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The Physiologist

A Publication for Physiologists and Physiology
Orr E. Reynolds, Editor

Volume 23, No. 1, February 1980

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AMENDMENTS TO THE APS BYLAWS

The following amendments to the Bylaws will be offered for vote at the Society Business Meeting, Tuesday, April 16, 1980. A two-thirds majority vote of the members present is required to amend the Bylaws.

Sectionalization of the Society

For several years, Council has been active in promulgating measures that will allow subject-area groups within the Society to exercise more control of Soceity affairs within their own sphere of interest.

One such move was the sectionalization of the *American Journal of Physiology*. Another has been the recognition of Specialty Group sections that are empowered to designate their own representatives on the Program Committee and advise other Society committees with respect to their area of interest.

The formation of these sections has been a somewhat informal process and although Statements(s) of Organization and Procedures for each has been published in *The Physiologist* as it was recognized by Council, there has been no provision for such organizational entities in the Bylaws.

Experience with the operation and contributions of the new sections has been quite good, and Council has conluded that it would be beneficial to recognize the existence of this level of Society organization in the Bylaws, to clarify the status of the existing sections and to promote the formation of new ones.

Existing sections recognized by Council are:

Gastrointestinal Physiology

Renal Physiology

Environmental, Thermal & Exercise Physiology

Comparative Physiology

Nervous System

ARTICLE X. *Society Sections and Affiliations*

SECTION 1. *Society Sections.* Upon acceptance of a Statement of Organization and Procedures by Council, any group of members of the Society may form a section which encompasses an area of physiology. Such sections shall:

a. Advise the Society on matters of interest to the specialty group represented by the section.

b. Assist the Society in organization of scientific meetings.

c. Nominate indivduals for membership on Society committees.

d. Be open to all members of the Society expressing an interest in section membership.

The Executive Secretary-Treasurer shall provide assistance to sections in the carrying out of section business.

Nothing in a section's Statement of Organization and Procedures may be construed as contradictory to the Constitution and Bylaws or Operational Guide of the Society.

SECTION 2. *Society Affiliations.* The Society shall maintain membership in such organizations as determined by Council.

AMENDMENTS TO AMENDMENT PROCEDURES

The existing Bylaws provide for their amendment only at the Spring Business Meeting of the Society. Various considerations now underway concerning venue of the FASEB Meeting might make this provision too restrictive in a future year. It, therefore, seems prudent to relax the requirement so as to allow amendments at any regular Business Meeting of the Society.

ARTICLE XIII. *Amendments*

SECTION 1. *Presentation.* Amendments to these Bylaws may be proposed in writing, by any regular member, to Council at any time up to three months in advance of the Spring business meeting, or at a business meeting of the Society. Such proposed amendments must be presented in writing at the following Spring business meeting for action by the Society.

SECTION 2. *Adoption.* These Bylaws may be amended at any Spring business meeting of the Society by a two-thirds majority vote of the regular members present and voting.

See page 2 for "Plan for Future Federation Meetings."

REPORT OF THE PRESIDENT

The following is a brief report of the last Business Meeting of the Society convened in New Orleans on October 18, 1979.

No committee reports were heard because these will be, or have been, published in *The Physiologist*. The membership status report and roster of new members have also appeared. The practice of posting committee reports prior to the Business Meeting initiated at the New Orleans Meeting will be continued. This procedure was established to permit an interchange between the membership and APS officers at the Business Meeting regarding committee reports even though these will no longer be presented orally.

The actions of Council can be summarized as follows:

Constitution and Bylaws: Council approved a proposed bylaw dealing with the formation of Sections of the Society. The proposed bylaw will be published in *The Physiologist* and will be subject to actions by Membership at the Business Meeting in Anaheim next April (See facing page). Council approved two amendments to amend bylaws at any Business Meeting rather than just the Spring Meeting, as is currently the case. This action was taken in anticipation of major changes in the format of FASEB meetings (see below).

Future Meetings: Council considered and adopted a proposal for future meetings of FASEB and of APS formulated by an ad hoc committee appointed by the President and chaired by him. The report of this committee which was composed of F.E. Yates, H.M. Goodman, O.E. Reynolds and Barbara Nichols, appears elsewhere in this Issue of *The Physiologist*. It provides for an annual APS meeting, modeled on our current Fall Meeting for the conduct of our Societal affairs and appropriate Scientific Programs. It also recommends the abandonment of the single, annual Spring Meeting of FASEB in favor of multiple, thematic, smaller Federation Meetings patterned on the highly successful "Intersociety Sessions" of old. (This plan, as noted elsewhere, was subsequently adopted by the Executive Committee of FASEB and by the Federation Board and will be implemented in 1986 when all prior commitments for the large Spring Federation Meetings will be undertaken by a new, intersociety program committe appointed by the President of FASEB and chaired by Dr. F. E. Yates.)

A recommendation at the Spring Business Meeting in Dallas that the APS membership be polled to determine attitudes toward meeting in States which have not ratified the ERA amendment. This was not implemented on advice of legal counsel because such a poll might be interpreted by the IRS as a violation of the regulation prohibiting "grass-roots" lobbying by tax exempt organizations.

Public Affairs: Council gave consideration to a proposal that the APS have a more active public affairs program specifically concerned with Physiology and Physiologists. At present, the APS contributes in major fashion to the public affairs of other organizations, notably FASEB, AAMC and AIBS but has no advocacy of its own. This seemed regrettable because, of all the biomedical sciences, Physiology is the most familiar to the public and to the members of Congress and annually receives special recognition in the form of a Nobel Prize. It was felt that these attributes should be capitalized upon to the advantage of research and education in Physiology.

FASEB: Major revisions of the Constitution and Bylaws of results of protracted efforts by member societies and their legal counsels were considered and approved. The revisions, which were subsequently ratified by the FASEB Board of Directors, permit new flexibility in the governance of FASEB and provide equitable mechanisms for its growth and dissolution.

Council invited the President of the American Society of Biological Chemists to meet with it in New Orleans for discussion of problems common to both Societies especially as these relate to FASEB activities. The increasing cost of our membership in FASEB, some $21.00 per APS member currently, the value of services received from FASEB in return and the increasing expense of the traditional, large Federation Meetings were of particular concern.

American Medical Research Expedition to Mt. Everest: Council agreed to sponsor this venture, which is being led by John West, without, however, making financial commitments to it at this time.

BUSINESS MEETING AT FASEB MEETING, ANAHEIM, CALIFORNIA, TUESDAY, APRIL 15, 1980

The Business Meeting of APS to be held at the time of the annual FASEB Meeting will follow the same pattern as at the 1979 Fall Business Meeting in New Orleans. It will be brief, consisting only of essential business and announcements. The Committee Reports, rather than being presented orally at the Business Meeting, will be posted for the duration of the FASEB Meeting at the Society's Headquarters Office, Inn at the Park, Executive Room 133. The Business Meeting will be followed by a forum. Topics of discussion in this forum will include:

1. Continuing Discussion of Format and Venue of APS Meeting.
2. Why Are Dues High and Where Is The Money Spent?
3. Role of APS in a Time of Increasing Specialization.

A PLAN FOR FUTURE "FEDERATION MEETINGS"

The following plan, proposed by Council, was adopted by the Executive Committee and Board of Directors of FASEB on November 29, 1979. Because FASEB has made commitments for its annual Spring meeting through 1985, this plan will not be fully implemented until 1986, although extensive experimentation with the new format will take place in the intervening years. Dr. F. E. Yates of APS has accepted the chairmanship of a new interdisciplinary intersociety program committee charged with the formulation of the new, thematic Federation meetings.

The Federation meetings have become increasingly large, unwieldy and expensive as the number of cities which can accomodate them have been reduced to one or two. The worthy objectives of interdisciplinary exchange are now but minimally served and can no longer be used to justify these massive gatherings.

There seems to be a consensus that smaller meetings are more conducive to scientific discourse, are generally more agreeable and are less expensive because a larger number of smaller, competing cities are able to accomodate them.

The virtues of both interdisciplinary exchange and smaller meetings without sacrifice of disciplinary interest can be attained by the following plan.

All FASEB societies will, if they so wish, have an independent annual meeting at which they will conduct their societal affairs. The fall meeting of the APS or the independent meetings of the ASBC could be appropriate models. These independant societal, disciplinary meetings could, but need not, be arranged by the OSM of FASEB. They could be held in conjunction with other societies and at any time of the year.

In addition, however, each member society, and in appropriate collaboration with others, will participate in one or more FASEB meetings organized along thematic rather than societal or disciplinary lines. Four large, overlapping themes designed to attract members of most biological disciplines could, at the outset, be the subject of as many annual meetings.

Each meeting will consist of symposia, plenary lectures and other invited components as well as of short communications. The latter will all be of the poster variety thus permitting virtually unrestricted opportunities for presentation.

Each thematic meeting will be organized by its own program committee comprised of representatives from participating societies. These need not be limited to members of FASEB. On the contrary, new intersocietal arrangements should be fostered by this thematic organization.

It is anticipated that the FASEB thematic meetings will be of a size (1,000-5,000 participants) to attract sufficient number of exhibitors to maintain registration fees at manageable levels. Each meeting should meet its own expenses.

It is recommended that such a plan be implemeted on an experimental basis at the Spring FASEB meeting scheduled for 1981 in Atlanta. Within the designated time frame and venue, with the possible addition of one additional half day making a total of 6, three thematic meetings of 2 days each will be organized by their respective program committees composed of members of relevant, participating societies inside and outside the Federation. Since the conventional array of exhibitors will be in attendance, this experiment will entail no financial risk while profferring the possibility of disengaging from the decreasingly viable traditional Spring meeting without sacrifice of interdisciplinary exchange on the one hand and societal cohesion on the other.

The initiation of thematic meetings, in sharp contrast to the maintenence of the status quo, will provide continuing and multiple opportunities for the evolution of meeting formats and for adaptation to the ever changing boundaries of the biological sciences.

HONORS AND AWARDS

David H. Hubel, M.D., and Torsten N. Wiesel, M.D. of the Department of Neurobiology at the Harvard Medical School were named the 1979-80 recipients of the Dickson Prize in Medicine from the University of Pittsburgh. The annual award honors distinguished scientific accomplishments and outstanding contributions to the progress of medicine.

The team's research work on understanding the mammalian visual system and the mechanisms by which sensory information is perceived and analyzed by the brain has resulted in novel and important evidence about processes underlying perception. Their pioneering studies have influenced diverse areas of research involving the central nervous system.

Dr. Hubel, a native of Ontario, joined the Harvard Medical School faculty in 1959. Dr. Wiesel earned his M.D. degree from Karolinska Institute in his homeland, Sweden and joined Harvard's faculty in 1959 and became Chairman of the Department of Neurobiology in 1973.

Doctors Hubel and Wiesel are co-recipients of numerous awards from professional and scientific societies. They include the Louisa Grass Horwitz Prize, the Karl Spencer Laskley Prize, the Friedenwals Award, and the Lewis S. Rosentiel Award for Basic Medical Research.

The Dickson Prizes are awarded to the foremost individuals in the United States in medicine and science. They were established by the estates of the late Joseph Z. Dickson, alumnus of Pitt's School of Medicine, and his wife, Agnes Fischer Dickson.

RALPH W. GERARD
(1900-1974)

Ralph Waldo Gerard became President of the Society after the Cleveland Meeting in 1951 and served in that capactiy at the Fall Meeting in Salt Lake City in 1951 and the Spring Meeting in New York in April 1952. He was born in Harvey, Illinois, October 7, 1900, and received from the University of Chicago a B.S. degree in 1919 and a Ph.D. in 1921. He then obtained the M.D. degree from Rush Medical College in 1925. He was Professor of Physiology at South Dakota University (1921–22) and had a National Research Council Fellowship in Europe from 1926 to 1927, working with Prof. A. V. Hill in London on heat production and then with Otto Meyerhof in Kiel on the oxygen consumption of stimulated nerve. Returning to the University of Chicago in 1928, he became a professor in the Physiology Department (1941–52). After three years as Professor of Neurophysiology and Physiology at the College of Medicine of the University of Illinois and a year in California, he became Professor of Neurophysiology at the Mental Health Research Institute in Ann Arbor in 1955.

Dr. Gerard is in great demand as a lecturer and consultant and has been concerned with science in human affairs as well as in creating and teaching new scientific knowledge. He has received many honors, including: medal from Charles University, in Prague; Order of the White Lion (4th class) of Czechoslovakia, honorary membership in the American Psychiatric Association and the Pan Hellenic Medical Association; membership in the American Academy of Arts and Sciences and the National Academy of Sciences; a D.Sc. fromt he University of Maryland in 1952; and an honorary M.D. from the University of Leiden in 1962, at the time of the XXII International Congress of Physiological Sciences. [This, the second honorary M.D. ever awarded by that distinguished University in its history, is well described in *The Physiologist* (6: 49, 1963).]

His professional interests started out in nerve physiology, especially nerve metabolism and the heat production of nerve (with A. V. Hill) and ended up in the behavioral sciences, omitting nothing in between. He is perhaps most widely known for his work on steady potentials in neuron masses: his pioneer study of evoked brain potentials and the demonstration that visual, auditory, and other impulses reach many structures, such as cerebellum; his demonstration of an extended "fixation time" for establishing a memory trace; his universally used capillary microelectrode; and his integrative approach to the nervous system and behavior, so well shown in the summary chapter of the Neurophysiology Section of the *Handbook of Physiology*. Aside from his many research papers and published lectures, he is the author of *Unresting Cells* (1940), *Body Functions* (1941), *Methods in Medical Research* (1950), *Food for Life* (1952), *Mirror to Physiology* (1958), and (with Cole) *Psychopharmacology; the Problem of Evaluation* (1959). A volume reporting the Leiden symposium which he organized, on "Information Processing in the Nervous System," is in press; and one reporting an extended interdisciplinary study of schizophrenia is in preparation. *Mirror* was the report of the Survey of Physiology which Dr. Gerard initiated and for which he served as chairman. He is a liberal in his attitude and is very articulate with the right words ready for any situation. At present he is Chairman of the National Committee (of the National Academy of Sciences) for IUPS and was chairman of the official delegation to the last General Assembly of IUPS in 1962 in Leiden.

Some of the more important events of Dr. Gerard's term of office were as follows: Dr. Chandler Brooks was appointed Chairman of a Committee on Motion Pictures; a Membership Committee was appointed with Fred Hitchcock as Chairman; the idea of associate members was further discussed; a committee was appointed, under Gerard as chairman, to carry out the Survey of Physiology and to seek funds for the purpose. Dr. Robert Gesell, at the New York meeting, created a great stir and probably some resentment by his charges of inhumanity to animals by physiological investigators. Dr. Gerard, as chairman, kept an explosive business meeting from exploding and succeeded in referring the matter to an ad hoc committee. The Society finally rejected the sweeping allegations of Dr. Gesell. At this time also Dr. Lee moved the Publications Office from the National Academy of Sciences to rented rooms in the Dupont Circle Building in Washington. Dr. Gerard was also chairman of a committee to revise the Constitution and By-Laws of the Federation. Finally, by vote of the Society, the President addressed a letter to Dr. Bronk of the National Academy of Sciences concerning problems of security, loyalty, and clearance. The letter expressed concern because of the "advancing encroachment upon the civil, academic and scientific freedoms" and hoped for some help from the National Academy.

In his address to the Society as Past-President, Dr. Gerard played the Elder Statesman and demonstrated his loyalty to the Society. "In Washington," he wrote, "our Society often is looked to as the leader in the biology group, due in large part to the quality of our representatives there. The Society has maintained interest in and exercised progressive leadership concerning problems of scientific statesmanship–witness the Survey. The Society has an inviolable tradition of democracy which is all too rare" (*Am. J. Physiol.* 171:695, 1952).

Taken from *History of the American Physiological Society - The Third Quarter Century, 1937-1962,* Wallace O. Fenn.

See also *R. W. Gerard* by Ben Libet and Orr E. Reynolds, *The Physiologist,* Vol. 17, No.2, May 1974.

BIOLOGY OF THE CHLORIDE CELL: JEAN MAETZ MEMORIAL SYMPOSIUM

In March 1980 a special issue of the *American Journal of Phsyiology: Regulatory, Integrative and Comparative Physiology* will be devoted to articles derived from a symposium on the "Biology of the Chloride Cell," which was held to honor the late Jean Maetz. The publication of an entire symposium in a single issue is a new venture for the *American Journal of Physiology.* It is another example of the flexiblity that is possible since the journals of the Society were reorganized.

The symposium focused on the integration of research information applicable toward understanding the function of the chloride cell, an acidophilic cell found in the gill epithelium and held to be the specific excretory (or secretory) unit responsible for the osmotic and ionic regulation of fish gills. The publication is divided into four sections on the anatomy, ultrastructure, physiology, and biochemistry of the chloride cell. Contributors to this special issue are P. Laurent, S. Dunel, F. E. Hossler, N. Naito, H. Ishikawa, C. W. Philpott, K. J. Karnacky, Jr., S. R. Hootman, C. Sardet, M. Pisam, J. Maetz, L. B. Kirschner, D. H. Evans, K. J. Degnan, J. A. Zadunaisky, M. S. Haswell, D. J. Randall, S. F. Perry, F. H. Epstein, P. Silva, G. Kormanik, M. Bornancin, G. de Renzis, R. Naon, J. P. Girard, P. Payan, and F. Conte.

The symposium was organized by Frank P. Conte; Bodil Schmidt-Nielsen served as symposium editor. The symposium was sponsored in part by the Commissariat a l'Energie Atomique Department de Biologie (France), the American Physiological Society, and the National Science Foundation (Grant PCM78-19027).

This pulication may be purchased separately or you may receive it as part of your annual subscription to the journal.

Place your order now for:

- Biology of the Chloride Cell: Maetz Symposium issue, $5.50 or
- 1980 Subscription (including Maetz Symposium issue) to

American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,

$30.00, U.S.; $33.00, Canada; $35.00, Elsewhere.

Send order and check to:

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American Phsyiological Society
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Bethesda, Maryland 20014

HALF-PRICE SALE - CIRCULATION HANDBOOK

The Heart, the first volume of the *Handbook of Physiology* section on the cardiovascular system was published this year. It will be followed by volumes on vascular smooth muscle, microcirculation, and peripheral and organ system circulation. These volumes will succeed the *Handbook of Physiology* section entitled *Circulation.*

A limited number of the original volumes, published in the 1960's, are available to members of the Society at half price.

Volume I- Covers the physiology of the heart, and its controls; blood volume. Sale price, $12.00

Volume II- Covers the functional morphology of vessels; blood flow. Sale price, $16.00

Volume III- Covers integrated aspects of cardiovascular regulation. Sale price, $16.00

Orders should be sent to the Subscription Office, American Physiological Society, 9650 Rockville Pike, Bethesda, MD 20014.

CONTINUING MEDICAL EDUCATION CREDIT

The ASPET Committee on Continuing Medical Education has certified ALL FASEB SCIENTIFIC SESSIONS for CME Category I Credit on an hour for hour basis to ALL PHYSICIANS REGISTERED for the FASEB meeting. To receive credit one must complete a CME credit form, available in the FASEB Headquarters Office (Convention Center, Orange Room 9) or the ASPET Office (Disneyland Hotel, Serrano Room). Completed forms are to be returned to either office during the meeting or mailed to ASPET, 9650 Rockville Pike, Betesda, MD 20014.

Third Annual Workshop on

PULMONARY PHYSIOLOGY AND FUNCTION TESTING IN SMALL LABORATORY ANIMALS

Satellite Conference to the Annual Meeting of the

AMERICAN THORACIC SOCIETY

Co-sponsored by

AMERICAN PHYSIOLOGICAL SOCIETY,

HEALTH EFFECTS RESEARCH LABORATORY U.S. ENVIRONMENTAL PROTECTION AGENCY

and

NATIONAL INSTITUTES FOR OCCUPATIONAL SAFETY AND HEALTH

May 17, 1980

The Johns Hopkins School of Hygiene and Public Health
Baltimore, Maryland

The one day program will cover the current state of the art of several pulmonary function tests potentially useful in evaluating lung damage in laboratory animals. Focus will be directed toward dynamic tests requiring body plethysmography, but there will also be discussion of other tests such as diffusing capacity and N_2 washout. Invited speakers will discuss each test, but the forum will allow extensive open discussion time for participants to address specific question or problems.

Further information can be obtained by calling or writing to:

Wayne Mitzner
Division of Environmental Physiology
The Johns Hopkins University
School of Hygiene and Public Health
615 N. Wolfe Street
Baltimore, Maryland 21205
(301) 955-3612

Sandy Iannotta
American Thoracic Society
1740 Broadway
New York, NY 10019
(212) 245-8000

Fill in form below and return with $15 registration fee (make checks payable to Johns Hopkins University) to The Hopkins University, School of Hygiene and Public Health, Division of Environmental Physiology, 615 N. Wolfe Street, Baltimore, Maryland 21205, Attn. Nola Graves.

REGISTRATION FORM

Name_____ Phone_____

Mailing Address _____

Check if accomodations in Baltimore are required ☐

Information on transportation between Baltimore and Washington, D.C. will be sent to registrants.

SOCIETY OF GENERAL PHYSIOLOGISTS

34th Annual Symposium
Organized by S. G. Schultz, M.D.
Univ. of Texas Med. Sch. at Houston
ION TRANSPORT BY EPITHELIAL TISSUES
Marine Biological Laboratory, Woods Hole, Massachusetts
4-7 September 1980

4 Sept.: Electrophysiology of Epithelial Tissues - Part 1
B. Lindemann, Univ. des Saarlandes, Homburg
S. Helman, Univ. of Illinois, Urbana
S. M. Thompson and S. G. Schultz, Univ. of Texas, Houston
A. Finn, Univ. of North Carolina, Chapel Hill

5 Sept.: Electrophysiology of Epithelial Tissues - Part 2
E. Fromter, Max - Planck - Inst. fur Biophysik, Frankfurt
S. Lewis, Yale Univ., New Haven
L. Reuss, Washington Univ., St. Louis
C. Clausen and J. M. Diamond, UCLA

6 Sept.: Applicaton of Ion-Selective Microelectrodes
W. McD. Armstrong, Indiana Univ., Indianapolis
M. Civan, Univ. of Pennsylvania, Philadelphia
R. Frizzell, Univ. of Texas, Houston
G. Giebisch, Yale Univ., New Haven

7 Sept.: Other Approaches
A. Taylor, Cornell Univ., New York City
A. W. Cuthbert, Univ. of Cambridge, Cambridge
R. Rick, Physiol. Inst., Munich
K. Mollgard, Univ. of Copenhagen, Copenhagen

Abstracts for posters dealing with epithelial transport and membrane physiology are invited by the Society. Abstracts from nonmembers must be sponsored by a member of the Society of General Physiologists. Deadline for receipt of abstracts will be June 1, 1980. Submit two copies, double spaced, including title, author(s) (designate nonmembers by *), institution, 300-word text, and grant acknowledgement, if any, along with a self-addressed postcard to: Dr. John S. Cook, Biology Division, Oak Ridge National Laboratory, P.O. Box Y, Oak Ridge, TN 37830.

Registration will be at the meetings. Further information will be distributed in Spring mailings to the Society.

NEWSLETTER FROM THE COMPARATIVE PHYSIOLOGY SECTION

There will be 54 presentations by comparative physiologists at the forthcoming Anaheim meetings, 31 in slide sessions and 23 in poster sessions. The details:

Comparative Physiology of Circulation and Respiration--I. Chaired by Al Bennett who will also present a 30-minute introductory talk on "Comparative Aspects of Respiratory Physiology." In addition to the introductory talk, there are 10 contributed papers.

Comparative Physiology of Circulation and Respiration--II. A poster session of 7.

Comparative Physiology of Ionic and Osmotic Regulation. A slide session of 11 contributed papers chaired by Lewis Greenwald.

Comparative Physiology of Temperature Acclimation and Adaptation. A slide session of 9 contributed papers chaired by Audrey Haschemeyer.

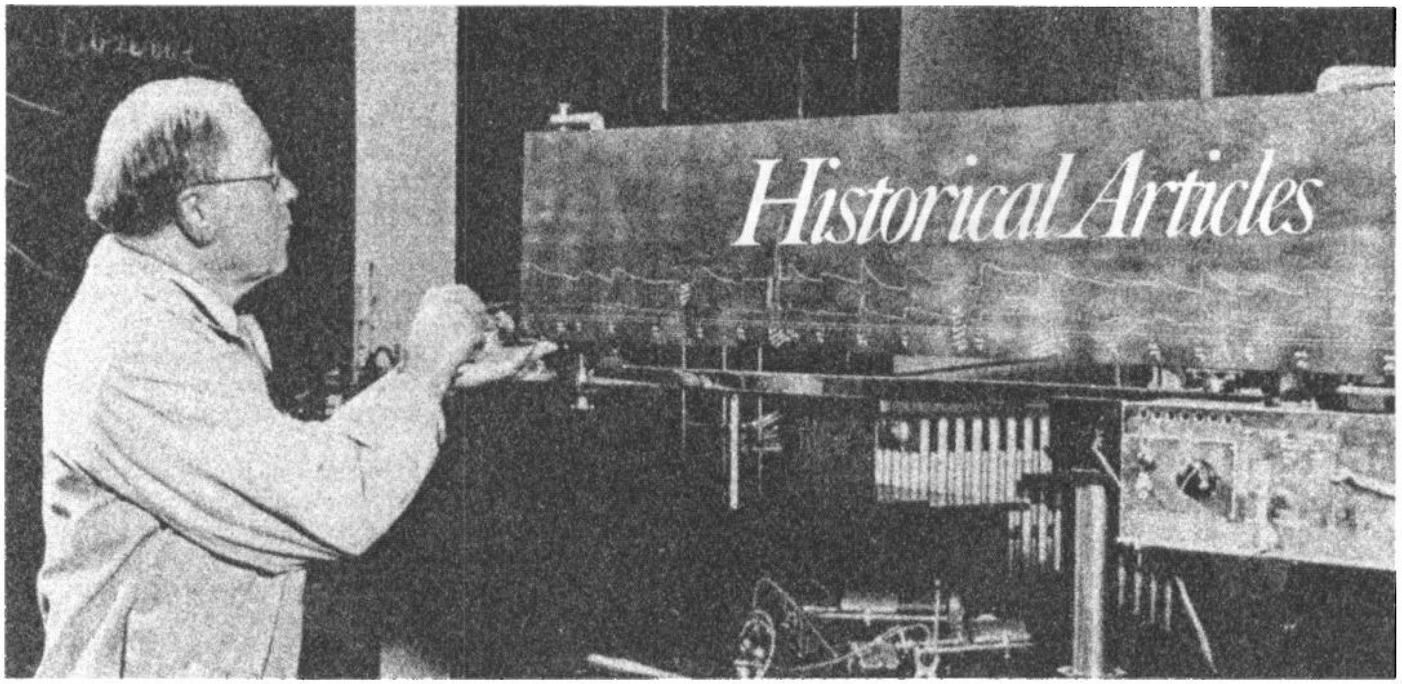
Feeding, Digestion, and Nutrition. A poster session of 10 comprising one fish and nine rat papers.

Comparative Physiology of Muscle. A poster session of 7 papers.

Although this was not a meeting emphasized by our section, the contributions are impressive in quality and quantity. No symposia are scheduled for this meeting by our section. The 1980 meeting that will be emphasized is the joint APS/ASZ meetings in the fall in Toronto.

In a rather evenly divided election, 39% of the CPS membership voted, and elected C. Richard Taylor as Councillor to replace Frank P. Conte.

Roger A. McNabb
Secretary



THE LIMITS ON SCIENCE

Mario Bunge
Foundations & Philosophy of Science Unit
McGill University Montreal H3A 1W7, Canada

ABSTRACT

Science is subject to constraints of three kinds: 1) physical, 2) biological and 3) social. Physical constraints deny us knowledge of certain kinds; biological constraints stem from the design of our brains. It is my view that neither of these limits to science compares in extent to those that arise socially, from the economic, cultural and political matrices of every scientific community. In this article I examine these social constraints in detail, and point out that science can and does die in the absence of social support. Greek science disappeared under just such circumstances. Optimistic arguments in favor of the view that scientific enterprise will prosper in the near future are examined, and shown to be weak. Evidence that we are already moving rapidly toward another eclipse of science is presented, and it is suggested that if science is to be saved, it will be because, through the public efforts of scientists - it comes to be regarded for what it is - an effort to understand reality, rather than as a dangerous and irresponsible handmaiden of power.

INTRODUCTION

Like every other human enterprise, scientific research has its drives and its bounds. Scientific research is motivated by curiosity and necessity, and it is subject to constraints of three kinds: physical, biological and social.

The physical constraints on scientific research consist in the impossibility, whether in principle or in practice, of obtaining certain information. For example, the upper limit on the speed of signals will forever prevent us from knowing, at any given instant, what is going on at the same time in distant places; and the geological, fossil and historical records are hopelessly incomplete.

The biological constraints on our cognitive activities boil down to this: we cannot outfox our own brains - and human brains, though marvellously competent at times, presumably are not the last evolutionary word. It is conceivable that there are, or will be, animals with an even larger and more plastic associative cortex, capable of learning much more quickly than we, as well as of inventing deeper and truer theories and more revealing experiments than any we could dream of.

Finnally, the social limitations on scientific research stem from the economic, cultural and political matrices of every scientific community. Science cannot escape its social environment, particularly if the latter is stifling. The social pressures on a scientific community can become so intense that science can stagnate, decay, or even disappear altogether. In fact it may well be that the Western world is entering a period of scientific decadence. If so, the decay originates in certain social constraints rather than in the physical or the biological ones and can be averted.

We propose to examine the above mentioned constraints on scientific research, as well as some indicators of an impending science crisis. Our analysis will include some reference to the role, now progressive, now regressive, that philosophy can play in the design of science policies and even in the conduct of scientific inquiry. However, we shall not touch on the philosophically important questions of whether the completion of science is around the corner, whether every cognitive problem can be investigated, or whether every problem that can be investigated is worth being investigated. We shall concentrate instead on the practical problem of the possible crisis in science and what to do about it.

1 PHYSICAL CONSTRAINTS

The physical limitations of science consist essentially in that not every desirable item of information is accessible. A few examples will make this clear and will show that such a limitation is

Mario Bunge is Professor of the Foundations and Philosophy of Science at McGill University. His papers and books have addressed topics in logic, epistemology, probability, dialectics, evolution, social structure, hierarchies and system theory, the nature of science and of physical theories (see list of books below). Impressed by the clarity and variety of his thought, Rodolfo Llinas and I invited him to participate in a workshop for the 10th Winter Conference on Brain Research, in 1977. He there presented ideas on hierarchies later published in the *American Journal of Physiology* (1). Since then I have had the pleasure of following his interests through correspondence. In 1978 I sent him an advance copy of an editorial on limits to knowledge I had prepared for the *American Journal of Physiology* (2), in which I point out some physical and biological limits to what we can ever know. Bunge replied by sending me an advance copy of the article that follows, then in press, in which the same topic is treated, but with a very different emphasis: the chief limit on knowledge, and science, is seen to be social. His viewpoint seemed so important, and right, that republication here seemed justified, so that the paper could be seen by our community of biological scientists. Dr. Reynolds agreed, and we are pleased to be able to call your attention to Bunge's concern about the future of scientific inquiry.

Perhaps the article that follows is too pessimistic, but there are many signs that it is correct in its assessment of our condition and our prospects as scientists during the last quarter of the twentieth century. The arguments and warnings deserve the attention of all who regard science as a cultural expression of man's creativity, beautiful for its own sake.

The article is based upon a talk given in February 1978 at the annual meeting of the American Association for the Advancement of Science, in a symposium on Limits on Scientific Progress. It was later published in Epistemologia (3).

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F. Eugene Yates, M.D., Editor
AJP: Regulatory, Integrative
and Comparative Physiology

Historical Articles

not merely a temporary shortcoming that can eventually be overcome. Our examples fall into two classes: events about which all information has been lost, and events about which any information will be too late.

A clear case of lost information is this. If the big bang cosmological hypothesis is true, then the history of the universe before the beginning of its expansion has left hardly any traces, so that we cannot possibly learn anything about that segment of eternity. A similar case, on a far more modest scale, is the loss of geophysical - in particular geological - information as a result of physical processes such as the melting of rocks. The destruction of prehistorical evidence and historical records is similar. For example, most of what is happening right now among us will never reasch our descendants because the chroniclers of the day - journalists, social scientists, and culture watchers - do not deem it important.

However, we may take heart recalling that we keep discovering or inventing new ways of finding and interpreting traces of past events. For example, until a few years ago nobody thought it possible to find evidence of primitive cells, and yet fossils of such, dating back to a couple of billion years, were eventually found. In other cases we come upon indirect evidence of past events. So, the historical record, though hopelessly incomplete, can be completed in part with some imagination.

In other cases there is no information to be had. For example, we cannot know what is happening right now in some remote corner of our galaxy, let alone in other galaxies. If and when the light signals accompanying some such events reach the present abode of mankind, they will give only a partial picture and one that may have no beholders. In other words, the events about which we can get information are those lying within our own light cone. However, what a single observer cannot attain, a few generations of observers, bound by a common tradition, may attain: some of the signals that are now leaving remote places will reach some of our descendants, so they may be able to know what is now going on there.

So much for knowledge of the past, i.e. knowledge proper. What about knowing the future; to what extent can we forecast? To the extent to which we know laws and present circumstances. Surely some of our predictions are bound to be stochastic, i.e. of averages, scatters, and the like. But this is no limitation if the processes themselves are stochastic, as is the case with quantum-mechanical, genetic, and social processes. For example, we cannot predict the exact trajectory of an electron because electrons have position distributions rather than precise positions. Likewise we cannot predict accurately the genetic make-up of a particular human baby, even if we could know the genic spectrum of its parents, because the parental genes mix randomly.

As for forecasts of social events, they are made all the time by governmental agencies of various descriptions. Surely many of them turn out to be false, either because of the proverty of social laws or because they are formulated by specialists who take into account only some aspect (e.g. the economy) while ignoring the others (e.g. the polity and the culture). However, reasonably accurate social forecasts can be made for short periods, particularly when they are of the self-fulfilling kind. Thus a government can predict that it will spend a certain amount because it is intent on exacting that amount in taxes. In general, the more controlled or planned a social sector, the easier it is to make forecasts about it, just because all the efforts in the sector converge on the success of the plan.

This applies even to certain types of scientific research, namely those conducted by well organized teams led by investigators with a clear research program. If they know what they want to

know, and feel that it can be found out with hard work and a bit of luck, then they can plan the investigation and request the necessary funds in the assurance that they will succeed to some extent. Hence any governmental agency willing to support well planned experimental research can count on a high yield - provided it does not meddle with the investigation itself, e.g. ask for specific results or demand so much paper work that no time is left for thinking. Of course that is not the way revolutionary theories or experimental procedures are produced; still, it is rather common and fruitful practice in experimental science. As for theoretical science, although no amount of planning and money wil replace talent, obviously encouragement - both moral and material - is welcome - again, with no strings attached.

To summarize: scientific research is subject to certain physical constraints. In fact some information is not to be had at all, while some is lost forever, and the future can be forecast only on the strength of considerable knowledge (of laws and circumstances). These constraints limit the amount of knowledge that can be won, without in any way slowing down the pace of scientific progress. We can know more and more about certain things while ignoring everything aobut others. We need not worry about all we shall never know provided we keep on finding out some of the infinitely many things that can be known.

2 BIOLOGICAL CONSTRAINTS

Despite all our advantages we humans are behind other species in a number of respects, such as strength and swiftness. Our greatest assets are our brains and our comparatively long life span. Ours is the most plastic of all known brains, and so the one capable of learning the most. And our maximum life span is long enough to learn lots of items, at any rate more than any other known species. Surely there is more to be learnt today than yesterday, but we do not have to learn everything that was known to our ancestors. We can learn to drive cars without first having to learn to drive buggies. The same holds, a fortiori, for science learning: most of the scientific ideas and data a student is supposed to learn are comparatively new. Thus most of the physics I learnt as a student did not exist in my grandfather's time. In sum, the limited (though comparatively long) life span of humans is not a severe restriction on our capacity to learn, and this because the progress of learning is not cumulative.

Much more serious a limitation on the human cognitive ability would seem to be our limited channel and storage capacity. But therse are not really disadvantages: if we were capable of admitting all stimuli, we would be unable to concentrate; and if we were capable of recalling every single experience we would hardly be able to try out new ideas. Blessed be our sensory and memory limitations, for they allow us better to create.

Anyway, the brain-information processor analogy is misleading, for our brains are not computers, not even computer-like. The human brain is a biosystem not an artifact, and moreover a highly unreliable system, for it does not deliver the same outputs every time it is presented with the same inputs. This is because the neural connectivity is variable. It is quite possible that the various mental functions are performed not by fixed circuits but by itinerant neural systems formed afresh every time a new task arises. If this is so, even though the number of neurons is finite, the number of neural systems capable of performing mental functions is practically infinite, in as much as that there is no limit to the number of percepts and concepts a person can have during her lifetime - even though, of course, the number would be finite.

The real limitation lies elsewhere and is to be found out by analogies with computers. It derives from our associative cortex's inability to function unless it is connected to the brain stem, the hypothalamus, the endocrine system, and the peripheral sensors. It is these extracortical systems that keep the associative cortex awake and active - and that at the same time are responsible for the nonrational streaks of human thought. We cannot think unless we are alert and motivated, but if we are alert we cannot avoid some distraction, and if we are motivated we cannot keep totally cool. In sum, pure reason is biologically impossible.

However, we often do manage to think rationally and to make rational decisions. But this is because we are not isolated. Sensory deprivation leads to hallucination, and social deprivation leads to mystical visions. I cannot think rationally all the time, nor can my partner, let alone my rival; but they correct me when I lapse, and all three together manage to set up - unwittingly to be sure - a self-correcting system within which we are sane, outside of which insane. Rationality, like language and morals, is social. (Robinson had learnt all three before his shipwreck.) The canons of rationality are those of argumentation and have evolved alongside the exchange of information, opinion, and valuation. Logic is a sort of collective conscience, and human knowledge is social or public property.

In short, the biological constraints on cognition, though quite real, are less formidable than they appear at first blush. First, because the human associative cortex is marvellously plastic and capable of spinning out new ideas without end. Second, because the scientist or the scholar need not, nay must not, rely exclusively on himself: he counts on all others. His personal limitations can be overcome by cooperation - with the living and the dead. What mankind knows, it knows collectively. (There is no isolated savant. Even a scientist working in seclusion is in touch with thousands of others through books and journals, If he is not, then he is not a scientist but a crackpot.)

Surely the human brain has limited capacities, but such limitations have not prevented mankind from making fantastic leaps since the neolithic revolution about 10,000 years ago. Since then man has learnt to think and do things undreamt of by his hunter and gatherer ancestors, even though he has undergone but little biological development. Modern science, which is less than four hundred years old, has been produced by brains with a neural organization similar to that of those which produced myths. What man does is conditioned not only by what he can do but also by what he chooses to do. If he decides to fabricate myths, he succeeds; if he decides to produce scientific knowledge, he succeeds as well. So, instead of deploring our biological limitations, let us celebrate our abilities and decide to make good use of them.

3 SOCIAL CONSTRAINTS

Scientific research is done by individuals composing a tightly knit system - the scientific community. In turn every scientific community is a subsystem of some culture or other, which is in turn a subsystem of some society, in turn a component of the international system. A society is formed by three main subsystems: its culture, its economy, and its polity - the latter being the control system, or the system concerned with managing or regulating all social activities. Each of these subsystems - the culture, the economy, and the polity - interacts with the other two. In particular, culture, and so science, is subject to economic and political pressures; and, of course, science is subject also to cultural pressures.

Far from being autonomous, then, science thrives or withers along with society. A peasant economy cannot support a vigorous experimental program in high energy physics; a totalitarian state does not tolerate free inquiry into political science; and a religion-oriented culture does not encourage studies on the origin of life, the evolution of the mind, or the

socioeconomic roots of religions. It does not follow that an industrial society, allied to a political democracy and a lay culture, will necessarily support scientific research. It will, provided the prevailing ideology is favorable to science, otherwise not. Nor does it follow that an underdeveloped economy, allied to an authoritarian polity and a backward culture, will necessarily stand in the way of scientific progress of some kind. It will support scientific research, sometimes at great sacrifice, as long as its ideology is friendly towards some science (usually natural rather than social).

It is a mistake to disregard ideology, for we are never free from it: every culture is centered on some ideology or other. If the prevailing ideology is science-oriented, one can expect vigorous support of scientific research. If it is indifferent to science, one may expect isolated scientific efforts but no massive, concerted and sustained effort, hence hardly any breakthroughs. If the prevailing ideology is ambiguous towards science - e.g. because it cherishes its practical spinoffs as much as fears the effects of its criticism of the ideology - then one may expect support of some kinds of scientific research but not of all. Finally, if the prevailing ideology is monolithically antiscientific, we can expect open hostility to the scientific community.

We must reckon with the prevailing ideology in a society if we wish to understand the control mechanism of scientific progress, for the ideology shapes the public attitude, which in turn codetermines the science policy. (The other determinant is the level of the economy.) In turn, the science policy regulates the two main contributions a society can make to its scientific community: the human and the material resources. (The tradition can always be borrowed from other cultures.) The human resources are of course the scientists, the science students, and their assistants -technicians, secretaries, librarians, etc. And the material resources are the buildings, instruments, machinery, libraries, funds, etc., used by the scientific community.

The future of science in any country depends then critically upon its science policy. This policy need not be stated explicitly: it may be as diffuse as the underlying ideology. It may be "up in the air," expressed only in practical ways, such as recruiting (or else discouraging) science students and scientific personnel, and subsidizing (or else throttling) research projects. Moreover any explicitly formulated science policy ought to sketch only the general lines, and even so by way of hint and encouragement rather than specific instruction. Here, as elsewhere, strict control inhibits creativity and promotes waste. Only routine work can be planned in all detail, and even here some room ought to be made for the unexpected emergency that calls for new insights -as, e.g., when hitherto standard materials become unavailable. If you want scientists to deliver their goods do not tell them what to do, let alone how to do it. Ask them instead what problems they would like to investigate and what they need to conduct their research - and ask their peers to evaluate the possible worth of the outcome. Detailed instructions can produce only short sighted research, which is unlikely to produce far sighted results.

I am advocating neither an authoritarian control of scientific research nor a *laissez faire* policy of unlimited hand outs. Since all resources are limited, no society can satisfy all the whims of its scientific community; some control is needed. But this control should be shared by scientists, for otherwise it is likely to distort the spirit of free inquiry and be wasteful. As Parkinson (1965, p. 116) notes, "The more resources have been devoted to projects the politician can understand - that is, to the development of discoveries already made and publicized - the fewer resources are available for discoveries which are now inconceivable in so much as they have not yet been made."

A science policy should be generous but not spendthrift. Extravagant generosity leads to waste, humbug, and empire-building. (Remember that power is proportional to the number of subordinates.) A good thing about the present budgetary squeeze on science in North America - the only good thing about it - is that only those who are motivated to study science for the fun of it, rather than for the prospects of affluence and security, will choose to do so. The scientific community will thus be spared many mercenary talents, and the scientific journals will carry fewer "pot boilers." This is the bright side of the current constraints on scientific development in North America. The dark side, is alas, the overriding one, the more so since, far from being a purely financial matter, it may reflect a deep change of attitude towards science, not just on the part of politicians but also on the part of the public, particularly the young. I find this development so disturbing that I propose to devote a separate section to it.

4 IMPENDING SCIENCE CRISIS?

Imagine yourself in the year 410 somewhere in the Roman Empire or what is left of it. Alaric and his Goths are sacking one of the two centers of power of the Mediterranean world. The Empire, which had been declining for two centuries, is crumbling down under the weight of its own iniquities and the unconcerted attacks of the barbarians from without and the Christians within. (Christianity had been proclaimed the official ideology one century before, and the Christians had proceeded to systematic destruction of all the treasures of the pagan culture, including scientific and philosophical manuscripts, and had persecuted the last pagan scholars.) People are surprised, shocked, frightened. Many are left without any means of livelihood, others fear for their lives. Those who can afford to, flee to the provinces. It is the end of civilized Europe.

