1954

The distribution of body water in the hypothermic dog.

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https://hdl.handle.net/2144/9150

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THE DISTRIBUTION OF BODY WATER IN THE HYPOTHERMIC DOG

by

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Submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
1954
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The author wishes to express sincere gratitude to Associate Professor A. H. Hegnauer for his patient instruction, guidance and assistance throughout the past several years. Professor Hegnauer, both as a gentleman and as a scientist, has his pupil's genuine admiration and respect.

The author also wishes to thank Dr. Roger Wright and Miss Jean Flynn for assistance in executing parts of this study and the Department of Biochemistry for the use of equipment.

Special thanks are offered to the author's wife for the preparation of this manuscript.
DEDICATION

A. M. D. G.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Illustrations</td>
<td>iii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>iv</td>
</tr>
<tr>
<td>Introduction</td>
<td>v</td>
</tr>
<tr>
<td>Section I. The Measurement of Body Water Compartments</td>
<td>1</td>
</tr>
<tr>
<td>Part A. Body Water</td>
<td>1</td>
</tr>
<tr>
<td>Hydrostatic and Osmotic Pressure</td>
<td>2</td>
</tr>
<tr>
<td>Transfer of Fluid</td>
<td>4</td>
</tr>
<tr>
<td>Part B. Plasma Volume and Red Cell Volume</td>
<td>5</td>
</tr>
<tr>
<td>Estimation of Plasma Volume</td>
<td>6</td>
</tr>
<tr>
<td>Estimation of Red Cell Volume</td>
<td>9</td>
</tr>
<tr>
<td>Part C. Measurement of Extracellular Water</td>
<td>12</td>
</tr>
<tr>
<td>Chloride, Sodium and Bromide Spaces</td>
<td>12</td>
</tr>
<tr>
<td>Thiocyanate Space</td>
<td>14</td>
</tr>
<tr>
<td>Inulin Space</td>
<td>14</td>
</tr>
<tr>
<td>Section II. Pathological Physiology of the Hypothermic Dog</td>
<td>17</td>
</tr>
<tr>
<td>Part A. General Metabolism</td>
<td>17</td>
</tr>
<tr>
<td>Part B. Survival</td>
<td>19</td>
</tr>
<tr>
<td>Part C. Abnormality of Physiological Function</td>
<td>22</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>22</td>
</tr>
<tr>
<td>Respiration</td>
<td>23</td>
</tr>
<tr>
<td>Cardiovascular System</td>
<td>25</td>
</tr>
<tr>
<td>Part D. Nature of Death</td>
<td>28</td>
</tr>
<tr>
<td>Section III. Water Studies in Hypothermia</td>
<td>32</td>
</tr>
</tbody>
</table>
Section IV. Procedure and Methods

General Procedure
Studies on Blood
Plasma Volume
Red Cell Volume
Thiocyanate Space
Total Water Content and Chloride Space of Tissues

Section V. Results of Original Research

Studies on Blood
Plasma Volume
Thiocyanate Space
Total Water Content and Chloride Space of Tissues
Red Cell Volume

Section VI. General Discussion

Section VII. Summary and Conclusions

Bibliography

Abstract
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>The Variation of Certain Anatomical Temperatures from that of the Rectum During Hypothermia in the Dog</td>
<td>39</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Plasma Mixing and Disappearance Curves of T-1824 Dye and Cells Tagged with Radioactive Phosphorous</td>
<td>42</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>Changes in Plasma Protein Concentration in the Hypothermic Dog. Copper Sulfate Method</td>
<td>51</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>Changes in Plasma Protein Concentration in the Hypothermic Dog. Refractometric Method</td>
<td>53</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Hematocrit of the Normothermic and Hypothermic Dog</td>
<td>47</td>
</tr>
<tr>
<td>Table 2</td>
<td>Percentage Change from Normal in Hematocrit in the Hypothermic Dog</td>
<td>48</td>
</tr>
<tr>
<td>Table 3</td>
<td>Water Content of Plasma in the Normothermic and Hypothermic Dog</td>
<td>50</td>
</tr>
<tr>
<td>Table 4</td>
<td>Percentage Change in Plasma Volume in the Hypothermic Dog</td>
<td>56</td>
</tr>
<tr>
<td>Table 5</td>
<td>Percentage Change in Thiocyanate Space in the Hypothermic Dog</td>
<td>58</td>
</tr>
<tr>
<td>Table 6</td>
<td>Water Content of Shoulder (Unimmersed) Muscle in the Normothermic and Hypothermic Dog</td>
<td>60</td>
</tr>
<tr>
<td>Table 7</td>
<td>Water Content of Hind Limb (Immersed) Muscle in the Normothermic and Hypothermic Dog</td>
<td>61</td>
</tr>
<tr>
<td>Table 8</td>
<td>Water Content of Cardiac Muscle in the Normothermic and Hypothermic Dog</td>
<td>63</td>
</tr>
<tr>
<td>Table 9</td>
<td>Water Content of Liver in the Normothermic and Hypothermic Dog</td>
<td>64</td>
</tr>
<tr>
<td>Table 10</td>
<td>Comparison of Red Cell Volume (RCV) Measured and Computed from Simultaneously Measured Plasma Volume (PV) and Hematocrit</td>
<td>66</td>
</tr>
<tr>
<td>Table 11</td>
<td>Percentage Change in Red Cell Volume in the Hypothermic Dog</td>
<td>68</td>
</tr>
</tbody>
</table>
INTRODUCTION

When a warm blooded animal is exposed to a cold environment mechanisms are called into play which function to produce and conserve body heat. Under conditions of moderate cold these mechanisms are usually effective. If however, the cold is extreme, the exposure prolonged and protection inadequate, the heat production mechanisms become exhausted and the heat conservation mechanisms insufficient. Heat loss then exceeds heat production, the body's store of heat diminishes and the body temperature falls. Decreases in body temperature of one or two degrees are fairly common and completely compatible with normal physiological behavior. Drastic reductions in body temperature are to varying extents detrimental to normal function. Eventually all temperature regulating mechanisms are overcome by the cold and the body tends to assume the ambient temperature. Such a state or condition of abnormally low body temperature is defined as hypothermia.

Although accidental hypothermia occurs often in peace time, its frequency is markedly increased during war. Indeed, the incidence of hypothermia among service men during the last World War was great enough to prompt the armed services to support research programs designed to study the abnormalities involved in this pathological state. Such a study, supported by the Air Force, has been in progress in this laboratory for the past several years. The experimentation has been carried out for the most part on the dog because of its typically mammalian anatomy and physiology and because of its general availability.
One of the more neglected aspects of hypothermia is the field of water distribution. The original work reported in this paper represents an attempt to investigate this problem. The object of this study was to make simple and direct measurements of the various body water compartments under normal conditions and in the hypothermic state, in order to discover whether water shifts occur with bodily cooling and the magnitude of these shifts, if they do occur. It is hoped that the results help to fill one of the larger gaps in scientific understanding of hypothermia.
SECTION I

THE MEASUREMENT OF BODY WATER COMPARTMENTS
Part A. Body Water

The animal body may be accurately considered as a complicated aggregation of highly specialized cells existing in a watery medium. Any part of the substance of the body must therefore be either intracellular or extracellular.

The extracellular component of the mammalian body has several subdivisions. The two principal subdivisions are the interstitial phase and the intravascular phase. The interstitial phase is that part of the extracellular substance which is found between the cells and outside of the blood vessels. The intravascular phase is that part of the extracellular substance always found within the blood vessels. In addition to the interstitial and intravascular phases there are several minor subdivisions of extracellular substance, e.g., synovial fluid, cerebrospinal fluid, and intraocular fluid, but these will be omitted from further discussion.

Separating the intracellular space from the extracellular space is a semipermeable cell membrane. Between the interstitial and intravascular phases is the likewise semipermeable endothelial vascular membrane. These membranes are selective as to the organic and inorganic solutes allowed to pass. They are, however, completely permeable to water, the universal medium of all bodily processes. For these reasons the composition of the three phases varies extensively but the total concentration of water at equilibrium is the same throughout all parts of the body. The intracellular fluid has potassium
as its chief cation with bicarbonate, protein and organic phosphate as its chief anions. Sodium is the chief cation of the interstitial and intravascular spaces. Chloride and bicarbonate are the chief anions of these spaces.

Hydrostatic Pressure and Osmotic Pressure

Transfer of fluid from one compartment to another involves two types of force, namely, hydrostatic pressure and osmotic pressure. Hydrostatic pressure is the pressure exerted by a fluid due to a mechanical force acting on the fluid. Thus the hydrostatic pressure within the blood vessels is due to the contractions of the heart. That of the interstitial and intracellular spaces is due to distention of the tissues. Under normal conditions the hydrostatic pressures of the intracellular and interstitial spaces are quite small in comparison with the hydrostatic pressure of the blood. The difference between two opposing hydrostatic pressures is called the effective hydrostatic pressure.

Osmotic pressure is not so easily defined. Peters (70) defines it as the expression in solutions of the same phenomenon which gives rise to pressure of a gas and obeys comparable laws. A gas in a non-rigid container can relieve its pressure by expanding its volume and that of the container. A solution in a non-permeable container cannot change its volume regardless of pressure but, if contained in a semipermeable membrane, its volume will change as the solvent passes the membrane. Thus a high pressure can be relieved by the passing of solvent into
the solution. The force with which a solution so tends to relieve its pressure is the osmotic pressure.

The osmotic pressure of a given solution is a function of the total number of osmotically active particles of solute in the solution. Osmotically active particles are those which are restrained by the barrier within which they are contained. They are non-diffusible, unable to distribute themselves evenly on both sides of a given barrier, and therefore they contribute to the osmotic activity of the solution. The resulting osmotic pressure follows the equation:

$$P = \frac{nRT}{V}$$

in which: 
- $P$ is the pressure,
- $n$ is the number of osmotically active particles,
- $R$ is the proportionality constant,
- $T$ is the absolute temperature,
- $V$ is the volume.

A semipermeable membrane is one which is permeable to solvent in every case. Degrees of semipermeability refer to the extent to which the membrane is permeable to solutes. Some membranes are semipermeable by virtue of the size of their "pores" in relation to the size of molecules. The vascular membrane is characteristic of this type. It is relatively impermeable to protein but completely permeable to all other constituents of plasma. Therefore the plasma protein concentration alone determines the effective osmotic pressure across the vascular membrane.
Other membranes are selectively permeable to ions because of the charge on the ions in relation to the charge on the membrane. Cellular membranes are in general of this nature. Such membranes are impermeable to certain ions as well as protein. Thus the effective osmotic pressure across the cell membrane is determined mainly by the concentrations of these non-diffusible ions and to a lesser extent by the concentrations of proteins.

Transfer of Fluid

It is generally accepted that transfer of fluid across the endothelial vascular membrane takes place according to Starling's theory. When the effective hydrostatic pressure across the membrane exceeds the effective osmotic pressure, fluid passes from the vascular to the interstitial space. In the reverse condition fluid passes from the interstitial space to the vessel. At the arterial end of a blood capillary the first condition is true. At the venous end the second condition holds.

When considering the passage of fluid from the interstitial space to the intracellular space, the hydrostatic pressure of these spaces becomes negligible. The transfer of fluid across the cell membrane depends solely upon the osmotic pressures. The effective osmotic pressure is the one measurable, universally active force in such transfer.
Part B. Plasma Volume and Red Cell Volume

The earliest and most direct experimental estimations of whole blood volume were the exsanguination studies made by Valentin (88) and later by Haller (47) a hundred years ago. Unfortunately, complete drainage of the small vessels, especially the capillaries, is not possible in this relatively simple technique and gross underestimations result. More recently McLain et al. (63) have employed intermittent bleeding and subsequent saline perfusion in an attempt to extract total circulating hemoglobin. By dividing this total hemoglobin by the hemoglobin contained in a unit volume of previously sampled blood, they computed the whole blood volume. Admittedly, this method is cumbersome, in so far as it involves the collection of large volumes of perfusate and many hemoglobin determinations.

