

The Harvey Lectures

Delivered under the auspices of
THE HARVEY SOCIETY
of New York, 1963-1964

SERIES 59

THE HARVEY LECTURES

DELIVERED UNDER THE AUSPICES OF
The HARVEY SOCIETY of NEW YORK

1963-1964

UNDER THE PATRONAGE OF THE NEW YORK
ACADEMY OF MEDICINE

BY

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SERIES 59

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THE HARVEY SOCIETY*

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I

This Society shall be named the Harvey Society.

II

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither Active nor Associate members. Associate and Honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to

* The Harvey Society was founded in 1905 and incorporated in the State of New York in 1955 as the Harvey Society, Inc. The Constitution is reprinted here for historical interest; its essential features have been included in the Articles of Incorporation and By-Laws.

the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

IV

The management of the Society shall be vested in an Executive Committee to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V

The Annual Meeting of the Society shall be held at a stated date in January of each year at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all meetings ten members shall constitute a quorum.

VI

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification to the members in writing.

THE HARVEY SOCIETY INC.

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

BY-LAWS

ARTICLE I

Purposes of the Society

SECTION 1. The purposes for which this Society is formed are those set forth in its Certificate of Incorporation as from time to time amended. It is not organized for pecuniary profit, and no part of the net earnings, contributions or other corporate funds of the Society, shall inure to the benefit of any private member or individual, and no substantial part of its activities shall be carrying on propaganda, or otherwise attempting, to influence legislation.

ARTICLE II

Offices of the Society

SECTION 1. The main office and place of business of the Society shall be in the City and County of New York. The Board of Directors may designate additional offices.

ARTICLE III

Members

SECTION 1. The members of the Society shall consist of the incorporators, members of the hitherto unincorporated Harvey Society, and persons elected from time to time. The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing or carrying on a major part of their work in the New York area at the time of

their election, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing or carrying on a major part of their work in the New York area at the time of their election. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered a lecture before the Society and who are neither Active nor Associate members. Associate and Honorary members shall not be eligible to office, nor shall they be entitled to participate by voting in the affairs of the Society. New active and Associate members shall be nominated in writing to the Board of Directors by an Active or Associate member and seconded by another Active or Associate member. They shall be elected at the Annual Meeting of the Society by a vote of the majority of the Active members present at the meeting. Active and Associate members who have remained in good standing for 35 years shall be designated Life members. They shall retain all the privileges of their class of membership without further payment of dues. Membership in the Society shall terminate on the death, resignation or removal of the member.

SECTION 2. Members may be suspended or expelled from the Society by the vote of a majority of the members present at any meeting of members at which a quorum is present, for refusing or failing to comply with the By-Laws, or for other good and sufficient cause.

SECTION 3. Members may resign from the Society by written declaration, which shall take effect upon the filing thereof with the Secretary.

ARTICLE IV

Meetings of the Members of the Society

SECTION 1. The Society shall hold its annual meeting of Active members for the election of officers and directors, and for the transaction of such other business as may come before the meeting in the month of January or February in each year, at a place within the City of New York, and on a date and at an hour to be specified in the notice of such meeting.

SECTION 2. Special meetings of members shall be called by the Secretary upon the request of the President or Vice-President or of the Board of Directors, or on written request of one-third of the Active members.

SECTION 3. Notice of all meetings of Active members shall be mailed or delivered personally to each member not less than ten nor more than sixty days before the meeting.

SECTION 4. At all meetings of Active members of the Society ten Active members, present in person, shall constitute a quorum, but less than a quorum shall have power to adjourn from time to time until a quorum be present.

ARTICLE V

Board of Directors

SECTION 1. The number of directors shall be seven, but such number may be increased or reduced, by amendment of the By-laws as hereinafter provided, within the maximum and minimum numbers fixed in the Certificate of Incorporation of any amendment thereto.

SECTION 2. The Board of Directors shall hold an annual meeting shortly after the annual meeting of the Society.

Special meetings of the Board of Directors shall be called at any time by the Secretary upon the request of the President or Vice-President or of one-fourth of the directors then in office.

SECTION 3. Notice of all regular and special meetings of the Board shall be given to each director at least one day before the meeting. Meetings may be held at any place within the City of New York designated in the notice of the meeting.

SECTION 4. The Board of Directors shall have and may exercise full power in the management and control of the business and affairs of the Society.

SECTION 5. Directors shall be elected by the members of the Society at the Annual Meeting or at special meetings and shall hold office until the next annual meeting and until their successors shall be elected. Vacancies occurring in the Board for any cause (including increase in the number of directors) may also be filled for the unexpired term by the majority vote of the directors

present at any meeting at which a quorum is present. Only Active members of the Society shall be eligible for membership on the Board of Directors.

SECTION 6. A majority of the Board as from time to time constituted shall be necessary to constitute a quorum, but less than a quorum shall have power to adjourn from time to time until a quorum be present.

SECTION 7. The Board shall have power to appoint individual or corporate trustees and their successors of any or all of the property of the Society, and to confer upon them such of the powers, duties or obligations of the directors in relation to the care, custody or management of such property as may be deemed advisable.