The victims of this huge social catastrophe feel sorry for themselves, but few mourn the collapse of the Western Empire or even that of its culture. Most had been slaves or citizens of subjected nations or both, so they did not mind the ruin of their masters. By that time few shared the ancient religion of Greece and Rome. Nor was there occasion to mourn the death of classical intellectual culture, for it had been agonizing for centuries. The Romans, superb engineers, administrators, politicians, and warriors, did not care much about the intellect. They produced no original mathematics, science, or philosophy. Not, of course, that they failed to be intellectually endowed: they just were not interested in these subjects. They valued other pursuits - such as acquiring wealth and power, and being on good terms with the gods that be.

Presumably, then, Alaric did not interrupt the proving of a single theorem, the recording of a single natural phenomenon, or the thinking up of a single scientific or philosophical theory. He entered Rome at a time when few individuals were still interested in intellectual work, and these as mere consumers rather than producers. Most of the intellectuals of the time were theologians or moralists: they chased what may be or what ought to be but not what is. One of those intellectuals, living in what is now Algeria, was Aurelius Augustinus, later to be called Saint Augustine. He was probably the most intelligent Roman citizen of his time, and reacted to the fall of Rome by writing a monumental defense of Christianity, *The City of God.* In this work, as well as in his earlier and no less admirable *Confessions,* Augustine drums up the essential message of the early Christians, namely this: We are here, in the earthly city, as resident aliens, passengers in transit to the everlasting City of God, which is to be the final residence of the faithful. Therefore let us not waste time on

worldly affairs but rather prepare ourselves for the transit. The City of Man is no more; the City of God awaits us.

In particular, writes Augustine echoing St. Paul, let us not be deceived by the Greek philosophers and scientists who sought to understand the physical world and human nature. Only Plato and his followers, in particular Plotinus, deserve any attention. The Christians prefer these to the others - i.e. the naturalists. He explains: "The others spent their wits in seeking out the causes of things, the means of learning, and the order of life: these (the Platonists), knowing God, found that in Him was both the cause of the whole creation, the light of all true learning, and the fount of all felicity" (Augustine, 1945, Bk, VIII, Ch.X).

The point of recalling those events and ideas is this. Science, mathematics and rationalist philosophy emerged in Greece in the 5th century B.C., flourished for a couple of centuries or less, and then declined steadily until, long before the Roman Empire was established, they were all but dead. Few cared about those components of classical culture: these were no longer regarded as valuable. And intellectural culture, a hothouse flower, dies if nobody cultivates it.

Leave now the dying ancient world and take a leap to the time and place of the *philosophies:* mid-18th century France. A new value system has emerged and prevails among the lesser nobility and the bourgeoisie. People want to enjoy life, to rise above poverty, to move about more freely, to learn about the world and human nature, to subdue nature and supp;ement it with artifacts. Augustine is all but forgotten, theology ridiculed, and the Church has become the greatest villain in history - *l'infame.* On the other hand Euclid and Archimedes, unknown to the Christian Fathers, are being honored and studied no less than Newton, the demigod who had revealed the last mysteries of the physical world. The best brains are discussing new technological, scientific, mathematical, philosophical, moral, economic, and political problems: they are building up a whole new world of ideas and are preparing the emergence of the modern nations. The burgeoning of intellectual culture and the optimism are such that many affirm confidently that the progress started a couple of centuries earlier will continue indefinitely at an accelerated pace.

The next century provides abundant confirmation of the hypothesis of relentless progress. People start to talk about the *law* of progress. (Thus Comte's "law" is that all nations pass necessarily through three stages: the theological, metaphysical, and scientific ones). Progress is seen and indeed occurs nearly everywhere: in social conditions and in politics, in the arts and in the sciences. Even the backward extra-European countries are forced to join in the triumphal march of progress. No limits are in sight, particularly as far as pure and applied science are concerned. Renan's *L'avenir de la science* is a best-seller and a flag. Only a few obscurantists feel nostalgia for the Middle Ages. All others, whether tories, whigs or socialists, have no doubt that tomorrow will be better than today. The belief in progress becomes part of the national ideology of a number of nations, not only France, Germany, Great Britain, and the U.S.A., but also of some underdeveloped countries: such as Brazil - whose motto is *"Ordem e progresso."*

This enthusiasm over progress lasts until 1914. The savage and senseless carnage shows that not much progress had been achieved after all, at any rate not in matters of feelings and morals. A pessimistic mood starts to grow, at least for a while. Oswald Spengler writes his influential *Decline of the West,* and some philosophers and historians unearth Vico's hypothesis of historical cycles. Now the law has changed from progress to life cycle. Finally the Nazis come and proclaim the West is in truth decadent but promise to save the Aryan race from the decline of everyone else. They establish the *Tausend-jahriches Reich,* which mercifullly lasts only 12. During this short period they manage to destroy the most advanced scientific community in the world -the one that created the theories of relativity and quantum mechanics in the course of two decades.

During the war that put an end to the Nazi savagery we were promised - by Roosevelt and Stalin, by the *Reader's Digest* and *Soviet Literature* - a resumption of the line of progress as soon as the war was won. Moreover we were promised heaven on earth: plenty, freedom, brotherhood, and continued scientific and technological advances - momentarily halted by the war effort. Numbed by our own propaganda, many of us failed to hear the atomic bomb explosions at Hiroshima and Nagasaki - or else heard tham as heralding the coming of the millenium of plenty, freedom, brotherhood, and overall progress. Such was our moral sensitivity, our political cunning and our historical perspective after the long-drawn and cruel war. True, soon thereafter the Cold War started and the coming of the millenium was postponed once more. But at the same time all the creative forces tied up by the world war were unleashed, and we did witness progress on almost all fronts except that of civil liberties. In particular we witnessed an unprecedented scientific and technological boom. This progress continued at a terrific pace until a few years ago and has given us, among many other things, high energy physics, molecular biology, and the computer. It was only in the mid-sixties that the first signs of slackening appeared.

Two indicators suggest the near end of the era of scientific and technological progress resumed in 1945. One is the radical shift in science policy effected towards the end of the 1960's by the U.S. government, which thenceforth reduced its support of basic science and favored instead medicine and certain branches of engineering. This policy is still in effect and has had two clear results. One is the discontinuation or at least weakening of a number of research projects in basic science. Another is the disheartening of many investigators and students; in particular, the latter have become disillusioned with science as a useful and valued way of life.

A second event that shook faith in the continued progress of pure and applied science was the student revolt against American involvement in the Vietnam war. Many of the angry students went farther and proclaimed their opposition to the entire "establishment." And not a few, mistaking the misuses of technology for technology itself, and technology for science, declared war on science as part of their offensive against the "establishment." Some even went as far as rejecting reason altogether and took refuge in various cults and in drugs - not realizing of course that in this way they eliminated themselves from all useful action.

The net result of these developments is that science no longer enjoys the unanimous support of the population in North America, the United Kingdom, and other areas. This is enough to fear that it may have entered a period of decay. To be sure, this crisis, if it exists, is not conceptual or methodological: it is social and psychological. Social because basic science is not allotted sufficient support. Psychological because there is widespread scepticism, particularly among the young, about the power of science to provide an understanding of reality as well as the means to improve our way of life. Science is even blamed for the deterioration of the environment and accused of being "the ideology of the ruling class." Do not underrate this counter ideology. It is being embraced not only by misguided and im-

mature critics of the powers that be, but also by some very talented young people. And it is no longer restricted to North America: only recently 40,000 Italian students met in Bologna chanting *"No al lavoro, no alla scienza."*

5 SCIENCE CRISIS: TEMPORARY OR TERMINAL?

It would seem than that a science crisis is impending, even though scientific productivity may go on undiminished for a few years because it is the outcome of projects started before the budgetary restrictions and the decline in student enrollment. If one can speak of a science crisis in the same breath as the energy crisis, the urban crisis, and the food crisis, then the question is whether it is a mere temporary slump or a deep ("structural") crisis - whether we can get out of it, as we did in 1918 and 1945, or whether it will become permanent and thus end up in the collapse, not just of science but of modern civilization.

Surely few if any have formulated the question in the above terms. Most people take modern civilization for granted and, with it, science and technology. So much so that, at least in academic circles, the very question whether science has a future is likely to raise plenty of eyebrows. But we should know by now that optimism, while strong when tied to truth, is debilitating when it rests on falsity. Instead of just counting the optimists, the pessimists, and the don't knows, we should try to examine what possible reasons there are for thinking that the impending science crisis, if real, is either temporary or terminal.

I can think of at least four arguments for the hypothesis that science is passing through a merely temporary slump. The first is that modern civilization needs science. Indeed, there is no technological advance without research, and much of technological research depends on basic science. It is a question not just of borrowing from past achievements, because many of them become obsolete and also because technology and the economy are constantly running up against new problems that call for new theories, procedures, and data. So, if modern civilization - or industrial society in any of its versions - is to go on, science must go ahead. This may be called the *argument from economy,* or from the *practical value of science.* It is persuasive provided one accepts the antecendent, i.e. the thesis that industrial society is worth preserving. Not so otherwise.

A second argument is this. Modern society is becoming increasingly a service society: it needs physicians and dentists, veterinarians and agronomists, electricians and electronics experts, accountants and programmers, and so on and so forth, in increasing numbers relative to workmen and farmers. And you cannot train them properly, let alone contribute to the improvement of their techniques, unless you have them master the fundamentals of science. This is the *argument from services,* which is really complementary to the previous argument rather than independent. Again, it holds water if and only if one cares for the service society.

A third argument is this. Scientists and their auxiliaries - technicians, librarians, etc. - have become a substantial part of modern society, so their needs and aspirations can no longer be ignored by any politician. Thus, the *argument from politics,* carries little weight, because scientists and, in general, intellectuals, are politically naive, can be led by the nose, and are harmless.

A fourth argument is that science has become the brain of modern culture. Modern schooling involves science curricula, and the public demands scientific literature: just look at the circulation of *Scientific American* and its parallels in other languages. This is the argument from the *cultural value of science.* It has a point but not a big one. Indeed, remember that millions of children and youngsters can pass through many schools without ever being exposed to any serious science courses; and remember that the readership of the scientific and parascientific literature is far more restricted than that of pseudoscientific and antiscientific publications.

All four above arguments come down to this: Modern civilization, West and East, involves science; hence, if it is to continue science has got to have a future. Unassailable. But who assures us that modern civilization will continue? There is no such guarantee: no law of continuity, let alone of progress. On the contrary, the prospects are currently rather bleak. For one thing the material resources, in particular the energy and mineral resources, may become exhausted before the right decisions and the lucky inventions have been made to replace them. Surely the depletion process may take a few centuries, but this only postpones the collapse. Surely, also, scientists and technologists may come up with something to avert it - but only if they feel motivated and get the necessary support. But even the present generation of scientists and technologists may not be renewable: after all, scientists are not trees, and they do not grow in hostile environments. Succeeding generations may lose interest in science and even in industrial society. If this happens we, or rather our offspring, are in for a new Dark Age.

Remember: Augustine and his contemporaries did next to nothing to preserve the ancient Graeco-Roman culture. They undermined it or, what amounts to the same, let it rot away. They could not have cared less for Greek mathematics, science, and naturalist philosophy - all of which had been buried in books for several centuries anyway. Could not something similar begin to happen right now under our own noses? Not that we have started to burn scientific memoirs or stone mathematicians. But we may have started sliding into corruption and indifference. Let me explain.

I submit that the barbarians are already among us and that they have already started to undermine the citadel of science. They are the uneducated, unemployed young people who have become disillusioned with the world of their parents and refuse to learn or enjoy whatever gave pleasure to them. But they are not the only ones to blame for the current science slump. Let us also count the parents, teachers and so-called spiritual leaders who have been unable to teach the values by which modern culture was built and enriched. Let us not forget the military leaders who manipulated science as a maidservant of war technology, no less than the scientists and technologists who served them. And the business leaders who claim to use scientific procedures to design and test their products when in fact they don't and as a consequence often sell us poison. And the labor leaders who could not care less for culture. And the politicians and civil servants who try to control the way scientists and technologists work, allegedly in the higher interest of the people but actually, in many cases, in their own. And those intellectuals who are perfectly satisfied with the *status quo* or, if dissatisfied, do not dare voice criticisms, let alone constructive proposals for reform. Last, but not least, let us count among the modern barbarians the barren scientific workers as well as those who produce only pot boilers. They contribute powerfully to the decline in the prestige of science by skirting deep problems, engaging only in short-term projects, and fearing new ideas: they are not the keepers of the sacred fire of research but just come close enough to keep warm. All these people are eroding public confidence in science and are thereby undermining the very core of modern civilization. They are the ones we must try to convert to the old and simple faith that science is good *for its own sake* not just because it brings wealth, health - or war.

6 CONCLUSION

The end of my analysis is this. First, although there are physical and biological limits to what man can know, they need not impede scientific progress: the collection of knowable facts is a nondenumerable subset of the total set of facts. Second, the really important constraints upon the evolution of science are social -economic, political, and cultural. We cannot learn faster, or get to know more deeply, than our society will allow us. Modern science is neither a matter of private erudition nor one of solitary speculation: it is a social concern involving millions of people and untold physical resources. If a society were to lose all interest in science there would remain neither human nor material resources to carry on scientific research. Third, we may be on the brink of a science crisis that could plunge us into a new Dark Age. To avert such a catastrophe we must portray for the public the true nature of science, so that it may continue to attract many of the brightest of the young, and deserve the support of enlightened administrators or politicians without making any false promises. To do so requires that we stop emphasizing science merely as the provider of bounty or the handmaiden of power. We must instead portray science for what it is: an effort to understand reality, an expression of the uniqueness of man, his desire to know.

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COMMENTS FROM BRIAN C. CURTIS, CHAIRMAN

Public Affairs and Public Informantion Committee

As Chairman of your Public Affairs and Public Information Committee, I applaud Professor Bunge's exhortation to communicate the true nature of science to the public, to administrators, and politicians.

HOW?

Interaction with youngsters: at their schools, as host in your lab, via videotape on public TV. What are you doing - why are you doing it - what satisfaction does it give you?

Interaction with scientific administrators at state and federal levels, preferably on one page. I, for one, was embarrassed by the 23 responses from the 18,000 FASEB members to Dr. Gil Omen's request (FASEB Newsletter, July 1978) for letters. There are many administrators of science at NIH, NSF, and FDA, to name a few agencies, who complain of a lack of communication with working scientists.

Communication with the Congress is vitally important because they provide our funding. Could you write, on one page, what you are doing and how it fits into a larger picture? Could you explain how your research enriches the education of your students? Could each of your students convey what their federally supported fellowship means?

Why one page? Attention spans are short on Capitol Hill -issues are many - days are short. One page also encourages us to distill our thoughts. All too many congressional offices have never heard from the scientists in their constituencies.

Porfessor Bunge's tone is, in my view, unduly pessimistic but certainly not far fetched. Are we going to sit by and allow the most sophisticated science establishment yet known to crumble for lack of communication? The Public Affairs and Public Information Committee cannot do the job alone. We need your help -now.

EPITHELIAL TRANSPORT SECTION

In the Spring of 1979 the Epithelial Transport Section Steering Committee under Chairman Sandy Helman drafted Bylaws and conducted elections by mail to approve the Bylaws and select officers for 1979-1981. The Bylaws were approved, Ernest Wright was elected Chairman and Arthur Finn, James Schafer and Kenneth Spring were elected to the Steering Committee. The Bylaws were subsequently submitted to Council for approval.

The 6th annual meeting of the group will be held at the Federation Meeting in Anaheim on Wednesday, April 16, at 8:00 p.m., 1980 in the Arcade Room at the Inn at the Park. George Sachs will speak and his topic will be "Epithelial Plasma Membrane Vesicles at the Frontier" and Robert Crane and Ulrich Hopfer will lead the discussion. All members and non-members are invited to attend.

At the Federation Meeting the symposium sponsored by the group and the Physiology Society will be held on Wednesday, April 16, at 1:30 p.m. and the topic will be "Epithelial Transport in Tissue Culture" and speakers include Dayton Misfeldt (Stanford), Marcelino Cereijido (Mexico, D.F.), Milton Saier (UCSD), Joseph Handler (NIH) and Chester Bisbee (UCB).

Ernest M. Wright
Chairman, Epithelial Transport
Section 1979-1980
Department of Physiology
UCLA, Los Angeles, CA 90024

NEWS FROM SENIOR PHYSIOLOGISTS

Ernest A. Siegel to Hal Davis:

I have prepared a survey of the use of guided electrodes for the study of subcortical areas and for production of circumscribed lesions in the human subcortex. This application of the Horsley-Clarke method to the hitherto more or leess unaccessible human subcortex initiated by me, together with my surgical friend Wycis, in 1947 has developed into a new neurological subspecialty. In recognition of this development, the German Neurological Association bestowed upon me the Erb medal, their highest award.

Gustav Eckstein to Horace Davenport:

I am in good condition. Recently they unveiled my John the Baptist head and I made a speech and felt the flattery like the October wind.

Anna Baetjer to Hy Mayerson:

I am still teaching and conducting a little research here at Hopkins. Force of circumstances at Hopkins many years ago diverted me from pure physiology to occupational health. Physiology has proven a very sound base for industrial health studies. I have enjoyed it immensely.

S. A. Asdell to Hy:

I live with my son and daughter deep in the country about 50 miles northwest of Washington, D.C. (Frederick, Maryland). I watch the growth of fruit, vegetables and flowers. Bird watching from the kitchen window is a favorite occupation. We have had friends and relatives visit us from England which provides some diversion.

Warren Rehm to Horace:

I retired June 1978 as Chairperson of the Department of Physiology and Biophysics at the University of Alabama in Birmingham. I wanted to continue my research and accepted the offer of Mat Freund at Southern Illinois University to become a visiting professor for the academic year of 1978 and 1979. It was a delightful year. I gave an advanced course on transport and gave some lectures in Mammalian Physiology. I highly recommend this type of visiting professorship to those senior physiologists who are about to reach retirement age. Following my year as visiting professor, I returned to my former university, University of Louisville where I am research professor in the department of medicine, section of nephrology, where I have been provided with an office and laboratory space. It is wonderful to be in the lab without administrative burdens.

Frederic A. Gibbs to Hal:

Erna and I are both well and keeping busy with EEGS recorded in our office, some on patients who come to our office but more transmitted by telephone from small hospitals in out-lying parts of Illinois and surrounding states. We also get quite a number sent to us by mail or UPS. We used to say that the time would come when every large hospital would have an EEG service. It never occurred to us that even the smaller hospitals could have an EEG service for their patients.

William C. Buchbinder to Bruce Dill:

Where do you find the comaradarie in any Society except APS where a Past President salutes one his unknown subjects.

I spend 80% of my time at the piano. That was my original venture prior to medicine and for many years and at frequent intervals, I did much chamber music of Shubert, Mozart and Brahms with excellent strings in the orchestra. I hope to continue this good pastime.

In eight years the APS will be 100 years old. Lets have music, for its achievements are deserving of it. Maybe I can participate. I'm just a good amateur and get away with it when strings drown me out. Come to think of it if there's music at the 100th anniversary maybe you'd better cancel out Buchbinder who will be 94.

In 1988 every living Past President will have to make a speech. Get ready.

Samuel L. Leonard to Hal:

When my wife and I are not traveling, I go to the office every day to read and consult with graduate students. Recently, I hypophysectomized a group of rats for some students and showed them how it was done. If research money ever becomes scarcer than it is now, future students better know how to do it on their own. This is only one example of the sort of activity that is very worthwhile.

Newell Stannard to Bruce:

In my view Physiology is the "Queen Mother" of the biomedical sciences and the "Queen Mother" is showing few if any of the marks of becoming a dowager. Perhaps it was starting with Wallace Fenn and Edward Adolph and before that a few months with Bruce Dill that nailed me to the field forever. We moved from Rochester to San Diego in 1977. I have an appointment at the University of California, San Diego as Adjunct Professor of Community Medicine and Radiology. The teaching demands have been light but the presence of a professional affiliation, library and colleagues is very important. It is a strong school with excellent students and I am proud to play a small part in it. I am preparing a book on the history of radioisotope research with emphasis on the biological effects and the "metabolism" of elements of special importance to the nuclear energy field. It is a three-year job and now I am in library research and the fun of interviewing many who "were there when." Emphasis will be on the 1940-75 era. Anyone with suggestions please write or phone me. My other major activity concerns the National Council on Radiation Protection and Measurements, based in Bethesda.

San Diego is a beautiful place and we try to take advantage of all the activities, cultural, sports, community and educational. We cannot tear around with abandon but would appreciate hearing from anyone from the "earlier days," including anyone in this area who might like a lunch table chat or other contact.

Leah Staling to Hy:

I retired in 1978 but was promptly reappointed as a Visiting Assistant Professor. In the establishment of a Myo-Oral Facial Pain Clinic in our Physiology Department (Univ. of Maryland Dental School, Baltimore), we have a "first" in Dental education, in a working liaison of preclinical and clinical disciplines. The Clinic grew out of my EMG research lab in physiology and is now well on the way to permanent incorportation in a teaching program that crosses all four years of dentistry, two years of dental hygiene, and continuing education for post-doctorates and clinicans, emphasizing basic neurophysiological correlation with occlusion (which is what dentistry is all about). Physiology is the

basis, in this health care field, as in all others, of all really enlightened and updated teaching approaches. I might add also it is just real good fun.

As for extracurricular activities, my avid interest in horticulture is manifested by a thriving tree farm on our twenty-seven acres. With family, friends, colleagues and students, life is indeed full and rewarding.

Ruth Conklin to Hal:

I am still going strong, much involved with Vietnamese refugees right now.

Phyllis Bott to Hal:

Thanks for your timely greeting on my untimely birthday. I'm still busy but the direction of my efforts has changed from basic to practical.

David E. Bass to Bruce:

Since my retirement from active research in June 1973, I have created a new career based on my earliest ambition and love, that of a teaching professor, with no interests other than serving the needs of students. In addition to course lectures, I provide guidance on academic, research and career goals to students ranging from undergraduate, pre-meds, pre-nursing, graduate and medical students. If I had a business card, it would read, "Utility Professor of Physiology - Have Course, will Travel. Short Term Appointments Accepted." I have been teaching a course in Human Physiology which is not team-taught. Team-teaching leaves much to be desired and may be compared to being a worker on an auto assembly line, and who never sees the finished product. From the student's viewpoint, there are certain advantages to having most of the lectures from a single insturctor. In my own case, I can, in early lectures, foreshadow concepts that will be discussed much later without having to wonder whether a fellow team-teacher will cover the later material as I envisioned it for the students. A case in point is treatment of blood gases and control of alveolar ventilation, with a foreshadowing of later lectures on renal regulation of acid-base balance; when details of the latter are discussed, I can confidently refer to the earlier lectures, and the earlier foreshadowing is firmly driven home to the class, along with current material.

I have been doing the foregoing for the past 6 years. Boston University, Simon Fraser University, University of Nevada, Las Vegas and now at University of Hawaii. I have no plans as yet for 1979-80 and will be receptive to interesting possibilities.

K. S. (Kacy) Cole to Bruce:

I resigned at NIH in 1966, was retired and rehired in 1970 and shook loose as emeritus in 1978 to come to San Diego. Each step has been more fun. I'm trying to tag along with cell and tissue impedances and noise - which are stepping out as is so much else on membranes. A bob-tailed autobiography was in *Ann Rev. Physiol.* in 1979. Also I decided that Hodgkin and Huxley didn't really need the responsibility for my voltage clamp given them in *Scientific American* - so I complained last June.

I've recently put together a Wanderer radio controlled glider, but I still can't fly the thing at Torrey Pines and have to be rescued almost every time it is up.

Ancel Keys to Horace:

I've been immersed for several years in my book, "Seven Countries - Death and Coronary Disease in Ten Years." It should be published this year. We have done some extensive traveling -lectures in Rio for the International Congress on Nutrition, Lisbon and Geneva, and to Athens for follow-up work on Greeks we examined in 1960, to Singapore to give a lecture and on to Hong Kong to rest before going to Japan to give a lecture inaugurating the new Noboru Kimura Medical Foundation. Back to Minneapolis at 15° below zero. We return to Italy for a longer time in the winter getting back to the States in March for six weeks. I coined the name "Minnelea" now official for our restricted area, following the example of Minneapolis which is coined from "Minne" meaning waters in the Chippewa language and "polis" from the Greek for a populace. "Elea" is the name of the Greek city about four miles down the coast from our villa, founded in 535 B.C. with vast ruins now being uncovered by archeologists. So we live at the "waters of Elea."

I never did much enjoy teaching but I think with pride of being a physiologist. Actually for many years my interest has been focussed on the epidemiology of heart disease and the relation of the plasma lipids to the diet. We are going back to the island of Crete to make follow-up examinations on some 400 survivors of the men we examined 19 years ago. The lifestyle of the Greeks is changing, notably in the diet and level of muscular work. I am more and more following the way of the farmers in the Mediterranean area - lots of physical work all year in the open air, diet low in meats and dairy foods, very high in vegetables and fruits. Margaret and I are working on a book aimed at readers in Italy and nearby Greece, southern France and Spain, on the simple Mediterranean diet as opposed to the vogue for richer meals on the U.S., northern Europe pattern.

Carl A. Dragstedt to Bruce:

How does it feel
To become eighty-four,
and still have good hope
That there may be some more?

It is the ninth inning,
And there are two outs,
And I've heard the "Strike-Two,"
Which the umpire shouts.

As the bat that I hold
Seems so heavy for me,
There may be some doubt
That I forestall "Strike-Three."

Do I feel short-changed,
Or have any complaint,
Am I fearful or sorry?
To be honest - I aint.

Jane Sands Johnson to Hal:

Two high points mark 1979 for me. I returned on December 6 from a two-month visit to the Orient. My Betty's daughters, Jane and Carol, went with me. They were wonderful companions as well as being ears, eyes, and baggage carriers. The girls and I were in Hong Kong, Macau, Taiwan, Korea, and Japan. We joined a tour and had eight days in Red China seeing three cities. On the return flight we had two days in Honolulu. The trip didn't overtire me and I know I'm a better person for having gone. We can learn a lot from Red. China.

The other high point - now I'm a great-grandmother.

Joseph H. Holmes to Hy:

I am still functioning as editor of the Journal of Clinical Ultrasound, but hope to relinquish these duties soon. I am trying to put together a history on the development of diagnostic ultrasound. Some of the early pioneers are still alive and able to fur-

nish historical material. We are putting together three tapes on the early history of ultrasound.

Other goals I would like to achieve include the completion of a study on polycystic kidney disease. We have collected data on some 600 patients or family members. From a better understanding of rate of cyst growth, achieved through continued observation, we might be able to prolong life of this group significantly.

Another goal would be the analyzing of a great amount of collected data on fluid intake in both the normal population and in those with various types of diseases. The primary problem is that of analysis and to assess the relative roles of thirst and drinking habit patterns in determining fluid intake in normals and in patients with cardiac disease, liver disease, renal stones, etc. Readjustment of fluid intake patterns is one approach in managing patients with heart disease, kidney disease and idiopathic edema to prevent the development of edema.

As an additional goal, there is the furtherance of ultrasound as a tissue characterization technique which may make it possible to study a tumor in vivo and predict precisely the nature of that tumor. One of the current problems in diagnosing tumors by ultrasound has been the fact that some tumors are echogenic and some are sonolucent. If the reason for these differences in echo patterns can be pinned down, then perhaps a more precise in vivo diagnosis of the type of tumor will be possible.

LAB COURSES AT WOODS HOLE

Marine Biological Laboratory, Woods Hole, Massachusetts, will conduct a series of residential laborartory courses as follows:

April 20--25, 1980 *Biological Electron Microscopy for Technicians,* Instructors in chief, Morton Maser, MBL, and Ann Scarborough, Louisiana State University; April 27-May 3, 1980, *Analytical and Quantitative Light Microscopy in Biology, Medicine, and Materials Sciences,* Shinya Inoue, MBL; May 4-9, 1980, *Freeze-Etching in Electron Microscopy,* Russell Steere, USDA, Beltsville; May 11-23, 1980, *Electron Microscopy in the Biological Sciences,* Blair Bowers, NIH, and Morton Maser, MBL; May 18-23, 1980, *Small Computers in Biomedical Research,* Larry Palmer, University of Pennsylvania. Application materials and additional information may be obtained from Admissions Office, MBL, Woods Hole, MA 02543; telephone 617-548-3705.

JOHN MERLE BRUHN
(1904-1978)

Each of us makes our unique journey through life. Some make big splashes; others proceed in a more subdued manner. John Merle Bruhn belonged to the latter category. He had a profound, yet often unknown to most, influence on the campus of the Medical Center of the University of Alabama in Birmingham.

John was born to Mary and Peter Bruhn of Flandreau, South Dakota on October 10, 1904. For his high school education, John went to the "big city" of Sioux Falls. Following graduation, he attended Carleton College in Northfield, Minnesota on an academic scholarship. During these years, the budding physiologist wanted to understand how mechanical gadgets worked. John developed and maintained an extraordinary ability with mechanical things and also became an excellent photographer using his abilities equally in the laboratory and around the world, especially its rivers, lakes and seas, which he loved.

Having been graduated with distinction from Carleton College in 1929, John Bruhn traveled to New Haven, Conn. where he matriculated in the Department of Physiology, School of Medicine, Yale University. Midway through his graduate career, in the depths of the depression, he married Ohio Wesleyan graduate Vera Grund in 1931. Two years later, at the time of his being awarded the Ph.D. degree, he was appointed Sterling Fellow in Physiology. In 1934 he became *the* resident physiologist at the Primate Biology Laboratories at Orange Park, Florida. When the Bruhns left New Haven, they expected to be back four years later, but instead in 1937 John accepted the position of Instructor in Physiology and Pharmacology in the (then two year) School of Medicine, University of Alabama.

While Dr. Bruhn was progressing through the professorial ranks, the School of Medicine became a four year degree granting institution and moved to Birmingham, Alabama in 1945. At that time Dr. Bruhn was Professor and Chairman of the Department of Physiology and Pharmacology. During the changes in 1948 which included establishment of the School of Dentistry, a separate Department of Pharmacology was established, John retaining his Chairmanship of Physiology until 1965. He retained, however, the chairmanship of the Admissions Committee of the School of Medicine, a position he held for 33 years.

Dr. Bruhn's love for physiology and his ability to expain in lucid detail the function of the human body benefited the thousands of medical, dental and graduate students who obtained their education in Birmingham.

Attendance at the Federation Meetings was one of his professional renewal activities and for many years John Bruhn never missed a meeting. Always unassuming but always willing to serve, at the time of his death in Birmingham on April 16, 1978, though retired, he was still actively serving as a member of the admissions committee and in other capacities as the administration made requests of him. This neurophysiologist truly served his discipline and his medical center.

In addition to his widow, Vera, his two children, Dr. John Richard and Mary Ellen, and six grandchildren, he left behind many friends and colleagues whose lives he enriched.

Geraldine M. Emerson
U.A.B. Medical Center
Birmingham, AL 35294

Editor's Note: A fuller statement of Dr. Bruhn's life and activities, with several photographs are on deposit in the APS Archives.

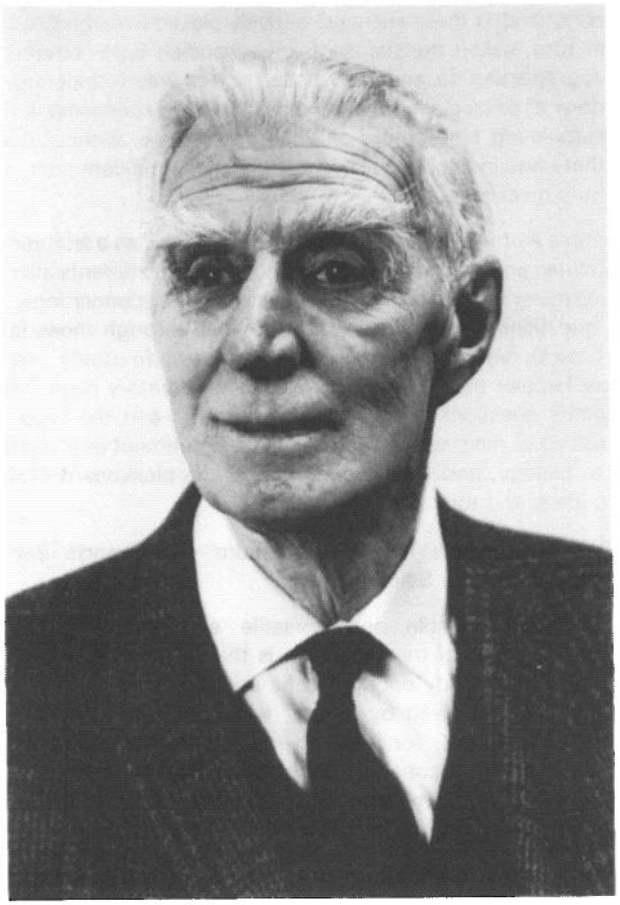
IN MEMORIAM

ALAN CHADBURN BURTON

(1904-1979)

Alan Burton, Professor-Emeritus at the University of Western Ontario and one of Canada's most distinguished biophysicists, died suddenly on June 27th, 1979. He was born in London, England, and received the B.Sc. degree with First Class Honors in Physics and Mathematics from the University of London. After teaching high school Physics in Liverpool he immigrated to Canada at 23 years of age, becoming a graduate student in Physics at the University of Toronto. He obtained the M.A. and Ph.D. degrees from Toronto, for research on 'Absorption spectra of the major planets' and 'Heating of electrolytes by high frequency currents,' respectively. The latter topic was then of great medical interest, since artificial fever by electromagnetic heating was in vogue, and it led him to join Professor Murlin, at the Department of Vital Economics, University of Rochester, in work on the heat exchange of man and animals. He did research on human calorimetry and became attracted to the virtually undeveloped field of Biophysics. During tenure of a Rockefeller Foundation Training Fellowship at the Medical School of the University of Pennsylvania, Burton undertook research with Professor Bazett on peripheral blood flow in man and learned, in the process, that 'Physiology is fun.' This was followed by four years as a research fellow at the Johnson Foundation for Medical Physics, University of Pennsylvania, working with Dr. Detlev Bronk on the study of human temperature regulation. On the outbreak of World War II, he returned to Canada and worked as a National Research Council Research Associate at the Banting Institute, Toronto, on aviation medicine and the development of protective clothing for all services. Much of this work was classified, but it was obviously considered significant, for he was later awarded the M.B.E. for his contributions during the war.

In 1945 Burton came, by invitation, to the Department of Medical Research, University of Western Ontario, and in 1948 was appointed Professor and Head of the new Department of Biophysics, the first such department in a Canadian Medical School. Under his leadership, Western became the first Canadian University to offer graduate degrees in Biophysics and he personally trained 14 M.Sc. and 22 Ph.D. students. Canada's first undergraduate program in Honors Biophysics was also started by him at Western in 1966 and the program still bears much of the imprint of its founder. His work received international acclaim and he is the only person to have been elected President of the American Phsyiological Society (1956), the Canadian Physiological Society (1959), and the Biophysical Society (1966). He served as Chairman of the Federation of American Societies for Experimental Biology (1957-58) and of the Canadian Federation of Biological Societies (1963). He received a Gairdner Foundation Award (1961), and the degrees (Honoris Causa) of LL.D. from the University of Alberta (1964) and D.Sc. from the University of Western Ontario (1974). Burton is known, internationally, for his work on temperature regulation, the physics of the circulation, the mechanics of the red cell and, more recently, on the fundamental mechanisms and epidemiology of human cancer. His book "Man in a Cold Environment," published with Otto Edholm in 1955, is still regarded as a classic in that area. His second book "The Physiology and Biophysics of the Circulation," published in 1965 and revised in 1972, was written for undergraduate students and has had a very wide acceptance, being translated into French, German, Italian, Spanish and Japanese. Although he



retired on the 30th of June 1970, he remained very active in undergraduate and graduate teaching, and in scientific research. His last paper was accepted for publication "as is" on May 3rd, 1979, and shortly before he died he made the final revisions to the manuscript for his third book "Understanding Human Cancer: The Physiological and Biophysical Point of View"; this monograph is now at the galley proof stage and is to be published shortly. It was on his way home from the laboratory that he collapsed and died, at the age of 75 years.

Alan Burton was one of the first physicists to become a biophysicist, and he summarized the challenges of this discipline in a paper written for Canadian Physicists (1953) entitled "Biophysics, Humble Pie for Inflated Physicists"! His unusual background and logical mind led to completely new and unconventional approaches to biological problems. During the early years at Western, when combined research seminars were held in the Medical School once a week, his exceptional ability to pose deceptively simple questions became legendary. At Medical Grand Rounds at Victoria Hospital, he could be counted on to enlighten clinicians and students with his penetrating questions and discussions of difficult clinical problems. Graduate students discovered that coffee break with Dr. Burton was the best course they ever took, a Socratic experience in the classical tradition. Dr. William Pace, who knew him well, has contributed the following delightful description of life in Burton's laboratory in the early 1950's:

"Having been brought up in the English tradition, Dr. Burton insisted that there be a tea period each morning and afternoon, created a space for this activity where none had been available and make it virtually mandatory that every graduate student attend faithfully twice daily. As you can well imagine, with his booming voice and great hulk of a body, he completely commanded these seances, actively picked everybody's brain in turn, asked questions of an outlandish type, offered encouragement to anyone whose project was foundering and drew all of us closer to each other. I never experienced a more stimulating time before or since and I knew, without doubt, that I was in the presence of not only a magnificent brain but a truly great person."

Burton's enthusiasm was infectious and his skill as a lecturer was renowned among undergraduate and graduate students alike. As an examiner he was dreaded, because of the disconcertingly simple questions he asked (peering the while through those bushy eyebrows); yet his fairness could be relied upon utterly. He was never happier than in an undergraduate laboratory class, asking students questions about their experiments and the wider implications of their results. He used each experiment as a 'window' on to biology, and always ended up at the chalkboard expounding ideas and topics for research.

One of Burton's favorite quotations was Francis Bacon's description of 'The Scientific Mind':

"A mind, nimble and versatile enough to catch the resemblances of things, which is the chief point, and at the same time steady enough to fix and discern their subtle differences, endowed by nature, with the desire to seek, patience to doubt, fondness to meditate, slowness to assert, readiness to reconsider, carefulness to set in order, and neither affecting what is new nor admiring what is old and hating every kind of imposture."

Burton exemplified much of this himself, except that he never was slow to assert! However, he showed a willingness to reconsider, and always put his opponents on their mettle to produce convincing arguments in support of their contentions. He will be most remembered by students for his ability to make understandable to non-physicists the application of physical laws to biology. He believed, and exemplified in person, that the arousal of interest is paramount to success in teaching. He made the study of physics of living things relevant, exciting and, above all, fun.

Dr. Burton is survived by his wife Clara, son Peter, two grandchildren, and a large community of scientific children and friends. It was his family's wish that memorial contributions be made to:

The Dr. Alan C. Burton Prize Fund,
c/o The University of Western Ontario,
London, Ontario, Canada.

This prize of $300 is awarded annually to the undergraduate student with the highest 'A' standing in Year III Biophysics courses, the courses Alan Burton loved most to teach. Each gift will be acknowledged and an official receipt issued by the University.

When I phoned a colleague of Dr. Burton's and broke the news to him, he said, reflectively, "That's really the end of an era, isn't it"? We have all shared this same awareness and have stopped in our tracks to reflect on Alan Burton - the man, his times and his achievements; not merely to admire or disapprove, but to learn what significant lessons we can learn from him as a man and as a medical scientist. His portrait, drawn by his former graduate student Dr. Alfred Jay, hangs in our Seminar room and the Quotation from Bacon is inscribed on the wall of the foyer of our Medical School. Each of us at Western owes much to Alan Burton, and his spirit will live on in the Department of Biophysics he created.

Alan C. Groom, Ph.D.,
Professor and Chairman,
Department of Biophysics,
The University of Western Ontario.

1. Biophysics, Humble Pie for Inflated Physicists. Alan C. Burton. Physics in Canada. *8*: 6-46 (1953).
2. A Virtue of Ignorance. A Biophysicist asks simple questions about medicine and Medical Research. A. C. Burton. Annals of the Royal Society of Physicians and Surgeons of Canada. *1*: 192-201 (1968).
3. Variety - the spice of science as well as of life. The disadvantages of specialization. A. C. Burton. Ann. Rev. of Physiol. 37, 1975.

SPLANCHNIC CIRCULATION GROUP

The Splanchnic Circulation Group was establshed in the Spring of 1979 in order to increase communication and cooperation between investigators interested in circulation of the liver and digestive organs. Currently, the group has 70 members including physiologists, pharmacologists, and clinical investigators. The second annual meeting of this group will be held on May 18, 1980 at the University of Utah Medical Center, Salt Lake City, Utah, during the Digestive Diseases Week. Topic of the meeting is Methodology of Splanchnic Circulation. Those who are interested in the meeting and/or in joining the group should contact:

Dr. C. C. Chou
Department of Physiology
Michigan State University
East Lansing, MI 48824

Editor: Orr E. Reynolds, Education Office, APS
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THE PHYSIOLOGY TEACHER

ADAPTATION OF RENAL AMMONIA PRODUCTION TO METABOLIC ACIDOSIS: A STUDY IN METABOLIC REGULATION

Leon Goldstein
Brown Universty
Division of Biology and Medicine
Providence, Rhode Island 02912

The human kidney excretes 30-50 mmol of ammonia per day (41). The main purpose of this excretion is the neutralization of hydrogen ions secreted by the renal tubules. During the metabolism of proteins, nucleic acids and phospholipids approximately 40-80 mEq of strong acids are produced per day; the sulfur-containing amino acids (methionine and cysteine) are oxidized ultimately to sulfate with the liberation of H^+ ions while the phospholipids and phosphoproteins are oxidized to phosphate, also liberating H^+ ions. These strong acids are neutralized by the buffer systems of the body, but the net effect of this neutralization process is to lower the buffer reserves and to increase the concentration of sulfate and phosphate in the body fluids. Since the total buffering capacity of the human body is about 1,000 mEq, only 20 times the amount of strong acid produced per day, maintaining the body's buffer reserves is critical for the acid-base balance of the individual. During disease states, such as diabetes mellitus, in which large amounts of metabolic acids (500 mEq/day) are produced, the situation is even more precarious (4).

The role of the kidneys in maintaining the body's buffer reserves is to eliminate the excess sulfate and phosphate or other acids such as ketone bodies, produced during metabolism, without loss of essential cations and to maintain buffer reserves at their normal levels. The manner in which the kidneys perform these tasks is fairly well understood (20,41). Briefly, the kidneys generate bicarbonate and hydrogen ions in the cells of the renal tubules by hydration of carbon dioxide. The hydrogen ions are eliminated in the tubular urine, in the form of titratable acid (mainly Na_2HPO_4 in normal urine) and ammonium ions (NH_4+), in exchange for sodium ions (and other cations) reabsorbed from the glomerular filtrate. The bicarbonate ions are simultaneously absorbed from the renal cells (along with reabsorbed sodium ions) into the peritubular capillaries and contribute to the body's buffer reserves.

The major source of ammonia excreted by the mammalian kidney is blood glutamine (37,51,53). This amino acid is extracted from the plasma and is deamidated as well as deaminated by the renal cells. The main pathway for renal glutamine deamidation-deamination is thought (17) to be the two-step reaction (shown in Fig. 1).

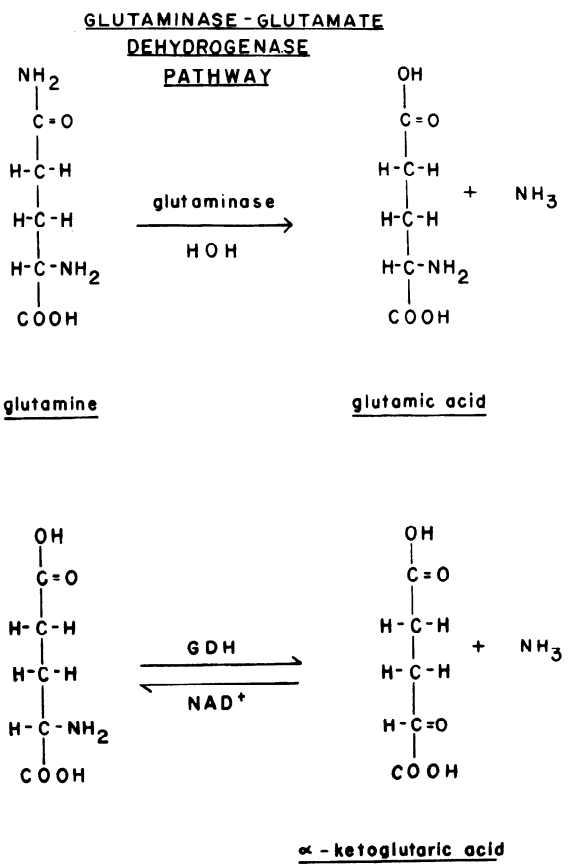

Fig. 1. Two-step reaction for the deamidation-deamination of glutamine in the renal tubule.

