Oxidative metabolic activity of cerebral cortex after fluid-percussion head injury in the cat

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To assess the metabolic and vascular effects of head trauma, fluid-percussion pressure waves were transmitted to the brains of anesthetized, paralyzed, and artificially ventilated cats. Changes in the redox state of cytochrome \( a_{3} \), as, and relative local blood volume were measured in situ by dual-wavelength reflection spectrophotometry of the cortical surface viewed through an acrylic cranial window implanted within the closed skull. Initial fluid-percussion impacts of 0.5 to 2.8 atm peak pressure produced consistent transient oxidation of cytochrome \( a_{3} \) and increases of cortical blood volume. These changes occurred despite the presence of transient posttraumatic hypotension in some cases. Also, impact-induced alterations of vascular tone occurred, independent of the presence or absence of transient hypertension in the posttraumatic period. These data demonstrate that hypoxia does not play a role in the immediate posttraumatic period in cerebral cortex, and are consistent with the idea that after injury there is increased cortical energy conservation. These data also support the concept that head trauma alters the relationship of metabolism and cerebral circulation in the period immediately after injury.

**Key Words** • acute head injury • cerebral energy • cortical metabolism • cytochrome oxidase • cerebrovascular reactivity

The clinical outcome of head trauma depends on the ability of the homeostatic mechanisms of the brain to preserve the functional integrity of its cells despite the disruptive influence of abnormal intracranial pressure gradients. One of the prime factors in central nervous system (CNS) homeostasis is the integrity of cell oxidative metabolism, specifically, the functioning of the mitochondrial respiratory apparatus, upon which the CNS is critically dependent. Activities demanding cellular energy, such as continued ion transport, synaptic function, or membrane synthesis and repair, require the continuous provision of oxidative energy. This latter depends upon mitochondrial integrity and continued vascular supply of substrates and oxygen.

Many studies have documented an impairment of cerebral circulatory mechanisms following head injury.\(^{1,2,10,29}\) However, the metabolic state of brain after head injury has not been well defined. In this report, changes in the reduction/oxidation ratio of the terminal member of the mitochondrial respiratory chain, cytochrome \( c \) oxidase (cytochrome \( a_{3} \)), were recorded before, during, and after head injury produced by the fluid-percussion model of Sullivan, et al.\(^{27}\) These recordings were made from the neocortex in situ by reflection spectrophotometry.\(^{6}\) Since cytochrome \( a_{3} \) is the final reactant with molecular oxygen in the scheme of energy conservation, its redox state provides an index of oxygen sufficiency and the turnover of the mitochondrial respiratory chain.\(^{1,6,21}\) We report here that head injury is accompanied by increased levels of oxidized cytochrome \( a_{3} \) together with increased blood volume. These data rule out the possibility that head-injury effects are due to oxygen insufficiency and lead to the conclusion that trauma-induced increases in energy demand and increased energy conservation characterize the early posttrauma period. A preliminary report of this work has been presented.\(^{9}\)

**Materials and Methods**

Seven mongrel cats weighing from 2.5 to 3.4 kg were used. Each was anesthetized with an intraperitoneal injection of sodium pentobarbital (32 mg/kg), and a polyethylene cannula was placed in a femoral
vein for the administration of supplemental doses of pentobarbital as required (10 mg/kg). A second cannula was placed in a femoral artery for continuous monitoring of systemic blood pressure, and a tracheostomy was performed. The cats were positioned in a head holder, and through a midline scalp incision holes were drilled over the parietal convexities exposing the dura bilaterally. A hollow metal injury screw, with an 11-mm inner diameter and right-angle configuration, was cemented over the right craniotomy with dental acrylic.\textsuperscript{24} The dura exposed by the left craniotomy was incised and reflected over the skull. An acrylic cranial window\textsuperscript{18} was then cemented over the left craniotomy using four radially placed stainless steel screws as anchors. Two screws were used as bipolar electrodes to record the electroencephalogram (EEG) across the 10-mm optical window. The cranial window was manufactured with four fluid conduits that communicated with the subdural space. These conduits could be sealed by external valve stems. One such conduit was used to monitor intracranial pressure (ICP) using a 0.58-mm inner diameter polyethylene tubing and a pressure transducer\textsuperscript{*} placed at the level of the third ventricle. The other conduits were used for clearing the exposed cortex by flushing with artificial cat cerebrospinal fluid (CSF).\textsuperscript{25}

