Study of Functional Recovery Produced by Delayed Localized Cooling After Spinal Cord Injury in Primates*

MAURICE S. ALBIN, M.D., M.Sc., ROBERT J. WHITE, M.D., PH.D.,
GASTON ACOSTA-RUA, M.D., AND DAVID YASHON, M.D.
Divisions of Anesthesia and Neurosurgery, Department of Surgery, and the Brain Research Laboratory, Case-Western Reserve University, School of Medicine at Cleveland Metropolitan General Hospital, Cleveland, Ohio

IN SPITE of the sophisticated advances in modern surgical therapy, the present day management of spinal cord trauma generally remains unsatisfactory. Current methods used in the treatment of spinal cord trauma consist of immobilization with skeletal traction, surgical decompression with or without incision of dural coverings, and immediate or delayed bone fusion to provide stabilization. Controversy even exists as to the merits of "conservative" treatment versus surgical decompression. Even among those favoring early laminectomy in patients displaying sensory-motor paralysis associated with a spinal fluid block, there is no unanimity of opinion whether the dura should be opened or left intact.

In reviewing other approaches to the problem of spinal cord injury, we were impressed with some of the work relating to the protective effect of cold on brain. It has been shown that hypothermia will decrease the cerebral metabolic demand, reduce brain volume, bring about a reduction in the inflammatory response to brain injury, and allow the brain to tolerate extended periods of circulatory arrest. It has also been demonstrated that total body cooling to 30°C will protect the spinal cord of the dog during occlusion of the thoracic aorta. "Because of the accessibility of the injured segment of the spinal cord during surgical laminectomy, an attempt has been made to assess the practical advantages of direct cooling to the traumatized area of the cord, in the belief that the advantages operant with cerebral cooling might also apply to spinal cord tissue.

To evaluate the effectiveness of hypothermia in experimental trauma to the spinal cord it was thought necessary to fulfill the following criteria:

1. The development of a predictable, quantitative method for producing irreversible spinal cord trauma in the control animal
2. The provision of a simple technique of rapidly and efficiently cooling the area of traumatized cord
3. The demonstration of significant functional differences in recovery from injury in the experimental group that was cooled when compared to the control group of animals.

We have shown in previous reports from this laboratory that it was possible to selectively reduce spinal cord temperature in the dog, such temperature reduction was not in itself injurious, and that significant recovery occurred in the dog when localized spinal cord cooling was carried out immediately after induced trauma.

This paper evaluates the effect of delayed localized cooling about the area of spinal cord injury in the subhuman primate, Macacus Mulatta.

METHOD

In this study, acclimatized Rhesus monkeys (Macacus Mulatta) ranging in weight from 7 to 11 lbs were used. All animals were anesthetized with 25 mg of intravenous sodium pentobarbital per kilogram of body weight, with supplements of 15 mg per kilogram when needed. To eliminate uninten-
tional bias on the part of an investigator, the procedures listed below were performed and evaluated by at least three investigators participating in this study.

**Technique of Injury.** Spinal cord trauma was produced by dropping a weight a known distance (through a virtually frictionless Teflon tube) on a contoured metal impounder resting on the spinal cord surgically exposed at the tenth thoracic vertebral level (T-10) with all investing membranes intact (Fig. 1). This method for producing impact injury provides a predictable quantitative model for studying spinal cord trauma with the resultant forces of injury calculated in gram centimeters of force (gcf).

**Normothermic Control Studies and Paraplegia Thresholds.** To determine the threshold for irreversible paraplegia, 15 monkeys were subjected to laminectomy at T-10. They were then divided into three groups of five monkeys each and subjected to the following forces of injury:

1. **Group 1.** 200 gcf (20 gm weight dropping 10 cm) followed by a 4-hour delay after injury
2. **Group 2.** 300 gcf (20 gm weight dropping 15 cm) followed by a 4-hour delay after injury
3. **Group 3.** 400 gcf (20 gm weight dropping 20 cm) followed by a 4-hour delay after injury.

After the 4-hour delay, the dura was incised and left open, the wounds closed aseptically, and the animals observed for at least 3 months. When the lowest threshold for irreversible paraplegia was determined, an additional eight monkeys were traumatized at this gcf and followed neurologically for at least 3 months.

**Localized Spinal Cord Cooling.** Twelve monkeys were subjected to localized spinal cord cooling after they had undergone laminectomy at T-10, exposure of the spinal cord, and incision of the dura. With the surgical area as a reservoir, cold isotonic saline entering between 2° to 5°C from a Mayo pediatric heat exchanger was continuously recirculated at a flow rate of 100 ml per min-

![Diagram of the device for producing impact injury](image-url)

