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BIOLOGICAL TRANSPORT of RADIOTRACERS

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Biological Transport of Radiotracers

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CRC SERIES IN RADIOTRACERS IN BIOLOGY AND MEDICINE

FOREWORD

This series of books on Radiotracers in Biology and Medicine is on the one hand an unbelievably expansive enterprise and on the other hand, a most noble one as well. Tools to probe biology have developed at an accelerating rate. Hevesy pioneered the application of radioisotopes to the study of chemical processes, and since that time, radioisotopic methodology has probably contributed as much as any other methodology to the analysis of the fine structure of biologic systems. Radioisotopic methodologies represent powerful tools for the determination of virtually any process of biologic interest. It should not be surprising, therefore, that any effort to encompass all aspects of radiotracer methodology is both desirable in the extreme and doomed to at least some degree of inherent failure. The current series is assuredly a success relative to the breadth of topics which range from in depth treatise of fundamental science or abstract concepts to detailed and specific applications, such as those in medicine or even to the extreme of the methodology for sacrifice of animals as part of a radiotracer distribution study. The list of contributors is as impressive as is the task, so that one can be optimistic that the endeavor is likely to be as successful as efforts of this type can be expected to be. The prospects are further enhanced by the unbounded energy of the coordinating editor. The profligate expansion of application of radioisotopic methods relate to their inherent and exquisite sensitivity, ease of quantitation, specificity, and comparative simplicity, especially with modern instrumentation and reagents, both of which are now readily and universally available. It is now possible to make biological measurements which were otherwise difficult or impossible. These measurements allow us to begin to understand processes in depth in their unaltered state so that radioisotope methodology has proved to be a powerful probe for insight into the function and perturbations of the fine structure of biologic systems. Radioisotopic methodology has provided virtually all of the information now known about the physiology and pathophysiology of several organ systems and has been used abundantly for the development of information on every organ system and kinetic pathway in the plant and animal kingdoms. We all instinctively turn to the thyroid gland and its homeostatic interrelationships as an example, and an early one at that, of the use of radioactive tracers to elaborate normal and abnormal physiology and biochemistry, but this is but one of many suitable examples. Nor is the thyroid unique in the appreciation that a very major and important residua of diagnostic and therapeutic methods of clinical importance result from an even larger number of procedures used earlier for investigative purposes and, in some instances, procedures used earlier for investigative purposes and, in some instances, advocated for clinical use. The very ease and power of radioisotopic methodology tempts one to use these techniques without sufficient knowledge, preparation or care and with the potential for resulting disastrous misinformation. There are notable research and clinical illustrations of this problem, which serve to emphasize the importance of texts such as these to which one can turn for guidance in the proper use of these powerful methods. Radioisotopic methodology has already demonstrated its potential for opening new vistas in science and medicine. This series of texts, extensive though they be, yet must be incomplete in some respects. Multiple authorship always entails the danger of nonuniformity of quality, but the quality of authorship herein assembled makes this likely to be minimal. In any event, this series undoubtedly will serve an important role in the continued application of radioisotopic methodology to the exciting and unending, yet answerable, questions in science and medicine!

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PREFACE

It has been said that life processes depend to some degree on biological transport. In effect, life itself could not exist without effective transport mechanisms, even in unicellular species, since nutrients must be transported into the cells and waste products, many of which are toxic, transported from them.

It was during the last few decades that this subject caught the attention of scientists trying to solve certain problems involved with life processes and disease. The need to understand how substances are brought together in the innermost part of cells, producing reactions that are in many cases completely incompatible with one another, and, at the same time, taking materials from the fluids surrounding the cells, secreting the products manufactured by cells, etc., created the need to develop new approaches to study these processes.

For our purpose we do not limit the problems of transport to determining the specific mode by which a solution or substrate passes from one side of the cell membrane to the other. It is not that we are trying to disregard the enormous role of transport in cellular function, but we recognize in addition that before a cell can incorporate a substance, this substance must be transported to the cell. This is quite a difficult problem in the human since body fluids are not only very complex in composition, but they may carry extraneous materials, such as drugs, which may influence chemically the substances introduced into the fluids through i.v. administration, intestinal absorption, etc.

This book attempts to explain many of these transport processes for radiolabeled tracers. We believe it is a timely topic since, in our need to know more about the behavior of radiotracers in the human body, transport is of prime importance in studying the disposition of these materials. It is our sincere hope that this book will stimulate scientists to study transport mechanisms of radiotracers as a means of creating more specific radiotracers which may be used in clinical diagnosis and therapy.