The animals were then removed from the head holder and placed on the injury table. Their heads were supported by the attachment of the central injury screw to the impounder cylinder described below. Each animal was paralyzed intravenously with tubocurare (0.15 mg/kg) and artificially ventilated with room air using a small-animal respirator.\textsuperscript{†} Arterial blood samples were drawn at this time and periodically throughout the experiments from the arterial catheter. These samples were analyzed\textsuperscript{‡} to confirm that PaO\textsubscript{2}, PaCO\textsubscript{2}, and pH values were being maintained within physiological ranges before and after impact. Rectal temperature was monitored with a thermometer probe and maintained at 37°C with an external heating pad.

Cerebral trauma was administered with the technique described by Sullivan, et al.\textsuperscript{27} A fluid wave of fixed duration and variable peak pressure was delivered to the intact dura by means of the hollow injury screw attached to the end of a saline-filled Plexiglas cylinder. A pendulum hammer was used to deliver a reproducible impact to the opposite cored end of that cylinder. A low-compliance strain gauge transducer,§ placed between the cylinder and screw perpendicular to the direction of pressure wave travel, allowed the waveform to be displayed on a storage oscilloscope and photographed for subsequent analysis.

Changes in the redox state of cytochrome a\textsubscript{a}s were measured by the dual-wavelength reflection spectrophotometry technique described by Jôbosis, et al.\textsuperscript{5} The method is based on the light absorption properties of the cytochrome components of tissue mitochondria. Since cytochromes absorb light more strongly in the reduced form than in the oxidized form, changes in redox ratios can be determined by measuring changes in reflected light intensity at a wavelength of maximal absorption difference between the reduced and the oxidized species of any of these mitochondrial components.

In the procedure used here, light from a 45-watt tungsten bulb was mechanically chopped to allow alternate presentation (at 30 Hz) to two monochromators. One monochromator was set to 605 nm, an absorption peak for cytochrome a\textsubscript{a}s. The other was set to 590 nm to provide reference compensation for changes in blood oxygenation and volume. The two monochromatic light beams were directed by fiberoptic light guides to the surface of the brain visible through the cranial window. Microscope optics with a 33-mm working distance and 3.2-mm diameter optical field were used to gather light returning from the tissue and focus it on a photomultiplier tube housed in the microscope barrel. Figure 1 illustrates the relationship of these components to the cranial window and central injury screw.

The signals representing the intensity of light reflected at 590 nm (the “reference” wavelength) and the difference between the intensity of light reflected at 605 nm and 590 nm (“sample” minus “reference”) were displayed on a chart recorder. The former signal provided a useful indicator of blood volume shifts. The latter signal provided an index of redox shifts of cytochrome a\textsubscript{a}s.\textsuperscript{28} The instrument was calibrated by making the reference-compensated difference signal equal to zero when the light path of the 605-nm monochromator was interrupted, allowing no “sample” but full “reference” light to reach the photomultiplier. Sample light was then allowed to illuminate the cortex, and its intensity adjusted to be equal to the reference intensity. The output signal was then considered to be 100% and all data were subsequently recorded as a percent of this full-scale value.

Each animal received from one to eight fluid-percussion impacts. If deflections in the reference light signal (indicating blood volume) suggested free blood in the optical field, measurements were suspended and

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\textsuperscript{*}Statham P23Db pressure transducer manufactured by Statham Instruments Co., 2230 Statham Boulevard, Oxford, California.

\textsuperscript{†}Small-animal respirator, No. 607, manufactured by Harvard Apparatus Co., 150 Dover Road, Millis, Massachusetts.

\textsuperscript{‡}Blood analysis system, BMS 3 Mk 2, manufactured by Radiometer-Copenhagen, 72 Emdrupvej, DK 2400, Copenhagen, Denmark.

\textsuperscript{§}Low-compliance strain gauge transducer manufactured by Statham Instruments Co., 2230 Statham Boulevard, Oxford, California.

\textsuperscript{5}Monochromator manufactured by Bausch and Lomb, Rochester, New York.

