THE PASSAGE OF SODIUM-24 AND RUBIDIUM-86 ACROSS THE
BLOOD-BRAIN BARRIER SYSTEM OF CANINES AT LOW
BODY TEMPERATURES

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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Denton, Texas

May, 1976

To evaluate the blood-brain barrier system in the pathogenesis of an irreversible hypothermic state in dogs, concentrations of $^{24}\text{Na}$ and $^{86}\text{Rb}$ were measured at body temperatures ranging from $37^\circ C$ to $16^\circ C$. A suppression of transport of sodium was demonstrated, followed by an increase as the temperature was lowered. The concentration of rubidium ion increased in concentration as the temperature fell.

These data indicate there may be a temperature threshold below which the blood-brain barrier system fails to maintain the internal environment of the central nervous system. The intimate relationship of several brain stem nuclei with the cerebro-spinal fluid indicates they may be at risk during profound cooling.
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CHAPTER I

INTRODUCTION

Tissue Hypothermia

The rate of cellular metabolism will vary proportionately with the environmental temperature in a manner analogous to the effect of temperature on the rate of any enzyme-mediated chemical reaction, with a few notable exceptions (10). For this reason, a simple hypothesis may be made: as the temperature decreases, the reaction rates of metabolism also decrease, and on a cellular level, the demand for oxygen, as a function of time, is reduced. With a diminished demand for oxygen at the tissue level during periods of lowered temperature, one can substantially reduce the blood flow to the tissue for longer periods of time without imparting serious damage to the tissue concerned, if it can resume normal function upon completion of rewarming (26).

Procedures involving circulatory stasis during deep hypothermia as an adjunct to elective cardiac arrest are approaching a routine status according to several workers in the field (13, 15, 16). The advantage to such a procedure circumvents the necessity for placing the patient on a cardiopulmonary bypass pump.
In higher mammals the tissue with the greatest oxygen demand and, therefore, with the greatest risk for possible irreversible damage due to anoxia is the tissue of the central nervous system (1, 8, 24). Thus any procedure involving a therapeutic episode of hypothermia used to retard tissue death due to anoxia or hypoxia must not produce irreversible brain damage.

Hibernation Studies

Classical investigations of hibernation led several early investigators to the assumption that producing an hypothermic state would reduce the tissue requirement for oxygen, and allow for prolonged circulatory stasis in non-hibernators (2, 12). The hibernating ground hog was the general animal of choice for these investigators. Although it is now thought that there are several factors contributing to the onset of hibernation, i.e., length of photoperiod, food availability, and seasonal hormonal changes, in addition to air and ground temperature, the important fact remains: when cold environmental temperatures do occur, the marmot retires to his abode and "sleeps" throughout the winter. The term sleep is used here because the animal enters into hibernation from a state of sleep, although periods of arousal may occur during hibernation (20). During this period of dormancy the body temperature of the marmot falls to 2-5°C (12). Shivering is not observed; the heart and
respiratory rate fall to a low level; and oxygen consumption is reduced tenfold (2, 12, 20, 22). The hibernator is also equipped with a feedback control thought to be located in the anterior hypothalamus which stirs him to activity if the temperature falls too low. This mechanism protects from frostbite or freezing solid (17).

Release from the state of hibernation probably involves several factors. As previously stated, the anterior hypothalamus plays an important role. Another factor concerns the so-called hibernation gland, actually discrete deposits of brown fat which might serve as an endogenous rewarming mechanism (4). These brown fat deposits have a prolific blood and nerve supply and at one time were thought to perform an endocrine function (4). This idea has been largely abandoned in favor of the idea that the lipids serve as an energy source during arousal from hibernation (20). Still more recent evidence points to a thermogenic role of brown fat based on increased membrane permeability of these cells, caused by sympathetic stimulation (9). Norepinephrine has been found to stimulate the Na-K dependent ATPase activity of these cells (27).

Bigelow (2) found circulation could be stopped for periods of one to two hours on marmots during their hypothermic states, and the animal could be successfully recovered. He began investigation into induction of hypothermia in mammals which normally exhibit no
such behavior and found many similarities in the physiology of cold, non-hibernating mammals and hibernators. Heart rate and respirations were greatly depressed, and prolonged circulatory stasis could be performed without serious impairment of the animal upon recovery.

