Neurosurgical use of human dura mater sterilized by gamma rays and stored in alcohol: long-term results

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Surgical experience with grafts of human dura mater sterilized by gamma rays and preserved in alcohol is reported in 804 cases. The method of graft preparation is a simple, cheap, and practical technique for making available a plentiful quantity of large and small pieces of dura to use for plastic reconstruction in everyday neurosurgical practice. Annual inspection for sterility and immunogenicity over an 18-year period indicates that this system of preservation is valid for an unlimited period of time.

KEY WORDS - dura mater - graft sterilization - graft preservation

Since 1895 when Abbe first utilized a rubber laminate in place of dura mater, numerous materials have been used as dural substitutes. However, reports on the clinical use of human dura mater first appeared in 1958, after extensive experimentation with various organic and inorganic materials, we reached the same conclusion as many other authors; namely, that the best dural replacement is human dura mater itself. This left the problem of finding suitable techniques for sterilizing and storing dura. We tried several procedures used by others, including lyophilization performed in collaboration with the Istituto Superiore di Sanità. In 1969, we developed a method consisting of sterilization with gamma rays and storage in polyethylene bags containing alcohol. Once it was established that this method was simpler and more suitable for maintaining unaltered the properties of the dura mater, we used it almost exclusively.

In 1979, lyophilized dura mater sterilized by gamma rays (Lyodura*) became commercially available and was widely used. More recently other products prepared by various methods of chemical dehydration, such as Tutoplast, have appeared on the market. However, because of the industrial preparation involved, these materials are expensive and this is one of the reasons we have continued to use the dural transplants prepared by our method.

Method of Dura Preparation

The dura mater is removed from the cadaver, stripped of its large vascular structures, and fashioned into pieces of various sizes, which are then washed thoroughly in running water. Following this they are put into glass containers with 70% ethyl alcohol which has previously been filtered through a millipore membrane (pore diameter 0.22 μ). Each graft is then individually sealed in a double polyethylene bag containing alcohol. Once it was established that this method was simpler and more suitable for maintaining unaltered the properties of the dura mater, we used it almost exclusively.

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* Lyodura manufactured by B. Braun, Melsungen AG, West Germany.
† Tutoplast manufactured by Lyofil-Pfrimmer GmbH, 852 Erlangen, West Germany.
‡ Gamma beam 650 apparatus provided by the Istituto Superiore di Sanità, Rome, Italy.
and sterile preservation. In addition, the grafts are ready for immediate use without the need to rehydrate them as in the case of lyophilized dura and, with preservation in alcohol, they retain the physical properties of fresh dura.

The method of preparation ensured the sterility of the grafts. Microbiological tests performed annually on a statistically significant number of samples from a batch prepared in 1967 and still in storage have consistently given favorable results, thus confirming that complete elimination of bacterial and fungal contamination has been achieved. Despite the good results of these tests, each batch of grafts is further checked for sterility before being used. Tests are performed according to the Farmacopea Europea and basically require a culture of the graft in media for aerobic and anaerobic Schizomycetes as well as for fungus. To rule out false-negative results as to the possible presence of germs “in metabolic stasis,” aliquots of samples under examination are cultured in 1% peptonic water to favor the reconstitution of any cellular structures that may have been only damaged by gamma irradiation. After incubation of these cultures for 5 days at 37°C, subcultures are made in the same media for definitive tests of sterility.

No cases of rejection after dural homotransplantation have been reported in the literature. This is correlated to the nature of the collagen tissue which, as it contains a great quantity of fiber but few cells, has scanty antigenic properties. However, since treatment of dural grafts with gamma rays does not rule out a possible increased antigenicity of the tissue and as, theoretically speaking, the radiation itself could give origin to new antigen configurations, it was thought worthwhile to test cases with suspected stimulation of B lymphocytes for the presence of circulating antibodies. While realizing that this is not the most sensitive procedure for detecting transplant rejection, it seemed a useful preliminary step. All the tests performed with the serum of 250 patients were negative.

Summary of Cases

In the Department of Neurosciences of the Rome University “La Sapienza,” dural homotransplants sterilized by gamma rays and stored in alcohol were used in 804 (7.7%) of 11,373 neurosurgical operations performed between January, 1967, and December, 1984 (Table 1). The grafts were usually applied using separate stitches or continuous silk sutures, and occasionally with biological adhesives such as Histoacryl blue and Bucrylate. Of the 804 patients, 755 (93.9%) had no complications. Forty cases (6.0%) had infections: 28 (3.4%) had wound infections, and 22 (3.1%) had meningitis. These complications, however, occurred mainly in patients who had surgery for cranial trauma, cerebrospinal fluid fistulas, and tumors of the cranial base which led to a connection being opened between the endocranium and the airways. In these circumstances, it can be reasonably assumed that the development of the infections was unrelated to the presence of the dural graft. This is confirmed by the fact that we found an equal incidence of septic complications in patients who had various types of surgery both with or without the use of dural plasties, when there were no clear reasons for contamination of the operative field.

In cases with early reoperation (less than 12 months after surgery), the phenomenon of fibroblastic infiltration and revascularization of the graft, which tends to assume all the features of normal dura, was observed. In 15 reoperations performed more than 6 months after the initial operation in patients previously treated with dural plasty using lyophilized dura or dura preserved in

TABLE 1
Analysis of 804 dural grafts using human dura mater sterilized by gamma rays and stored in alcohol

<table>
<thead>
<tr>
<th>Reason for Operation</th>
<th>No. of Cases</th>
<th>Complication-Free</th>
<th>Wound Infection</th>
<th>Meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>neoplastic infiltration</td>
<td>427</td>
<td>411</td>
<td>11</td>
<td>2.5</td>
</tr>
<tr>
<td>traumatic lesion</td>
<td>115</td>
<td>102</td>
<td>10</td>
<td>8.7</td>
</tr>
<tr>
<td>cerebrospinal fluid fistula</td>
<td>94</td>
<td>82</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>myelocele &amp; encephalocele</td>
<td>63</td>
<td>57</td>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>surgical laceration &amp; other</td>
<td>105</td>
<td>103</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>totals</td>
<td>804</td>
<td>755</td>
<td>28</td>
<td>3.4</td>
</tr>
</tbody>
</table>

§ Histoacryl blue manufactured by B. Braun, Melsungen AG, West Germany; Bucrylate manufactured by Ethicon, Inc., Somerville, New Jersey.
Preservation of human dura mater

alcohol, the dura preserved in alcohol was observed to be better adapted in most cases: macroscopically, it appeared better preserved and its features were closer to those of the host dura. The lyophilized dura, on the other hand, appeared to be altered and transparent as if it were in the process of being reabsorbed. Histologically, the lyophilized dura presented disorganized and fragmented bands (Fig. 1), whereas the graft preserved in alcohol had a looser structure than the host dura even though it showed neither fragmentation nor disorganization of the bands (Fig. 2).

In none of the reoperations was there adherence between the dural graft and the encephalic or spinal structures. In a few cases there was noticeable granulation of the soft epicranial tissue above the graft when this was exposed in 3- to 4-sq cm areas without a bone table.

In conclusion, there are advantages in the use of dura mater preserved in alcohol that allow wide use of these grafts. The availability of ready-to-use dural grafts of all sizes (smaller and larger than the standard sizes commercially available) at a reasonable cost means that they can be utilized not only when there is a real loss of dural tissue but also when a paucity of the host dura makes closure difficult or not sufficiently watertight. A final advantage is that the small pieces left over from dural grafts may be reutilized by repeating the process of sterilization by gamma rays and preservation in alcohol.

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