Acta Neuropathol. (Berl.) 47, 1–11 (1979)

Acta Neuropathologica

Original Works

The Morphopathologic Substrates of Concussion?

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Summary. Neuronal inundation with i.v. infused horseradish peroxidase was studied following concussive brain injury by means of both light and electron microscopy. In animals sustaining mechanical brain injury of insufficient intensity as to elicit either microscopic intraparenchymal hemorrhage or other neuropathological change, yet of sufficient intensity as to provoke a physiological concussive response, vascular peroxidase exudation concomitant with neuronal peroxidase inundation occurred throughout the raphe and reticular core. Initially such inundated neurons were totally flooded with the tracer and as such appeared reminiscent of cells visualized in Golgi preparations. However, over the course of a 24-h period these peroxidase flooded neurons apparently organized the peroxidase into vesicles and vacuoles which assumed a perinuclear position from where the peroxidase ultimately reached both the nucleus und nucleolus. It was remarkable that these events occurred without any evidence of subcellular alteration. We interpret such initial inundation with this protein tracer, its ultimate reorganization, and its nuclear and nucleolar uptake as being consistent with some form of subtle and transient neuronal perturbation. We speculate that as such this neuronal perturbation may constitute a morphological correlate of the concussive episode.

Key words: Experimental Concussion – Peroxidase, neuronal uptake – Electron microscopy

In the genesis of coma following blunt trauma to the head, much emphasis has been laid on neuronal damage in the brain stem affecting nuclear groups in the reticular formation (Brown et al., 1972; Chason et al., 1958, 1966; Friede, 1961; Groat et al., 1944). A recent theory on the pathology of head injury by Ommaya and Gennarelli (1974) has proposed that in diffuse acceleration-deceleration injury the brain is progressively and concentrically injured from without to within as the force increases. Thus, primary brain stem damage can occur only in the presence of diffuse hemispheric damage (axonal disruption); thus, although hemispheric damage can occur without brain stem injury, the converse cannot occur.

One of the main problems of interpreting traumatic brain damage post mortem is the difficulty of distinguishing primary impact damage from secondary damage due to ischemia and brain shift. This is of particular importance in brain stem lesions. In a carefully selected series of cases, Hume Adams and his colleagues (1977) have provided considerable support for Ommaya's centripetal theory of brain injury by finding no evidence of primary brain stem damage but considerable hemispheric injury in a group of patients who remained comatose following blunt head injury and had no evidence of raised intracranial pressure or brain shift. In no case have these investigators observed primary brain stem lesions in the absence of diffusely spread hemispheric damage.

We have found some support in vivo for this view by demonstrating that in comatose head injury patients, decerebrate rigidity and prolonged coma were most closely correlated, not with abnormal brain stem electrical activity (far field auditory potentials), but with hemispheric dysfunction (abnormal somatosensory and visual evoked potentials) (Greenberg et al., 1977).

This view entirely fails, however, to explain the phenomenon of temporary loss of consciousness following milder head injury in which the patient makes an evidently complete recovery and no overt neuronal cellular or vascular damage occurs. It would seem reasonable to seek a physiological or ultrastructural substrate in the brain stem in order to explain such temporary loss of consciousness after blunt head injury.

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0001-6322/79/0047/0001/$2.20

In regard to this issue of concussive head injury, we have recently studied cerebrovascular permeability to horseradish peroxidase in a fluid percussion model of blunt head trauma in cat (Povlishock et al., 1978b). In this model, severe pressure pulses of 4.0 atmospheres (3,000 mm Hg) and above produce visible brain stem injury consisting of parenchymal hemorrhage associated with loss of brain stem reflexes, and apnea with disappearance of EEG activity (Sullivan et al., 1976). Milder injuries in the range of two atmospheres produce a temporary loss of consciousness with brief apnea, elevated blood pressure, and a temporary suppression of EEG activity, but no visible brain damage at the light microscopic level. At this milder level of injury, we have described an increased vascular permeability to i.v. injected horseradish peroxidase and as such have noted that the increased vascular permeability was confined to the raphe and reticular core of the brain stem (Povlishock et al., 1978b). Serial section light and electron microscopic analysis failed to reveal any evidence of mechanical vascular disruption; yet, in these animals the protein apparently reached the parenchyma through a dramatic form of transendothelial vesicular transport. Such vesicular activity ferried the peroxidase from the vascular luminal surface to extrude it into the perivascular basal lamina from where the reaction product was free to flood the interstices of the surrounding brain stem parenchyma. Concomitant with this peroxidase spread into the parenchyma, both raphe and reticular neuronal somata and appendages were observed to be diffusely flooded with the protein tracer (Povlishock et al., 1978a). Comparable neuronal flooding with peroxidase has been described after topical application of horseradish peroxidase in the cerebral cortex and it was suggested that such flooding was linked to cell membrane damage (Turner and Harris, 1974; Vanegas et al., 1978). Furthermore, Keefer (1978) and Vanegas et al. (1978) have recently suggested that such peroxidase flooding may not necessarily represent permanent cell damage, but rather may reflect only a transient membrane perturbation. Thus, in view of such a statement, the contention arises that the peroxidase flooding of the reticular and raphe neurons occurring in low-grade concussive injuries uncomplicated by hemorrhages, may be linked to subtle neuronal membrane alterations which may be of a transient nature. The implications of such a contention are provocative and perhaps identify the morphological substrates of concussive injury. Due to the significance of such a hypothesis, the present study investigates neuronal flooding with horseradish peroxidase in response to concussive injuries of severity insufficient to cause either intraparenchymal hemorrhage or other relevant neuropathological change. The fate of these peroxidase flooded cells as well as the fate of the peroxidase contained therein will be evaluated over a 24-h period to determine if such flooded cells are only transiently perturbed or are they irreversibly damaged.

