Experimental head injury in the rat

Part 1: Mechanics, pathophysiology, and morphology in an impact acceleration trauma model

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Impact acceleration was used to elicit cerebral concussion in the albino rat. The pathophysiological response and morphological damage from the concussion were studied in groups of animals. The animals were grouped according to impact velocity (6–11 m/sec), and the threshold and different degrees of the concussive response were established. The concept of concussion as primarily a neuronal, functional disturbance was confirmed. However, this reaction was readily influenced by respiratory and circulatory changes, and morphological damage.

Defined reactions could be elicited in groups of animals according to impact velocity. Thus, the model appeared well standardized for further studies of concussion pathophysiology, expressed in terms of cerebral energy metabolism and blood flow.

KEY WORDS □9 experimental concussion □9 impact acceleration □9 concussion pathophysiology □9 morphology

The overall pathophysiology of head injury is very complex. In most instances both the regional effects upon the central nervous system and the systemic reactions must be considered. The most frequent cerebral symptoms of blunt head injury are those of cerebral concussion, which, according to the common use of the term, implies a short-lasting disturbance of cerebral function without morphological changes, visible by gross examination or light microscopy. However, such a concept of concussion includes additional factors, including mechanical effects upon the cerebral vasomotor function. Systemic effects also occur, the most important being neurogenic interference with respiratory and circulatory functions. The threshold of trauma causing concussion is very close to that causing gross anatomical damage. Thus, focal brain lesions and hemorrhages may readily influence the pathophysiological events of concussive head trauma.

Clinically, the main features of cerebral concussion are transient loss of consciousness and amnesia. Experimentally, immediate motor, respiratory, and circulatory effects, and loss of reflex functions have been described. In their classical study, Denny-Brown and Russell explained these effects of trauma in terms of mechanically induced paralysis of function. On the other hand, Walker, et al., found electroencephalographic (EEG) evidence of excitation during the immediate trauma response. Neither of these theories is sufficient to ex-
plain the fundamental biological mechanisms underlying the phenomenon, and the physicochemical basis of concussion is still unclear. Further, it is not known whether such a functional concussion response is a uniform, all-or-none response, or a reaction that can attain different degrees of severity, thus explaining the range of clinical conditions observed. It is obvious that the early complicating factors mentioned may be involved and cause a prolonged comatose state, as well as conditions like primary brain-stem lesions and posttraumatic ischemic lesions. The cerebral blood flow (CBF) might be an important factor within this pathophysiological complex. Although the mechanical stress upon the vascular tree should be as extensive as that on neural tissue, there is little information available about the CBF in the acute stages of trauma.

It should be added that the definition of concussion as a purely functional change has also been questioned. Hemorrhagic lesions and chromatolytic cell changes appear with experimental concussion. Oppenheimer found the early appearance of microglial clusters, especially in the brain stem, in cases of light concussion in man. Symonds concluded that the term “concussion” should not be confined to a purely functional syndrome, but should include conditions of neuronal damage, since such lesions were considered more or less inevitable in any injury.

In most clinical concussive head injuries the impact affects the freely movable head, and both acceleration and skull deformation, as well as movement at the craniospinal junction may result. Taking into consideration these facts, and the complex pathophysiology of head trauma, we have designed a model in which the effects of a highly standardized impact acceleration can be studied in the albino rat. The rat is well suited for quantitative monitoring of the physiological parameters that constitute the response to trauma. Furthermore, it has been extensively used for the study of cerebral energy metabolism and blood flow parameters that are essential for the understanding of the pathophysiology of cerebral concussion.

