THE PHYSIOLOGY OF INDUCED HYPOTHERMIA

PROCEEDINGS OF A SYMPOSIUM

28–29 October 1955

convened by

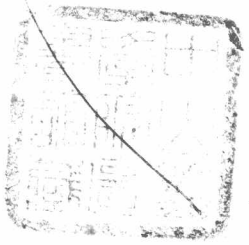
The Division of Medical Sciences

National Academy of Sciences—National Research Council

with the sponsorship of

The United States Army, Navy, and Air Force

Robert D. Dripps, M.D.
Chairman and Editor



This Symposium was undertaken by the National Academy of Sciences—National Research Council as a result of discussions with the Research and Development Division, Office of the Surgeon General, U. S. Army. The costs of the meeting were defrayed jointly by the Army, Navy and Air Force, and the Army contributed additional funds to support publication of these Proceedings.

The Symposium was organized and conducted under the auspices of four subcommittees of the Committee on Medicine and Surgery, in the Academy-Research Council's Division of Medical Sciences—the Subcommittee on Anesthesia, the Subcommittee on the Cardiovascular System, the Subcommittee on Shock, and the Subcommittee on Trauma.

Dr. R. D. Dripps served as Chairman of a planning conference on 21 March 1955, as well as of the Symposium, and also as editor-in-chief of these Proceedings. Lt. Col. W. H. Crosby, Jr., and Drs. M. E. De Bakey, A. H. Hegnauer, F. J. Lewis, F. D. Moore, and W. H. Muller, Jr., presided at various sessions of the Symposium. In the Division of Medical Sciences, staff responsibility was borne chiefly by Lt. Cdr. C. D. West, who organized the Symposium under the guidance of Dr. M. H. Sloan and Dr. Thomas Bradley, and by Dr. D. E. Copeland, who assembled and prepared the manuscript for Dr. Dripps. Mr. Frank M. Holz was retained to complete the final editing of the Proceedings and prepare them for publication.

Some regrouping of the papers was found desirable. The discussions have also been rearranged and much condensed. A brief report by Capt. T. G. Barila concerning work then in progress was later withdrawn at his own request. Since the work of Drs. R. K. Andjus, J. E. Lovelock, and A. U. Smith was presented chiefly by means of a motion picture, a summary of the film and relevant addena were submitted after the Symposium. The five articles of Review and Appraisal were also prepared subsequent to the meeting.

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FOREWORD

PHILIP S. OWEN

In his introduction the Chairman, Dr. Dripps, points out how this conference arose from small beginnings. We thought originally that interest in this subject would be rather limited and specialized. It has proved otherwise, and we owe a measure of apology to many whom we had, of necessity, to turn away.

On behalf of the Division of Medical Sciences of the National Academy of Sciences—National Research Council I want to express our appreciation to Dr. Dripps and to the members of the several committees who worked long and hard in the preparation of this conference, and to the participants who bore with us in these preparations. They are too numerous to thank individually here. I want especially to thank our guests who came from abroad—from Britain, Sweden, France, Holland, and Yugoslavia. I hope they found their journey rewarding.

Finally, we are most grateful to the Armed Forces who together, not only through their interest in the practical application of cold to problems of medical practice but also through their concern with the fundamental underlying physiological mechanisms, have sponsored and made possible this meeting. The three services have contributed jointly to the support of the Symposium, but particular credit is to be given to the Army for giving initial impetus to the program and for its generous support of this publication.

INTRODUCTION

ROBERT D. DRIPPS

The interest of the Armed Forces in hypothermia was aroused by the possibility of using lowered body temperature to prevent the onset of shock, to treat shock once it had developed, and to permit surgical intervention on patients who might be unable to tolerate the stress of anesthesia and operations at normal body temperature. The initial interest in hypothermia was therefore primarily of a practical nature.

