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CARL VERNON MOORE

1908–1972

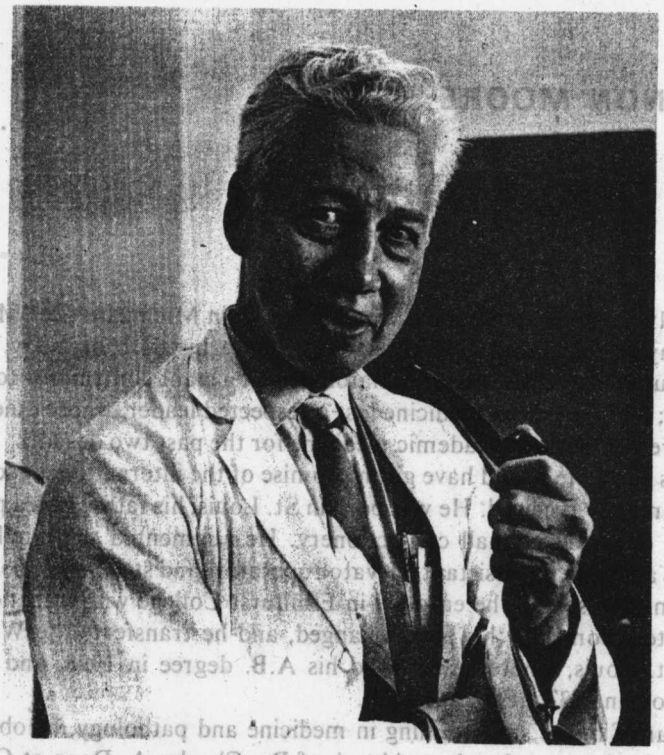
On August 13, 1972, while vacationing with his family in Michigan, Carl Moore died of a massive myocardial infarction eight days before his 64th birthday. With his death, many of us lost a warm friend; hematology lost a giant contributor to its era of greatest growth; and American medicine lost a respected leader, whose quiet counsel had helped weave the fabric of academic medicine for the past two decades.

Little of his early life would have given promise of the international acclaim that Carl Moore ultimately received. He was born in St. Louis; his father was a policeman and his mother operated a small confectionery. He augmented the family income with odd jobs as a pharmacy assistant, elevator operator, and steel mill laborer. After graduation from high school, he enrolled in Elmhurst College with intentions of becoming a minister. Somehow his plans changed, and he transferred to Washington University in St. Louis, where he received his A.B. degree in 1928, and his M.D. degree with honors in 1932, at the age of 23.

After a year of house staff training in medicine and pathology, he obtained his early research experience under the guidance of Dr. Charles A. Doan at Ohio State University. It was there that he first published studies on serum iron and iron absorption, launching a career that won him worldwide acclaim as an imaginative and thorough investigator. After two more years on the staff at Ohio State, Carl Moore was brought back to Washington University in 1938 by David Barr, then Professor of Medicine, who recognized the promise of this new breed of physician who could combine clinical medicine with biochemical physiology and apply them to the fledgling specialty of hematology.

By 1946 he had risen to full professor in medicine and had established a warm and effective working relationship with W. Barry Wood, who had succeeded Dr. Barr in 1942. Carl Moore, in turn, succeeded Dr. Wood as Chairman of Medicine at Washington University—a position he held until his death.

His early career as a productive investigator was most notable for his pioneering studies in iron metabolism and iron nutrition, in collaboration with Virginia Minnich and Reubenia Dubach, among others. However, in the more than 150 scientific articles that he authored or co-authored, he covered a broad cross section of



Carl Vernon Moore, 1908–1972

hematology, including a notable paper with Bill Harrington on the mechanism of immune thrombocytopenias. Few people realize how frighteningly close medicine came to losing both of these investigators in their zeal to nail down by self-experimentation the fact that thrombocytopenia could be transmitted by a plasma factor.

Inevitably, work in the laboratory gave way to the increasing responsibilities associated with a rapidly expanding Department of Medicine. Drawn by the excitement and enthusiasm that their chief instilled in his department, many young investigators were encouraged to build their programs with a spirit of inquiry and cooperation that Carl Moore assumed to be the natural state of things. He created a splendid environment. He led by outstanding example. His office was always open; colleagues, house officers, fellows, students, and even the dishwasher all had ready access to him, despite his overburdened schedule. He also exemplified his dictum that "if you work in a university your first commitment is to teach." He never failed to prepare carefully for even the most informal of rounds, seeking a bit of new factual information to impart, rather than following the easy path of authoritative, anecdotal medicine. His lectures and his writings were models of simplicity, order, and thoroughness. In this environment, a department of distinction was fashioned. From it emerged one university president, three vice-presidents, three deans, seven department chairmen, and 12 directors of hematology in American schools, plus many people of stature in foreign countries.

