EXPERIMENTAL BRAIN INJURY DURING HYPOTHERMIA*

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Morbidity and mortality following acute trauma to the brain are often the result of fulminating, severe cerebral edema. Any procedure that could reduce the magnitude of edema would facilitate the management of such injuries. Hypothermia is known to decrease the volume of normal brain and to protect against infarction following permanent interruption of major vascular channels.11–13 This study was undertaken to determine the effect of hypothermia upon the pathogenesis of experimental brain injury and, in particular, the effect of reduction of temperature upon the size of a pathologically swollen brain.

METHODS

Forty mongrel dogs, weighing between 11.0 and 23.4 kg., and unselected as to age and sex, were used in this investigation. Acute and chronic experiments were done. In the acute group, brain injuries were inflicted in pairs of animals. One dog of each pair was normothermic at the time of injury, and the other was hypothermic, 25°C. or less, at the time of injury. Each pair was sacrificed at given intervals following the injury and the data to be described below were obtained.

In the chronic experiments, a control group of animals was subjected to brain injury at normal body temperature. A second group was injured during hypothermia; their temperature was maintained at 25°C. or less for 18 hours, and then they were rewarmed. Both groups were observed for life expectancy and survival.

At the start of each experiment, each animal received long-acting Benzathine penicillin G, 600,000 units, intramuscularly. Diphenylhydantoin sodium, 0.4 gm. daily, was given to suppress convulsions; this was continued through the 5th post-traumatic day in surviving animals.

Anesthesia was achieved with intravenous Pentobarbital Sodium, 30 mg./kg., and the dogs were intubated with auffed No. 38 Fr. endotracheal catheter. The catheter was attached to an automatic positive-negative pressure closed-system respirator which delivered 100 per cent oxygen at a rate of 24 respirations per minute. The positive pressure was adjusted between 7 and 11 mm. Hg and the negative pressure was set between 1 and 4 mm. Hg in order to maintain a tidal exchange of


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200–400 ml. A thermistor was inserted 200 mm. into the esophagus for the recording of body temperature. The animals that were to receive brain injuries at normal body temperature were maintained in the 35–39°C. range, with the aid of a heating pad when necessary. In the hypothermic group, reduction of temperature was effected by immersion to the shoulders in ice water. The injury was inflicted when the body temperature reached 25°C.

Standardized brain injuries were produced by a modification of the method of Clasen and his co-workers. The animals were placed in the right lateral recumbent position. The left hemiceranium was exposed by reflecting the overlying scalp and musculature, using aseptic technique (Fig. 1). An imaginary line was projected along the prominence of the convexity between the apices of the frontal and parietal bosses. A burr hole, \( \frac{3}{8} \) inch in diameter, was made two-thirds of the way from the frontal boss along this line. An insulating plastic bushing was inserted into the burr hole. The tip of a brass cylinder was placed through the bushing at such a depth as to make intimate contact with the intact dura mater beneath. The diameter of the tip of the cylinder was 1 cm. With the instrument fastened in place, liquid air, at a temperature of \( -196°C \), was poured into the cylinder. The cylinder was left in situ exactly 8 minutes. The instrument and bushing were removed and the bony defect was filled with bone wax and was covered with steel screening. The muscle and scalp were closed in layers. Thus, a brain injury was created which, for practical purposes, could be called a closed head injury.

The animals were sacrificed at appropriate intervals with an overdose of Pentobarbital Sodium. At the time of sacrifice or death, the brain was removed immediately and was treated as follows. The brain stem was sectioned at the caudal end of the 4th ventricle and through the midbrain at the level of the tentorium. The supratentorial portion of the brain was cut in the mid-sagittal plane. Each of the three segments was weighed to the nearest 0.1 gm. They were then displaced volumetrically to determine their total volume. The density of the brain was calculated and the percentage increase in size of the injured side of the brain as compared to the
uninjured side was determined. The brains were fixed in 10 per cent isotonic sodium acetate formalin. They were removed 7 days later, and the volumes were reestimated in order to detect any change in size. The brains were cut at 5-mm. intervals in the coronal plane. In the sections where the lesion appeared, the surface areas of the anterior and posterior aspect of the lesion were traced onto cellophane. The two areas were measured by planimetry, the average area was calculated, and the volume of the lesion was determined by multiplying the average sectional surface area by the thickness. The total volume of brain injury was calculated as the sum of the volume of the lesion of each individual section. This "fixed lesion volume" was then corrected to a "fresh lesion volume" by a factor calculated from the difference in total volume of the brain measured at the time of death as compared to the total volume estimated after fixation. Appropriate sections of brain were processed further for histological examination.