Hypothermia

In his textbook *Modern Surgical Technic*, Thoreck (25) described a procedure that he called refrigeration anesthesia. This was a local anesthetic for amputations and other procedures involving the extremities, the patient being only mildly sedated with a narcotic analgesic. Dr. Thoreck felt the procedure had considerable potential for it afforded the only known anesthesia which prevented shock.

Other investigators and clinicians have since expanded these earlier ideas and incorporated them into surgical procedures (24). Mohri (15) described a technique for reparation of congenital heart defects in infants using deep stages of hypothermia to prevent brain damage during cerebral hypoxia.

With the advent of hypothermia as a standard procedure, the study of the physiological changes becomes of increasing importance. Various investigations into the physiological perturbations accompanying hypothermia have largely been centered around two areas: the cardiovascular system and the central nervous system. Studies
concerning cardiac abnormalities associated with hypothermia are indeed of foremost importance since the heart is grossly affected by cold temperatures. Fibrillation, cardiac standstill, and loss of spontaneous contractility at low temperatures are common events (5, 7, 21).

Hypothermia and the Central Nervous System

The effects of hypothermia on the central nervous system have also been investigated (6, 11, 23, 26, 14) to establish the effect of temperature on the substance of the brain, the cerebral circulation, and the blood brain barrier.

The CNS has an absolute requirement for oxygen and therefore cannot tolerate any oxygen debt or, consequently, hemostasis. The intention of deep body cooling is not to establish an oxygen debt tolerance where none previously existed, but rather to reduce the metabolic load of the brain and hence reduce its demand for oxygen and other substrates, most notably glucose (3). The brain, unlike heart or muscle, does no mechanical work, and unlike liver or kidney does no significant metabolic work, other than that required to maintain its own environment. The main requirement for energy is on the basis of maintaining electrical and concentration gradients, largely to allow for spontaneous, non-volitional activity. It is
interesting to note that the Cerebral Metabolic Rate (CMR) does not significantly decrease during sleep (19).

During cooling it has been noted that the brain's excitability and spontaneous activity decreases--the EEG activity at $22^\circ C$ is only 10 per cent of that observed at normothermia. High energy phosphates and glycogen levels are maintained or elevated during hypothermia (3, 18).

Hypothermia also has been found to reduce experimentally produced increased intercranial pressure in dogs, since it is believed to slow the rate of Cerebro-spinal fluid production (3).

The Blood-Brain Barrier and Cerebro-spinal Fluid

Evidence for a physiologically active physical barrier between the circulation and the formed cerebro-spinal fluid has been noted since the late 1800's. It was observed that certain supravital dyes which stained all other tissues in the body failed to enter the central nervous system (11, 22). As presently understood, regulation of transport of water, metabolites, and electrolytes occurs against a concentration gradient in the area known as the blood-brain barrier. The composition of this selective ultrafiltrate is an important factor in determining the degree of activity and efficiency of this complex system. The cerebro-spinal fluid plays a dual role in the central nervous system. The first and least complex is a structural role, providing an aqueous
medium in which the brain and spinal cord "float" and are protected from damage due to sudden violent movements. The second, which is more complex and less well understood, is a functional role of providing nourishment to some extent, providing the proper environmental electrolyte solution for the most efficient transmission of action potentials, providing a medium through which receptors in the deep nuclei of the brain can react to changes in pH, pO₂, pCO₂, osmodarity, and possibly hormonal transmission to areas of the brain (1, 8, 22).

The cerebro-spinal fluid is elaborated by a complex system known as the choroid plexus. The choroid plexus is located in the lateral ventricles, which compose the major portion, and also the third and fourth ventricles. The structural makeup of the choroid plexus is of three parts: the endothelium of the capillaries entering the plexus, the epithelium of the cells of the choroid plexus in contact with the inner side of the ventricles, and an intervening basal lamina. The cells of the choroid epithelium are thrown into elaborate folds to form a complex junctional apparatus at the base of the cells in contact with the basal lamina. The vascular part of the choroid plexus is formed by an invagination of the vascular pia mater; thus the core of a choroid villus contains wide capillaries and connective tissue. The walls of the capillaries are fenestrated and quite porous in contrast to the tight junctions of the choroid epithelium and the dense basal lamina.
The cells of the choroid epithelium appear as cuboidal in shape, with a spherical centrally located nucleus, numerous mitochondria in their abundant cytoplasm and irregular microvilli on their free edge in contact with the cerebro-spinal fluid. The numerous mitochondria would seem to indicate a prolific amount of energy requiring processes, undoubtedly related to active transport.

