

Diffuse Axonal Injury in Infants With Nonaccidental Craniocerebral Trauma

Enhanced Detection by β -Amyloid Precursor Protein Immunohistochemical Staining

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● **Objective.**—Accurate identification of diffuse axonal injury is important in the forensic investigation of infants who have died from traumatic brain injury. β -Amyloid precursor protein (β -APP) immunohistochemical staining is highly sensitive in identifying diffuse axonal injury. However, the effectiveness of this method in brain-injured infants has not been well established. The present study was undertaken to assess the utility of β -APP immunohistochemistry in detecting diffuse axonal injury in infants with either shaken baby syndrome or blunt head trauma.

Materials and Methods.—Archival formalin-fixed, paraffin-embedded blocks from infants (<1 year old) with shaken baby syndrome (7 cases) and blunt head trauma (3) and blocks from 7 control cases that included nontraumatic cerebral edema (1), acute hypoxic-ischemic encephalopathy (1), and normal brain (5) were immunostained for β -APP. A semiquantitative assessment of the severity of axonal staining was made. Corresponding hematoxylin-eosin-stained sections were examined for the presence of axonal swellings.

Results.—Immunostaining for β -APP identified diffuse axonal injury in 5 of 7 infants with shaken baby syndrome and 2 of 3 infants with blunt head trauma. Immunoreactive axons were easily identified and were present in the majority of the sections examined. By contrast, hematoxylin-eosin staining revealed axonal swellings in only 3 of 7 infants with shaken baby syndrome and 1 of 3 infants with blunt head trauma. Most of these sections had few if any visible axonal swellings, which were often overlooked on initial review of the slides. No β -APP immunoreactivity was observed in any of the 7 control cases.

Conclusions.—Immunostaining for β -APP can easily and reliably identify diffuse axonal injury in infants younger than 1 year and is considerably more sensitive than routine hematoxylin-eosin staining. We recommend its use in the forensic evaluation of infants with fatal craniocerebral trauma.

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The brains of individuals with closed head injury are frequently subjected to considerable acceleration-deceleration forces that can cause widespread “shear” damage to axons, namely, diffuse axonal injury (DAI).¹ Although DAI can occur in both adults and children, the brains of young infants are thought to be particularly susceptible for a variety of reasons, including the relatively large size of the head, weakness of neck musculature, larger subarachnoid space, and higher brain water content.^{2,3} Infants who have sustained intentional whiplash movement of the head, commonly referred to as the shaken baby syndrome (SBS), almost invariably have neuropathologic findings indicative of acceleration-deceleration injury.^{2,4} These changes include subdural and subarachnoid hemorrhages, bilateral retinal hemorrhages, cerebral edema, and DAI. Victims of SBS often become fatalities, and survivors suffer developmental delay.⁵

Diffuse axonal injury can be detected at autopsy with use of conventional histologic staining methods, such as hematoxylin-eosin (HE) or silver impregnation techniques, which demonstrate the characteristic axonal retraction balls or swellings.¹ However, axonal swellings are often difficult to recognize in HE sections, so this method alone may entirely miss or markedly underestimate the severity of DAI in a given case. Axonal swellings are easier to identify with silver stains, but since this method labels both normal and damaged axons, otherwise normal irregularities in axon diameter could be misinterpreted as evidence of axonal injury. Also, some silver techniques (eg, Bodian) do not stain all axonal swellings.⁶ Immunohistochemical staining for neurofilament subunits⁷ and ubiquitin⁶ has also been used to improve detection of DAI in tissue sections, but neither has proved ideal.⁸

Recently, immunohistochemical staining for β -amyloid precursor protein (β -APP) is extremely sensitive in identifying damaged axons and has emerged as the method of choice for detecting DAI.^{8–13} A constituent neuronal transmembrane glycoprotein, β -APP is transported by fast anterograde axoplasmic flow.¹⁴ In DAI, either tearing or mechanical stress to the axon may lead to irreversible damage to the cytoskeleton and disruption of normal axonal transport,¹⁵ the latter causing transported proteins, including β -APP, to accumulate within the axon at or near