Since the early part of this century most estimations of whole blood volume, plasma volume and red cell volume have been based on the dilution principle. In essence this principle is as follows: A known quantity \( N \) of a substance is introduced into its diluting compartment. When mixing is complete and distribution uniform, a sample of the compartment is withdrawn and the quantity of substance \( n \) in a unit volume of compartment determined. The total volume \( V \) of the compartment is calculated from the equation \( V = \frac{N}{n} \). This principle is also used for the estimation of total body water and of extracellular space.

Ideally the introduced substance should be:
(a) uniformly distributed in the compartment to be measured,
(b) limited entirely to that compartment,
(c) metabolically inert, and
(d) non-excretable.

Needless to say, these conditions are not completely fulfilled by any of the methods presently available. In all methods metabolic activity, excretion, phagocytosis and slow diffusion across the limiting membrane result in a progressive disappearance of the introduced substance. If, however, these processes are sufficiently slow, the rate of disappearance will permit extrapolation back to zero time on a concentration-time graph. The theoretical concentration at zero time, the time of injection, is assumed to be the concentration of substance thoroughly mixed and without loss.

Estimation of Plasma Volume

Materials used in the estimation of plasma volume are of two classes:

(a) substances with molecular weights similar to those of the plasma proteins,
(b) substances with a strong affinity for combining with plasma proteins.

The first group is comprised mainly of substances well known as plasma substitutes or expanders, e.g., acacia, polyvinylpyrolidone and dextran. The use of the latter two substances is quite new and relatively unexplored.

The second group is comprised of various dyes and of radioactive iodine. Brilliant vital red dyes were investigated
rather thoroughly by Hooper et al. (56). They found that small amounts of hemoglobin, released by hemolysis, caused serious error in the accuracy of these red dyes. Dawson, Evans and Whipple (24) therefore proposed the use of a blue azo dye, obtained from the reaction between orthotoluidine and 1,8 amino-naphthol 2,4 sulfonic acid. The dye has since become known as T-1824 or "Evans Blue." In as much as this dye was employed in the plasma volume studies to be reported in this paper, it may be helpful to elaborate somewhat on this method.

When injected into plasma, T-1824 attaches to albumin and, when concentrations are very high, to globulin (72). The early part of the plasma dye concentration-time curve is controversial. It is agreed that the curve is composed of two components: an early mixing phase and a subsequent disappearance phase. The time at which the mixing phase ends and the disappearance phase begins is apparently variable. In man the mixing phase has been reported to last anywhere from six to twenty minutes (37,40,66,83). In the dog similar mixing times have been reported. Miller (64), however, in a careful investigation of the problem found that mixing was not complete in his dogs until forty to fifty minutes after injection. Data obtained in this laboratory agree with those of Miller.

The second part of the dye concentration-time curve reflects the rate of removal of dye from the plasma. Prescinding from fluid shifts into or out of the plasma, this removal depends on several factors:
(a) Chemical destruction of dye.
(b) Phagocytic removal and excretion of dye.
(c) Passage of dye into the lymph.
(d) Loss of plasma albumin.

(a) Practically nothing is known of the possibility of chemical destruction of dye in the body (74).
(b) The dye is removed phagocytically by the reticulo-endothelial system (37) and appears in the bile thirty minutes after injection (64). It is not excreted via the kidneys, nor does it appear in the gastric or pancreatic juice (60).
(c) Passage of dye into the lymph has been reported (16, 19, 32) but Miller (64) found that ligation of the thoracic and cervical lymph ducts in dogs does not alter the rate of disappearance for five hours after injection.
(d) Studies with radioactive iodine show that the rates of disappearance of dye from the plasma and of albumin from the plasma are practically equal and when represented graphically, the disappearance slopes are parallel (58). It appears then that albumin turnover is the chief contributor to the dye disappearance curve.

Average rates of disappearance of dye in man are of the order of 5% to 6% for the first hour (37, 66) and 50% in the first twenty-four hours (37, 60, 66). In the dog, however, mean rates have been reported to be 8.8% (42), 20% (19) and 25% (64) during the first hour. Some of these figures are based on dye concentration-time curves, wherein plasma sampling did not extend beyond sixty minutes after injection. If the series of samples
had been prolonged so as to cover another hour, a less steep disappearance slope might have been obtained. In this laboratory the mean rate of dye disappearance for the first two hours was 4% per hour.

Estimates of plasma volume are usually made by extrapolation of the disappearance slope back to zero time. Successive determinations in the same subject usually involve (a) successive injections, (b) extrapolation of the total dye concentration-time curve back to zero, and (c) subtraction of the dye concentration immediately before the second injection from this total concentration. Gregersen (41) proposed the use of a single determination of dye concentration ten minutes after injection in the estimation of plasma volume. Thus repeated estimates could be made with repeated injections and samples withdrawn just before and ten minutes after each injection. This was proposed as a handy clinical technique but never attained widespread experimental acceptance.

The use of albumin tagged with radioactive iodine in the estimation of plasma volume eliminated two sources of error present in the dye method; (a) loss of dye by phagocytosis, and (b) loss of dye before the dye has become firmly bound to albumin. Simultaneous determinations by these two methods have shown plasma volumes to be about 5% greater with T-1824 (58). Disappearance rates and curves are, however, equal and parallel (58).

Estimation of Red Cell Volume

Red cell volume has been estimated by the dilution tech-
Technique involving the use of carbon monoxide, radioactive materials and in man, differential cell typing.

The carbon monoxide method was first reported by Grehant and Quinquand in 1882 (44). In this technique labelling of hemoglobin may be done in vitro or in vivo by the inhalation of a known quantity of carbon monoxide. The rapid loss of carbon monoxide, chiefly via the lungs, and the large volume of carbon monoxide or of carbon monoxide labelled cells which is required have discouraged the recent use of this method.

Ashby (3) introduced the method of differential cell typing. Group O cells are injected into a non-O subject. Time is allowed for complete mixing and samples subsequently withdrawn. The non-O cells are agglutinated and the remaining O cells counted. The total number of O cells injected divided by the number of O cells per unit volume of red cells gives the total volume of red cells. The method is limited to man and requires careful cross matching and Rh typing.

Radioactive isotopes used in the estimation of red cell volume include those of iron (Fe\(^{55}\) and Fe\(^{59}\)), phosphorous (P\(^{32}\)) and recently, chromium (Cr\(^{51}\)). In all cases, a known quantity of radioactivity, determined on a Geiger Muller counter, is tagged on to red cells in vivo or in vitro and the tagged cells injected into the subject. The dilution of these cells and their radioactivity is proportional to the red cell volume.

Both isotopes of iron are incorporated into hemoglobin and, when once so incorporated, are not exchanged. Moreover, the time required for decay of 1% of initial activity of Fe\(^{59}\)
is 310 days. For Fe\textsuperscript{55} this time is thirty-three years. For all practical purposes then, radioactivity within a given red cell remains constant. Disappearance of activity from the circulating red cell volume depends solely on the rate of degeneration of red cells.

The use of radioactive phosphorous (P\textsuperscript{32}) involves the tagging of some, as yet unknown, intermediate in carbohydrate metabolism. It is advised that \textit{in vitro} tagging be carried out in the presence of glucose, as well as NaP\textsubscript{4} and that the tagged cells be washed to eliminate any P\textsuperscript{32} not firmly bound within the cell (73). This procedure eliminates the necessity of separating cells from plasma in the withdrawn samples. Unlike radioactive iron, P\textsuperscript{32} is not firmly bound within the cell. Disappearance curves reported by Reeve and Veall (75) and substantiated by data obtained in this laboratory indicate a mean rate of loss of activity of 6% per hour in the first two hours. Proper extrapolation from a disappearance slope eliminates serious error due to this loss of activity. On the other hand, the weak beta emissions of P\textsuperscript{32} in contrast to the strong gamma emissions of Fe\textsuperscript{55} and Fe\textsuperscript{59} make the P\textsuperscript{32} technique much less hazardous and much less expensive.
Part C. Measurement of Extracellular Water

The physiologic importance of the extracellular space stems from the fact that it represents the true environment, the "internal environment" in which the body cells exist, function, reproduce and perhaps die. The maintenance of a normal composition and adequate magnitude is therefore a necessity. Measurement of extracellular space is, however, difficult, much more difficult than measurement of its chief subdivision, plasma.

Chloride, Sodium and Bromide Spaces

The first attempt to measure extracellular space consisted of histological examinations of frozen preparations of muscle (31). This, of course, was an estimate of interstitial space only and indicated values of 15% to 17% of the total muscle mass.

Overton (67) demonstrated that chloride could be easily removed from frog hind limb muscle by perfusion with isotonic glucose, while potassium remained behind some limiting barrier. Moreover, muscle immersed in isotonic glucose soon lost its chloride, without losing potassium (31). Since glucose could not enter muscle cells, it could replace only extracellular solutes. It was therefore concluded that all chloride was extracellular. Fenn, Cobb and Marsh (31) further observed that if all the chloride in muscle tissue was limited to the 15% of the total mass, which was suggested as extracellular space by histological studies, then the concentration of chloride in this space
would be equal to its concentration in an ultrafiltrate of plasma.

Accepting these conclusions, one can compute the chloride space in a given tissue from the total chloride in that tissue and the concentration of chloride in the plasma. Likewise, the total chloride space in the body can be computed from the total body chloride and the concentration of chloride in plasma. The general formula is:

\[
F = \frac{(\text{Cl}^-)_t}{(\text{Cl}^-)_s}
\]

in which: 

- \((\text{Cl}^-)_t\) represents the total amount of chloride in the given tissue or in the body,
- \((\text{Cl}^-)_s\) represents the total concentration of chloride in the plasma, and
- \(F\) represents the volume of chloride space.

Similarly, sodium was originally thought to be distributed solely in extracellular water and sodium space, similarly computed, was adopted as an index of extracellular water.

However, if both sodium and chloride are ideally distributed in extracellular space then the sodium to chloride ratio in all tissues would be the same as that in plasma. Actually, the sodium to chloride ratio in most tissues, especially bone, cartilage and skeletal muscle is higher than that of plasma, indicating a certain amount of intracellular sodium in these tissues (48,61). On the other hand, the ratio of sodium to chloride in the gastric and intestinal mucosa, salivary glands, erythrocytes and connective tissue is lower than that of plasma,
indicating intracellular chloride \((2,48,61)\). In spite of these apparent errors radioactive chloride and radioactive sodium are employed frequently in estimating extracellular water.

Bromide, which is distributed in the tissues much like chloride, has also been used to estimate extracellular space. Obviously its disadvantages are similar to those of chloride.

**Thiocyanate Space**

In 1937 Crandall and Anderson \((21)\) proposed the use of thiocyanate as an indicator of extracellular space. It has since become the most widely used method. Advantages include simplicity of determination, rapid equilibration and slow renal excretion. Like chloride, thiocyanate enters red cells \((21)\), cells of the gastric mucosa and, in view of its large volume of distribution, probably other cells of the body. The method was originally proposed as a means of studying changes in extracellular volume. Even this application has been challenged by several investigators \((59)\) who claim \((a)\) that cellular permeability to thiocyanate increases in certain pathological states, and \((b)\) that thiocyanate space is not constant in the same animal under identical conditions. The pathological states enumerated have been limited for the most part to infectious diseases. Moreover, variability among animals in a given series is usually reasonably low. In a series of dogs studied in this laboratory the coefficient of variation was of the order of \(9\%\).

**Inulin Space**

In order to avoid errors introduced by the entrance of electrolytes into cells, the use of neutral molecules, to which
the cell membrane was impermeable, was proposed. The carbohydrates sucrose, mannitol and inulin have been investigated. These present two disadvantages, (a) very difficult analytic techniques, and (b) rapid renal excretion.