SECTION 8. The directors shall present at the annual meeting a report, verified by the President and Treasurer, or by a majority of the directors, showing the whole amount of real and personal property owned by the Society, where located, and where and how invested, the amount and nature of the property acquired during the year immediately preceding the date of the report and the manner of the acquisition; the amount applied, appropriated or expended during the year immediately preceding such date, and the purposes, objects or persons to or for which such applications, appropriations or expenditures have been made; and the names of the persons who have been admitted to membership in the Society during such year, which report shall be filed with the records of the Society and an abstract thereof entered in the minutes of the proceedings of the annual meeting.

ARTICLE VI

Committees

SECTION 1. There may be an Executive Committee consisting of three of the members of the Board of Directors to be elected by its Board of Directors at its annual meeting. The President shall, ex officio, be the Chairman of the Executive Committee.

SECTION 2. The Executive Committee shall fix its own rules of procedure. Meetings of the Executive Committee shall be held at

such times and places as it may determine. Notice of all meetings shall be given to each member of the Executive Committee at least one day before the meeting. A quorum for the transaction of the business of the Executive Committee shall consist of one-third of its members, but in no case fewer than two, then in office.

SECTION 3. Subject to the direction of the Board of Directors, the Executive Committee shall have the immediate charge, management and control of the activities and affairs of the Society, and it shall have full power, in the intervals between the meetings of the Board of Directors, to do any and all things in relation to the affairs of the Society and to exercise any powers of the Board of Directors.

SECTION 4. The Board of Directors or the Executive Committee may appoint from time to time such other committees, as the Board of Directors or the Executive Committee may deem advisable, and each such committee shall exercise such powers and perform such duties as may be conferred upon it by the Board of Directors or the Executive Committee, subject to the continuing direction and control of the Board of Directors and the Executive Committee.

ARTICLE VII

Officers

SECTION 1. The officers of the Society shall consist of a President, a Vice-President, a Secretary and a Treasurer, and such other officers as the Board of Directors may from time to time determine. All of the officers of the Society shall be members of the Board of Directors.

SECTION 2. The President shall be the chief executive officer of the Society and shall be in charge of the direction of its affairs. The other officers of the Society shall have the powers and perform the duties that usually pertain to their respective offices, or as may from time to time be prescribed by the Board of Directors.

SECTION 3. The officers and the directors shall not receive, directly or indirectly, any salary or other compensation from the Society, unless authorized by the concurring vote of two-thirds of all the directors.

SECTION 4. The officers shall be elected at the annual meeting of the Active members. All officers shall hold office until the next annual meeting and until their successors are elected or until removed by vote of a majority of all the directors. Officers must be Active members of the Society.

ARTICLE VIII

Fiscal Year—Seal

SECTION 1. The fiscal year of the Society shall be the calendar year.

SECTION 2. The seal of the Society shall be circular in form and shall bear the words "The Harvey Society, New York, Corporate Seal."

ARTICLE IX

Amendments

SECTION 1. These By-laws may be added to, amended or repealed, in whole or in part, by the Active members or by the Board of Directors, in each case by a majority vote at any meeting at which a quorum is present, provided that notice of the proposed addition, amendment or repeal has been given to each member or director, as the case may be, in the notice of such meeting.

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TRANSPORT OF ELECTROLYTES AND WATER ACROSS EPITHELIA*

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I. INTRODUCTION

AMONG the most intriguing properties of living organisms, as well as of the individual cells of which they are composed, is that of being able to take up and excrete a variety of substances in a highly selective way. When these processes are analyzed it turns out that some of the transport phenomena can be explained in terms of well known physical processes like diffusion, osmosis, distribution according to electrical potentials, etc. There remain, however, a certain number of cases where the transport systems seem to proceed in a manner distinctly different from that predicted on the basis of the observable physical forces. We must then assume that chemical reactions in the cells provide the energy necessary, and we use the term active transport for these processes. So far, to my knowledge in no case of active transport has the molecular mechanism been fully clarified. One might, therefore, be justified in asking what is gained by using the term. As far as I can see the advantages are of two kinds. First, the singling out of certain transport systems as being active may help focus the interest of biochemists upon chemical events in the cells which deserve further study. But secondly, once the active transport systems have been singled out, the remaining transport phenomena may be described in a coherent way according to known physical laws.

Thus the concept of active sodium transport in the cases, for instance, of nerve and muscle, has led to a unified theory for the origin of resting potentials, whereas, in the cases of several epithelial tissues, it has led to an explanation of net electrolyte

* Lecture delivered September 19, 1963.