Materials and Methods

Experimental Animals

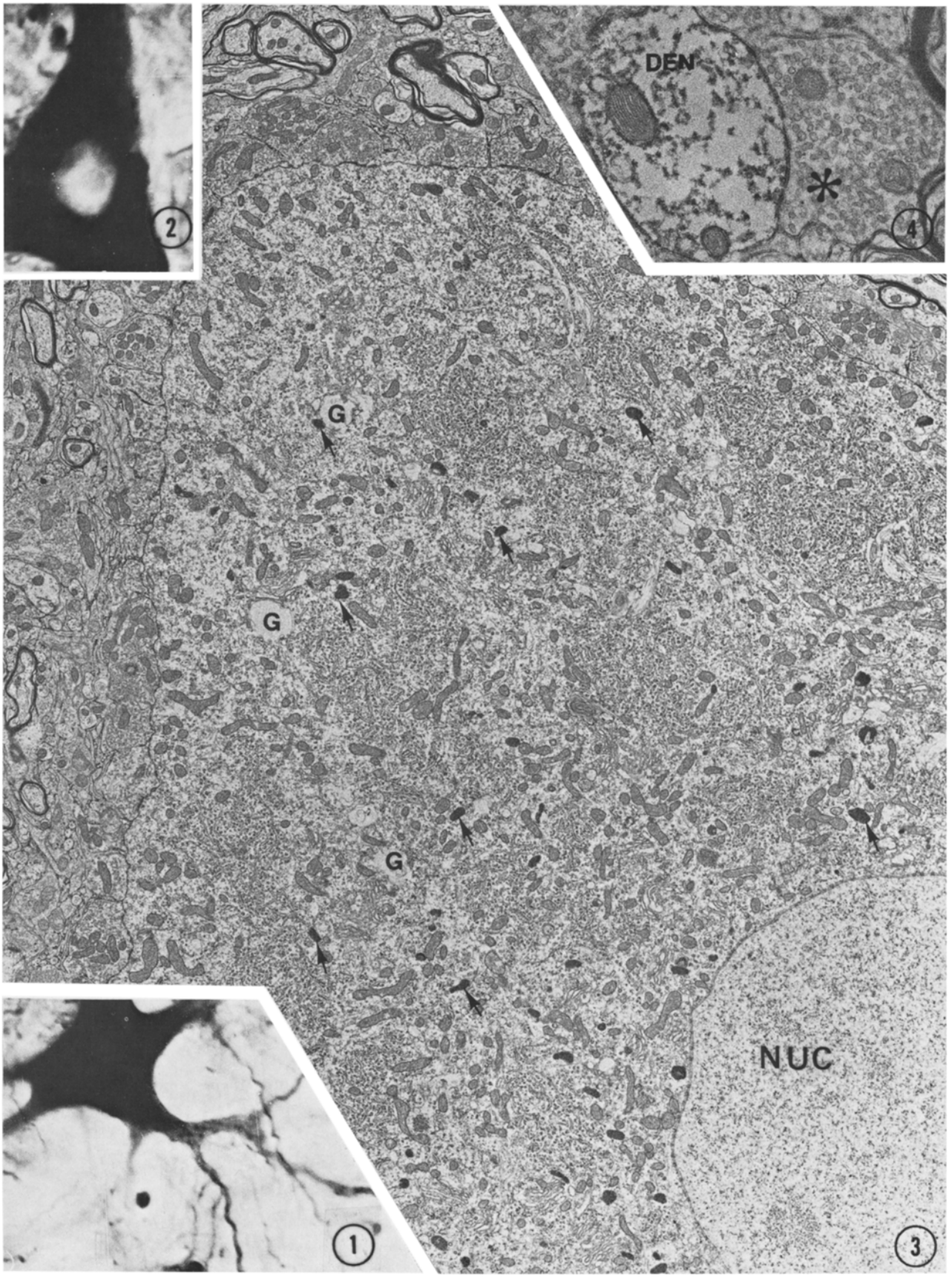
In all, 22 cats weighing 2.5–4.0 kg were used in the present study. Each animal was anesthetized i.v. with a solution of 1% sodium Brevital in normal saline and was subsequently prepared for the induction of mechanical brain injury in accordance with our previously described protcols (Povlishock et al., 1978b; Sullivan et al., 1976). The femoral artery was cannulated to monitor blood pressure and a tracheostomy was performed. The animal was placed in a stereotaxic frame, and via the use of a high speed electric drill an 11 mm diameter craniectomy centered over the sagittal sinus was performed. Within this craniectomy a hollow, metal central injury shaft was insetted and was fixed into place with dental acrylic cemnt. Once the acrylic hardened, the central injury shaft was tightly connected to a transducer housing which in turn was affixed to a plexiglass cylinder which was closed at the opposite end by a plexiglass cork mounted on 0 rings. The whole system was then filled with saline at 37° C. At this point the animal was removed from the stereotaxic device and transferred to a supporting frame. The animals were then paralyzed with continuous i.v. infusion of 0.2% solution of succinylcholine chloride. The paralyzed animals were maintained on controlled ventillation on air with added O_2 so that the normal arterial blood gases were maintained in the following range: pCO_2 = 35 mm Hg ± 3 mm Hg; pO_2 = 150 mm Hg; ± 50 mm Hg; pH = 7.35. The temperature was maintained at 37° C by a heating blanket. When the blood pressure, pulse, temperature, and arterial blood gasses were stabilized within the normal range, the animals