Mannitol and sucrose are never completely recovered in the urine, indicating partial metabolism, for which correction is impossible (26,57,80). Inulin, on the other hand, is metabolically inert, since it is recovered quantitatively in the urine (36,79,80). However, its very rapid excretion prevents uniform distribution throughout the extracellular space when administered in a single injection. The use of inulin in the intact animal, or in man, therefore requires the maintenance of a steady infusion which compensates for renal excretion. Thus a constant plasma level is attained and uniform concentration exists throughout the entire space. Once equilibrium is established a single sample of blood is withdrawn. All the inulin in the body at the time of the blood sample is then collected. The inulin space is computed by dividing the total body inulin by its concentration in the plasma. The time of infusion necessary to insure uniform distribution of inulin is two hours in the dog and five hours in man. Although the long equilibration period constitutes a practical disadvantage, the inulin method is theoretically the most reliable method for estimating extracellular space.

Smallest estimates of extracellular space have been obtained in the dog with the use of inulin. Chloride has given the next smallest estimates, followed by bromide and sodium.
The use of thiocyanate has resulted in the largest estimates. In man, the smallest estimates have also been obtained with the use of inulin. The next smallest estimates resulted from the use of chloride, followed by mannitol, sucrose, bromide, thiocyanate and lastly sodium.
SECTION II

PATHOLOGICAL PHYSIOLOGY OF THE HYPOTHERMIC DOG
Part A. General Metabolism

In general, chemical reactions which proceed at measurable rates are accelerated by heat and slowed down by cold. According to the law of vant'Hoff the rate of an enzymatic reaction increases two or three fold with a rise in temperature of ten degrees centigrade. Likewise, a decrease in temperature of ten degrees will reduce the rate of an enzymatic reaction to about one half of its control rate. Considering the body cell as a physicochemical unit composed of numerous such reactions, one would expect the sum total of all chemical activity of a given cell to decrease as the temperature of the cell decreased. In other words, one would expect cellular metabolism to decrease as cellular temperature decreases. Furthermore, since the body of the dog is an aggregation of individual cells, one would anticipate an overall depression of metabolism in the hypothermic dog. With qualification, one can say that this is the situation.

The situation is complicated, however, by the occurrence of reflexes evoked in response to the cold stimulus by which cellular metabolism is increased in certain tissues. The overall metabolism of the dog in hypothermia is determined by the algebraic sum of the direct depressing effect of the cold and the reflex increase in activity in certain tissues. The depressing effect of the cold is progressive, i.e., as body temperature decreases cellular depression becomes more marked. For example, Brokaw and Penrod (15) offered evidence which suggested that liver metabolism was progressively depressed in the hypothermic dog. Reflex increases in activity, on the other
hand, are temporary. The most dramatic example of these reflexes is the phenomenon of shivering. Increases in metabolism of skeletal muscle cells as great as 300% to 400% of normal can occur in the intensely shivering animal. The maximum intensity of reflex shivering occurring in a given experiment is greatly influenced by the depth of anesthesia under which the procedures are carried out. In fact, profound general anesthesia can abolish all reflex shivering (68). In such cases overall metabolism shows only the progressive depression due to the cold. More frequently the pattern of overall metabolic change consists of an initial increase, ascribable chiefly to shivering, followed by general depression which progresses to the animal's death.

In the absence of oxygen debt, metabolic changes are reflected in changes in total oxygen consumption. Grosse-Brockhoff and Schoedel (45) reported a temporary increase in oxygen consumption averaging 300% to 400% in the hypothermic dog. The peak increases were reached when the dog's rectal temperature was 33°C-30°C. Prec et al. (71) and Penrod (68) reported similar findings. The latter author correlated these increases with observed shivering. In dogs shivering violently, oxygen consumption increased maximally. Intermediate increases in oxygen consumption were correlated with moderate shivering. At rectal temperatures of 23°C, when shivering had invariably ceased, oxygen consumption was invariably below normal. Hegnauer and D'Amato (51) extended these observations to lower temperatures and found the average oxygen consumption became reduced to only 15% of normal at 17°C.
Part B. Survival

In a critical review of studies dealing with the limit of survival of the dog in hypothermia it is most important to consider the possible effects of the more common extraneous experimental procedures. First and foremost of such procedures is the use of general anesthesia. Another is the use of intracardiac catheters.

Anesthesia is desirable for several reasons. The pain associated with drastic reduction in body temperature in conscious humans has been reported to be excruciating (1). The perception of pain of similar intensity must be assumed for the dog. Moreover, violent psychomotor reactions, including shivering, which are induced by abrupt exposure to cooling procedures (usually immersion in cold water) can prevent reduction in body temperature for relatively long periods of time (82).

In spite of the desirability of anesthesia in these experiments, a definite effect of the anesthetic agent on survival must be admitted. The most detrimental action of these drugs in hypothermia is respiratory depression. In the deeply anesthetized hypothermic dog respiration invariably ceases at a rectal temperature $5^\circ-10^\circ$C higher than that at which cardiovascular crisis occurs. In the lightly anesthetized dog (e.g., under light ether anesthesia) respiration very frequently continues down to and even beyond cardiovascular failure (49).

There is abundant evidence that cardiovascular failure always precedes respiratory failure in the unanesthetized hypothermic
smaller animal, e.g., the rat (23, 28). It has therefore been assumed that in the hypothermic dog, cessation of respiration prior to cardiovascular failure is due to the anesthetic agent employed rather than cold per se. The validity of this assumption has not been conclusively demonstrated in the dog. Nevertheless, in the studies of survival mentioned below, free access to artificial respiration was permitted, thereby eliminating as contributory lethal factors not only the depressing action of the drug on respiration but also the possible depression by the cold. However, differences in the effects of anesthetic agents, even with respiratory effects controlled, appear in the hypothermic dog.

Thus Crismon (22) using pentobarbital was unable to revive dogs cooled below 24°C rectal temperature while Hook and Stormont (55) successfully revived dogs from temperatures as low as 17°C after ether and sodium barbital anesthesia. Haterius and Maison (49) cooled dogs frequently to rectal temperatures of 14°C-15°C with subsequent successful revival. They observed that pentobarbitalized dogs died at higher rectal temperatures than dogs cooled under thiopental, ether or cyclopropane but offered no quantitation of this difference. Woodruff (90), using pentobarbital, was unable to cool his dogs below 23°C. However, his experiments involved cooling dogs to 23°C-25°C and maintaining them at these low temperatures as long as possible. In these studies, body temperature alone does not give a true indication of total cold stress. Prolongation of the hypothermic state could conceivably exhaust the ability of the
dog to survive extremely low temperatures. Except for the work of Woodruff, prolonged cooling has not been investigated in the dog.

Gollan et al. (39) were unable to revive dogs cooled under thiopental anesthesia from rectal temperatures below 27°C. These results could be attributable to their unique extracorporeal cooling technique.

Bigelow et al. (12) observed that dogs cooled by immersion with divinyl ether anesthesia "faired better" during the cooling process than dogs cooled under diethyl ether or barbiturate anesthesia. Again, quantitative data as to survival temperatures were not reported.

A controlled study on the comparative effects of pentobarbital, thiopental and diethyl ether on survival was recently completed in this laboratory. The animals were subjected to no experimental procedure other than anesthetization, cooling, and when necessary, artificial respiration. The mean lethal rectal temperature of dogs cooled under pentobarbital was 18.5°C. This differed significantly from the mean lethal rectal temperature of the etherized dogs (15.2°C) and the dogs under thiopental (15.7°C). Thus, even apart from respiratory effects, pentobarbital appears to be more deleterious than ether or thiopental to the hypothermic dog.
Part C. Abnormality of Physiological Function

Central Nervous System (CNS)

To date, no controlled study of CNS function in the hypothermic dog has been reported. However, observations of this function made incidentally in this laboratory have been summarized by Hegnauer and Penrod (53). In general, these observations are similar to those made by Britton (14) on the hypothermic cat.

Evaluation of the effect of cold on the nervous system in the hypothermic dog is most difficult because of the depression imposed by the anesthetic agent used. In general, the use of inhalation anesthetics is less detrimental to the CNS than the use of barbiturates. The effective concentration of a volatile anesthetic in the dog can be blown off within a few minutes after removal of the agent. Barbiturates or other systemicly administered anesthetics when once administered cannot be withdrawn and must be excreted or metabolized. These latter processes require considerable time. As a result, in deep hypothermia after cessation of administration of the anesthetic agent, there is less systemic anesthetic, hence less CNS depression from this source when inhalation anesthesia is employed.

In spite of this point of criticism some observations have biological significance. For example, the knee jerk is sluggish and has a long latency in deep hypothermia, indicating slow conduction through peripheral nerve and/or cord. This re-
flex is usually abolished within a degree or two of the lethal temperature, varying with the lethal temperature under various anesthetics. Muscle tone in the hypothermic dog is usually abolished just prior to abolition of the knee jerk.

Respiratory reflexes are integrated in the medulla. Under ether anesthesia spontaneous respiration is frequently observed at cerebral temperatures of 18°C-19°C, which correspond to rectal temperatures of 13°C-14°C. Shivering, which involves a functional hypothalmnus, has invariably ceased by the time the cerebrum reaches 23°C (20°C rectal temperature). The lower limit of cortical and thalamic function in the hypothermic dog appears, from rewarming experiments, to be in the cerebral temperature range of 25°C-27°C (22°C-25°C rectal temperature). Thus, the brain undergoes a descending narcosis.

Since the spinal cord is immersed in ice water in these experiments, its temperature at any given time in the cooling process is considerably lower than that of the unimmersed brain. Although the function of the cord is lost earlier in time than the function of the medulla, it is lost at a lower tissue temperature. Hence the descending narcosis is progressive even to the cord.

Respiration

Quantitation of external respiration and total oxygen consumption in hypothermia is most significant when considered in relation to tissue requirements. Marked depression of rate and depth of breathing and of total oxygen consumption has been
reported by several authors (12,68,71). However, strong evidence in favor of adequate tissue oxygenation was offered by Penrod (69), who found essentially normal arterial and venous oxygen contents and a normal arterial-venous oxygen difference in the dog at 20°C. Hegnauer and D'Amato (51) later found the same normal picture at 17°C. In general these dogs incurred no oxygen debt during cooling and no compensatory rise in oxygen consumption was observed on subsequent rewarming. There was one exception. One dog in deep hypothermia apparently incurred oxygen debt due to temporary cardiovascular failure. Blood samples withdrawn several minutes after recovery from this crisis, while the dog was still cooling, revealed a normal arterial oxygen content but a very low venous oxygen content, hence a marked increase in the coefficient of utilization. A similar condition arose during rewarming and similar observations were made. It therefore appears that in the hypothermic dog, tissues in need of oxygen can extract additional oxygen from the blood. The fact that a normal arterial-venous oxygen difference exists in deep hypothermia indicates that in hypothermia there is no want of oxygen in the tissue cells. The above results contradicted the prediction of von Werz (89). He claimed that the increased affinity of hemoglobin for oxygen in the cold (the shift of the oxygen dissociation curve to the left) would prevent adequate dissociation, hence adequate tissue oxygenation. However, a decrease in pH is known to favor oxygen dissociation, i.e., to shift the curve to the right, to affect the curve in a manner opposite to that of cold. Hegnauer and Penrod (53) there-
fore hypothesized that a decrease in blood pH could compensate, at least partially, for the effect of cold on dissociation. Measurements revealed a progressive decrease in blood pH from a normal of 7.4 to 6.9 at a heart temperature of 16°C (rectal temperature of 14°C). These values were quite close to those, which according to computations would be sufficient to compensate entirely for the effect of cold on dissociation.

Cardiovascular System

Hook and Stormont (55) reported a sinus bradycardia in the dog at rectal temperatures of 17°-20°C. Woodruff (90) observed progressive bradycardia down to rectal temperatures of 23°C. Grosse-Brockhoff and Schoedel found that this bradycardia was not influenced by vagotomy nor by atropinization. These observations were confirmed by Haterius and Maison (49). Hegnauer and Penrod (53) observed a slight, temporary increase in heart rate followed by a gradual depression. Others (12,71) have since substantiated these results.