In this report, the mechanical features of the trauma model are described, and the relationships between impact velocity and physiological and morphological effects determined. The main purpose was to determine quantitatively the components of the concussive response in the hope of elucidating fundamental pathophysiological problems. We have also used this model to further study cerebral energy metabolism and blood flow in concussive head injury. Materials and Methods

Mechanics of the Model

The impact was delivered by a piston accelerated by compressed nitrogen. Similar devices have previously been extensively used. The piston weighed 600 gm, and its tip (diameter 10 mm) was covered by a hard rubber cap 2 mm thick. The velocity of the piston at the moment of impact could be varied between 5 and 12 m/sec by adjustment of the applied gas pressure. Repeated calibrations during the experimental periods showed a fairly constant relation between gas pressure and velocity, the latter varying within a range of less than ± 0.2 m/sec at defined settings of the gas pressure.

The rat was placed on its back in a modeled plaster bed with a hole for the piston corresponding to the occipital crest of the rat’s
Experimental head injury in the rat head. Since the rats were of equal size, the site of impact and the position of the head and neck of the rat were exactly defined. The head was accelerated upward by the piston combined with an initial backward rotation with a marked retroflexion in the head-neck junction and the upper part of the cervical spine. The piston was braked at a level of 10 mm above the initial impact level. The movement of the head, neck, and, subsequently, the body was unrestricted until stopped by a soft cushion 7 to 10 cm above the plaster bed. Figure 1 shows the principle features of the device, the position of the rat, and the movement of the head as analyzed from high-speed films. Both living animals and cadavers equipped with markers fixed in the upper incisors, the external auditory meatus, the occipital crest, the atlantooccipital membrane, and the spinous processes of the cervical vertebrae, were filmed with a Hitachi high-speed camera, at 5000 to 7000 pictures per second.

In the actual study impact velocities of 6, 7, 8, 9, 10, and 11 m/sec were used.

**Experimental Technique**

Male Wistar rats weighing 300 to 400 gm were used in the study. Anesthesia was induced with divinyl ether or halothane. The rats were intubated endotracheally, and a gas mixture containing 25% oxygen in nitrous oxide was administered. Twenty-one spontaneously ventilating animals were maintained on a low concentration of halothane (0.5%). Fifty animals were paralyzed with repeated small doses of suxamethonium chloride (Celocurin), and artificially ventilated with a volume- and flow-regulated respirator. Spontaneous breathing was monitored by means of an elastic strain gauge transducer,* wrapped around the chest, or by registration of the intrathoracic pressure via a small balloon in the esophagus. The tail artery and vein were cannulated for arterial blood sampling, continuous monitoring of arterial blood pressure, and for intravenous injection of drugs.

After preparation of the experiment, which usually took 10 to 15 minutes, the animals were placed in the plaster bed for 15 to 30 minutes until a steady state was reached with regard to anesthesia and ventilation. Blood gases were kept within normal ranges (arterial oxygen pressure (PaO₂) 90 to 110 mm Hg, arterial carbon dioxide pressure (PaCO₂) 35 to 40 mm Hg). The rectal temperature was kept close to 37° C (range 36.5° to 37.5° C).

The reaction to trauma was first studied in a group of 21 animals that were unparalyzed and spontaneously breathing. In order to get sufficient relaxation to keep the animal in place in the plaster bed, 0.5% halothane was added to the nitrous oxide and oxygen mixture. The anesthetic gas mixture was withdrawn and the rat started to breathe room air just before the blow was delivered. If no blow was given, a simple pain stimulus (tail or nose pinching) aroused the animal, which moved and turned onto its feet within 30 seconds after withdrawal of the anesthetic gases. Thus, in animals so handled, the reaction to trauma should be very similar to that of an unanesthetized animal.

To further standardize the mechanical model by excluding spontaneous muscle activity and movement at the moment of impact, a series of 50 animals were hit while relaxed by suxamethonium chloride. The dose was adjusted so that weak muscle activity returned just before the blow in 14 animals, or within a few minutes after the blow in 36 animals. Just before the blow nitrous oxide was replaced by nitrogen in the insufflated gas mixture, keeping the oxygen concentration constant at 25%. Artificial respiration was maintained until sufficient spontaneous respiration was resumed.