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Case No.	Age, Mo/Sex	Cause of Death	Manner of Death	Approximate Survival, h	Brain weight, g	Subdural Hemorrhage	Subarachnoid Hemorrhage	Retinal Hemorrhage	Other Findings
1	3/M	SBS	HOM	0	530	Y	Y	Y	White matter gliosis, mild
2	10/F	SBS	HOM	53	1150	Y	Y	Y	CE, pulmonary infarct
3	4/M	SBS	HOM	51	950	Y	Y	Y	CE, AHE, rib fracture
4	2/M	SBS	HOM	68	640	Y	Y	Y	CE, AHE, SGH, rib fracture
5	2/F	SBS	HOM	24	600	Y	Y	Y	CE, rib fracture, abdominal injuries
6	3.5/M	SBS	HOM	6	785	Y	Y	Y	AHE
7	7/M	SBS	HOM	2544	?	Y	Y	Y	Severe neuronal loss, gliosis
8	4/M	BHT	HOM	0	710	Y	Y	?	Brain lacerations, skull fracture
9	4/F	BHT	HOM	0	700	Y	Y	?	Brain lacerations, skull fracture
10	2/M	BHT	HOM	0	370	Y	Y	Y	Skull and rib fractures, old brain injury
11	2/F	SUF	ACC	0	478	N	N	?	Normal brain
12	1/M	SUF	ACC	0	530	N	N	?	Normal brain
13	12/F	DRW	HOM	0	1420	N	N	?	CE, scalp and skin contusions
14	23/M	PNEU	NAT	N/A	1325	N	N	N	Normal brain
15	11/M	PNEU	NAT	N/A	970	N	N	N	Normal brain
16	22/F	PNEU	NAT	20	1150	N	N	N	CE, AHE
17	12/F	SIDS	NAT	0	880	N	N	N	Normal brain

* SBS indicates shaken baby syndrome; BHT, blunt head trauma; SUF, suffocation; DRW, drowning; PNEU, pneumonia; SIDS, sudden infant death syndrome; HOM, homicide; ACC, accident; NAT, natural; N/A, not available; ?, not known; Y, yes; N, no; CE, cerebral edema; and AHE, acute hypoxic-ischemic encephalopathy.

the site of injury. Immunostaining for β -APP is inherently superior to both silver impregnation and neurofilament immunostains since only injured axons are labeled by the β -APP method and more subtle degrees of axonal injury (not only axonal swellings) can be identified.¹¹ In addition, β -APP immunostaining can be observed as early as 2 hours after injury,¹² whereas a postinjury survival interval of at least 12 to 24 hours is required in order for axonal swellings to be identified by HE,¹ silver stains,¹ or immunostaining for ubiquitin⁶ or higher-molecular-weight neurofilament subunits.⁷

Relatively few studies have evaluated the effectiveness of various histologic methods, including immunohistochemical staining, in detecting DAI in infants younger than 1 year. Although most published series have included occasional children or young infants with DAI, only Vowles et al¹⁶ specifically addressed this issue. They used several different silver impregnation methods to investigate DAI in 9 infants (≤ 5 months old) who had sustained fatal, nonaccidental closed head injury. They found that most silver stains, with the exception of the Naoumenko and Feigin technique,¹⁷ failed to identify DAI, which they attributed to the smaller, more variable diameter and incomplete myelination of axons at this age. To our knowledge, the effectiveness of β -APP immunohistochemical staining to detect DAI in young infants has not been investigated systematically. In this study, we evaluated β -APP axonal immunostaining in a series of infants younger than 1 year whose death was attributed to either SBS or blunt head trauma (BHT).

