Pediatric and Developmental Pathology 7, 595–600, 2004
DOI: 10.1007/s10024-004-0041-0

Heat-Accelerated Fixation and Rapid Dissection of the Pediatric Brain at Autopsy: A Pragmatic Approach to the Difficulties of Organ Retention

CIARA BARRETT,[1,2] FRANCESCA BRETT,[3] DAVID GREHAN,[1,2] AND MICHAEL B. MCDERMOTT[1,2]*

[1]Department of Histopathology, Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland
[2]The Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin 4, Ireland
[3]Department of Neuropathology, Beaumont Hospital, Dublin 9, Ireland

Received April 19, 2004; accepted July 13, 2004 published online December 6, 2004.

ABSTRACT

We investigated whether it is possible to accelerate the examination of a pediatric brain at autopsy and thus facilitate its return to the body before a funeral without compromising the quality of the neuropathologic examination. Accelerated fixation and next-day dissection of the brain was performed in selected cases over a 2-year period by using a microwave histologic tissue processor (MicroMed T/T MEGA, Milestone, Sorisole, Italy). Direct comparison of the histologic appearance and immunohistochemical reactivity of 2 cases, 1 fixed by conventional methods and 1 fixed with the accelerated method, was performed in a blinded fashion by a specialist neuropathologist. Examination of rapidly fixed brain by conventional thin coronal sections was readily achieved. There was no appreciable difference between tissue sections stained with hematoxylin and eosin and prepared from conventional formalin-fixed cortical and cerebellar brain tissue and that fixed by rapid heat acceleration. Immunocytochemical studies were not adversely affected by the accelerated heat-fixation process of tissue. Heat-accelerated fixation is a potential method of speeding up the examination of the brain at autopsy without unduly compromising the quality of the neuropathologic examination.

*Corresponding author, e-mail: Michael.mcdermott@olhsc.ie

Key words: autopsy, brain, rapid fixation, children

INTRODUCTION

In recent years, retention of whole organs after postmortem examination has become a contentious issue in Ireland and throughout the world. As a consequence of an inquiry into the deaths of children following heart surgery at the Bristol Royal Infirmary, there was extensive public and media disquiet regarding the failure to provide specific information on this practice to parents before the performance of an autopsy. A flood of private and public inquiries ensued, prompted by similar disclosures at other hospitals and other countries. The result of these inquiries has been sweeping change in the process of transmitting information, obtaining consent, and ultimately disposing of organs after postmortem examination [1,2].

The most common reason for the retention of a whole organ is in the setting of central nervous system (CNS) disease. Although it is

possible to examine and dissect the brain at the time of autopsy, this is significantly less sensitive than examination after a period of formalin fixation [3]. Because the brain of a child has higher water content, it is softer than that of an adult [4]. Therefore, it is even more difficult to examine fresh, and there is an even greater necessity for prior fixation to facilitate a detailed examination. By convention, the preferred method is immersion in formalin for a period of 2 to 3 weeks [5].

Delaying a funeral for a period of weeks to allow such an examination to be completed has not proved to be acceptable to parents attending this hospital, but the overwhelming majority have requested the return of any organs retained at some future point for burial. The conflict between the requirements to perform a postmortem to a satisfactory professional standard for parents, hospitals, and coroners and the wishes of families for the return of organs without undue delay of funeral services has prompted a search for methods to accelerate the examination of the CNS without unduly compromising the quality of the examination.

One proposal involves heat-accelerated tissue fixation with the use of a modified microwave tissue processor. This method produces a level of tissue fixation that, although not directly comparable to conventional methods, is sufficient to facilitate a satisfactory dissection and gross examination within 24 to 48 h and thus not impose a prolonged delay on funeral arrangements. We report our experience with this technique over the past 2 years.

METHODS

Between January 1, 2001 and December 31, 2002, 68 autopsies were performed at our institution. CNS examination was performed in 53 cases, with 10 referred for specialist examination to a neuropathologist. Ages ranged from 1 day to 13 years (mean 20.8 months), and brain weights ranged from 280 to 1730 g (mean 836 g). In 40 of the 53 cases, the brain was fixed with accelerated heat fixation, and brains for the remaining 13 were conventionally fixed in formalin over a 2- to 3-week period.

