PERMEABILITY CHARACTERISTICS AND ION SELECTIVITY IN THE BASAL LAMELLA OF *ASCARIS SUUM*

By

MANUS JOSEPH DONAHUE

Bachelor of Science in Biology
Villanova University
Villanova, Pennsylvania
May, 1968

Master of Science
North Texas State University
Denton, Texas
August, 1974

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of the Oklahoma State University
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Thesis Approved:

Calvin G. Beames, Jr.
Thesis Adviser

John R. Sauer

David P. Jennings

Richard L. Essenberg

Norman N. Durham
Dean of the Graduate College

What a scientist does is compounded of two interests: the interest of his time and his own interest. In this his behavior is no different from any other man's. The need of the age gives its shape to scientific progress as a whole. But it is not the need of the age which gives the individual scientist his sense of pleasure and of adventure, and that excitement which keeps him working late into the night when all the useful typists have gone home at five o'clock. He is personally involved in his work, as the poet is in his, and as the artist is in the painting. Paints and painting too must have been made for useful ends; and language was developed, from whatever beginnings, for practical communication. Yet you cannot have a man handle paints or language or the symbolic concepts of physics, you cannot even have him stain a microscope slide, without instantly waking in him a pleasure in the very language, a sense of exploring his own activity. This sense lies at the heart of creation.

Jacob Bronowski
Science and Human Values

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Even in the best of times our days are numbered; how fortunate then, to spend three years in an enjoyable quest which lay "at the heart of creation." These years were made enjoyable because all the faculty members and all the graduate students in the department made themselves available for constructive analytical seminars as well as social relaxing seminars.

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CHAPTER I

INTRODUCTION

Ascaris suum and Ascaris lumbricoides are parasitic nematodes which inhabit the upper small intestine of swine and man, respectively. The adult female worm is approximately 25 cm long and its intestine is essentially a straight tube which runs the length of the organism. This intestine can be dissected easily from the worm. Using light microscopic techniques, the intestine is found to contain tall columnar cells resting on a thick basal lamella, i.e., basement membrane. The membrane can be freed from the epithelial cells by ultrasonic treatment and is found to be relatively tough and easy to manipulate.

Basement membranes, in general, are extracellular which function as ubiquitous support structures for all epithelial and endothelial cells (54). Recently, they have been thought to function as barriers to the passive diffusion of electrolytes and non-electrolytes. The intestinal basement membrane of Ascaris suum is considered as a supportive structure for the columnar epithelial cells which rest upon it (43). In the nutrition of the worm the foodstuffs which are absorbed by these same epithelial cells find the basement membrane as a barrier to their passage into the pseudocoelomic fluid of the worm.

Previous investigators [Beames (3), Merz (35), Castro (7), and von Brand (62)] have been involved with the elucidation of the mechanisms by which carbohydrates and fatty acids are absorbed by the intestine of

Ascaris. The movement of carbohydrates and fatty acids from the luminal to the pseudocoelomic side of the intestine has been investigated with in vitro sac preparations and radioactively labelled substrates (3) and by short circuit current techniques (35). Since it is known that the foodstuffs are absorbed by the intestine of Ascaris and that these same foodstuffs are found in the pseudocoelomic cavity, it is obvious that these materials pass through the basement membrane.

The biochemical nature of this basement membrane has been investigated. Chemically it is a glycoprotein containing 91% amino acids and 4.9% carbohydrate (43). It was further discovered that this basement membrane is dissimilar from those basement membranes of vertebrates in that it contained no identifiable strands of collagen fibrils, no detectable sialic acid and no phosphorus containing compounds. Collectively, this biochemical evidence and the ultrastructural work of Peczon, et al. (43) indicates that the basement membrane is a fine felt work with no collagen fibrils; it may have a net positive charge because of the lack of detectable sialic acid; and finally, movement through this basement membrane most probably is by passive diffusion since no adenosine triphosphate (ATP) was detected for an active transport process to occur.

To gain more insight into other possible means of transport of solutes through the membrane it was thought that a more complete understanding of the physical characteristics of the basement membrane was needed. Physical characteristics such as, the average pore radius of the membrane, the filtration coefficient, the reflection coefficient, the apparent solute permeability coefficient, and the net ionic charge in the pores of the membrane would allow characterization of the basement membrane as a diffusion and ion selective barrier. The elucidation of

these permeability characteristics of the intestinal basement membrane of *Ascaris* is the concern of this thesis.