**Posttraumatic Observations**

In the unparalyzed animals concussion was defined as loss of motor reaction to pain stimuli. In addition, the immediate reaction to trauma was characterized by an abrupt blood pressure increase, short-lasting convulsive activity, and often, apnea. In paralyzed animals the same type of blood pressure response was seen, and the recovery of reaction to pain stimuli could be evaluated from the appearance of a short-lasting blood pressure increase, characteristically seen in the alert animal on pain stimulation.

During the recovery phase, corneal reflexes and reaction to pain stimuli were repeatedly

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*Strain gauge transducer manufactured by AB Dirigo, Box 30045, S-400 43 Gothenburg 30, Sweden.
TABLE 1

General response and morphological findings 30 to 60 minutes after acceleration head trauma at various impact velocities

<table>
<thead>
<tr>
<th>Impact Velocity (m/sec)</th>
<th>No. of Rats*</th>
<th>Concussion</th>
<th>Mortality</th>
<th>Survival State†</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6 (3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>20 (4)</td>
<td>17</td>
<td>—</td>
<td>—</td>
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<tr>
<td>8</td>
<td>10 (4)</td>
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<td>2</td>
<td>—</td>
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<td>9</td>
<td>18 (1)</td>
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<td>5</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>11 (6)</td>
<td>11</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>6 (0)</td>
<td>6</td>
<td>4</td>
<td>—</td>
</tr>
</tbody>
</table>

*Number of spontaneously ventilating animals not given artificial support indicated within parentheses.
†Coma denotes total unresponsiveness, while stupor-lethargy includes various states of reduced responsiveness and spontaneous activity.

tested, and the time when the animals righted themselves was noted. Return of spontaneous respiration was also followed in the rats with artificial respiration, either by monitoring chest movements during artificial respiration, or by interruption of the artificial ventilation for 10 to 15 seconds every minute.

Morphological Observations

The surviving animals were usually sacrificed after 30 to 60 minutes by intravenous injection of potassium chloride or phenobarbital in a dose causing immediate heart arrest. Long-term survival was not studied since pilot studies showed a high frequency of respiratory complications, obviously as a result of intubation trauma. Only six animals were allowed to survive for 3 to 6 days. After death the skull was examined for fractures. The brain and upper cervical cord were carefully removed. The occurrence and extent of extracerebral hemorrhages were noted. The specimen was immediately fixed in 6% formaldehyde. In some animals the whole spinal cord was removed after fixation in situ. In all, 22 brains and spinal cords were microscopically examined. Staining methods included hematoxylin and eosin, Azan, Mallory, and myelin staining according to Mahon. The surface, and cross sections 1 to 2 mm thick of the other brains were examined in a dissection microscope. Pulmonary edema was assessed from the macroscopic appearance of the lungs, and by weighing the lungs to calculate the lung-to-body weight ratio (normal value < 0.5%).

Results

The frequency of production of concussion, survival rate, quality of survival, and morphological findings are summarized and related to impact velocity in Table 1. The data from all the rats are listed together, since no apparent differences were found between spontaneously breathing and artificially ventilated animals, except for the occurrence of apnea in the former group.

To elicit concussion causing loss of motor reaction to painful stimuli, velocities above 6 m/sec were necessary. At impact velocities of 8 m/sec or higher, concussion was invariably obtained.

Morphological Findings

The morphological damage became progressively worse with increasing impact velocity. Concussion could occur without visible lesions but, even in the 7 m/sec injury group, about 50% of the rats showed some degree of subarachnoid hemorrhage (SAH) primarily in the occipital cistern. With an injury of 9 m/sec or more, SAH was very frequently observed and more marked, often extending to the basal parts of the anterior fossa and along the cervical spinal cord. Next in frequency were fractures of the occipital bone. With 7 to 9 m/sec force injuries, these
were usually thin linear fractures, but at higher impact velocities comminuted depressed fractures were seen, often continuing to the base of the skull. No fractures of the cervical spine occurred. Brain lesions appearing as microscopic perivascular hemorrhages, or gross hemorrhages, were seen at impacts above 9 m/sec velocity. Bleeding was located in the brain stem. Microscopic hemorrhages were also seen in the spinal cord. Lesions of the cerebellum were seen under depressed fractures. No supratentorial cerebral lesions were found. Six animals from the 7 to 8 m/sec injury groups that survived in good condition were allowed to live for 3 to 6 days. Microscopically, no evidence of cell change or axon tearing was found.