The MicroMed T/T MEGA (Milestone, Sorisole, Italy) microwave is designed for the histopathology laboratory to rapidly fix, decalcify, stain, and process tissue specimens. The instrument consists of an 800 magnetron microwave unit interfaced to a terminal control unit that can store up to 30 programs in which the operator can change power, time, and temperature. The microwave has the capacity to hold a 5-L container and retaining basket. The brain was placed in the basket, which at its base had cotton wool soaked with formalin. The basket was placed into the Histomodule (container), and 10% buffered formalin was poured into the Histomodule until the brain was fully submerged. Cotton wool was put on top of the brain to avoid drying as the brain may float in the formalin. The Histomodule with its lid was then placed in the MicroMed T/T microwave and the program was activated.

The brain fixation program involved 2 steps. The first step raised the temperature of the formalin to 50°C by using a power setting of 600 W for 20 min. The second step used a 300-W power output for 6 h that maintained the temperature of the formalin at 50°C. After completion of the program, the brain was left in the cooling formalin for an additional 12 h. The brain was then taken from the Histomodule, washed in running water, and cut into coronal slices at 1-cm intervals with metal guides in conventional fashion. Blocks taken from the brain were left to fix for another 24 h before processing to paraffin.

Tissue blocks from the CNS, regardless of the method of fixation used, were processed with a 48-h slow-processing program on a standard Tissue-Tek VIP Processor (Miles Scientific, Naperville, IL, USA).

For the purposes of this study, 2 of the 53 autopsies that involved examination of the brain were subjected to additional hematoxylin and eosin (H&E) and immunohistochemical staining. The cases were chosen to reflect an example of brain fixation using rapid heat acceleration in the MicroMed T/T Microwave tissue processor and an example of conventional prolonged formalin fixation. From each case, 24 slides, 12 of cortex and 12 of cerebellum, were cut and stained with H&E. Immunohistochemical stains for glial fibrillary acid protein and neurofilament (Table 1) were

Table 1 Immunohistochemical antibodies

Antibody	Supplier	Antigen retrieval	Dilution
Glial fibrillary acid protein	DAKO, Buckinghamshire, UK	Trilogy antigen retrieval (Cell-Marque) and pressure cooker	1:3000
Neurofilament 200 (n-52)	Sigma, St. Louis, MO, USA	Trilogy antigen retrieval (Cell-Marque) and pressure cooker	1:1600