CHAPTER II

LITERATURE REVIEW

Background

Ascaris suum is a relatively large nematode. The adult males range from 10 to 31 cm and the adult females range from 22 to 35 cm. They have a smooth, relatively perfect cylindrical shape of the body, finely striated cutical, and conical anterior and posterior extremities. Ventrally these are curved papillated posterior extremities in the male with two spicules. Both males and females have a terminal mouth with three oval lips. There are paired reproductive organs in the posterior two-thirds of the female and a single long tube in the male (5).

The species is essentially a tube within a tube. The outer tube, the cutical, consists of an outer keratinized cortex. Beneath the cortex is a thick matrix layer, the outer part of which contains branching canals that extend into the cortex. The basal layer is composed of three strata of collagen fibers that cross each other obliquely (1). The inner tube is essentially a straight intestine running the length of the animal. These two "tubes" are separated by the pseudocoelomic cavity. It is derived from the blastocoel of the embryo (1).

The intestinal wall of Ascaris consists of a single layer of palisade-like high columnar cells. The luminal surface of these cells is crowned by microvilli (43) and the cells extend from the thick basal lamina on the pseudocoelomic side to the lumen of the intestine.

The basal lamella, basement membrane, is a ubiquitous support structure of all epithelial and endothelial cells (4, 43, 54). Recently, it has been thought to function as a physiological barrier to the passage of electrolytes and nonelectrolytes, thus acting as a selective ultrafilter (54). Biochemical characterization, i.e., amino acid and carbohydrate composition of the basement membrane, has been determined for several vertebrate glomeruli (52), Bowman's membrane of the kidney (30), the lens capsule of the eye (30), Descement's membrane of the cornea (24, 30), the choroic plexus membrane of the brain (30), the alveolar membrane of the lung (30) and the intestinal basement membrane of Ascaris (43). These membranes are all glycoproteins in nature (52).

The cutical of Ascaris is essentiahly impermeable to sugars (7, 8). The intestinal wall however, is permeable to sugars and the microvilli increase the absorptive surface of the intestine 75-90 times in Ascaris (62). Castro and Fairbairn (7) have shown that glucose can be absorbed against a concentration gradient. Beames (2) showed the same for 3-O-methylglucose, a non-metabolizable sugar, and fructose. He reports very limited movement of galactose.

The most apparent function of the basement membrane is a supportive one in which it serves as a micro-skeleton upon which the cells rest. As indicated earlier, however, they also play a vital physiological role as selective filters. An example of such a membrane is the renal glomerular basement membrane which anatomically is the only complete barrier between the plasma and its filtrate. It is composed of three layers: a) the lamina rara interna, b) the lamina densa, and c) the lamina rara externa. The absolute and relative thickness of these three layers varies with the species and with age (66, 67). The lamina densa appears to be the

effective barrier in the filtration process (26, 49).

The alveolar basement membrane probably plays a role in regulating gas exchange in the lung (32), and the basement membrane of the choroid plexus appears to be an important component of the blood-brain barrier (11, 12, 47). The lens capsule of the eye is another good example of a basement membrane which serves a role both as a supportive structure and as a filter. It obviously contributes to the shape of the lens and probably influences the flux of material through the lens capsule, that is, from the aqueous to the vitreous humors (23, 52). The blood-testis barrier is yet another physiological barrier that has as its functioning component the basement membrane (25). In the mammalian testis, the germ and Sertoli cells are surround by the boundary tissue which separates them from the intertubular medium. The boundary tissue, i.e., blood-testis barrier, may be subdivided into four layers in the adult: a) the internal non-cellular layer, b) the internal cellular layer, c) the external non-cellular layer, and d) the external cellular layer. The internal non-cellular layer, i.e., the basement membrane, ensures fluid regulation from the intertubular medium towards the lumen of the seminiferous tubules. The difference in the composition of the testicular fluid and the blood plasma (5) suggests that the boundary tissue, most probably the basement membrane, plays a major role as the filtering barrier (25).

Pappenheimer (40, 41, 42) had earlier been concerned with the diffusion through peripheral capillary membranes, the lamina densa of the renal glomerulus, the blood-brain barrier, and inert artificial membranes. He reported that the penetration of capillary walls by water and dissolved substances appears to take place solely by processes which

require no energy transformation on the part of the capillary endothelial cells. The rates of net fluid movement across the capillary wall has been shown to be simply proportional to the differences between the hydrostatic and osmotic forces acting across the capillary membrane (39).

Pappenheimer (41) developed methods for the determination of the average pore radii of membranes and to describe restricted diffusion and molecular sieving through the walls of membranes. Pappenheimer's method of determining the average pore radius of a membrane takes two factors restricting the diffusion of spherial molecules through cylindrical pores into consideration. The first condition was that the permeating molecule must pass through the opening of the pore without striking its edges, and secondly, viscous resistance inside the pore should be as if no pore were present. In Pappenheimer's method the pore radius measurements are dependent upon the filtration coefficient (Lp) which is determined by establishing a pressure gradient across the membrane.