I acknowledge most gratefully the contributions of the authors and all those who inspired and encouraged the preparation of this book.

Lelio G. Colombetti

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Dr. Colombetti graduated from the Litoral University in his native Argentina with a Doctor in Sciences degree (summa cum laude), and obtained two fellowships for postgraduate studies from the Georgetown University in Washington, D.C., and from the M.I.T. in Cambridge, Mass. He has published more than 150 scientific papers and is the author of several book chapters. He has presented over 300 lectures both at meetings held in the U.S. and abroad. He organized the First International Symposium on Radiopharmacology, held in Innsbruck, Austria, in May 1978. He also organized the Second International Symposium on Radiopharmacology which took place in Chicago in September, 1981, with the active participation of more than 500 scientists, representing over 30 countries. He is a founding member of the International Association of Radiopharmacology, a nonprofit organization, which congregates scientists from many disciplines interested in the biological applications of radiotracers. He was its first President (1979/1981).

Dr. Colombetti is a member of various scientific societies, including the Society of Nuclear Medicine (U.S.) and the Gesellschaft für Nuklearmedizin (Europe), and is an honorary member of the Mexican Society of Nuclear Medicine. He is also a member of the Society of Experimental Medicine and Biology, the Coblenz Society, and the Sigma Xi. He is a member of the editorial boards of the journals *Nuklearmedizin* and *Research in Clinic and Laboratory.*

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TABLE OF CONTENTS

Chapter 1

BIOLOGICAL TRANSPORT: AN HISTORICAL VIEW

Lelio G. Colombetti

TABLE OF CONTENTS

I. INTRODUCTION

The Isua rocks of Greenland, which have been radiometrically dated as 3,800 million years old, contain carbon compounds in which the isotope ratios are similar to those resulting from the activity of living organisms. This finding is not, of course, an assurance that life existed that long ago, and the inference drawn by some investigators, that those rocks components are of biogenic origin has been challenged by others. There is no dispute, however, about the authenticity of fossilized cellular organisms present in Australian stromatolites 3,400 million years old. The species in the stromatolites appear to have mastered the metabolic trick of photosynthesis; by that time, then, life forms had become so complex that they depended absolutely upon transport, perhaps mechanistically simple, but efficient enough to enable the cells to capture the nutrients necessary to keep them alive. It seems quite safe to say that transport is by no means a new phenomenon.

By comparison, the scientific study of biological transport is of recent origin, and it is interesting to note that the first approaches to this discipline have been directed at the properties and functions of the cell membrane, not at how materials are transported to the boundary of the cell. We consider that it is important to know not only how a substrate is transported into the cell and how the products of metabolism are transported from the cell, but also how the substrates reach the immediate neighborhood of the cell. All transport functions in unicellular organisms depend on the cellular membrane, but for highly developed organisms the problem is much more complicated, since the substances to be transported to the interior of the cell have to be transported first to the cell in the biological fluids. It is very important, therefore, to examine the ways in which the transport mechanisms are influenced by variations in the composition of these fluids. In the case of man, the body fluids are highly complex. The principal fluid, the plasma, which is the liquid portion of circulating blood, contains several hundred different components,[1,2] the most important groups being (1) the proteins, including albumin, fibrinogen, and several globulins (α_1, α_2, β_1, β_2 γ); (2) nutrients, such as carbohydrates, amino acids, lipids, etc.; (3) inorganic salts, including chlorides, carbonates, sulfates, phosphates, etc., of sodium, potassium, calcium, magnesium, etc.; (4) gases, such as oxygen, nitrogen, carbon dioxide, etc.; (5) metabolic waste products; and (6) special substances such as hormones, enzymes, etc. It is not surprising, therefore, that this complex system may influence the transport of a tracer used to study the functions of the body.

In reviewing the development of the study of transport, it is convenient to divide the subject into two parts: transport into the cell and transport in body fluids.