Fig. 1. This insert displays a peroxidase inundated neuron seen immediately after a low intensity concussive brain injury. Note that such a cell is reminiscent of one visualized in a Golgi preparation. (2.2 atm lesion, 3-min survival, cleared section) ×260

Fig. 2. This unstained plastic section clearly demonstrates neuronal inundation with peroxidase. Note that the reaction product is conspicuous within the cell cytoplasm, while being absent in the nucleus. (2.2 atm lesions, 3-min survival) ×780

Fig. 3. This electron micrograph is from the same neuron shown in Fig. 2. The peroxidase reaction product is diffusely spread in the cytoplasm as well as being localized within peroxidase containing vesicles and vacuoles (*arrows*). Note that despite occasional dilation of the Golgi apparatus (*G*), the cell otherwise appears normal and the nucleus (*NUC*) displays no evidence of peroxidase inundation. (2.2 atm lesion, 3-min survival) ×8,320

Fig. 4. This micrograph shows that neuronal peroxidase flooding is so complete that even a distal dendritic segment (*DEN*) here contacted by an axon terminal (*asterisk*) clearly reveals the electron dense reaction product. (2.4 atm lesion, 3-min survival) ×22,880



Figs. 1—4

received an i.v. injection of 50–75 mg/kg of horseradish peroxidase, Sigma Type VI, diluted in 3 ml of saline. Five minutes after the peroxidase administration the animals were subjected to mechanical brain injury produced by a 4.8 kg pendulum falling from a predeterminded height to strike the 0 ring mounted plexiglass cork on the injury device. The cork's compression induced a brief, (20 ms) hydraulic pressure transient which was conducted in turn through the saline filled plexiglass cylinder, transducer, and central injury shaft to elicit the elastic deformation of the brain. The severity of the fluid-percussion injuries, as determined via a transducer and recorded on a storage oscilloscope, ranged from 2 to 2.5 atm. In all animals, such injuries elicited an elevated blood pressure and a temporary suppression of EEG activity. After injury, brain tissue was harvested after 3 min, 30 min, 2 h, 6 h, 12 h, and 24 h of survival. In those animals surviving for 6,12, and 24 h the infusion of succinylcholine chloride was ceased and Brevital was administered to allow for the surgical removal of the injury shaft and the closure of all wounds. After such a regimen the animals recovered and appeared normal. After the designated survival times, the animals were perfused transcardially with 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 m Na-phosphate buffer. The brain was left in situ for 2 h after the perfusion, at which point the brain was removed and the brain stem divided into mesencephalic, pontine, and medullary segments which were washed for an additional 2 h in chilled (4° C) Na-phosphate buffer. The brain stem segments were then serially cut at a thickness of 40 μm with an Oxford vibratome. The serial sections were collected in ordered compartments of divided plastic trays which contained chilled (4° C) Na-phosphate buffer, and these sections were washed for an additional 2 h in several changes of phosphate buffer.

Next, the sections were incubated for 45 min at 4° C in 0.05 M tris-HCl buffer containing 5.0 mg of 3-3′ diaminobenzidine per 10 ml of buffer and a final concentration of 0.01% of hydrogen peroxide (Graham and Karnovsky, 1966). The sections were then washed for another 2 h in the phosphate buffer. Alternate serial sections were then either dehydrated, cleared, mounted, and cover-slipped for light microscopic analysis or were further processed for electron microscopy. That tissue processed for light microscopy was most valuable in revealing selective leakage sites containing peroxidase inundated neurons for ultrastructural observation in the adjacent serials. For electron microscopy the appropriate sections were trimmed to include the zone of leakage and neuronal flooding and these tissue segments were postfixed in 2% buffered osmic acid for 2 h, dehydrated in graded ethanols and propylene oxide, and embedded in Epon 812. Thick sections were cut with glass knives and examined to identify the anatomic site of the peroxidase leakage and neuronal inundation. The desired areas were selected and serial ultrathin sections were cut with a diamond knife, picked up on Formvar coated, slotted grids, stained with uranyl acetate and lead citrate, and examined in a Hitachi HU-12 electron microscope.

Control Animals

In that the basic premise of the present study rests upon the contention that neuronal inundation with peroxidase is an atypical occurrence related to the concussive injury, several meticulous control studies were undertaken to insure that neuronal flooding with peroxidase could not occur under other conditions.

Control 1

In order to insure that any observed neuronal flooding was directly related to horseradish peroxidase inundation and not to the presence of some traumatically released diaminobenzidine positive product, in three animals the above mentioned experiments were conducted without the administration of horseradish peroxidase.