**Fig. 1.** Device for producing impact injury: teflon dropping tube is 30 cm long with perforations every cm starting at the 10.0 cm level. This is attached to metal impounder saddle that rests on lateral sides of vertebral body to give stability to dropping tube. Metallic impounder fits convexity of posterior surface of spinal cord and is placed in saddle. Teflon tube is loaded with weight and held in place by weight-restraining pin which is placed through desired centimeter perforation hole. In this manner, weight can be dropped any preset distance on impounder, delivering a force calculated in gram centimeters. A small rounded spirit level (not shown here) is used to align the dropping tube perpendicularly to spinal cord. This spirit level easily fits on top of the dropping (tube).
ute over the exposed cord (Fig. 2). The 12 monkeys were then divided into two groups:

**Group 1. Physiological study (5 monkeys).** Cooling was carried out for 3 hours, and temperature measurements with a YSI thermistor system were then sampled from the cord substance, inflow and outflow perfusate, brain, and mid-esophagus. Arterial blood pressures and pulse rates were obtained from a catheter placed in the lower aorta via the femoral artery and connected to a Statham strain gauge leading to a Grass Polygraph. Heart rates and electrocardiograms were monitored with appropriate leads connected to an electrocardiograph preamplifier.

**Group 2. Control cooling study (7 monkeys).** Five monkeys were subjected to localized cord cooling for 3 hours, and two for 6 hours. After termination of cooling, the dura was left open, the wounds closed, and the animals followed neurologically for at least 3 months.

**Neurological Evaluation.** Motor function was judged by Tarlov's scale for grading the recovery of motor function of the hind limb: (0) = no voluntary movement; (1) = perceptible movement of joints; (2) = good movements at joints but inability to stand; (3) = ability to stand and walk, (4) = complete recovery.

The animals were observed in their cages, tested on a table, and placed midway on an 8-foot pole to evaluate their climbing ability. Studies on locomotion were further carried out by observing the animals in a small room. Tactile stimulation was carried out by squeezing the skin of the lower extremities with a hemostat, the pain reaction judged by the degree of withdrawal of the extremity as well as the animals' facial expression and general body response. Placing reaction was tested by moving the animals' hind legs toward the edge of a table and allowing the toes to make contact with it. Periodic movies on 16 mm color film were taken of all animals so as to continually document their progress.

**Animal Care.** These animals with spinal cord lesions received close attention and were under continuous veterinary surveillance. Their bladders were expressed every 4 to 6 hours, cages cleaned frequently during...
the day, and the animals were washed and cleaned each day. Daily body weights and temperatures were taken, analgesics given if needed, antibiotics given postoperatively, and multivitamin supplements added to their diet.

Results

Normothermic Control Studies and Paraplegia Thresholds. The five Group 1 animals (200 gcf) showed complete return of function within 72 hours after injury and demonstrated no neurological residual. They had no difficulty in climbing and running, placed well, and responded to tactile stimulation.

The five Group 2 animals (300 gcf) and the five Group 3 animals (400 gcf) all developed an irreversible paraplegia when followed for at least 3 months. Since 300 gcf represented the lowest threshold force for paraplegia, an additional eight monkeys were then traumatized and these also became irreversibly paraplegic.

In the 13 animals injured with 300 gcf (Table 1) the cords appeared blueish-red within minutes after impact injury as viewed through the dura. When the dura was opened after the 4-hour delay, the cord appeared swollen and often nonpulsatile, the pia arachnoid hemorrhagic. In these animals, the period of “spinal shock” ranged from 2 to 5 weeks after impact injury; during this time the lower extremities remained flaccid, with loss of all somatic sensation below the level of the lesion and absent reflexes. When viewed 3 months after injury, nine of the 13 had developed spastic paraplegia in flexion, with complete sensory loss; only one in this group gave evidence of perceptible movement at the joints.

Two animals developed a spastic paraplegia in extension at the end of the 3-month observation period, with no response to sensory stimulation and no voluntary activity.

Two animals remained essentially flaccid at termination of the 3-month period, manifesting no motor or sensory responses.

Localized Spinal Cord Cooling. Results in the five animals in the physiological study (Fig. 3) showed that, from a preperfusion mean temperature of 37.0°C ± 0.5°C, it took an average time of 20.0 min ± 1.0 min to reach 10°C within substance of cord. Brain and esophageal temperatures were at or near normothermic levels, with a maximal drop of 2.0°C occurring in one animal. Mean arterial blood pressure showed a slight decline upon initiation of cold perfusion and then quickly returned to preperfusion levels for the remainder of the experiments. Heart rates and the electrocardiogram showed no significant changes from preperfusion levels.

In the Control Cooling Study, within 24 hours after termination of the procedure no neurological deficits could be found in the five monkeys subjected to 3 hours of cooling

**TABLE 1**

<table>
<thead>
<tr>
<th>Animal No</th>
<th>1st wk*</th>
<th>2nd wk</th>
<th>3rd wk</th>
<th>4th wk</th>
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*0 = no voluntary movements; 1 = perceptible movement of joints; 2 = good movement at joints but inability to stand; 3 = ability to stand and climb; 4 = complete functional recovery; FP = flaccid paraplegia; SPF = spastic paraplegia in flexion; SPE = spastic paraplegia in extension; S = sensation; - = absent; + = present
and the two monkeys subjected to 6 hours of cooling.

