Ependymal-choroidal cells in cerebrospinal fluid

Increased incidence in hydrocephalic infants

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Samples of cerebrospinal fluid removed from 66 patients with various non-neoplastic disorders during pneumoencephalography or ventriculography were examined cytologically using the method of Millipore filtration. In addition to leukocytes and other types of cells, clumps of ependymal or choroidal cells were frequently noted; they were found most often and in greatest numbers in hydrocephalic infants. The authors hypothesize that these cells are constantly being shed and replaced, especially during infancy, and that their exfoliation is increased if the ventricles are stretched by hydrocephalus. It is important that one recognizes the nature of these clumps of ependymal or choroidal cells to avoid misidentifying them as leukocytes or tumor cells.

KEY WORDS cerebrospinal fluid cytology hydrocephalus ependyma choroid plexus

DURING the course of a cytological study of the dissemination of neoplastic cells into the cerebrospinal fluid (CSF), unusual clumps of cells were noted in some of the CSF specimens. These clusters of cells were confused at first with clumps of tumor cells, but when more were encountered they were noted to resemble ependymal or choroidal cells. The present investigation was then instituted to determine the characteristics of these cells and their frequency in the CSF of patients with no neoplastic disease.

Materials and Methods

We selected 66 patients with various non-neoplastic disorders (Table 1). Samples of CSF were collected from these patients during pneumoencephalography or ventriculography. The CSF samples in 36 of these cases were collected in two or three increments and the fluid initially removed was kept separate from the fluid removed later in the air study. In all, 127 CSF specimens were examined.

Within an hour of collection, each fluid specimen, varying from 1 to 180 ml in volume, was drawn through a separate Millipore filter with a pore size of 5μ. The filters were fixed immediately in Carnoy's solution, and then stained by a modified Papanicolaou technique and mounted on in-

*Millipore filter SMWP 025 manufactured by Millipore Filter Corporation, Bedford, Massachusetts.
TABLE 1

Incidence of ependymal or choroidal cell clumps in cerebrospinal fluid

<table>
<thead>
<tr>
<th>Age (Average)</th>
<th>Diagnosis</th>
<th>Source of CSF*</th>
<th>No. of Cases</th>
<th>Specimen Size (ml)</th>
<th>E-C</th>
<th>E-C Clumps per ml</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
<td>Range</td>
<td>Average</td>
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<tr>
<td>7 mos</td>
<td>hydrocephalus, communicating</td>
<td>PEG</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5 mos</td>
<td>hydrocephalus, obstructive</td>
<td>VG</td>
<td>3</td>
<td>3</td>
<td>2 to 110</td>
<td>36</td>
</tr>
<tr>
<td>6 mos</td>
<td>seizures</td>
<td>PEG</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>9 mos</td>
<td>other disorders</td>
<td>VG</td>
<td>2</td>
<td>1</td>
<td>8 to 18</td>
<td>12</td>
</tr>
<tr>
<td>15 mos</td>
<td>hydrocephalus, obstructive</td>
<td>PEG</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>3 yrs</td>
<td>seizures</td>
<td>VG</td>
<td>2</td>
<td>0</td>
<td>3.15</td>
<td>9</td>
</tr>
<tr>
<td>3 yrs</td>
<td>other disorders</td>
<td>VG</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10 yrs</td>
<td>seizures</td>
<td>VG</td>
<td>1</td>
<td>0</td>
<td>50</td>
<td>50</td>
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<tr>
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<td>other disorders</td>
<td>VG</td>
<td>1</td>
<td>0</td>
<td>8 to 11</td>
<td>9</td>
</tr>
<tr>
<td>15 yrs</td>
<td>hydrocephalus, communicating</td>
<td>PEG</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>14 yrs</td>
<td>seizures</td>
<td>VG</td>
<td>2</td>
<td>2</td>
<td>2 to 12</td>
<td>7</td>
</tr>
<tr>
<td>16 yrs</td>
<td>other disorders</td>
<td>VG</td>
<td>5</td>
<td>2</td>
<td>1,800</td>
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<td>seizures</td>
<td>VG</td>
<td>2</td>
<td>1</td>
<td>5 to 10</td>
<td>7</td>
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<td>37 yrs</td>
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<td>VG</td>
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<td>5</td>
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<td>VG</td>
<td>2</td>
<td>2</td>
<td>1,53</td>
<td>55</td>
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</tbody>
</table>