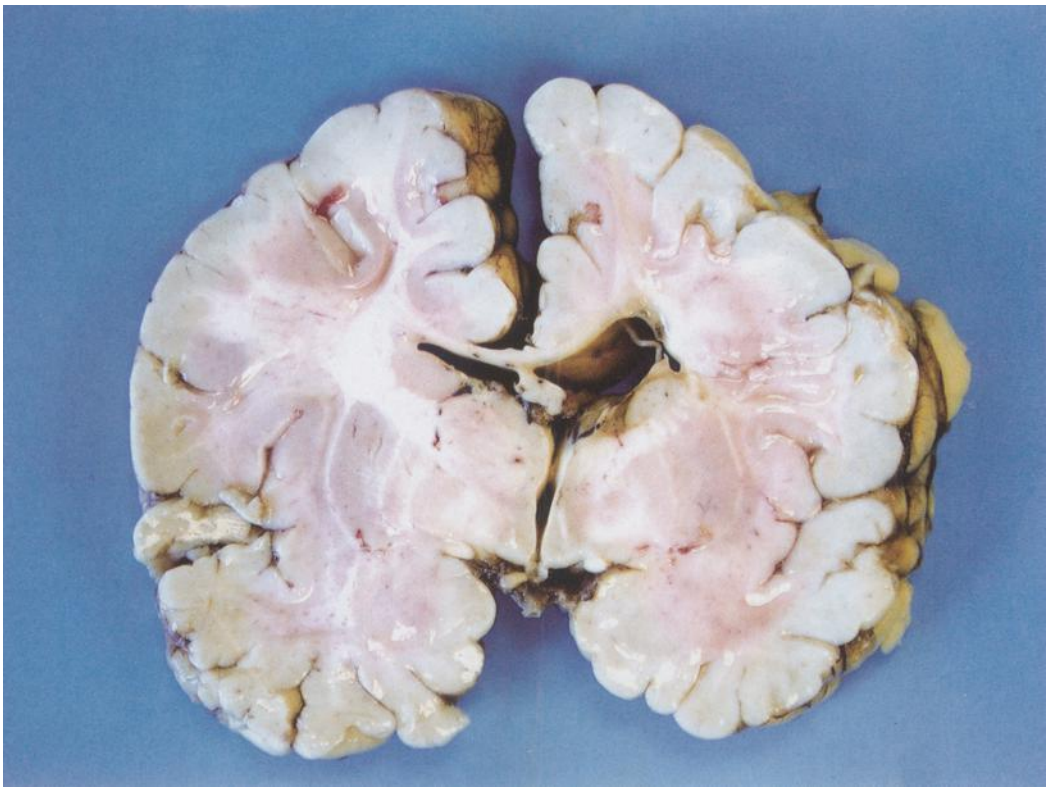

Figure 1 Coronal slice through brain 24 h after of heat-accelerated fixation. The peripheral rim of tissue is sufficiently fixed to permit satisfactory sectioning at 1-cm intervals.

performed on the tissue by using the Vectastain Elite detection system (Vector Laboratories, Burlinhame, CA, USA).

Slides from these 2 cases were reviewed by a specialist neuropathologist who was blinded to the method of fixation used.

RESULTS

Examination of rapidly fixed CNS by conventional thin coronal sections was readily achieved. Although the center of the brain was obviously not fixed, the presence of a rim of firm fixed tissue facilitated the dissection process and allowed the dissector to take tissue blocks within 24 h of the autopsy (Fig. 1). Longer periods were associated with greater fixation, but a satisfactory gross examination of the CNS was achieved when parental wishes did not allow retention beyond 24 h.

Subsequent microscopic examination of the brain was not compromised by the method of fixation used. Even in the cases subjected to formal assessment by a neuropathologist, no appreciable difference was found between H&E tissue sections prepared from conventional formalin-fixed deep gray matter structures, cerebral cortex, and cerebellar brain tissue and those fixed by rapid heat acceleration using the MicroMed T/T microwave processor (Fig. 2). Tissue subjected to heat fixation by microwave irradiation therefore preserved histologic detail that was comparable to conventional formalin fixation.

Immunohistochemical studies were not adversely affected by the accelerated heat-fixation