MATERIALS AND METHODS

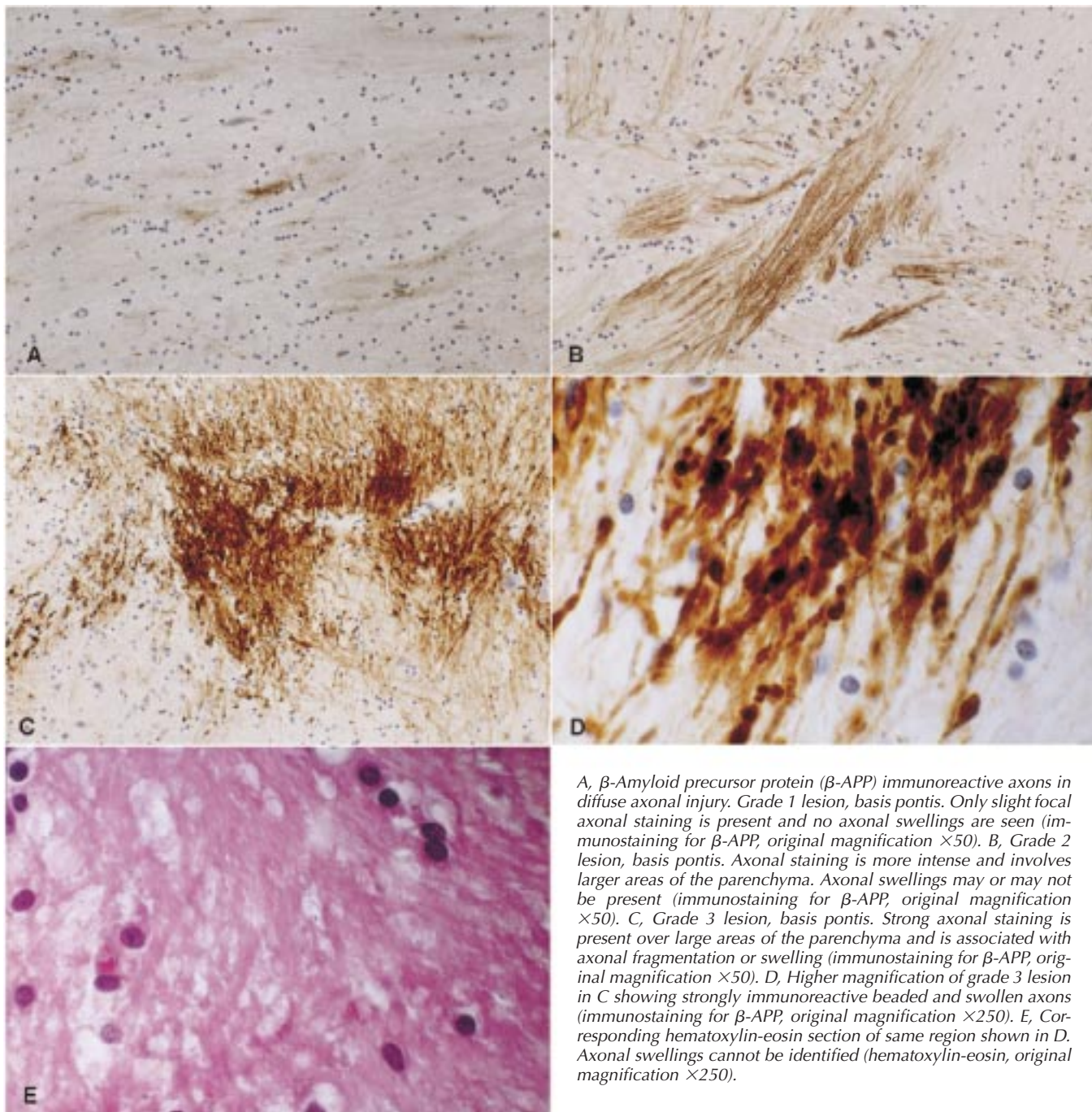
Case Material

Archival paraffin-embedded blocks of autopsy brain sections were obtained from the Metropolitan Dade County Medical Examiner Department, Miami, Fla; Palm Beach County Medical Examiner Department, Palm Beach, Fla; Office of the Chief Medical Examiner, Boston, Mass; and the University of Massachusetts Medical Center, Worcester. All brains had been fixed in 10% buff-

ered formalin for 2 to 3 weeks prior to sectioning. The anatomic areas sampled for histologic examination usually included representative blocks of cerebral cortex, subcortical white matter, basal ganglia and internal capsule, midbrain, pons, and medulla; only a few cases included blocks of hippocampus, thalamus, corpus callosum, and cerebellum. The cases included infants with well-established SBS (7 cases), nonaccidental BHT (3), accidental suffocation (2), drowning (1), pneumonia (3), and sudden infant death syndrome (1) (Table 1). The infants with SBS (cases 1–7) ranged in age from 2 to 10 months. All SBS cases had a clinical history of whiplash shaking of the head and had autopsy findings of subdural, subarachnoid, and bilateral retinal hemorrhages but did not have gross neuropathologic changes suggestive of DAI, such as focal tissue tears, hemorrhages, or gliding contusions. The recorded time intervals between the shaking episode and death ranged from 0 to 2544 hours. The infants who died from nonaccidental BHT (cases 8–10) ranged in age from 2 to 4 months. All had skull fractures, subdural and subarachnoid hemorrhages, and cerebral lacerations or contusions. None survived long enough to be hospitalized for their injuries. Neither the SBS cases nor the BHT cases showed evidence of herniation. The infants who died from suffocation, drowning, sudden infant death syndrome, and pneumonia ranged in age from 1 to 23 months. The brains were normal in all but 2 of these infants (cases 13 and 16), both of which had cerebral edema and, in case 16, also acute hypoxic-ischemic injury.