Renkin (48) corroborated Pappenheimer's method by determining the relative geometric pore area per unit path length ($A/\Delta x$) for various molecules of graded molecular size. He graphically determined the actual geometric pore area per unit path length ($A_o/\Delta x$) and used this value to calculate the pore radius of an artificial membrane. Subsequent authors have determined the pore radius of cellulose membranes (14) and Vargas (58) calculated filtration coefficients by using hydrostatic and osmotic pressure gradient methods to determine the pore radius of giant squid axons.

Goldstein and Solomon have developed a method for determining the average pore radius of a membrane which plots ($1-\sigma$) one minus the Staverman coefficient (55) as a function of the radius of the permeating

molecule for various equivalent pore radii. Goldstein and Solomon making use of Renkin's (48) equation for filtration and Durbin's (14) correlation of the Staverman coefficient with osmotic pressure, plotted a family of theoretical curves with the equivalent pore radius as the parameter. The experimentally determined 1-σ values of the radii of the test molecule was plotted and the best fit curve of those values determined the equivalent pore radius of the membrane.

Goldstein and Solomon (18) determined the pore radius of human red blood cells by osmotic pressure measurements to be 4.2 Å. Villegas (61) using radioactive ethylene glycol, glycerol and water determined the $A/\Delta x$ for both the axolemma and Schwann layer in the squid nerve fiber. He found these structures to function as diffusion barriers and to have an effective pore radius of 4.25 Å in the axolemma (59, 60). Vargas (57) using the approach of Durbin and Solomon (13) estimated the equivalent pore radii of rabbit heart capillaries to be about 35 Å. Vargas also determined the filtration coefficient (L_p) and the reflection factor (σ) for these isolated perfused rabbit hearts. Phillips (44) determined the pore radius in the rectal intima of the desert locust to be 6.5-8.0 Å. Much more recently Owen (38) has calculated the σ, L_p, and ω (the product of the permeability coefficient, the gas constant and the absolute temperature) for the dog, cat, beef and human red cells using permeant molecules.

Thermodynamics

Kedem and Katchalsky (28, 29) present arguments for the need for three coefficients to fully characterize the permeability of a membrane to a particular solute-solvent system. These coefficients are: a) omega

(ω), which describes the solute membrane interaction, b) the filtration coefficient (L_p) which describes the permeability of the membrane to the solvent and c) the reflection coefficient or Staverman coefficient (σ) which describes the relative rate of flux of solute and solvent across the membrane.

The development of these coefficients as a quantitative description of the transport phenomena rests ultimately on basic principles of thermodynamics. A brief review of thermodynamics and the derivation of these coefficients follows (14, 27, 28, 29, 56).

The first law of thermodynamics is the law of conservation of energy. Mathematically and in the differential form it is:

$$dU = dQ - dW \tag{1}$$

where dU is the internal energy of the system under consideration, dQ is the heat gained by the system, and dW is the work done by the system on the surroundings.

The work term in the equation can take on different forms. If the volume of a system at a pressure P changes by an amount dV, the work performed is then equal to PdV. If an amount, de, of electrical charge is transferred into a system at an electrical potential Ψ, work is then equal to $-\Psi de$. The transfer into a system of dn_i moles of substance i at a total chemical potential μ_i, yields work that is equal to $-\mu_i dn_i$. Thus dW in Equation 1 above can be expressed:

$$dW = PdV - \Psi de - \mu_i dn_i \quad . \tag{2}$$

The second law of thermodynamics states that reactions go spontaneously in the direction that will lead to an increase in the randomness of

the system or entropy (S). For a system undergoing a reversible change, the change in entropy is defined by the heat gained (dQ) divided by the absolute temperature T and

$$dS = \frac{dQ}{T} \quad . \qquad (3)$$

Introducing Equations 2 and 3 into Equation 1, yields:

$$dU = TdS - PdV + \Psi de + \Sigma i \mu_i dn_i \quad . \qquad (4)$$

The Σi denotes summation over all components of a system and this expression is the Gibbs equation.