His wisdom and good judgment were utilized by his University in other roles, as Dean of the Medical School from 1954 to 1955, and in the crucial job of first Vice-Chancellor for Medical Affairs in 1964 and 1965.

The qualities that led to his development of a distinguished Department of Medicine were recognized by national societies, foundations, and governmental agencies, to whom Carl Moore gave unstintingly of his time. It is hard to gauge the full impact of his influence in the highest councils of medical science and education. He served as president of the Central Society for Clinical Research; president of the American Society of Clinical Investigation; president of the Association of American Physicians, president of both the American and International Societies of Hematology, member of the august National Academy of Sciences and National Academy of Medicine, and fellow of the American Academy of Arts and Sciences.

Honors included the Joseph Goldberger Award in Clinical Nutrition; the Alumni Award, Washington University; Modern Medicine Award for Distinguished Achievement; the John Phillips Memorial Award of the American College of Physicians; the Abraham Flexner Award of the Association of American Medical Colleges, plus numerous distinguished name lectureships in this country and around the world.

Modestly detached from this imposing, and grossly incomplete, listing of honors and achievements was a man noted for his warmth, simplicity, humility, integrity, and compassion. He looked on medicine and his daily work in the medical center as a privilege and a joy. He was always willing to drive himself a little harder to achieve the goals he identified as being worthwhile. He had a disarming simplicity and directness to his speech, with a knack for saying the proper thing; his writing reflected this simplicity, with careful elimination of all that was wordy, flowery, or trite. He was profoundly thoughtful and considerate. Many people, awed by his position, his international reputation, and his leonine visage, were not privileged to see his pervading warmth, sensitivity, and attention to small details that helped brighten the lives of the friends he made wherever he went.

Perhaps his philosophy can best be summed up by a brief statement he made to students and parents at a senior awards ceremony in 1966. "It seems to me that when it comes time for a man to hang up his hat and quit, what will seem of greatest importance will not be the stature he has achieved in society or the money he has in the bank—but the contributions he has been able to make to other people. Those of us in medicine enjoy the great good fortune of being surrounded by opportunities to be helpful as physicians, or as teachers, or as investigators. Few other people are so fortunate."

To the memory of Carl Moore, mentor, colleague, and friend, this volume of Progress in Hematology is respectfully dedicated.

Elmer B. Brown

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Introduction

As in the past, the purpose of this volume of PROGRESS IN HEMATOLOGY is to provide timely, authoritative reviews in varied disciplines of interest to the hematologist. With the rapid proliferation of information, most of us are increasingly aware of the difficulties in sorting fact from artifact in our own narrow fields of endeavor, and are appalled at the task in more remote areas of our interest. To help solve this problem, authorities have been invited to share their knowledge and critical appraisal of developing information in their fields of competence. No attempt has been made to cover all advances in hematology in a systematic fashion. Instead, the editor has tried to select topics that have not been reviewed extensively or recently elsewhere, and has relied heavily on the judgment of the reviewers about topics that are ripe and appropriate for review. In some cases, such as the home management and prophylaxis of hemophilia, human marrow transplantation, and the use of cyanate in sickle cell disease, the clinical application of these procedures is preliminary and may not be able to be completely evaluated for several years. However, these reviews serve to alert people to exciting developments that are on the threshold of true progress.

With the death of Carl Moore at the time when initial plans for this volume were being formulated, the great weight of editorial responsibility has fallen on me to continue the work we have shared through four previous volumes. It is a pleasure to acknowledge the splendid cooperation of all the contributors who have done so much to lighten the load.

Elmer B. Brown

Contents

Eugene P. Orringer, M.D., and
John C. Parker, M.D.

Ion and Water Movements in Red Blood Cells

This review intends first to call attention to recent progress in several areas of red blood cell (RBC) transport physiology. Secondly, it presents a practical classification of some clinical syndromes in which cations move abnormally across the RBC membrane.