Immunohistochemistry

Sections from the paraffin blocks were cut at 4 μ m, heated at 60°C for 30 minutes, and then deparaffinized and hydrated through a series of xylenes and alcohols. The sections were then subjected to antigen retrieval by microwaving in 0.01 mol/L citrate buffer (pH 6.0) for 5 minutes at 800 W. Following replenishment of this solution, the slides were microwaved again for an additional 5 minutes and then allowed to cool for 20 minutes. Immunohistochemical staining for β -APP was performed on a TechMate 1000 (Ventana Medical Systems, Tucson, Ariz) automated immunostainer using the avidin-biotin complex procedure. The specific monoclonal antibody used (clone 22C11, Boehringer Mannheim, Indianapolis, Ind) recognizes an N-terminal epitope (amino acids 60–100) of the β -APP protein. The primary antibody was used at a dilution of 1:160. Following a hydrogen



A, β -Amyloid precursor protein (β -APP) immunoreactive axons in diffuse axonal injury. Grade 1 lesion, basis pontis. Only slight focal axonal staining is present and no axonal swellings are seen (immunostaining for β -APP, original magnification $\times 50$). B, Grade 2 lesion, basis pontis. Axonal staining is more intense and involves larger areas of the parenchyma. Axonal swellings may or may not be present (immunostaining for β -APP, original magnification $\times 50$). C, Grade 3 lesion, basis pontis. Strong axonal staining is present over large areas of the parenchyma and is associated with axonal fragmentation or swelling (immunostaining for β -APP, original magnification $\times 50$). D, Higher magnification of grade 3 lesion in C showing strongly immunoreactive beaded and swollen axons (immunostaining for β -APP, original magnification $\times 250$). E, Corresponding hematoxylin-eosin section of same region shown in D. Axonal swellings cannot be identified (hematoxylin-eosin, original magnification $\times 250$).

peroxide block of endogenous peroxide and a serum blocking step, the slides were incubated with the primary antibody for 45 minutes followed by brief buffer washes and then incubation in a cocktail of biotinylated anti-mouse IgG/IgM and anti-rabbit IgG (Ventana) for 30 minutes. The sections were washed, incubated in avidin-biotin complex (Ventana) for 30 minutes, washed, and then reacted with diaminobenzidine and hydrogen peroxide to visualize the end product. Sections were counterstained with hematoxylin. A duplicate set of slides stained in the same manner but with use of normal mouse serum for the β -APP antibody served as a negative control.

A semiquantitative determination of β -APP axonal immunoreactivity was made with use of a modification of the grading system proposed by Gentleman et al.¹¹ In our scheme, grade 0

represented complete absence of axonal staining. Grade 1 was assigned when axonal staining was slight and no axonal swellings were discerned (Figure, A). Grade 2 was used when axonal staining was more intense and/or a larger proportion of a particular anatomic region was involved (Figure, B). Axonal swellings could also be found but were not required. Grade 3 was reserved for sections showing extensive β -APP immunostaining throughout large areas in association with axonal fragmentation or swelling (Figure, C and D). Each section was analyzed concurrently by 2 of us (A.M.G., T.W.S.), and consensus was reached. Cases 1 through 5 had been previously submitted as consultation cases before this study was performed; thus, we were fully aware of the clinical history and pathologic findings in these cases; cases 6 through 13 (submitted by M.D.B.) were analyzed blindly with-

Table 2. Diffuse Axonal Injury: Comparison of β -Amyloid Precursor Protein (β -APP) Immunostaining With Hematoxylin-Eosin (HE) Staining*