As clinicians began the practice of reducing the body temperature of patients, it became evident that much additional information was needed on the fundamental alterations in function which accompanied hypothermia. Until these physiologic factors were understood, the use of hypothermia would remain largely empirical. Furthermore, certain primary hazards associated with the lowering of body temperature were soon recognized. Rather than approach these in the clinic by trial and error, it seemed essential to enlist the aid of individuals with a basic orientation in various of the medical sciences.

This conference was consequently designed to bring together clinicians with a practical experience in the problems of induced human hypothermia and research workers with a broad background of related interests. It was our hope that the more important questions in hypothermia would be delineated, that profitable areas of investigation would be outlined, and that through the stimulus of the conference some would return to the laboratory for a fresh look at this intriguing subject.

The large number of formal presentations and the breadth of the subject limited the time available for free discussion, appraisal, and synthesis of the material. In consequence, several of the participants were asked to review the data presented and to offer for inclusion in these pages an evaluation of specific sections. It was hoped that the reviewers would call attention to conflicting data and opposing theories; that the sources of such differences would be pointed out in terms of such variables as differences in technique, experimental design, species studied, temperature range, and degree and type of anesthesia; and that directions for future work would be indicated. This has been attempted by J. W. Severinghaus and S. M. Horvath for the general physiological aspects of hypothermia; by C. McC. Brooks and B. F. Hoffman for cardiac irritability; and by R. D. Dripps for the clinical and technical phases of induced hypothermia in man. These will be found at the end of the particular sections concerned.

I should like to take this opportunity to acknowledge the tremendous amount of effort expended by Drs. Charles D. West and D. Eugene Copeland of the staff of the Division of Medical Sciences of the Academy-Research Council in the preparation of this conference. Their help has been invaluable.

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PART II—THE EFFECTS OF HYPOTHERMIA ON SPECIFIC SYSTEMS

PART I

SOME CONSIDERATIONS OF PHYSICOCHEMICAL FACTORS IN HYPOTHERMIA

DUGALD E. S. BROWN

A consideration of physicochemical factors in hypothermia is a complex assignment. The concept of homeostasis, so well developed by the late Prof. Cannon and stemming from Claude Bernard's vision of the milieu interior, is sufficient to give anyone a conservative view on the direct role of physical chemistry in hypothermia. I am honored at being given this opportunity to open problems for consideration, but I am approaching this assignment chiefly as a physiologist with a strong interest in physicochemical biology.

The regulation of intra-animal affairs and the maintenance of homeostasis, in the final analysis, rests at the cellular level. If the cardiac output and the chemical composition of the blood are sufficient to meet the cellular requirements, the regulatory mechanisms will remain effective and the survival of the animal will be assured. On the other hand, when the rate of oxygen utilization exceeds the rate of oxygen transport, cellular activities are reduced and regulation is impaired.

In induced hypothermia, the low temperature slows the rates of all processes and modifies the action of metabolites and other substances. This in itself is not necessarily harmful, as shown by the true hibernator, but will become disastrous as soon as anoxia and chemical imbalance begin to develop. The excellent experiments of Gollan *et al.* (1955) where dogs, provided with an adequate composition and circulation of blood by artificial means, survived cooling to 1.5° C., point directly to circulatory failure as the limiting condition in hypothermia with anoxia and chemical imbalance as primary agents modifying the activity of the regulatory cells.

The basic physicochemical considerations in hypothermia thus relate to the laws governing the dependence of cellular activities and their enzymatic reactions on temperature, ions, metabolites and drugs. Of particular importance are such cellular phenomena as excitability, rhythmicity and contractility. In regulating the oxygen transport these can act interdependently, since their specific rates are set at complementary levels. When the temperature is lowered, the rates are reduced in accordance with the temperature coefficients of the respective processes. In such an interdependent system its effectiveness at any temperature depends on the actual relative rates of the processes and on their temperature coefficients.

In both the true hibernator and the non-hibernator there is every reason to expect comparable values for the temperature coefficients of the cellular reactions. For several hundred processes, including rates of diffusion, cardiac rhythms, and numerous enzyme reactions, the Arrhenius U. values range from U. 3,000 to 25,000 with a large number of processes grouped at U. 6,000, 12,000 and 16,000 (Morales, 1947).