Classical thermodynamics provides only a set of inequalities that indicate the direction of irreversible processes. To develop a quantitative description of spontaneous processes we must look to nonequilibrium thermodynamics. The first concept in this theory is that the total entropy change of a system (dS) can be divided into two parts, an exchange of entropy between the system and its surroundings (deS) and an internal production of entropy (diS) due to irreversible processes taking place within the system. Thus:

$$dS = deS + DiS \quad . \qquad (5)$$

For a closed system Equation 5 is equivalent to

$$deS = \frac{dQ}{T} \qquad \text{and} \qquad diS = 0 \quad . \qquad (6)$$

The assumption is then made that the total change in entropy of a system can be described by the Gibbs equation (Equation 4) even though the system is not at equilibrium. This assumption has limited range in its validity and cannot be used if the system is not near equilibrium.

Thus:

$$TdS = dU + PdV - \Psi de - \Sigma i \mu_i dn_i \quad ,$$

and if Ψ is zero, then:

$$TdS = dU + PdV - \Sigma i \mu_i dn_i \quad . \tag{7}$$

The total entropy change (dS) for any system can be evaluated from Equation 7 and the quantities deS and diS can be determined. In all cases, the time derivative of diS (the rate of internal entropy production) is composed of a sum of products which has the form:

$$\frac{diS}{dt} = \Sigma i J_i X_i \tag{8}$$

where J_i equals flows and X_i equals the thermodynamic forces, i.e., conjugate forces.

In the case of a two component system in which two solutions of the same solvent and solute are separated by a membrane, the entropy production per unit time diS/dt can be written:

$$\frac{diS}{dt} = \frac{1}{T} (\mu_w^\circ - \mu_w^i) \frac{dN_w^i}{dt} + \frac{1}{T} (\mu_s^i - \mu_s^i) \frac{dN_w^i}{dt} \tag{9}$$

where μ denotes the chemical potential (of the solvent, subscript w, and solute subscript s and superscript $\circ$ and i denoting the system on the outside and inside of the membrane), dN_w^i/dt and dN_s^i/dt represents the number of moles of solvent and solute, respectively, entering the inner compartment per unit time.

The dissipation function (ϕ) is a measure of the rate of change of iS of an irreversible process and is given by TdiS/dt. Using ϕ per unit

area, Equation 9 becomes:

$$\phi = T \frac{1}{A} \frac{diS}{dt} \quad ,$$

and when

$$\dot{n}_w = \frac{1}{A} \frac{dN_w^i}{dt} \qquad \text{and} \qquad \dot{n}_s = \frac{1}{A} \frac{dN_s^i}{dt} \quad ,$$

then the expression simplifies to:

$$\phi = (\mu_w^\circ - \mu_w^i)\, \dot{n}_w + (\mu_s^\circ - \mu_s^i)\, \dot{n}_s \quad . \qquad (10)$$

The dissipation function, Equation 10, is the sum of products of flows per unit area ($\dot{n}_w$ and $\dot{n}_s$) and their corresponding forces, the differences in chemical potential.

The dissipation function, Equation 10, constitutes a special case of the general expression:

$$\phi = \Sigma i J_i X_i$$

where ϕ is the local entropy production, J_i is again flow and X_i the conjugated forces. The choice of flows and forces is arbitrary so long as the sum of their products is the same dissipation function.

For a system where the chemical potentials approximate those for ideal solutions, the following relationship applies:

$$\mu^\circ - \mu^i = \bar{v}\Delta p + RT\Delta \ln \gamma c_s \quad . \qquad (11)$$

Here $\bar{v}$ is the partial molar volume, Δp is the difference in pressure between the outer and inner compartment, γ is the activity coefficient of the solute, and c_s is the concentration of the solute. In the case of dilute solutions, where the activity coefficient goes to one, we may

rewrite Equation 11 for the solute to read:

$$\mu_s^\circ - \mu_s^i = \bar{v}_s \Delta p + RT\Delta \ln c_s = \bar{v}_s \Delta p + RT \frac{\Delta c_s}{\bar{c}_s} \qquad (12)$$

where $\Delta c_s = c_s^\circ - c_s^i$ and $\bar{c}_s$ is a mean of the concentrations of the solute in the two compartments given by:

$$\frac{\Delta c_s}{\bar{c}_s} = \ln \frac{c_s^\circ}{c_s^i} \quad .$$

If,

$$\frac{\Delta c_s}{c_s^i} << 1 \quad ,$$

then

$$\bar{c}_s = \frac{c_s^i + c_s^\circ}{2} \quad .$$

The corresponding equation for the solvent is:

$$\mu_w^\circ - \mu_w^i = \bar{v}_w \Delta p - RT \frac{\Delta c_s}{\bar{c}_w} \quad . \qquad (13)$$