Systemic arterial blood pressure, like pulse rate, undergoes an initial slight increase due mainly to intense vasoconstriction followed by a progressive drop somewhat dependent on cardiac output and pulse rate (53).

Cardiac output in the hypothermic dog has been shown to parallel oxygen consumption (12,50,71). It is therefore related to shivering and dependent upon depth of anesthesia. In view of the essentially normal arterial-venous oxygen difference in hypothermia, it appears that changes in cardiac output consti-
tute the chief mechanism by which tissue oxygenation is regulated. As tissue demands for oxygen increase (e.g., with shivering), cardiac output increases. When tissue demands are depressed in deep hypothermia, cardiac output diminishes.

Electrocardiographic studies in deep hypothermia by Hook and Stormont (55) revealed prolongation of the P-R interval and of the QRS complex, indicating depression of the conducting mechanisms by the cold. In addition they observed changes in the ST segment and in the T wave, the latter becoming diphasic or deeply and bizarrely inverted. Subsequently, others (45,71) reported voltage changes, heart block and complete disappearance of the P wave.

Haterius and Maison (49) observed irregularities of heart rate in an otherwise sinus rhythm at rectal temperatures of 20°C and higher but reported no electrocardiographic study of these. Hegnauer et al. (54) and Prec et al. (71) identified these irregularities as ventricular ectopic beats arising from one focus or from many foci. These often precipitated ventricular fibrillation in the cold and occasionally in rewarming from low temperatures. Idioventricular beats have been observed at even higher temperatures in the hypothermic dog (39). More will be said of these phenomena in the next part of this section. It will suffice for the present to say that electrocardiographic studies on the hypothermic dog reveal (a) depression of the conducting mechanisms of the heart by the cold, and (b) the origin in the cold heart of idioventricular beats, often leading into fibrillation.
Hegnauer et al. (54) and more recently Berne (10, 11) found increased duration of systole and increased duration of isometric relaxation in deep hypothermia, indicating a direct depression by the cold of the contractile mechanisms of the myocardium. Moreover, Hegnauer pointed out that if the heart rate at normal temperature is slowed to a rate similar to a representative rate in deep hypothermia, the activity phase occupies a much smaller percentage of the total cycle at normal temperature than at the cold temperature. For a given heart rate the cold heart spends a greater time under stress.

Hegnauer et al. (54) reported increased right atrial pressures and left ventricular pressures in deep hypothermia. These elevated pressures were invariably accompanied by ventricular extrasystoles and frequently followed by ventricular fibrillation. The occurrence of extrasystoles and fibrillation were later ascribed to the presence of intraventricular catheters (52). Bigelow (12) reported high venous pressures in hypothermic dogs most of which died in ventricular fibrillation. He concluded that high venous pressures precipitated fibrillation. The evidence indicates however, that elevated pressures of this type are the effect rather than the cause of cardiac irregularities (ectopic beats). Because of their common origin, elevated intraventricular, intra-atrial and venous pressures and ventricular fibrillation often accompany one another. In the absence of extrasystoles Berne (11) found no increase in left intraventricular or right atrial pressure. He concluded that in the absence of extrasystoles, no myocardial weakness occurs in hypothermia.
Part D. Nature of Death

In Part B of this section it was pointed out that anesthetic agents, when used in experimental hypothermia, depress the respiratory reflex even to the point of hastening complete cessation of breathing movements. For this reason, most studies of death in the hypothermic dog have been carried out with free access to artificial respiration. Attention therefore has been focused on the cardiovascular system and the factors which effect its eventual failure.

Terminal cardiac events in the hypothermic dog can be divided into three types. In Type A there is an uncomplicated slowing of the heart rate and a progressive lengthening of diastole until diastole becomes infinitely long. The heart is then said to be asystolic and the cardiac termination is classified as "asystole." This type of termination rarely occurs at rectal temperatures above 17°C.

In marked contrast to the above is Type B. In this type of cardiac termination a relatively rapid sinus rhythm is interrupted by few or many idioventricular beats, one of which leads directly into ventricular fibrillation. Such termination is called "true ventricular fibrillation" or simply "ventricular fibrillation." "Ventricular fibrillation" occurs invariably above rectal temperatures of 17°C and most frequently around 20°C.

The third type of cardiac termination contains both asystolic and fibrillatory components. The usual picture consists of a gradual slowing of the heart rate to three or four beats per minute, a long interval of asystole and finally, interrupting
the asystole, sluggish fibrillation. Etiologically, this type is thought to be related more closely to "asystole" than to "true ventricular fibrillation" but is referred to by the combination of terms, "asystolic fibrillation." It is common practice in this laboratory to classify fibrillatory terminations which occur at heart rates of fifteen or more beats per minute as "true ventricular fibrillation" (Type B). Fibrillatory terminations occurring from heart rates less than fifteen beats per minute are arbitrarily classified as "asystolic fibrillation."

Govino et al. (20) have shown that the relative frequency with which these types occur in the hypothermic dog varies with the anesthetic agent employed. Under pentobarbital anesthesia (30 mg/kg., intraperitoneally) 60% of deaths were classified as "ventricular fibrillation" (Type B). The remaining 40% were classed as "asystole" (Type A) or as "asystolic fibrillation" (Type C). Only 30% of deaths were classified as "ventricular fibrillation" when the experiments were carried out under ether (inhalation) or thiopental (5%, intravenously as required to maintain surgical anesthesia). It would be of great value to know the relative frequency of these types of cardiac termination in the unanesthetized hypothermic dog. The only known effect of these anesthetic agents on the myocardium is one of depression. It is conceivable, therefore, that the tendency toward arrhythmias and "ventricular fibrillation" observed in the anesthetized hypothermic dog is actually a partly masked effect, the result of depression by the anesthetic of a much greater
tendency induced by the cold.

The exact nature of death, i.e., the series of causes leading to any or all of these three types of cardiac termination, is not known. Von Werz (89) postulated progressive cardiac ischemia due to a shift of the oxygen dissociation curve to the left. Coronary arterial-venous oxygen studies indicate that at lethal temperatures the myocardium, as a whole, is adequately oxygenated (51, 69). For the same reason, the formation in the myocardium of localized anoxic areas, which give rise to areas of increased excitability and ectopic activity, is difficult to establish as a cause of "ventricular fibrillation."

Increased venous pressure has been suggested by Bigelow (12) as the cause of "ventricular fibrillation." In his experiments such fibrillation was prevented and, in one case, reversed by venesection. No substantiation of these results has been reported. Completely negative results were obtained with venesection in this laboratory.

Endogenous epinephrine and its effect on a hyperexcitable myocardium have been considered as possible factors in the occurrence of "ventricular fibrillation." However, administration of exogenous epinephrine at near lethal temperatures has no effect or a beneficial effect on survival in hypothermia (20). Adrenergic blockade has no beneficial effect (20).

Undoubtedly, the phenomenon of "true ventricular fibrillation" in hypothermia is related in some way to an increased myocardial excitability. Preliminary investigations of electri-
ocal threshold of ectopic beats suggest that an increased myocardial excitability does exist. The mechanism underlying this increased excitability has not been revealed. One mechanism, which must be considered as a possibility, is the loss of intracellular potassium from the cardiac fibers. The presence or absence of this factor in the hypothermic dog can be determined by measurements of the coronary arterial-venous potassium differences.

The causes underlying "asystole" are similarly not clear. Conceivably, the direct slowing effect of the cold on the metabolic processes underlying myocardial contraction could progress to the point at which energy production failed to reach contractile threshold. If this were the entire explanation, rewarming the animal should be sufficient to restore the asystolic heart to normal function. Complete cardiovascular recovery has been observed in this laboratory in a hypothermic dog whose electrocardiographic recordings revealed no heart beat for over five minutes. As far as the cardiovascular system is concerned, this type of termination is much like a state of suspended animation.
SECTION III

STUDIES ON WATER DISTRIBUTION IN HYPOTHERMIA
Relatively little experimental work has been carried out in the field of water distribution in hypothermia. Isolated clinical observations have been reported but there have been few extensive studies.

Several clinicians, notably Woodruff (90) and Talbott (85) have reported edema of the lungs in patients dying of hypothermia.

Smith and Fay (81) reported occasional hemoconcentration in cancer patients whose deep rectal temperatures ranged from 32°C to 23°C.

Talbott, Consolazio and Pecora (86) observed tissue water changes in hypothermia. They reported a total water percentage below the average found in normal autopsy subjects in muscle, liver, kidney and brain but a higher water percentage in heart muscle. They also found a concentration of sodium below average in all the organs mentioned. This led them to believe that in these victims of hypothermia there was a generalized decrease in extracellular water.

The work of Barbour, concerning the homeostatic control of water distribution, touched occasionally upon hypothermia. For example, in 1924 Barbour and Tolstoi (7) reported the results of several experiments in which dogs were cooled to rectal temperatures of about 33°C. In two cooled, but otherwise normal, dogs they observed increases in total blood solids of the order of 10% over the control values. One dog, in which the spinal cord had been transected at the sixth cervical segment, showed no significant change in total blood solids in
hypothermia. Similarly, one decerebrate hypothermic dog revealed no change in total blood solids. From these four dogs the authors concluded that the intact animal possessed a "water shifting mechanism" under the control of the central nervous system.

In 1934 Barbour and Gilman (5) reported the immersion of a cat in 10°C water for ten minutes. No body temperatures were recorded. An increase in serum specific gravity of 5% over the control value was observed. The authors stated by way of discussion that this type of change could be eliminated by a variety of procedures which interfere with proper function of the central nervous system. Six years later, Barbour (4) found percentage increases in the serum osmotic pressure of cats equal to the increases in specific gravity previously reported. In addition, intracellular water was computed by subtracting total chloride space from total water volume for a group of seven normal cats and a group of seven cats exposed to temperatures varying from +7°C to -4°C. The average intracellular water content in the cold cats was 7% greater than that of the normal cats. From these two experiments Barbour concluded that the acute movement of water into the cells was due to an accumulation of metabolites in the cell. In this same report the author states that no significant difference in osmotic pressure was observed between cats exposed to -4°C with shivering and cats exposed to +7°C environmental temperature without shivering. No quantitative data were submitted to establish this point, but the author concluded that the shivering
mechanism was not responsible for the increase in intracellular metabolites and the consequent attraction of water into the cells. No further exploration of the possible role of shivering on these water shifts was undertaken by Barbour in spite of continual references to the "water shifting mechanism under the control of the central nervous system" in hypothermia.

The work of Barbour, discussed thus far, involved only moderate reduction of body temperature. In 1943 Barbour, McKay and Griffith (6) reported a study of water shifts in hypothermia which involved more drastic reduction in temperature. The study consisted of three parts:

(A) Monkeys were wrapped in half inch coiled tubing through which 7°C water circulated. In this way they were chilled to 23°C.

(B) Rats were exposed to a cold room (3°C to 4°C) for one hour, confined only by individual cages, hence free to move about during the exposure.

(C) Another group of rats was chilled in a manner similar to the monkeys.

In the monkeys chilled to 23°C the authors found an initial rise in serum protein of 5.1% of the normal level and in serum chloride of 4.1% of the normal. This initial increase was followed by a fall in both protein and chloride which persisted to the end of chilling. Rewarming augmented the fall so as to result in a maximum decrease of 8% of the normal in protein and 2.7% of the normal in chloride.

The free rats, cooled to an average rectal temperature
of 22.4°C showed an increase in serum protein and serum chloride. Intracellular water content of the liver, muscle, brain and minced carcass also increased. Total water content increased in muscle, brain and minced carcass but not in liver.

The rats which were chilled by coil, cooled to an average rectal temperature of 18.9°C. The serum protein, serum chloride, total and intracellular water of these rats were above normal but lower than those of the other group.

The increases in total water described above, especially those observed in the rats cooled to 22.4°C are difficult to understand. In order to produce an increase in total water of the magnitude reported, e.g., 9% in minced carcass, practically the entire plasma volume would have to desert the vascular system for the tissues.