Control 2

In order to ascertain whether the raphe and reticular neurons can normally flood with peroxidase in response to a high intracellular concentration of the tracer induced via retrograde axoplasmic flow, such a protocol was so devised. In such experiments a 50% horseradish peroxidase solution containing 2% dimethyl sulfoxide (DMSO), a known accelerator of peroxidase uptake (Keefer, 1978), was injected via a microliter syringe into the dorsolateral funiculus and ventral horn of the lumbar cord of six cats. Such injection sites were chosen as they contained either axons or terminals whose cell bodies of origin are found within the raphe and reticular nuclei (Basbaum et al., 1975; Nyberg-Hansen, 1965). Subsequent to the injection, the animals were allowed to survive for a period of 48 h in order to allow for maximal neuronal uptake of the peroxidase tracer. After this survival period, the animals were then perfused with aldehydes and processed for both the light and electron microscopic visualization of the horseradish peroxidase reaction product in accordance with these protocols previously outlined.

Control 3

Lastly, to insure that the reticular and raphe neurons cannot normally flood with peroxidase in response to a high extracellular concentration of the tracer, such a control study was considered. Unfortunately, as the reticular and raphe neurons cannot be directly exposed to a high extracellular peroxidase concentration without the use of invasive topical applications, the response of these neurons to extracellular peroxidase could not be truely assessed. However, in that all brain stem neurons should respond to a high extracellular concentration of peroxidase in a similar fashion, those superficial brain stem nuclear groups, which could readily be reached by high concentrations of peroxidase diffusing from the subarachnoid space, were employed to test the effect of such peroxidase exposure. To this end, horseradish peroxidase was slowly infused into the cisterna magna of six cats, all of which were monitored through a ventricular needle, in order to insure that no modification of intracranial pressure occurred concomitant with the peroxidase infusion. Subsequent to peroxidase infusion these animals were allowed to survice for periods ranging from 1–3 h at which point they were perfused with aldehydes and processed for the light and electron microscopic visualization of the horseradish peroxidase reaction product.

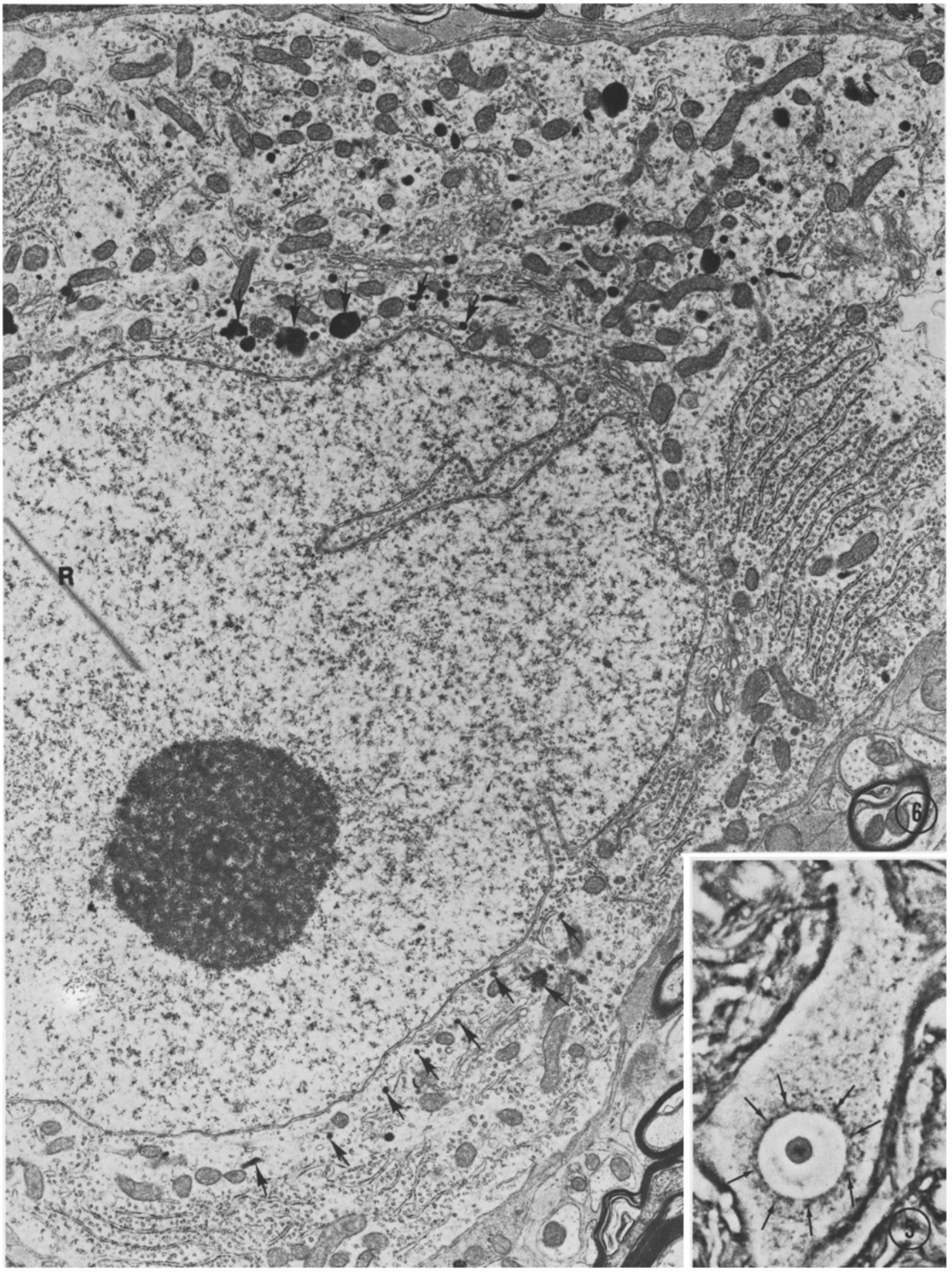
Results

Experimental

At both 3 and 30 min following traumatic injury, numerous peroxidase flooded neurons could be recognized at the light microscopic level within the

