WATER CONTENT OF THE BRAIN AFTER CONCUSSION AND ITS NONCONTRIBUTORY RELATION TO THE HISTOPATHOLOGY OF CONCUSSION*

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Studies of concussion of the brain and spinal cord in experimental animals have demonstrated structural alterations in nerve cells of a permanent nature and significant magnitude. In guinea pigs and cats it was found that some of the larger neurons of the brain stem as well as the spinal cord underwent subtle structural alterations immediately after striking an adequate blow. Within 24 hours these same large neurons as well as many smaller cells began to undergo degenerative changes. Primary motor and sensory neurons were relatively unaffected. The greatest structural change appeared in interneurons of the reticular formation, vestibular nuclei and red nuclei. The chromatolysis resulting from concussion was shown to be different from that which followed asphyxiation and that which occurred as a result of axon section. Concussion was differentiated from contusion and skull fracture. Hemorrhages and vascular changes were not observed after simple concussion of the brain or spinal cord. After a series of concussive blows it was shown that permanent cell loss in certain nuclei of the brain stem amounted to more than 50 per cent.

Our experimental investigations of brain and spinal cord concussion suggested that the severe and irreversible cell changes were due to primary injury at the time of concussion. This theory was supported by the observation of immediate cytoplasmic disorganization during concussion and was strengthened by failure to observe afterwards any histological evidence of edema or vascular alterations that might have been primary to the cytopathology characterizing postconcussion. However, it was reported by some investigators that swelling of the brain of experimental animals occurred after concussion and that an increase in cerebrospinal fluid pressure was demonstrable after head trauma. Others, notably Ferraro, claimed to have found numerous cytopathological changes following edema produced experimentally. Thus, it became evident that we should have to test the possibility that the cytopathological changes and loss of cells that we have re-

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† It should be noted that in a recent review of the subject of cerebral concussion the statement that Windle, Groat and Fox "failed to find any immediate histological change" is in error.
ported might have been brought about by an increase in pressure upon the brain due to swelling or edema rather than to a primary injury of the nerve cells themselves. That is the concern of the present report.

Guinea pigs were used for this study because methods of producing concussion had been perfected and histopathological changes in the brain had been most carefully worked out in this species. The first task was to devise an accurate method for detecting small changes in water content of the brain which might be encountered after concussion.

The brain was placed in a previously tared beaker and weighed. It was immediately covered with 25 cc. of acetone under which it was subdivided into pieces of approximately 2 c.mm. volume to facilitate removal of the water. The material was allowed to stand in acetone for 60 minutes. The fluid was then poured into another tared beaker and a second 25 cc. portion of acetone was poured over the tissue. After 45 minutes this was decanted into the beaker containing the first 25 cc. portion. A third extraction with 25 cc. of acetone was performed, this time for 30 minutes, and the supernatant fluid was added to the first two portions.

The beaker containing the acetone washings was placed in an oven at 70°C. and the beaker containing the dehydrated, acetone insoluble brain residue (including phospholipids) was placed in a vacuum oven at 60°C. As soon as the acetone had evaporated (about 2½ hours) from the beaker containing the water, fats, cholesterol and its esters and galactolipids, it also was placed in the vacuum oven. Frequent weighings determined that the substance in the beakers reached constant weight within 24 to 28 hours. The low temperature, low air pressure and short time in the oven were used to minimize oxidation of phospholipids. It was found that the dried residues increased slightly in weight if allowed to stay in the oven longer than 36 hours.

Methods of killing the guinea pig and removing its brain were standardized. The untreated animal was decapitated on a guillotine. The skin and muscles of the head were quickly removed and an incision was made through the atlanto-occipital membrane severing the spinal cord about 1.5 to 2 mm. caudal to the obex of the medulla oblongata. The bone was promptly removed over the cerebellum and then over the cerebrum. The dura was laid back and the brain quickly scooped intact from the cranium with a flat, blunt instrument. It was placed immediately in the beaker provided for it. The total time elapsing between decapitation and weighing the specimen was 1½ to 2 minutes.

Preliminary experiments were performed to ascertain the accuracy and uniformity of this method for quantitative determination of water in brain

<table>
<thead>
<tr>
<th>Animal*</th>
<th>Wt. of Half Brain (gm.)</th>
<th>Water Content of Half Brain (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.8064</td>
<td>78.66</td>
</tr>
<tr>
<td>A'</td>
<td>1.9475</td>
<td>78.67</td>
</tr>
<tr>
<td>B</td>
<td>1.8101</td>
<td>78.69</td>
</tr>
<tr>
<td>B'</td>
<td>1.8355</td>
<td>78.66</td>
</tr>
<tr>
<td>C</td>
<td>1.8400</td>
<td>78.70</td>
</tr>
<tr>
<td>C'</td>
<td>1.8243</td>
<td>78.67</td>
</tr>
</tbody>
</table>

* Halves of three brains are indicated by A and A', B and B', C and C'.

TABLE 1
Comparison of water content of brain halves

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