Introducing Equations 12 and 13 into the dissipation Equation 10, and rearranging we get:

$$\phi = (\dot{n}_w v_w + \dot{n}_s v_s)\ \Delta p + \left(\frac{\dot{n}_s}{\bar{c}_s} - \frac{\dot{n}_w}{\bar{c}_w}\right) RT\Delta c_s \quad . \qquad (14)$$

Note that in Equation 14 the dissipation is represented by a new set of forces and flows. The new forces $X_1 = \Delta p$ and $X_2 = RT\Delta c_s$ are the forces usually employed in permeability studies, Δp is the hydrostatic pressure, while $RT\Delta c_s$ is the driving force of Fick's Law. The conjugate flows are the total volume flow per unit area,

$$J_v = \dot{n}_w \bar{v}_w + \dot{n}_s \bar{v}_s \quad ,$$

and the relative velocity of the solute versus solvent which is a measure for exchange flow,

$$J_D = \frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w} \quad .$$

The general theory of irreversible thermodynamics is based on the assumption that the flows, J, are functions of all forces operative in the system and that if the forces are sufficiently small, the dependence is linear. Thus, in the case of two flows, J_1 and J_2, dependent on two forces, X_1 and X_2, the relation between the J's and X's is given by:

$$\begin{aligned} J_1 &= L_{11}X_1 + L_{12}X_2 \\ J_2 &= L_{21}X_1 + L_{22}X_2 \end{aligned} \qquad (15)$$

where the L's are called the phenomenological coefficients.

The phenomenological coefficients are correlated by the law of Onsager which requires the equality of the cross-coefficients.

$$L_{12} = L_{21} \qquad (16)$$

Writing Equation 15 in the notation of our system where L_p, the filtration coefficient, equals L_{11} and Δp is X_1 and $RT\Delta c_s$ is the osmotic pressure gradient, etc., the following holds true.

$$J_v = L_p \Delta p + L_{pD} RT \Delta c_s$$

and (17)

$$J_D = L_{Dp} \Delta p + L_D RT \Delta c_s \quad ,$$

with

$$L_{Dp} = L_{pD} \quad , \qquad (18)$$

and since

$$\pi = RT\Delta c_s \quad ,$$

we may rewrite Equation 17 to read:

$$\begin{aligned} J_V &= L_p\Delta p + L_{pD}\Delta\pi \\ J_D &= L_{Dp}\Delta p + L_D\Delta\pi \quad . \end{aligned} \tag{19}$$

Membrane Properties

I will now correlate the significance of each of these phenomenological coefficients with the ability of the basement membrane to function as a barrier to diffusion.

Consider an experiment in which the concentration of the solute is the same on both sides of the membrane, and the osmotic pressure is zero. If a pressure difference is maintained, there will be a volume flow J, which is a linear function of Δp. The proportionality coefficient relating J_V to Δp is L_p, which is the filtration coefficient of the membrane. A hydrostatic pressure at an osmotic pressure of zero can produce not only a volume flow, but also a diffusional flow.

$$J_D = L_{Dp}\Delta p + L_D\Delta\pi$$

where

$$\Delta\pi = 0 \quad ,$$

then

$$J_D = L_{Dp}\Delta p \quad .$$

So, with $\Delta\pi = 0$, we get a J_v and J_D. The J_D, the movement of the solute relative to the solvent, is induced by a hydrostatic pressure and is called ultrafiltration. The coupling coefficient L_{Dp} is called the ultrafiltration coefficient and is a measure of the ultrafiltration properties of the membrane.

An alternative experiment is one in which different solute concentrations exist in two compartments ($\Delta\pi \neq 0$) and the hydrostatic pressure $\Delta p = 0$. The difference in osmotic pressure causes a diffusional flow characterized by the coefficient L_D, which represents a diffusional mobility per unit osmotic pressure. Thus

$$J_D = L_{Dp}\Delta p + L_D\Delta\pi \quad ,$$

but at $\Delta p = 0$; so

$$J_D = L_D\Delta\pi \quad .$$

There is also a volume flow caused by an osmotic pressure difference at $\Delta p = 0$. So that,

$$J_v = L_p\Delta p + L_{pD}\Delta\pi \quad ;$$

so

$$J_v = L_{pD}\Delta\pi \quad .$$

This is the osmotic flow and L_{pD} is the osmotic coefficient.