Case No.	Cause of Death	Subcortical White Matter	Internal Capsule/ Basal Ganglia	Hippocampus	Midbrain	Pons	Medulla
1	SBS	1†/N‡	2/N	0/N	3/N	1/N	N/A
2	SBS	1/N	3/N	3/N	N/A	3/N	N/A
3	SBS	0/N	1/Y	N/A	1/N	3/Y	N/A
4	SBS	N/A	3/N	N/A	3/N	3/N	3/Y
5	SBS	0/N	N/A	N/A	N/A	2/N	2/Y
6	SBS	0/N	0/N	N/A	N/A	0/N	N/A
7	SBS	0/N	N/A	N/A	0/N	0/N	0/N
8	BHT	N/A	0/N	N/A	0/N	N/A	0/N
9	BHT	0/N	1/N	N/A	1/N	N/A	N/A
10	BHT	1/N	3/Y	3/N	N/A	3/Y	3/N
11	SUF	0/N	N/A	N/A	N/A	N/A	0/N
12	SUF	0/N	0/N	0/N	0/N	N/A	0/N
13	DRW	0/N	0/N	N/A	N/A	N/A	0/N
14	PNEU	0/N	0/N	N/A	N/A	N/A	0/N
15	PNEU	0/N	N/A	N/A	0/N	0/N	N/A
16	PNEU	0/N	0/N	N/A	N/A	0/N	N/A
17	SIDS	0/N	N/A	0/N	N/A	0/N	0/N

* SBS indicates shaken baby syndrome; BHT, blunt head trauma; SUF, suffocation; DRW, drowning; PNEU, pneumonia; SIDS, sudden infant death syndrome; and N/A, section not available.

† β -APP grade: 0 indicates no axonal staining; 1, slight axonal staining, no swellings; 2, increased axonal staining intensity or more axons stained, no or few swellings; 3, extensive damage throughout large areas of white matter; axonal swellings.

‡ Axonal swellings observed on HE stain (yes [Y]/no [N]).

out prior knowledge of either the clinical or the pathologic findings.

For every brain section stained with β -APP, a counterpart HE-stained section was examined for the presence of axonal swellings. Although an initial attempt was made to precisely match the sites of β -APP immunoreactivity with any axonal swellings seen in the HE-stained sections, this proved unsatisfactory. We therefore chose simply to record the presence or absence of axonal swellings in any region of the corresponding HE-stained slide (Table 2).

RESULTS

Shaken Baby Syndrome

Immunohistochemical staining for β -APP showed evidence of DAI in 5 of 7 cases of SBS (cases 1–5, Table 2). Most importantly, some degree of axonal immunoreactivity was observed in almost all sections examined from each case, although blocks of the same or similar anatomic region were not always available for comparison in every case. The most severe degree of DAI (grade 2–3) was observed in the internal capsule, midbrain, pons, and medulla, with less severe damage (grade 1) present in the subcortical white matter. Evidence of DAI was also present in other regions of the basal ganglia (eg, interface between globus pallidus and putamen, pencil fibers of putamen, external capsule) and in the thalamus. Within the brainstem, the specific anatomic regions that most often demonstrated DAI (all cases combined) included midbrain tegmentum, substantia nigra, cerebral peduncles, transverse pontine fibers, descending tracts in basis points, superior, middle, and inferior cerebellar peduncles, medial lemniscus, hilum of inferior olive, and lateral medullary tegmentum. Blocks of the corpus callosum were not available in any of the SBS cases. By contrast, the corresponding HE-stained sections showed axonal swellings in only 3 of the 5 SBS cases that had extensive DAI demonstrated immunohistochemically (cases 3–5). In these 3 cases, the axonal swellings were observed in few of the HE-stained slides and were generally limited to very small regions of the respective sections. Often no axonal swellings were

seen within a region that had obvious, often intense axonal β -APP immunopositivity (Figure, D and E). Two SBS cases (cases 6 and 7) showed no evidence of DAI by either β -APP immunohistochemical or conventional HE histologic methods.

Blunt Head Trauma

The β -APP-immunoreactive axons indicative of DAI were observed in 2 of the 3 cases of BHT (cases 9 and 10, Table 2). Almost all the immunostained sections from the 2 BHT cases showed some degree of DAI, which generally involved the same anatomic regions as in the SBS described above. By contrast, only case 10 had axonal swellings visible in the HE-stained sections. The axonal swellings were extremely difficult to find and were evident in only 2 of the 5 HE slides available for review in this case. One BHT case (case 8) showed no evidence of DAI by either β -APP immunohistochemical or conventional HE histologic methods.

Nontraumatic Control Cases

The infants who died from suffocation (cases 11 and 12), drowning (case 13), pneumonia (cases 14–16), and sudden infant death syndrome (case 17) showed both complete absence of β -APP axonal immunostaining as well as absence of visible HE-stained axonal swellings in all sections examined.