The cerebro-spinal fluid contains dissolved oxygen, glucose, some small proteins, amino acids, and several inorganic ions. Sodium ion is actively transported past the villi (6); rubidium ion is selectively excluded (26). Rubidium has been shown to be an effective tracer of regional blood flow presumably exchanging freely with potassium (26). However, under normal conditions rubidium will not pass the blood-brain barrier (26). In a comparative study of hibernating ground squirrels and rats, Wells (26) found rubidium would not pass into the cerebro-spinal fluid of squirrels at any temperature; however, in rats cooled to a rectal temperature of 16°C, appreciable amounts of radioactive rubidium were found to have penetrated into the brain substance.

Statement of the Problem

The purpose of this investigation was two-fold. The first phase was an attempt to demonstrate a decrease in cellular metabolic rates at the level of the choroid epithelium using sodium ion as the
indicator. The purpose was to show the involvement of this aspect of the central nervous system in the hypothermic experience. The second phase of this investigation was to place an element, rubidium, into the circulation which normally does not cross the choroid epithelium and to observe any subsequent change in membrane permeability through the appearance of that element within the cerebro-spinal fluid.

The sodium ion tracer also served as an indicator that the choroid plexus was adequately perfused and that any absence of rubidium in the CSF was not a result of decreased blood flow to the choroid plexus during hypothermia.
CHAPTER BIBLIOGRAPHY


CHAPTER II

MATERIALS AND METHODS

Ten mongrel dogs, weighing an average of 15-17 kilograms were selected for this study. All animals were observed for two weeks prior to their use to ascertain that they were free from disease and in good health.

At the beginning of each experiment the animals were given four-hundredths of a milligram of atropine sulfate to dry nasopharyngeal secretions and then anesthetized with thirty-three milligrams per kilogram of sodium pentobarbital. A cuffed endotracheal tube was inserted into the trachea and the cuff inflated in the likely event it became necessary to assist respiration. The animal was then clipped along the abdomen and thorax to facilitate heat loss and shaved along the proposed surgical areas: the left femoral triangle and the lumbar region of the back.

An incision was made in the left femoral triangle, the exact location of the cut being determined by palpation of the left femoral artery. The fascia was cleared down to the artery and the accompanying femoral vein. The vein was cleared of fascia for a length of three
to six centimeters. All collateral vessels were ligated and cut free from the main femoral trunk. Ligatures were placed around each vessel, proximally and distally, the distal ligature being tied and the ends left long at this point. A short length of Intramedic polyethylene cannula, with the same approximate diameter as the isolated vein was prepared by priming the cannula with isotonic saline containing 5 per cent heparin (v/v 3 mg/cc) and clamped at one end by rubber-shod straight Kelly hemostats. The animal was then given an intravenous injection of heparin (five milligrams per kilogram body weight) to prevent blood clotting. The femoral vein was then cannulated proximally through a small diagonal cut in the vein, into which the cannula was inserted for a distance of several centimeters. The proximal ligature was tied tightly around the vein and cannula to hold it into place. All future injections were made through this cannula. The artery was treated identically for the withdrawal of blood samples.

A standard lead II of an electrocardiograph was continuously monitored on a Dallons model CMS cardioscope with an accompanying cardiographometer.

A Yellow Springs Instrument Company thermistor probe was inserted rectally to a depth of 15 centimeters. The probe was coupled to a YSI telethermometer to continuously monitor temperature.
Immediately prior to cooling the animal was given an injection of ten gamma per kilogram of prostigmine to enhance vagal tone and decrease the chances of ventricular fibrillation (2). Animals which were destined to remain isothermic were treated as above. However, they were not subjected to the immersion treatment described below. In this way control levels were established.

Cooling was accomplished by placing a salted ice pack into a cooling basin eighteen inches deep and forty inches in diameter produced by the Schleuter Manufacturing Company, St. Louis, Missouri. The animal was placed on its side on the ice pack. Additional ice packs were used as needed to totally cover the exterior surface of the animal. Often times at various stages of the immersion the animals would commence violent shivering to maintain body heat. Oxygen consumption increases dramatically at this point which negates any beneficial effect of hypothermia. For this reason, animals were given Flaxedil (Gallamine triethiodide) img/kg as necessary to prevent shivering.