II. TRANSPORT INTO CELLS

The study of mechanisms by which materials are transported into cells began with observations of the permeability of plant cell membranes in the mid-19th century by Naegely and Cramer.[3] These investigators found that the surfaces of plant cells were permeable to the pigments from cherry juice. In 1895, Overton demonstrated the selective permeability of plant cells to organic solutes,[4] later extending his original studies of plants to the permeability of muscle cells in animals.[5] About the same time, Nagano reported that pentoses are absorbed from the gut more slowly than hexoses, an unexpected phenomenon considering the molecular size of these compounds, suggesting that the difference in the speed of absorption was due to selective transport mechanisms.[6]

The classical work of Van Slyke and Meyer, done in 1913, showed that after an infusion of hydrolyzed casein into the bloodstream, the amino nitrogen level of several

tissues became greater than that in the blood; in other words, the amino acids entered those tissues against the concentration gradient, indicating an active transport mechanism.[7] In 1916, Bang reported similar results upon infusing glycine or alanine solution in the dog, though infusion of lysine did not produce the effect, demonstrating that certain amino acids are selectively transported.[8] At the same time, Constantino demonstrated that red cells have a higher concentration of amino nitrogen than the serum, also indicating the existence of an active transport mechanism.[9] This phenomenon was later confirmed by other investigators, including Hamilton and Van Slyke,[10] Johnson and Bergeim,[11] Stein and Moore,[12] and Abderhanden and Kürten,[13] who suggested that the accumulation of amino acids in the red cells represented the binding of these amino acids to other cellular components. The accumulation and the state of free or bound amino acids in the red cells were debated for many years until Christensen showed that lysed erythrocytes of the duck did not bind alanine or glycine, even though these amino acids were concentrated to levels higher than those found in the cellular environment.[14] This conclusion was later revised by Christensen because, as he explained, the number of binding sites required to explain the high levels of amino acids found in the erythrocytes was in excess of any reasonable value.[15] Gorter and Grendel compared the surface of erythrocytes with a monomolecular film obtained by spreading the extracted total cellular lipids, creating the concept of a "cellular enclosing layer".[16] This idea was later modified into that of a cell membrane consisting of a double layer of lipid molecules with hydrophobic endings adhering back to back and hydrophilic endings facing the intra- and extracellular environments. This model of biological membranes later received strong support from electron microscopy.

The simplest transport process is diffusion through the cell membrane. Certain compounds and ions can engage in this process; special consideration should be given to solutes having a lipophilic nature. As shown by Collander, the lipophilic nature of some small organic molecules facilitates their entry into plant cells, with a rate of entry proportional to the partition coefficient between oil and water.[17]

In a second process, the entry of certain solutes into the cell has been found to be facilitated by a mediator, even though the solute does not become more concentrated inside the cell than outside. In the simple diffusion case, the rate of entry depends directly upon the concentration of solute outside the cells, while in the facilitated transport, a large concentration of solute could saturate the transport mediator and cause the proportionality to be broken. Some evidence of the facilitated transport mechanism was obtained by Ege and Cori.[18,19]

The intestinal absorption of some amino acids administered at the same time, such as alanine and glycine, is much lower than expected, as was demonstrated by Cori.[19] This fact could result from competition for absorption by a common transport mechanism, unless during these experiments the transport mediator was saturated, as was shown to occur during glucose resorption by the renal tubule in the dog.[20]

There is yet another mechanism of transport of solutes into cells, in which the solute reaches a larger concentration on one side of the cell membrane than the other. Since this higher concentration requires the expenditure of energy by the cells, it was called "active transport". The needed energy may derive from direct hydrolysis of a high energy compound, such as adenosine triphosphate (ATP), or from the coupled movement of another solute that has previously been concentrated against its electrochemical gradient. In the latter case, the second solute may be a metal ion, most frequently an alkali metal ion, as has been proved by Riggs et al.[21] for the uptake of sugars.

Several investigators [23-27] have reported that certain neutral depsipeptides (such as valinomycin), macrolide actins (such as nonactin), or polypeptides (such as gramicidin) induce the transport of alkali metal cations into the mitochondrial matrix against a concentration gradient at the expense of energy generated by electron transport or the

hydrolysis of ATP. Coincident with this uptake there is an ejection of protons and a swelling of the particles due to a simultaneous uptake of water. On the other hand, the addition of some monocarboxylic polyethers (such as monensin, nigericin, or synthetic polyethers) induces permeability of cations, which then diffuse passively along the concentration gradient from the matrix to the outside. Coincident with this diffusion of alkali cations outward, there is a migration of protons inward and an extrusion of water.

Mechanisms for active transport of cations have been reviewed by Eiseman[28,29] and Pressman.[24,25] These investigators proposed four mechanisms by which such a selective permeation of cations may take place

1. Coordination of a negatively charged ligand with the cation to form an ion pair that can cross the membrane
2. Prior penetration of the ligand into the membrane, in this way permitting the cation to penetrate by ion exchange through fixed, negatively charged pores or channels
3. Formation of a positively charged complex between a neutral, polar carrier that can surround the cation
4. If the carrier molecule, as in (3), becomes fixed to the membrane by a mechanism similar to (2), one can visualize the permeation through a fixed, neutral polar pore.