In terms of the temperature coefficient, or Q_{10}, these are grouped at Q_{10} 1.0, 2 and 3, thus indicating that the rates of the reactions involved would increase in this proportion for a rise in temperature of 10° C. or, in relation to hypothermia, a

decrease in the same proportion. In general, the rates of metabolic and rhythmical processes exhibit a Q_{10} of 3, the rates of contraction a Q_{10} of 2, and the rates of most physical processes such as diffusion, a Q_{10} of 1. As a result, when the temperature is lowered the rates of metabolic and rhythmical processes decrease two to three times as much as the rate of diffusion of the metabolites.

In both the hibernator and non-hibernator it would be expected that the temperature coefficients for identical processes would be the same. The factor contributory to the ready survival of the hibernator would be that the reaction rates of the various cellular processes have a better relative setting at 38° C. Thus on cooling, although the rates decrease, the relative values are sufficient for the over-all effectiveness of the system.

In the non-hibernator it appears that the rate setting of the processes is quite different. The end result is that, although the Q_{10}'s are the same, a lowering of the temperature reduces the rates of certain reactions to a level where they can no longer contribute effectively as members of an interdependent reaction system.

Considering that such a system is acting on events within cells, it could lead, for example, to cessation of the cardiac rhythm at 13° C. in the dog, whereas the beat of the hamster could continue at a much lower temperature. At the systemic level, acting between the heart and the nervous system, it could interfere with the nervous regulation of the heart.

In accordance with the foregoing, the control of induced hypothermia would rest on the extent to which the rates of cellular processes and their temperature coefficients could be controlled. The extent to which this could be accomplished naturally depends on a sound understanding of the cellular phenomena involved and the laws governing their susceptibility to temperature, ions and drugs.

The most significant physicochemical development bearing upon intracellular enzymes stems from the studies of F. H. Johnson and co-workers * on bacterial luminescence. In an extensive investigation of the luminescence reaction *in vivo,* its dependence on temperature, pressure and various chemical agents was established and the kinetics described in terms of the Glasstone-Eyring theory of absolute reaction rates. Recently the essential enzymatic proteins were isolated from the bacterium *Achromobacter fischeri* and light emission found to occur in the presence of FMN (flavin mononucleotide), reduced DPN (dihydrodiphosphopyridine nucleotide), and palmitic aldehyde. When this system was studied in relation to temperature, pressure and inhibitors, it was found to behave similarly to the system *in vivo* (Strehler and Johnson, 1954).

For many years it has been the hope of both physiologists and physical chemists that a specific cellular process, enzymatically controlled, could be duplicated by the isolated enzymatic proteins *in vitro*. It seems that this is being approximated in bacterial luminescence. Assurance is thus given that the physicochemical analyses of intracellular reactions can provide valuable information on the properties of the underlying enzymatic reactions which control the wide spectrum of cellular reactions. In relation to the regulation of hypothermia and its control, there is thus a good

* An extensive treatment of the physical chemistry of enzymatic and cellular processes in relation to temperature, pressure and chemical agents will be found in F. H. Johnson, Henry Eyring and M. J. Polissar: The Kinetic Basis of Molecular Biology, John Wiley and Sons, Inc., New York, 1954.

reason to consider the luminescence reaction as the prototype of many groups of intracellular processes, particularly in relation to temperature and chemical agents.

The temperature relations of the luminescence reaction are typical of numerous biological processes. With increasing temperature, the rate of the reaction increases in accordance with the Arrhenius equation, reaches a maximum, and then decreases (Brown, Johnson, Marsland, 1942). At low temperatures, the rate is determined by the luminescence reaction with U. 16,000, while at high temperatures the rapid decrease in rate is controlled by the reversible thermal denaturation (RTD) of the enzymatic proteins. The rate at intermediate temperatures then depends on the interplay between these opposing reactions. Concerning the RTD, a sufficient body of evidence has accumulated to consider that it depends on a reconfiguration of the protein enzymes, this being attended by a large increase in volume (ΔV 80–100 cc.) and a heat equilibrium (H 50,000–80,000 cal.).