REVIEW OF SELECTED TOPICS

Control of Cell Water Content [19, 82, 172]

The magnitude of the forces tending to draw water into the RBC can be demonstrated by considering an imaginary cell which retains within its cytoplasm hemoglobin, organic phosphates, glutathione, and enzymes—all at the concentrations existing in normal human RBC. The membrane of this hypothetical cell is freely permeable to Na, K, small anions, and water. The cell is allowed to come into equilibrium with a large volume of plasma, with the restriction that its volume, and therefore the concentration of its retained contents, is constant. Table 1 shows that at ionic equilibrium the cell would have a hypertonic interior. A hydrostatic pressure of about one atmosphere would have to be exerted across the cell to keep water from entering. The magnitude of this pressure is directly related to the concentration and charge of the impermeant cell contents. In normal human RBC, however, the cytoplasm is isosmotic with plasma [196]; there is no hydrostatic pressure gradient between the plasma and the cell interior [136]. The principal cations in the cell-plasma system, Na and K, are far from equilibrium (Table 1). The features of the human RBC which differentiate it from the hypothetical cell are the following: First, its membrane is sparingly permeable to cations; when its leakiness to cations is experimentally increased, ions flow to equilibrium, and the cell takes on water [16, 126, 150]. Second, the cell pumps cations "uphill," resisting their movements toward equi-

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Table 1
Donnan Equilibrium versus Osmotic Equilibrium

	Plasma	Hypothetical Cell	Human RBC
Concentration of permeant ions (mEq/l water)			
Na^+	150	177	15
K^+	5	6	140
Anions	155	131	108
Magnesium ion (mEq/l water)	—	—	5
Osmolarity (mOsm/Kg water)			
Permeant ions	285	289	242
Other solutes	5	45	48
Total solutes	290	334	290

Solute distribution between an idealized plasma and a hypothetical cell at Donnan equilibrium. All permeant ions are regarded as monovalent. The contents of a human RBC [42] are included in the right hand column for comparison. Like the human RBC, the hypothetical cell is regarded as containing 52 impermeant anion equivalents per liter cell water [42]. Its other constituents were calculated by simple Donnan theory [19, 53, 182] in which (a) electroneutrality obtains, (b) the cell/plasma ratio of each species of permeant anion is equal to the plasma/cell ratio of the various permeant cations, (c) the hematocrit is very low, and (d) the osmotic coefficient of the permeant ions is 0.92 [42].

librium. In some species when the pump is blocked, as with digitalis, the cell swells [176]. The constancy of the cell volume depends on the interaction of pump and leak in sustaining the Na-K gradients. In at least two types of RBC the ion and water content can be completely defined by numerical values for Na-K pumps and leaks [176].

The driving forces which tend to move ions to equilibrium in the cell-plasma system are not completely described by expressions of concentration or chemical activity; the electrical potential gradient across the membrane must also be considered. The unequal distribution of ions between cytoplasm and plasma gives rise to a voltage whose magnitude is determined by the relative permeability of the membrane to the various ion species. This voltage is stated mathematically by the Goldman or constant field equation [53, 57].

$$E = RT/F \ln \frac{P_K(K_0) + P_{Na}(Na_0) + P_{Cl}(Cl_i)}{P_K(K_i) + P_{Na}(Na_i) + P_{Cl}(Cl_0)}, \qquad \text{(Equation 1)}$$

where E is the transmembrane potential, R the gas constant, T the absolute temperature, F the Faraday, P_X the permeability of the membrane to ion X, (X_0) and (X_i) the concentrations (more correctly, the activities) of ion X in plasma and cell water, respectively.

In human RBC the values of P_{Na} and P_K are so small in relation to P_{Cl} that the cation terms may be neglected, and the equation reduces to

$$E = RT/F \ln \frac{(Cl_i)}{(Cl_0)}. \qquad \text{(Equation 2)}$$

This means that the ratio of cell to plasma chloride can be used to compute the voltage across the membrane. In the few instances in which electrodes have been placed in RBC, the cytoplasm has had a potential of about −7 mV relative to the plasma, agreeing well with the prediction from the chloride gradient [77, 92]. A major determinant of cell chloride concentration, and hence of this voltage, is the concentration and charge of impermeant intracellular solutes such as hemoglobin and organic phosphates [27, 54]. In a later section we consider some circumstances in which the cell's permeability to K is so large that the reduction of Equation 1 to Equation 2 is not valid.

There have been several recent contributions to this general theory of cell volume control. The first of these have to do with ion and water equilibria.