RESULTS

Acute Brain Injury at Normal Body Temperature. Brain injuries were inflicted in 13 control dogs at normal body temperature. Lesions were created with a mean volume of 9.20 ml. (S.D. ± 0.98) by allowing an 8-minute contact time of instrument to dura mater. This size injury was chosen because preliminary studies had indicated that such a lesion was uniformly fatal in dogs weighing 10–20 kg. This injury was considered to be, in effect, a closed injury, since dura mater was left intact and the skull defect was closed solidly immediately following the production of the lesion.

The extent of injury is demonstrated in Figs. 2 and 3. The lesion was

![Image](image_url)
sharply circumscribed and resembled grossly a cerebral contusion or hemorrhagic infarction. In addition, if sufficient time had elapsed before death, typical post-traumatic secondary changes developed. These were: edema of the entire injured hemisphere, shift of the internal structures across the midline, ipsilateral ventricular collapse with contralateral dilatation, transtentorial herniation, incisural and aqueductal block, and brain-stem compression, the probable eventual cause of death.

Fig. 3. Coronal sections of a dog brain, normal body temperature, 7½ hours after injury. Note: ridging of unci bilaterally (row 2, section 3), herniation of the suprasplenial gyri beneath the corpus callosum (row 3, section 1) and occlusion of the aqueduct of Sylvius (row 4, section 1).

Since the injury was inflicted under general anesthesia, the immediate post-traumatic "clinical" manifestations could not be observed. However, when survival time exceeded the effect of anesthesia, it was usual to observe the following. The animals regained consciousness for a short period of time or they were semicomatose. If conscious, they were lethargic but made feeble attempts to stand up. The limbs contralateral to the injured hemisphere were paretic and a contralateral visual-field defect was demonstrable. The animals became progressively less responsive and ultimately lapsed into coma. Pupils were initially constricted but became dilated and fixed terminally. Bradypnea and bradycardia preceded the development of Cheyne-Stokes respiration, apnea, cardiovascular collapse and death.
Histologically, the lesions were characterized by widespread destruction of the cellular elements, especially the neurons, complete loss of cytoarchitectural markings, interstitial edema, vascular dilatation, capillary and venous thromboses, perivascular diapedesis, petechiae, and coalescent hemorrhages (Fig. 4). If the lesion was more than 4 hours old, an intense inflammatory reaction occurred, polymorphonuclear leukocytic elements predominating, with maximum intensity being manifest within 12 hours. It is of interest that brain-stem hemorrhages were rare despite evidence of severe brain-stem compression.

![Image of histological lesion](image)

**Fig. 4.** Microphotograph of the lesion, normal body temperature, 12 hours after injury. Cresyl violet, ×32.

**Acute Brain Injury during Hypothermia.** The identical method was used to produce brain injuries in 13 hypothermic dogs. Mean body temperature was 24.1°C. at the time of injury. Grossly, the lesion had a scalloped appearance with the hemorrhage tending to follow the cortical gray markings while sparing the white matter (Fig. 5). This was in contrast to the wedge-shaped, completely hemorrhagic appearance, both of the gray and white matter, in the normothermic lesions. Cerebral edema secondary to injury was minimal, as may be seen by comparing Fig. 5 with Fig. 3.

The mean volume of the lesion was 9.50 ml. (S.D. ± 1.46). Thus, the size
of the lesion was not altered significantly by the use of hypothermia, although the standard deviation was greater. The latter was probably ascribable to an increased difficulty in measuring the lesion, since the estimate of traumatized brain included the hemorrhagic and nonhemorrhagic portions of the lesion. The latter were sometimes difficult to delineate.