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The Initial Trauma

The mean systemic blood pressure before impact was 121 ± 10 (mean ± SE) for six of the seven cats prepared. The arterial pH was 7.38 ± 0.03 and the PaCO₂ was 28 ± 2 torr. The seventh cat was hypotensive at the moment of impact (65 torr) and acidic (pH 7.20). Interestingly, the trauma-induced changes in cytochrome a,a₈ redox level and cortical blood volume in this seventh animal were consistent with those of the remaining six, and were included in the analysis. The preinjury PaO₂ was greater than 95 torr in all cases.

The magnitude of the initial fluid-pressure wave was varied from 0.5 to 2.8 atm. In five of the cats, the impact was greater than 2.5 atm. The duration of the fluid waves ranged from 30 to 35 msec and their configuration was similar to those reported previously for this technique.⁷

In six of seven cases, a systemic blood pressure change occurred immediately after impact. Four animals showed an increased systemic blood pressure (Fig. 2), and two showed a decrease (Figs. 3 and 4 upper). The decreases were of 15 and 25 torr, and the increases ranged from 15 to 130 torr (mean 72 torr). Peak pressure changes occurred within 1 minute and returned to the pretrauma baseline within 6 to 20 minutes. Persistent posttraumatic hypotension was not seen.

Intracranial pressure ranged from 3 to 12 torr (mean 6.6 torr). After fluid-percussion injury, there were no ICP changes in three animals, but slow decreases occurred in two animals. The remaining two animals had a transient increase in ICP to peaks of 15 and 23 torr within 1.5 minutes, followed by recovery within 1 hour. There was no apparent correlation between the direction of systemic blood pressure and ICP responses.

Electroencephalographic activity was suppressed after the impact for only from 24 to 72 seconds. This suppression was mild in the two animals that received less than 2.5-atm peak impact pressure. There was loss of high-voltage sharp activity but minimal change in the basic frequencies. Moderate changes (loss of high-voltage sharp activity and increased slowing) and marked changes (overall suppression of amplitude with obvious slowing) were seen in the remaining cases. Total suppression of EEG activity or persistent changes were not seen. This is demonstrated in Fig. 3 on a compressed and expanded time scale.

In all cats, the initial fluid-pressure impact was followed by a transient increase in the level of oxidized cytochrome a,a₈ and an increase in the blood volume of the optical field. As comparison of Figs. 2 and 3 or 4 upper shows, this response occurred independently of impact-induced hypertension or hypotension. In four cases, this oxidation peaked within 41 seconds to 3.5 minutes, and returned to baseline within 10 to 18 minutes (Fig. 2). In three cases, this peak oxidation occurred sooner (from 10 seconds to 1.5 minutes), and returned to a level more reduced than the original baseline (Fig. 4 upper). In two animals, there was a secondary suppression of the EEG that coincided with the period of peak cytochrome a,a₈ oxidation (Fig. 3).

In all cats, the initial impact produced an increase in blood volume. This increase peaked between 14 seconds and 1.9 minutes. After this peak increase, a new steady-state optical signal level (absorption at 590 nm) was established at between 1 and 15 minutes. Again, this increase was seen in those animals exhibiting both increases or decreases in systemic blood pressure after fluid-percussion trauma.

Repetitive Head Trauma

In all, 21 fluid-percussion impacts were delivered. These ranged in intensity from peak pressures of 0.5 to
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3.7 atm and in duration from 23 to 36 msec. Changes in blood pressure, ICP, and EEG were not outside the range of the responses to initial impact. Twelve of 14 repeat impacts resulted in cytochrome $a,a_3$ oxidation that appeared similar to the subgroup of seven responses to initial impact described above. In two cases, however, impact trauma was accompanied by an increased level of reduced cytochrome $a,a_3$. An example of this response is shown in Fig. 4 lower. The change from the more usual oxidative response of cytochrome $a,a_3$ after trauma to cytochrome $a,a_3$ reduction cannot be explained by systemic blood pressure effects since the systemic blood pressure response to trauma increased in one of these cases (110 increased to 130 torr) and decreased in the other (Fig. 4 lower). It should be noted, however, that this initial reduction of cytochrome $a,a_3$ was only seen after the sixth and eighth impact in a single animal, and hence may not represent a normal physiological response characteristic.