*PEG = pneumoencephalogram; VG = ventriculogram.
†Number of patients with clumps of ependymal or choroidal (E-C) cells per number of specimens.

dividual glass slides for microscopic examination. In each instance the entire filter was examined. The characteristics of the cells encountered were noted, and the numbers of clumps of ependymal or choroidal cells were counted. Partially degenerated cells were disregarded.

Results

As expected, most of the cells seen in the CSF specimens were leukocytes. Because of the volumes of the CSF specimens, there were relatively large numbers of leukocytes present on almost all of the filters. For example, filtration of a 4 ml sample containing three leukocytes per mm³ deposited 12,000 cells on the filter.

Histiocytes, arachnoidal cells, and squamous epithelial cells were seen less frequently (Fig. 1). Various kinds of debris were common, and a few fibrin clots, capillaries, and fragments of muscle were also seen. The erythrocytes in the CSF specimens either were drawn through the filter or were lysed by the Carnoy's fixative.

The cells designated as ependymal or choroidal cells were cuboidal to round in shape, with poorly defined cell outlines and well defined round or oval vesicular nuclei. They were somewhat larger than lymphocytes, were uniform in size, shape, and appearance, and occurred most frequently in clusters of three to 50 or more cells (Fig. 2). The filter interfered with detailed examination of the internal structure of the cells; cilia and blepharoplasts were not identified. From the separate microscopic study of fixed histological specimens of human ependyma and choroid plexus, and the cytological examination of washings of such structures, one of us (GLO) feels that most of the cells encountered in the present investigation were ependymal cells.

The incidence of clumps of ependymal-choroidal cells was tabulated according to patient age, diagnosis, and method of fluid removal (Table 1). These cell clumps were found most frequently in the CSF from the youngest patients with hydrocephalus.

Of the 36 patients who had CSF samples removed in two or three aliquots, 30 had the
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**Fig. 1.** Photomicrographs of samples of spinal fluid removed by lumbar puncture. *Left:* Sample from a 14-year-old boy, 8 days after a craniotomy. The clump of histiocytes (below) is readily identified by the granular phagocytized material and vacuoles within their cytoplasm. A single lymphocyte is seen above. *Right:* Sample from a 35-year-old woman. Squamous epithelial cells are present, with abundant cytoplasm and well-defined cell outlines. Millipore filter, \( \times 1750 \).

same types of cells in each specimen. There were no striking differences noted between the numbers of ependymal-choroidal cells in the earliest specimens removed and the numbers of these cells in the specimens removed at the end of the air exchange. The remaining six patients had ependymal-choroidal cells in some of the specimens but not in others; there was no recognizable pattern for this occurrence.

**Discussion**

In a histological section, ependymal cells are cuboidal to columnar in shape. They normally are tightly arranged into a layer that is one or two cells thick, forming a lining for the intracranial ventricular system and the central canal of the spinal cord. These cells may have one or more processes extending into the underlying nervous tissue and frequently have one or more cilia extending from the intracellular blepharoplasts out into the adjacent ventricular, aqueductal, or central cavity. The walls of the ependymal cells and their intracellular blepharoplasts may be difficult to delineate using the hematoxylin and eosin stain and routine light microscopy. In contrast, their round or oval vesicular nuclei can be stained easily and are usually well demarcated.\(^1,3\)

On the choroid plexus, the ependymal lining is modified and plays an important role in the formation of cerebrospinal fluid. These choroidal cells are cuboidal to low columnar in shape, and rest in a layer one cell thick on a basement membrane. They do not have cellular processes extending into the underlying nervous tissue, but often have cilia. Their

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cell outlines are usually sharp, and their nuclei tend to be smaller, rounder, and darker than the usual ependymal nuclei.\(^1,21\)