COMMENT

In cases of suspected fatal child abuse, autopsy findings are crucial in determining whether the manner of death was due to natural causes, unintentional injury, or homicide. The perpetrators of fatal child abuse often contrive explanations for an infant's death, claiming it to be "natural" or "accidental."¹⁸ When an infant's death is considered to be the result of intentional craniocerebral trauma, the mechanism of injury usually involves vigorous, often repetitive shaking (SBS), BHT, or a combination of the two. Although these cases frequently have associated pathologic

ic findings, such as subdural, subarachnoid, and retinal hemorrhages suggesting a likely mechanism of injury, the presence of DAI remains the best and most specific indicator of the acceleration-deceleration forces sustained by the brain itself under these circumstances.⁴ From the medical-legal perspective, the presence of DAI, even if few other neuropathologic changes are evident, might more likely favor a homicidal or accidental rather than natural manner of death. Thus, any method that facilitates detection of DAI would have considerable importance in the forensic evaluation of fatal child abuse.

In this study, we have shown that β -APP immunohistochemical staining is a highly sensitive technique for identifying DAI in infants younger than 1 year. Our findings are essentially identical to those observed by other investigators in older individuals using the same or similar methods.⁸⁻¹³ Not surprisingly, β -APP immunostaining is considerably superior to conventional HE staining in identifying not only the presence but also the extent or severity of DAI in a given case. Although we did not compare the effectiveness of β -APP immunohistochemical staining with other staining methods that have been used to detect DAI, Gentleman et al¹¹ clearly demonstrated the superiority of β -APP immunostaining over silver impregnation in detecting DAI in their series.

Despite the advantages afforded by β -APP immunostaining, there are some limitations and potential pitfalls that must be considered. Both the time interval between injury and death and the state of cerebral vascular perfusion during this period may influence the ability of this method to detect DAI. Prior studies have shown that β -APP-immunopositive axons are not likely to be observed if the survival period between injury and death is less than 2 hours.¹² This may reflect both the relative insensitivity of the immunohistochemical technique to detect very small amounts of β -APP plus the fact that accumulation of β -APP is an evolving, time-dependent process.^{12,19} However, it should also be emphasized that in some cases the true survival interval may not be accurately known and may not be reflected by hospital, police, or emergency transport records. Also, in some SBS cases, episodes of shaking could have occurred well before actual death of the infant and certainly within the 2-hour period when injured axons could become detectable by β -APP immunostaining. Thus immunohistochemical evaluation of DAI should not be omitted simply because the survival interval is assumed to be too short.

Second, it is important to recognize that, for β -APP to accumulate within an injured axon, there must be an intact axoplasmic transport mechanism, which is an energy-dependent process.²⁰ In some cases, the initial brain injury could be complicated by the early development of severe hypoxia-ischemia due to either systemic cardiopulmonary arrest or impaired cerebral vascular perfusion resulting from increased intracranial pressure secondary to cerebral edema.¹ If this occurs within the critical 2-hour period following injury, the resulting impairment of axoplasmic transport would allow very little, if any β -APP to accumulate.¹⁰ On the other hand, β -APP immunoreactivity could still be observed in injured axons if hypoxia-ischemia occurred concomitant with or following this critical time period. Bearing in mind that the histologic recognition of acute ischemic cell injury requires a 12- to 24-hour survival period with intact cerebral vascular perfusion,²¹ it is not surprising that 3 of our cases of SBS had both

widespread acute hypoxic-ischemic encephalopathy and immunohistochemical evidence of DAI.

Although theoretically it might be possible for hypoxia-ischemia alone to cause sufficient accumulation of β -APP within axons that could be detected immunohistochemically, we did not observe this in our control (case 16) or in other cases of nontraumatic acute anoxic encephalopathy studied subsequently (unpublished data). We postulate that under these circumstances, axoplasmic flow would be more uniformly impaired along the entire axon, thus preventing focal accumulation of any transported substances at specific sites in the axon. Whatever β -APP remained would be so evenly distributed over the length of the axon that its immunohistochemical detection would be difficult. Furthermore, we often observed higher than normal β -APP immunoreactivity within neuronal cell bodies in these cases, which suggests that severe anoxia may completely shut down the axonal transport mechanism leading to excessive accumulation of β -APP within the soma where it is synthesized.