Fig. 5. This phase contrast light micrograph of a plastic thick section demonstrates a peroxidase containing neuron. Note that the peroxidase (*arrows*) has assumed a distinct perinuclear position. (2.2 atm lesion, 12-h survival) ×788

Fig. 6. An electron micrograph of a neuron, comparable to that neuron displayed in Fig. 5, again manifests the peroxidase reaction product in perinuclear vesicles and vacuoles (*arrows*). The cytoplasmic organelles appear normal and an intranuclear rodlet (*R*) can be recognized. (2.2 atm lesion, 12-h survival) ×12,600



Figs. 5 and **6**

regions of vascular peroxidase exudation. As such these neurons were confined to the raphe and reticular core. With injuries of increasing severity (2.0→2.5 atm) an increasing number of neurons demonstrated peroxidase inundation. Such neurons were so flooded with peroxidase that their somata, dendritic trees, and axons were readily visible and they appeared reminiscent of cells visualized in Golgi preparations (Figs. 1 and 2). At the fine structural level these flooded cells demonstrated the peroxidase reaction product both diffusely spread throughout the neuronal cytoplasm as well as being localized within vesicles and vacuoles (Fig. 3). Peroxidase inundation of these neurons was so complete, that their peroxidase laden distal dendritic segments scattered throughout the neuropil could be readily recognized (Fig. 4). These observations were confirmed in both stained and unstained sections to eliminate any controversy surrounding the identification of the horseradish peroxidase reaction product from the normal cellular complement of lysosomes and lipofuscin granules. Careful examination of the neuronal cell membranes failed to reveal any evidence of physical damage. Although the neuronal cytoplasm displayed occasional dilated and distended Golgi saccules, all other cytoplasmic constituents appeared unremarkable despite the presence of peroxidase flooding.

Within 2 h of the traumatic brain injury both light and electron microscopic examination revealed flooded neurons within the raphe and reticular core; however, both the number of neurons flooded as well as the intensity of the reaction product contained therein appeared reduced. Serial ultrathin analysis confirmed our previous observation that despite peroxidase flooding such neurons appeared otherwise normal, and now dilated Golgi saccules could no longer be recognized.

Within 6 h of brain injury despite the continued presence of peroxidase in the extracellular space few flooded neurons could be positively identified at the light microscopic level and within these neurons the intensity of the peroxidase reaction product appeared markedly reduced. Ultrastructural examination of these cells revealed that their cytoplam now appeared more electron-lucent and that a proportionally larger number of peroxidase containing vesicles and vacuoles could now be recognized. In that no evidence of cellular degeneration was noted, the fate of those flooded neurons recognized immediately after trauma appeared questionable. However, those same loci, which immediately after trauma displayed numerous inundated neurons, now demonstrated numerous neurons with an electron-lucent cytoplasm laden with peroxidase containing vesicles. Such peroxidase vesicles were readily visualized with phase microscopy and this observation was confirmed at the electron microscopic level.

Twelve hours after traumatic brain injury, no evidence of neuronal flooding was observed; yet, in those loci, which immediately after trauma displayed inundated neurons, numerous neurons laden with horseradish peroxidase containing vesicles, comparable to those observed at the sixth post-traumatic hour, were observed. However, now these peroxidase containing granules had assumed a distinct perinuclear orientation (Figs. 5 and 6). Ultrastructural examination confirmed the perinuclear localization of the peroxidase and again demonstrated that such neurons appeared otherwise normal (Fig. 6). One unique aspect of these neurons, however, was the presence of numerous intranuclear rodlets of various size and configuration (Fig. 6).

Within these same loci 24 h after the traumatic brain injury, the peroxidase reaction product was now observed to be both diffusely spread in neuronal nuclei as well as being selectively sequestered within nucleolar vacuoles (Figs. 7 and 8). Light microscopy readily demonstrated the intranuclear and intranucleolar location of the peroxidase reaction product (Fig. 7), which electron microscopic examination precisely localized (Fig. 8). In conjunction with this nuclear and nucleolar peroxidase uptake, no ultrastructural manifestation of any cellular alteration was noted (Fig. 8). Nuclear rodlets were again visualized.