*Tutorial Lecture presented October 17, 1979 at the APS Fall Meeting, New Orleans.

Both the deamidation reaction catalyzed by phospate-dependent glutaminase and the deamination reaction catalyzed by glutamic acid dehydrogenase take place in the mitochondria of renal cells (13, 29,31). Several other enzymes have been proposed to play some role in renal glutamine deamidation-deamination (7,39,55) but their functional and quantitative significance is unclear at this point.

Continued metabolic acidosis leads to progressive increases in both the production of urinary ammonia and the extraction of glutamine by the kidney. Although the adaptation may take days to reach a maximum (41) it can be observed within hours after initiation of metabolic acidosis (22). The mechanism by which this adaptation takes place is still uncertain; the purpose of this lecture is to present past and recent theories that have attempted to explain how this adaptation might occur.

EXPERIMENTAL APPROACH

Studies on the mechanism of adaptation of renal ammonia production and glutamine deamidation-deamination to metabolic acidosis have employed a variety of in vivo and in vitro techniques. Experiments in intact animals have defined the in vivo responses and have established the foundation for in vitro investigations. Measurements of ammonia excretion, renal extraction and release of nitrogenous compounds and the fate of ^{15}N and ^{14}C-labeled tracers have clearly shown that the increase in renal ammonia production during acidosis is due to stimulation of the rate of glutamine deamidation-deamination in the renal cells (37,51,53). However, it is unlikely that in vivo techniques will provide much more insight into the mechanism of this adaptation since in vivo studies are complicated by a multitude of uncontrollable renal and extra-renal factors that can influence renal ammonia production in the whole animal. Therefore, several in vitro approaches have been used in the attempt to discover the mechanism(s) by which metabolic acidosis leads to an increase in renal ammonia production.

The techinque of freeze-clamping tissue (57) has been used in the determination of the levels of glutamine metabolites in the intact kidney. With this technique the kidney is either freeze-clamped in vivo or quickly excised and immediately freeze-clamped. The kidney is kept frozen while being powdered and treated with acid. The neurtalized acid extract is assayed for metabolite levels by specific enzymatic techniques. This procedure allows one to stop renal metabolism in less than a second and then assay the levels of metabolites in renal cells in the absence of interfering enzyme activities.

An in vitro preparation which closely mimics the operation of the kidney in vivo and allows complete control over extra-renal factors is the isolated perfused rat kidney (27). Although this system suffers from problems such as unphysiologically high flow rates it does provide the investigator with the opportunity to study ammonia metabolism in the intact organ uncomplicated by uncontrolled extra-renal factors. The preparation at the next level of physiological disintegration is the isolated renal cortical slice. Although almost all semblance of normal renal function is lost in this preparation it does offer the advantage of ease of handling and retains most of the metabolic functions of the intact kidney.

Enzyme activities are best measured in the absence of permeability barriers for substrates and products. Therefore, cell-free preparations such as tissue homogenates and extracts have been used in enzyme assay experiments.

Isolated renal mitochondria remain physiologically intact for up to an hour or more at 0°C. One can examine events going on in mitochondria under strictly defined and controlled conditions. They have been employed in the study of the transport of glutamine across the inner mitochondrial membrane and of the regulation of both transport and metabolism of glutamine at the mitochondrial level.

THEORIES ON THE MECHANISM OF ADAPTATION OF RENAL AMMONIA PRODUCTION AND GLUTAMINE DEAMIDATION-DEAMINATION

Assuming that the glutaminase-glutamate dehydrogenase pathway is the major route of conversion of glutamine to ammonia in the mammalian kidney, then the activity of this pathway must be controlled in vivo since the rate of conversion of glutamine to ammonia is stimulated several fold by metabolic acidosis. Thus, one or more factors regulating glutaminase and glutamate dehydrogenase in renal cells must be altered by acidosis. As shown in Fig. 2 there are several possible regulators of these two enzymes in vivo: substrates, cofactors, enzyme and product concentrations as well as the system that transports glutamine across the inner mitochondrial membrane. The major theories that have attempted to explain the mechanisms of adaptation of renal ammonia production to acidosis have been concerned with changes in enzyme levels, product concentrations and mitochondrial glutamine transport.

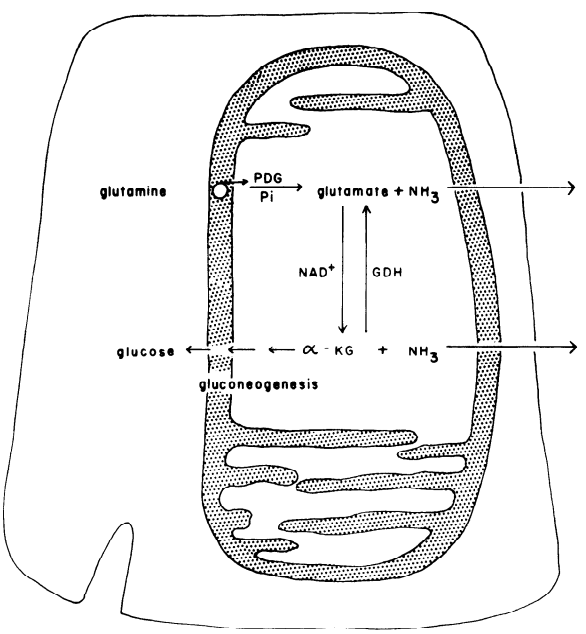

Fig. 2. Hypothetical scheme for the uptake, deamidation and deamination of glutamine by renal mitochondria. PDG, phospate-dependent glutaminase; GDH, glutamate dehydrogenase; NAD^+, nicotinamide adenine dinucleotide (oxidized); α-KG, α-ketoglutarate.

Enzyme concentration

Several studies have shown that the level of glutaminase in the kidneys of at least two rodents, the rat and the guinea pig, is altered by changes in acid-base balance. Davies and Yudkin (14) reported that prolonged acidosis stimulated the ability of rat renal cortical slices to convert glutamine to ammonia, and prolonged alkalosis had the opposite effect. Rector et al. (47) subsequently found the continued administration of NH_4Cl to rats produced parallel increases in the rate of ammonia excretion in vivo and the activity of renal phosphate-dependent glutaminase activity measured in kidney homogenates. On the basis of these two experiments, it was proposed that the adaptations of renal ammonia excretion and production were due to a rise in the level of

renal glutaminase. This hypothesis was supported by similar experiments done with the guinea pig (24). However, the hypothesis received a significant set back when it was shown that prolonged metabolic acidosis in the dog produced a significant elevation in renal ammonia production without any effect on the level of renal glutaminase assayed in tissue homogenates (46). However, one could still argue that the enzyme level might have increased in only one segment of the dog nephron and that this increase could not be detected by assay of glutaminase activity in a homogenate of whole kidney or even cortex. But, the hypothesis was dealt another blow by experiments (15) showing that administration of actinomycin D completely inhibited the acid-induced rise in renal glutaminase with no significant effect on ammonia excretion in the rat (Fig. 3). It may be concluded, therefore, that the adaptation in renal ammonia excretion to metabolic acidosis is not due to an elevation in glutaminase level in the kidneys of adult mammals. Furthermore, recent experiments clearly demonstrate that the magnitude of the response of ammonia excretion to an acid load is not affected by the level of glutaminase in the rat kidney (38).

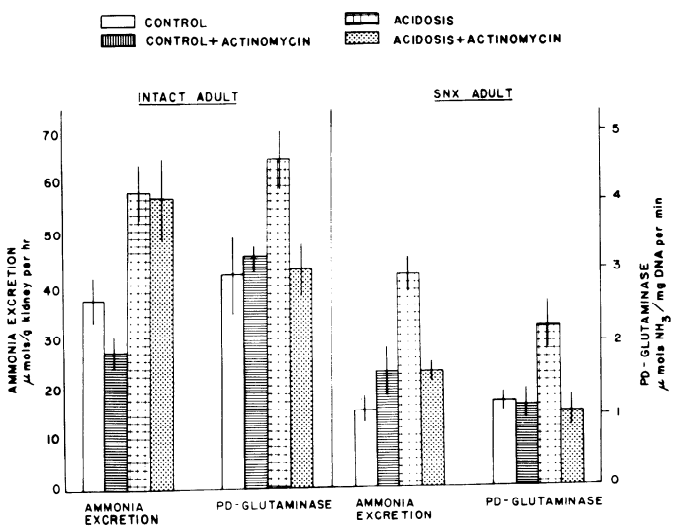

Fig. 3. Contrasting effects of actinomycin D on the response of renal ammonia excretion to metabolic acidosis in intact and subtotal nephrectomized (SNX) rats. Drawn from data in references 6 and 15.

Although an increase in the level of renal glutaminase does not seem to be a prerequisite for the adaptation of renal ammonia production to metabolic acidosis in the intact adult kidney, the situation appears to be different in the developing and regenerating rat kidney. The response of renal ammonia excretion to an acute acid load is significantly lower in developing rats than in adult animals (18). Several biochemical factors that might be responsible for the limited response of ammonia excretion in neonatal kidneys have been examined (23). The one that correlates best with the maturation of the response of ammonia excretion to acidosis is the postnatal increase in renal glutaminase activity. Prolonged metabolic acidosis both improves the response of renal ammonia excretion and induces a rise in the level of renal glutaminase in the infant rat kidney (5). In contrast to the situation observed in adult rats, administration of actinomycin D. completely blocks the adaptation of renal ammonia excretion to prolonged metabolic acidosis in infant rats as well as inhibits the rise in renal glutaminase level (5). This data suggests that a rise in the level of renal glutaminase is a prerequisite for the adaptation of renal ammonia excretion in the developing rat kidney.

Another condition in which the level of glutaminase in the kidney appears to limit the response of ammonia excretion to acidosis occurs in the regenerating rat kidney. Studies (6) done on subtotalnephrectomized (SNX) rats, in which all of one kidney and about half of the other are surgically removed, show that both the response of ammonia excretion to an acid load and the level of phosphate-dependent glutaminase are significantly reduced in the remnant kidney. Furthermore, the ammonia response is blocked when actinomycin D is given to inhibit glutaminase induction (Fig. 3). Thus, it appears that the induction of renal glutaminase is an important component of the adaptation of ammonia excretion to acidosis under conditions in which the enzyme content is low to begin with, and that a certain level of enzyme activity must be reached before a normal ammonia response can occur.

Glutamate dehydrogenase activity, assayed in tissue homogenates, is also elevated by prolonged metabolic acidosis (50). It is unlikely, however, that this increase plays a significant role in the adaptation of renal ammonia production for two reasons. First, this enzyme catalyzes a reversible reaction (Fig. 1) and an increase in its activity would accelerate both amination and deamination reactions simultaneously. Second, actinomycin D administration produced a general inhibition of protein syntheses in the experiments described above. It is likely, therefore, that the rise in glutamate dehydrogenase was inhibited during the time that renal ammonia excretion was increased.

Feed-back regulation of renal ammonia production

The second hypothesis on the mechanism adaptation of renal ammonia production to acidosis deals with the feed-back inhibition of renal glutaminase activity by the reaction product glutamic acid. Enzyme activities are often markedly affected by the products of the reactions that they catalyze (36). The effect of the product on the enzyme activity may be due to either mass action (in the case of immediate products and reversible reactions) or allosteric effects (in the case of irreversible reactions). A product of the glutaminase reaction with potent inhibitory allosteric effects on renal glutaminase activity is glutamic acid (16,48). Renal phosphate-dependent glutaminase activity is inhibited 50% at 1 x 10^{-3} M; the inhibition is of the mixed type: the Km for glutamine is elevated while the V_{max} is lowered (25). At the concentartion of glutamic acid found in the cells of the rat kidney (approximately 5 mM) the enzyme is inhibited about 90% by glutamic acid. Thus, glutamate could act as an effective regulator of renal glutaminase in vivo.

Induction of severe metabolic acidosis by administration of a high dose of NH_4Cl (e.g. 20 mmoles/kg) causes a sharp drop in the concentration of glutamate in the rat kidney (16,28,35) which roughly parallels the rise in renal ammonia excretion during the first 24 hours of acidosis (16). Prolonged administration of NH_4Cl beyond 24 hours keeps glutamate levels in a relatively constant, depressed state, while ammonia excretion continues to rise (Tabel 1). Thus, the two parameters no longer appear to be related after 24 hours of acidosis. Since glutamate is an inhibitor of renal glutaminase activity and its concentration is significantly altered during metabolic acidosis it has been hypothesized that the acid-induced fall in renal glutamate concentration may play a role in the adaptation of renal ammonia excretion to acidosis by decreasing the inhibition of renal glutaminase activity within the renal cells (16,45).

The feed-back inhibition hypothesis has been tested by several groups, but the results have not led to a general agreement as to whether or not the hypothesis is valid. In support of the hypothesis it has been shown that if the acid-induced fall in renal

glutamate concentration is prevented by administration of exogenous sodium glutamate, then acute acidosis does not increase ammonia excretion in rats (16). However, in a similar experiment administration of glutamate has been found not to inhibit ammonia excretion in acidotic dogs (10). Renal glutamate concentrations were not measured in the latter study, so that it is not certain that the glutamic acid administered to the dogs actually permeated the kidney cells and prevented the acid-induced fall in renal glutamate concentration. Administration of fluorocitrate has recently been shown to both increase renal glutamate concentrations and decrease ammonia excretion in dogs (8). Infusion of α-ketoglutarate reduces the inhibitory action of fluorocitrate on both renal glutamate concentrations and ammonia production. Although these experiments tend to support the glutamate feedback inhibition hypothesis it must be pointed out that both treatments probably altered the renal concentration of α-ketoglutarate in addition to glutamate and that α-ketoglutarate itself has been shown to have an inhibitory effect on renal mitochondrial ammoniagenesis (22).

TABLE 1: Effects of prolonged acidosis on renal glutamate concentration and ammonia excretion

	Day 0*	Day 1	Day 2	Day 3
Glutamate concentration (μmoles/g dry wt)	33 ± 2	23 ±1†	22 ± 3‡	27 ± 1‡
Ammonia excretion (μmoles/100 g bw X hr)	54 ± 7	86± 10‡	116 ± 6†	125 ± 10†

Values are mean ± S.E. of six animals per group.
*Acidosis induced by administration of 40 mmoles NH_4Cl/kg X day.
†Significantly different from control group ($p < 0.01$).
‡Significantly different from control group ($p < 0.05$).
Data from reference 25.

The evidence against the feed-back inhibition hypothesis is significant. As noted above, renal glutamate concentration does not continue to fall after the first 24 hours of acidosis, but ammonia excretion increases for several days. Furthermore, mild acidosis can produce significant increases in renal ammonia excretion and production in rats without significant alteration in total renal glutamate concentration (9). This latter observation appears to contradict the feed-back inhibition hypothesis. However, it is possible that mild acidosis may produce a localized change in glutamate concentration, e.g. either in the mitochondria or in a single nephron segment, that could have a significant effect on renal ammonia production but not be detected in an assay of total renal glutamate content.

It is clear that no convincing case can be made for or against the feed-back inhibition hypothesis in the regulation of renal ammonia production. Before this hypothesis can be accepted or rejected more information is needed on the effects of acid-base disturbances on glutamate concentrations in the mitochondria of specific nephron segments involved in the renal ammonia responses.

The cause of the fall in renal glutamate concentration during metabolic acidosis is not known for certain. The fall in glutamate maybe due to the stimulation of renal gluconeogenesis that occurs during acidosis (2,3,26). Since glutamate is an excellent substrate for gluconeogenesis (32), stimulation of this latter process would tend to lower glutamate concentration in the renal cells. However, it is possible to inhibit gluconeogenesis without significant effect on ammonia production in rat renal cortical slices incubated with glutamine in vitro (44). Thus, an alternative explanation for the acid-induced fall in renal glutamate concentration has been proposed. It has been suggested that the ratio of $NAD^+/NADH$ within renal mitochondria regulates renal glutamate concentration by the effect of this ratio on mitochondrial glutamate dehydrogenase activity (44). Although one group of investgators have reported an appropriate change in the $NAD^+/NADH$ in the kidneys of acidotic rats (42,43) two other groups (23,28) have failed to confirm this finding. Thus, the roles of both renal gluconeogenesis and mitochondrial $NAD^+/NADH$ ratio in the control of renal glutamate concentration, and therefore in glutamine deamidation-deamination, are still unclear.

There is a growing body of evidence which indicates that metabolic acidosis produces an increased flux of glutamate through the glutamate dehydrogenase (GDH) pathway. Slices prepared from the kidney cortex of acidotic rats deaminate (and deamidate) glutamine faster than slices from control rats (45). These effects are consistent with a stimulation of glutamate deamination via the GDH reaction as a consequence of increased gluconeogenesis. However, it has recently been reported that glutamate deamination is markedly stimulated in renal mitochondria isolated from acidotic rats (49,54). Since the step in gluconeogenesis thought to be stimulated by acidosis is located in the cytoplasm, i.e. the conversion of cytoplasmic oxaloacetate to phosphoenolpyruvate, it is highly unlikely that the increased flux through the GDH pathway is isolated 'acidotic' mitochondria is related to any change in gluconeogenesis. Thus, the cause of the increased flux through the GDH pathway is not known; it does not seem to be due to an alteration in equilibrium conditions associated with the GDH reaction (49).

Regulation of mitochondrial glutamine transport

In 1972 Pitts (40) suggested that permeability of the mitochondrial membrane to glutamine might be increased during metabolic acidosis and thus allow glutamine to penetrate more rapidly from the cytoplasm to the site of deamidation-deamination inside the mitochondrial membrane. The underlying assumptions in this hypothesis are that one or both of the mitochondrial membranes (inner or outer) are a permeability barrier to the passage of glutamine and that a carrier-mediated entry of glutamine into the mitochondria is limiting in the overall metabolism of glutamine by renal mitochondria. Although it is highly unlikely that the outer mitochondrial membrane acts as a glutamine permeability barrier there is experimental evidence to support the idea that the inner mitochondrial membrane acts as a rate-limiting barrier for the entry and metabolism of glutamine. First, the inner membrane of mitochondria isolated from a variety of tissues have been shown to be permeability barriers for a variety of polar metabolites; studies done in the past 10 years have shown that a number of transport systems are present in the inner membranes of these mitochondria that facilitate the passage across these membranes (30,33). Second, sonic disruption of pig kidney mitochondria significantly increases the rate of glutamine deamidation by these organelles (11). Third, structural analogues of glutamine decrease both the rate of entry and the subsequent deamidation of glutamine in isolated rat renal mitochondria (19). Finally, α-ketoglutarate, which has no effect on rat renal glutaminase activity can inhibit glutamine entry and deamidation in these organelles (22).

The nature of the glutamine transport system in the inner mitochondrial membrane is not at all clear. For example, there is disagreement as to whether glutamine is transported intact from the extramitochondrial to intramitochondrial compartment during its passage across the inner mitochondrial membrane (1,12). Since phosphate-dependent glutaminase appears to be present within the inner mitochondrial membrane with the active site fac-

ing the mitochondrial matrix (13), it is possible that glutamine transported across the inner membrane is deamidated as soon as it reaches the matrix side of this membrane. However, until some way is discovered to inhibit the glutaminase enzyme without affecting the glutamine transport system, the exact nature of the transporter will remain unknown.

The transport of glutamine across the inner membrane of renal mitochondria has been shown to be adaptive. Chronic (one week) metabolic acidosis produces a two or three-fold increase in the rate of glutamine transport and deamidation by rat (1,19) and dog (52) renal mitochondria assayed in vitro. However, short-term (3 hours) marked acidosis or subchronic (2 days) mild acidosis produces only a modest (<50%) increase in glutamine transport and deamidation capacities of isolated rat renal mitochondria (1,19), even though similar treatments produce marked (two or three-fold) increases in renal ammonia production in vivo (22). Therefore, the acidosis-induced increase in renal mitochondrial glutamine transport capacity may play an important role in the long-term adaptation of renal ammonia production to metabolic acidosis but would appear to play only a minor role in the initial adaptation. Thus, if indeed the glutamine transport system is involved in the early adaptation of renal ammonia production the renal cells must use some way of modulating the activity of this system independent of changes in the number of 'carriers' within the membrane.

In a systematic study (21) on the effects of cellular metabolites on the transport and deamidation of glutamine by isolated rat renal mitochondria it was found that both transport and deamidation significantly and specifically were inhibited by physiological concentrations of α-ketoglutaric acid (Fig. 4). Several other metabolically important organic acids did not have an inhibitory effect on glutamine transport when tested at three-times the α-ketoglutarate concentration. The inhibition of mitochondrial glutamine transport by α-ketoglutarate is due to a decrease in affinity of the carrier for glutamine in the presence of the ketoacid. Studies with a rapid-flow, rapid-mixing technique, in which initial rates of glutamine transport could be measured at times as short as one sec (22), showed that physiological concentrations of α-ketoglutarate produced a two-fold increase in the transport K_m of glutamine with little effect on the V_{max} (Fig. 5). The K_m was raised from 2.6 mM, a value similar to the concentration of glutamine in renal cells, to 5.4 mM which is outside the physiological range of glutamine concentrations. If similar conditions pertain in vivo then glutamine entry into rat renal mitochondria would be markedly inhibited by α-ketoglutarate in vivo.

Acid-base changes in vivo produce marked and rapid alterations in the concentrations of α-ketoglutarate in the renal cells (9,34,35). As shown in Fig. 6 both metabolic and respiratory acidosis and metabolic alkalosis cause significant changes in renal α-ketoglutarate concentrations as assayed in rat kidneys freeze-clamped one hour after initiation of treatment. Although the concentrations of other metabolites were altered as well none changed to the same degree or as consistently as α-ketoglutarate (Fig. 6). Thus it appears that acid-base changes may have a direct, specific effect on renal α-ketoglutarate metabolism. If this were true it would tend to support the idea that the ketoacid is an important regulator of renal mitochondrial glutamine transport and metabolism (Fig. 7). In this connection it is interesting to note that infusion of α-ketoglutarate into acidotic dogs increases the renal concentration of α-ketoglutarate and reduces the elevated level of renal ammonia production (56).

In summary, three theories on the mechanism of adaptation of renal ammonia production to metabolic acidosis have been postulated. The first deals with the role of enzyme adaptation in

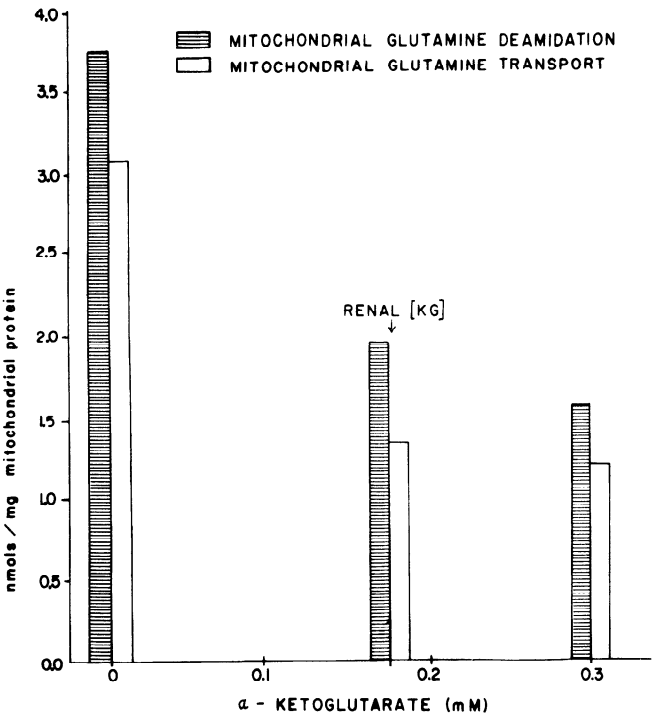

Fig. 4. Ketoglutarate inhibition of glutamine deamidation and transport (matrix accumulation glutamine carbon) by isolated rat renal mitochondria. Drawn from data in reference 21.

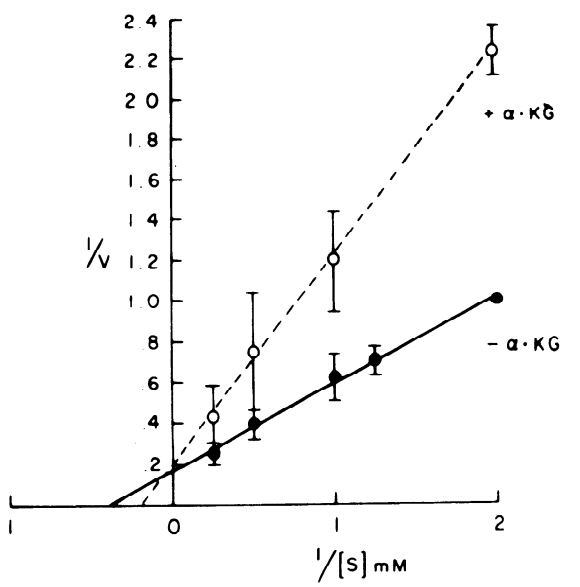

Fig. 5. Lineweaver-Burke plot of reciprocal glutamine concentration vs. accumulation rates in presence and absence of 0.3mM α-ketoglutarate. 1/v is expressed as 1/nmol per mg protein sec $^{-1}$. From Goldstein and Boylan, *Am. J. Physiol.* 234:F514, 1978, Fig. 4.

the response of ammonia excretion to acidosis. The results of experiments designed to test this hypothesis indicate that enzyme adaptation cannot play an important role in the kidneys of adult rats. However, it appears that enzyme adaptation may be an important component of the renal response to acidosis under conditions when enzyme content may be limiting for the deamidation-deamination of glutamine: in the developing and regenerating kidneys.

The second theory is concerned with the role of feed-back inhibition by the end-product glutamic acid. The initial observation

that the concentration of this potent glutaminase inhibitor fell rapidly during metabolic acid gave the theory great appeal. However, subsequent, extensive investigation has failed to unequivocally support or negate the theory.

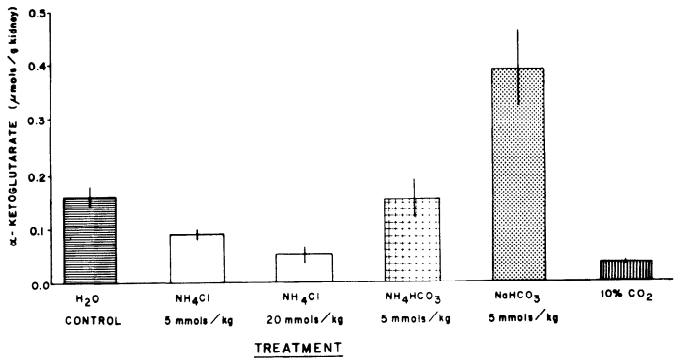

Fig. 6. Changes in mean kidney metabolite levels expressed as percentage change from mean control (water) values. Asterisks indicate changes that are statistically significant ($P < .05$). From Boyd and Goldstein, *Am. J. Physiol.* 236:E289, 1979, Fig. 2.

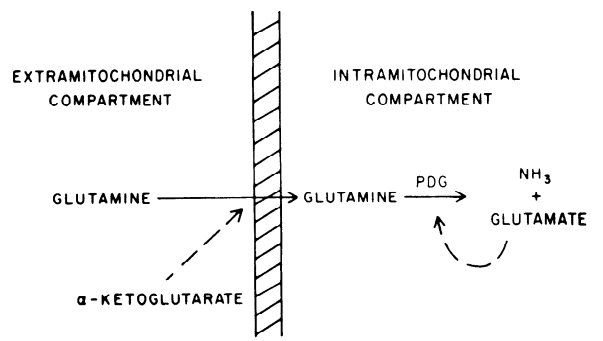

Fig. 7. Hypothetical model for regulation of glutamine uptake and deamidation by rat kidney mitochondria. Dashed lines indicate possible sites of inhibition. From Boyd and Goldstein, *Am. J. Physiol.* 236:E289, 1979, Fig. 1.

The most recent theory centers around the role of mitochondrial glutamine transport in the response of renal ammonia production to acidosis. Although current experimental evidence suggests that this system may play an important role it is still too early to fully assess the full significance of these studies.

As with all biological responses, there is probably more than one mechanism that is involved in producing the ammonia response. Depending on factors such as the duration of the stimulus, the age of the animal and the particular species each of the mechanisms proposed in the theories presented above plays some role, major under some conditions and minor under others.

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VASOTOCIN SECRETION AND ACTION IN THE TOAD: A LABORATORY EXERCISE

Patrick Eggena
Department of Physiology and Biophysics
Mount Sinai School of Medicine
of the City University of New York, New York 10029

INTRODUCTION

Last year we introduced a new laboratory exercise on the Mechanism of Antidiuretic Hormone Secretion and Action into our General Physiology Course. This paper describes the experimental procedure as it was printed in the student syllabus and discusses the results obtained by 102 first year medical students.

LABORATORY: VASOTOCIN SECRETION AND ACTION IN THE TOAD

PURPOSE: The aim of this experiment is to demonstrate the release of antidiuretic hormone from the posterior pituitary and its action on the permeability to water of the toad urinary bladder. The antidiuretic hormone employed by amphibia is vasotocin; man uses vasopressin. The toad urinary bladder is phyllogenetically related to the collecting duct of man, and it performs a similar function in water conservation. Both, the secretion of vasotocin and its antidiuretic action, can be studied with *in vitro* preparations of the posterior pituitary and urinary bladder by methods adapted from P. Eggena and A. Polson, *Endocrinology,* 94: 35, 1974. The laboratory exercise is scheduled for a three hour period. The exercise is preceded by a 25 minute videotape illustration of the methods. Each student is expected to plot his/her results, to briefly answer the appended questions, and to hand in a signed copy to a laboratory committee for review and comment. Students perform the experiment in pairs.

MATERIALS:

Each pair of students will have at their bench the following:

a) 2 hollow glass rods
b) No. 3 black braided surgical silk, 2 pieces about 10 inches
c) small beaker (25 ml) with Ringer's fluid
d) 3 plastic test tubes with snap-on tops (or equivalent)
e) 1 disposable pipette (1 ml)
f) 3 Pasteur pipettes with suction bulb

Each Central Station (10 pairs of students) will have the following:

a) 1 semi-microbalace (+1 mg accuracy)
b) 2 evaporating dishes for wiping bladders (1 for each hemi-bladder)
c) 40 beakers (50 ml size) with labels
d) 1 liter Ringer's fluid of the following compostion, in mM/L: NaCl, 110; KCl, 3.5; $CaCl_2$, 1; $MgCl_2$, 1; dextrose, 5.5; Tris (hydroxymethyl) aminomethane-HCl, 10; pH, 7.4; tonicity, 235 mOsm/L.
e) 1 liter of dilute (1:5) Ringer's fluid (made by adding 800 ml of distilled water to 200 ml of full strength Ringer's fluid)
f) 1 liter of 55 mM KCl medium of the following composition, in mM/L: NaCl, 55; KCl, 55; $CaCl_2$, 1; $MgCl_2$, 1; dextrose, 5.5; Tris (hydroxymethyl) aminomethane-HCl, 10; pH, 7.4; tonicity 235 mOsm/L.
g) 3 disposable syringes (10 ml) with needle (18 gauge) and 4 inch long polyethylene tubing attached.
h) 2 foot long wire (or rod) attached to ring-stands on which 20 bladders can be suspended via their glass stalks.
i) 1 ampule of synthetic vasopressin (1 ml containing 0.1 mg AVP)
j) 1 Eppendorf pipette with plastic tip for dispensing 25 λ of vasopressin solution.
k) 10 toads, *Bufo marinus,* female, medium size (about 250 gm)
l) 1 guillotine
m) 2 bone clippers (small)

METHODS:

Toads are decapitated with a guillotine by an instructor, and the spinal reflexes to the trunk are destroyed by moving a long needle down the spinal chord. Two students will receive one toad prepared in this manner.

I. *Brain Dissection and Incubation of the Pituitary Gland*

a) Using the small bone clippers remove the bone overlying the cranial fossa as illustrated in the video-tape. Be careful not to destroy the brain; have an instructor help you if there is any question how to do this. Once the brain is exposed dorsally, gently lift upward on the spinal chord and medulla with broad forceps and cut with fine scissors the blood vessels and nerves which keep the brain anchored in the cranium. As you lift upward on the spinal chord while cutting close to the bone of the floor of the cranium, you will see the pituitary gland come into view. Since the toad does not have a deep sella tursica, it is possible (with some practice) to cut the adhesions between the pituitary and the cranium without rupturing the delicate pituitary stalk. However, in the present experiment do not attempt to free-up the pituitary gland before removing the brain from the cranium. Just lift up on the brain, cut the frontal lobes, and discard the brain. Now look carefully in the cranial fossa for the pituitary gland, which should still be attached to the sella tursica. Remove the pituitary gently with fine forceps and place in a test-tube.

b) *Washing the Pituitary Gland*

Gently wash pituitary by adding about 1 ml Ringer's fluid to the test tube. Remove the wash carefully with a Pasteur pipette, leaving the gland in the tube. Discard wash and repeat once.

c) *Incubation of Pituitary in Ringer's Fluid*

Add exactly 1 ml Ringer's fluid measured with a disposable pipette to the tube. After 15 minutes, remove only the Ringer's fluid using a Pasteur pipette and transfer it to the second plastic test tube.

d) *Incubation of Pituitary in 55mMKCl Medium*

Now add 1 ml of 55 mMKCl Medium to the tube containing the pituitary gland. After another 15 minutes, transfer this solution to the third tube, still leaving the pituitary in the original tube.

II. *Urinary Bladder Dissection*

The urinary bladder of the toad consists of two lobes which are transparently thin and may occupy two-thirds of the abdominal

cavity in the well hydrated animal. Take care not to cut the bladder or to injure it by picking it up with forceps.; use your fingers when handling the bladder!

Enter the abdominal cavity by making a midline incision. This is done most readily by picking up the skin at the xyphoid process, cutting a small hole with large scissors, and then extending this incision downward just short of the pubic symphysis with scissors. Lift upward on the abdominal skin and musculature while cutting, so that the bladders glide out off the way of the scissors to the sides. In order to increase exposure cut laterally from the xyphoid process in both directions.

Gently lift up on the tip of one half-bladder, so that the urine contained in it flows into the contralateral half-bladder. Using the fine scissors cut the peritoneal relfection, which anchors part of the bladder sac to the abdominal wall, and then cut transversily across the bladder neck and remove the half-bladder to a small beaker containing Ringer's fluid. Next free-up the other half-bladder and remove it to the same beaker with Ringer's fluid.

III. *Suspending The Bladder as a Sac*

Using your fingers and keeping the bladder moist with Ringer's fluid, manipulate the half-bladder so that it covers the end of a glass stalk like a stocking. Be sure that the urine side of the bladder (mucosal side) is next to the glass. (This will be the case if you do not invert the bladder). Keeping the bladder gently stretched (about 5 cm), have your partner place a square knot with #3 surgical silk around the bladder neck and stalk. While keeping a small amount of traction on the single square knot, pull the bladder downward to the end of the glass stalk and secure a second square knot. Now use a syringe to fill the bladder with about 7 ml of diluted Ringer's fluid (1:5 dilution). Use the syringe to suck out air bubbles. Now suspend the half-bladder by the hook on the glass stalk in 25ml of full-strength Ringer's fluid. Repeat this procedure for the other half-bladder. (Note: if the two bladder sacs are markedly different in size, retie the larger one so that it approximately matches the smaller one).

IV. *Measuring the Permeability to Water of the Bladder*

The rate of water movement along an osmotic gradient from the inside to the outside of the bladder wall is measured by weighing the bladder by its stalk periodically on a semi-micro balance. The bladder is lifted out off the Ringer's fluid by its stalk, wiped on "four" sides by moving it over the edge of an evaporating dish, and suspended on a hook in the balance. *Do not weigh the bladder yourself. Each balance will be manned by an instuctor, who will do all the weighing for you.* The bladder is rapidly weighed to the nearest 1 mg. Care is taken not to touch the pan of the balance with the wet bladder and not to keep the bladder exposed to air for longer than necessary to do the weighing. Once weighing has started no adjustments in the tare of the balance can be made. The exact time of the weighing is noted, and the rate of weight loss of the bladder is calculated in terms of mg/min/half-bladder.

RESULTS:

I. *Baseline Permeability to Water*

Weigh half-bladder "A" and half-bladder "B" and list the weights and time of weighing in Table I. After 15 to 30 minutes (depending on the availability of the balance) weigh the bladders again and note the new weights and time in Table I. Calculate the rate of weight loss of each half-bladder in terms of mg/min/half-bladder, and plott your results in Figure 1. Use whatever type of plott you feel best illustrates your findings. The baseline permeability of the bladder should be much less than 10 mg/min/half-bladder. If you have a high baseline permeability, have an instructor check your bladder for leaks, before continuing with the experiment.

II. *Response of Bladder to Pituitary Incubation Medium*

Add 1 ml of the Ringer's fluid in which the pituitary gland had been incubated for 15 minutes (see Methods, I, c) to the outside bathing medium of half-bladder "A." Add 1 ml of the KCl medium in which the pituitary gland had been incubated for 15 minutes (see Methods, I, d) to the outside bathing medium of half-bladder "B." After 15 to 30 min. weigh the bladders again and note the weights and time in Table I, and again plott your findings in Figure 1.

III. *Response of Bladder to Synthetic Vasopressin*

Add 25 λ of the vasopressin solution to the outside bathing media of half-bladders "A" and "B." The final concentration of vasopressin in the 25 ml bath is thus $1x10^{-7}M/L$. After 15 to 30 min. weigh the bladders again and note the weights and time in Table I, and again plot your results in Figure 1.

TABLE 1

Desk #: _____

Name: _____

TABLE I

BLADDER WEIGHT (mg)

TIME	"A" Bladder	"B" Bladder

FIGURE 1

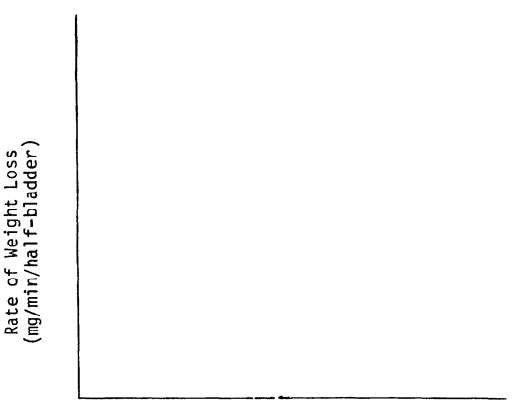


TABLE II

TOAD BLADDER PERMEABILITY TO WATER
(mg/min/hemibladder)

STUDENT GROUP No.	BASELINE "A"	BASELINE "B"	PITUITARY INCUBATION "A"-Na-Ringers	PITUITARY INCUBATION "B"K-Ringers	VASOPRESSIN "A"	VASOPRESSIN "B"
1	2	2	3	17	41	52
2	0	0	4	9	13	34
3	3	2	1	23	123	62
4	3	1	2	12	48	63
5	5	4	13	29	59	54
6	2	6	7	37	58	64
7	3	2	1	23	69	33
8	2	5	4	8	28	34
9	3	1	13	64	48	54
10	3	2	16	37	125	79
11	1	1	9	32	61	65
12	2	2	37	126	250	100
13	4	1	4	32	46	60
14	4	3	12	42	30	48
15	5	4	11	28	48	47
16	3	2	16	37	125	79
17	2	5	4	9	28	34
18	0	0	31	39	62	52
19	3	5	3	5	13	14
20	10	6	65	106	6	46
21	10	8	1	30	26	50
22	9	5	10	18	55	50
23	4	6	4	9	9	14
24	3	5	8	11	47	48
25	8	6	9	20	53	50
26	6	9	9	15	13	24
27	4	10	2	15	12	55
28	9	9	49	49	76	38
29	10	14	1	32	27	51
30	8	10	5	12	10	60
31	2	4	47	8	50	15
32	4	2	9	5	22	6
33	1	2	1	1	3	1
34	6	leaky	3	leaky	49	leaky
35	0	leaky	0	leaky	29	leaky
36	10	19	--	30	50	75
37	leaky	1	leaky	17	leaky	28
38	0	leaky	8	leaky	28	leaky
39	leaky	1	leaky	12	leaky	50
40	leaky	4	leaky	9	leaky	23
41	leaky	4	leaky	8	leaky	25
42	leaky	0	leaky	13	leaky	51
43-51	"A" and "B" hemibladders leaky or no response					

Desk #: _____

Name: _____

DISCUSSION: Please answer the following questions briefly.

1. Where is vasotocin (or vasopressin) synthesized and where is it stored?

2. Name a few of the physiological stimuli which cause release of vasotocin (or vasopressin).

3. Was a 55 mM KCL medium effective in releasing vasotocin from the isolated toad pituitary in your experiment? If yes, why?

4. Do you think, that you might have triggered vasotocin release from the isolated putuitary with hypertonic Ringer's fluid?

5. Why is it necessary to include $CaCl_2$ in the 55 mM KCl medium?

6. Functionally the toad urinary bladder is most closely related to which part of the mammalian nephron?

7. Can a toad concentrate its urine beyond the concentration of its plasma?

8. Which plasma membrane, the basal-lateral or the apical, is rendered more permeable to water with vasotocin?

9. How does vasotocin (or vasopressin) bring about this change in membrane permeability?

COMMENTS

The isolated toad urinary bladder is quite impermeable to water in the absence of antidiuretic hormone. A person with a little experience in mounting, wiping, and weighing bladders will usually record a baseline weight loss of 1-2 mg/min/hemibladder. Our students obtained quite respectable baseline permeabilities below 10 mg/min/hemibladder in most groups (see Table II). This was their first exposure to a toad bladder experiment and bladders were not always handled as gently as necessary or weighed with the required care, so that baseline permeabilities were a little higher than in experienced hands. Not infrequently bladders were found to be grossly "leaky." This resulted either from puncturing the bladder during the dissection or from improper mounting of the bladder to the glass cannula.

Most students observed little or no increase in bladder permeability to water when Ringer's fluid, which had bathed the pituitary gland, was added to the outside bathing medium. Extensive manipulation of the pituitary gland during the isolation procedure will usually cause some vasotocin to be released. Most of this vasotocin is removed by gently washing the puituitary in Ringer's twice, as was the protocol in the present study. When the isolated pituitary gland is exposed to high concentrations of potassium, the axon terminals of the preoptico-neuro-hypohyseal neurons are depolarized and vasotocin is released by the process of exocytosis. Calcium ions are required for excitation-secretion coupling. Most students observed a marked increase in water permeability of bladders exposed to potassium-Ringers, which had bathed the pituitary gland for 15 min.

Virtually all students observed a marked increase in bladder permeability to water when synthetic vasopressin was added to the outside bathing medium. This final step in the protocol was designed to ensure that students had not inverted the bladders during mounting or had failed to establish an osmotic pressure gradient across the bladder wall by using the wrong solutions. Since the concentration of vasopressin employed is supramaximal, the student has a sense of what the optimal permeability state of the bladder is in relationship to the response previously observed with vasotocin released from the pituitary gland.

Most students completed the experiment in 2 hours. There was a general concensus among faculty and students that this laboratory excercise was a good learning experience.

COURSE IN DESIGN AND ANALYSIS OF SCIENTIFIC EXPERIMENTS

Massachusetts Institute of Technology will offer a one-week elementary course in Design and Analysis of Scientific Experiments, June 23-June 28, 1980. Applications will be made to the physical, chemical, biological, medical, engineering, and industrial sciences, and to experimentation in psychology and economics. The course will be taught by Professors Harold Freeman and Paul Berger. Further particulars may be obtained by writing to the Director of the Summer Session, Room E19-356, Massachusetts institute of Technology, Cambridge, Massachusetts 02139.