The recognition that this typical temperature relation involves at least two quite distinct reactions has opened the way to an understanding of the action of various agents on cellular processes and enzymatic reactions *in vitro*. As a result of a most extensive study of the action of inhibitors, F. H. Johnson and co-workers concluded that in the simplest cases these agents fall into two classes, designated as Type I and Type II. Among the Type I compounds are agents such as sulphanilamide and certain anticholinesterases. These, it seems, combine with the prosthetic group of the enzymes and, since the degree of association is temperature-dependent, they become more effective at lower temperatures. In view of their mode of action, the Type I compounds tend to compete with the substrate and are thus influenced by variations in the effective substrate concentration.

The Type II compounds, which include a large number of narcotics, encourage the RTD and are thus greatly potentiated by a rise in temperature. Certain agents, such as quinine, exhibit both Type I and Type II effects, indicating in all probability that they are acting at more than one locus. The effectiveness of such agents is at a minimum at intermediate temperatures but increases when the temperature is raised or lowered.

During recent years, the conclusions drawn from studies on bacterial luminescence have been found to be applicable to many phenomena, such as growth, disinfection, cardiac rhythmicity, contraction, cell division, and amoeboid motion. It seems certain that knowledge brought to light in this long series of studies may have an important bearing on the role of chemical agents in induced hypothermia. To allay any doubts, the results of Overton on the anesthetization of tadpoles by ethyl alcohol may be mentioned. Here tadpoles, anesthetized at 20° C., tend to revive on being cooled. Since this is the typical action of a Type II compound, Johnson and Flagler (1951) argued that compression by reducing the volume of the protein enzymes should revive the animals and proceeded to perform experiments to test the matter. The results were as expected: on compression, anesthetized salamander larvae resumed swimming, and on subsequent decompression they became inactive.

The results of this simple but critical experiment give clear evidence that reactions, similar to the luminescence reaction in their basic physicochemical relations, are involved in the responses of a vertebrate to temperature and anesthetics.

Another sort of reaction, differing from the luminescence type only in that the

reversible thermal denaturation is absent, is typical of many cellular processes and enzymic reactions. In these the logarithm of the rate increases linearly with the reciprocal of the absolute temperature until irreversible thermal denaturation ensues more or less abruptly at a temperature near or above the upper physiological limit. Since the reversible thermal denaturation is absent, inhibitors (Type I) act primarily by combining with the prosthetic group, competing with the substrate at this site. As a result, their potency tends to increase progressively with a lowering of the temperature.

Serum cholinesterase exemplifies the above type of process. In the absence of an inhibitor the rate of the cholinesterase of dog or human blood serum varies with temperature in accordance with the Arrhenius relation, beginning to show some irreversible denaturation above 30° C. (Bach, *et al.*, 1951; Robert, *et al.*, 1951). When an inhibitor such as quinine is present, the inhibition at low temperatures is much greater and the temperature coefficient is increased accordingly. It is significant, however, that if a series of quinine derivatives is compared the degree of temperature sensitivity varies widely, the inhibition of certain members being independent of temperature over the physiological range (Lawler, Brown, unpublished). An anticholinesterase with the latter characteristics would be particularly useful in the regulation of induced hypothermia.

The foregoing physicochemical considerations are sufficient to illustrate the types of reactions underlying cellular processes and to show the manner in which they are influenced by temperature and chemical agents. In induced hypothermia, under conditions of a natural circulation, the chemicals subject to experimental control are drugs and such substances as might be used in an attempt to sustain the electrolyte balance. In relation to their use, the above discussion may serve to emphasize the fact that one group of substances (Type I) combines with the prosthetic group of the enzyme and is usually potentiated by a lowering in temperature, but it is possible to have inhibitors that act independently of temperature. The additional fact is that enzymes which exhibit a reversible thermal denaturation are also inhibited by substances such as ether and narcotics (Type II), the inhibition tending to decrease with a lowering of the temperature. This could be a disturbing factor in hypothermia but could be eliminated if anesthetics acting independently of temperature could be employed.