Embryologically, the medullary epithelium lining the central canal of the developing neural tube is the precursor of both neurons and neuroglia. In newborn animals, this process may still be observed, and it is not uncommon to see heavy subependymal collections of differentiating spongioblasts and neuroblasts during the first year of life. In these areas, the ependymal lining may be several layers thick.\(^3,29\) In other words, histological evidence exists to support the concept that the ependyma is a dynamic and changing structure during infancy. Furthermore, Shuangshoti and Netsky presented convincing documentation that the choroidal epithelium of human embryos and fetuses proliferates, stratifies, and desquamates its superficial cells into the ventricular CSF.\(^29\)

Various investigators have found that the normal leukocyte count in the cerebrospinal fluid of children under one year of age may be as high as 30 per mm\(^3\) or more,\(^2,10,26\) and in the CSF of premature or immature infants it may be even higher.\(^10,28\) Since the more exact cytological techniques were not employed in the studies cited, it is conceivable that some of the counted cells were actually ependymal or choroidal cells, reflecting increased exfoliation of these cells during infancy.

With the development of hydrocephalus, there is a stretching of the walls of the ventricular system, and frequently areas of flattened ependymal cells are broken by gaps in which no lining is present.\(^9,21,27\) The choroid plexus also undergoes atrophic changes.\(^21,27\) Under these conditions, one might expect to encounter increased desquamation of ependymal and choroidal cells into the cerebrospinal fluid, and this process might be temporarily intensified by the mechanical agitation of the air-fluid exchange during ventriculography or pneumoencephalography. No mention of such a phenomenon is made in standard texts dealing with CSF.\(^7,9,10,20\)

In the literature relating to CSF cytology, several investigators have mentioned that they have occasionally encountered ependymal and

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**Fig. 2.** Photomicrographs of 8 ml samples of ventricular fluid removed during ventriculogram. **Upper Left:** Sample from an 8-month-old boy with obstructive hydrocephalus, showing a small clump of ependymal or choroidal cells. **Upper Right:** Sample from a 3-month-old boy with communicating hydrocephalus. Part of a large clump of ependymal or choroidal cells is present. **Lower Right:** Sample from a 7-month-old boy with communicating hydrocephalus. A clump of ependymal or choroidal cells is seen. Millipore filter, × 1050.
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choroidal cells, primarily in ventricular rather than spinal CSF.4-6,11-14,19,22,24,26,28,35,37,38 Kolar and Zeman38 noted increased numbers of these cells after trauma, surgical intervention, and ischemic infarction, and El-Batata6 found a greater incidence in association with postoperative intracranial inflammation.

Wiersbitzky and Wiersbitzky38 found similar cells in the CSF of nine of 12 children with hydrocephalus and meningomyelocele, but in only one of 16 children with uncomplicated hydrocephalus and one of 26 children with other disorders. Ependymal and choroidal cells were not recognized by Rautenbach and Tischer25 in ventricular fluid samples from 38 children with hydrocephalus (examined cytologically by Sayk's sedimentation method), though such cells may have been mistakenly classified as reticulohistiocytes. Similarly, Weissbach84 encountered clusters of unusual cells in the lumbar CSF of five infants that had been treated with spinoperitoneal shunts, but did not identify these as ependymal-choroidal cells.

Bots and co-workers4 found ependymal cells in the CSF of patients with hydrocephalus; they attributed this phenomenon to "chronic irritation," and only mentioned it in passing. Simon38 encountered clumps of cells in the CSF of hydrocephalic patients which he thought were ependymal and choroidal cells because of their microscopic similarity to cells scraped from the ependymal lining and the choroid plexus. Neither Bots, et al., nor Simon quantified their findings. In our investigation, the unexpected frequency with which ependymal-choroidal cells were encountered may have been due to the use of the Millipore filter, which probably trapped almost all such cells that were present, and to the investigation of relatively large volumes of CSF.

No matter what their relationship to infancy and hydrocephalus, however, it is important that these "normal" ependymal or choroidal cells be recognized so that they are not confused with leukocytes or tumor cells. Their uniform size, shape, and appearance, their moderately abundant cytoplasm, their delicate nuclear chromatin, and their occurrence in clumps of three to 50 or more cells are their identifying features.

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References


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