LEARNING THE CARDIAC CYCLE: SIMULTANEOUS OBSERVATIONS OF ELECTRICAL AND MECHANICAL EVENTS

Richard Alec Kenney
and
Mary Anne Bassett Frey
Departments of Physiology
George Washington University
School of Medicine
Washington, D.C. 20037

Wright State University
School of Medicine
Dayton, Ohio 45435

Physiology students are not uncommonly confronted at an early stage of their exposure to the cardiovascular system with the highly complex description of the events of the cardiac cycle embodied in the classical Wiggers diagram. Understanding of the inter-relations of events is made the more difficult for the student tobecause much of the information lies outside of their own experience. However, we have found that it is possible for students to establish an integrated understanding of the electrical and mechancial events of the cardiac cycle by measuring systolic time intervals. Essentially this involves simultaneous recording of the ECG, a phonocardiogram and the contour of the carotid pulse. The equipment necessary is a multi-channel photographic recorder (our experience is based upon an Electronics for Medicine DR-12 or VR-6), disposable ECG electrodes and a pair of microphones (PS-1-B or similar). The protocol which follows is accomplished in a single laboratory period and has as its end-product a time-scaled diagram of the cardiac cycle in a supine resting individual and data on the changes produced by some simple stresses. These then form the basis for discussion of the mechanisms of cardiac response to changing loads.

LABORATORY EXERCISE

The timing of events of the cardiac cycle

Introduction

Throughout the cardiac cycle information is being generated by the heart and transmitted to the body surface where it appears as electrical potentials, physical movements or pulses and vibrations or sounds. These varieties of information can be transduced into electrical signals displayed on an oscilloscope or recorded as hard copy by a galvanometer. Analysis of the records so obtained permits study of the cardiac cycle in terms of the timing of events such as the excitation of the cardiac muscle, the opening and closing of valves and the transmission of pressure waves and blood flow to the periphery.

Before coming to class you should read

(Insert references from text in use)

Protocol for the Laboratory Exercise

Students should organize into groups of four. One will serve as the subject, one will be responsible for labelling records, one will operate the recorder and one will be responsible for adjusting the transducers and assisting the subject. Females who are designated as subject may wish to bring a bathing suit top or halter. A stethoscope will be required, and a pocket calculator will be helpful.

Equipment

(i) Recordings will be made on a photographic recorder and its operation will be demonstrated to you.

(ii) Transducers will be connected to the recorder ready for use.
These will be
a) a three-lead ECG cable fitted with disposable silver electrodes
b) a microphone for the recording of heart sounds (marked "sounds")
c) a microphone for transducing pulsations of the carotid artery (marked "pulse").

(iii) Other items provided include:
Straps to hold microphones in place
Alcohol swabs and electrode paste for ECG
Plastic ruler with millimeters indicated
Timer
Cot or table for supine and seated recordings
Sphygmomanometer (stethoscope)
Hand dynamometer
Plastic dish pan
Ice and water
Thermometer
Towel

The amplifiers of the recorder will have been adjusted to give an appropriate sensitivity and frequency response. Do not change any of the settings without first consulting the instructor. The recorder will also have been set to provide a record at a speed of 100 mm/second.

Do not change this recording speed.

PART I: OBTAINING THE RECORDINGS

1. *Recording the carotid pulse contour*
Have the subject lie supine for 10 minutes.

Palpate the pulsations of the carotid artery high in the neck and whilst doing this also feel the radial pulse. Do you notice any time lag between the pulses or any difference in character?

Move your fingers over the course of the carotid artery to identify the point of maximum pulsation and place the pulse microphone directly over this point. Secure the microphone around the neck with the velcro strap firmly enough so that the microphone will not shift but not so tightly as to compress the artery or be uncomfortable. Observe the record being produced on the monitor oscilloscope. You may need to adjust the position of the microphone slightly to obtain a clear record with a sharp upstroke and a well defined notch in the down stroke. Run the camera for 15 seconds to obtain a hard-copy record. Examine the

record. This wave form recorded from the carotid artery is very similar to one recorded from the aorta but is displaced in time because of transmission of the pulse from its origin to the point of recordings (about 15 cm). The steep upstroke of the pulse contour results from the ejection of blood from the left ventricle into the arterial system; the notch on the down stroke (the dicrotic notch or incisura) reflects the cessation of ejection of blood and closure of the aortic valve. The time therefore from the start of the steep upsweep to the notch is the period during which blood is being ejected from the ventricle, the Left Ventricular Ejection Time (LVET). Measure the LVET for 3 to 4 consecutive beats using the following formula:

$$\text{LVET (msec)} = \frac{\text{distance from upstroke to notch (mm)}}{\text{Recording speed (mm/sec)}}$$

Calculate the mean LVET.

2. *Recording the electrical activation of the ventricle*

Before ejection of blood can take place the ventricular muscle is excited to contract by the depolarization spreadings into it. The time course of this depolarization can be recorded by the electrocardiograph. Recall the anatomy of the heart and the direction taken by the spreading depolarization as it enters the ventricular septum. The clearest record of this (i.e., the Q wave) can usually be obtained if electrodes are arranged in the general pattern of Lead II. Take the ground electrode and fix this in the upper left quadrant of the chest in the midclavicular line and 8-10 cms above the nipple. The "negative" electrode is fixed in the symmetrical position on the right side. Fix the positive electrode in the left anterior axillary line at about the 8th intercostal space. Run the camera for 15 seconds and examine the trace. Identify the P, Q, R, S and T waves and observe the relation of the QRS complex (ventricular depolarization) to the start of ejection as shown on the carotid pulse. It is convenient to use the ECG trace to measure the heart rate or in this context the beat-to-beat interval. Mark the start of the Q wave on 3 or 4 consecutive beats and measure the distance from each Q wave to the next succeeding one.

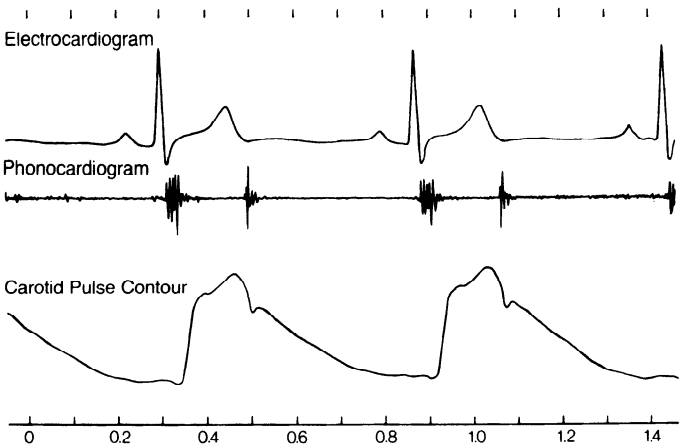
$$\text{Q-Q interval (msec)} = \frac{\text{Q-Q distance (mm)}}{\text{Recording speed (mm/sec)}}$$

$$\text{or heart rate (beats/min)} = \frac{\text{60,000 (i.e. msec/minute)}}{\text{Q-Q interval (msec)}}$$

Determine the time which elapses from the start of the Q wave to the start of ejection. What occurs?

3. *Recording the heart sounds*

Sounds which can be heard (or detected) at the chest wall are generated in a number of ways: (i) by vibrations in the tense wall of the contracting ventricle, (ii) by movement of the valves of the heart, (iii) by vibrations in the wall of the aorta, (iv) by turbulent flow of blood set up when fluid flows at high velocity through a narrow orifice or when the smooth flow of blood is interrupted by closure of valves. Since we are examining events occurring in the left ventricle we need to record from the chest wall in a position which will optimize the sounds produced by the aortic valve. This will usually be in a parasternal position in the 2nd intercostal space. Listen with your stethoscope in this area and find the spot where the clearest sounds can be heard. Place the sounds microphone over this spot and fasten it firmly in place with a velcro strap around the chest. Observe the monitor oscilloscope and identify the 1st and 2nd sounds. If necessary adjust the position of the microphone. Run the camera for 15 seconds and examine your record. Are there any 3rd or 4th sounds? Notice carefully the relation in time of the sounds to the ECG and to the carotid pulse contour (Figure 1).



The 2nd heart sound begins a little before the dicrotic notch of the pulse tracing. In fact these two things have a common origin, closure of the aortic valve, but are separated in time because while the sound is generated at the valve, transmission of the effect of valve closure to the carotid artery takes a few msec.

The beginning of the 1st heart sound is produced by the closing of the mitral valve. This occurs as ventricular contraction first raises ventricular pressure above that in the atrium and so is very close to the start of ventricular contraction. The time from the start of the 1st sound (S_1) to the start of the 2nd (S_2) is thus an acceptable measure of the total duration of ventricular contraction. This time is greater than LVET since the ventricle must first generate tension by isovolumic contraction and thereby overcome the pressure in the aorta before ejection of blood can occur. In other words, the ejection portion of ventricular systole does not begin until ventricular pressure exceeds aortic pressure.

S_1-S_2 (msec) = LVET + isovolumic contraction time (IVCT). Make measurements of S_1-S_2 on 3 or 4 beats, measure LVET for the same beats and calculate IVCT.

Another measurement that can now be made is the duration of Electro-Mechanical Systole (EMS) which is the time between entry of excitation into the ventricular system and the termination of ejection. Draw a vertical line through the start of the Q wave and another through start of the 2nd sound and measure this interval.

$$\text{EMS (msec)} = \frac{\text{Q-}S_2\text{(mm)}}{\text{Recording speed (mm/sec)}}$$

What events are occurring during EMS? If one subtracts from EMS the LVET, the Pre-Ejection Period (PEP) is calculated which includes the time involved in excitation of the ventricle and the IVCT.

Measure from the start of S_2 to the next succeeding Q wave. This is the Electro-Mechanical Diastole (EMD) and includes a brief period of isovolumetric relaxation of the ventricle, the period of passive ventricular filling and atrial contraction. [Note: these fractions of EMD cannot be obtained from the records which are being made in this experiment but can be measured from a tracing of the apex beat. This is a record made by placing a pulse microphone on the chest wall over the point of maximal impact of the apex of the ventricle. If time permits attempt to record the apex beat together with the ECG and the heart sounds. Discuss the analysis of your record with the instructor.]

These measurements which you have just made, EMS, LVET, PEP and IVCT, and referred to as the systolic time intervals and are commonly used in the clinical examination of the heart. The least reliable of these measurements is IVCT since the exact point of onset of the first heart sound is often difficult to discern. For this reason more clinical reliance is placed upon PEP which correlates well with IVCT under most circumstances. What factors could change the duration of PEP? of LVET? of IVCT?

Run 15 seconds of record with the subject still supine to serve as a control record for the remainder of the exercise during which you will examine how the cardiovascular system responds to some everyday stresses. The information you obtain here will help you to answer the questions above.

PART II: OBSERVATIONS DURING STRESS

1. *Sitting*

   With the subject supine, measure the arterial pressure by auscultation. Have the subject assume a sitting position on the side of the couch. Check that you are still producing clear records; adjust the transducers if necessary. After 2 minutes, measure arterial pressure and then run 15 seconds of record.

2. *Standing*

   Have the subject stand quietly for 3 minutes; measure arterial pressure and run 15 seconds of record. Allow the subject to sit quielty for 10 minutes. (During this time, start measuring the systolic time intervals that you have already recorded.)

3. *Static (Isometric) Exercise*

   Isometric exercise involves a change in tension developed by muscles with no change in muscle length. Many functions we regularly perform involve isometric exercise. These include lifting, pushing, even riding a bicycle. There is a marked change in the cardiovascular system in response to isometric or "static" exercise. The resultant increase in arterial blood pressure challenges the heart as an increased afterload to which it must adjust.

   Using the hand dynamometer, first squeeze maximally to determine maximum voluntary contraction (MVC). Calculate 40% of MVC. The subject maintains a handgrip at 40% MVC for 2 min or until fatigued. Do not hold breath during experiment and endeavor to sit still so the microphone will remain in place. Experimenter should measure heart rate, systolic time intervals and blood pressure before exercise (above) and at 2 minutes of isometric exercise. If subject feels unable to maintain the handgrip for the full period, subject should so indicate so a record can be made before release. If subject is right-handed, blood pressure should be measured in the left arm. The student should start inflating the blood pressure cuff 30 seconds prior to time of measurement; this will ensure taking the pressure on time.

   After isometric exercise, a 15 minute recovery period should be allowed before the next test. Again measure heart rate, systolic time interval, and blood pressure in a sitting subject.

4. *Cold Pressor*

   Cold is another commonly encountered stress. The increase in arterial pressure when an individual puts his hand or foot in cold water puts an added afterload on the heart.

   After recovery from the preceding experiment, subject immerses their hand in a pail of water at approximately 4°C for one minute. Subject must not hold breath during experiment. Examiner records heart rate, systolic time intervals and arterial pressure preimmersion and after 60 sec immersion. If subject is unable to maintain the immersion they should so indicate so a record can be made before removal from the water.

   (We have also used face immersion and Valsalva maneuver as a part of this laboratory experience. The Valsalva is particularly valuable in illustrating the baroreceptor reflex. This, however, requires a continuous recording so we determine only heart rate on a beat-by-beat basis.)

   (We provide each student with a blank data sheet for recording the following variables: Q-Q (msec), HR (bpm), P-R (msec), Q-T (msec), EMD (msec), EMS (msec), S_1-S_2 (msec), LVET (msec), PEP (msec), IVCT (msec), S_2-C_D (msec), SBP (mmHg), and DBP (mmHg) during the following conditions: supine control, standing, sitting control, isometric hand grip, sitting control and cold.)

Summary of Definition of Terms

R-R Interval (Q-Q interval) = time elapsed from start of one depolarization of the ventricle to start of the subsequent depolarization = *Cardiac Cycle.*

Left Ventricular Ejection Time (LVET) = time during one Cardiac Cycle when the heart is ejecting blood. Can be estimated noninvasively from the carotid displacement pulse as the time elapsed from the rapid upstroke to the dicrotic notch.

Electro-Mechanical Systole (EMS) = time for electrical depolarization of the ventricle plus isovolumic contraction and ejection periods. Measured from Q wave of ECG to first high frequency component of second sound of phonocardiogram.

Pre-ejection Perod (PEP) = EMS - LVET.

Mechanical Systole/S_1-S_2/Period = contraction periods of ventricle. Measured on phonocardiogram from the first to the second heart sounds.

Isovolumic Contraction Time (IVCT) = /S_1-S_2/ - LVET.

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PROLOGUE TO
CAPILLARY PERMEABILITY AND MECHNISM OF TRANSPORT

A SALUTE TO PROFESSOR HYMAN S. MAYERSON

The idea for a symposium on "capillary permeability and mechanisms of transport" germinated while waiting in a hotel bar for the airport limousine after the Fall St. Louis meetings. Drs. Taylor, Diana and I were swapping Gulf water fish stories when quite naturally the theme reeled to restaurants in New Orleans, the site of the '79 gathering. A serious note diverted our questitory fantasies: and appropriate topic for the stellar slots on the program, the symposia. Traditionally, local expertises are featured be it workshop, symposium or tutorial lecture. My two permeant colleagues quickly filtered down to their vested interest in transcapillary exchange. In the midst of their fluid repartee, I dragged in the thought that a program in New Orleans covering concepts of "stretch pore" "bulk flow" and the like should salute Hy Mayerson. Hy, a past-president of the society was chairman of physiology at Tulane, the host institution, when over a quarter of a centruy ago, APS last met in the Crescent City. Since his retirement Hy has remained active, serving as correspondent for the Senior Physiologist.

As a former student in Mayerson's department, I was pleased when the Program Committee bought the package; a full day symposium, a tribute to Professor Mayerson and chaired by two of the area's good-old boys. The morning session not only gives tribute to Professor Mayerson but also gives an update on a number of concepts he, at an earlier date, had introduced into the literature. The afternoon program continues with an expose of current thoughts at the forefront of the field. Although Hy himself could not attend because of a momentary setback in health, his paper reminiscing on people, places and contemplations was read. He missed very much not being able to attend. However, Hy regretted equally if not more, having to cancel the Senior Physiologist Luncheon, scheduled for Antoine's.

John W. Manning, Ph.D.

PROFESSOR H. S. MAYERSON, PH. D. - THE MAN

Karlman Wasserman

Harbor-UCLA Medical Center

It is indeed appropriate that we honor Dr. Hymen S. Mayerson today at this symposium on capillary permeability. It was under Hy Mayerson's tutelage and guidance that the initial work on using lymphatics to study capillary permeability was started in the Physiology Department of Tulane University School of Medicine. By use of appropriately labelled macromolecules and studying the kinetics of their disappearance from blood and appearance in lymph, a new understanding of the role of lymphatic and interstitial protein turnover and their relationship to plasma proteins leaked from the circulation emanated.

But it is Dr. Taylor's responsibility to tell you about Dr. Mayerson's research. My responsibility is to tell you about Dr. Mayerson, the man. The time is short for all that I would like to say about Hy Mayerson. However, I will try my best to highlight certain characteristics of this special physiologist in the time assigned.

I first met Hy Mayerson in the Fall of 1948 when I arrived at Tulane to start graduate work in Physiology. I was young and apprehensive about this new experience in this big university, having done my undergraduate work in a small college. Hy quickly put me at ease, with such warmth and concern for my graduate program that I felt him as a wise, all-knowing father figure; as I would later discover, this was how both students and faculty, alike, viewed him. He was the man you went to if you had a problem. He had a sympathetic ear and provided sage advice, whether it related to scientific problems, teaching problems or even personal problems.

But before going into further detail on the characteristics that enabled Hy Mayerson to be a department chairman and a most respected member of the Tulane Medical School faculty and to be recipient of so many outstanding recognitions as a physiologist and teacher, allow me to recount some of the highlights of his early life.

Hy's father migrated from Austria at the age of 17 in the latter part of the 19th century. He settled in Woonsocket, Rhode Island, where he opened a general store. He then sent back to Austria for a bride and got in return a Rabbi's daughter. His mother's background put a high premium on learning, scholarship and teaching and this she transmitted to Hy, her oldest son.

Hy was born on Sept. 10, 1900 in Providence, R.I., as the second child and first son of this immigrant couple (Table 1). He had his primary and secondary education in Providence. While in high school, he managed the school's baseball team, becoming manager by default, as the story goes. Hy is lefthanded and the school didn't have enough lefthanded fielders' gloves to go around. But possibly in this setting, Hy began to learn how to get people to work together and recognize their capabilities (I gather that his team did well). Hy entered Brown University in 1918 and studied biology. After graduation from Brown, he started graduate work in physiology at Yale. One year after he received his Ph. D., Dr. Henry Laurens, one of the more senior department members, was invited to take the Chair of Physiology at Tulane, and Dr. Laurens invited young Mayerson to accompany him as a member of the department.

Hy married his wife, Caroline, a New Orleans girl, several years later and they have two children, Peter and Mary. Peter is now 46 and a very successful practicing psychiatrist in Denver with a family of his own. Peter describes Hy as being a warm, kind, loving and infinitely patient father.

In 1945, Hy became Chairman of the Department and held that position for the next 20 years until his retirement at age 65, after 39 years on the Tulane faculty. Hy, however, still had too much to contribute and didn't retire from work. He took a full-time position as associate director in charge of research and education at Touro Infirmary, the second largest hospital in New Orleans. He continued in this position for 10 years, when he again retired. However, Hy continues to contribute on a voluntary basis in two important areas. He served on the Board of Directors of Dominican College in New Orleans until earlier this year. In fact, he was chairman of the committee that recruited its current president. A second area in which Hy continues to work is on the Senior Physiologists' Committee with Bruce Dill. I know that we all enjoy reading in the Physiologist the interesting letters that he and Bruce generate from correspondence with retired physiologists.

Hy received many honors during his long and illustrious career. I have listed what I believe to be the more outstanding ones. Certainly it is a tremendous honor to be elected by one's peers at a national level to the presidency of the American Physiological Society. That same year Hy was awarded an honorary Doctor of Science degree from Brown to go along with the Ph.D. that he received from that university 37 years earlier. Hy also served as President of the Federation,

another great trust. Hy generously gave of his time. He accepted challenges to help out when asked, working on Boards of Directors of a number of volunteer health agencies.

But, importantly, Hy was a stabilizing factor at Tulane. The New Orleans Item published a profile of Hy Mayerson, as an educator, in 1951. In their opening paragraph, they stated, "When Tulane University established an award for the 'most cooperative member of the Tulane faculty' there is little doubt that the first recipient will be Hymen Samuel Mayerson, Ph.D. ....." Yes, when a cool head was needed to resolve a serious problem, the Dean called on Hy. The hottest issue of the mid-50's at Tulane was the planned new building to allow the basic science departments of the Medical School, then housed on the uptown campus in the Richardson building, to move downtown with the rest of the Medical School. Hy was put in charge of the building committee and in this way made one of his more lasting contributions to Tulane.

To this point, I've described a man of intelligence, very diplomatic and coolly self-confident. Figure 1 shows Matt Bach (L.M.N.Bach) and Hy sitting together in Hy's office in 1950. Matt, now Associate Dean at the University of Nevada School of Medicine, related to me an amusing story which characterized Hy's sensitivity.

Matt relates, "Dr. Mayerson always fulfilled my understanding of the definition of a gentleman. This first came home to me during my first year as an instructor in physiology (circa 1946). He had asked me to assist him in a demonstration of shock in the dog for the medical class. Lack of funds in those days obligated us to use old wine and vinegar jugs for our solutions. I had made up all of the required fluids, but my labelling was inadequate - or perhaps it was my vision. In any case, Dr. Mayerson announced to the class that we would now infuse histamine and observe the blood pressure responses. I began the infusion and the blood pressure obediently began to decline. Then he described the phenomenon that was occurring as the students watched in awe, 'Now, you can see the pressure is going down....is continuing to drop....we should see it begin to rise again as reflexes take hold....it is dropping a little further....and a little further',as the dog took its final agonal breath. 'Well,' Hy related, 'I guess it isn't going to rise after all.' Then Hy divined that I had run in the wrong infusion (it turned out to be citrate which was used as the anticoagulant to keep the blood in the arterial pressure cannula from clotting); but being keenly aware of my confusion, he deftly laid the blame on some obscure law of nature."

Along the same vein, John Hampton, who is now professor and head of the biological sciences department at California Polytechnic State University and who received his Ph. D. under Hy's guidance and was on the faculty in the Physiology Department from 1948 to approximately when Hy retired, relates, "I would not deny that he has influenced me in professional ways, for which I'm grateful. However, it is much more the personal and human involvement that I remember and cherish. I think his personal values place the human interaction above all else."

Jack Ginsburg (professor of physiology at Georgia School of Medicine) related to me the lasting impact that Hy has had on his approach to teaching and learning. "I do not recall the specific exercise that we were performing; it was cardiovascular in nature; it was a dog study and my results were definitely at odds with what I had predicted from my attendance at lecture and my readings in Best and Taylor. I sought assistance from Hy, who was in the lab that afternoon, complaining to him about the response of our dog to the experimental procedures and my conviction that 'things were going wrong'. Hy's reply was, 'Jack, no matter what the textbook says, the dog is always right!' He then made me go step-wise through the experimental procedures that we were employing and compare them to those described in the textbook noting the differences in techniques, anesthesia and such that could explain the discrepancy between the results of my experiment and our predictions based on my first reading of the text material. This experience served, for me and for other students as well, to set the stage for graduate study, as we came to realize over the ensuing months that all experimental observations, and therefore interpretations, are conditioned by the experimental design under which they are made. It was a good introduction to the basic scientific approach and I have, on more than one occasion, quoted to more recent students of physiology, those remarks made to me by Hy in the spring of 1950."

Hy had a wonderful, dry sense of humor that fitted well with his unflappable demeanor. Jack reminds me how it was about impossible to "get Hy's goat". In an attempt to ruffle his composure, several of the more playful graduate students in the early 1950's played a prank on Hy. While Hy was off at the Spring meetings, a collection of photos of French Quarter exotic dancers, all appropriately autographed, were substituted for autographed pictures of great physiologists which hung in Hy's office and which he cherished. Lili Christine, who was popular then as the "cat girl", was prominently featured. All the day of Hy's return, we waited for a reaction which, of course, never came. At the day's end, just as Hy was leaving, he casually remarked, "I would appreciate the reappearance of my old friends in their accustomed place on my office wall". (We knew that he meant it, the pictures were replaced and he remained "unflapped".)

Hy did much of his teaching as dog demonstrations. Jay Friedman (Professor of Physiology at Indiana) reminded me of the frequent surgical crises that occurred while Hy was cannulating the femoral artery to measure blood pressure while simultaneously talking to an ampitheater full of students. Once, the cannula came loose, Jay recalls, and Hy received an explosive hemorrhage directly into the face. Without dropping a word or changing pace, he stopped the hemorrhage, wiped his glasses, then his face, and finished his presentation amid great applause.

Hy took his teaching responsibilities very seriously. All of his lectures were well planned and beautifully presented. He gave more lectures

than anyone else in the department. He was also careful not to schedule meetings at times that might conflict with his teaching, despite having a large number of intra- and extra-mural meetings to attend. I think that all of his former faculty members would agree that Hy's traits as a teacher, department chairman and a person were a model which we would all love to emulate. He always gave of himself for others and, fortunately, he had much to give.

TABLE 1. Landmark Events in H. S. Mayerson's Academic Life

Born:	September 10, 1900 Providence, R. I.
Education:	Brown - A.B. - 1922 Yale - Ph.D. - 1925
Positions:	Instructor in Physiology Yale, 1925-1926 Instructor, Asst. Prof., Assoc. Prof., Physiology, Tulane, 1926-1945 Professor and Chairman of Department of Physiology, Tulane, 1945-1965 Associate Director, Touro Infirmary, 1965-1975 Board of Trustees, St. Mary's Dominican College, 1973-1979 Senior Physiologists Committee, Current
Honors:	President, American Physiological Society 1962 (many other positions of responsibility in Society) Honorary D.Sc., Brown, 1962 President, Federation of American Societies for Experimental Biology, 1963-1964
Editorial Boards:	Physiological Reviews Circulation Research
Board of Directors:	Louisiana Heart Association American Heart Association Cancer Assoc. of Greater New Orleans St. Mary's Dominican College, Board of Trustees, 1973-1979

Figure 1. Picture taken in 1950 of Dr. H. S. Mayerson (right), Chairman of Department of Physiology, Tulane University School of Medicine, and Dr. L. M. N. Bach, Neurophysiologist in the Department. Dr. Mayerson (an ardent pipesmoker) is feeding one of his pipes while conferring with Dr. Bach.

DR. MAYERSON, THE SCIENTIST

Aubrey E. Taylor

University of South Alabama School of Medicine, Mobile, AL.

The mark which a great scientist leaves on his field of research can be measured by many parameters; however, the most obvious and lasting gift which is endowed to the next generation of scientists is his ability to define and systematically evaluate difficult physiological problems. Dr. Mayerson has produced many research accomplishments in the cardiovascular field, but today we wish to focus on his tremendous contributions to the field of lymphatic function and capillary macromolecule permeability (1).

As this symposium progresses, it will become quite evident that each speaker is expanding either an idea or concept which was originally developed within Dr. Mayerson's classical studies dealing with the kinetics of dextran and plasma protein exchange between lymph and plasma (2).

Figure 1 (2, 3, 7) represents a study from Dr. Mayerson's laboratory in which dextran (mw = 35,000) was injected into the plasma and subsequently sampled from liver, intestine and cervical lymphatics at different time intervals. The most important point to make concerning this figure is that a differential of permeability exists between different capillary beds and that this permeability difference can be detected by using lymph concentrations of either graded molecular weight substances such as dextrans or endogenous plasma proteins (4, 6, 7, 8, 9, 10). Drs. Granger and Brigham will present their work on protein permeability in intestinal and pulmonary circulations respectively, using lymph protein flux measurements which represent an extension of the earlier techniques developed by Dr. Mayerson. Although the mathematical approaches may be different, the basic approach of utilizing lymphatic protein fluxes to analyze capillary permeability remains unchanged.

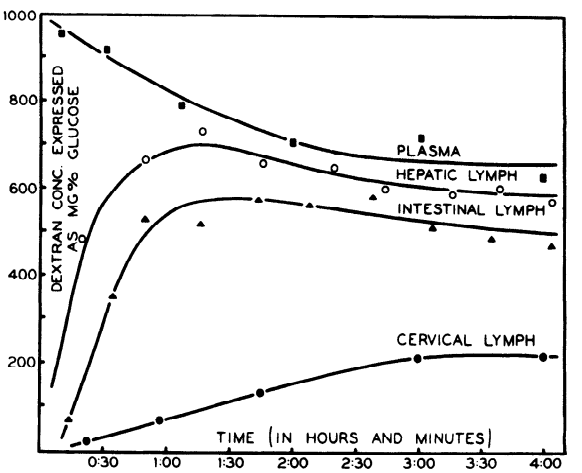

Figure 1. Plot of dextran (35,000 molecular weight) concentration in plasma (■), hepatic lymph (O), intestinal lymph (▲) and cervical lymph (●). Note that the concentration of dextran in hepatic lymph equilibrates at a level very similar to plasma and the time course is very rapid (equilibration time, 1 hour). The intestinal concentration is somewhat lower and requires one and one-half hours to equilibrate. Conversely, cervical lymph requires three hours to equilibrate and has a concentration approximately 1/4 that of plasma.

Figure 2 is another classical figure obtained in Dr. Mayerson's laboratory which again demonstrates the selectivity of different capillary beds to dextrans. Another important consequence of these

studies was that it appears that large molecules (greater than 250,000 molecular weight in intestinal lymph and greater than 150,000 molecular weight in cervical lymph) appeared to cross capillaries without any apparent restriction. Although the concentration of the large dextrans in lymph was low, there did not appear to be any greater restriction with increased molecular size, even when molecular weight was doubled. This finding led Dr. Mayerson to introduce the concept of vesicular transport as a means of transferring macromolecules across the capillary wall, and the term "cytopempsis" became associated with that phenomenon in the physiological literature. Today, you will hear both Drs. Renkin and Chinard discuss the concept of vesicular transport as related to their work, and Dr. Mayerson can rest assured that the controversy of "large pores" versus "cytopempsis" is still a very lively one today. In fact, it will be quite interesting to compare Dr. Renkin's present thoughts on vesicular transport to one of his earlier papers which was presented at the first conference on lymph and the lymphatic system which Dr. Mayerson chaired in 1965: "The bulk of the volume of lymph -- the water and low molecular weight solutes -- originates by filtration and diffusion through the small pore system. The greater part of the protein and other macromolecular constituents is derived from plasma by way of the large pore or vesicular transport system." I am certain that Dr. Renkin will end his paper with a similar note today. Dr. Chinard will show evidence that transport by "cytopempsis" is enhanced when Starling forces are altered, e.g., dilution of plasma proteins or increases of capillary pressure.

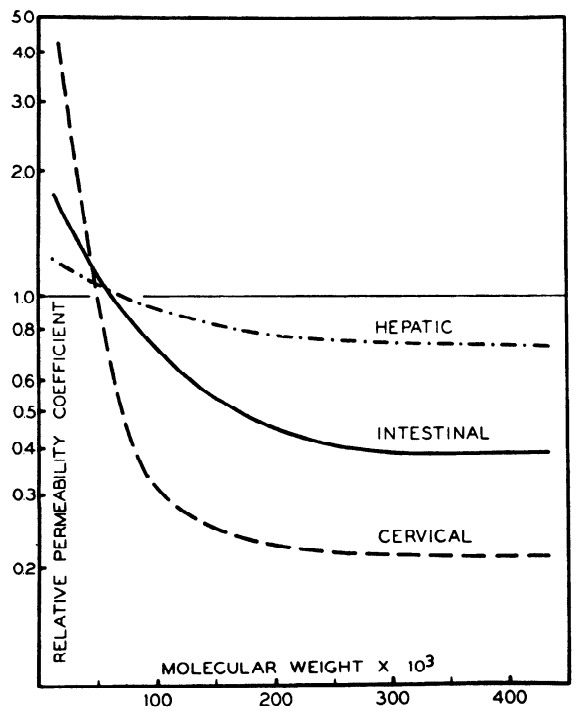

Figure 2. Plot of relative permeability ratios of different dextran fractions to albumin as a function of molecular weight. Note that the relative permeability becomes constant for the high molecular weight dextrans.

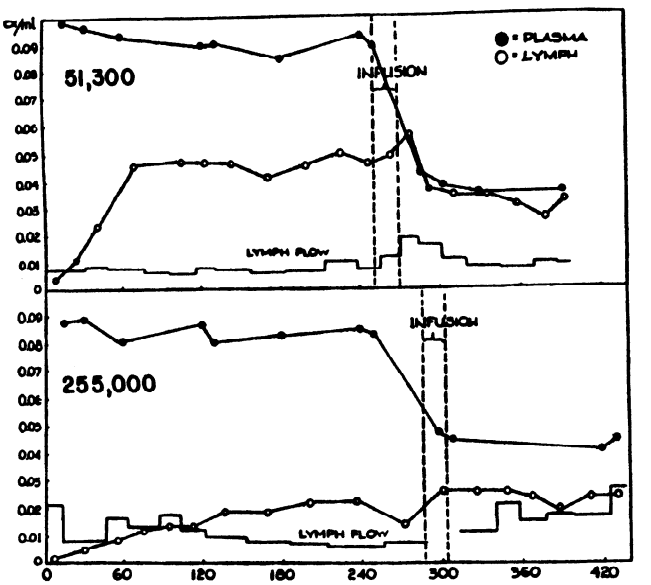

Figure 3. Plot of plasma (●) and thoracic duct lymph (O) concentrations of two different molecular weight dextrans (51,300 and 255,000) as a function of time in minutes. At the portion of the curve marked infusion, 40 ml/kg of a 5% albumin solution was infused into the animals. Note the difference between the lymph to plasma ratios following the infusion. Since lymph flow was approximately the same after the infusions (.25 - .50 ml/10kg body weight) then the permeability must have increased following the infusion.

Figure 3 is a modification of a figure from another of Dr. Mayerson's studies. In this study, dextrans of low and high molecular weight (51,300 and 255,000) were introduced into the plasma. Lymph to plasma ratios (L/P) and lymph flow were then measured in the thoracic duct as a function of time. After these parameters reached a steady state, L/P's of 0.5 and 0.25 were measured for the small and large molecular weight dextrans, respectively. At the arrow, a 5% albumin solution (40ml/kg) was infused systemically and lymph flow and L/P of the dextrans were again determined as a function of time. Following the plasma volume expansion, the lymph to plasma ratio of the small dextran was increased to approximately one and the L/P of the large dextran was increased to 0.625. Dr. Mayerson interpreted this finding as indicating that the capillary permeability had increased due to the increased vascular pressures widening existing "pores" and the term "stretched pore" was applied to this phenomenon (5, 7, 9).

Dr. Fishman will present his data in this symposium which deals with the phenomena of "stretched pores" in the pulmonary circulation. Needless to say, Dr. Mayerson had early on indicated that the "stretched pore" phenomenon was not confined to the systemic circulation, but was also always present in the pulmonary circulation following plasma volume expansion and may be one of the principle problems associated with the development of alveolar edema following elevation of left atrial pressures.

As the symposium develops, the impact of Dr. Mayerson's classical studies on the present state of the art will be self-evident, since the papers deal with selectivity, "cytopempsis" and stretched pores as applied to different capillary exchange systems. I am reminded of a recent statement made by Dr. Norman Staub who helped many of us to rediscover the lymphatic system: "The work of Dr. Mayerson was the major reason that I became interested in the lymphatic system and developed the sheep lung lymph fistula model as a tool to investigate pulmonary capillary permeability."

Every worker in the field of lymph function and capillary permeability would respond similarly to Dr. Staub when evaluating Dr. Mayerson's impact on the field of large molecule exchange, i.e., Professor Mayerson's contributions have had a lasting impression on all studies dealing with the movement of proteins between plasma and lymph, and his studies still provide us with the basic tools with which to answer the questions he so eloquently posed a quarter of a century ago. This, to me, represents the utmost relative to achievement in any particular field of endeavor.

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A Chance to Reminisce

Hymen S. Mayerson

1140 Seventh Street, New Orleans, LA 70115

Fellow capillarians and lymphomaniacs - greetings! It is indeed a signal honor and affectionate tribute for you to dedicate this session to me. I am deeply appreciative and touched.

I suppose this all started last Christmas vacation when John Manning and his lovely spouse paid us their usual Christmas visit. We talked of many things and late in the conversation, John casually mentioned that he was on the program committee, and that they were considering a session in my honor - I made the usual remonstrances - John didn't say much but I realized that he had evaluated my mental and physical condition, and that he had decided that I was fit enough to do it!

The next step was a letter from Aubrey Taylor with an outline of the program which started with a distinguished lecture by me. I immediately wrote him that this was impossible - I could not give a "distinguished" lecture simply because I had been away from Academia since 1965 - helping run a 560 bed teaching hospital, Touro Infirmary- and the only lecturing I had done there was certainly not distinguished.

Aubrey nicely took the hint and prepared the present program. I told him I'd just like to reminisce.

I suppose every investigator, at some time, has asked himself, "How did I ever get into this mess?" As some of you may remember, I came down from Brown University to New Haven and Yale in 1922. Lafayette Mendel was Chairman of the Department of physiology which was then divided into Chemical Physiology and Physical Physiology, Henry Laurens being Chairman of the latter subdivision. Most important, 1922 was the year August Krogh came to Yale and delivered the Silliman Lecture on capillaries. He was a magnificant speaker. He showed one marvelous slide of capillaries after the other and I was most excited - particularly when he said to me after the lecture that I should give some thought to working on capillaries since so few investigators were interested in this field. But I was then working on the effects of light and later got interested in the effects of posture on circulation which later led to the development of the G-suit.

But the capillaries were always in the background! I tried photographing them without too much success until at one juncture I was fortunate to borrow a camera from Dr. Kearney, my son's physician, whose hobby was photography. I got some fine pictures of rat mesenteric capillaries and some in the dog but again got sidetracked because on one of those days, I was drinking coffee with Dr. Champ Lyons, who was then at Tulane in the Department of Surgery. We got around to some of his war experiences in the Mediterranean theater where he had given large transfusions to wounded men and followed plasma volumes with the dye T1824. His data indicated that much of the transfused volume was not being retained. I knew that the problem of "lost plasma" was not a new one, but I had no really satisfactory answers. So we decided to get some answers, and in no time at all a project was born. We applied for funds from the Research and Development Command of the Surgeon General's Office, received them and they continued to generously support us for the rest of our scientific course.

Fortunately, we were working at the right time. Isotopes were just becoming available and our first job was to develop methods of accurately measuring blood volumes, using human serum albumin iodinated with radioactive iodine (I^{131}) for plasma volume and red cells labelled with radioactive phosphorus (P32) for measurement of red cell volumes. Armed with these reasonably quantitative methods and with the assistance of two fellows, John H. Clark and Woodrow Nelson, who came back from the war for training in surgery, we invaded the clinics. Few patients were sacred. We became particularly interested in the data from chronically debilitated patients, who usually showed low blood volumes and we suggested that these individuals were suffering from "chronic shock". In doing so, we brought down the wrath of colleagues, as some of you recall, who complained that modifying an indefinable term like shock was "confusion worse confounded". However, I do think it emphasized the importance of actually measuring blood volumes in these and other patients rather than relying solely on the usual concentration measurements. We made many other interesting observations, but most important, we confirmed the previous empiric observations that when large quantities of blood were given, the plasma appeared to leave the blood stream and only the red cell portion remained. This implied that the plasma proteins were leaking out of the capillaries. But capillaries, as I indicated previously, were supposed to be relatively impermeable to molecules as large as protein. It is true that Drinker had been insistent that so called "normal"

healthy capillaries leaked protein. Couriously enough, it was a hard idea to sell and most textbooks of physiology continued to hedge the issue by saying that significant protein leakage could occur, but only when capillary permeability had increased over "normal" for one or another reason.

Drinker mustered considerable evidence to support his argument but much of it was necessarily indirect, for he had no satisfactory way of labelling plasma proteins and following their leakage from capillaries. Time had given us an advantage. With the availability of isotopes, it was apparent that we could label proteins and not only quantitatively follow their disappearance from the blood stream but could also trace them to lymph. This then became the next phase of the problem - to learn what we could about the permeability of blood capillaries. In passing, we had another important advantage over Drinker - polyethelyne catheters - which made even the most obstinate lymphatic accessible.

I had spent several summers at Woods Hole and had listened with awe at some of Drinker's lectures. I came back and wrote up a demonstration for our medical students in which we fed a dog with cream, isolated the thoracic duct, compared clotting of lymph and blood, massaged the abdomen, noted respiratory effects, infused 10 p.c. sucrose and so on. I had become reasonably adept at isolating the thoracic duct, wherefore it was a perfectly natural thing for Karl Wasserman to agree that we study lymph - and we went at it with a vengeance. Our early studies had to do with lymph from the thoracic duct and we drew various conclusions from the data collected. Grotte, working in Upsala, Sweden, on more or less the same problem, objected to our various conclusions (correctly, I believe, because they were based on data derived from only thoracic duct lymph).

He pointed out that the thoracic duct lymph in the anesthetized dog is derived mainly from the intestines and liver and that the great permeability of the liver may constitute a special outlet mechanism, i.e., after large infusions of colloidal solutions, the increase in macromolecular concentration measured in thoracic duct lymph may be due to a relatively greater increase of lymph flow from the liver than from the other sources of thoracic duct lymph having a lower permeability to macromolecules. To answer this objection, we undertook to repeat the experiments but collected lymph from the right duct. In the quiescent, anesthetized dog the amount of lymph collected from the right duct expresses the lymph delivery from the contracting heart and the moving lungs. Right duct lymph resembles cervical and leg lymph in that it has a low protein concentration (0.5-3.5 qm. p.c.).

Although Drinker described a procedure for isolating and cannulating the right lymph duct which seemed simple enough, in my hands, this was no easy task. I had reached the point where I could find the right duct reasonably well at the beginning of a summer session when we usually got an influx of bright young medical students who wanted to spend the summer learning to do research. One of these, Hoke Shirley, allowed that he would like to work in the laboratory but only if we could challenge him with a really difficult problem. He had a buddy, Gordon Wolfram, who, he said, was equally good. I sold them the right duct problem then and there. When they were ready to do their first experiment, I carefully absented myself from the laboratory and came back later in the day prepared to console them and hold their hands. But you may have guessed what happened. They not only did a most successful experiment that day but did six successive successful experiments before they missed.

Their results, we believe, answered Grotte's criticisms, since the abdominal viscera were not implicated. They emphasized the correctness of our previously formulated concept that infusions producing plasma volume expansion cause capillary "pores" to enlarge or stretch with a resultant increased leakage of large molecules. This was a possible explanation for the "lost plasma" phenomenon and the answer to the question which stimulated the beginning of the work some ten years previously. Large infusions not only engorge the capillaries and raise filtration pressure but the pores stretch and permit increased leakage of macromolecules.

We have been subjected, over the years, to much kidding about our "stretched pores". But I notice today, that Al Fishman is now stretching pores in the lungs - we couldn't be far wrong.

During our early work on capillaries, we had a nice visit from Gene Renkin. He was then working with Gene Landis and John Pappenheimener at Harvard. Gene spent several days with us and made valuable suggestions about our data. We enjoyed his visit very much. Several days after he left, I got a small package from him which turned out to contain a slide labeled Neg. #3-407-5. Here it is! It was the first inkling of his art as well as his science. You've all since seen others of his cartoons labelled as graphs in his publications. Gene, its the one and only time I have been characterized as "devilish"!

And let's get back to Karl who was the other inspiration for much of the work. As I indicated, our research funds came from the Research and Development Fund and we made periodic reports to the Committee on Shock of the National Research Council. The committee was composed of the "great white fathers" - mostly eminent surgeons - Jake Fine of Harvard, "Dusty" Rhodes of Pennsylvania, etc. It was a formidable group. Well, one day I suggested to Karl that since he had done most of the work on the project we needed to report, that he come to Washington and give the report. Karl jumped at the chance of a free trip to Washington. That evening, at the hotel, Karl began to complain about not feeling well and didn't know whether he could really give the paper the next morning. I wasn't bothered, I knew the symptoms! At any rate, I told him at breakfast that he had to give the report. You know the rest. He got up - started running - did a beautiful job- and has been running ever since. I don't know whether you all know that Karl ran in the last Boston Marathon - I don't know where he finished - but he vows he'll be back.