Controls

Control 1

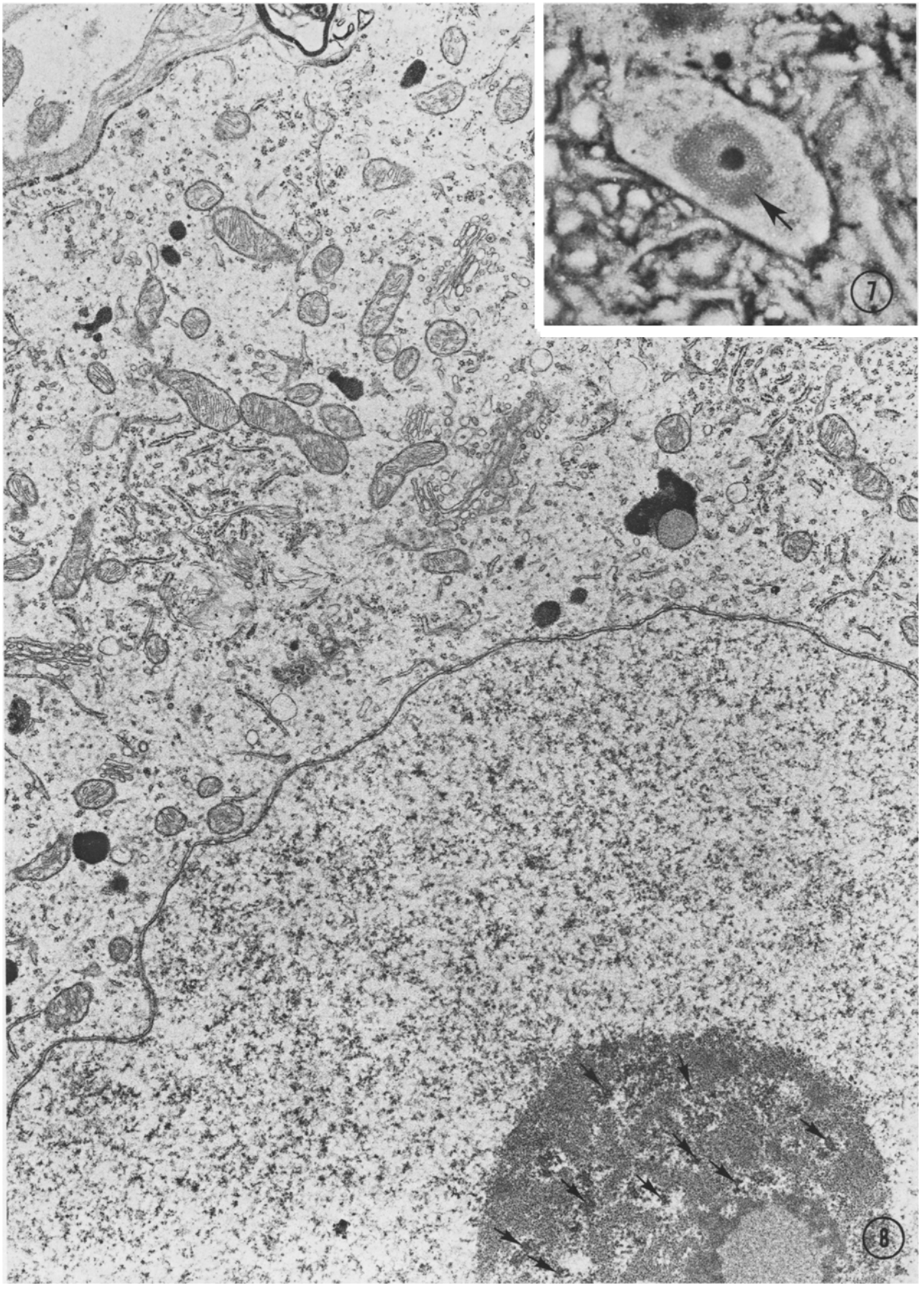
In those animals in which the peroxidase administration was withheld, no evidence of a diaminobenzidine positive reaction product was noted. At either 2 h, 12 h, or 24 h of survival no reaction product could be recognized within the raphe and reticular somata and/or their nuclei. Such a negative finding was consistent in all sections viewed at both the light and electron microscopic level.

Control 2

Those animals, who had received peroxidase injections within the dorsal longitudinal fasciculus and anterior

Fig. 7. Peroxidase is here visualized within both the nucleus (*arrow*) and nucleolus of this plastic thick section. (2.2 atm lesion, 24-h survival) ×405

Fig. 8. Ultrastructural examination of the same neuron shown in Fig. 7 demonstrates the peroxidase reaction product both sequestered within nucleolar vacuoles (*arrows*) as well as being dispersed in the nucleoplasm. Additionally note that all ultrastructural cellular features appear normal. (2.2 atm lesion, 24-h survival) ×22,680



Figs. 7 and **8**

horn of the spinal cord, displayed the peroxidase reaction product within both the raphe and reticular neurons. The reaction product, however, never flooded these cells but rather was confined to cytoplasmic vesicles and vacuoles (Figs. 9 and 10). Never was the peroxidase reaction product localized within either the neuronal nuclei or nucleoli (Figs. 10 and 11).

Control 3

When peroxidase was infused into the cisterna magna, the protein tracer readily penetrated the glia limitans and entered the superficial brain stem parenchyma, where it coursed throughout the extracellular compartment. Once within the medullary extracellular space, no evidence of neuronal flooding with peroxidase was observed irrespective of the peroxidase concentration and/or survival time. The only evidence of neuronal peroxidase uptake was the presence of intracytoplasmic peroxidase containing vesicles and vacuoles whose accumulation was apparently linked to the endocytosis of the protein tracer at the neuronal, dendritic, and axonal membranes. In such neurons no evidence of peroxidase uptake by either the nucleus or nucleolus was visualized.