Except for the expenditure of energy in the "active transport" mechanism, it can be accepted that "facilitated diffusion" and "active transport" are nearly the same. Osterhout,[30] and later Widdas and Rosenberg,[31,32] introduced the idea that a "mobile carrier" shuttles back and forth through the cell membrane, binding a solute molecule at the cell exterior and carrying it through the membrane to the interior of the cell, where the solute is released. Ussing proved experimentally the motility of the carrier as well as the phenomenon called "exchange diffusion".[33] During "exchange diffusion" the carrier works in both directions, translocating a solute molecule to the cell interior, where the solute is released and another molecule is bound. The carrier then diffuses back to the cell exterior carrying the second solute, which is then released. The process can now start again, since the carrier is not wasted, and will have the same chemical characteristics, at the end of the process. Different models have been proposed to explain how the mechanism works. Danielli proposed that the process was due to either the concentration of a protein or the rotation of a macromolecule,[34,35] while Patlak suggested that the translocation must be explained by a swinging effect of a gate, in which the external molecule causes the gate to swing inward, while another solute molecule will cause the opposite effect.[36]

The constancy of internal cellular composition, despite marked fluctuations in the concentration of solutes in the surrounding medium, is evidence that selective and active processes maintain the internal balance of cell composition. The observation that cells can regulate their internal solute concentrations was made in 1948 by Liebig. He noticed that muscle tissue contains a much higher concentration of potassium and much lower concentration of sodium than does the blood; this indicated that a selective transport mechanism for these two ions exists.

Mathematical techniques were first applied to the study of biological transport by Wilbrandt and Rosenberg[37] and by Widdas[38,39] in the early 1950s, and soon they became major contributions to the further description of the transport processes.[40]

The specificity and discrimination of transport system were recognized slowly. In the late 20s, Wilson reported that there was no difference in the rates of absorption of L-alanine and DL-alanine by the small intestine,[41] while Chase obtained similar re-

sults with D- and L-leucine, isoleucine, and valine.[42] Years later, Schofield reported that DL-serine was absorbed much faster that DL-isoserine, suggesting that the amino acid group must be on the α-carbon for an optimal rate of absorption, and that the small intestine maintains a degree of specificity for the transport of neutral amino acids.[43]

A general mechanism for amino acid transport into cells was proposed by Meister and co-workers.[44] Absorption of amino acids occurs chiefly in the small intestine and is an energy-requiring process. Several transport systems, with a high degree of specificity, function in the absorption of amino acids. The L-isomers are more rapidly absorbed than are the experimentally administered D-isomers. In general, the neutral and the more hydrophilic amino acids are more rapidly absorbed than are the basic and the more hydrophobic amino acids. Thus the absorption of leucine present in relatively high concentrations diminishes the absorption of isoleucine and valine, contradicting the results obtained by Chase in the middle 1940s. The mechanism for amino acid transport is quite complex, involving the action of few enzymes.[45]

The application of chemical and biological techniques to the study of transport permitted the identification of various membrane-bound proteins having the necessary characteristics to act as transport mediators. One of the biological techniques applied to these studies is the inhibition of B-galactoside transport in *Escherichia coli* by the sulfydryl reagent *N*-ethylmaleimide. By using a culture of this bacterium, which can duplicate in number every 20 min in a simple medium containing glucose and inorganic salts, these studies can be easily made. It was also found that in these cases, the transport substrate protected the cells from inactivation by the reagent.[46]

Another technique utilized to make these studies is to provoke the release of small groups of proteins from the bacteria, using a "cold osmotic shock technique" developed by Neu and Heppel in the middle 60s.[47,48] The microbial cells, suspended in a concentrated solution of glucose, are washed and rapidly resuspended in distilled water containing a magnesium salt. The cells loose proteins, some of which are involved in transport processes, while the cells remain viable. The treatment of *Escherichia coli* resulted in a loss of transport activity for some amino acids, including leucine and isoleucine.[49]