Motor Response

In addition to the immediate unresponsiveness to pain, the reaction of concussion was characterized by general motor phenomena and blood pressure changes, while rhythmic respiration was not invariably affected. The motor phenomena consisted of short-lasting tonic-clonic fits most marked in the hindlegs and usually lasting less than 1 minute, that is, slightly shorter than the period of elevated blood pressure. At the end of this period, pain stimuli applied to the hind limbs enhanced clonic spasms and could induce reflex spasms in a pattern similar to that seen in decerebrate automatism in man. The convulsive period of 20 to 60 seconds was followed by a period of relaxation and unresponsiveness to pain. In the 7 m/sec injury group, this period was very short, but after more severe trauma it was prolonged or permanent.

Respiration and Cardiovascular Response

Respiration was not always abolished in concussion. When affected, it was more rapidly restored than the reaction to pain stimuli. Three of 18 rats in the 9 m/sec injury group, and two of 11 rats in the 10 m/sec group never regained spontaneous respiration but survived by means of artificial ventilation. Recovery of spontaneous respiration was seen as late as 6 minutes after the trauma.

The cardiovascular response was a very distinct feature of concussion. As seen from the examples in Fig. 2, concussion was characterized by a linear increase in the mean arterial blood pressure (MABP) during the first 2 to 3 seconds, followed by a period of continuously elevated MABP and high pulse pressure with gradual return within 30 to 120 seconds. Figure 3 shows that the duration of MABP elevation was longer after the 9 m/sec impact than after 7 m/sec velocity, while at 10 m/sec and, especially, at 11 m/sec the reaction was short-lasting or absent. The MABP reached maximum values of 180 to 220 mm Hg as compared to control levels of about 120 mm Hg. The most pronounced in-
Increases in blood pressure usually lasted the longest. During the next few minutes the MABP often dropped to slightly subnormal levels.

The more detailed responses, as shown in Fig. 2, indicate further differences between impact velocities. Thus, the response to 7 m/sec injury was usually associated with distinct bradycardia, often starting 2.5 seconds after the blow; at 9 m/sec, this response was often absent. The response to 11 m/sec injury was quite different with slight or no elevation of blood pressure, followed by a rapidly developing decrease.

**Morbidity and Mortality**

The immediate mortality was due to apnea (in the unventilated group) or to fulminant pulmonary edema. In many animals in the 8 to 11 m/sec injury groups, death from apnea was evidently prevented by artificial respiration during the first minutes after the trauma. In the 11 m/sec injury group, in which all animals were artificially ventilated, four deteriorated within a few minutes with rapidly decreasing blood pressure without developing pulmonary edema, and died in a condition of circulatory collapse.

The quality of survival was good in most animals in the 7 m/sec injury group, while at higher impact velocities there was an increasing proportion of survivors with stupor and coma. The pulmonary edema was evaluated from gross examination and weight.

![Fig. 2. Typical blood pressure responses to acceleration trauma of different impact velocities.](image)

![Fig. 3. Duration of the concussive blood pressure response at different impact velocities.](image)
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FIG. 4. Time of recovery of the response to pain after blows of different velocities. Open circles: spontaneously ventilating animals; filled circles: artificially ventilated animals. C: Animals still comatose 30 minutes after impact. D: Animals dead before 30 minutes, without recovery of pain response.

of the lungs. In some cases it was fulminant, leading to hypoxia and circulatory failure within a few minutes. In some animals, however, it was less severe and compatible with survival. Intermittent positive pressure respiration in some cases improved pulmonary function.