Discussion

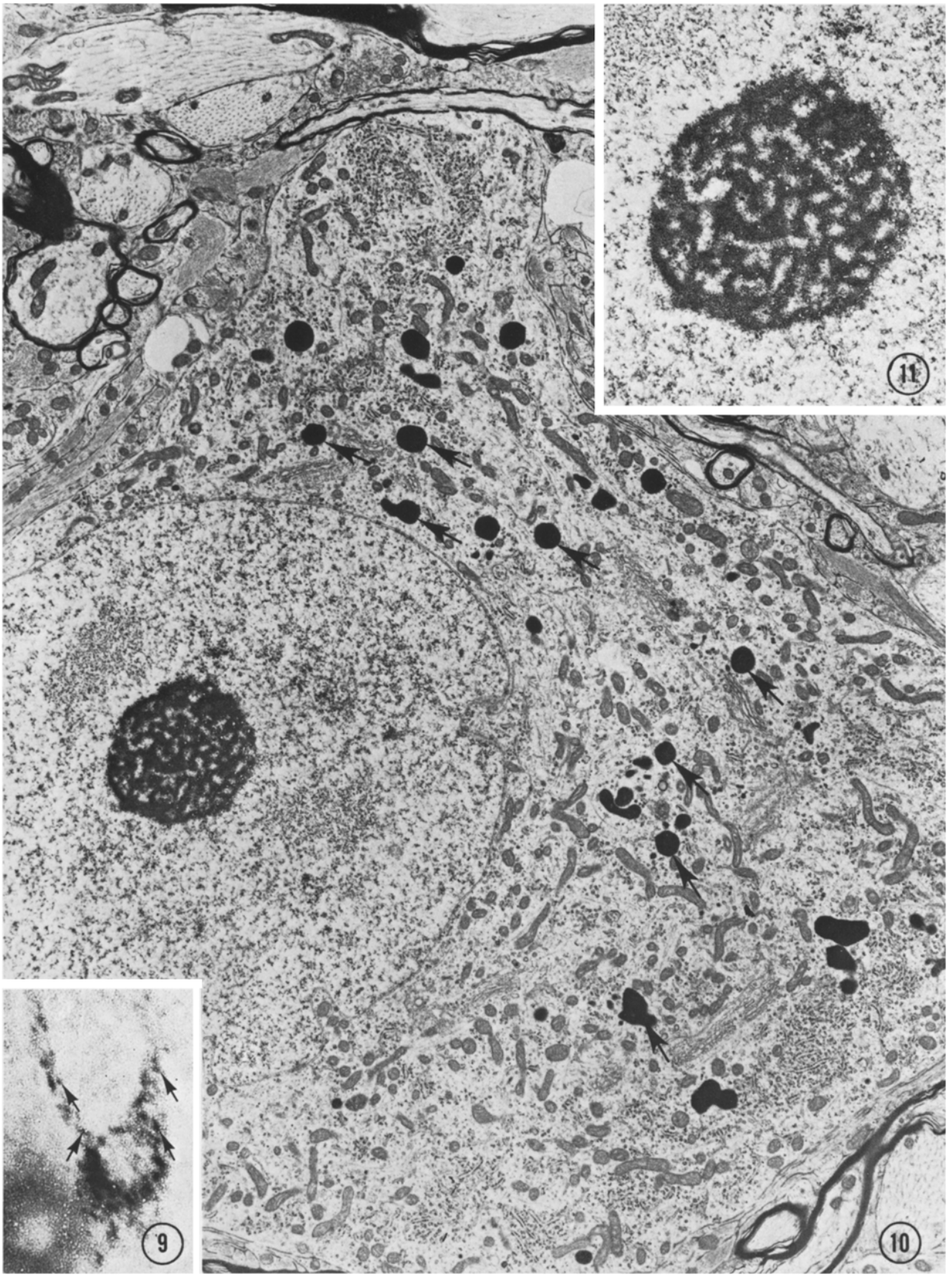
The results of the present study suggest that concomitant with traumatically induced blood-brain barrier dysfunction there occur transient neuronal perturbations reflected by local neuronal inundation with peroxidase. Comparable neuronal flooding with various tracers such as Evan's blue, trypan blue, fluorescein and horseradish peroxidase has been described under a host of experimental and pathological conditions including concussive brain injuries (Griffiths and Miller, 1974; Hamberger and Hamberger, 1966; Klatzo et al., 1962; Olsson and Hossman, 1970; Persson et al., 1976; Rinder and Olsson, 1968; Sasaki and Schneider, 1976). For the most part these studies were confined to the light microscopic level and considered only the acute response of the inundated neurons which most investigators believed irreversibly damaged due to the disruption of their cell membranes. The present report represents a novel departure from these previous descriptions of neuronal inundation with tracer in that it clearly suggests at both the light and electron microscopic level that such inundation occurring with concussive injury is only a transient and nonlethal perturbation. Thus, the implication arises that neurons subjected to both concussive and perhaps other insults may be only transiently perturbed and thereby theoretically only temporarily dysfunctional. Certainly such a contention of subtle and transient neuronal perturbation in concussive injury is a departure from the classical theories of brain injury which ascribed traumatic neuronal dysfunction solely to either the direct physical shearing of neuronal appendages (Friede, 1961; Groat et al., 1944; Strich, 1956, 1961; Windle et al., 1944) or insults secondary to hemorrhagic lesions (Denny-Brown and Russell, 1941; Friede, 1961), which as such were meticulously avoided in the present investigation. That such neuronal inundation with peroxidase is an event linked to the concussive injury rather than an artifactual occurrence is convincingly attested to by our control studies. Neither high concentrations of extracellular horseradish peroxidase nor retrograde axoplasmic uptake of the protein tracer elicited a response reminiscent of that visualized in the peroxidase flooded neurons. Thus, there is a strong suggestion that the neuronal inundation with peroxidase occurring with trauma is directly related to the concussive injury and not secondary to other phenomena such as either high intra- or extracellular peroxidase concentrations or the toxicity of the protein tracer.