An excessively long period between injury and death may likewise pose a problem with regard to the immunohistochemical detection of DAI. It is unclear how long β -APP immunoreactivity may persist. Some axons may be repaired with subsequent restoration of axoplasmic flow, whereas others may continue to degenerate and eventually disappear.¹² Blumbergs et al²² demonstrated β -APP axonal staining in individuals with mild head injury who survived as long as 99 days. However, few if any stainable axons might be present in more severe degrees of cranio-cerebral trauma where extensive DAI may be compounded by supervening hypoxic-ischemic injury or other focal damage. This is probably the best explanation for the lack of β -APP immunoreactivity seen in the SBS infant (case 7) who survived 106 days.

Adequacy of sampling must be considered when evaluating β -APP immunostaining or other methods used to detect DAI. Although DAI by definition involves many diverse regions of the brain, both conventional histologic and immunohistochemical techniques have shown that certain sites are particularly vulnerable.¹ McKenzie et al¹² found that axonal β -APP immunoreactivity was most often present in the brainstem (95% of cases), in the internal capsule and thalamus (80%), and in parasagittal white matter and corpus callosum (70%). However, involvement of a particular site in a given case may show considerable variation. For example, McKenzie et al¹² noted that several of their cases had β -APP-immunopositive axons in the internal capsule or thalamus but not in the brainstem. Geddes et al¹³ found that a DAI "screening" protocol limited to 3 sites (parasagittal white matter and corpus callosum, internal capsule, rostral brainstem) failed to identify DAI in 59% of their cases in which it was present by more extensive sampling. In our retrospective series, the actual number of sites sampled in the SBS and BHT cases was relatively small. Yet we successfully demonstrated DAI in the majority of these cases. Furthermore, it is unclear whether our failure to observe DAI in 3 of our cases was related to sampling or other factors, such as time interval or state of vascular perfusion. From a practical perspective, an initial, limited sampling protocol may be adequate if DAI is present in these blocks. However, since tissue samples are sometimes discarded or otherwise become unavailable, it is not unreasonable to suggest that a more

extensive sampling for DAI as outlined by Geddes et al¹³ be carried out at the time of brain sectioning.

When selecting blocks for histologic evaluation of DAI, it is also important to be aware that any focal destructive lesion, be it an infarct, contusion, or hemorrhage, may cause localized axonal injury that leads to accumulation of immunoreactive β -APP.^{10,13,23} Certain structures, such as the brainstem, may be particularly vulnerable to the secondary effects of increased intracranial pressure. Thus, care must be taken during sampling to avoid such lesions or to be aware of their presence on subsequent microscopic review of HE-stained sections so as not to misinterpret axonal immunoreactivity as DAI at these sites.

As a final consideration, one must always bear in mind that failure to identify DAI by β -APP immunostaining or any other method in a case of suspected infant abuse may simply be attributable to the fact that the presumed head injury either did not occur or was of insufficient magnitude to cause DAI. The conclusion that DAI is not present, however, should not be made until all other possible explanations for lack of immunostaining, as discussed above, have been adequately addressed.

In conclusion, β -APP immunostaining appears to be an effective method for detecting DAI in infants who have suffered traumatic brain injury and thus may be an important adjunct in the forensic evaluation of infants with suspected nonaccidental craniocerebral trauma, including SBS and BHT. However, our investigation was based on a relatively small number of cases, and further studies of a greater number and variety of cases are clearly warranted.

Potential interpretative difficulties that may arise when using this method can be avoided if the following caveats are kept in mind: (1) β -APP immunostaining will not reliably detect DAI if the time of survival is less than 2 hours; (2) adequate cerebral vascular perfusion must have occurred during the initial period of survival to ensure adequate function of the axoplasmic transport system; (3) β -APP-immunoreactive axons may not be observed if an excessively long time has elapsed between initial injury and death; (4) an adequate number of brain regions known to be susceptible to DAI must be sampled for immunohistochemical staining; and (5) β -APP axonal immunoreactivity should not be interpreted as DAI if it is found in close proximity to other focal destructive lesions, such as hemorrhages, infarcts, or contusions.

Note.—Subsequent to acceptance of this manuscript for publication, we have become aware of an additional study utilizing β -amyloid immunohistochemistry to detect diffuse axonal injury in shaken baby syndrome by Shannon et al.²⁴

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