In turning to a consideration of cellular processes, such as excitability, rhythmicity, contractility and secretion which may be the target of metabolites and other agents in hypothermia, it may be stated that they reflect the behavior of enzymatic systems in their temperature dependence. This is shown in the case of the cardiac rhythm which Landau and Marsland (1952) have found to be controlled by a process showing a temperature and pressure dependence resembling in its main characteristics the luminescence reaction. The contractility of auricular strips of the turtle also shows a dependence on events which increase with temperature to an optimum and then decrease, the latter decrease, as in the case of luminescence, being reversed by high pressure.

The major problem with which we are faced in dealing with such complex cellular processes is insufficient knowledge on which to decide whether the potentiation of some processes results from the inhibition of a recovery process or an increased

activation. Or for that matter whether a reduction in activity with increasing temperature depends on the increase in rate of some interfering reaction or a reversible thermal inactivation of a controlling enzyme. In the former a Type I inhibitor could lead to an increase in activity while in the latter a Type II would increase the inhibition. Clearly the extent to which the physical chemistry of enzymes may be applied to cellular processes is limited by our knowledge of cellular physiology.

In hypothermia considered at the cellular level the impairment of any cellular activity arises when the energy essential for function is impaired either for the primary cause of anoxia or the secondary cause of an insufficient electrolyte balance across the cell membrane, both stemming from an inadequate composition of the extracellular fluid. In the light of the body of evidence on cellular function now available, it seems certain that the locus of action of the above agents is on the cell membrane. By acting there they tend to limit its capacity (a) to maintain the electrical potential, (b) to excite, (c) to induce activation, and (d) to determine the duration of the active state. Since the cardiac contractility is of such importance in hypothermia it is appropriate to consider its temperature dependence and the extent to which it depends on excitation and the activation cycle.

The significant fact concerning the effect of temperature on the isometric tension developed by an isolated strip of heart muscle is that all vertebrates exhibit a temperature optimum. Thus the frog, turtle and cat have optima at 0°, 10° and 22° C., respectively, the tension diminishing at lower or higher temperatures. In an unfatigued heart the tension developed at the optimum temperature is the maximum which can be developed at any temperature and pressure or in the presence of drugs such as β strophanthin. If the rate of stimulation is increased above a certain limit, the tension at temperatures below or at the optimum is reduced. But above the optimum temperature, where treppe exists, the maximum tension may be attained provided a suitable rate of stimulation is employed at each temperature (Hajdu and Szent Györgyi, 1952; Twente, 1955).

Perhaps the most important fact with respect to tension and temperature is that when the heart is treated with β strophanthin or if the Ca^{++} is sufficiently increased, the tension increases with temperature to the maximum level and maintains this value at increasingly higher temperatures over the physiological range and treppe is non-existent. In the turtle the maximum tension obtains from 9° to 34° C. When the heart is in this state it requires a much higher rate of stimulation before the tension is reduced. In the mammal the maximum tension is reached at about 22° C. and it would be expected that increased Ca^{++}, digitalis, or β strophanthin would cause this tension to be sustained up to 38° C., provided that the rate of beat were suboptimal.

The view has been held by some that a heart *in situ* under normal physiological conditions does not exhibit treppe. Although this may be so, it is certain that the "treppe state" is readily induced by rather minor changes in the composition of the extracellular fluid and it is quite probable that it would appear during progressive hypothermic failure. If this were the case, it would be a very unfavorable situation since the isometric tension becomes very dependent on heart rate. The fact that digitalis, alkaloids, cortisone and other agents tend to stabilize the tension with