Histologically, the differences in the hypothermic lesions as compared to the normothermic lesions were even more marked (Fig. 6). The cortical architecture was better preserved, cellular elements showed less evidence of injury, albeit definite degenerative changes were found which may or may not have been reversible in nature. Interstitial edema was reduced, and vascular dilatation and hemorrhage were much decreased. Inflammatory reaction to injury was negligible. Cellular destruction and hemorrhage continued to a maximum degree at 24 hours. It is noteworthy that the inflammatory reaction was still absent or minimal even in lesions 36 hours old, the longest period of observation in this study.

Effect of Hypothermia on Cerebral Edema Following Injury. Standardized brain injuries were produced in 13 pairs of dogs, one of each pair being normothermic at the time of injury, the other being hypothermic. At predetermined intervals following injury, each pair of animals was sacrificed and
the brains were removed for examination. If the animals died before sacrifice, the same procedure for examining the brains was followed. The brains were sectioned and their size was determined both gravimetrically and volumetrically, as described under "Methods." The weight of the injured hemisphere was compared with the weight of the uninjured hemisphere. The cerebral hemispheres of normal, uninjured dogs showed no significant difference in size when compared with each other. Therefore, in the experimental

animals, an increase in weight of the injured hemisphere over its uninjured mate was interpreted as indicating the presence of an abnormal accumulation of fluid. Clasen and his co-workers\textsuperscript{6} studied intensively the whole weights, water contents, dry-wet weight ratios, blood contents, etc., of injured brains, and they, too, concluded that measurements of the weight were the best index of formation of edema.

Within the first hour following injury, there was a precipitous increase in the size of the injured hemispheres of both normothermic and hypothermic dogs. The increase in the normothermic dogs was greater initially and became progressively larger with the passage of time (Fig. 7). Six of the 13

Fig. 6. Microphotograph of the lesion, hypothermia, 12 hours after injury. Cresyl violet, $\times32$. 
normothermic dogs died during the experiments. An initial increase was also observed in the hypothermic dogs, but the magnitude was not as great and the change thereafter was small for a period of observation up to 36 hours. Two of the 13 hypothermic dogs died before the predetermined time for sacrifice.

Effect of Hypothermia Upon Life Expectancy and Mortality Rate. Standardized brain injuries were created in 10 normothermic control dogs for the purpose of determining survival time. All 10 died. The animals survived between 2 and 19 hours with a mean life expectancy of 8.3 hours (S.D. ±5.41).

Lesions were produced in 7 hypothermic dogs. Their body temperatures were maintained at 25°C. or less for 18 hours; they were then rewarmed and observed. An 18-hour period of hypothermia at 25°C. was selected as this was found to be the longest period compatible with survival upon rewarming in normal uninjured dogs. Two of the 7 animals survived; they were sacrificed at 14 and 21 days following the injury. The remaining 5 dogs died; average survival time in these 5 animals was 45 hours, ranging between 27 and 90 hours.

Pathologically, it was possible to compare the lesions in the 2 survivors of this series with lesions of the same age from earlier experiments in which
nonfatal smaller injuries had been produced at normal body temperature. It was seen that the "protective influence" of hypothermia had still prevailed. The areas of the injury in the normothermic dogs were completely necrotic and surrounded by a dense, tough, fibrous capsule. By contrast, viable elements were found within the lesions in the hypothermic animals and the reactive attempt to wall off the injured area was mild and incomplete.

Despite a five-fold increase in survival time, this sample did not demonstrate a statistically significant decrease in mortality rate. However, it appeared that if the degree of protection provided could have been increased only slightly, more animals would have survived.

DISCUSSION

The method of injury described in these experiments has been utilized in principle by several investigators in the past. Its greatest proponents have been Hass and Taylor and, more recently, Clasen and his co-workers, who have modified the technique to provide more nearly accurate quantitation of the extent of injury. This type of injury is not entirely analogous to that pathology seen subsequent to the usual form of trauma when the head comes in contact with another object; yet, in many respects, this lesion is preferable from an investigative viewpoint. Not only does it allow control of the dimensions of the lesion, but it avoids the complicating variables inherent in direct trauma to the head; namely, forces, velocities, deformation of the skull, mass movements of the brain, transmitted energies and their pathological sequelae, contrecoup injury, laceration, and hemorrhage. Instead, a "pure" lesion is produced for study which approximates a cerebral contusion.