Another case is one in which the membrane is completely impermeable to the given solute, that is, for the case of the ideal semipermeable membrane. Here fewer coefficients are required to characterize the membrane and only one phenomenological coefficient is necessary. In this

case the flow of volume J_v is given, exactly by the flow of solvent alone. There is no flow of solute. The relative flow of solute versus solvent is also given by the flow of water alone. However, J_D now has a negative sign, for if the flow of water is positive the relative flow of solute versus solvent is in the opposite direction. Thus:

$$J_v = -J_D$$

and substituting into Equation 19 and rearranging gives:

$$(L_p + L_{Dp})\,\Delta p + (L_D + L_{pD})\,\Delta\pi = 0 \quad . \tag{20}$$

Equation 20 can be satisfied for all values of Δp and $\Delta\pi$ only if the terms within the parentheses are always zero. Therefore:

$$L_p = -L_{Dp} \quad \text{and} \quad L_D = -L_{pD} \quad ,$$

and since $L_{pD} = L_{Dp}$ (Equation 18, Onsager's Law), we have

$$L_p = L_D = -L_{Dp} = L_{pD} \quad . \tag{21}$$

A single phenomenological coefficient suffices then to characterize an ideal semipermeable membrane. Flow across such a membrane will be either hydrostatic, given by the pressure gradient Δp and the phenomenological coefficient $L_p(-L_{Dp})$, or osmotic, given by the osmotic gradient $\Delta\pi$ and the identical coefficient $L_D(-L_{pD})$. Thus the rates of hydrostatic flow and osmotic flow are equal.

For "leaky" membranes Equation 21 will not hold and the movement of the solute must be taken into account. In order to describe the relative rates of solvent and solute permeabilities, Staverman (55) has defined the reflection coefficient, σ, which is the ratio $-L_{pD}$ to L_p, that is,

the ratio of the osmotic coefficient to the pressure-filtration coefficient,

$$\sigma = \frac{-L_{pD}}{L_p} \quad . \tag{22}$$

For an ideal semipermeable membrane σ from Equation 22 is equal to unity. In a coarse, non-selective membrane, there can be no ultrafiltration and σ is zero. The Staverman factor is thus a measure of the semipermeability of the membrane to a given solute.

The following experiment is an example of how one may determine σ. A pressure difference Δp is exerted across a permeable membrane that separates two very large and well stirred compartments containing the same solution. The solvent under pressure passes through faster than the solute (sieving). However at time zero (see Equation 17),

$$J_v = \dot{n}_s v_s + \dot{n}_w v_w = L_p \Delta p \tag{23}$$

$$J_D = \frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w} \quad . \tag{24}$$

Equations 23 and 24 may be added and the results divided by Equation 23 to give

$$\frac{\dot{n}_s}{J_v}\left(\bar{v}_s + \frac{1}{c_s}\right) = 1 + \frac{L_{Dp}}{L_p} \quad . \tag{25}$$

As before, $L_{Dp} = L_{pD}$. The ratio L_{Dp}/L_p is $-\sigma$. For dilute solutions $\bar{v}_s$ is negligible in comparison to $1/c_s$ and Equation 25 becomes

$$\sigma = 1 - \frac{\dot{n}_s}{J_v c_s} \quad . \tag{26}$$

The quotient $\dot{n}_s/J_v c_s$ is equal to the moles of solute passing through the membrane per unit time, divided by the moles of solute arriving at the membrane during that time. This ratio may be equated to the ratio

A_{sf}/A_{wf}, where A_{sf} is the effective pore area available to solute molecules and A_{wf} to solvent molecules. Substituting into Equation 26, it becomes

$$\sigma = 1 - \frac{A_{sf}}{A_{wf}} \quad .$$

The ratio A_{sf}/A_{wf} for any given solute molecule is given by the ratio (A/A_o) solute/(A/A_o) water.

(A/A_o) solute can be calculated from Renkin's (48) equation:

$$\frac{A}{A_o} = (1 - \frac{a}{r})^2 \ (1 - 2.10 \ (\frac{a}{r}) + 2.09 \ (\frac{a}{r})^3 - 0.95 \ (\frac{a}{r})^5) \quad ,$$

where r is the pore radius and a the radius of the penetrating molecule. A is the effective area of the opening and A_o the cross-section opening of the pore.

Now the coefficient L_D must be translated into a more conventional form because as it stands, it describes the exchange flow of solute versus solvent, a quantity that cannot be directly measured. What is most easily measured is the rate of flow of solute across the membrane, which can be symbolized as J_s, the flux of solutes. In terms of our previous fluxes J_v and J_D, J_s is

$$J_s = (J_v + J_D)c_s \quad . \qquad (27)$$

Since $(J_v + J_D)$ gives the total flow of the part of the volume of the solution that is occupied by the solute, and the product of this volume and c_s, the volume concentration, it then gives the total number of moles of solute that crosses the membrane. Using Equations 17, 18 and 22 and substituting into Equation 27 and rearranging yields

$$J_s = (1 - \sigma) \ J_v c_s + (L_D - \sigma^2 L_p) \ c_s \ RT\Delta c_s \quad .$$

In the particular case of zero volume flow ($J_v = 0$), J_s is directly proportional to Δc_s.