And then there was the invitation from Art Guyton to come to Mississippi and tell of our work. I don't remember what I told the group, but I do remember the fine dinner with Mrs. Guyton and the large, attractive family. I was taking the train back to New Orleans, and Art took me to the station. The train was late and Art expounded on what I reported. I told Art that Drinker emphasized that the primary function of the lymphatic system was to return protein to the blood stream - that which had leaked out. He immediately began going off into "space" - what about negative pressure of interstitial fluid - why isn't the function of lymph to maintain negative pressure of the interstitial fluid by removing protein from the tissue spaces while the blood capillaries perform the function of removing excess fluid by osmosis? So there you are -

One of the most acute rememberances is the awe with which people look at their first sample of lymph. I was asked by my friends in Oak Ridge at one point to come up and show one of their surgeons how to cannulate the thoracic duct. I took John Hampton with me to help. When I got there, the young surgeon produced the animal - and I learned that we were to operate in the large amphitheater because everyone wanted to see it. Well, John was sweating - he was worried, for he had seen me miss so many times. But there was the duct and in no time we had a test tube of thoracic duct lymph - which now was passed around to the assembled staff who looked with wonderment and awe. So this was lymph! And I have had many similar experiences. Somehow lymph still has a fascination as something esoteric and strange.

Lastly, I suppose the most gratifying experience, and I have had a lot of fun over the years, was the organization of the first conference on lymph held in New Orleans in 1965, the year of my retirement from the Tulane Medical School. We were able for the first time to bring some of the foreign workers to this country, Kimmonth from London, Strauli from Switzerland, Courtice from Australia, and Foldi from Hungary. It was followed by a second conference in Miami, and by the formation of the Society. While the accents were different, the language was the same, for no matter how you say it a lymphatic is a lymphatic - is a lymphatic, etc. The conference brought together the first good bibliography on lymphatics. And lymphology had come to its own - and it was a damned nice way of ending a career.

One amusing incident regarding the second conference. Dr. Viamonti called me in great distress from Miami early in 1968. How the devil, he asked, had I gotten money to support the New Orleans Conference - he had repeatedly been turned down. I suggested that perhaps he wasn't talking to the right people. How well did he know the secretaries of the various sections and committees? He took the hint, and we got the money.

Again, my friends and colleagues, thank you for your thoughtfulness and kindness in planning this session. I shall listen carefully to all you have to say and be grateful that you wanted me here to listen and learn.

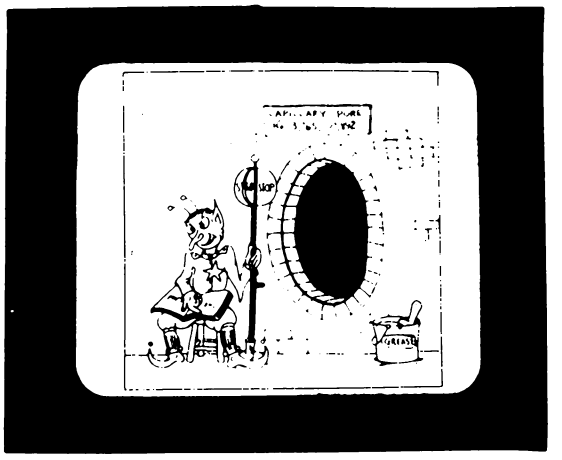

Fig. 1 Eugene Renkin's illustration #3-407-5 of a capillary pore.

ESTIMATIONS OF PERMEABILITY PROPERTIES OF PULMONARY CAPILLARIES (CONTINUOUS ENDOTHELIUM)

Kenneth L. Brigham

Pulmonary Circulation Center
Vanderbilt University School of Medicine
Nashville, Tennessee

In an elegant set of experiments published in 1962, Professor Mayerson and his colleagues demonstrated that molecules as big as insulin (M.W. 6,000) and bigger, traveling in lymph through the popliteal node, do not exchange with blood supplying the node (1). They cannulated afferent and efferent lymphatics and the nodal vein, and perfused through the afferent lymphatic with solutions containing different sized molecules while collecting efferent lymph and blood. Albumin (M.W. 70,000) and inulin were recovered entirely in efferent lymph while smaller molecules ranged partly in lymph and partly in plasma.

I presume that the motive for those experiments was the conviction that important information about transvascular solute exchange in a microcirculatory bed can be gained by measuring the flow and composition of lymph if solute concentrations accurately reflect concentrations in the microvascular filtrate. Dr. Mayerson's experiments and several other studies indicate that that assumption is a reasonable one (2,3).

To study lung microvascular permeability to proteins, we have used a chronic sheep preparation, conceived by Staub (4), in which the efferent duct of the caudal mediastinal lymph node is cannulated and the tail of that node resected to eliminate lymph not originating in the lungs (4,5). Responses of this preparation to mechanical elevations of left atrial pressure have been well-characterized (6), and we have concentrated on manipulations which may increase lung microvascular permeability (7).

Figure 1 shows the effects of infusing E coli endotoxin on pulmonary vascular pressures and lung lymph in an unanesthetized sheep (8).

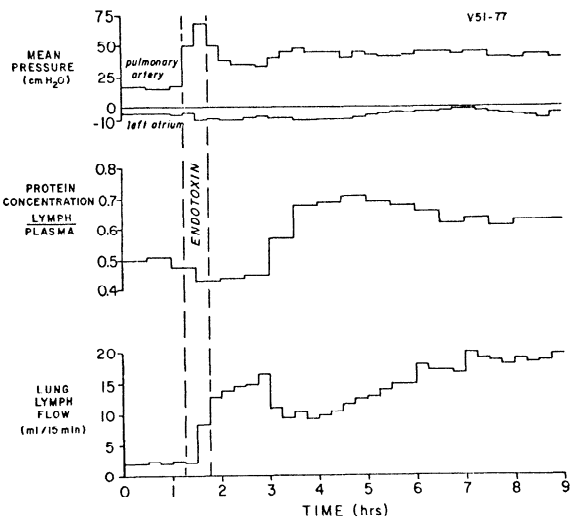

Figure 1: Responses of pulmonary vascular pressures and lung lymph to an intravenous infusion of E. coli endotoxin in an unanesthetized sheep.

The late phase of the response, where vascular pressures are stable and there is high flow of protein rich lymph, is typical of increased vascular permeability (7). That response is similar to that reported by Wasserman, Loeb and Mayerson when they infused 40 ml/kg of a 5% solution of human albumin into dogs while measuring thoracic duct lymph flow and protein concentration (9). Because when lymph flow increases in response to elevated hydrostatic pressure, lymph protein concentration always falls, high flow of protein rich lymph, at least in the lung, must indicate increased permeability.

Wasserman, Mayerson and Loeb also studied the sieving characteristics of the systemic blood-lymph barrier by infusing dextrans of different sizes into dogs and observing their partitioning between blood and thoracic duct lymph (9). We have made similar efforts by separating endogenous plasma and lymph proteins electrophoretically (10). Figure 2 compares the baseline steady state relationships between

lymph/plasma concentration and molecular weight for dextrans in thoracic duct lymph and for endogenous proteins in lung lymph in sheep.

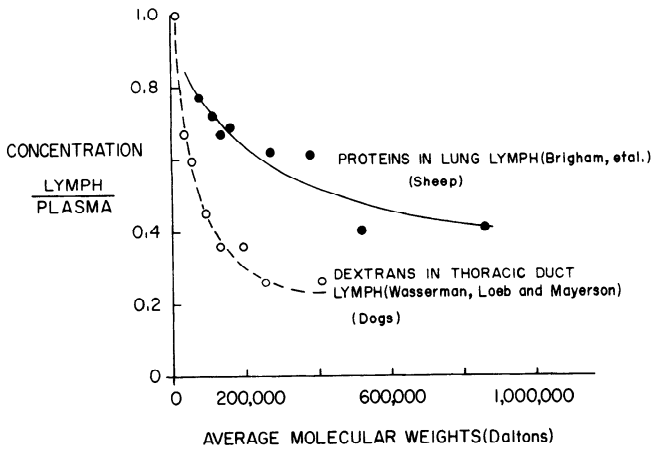

Figure 2: Relationships between lymph/plasma concentrations and molecular weight dextrans in thoracic duct lymph in dogs and proteins in lung lymph in sheep.

In both cases, sieving of molecules between plasma and lymph is indicated by decreasing lymph/plasma concentrations with increasing molecular weight. From this comparison, it appears that there is greater overall sieving of macromolecules in the systemic circulation of anesthetized dogs than in the lungs of unanesthetized sheep.

What happens to the sieving characteristics of the blood-lymph barrier in the lung when microvascular permeability is increased? We have measured lung lymph flow and lymph and plasma concentrations of eight endogenous protein fractions during steady state responses to several interventions which appear to increase lung vascular permeability. To compare lymph transport of different sized proteins with different plasma concentrations, we have compared steady state relationships between lung lymph clearance (lymph flow x lymph/plasma concentration) and Einstein-Stokes radius (7). Figure 3 shows such relationships for baseline, mechanically increased pressure and three interventions which increase permeability: histamine infusion (11), infusion of Pseudomonas bacteria (5) and infusion of E. coli endotoxin (8).

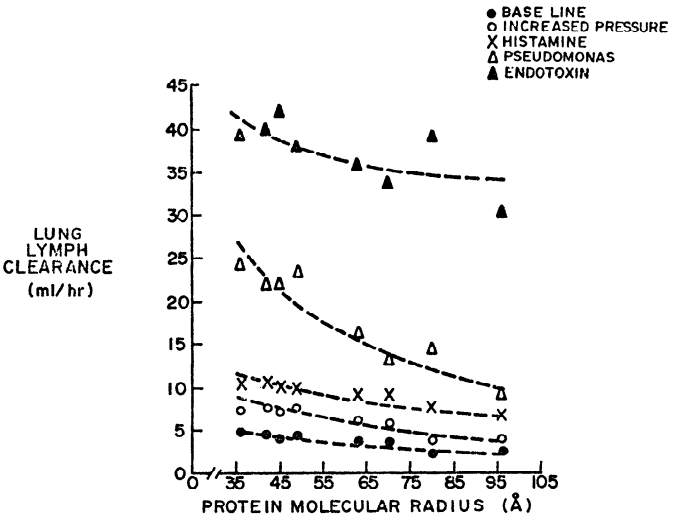

Figure 3: Sheep lung lymph clearance (flow x lymph/plasma concentration) of eight endogenous protein fractions during steady state baseline, increased vascular permeability.

Each of the interventions increases clearance of all of the proteins. Clearance of all of the proteins is increased much more when permeability is increased than when pressure is mechanically elevated. Even when permeability is increased (and regardless of the cause), lung lymph clearance still decreases as protein molecular radius increases, that is, microvascular sieving of macromolecules persists during increased permeability. We have interpreted these data as evidence that lung microvessels may leak proteins and fluid more readily than normal without severe alterations in their structure (12). Such an interpretation is consistent with equivalent pore theory and with the experimental observation that increased lung vascular permeability is often rapidly reversible (5,8,11).

In addition to the qualitative inferences about the structure of exchanging vessels in the lung discussed so far, it may also be possible to make quantitative inferences from lymph data. Several attempts have been made to calculate overall membrane coefficients {permeability-surface area (PS); reflection coefficients (σ) directly from lymph data (13,14,15)}. If the walls of exchanging vessels behaved as a simple homoporous membrane, this should be possible. But, at least in the lung, the filtering characteristics of exchanging vessels are not those of simple homoporous membrane (12). It is possible to construct theoretical multiple pore models which accurately predict lung lymph flow and protein concentrations. Such models promise specific information about

the function of the lung microcirculation.

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PERMSELECTIVITY OF INTESTINAL CAPILLARIES

D. Neil Granger and Aubrey E. Taylor

Department of Physiology, University of South Alabama

INTRODUCTION

The partition of fluid between intravascular and extravascular compartments is largely governed by hydrostatic and osmotic forces acting across the capillary wall. The degree to which capillaries restrict the passage of macromolecules (plasma proteins) is also of fundamental importance in controlling the rate and direction of transcapillary fluid movement. The restrictive properties of capillaries is particularly important in transporting organs such as intestine and kidney where large quantities of water and solute are removed from the interstitial spaces predominantly by the transcapillary oncotic pressure gradient (10).

This treatise focuses on the restrictive properties of intestinal (fenestrated) capillaries to macromolecules. The first section provides an overview of the ultrastructural-functional correlates in the transendothelial exchange of water-soluble macromolecules. In the second section we examine the available physiological data on blood-lymph transport of macromolecules. Finally, permeability estimates which are applicable to transcapillary fluid balance and the Starling hypothesis are discussed.

STRUCTURAL-FUNCTIONAL CORRELATES

The capillaries of the intestinal wall are structurally heterogeneous. The vessels of the mucosa are of the fenestrated type and represent by far the predominant component of the entire capillary bed. The vessels of the muscularis have a continuous endothelium, yet their contribution to the overall permeability of the intestinal capillary bed is considered negligible. Within the villi, fenestrated capillaries are concentrated immediately (0.5μ) under the epithelium and the fenestrations are preferentially oriented toward the base of the epithelium. The fenestrae are circular openings in the endothelium with a diameter of 250-600 Å. Over sixty percent of the fenestrae are provided with an aperature or diaphragm of unknown porosity. Intercellular junctions or "gaps" are infrequent and considered to play a minor role for solute and water exchange relative to the fenestrae. The basement membrane surrounding the intestinal capillaries is formed by a layer of fine fibrillar material similar to that surrounding other capillaries (4, 5, 14, 19, 20).

Electron-dense molecules of known dimensions have been used to identify the structural equivalents of the small and large "pores" predicted by the pore theory of capillary permeability. For the fenestrated capillaries of the intestine the tracer molecule studies suggest that the large pores are represented by that fraction of the fenestral population without diaphragms while the small pores are located in all other fenestrae, the size-limiting structures being the porosity of the diaphragms. No probes have been shown to enter the pericapillary space via the intercellular junctions. There is a transient accumulation of tracer particles (diameter > 200 Å) at the basement membrane suggesting that this structure may also be a component of the large pore system.

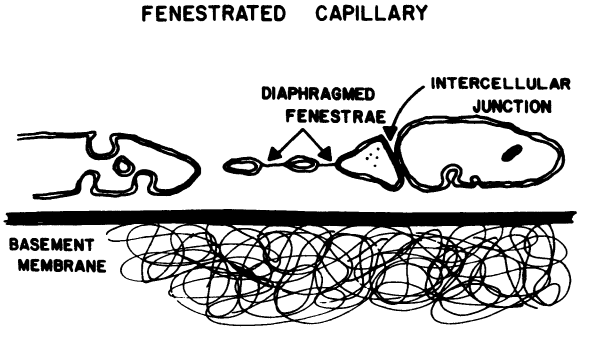

Figure 1. Diagrammatic representation of the structural equivalents of the "small" and "large" pore systems in the fenestrated capillaries of the intestine (modified from ref. 18).

Although vesicular transport does account for some of the transcapillary exchange of tracer molecules, it appears that transport by vesicles is approximately 8 times slower than exit through the fenestrae (5, 6, 19, 20). Figure 1 summarizes the proposed structural equivalents of the pathways followed by macromolecules across the wall of the fenestrated capillaries in the intestine.

PHYSIOLOGICAL ESTIMATES OF CAPILLARY PERMEABILITY

A general approach to studying the restrictive properties of capillaries involves measuring the concentration of various test substances in plasma and lymph and to assume that the lymph-plasma concentration ratio (L/P) under steady-state conditions provides an estimate of the capillary permeability to the molecule. An inherent assumption in this type of analysis is that the concentration of the test substance in lymph is dependent on its concentration in the capillary filtrate and on the relationship of the capillary pore and solute sizes. By obtaining the relationship between L/P and molecular radius for several solutes one can approximate the size and distribution of capillary pores. Figure 2 illustrates the relationship between L/P and solute radius for long-chain polymer molecules (dextrans) and plasma proteins in intestinal lymph collected at a normal capillary filtration rate (lymph flow) (1, 8, 9, 15). The data in Figure 2 show a steep fall in the permeation in the molecular size range below 60 Å. Above 60 Å there is an extension of residual permeability with little decrement in L/P as far as 135 Å. In his classical study on intestinal capillary permeability, Mayerson (15) explained his L/P data according to the pore theory by a system of pores of 110 Å radius and capillary leaks > 140 Å radius. The small pore system was considered to account for approximately 78% of the total capillary pore area while the leaks account for the remaining 22%. In addition, Mayerson (15) offered the possibility that vesicular transport could account for the constant residual permeability (L/P) over the high range of molecular size. The more recent analyses (1, 9) of L/P data from intestine have been explained according to the pore theory by a system of "pores" and "leaks" which are smaller than that predicted by Mayerson; however, the restrictive properties of the fenestrated capillaries of the intestine continue to fall between that of the highly permeable liver capillaries and the relatively impermeable muscle capillaries.

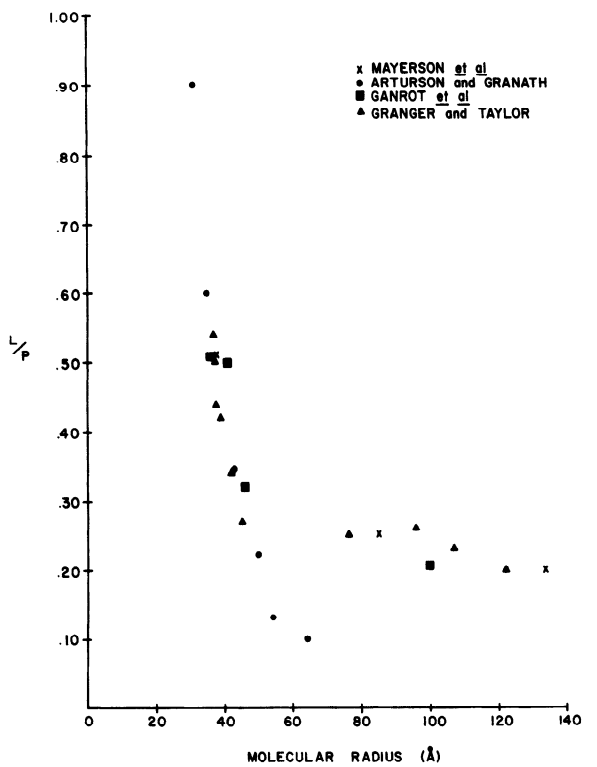

Figure 2. Steady-state relationship between lymph:plasma concentration ratio of various macromolecules and molecular radius at normal lymph flow.

OSMOTIC REFLECTION COEFFICIENTS

According to the modified Starling hypothesis, transcapillary filtration rate (J_v) can be described by the expression:

$$J_v = K_{f,c}[(P_c - P_t) - \sigma_d(\pi_p - \pi_t)] \quad . \; . \; .(1)$$

where $K_{f,c}$ = capillary filtration coefficient
P_c = capillary hydrostatic pressure
P_t = interstitial fluid pressure
σ_d = osmotic reflection coefficient for plasma proteins
π_p = plasma oncotic pressure
π_t = tissue oncotic pressure

There are two parameters in the Starling equation which relate the permeability of the capillary wall to transcapillary fluid exchange, $K_{f,c}$ and σ_d. The osmotic reflection coefficient more specifically relates the degree of macromolecule restriction by the capillary wall to osmotically induced water movement across the capillary and, as such, is the most significant measure of macromolecule permeability in relation to transcapillary fluid exchange. An osmotic reflection coefficient of 0.70 means that the macromolecule striking a pore has a 70% probability of being reflected and a 30% probability of passing through. The greater the pore size, the

less the reflection coefficient such that σ_d = 1.0 in a perfect semi-permeable membrane and σ_d = 0 across a membrane where the solute traverses as easily as water. Capillaries with a σ_d = .70 for plasma proteins would allow for 70% exertion of the colloid osmotic pressure across their membranes.

It has long been assumed that σ_d is greatest in continuous type capillary beds, intermediate in fenestrated capillary beds and lowest (approaching zero) in the discontinuous type capillaries based on ultrastructural estimates of pore size. Based on the dimensions of fenestrae one would estimate an osmotic reflection coefficient for molecules the size of albumin less than 0.10 in intestinal capillaries. Comparable values of σ_d are predicted from pore distributions proposed using the relationship of L/P and molecular radius.

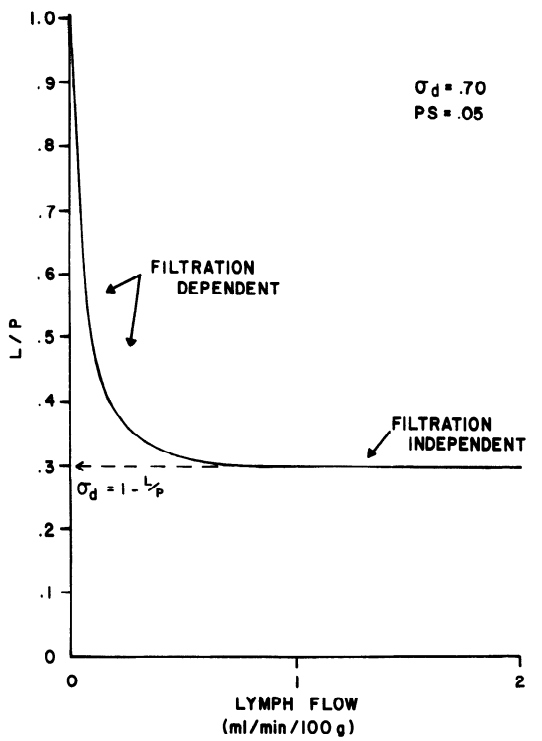

Figure 3. Theoretical relationship between L/P and lymph flow derived from equation 2. The osmotic reflection coefficient (σ_d) can be estimated using 1-L/P when L/P is filtration independent.

Two physiological methods are currently available for estimation of σ_d: (1) the osmotic transient method and (2) the lymphatic protein flux approach. Although σ_d for small lipid insoluble substances (e.g., glucose) have been determined, estimation of σ_d for macromolecules such as albumin has been technically difficult using the osmotic transient method in the blood perfused small intestine (11). The osmotic reflection coefficient of total plasma proteins and various proteins of known molecular size have been obtained in the small intestine using lymphatic protein flux data. Figure 3 illustrates the method employed to estimate σ_d using steady-state L/P and lymph flow values. The theoretical curve in Figure 3 was derived from the equation (17):

$$L/P = \frac{1 - \sigma}{1 - \sigma_e - x} \quad \ldots (2)$$

where $x = (1-\sigma)J_L/PS$ and, J_L and PS are the lymph flow and permeability-surface area product, respectively. Figure 3 illustrates that as lymph flow (capillary filtration rate) is increased, L/P decreases rapidly (filtration dependent) and then becomes relatively constant as L/P approaches a minimal value (filtration independent). Studies from our laboratory indicate that 1 - L/P at the level where L/P is filtration dependent provides an estimate of the osmotic reflection coefficient (2, 9). Experimentally, the relationship between L/P and lymph flow was acquired by measuring intestinal lymph and plasma protein concentrations and lymph flow at portal venous pressures of 0, 10, 20 and 30 mmHg.

Table I. Osmotic reflection coefficients of intestinal capillaries to various endogenous macromolecules.

Molecular Radius (Å)	Osmotic Reflection Coefficient (σ_d)
37	.90
39	.94
45	.96
96	.98
120	.99

Data acquired from ref. 9.

Figure 4 depicts the relationship acquired between L/P for total plasma proteins and lymph flow in the cat small intestine under control conditions. Using 1-L/P when L/P is filtration independent, a value of 0.92 is acquired for the osmotic reflection coefficient. The osmotic reflection coefficient was also determined for several endogenous plasma protein fractions of known molecular size using the relationship between L/P and lymph flow. The values of σ_d for several

endogenous plasma protein fractions are presented in Table I. The distribution of osmotic reflection coefficients acquired for the various plasma protein fractions (Table I) is consistent with two equivalent pore populations, i.e., a small pore system of 50-60 Å and a large pore system of 100-130 Å.

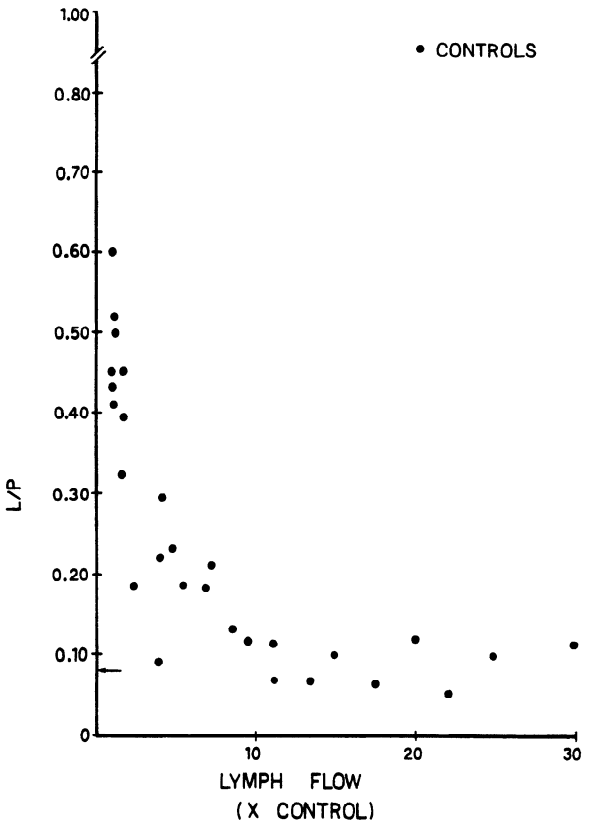

Figure 4. Steady-state relationship between intestinal L/P (total proteins) and lymph flow.

Several humoral agents including bradykinin histamine and glucagon and hyperosmolarity decrease the osmotic reflection coefficient of intestinal capillaries thereby favoring protein leakage and enhancing capillary filtration (11, 13, 16). Figure 5 illustrates the effect of intra-arterial infusion of glucagon and intraluminal placement of bile with oleic acid on the relationship between intestinal L/P (total plasma proteins) and lymph flow. It is evident from the data presented in Figure 5 that both glucagon and fat absorption decrease the osmotic reflection coefficient for total plasma proteins and increase the permeability of intestinal capillaries. Estimates of the osmotic reflection coefficient coupled to CFC measurements are particularly useful for assessing the mechanism by which various humoral agents and physiological conditions alter transcapillary fluid balance. For example, both isoproterenol and bradykinin increase the intestinal capillary filtration coefficient, yet bradykinin decreases the osmotic reflection coefficient while isoproterenol does not (13). Such finding suggest that the increase in capillary filtration coefficient (and transcapillary exchange) with isoproterenol must result from an increase in capillary surface area.

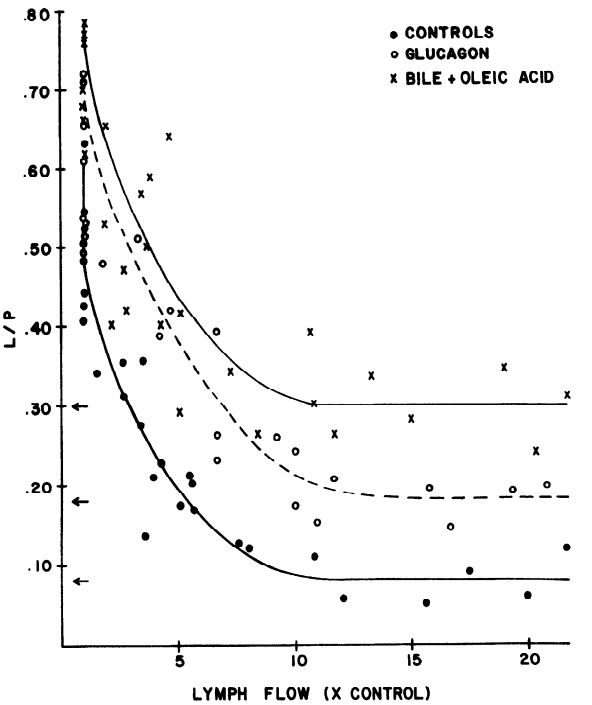

Figure 5. Effect of glucagon and fat absorption on the relationship between lymph flow and L/P.

CONCLUSIONS

The fenestrated capillaries of the intestinal mucosa are perceived by many physiologists to be "leaky" due to the normally high lymph protein concentration coupled to ultrastructural estimates of the dimensions of the fenestrae. However, physiological estimates of the osmotic reflection coefficient indicate that intestinal capillaries are more restrictive to endogenous macromolecules than lung, liver and subcutaneous tissue capillaries (12, 18, 21). These findings suggest that an analysis of lymph to plasma protein concentration ratios at normal capillary filtration rates can only provide qualitative

information which is insufficient to delineate regional differences in capillary permeability. Thus, the influence of various factors such as capillary heteroporosity, and capillary surface area limits the applicability of L/P data at normal filtration rates for development of pore models (2, 9, 21). It is now evident that the most thermodynamically sound approach for acquiring a measure of capillary permeability,which can be applied to Starling hypothesis,using lymphatic protein flux data is to attain the condition where L/P is independent of capillary filtration rate. Application of this approach to intestinal capillaries indicates that the restrictive properties of the fenestrated capillaries allow for exertion of 92% of the total transcapillary oncotic pressure gradient across the capillary wall. These data, coupled to the extreme selectivity of glomerular and peritubular capillaries to macromolecules (3, 7) suggest that fenestrated capillaries in general are more or, at least, equally restrictive to macromolecules than the continuous type capillaries (with the exception of the blood-brain barrier). Although the precise location of the limiting restrictive barrier in glomerular capillaries is relatively well known (3), the major restrictive barrier of intestinal capillaries which accounts for the high osmotic reflection coefficient remains uncertain. From the available ultrastructural tracer data it is tempting to suggest that porosity of the diaphragms which cover over 60% of the fenestrae is the rate limiting barrier to diffusion and convection of macromolecules in the intestine. However, until the restrictive properties of the diaphragms are delineated, one ca-not exclude the basement membrane as the limiting barrier.

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STRETCHED PORES, BLAST INJURY, AND NEUROHEMODYNAMIC PULMONARY EDEMA

A. P. Fishman, and G. G. Pietra

University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

The concept of "stretched pores" can be traced to three separate beginnings: 1) a hypothesis to explain the pulmonary edema that follows experimental blast injury or the massive administration of epinephrine [3]; 2) the results of systematic physiologic experiments designed to explore the concept [31]; and 3) the visualization by electron microscopy of the passage of large macromolecules between endothelial cells during experimental hemodynamic pulmonary edema [24,30]. Once the idea took hold, it also proved useful in explaining earlier observations that otherwise were perplexing.

The Systemic Circulation

Almost a half-century ago, Landis et al had difficulty in explaining the relationships that they demonstrated between filtration pressure and capillary permeability in the human arm [17]: when venous pressure was increased moderately, i.e. to levels of 40 to 60 mm Hg, the ultrafiltrate appeared to be virtually devoid of protein; but, when the venous pressure was raised further, i.e. to 80 mm Hg, considerable quantities of protein did appear in the ultrafiltrate. Thus, at low to moderate capillary pressures, the permeability of capillaries in the human arm seemed to be low and invariant; in contrast, at very high capillary pressures, permeability to blood proteins increased dramatically. Although microinjection studies had shown that the frog's capillary could stretch enough under high pressure to allow spurts of india ink to traverse the capillary wall without tearing [15], it was easier at that time to picture tearing rather than stretching of the capillary wall since the capillary wall was popularly regarded as a rigid tube, a mosaic of endothelial cells held together by a cement substance that was interrupted at intervals by gaps for transmural exchange [5, 16].

Over the subsequent decades, confidence dwindled in the role of intercellular cement as the major determinant of capillary permeability [19,20,34]. Even when the model of the non-fenestrated capillary was developed [23], it made no provision for any play in the capillary wall. The endothelium was envisaged as though it were a semi-permeable membrane made of collodion; pores within the wall made it possible for small, lipid-insoluble molecules to cross by diffusion. Two populations of pores could account for the physiologic observations: "small pores", about 4.0 to 4.5 nm in radius, occupying about 0.1 percent of the capillary surface, offered moderate resistance to diffusion for substances of molecular weight from 1 to 10,000 daltons; "large pores" were invoked to explain the passage of larger macromolecules [9]. In the 1960's, the "small pore" system of the physiologists was equated with the intercellular junctions between adjacent endothelial cells [12, 13]; the "large pore" system was attributed to pinocytotic vesicles [2, 20].

In these representations, the capillary endothelium was held to be an inflexible barrier. This construct had the advantage that the capillary wall could be regarded as a membrane in which the diffusion coefficient was restricted and in which molecular sieving could be depicted by relating pores of constant size to blood constituents and tracer molecules of constant molecular weight. Strong support for the idea of an inflexible barrier was provided by experiments involving the manipulation of hydrostatic and oncotic pressures in the hind limb of the cat and dog [23]. In this preparation, no permeability to protein could be demonstrated at capillary pressures that ranged from 12 to 22 mm Hg. Unfortunately, the experiments afforded little insight as to what would happen at higher pressures.

In the 1950's, Mayerson, Wasserman, Shirley and co-workers undertook a systematic exploration of the idea of "stretched pores" [31,36]. Relying heavily on labelled albumin and dextrans of known particle size, they showed that when the circulating blood volume was normal, only the small macromolecules left the blood stream. On the other hand, expansion of the circulating blood volume allowed the larger particles to cross the capillary wall and to appear in the lymph. They attributed the consequences of expanding the blood volume to an increase in the size of capillary "pores" and envisaged a "labile capillary 'pore' size", that could change when the plasma volume enlarged sufficiently [36]. Although some reservations were expressed about this explanation when it was first promulgated [9], further experiments by the same group seemed to dispel these misgivings [31].

The Pulmonary Circulation

In 1954, Cassen and Kistler proposed that the pulmonary edema produced either by blast injury [Fig. 1] or by the massive injection of epinephrine to a sudden and tremendous surge in pulmonary capillary pressure "to the point where blood plasma exudes freely through spaces between the cells, which spaces are caused by the stretching of the elastic intracellular processes..." [3]. In 1968, cytochemical techniques for the visualiza-

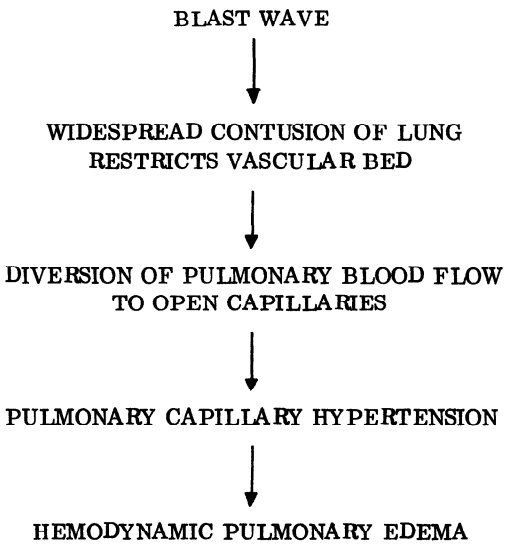

Figure 1. Pulmonary Edema in Blast Injury of the Lungs [14].

tion by electron microscopy of injected peroxidative enzymes [28,29] made it possible to scrutinize pulmonary capillary pores directly. But, it took a series of steps to unravel how the pores operated. Thus, the initial observations in mice by Schneeberger-Keeley and Karnovsky [30] showed that horseradish peroxidase [mw of 40,000 daltons] passed readily between the endothelial junctions of pulmonary capillaries. This easy passage of the macromolecular tracer was difficult to explain. Shortly thereafter, Pietra, Szidon, Leventhal and Fishman [24], using stroma-free hemoglobin [mw of 68,000 daltons] as the ultrastructural tracer in the dog lung, failed to observe this easy passage as long as capillary pressures were normal or near-normal. Indeed, only when pulmonary capillary pressures exceeded at least twice normal could they reproduce the picture of Schneeberger-Keeley and Karnovsky in mice. Horseradish peroxidase and myoglobin [mw of 17,000 daltons] in the dog lung behaved qualitatively like stroma-free hemoglobin. The apparent disparities between the mouse and dog studies were soon reconciled: when the volume of injectate was too small to raise capillary blood volume [and presumably hydrostatic pressure] to the point of stretching the endothelial junctions, the tracer appeared to be confined to the capillary lumens; conversely, when the blood volume was greatly expanded, interendothelial junctions stretched, allowing ready passage of macromolecules from capillary lumen to interstitial space [7]. Thus, the direct observations of stretched pores in the pulmonary circulation provided anatomical substantiation of the physiologic evidence for stretched pores in the systemic circulation. That the degree of stretching is limited is suggested by the failure of extraordinarily-high molecular weight tracers to diffuse through endothelial junctions even when the volume of injectate was quite large [29]. Moreover, that more than molecular size is involved is indicated by the fact that myoglobin, a smaller molecule than horseradish peroxidase, did not traverse the interendothelial junctions at a level of pulmonary capillary pressure that caused horseradish peroxidase to do so [7]. Although these studies did not exclude completely a role for pinocytosis [2], the rapidity and route of passage clearly indicated a predominant role for the interendothelial junctions. Nor did they clarify the role played by other influences, such as electrostatic charges on the endothelium and on the test substances in enhancing or retarding transendothelial passage [1,4,11,26].

Alveolar Pores

Alveolar capillaries resemble muscular capillaries morphologically. But, they differ strikingly in the way that they are incorporated into the surrounding tissue. An important element in this architecture is the creation of thick and of thin sides to the alveolar-capillary barrier. This arrangement has several implications for the exchange of constituents between the plasma and the structures on the two sides of the barrier: 1) on the thin side, where fusion of the alveolar and endothelial basement membranes is the remnant of the interstitial space, the plasma in the capillary is closely approximated to, and probably exchanges with, the surfactant layer that lines the alveoli; conversely, on the thick side, the interstitial space constitutes an intermediary zone between the capillary and the alveolus, 2) the mounting of the capillary by fused basement membranes on the one hand, and by the matrix of the interstitial space on the other, may act as shock absorbers, i.e. to absorb undue perturbation and deformation during breathing, and 3) the disposition of the capillary within the alveolar septum protects the gas-exchanging surfaces by using the interstitial space on the thick side for drainage of excess water from the alveolar interstitial space [7].

Another ultrastructural feature of the air-blood barrier is that alveolar pores are structurally more complex, and functionally less permeable and deformable than endothelial pores [28,32]. Indeed, the alveolar epithelium, rather than the capillary endothelium, constitutes the principal barrier to alveolar flooding. The location of the less-permeable alveolar barrier on the outer aspect of the interstitial space serves to direct those plasma constituents that manage to enter the interstitium towards lymphatics and large peribronchial sumps rather than into alveoli. And, as a corollary of this arrangement, hemodynamic alveolar edema may be regarded as a breakdown in the alveolar barrier that follows overwhelming of the interstitial drainage system for water and proteins. Freeze-fracture replicas of normal alveolar pores has suggested that alveolar edema is associated with severe derangement in the ultrastructure of the poorly deformable pores [28]. However, this observation is difficult to reconcile with the ready re-

versibility of alveolar edema. One possibility is that reabsorption of alveolar fluid takes place via intact alveolar segments rather than those involved in the pathogenesis of the alveolar edema. Moreover, the process of reabsorption may resort to mechanisms other than that involved in the formation of the edema. For example, pinocytosis may play a larger role in reabsorption of macromolecules than in the pathogenesis of pulmonary edema.

Neurohemodynamic Pulmonary Edema

As was indicated at the start of this paper, the idea that the stretched pore phenomenon might have clinical implications was originally prompted by observations on experimental blast injury and massive epinephrine infusion [3]. The mechanism causing pulmonary edema in these experimental circumstances was thought to begin with intense and generalized systemic vasoconstriction that displaced the circulating blood volume from venous reservoirs into the heart and pulmonary circulation; the increase in pulmonary venous and capillary pressures was inordinate because of the lesser distensibility of the left - than of the right - atrial reservoir [21].

This hypothesis was greatly strengthened by the ultrastructural demonstrations that a large increase in pulmonary capillary pressure caused by expansion in pulmonary blood volume caused pulmonary capillary endothelial pores to widen sufficiently to enhance the exit of large macromolecules from the capillary [12,24]. Since neurohemodynamic pulmonary edema, as well as the pulmonary edema that follows blast injury or epinephrine infusion, involves intense hyperactivity of the sympathetic nervous system - predominantly the alpha-adrenergic component - the possibility exists that stretched pores are operative in all three circumstances.

Neurohemodynamic pulmonary edema has been produced experimentally, and occurs spontaneously after certain injuries to the central nervous system [22]. The usual picture is one of massive sympathetic nervous discharge [18,25]. Often, but not invariably, the hypothalamus is the site of injury. The pulmonary edema generally occurs soon after the injury, progresses rapidly and, if it is not arrested, produces alveolar edema in which fluid collected from the airways has a high protein content [33]. Characteristically, pulmonary venous [and capillary] pressures are dramatically increased. For example, after the intracisternal injection of thrombin and fibrinogen [27], pulmonary venous pressures of the order of 75 mm Hg have been recorded within 10 to 120 secs. An increase in pressure of this magnitude would be expected to stretch pulmonary capillary pores and to deposit excess fluid in the interstitium of the lungs for more gradual clearance after capillary pressure has returned to normal. As the massive discharge subsides, capillary hydrostatic pressures return to normal [10].

The level of capillary pressure that will disrupt rather than widen interendothelial junctions, or directly damage endothelial cells, is unknown. Nor is it clear if the two sides of the alveolar capillary barrier - the thick and the thin - are equally affected. Indeed, it is not unreasonable to picture pores on the thick side being stretched by a burst in capillary pressure while the fused membranes on the thick side are being torn. Unfortunately, this notion is not easily tested experimentally.

Another element in considerations of alveolar edema is the fact that alveolar epithelial pores are less permeable to macromolecules and less deformable than the first barrier to edema, i.e. the endothelial pores. The chemical and electrostatic composition of the alveolar interstitial space may constitute a second obstacle to the formation of alveolar edema by restraining, sorting and redirecting plasma constituents towards the lymphatics and larger interstitial spaces. The alveolar epithelium is not only the third impediment to the passage of plasma constituents into alveoli but also provides the major alveolar water- and protein-proofing.

Protein-rich fluid harvested from the airways has been widely accepted as evidence of damage to the alveolar-capillary barrier [6, 8, 35]. However, in view of the uncertain effects of a large increment in capillary pressure on alveolar-capillary wall, it is conceivable that sufficient stretching of endothelial and epithelial pores, per se - without tearing of pores or damage to the lining cells - could result in protein-rich edema fluid. This conclusion implies that the effects of extreme hemodynamic stretching of pores may mimic those of alveolar-capillary barriers that "leak" protein because of injury. It also raises the prospect that the alveolar pulmonary edema which follows an extraordinary burst in capillary pressure in response to intense sympathetic stimulation represents a combination of stretched pores and alveolar-capillary damage in proportions that are currently not quantifiable.

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TRANSPORT OF PROTEINS BY DIFFUSION, BULK FLOW AND VESICULAR MECHANISMS

Eugene M. Renkin
Department of Human Physiology
School of Medicine
University of California
Davis, CA 95616

Large molecules may find their way across the vascular endothelium by exchange of cytoplasmic vesicles between its surfaces, and by way of openings through the cells (open fenestrae, vesicle chains) or between them (7,11,12). Another possibility is that they may be transported by direct interaction with the endothelial cell surface much as Scow et al (13) proposed for chylomicron lipids. Electron-microscopic studies can identify pathways, and can suggest the kinds of substance which a given pathway can transport. However, only measurements on living tissues can provide information on transport rates of specific substances. The question I would like to explore today is how such measurements can be critically evaluated in relation to morphological transport pathways.

For my exploration, I shall use a collection of experimental data on blood-lymph transport of plasma proteins in the dog's paw. Lymph flow was increased by stepwise elevation of venous pressure. Lymph flow and lymph and plasma concentrations of albumin (A), immunoglobulin-G (G) and fibrinogen plus macroglobulin (F) were measured by electrophoresis. The methods and data have all been published as well as a more rudimentary attempt at analysis (9,10).