It is established that hypothermia reduces the blood flow and metabolic rate of normal brain. Elements of these parameters are operative in the pathogenesis of brain injury; therefore, the changes induced by hypothermia should theoretically prove advantageous in limiting post-traumatic sequelae by decreasing the volume of the brain and intracranial pressure. Furthermore, a reduction in metabolic rate might act as a protective influence against injury by inhibiting the post-traumatic inflammatory reaction.

Hypothermia did affect markedly this experimental form of trauma. Both the primary and secondary components of injury were modified. The mechanism by which this was achieved is not known. However, one conclusion seems evident; that is, that the post-traumatic inflammatory reaction is depressed or even prevented by a reduction in temperature.

Analysis of the data from these experiments provides direct evidence supporting this hypothesis. There was a relative preservation of the cellular elements and cytoarchitecture within the lesions of the hypothermic dogs as compared to the almost complete destruction observed in the lesions of the normothermic dogs. There was a lack of leukocytic response, even 36 hours after injury, in the acute hypothermic dogs, and there was only a mild reac-
tive gliosis in the survivors of the chronic experiments. Although an initial post-traumatic increase in the size of the brain was observed, accumulation of fluid of edema thereafter was small. It is felt that this initial increase represents an increase in volume caused by extravasation of blood and fluid into the lesion itself. This is in keeping with the data of Brierley, who found a sharply elevated concentration of radioactive phosphorus and di-iodo-fluoroscein in similar type injuries within 15–30 minutes of their occurrence. He interpreted his data as indicating an increase in local volume of blood caused by vasodilatation and an excessive accumulation of blood and fluid within the injured tissue caused by a change in permeability of the blood vessels. The progressive secondary increase in size of the brain in the normothermic animals of the present study is attributable to the development of edema in the rest of the hemisphere in response to trauma. The almost complete attenuation of the secondary increase in the hypothermic dogs is thought to represent a direct effect of reduction of temperature on this response.

This study presents a method for observing the primary and secondary effects of an isolated, circumscribed injury of the brain. It also provides evidence of protection by cold against the sequelae of such lesions. It must be emphasized that such protection was demonstrated under artificial conditions. This study tested the effects of reduction of temperature on experimental lesions with the temperature at 25°C. at the time of injury. The only variable was the duration of hypothermia after production of the lesion. No attempt was made to determine the influence of delay between time of injury and the induction of hypothermia, rates of cooling and rewarming, and the level of reduction of temperature. It is left for future investigation to delineate these parameters.

This is not to be construed as an endorsement of the 25°C. level of hypothermia for clinical use. This still remains an experimental range of reduction of temperature, one not generally considered safe for use in man. The results of this investigation serve only as an index of the potential therapeutic value of reduction of temperature. However, it is hoped that further study will make possible the safe utilization of lower temperatures, thereby allowing clinical application of the principles demonstrated in this investigation.

SUMMARY AND CONCLUSIONS

Standardized brain injuries were inflicted in groups of normothermic and hypothermic dogs. Acute brain injuries at normal body temperature resulted in wedge-shaped, hemorrhagic lesions with edema of the affected hemisphere, shift of internal structures across the midline, transtentorial herniation, and brain-stem compression. Post-traumatic inflammatory reaction in the form of cerebral edema and leukocytic response was marked and progressive. When brain injuries of the same size were produced with the body temperature 25°C. or less, the lesion was only partially hemorrhagic and post-traumatic cerebral edema with its pathological sequelae was markedly
reduced. The leukocytic inflammatory response was absent or minimal even 36 hours after injury. Cerebral edema developed to a mild degree immediately following injury but thereafter progression was small. Seven dogs with brain injuries were subjected to hypothermia at 25°C or less for 18 hours before rewarming. Two survived and 5 died. Those that died survived 5 times longer than their normothermic equivalents.

It was concluded, therefore, that hypothermia protects against acute experimental brain injury by limiting the response to trauma.

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