The time course of the recovery, as estimated from the reaction to pain stimuli, is illustrated in Fig. 4. After blows at 7 m/sec velocity, the rats usually reacted to pain again within 2 to 3 minutes. Blows at 9 m/sec velocity produced unresponsiveness lasting 3 to 10 minutes, but in some cases the coma outlasted the observation period of more than 30 minutes. The surviving animals in the 10 to 11 m/sec injury groups were also comatose for long periods of time.

The righting reaction usually followed the recovery of response to pain within a few minutes. The corneal reflex usually reappeared simultaneously with the response to pain stimuli, but was sometimes inconsistent and exhaustible.

Discussion

Various experimental models have been used to study different aspects of head trauma, and different biomechanical principles have been pointed out in the literature. Generally, various types of focal brain damage and hemorrhage depend on specific characteristics of the impact mechanisms and anatomical features of the species used, while the concussive response can be regarded as a more universal type of reaction that can be elicited by a variety of impact mechanisms in a great many species, including rats. Denny-Brown and Russell first pointed out two basic mechanisms by which concussion can be produced. In their cat model an acceleration of the movable head by an impacting mass with a velocity of more than 8 m/sec was required to produce acceleration concussion. This type of impact did not cause any substantial increase of the intracranial pressure (ICP) in contrast to another commonly used trauma model, a blow to the fixed head, which by compression causes a sudden increase of ICP (compression concussion). However, Gurdjian and coworkers showed that an increase of ICP was an essential component whether blows were delivered to the fixed or to the movable head. The concussive effect of impact acceleration has been attributed both to a direct deformation of tissue due to movement in the head-neck junction and to pressure gradients within the skull and at the craniospinal junction. These gradients, produced by the inertia of the brain or by skull compression, probably also exert their effects by tissue deformation. Compression concussion has been widely used as a method of an exactly defined trauma, usually with a device by which the pressure pulse is applied via a column of fluid.
in direct communication with the intracranial space.\textsuperscript{40} Although this model appears somewhat artificial, the concussive response is very similar to that produced by impact acceleration of the intact head. Probably the final effects in both models are shear strains in the brain-stem region, which, according to the opinion of most authors,\textsuperscript{10,14,25,26,66,69} is the region predominantly involved in concussion. However, Ommaya and Gennarelli\textsuperscript{52} recently proposed that in cerebral concussion the rotational components of accelerative trauma exerted maximum effects in the periphery of the hemispheres. This model, which involves a very restricted and guided acceleration-deceleration, may not be fully representative of clinical concussion.

The present method probably induced its main tissue strain via a direct deformation of the brain stem. Head-neck movement within the physiological range is known to distort the brain stem and spinal cord to a remarkable degree in man.\textsuperscript{4} Skull deformation with compression may have contributed, but pressure gradients and shearing strains due to inertia probably had negligible effects. The velocity of the piston at impact necessary to produce concussion (7 m/sec) agreed well with the values reported in other studies.\textsuperscript{10,65,70} However, it should be emphasized that the mechanics of the model can only be defined operationally from its specific experimental design.

The syndrome produced fulfilled the accepted criteria of experimental concussion: loss of responsiveness to external stimuli, loss of reflex functions such as corneal reflexes and righting reactions, respiratory and cardiovascular effects, and motor phenomena. These changes can all be symptoms of brain-stem dysfunction but additional involvement of other parts of the central nervous system is possible. The symptoms appeared to be an all-or-none response at a threshold of impact velocity of 6 to 7 m/sec. Above this threshold, however, differences between the trauma groups were seen. Thus, the immediate blood pressure reaction was more pronounced in the 9 m/sec injury than in the 7 m/sec injury group. Also the increasing duration and severity of the other functional disturbances studied indicate a graded response above the concussion threshold. In addition, however, the prolonged comatose state in part of the 9 m/sec injury group raises suspicion as to the early appearance of complicating factors.