When the body temperature of the experimental animal had fallen below 27°C, respiration was generally assisted. As the animal approached the desired temperature and appeared stable, an injection of $^{24}$NaCl and $^{86}$RbCl was flushed into the intravenous catheter with ample amounts of normal saline. An aliquot of each of
the injected isotopes was retained to calculate the total amount of activity injected.

Thirty minutes after injection the animal was removed from the ice bath for a spinal tap. During this procedure the animal was placed on its side and the hind legs brought around the fore legs and tied behind the neck with a length of gauze bandage. The posterior superior iliac spines were palpated and a line made between them fell upon the fourth lumbar vertebrae. The space between the fourth and fifth lumbar vertebrae was palpated, and an eighteen-to twenty-two-gauge trochar needle, four inches long with a close fitting stylette, was inserted through the skin with a quick jab. The rostral aspect of L-5 was closely followed with the needle; using this technique the largest opening between the two vertebrae could be entered. The needle was then passed through the dura and arachnoid membranes into the sub-arachnoid space of the cauda equina. The operator was made aware of the entrance of the needle into this space by a characteristic jerk of the hind limbs when the nerve roots were disturbed. The withdrawal of the stylette at this point permitted the outflow of cerebro-spinal fluid, a few drops of which appeared immediately (I). Two or three cubic centimeters were drawn into a clear glass syringe and transferred to a sample collection tube.
A sample of blood was obtained by cardiac puncture. The blood was placed in a collection tube and centrifuged to separate the formed elements from the plasma.

Three five-microliter samples each of plasma and cerebrospinal fluid were taken and spotted on an icm diameter disc of filter paper. These discs were dried for five minutes at 80°C, then placed in individual Beckman disposable scintillation vials. The time was noted, and the samples were counted on a Beckman 100 L-S scintillation counter.

Preliminary investigation indicated that it was impossible to discriminate between the energies of a mixture of the two isotopes, $^{24}$Na and $^{86}$Rb. A differential decay technique was thus employed. This was possible because of the differences in the half lives of the two isotopes, that of $^{24}$Na being fifteen hours, whereas $^{86}$Rb enjoyed a longevity of eighteen days. Thus the samples were counted again nine days after their collection. At this time $^{86}$Rb had undergone one-half of one-half life whereas sodium approached complete decay at 14.5 half lives (3).
CHAPTER BIBLIOGRAPHY


CHAPTER III

DATA AND RESULTS

Technique

The movement of inorganic ions of sodium and rubidium across the epithelium of the blood-brain barrier was monitored by the periodic isotope activity determinations of each tracer ion in the cerebro-spinal fluid and plasma. These activity determinations were organized and averaged according to temperature ranges and the level of artificial hypothermic treatment. In most cases the time of determination was arbitrarily fixed at thirty minutes after injection of the isotopes. However, in some instances, later samples were also obtained, but in every case a thirty-minute sample was taken. Also in every case the injection was made after the animal had reached a satisfactory temperature and was stable at that temperature.

The results would certainly have been more satisfactory had it been possible to obtain continuous timed samples of cerebro-spinal fluid throughout cooling, and this was indeed the naive intention of the investigator. However, it was soon learned that the lumbar puncture
preparation would not remain patent with the slightest manipulations of the animal. Several attempts were made to insert a small gauge polyethylene catheter into the spinal needle and from there up into the sub-arachnoid space rostrally for a distance of several centimeters, with the assumption that the more piable tubing would cause less damage to the delicate pia mater surrounding the cord. However, these attempts proved to be futile, for after a period of time varying from five to thirty minutes the CSF samples taken could be seen to be contaminated with copious amounts of blood, which was evident on macroscopic inspection. Needless to say, any contamination with blood rendered the samples valueless for the purposes of this investigation.

Plasma Versus CSF Activities

To facilitate the reporting of data, the activities of radioactive sodium ions are expressed as a ratio of the activity found in the spinal fluid to that activity of the serum, the ratio hereafter being referred to simple as SF/S(Na). The data for rubidium eighty-six is reported identically as SF/S(Rb).