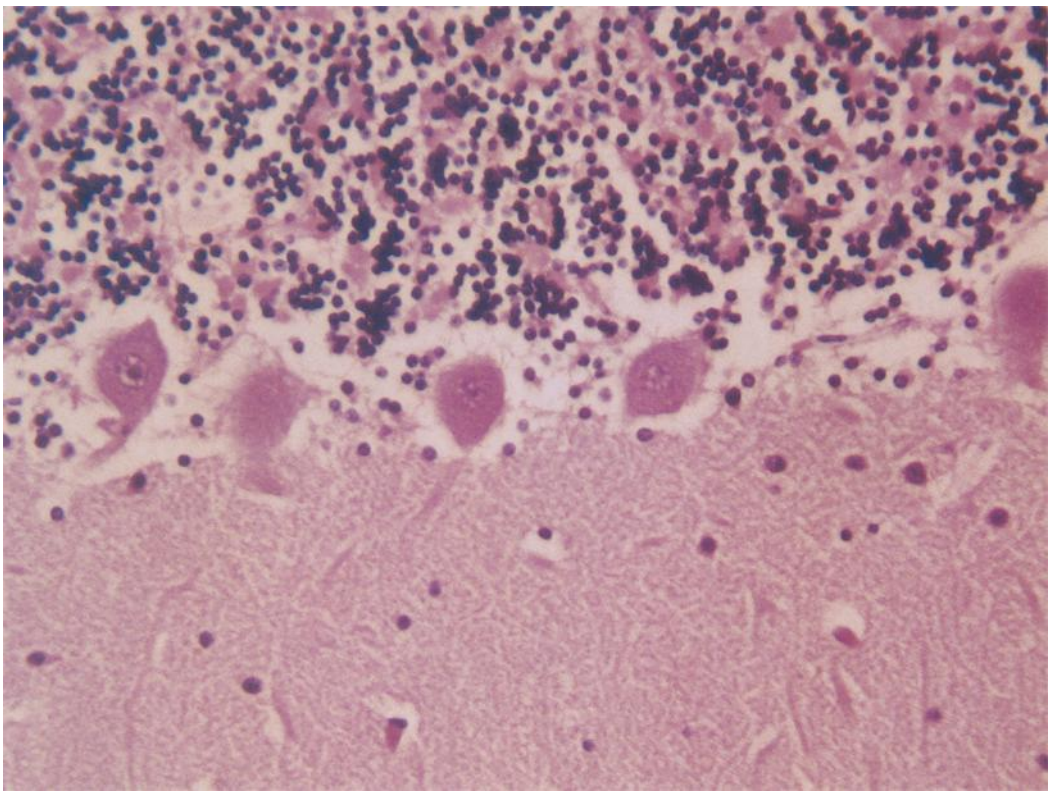

Figure 2 Cerebellar section of rapidly fixed brain showing satisfactory morphology (hematoxylin and eosin stain).

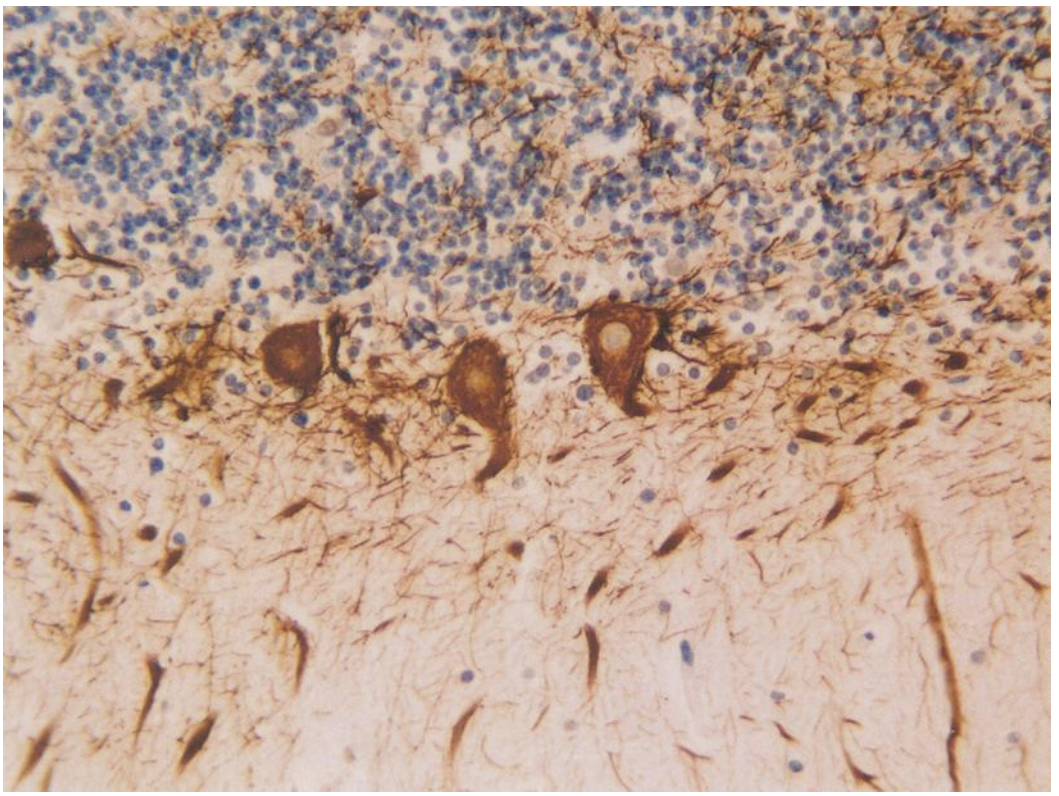

Figure 3 Neurofilament from a cerebellar section of a rapidly fixed brain.

process (Fig. 3). The antigens examined were detectable in the microwave-fixed tissue at the same antibody dilutions used after conventional fixation. Immunohistochemical stains after rapid fixation appeared sharper and more distinct than those seen in conventional formalin-fixed tissue.

DISCUSSION

The gold standard for examination of the brain postmortem is a detailed dissection after a period of formalin fixation. It is possible to examine the brain in the fresh state but at the expense of a substantial decrease in sensitivity [3]. Its significantly higher water content makes an immediate dissection of a pediatric brain even more problematic than the adult counterpart and renders such an assessment of only limited value. As such, fresh dissection should be regarded as the exception rather than the rule, and many professional bodies have incorporated such a recommendation into their practice guidelines [5–8]. Some investigators have gone so far as to suggest that the dissection of a fresh brain represents professional negligence [3].