In 1951 Rodbard et al. (76) reported measurements of plasma and extracellular volumes in experimental hypothermia. Plasma volume, as determined by T-1824 was reduced by over 30% in chicks and rabbits cooled to rectal temperatures of 25°C. Extracellular space as determined by thiocyanate was reduced by an equal percentage. Moreover, thiocyanate space increased to normal values upon subsequent rewarming. Plasma volume was not measured in the rewarmed animal.

Measurements of extracellular space were also made in nephrectomized rabbits with inulin. A single injection of inulin was made at normal temperatures and its volume of distribution determined by dilution first at normal temperature, then at 25°C rectal temperature. No change occurred in inulin space.
When, however, the injection of inulin was made at 25°C and its volume of distribution determined first in the cold rabbit and subsequently after rewarming, then the volume of distribution of inulin increased. The authors reasoned that if the apparent loss of extracellular space with cooling were due to a flight of water into the cells of the body, then the volume of distribution of inulin, injected only at normal temperature, would have decreased with cooling. They concluded that the lost plasma was still within the vascular tree, but locked in capillary beds, which were inaccessible to blood borne indicators. The inaccessibility of these vascular beds rendered a corresponding percentage of interstitial space unavailable for measurement in the cold.
SECTION IV

PROCEDURE AND METHODS
The investigations concerning water distribution in hypothermia reported in this and the following sections are divided into five groups:

1. studies on blood, comprised of determinations of hematocrit, plasma water content and plasma protein concentration;
2. measurements of plasma volume;
3. measurements of red cell volume, i.e., circulating red cell mass;
4. measurements of thiocyanate space, and
5. measurements of total water content and chloride space in skeletal muscle, cardiac muscle and liver.

General Procedure

All experiments were performed on unselected, apparently healthy, mongrel dogs. Individual weights of these dogs ranged from seven to eighteen kilograms, but the majority of weights fell between ten and fourteen kilograms.

The anesthetic agent employed was either thiopental or pentobarbital. In the former case a 5% solution of thiopental sodium was initially titrated into a vein of the forelimb until the upper plane of surgical anesthesia was attained. During the course of the experiment additional thiopental was administered intravenously or intra-arterially as needed to maintain this level of anesthesia. In other experiments pentobarbital sodium was administered intraperitoneally, the dose being thirty milligrams per kilogram of body weight. The level of anesthesia resulting from this constant dose was not so consistent as that resulting from the intravenous injection of thiopental. Usually, immediate anesthesia with pentobarbital was somewhat deeper and
longer lasting. Additional pentobarbital during the course of the experiment was rarely required.

Preimmersion surgery was generally limited to through-and-through cannulation of one or both common carotid arteries and exposition of one or both external jugular veins. Intravenous heparin in a dose of five milligrams per kilogram of body weight was sufficient to prevent clotting in the cannulae and coagulation in blood samples subsequently withdrawn for analysis. Deep rectal temperature was determined by means of a thermocouple inserted to a depth of ten centimeters. The thermocouple was connected to a Leeds-Northrup Speedomax upon which the temperature was recorded every twenty-four seconds.

Reduction in body temperature was effected by the immersion method. The anesthetized dog was strapped in the supine position to a dog board. After preliminary preparations and control observations were completed, both dog and board were lowered into a tub filled with iced water. Immersion was such that only the head, neck and ventral thorax remained above water. This position was originally chosen by Haterius in order to simulate the position assumed by one kept afloat in deep waters by a life preserver of the "Mae West" type. An inevitable result of this type of cooling system is the establishment of a surface to core temperature gradient. Since the more peripheral parts of the immersed body come into more immediate contact with the cold water, at any given time in the cooling process they are colder than the inner structures. Moreover, in addition to this surface to core gradient, there
is a cephalo-caudal gradient. The unimmersed head, for example, is warmer than the immersed trunk.

In these experiments rectal temperature was the only temperature measured. Previous work in this laboratory established definite temperature relationships between various anatomical parts of the hypothermic dog. Since these relationships have important bearing on some of the results obtained, they are illustrated graphically in Figure 1. The temperatures of the heart and rectum are fairly close throughout most of the cooling process. The temperature of the thigh, however, deviates markedly from that of the rectum from the onset of cooling. This is quite understandable in view of the facts that heat loss occurs at the surface of a structure and that the thigh presents a much greater surface-mass ratio than does the whole trunk. At any given time in immersion cooling, the temperature of the deep muscles of the thigh is considerably lower than that of the rectum.

Studies on Blood

Hematocrit:

Hematocrit was studied both in random groups of dogs and in a series of dogs before and after cooling. Random group studies consisted of determinations made on a group of seven anesthetized dogs at normal body temperature, a group of seven dogs cooled to and sacrificed at rectal temperatures of 28°C, and a group of eleven dogs cooled to and sacrificed at a rectal temperature of 20°C. In connection with blood volume experiments, hematocrits were also determined on thirty-four anesthetized
Figure 1. Variation of Certain Anatomical Temperatures from that of the Rectum During Hypothermia in the Dog.
dogs, first at normal body temperature and then at rectal temperatures of 23°C to 20°C. In both studies the hematocrit was measured by centrifugation in Wintrobe tubes at 3000 revolutions per minute for thirty minutes. The "buffy coat" was not included in observing the packed cell mass and no correction was made for trapped plasma.

Plasma Water Content:

Plasma water content was determined in random groups of dogs by drying for forty-eight hours in an oven at 95°C. The groups were comprised as follows: (a) eight anesthetized dogs sacrificed at normal rectal temperature; (b) seven dogs cooled to and sacrificed at rectal temperatures of 28°C; (c) seven dogs cooled to and sacrificed at rectal temperatures of 20°C; five dogs cooled to rectal temperatures of 20°C and maintained at that temperature for periods of time ranging from thirty minutes to five hours; (e) seven dogs cooled to death, which in these experiments occurred between rectal temperatures of 17.6°C and 15.3°C.

Plasma Protein Concentration:

Plasma protein concentrations were first studied by the copper sulfate method of Moore and Van Slyke (65). In this relatively simple technique plasma is dropped into stock solutions of copper sulfate of known specific gravity. The specific gravity of the solution, in which the drop of plasma remains briefly suspended, is equal to the specific gravity of the plasma. Plasma protein concentrations is then computed from the formula:
grams of protein per 100 cubic centimeters = 343 \( (G - 1.007) \)
of plasma

in which: \( G \) represents the determined specific gravity,

1.007 represents the specific gravity of plasma with no protein, and

343 represents the factor, equivalent to an increase in protein of 1 gram per 100 cubic centimeters.

Changes in plasma protein concentration were later studied refractometrically in connection with tissue water and chloride experiments. In nine dogs serial determinations were made at several points during cooling. The method involved direct measurement of the refractive index of plasma and computation of plasma protein concentration from the formula:

\[
\text{grams of protein per 100 cubic centimeters} = \frac{R_s - (R_{H_2O} - 0.0022)}{0.00201}
\]

in which: \( R_s \) represents the reading obtained with plasma,

\( R_{H_2O} \) represents the reading obtained with distilled water,

0.0022 represents the assumed reading due to crystalloids in plasma, and

0.00201 represents the assumed reading due to 1 gram of protein per 100 cubic centimeters of plasma.

**Plasma Volume**

Plasma volumes were measured by the T-1824 dye dilution technique. The time required for complete mixing of dye in the plasma was determined by plotting the concentration of dye in
successive samples of plasma against the time intervals at which these samples were drawn after a single injection of dye. Thus mixing was found to be complete in thirty to forty minutes in four anesthetized dogs at normal body temperature, in agreement with the findings of Miller (64). In seven dogs at rectal temperatures of 23°C to 20°C, mixing periods were found to be forty to sixty minutes. A typical dilution curve obtained at normal temperature is presented in Figure 2. The cause of the initial plateau, occurring at ten to twenty minutes after injection, is not known. Evidence for a similar temporary plateau is found in the literature (42) and has been obtained in the unpublished work of others (73). Indeed, failure to extend investigations of mixing time beyond twenty minutes may have been responsible for the assumption, made in the earlier days of this technique, that mixing was complete in ten minutes.

Using these mixing periods, plasma volumes were determined in fifteen anesthetized dogs at normal body temperature. Computation, made according to the formula presented in Section I, were based on the average dye concentration in plasma samples drawn thirty, thirty-five and forty minutes after injection. Each dog was then cooled to a rectal temperature of 23°C and removed from the bath. Dye was again injected and samples of plasma withdrawn fifty, fifty-five and sixty minutes later. The dye concentration immediately before the second injection subtracted from the average concentration in the samples withdrawn after the second injection gave the concentration of dye
Figure 2. Plasma Mixing and Disappearance Curves of T-1824 Dye and Cells Tagged with Radioactive Phosphorous.
in these samples due to the second injection. The plasma volume in hypothermia was computed from the dye concentration due to the second injection.

During the time required for mixing of dye in the cold, rectal temperatures generally fell two or three degrees, perhaps as a result of a redistribution of heat from the warmer core to the colder periphery. Plasma volumes under these conditions were presumed to be referable to the final temperature (21°C to 20°C).

Inasmuch as shorter mixing periods have been used frequently in measuring plasma volume, nine experiments were performed in which plasma volumes were measured using mixing times of ten minutes at normal body temperature and twenty minutes at the cold temperature.

**Red Cell Volume**

Circulating red cell volumes were measured by the radioactive phosphorous (P32) technique. Previously drawn red cells (5cc. to 10cc.) were incubated at 37°C with approximately 100 microcuries of radioactive phosphorous in the form of phosphate and in the presence of glucose for two or three hours. The tagged cells were washed four times with saline. Analyses showed that the third and fourth washings contained practically no radioactivity, thereby indicating complete binding of the phosphorous within the cell.

Times required for complete mixing of cells were found to be thirty to forty minutes at normal temperature and fifty to sixty minutes at the cold temperature. A representative
curve is reproduced in Figure 2.

Red cell volumes were thus measured in seven dogs at normal temperature and then at rectal temperatures of 20°C, the procedure being the same as that followed in the plasma volume measurements.

Thiocyanate Space

Total extracellular space was estimated from the volume of distribution of thiocyanate. Mixing periods similar to those observed for T-1824 and tagged cells were found for thiocyanate in two dogs at normal temperature and in two dogs at 20°C rectal temperature. Subsequent measurements of thiocyanate space were made in six dogs, following a procedure similar to that described above for plasma and red cell volumes.

Total Water Content and Chloride Space of Tissues

Total water content and chloride space was measured in skeletal muscle of the thigh (representing immersed musculature), in skeletal muscle of the shoulder (representing unimmersed musculature), in cardiac muscle and in liver. Total water was determined by drying for forty-eight hours in an oven at 95°C. Total tissue chloride was determined by the open Carius method as modified by Sunderman and Williams (84), and chloride space computed according to the formula presented in Section I. Assuming chloride space and extracellular space to be identical in these tissues, intracellular water content was computed by subtracting the former from the total water content.
These measurements and computations were made on a total of thirty-four dogs grouped as previously described in this section under plasma water studies.
SECTION V

RESULTS OF ORIGINAL RESEARCH
Studies on Blood

Hematocrit:

One of the more consistent observations in hypothermia is that of hemoconcentration (53). In humans, cooled by the Germans at Dachau, blood counts increased by 20% of the normal (1). Assuming no change in single corpuscular volume this would be reflected in an increase in hematocrit of similar magnitude. The sole negative report has been that of Rodbard et al. (76) who observed no significant difference between the hematocrit of normal chicks and rabbits and that of chicks and rabbits cooled to 25°C.

Table 1 summarizes the results of random group studies of hematocrit in the dog. The average hematocrit of the group of dogs cooled to 28°C rectal temperature was 30% higher than that of the group of normal dogs. The average hematocrit of the dogs cooled to 20°C was higher than that of the normal dogs by about 25%. While both groups of hypothermic dogs differed significantly from the control group, they did not differ significantly from one another.

Changes in hematocrit occurring in a series of dogs subjected to immersion hypothermia are summarized in Table 2. As in the random group studies, marked increases in hematocrit were observed in hypothermia. The average change at 20°C in this series was a slightly lower increase than that observed at the same temperature in the previous study.