The blood volume increased after impact in 19 of 21 cases. Blood volume decreases were seen after the seventh and eighth impact in an animal that was given eight separate blows. This was the same animal that gave the unusual response of cytochrome $a,a_3$ reduction after multiple trauma. The systemic blood pressure response after these two impacts was a decrease of 50 torr from baselines of 120 and 130 torr. This blood volume decrease is seen in Fig. 4 lower.

**Histological Examination**

A macroscopic review of all the formalin-fixed brains revealed few lesions in the cortex. Three brains showed some degree of swelling or contusion in the area directly under the implanted window, obviously related to the displacement of the brain toward the contralateral side of the injury impact. Only one brain showed no evidence of damage upon analysis of coronal sections. In general, macroscopic observation revealed extensive subarachnoid hemorrhage, most marked in relationship to the brain stem, the basal cisterns, and the cerebellum.

Coronal sections showed petechial hemorrhages.
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**FIG. 3.** Polygraph recordings of the oxidative response to initial fluid-percussion injury (dotted line) when systemic hypertension did not occur. The electroencephalographic activity (EEG) was transiently suppressed after impact. The EEG at Points A to E is shown on an expanded time scale to show the suppression at Point B. In this animal, a minor slowing occurred at the point of maximal cytochrome $a_a$ oxidation; Point D. Each expanded EEG tracing represents 12 seconds of recording. BL VOL = blood volume; ICP = intracranial pressure; BP = blood pressure; f.s. = percent of the full-scale optical signal (see text).

within the cerebellum, most conspicuously in the vermis and in the white matter of the deep cerebellar nuclei. Cell damage was exemplified by an increase in Purkinje cell acidophilia. Petechial hemorrhages were observed in the dorsolateral region of the medulla and at the level of the superior and inferior colliculi. Additionally, substantial bleeding was observed within the midregion of the diencephalon just dorsal to the mamillary bodies and, in two brains, in the dorsolateral aspect of the pons. There were scattered petechial hemorrhages in the corpus callosum and the dorsal hippocampus.

**Discussion**

The acute effect of moderate fluid-percussion impact to cat neocortex upon the redox state of cytochrome $a_a$ consisted of a transient increase in the ratio of oxidation to reduction. This oxidation, together with an increase in the volume of hemoglobin within the optical field, occurred independently of changes in systemic blood pressure. In fact, in some cases, impact resulted in increased systemic blood pressure while in others systemic blood pressure was transiently decreased.

This oxidative response of cytochrome $a_a$ after impact injury rules out the possibility that hypoxia plays a role in the posttrauma situation, at least in its acute stages. This follows from previous reports of experiments done in vitro and in vivo showing that decreased oxygen tension or ischemia are accompanied by increased levels of reduced cytochrome $a_a$.

The question remains as to the cause of the cytochrome $a_a$ oxidative response following impact insult. One possibility is that impact could produce a condition of increased energy conservation resulting from increased energy demand. Previous reports have demonstrated that increased energy demand induced by evoked cortical activity, spreading depression, and seizures is also accompanied by oxidations of cytochrome $a_a$. The consistent transient oxidation observed to peak from 0.26 to 2.32 minutes after ini-
Although most investigators in the field report consistent hypertensive responses, it is interesting to note that the reports by Meyer, et al., 16 and Nilsson and Pontén 18 attest to both hypo- and hypertensive responses. The pial vessel dilation, and hence volume increase, is temptingly attributable to abrupt hypertension. However, by characterizing those occasionally observed hypotensive responses as abnormal chance occurrences, we express some reservation for what might well be an oversimplified mechanism of action. With defective autoregulation and the presence of systemic hypotension, pressure-passive changes in flow would likely lead to decreased perfusion and decreased tissue oxygen availability in some cases. Consistent oxidations of cytochrome $a_d$ despite systemic hypotension suggest that increased tissue oxygen content may not be responsible for the oxidation in all cases.