The mechanism by which the peroxidase floods these neurons appears uncertain. Careful examination of serial ultrathin sections failed to reveal any evidence of membrane disruption in either axons, somata, or dendritic processes of inundated neurons. Although no direct mechanical disruption of the inundated neurons was noted in our study, we must acknowledge that never in the course of this study were serial thin sections made along the complete axonal length and dendritic domain of a flooded neuron, as such an undertaking would have constituted a most tedious and virtually impossible task. LaVail and LaVail (1974) and Adams and Warr (1976) have demonstrated that surgical axonal ligation followed by topical horseradish peroxidase application could elicit peroxidase inundation of those neurons whose axons were ligated. Thus, it is

Fig. 9. A light micrograph of a reticular neuron in which the peroxidase (*arrows*) has reached the cell cytoplasm via retrograde axoplasmic flow from a spinal cord injection site. (Control) ×728

Fig. 10. An electron micrograph of the same neuron shown in Fig. 9 demonstrates that the peroxidase reaction product is confined to numerous peroxidase containing vesicles and vacuoles (*arrows*). Note that under these conditions of retrograde neuronal peroxidase filling, no peroxidase reaction product can be seen within either the cell nucleus or nucleolus. (Control) ×9,360

Fig. 11. An enlargement of the nucleolus shown in Fig. 10 clearly demonstrates the absence of any reaction product within the nucleolar vacuoles. ×16,640



Figs. 9–11

conceivable that in concussive brain injury distal dendritic and axonal shearing could have occurred and thereby provided the corridor for the peroxidase infusion. Despite the potential for such a problem, however, we believe that our failure to observe such a phenomenon in the large number of tissue samples employed in this study would preclude the possibility of such an event. Also, perhaps of consequence to this issue of peroxidase flooding are questions regarding the timing of our studies and the inherent size of the peroxidase tracer which normally approximates 6 nm. It is possible that the neuronal inundation with peroxidase was an extremely rapid event, and thus, was not detected in the temporal sequence of our study. Additionally, macromolecules the size of peroxidase could penetrate the proteolipid cell membrane of these flooded neurons through relatively small defects unnoticed by routine electron microscopy. Irrespective, however, of that route by which the peroxidase floods neurons, the fact remains that those neurons of the raphe and reticular core, which initially flood with the tracer, do not suffer irreversible damage and ultimately do well. This fact is borne out by our consistent failure to identify any evidence of ultrastructural alterations within flooded neurons as well as by the fact that with time such flooded neurons apparently organize the protein tracer into peroxidase containing vesicles and vacuoles in a manner consistent with normal cellular function (Turner and Harris, 1974). The ultimate passage of the peroxidase into the neuronal nuclei and nucleoli as reported in the present study was an unexpected finding and to the best of our knowledge has not previously been reported in any study employing horseradish peroxidase. This nuclear and nucleolar peroxidase uptake coupled with our report of numerous intranuclear rodlets within such neurons suggest the possibility of a traumatically induced, excited neuronal state. Such a contention is based upon the fact that the presence of nuclear rodlets has been linked to the activity of neurons. Seïte et al. (1971) reported that the occurrence of such rodlets dramatically increased in sympathetic chain ganglia subjected to electrical stimulation. They postulated that the increased number of rodlets in these cells was linked to their temporarily excited, functional state and increased metabolic demands. The fact that these rodlets were rarely seen in both our control studies as well as those performed by Bowsher and Westman (1970, 1971) suggests that their frequent occurrence in such inundated neurons constitutes an atypical occurrence perhaps linked with these neurons traumatically altered state. The presence of such rodlets coupled with the presence of peroxidase within the nucleolar vacuoles near RNA assembly sites of the pars fibrosa are consistent with the possibility of some form of excited neuronal state, perhaps reflecting accelerated synthetic mechanisms. Since again these morphological events are not accompanied by any form of ultrastructural alteration and as their significance in normal cells is poorly understood, it is difficult to comment upon their significance in these traumatized neurons. However, it is indeed intriguing to speculate that although concussive brain injury elicits no manifest ultrastructural change, it may evoke an avalanche of metabolic consequences here reflected in the nuclear/nucleolar uptake of peroxidase and the appearance of nuclear rodlets.

Our observation of a post-traumatic elevated blood pressure and EEG suppression followed by an apparently normal recovery conforms to the concept of a transient dysfunction within the reticular system (Ward, 1966). The observed transient flooding of the reticular neurons with peroxidase is perhaps related to transient cellular dysfunction, which as such is consistent with the brief physiological abnormalities and changes of consciousness occurring with concussion. Additionally, the rather subtle and nonlethal neuronal changes associated with this inundation with tracer are consistent with the fact that in classical descriptions of concussion overt cellular damage has not been frequently recognized. Thus, we believe that perhaps the neuronal inundation with peroxidase here seen within the reticular and raphe core constitutes a morphological correlate of concussion.

Acknowledgement. We wish to thank Mr. Thomas Coburn and Mr. and Mrs. Gilbert Allen for their technical assistance during the course of this project. Additional thanks are extended to Miss Ellen Hawthorne for her help in the preparation and typing of this manuscript. This work was supported by PHS Grant NS-12587-04.

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Received November 28, 1978/Accepted January 18, 1979