Other workers have inferred that increases in hematocrit similar to those measured in these studies, were due to con-
Table 1.
Hematocrit of the Normothermic and Hypothermic Dog (Random Groups)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Dogs</th>
<th>Ave. Hct. %</th>
<th>S. D. %</th>
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<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>40.5</td>
<td>± 5.0</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C</td>
<td>7</td>
<td>52.7*</td>
<td>± 5.3</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C</td>
<td>11</td>
<td>49.8*</td>
<td>±12.4</td>
</tr>
</tbody>
</table>

* Differences from control group are statistically significant (P < .05, random group, t test).
Table 2.

Percentage Change from Normal in Hematocrit in the Hypothermic Dog

<table>
<thead>
<tr>
<th>Number of Experiments</th>
<th>Range %</th>
<th>Ave. Change %</th>
<th>S. E. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>-1.3 to +54.2</td>
<td>+17.8</td>
<td>±1.7</td>
</tr>
</tbody>
</table>
traction of the spleen or to loss of plasma from the blood to the tissues. It is hoped that the nature of this hemoconcentration will be elucidated somewhat by the results presented in this section.

Plasma Water Content:

Determinations of plasma water content under control and hypothermic conditions (Table 3) revealed no overall change in plasma water content in hypothermia. A transient decrease was observed in the early stages of cooling but normal values were reestablished before rectal temperatures of $20^\circ C$ were attained. The decrease in water observed in the group cooled to $28^\circ C$ corresponds to an increase in plasma solids of 15% of the control value. Increases in plasma solids of 10% have been reported in cats cooled to $34^\circ C$ to $33^\circ C$ (5).

Plasma Protein Concentration:

Studies of changes in plasma protein concentration in hypothermia yielded data which generally agree with the above measurements of plasma water content.

Figure 3 illustrates graphically the results of plasma protein determinations made by the copper sulfate method. It should be pointed out that in this study, determinations were made at normal temperature, at $25^\circ C$ and at $20^\circ C$, as opposed to the measurements of water content which were made at normal temperature, at $28^\circ C$ and at $20^\circ C$. This difference in temperature accounts for the quantitative difference which will be noted between the results of the plasma water studies and the
Table 3.

Water Content of Plasma in the Normothermic and Hypothermic Dog

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Dogs</th>
<th>Average Plasma Water %</th>
<th>S. D. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>92.7</td>
<td>±0.4</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C</td>
<td>7</td>
<td>91.5*</td>
<td>±1.1</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C</td>
<td>9</td>
<td>92.6</td>
<td>±0.8</td>
</tr>
<tr>
<td>Held at 20°C before sacrifice</td>
<td>5</td>
<td>92.4</td>
<td>±0.3</td>
</tr>
<tr>
<td>Cooled to death</td>
<td>7</td>
<td>92.6</td>
<td>±0.5</td>
</tr>
</tbody>
</table>

* Difference from control group is statistically significant (P < .05, group means, t test).
Figure 3. Changes in Plasma Protein Concentration in the Hypothermic Dog. Copper Sulfate Method.
results of plasma protein determinations made with copper sulfate.

Although five of the experiments presented in Figure 3 revealed no change and one a decrease in plasma protein concentration at 25°, the average change was an increase of 5% of normal, a statistically significant change according to paired data analysis. Between rectal temperatures of 25°C and 21°C, plasma protein concentration tended to return toward normal. As a result, the average concentration at 21°C was not significantly different from normal. Thus a transient increase in plasma protein concentration was demonstrated in the hypothermic dog. The study failed, however, to reveal an increase of protein similar in magnitude to the increase in plasma solids computed from the observed decrease in plasma water. In addition, the study failed to demonstrate the rectal temperature at which the peak increase in plasma protein occurred. Therefore, serial determinations of plasma protein concentration were carried out refractometrically in nine dogs. Temporary increases were seen in all nine experiments (Figure 4). Two dogs revealed increases of a magnitude much greater than any of those seen in the previous study. In general, the results indicate that the peak increase in plasma protein concentration may occur at any rectal temperature between 33°C and 24°C. In those dogs in which increases occurred at relatively high rectal temperatures, reversals of these increases were complete by 20°C. In those dogs in which increases in plasma protein concentration were delayed, reversals were nevertheless
Figure 4. Changes in Plasma Protein Concentration in the Hypothermic Dog. Refractometric Method.
well underway at 20°0. The average response at 28°0 rectal temperature was an increase of 12% of the control level. This compares well with the 15% increase in plasma solid content computed from the plasma water measurements made at the same rectal temperature.

It must be borne in mind that temporary increases in plasma solid content and plasma protein concentration of 15% and 12%, respectively, correspond to decreases in plasma water of less than 2%. Such changes can be ascribed to (a) an actual loss of water from the plasma; (b) an increase in total circulating protein; or (c) a combination of (a) and (b). In view of the rapidity and brevity of change the first explanation seems more probable. If it is further assumed that this 2% of plasma water is distributed evenly to the body tissues, the consequent increase in tissue water would be negligible and undetectable by the analytical methods employed in tissue studies reported in this paper. The transitory nature and the minimal magnitude of plasma water loss in hypothermia do, however, establish one point: the marked increases in hematocrit observed at rectal temperatures of 20°0 cannot be ascribed to losses of plasma water as such.
Plasma Volume

Determinations of plasma volume were made with T-1824. When mixing periods of forty and sixty minutes were employed at normal and hypothermic temperatures, respectively, the average plasma volume was found to be 54.5 ± 8.4 ml./kgm. at normal temperature, and 45.5 ± 8.9 ml./kgm. at rectal temperatures of 21°C to 20°C. The results of individual experiments are summarized in Table 4. The average response obtained with forty and sixty minute mixing periods was a decrease of 16% from the normal plasma volume, a slightly greater decrease than that obtained with mixing periods of ten and twenty minutes.

Since in a large series of experiments plasma water content and plasma protein concentration are essentially normal at rectal temperatures of 21°C to 20°C, then the shrinkage of plasma volume which occurs at these low temperatures is due to a loss of "whole plasma." Both watery and solid phases (including protein) of 16% of the normal plasma volume are removed from the circulating stream in the deeply hypothermic state. It is possible to explain this loss by (a) escape of plasma to the interstitial and/or intracellular spaces; (b) an isolation of plasma within vessels through which circulation has ceased; or (c) a loss of water with concomitant storage of protein. The results of measurements of thiocyanate space, tissue chloride space and total tissue water tend to negate the first explanation.
Table 4.

Percentage Change in Plasma Volume in the Hypothermic Dog

<table>
<thead>
<tr>
<th>Mixing Periods Minutes</th>
<th>Number of Experiments</th>
<th>Range %</th>
<th>Average ±S. E. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40* and 60**</td>
<td>15</td>
<td>0 to -27</td>
<td>-16.5 ± 2.3</td>
</tr>
<tr>
<td>10* and 20**</td>
<td>9</td>
<td>-5 to -18</td>
<td>-13.4 ± 1.3</td>
</tr>
</tbody>
</table>

* at normal body temperature
** at rectal temperature of 21°-20°C
Thiocyanate Space

In six dogs, the average volume of distribution of sodium thiocyanate was $245 \pm 24.4$ ml./kgm. at normal body temperature and $203 \pm 22.1$ ml./kgm. at rectal temperatures of $21^\circ C$ to $20^\circ C$. One will note that percentagewise, the average change in thiocyanate space and the average change in plasma volume in hypothermia (Table 5) are equal. A similar observation was made by Rodbard et al. (76) on hypothermic rabbits. The latter authors ascribed this to a clamping down of certain vascular beds which became inaccessible to T-1824. This closing of vessels made corresponding percentages of interstitial space unavailable to injected thiocyanate. However, in experiments in which thiocyanate space and plasma volume were measured simultaneously in the dog, percentage decreases in the two volumes were not always the same. It must be pointed out, on the other hand, that in these simultaneous measurements, a marked decrease (at least 10% of normal volumes) in either space was never observed in the absence of a marked decrease in the other.

In terms of absolute quantities, the measured decreases in plasma volume and in muscle chloride space (to be considered next) can account for about two-thirds of the total decrease in thiocyanate space. If one postulates nearly complete cessation of blood flow through the skin in hypothermia and consequent unavailability of skin water for thiocyanate distribution, most of the remaining one-third can be accounted for. The
Table 5.

Percentage Change in Thiocyanate Space in the Hypothermic Dog

<table>
<thead>
<tr>
<th>Number of Experiments</th>
<th>Range %</th>
<th>Average ± S. E. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-9 to -23</td>
<td>-17 ± 2.5</td>
</tr>
</tbody>
</table>
microscopic investigations of Bigelow et al. (12) of the conjunctival vessels in deep hypothermia revealed cessation of flow through vessels as large as sixty micra in diameter.
Total Water Content and Chloride Space of Tissues

Total water content and chloride space of skeletal muscle, cardiac muscle and liver were measured in a group of normal dogs and in several groups of hypothermic dogs. Intracellular water was computed by subtracting chloride space from total water content.

The long held concept that chloride ions in mammalian tissue are confined largely or entirely to interstitial spaces has been repeatedly challenged in recent years (25,27,34,35). However, except for certain theoretical considerations and certain in vitro studies involving hypertonic solutions and high potassium chloride concentrations (13,18) there is little to indicate that chloride ions penetrate muscle fibers in vivo under conditions of physiological plasma electrolyte levels. Justification for the use of chloride space as an estimate of interstitial space in these tissues is based on the absence of a more accurate method applicable in this type of experiment.

The data of Tables 6 and 7 reveal temporary increases in intracellular water at the expense of the extracellular space in skeletal muscle. These shifts are at least qualitatively similar in the unimmersed shoulder muscle and in the immersed hind limb muscle in spite of the fact that the latter may be 5°C to 10°C colder than the former. Both shifts are maximal by rectal temperatures of 28°C and are maintained with cooling to 20°C. The maintenance of the animal at 20°C or further cooling to 17°C result in some reversal of the original water shift directly to the plasma, since chloride space
Table 6.
Water Content of Shoulder (Unimmersed) muscle in the Normothermic and Hypothermic Dog

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>767 ± 8.8</td>
<td>115 ± 19.4</td>
<td>652 ± 19.8</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C.</td>
<td>7</td>
<td>763 ± 12.0</td>
<td>88 ± 10.4* (-24%)</td>
<td>675 ± 12.3* (+4%)</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C.</td>
<td>9</td>
<td>769 ± 7.5</td>
<td>91 ± 15.3* (-21%)</td>
<td>678 ± 13.5* (+4%)</td>
</tr>
<tr>
<td>Held at 20°C before sacrifice</td>
<td>5</td>
<td>754 ± 11.5</td>
<td>91 ± 11.9* (-21%)</td>
<td>663 ± 13.3</td>
</tr>
<tr>
<td>Cooled to death</td>
<td>7</td>
<td>751 ± 8.2* (-2%)</td>
<td>95 ± 12.7* (-17%)</td>
<td>656 ± 17.8</td>
</tr>
</tbody>
</table>

* Differences from control groups are statistically significant (random group, t test). These differences, expressed as percentage of control value are given in parentheses.
Table 7.

Water Content of Hind Limb (Immersed) Muscle in the Normothermic and Hypothermic Dog

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>751 ± 15.2</td>
<td>119 ± 18.4</td>
<td>632 ± 12.3</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C</td>
<td>7</td>
<td>753 ± 15.3</td>
<td>93 ± 16.9*</td>
<td>659 ± 14.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-22%)</td>
<td>(+4%)</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C</td>
<td>9</td>
<td>759 ± 14.4</td>
<td>102 ± 12.3*</td>
<td>657 ± 11.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-14%)</td>
<td>(+4%)</td>
</tr>
<tr>
<td>Held at 20°C before sacrifice</td>
<td>5</td>
<td>759 ± 10.8</td>
<td>102 ± 19.2</td>
<td>657 ± 23.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-14%)</td>
<td></td>
</tr>
<tr>
<td>Cooled to death</td>
<td>7</td>
<td>748 ± 11.4</td>
<td>92 ± 16.7*</td>
<td>654 ± 25.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-22%)</td>
<td></td>
</tr>
</tbody>
</table>

* Differences from control groups are statistically significant (random group, t test). These differences, expressed as percentage of control value are given in parentheses.
remains subnormal in volume. The transient nature of these shifts and their occurrence at or before 28°C suggest that the causative factor is one of muscular activity in the form of shivering, the pattern of which is similarly transient. Temporary increases in intracellular water of a similar magnitude have been reported for the hypothermic rat (6).