Figures 1 through 10 illustrate the relationships of the mean levels of isotopic sodium and rubidium with decreasing temperatures, based on a minimum of three samples per animal. The general trend displayed was a conspicuous decrease in the SF/S ratio of sodium
Fig. 1--Histogram Showing Comparative SF/S Ratios at 37°C
Fig. 2--Histogram Showing Comparative SF/S Ratios at 27°C

<table>
<thead>
<tr>
<th>Element</th>
<th>SF/S Ratio</th>
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<tr>
<td>Na-24</td>
<td>1.747</td>
</tr>
<tr>
<td>Rb-86</td>
<td>0.051</td>
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Fig. 3--Histogram Showing Comparative SF/S Ratios at 25°C
Fig. 4--Histogram Showing Comparative SF/S Ratios at 24°C
Fig. 5--Histogram Showing Comparative SF/S Ratios at 23°C
Fig. 6--Histogram Showing Comparative SF/S Ratios at 22°C
Fig. 7--Histogram Showing Comparative SF/S Ratios at 21°C
Fig. 8--Histogram Showing Comparative SF/S Ratios at 20° C
Fig. 9--Histogram Showing Comparative SF/S Ratios at 19°C
Fig. 10--Histogram Showing Comparative SF/S Ratios at 16°C
activity and a gradual increase of the SF/S ratio of rubidium activity.

As the body temperature was forced down from 37°C to 16°C, there was a decline in the amount of sodium-24 in the cerebro-spinal fluid to less than one per cent of its normothermic concentration. This gradual decline is tabulated in Table I as a ratio of activity, the SF/S ratio. The ambient temperature ratio mean is 1.32, which suggests that the tracer sodium ion was being slightly concentrated in the spinal fluid. After a decline in temperature to the 25 to 27°C range the SF/S ratio is 1.44, again suggesting a concentration in the spinal fluid. At a temperature range of 22 to 24°C the SF/S ratio dropped to 0.506, then further decreased to 0.168 in the 20 to 21°C range, indicating more of the tracer ion was remaining in the blood than was released to the cerebro-spinal fluid. In the 16 to 19°C range there was an increase of the SF/S sodium ratio to 0.563.

The rubidium ion concentration increased tenfold in the cerebro-spinal fluid, beginning at temperatures ranging from 21 to 22°C and continuing through the lowest temperature ranges recorded in this series of experiments. As shown in Table I, this increase continues until the ratio of rubidium exceeds that of sodium. The mean Rb SF/S activity ratio at 37°C was 0.012, increasing to 0.049 in the 25 to 27°C range, 0.030 in the 22 to 24°C range, then
dramatically increasing to 0.306 in the 21 to 20°C range, and finally 0.420 in the 16 to 19°C range.

**TABLE I**

A COMPARISON BETWEEN THE SPINAL FLUID/SERUM RATIOS OF Na²⁴ AND Rb⁸⁶ AND THE VARIOUS DEPTHS OF HYPOTHERMIA AT WHICH THEY WERE EXAMINED

<table>
<thead>
<tr>
<th>Temperature (Centigrade)</th>
<th>Depth (37°C-t)</th>
<th>Na²⁴ SF/S</th>
<th>Rb⁸⁶ SF/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0</td>
<td>0.410</td>
<td>0.000</td>
</tr>
<tr>
<td>27</td>
<td>10</td>
<td>1.747</td>
<td>0.051</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>1.148</td>
<td>0.047</td>
</tr>
<tr>
<td>24</td>
<td>13</td>
<td>0.570</td>
<td>0.026</td>
</tr>
<tr>
<td>23</td>
<td>14</td>
<td>0.900</td>
<td>0.022</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
<td>0.049</td>
<td>0.042</td>
</tr>
<tr>
<td>21</td>
<td>16</td>
<td>0.195</td>
<td>0.433</td>
</tr>
<tr>
<td>20</td>
<td>17</td>
<td>0.142</td>
<td>0.179</td>
</tr>
<tr>
<td>19</td>
<td>18</td>
<td>0.860</td>
<td>0.684</td>
</tr>
<tr>
<td>16</td>
<td>21</td>
<td>0.267</td>
<td>0.156</td>
</tr>
</tbody>
</table>

Figure 11 is a linear regression plot of the SF/X isotopic ratios against temperature. The line extrapolated to represent sodium has a positive slope of 0.058 indicating a very gradual inclination, or in this case a decrease in the ratio as the temperature was lowered. The line representing rubidium has a negative slope of 0.016 which indicates a line very close to parallel to the horizontal axis, representing very little change in the SF/S ratio. Again in this
Fig. 11--Regression Plot of SF/S Ratios Versus Increasing Temperature
case the downward slope of the line indicates an overall increase in the SF/S ratio, hence an increase of Rb$^{86}$ in the cerebro-spinal fluid at hypothermic levels.