The coefficient of proportionality is $(L_D - \sigma^2 L_p)c_s RT$, which is of the same form as a conventional permeability constant and hence is directly measurable. The term $(L_D - \sigma^2 L_p)c_s$ can be replaced by the symbol ω. The coefficient ω then is the membrane permeability to solute under conditions of zero volume flow. Since $\Delta\pi = RT\Delta c$, ω is related to the classical permeability coefficient P_s as follows:

$$P_s = \omega RT$$

Thus ω, σ, and L_p are three independent parameters all of which are obtained experimentally and from which L_p, L_D, and L_{pD} can be derived; ω, σ, and L_p thus may be used to characterize the basement membrane of Ascaris suum as a barrier to diffusion.

Osmotic Pressure

The osmotic pressure depends on the activity of the solvent:

$$\pi = -\frac{RT}{V_s} \ln a_s \quad . \qquad (28)$$

An approximate equation developed by van't Hoff (19) gives the osmotic pressure remarkably well, in terms of the molar concentration of the solute. It has the form:

$$\pi = RT(A) \quad . \qquad (29)$$

I will develop this equation here to demonstrate mathematically that the activity of a solute becomes almost identical to its concentration at low concentrations. Similarly, the mole fraction of the solvent has been

found to be a suitable approximation of its activity for dilute solutions, the standard state of the solvent in this case being pure solvent.

The mole fraction of solvent, X_s, is given by:

$$X_s = \frac{n_s}{n_a + n_b + n_c + \cdots + n_s}$$

where the terms n_a, etc., refer to the total number of moles of a, b, etc.

An equation similar to that for the mole fraction of the solvent can also be written for the mole fraction of each component of a solution; for example,

$$X_a = \frac{n_a}{n_a + n_b + n_c + \cdots + n_s} \quad .$$

The mole fraction of all the component parts must add up to one. Hence:

$$X_s = 1 - (X_a + X_b + X_c + \cdots) \quad .$$

Furthermore, for dilute solutions far larger numbers of solvent molecules than solute molecules are present. For example, for a liter of 0.1 M sucrose solution, $n_s = 55.4$, $n_a = 0.1$.

Therefore we can introduce the approximation

$$X_a \simeq \frac{n_a}{n_s} \quad ,$$

and also the approximation

$$V \simeq n_s \bar{v}_s \quad ,$$

neglecting the contribution of the solutes to the volume. Hence:

$$X_a \simeq \frac{n_a}{n_s} \simeq n_a \left(\frac{\bar{v}_s}{v}\right) = \bar{v}_s(A) \quad .$$

This equation permits us to say that the mole fraction of any solute is approximately the product of the partial molar volume of the solvent and the concentration of the solute for very dilute solutions.

Writing a similar expression for each solute, and when these expressions are all summated, we can state the activity of the solvent in terms of the concentrations of the solutes:

$$a_s \simeq 1 - \bar{v}_s((a) + (b) + (c) + \cdots) \quad .$$

Substituting into Equation 28, one gets:

$$\pi = -\frac{RT}{\bar{v}_s} \ln[1 - \bar{v}_s((a) + (b) + (c) + \cdots)] \quad . \qquad (30)$$

In the expression $-\ln(1 - x)$, when x is small, then:

$$\text{if } x = 0.1, \text{ then } -\ln(1 - x) = -\ln 0.9 = 0.105$$

$$\text{if } x = 0.01, \text{ then } -\ln(1 - x) = -\ln 0.99 = 0.0101$$

$$\text{if } x = 0.001, \text{ then } -\ln(1 - x) = -\ln 0.999 = 0.00100 \quad .$$

As x becomes smaller, $-\ln(1 - x)$ is approximately equal to x. If one makes the corresponding substitution of

$$\bar{v}_s((a) + (b) + (c) + \cdots)$$

for

$$-\ln(1 - \bar{v}_s(a) + (b) + (c) + \cdots)$$

into Equation 2, we then get

$$\pi = RT((a) + (b) + (c) + \cdots) \quad ,$$

and for a single solute we get

$$\pi = RT(a) \quad .$$

In calculating the filtration coefficient in my experiment on the basement membrane I will use only dilute solutions of sucrose to obtain an osmotic pressure gradient. I will use the van't Hoff (19) equation as an easy approximation for the activity of the solvent.