Increase of blood pressure and bradycardia have usually been explained as symptoms of a stimulation of sympathetic and vagal centers.\textsuperscript{29} However, the present findings of a latency in the development of the bradycardia response and the disappearance of this response at more severe trauma levels, suggest that the bradycardia of concussion may be a baroreceptor reflex, which can be extinguished in more severe brain-stem insults. An intravenous bolus of epinephrine exactly reproduces the blood pressure reaction and the bradycardia. The pulmonary edema can be reproduced in this way, too, indicating sympathetic discharge as the essential pathogenetic mechanism in this type of neurogenic pulmonary edema.\textsuperscript{2,43} The respiratory and cardiovascular responses emphasize the importance of systemic factors in the immediate concussion reaction. Thus, some spontaneously ventilating animals died from apnea, and pulmonary edema was a common phenomenon in the artificially ventilated group.

Respiratory dysfunction is apparently an essential component of severe head trauma in humans. Hypoxemia is a common finding in newly admitted patients with severe head injuries.\textsuperscript{5,16,36,38} The basic nature of the concussive reaction may be discussed on the basis of the present findings. The old theory of compression ischemia\textsuperscript{68} dominated the discussion for many years, despite early evidence against it.\textsuperscript{57,74} It should not need further consideration. The term "paralysis," as used by Denny-Brown and Russell,\textsuperscript{10} essentially means reflex paralysis, namely, loss of reflex function, and does not offer any explanation of the physicochemical mechanisms. On the other hand, the theory of Walker, \textit{et al.},\textsuperscript{98} that concussion is an immediate excitation similar to the neuronal firing in epilepsy, deals with more basic mechanisms. An EEG pattern suggesting excitation has also been found by others,\textsuperscript{19,75} but most authors report EEG flattening and slowing in experimental concussion.\textsuperscript{13,32,74} In support of Walker's theory, Nelson, \textit{et al.},\textsuperscript{47} and Meyer, \textit{et al.},\textsuperscript{48} using compression models, found evidence of increased energy and oxygen consumption, respectively. No such studies have been done in acceleration-trauma models. The cardiovascular response and motor phenomena of the present concussion model appear excitatory. Also the more general clinical characteristics of concussion
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with the instantaneous change of function, including occurrence of retrograde amnesia, strongly suggest sudden depolarization as the immediate neuronal reaction to trauma. The blood pressure response and motor phenomena indicate a proceeding activity that is self-sustaining once excitation is started. We found that this reaction could be stopped by more severe trauma inducing vasomotor paralysis, as seen in the 11 m/sec injury group.

The morphological examination showed that concussion can be elicited without the production of gross tissue damage. This is in agreement with the findings of earlier authors. In the animals surviving several days no evidence of chromatolytic cell change or axon tearing was found, but the group was too small to allow firm conclusions. However, the morphological study further illustrated that there is a very narrow border zone between trauma causing only functional changes and that with morphological damage. In this model, SAH was the most common complication. Possibly some SAH is very common also in clinical concussive head injury. In the 7 m/sec injury group the occurrence of SAH did not seem to have any influence on the clinical course. On the other hand, the SAH that usually accompanied the 9 m/sec trauma might be a factor of pathogenetic significance with possible effects on CBF seen angiographically as vascular narrowing. In the present model we evidently have to accept that SAH occurs in connection with the concussion; its influence needs to be further evaluated. The localization of the intracerebral lesions in more severe trauma again indicates the brain stem to be the site of the most pronounced tissue stress.

Summary

The present acceleration model seems well suited for the production of a defined concussion response. The effect was probably mediated via shear stress along the brainstem axis. Concussion could be produced without visible morphological damage at impact velocities of 7 to 9 m/sec, but SAH was a common complication. Intracerebral lesions were not seen until higher impact velocities were used. The results justify a concept of concussion, the essence of which is an immediate change of neuronal function elicited by mechanical stress, and possibly involving a graded excitatory response. However, this cerebral response is readily accompanied by important effects of systemic reactions or local vascular complications, as well as focal tissue damage. Thus, the acute clinical symptoms may reflect a process involving many factors, and difficulties may arise with regard to their definition.

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