Figure 12 is a compilation of the histograms of the sodium SF/S values placed on one axis. Figure 13 shows a similar treatment of the rubidium data. Apart from graphically showing the wide scatter of the points involved, they illustrate some rather intriguing similarities, as is further demonstrated in Figure 14, a composite of the two previous diagrams.

The line representing sodium roughly resembles a bimodal curve. The first downward slope could demonstrate the decrease of sodium ion transport viewed as a function of temperature reducing the reaction rate of the active transport of sodium. The interval between 20-22°C could be viewed as a stable phase of diminished sodium ion pump activity in the cooled state. However, the second peak, occurring in the 16-19°C interval might well represent a point at which sodium ions are freely passing a dysfunctional blood-brain barrier system. Further evidence of this would be the appearance of increased amounts of rubidium ion which also appear in this interval.
Fig. 12--Distribution of Concentrations of Sodium Ions at the Temperatures Investigated
Fig. 13--Distribution of Concentrations of Rubidium Ions at the Temperatures Investigated
Fig. 14--Composite Graph Showing the Relationship of the Change in Activity of Sodium and Rubidium with Decreasing Temperature
CHAPTER IV

CONCLUSIONS AND SUMMARY

Conclusions

The observed decrease in sodium and increase in rubidium in the cerebro-spinal fluid during hypothermia substantiate the hypothesis by Wells (15) that during hypothermic treatment there appears to be a change in the permeability of the blood-brain barrier system with regard to rubidium ion. This further indicates the possibility of a cortical temperature at which the barrier system loses integrity and becomes permissive to the rubidium ion and perhaps other entities, a theorem also postulated by Wells (15, 16).

The wide variability of the results, which have been reported by other investigators in studies on rats, was also observed and recorded in this investigation. Wells rationalizes this variability on the basis of his experimental technique, i.e., the placement of a cannula in the aortic arch, the method of determining the time between injection and sacrifice, and the individual idiosyncrasies of the animals' responses to hypothermic treatment. However, Bigelow, in his classic work on oxygen utilization at low body temperatures,
demonstrated a surprisingly close relationship between temperature and oxygen utilization, in view of the fact that the dogs used were of varying size, breed, and age.

In this particular investigation the age of the animal might well assume some importance as it has been noted that premature babies behave as poikilotherms in their tendency to assume environmental temperatures, and in some studies it has been noted that there is an increased resistance to cold in young subjects. Unfortunately, during this study, it was not possible nor considered feasible to choose animals selectively by age groupings, although such a study would not be without considerable merit. Thus, some of the variability noted could well be due to an age factor. The wisdom of waiting for a fixed time interval between injection of the isotopes and withdrawal of the sample may be questioned. Wells (15) felt a fixed number of heartbeats, 200 in this case, detected by an EKG, more accurately depict a physiologic interval. However, it was noticed early in the experimental process that rubidium was "cleared" from the circulation rather rapidly, presumably by the kidney or bound, either to a tissue component or plasma proteins. Therefore, one would have to strive for the interval which would give adequate mixing throughout the system, but prior to the time required for the animal to deplete the rubidium below acceptable levels. To meet these requirements, a
time interval of thirty minutes was chosen. Although this time period may have diminished the over-all validity of the noted decrease in sodium ion transport, it gave a reasonably accurate picture as far as both ions together were concerned.

With these concepts of clearance or binding, one could further explain variability in the results on the presumably different rates at which animals had accomplished clearing. At 16°C, 19°C, 20°C, and 21°C, the $SF/S$ activities of sodium were 0.156, 0.684, 0.179, and 0.433 respectively, thus giving the appearance of an indiscriminant passage, or a partial breakdown of the blood-brain barrier system. Also, since these are expressed as ratios, the plasma may have been partially cleared of rubidium, thus giving the appearance of a greater value.

Bigelow (1) has previously shown how oxygen consumption and overall body metabolism decreases during hypothermia. Because of this decrease in metabolic rate, one could expect the results shown in Figure 11 indicating an initial overall decrease in the $SF/S$ activity of sodium ions.

Barbou (2) indicated there was a shift of body fluids from the plasma into the cell during hypothermia. If this were the case, one could expect a decrease in the circulating blood volume and an overall decrease in sodium and rubidium as they entered the tissues.
However, in a more recent investigation Farrand and Horvath (4) report an increase in blood volume during moderate hypothermia, i.e., 27°C.