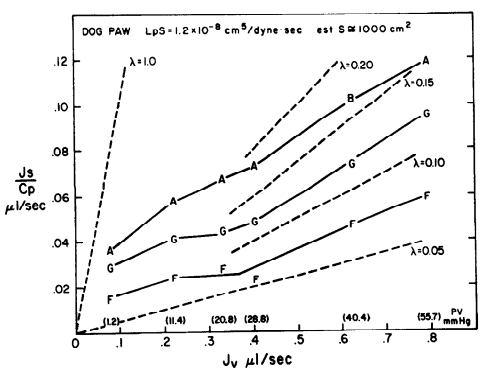
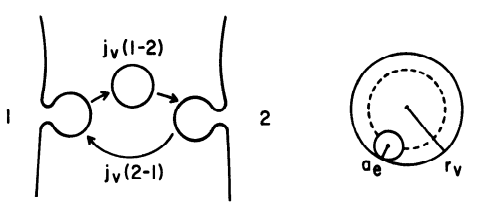


Figure 1 shows that data plotted for each protein as its plasma clearance (net protein flux into lymph Js ÷ plasma concentration Cp) against lymph flow (Jv). In the range of venous pressures covered by this graph, lymph flow increased linearly with pressure; the calculated hydraulic conductivity was 1.2×10^{-8} cm^5/dyne·sec for an estimated capillary surface area of around 1000 cm^2/paw. The points shown are averages of 8 experiments. The sets of data points at the two lowest Jv's represent lymph flows in the "control" range, all others are at elevated pressure and flow. (Venous pressures in mm Hg are indicated below each set. The dotted line of slope = 1 represents the limit of no restriction to transport of solute: complete coupling of solute and volume fluxes. The dotted lines of smaller slope indicate various degrees of partial coupling.) The principal characteristics of these data are as follows: 1) At all pressures and flows, protein clearance decreases with increasing molecular size. 2) Clearance for all proteins increases with volume flow, with limiting slopes in the range of 0.10 to 0.06. 3) Straight lines drawn through the experimental points for each protein do not go through the origin, but have definite ordinate intercepts. These decrease with increasing molecular size by a larger factor than the limiting slopes. 4) At venous pressures below 45 mm Hg, protein clearances remained constant at the values shown for 1 to 3 hours. At higher pressures there was often a sudden rise in protein flux after a lag of 1/2 to 1 hour. The points shown on the graph for the highest venous pressure elevation represent data collected before this happened. Landis et al (5) reported an increase in permeability of vessels in human limbs to plasma proteins at venous pressures of 60 mm Hg and higher. Though not as striking as the change at higher pressure, there seems to be a distinct increase in the slope of each curve starting around 30 mm Hg.

A. Transport by vesicular exchange

To what extent can this data be accounted for by vesicular transport mechanisms? Consider the exchange of vesicles across the endothelium (Fig. 2, below).



$J_V \rightarrow$
$J_S \rightarrow$

$$\phi = \left(1 - \frac{a_e}{r_v}\right)^3 \qquad (1).$$

Let jv represent the total volume of vesicular contents moving from one surface to the other per unit time: ($n\ 1.33\ \pi\ r_v^3$, where n = number of vesicles making a complete transit in one direction, r_v = vesicle internal radius and π = 3.14). The center of a spherical solute molecule will be excluded from a shell of thickness equal to its radius (a_e); thus there will be a concentration ratio ϕ less than one between vesicular fluid and that on either side of the cell (3).

A-1. Symmetrical exchange

If movement of vesicles is symmetrical: $jv(1\rightarrow2) = jv(2\rightarrow1)$, then vesicular solute flux,

$$Js = jv\phi\ (C_1 - C_2) \qquad (2).$$

Symmetrical vesicular exchange cannot produce a net volume flux. Since the flow of lymph represents a steady unidirectional volume flux, this must occur either by asymmetrical movement of vesicles or by another pathway (e.g. pores, cell membranes). For the purpose of examining the characteristics of solute transport by symmetrical exchange of vesicles, let us assume that net volume flux takes place through an unspecified pathway impermeable to macromolecular solutes. In this case, it can be shown (3) that

$$R_s = \frac{jv\phi s}{jv\phi s + Jv} \qquad (3),$$

where R_s is the lymph/plasma concentration ratio of solute s and Jv is the net volume flux (lymph flow). Solute clearance,

$$\frac{Js}{C_1} = Jv\ \frac{jv\phi s}{jv\phi s + Jv} \qquad (4).$$

For vesicles of 250 Å internal radius, calculated values of ϕs are as follows:

ALBUMIN	a_e = 36Å	ϕ = 0.63
GLOBULIN	56	0.47
FIBRINOGEN/MACROGLOBULIN	100	0.22

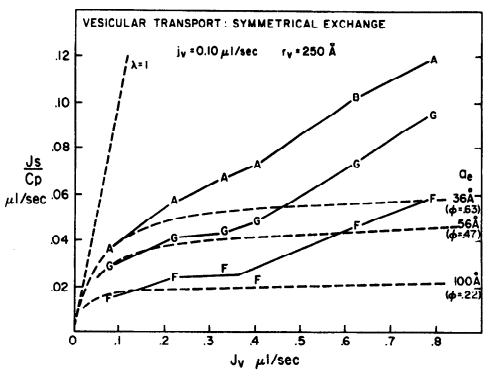


Figure 3 shows three curves drawn for jv = 0.10 µl/sec, a value arbitrarily chosen to fit the low - Jv ends of the data curves.

Symmetrical vesicular exchange can transport albumin, globulin and fibrinogen/macroglobulin at rates corresponding to those observed at low lymph flows but it cannot account for the increase in solute fluxes with increasing flow. In the low Jv range, the degree of restriction of F and G with respect to A can be accounted for by exclusion of equivalent sphere molecules from 250 Å radius vesicles.

A-2. Asymmetrical exchange

If fluxes of vesicles in the two directions are not the same, there will be a net volume flux with a component of solute flux coupled to it in proportion to the vesicular partition ratio ϕ. Since Jv was increased in these experiments by elevation of intravascular pressure, it is necessary to assume that the asymmetry of vesicular flux is a function of intravascular pressure. Johansson (4) has recently suggested such a relation on morphometric grounds. If we represent the asymmetrical component as kJv, where k represents the fraction of total net volume flux carried by the vesicles, this flux is superimposed on the symmetrical exchanges, jv. Since both components of vesicular flux share a common ϕ, for the total solute flux we may write

$$Js = jv\phi s\ (C_1 - C_2) + kJv\phi sC_1 \qquad (5)$$

$$R_s = \frac{jv\phi s + kJv\phi s}{jv\phi s + Jv} \qquad (6)$$

$$\frac{Js}{C_1} = Jv\ \frac{jv\phi + kJv\phi s}{jv\phi s + Jv} \qquad (7).$$

It is clear from this relation that if all volume flux is carried by asymmetrical vesicular transport (k=1) the limiting slopes of Js/C_1 vs Jv will be equal to ϕ:0.63, 0.47, 0.22 for A,G and F respectively.

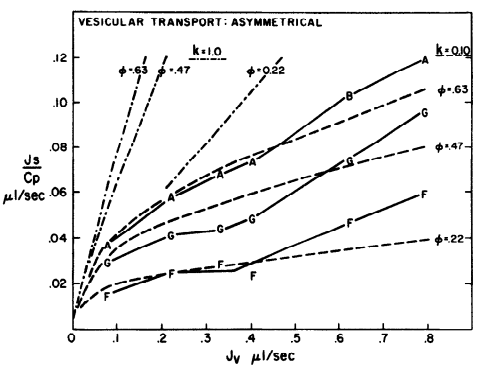


Figure 4 shows curves drawn according to eq. (7) for these values (k=1.0, above left). Their slopes are much too large to account for the experimental observations. However, if only one-tenth of the total volume flux is due to asymmetrical vesicular exchange, a fairly good match to the experimental curves is obtained (k=0.10, right margin). If k and r_v are

adjusted to fit A and F, the curve for G is a bit too high in the low Jv region; however, other factors than geometric exclusion might have some influence on partition coefficients. Data on other molecules are needed to resolve this question. It is also important to know more about the effects of hydrostatic pressures between 10 and 30 mm Hg on the size and distribution of endothelial vesicles.

B. Transport through pores.

I shall leave vesicular transport at this point and turn to "pores". In this category I include all open channels through which flow of water can take place: intercellular channels, open fenestrae, chains of vesicles. We can distinguish among these alternatives only by degrees of restriction to molecules of different sizes.

Transport of solutes through pores may take place by convection and diffusion. The parameters for these two modes of transport are related through pore geometry. Volume flow through an assemblage of n cylindrical pores, radius r_p, length x,

$$Jv = \frac{n(3.14)r_p^4}{8\eta x} \Sigma(\Delta P - \sigma\Delta\pi) \qquad (8)$$

where n is the viscosity of fluid within the pore, $(\Delta P - \sigma\Delta\pi)$ represents the sum of all effective hydrostatic-osmotic pressure differences (P = hydrostatic pressure, σ = solute reflection coefficient, π = solute osmotic pressure) across the membrane. The coefficient of the pressure terms in equation (8) may be defined as the hydraulic conductivity - surface area product, L_pS. Solute flux by diffusion,

$$Js,d = D^*_s \frac{n(3.14)r_p^2}{x}(C_1 - C_2) \qquad (9)$$

where D^*_s is the effective diffusion coefficient of solute in the pore. This is less than the free diffusion coefficient (D_s) if the solute molecule is dimensionally comparable to the pore. For spherical molecules in cylindrical pores,

$$\frac{D^*_s}{D_s} = \left(1 - \frac{a_e}{r_p}\right)^2 F\left(\frac{a_e}{r_p}\right) \qquad (10)$$

where a_e = molecular radius and r_p is the pore radius. F represents a specific hydrodynamic function (8). The coefficient of $(C_1 - C_2)$ in equation (9) may be abridged as a permeability - surface area product, PS.

Solute flux by convection (solvent drag) is proportional to volume flux according to the relation

$$Js,v = Jv(1 - \sigma_s)\bar{C}_s \qquad (11)$$

where σ_s is the solute reflection coefficient, and $\bar{C}_s$ the mean solute concentration in the pore. For spherical molecules in cylindrical pores,

$$(1-\sigma s) = \left[2\left(1-\frac{a_e}{r_p}\right)^2 - \left(1-\frac{a_e}{r_p}\right)^4\right] G\left(\frac{a_e}{r_p}\right) \qquad (12)$$

where G is a specific hydrodynamic function (8).

For combined diffusion and convention transport (2,6,8):

$$R_s = \frac{(z \coth z)\,PS + 1/2(1-\sigma s)Jv}{(z \coth z)\,PS + 1/2(1+\sigma s)Jv} \qquad (13)$$

where $z = (1-\sigma s)Jv/2PS$

$$\frac{Js}{C_1} = JvR_s \qquad (14)$$

For a given pore radius, $(1-\sigma)$ and PS bear a fixed relation to each other, as determined by equations (8) - (12).

Can we fit the experimental data of Fig. 1 to a pore transport model? The limiting slopes of the curves Js/C_1 vs Jv at high values of Jv approximate $1/\sigma$ - 1 (1,9,14). From the data of Fig. 1, we may estimate for

A (a_e 36Å),	λ = .106	σ = .904
G (56),	= .094	= .914
F (100),	= .064	= .940.

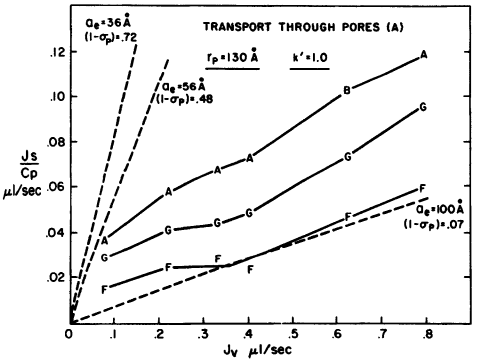


Figure 5A shows that these values are not compatible with all the volume flux being carried through pores of one size. For example, if pores of 130Å radius are assumed, σF is calculated to be 0.93, which is close to that observed, but σG = .52 and σA = .28. For pores of this size, transport of A and G would be much greater than observed.

The ordinate intercepts of the experimental curves are approximately in the ratio of 3:2:1 for A,G, and F respectively. Such a ratio of PS values suggests a pore radius of about 300Å, for which σ's would be 0.06, 0.14 and 0.36, in the same order.

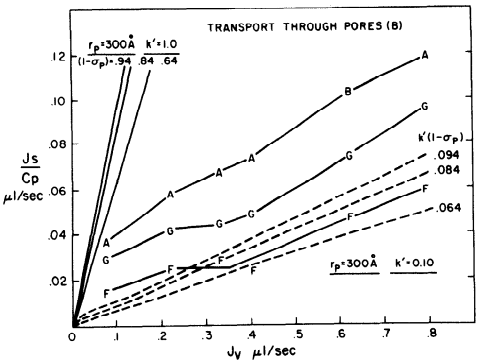
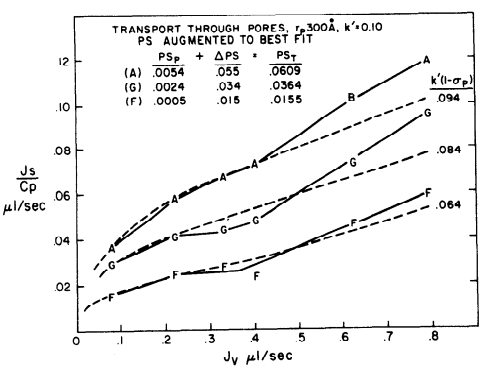


Fig. 5B, above, shows that if all Jv goes through pores of this size, solute flux in relation to volume flux will be far greater than observed (solid lines, upper left). However, if only one-tenth of Jv goes through 300Å pores (k'=0.1), and nine-tenths goes through pathways impermeable to protein, a good approximation to the limiting slopes can be obtained (dashed lines, lower right).

The high-Jv fibrinogen data is closely approached, and small adjustments of k' and r_p could improve the fit further. However, the low-Jv fibrinogen data cannot be fitted by curves of this kind, nor can the elevation of A and G curves above the F curve be accounted for. This failure is due to the very low values of PS attributable to pores of this size accounting for only one-tenth the overall hydraulic conductivity.

C. Multiple pathway transport.

We can produce a much better fit to the data if we arbitrarily augment the pore PS to an appropriate level (Fig. 6).



Both limiting slopes and elevations can be matched reasonably well for Jv's up to 0.4 µl/sec. However, the nature of the model requires that the additional "PS" be attributed to a non-porous pathway - that is, that the movement of solute molecules not be directly coupled to net movement of fluid. What kinds of mechanisms might satisfy this requirement? Let us assume that the system of 300Å pores we have described exists. The following increments of total PS over that of the pores alone are required: A .0546, G .0340, F .0150 µl/sec.

C-1. Phase-transfer diffusion.

It is possible that the molecules become associated with the endothelial cell membranes and diffuse through them; or diffuse laterally in them through intercellular junctions, as Scow et al (13) have proposed for plasma lipids. The ratios of PS increment to free diffusibility of the solutes are nearly constant: A .0058, G .0052 and F .0047. This is compatible with the notion of independent movement. However, there is presently no direct evidence of membrane binding or other association which would support this mode of transport.

C-2. Symmetrical vesicular turnover.

We can combine the pore and vesicle models by bringing in vesicular exchange to account for the augmentation PS, since PS is dimensionally equivalent to $jv\phi$. For 250Å vesicles, an exchange flux jv = 0.07 µl/sec would yield the following values of $jv\phi$: A 0.044, G 0.033 and F 0.0154 µl/sec. The latter two are close to the values obtained (as PS) by curve fitting. The value for A is distinctly smaller.

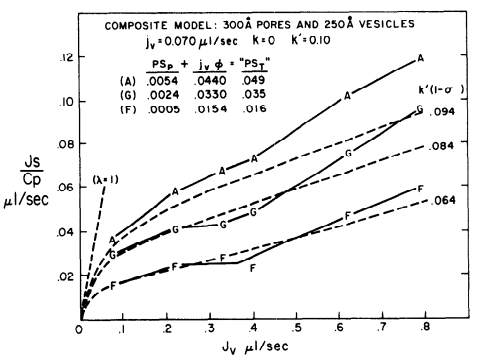


Figure 7 shows that the overall fit of this model is not so good as that obtainable by arbitrary assignment of PS, but then there are fewer degrees of freedom. Perhaps albumin shows slight association with the internal coats of vesicles, diminishing its exclusion slightly, or maybe a smaller system of pores (responsible for some part of the 0.9 LpS not attributed to the large pores) is slightly permeable to albumin.

C-3. Asymmetrical vesicle exchange and large pores.

One of the most interesting possibilities for further exploration is that the fraction of volume flux coupled to transport of large molecules might be divided between asymmetrical vesicular transport and large pores (the sum of k and k' would be 0.10). Further information of the effects of hydrostatic and colloid osmotic pressure differences on the distribution of endothelial vesicles would be helpful in evaluating the roles of these two processes.

SUMMARY

Large pores alone cannot account for the transport of plasma proteins across vascular endothelium of the dog's paw. This is true even if a large fraction of volume transport is assigned to small-pore pathways or to cell membrane pathways impermeable to protein. Vesicular exchange, or some form of molecular diffusion not linked to volume flow must carry the bulk of transported albumin, globulin and fibrinogen and macroglobulin. Asymmetrical exchange of vesicles proportional to transmembrane differences of hydrostatic and osmotic pressure might account for up to one-tenth of the volume fluxes observed when these forces are varied, and for much of the coupling of macromolecules transport to net volume flow. Further study of the effects of hydrostatic and osmotic forces on the distribution and dimensions of endothelial cell vesicles is needed to distinguish between pore and vesicle transport.

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PULMONARY ENDOTHELIAL AND EPITHELIAL VESICULATION AS A RESPONSE TO INCREASED BLOOD-TO-TISSUE FILTRATION

Francis P. Chinard

Departments of Medicine and Physiology
CMDNJ-New Jersey Medical School
Newark, New Jersey 07103

Chinard, F.P. Pulmonary endothelial and epithelial vesiculation as a response to increased blood-to-tissue filtration. Depts. of Medicine and Physiology, CMDNJ-New Jersey Medical School, Newark, N.J. 07103. - The conventional view of blood-to-tissue filtration is of protein-poor fluid crossing by way of large solute restrictive pores at endothelial cell junctions. Hydrostatic pressure drop presumably occurs at the pores but is not defined across endothelial cells. If filtration is zero, chemical potentials of water in blood, cells, interstitium must be equal (equilibrium with respect to water) but hydrostatic pressures need not be equal since differences of solute concentrations can be maintained. In isolated perfused lung preparations, DeFouw and Berendsen (1978,1979) have reported marked increases of vesiculation following hydrostatic or "oncotic" edema. It is proposed that intracellular hydrostatic pressures are less than tissue pressures and that initiation of vesiculation is brought about by pressure differences at the plasmalemma.

pulmonary edema, pinocytotic vesicles

For the lungs, a relationship considered by some descriptive of fluid and small solute movements from blood to interstitium and thence to lymph is:

$$F_v = k S(\Delta P - \sigma \Delta \Pi) = F_L \quad (1)$$

where F_v is the filtration rate, k is a coefficient which concerns the barrier properties, S is surface area, ΔP is the difference of the vascular (P") and tissue hydrostatic (P') pressures, $\Delta \Pi$ is the difference of the "oncotic" pressures of plasma and of tissue fluid, F_L is lymph flow rate and σ, a lumped reflection coefficient, for all proteins must have values $1 > \sigma > 0$ to account for the presence of proteins in lymph. There are some assumptions involved in the use of such a relationship. Among these assumptions are: 1) the fluid that crosses the endothelium has the composition of plasma with most if not quite all of the proteins removed; 2) differences of protein concentrations but not of small solutes affect the rate of fluid passage; 3) a single lumped coefficient is sufficient to describe the barrier properties with respect to filtration over the entire surface of the microvasculature; 4) a single lumped reflection coefficient is sufficient to describe the interaction of all proteins with the entire surface. An additional assumption is often made to the effect that fluid passage occurs through slits or pores at the cell junctions.

Locally applicable simplified but more accurate description of substance flux is provided by relationships based on the thermodynamics of irreversible processes. Thus, for water we have:

$$J_v = L_p[\Delta P - RT\sum_i \sigma_i \Delta c_i \quad (2)$$

Where J_v is volumetric flux (considered equal to water flux) across unit area, L_p is the filtration coefficient per unit area, ΔP has the same meaning as above, R is the gas constant, T is temperature, and Δc_i is the concentration difference in plasma and in interstitial fluid of each of the i constituents of the system except water. The system is assumed to be isothermal. For a given solute, s, we have:

$$J_s = \omega_s RT \Delta c_s + J_v(1-\sigma_s)\bar{c}_s \quad (3)$$

where J_s is the flux of solute, ω is the permeability coefficient (again, a unit area characteristic), Δc_s is the concentration difference as above, σ_s is the reflection coefficient of the solute concerned and $\bar{c}$ is its average concentration across the barrier For $\omega_s RT$ we can substitute P_s. There are as many values for σ and again as many for P for a given unit area of barrier as there are constituents of the system unless it be demonstrated experimentally that there are equalities. Thus, if it were demonstrated that the reflection coefficients of all small solutes normally present in plasma were equal and zero at the site of passage then expression (2) could be rewritten as

$$J_v = L_p[\Delta P - RT\sum \sigma_{protein} \Delta c_{protein}] \quad (4)$$

and assuming that only albumin need be considered we get an expression similar to (1), namely

$$J_v = L_p[\Delta P - \sigma_{alb} \Delta \Pi_{alb}] \quad (5)$$

Pride of place can not be given to "oncotic" pressure as in expression (1) without consideration of the limitations indicated above.

If we set F_L and hence J_v to zero in expressions (1), (2) or (4), we do not necessarily establish equilibrium with respect to water between plasma and the interstitium. The condition for that equilibrium is that the chemical potentials of water in the two compartments be equal. For the chemical potential of water in plasma, $\mu"_w$, relative to water in a given standard state μ°_w, we write

$$\mu"_w - \mu^\circ_w = \bar{V}_w(P" - P^\circ) + RT \ln a"_w / a'_w \quad (6)$$

where the a's denote activities and the superscript "o" refers to properties in the standard state. For water in the interstitium, μ'_w, we write

$$\mu'_w - \mu^o_w = \bar{V}_w (P' - P^o) + RT \ln a'_w / a^o_w \quad (7)$$

Thus, for the difference of the chemical potentials of water, we have

$$\mu''_w - \mu'_w = \bar{V}_w (\Delta P + RT \ln a''_w / a'_w \quad (8)$$

If this difference is not zero, there will be net flux of water at a rate described by (1), (2), (4) or (5) unless the σ's are unity. In that event, the rate is proportional simply to the difference of the chemical potentials, or

$$J_v = L_p \Delta \mu_w \quad (9)$$

I would like to consider now the passage of water across the pulmonary endothelial barrier and the consequences of an increase in filtration rate. Consider Figure 1 which schematically illustrates the so-called gas-exchanging portion of the air-blood barrier from the classical approach of net flux restricted to the junctions. The endothelial and epithelial cells can initially be considered as blobs of material responsive neither to pressure nor to solute concentration changes. Under control conditions, with F_L approaching zero, $P'' > P'$ and necessarily $a''_w/a'_w > 1$. An increase of filtration rate can be achieved either by increasing ΔP or by decreasing the solute concentration differences across the barrier. With J_v increased from changes of either or both factors, run off reflected by an increase of F_L may not be immediate. In that case we can expect a pressure build-up in the interstitium. Thus, P' would increase. Hence, the surfaces of the endothelial and epithelial cells resting on the basal lamina or basement membranes would experience an increased pressure at the interface.

Consider now a somewhat more realistic model in which the endothelial and epithelial have intracellular hydrostatic pressures and compositions as indicated in Figure 2. Several possibilities can be considered for the values that could be taken on by these determinants of the chemical potential of intracellular water. Consider first the equilibrium situation, with respect to endothelial cell water. For its chemical potential, $\mu^{c_1}_w$, we have

$$\mu^{c_1}_w - \mu^o_w = \bar{V}_w (P^{c_1} - P^o) + RT \ln a^{c_1}_w / a^o_w \quad (10)$$

If equilibrium obtains in the three compartments with respect to water, then

$$\mu''_w = \mu^{c_1}_w = \mu'_w \quad (11)$$

and

$$\mu''_w - \mu^{c_1}_w = \bar{V}_w (P'' - P^{c_1}) + RT \ln a''_w / a^{c_1}_w \quad (12)$$

It is obvious that with P'' and a''_w known and fixed, P^{c_1} and $a^{c_1}_w$ must vary reciprocally but are not fixed by the values of P'' and a''_w. If $P^{c_1} = P''$, we must have $a''_w = a^{c_1}_w$ and the cytoplasm thus has the same pressure and water actvity (possibly total solute concentration as plasma). The pressure drop ΔP, assumed to occur at the junction must also occur at the abluminal interface of the endothelial cell and the interstitium.

Consider now epithelial cell. Here, at equilibrium with respect to water, we have

$$\mu'_w - \mu^{c_2}_w = \bar{V}_w (P' - P^{c_2}) + RT \ln a'_w / a^{c_2}_w = 0 \quad (13)$$

If $P^{c_2} = P^{alv}$, then the pressure drop must be also at the abluminal interface of epithelial cell and the interstitium. P^{alv} is gas phase pressure.

The setting of the intracellular pressures at values found at the vascular and alveolar interfaces has no more basis than the presence of structurally supportive elements in the basement membrane. If these assumptions are correct, at equilibrium

$$P'' = P^{c_1} > P' < P^{c_2} = P^{alv} \quad (14)$$

There are no data available on the internal pressures of pulmonary endothelial and epithelial cells. Suffice it to emphasize that equality of intra-and extracellular pressures is not a requirement for equilibrium with respect to water. Indeed, with appropriate adjustment of the activity terms $a^{c_1}_w$ and $a^{c_2}_w$ both P^{c_1} and P^{c_2} could be less than P', which is considered by some to be subatmospheric itself. Active transport out of cells may also be important in regulating a^c_w and secondarily P^c. These considerations provide a basis for an examination of the responses encountered in the development of pulmonary edema.

In the course of studies of specific barrier parameters of the lung endothelium and epithelium with the late Dr. William Perl (see Perl et al., 1975 and 1976 for some of the results of these studies) I was fortunate enough to interest two young anatomists at my medical school in studying quantitatively the morphologic changes occurring in pulmonary edema. Because many of the functional studies were carried out in isolated perfused preparations and because hydrodynamics and fluid composition could be much more controlled, we elected to carry out the first portion of the morphometric studies on isolated perfused dog lung preparations. A substantial portion of these studies has now been published (DeFouw and Berendsen, 1978,1979). Three series of experiments have been carried out. The first is a morphometric study of isolated perfused lungs which did not become edematous i.e., the controls. The second is a study of such lungs made to become edematous by increase of the microvascular pressure. The third is a similar study of lungs made to become edematous by decreases of the solute (albumin) concentrations. In Table 1, are shown general structural parameters from the published series. As expected, the interstitial thicknesses are larger in the edematous lungs than in the controls whether the edema has resulted from microvascular hydrostatic pressure increases (the equivalent of cardiogenic edema) or from decreases of protein concentration in the perfusion fluid (the equivalent of "oncotic" edema). Compared to the increased thickness of the interstitium, the increased thicknesses of the endothelial and epithelial (Type I) cells are insignificant. In Table 2 are shown the changes which occured in the overall cytoplasmic volume densities of the vesicles and in their distributions. The volume densities in the control lungs are much greater in the endothelial than in the epithelial cells (ratio of approximately 3.7).

In both the "oncotic" edema and the hydrostatic edema there is marked increase in the volume densities of both endothelial and epithelial cells. The ratio drops to about 2.75 and indicates a greater proportional increase of vesicle density in the epithelial cells than in the endothelial cells. These increases of vesicle volume density occur without significant change of the diameters of the vesicles. Therefore, we are dealing with an increased number of vesicles rather than with an increased volume of individual vesicles.

The increase in the number of vesicles in both endothelial and epithelial cells is greater at the interfaces of cells and interstitium (abluminal surfaces) then at the interfaces of cells and alveoli or of cells and vascular compartment (abluminal surfaces). From functional considerations presented above, it is obvious that increasing microvascular pressure and decreasing macromolecular solute concentration have a common resultant: an increase in outward filtration rate. In these preparations, the increased outward filtration is accompanied by striking edema, limited to the portions of the parenchyma where epithelium and endothelium are not closely opposed. Thus, an increased tissue pressure is likely in these regions of the lung tissue where epithelium and endothelium are closely opposed and where there is a restrictive basement membrane. I propose this presumed increase tissue pressure as the link of the hydrostatic and "oncotic" edemas and as the determining factor in the resultant increased vesiculation (Chinard quoted by DeFouw and Berendsen, 1979 and Chinard, 1979). Whatever the initiating factor is, if not the increased tissue pressure, it is difficult to see how increased microvascular pressure and decreased colloid or oncotic pressure in the microvascular compartment could both bring about a similar effect in the cells. Both changes affect the chemical potential of water similarly and thus must lead to the increased filtration rate with its secondary effects.

The question of intracellular pressure must now be addressed. If cell hydrostatic pressures, P^{c_1} and P^{c_2}, are greater than the interstitial pressure, P', it difficult to see how an increase of P could make a dent in the plasmalemma that could lead to new vesicle formation. However, intracellular pressure, P^{c_1} and P^{c_2}, could be set at pressures less than P' by maintaining $a^{c_1}_w$ and $a^{c_2}_w$ greater than $a^{'}_w$. As pointed out above, P', tissue pressure, is considered by some to be less than atmospheric pressure. Intracellular pressures would be less than this. It is not necessary, of course, that these intracellular pressures be everywhere the same within a given cell. Indeed, one could construct an hypothesis involving local decreases of intracellular pressures as a result of outward solute transport which would be followed by passive water movement and consequent boundary displacement (Figure 3). Locomotion of cells such as amoebae presumably has a component of heterogeneity (non-uniformity) of intra-cellular pressures. Localized solute pumps could produce the required pressure difference across the bilayer. In this connection, the report of Mooi et al, 1978 on the production of marked pulmonary endothelial cell swelling with furosemide (Lasix) is of interest). As caveolae, still attached to the luminal or abluminal surfaces and with the operculum or shield as the only separation between cavity and lumen, hydrostatic pressures in the cavities would probably be the same as the adjacent extracellular compartment. Intracellular vesicles, if not connected, could have the same internal pressure as the surrounding cytoplasm (see, for example, Tanford, 1979). These are important considerations which must be put aside for another time and place. In any event, maintenance of $a^{c_1}_w$ and $a^{c_2}_w$ at values greater than $a^{'}_w$ would require expenditure of energy. The greater utilization of glucose by lung tissue as it becomes edematous (Tierney, et al., 1977) could be related to such phenomena. Specifically, the increase of vesiculation is not associated with a significant increase of either endothelial or epithelial volume. There must, therefore, be a smaller intracellular "free" volume as the fraction of intracellular space occupied by vesicles increases. Cytoplasmic constituents, excluded from entering the domain of the vesicle lumen which is presumably of extracellular origin, would be increased in concentration and a^{c}_w would decrease. Maintenance of volume could be achieved by pumping out solutes to restore a^{c}_w to its previous values.

The increased vesiculation could be considered a second line defense mechanism against pulmonary edema that would become operative as the filtration rate extended the rate of lymph flow and resulted in an increase of tissue pressure. Under ordinary circumstances, vesiculation would be initiated mainly at the vascular interface and, presumably to a lesser degree since there are fewer epithelial vesicles, at the alveolar interface. With increased vesiculation, increased transport of macromolecules could well be envisaged as occurring in the direction blood to tissue, particularly in those situation where there was an increase in the hydrostatic pressure, P", in the vascular lumen. The net flux of water and solutes may well be as conventionally postulated, although without much direct evidence, at the cell junctions. The viewpoint being introduced here is compatible, however, with the concept that septal edema is the result of net fluid flux at the venular ends of the capillaries, perhaps at the level of the terminal bronchioles and transmitted in a retrograde fashion to the alveoli through both the thick and thin portions of the interstitium but perhaps even under the surfactant (Fig.4). Junctional passage at the level of the capillaries and changes in junctional characteristics are not excluded but are not essential to these considerations. In any event, as suggested by others, the stretched pore phenomenon could be related to vesicles as such or to a vesicular shuttle.

In brief, these are two aspects to this proposal. One is that endothelial and epithelial intracellular hydrostatic pressures may be less than tissue pressures. The other is that increased tissue pressure is an initiating factor in the formation of vesicles. Local plasmalemma structural heterogeneities may, of course, be involved. The proposal is an evolving working hypothesis that is incomplete but has testable features.

In conclusion, I would like to indicate my respect and admiration for Dr. Mayerson and his work and my appreciation of the stimulus he has provided to this field.

TABLE 1

Structural parameters of isolated perfused dog lung preparations: thicknesses [a]

	Interstitium μm	Epithelium μm	Endothelium μm
control	0.64 ± 0.03	0.32 ± 0.01	0.34 ± 0.01
"oncotic" edema	0.83 ± 0.03	0.35 ± 0.01	0.33 ± 0.01
hydrostatic edema	0.89 ± 0.04	0.35 ± 0.01	0.37 ± 0.01

[a] All values in μm ± 1 S.E.M. Data from DeFouw and Berendsen (1978,1979)

TABLE 2

Structural parameters of isolated perfused dog lung preparations: vesicles, volume density and distribution [a]

	Type I Epithelial				Endothelial			
	Overall volume density	Luminal	Cyto-plasmic	Abluminal	Overall volume density	Luminal	Cyto-plasmic	Abluminal
control	7.0 ± 0.73	16.0	72.0	12.0	26.0 ± 1.4	13.0	73.0	14.0
"oncotic" edema	15.0 ± 1.8	20.0	59.0	21.0	42.0 ± 5.2	20.0	59.0	21.0
hydrostatic edema	17.0 ± 3.9	21.0	55.0	24.0	46.0 ± 3.4	19.0	55.0	26.0

[a] All values are percentages ± 1 S.E.M. Data from DeFouw and Berendsen (1978,1979)

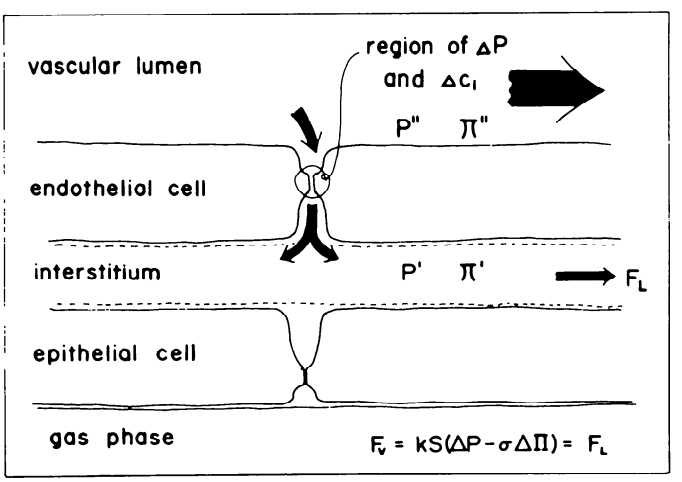

Figure 1. Conventional view of net flux of water and small solutes through slits or pores at cell junctions. The cells are ignored. The dashes indicate basement membrane or basal lamina. The continuous line below the epithelial cells indicates the surfactant layer. The term Δc_i here refers only to the solutes with reflection coefficients greater than zero.

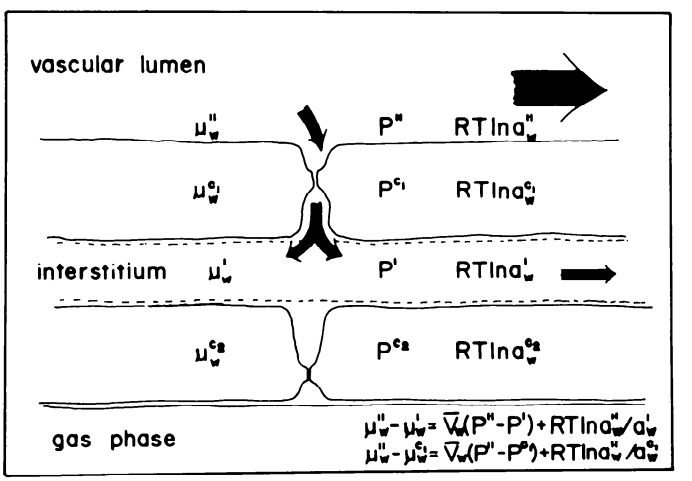

Figure 2. Conventional view modified to incorporate all solutes. The rate of passage water from vascular lumen to interstitium is directly proportional to the difference of the chemical potentials, $\mu'' - \mu'_w$, only if solute reflection coefficients are unity. Similar considerations apply to passage of water to cells.

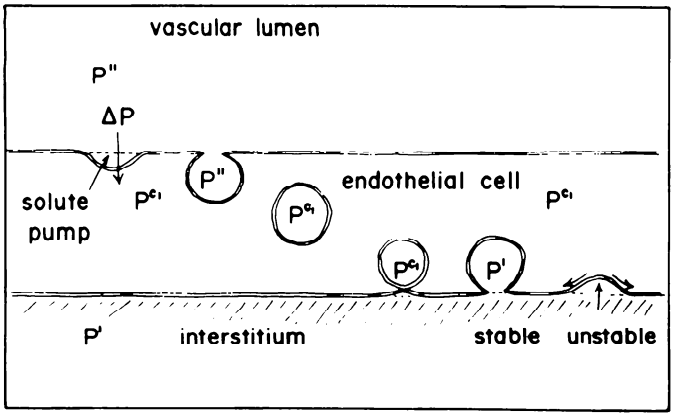

Figure 3. Initiation of vesicles as depressions in plasmalemma occurring at local heterogeneity as result of pressure difference across barrier. Pressure difference may be hydrostatic as $P'' - P^{c_1}$ or could be local as result of outward pumping of solutes with secondary outward water flux. The initial indentation may be unstable while the fully formed caveola with its operculum and the free vesicle are more stable configurations. The pressure in the caveolae is considered to be the same as in the adjacent extracellular phase. The pressure in the vesicle is considered to be the same as the local intracellular pressure, P^{c_1}.
For discussions involving local cell membrane heterogeneities see Bretscher (1976) Meyer (1978) and Wiegel (1979).

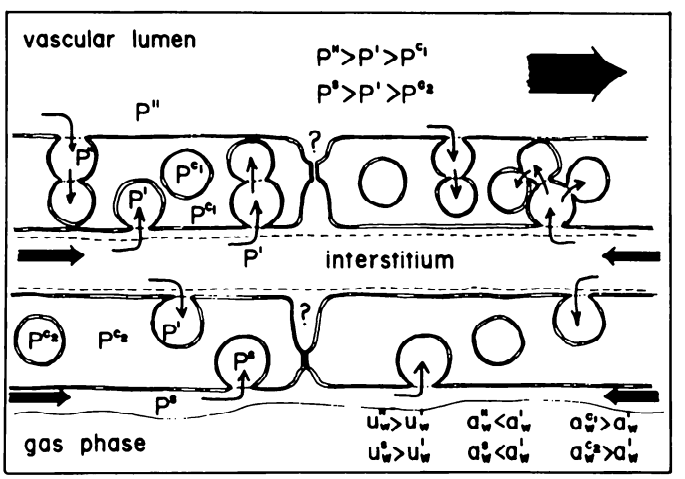

Figure 4. Unconventional view. Vesicles may exist as caveolae, as fused vesicles in series or in parallel, as free single vesicles in the cytoplasm. The latter may represent the vesicular shuttle. In lungs developing edema, increase of interstitial fluid may result from retrograde flux from more distal portions of the microvasculature and from retrograde flux under the surfactant layer. Postulated hydrostatic pressure relationships are as indicated. A role for the junctions is neither excluded nor necessary.

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INTRODUCTION: SYMPOSIUM ON CAPILLARY PERMEABILITY AND MECHANISMS OF TRANSPORT, SESSION II

John N. Diana

Department of Physiology and Biophysics, LSU School of Medicine in Shreveport, Shreveport, Louisiana 71130

The afternoon session of the symposium on Capillary Permeability and Mechanisms of Transport, in honor of Professor Hy Mayerson, was organized to compliment and extend the presentations of the morning session. The general aim of the program was to present information that had the characteristics of being reasonably specific for a given microvascular network but which also had broad general implications in the analysis and interpretation of microvascular transport mechanisms.

The first paper presented information which summarized the direct evidence that the movement of small lipid insoluble molecules across skeletal muscle capillaries (MW<5000) is not affected by net filtration of fluid from plasma to tissue. That is, the convective transport or solvent-drag effect is not important. Solvent-drag or convective transport is, however, a significant factor contributing greatly to the total movement of large molecules (>40,000 MW) such as albumin across skeletal muscle capillaries. The point was emphasized that unless microvascular hemodynamics are known, or precisely controlled, information about capillary permeability changes using lymph/plasma ratios of proteins is subject to severe question. Using data in the literature relating to histamine "permeability" changes, the point was emphasized that microvascular hemodynamics must be accounted for before any quantitative assessment of changes in capillary wall integrity can be made. The second part of this presentation used a capillary model to show that perfusion heterogeneity will underestimate permeability-surface area products when the single injection multiple-indicator-diffusion method is employed. It was suggested that perfusion heterogeneity may account for the observation that restricted diffusion of lipid insoluble substances in skeletal muscle capillaries is not found with the indicator diffusion method.

Dr. William Paaske presented data which indicated that the average permeability of continuous type capillaries is identical whether located in skin, subcutaneous adipose tissue or skeletal muscle. He proposed that the available evidence would indicate a $_{o}$ transendothelial capillary "pore" radius of 200-400Å, which does not offer restriction to diffusion for small lipid insoluble molecules. He then proposed that the barrier which discriminates between different molecular weight solutes may be the characteristics of the interstitial compartment.

Dr. Harris Granger talked about the special characteristics of the liver microvasculature and how net water and protein flux is regulated in this organ. He presented data showing a transsinusoidal pressure difference of approximately 1 mmHg which provides the net force for movement of fluid from plasma to tissue. The lack of any protein oncotic pressure difference between capillary and tissue would indicate that protein movement is primarily by convective transport (solvent-drag). Since net fluid transfer from blood to tissue in the liver also depends on osmotic and hydrostatic pressures in the peritoneal cavity, consideration was made of the influence of these factors on the overall fluid balance in the liver. Dr. Granger relates his findings to the pathophysiology of ascites.

Mechanisms related to vesicular transport was the topic of Dr. William Joyner's presentation. Endothelial cells cultured from human umbilical veins by collagenous perfusion were used to study macromolecular accumulation for varying albumin molecules and horseradish peroxidase. These cells accumulated each of the molecules in a time and temperature depended fashion. Internalization was inversely related to the molecular radius. Varying the external concentration of albumin monomer, the binding capacity and maximum internalization were determined for this process of endocytosis.

Dr. Philip Watson considered the role of the capillary endothelium, the basement membrane, the interstitial space and the lymphatic vessel wall as serial barriers in blood to lymph movement of molecules. He concluded that the present available data support the hypothesis that the interstitial matrix may be the only site where restricted diffusion of large molecules can occur.

The last presentation, by Dr. Curry, presented another picture of the capillary barrier to diffusion and challenged the classic pore theory. He presented the hypothesis that the permeability of the capillary wall to hydrophilic molecules may be determined by a dense fiber matrix which surrounds the endothelial cells (luminal side as well as extraluminal side and within the gap between endothelial cells). Using this model, he shows good correlation between theory and experimentally derived values for diffusion across microvascular beds. He speculated that a change (decrease) in fiber volume could account for the increased capillary permeability to molecules which has been observed in some animal studies.

INFLUENCE OF SOLVENT-DRAG AND PERFUSION HETEROGENEITY ON MASS TRANSPORT IN SKELETAL MUSCLE CAPILLARIES

John N. Diana and Bernard P. Fleming

Department of Physiology and Biophysics
LSU School of Medicine in Shreveport
Shreveport, Louisiana 71130

In the isolated, skinned dog hind-limb perfused at constant flow, studies were made of the effect of fluid filtration on transport of small lipid-insoluble molecules from blood to tissue. The results indicate that solvent-drag effects on solute exchange are not important and very small in comparison to the flux by free diffusion. Solvent-drag effects appear to become increasingly more important with increasing molecular size. The effects of perfusion heterogeneity on net solute exchange was studied using a multi-capillary model. Perfusion heterogeneity gives rise to greater underestimates of PS-product for small flow limited solutes than for larger solutes and, therefore may explain the apparent absence of restricted diffusion found with the single injection multiple indicator dilution technique. Drug induced alterations in the distribution of flow may also provide explanations for observed changes in PS-product even in situations where surface area or permeability are unchanged.