III. TRANSPORT IN BODY FLUIDS

The classical experiment in which Georg Hevesy, in the early 1920s, demonstrated the transport of radioactive lead in bean seedlings, was probably the first time in which a radioactive tracer was used to demonstrate transport in biology.[50] Similar experiments were later repeated by Hevesy in animals. In the mid-1920s, Yens, a physicist working in New York, used radiotracers to determine the blood circulation in humans, and to the best of our knowledge this is the first time in which a radiotracer was used to study transport in humans.[51]

The study of transport in body fluids using nonradioactive tracers is older. In effect, transport in body fluids was studied by the end of the last century by Steward, who used indicator dilution techniques to measure transit times and flow through the vascular system.[52-55] Chinard et al. established, in the middle 1950s, the multiple-tracer indicator-dilution techniques to study water and solute exchange in organs.[56-58]

The transport of radiotracers in body fluids may be affected by many factors, among them the route of administration, carrier concentrations, stimulation and depression of the transport system by disease or any other metabolic change, such as fasting, medication, etc. Most radiotracers are administered intravenously; therefore, the first consideration of transport is the binding of the radiotracer to blood components. Blood, like most of biological entities, is very complex in nature, having a large number

of different types of components; some of these components are needed to maintain life, others are products of metabolism, waste materials in the process of being discarded. The concentration of these waste products in the blood may vary during disease states, together with the medication used to fight disease. All these components, the naturally occurring and those administered to a patient, may influence the transport of the radiotracers by modifying the radiotracer or by producing unexpected bindings.

Thyroid hormones enter the bloodstream and bind to thyroid-binding globulins (TBG), thyroid-binding prealbumin (TBPA), and thyroid-binding albumin (TBA).[59-60] The binding and debinding of thyroid hormones in plasma was studied by Hillier, who found that debinding is fast relative to the capillary transient time in the brain, as well as in the liver.[61-62] Pardridge found that there is a difference in transport depending on the type of binding: albumin-bound T_3 or T_4 is transported into the brain or the liver, while TBG-bound T_3 is transported only into the liver, and TBPA-bound T_4 is not transported to the liver.[63].

Metallic ions were introduced in nuclear diagnostic studies many years ago. In effect, the first studies in transport were done using radioisotopes of lead,[50] but these ions were not used widely until a few years ago. These ions do not remain free once they are introduced into the bloodstream, but bind immediately to plasma proteins. It was found that once injected, ^{67}Ga-citrate is bound to proteins, particularly transferrin, which not only plays an important role as a carrier for gallium in the plasma, but also appears to be essential for the incorporation of gallium into tumor cells.[64-68] Besides transferrin, gallium ions bind to at least three other iron-binding molecules: lactoferrin, ferritin, and siderophores. The relative affinity of gallium to ferritin is not known, but the relative affinity to lactoferrin was found to be higher than to transferrin.[68] Ferric ions easily displace gallium from its transferrin binding, and to a lesser extent from lactoferrin.[69] If large quantities of iron are administered before or coincident with ^{67}Ga-citrate, tumor localization of ^{67}Ga is inhibited, proving that transferrin plays an important role in the transport of gallium to the tumor cell.[70]

Radioindium ion was found to bind to transferrin when administered, becoming a blood pool label,[71] but it does not enter into a metabolic pathway as iron-labeled transferrin does. Indium ions have been used to label a series of compounds for medical applications; for example, ^{111}In was introduced as a label for bleomycin in nuclear diagnostic studies. After the labeled compound is introduced into the bloodstream, however, a competition occurs between the labeled bleomycin and free ions present in the plasma. Copper ions, for example, not bound to ceruloplasmin, remove indium from bleomycin, and indium ions becomes attached to beta-globulins.[72]

Pertechnetate is loosely bound to plasma proteins. This binding is affected by temperature and pH, and Hays et al. demonstrated that the binding may be reduced in disease states.[73] Since binding to proteins is weak, pertechnetate easily leaves the plasma compartment to cross the endothelium.

Radioiron ions are bound to transferrin, and may be transferred to intracellular ferritin via a collision mechanism occurring in the cell membrane.[74] After transferrin enters the cell and releases the iron ion, it returns across the cell membrane to the plasma and is ready to repeat the operation.[75]

Copper ions were found to label both ceruloplasmin and albumin,[76] preferentially ceruloplasmin, which is the specific binding material for copper ions. About 50% of the calcium ions circulate in the unbound state, while the other half are bound to proteins.[77]

Free fatty acids, when injected, are tightly bound to albumin, being distributed over multiple binding sites.[78] These sites of varying affinity probably favor the transport of fatty acids to different types of tissues. Cholesterol is bound by plasma lipoproteins.[79]