In a qualitative sense RBC's behave as osmometers. They shrink in hypertonic salt solutions and swell in dilute ones [149]. Quantitative studies indicate that they are not perfect osmometers, and their deviation from ideal behavior has suggested to some that part of the water in the cell is bound or compartmentalized, so that it does not move freely in response to changes in the medium solute concentration. Gary-Bobo and Solomon have presented evidence to explain the apparently anomalous behavior of RBC on the basis that the state of ionization of hemoglobin molecules is greatly influenced by their proximity to one another within the cell [42, 167]. In an osmotically shrunken cell the hemoglobin is less negatively charged that it would be in a normal cell at isotonicity; in a swollen cell the hemoglobin is more anionic than normal. As the cell changes volume, rapidly diffusible anions move to preserve electroneutrality in the cytoplasm. The effect of these anion movements on water distribution explains the observations which were used to argue that the RBC is not a perfect osmometer. This property of hemoglobin makes it unnecessary to postulate an osmotically unavailable water compartment in the cell. Furthermore, it presents a mechanism by which osmotic signals can be converted into electrical signals.

Another variable which influences the cell's content of impermeant solute is its metabolic state. Glycolytic intermediates are, for the most part, negatively charged organic phosphate compounds that do not penetrate the cell membrane. The most abundant organic phosphates in human RBC are adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG). In two recent studies [21, 119] emphasis has been placed on the importance of organic phosphate compounds as determinants of cell water content. The water which accumulates in energy-depleted cells can be driven out by metabolic resuscitation, even when the cation pump is blocked [119]. Experimental loading of cells with ATP or 2,3-DPG has important consequences for cell ion and water content: coincident with a rise in organic phosphates, the cell shrinks as highly diffusible monovalent anions leave to make room for polyvalent solutes which have much less osmotic activity per charge. The voltage within the cell becomes more and more negative and this change increases the driving force for entry of cations, which because of their slow permeance accumulate gradually, bringing water back into the cell. These ion and water shifts are all predictable consequences of the metabolically-induced alteration in cell solutes [72]. They underscore the influence of impermeant cell ions on volume homeostasis.

There is an increasing body of evidence that control of water content in some

RBC is quite independent of the classical Na-K pump. Kregenow [85, 86, 86a] has demonstrated that duck RBC can correct osmotically induced changes in their volume by adjusting their ion content, even though the pump is effectively blocked by ouabain. A similar effect is observed in human RBC, although participation of the pump in the volume response has not been excluded [132, 133]. Dog RBC are of particular interest in this regard because they have a high intracellular Na content and by all criteria lack an ouabain-sensitive pump. Changes in water content have a pronounced and specific effect on Na and K movements in dog red cells [59, 124]. Shrunken cells are highly permeable to Na and tight to K, while in swollen cells just the reverse pattern is seen. This property does not explain how they protect themselves from osmotic destruction, however. In a recent study dog RBC were labeled with ^{51}Cr and incubated in a hypertonic salt solution to raise their concentration of NaCl and water [120, 121]. The labeled, swollen cells could be distinguished from untreated cells by density gradient separation. Upon reinjection into the donor dog, the swollen cells slowly returned to normal density, thus demonstrating their capacity for volume adjustment. In vitro studies have suggested that dog RBC possess a means of extruding NaCl which is insensitive to ouabain and requires extracellular calcium plus an energy source such as glucose [122, 122a]. Work in isolated renal tubules [143] and amphibian bladders [74] points to a similar type of volume-regulatory mechanism which operates independently of the ouabain-sensitive pump.

Models of the Pump

There have been several recent reviews dealing in detail with the digitalis-sensitive, ATP-driven, Na-K exchange pump and its metabolic counterpart, Na,K-ATPase [1, 22, 43, 62, 127, 163, 188]. A model which summarizes some of the data is shown in Figure 1. Three Na ions are carried out of the cell as the terminal phosphate of one ATP is transferred to a carboxyl group in a membrane protein [80]. Hydrolysis of the phosphorylated intermediate (a process inhibited by digitalis) is associated with the inward transport of two K ions. Normally there is thought to be a

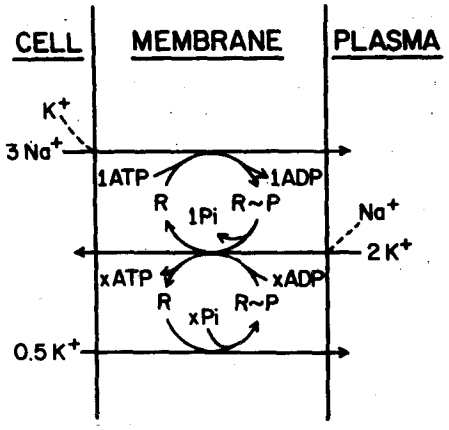

Fig. 1. Model of the Pump. R represents a lipoprotein in the membrane which becomes phosphorylated (R ≈ P) and dephosphorylated as Na and K ions are carried across the membrane.