Solvent drag. Because the movement of water and small lipid insoluble molecules across the capillary endothelium occurs simultaneously and via the same pathway, there is the possibility that coupling may occur between the volume flow of fluid and diffusial solute fluxes. The early studies by Kruhoffer (16) and Pappenheimer, Renkin and Borrero (20) support the idea that bulk fluid movement has little effect on net capillary exchange of small molecules. Hyman et al (11), Lundgren and Mellander (18) and Aberg (1) have produced experimental results which support the idea that fluid movement will augment net solute exchange of small molecules across capillary walls. The studies by Pappenheimer (19), Grotte (10) and Perl (22) do suggest, however, that bulk fluid flow may increase the transport of large molecules across capillary walls. More recently the studies by Fleming and Diana (7) and Rippe, Kamiya and Folkow (30) provide strong evidence to support the notion that bulk fluid movement does not influence the net transport of small molecules from blood to tissue across skeletal muscle capillaries. Rippe, Kamiya and Folkow (31) also report that, in rat hindquarters, the major component (70%) of transmicrovascular albumin passage results from convective transport (solvent-drag) and not from passive diffusion (30%) or vesicular transport (negligible effect). It is worthy of note that, in the above studies where solvent-drag has minimal influence on net exchange of small solutes, the situation was one where blood to tissue transport was being measured. Where effects of solvent-drag showed appreciable influence on net exchange of small solutes the studies performed have looked at molecules from tissue to blood. If this observation is indeed correct then a new area of inquiry into capillary exchange must be opened up to explain the difference in the way solute movement occurs across, what would semmingly appear to be, the same capillary endothelial barrier. The concern to be presented in this report, however, relates to the wide acceptibility of the notion that a net increase in protein transport from blood to tissue (or lymph) must necessarily indicate that capillary permeability has increased. Within the pore theory of capillary permeability this would mean that either the existing small or large pores increased their radius or there was recruitment of a greater number of venular large pores. The effect of convective transport on total protein movement has been largely ignored in these studies, and it is the thesis of this report that convective transport plays a major role in lymph protein accumulation. If convective transport is to have an appreciable effect on the transport of protein from blood to lymph the Starling balances of forces must be directed in a manner which favors net filtration of fluid. That is, capillary hydrostatic pressure, tissue hydrostatic pressure, plasma protein oncotic pressure or tissue protein oncotic pressure must be changed, either singularly or collectively, in a manner which increases net filtration of fluid from blood to tissue.

First, what experimental data supports the assumption that convective transport is of real concern in the analysis of capillary permeability changes. Table 1 lists some recent studies where histamine has been used to change "capillary permeability" in either skin and/or skeletal muscle preparations.

The reports listed in Table 1, as well as others, show three distinct facts. 1) There is a linear relation between increased capillary hydrostatic pressure and increased lymph flow. 2) There is a linear relation between increases in venous (or capillary) hydrostatic pressure, capillary filtration and lymph flow. 3) Protein clearance will be directly proportional to lymph flow (28) (30). It follows that any change in microvascular hemodynamics which favors net filtration and a change in lymph flow will alter total protein transport (and the net amount of protein found in lymph) via

TABLE 1. HISTAMINE EFFECTS ON FLUID MOVEMENT AND PROTEIN TRANSPORT

Reference	Dose Histamine Route Preparation	Weight or Volume Change During Histamine Infusion	Macromolecular Transport
Rippe et al Acta Physiol. Scand. 103:252-262, 1978.	30-60 µg/ml i.a. Rat hindparts	↑ approx. 1 ml/min·100g	↑ Albumin Clearance
Joyner et al Microv. Res. 7:19-30, 1974.	1-100 µg/paw/30 min s.c. dog paw 5-20 µg/min i.a. dog paw	not measured (↑ Lymph Flow)	L/P Ratio Dextran 110 Total Protein
Carter et al Microv. Res. 7:31-48, 1974.	12-18 µg/paw/30 min s.c. dog paw	not measured (↑ lymph flow) 3x	L/P Ratio Albumin Dextran 110
Renkin et al Microv. Res. 7:49-60, 1974.	11 µg/paw/30 min s.c. dog paw	not measured (↑ lymph flow) 3x	L/P Ratio Total Protein Dextran 110
McNamee and Grodins Am. J. Physiol. 229:119-125, 1975.	6.5 µg/min/100g i.a. gracilus muscle	isogravimetric	Albumin Clearance 24x
Haddy, Scott, Grega Am. J. Physiol. 223:1172-1177, 1972.	5.40 µg/min i.a. dog forelimb	↑ 10-11g/min	↑ Lymph Protein
Marciniak et al Am. J. Physiol. 233:H148-H153, 1977.	4-64 µg/min i.a. whole dog forelimb	↑ edema	↑ Lymph Protein
	4-64 µg/min i.v.	↓ edema	⟷
	400-800 µg/min i.a. (left vent) (hypotension)	↔ edema	⟷
Marciniak et al Am. J. Physiol. 234:180-185, 1978.	4 µg/min CF, CP* i.a. dog forelimb	↑ Lymph Flow ↑ Weight	↑ Lymph Protein
	Histamine + Norep 4 µg/min CF i.a. dog forelimb	↑ Lymph Flow ↑ Weight	↓ Lymph Protein
	Histamine + Norep CP i.a. dog forelimb	↓ Weight ↔ Lymph Flow	↔ Lymph Protein
	Histamine + isoproterenol 3 µg/min CF i.a. dog forelimb	↔ Weight ↔ Lymph Flow	↔ Lymph Protein
	Histamine + isoproterenol CP	↑ Lymph Flow ↑ Weight	↔ Lymph Protein
Baker, C. J. Pharm. Exp. Therap. (In Press)	5 µg/Kg/min or 60 µg/Kg/min i.a. dog gracilus muscle	↑ Volume 0.15 ml/min	↑ Albumin Clearance
Renkin et al Microv. Res. 7:49-60, 1974	Histamine and ↑ Venous Pressure	↑ Lymph flow 6x	↑ Clearance of Total Protein and Dextran 110
Renkin et al Microv. Res. 14:191-204, 1977.	↑ Venous Pressure (no histamine)	↑ Lymph flow 5x	↑ Clearance of Total Protein

*CF = Constant flow perfusion; CP = Constant pressure perfusion

solvent drag. The Marciniak report even suggests that in those situations where capillary pressure and thus net filtration may be reduced (e.g. situations where there is activation of the sympathoadrenal system) that the net protein found in lymph is accordingly not changed or reduced. This is further supported in dog forelimb studies where histamine and norepinephrine were infused simultaeously.

From the work on Renkin et al (28) it was shown that, in the dog paw, the convective component of the blood-lymph transport under normal conditions (i.e. venous pressure not elevated, no vasoactive agents) comprised 30% of the total unidirectional protein flux. Rippe et al (1979) have stated that in the normal rat hindlimb as much as 70% of the total blood-tissue protein flux may be coupled to transcapillary fluid movement. Thus, the rate of transendothelial protein movement in normal physiological conditions depends to a significant degree on the state of fluid balance in the tissue studies. If the results of these studies are extended, to experimental situations where vasoactive agents or physiolgic stresses are applied which may promote net filtration of fluid and/or changes in endothelial porosity, then consideration must be given to the convective component as well as the diffusive component of the protein transport.

The total blood lymph protein flux can be separated into two mechanistically distinct components diffusive and convective (solvent-drag) as follows (14)(23):

$$J_S = PS(C_p - C_L) + \tfrac{1}{2}(1-\sigma_f)L(C_p + C_L) \qquad (1)$$

where:

J_S = total blood-lymph flux of protein (gl sec)

PS = average endothelial permeability-surface area product for protein: including molecular diffusion and vesicular exchange (cm^3/sec)

f = average solvent-drag reflection coefficient for protein

L = total lymph flow rate (cm^3/sec)

C_p and C_L = concentration of protein in plasma and lymph, respectively ($glcm^3$)

The first term on the right-hand side of eqn (1) represents the diffusive (or dissapative) component and the second term describes the convective (or solvent drag) contribution to the total blood-lymph protein transport. Employing eqn (1) and expressing the steady-state protein flux in the form of LC_L one can arrive at the sieving relation (Perl, 1975):

$$R = \frac{C_L}{C_p} = \frac{PS + \tfrac{1}{2}(1-\sigma_f)L}{PS + \tfrac{1}{2}(1+\sigma_f)L} \qquad (2)$$

The plasma lymph clearance of protein is given by:

$$LR = L\ \frac{PS + \tfrac{1}{2}(1-\sigma_f)L}{PS + \tfrac{1}{2}(1+\sigma_f)L} \qquad (3)$$

Renkin et al (28) have employed the above formulation to analyze lymph/plasma protein ratios in dog paw at various states of fluid balance. They were able to arrive at estimates for PS and σ_f for six plasma proteins, total protein and Dextran 110. Utilizing their approach we attempted to predict the influence of the convective contribution to the net protein flux in conditions resembling those observed with application of histamine. A vasoactive agent such as histamine is capable of producing changes in microvascular hemodynamics and is purported to alter endothelial permeability to large molecules although the data for the latter is conflicting. Specifically, when histamine is applied to skin or skeletal muscle changes in transcapillary fluid movement and/or endothelial protein permeability may occur.

Using data reported in the literature one can estimate the relative importance of the changes to be expected in the convective component of the increased blood-lymph protein exchange with histamine (28)(30). In the normal (control situation approximately 23% of the total protein movement is coupled to transcapillary fluid movement. With application of an effective dose of histamine, there is observed an increased permeability-surface area product for protein and an increased rate of lymph flow which reflects on increased transcapillary fluid movement. The only estimate available for the value of the solvent drag reflection coefficient during histamine application is from Renkin (29) which showed no change from control. To illustrate our point, however, we have calculated the protein clearance with an assumed value of σ_f for histamine of 0.70 (normal σ_f = .83). Assuming such a "permeability change with histamine it can be shown that the convective component of protein transport will increase to a greater degree than the diffusive component. In Table 2, the total blood-lymph clearance of protein is predicted to increase approximately 6-fold from 0.30 to 1.75 x 10^{-4}ml/sec. In the normal solution in the dog paw only 23% (0.07 x 10^{-4}ml/sec) of the total exchange is coupled to transcapillary fluid movement whereas with histamine 34% (0.59 x 10^{-4} ml/sec) of the total protein transport is convective. As the state of fluid balance in a histamine-treated tissue is altered so is the lymph-plasma protein concentration and protein clearance. As lymph flow (or equivalently fluid filtration in the steady state) is increased in a histamine-treated tissue, R may actually decrease to a value less than that observed in the control case. (R= C_L/C_p = concentration of protein in lymph/concentration of protein in plasma.) At the same time total protein clearance (RL) increases substantially. The point to be made is that the value observed for lymphatic protein concentration or blood-lymph protein clearance in a histamine-treated tissue depends critically on the state of fluid balance. Therefore, it is very difficult to quantitatively evaluate changes in protein permeability with a vasoactive agent such as histamine unless care is taken to account for alterations in tissue fluid exchange at the same time. This implies that microvascular hemodynamics must be carefully monitored and quantitatively assessed.

TABLE 2. Influence of Convective Transport on Blood-Lymph Protein Transport in Normal and Histamine-Treated Dog Paw

	DIFFUSIVE					TOTAL		
	PS[1]	L[1]	R	RL[1]	σ_f	R	RL[1]	% Convective Transport
CONTROL[2]	0.33	0.77	0.30	0.23	0.83	0.39	0.30	23%
HISTAMINE	1.66[3]	0.77	0.69	0.53	0.70	0.77	0.59	10%
		2.00	0.46	0.91	0.70	0.59	1.17	22%
		3.85[3]	0.30	1.16	0.70	0.45	1.75	34%
		10.00	0.14	1.42	0.70	0.31	3.11	54%

[1]Units for PS, L and RL are ml/sec x 10^{-4}

[2]Data for control PS, L and σ_f are taken from (28).

[3]Data for histamine-induced changes in PS and L are taken from (29).

This leads us to the final point to be made in this section of the report. This point addresses the indirect evaluation of capillary pressure changes in organ systems. The overall assessment of whether average microvascular pressure increase or decrease cannot be made from measurement of total organ vascular resistance as is frequently assumed. In isolated organs perfused under conditions of constant pressure (natural perfusion) or constant flow (pump perfusion), there may be large changes in capillary hydrostatic pressure subsequent to arteriolar vasodilation or vasoconstriction without significant alterations in the large vessel pressure difference or organ flow. This has been shown by Folkow and Neil (8) and is re-emphasized in Figure 1.

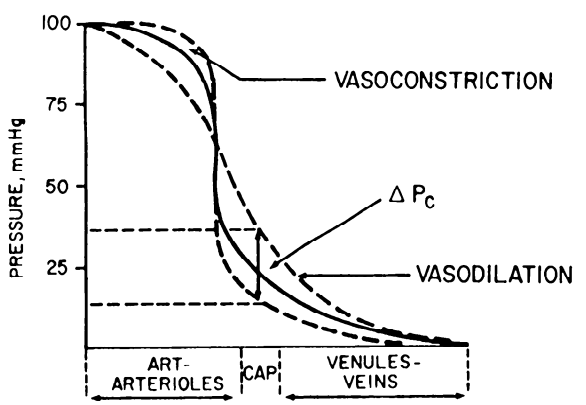

Fig. 1. Pressure Profiles of a normal cardiovascular system. A range of approximately 20 mmHg capillary pressure may vary during vasodilation and vasoconstriction.

Especially this appears to be true with histamine vasodilation since large shifts in blood flow between skin and muscle were seen in isolated dog hindlimb preparations (not treated with papaverine) perfused at either constant flow or constant pressure (4)(5). Only when the organ is maximally vasodilated with papaverine (or some other agent) and perfused at constant flow can it be even remotely assumed that total peripheral resistance may reflect intrinsic changes in capillary hydrostatic pressure. Even under these conditions the assumption may be inaccurate because of the possibility of regional redistribution of flow between microvascular units which could obtain from changes in transmural pressure differences across the walls of microvascular vessels. From these considerations, it is concluded that the rate limiting step for real progress in our understanding of capillary permeability alterations may well be the ability, and technology, to measure overall microvascular pressure changes in the whole organ vascular system.

PERFUSION HETEROGENEITY. The assumptions implicit in the use of the Kety-Renkin-Crone (2)(15)(26) relation for the estimation of capillary permeability surface area product (PS) are highly unrefined. The validity of the prediction of PS product from the equation:

$$PS = -F \log_e (1 - E) \qquad (4)$$

(where, F - flow of solute carrying fluid, and, E = steady state extraction of a diffusible solute) depends critically on the PS/F ratio being identical for each exchange vessel in the entire organ. This assumption of uniform PS/F ratios is not borne out by the results of other studies. Microvascular heterogeneity can be of several different types, all of which are known to exist in mammalian tissues. Geometrical nonuniformities would result from differences in length, radius and intercapillary distance among vessels comprising an organ's microcirculation (6)(24). Perfusion heterogeneity has been shown to occur by different patterns of flow and flow rates among capillaries of the same tissue (9)(12)(17). Regional variations in flow rate to large groups of capillaries in localized areas of a tissue have been suggested by direct observation techniques (32), by regional differences in the deposition of tracer substances (21) and by a stead-

ily declining fractional rate in the washout of small non-electrolytes from skeletal muscle (3). It has also been suggested that a classification into well perfused and poorly perfused regions of tissue could explain the observed data in isolated hindlimb preparations (25)(27). These considerations strongly suggest the possibility that non-uniformity of exchange characteristics may exist in all tissues, but certainly in skeletal muscle.

To investigate the exchange characteristics to be expected from a heterogeneous group of capillaries, a simple model was developed. For an exchange unit consisting of N capillaries in parallel with one another, the total steady-state extraction (E) of solute can be expressed by the following equation:

$$E = 1 - \sum_{i=1}^{n} f_i \exp - \frac{P_i S_i}{F_i} \qquad (5)$$

where, E - total tissue fractional extraction of a given test solute; P_i = permeability of capillary i for the specified test solute; S_i = surface area of capillary i; F = flow of solute-carrying fluid through capillary i; f_i = fractional flow of solute carrying fluid through capillary i. The model developed consisted of five hypothetical capillaries in parallel with one another (i = 1,5).

Two test solutes were used. For the first solute (denoted by subscript I in Table 3) the total PS-product for the tissue was set at 4 cm^3/min. For the other solute (donated by subscript II in Table 3) the overall PS-product was set at 1 cm^3/min. Each individual capillary was assumed to have a PS-product of 0.8 cm^3/min for solute I and 0.2 cm^3/min for solute II. That is, the PS-product for these two solutes are equally divided among all five vessels. In the model the P_iS_i for all capillaries was kept constant to better locate the effects of nonuniform flow changes. Two types of perfusion heterogeneity were considered. The first type of flow distribution was simple "recruitment" of new capillaries with increasing flow rates and perfusion pressures. In this type of flow heterogeneity the new capillaries which opened up received an equal share of the total flow through simple redistribution. The second type of flow distribution was called perfusion nonuniformity where changes occurred in total organ flow. In this latter case, all capillaries were open to flow but the flow was not uniformly distributed and, therefore, the fractional flows (f_i) were not equal.

The results of the model studies show that with either type of flow heterogeneity, in fact in any and all cases of flow nonuniformity, the computed value for PS (Eqn. 5) is less than that value which would be calculated if P_iS_i/F_i ratios were all identical as required by the assumption for use of Eqn. 4.

Two important considerations arise from these results. First, the use of multiple indicator diffusion data may, in a great variety of cases, lead to serious underestimates of capillary permeability. The extent of the underestimate of PS from flow heterogeneity is more serious for the more highly permeable substances. This is shown in Table 3. Take the case where the total flow is 2 ml/min (lines 4-6, Table 3). Case A describes the situation where each capillary is characterized by an identical PS/F ratio. The fractional flow to each capillary is 20% of the total flow. This is the situation which would obtain with the single capillary model. Case B describes the situation of perfusion heterogeneity where all capillaries are open but the fractional flows (f_i) to each capillary are not equal (non-uniform distribution of flow). Fractional flow to capillary 1=60% of the total, while 40% is spread out among the other 4 capillaries at 10% of the total flow for each. Case C describes the situation where there is simple recruitment of capillaries with increasing flow rates. Note that for the smaller molecule (high permeability) the estimate for permeability (PS_I^d) is 2.3/4.0 = 58% of the true permeability. For the larger molecule (low permeability) the estimate of PS is 0.85/1.00 or 85% of the true value (PS_{II}). The two hypothetical solutes only differ by a factor of 4 in permeability. The inability to find evidence for restricted diffusion of small molecules with the indicator diffusion technique may relate to the fact the permeabilities of small molecules are being underestimated to a greater degree than those of the large molecules. Restricted diffusion is simply being masked. Second, it may be invalid to suggest that a change in either permeability or surface area has occurred in response, for example, to a drug induced change in PS product. Such a change could easily occur from an alteration in flow without any change in perfused surface area or permeability. From a practical standpoint, the measurements of capillary filtration coefficients (capillary hydraulic conductivity) are less sensitive to changes in flow distribution and may thus provide better insight into alterations in permeability and surface area. These data do suggest that PS changes may be better assessed using large test solutes with the indicator diffusion technique. (Supported by Grant HL21356 and HL23196 from NHLBI).

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TABLE 3. EFFECTS OF PERFUSION HETEROGENEITY ON MICROVASCULAR EXCHANGE FUNCTION

		f_1[b]	f_2	f_3	f_4	f_5	E_I[c]	E_{II}	PS_I[d]	PS_{II}	C_I[e]	C_{II}	PS_I/PS_{II}
(ml/min)									(cm^3/min)		(ml/min)		
A	1	.20	.20	.20	.20	.20	.98	.63	4.0	1.0	.98	.63	4
B	1	.80	.05	.05	.05	.05	.71	.37	1.2	.47	.71	.37	2.6
C	1	1	0	0	0	0	.55	.18	.80	.20	.55	.18	4
A	2	.20	.20	.20	.20	.20	.86	.39	4.0	1.0	1.7	.79	4
B	2	.60	.10	.10	.10	.10	.68	.34	2.3	.85	1.4	.69	2.7
C	2	.50	.50	0	0	0	.55	.18	1.6	.40	1.1	.36	4
A	3	.20	.20	.20	.20	.20	.74	.28	4.0	1.0	2.2	.85	4
B	3	.33	.17	.17	.17	.17	.71	.27	3.8	.96	2.1	.82	3.9
C	3	.33	.33	.33	0	0	.56	.18	2.4	.60	1.7	.54	4
A	4	.20	.20	.20	.20	.20	.64	.22	4.0	1.0	2.5	.88	4
B	4	.25	.19	.19	.19	.19	.63	.21	3.9	.96	2.5	.85	4
C	4	.25	.25	.25	.25	0	.56	.18	3.2	.80	2.2	.72	4
	5	.20	.20	.20	.20	.20	.56	.18	4.0	1.0	2.8	.91	4

[a] Total organ flow.

[b] Fractional flow through an individual capillary.

[c] Extraction computed from, $E=1-\frac{C_i}{C_a}=1-vi\exp\left(\frac{P_iS_i}{P_i}\right)$

[d] Permeability-surface area product computed from, $PS=^{i}-F\cdot\log_e(1-E)$

[e] Capillary clearance computed from, $C=F\cdot E$

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PERMEABILITY OF CAPILLARIES IN MUSCLE, SKIN, AND SUBCUTANEOUS TISSUE

William P. Paaske

Dept. of Internal Medicine P
Rigshospitalet
University of Copenhagen
Denmark

INTRODUCTION

The theories of restricted diffusion and equivalent pore have been extensively applied to the analysis of data obtained from experiments on transcapillary solute flux and solvent flow even if it can be doubted whether the theories are adequate descriptive means. The microvascular exchange barrier is considered analogous with an artificial membrane provided with water-filled, circular, cylindrical pores through which rigid, spherical molecules pass from one well-mixed compartment to the other. The effective pore area for a test molecule is considered smaller than the actual pore area due to the combined effect of steric exclusion and friction. The exclusion is due to steric hindrance of permeation af molecules into the pore. When filtration is zero the effective pore area, S_D, is given by:

$$S_D = \pi(r - a)^2/\pi r^2 = (1 - (a/r))^2$$

(1) where a and r are molecular and pore radius respectively.

The friction effect is a function of all hydrodynamic interactions between pore, solvent (water), and test molecule. The dimensionless correction factor, $1/K_1$, is the frictional resistance to diffusion in free solution expressed as a fraction of the frictional resistance to diffusion in the pore (see for example (2)). At zero filtration:

$$D'/D = S_D(1/K_1)$$

where D' is the restricted diffusion coefficient, and D is the free diffusion coefficient in water. Numerically,

$$1/K_1 = 1 - 2.104(a/r) + 2.09(a/r)^3 - 0.95(a/r)^5$$

(3, 4).

If two test solutes with permeability coefficients P_1 and P_2 pass the membrane proportional to their free diffusion coefficients in water we have:

$$P_1/P_2 = D_1/D_2$$

which indicates a pore radius much larger than the radius of test molecule, *i. e.*, the combined effect of steric exclusion and friction is negligible. Usually, P is calculated from the permeability - surface area product, PS (= CDC, the capillary diffusion capacity), which can be determined experimentally. If restricted diffusion is present in various degrees for the test molecules we have:

$$P_1/P_2 = (k_1/k_2)(D_1/D_2)$$

where $k = D'/D = S_D(1/K_1)$. If the molecular radius of each test solute is known, the equivalent pore radius can be calculated.

The isogravimetric osmotic transient experiments of Pappenheimer, Renkin, & Borrero (5) on the isolated, denervated cat hindleg preparation provided determinations of P for a series of smaller hydrophilic solutes. Restricted diffusion was found to be present, and the data were compatible with an equivalent pore radius of 30 Å. However, no account was taken with respect to the osmotic reflection coefficient, σ. In fact, Pappenheimer *et al.* had determined P/σ instead of P. Several attempts have been made to "correct" the P/σ data in order to obtain actual P values, but the results are uncertain. Yet, the 30 Å pore radius estimate has till now had influence on the current concepts of microvascular exchange across the diffusion barriers of the capillaries in peripheral tissues.

With methods based on diffusion principles, exclusively, doubt has been cast regarding the correctness of the 30 Å estimate. Crone (6), *e. g.*, was not able to demonstrate restricted diffusion to inulin as compared to sucrose in the dog hind limb preparation. These results consequently indicated a much larger equivalent pore radius than 30 Å. Similar experiments on the heart also point towards a larger pore radius (7, 8).

Noteworthy is the fact that the experiments of Pappenheimer *et al.* as well as those of Crone had been performed in hind limb preparations containing all the peripheral tissues.

The capillaries in skin, subcutaneous adipose tissue, and skeletal muscle are all of the so-called continuous (non-fenestrated) type. They are formed of a one-layered endothelium surrounded by a continuous basement membrane. The individual endothelial cells are positioned closely towards each other. The characteristic intracellular structure is cytoplasmic vesicles that may fuse to form patent transendothelial channels (9). Occasionally, one vesicle may be open at the same time towards the capillary lumen and the interstitial space. In some studies with electron-micrographic indicators the intercellular space, the slit, has been determined to be about 37 Å at the narrowest location whereas other investigations have suggested that the slit is completely obliterated over some distance. Recent experimental evidence seems to suggest that a fibrous network within the pore may be of importance for the transport of solutes and solvent through the extracellular pathway (10). Possibly, electrostatic restriction occurs in the pore towards charged indicators (11).

Several investigations with the isogravimetric osmotic transient method are in excellent concert with the concept that transcapillary exchange of smaller hydrophilic solutes occurs through a 30 Å intercellular slit, but the indicator diffusion experiments consistently point towards a much larger pore size for this "small pore" system. The discrepancy between these earlier findings initiated the present series of investigations that were carried out with a method based on indicator diffusion principles. The tissues under study (skin, subcutaneous adipose tissue, and skeletal muscle) all have capillaries of the continuous type, and it must be emphasized that each experiment was performed on preparations containing only one of these peripheral tissues.

The problems investigated were:

1) What is the capillary permeability for smaller hydrophilic molecules in skin, subcutaneous adipose tissue, and skeletal muscle?

2) Does the continuous capillary exhibit the same permeation characteristics regardless of the tissue in which it is located?

3) Through which transendothelial pathways do smaller hydrophilic molecules permeate the microvascular exchange membrane in capillaries of the continuous type?

4) Does recruitment of skeletal muscle capillaries occur with functional increase of blood flow.

METHODS

The single injection method of Sejrsen (12, 13) was used together with the electronic data processing equipment of Paaske & Nielsen (14, 15). An arterial bolus injection of a diffusion limited test substance is made close to the arterial inflow of the preparation. If the indicator is gamma emitting the response curve can be recorded either externally by a scintillation detector (residue detection) or by venous sampling (outflow detection). If the indicator is either beta emitting or a substance whose concentration in the outflowing blood can be determined by other means, outflow detection is feasible. When the bolus traverses the organ it will be separated in an extracted fraction, E, and a complementary fraction, 1, which is transmitted intravascularly without entering the interstitium. As shown by Sejrsen the response curve can be analysed to give E and $\bar{t}(iv)$, the intravascular mean transit time. If plasma flow, f(pl), is measured directly, PS can be calculated from:

$$PS = -f(pl) \cdot K \cdot \ln(1 - E)$$

(ml/100 g·min) where K is a factor taking into account that the driving force for electrically charged molecules is not only the concentration gradient in the water phases but also the electrical potential difference across the membrane. In cases where f(pl) cannot be measured directly we have:

$$PS = -(1/\bar{t}(iv)) \cdot V(iv)_b \cdot (1 - Hct) \cdot K \cdot \ln(1 - E)$$

(ml/100 g·min) where $V(iv)_b$ is the blood volume of the organ, and Hct is the haematocrit value.

For skin, the rabbit abdominal cutaneous tissue preparation was used, which provides a recirculating, autoperfused, multiple inlet, multiple outlet system (16, 17).

For adipose tissue, the rabbit inguinal fat pad preparation was employed which provides a non-recirculating, autoperfused single inlet, single outlet system (14, 15, 18).

Further, the cat gastrocnemius muscle preparation was used with and without electrical stimulation. In this case we have a non-recirculating, autoperfused single inlet, single outlet system (12, 13, 19, 20).

The indicators were ^{51}Cr-EDTA (^{51}Cr-ethylene-diamine-tetraacetate), molecular weight, MW, 341.2, $D = 0.70 \cdot 10^{-5}$ cm^2/s, a = 4.8 Å, ^{57}Co-B12 (^{57}Co-cyanccobalamin), MW = 1353.5, $D = 0.39 \cdot 10^{-5}$ cm^2/s, a = 8.4 Å, and ^{14}C-inulin (Inulin(^{14}C)carboxylic acid), MW = ca. 5500, $D = 0.22 \cdot 10^{-5}$ cm^2/s, a = 14.8 Å.

RESULTS

The results are presented in Table I. The results from skeletal muscle are also presented in Fig. 1 (19, 20 - compiled data).

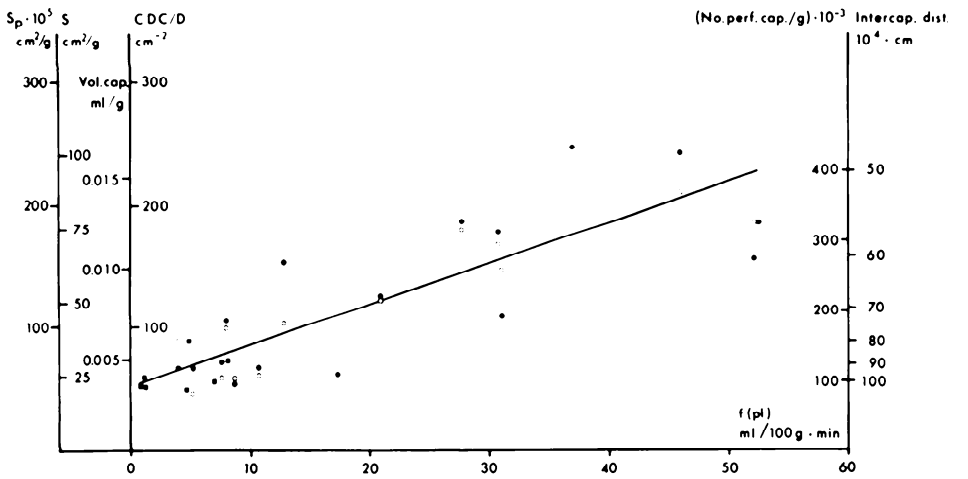

Fig. 1. S_p : Pore area, for other abb. see text. Open circles: EDTA, closed circles: B12, filled squares: inulin.

Table I

Tissue	Test Molecule	P $\cdot 10^5$ cm/s	Ref.
Skeletal muscle	EDTA	1.02	(19)
	EDTA	1.06	(13)
	B12	0.59	(19)
	Inulin	0.20	(20)
Adipose tissue	EDTA	0.95	(14,15)
	B12	0.52	(18)
Cutaneous tissue	EDTA	0.88	(16)
	B12	0.56	(17)
Myocardium	EDTA	1.08	(21)

The capillary surface area, S, was estimated at 70 cm^2/g in skeletal muscle and cutaneous tissue and 35 cm^2/g in adipose tissue.

The ratio PS(EDTA)/PS(B12) was 1.81 in skin, 1.61 in adipose tissue, and 1.79 in skeletal muscle. PS(EDTA)/PS(inulin) was 3.21 in skeletal muscle, where PS(B12)/PS(inulin) was 2.04. The ratio between the free diffusion coefficients was:
D(EDTA)/D(B12).....1.79
D(EDTA)/D(inulin).....3.18
D(B12)/D(inulin).....1.77

DISCUSSION

The permeability coefficients indicate that the capillary permeability for a smaller hydrophilic solute is identical for all practical purposes in the tissues under study, and from the results it seems reasonable to suggest that the average permeability of the continuous capillary is uniform regardless of the tissue in which it is located.

Restricted diffusion for the larger molecules as compared to the smaller was not present to any significant degree in either tissue. Consequently, the results are incompatible with the belief that the transcapillary flux of smaller hydrophilic solutes occurs through a 37 Å intercellular slit. A much larger pore radius of 200 - 400 Å must be responsible for the finding that the indicators permeate the microvascular exchange area proportionally to their free diffusion coefficients in water. The fused vesicles forming the system of patent transendothelial channels might prove to be the morphological basis for the pathway as the narrow interendothelial slit cannot explain the present findings. One might speculate that the capillary membrane acts as a barrier only due to the fact that the endothelial cells reduces the exchange area whereas the functional pores do not exhibit any resistance to diffusion. The net selectivity of the total microvascular exchange barrier may depend on interstitial factors rather than membrane properties. Also, the graded exclusion and heterogenous distribution of solutes in the interstitium as evidenced by the multiexponential washout of the in-

dicators from this space might be due to the two-phase nature of the interstitium. Further, the multiexponential washout from the interstitial space shows that the assumption of a well-mixed interstitial compartment used in the isogravimetric osmotic transient method is not fulfilled.

As shown by Krogh (22) the number of open muscle capillaries per unit cross-sectional is considerably larger during maximum perfusion than the number perfused during rest. When blood flow through the organ is increased this is due to either an increase of linear velocity of the blood through the open capillaries or by opening up of more capillaries ("recruitment"). The present results (Fig. 1) show that PS for the indicators increases about 3.5 times within the experimental plasma flow range. PS-ratio was constant for the indicators within the perfusion range. Assuming P to be constant the finding of a constant PS-ratio seems to be a reasonable indication of the fact that recruitment does in fact occur with increased plasma flow.

The intravascular blood volume was found constant at about 3 ml blood/100 g skeletal muscle - regardless of the perfusion rate. If it is assumed that the capillary radius is 3.2 μm, that the length of the capillary is 1200 μm, and that $1.2 \cdot 10^5$ capillaries per g are perfused at rest, the capillary volume can be estimated at 0.0039 ml/g (i. e. 12 per cent of the total blood volume of the muscle). If the number of perfused capillaries increases with a factor of about 3.5 and the vascular volume is constant, it can be assessed that the capillary volume perfused during maximum work will be as large as 40 per cent of the total blood volume of skeletal muscle. This implies that the volume of another section of the vasculature must be correspondingly decreased (veins and venules).

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PROPERTIES OF MACROMOLECULAR ACCUMULATION IN ENDOTHELIAL CELLS AND THEIR POTENTIAL ROLE IN VESICULAR TRANSPORT

William L. Joyner, Stephen P. Bruttig and Knut Wallevik*

Dept. Physiol.-Biophys., Univ. Nebr. Coll. Med., Omaha, NE 68105
*Finsen Laboratory, The Finsen Institute, Copenhagen, Denmark

Using endothelial cells cultured from human umbilical veins, this study was designed to investigate the kinetics of macromolecular accumulation, binding and internalization. These cells accumulated horseradish peroxidase (HRP, A_e=30 Å) and human serum: albumin-monomer (ALB-M, A_e=36 Å), albumin-denatured (ALB-D, A_e=40 Å) and albumin-polymerized (ALB-P, A_e=152 Å) in a time-dependent manner. The initial phase was not attenuated by lowering the incubation temperature; whereas, the second phase was significantly depressed. Internalization was inversely related to the molecular radius; whereas, binding was greater for HSA-P. Vesicular radius, considering restricted entry of these macromolecules into the vesicles, was 200-250 Å. By varying the external concentration (1-10 mg/ml) of ALB-M, the binding capacity ($8x10^6$ mol·cell^{-1}) and maximal internalization ($39x10^{-4}$ μg·hr^{-1}·cell^{-1}) were determined. These present experiments describe some aspects of the process of vesicular transport for macromolecules and this process of endocytosis in endothelial cells involves both binding and engulfment for these macromolecules. (NIH HL 19455)

Nature of Capillary Barrier

Plasma to lymph transport of macromolecules has been the topic of numerous investigations in the last few years (1,2,3). One recent review (4) describes potential, multiple-type pathways which have been invoked to quantitate this transport process. After Grötte (5) and Mayerson and colleagues (6) described the appearance of large dextran fractions and proteins in lymph, the initial postulate for the exchange of large molecules was an extension of the filtration- and concentration-dependent small pore model. Recent anatomical (7) and some physiological studies (8) have described the presence of large gaps in postcapillary venules. The appearance of these postcapillary gaps may depend upon the presence of permeability-altering agents (e.g., histamine) or tissue injury. Further physiological investigations (9) questioned the validity of this large pore concept as the major pathway for the exchange of large molecules. Using techniques to alter the potential formation of large pores and filtration forces, these investigators characterized the transport mechanism for macromolecules as a vesicular process which was primarily independent of convective mechanisms. Anatomical studies have described the existence of vesicles in endothelial cells and have defined the kinetics and specificity of this pathway (10,11). Calculations of transport dynamics from morphological studies have coincided with those obtained from physiological studies (12). This parallel transport pathway is dependent upon the concentration gradient for the diffusing molecule and has been described as a dissipative process (1); whereas, the other could occur through some intercellular route dominated by convective forces. Thus, the separation of these two processes using conventional, *in vivo*, techniques has definite limitations.

Endocytotic processes have been investigated in various types of isolated cells (13) and have been given a wide variety of classifications, e.g., pinocytosis, phagocytosis, etc. (14). One common feature of these processes is membrane invagination with subsequent engulfment of the extracellular material either free in the invaginated cavity or bound to the invaginated membrane. Two prominent endocytotic processes have been labeled pino- and phago-cytosis; however, a clear distinction between these two processes has yet to be described. Some ascribe an engulfment of larger molecules, a greater sensitivity to metabolic inhibition, and a cell density inhibition as characteristic of the latter process (15). These processes have been investigated in macrophages and other types of reticulo-endothelial cells (15). A few (16,17) studies concerning the accumulation of large molecules by endothelial cells, in culture, have appeared. Even though there are deficiencies in using cultured endothelial cells to study the process of endocytosis as it relates to vesicular transport, *in situ*, the advantages of investigating the kinetics of the endocytotic processes in a controlled environment should lead to a better understanding of this dynamic function for endothelial cells.

Macromolecular Endocytosis in Endothelial Cells

Endothelial cells isolated from human umbilical veins by collagenase perfusion were maintained in culture flasks containing Lewis media (Medium 199, GIBCO, pH-7.2) with 10-20% fetal calf serum (18). At confluency (7-10 days), the cells were in a monolayer attached to the flask and the average number of cells per cm^2 was $6x10^4$. Endothelial cell characterization was completed by phase contrast and electron microscopy, metabolic activity, and antigen/antibody staining (19).

Time and temperature dependent studies were performed on endothelial cells, which were prewashed and counted, by incubating the cells at 37°C and 4°C for 15,30, and 120 min. The incubation medium contained the following proteins iodinated with carrier-free ^{131}I or ^{125}I using monchloride:human serum albumin monomer (ALB-M), human serum albumin denatured (ALB-D) and human serum albumin polymer

(ALB-P). These macromolecules have been structurally characterized by Wallevik (20). In some experiments horseradish peroxidase (HRP) was used. After the incubations, the cells were washed (5x) with cold phosphate-buffered saline, lysed with Triton X-100 (0.05% in distilled water), and scraped from the flask in 3.0 ml aliquots. The total amount of radioactive tracer activity was determined on both the incubation medium and the cell lysate. Aliquots (2.0 ml) of each sample were placed in a gamma counter (Nuclear Chicago 1195) and from the disintegrations per minute along with the specific activity of the tracer, the total concentration (moles per liter) or amount (moles) determined. An enzymatic assay using O-dianisidine and the development of a colored product at 460 nm was used to determine the concentration of HRP. Accumulation of each macromolecule by the endothelial cells was increased with the time of incubation at 37°C (Figure 1). When the cells were

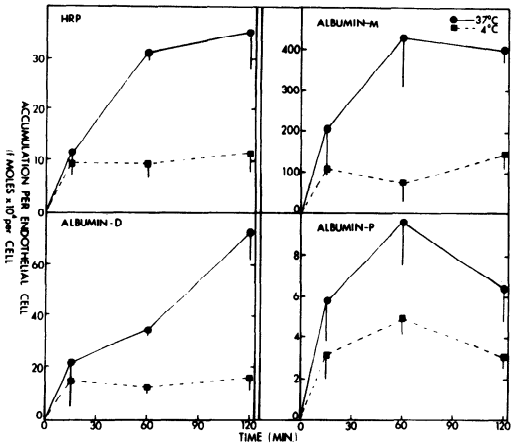

Fig. 1. Accumulation of macromolecules.

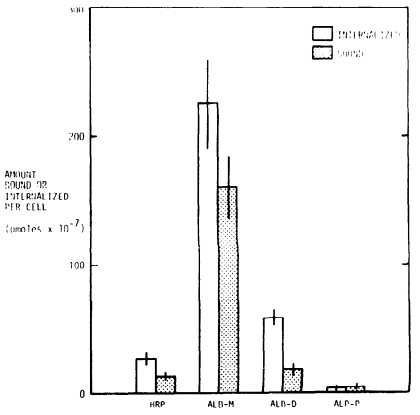

Fig. 2. Binding and internalization.

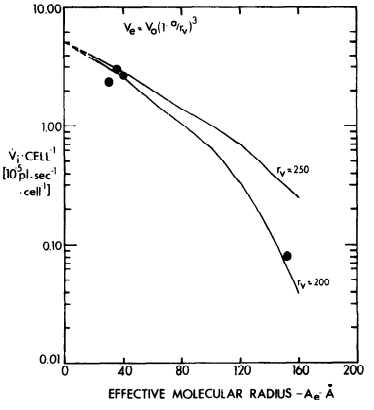

Fig. 3. Clearance of macromolecules.

incubated at 4°C the initial accumulation at 15 min was not statistically different from that at 120 min. Linear regression analysis of the 15-120 min incubations predicted a slope which was not different from zero. It appeared that for most of the macromolecules, the accumulation was saturated at 60 min. The accumulation process was described by two phases: 1) an initial phase which is rapid and not dependent upon temperature--adsorption (binding) of the macromolecule to the cell surface, 2) a slower phase which is attenuated by temperature--engulfment (internalization) of the molecules by the cells (15). Thus, endothelial cells bind each of the macromolecules at a rate which is saturated within 15 min and the relative amounts bound are: 20-25% for HRP, 30% for ALB-M, 30% ALB-D, and 50% for ALB-P of the accumulated load (Fig. 2). The rate of binding is greater for ALB-P and decreases with the molecular weight of the molecule. The amount engulfed or internalized is inversely related to the molecular weight of the molecule and if this is a diffusive process and has reached equilibrium, the effective volume of the substance cleared from the medium at a specific time can be calculated (Fig. 3). The rate of volume clearance is plotted against the effective molecular radius of the molecule and according to the properties of steric restriction, lines of best fit were determined. These data indicate that the internal radius of the vesicle, presumeably a sphere, required to selectively restrict molecules with diffusing radii greater than 30 Å is approximately 200 Å. The ordinate intercept of this curve represents the total volume engulfed by the endothelial cells per unit time ($5x10^{-5}$ pl/sec/cell). If the surface area of an endothelial cell is $1.5x10^{-5}$ cm^2 (11,18) then the total amount engulfed per unit time per cm^2 endothelial cell surface area is 3.3 pl/sec/cm^2. Renkin (4) calculated that for the dog paw the actual volume exchanged by vesicular transport was $2x10^{-8}$ cm^3/sec/cm^2 endothelial cell surface area (200 pl/sec/cm^2). The figure obtained from cellular data is 10-100 times lower than that calculated from whole tissue experiments. Also, assuming an internal vesicular radius of 200 Å and calculating the vesicular volume ($3.4x10^{-5}$ pl) for a sphere, the number of vesicles transferred per unit time per unit endothelial cell surface area can be calculated from the present data (10^5 vesicles/sec/cm^2). This value is lower (100-1000x) than that calculated (10^8 vesicles/sec/cm^2) by Renkin (4). Even taking into account the internalized albumin coat surrounding the vesicle, the vesicular turnover time per unit endothelial cell

surface area was increased by only 3-5 times.

Further studies were completed using only ALB-M as the external tracer molecule. The cells were postfixed in gluteraldehyde and scraped from the flasks. The accumulation of ALB-M was dependent upon both time and temperature (Fig. 4). Incubation at 4°C depressed albumin accumulation after

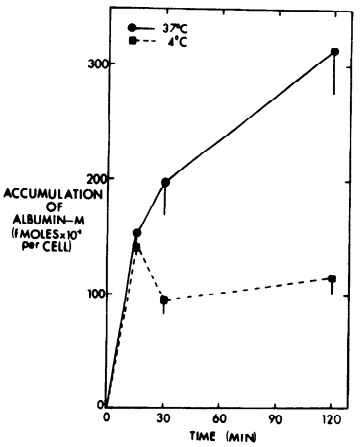

Fig. 4. Accumulation of ALB-M.