The data of Table 8 indicate that a shift of water into the cells occurs also in the ventricular myocardium. In this case, however, the phenomenon is progressive, increasing as cooling proceeds to terminus. Moreover, the rise in intracellular water is not solely at the expense of the chloride space, since the groups held at 20°C and cooled to death exhibited increases in total water content. The nature of causative factors is not clear.

Data concerning changes in water distribution in the liver of the dog in hypothermia are contained in Table 9. An apparent shift of water into the cells was observed in those animals held at 20°C and in those cooled to death. Fenn (29) showed that water was deposited with glycogen in the liver. The ratio of extracellular water deposited with glycogen was smaller than the ratio already present, resulting in an overall decrease in extracellular water as glycogen was deposited. One would, therefore, expect that the glycogenolysis which occurs in hypothermia (9) would be accompanied by an overall increase in extracellular water. In the absence of glycogen measurements, liver water studies mean little. Barbour, McKay and Griffith
Table 8.
Water Content of Cardiac Muscle in the Normothermic and Hypothermic Dog

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>774 ± 3.9</td>
<td>216 ± 16.7</td>
<td>558 ± 15.5</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C</td>
<td>7</td>
<td>771 ± 10.8</td>
<td>199 ± 14.8</td>
<td>573 ± 13.5</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C</td>
<td>9</td>
<td>777 ± 8.0</td>
<td>192 ± 26.6*</td>
<td>584 ± 22.2*</td>
</tr>
<tr>
<td>Held at 20°C before sacrifice</td>
<td>5</td>
<td>788 ± 4.8*</td>
<td>123 ± 9.3*</td>
<td>665 ± 11.7*</td>
</tr>
<tr>
<td>Cooled to death</td>
<td>7</td>
<td>785 ± 5.9*</td>
<td>143 ± 24.8*</td>
<td>642 ± 23.3*</td>
</tr>
</tbody>
</table>

* Differences from control group are statistically significant (random group, t test). These differences, expressed as percentage of control value are given in parentheses.
Table 9.

Water Content of Liver in the Normothermic and Hypothermic Dog

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>701 ± 14.3</td>
<td>229 ± 19.0</td>
<td>472 ± 19.6</td>
</tr>
<tr>
<td>Sacrificed immediately at 28°C</td>
<td>7</td>
<td>714 ± 20.1</td>
<td>233 ± 19.2</td>
<td>481 ± 23.8</td>
</tr>
<tr>
<td>Sacrificed immediately at 20°C</td>
<td>9</td>
<td>711 ± 9.2</td>
<td>247 ± 15.4</td>
<td>465 ± 22.0</td>
</tr>
<tr>
<td>Held at 20°C before sacrifice</td>
<td>5</td>
<td>712 ± 7.9</td>
<td>190 ± 20.0*</td>
<td>519 ± 13.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-17%)</td>
<td>(+10%)</td>
</tr>
<tr>
<td>Cooled to death</td>
<td>7</td>
<td>710 ± 16.3</td>
<td>202 ± 19.2*</td>
<td>508 ± 33.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-12%)</td>
<td>(+8%)</td>
</tr>
</tbody>
</table>

* Differences from control group are statistically significant (random group, t test). These differences, expressed as percentage of control value are given in parentheses.
(6) found a similar increase in intracellular water in the liver of hypothermic rats and attributed the increase to a "protective neurogenic reflex" in response to cold exposure.
Red Cell Volume

In 1942, Hahn et al. (46) demonstrated that the red cell volume measured by radioactive iron was 15.7% smaller than the red cell volume simultaneously computed from the measured plasma volume and hematocrit. Brilliant vital red dye was used in the estimation of plasma volume. The hematocrit was determined by centrifugation of blood withdrawn from the jugular vein. Assuming valid measurements of red cell and plasma volumes and arguing from hemodynamic principles, they attributed this discrepancy to an error in determining the true cell to plasma ratio in the body as a whole. In essence, the authors contended that the ratio of cells to plasma in blood drawn from large vessels is larger than the overall ratio of cells to plasma in the whole body. Thus computations of red cell volume, based on the large vessel hematocrit, will result in proportionate overestimates. This inaccuracy on the part of the large vessel hematocrit must not be confused with the slight error introduced by plasma trapped in the column of packed cells. This latter error is usually correctable by multiplying the observed hematocrit by 0.95 or 0.96.

Table 10 is a summary of all reported data from the time of Hahn's report to date, concerning the discrepancy between red cell volumes simultaneously measured and computed. The only studies which do not reveal sizeable differences are those in which carbon monoxide was used in determining red cell volume. The chief criticism of the carbon monoxide method has always been its tendency to overestimate true volume because of
### Table 10.

Comparison of Red Cell Volume (RCV) Measured and Computed from Simultaneously Measured Plasma Volume (PV) and Hematocrit

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Subject*</th>
<th>No. of Exp.</th>
<th>RCV Method</th>
<th>PV Method</th>
<th>Factor of RCV Trapped to Plasma</th>
<th>Computed</th>
<th>Ratio of RCV Measured to RCV Computed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hahn (1942)</td>
<td>dog</td>
<td>18</td>
<td>Fe</td>
<td>Vital</td>
<td>.95</td>
<td>.85</td>
<td></td>
</tr>
<tr>
<td>Hopper (1946)</td>
<td>dog**</td>
<td>16</td>
<td>CO</td>
<td>T-1824</td>
<td>.96</td>
<td>.93</td>
<td></td>
</tr>
<tr>
<td>Hopper (1946)</td>
<td>man</td>
<td>9</td>
<td>CO</td>
<td>T-1824</td>
<td>.96</td>
<td>.98</td>
<td></td>
</tr>
<tr>
<td>Root (1946)</td>
<td>dog**</td>
<td>19</td>
<td>CO</td>
<td>T-1824</td>
<td>.96</td>
<td>.98</td>
<td></td>
</tr>
<tr>
<td>Root (1946)</td>
<td>man</td>
<td>11</td>
<td>CO</td>
<td>T-1824</td>
<td>.96</td>
<td>.99</td>
<td></td>
</tr>
<tr>
<td>Mayerson (1948)</td>
<td>man</td>
<td>6</td>
<td>P32</td>
<td>T-1824</td>
<td>.915</td>
<td>.97</td>
<td></td>
</tr>
<tr>
<td>Hopper (1946)</td>
<td>man</td>
<td>13</td>
<td>P32</td>
<td>T-1824</td>
<td>.95</td>
<td>.92</td>
<td></td>
</tr>
<tr>
<td>McLean (1951)</td>
<td>dog</td>
<td>14</td>
<td>Bleeding Hb</td>
<td>T-1824</td>
<td>none</td>
<td>.83</td>
<td></td>
</tr>
<tr>
<td>Wasserman (1951)</td>
<td>man***</td>
<td>30</td>
<td>P32</td>
<td>T-1824</td>
<td>none</td>
<td>.84</td>
<td></td>
</tr>
<tr>
<td>Gibson (1945)</td>
<td>man</td>
<td>40</td>
<td>Fe</td>
<td>T-1824</td>
<td>.96</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>Gibson (1946)</td>
<td>dog</td>
<td>40</td>
<td>Fe</td>
<td>T-1824</td>
<td>.96</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>Gibson (1946)</td>
<td>dog****</td>
<td>8</td>
<td>Fe</td>
<td>T-1824</td>
<td>.96</td>
<td>.77</td>
<td></td>
</tr>
<tr>
<td>Barnes (1948)</td>
<td>man***</td>
<td>12</td>
<td>Ashby</td>
<td>T-1824</td>
<td>.95</td>
<td>.92</td>
<td></td>
</tr>
<tr>
<td>Barnes (1948)</td>
<td>man</td>
<td>8</td>
<td>Ashby</td>
<td>T-1824</td>
<td>.95</td>
<td>.94</td>
<td></td>
</tr>
</tbody>
</table>

* State of all subjects normal unless otherwise indicated
** Normal and splenectomized
*** Patients
**** After bleeding and transfusion
its rapid excretion and oxidation to carbon dioxide. This tendency may have been sufficient to nullify the discrepancy which would otherwise have appeared. Mayerson (62) based his computations on a hematocrit correction factor for trapped plasma of 0.915, which was suggested by Chapin and Ross (17). Inasmuch as this factor has never been substantiated and has not been generally accepted, the data of Mayerson were recomputed, using a factor of 0.96. In general, red cell volumes measured by a variety of methods appear to be 10% to 20% smaller than red cell volumes computed from plasma volumes and hematocrits.

From fourteen of the previously described plasma volume measurements red cell volumes were computed as above. Calculations were based on the hematocrit of blood withdrawn from the common carotid artery. Computed volumes averaged $55.5 \pm 14.8$ ml./kgm. at normal temperatures and $66.4 \pm 20.3$ at rectal temperatures of $21^\circ$C to $20^\circ$C. Table 11 shows that the results of individual experiments ranged from -8% to +53% of the normal volume. The average computed change in deep hypothermia was +23%.

However, in seven experiments in which red cell volumes were measured with radioactive phosphorous, these volumes averaged $40.9 \pm 7.1$ ml./kg. at normal body temperature and $40.1 \pm 9.4$ ml./kg. in hypothermia, clearly demonstrating the absence of consistent volume change. Individual responses (Table 11) varied from -9% to +8%. If the discrepancy between measured and computed red cell volumes were constant at all temperatures
### Table 11.

Percentage Change in Red Cell Volume in the Hypothermic Dog

<table>
<thead>
<tr>
<th></th>
<th>Number of Experiments</th>
<th>Range %</th>
<th>Ave. ±S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computed from Plasma Volume and Hematocrit</td>
<td>14</td>
<td>-8 to +53</td>
<td>+23 ±4.1</td>
</tr>
<tr>
<td>Measured Directly</td>
<td>7</td>
<td>-9 to +8</td>
<td>-2.6 ±2.1</td>
</tr>
</tbody>
</table>
in a given dog, percentage changes or the lack of change in hypothermia would be equally evident from measurements and computations. The data contained in Table 11, therefore, indicate that in hypothermia the discrepancy between computed and measured RCV becomes greater. Moreover, at normal temperature the ratio of average measured to average computed red cell volume was 0.78. In hypothermia the ratio was reduced to 0.60. Correcting the observed hematocrit for trapped plasma by the usual factor of 0.96, these values became 0.83 and 0.65 respectively. If the discrepancy is due to an unequal distribution of cells between larger and smaller vessels, as proposed by Hahn, then the greater discrepancy in hypothermia suggests a redistribution of cells and plasma, in which blood in the larger vessels becomes more concentrated, while blood in the smaller vessels becomes more dilute.
SECTION VI

GENERAL DISCUSSION
Temporary shifts of water into tissue cells in hypothermia have been attributed by others to a reflex increase in metabolism which is eventually depressed by the cold (6). The identification of this reflex with the phenomenon of shivering has been denied although the reported evidence supporting this denial is meagre and evidence in favor of any other physiological mechanism entirely lacking. Increased intracellular water in response to increased activity has been demonstrated conclusively in skeletal muscle of the dog by Barcroft and Kato (6), of the rat by Fenn and Jobb (30) and of the cat by Tipton (87). Inasmuch as maximal shifts of water into skeletal muscle cells occur at or before 28°C, the rectal temperature at which shivering is maximal, a casual relationship is strongly suggested. The slight loss of water from plasma at 28°C is probably part of the same phenomenon. The complete reversal of these shifts may be delayed by extremely slow removal of metabolites due to the very sluggish blood flow at low body temperatures (12). Postulation of any mechanism, other than shivering, to explain these temporary increases in intracellular water in skeletal muscle seems unnecessary.

