful comparisons were between first and subsequent biopsies from the same patient. Hypertrophy of astrocytes

![Image of an astrocyte](http://adc.bmj.com/)

**Fig. 1.**—Case 7. Electron micrograph of an astrocyte in the white matter of the first biopsy, showing pale cytoplasm containing fine cytoplasmic fibrils (F); nucleus (N). (x 6900.)
was seen in the second biopsies of Cases 5, 7, and 8 when compared with their first biopsies.

Although both Cases 5 and 7 had shunt revisions after infections, no inflammatory cells were seen in the biopsies taken at the revision operations. Comparison of the ultrastructural appearances of the white matter in two biopsies from Case 7 did, however, show several aspects of tissue damage. In the first biopsy the tissue was compact with little extracellular space between the myelinated fibres. The fibrillary astrocytes had pale cytoplasm with loosely packed sheaths of fibrils (Fig. 1). A similar region in the white matter from the second biopsy, taken after a period of ventriculitis and acute hydrocephalus, showed an increase in the extracellular space. The astrocytes were hypertrophic (Fig. 2) with moderately dense cytoplasm containing coarse fibrils and scattered glycogen granules. The astrocyte nuclei were eccentric with some peripheral condensation of chromatin, and there was also an increase in endoplasmic reticulum and ribosomes in the perinuclear cytoplasm. Occasional small corpora amylacea were also observed in this biopsy (Fig. 3). Evidence of recent axonal degeneration was seen in the form of axon balloons (Fig. 4) and macrophages containing myelin debris. These latter features suggest acute damage to the white matter, whereas the astrocytosis is more indicative of past tissue damage. Dilation of the perivascular Virchow-Robin spaces in the white matter (Fig. 5) was seen in 5 patients; this is also indicative of cerebral atrophy due to previous tissue damage.

Focal collections of microglia or foamy macrophages were seen occasionally in other biopsies. Amorphous osmophilic droplets were observed in some astrocytes by electron microscopy in Case 7, but these appear to be a normal feature of the developing white matter (Tuthill, 1938) and have been described in previous biopsies of hydrocephalic children (Weller et al., 1969).

A steady increase in myelin content was seen in the white matter with age, but no useful quantitative estimation of myelination was possible due to lack of suitable controls. In a previous series of biopsies (Weller and Shulman, 1972) there was myelination

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**Fig. 2.** Case 7. Second biopsy showing part of a hypertrophic astrocyte in the white matter with an eccentric nucleus (N). Fibrils (F) are more tightly packed; there are glycogen granules in the cytoplasm (G) and abundant organelles are seen in the perinuclear region. (× 6900.)
of astrocyte processes, but this was not observed in the present series.

**Discussion**

The histological appearances in the 18 cerebral biopsies examined in this study varied depending upon the age of the patient, duration and severity of the hydrocephalus, and ensuing complications. However, the major tissue changes appeared to be in the periventricular white matter and could be detected even when the immediate subependymal tissue was not included in the biopsy. In acute hydrocephalus there was direct evidence of axonal degeneration and myelin breakdown, whereas in the children with less acute, but long-standing ventricular enlargement, there was cerebral atrophy and astrocytic scarring of the white matter. The pattern of changes was similar to that seen in experimental animals where the major damage to the brain was detected during the acute phase of high pressure ventricular enlargement (Weller et al., 1971; McLone, Bondareff, and Raimondi, 1971).

If the major damage to the periventricular white matter occurs in the acute stages of hydrocephalus, this would explain the better intellectual development in children who are treated early. Once there is axonal degeneration, however, the damage to the nerve fibres is almost certainly irreversible as little effective regeneration takes place. The neural tissue is therefore replaced by glial scar tissue which may in some cases account for a high proportion of the bulk of the white matter (Weller and Shulman, 1972). Ventricular shunting will do nothing to replace the lost neural tissue in the gliotic brain despite the shrinkage of the ventricles and the increase in thickness of the cerebral mantle.

Further study of cerebral biopsies taken during ventricular shunt operations will help to define the pattern and incidence of tissue damage in hydrocephalic children. Individual biopsies may ultimately contribute useful data for estimating the patient's future intellectual development.

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REFERENCES


Correspondence to Dr. R. O. Weller, Department of Pathology (Neuropathology), Southampton General Hospital, Tremona Road, Southampton SO9 4XY.