15 minutes, but the total accumulation of ALB-M was increased 10 fold. This did not affect the percent of the total amount accumulated (30%).

The effect of varying the external albumin monomer concentration on the accumulation at 37° and 4° C by endothelial cells is described in Fig. 5.

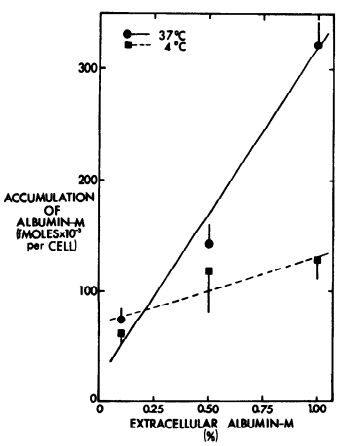

Fig. 5. Concentration-dependent accumulation of ALB-M.

ALB-M accumulation was not significantly depressed by incubation at 4°C until the external concentration was greater than 0.5% ($7x10^{-8}$ moles/ml). ALB-M accumulation at 4°C appeared to be saturated when the external concentration was greater than 0.5%. If the 4°C incubations represent binding of the molecules to the cell surface, analysis of the binding characteristics can be accomplished using Scatchard plots (22). This analysis relates the amount of material bound to the cell surface to the amount available (Table 1). Plotting the

TABLE 1.

SCATCHARD ANALYSIS OF ALBUMIN-M BINDING TO ENDOTHELIAL CELLS

EXTERNAL ALBUMIN (MOLES · ML^{-1})	2×10^{-8}	7×10^{-8}	15×10^{-8}
BOUND ALBUMIN (MOLES · CELL^{-1})	80×10^{-18}	119×10^{-18}	127×10^{-18}
BOUND/FREE (ML · CELL^{-1})	53×10^{-10}	17×10^{-10}	9×10^{-10}

BINDING CAPACITY = 137×10^{-18} MOLES · CELL^{-1} = 8×10^{6} MOLECULES · CELL^{-1}.

APPARENT AFFINITY COEFFICIENT = 9×10^{4} L/MOLE.

ratio of the amount bound per cell to that available for binding (free) in ml/cell to that bound/cell (moles/cell), the binding capacity and the apparent affinity of the cell for the substance can be calculated. The relation was linear (r=0.99) and the slope of the line (Ka) was $9x10^4$ 1/mole. The binding capacity of ALB-M to endothelial cells was $8x10^6$ molecules/cell. Both of these values are 10-100 times less than similar values obtained for albumin in macrophages (15). If the surface area for an albumin molecule ($5x10^{-13}$ cm^2) is calculated for an ellipsoid with a short radius of 22 Å and a long axis of 72 Å, then a theoretical total surface area for the binding of albumin to the endothelial cell can be estimated from the binding capacity. This value was found to be $4x10^{-5}$ cm^2 and is within the range of reported values for endothelial cell surface area (11). Thus, it appears that 4°C incubation does reflect the binding of albumin to the external surface of endothelial cells and that these cells are capable of ingesting more albumin than can be accounted for by binding alone.

Potential Role in Transcapillary Exchange.

Studies concerning the accumulation of macromolecules by endothelial cells, in culture, and their use in postulating the potential role of a transendothelial transport system, in vivo, are well recognized. However, specific stimuli of the multipathway system for macromolecular transport are not mutually exclusive; thus, one is usually left with theoretical maneuvers designed to separate one pathway from the other. Using data for albumin transport in isolated endothelial cells ($1.61x10^{-4}$µg/cell/day), the total amount of albumin in thoracic lymph (47.6 g/day), and the postulated endothelial cell density ($6.67x10^4$ cells/cm^2 cell surface area), the total capillary surface area required to match the endothelial transport capacity with that appearing in thoracic lymph is 443 m^2. This value closely approximates reported values (22). Does this imply that all albumin transport is via intraendothelial passage, i.e., vesicular transport? Probably not, since the same calculations described from Lassen's et al. (3) measurements of transcapillary escape rate for albumin produces a value which is apparently 3-4 times larger. Also, it is probable that the rate of albumin accumulation in isolated endothelial cells is much slower than that described from morphological studies. Thus, further studies are required to accurately describe the rate and other kinetic aspects of this accumulation process and the role of binding to transport as well as the possible

catabolic role of the endothelial cell for various macromolecules.

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CONSECUTIVE BARRIERS TO MOVEMENT OF WATER AND SOLUTES ACROSS THE LIVER SINUSOIDS

Harris J. Granger and Glen A. Laine
Department of Medical Physiology

College of Medicine, Texas A&M University
College Station, Texas 77843

In most tissues of the body, the transmicrovascular flux of water and protein into the interstitium is exactly matched, under normal conditions, by lymphatic removal of these materials. The liver represents a dramatic departure from this simple arrangement. In the liver, the interstitium also communicates directly with the potential space of the peritoneal cavity. The interaction of the peritoneum with the hepatic interstitium and lymphatics has a profound influence on transsinusoidal fluid movement and, in turn, impacts directly on overall regulation of body fluid balance.

HEPATOPERITONEAL FLUID SYSTEM

Figure 1 illustrates the forces and flows which mediate fluid dynamics in the hepatoperitoneal complex. Under normal conditions, water and protein continuously move from the sinusoids into the hepatic interstitium. The build-up of interstitial fluid is prevented by the operation of two outflow mechanisms-lymphatic removal and surface transudation. The surface transudate enters the peritoneal cavity; in turn, peritoneal fluid is continuously absorbed by lymphatics and capillaries imbedded in the peritoneal lining.

According to Figure 1, the hepatoperitoneal fluid system is characterized by at least 4 interfaces - sinusoid/interstitium, interstitium/lymph, interstitium/peritoneal cavity, peritoneal cavity/parietal peritoneum. At each of these interfaces, the rate of convective flow (J_v) is given by

$$J_v = K_f \cdot [(P_1 + P_2) - \sigma (\pi_1 - \pi_2)] \qquad (1)$$

where $(P_1 - P_2)$ and $\sigma (\pi_1 - \pi_2)$ are the hydraulic and effective oncotic pressure differentials, respectively; σ is the osmotic reflection coefficient of plasma proteins; and K_f is the membrane filtration coefficient. The rate of protein movement (J_p) across each interface is described by

$$J_p = (1 - \sigma)[(C_1 + C_2)/2] \cdot J_v + PS(C_1 - C_2) \qquad (2)$$

where PS is the permeability·surface area product of the plasma proteins. The first term in equation 2 represents convective transport of protein; the diffusive component is given by the second term. To fully characterize the quantitative basis of hepatoperitoneal fluid balance, the magnitude of each force, flow and membrane parameter at each interface must be ascertained. In addition, the compliance of the vascular, interstitium and peritoneal cavity must be quantified to define the role of the hepatoperitoneal system in overall control of fluid balance.

SINUSOID/INTERSTITIUM INTERFACE

In the dog liver, interstitial pressure averages 5.8 mmHg and the sinusoidal hydrostatic pressure ranges between 5.8 and 7 mmHg. Hence, the transsinusoidal hydraulic pressure differential is 1.2 mmHg or smaller. An effective transsinusoidal oncotic differential is probably non-existent under normal conditions; the lymph protein concentration is 95% of the plasma level and estimates of the reflection coefficient yield values equal, or close, to zero. Utilizing these measurements along with equations 1 and 2, we conclude that 1) the net transsinusoidal driving force is primarily hydrostatic in nature and 2) protein transfer across the sinusoids occurs by convection (7). The filtration coefficient of the sinusoid lining must be very large; the total surface area of sinusoidal endothelium is extensive and is perforated by holes ranging from 400 to 6000 Å is diameter (9). The best plethysmographic estimate of K_f (5) for the sinusoidal membrane (e.g., 0.3 ml/min/100g/

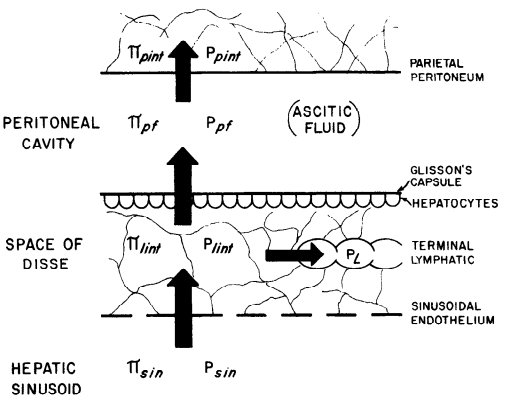

FIGURE 1: Pressures and flows which mediate the fluid dynamics in the hepatoperitoneal system: πpint and Ppint are osmotic and tissue pressures in the parietal peritoneal interstitium, respectively. πpf and Ppf are osmotic and hydrostatic pressures in the peritoneal cavity, respectively. πlint and Plint are osmotic and tissue pressures in the hepatic interstitium, respectively. PL is the hydrostatic pressure in the hepatic lymphatics. πsin and Psin are osmotic and hydrostatic pressures in the hepatic sinusoid, respectively. (From Circ Res 45:317-323, 1979.

mmHg)may underestimate the true value by an order of magnitude due to lack of an accurate assessment of the net transsinusoidal pressure differential.

INTERSTITIUM/LYMPH AND INTERSTITIUM/PERITONEAL CAVITY INTERFACES

The transport of molecules across the endothelial lining of the terminal lymphatics in the liver has not been studied in a direct fashion. Most investigators usually assume that this interface offers no restriction to protein movement (3, 4). Thus, lymph formation is viewed as being determined exclusively by the hydrostatic differential operating across the lymphatic wall. The rate of surface transudation across Gilsson's capsule should be influenced by the hepatic interstitial and intraperitoneal pressures. Indeed, the rate of formation of peritoneal fluid is modulated by intraperitoneal pressure when portal and hepatic venous pressures are maintained at constant levels (10). Although hepatic lymph and surface transudate contain equal concentrations of plasma proteins at high sinusoidal pressures (2), the possibility of molecular sieving at the liver surface under normal pressure condition cannot be excluded. Indeed, the parallel-series arrangement of sinusoidal, capsular and lymphatic barriers may complicate determinations of membrane parameters, especially since the three structures appear to possess permeabilities of similar magnitude. For example, sieving at the sinusoid would tend to cause a reduction of interstitial protein concentration during elevation of transsinusoidal filtration rate. A concomitant increase in transcapular filtration would elevate interstitial protein concentration if sieving occured at Glisson's membrane. Consequently, lymph/plasma concentration ratios would tend to remain constant as transsinusoidal filtration was elevated, especially if a large fraction of the sinusoidal flux crossed the liver surface.

THE PERITONEAL CAVITY AND ITS INTERFACE WITH THE PERITONEAL WALL

At a given instant, peritoneal fluid volume is dependent on the rate of surface transudation and the rate of fluid absorption by the parietal peritoneum. Peritoneal fluid absorption is influenced by the prevailing peritoneal pressure. Peritoneal pressure, in turn, is determined by peritoneal fluid volume and the compliance of the peritoneal wall. In the dog, peritoneal compliance averages 10 ml/kg/mmHg (1); translated to the 70kg human, this means that 700 ml of fluid must accumulate in the cavity to produce a 1 mmHg rise in intraabdominal pressure. Thus, by serving as an extension of the hepatic interstitium, the peritoneal cavity provides a large potential space for spillage of excess plasma.

Because peritoneal fluid is absorbed by an iso-oncotic mechanism, it has been suggested that the lymphatics of the peritoneal wall may play a major role in the absorption process (8). However, the contribution of the capillaries of the peritoneal lining remains unknown and therefore cannot be discounted.

PATHOPHYSIOLOGY OF ASCITES

Excess accumulation of fluid in the peritoneal cavity occurs when sinusoidal hydrostatic pressure rises to high levels (Figure 2). Ascites usually is precipiated by restriction of hepatic venous outflow due to a rise in caval pressure or an elevation of hepatic venous resistance (6). Under these conditions, transsinusoidal water and protein fluxes rise. Although the lymphatic removal of interstitial fluid increases, the rate of formation of peritoneal fluid also accelerates. Consequently, peritoneal fluid volume increases until peritoneal pressure is elevated to a level sufficient to adequately augment reabsorption by the peritoneal lining. In the new steady-state, all water and protein fluxes are elevated and the peritoneal cavity is distended. In a clinical setting, the distended abdomen reflects altered hepatoperitoneal dynamics. If current concepts of sinusoidal permeability and peritoneal fluid reabsorption are correct, hyperoncotic therapy will neither reduce transsinusoidal filtration rate nor accelerate the reabsorptive process at the parietal peritoneum. On the other hand, paracentesis simply provides a temporary interruption of a steady-state which the hepatoperitoneal fluid system will seek to reestabilish. From a physiological standpoint, the best solution is to eliminate the cause of sinusoidal hypertension.

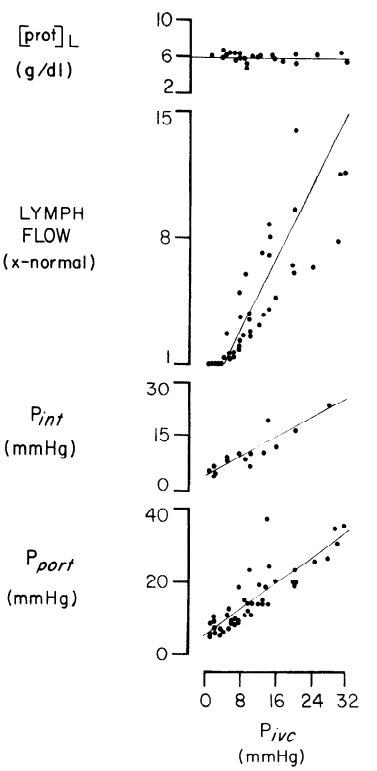

FIGURE 2: Effect of increased inferior vena cava pressure (Pivc) on protein concentration in liver lymph, hepatic lymph flow, hepatic interstitial pressure (Pint) and portal pressure (Pport). Surface transudation rate (not shown) increased with each elevation of caval pressure. (From Circ Res 45:317-323, 1979).

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THE INTERSTITIAL MATRIX AS A BARRIER IN BLOOD-TO-LYMPH SOLUTE MOVEMENT

Philip D. Watson

Department of Physiology, School of Medicine
University of South Carolina, Columbia, SC 29208

When an extracellular solute such as sucrose or albumin moves from plasma to lymph, it must cross several distinct barriers. These are the capillary wall, the basement membrane, the interstitial space and the wall of the lymphatic capillary. They are arranged in series and in theory at least any one could provide part of the diffusion barriers suggested by the experimental data. The issue to be considered here is the relative importance of each of these barriers in the total resistance to movement.

Traditionally the view has been that the capillary wall has by far the greatest resistance, so that the steady-state concentration profile for a large molecule such as albumin drops rapidly within the capillary pores, and the concentration within the pericapillary interstitial fluid is close to that of the lymph. However the biochemists, such as Ogston (16) and Laurent (13) have suggested that the interstitial macromolecules, collagen and the proteoglycans, might significantly influence solute movement, especially that of the larger molecules. If this is the case then the question can be asked: how much of the transport resistance presently ascribed to the capillary wall is actually created by the interstitial matrix and basement membrane? [There seems to be no question that the terminal lymphatics are freely permeable to both large and small solutes (22), and probably have a very small resistance compared to the total amount. Very little is known about the transport properties of the basement membrane, so for simplicity the basement membrane resistance will be lumped with that of the capillary wall.]

If the interstitial space has a significant resistance to movement, then as soon as a solute leaves the capillary its extravascular movement is hindered by this resistance, and the pericapillary solute concentration rises more rapidly than it would in the absence of interstitial resistance. Hence most experiments, which are intended to measure capillary permeability, are in fact measuring both capillary permeability and interstitial permeability simultaneously (permeability-surface area product is the inverse of resistance). In most cases it is assumed that the visually-obvious capillary wall is the only significant resistance. It would be expected that some experiments weight the interstitial contribution more than others, the weighting depending on the distance travelled from the capillary during the measurement period. The plasma-to-lymph transport measurements probably are influenced the most by interstitial resistance and the single-injection multiple-indicator measurements the least.

Before considering the data which separates the two contributions to solute hindrance, the meaning of the term 'resistance to diffusion' will be defined more exactly. For steady-state diffusion between two regions Δx apart, Fick's Law gives

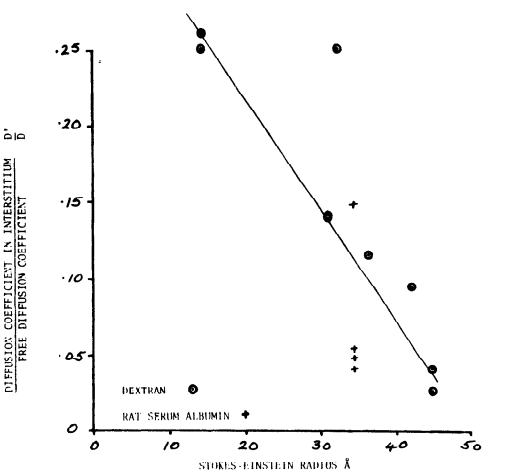
$$\text{Solute flux rate} = \frac{D'A}{\Delta x} \times \text{Concentration difference.}$$

where D' is the local diffusion coefficient ($D' < D$, the free diffusion coefficient), and A is the area through which solute moves. By analogy with Ohm's Law:

$$\text{Resistance to diffusion,} = \frac{\Delta x}{D'A}$$

Hence a barrier can reduce diffusion rates in two ways, by decreasing $A/\Delta x$, or by reducing D'. The latter case is called restricted diffusion, and may occur because the barrier has pore dimensions or average interfiber distances comparable to that of the diffusing molecule.

The usual view is that the capillary wall has both a very small area for diffusion relative to the capillary surface area, Pappenheimer suggested .2% (17), and has pores small enough to create highly restricted diffusion for the plasma



Reduction in diffusion coefficient in the rat mesentery interstitium. Data of Fox and Wayland. Note that the ratio may be extrapolated to zero around 50 Å radius.

Figure 1

proteins. The interstitial space has usually been regarded as having a very large $A/\Delta x$ and a D' close enough to D such that $\Delta x/D'A$ for the extravascular-region was insignificant compared to that for the capillary wall.

There have been many studies suggesting inhibited extravascular movement (see ref 18), but the most pertinent evidence that the interstitial matrix plays a significant role in plasma-lymph transport are the diffusion measurements of Fox and Wayland (8). They measured the diffusion coefficients of fluorescent-labelled albumin and dextran in the rat mesentery, and their data are shown in figure 1. Despite the scatter it is clear that all the solutes show restricted diffusion, $D' < D$, and that the larger molecules show more restriction than the smaller. Fox and Wayland point out that all the likely experimental errors would tend to increase the measured value of D'. The amount of restriction is consistent with the mesentery behaving like a system of pores of approximately 50 Å radius. This is ,of course, the size of the so-called small pores found in capillary permeability measurements (10).

Large molecules such as the gamma globulins and fibrinogen are found in lymph in significant quantities (4,10). Clearly if the interstitial space contained only a system of 40-70 Å pores, then these large proteins could never reach the lymph. Preferential or free-fluid channels which could carry the larger solutes have been seen by McMasters and Parsons (14), Wiederhielm (21) and Nakamura and Wayland (15); and McMasters observed that the pathways followed collagen fibers. While channels are observed in regions without collagen (5), it is interesting to observe that these fibers do have the right internal dimensions to create preferential or free-fluid channels. Collagen fibers are made of collagen fibrils with gaps between the fibrils of several hundred angstroms, and the probable arrangement of the ground substance and the collagen fibers is shown in figure 2, taken from Zweifach and Silberberg (22). Notice that in the opinion of those authors no ground substance is found within the collagen fibers. This is essential if the fibers are to act in non-sieving manner.

If we hypothesize that the hyaluronate and proteoglycans of the ground substance create the 50 A pore system found by Fox and Wayland, and that the interior of the collagen fibers or some other system provides a preferential or free-fluid pathway, then we have an interstitial space transport system which could provide the small and large pore systems used to explain the steady-state plasma-to-lymph studies of Grotte (10) and many others (2,4).

The two-part arrangement just described closely resembles that of a gel chromatography column. This possibility was first mentioned by Arturson et al (2) and Watson & Grodins (19) showed that the plasma-to-lymph dextran data of Garlick and Renkin (9) was consistent with the gel-column model. Recently Watson, Bell and Renkin have tested the gel-column hypothesis further (20), and have shown that larger molecules approach equilibrium in lymph faster than smaller ones, strongly supporting the gel-column structure. Figure (3) shows the more rapid equilibration of tracer fibrinogen compared to tracer albumin in lymph following a step in tracer plasma concentration. This is exactly the behavior of a gel chromatography column loaded with a step instead of a pulse, with the larger molecules preceeding the smaller. The precession of fibrinogen is physiological evidence for free-fluid channels running between plasma and lymph.

While the Fox and Wayland data provide direct measurements of D', there are no direct measurements for $A/\Delta x$ for the interstitial space. However, it is possible to calculate $A/\Delta x$ values for plasma to lymph transport from the data of Garlick and Renkin (9), and the model of Watson and Grodins (19). From Table 6 of the latter paper, the total interstitial area for sucrose diffusion is 20 sq cm for 24 grams of tissue (9), and the value of Δx was 0.2cm. This gives a value of $A/\Delta x$ per 100g tissue of approximately 400 cm. Based on more recent data (3), this value may be too low and a more conservative value of 1500 cm is calculated in the appendix.

It has been suggested above that the properties necessary to explain both the dynamic and steady-state plasma-to-lymph transport data for dextrans and proteins can be found in the interstitial matrix. This raises questions which are the opposite in emphasis to the question at the start of this paper: how much does the capillary wall contribute to the total resistance to transport? Is there evidence that these properties are also found in the capillary wall? To answer these, data are needed on D' and $A/\Delta x$ for the capillary wall which were obtained in a manner which excluded possible interference from the surrounding matrix. It might be thought that the single capillary data from the frog mesentery is immediately applicable, but this must be used with care because even mesenteric capillaries are embedded in a matrix of collagen and ground substance (W.G. Frasher, Jr., personal communication).

Small molecule movement at the capillary wall however, should be relatively insensitive to an extravascular matrix with an equivalent pore

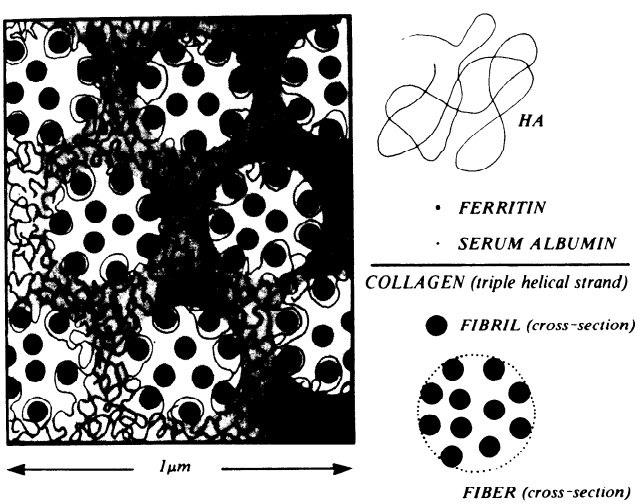


Figure 2. Diagram of Wharton's Jelly, ref. 22.

radius of 50 A. From a measured potassium permeability value of 67 X 10^{-5} cm /sec in a single capillary and the free diffusion coefficient for that ion, Crone (6) calculated that the pore area available for transcapillary transport was only 0.3% of the capillary surface area, which suggests there might be a large resistance to diffusion. If we assume 7000 sq. cm. of capillary per 100g of tissue (17), then for potassium, the resistance to diffusion $\Delta x/D'A = 1/(7000 \times 67 \times 10^{-5}) = 0.21$ sec/cm. The corresponding figure for the interstitial matrix is $1/(400 \times 1.8 \times 10^{-5}) = 139$ sec/cm, or 37 sec/cm for the more conservative value of $A/\Delta x$. These figures suggest that when the equilibration of the whole tissue is considered, the resistance of the capillary wall to small molecules is very much less than that of the interstitial space. Even if the conservative estimate of $A/\Delta x$ for the interstitium were out by a factor of 100, the resistance there would still be larger than that at the capillary wall. The large difference in he $A/\Delta x$ values comes mainly from the Δx contribution, being 10^{-4} cm for the capillary wall and 0.2 cm for the interstitial space. The latter result is a direct consequence of Garlick and Renkin's finding a 40 minute time constant for sucrose equilibration in lymph.

The second consideration is whether restricted diffusion ($D' < D$) occurs at the capillary wall for larger solutes. By assuming the resistance to water flow resides in the capillary wall, and combining the filtration coefficient data with the permeability data, Crone calculates that the capillary pore radius is approximately 110 Å. If the interstitial matrix presents any resistance to water flow at all, and it has been suggested that this might be considerable (11), then the value for the pore radius must rise. Hence the 110 Å value probably is a lower limit of pore radius, which implies there is little or no restricted diffusion to molecules of physiological interest, including serum albumin. Curry has similar permeability data obtained by a different technique (7). To the author's knowledge there are no experiments demonstrating restriction to diffusion where the observed restriction could only be ascribed to the capillary wall.

The data presented suggests that the interstitial matrix may be the only site of the restricted diffusion of large molecules, and is the principal resistance to movement between plasma and lymph. This of course is the exact opposite of the conventional view. The conclusion concerning the site of restricted diffusion is very dependent on the Fox and Wayland data, and their results should be verified independently. The resistance to diffusion conclusions depend strongly upon Garlick and Renkin's data on the slow equilibration of sucrose. Kruhoffer (12) and Watson, Bell and Renkin (unpublished) found similar results. It should be pointed out that much of the existing data are consistent with either view, and cannot be used to distinguish between them.

If the new hypothesis is correct, then two other observations which have given difficulty may be explained. The single-injection method of measuring capillary permeability gives results which do not show restricted diffusion (1,6). In this method the solutes do not have time (< 30 seconds) to penetrate far into the tissue, so the method will mainly measure the influence of the capillary wall. The absence of restricted diffusion in the single-injection method data is consistent then with the non-restricting capillary model.

The second observation was made by Pappenheimer, Renkin and Borrero (17). They increased the plasma colloid osmotic pressure stepwise, by the addition of various proteins to the perfusate of an isolated hindlimb. Instead of the expected sudden rise in isogravimetric capillary pressure, they found that it took approximately 20 minutes for the protein to have its full osmotic effect, $2\frac{1}{2}$ times longer than the tissue washout time. If the interstitial matrix were the site of the restriction to diffusion, it would also be the site of the osmotic action of the plasma proteins. To reach the osmotic barrier the added protein has to travel through the capillary wall to reach the matrix, and this will take time. Pappenheimer's observations, previously unexplained, are consistent with the restricting matrix hypothesis.

A new hypothesis has been presented in which the interstitial matrix is the principal resistance to diffusion, the site of restricted diffusion and the osmotic barrier. The hypothesis is consistent with all the transport data to which it has been compared, and it can explain observations that are inconsistent with the conventional view. There are however some regions of importance which have not been explored in detail. These include water balance and the distribution of flow resistance. No reason is apparent why the hypothesis, or a modification of it, could not be consistent with the water movement observations.

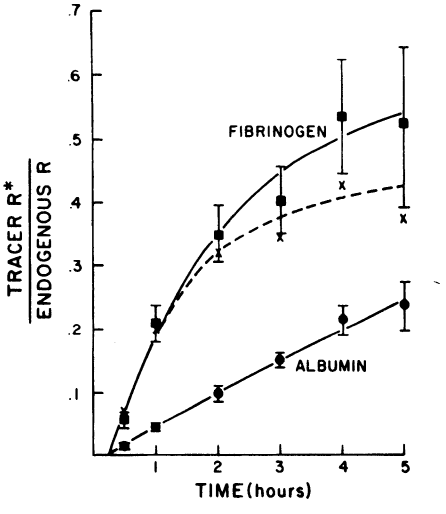


Figure 3. The solutes are equilibrated when TRACER R*/ENDOGENOUS R equals one. The dashed line represents the fibrinogen data corrected for contaminants.

APPENDIX

The values of A ($A = A_F + A_G$) in table 6 of ref (19) were obtained by choosing Δx and calculating A from $V = A \times \Delta x$, where V = 4 ml. The value of Δx was adjusted until a sucrose time constant of 40 minutes was obtained. More recent data of albumin and fibrinogen equilibration (19) suggest that the dextrans were not in steady-state as Garlick and Renkin supposed. This suggests that the interstitial volume of 4 ml, which was calculated from the dextran equilibration, may be too small. This could significantly affect the value of $A/\Delta x$.

Bell, Watson and Renkin (3) measured a sucrose volume of approximately 12 ml per paw in the same preparation using dogs of approximately twice the weight of those of Garlick and Renkin. Using 12 ml as the upper limit, a new value of $A/\Delta x$ may be estimated as follows.

The time constant of equilibration for a well-mixed volume V, of cross-sectional area A and length Δx, drained by lymph flow rate L is given by

$$\tau = \frac{V}{\frac{DA}{\Delta x/2} + L}$$

Using the values from table 6, ref (19), τ = 45 mins., which is close to the 40 minute value obtained from the distributed model. Using this equation and $A = V/\Delta x$, values of A and Δx may be calculated for different values of V. When V = 12 ml, Δx = .18 cm and A = 67 sg cm, giving an $A/\Delta x$ of 370 cm per 24g of tissue, or approximately 1500 cm per 100 g. This is still very much less than $A/\Delta x$ for the capillary wall.

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IS THE TRANSPORT OF HYDROPHILIC SUBSTANCES ACROSS THE CAPILLARY WALL DETERMINED BY A NETWORK OF FIBROUS MOLECULES?

Fitz-Roy E. Curry
Department of Human Physiology
School of Medicine
University of California
Davis, CA 95616

The striking similarity between the ultrafiltration properties of collodion membranes and the walls of capillary blood vessels gave rise to the pore theory of capillary permeability. Pappenheimer and his colleagues introduced the idea of an equivalent pore radius to describe the frictional resistance on water and hydrophilic solutes within capillary and artificial ultrafiltration membranes (Fig. 1A) (1,2). The solute-membrane interaction is described in terms of the steric exclusion of the solute by the pore entrance and the increased drag on a spherical molecule as it moves within a cylindrical pore or rectangular slit (see 3 for review). The idea is similar to the use of an Einstein-Stokes radius to characterize free diffusion. Physicochemical investigations have established the relation between the latter quantity and the molecular weight and molecular structure of a freely diffusing solute, but we are still at a rather primitive stage in our understanding of the relationship between the magnitude of the equivalent pore radius and the physical and chemical nature of the transcapillary pathway.

The size limiting structures within transcapillary channels are now believed to be molecular networks within the pathways identified by conventional electron microscopic fixation and staining procedures. Both Palade et al (4) and Wissig (5) emphasize this point, even though the question concerning the actual site of the channels (specialized intercellular junctions or chains of vesicles) remains unresolved.

One clue to the nature of one of the structures determining normal capillary permeability is the observation that the perfusion of capillaries with solutions completely free of protein leads to a large increase in capillary permeability (6). In frog mesenteric capillaries we have shown that the effect on hydraulic conductivity can be rapidly reversed by the addition to the capillary perfusate of albumin, γ globulin or hemoglobin (7). We also examined the ultrastructure of capillaries perfused without protein and could find no conspicuous abnormalities (8). We therefore concluded that plasma proteins combine reversibly with some electron lucent component of the capillary wall to increase the frictional resistance to the transcapillary exchange of hydrophilic molecules.

Recently, evidence that ferritin molecules are excluded from the luminal vesicles and the luminal surface of the endothelial cells has been presented by Michel and his colleagues. On the basis of this finding, Michel has reintroduced the idea that the molecular sieving properties of the capillary wall may reside in an endocapillary layer rather than in the dimensions of channels through or between the cells (9,10). It is suggested that the endocapillary layer is a three dimensional network formed by the fibrous chains of the glycoproteins which are part of the plasma membrane of the endothelial cells (Figure 1B). The latter may be identified with the ruthenium red staining layer described by Luft (11). The coat not only appears to line the luminal surface of the capillary but also appears to fill the wide regions of the intercellular cleft and to merge with the basal laminae. It is found covering the fenestrae when these are present and can also be seen lining the cytoplasmic vesicles (12).

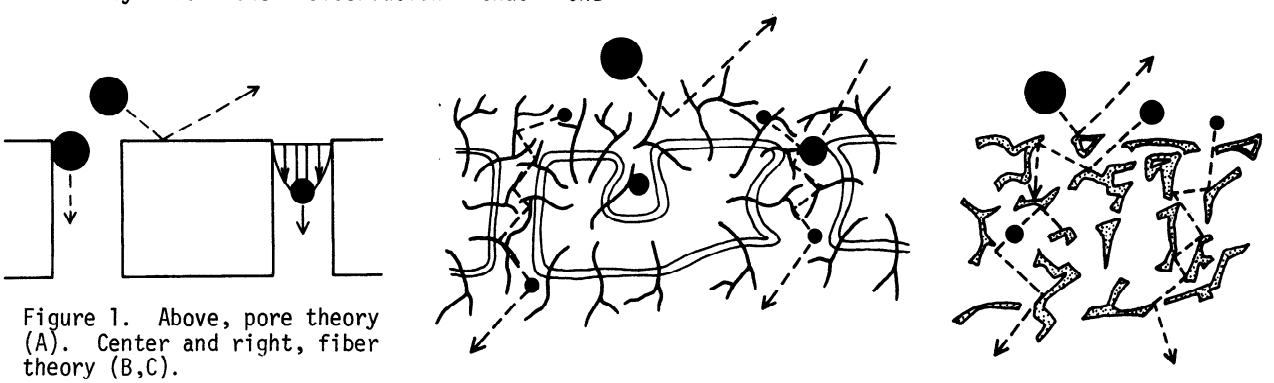


Figure 1. Above, pore theory (A). Center and right, fiber theory (B,C).

In this paper I will use a quantitative description of transport through such a network to examine the permeability and sieving properties of both the capillary wall and cellulose membranes.

Theory

The analysis of the transport of water and solutes through the porous region of the membrane requires expressions for the hydraulic conductivity, Lp, the permeability, P, and the osmotic reflection coefficient, σ, of the membrane. No systematic description of the coefficients in terms of the radius of the fibers, r_f, and the length of fiber per unit volume of the network, ℓ, has been available. Recently I have collaborated with Dr. C.C. Michel to develop a fiber matrix theory of capillary permeability as summarized below.

The porous region of the capillary has an area Ap and a thickness ΔX. Ogston's equations for the exclusion (13) and diffusion (14) of a spherical solute in a fibrous network were combined to give an expression for the permeability coefficient in a fibrous membrane:

$$P = \frac{Ap}{\Delta X} \exp-(1-\varepsilon)\left(\frac{2a}{r_f}+\left(\frac{a}{r_f}\right)^2\right)D_o \exp-(1-\varepsilon)^{0.5}\left(1+\frac{a}{r_f}\right) \quad (1)$$

ε is the void volume equal to $(1-\pi r_f^2 \ell)$. The first exponential term is the membrane partition coefficient, represented in equations below by ϕ. The second describes the fractional reduction in the free diffusion coefficient D_o.

An expression for the osmotic reflection coefficient is also derived from the partition coefficient:

$$\sigma = (1 - \phi)^2 . \quad (2)$$

Anderson and Malone (15) first derived the above relation for a cylindrical pore but recent theoretical and experimental studies (Anderson, personal communication) demonstrate a much more general application.

Sullivan and Hertel (16) confirmed the application of the Carman-Kozeny equation to the flow of water through random arrays of macroscopic fibers. The corresponding expression for the hydraulic conductivity is:

$$L_p = \frac{A_p}{\Delta X} \cdot \eta \frac{\varepsilon^3}{(1-\varepsilon)^2} \frac{r_f^2}{4K} \quad (3)$$

η is the viscosity and K is a parameter dependent upon the fiber geometry. For random arrays of fibers with void volumes up to 0.95, Sullivan and Hertel found K to lie within the limits of 5.0 to 6.0, but to increase rapidly in magnitude as $\varepsilon \to 1$.

Application of the fiber matrix theory to cellulose membranes.

The remarkable electron micrographs obtained using the metal shadowing technique of Helmcke in the early 50's showed the structure of cellulose membranes as a network of coarse interconnecting rods below a surface "skin" penetrated by irregular openings (17). Helmcke's representation of the mechanism of permeation of a solute through such a membrane is shown in Fig. 1C; the selectivity of the membrane is determined by the statistics of solute interactions with the membrane network. Although modern scanning electron micrographs confirm this picture, the finest details of the membrane structure still lie below the limits of resolution of present instruments. Nevertheless, it would seem that the structure determining the transport properties of the cellulose membranes studied by Renkin (2) and Durbin (18) is more closely described as a network of interconnected cellulose fibers than as cylindrical pores penetrating an otherwise impermeable barrier. I have attempted to describe the permeability coefficients of these membranes in terms of the fiber matrix theory.

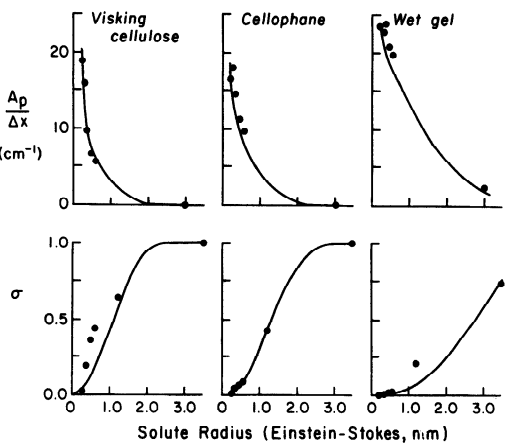


Figure 2 above shows the permeability coefficients (Renkin) and osmotic reflection coefficients (Durbin) of Visking cellulose (ε=0.89), cellophane (ε=0.93) and wet gel (ε=0.98) plotted as a function of the molecular size of the test solute. Permeability coefficients are expressed as the apparent area for diffusion equal to P/Do. The solid lines are calculated using the fiber matrix theory, Eqns. 1 and 2. The fiber radius in all calculations is 0.4 nm, as measured for cellulose derivatives in sedimentation experiments (14). For each membrane, the value of the void volume, ε, which best described the measured membrane filtration coefficient (Eqn. 3) was first calculated, then substituted into Eqns. 1 and 2. There is no simple relation between the calculated magnitude of ε and the measured water content of the membrane; an indication of the gross heterogeneity of the membrane structure. Several features of the relations are note worthy:

(i). The value of ε calculated from the

expression for L_p provides a consistent description of the membrane permeability and osmotic reflection coefficients in each membrane.
(ii). Values of ε and r_f predict an upper limit to the permeability of the membrane which is consistent with the observed limit of permeability for each membrane.
(iii). The sigmoidal shape of the relation between the reflection coefficient and the molecular size, described by modern revisions of the hydrodynamic pore theory, is preserved. Furthermore, the fiber matrix model describes the osmotic reflection coefficients for solutes with radius larger than 1 nm more closely than the hydrodynamic pore theory which underestimates these coefficients (3). Although further studies on the correlation between structure and function of membranes manufactured from fibrous materials is required, this first attempt to test the fiber model provides an encouraging basis from which to examine Michel's hypothesis that a network of glycoproteins determines the transport properties of the capillary wall.

The Permeability of the Capillary Wall.

Before examining the fiber matrix theory, I would like to review some recent applications of the pore theory to capillary permeability.

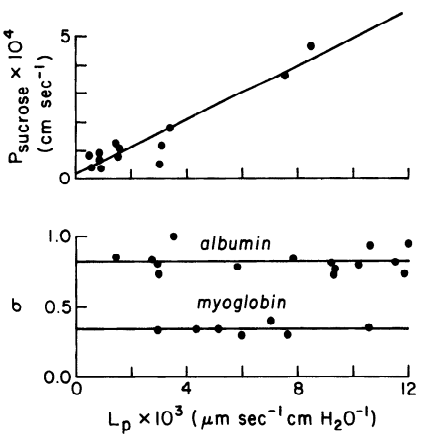
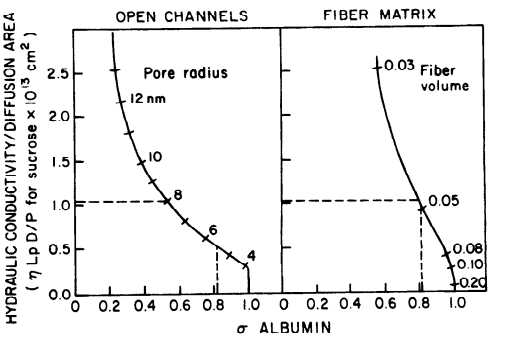


Figure 3 above summarizes measurements of the filtration coefficients, permeability coefficients (19) and osmotic reflection coefficients (20) in individually perfused capillaries of the frog mesentery. Additional o data was kindly provided by Dr. C.C. Michel. The sample includes some arteriolar and venous capillaries. Over a tenfold range of hydraulic conductivities, the porosity of the capillary wall appears to be relatively constant when assessed either as the osmotic reflection coefficient to albumin and myoglobin or as the constant proportion between a change in the hydraulic conductivity of a capillary and the corresponding change in sucrose permeability.

The relation between the ratio $L_p/P_{sucrose}$ and the albumin osmotic reflection coefficient calculated from the pore and fiber matrix theories is given by the solid lines in Fig. 4. In part A the parameters on the curve are the pore sizes; in B, fractional fiber volumes $(1-\varepsilon)$.

Figure 4A 4B



When the pore theory is used to evaluate the porosity from Fig. 4A a discrepancy is found: the equivalent pore radius calculated from the albumin (and myoglobin) reflection coefficients is 5.5 nm, whereas that calculated from the $L_p/P_{sucrose}$ ratio is 8 nm. An even larger figure (11 nm) was calculated from the ratio of L_p/P for potassium ions by Crone et al (21). It is unlikely that the discrepancy can be accounted for in terms of the charge on the macro molecules since the isoelectric point of albumin is 5.16 whereas that for myoglobin is 7.8. These results also indicate structures in the capillary whose frictional resistance to the movement of water and solutes is different from that of a cylindrical pore.

A fiber radius of 0.6 nm, the radius of fibers in a sulphated proteoglycan (14) was used to construct the curve in Fig. 4B. Both the $L_p/P_{sucrose}$ and the albumin reflection coefficients are consistent with a fiber volume of 5%. The myoglobin σ calculated from these network parameters is 0.31, also in agreement with the measured value. The same values of r_f and ε may be used to calculate permeability coefficients for potassium and sodium chloride which closely match published values (19,21). Experimental values for the $L_p/P_{sucrose}$ ratio and σ albumin from mammalian skeletal muscle and heart capillaries may also be described using a fiber radius of 0.6 nm and a fiber volume of 7-8%. The diffusion of all solutes is restricted.

Since we know so little about the molecular structure of the endothelial surface coats (see for example 22), I have explored the predictions of the fiber matrix model using other fiber radii. As r_f is increased, the solid curve in Fig. 4B tilts to the left, and a discrepancy similar to that described for the pore theory is apparent for fiber radii greater than 1 nm. Within the limitations of our present theory, it seems that only fiber radii less than 1 nm can account for the observed permeability properties of the capillary wall to molecules up to the size of albumin. It is also difficult to

imagine a mechanism which will change r_f; changes in c due to the making and breaking of cross links between fibers or the swelling and shrinkage of the fiber network are more likely. Such a mechanism may account for the effect of absorbed plasma proteins on capillary permeability. For example, the hydraulic conductivity of hindlimb capillaries increases approximately threefold when proteins are removed. The 30% increase in pore radius which would account for the increased L_p is too small to describe the large reduction in the osmotic effectiveness of clinical dextran in this preparation in the absence of plasma proteins (8). On the other hand, a halving of the fiber volume from 7 to 3% accounts for a threefold increase in L_p and a reduction of the reflection coefficient of a solute the size of albumin from 0.9 to close to 0.5.

SUMMARY

The hypothesis that the permeability properties of the capillary wall to hydrophilic solutes are determined by a network of fibrous molecules has been investigated using the fiber matrix theory. A network composed of fibers 0.6 nm in radius and occupying less than 8% of the network volume has permeability properties which conform to those measured for the continuous capillaries of frog mesentery and mammalian skeletal and heart capillaries. An independent test of the theory using cellulose membranes was made. Further experiments to measure L_p, σ and P in capillaries perfused with and without proteins in the perfusate may elucidate a mechanism for maintaining normal capillary permeability.

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