The observed redistribution of water in cardiac muscle is less readily explained. The work output per stroke and minute may be readily calculated by application of the accepted formula

\[ W = QR \]
in which: \( q \) represents the stroke volume, and

\[ R \]

represents the mean arterial pressure.

Under pentothal anesthesia and at normal body temperature the stroke volume for dogs of the weight range here employed averages 9 ml. (50) and the arterial pressure is 130 mm. Hg. (53). The work is then \( 9 \times 0.13 \times 13.6 = 15.9 \) gram meters per stroke or \( 15.9 \times 150 \) (heart rate) = 2,380 gram meters per minute. At a rectal temperature of 20°C these values are reduced to \( 10 \times 0.075 \times 13.6 = 10.2 \) gram meters per stroke and \( 10.2 \times 35 \) (heart rate) = 357 gram meters per minute. Thus the work per stroke is reduced by 33% and per minute by 85%.

If the oxygen consumption of the heart is affected proportionately to the same degree as that of the whole animal, then at 20°C it is reduced by 75% (68). The figures for work output per stroke suggest that in hypothermia the heart is under greater stress even after shivering has ceased, although, when calculated per unit of time this is not apparent. In the absence of direct measurements of cardiac oxygen consumption in hypothermia, one cannot evaluate the degree of cardiac stress which hypothermia may impose. In any event, the increase in intracellular water in the heart of the hypothermic dog is of interest in view of possible electrolyte changes which may accompany this water shift.

The marked increase in hematocrit, characteristic of the hypothermic dog, appears to be due to two factors. The greater part of the increase is attributable to an actual loss of circulating plasma, without change in circulating
red cell volume. The observed decrease in plasma volume can of itself account for an increase in hematocrit of about 12%. The remainder of the hematocrit increase is apparently attributable to redistribution of cells and plasma still within the circulating stream.

The whereabouts of the lost plasma remains a question. A transfer of 16% of the normal plasma volume into the tissue of the body could have easily gone undetected in these studies. Indeed, the magnitude and variability of total tissue water in the dog are such as would require a shift of at least 30% of the plasma into the tissue spaces, in order to result in a statistically significant increase in random group analysis. However, the average loss of thiocyanate space which, percentage wise, equals the average plasma loss, cannot be explained entirely on the basis of a shift into the cells. Observed shifts into cells added to plasma loss can account at best for only two-thirds of the decrease in thiocyanate space. The fate of the remainder of the lost thiocyanate space is also open to speculation. Rodbard thinks that blood vessels clamp down so as to exclude from circulation a certain percentage of the blood volume and a corresponding percentage of extracellular space. His hypothesis is supported by the observation that rewarming rapidly restores both plasma volume and thiocyanate space to normal.

The phenomenon of redistribution of circulating cells and plasma, whereby blood in larger vessels becomes more concentrated while blood in the smaller vessels becomes more
dilute, is well known. Gibson et al. (38) observed such redistribution in dogs in shock, induced by a variety of procedures. Indeed, it seems to be a characteristic effect of circulatory disorders in which systemic flow is relatively sluggish. As a result, in such pathological states the large vessel hematocrit does not bear the same quantitative relationship to the whole body hematocrit as it does under normal conditions. Moreover, this new relationship is unpredictable, depending on the circulatory dynamic state at the time at which measurements are made. Thus the use of the large vessel hematocrit in calculating red cell volumes in circulatory states other than normal is inadvisable and may even be dangerous.

In general, the changes in water distribution observed in the hypothermic dog appear to be explicable on the basis of compensatory physiological mechanisms such as shivering and intense vasoconstriction. Redistribution of cells and plasma results from the slowed systemic circulation. With the possible exception of increases in intracellular water of cardiac muscle, the observed changes are, in terms of survival, not dramatic.
SECTION VII

SUMMARY AND CONCLUSIONS
The following investigations were made in the hypothermic dog:  
(A) Determination of the hematocrit of large vessel blood, determination of plasma water content and of plasma protein concentration;  
(B) Estimation of plasma volume;  
(C) Determination of thiocyanate space;  
(D) Determination of total water content and chloride space of skeletal muscle, cardiac muscle and liver;  
(E) Estimation of red cell volume.

The following observations were made:  
(A) Increases in large vessel hematocrit of 13% - 25% of the normal value were observed. A transient decrease in plasma water was observed at a rectal temperature of 28°C, below which normal content was restored. This rectal temperature corresponds to that at which shivering is near maximal in the hypothermic dog. Plasma protein concentration also increased temporarily, the peak increase occurring at any temperature between 33°C and 23°C. The average increase in protein concentration at 23°C agrees well with the average increase in plasma solids, computed from the plasma water decrease observed at the same rectal temperature.  
(B) Plasma volume changes ranged from no change to a decrease of 27% of the control volume, the average change being a decrease of 16%.  
(C) Changes in thiocyanate space ranged from a decrease of 9% to a decrease of 23% of the control space, the average decrease being 17% of the control space.
(D) Intracellular water in skeletal muscle increased at the expense of the muscle chloride space. This shift of water reached maximum at or before 28°C rectal temperature, and was still present at 20°C. Holding the animal at 20°C or further cooling resulted in a reversal of this shift. The shift, like that of the plasma water at 28°C, coincides in time and in rectal temperature with the onset, peak and subsequent depression of shivering.

Intracellular water in cardiac muscle, on the other hand, increased progressively as the animal was cooled to death. Intracellular water in the liver also increased progressively.

(E) Direct measurement of circulating red cell volume revealed no consistent change in deep hypothermia. Computation of red cell volume from plasma volume and large vessel hematocrit, however, predicted increases in red cell volume averaging 23%. The discrepancy between measured and computed red cell volumes appears to be due to a misrepresentation of the true cell-plasma ratio in the body by the hematocrit of blood withdrawn from a large vessel.

The following conclusions are drawn:

(1) The plasma, which is lost from the circulation, is lost as "whole plasma." Its fate is not known, although transfer into the tissue seems improbable.

(2) The loss of thiocyanate space is attributable in part to shifts of water into cells. The remainder of the decrease is probably due to intense constriction in certain
vascular beds making these beds and associated interstitial spaces unavailable for distribution of thiocyanate.

(3) Temporary shifts of water into the cells of skeletal muscle, as well as the temporary shift of water out of plasma at 28°C rectal temperature, are very probably due to increased work on the part of the muscle fibers in the form of shivering. The causative factors involved in the shift of water into the cells of cardiac muscle and of liver are not known.

(4) There occurs in hypothermia a redistribution of cells and plasma in which the blood of larger vessels becomes more concentrated, while the blood in the smaller vessels becomes more dilute. This redistribution increases the discrepancy between the large vessel hematocrit and the hematocrit of the body as a whole. Thus the large vessel hematocrit is entirely unreliable in the computation of red cell volume from plasma volume.

(5) The redistribution of cells and plasma together with the actual loss of plasma in hypothermia are responsible for the characteristic marked increase in hematocrit observed in the hypothermic dog.
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The Hypothermic Dog

The direct effect of reduction in body temperature on general metabolism in the dog is one of depression. This is a progressive phenomenon, increasing as body temperature falls. However, in the early stages of hypothermia there simultaneously occurs in certain tissues marked increases in activity which are reflex in nature. The overall metabolism, indicated by total oxygen consumption, at any given time in the cooling procedure is therefore determined by the algebraic sum of the effects of direct depression of some cells and of reflex stimulation of other cells by the cold.

The ability of the dog to survive severe reduction in temperature appears to be influenced by the anesthetic agent under which cooling procedure is carried out. In general, ether (by inhalation) interferes least with respiratory and cardiovascular function in deep hypothermia. Of the barbiturates, pentobarbital seems to predispose the dog to death at relatively high body temperatures.

Reduction in body temperature in the dog is characterized by descending narcosis of the central nervous system. In terms of tissue temperature, cortical, thalamic, hypothalamic, medullary and finally cord functions are lost in that order as cooling progresses. Although external respiration is markedly depressed, evidence indicates that tissues are adequately oxygenated at very low body temperatures and that the tissues at these low temperatures can obtain additional oxygen, as required, by increasing the coefficient of utilization.
Cardiac physiology in the hypothermic dog is characterized by progressive depression of both conductile and contractile mechanisms. In the absence of idioventricular beats, myocardial weakness is not present. Three types of cardiac terminal events can be described. Frequently, in the hypothermic dog a normal, relatively rapid sinus rhythm is interrupted by ectopic beats, one of which precipitates ventricular fibrillation. In other experiments the bradycardia induced by hypothermia progresses to permanent asystole. In still other experiments relatively long bouts of asystole occur, the last of which is terminated by sluggish fibrillation. Asystole in hypothermia is probably intimately related to the depression of conductile and contractile mechanisms. Ventricular fibrillation is probably related to increased myocardial excitability, present in many but not all experiments. It is conceivable that this increased myocardial excitability is opposed to a variable extent by another factor, the depressing effect of the anesthetic agent employed. This would help to explain the differences in survival limit and type of death observed with the various anesthetic agents.

Water Studies in Hypothermia

Increases in serum specific gravity and in intracellular water of certain tissues have been observed in the cat and in the rat. The early occurrence and subsequent reversal of these changes and their relationship to the integrity of the central nervous system, prompted their description as "reflex protective mechanisms, nervous in nature and eventually depressed by
the cold." Identification of these reflexes with shivering was denied, although evidence supporting the denial is meagre and evidence supporting any other mechanism entirely lacking. In addition to these changes in plasma specific gravity and in intracellular water, decreases in plasma volume and thio­cyanate space have been observed. These latter changes were found in hypothermic chicks and rabbits and were ascribed to the clamping down of vascular beds, which consequently became locked out of the circulating stream and inaccessible to blood borne indicators.

Experimentation

The following investigations were made in the dog at normal body temperature and in hypothermia:

(A) Hematocrit of blood drawn from the carotid artery was determined by centrifugation in Wintrobe tubes at 3000 revolutions per minute. Plasma water content was determined by drying in an oven at 95°C for forty-eight hours. Plasma protein concentration was determined first by the copper sulfate method of Moore and Van Slyke and subsequently by refractometric measurements.

(B) Plasma volume was estimated by the dilution method involving T-1824 (Evans Blue).

(C) Thiocyanate space was measured by the dilution technique.

(D) Total water content of skeletal muscle, cardiac muscle and liver was determined by drying as in (A). The chloride content of these tissues was measured by the open Carius
method. From these measurements chloride space and intracellular water were computed.

(E) Circulating red cell volume was estimated by the dilution of injected cells tagged with radioactive phosphorous.

Results:

The following observations were made:

(A) Increases in large vessel hematocrit of 18% to 25% of the normal value were observed. A transient decrease in plasma water was observed at a rectal temperature of 28°C, below which normal content was restored. This rectal temperature corresponds to that at which shivering is near maximal in the hypothermic dog. Plasma protein concentration also increased temporarily, the peak increase occurring at any temperature between 33°C and 23°C. The average increase in plasma protein concentration at 28°C agrees quite well with the average increase in plasma solids, computed from the plasma water decrease observed at the same rectal temperature.

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(D) Intracellular water in skeletal muscle increased at the expense of the muscle chloride space. This shift of water reached maximum at or before 28°C rectal temperature and was still present at 20°C. Holding the animal at 20°C or fur-
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BIography

Henry E. D'Amato was born in Shrewsbury, Massachusetts, on October 3, 1928, the son of Palmerino and A. Lillian (Corazzini) D'Amato. He received his elementary education in the Shrewsbury public schools. In June of 1945 he graduated from St. John's High School in Worcester, Massachusetts. Four years later he received his A. B. from the College of the Holy Cross in that same city. In June of 1951 he received his A. M. from Boston University.