Changes in autopsy consent and the delivery of information to parents that have followed the public reaction to the revelations at Bristol have had inevitable consequences for postmortem practice. Many centers have reported a decrease in postmortem rates, and discussion with colleagues suggests that an increased number of the postmortem examinations that are performed have had limitations imposed that affect the quality of information that may be obtained. These limitations most frequently involve forgoing an examination of the CNS. Although this often reflects a parental desire to avoid an incision on the scalp, it may also reflect a family that is unwilling to countenance the prolonged retention of the CNS in circumstances in which the pathologist is reluctant to perform a fresh examination when the pathologist knows may have less value. The pathologist may be put in an even more invidious position in the setting of a coroner's autopsy when a family is unwilling to entertain the retention of the brain beyond the funeral and the coroner is insisting on a complete and thorough postmortem.

Where clinical circumstances dictate, retention of the brain for formalin fixation and subsequent detailed examination remains the most appropriate course of action. In many cases, a detailed explanation of the benefits of such an examination to parents by the pathologist will lead to their acquiescence. Our experience of the pathologist in this role as the principal conduit of information to families about postmortem examinations has been reported [9]. However, there are other families that are unwilling or unable to consider such a course of action, leaving the pathologist with the choice of performing an immediate and potentially professionally unsatisfactory examination or forgoing any examination of the CNS. There are also occasions when the insistence of the coroner on an examination of the brain as a component of a complete postmortem examination is less easily justified, e.g., when the cause of death is known or at least highly likely to be a consequence of pathology in the thorax or abdomen, such as perioperative death in a child with congenital heart disease. Clearly, retention for detailed examination in such a case is the professionally comprehensive course of action and will best serve the autopsy in its multiple roles including audit, but is the only way to satisfy the professional imperative at the expense of the wishes of the family?

Faced with this dilemma, we sought a third alternative in which the wishes of the pathologist to perform a professionally satisfactory examination of the pediatric brain postmortem could be accommodated alongside the wishes of a family to have all whole organs returned to the body before the funeral.

The use of heat to accelerate reactions in the laboratory is well established [10]. Most pathologists and their medical laboratory science colleagues are familiar with the benefit that accrues from placing a recently obtained fresh and hemorrhagic biopsy in a conventional oven for a short period before dissection and processing, especially when the biopsy has arrived at 4:30 PM and the processor starts at 5 PM! However, fixing whole organs on a hot plate or in a conventional laboratory oven is not practical due to size constraints and the absence of ventilation for hazardous formalin fumes.

Microwave devices for accelerating histologic procedures are increasing within pathology laboratories and have found particular favor for antigen retrieval in immunohistochemistry. With minor modifications of the container, we were able to use a commercial microwave tissue processor/laboratory station to provide accelerated fixation of the brain. Although in no way comparable to conventional prolonged formalin immersion for 2 weeks or longer, the fixation provided by this mechanism did produce sufficient fixation to allow 1-cm serial sectioning and observation of an intact cut surface, something that would be essentially impossible for the fresh small pediatric brain. Histologic sections that are obtained at that time may be subjected to further fixation. In this study, we detected no significant differences between histologic sections prepared after conventional fixation and those prepared after accelerated fixation. The procedure had the added unanticipated benefit of improving the immunohistochemical staining properties of the tissue, presumably as a result of the antigen retrieval effects of the heating process. The additional advantage of the processing laboratory station is the ability to program, maintain, and record a continuous temperature profile and the existence of a suitable inbuilt ventilation system.

An obvious limitation of this study is the small number of cases that were subjected to more rigorous immunohistochemical examination, but consent restrictions prevented any extension of the study beyond the cases examined. Larger studies will be necessary to confirm the observations in this small group. Future studies should also address the effect of prolonging the period of exposure to microwave fixation. Similarly, our experience is limited to the setting of infants and children and cannot necessarily be extrapolated to the setting of fetal and perinatal autopsies where the brain is smaller and even softer or to adult practice where the brain is larger and intrinsically firmer.

When a child's death is a consequence of known or suspected neurologic disease, we support the conventional methods of fixation and examination. However, when the expectation of neurologic findings is lower and/or the family has strong wishes for return of organs before the funeral, heat-accelerated fixation and next-day dissection appear to offer a potential compromise that maintains the professional integrity of the pathologist and respects the wishes of the parents.

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