**Spinal Cord Trauma and Localized Cooling.** The same type of hemorrhagic, edematous spinal cord noted in the normothermic control studies was seen in this group of 14 monkeys upon incision of the dura after the 4-hour delay (Table 2). After the cooling period ended, it was frequently noted that the injured spinal cord mass was reduced in size. Excellent return of function as demonstrated by the ability to run, climb, and place was evidenced by seven monkeys within 3 days. Three animals had mild deficits in placing, with bilateral leg weakness which cleared within 1 week; a similar type of deficit cleared in another three monkeys by the end of the second week. In one animal, mild bilateral leg weakness with slight impairment in placing persisted through the end of the 3-month observation period, although this animal was able to climb with good facility.

**Discussion**

Localized cooling to intrinsic cord temperatures of $10^\circ$ did not produce any deleterious effect. Body core and brain temperatures remained stable and showed little deviation during prolonged localized spinal cord cooling. The cooling technique is simple in design and implementation and utilizes a closed perfusion circuit that can be easily sterilized with the perfusate remaining sterile under operating conditions.

Under the design of experimental injury used here, localized spinal cord cooling has been effective in arresting the development of severe neurological damage due to spinal cord injury when instituted 4 hours after trauma. It is possible that the protective mechanism of cooling involves the reduction of edema, the lowering of metabolic demand in the injured area, and hence a greater tolerance to ischemia. The findings of a hemorrhagic and edematous spinal cord 4 hours after injury is in line with the observation of Allen who noted that spinal cord edema in the canine following impact injury became maximal about 4 hours after injury. It is recognized that in this study the spinal cord of the Rhesus monkey has been subjected to a very specific type of injury and that the minimal force needed to produce an irreversible paraplegia in the experimental animal was used. We do not know the acute compression force exerted on cord from the impact injury and assume that the factor of mechanical deformation might become significant if higher impact injury forces were used.26
From these observations it may be assumed that the development of spinal cord edema as a sequela of impact injury was an important factor in producing the sensorimotor paralysis found in the normothermic controls. This is in agreement with Allen and Freeman and Wright who used this impact injury technique in experiments on animals. The swelling of the spinal cord against a relatively inelastic dura has been cited by Scarf as a contributing cause of neurological damage in the human.

Under our experimental conditions, it is possible that the impact injury caused intrinsic damage to the vascular supply of the affected area and that the concomitantly developing edema contributed to the development of ischemia. This damaged portion of the spinal cord would then have a compromised vascular perfusion probably enhanced by the segmental nature of the spinal cord blood supply with its relative lack of anastomotic channels.

Although it has been observed that a significant reduction in the caliber of cerebral arterioles occurs during hypothermia to 30°C, we have noted that the normal homeostatic regulatory mechanisms involved in cerebrovascular tone were abolished when temperatures below 12°C were reached in the donor-perfused isolated monkey brain. Below 12°C, flow rates through the isolated monkey brain equalled or even exceeded those measured during normothermia. With this in mind, one may speculate that vasospasm occurring among the injured vessels in the traumatized area of cord would be overcome (by reaching 10°C) and allow an adequate flow through these vessels and possibly those with marginal injury.

The mechanism by which hypothermia exerts its protective effect can be partially explained by the marked reduction in metabolic activity and demand of the cooled tissue. Evidence has been accumulating recently to show that the application of cold to cells also has a profound effect on the ultrastructural arrangement. The microtubules (or neurotubules in nerve cells) are cytoplasmic bodies that are thought to provide the motive force and directional guidance for particle flow and metabolites in both dendrite and axon, as well as having form building function. Under low temperature conditions, structural alterations occurred with depolymerization of the microtubules. This process is reversible with polymerization developing on rewarming. Thus, hypothermia has been found to have important structural effects on the cell itself which possibly prevents untoward ionic shifts or the entrance of deleterious metabolites.

The type of impact injury we have produced has responded well to localized perfu-
Localized Cooling for Spinal Cord Injury

16. PONTIUS, R. G., and DEBAKEY, M. E. Hypo-

Summary

1. In the Rhesus monkey, localized spinal cord cooling with a simple perfusion unit produced effective selective reduction of cord temperature to 10° C.
2. This drop in spinal cord temperature caused no appreciable change in cardiovascular dynamics or in brain and body core temperatures.
3. Thirteen monkeys had irreversible lower extremity paraplegia following subjection of the spinal cord at T-10 to an impact injury force of 300 gcm and incision of the dura after a 4-hour delay.
4. Fourteen monkeys showed an excellent return of neurological function within 1 month from subjection to an impact injury force of 300 gcm (after laminectomy at T-10) followed by incision of the dura 4 hours later and localized spinal cord cooling for 3 more hours.
5. This protective effect of cooling was presumed to result from a reduction in spinal cord edema, a decreased metabolic demand, and a better hemodynamic perfusion through a vascularly compromised area of injury.

References


32. White, R. J., and Albin, M. S. Perfusion characteristics of the isolated brain. 1968 (to be submitted for publ.).