and water transfer as well as accounting for the transmembrane potentials. But no chain is stronger than its weakest link. How good is the evidence that the active transport of sodium really exists? Can the observed facts be explained in any other way? Let us consider the distribution of potassium and sodium between a body cell and its surrounding extracellular fluid. Typically, the cellular potassium concentration is many times that of the surrounding medium, whereas the cellular sodium is present in a much lower concentration than outside. Obviously, one might ascribe this to a specific binding of potassium and a specifically low "solubility" for sodium in the cytoplasm, although such a view is hard to reconcile with the measured intracellular potentials which show that the intracellular potassium is usually close to Donnan equilibrium with that of the outside. However, the interior of most cells, as we are coming to know it from electron-microscopic pictures, is certainly so complex that the concept of a uniform concentration of readily diffusible potassium may be a conclusion that has to be accepted, although with some astonishment, but it is hardly the firm ground on which the theory of active sodium transport can rest. More direct evidence for the existence of active transport is obtained from the study of organs like intestine, kidney tubules, amphibian skin, in which massive amounts of electrolytes undergo unidirectional transport. In these cases the transport takes place between dilute watery solutions, for which good estimates of activity coefficients can be obtained. Furthermore, whereas the ion transport necessary to maintain the electrolyte composition of, for instance, a muscle fiber, is likely to consume a very small fraction of its total metabolism, it stands to reason that the large amounts of electrolytes transported across certain epithelia must consume a sizable fraction of their energy output. Reasons of this type induced our group in the years just after the war to try to develop methods for distinguishing between active and passive transport through epithelial organs. After some shopping around, the isolated surviving frog skin was chosen as the "testing ground" for the methods. Although, today, much is known about the active sodium transport of other organs, the frog skin is probably better known in this respect than any other tissue. It may, therefore, be appropriate to discuss at some length the

evidence on which we base the conclusion that the sodium transport in the frog skin is active.

II. THE ISOLATED FROG SKIN AS AN EXPERIMENTAL OBJECT

For more than a century the frog skin has been one of the favorite objects of electrophysiologists. As long ago as 1848, Du Bois-Reymond observed that it maintained an electrical potential difference between the inside and the outside, the inside normally being positive relative to the outside. In 1904, Galeotti demonstrated that sodium (or lithium) ions were necessary for the maintenance of the potential. In order to explain his finding, he postulated that the diffusion coefficients for these two ions were greater for the direction from the outside inward than in the opposite direction. Although this explanation was ridiculed at the time, since it seemed to violate the second law of thermodynamics, he was, in a way, not far from the truth. The net electrolyte transport of the frog skin was first observed by Huf (1935), who noticed that a frog skin bathed with Ringer solution on both sides will transport chloride from the outside in. Although he did not analyze for sodium, Huf assumed the process to be an active transport of sodium chloride. Shortly afterward, Krogh (1937, 1938) found that salt-depleted frogs would take up chloride and sodium ions through the skin even from solutions as dilute as $10^{-5} M$ with respect to NaCl. In our preliminary experiments (Ussing, 1949a) with isolated frog skins we could demonstrate that the isolated frog skins were able to transport sodium against a steep concentration gradient. Thus even when the inside medium was Ringer and the outside medium $\frac{1}{100}$ Ringer, the inward sodium flux (as measured with Na^{24}) was greater than the outward flux. Furthermore, these experiments showed that the transport of chloride ions inward might be a consequence of the electric potential difference. Calculations showed that the potential was sufficient to raise the electrochemical potential of the chloride ions of the outside solution over that of the inside medium, viz., $\bar{\mu}_{\text{Cl}(o)} > \bar{\mu}_{\text{Cl}(i)}$. For the sodium ion, however, we obtained $\bar{\mu}_{\text{Na}(o)} \ll \bar{\mu}_{\text{Na}(i)}$. In plain words this meant that the sodium ion had to overcome the combined effects of the concentration gradient and the electrical potential difference, both of which oppose its inward transport. Thus ac-

according to the criterion of Rosenberg (1954): "Active transport is a transport against the electrochemical potential," the sodium transport must be active.

One might argue, however, that the active process was a transfer of, for example, ion pairs of sodium chloride. If so, it might not be meaningful to consider the transport of one ion as active and the other as passive. Therefore it was necessary to develop methods for demonstrating that sodium and chloride move independently through the frog skin.

III. THE FLUX RATIO EQUATION

The net transport of sodium and that of chloride under most conditions are usually of the same order of magnitude and so small that their accurate determination is difficult. The kinetics of the ionic fluxes, as determined with tracers, seemed more promising. In the case of a multilayer membrane like an epithelium, the unidirectional isotopic fluxes can be predicted to be very involved functions of the membrane structure as well as of the ionic concentrations and electric potentials of the bathing solutions. It can be shown, however, that for an ionic species that does not interact with other moving particles, the flux ratio (the ratio between the simultaneously determined inward and outward fluxes) is independent of the membrane structure, being determined solely by the difference between its electrochemical potentials in the bathing solutions.

Thus we have for a passively diffusing ion

$$RT \ln (M_{in}/M_{out}) = \bar{\mu}_o - \bar{\mu}_i$$

or, disregarding the activity coefficients

$$M_{in}/M_{out} = (c_o/c_i)e^{(EF/RT)}$$

(Ussing, 1949b; Teorell, 1949), where M_{in} is the inward flux; M_{out} , the outward flux; E , the skin potential; F , the Faraday; R , the gas constant; and T , the absolute temperature.

Clearly this equation describes an idealized situation since the complete absence of interaction with other moving particles is impossible. Nevertheless, the above simple flux ratio equation de-