Recently, Wells (16) has indicated that the amount of time spent at a given level of hypothermia was far less important than the level of hypothermia itself, in regard to the integrity of the blood-brain barrier system to a rubidium ion tracer. This would explain the lack of significant differences in SF/S activity observed in this investigation in animals from which samples were taken at times later than thirty minutes.

Figures 12 and 14 illustrate a paradoxical increase of sodium SF/S activity after the preliminary decrease. It has been found in other studies (5, 6, 8, 10, 14) that a prolonged and intense exposure to hypothermia (16°C) resulted in varying degrees of cerebral edema in rats and dogs. Investigation of these edematous brains indicated that there was increased amounts of intracellular and extracellular sodium and water. This has been explained as a temperature sensitivity of the sodium-potassium enzyme systems at the membrane level (17). It is apparent that the increase of one of these edematous components is dependent on the other, i.e., either sodium transport is increased and water diffuses in to effect an osmotic balance, or water ratios in the CSF are increased and sodium ion is pulled in
osmotically. It is unlikely that sodium transport would suddenly increase after a steady decline; however, an increase in the water component could be explained by the description of the junctional complexes at the base of the choroid epithelium. This idea is further substantiated by Figures 13 and 14 which illustrate a dramatic increase in rubidium ion concentration occurring at this same time.

If there is a loss of integrity of the blood-brain barrier as indicated by these results, the effects of such a loss should be explored. Two situations immediately arise as life threatening to the organism. Merrill (9) has shown by a stereotaxic mapping technique that the cell bodies responsible for initiating the inspiratory and expiratory impulses of respiration are located in the lateral aspects of the medulla in close contact with the floor of the fourth ventricle. The rapid influx of ions into the pneumotoxic center would conceivably impair the function of the neurons responsible for the initiation of the respiratory cycle. Also Meyers (11) has demonstrated a decrease in the body temperature of Rhesus monkeys by perfusing the brain ventricles with artificial cerebro-spinal fluid which was high in Ca\textsuperscript{++} ion concentration (10.4 mM Ca\textsuperscript{++}). This decrease in temperature was presumably related to the effect of increased ionic strength on the hypothalamus and indicates that a rapid change in ionic balance might seriously impair an animal's ability to rewarm.
The choroid plexus has been shown to be very sensitive to increases in CO$_2$ (7), and it should be pointed out that the demonstrated loss of integrity of the blood-brain barrier could result from such an increase. This increase CO$_2$ effect would be an autolytic process involving the epithelial cells of the choroid plexus. Although this does exist as a possibility, it does seem unlikely, as the animals were all exposed to moderate hyperventilation after cooling to approximately 28°C. Hypocarbia also functions to reduce the incidence of ventricular fibrillation (7).

Summary

1. Hibernation, a naturally occurring state in some mammalian species, has been the model for introduction of an unnatural cold state in homiothermic species.

2. Early investigations demonstrated a decrease in oxygen consumption by tissues during hypothermia, and later studies have involved prolonged periods of circulatory stasis with successful recovery using this treatment.

3. This investigation was carried out in an attempt to illustrate changes in the blood-brain barrier system, with the possibility that elements injurious to the central nervous system enter the CSF during hypothermia.
4. Two tracer elements were used, sodium-24, which normally passes the blood-brain barrier system and enters the CSF, and rubidium-86, which is normally excluded.

5. Animals were cooled to a pre-selected temperature in a range from 16°C to 27°C, injected with isotopes and after thirty minutes blood and cerebro-spinal fluid withdrawn and subsequently analyzed for activity.

6. The results obtained indicated an erratic decline of sodium ion concentrations in the CSF during hypothermia to 22°C deep body temperature; this was followed by a gradual reappearance of the sodium in the CSF. As the sodium ion levels began to regain in quantity, there was a concurrent increase in the rubidium ion activity in the CSF.

7. These results support the hypothesis that a critical temperature or temperature range might exist, beyond which the potential damage to the central nervous system outweighs the advantages of prolonged circulatory stasis. However, rewarming experiments on dogs under the conditions of this investigation need to be performed to substantiate this hypothesis.

8. Future studies suggested by these results would be a comparative study of the ultrastructure of the choroid epithelium and basal lamina of hibernating and non-hibernating animals to assess
the change or damage that is occurring at the cellular level and below.
CHAPTER BIBLIOGRAPHY


BIBLIOGRAPHY