There is sufficient evidence to support the assumption that a defect of autoregulation existed after fluid-percussion injury in the current study. Autoregulation has been shown to be impaired after compression-concussion injury at levels too mild to cause suppression of electrocortical activity recorded adjacent to the region of impact, alteration of blood-brain barrier permeability to dye, or morphological alteration by light or electron microscopy. 16, 18, 20, 28 Disturbances of autoregulation result in pressure-passive flow changes. These have been described in mild and severe experimental compression concussion. 18 With mild concussion, increased systemic arterial pressure was observed with a concomitant increase in CBF without changes in cerebral vascular resistance (CVR). Severe concussion produced a decrease in arterial pressure with a lowering of CBF; CVR showed a paradoxical increase indicative of defective autoregulation. The transient suppression of the EEG after all impacts delivered in the current study is evidence that sufficient trauma occurred to compromise cerebrovascular autoregulation.

Increased cerebral blood volume has previously been reported after experimental head trauma. 14 Related observations of vessel dilation induced by head trauma have been made through a Lucite calvaria. 26 Direct examination of pial artery diameter through a cranial window, similar to the type used in this study, showed dilation induced by fluid-percussion injury that persisted after the systemic response to trauma had abated. 7, 20, 30 Although similar percussion intensities were used (1.4 to 3.4 atm), a larger and more consistent systemic blood pressure increase was produced (minimum mean increase of 87 torr for trauma less than 2.5 atm). These studies showed that transient increases in systemic blood pressure could decrease the responsiveness of pial arteries to hypocarbia 7, 30 and hypotension induced by volume depletion. 29 These changes, as well as endothelial damage observed by transmission and scanning electron microscopy, were absent if the trauma-induced transient hypertension...
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was abolished by systemic blood volume depletion with or without ATP infusion. These data suggest that there is a cerebral vascular lesion due to a transient rise in systemic arterial pressure.

Vascular reactivity to CO₂ as defined by regional CBF changes induced by hypercarbia was reduced by percussion trauma between 1.15 and 1.9 atm and abolished at impacts above 2.0 atm. This effect was present if systemic blood pressure increases were prevented by infusion of ATP, suggesting that there is an impact-induced alteration of vascular reactivity to hypercarbia independent of hypertensive effects.

In the current study, both hypertensive and hypotensive responses were seen with the initial impacts at 0.5 and 2.8 atm. Cortical blood volume always increased and stabilized at a higher level. This increase of blood volume must reflect a relaxation of vascular tone secondary to the trauma. It does not give an indication of CBF. With defective autoregulation, pressure-passive changes in CBF would be expected. With the increases and decreases of systemic blood pressure observed in this study, consistent increases in CBF would not be expected. The finding of consistent oxidation of cytochrome a,a₃ would then correlate more with increased energy utilization from trauma-provoked neuronal excitation than with a perfusion-dependent rise in tissue oxygen availability.

It should be noted that the magnitude of systemic blood pressure responses to cerebral percussion was variable and was smaller than those previously obtained after impacts with the fluid wave method. The source of these autonomic changes after head impact is generally considered to be the result of the focusing of the destructive forces of the pressure wave in brain-stem structures. The lack of consistent systemic blood pressure responses to impacts in the range of 2.8 atm suggests that the degree of cerebral trauma in our animals was below what one would expect from pressure waves of similar amplitude in different laboratories using the same technique. Sullivan, et al. reported that increases in blood pressure were abolished by high cord transection. Saunders, et al., did not report blood pressure responses to impacts between 1.9 and 2.9 atm, but did report increases of 34 torr lasting from 1 to 4 minutes after peak impacts of 1.15 to 1.9 atm. Persistent secondary systemic hypotension was observed with impacts above 2.9 atm, but not after impacts of the lower range. Wei, et al. found increases in systemic arterial pressure resulting from trauma in the range of 1.4 to 3.4 atm that increased from 26 to 98 torr as the trauma was increased. Consistent posttraumatomc hypotension was also seen 30 to 60 minutes after impacts equal to or greater than 2.5 atm. This result is to be compared with the findings of Saunders, et al., that neuro-pathological changes at the light microscopic level are not found until the level of impact exceeds 2.9 atm.

Nevertheless, the lack of consistency in blood pressure effects of trauma strengthens the conclusion that, independent of changes in systemic blood pressure, consistent increases in the ratio of oxidized to reduced cytochrome a,a₃ and consistent increases in cortical blood volume during the acute stages of fluid-percussion impact are an early effect of head trauma. This oxidation rules out the possibility that hypoxia plays a role in the immediate posttraumatic period, and supports the concept of increased energy utilization at the moment of concussion.

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References


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