Diffusion and Pore Area

Simple diffusion is the migration of a solute from an area of higher concentration to an area of lower concentration, and is a result of the random motion of the solute molecules. According to the theoretical treatment of Fick (16), the amount of substance diffusing through a cross-section of area, A, is directly proportional to the concentration across this section; expressed mathematically, Fick's first law states:

$$\frac{ds}{dt} = - DA \frac{dc}{dx} \quad . \qquad (31)$$

The differential ds/dt expresses the rate of transport of the substance in moles, crossing in time dt; the differential dc/dx is the concentration gradient at the point considered and D is the diffusion coefficient in cm^2/sec.

The diffusion component of flux can be evaluated by applying the general relationship

$$\frac{ds}{dt} = - \frac{DA}{RT} \frac{\Delta p}{\Delta x} \quad ,$$

since

$$\Delta\mu = \bar{v}\Delta p$$

and

$$c\bar{v} = 1 \quad .$$

The factor $DA/\Delta x$ can be evaluated after observing the diffusion of tritiated water across the membrane from the relationship

$$\frac{ds}{dt} = DA\,\frac{dC}{dX} \quad .$$

An integrated form of Fick's Law for a system where the volume of the two compartments is not equal is the following:

$$\frac{A}{\Delta x} = \left(\frac{v_1 v_2}{v_1 + v_2}\right)\frac{1}{D \cdot t}\ln\frac{C_0}{C_0 - C_1\left(1 + \frac{v_2}{v_1}\right)} \quad . \qquad (32)$$

Here, $A/\Delta x$ is the apparent diffusion area per unit path length for each diffusing solute, v_1 and v_2 are the volumes of the chambers, t is time in seconds, and C_0 and C_1 are the initial and final solute concentration in the chambers. One can estimate the pore radius of a membrane from this information but using Pappenheimer's (26) equation,

$$r_p = 2\sqrt{\frac{2L_p\eta}{\frac{A_o}{\Delta x}}} \quad . \qquad (33)$$

Here L_p is the filtration coefficient and η the viscosity of water for estimating pore size.

Goldstein and Solomon (18) developed a method for determination of the average pore radius which is not dependent upon determinations of the filtration coefficient (L_p). The σ coefficient is calculated for the

individual permeating substances. Using Renkin's (48) equation for filtration, Goldstein and Solomon (18) express the ratio of the apparent area for filtration of the solute (A_{sf}) to the apparent area for filtration of water (A_{wf}).

$$\frac{A_{sf}}{A_{wf}} = \left[\frac{[2(1 - \frac{a}{r})^2 - (1 - \frac{a}{r})^4][1 - 2.104 \frac{a}{r} + 2.09 (\frac{a}{r})^3 - 0.95 (\frac{a}{r})^5]}{[2(1 - \frac{a_w}{r})^2 - (1 - \frac{a_w}{r})^4][1 - 2.104 \frac{a_w}{r} + 2.09 (\frac{a_w}{r})^3 - 0.95 (\frac{a_w}{r})^5]} \right]$$

where, a is the molecular radius of the permeating molecule, r is the equivalent pore radius, and a_w is the molecular radius of water.

Durban (14) shows further that

$$1 - \sigma = \frac{A_{sf}}{A_{wf}} \quad .$$

Goldstein and Solomon plot $(1 - \sigma)$ as a function of the radius of the permeating molecule for various equivalent pore radii. A family of theoretical test curves are then determined with the equivalent pore radii as the parameter. The best fit curve for the test molecule is then determined.

CHAPTER III

MATERIALS AND METHODS

Collection and Preparation of the Tissue

Adult female Ascaris suum are collected at the packing house and transported to the laboratory in a basal saline (21) maintained between 32 and 39° C. The worms are used experimentally within six hours. The intestine is removed from the individual worms, placed in a dish of warm saline and sliced open. Ribbons of the intestine are then isolated that are 0.5 cm wide and 1.0 cm or more in length. The layer of epithelial cells can be separated from the basement membrane either by sonication, by increasing the sodium chloride concentration in the holding medium, or by shaking the intestine in a suspension of glass beads. The isolated basement membrane is then clamped into the diffusing chamber.

Diffusion Chamber

The diffusion chamber is a modified Ussing chamber (see Figure 1) and the membrane is positioned between the two compartments of the chamber. The opening between the two compartments of the chamber is 0.5 cm wide and 1.0 cm long. The apparatus is equipped with a gas lift circulating system which provides adequate perfusion, i.e., reduction of the unstirred layer to a minimum, for each surface of the tissue.

INCUBATION CHAMBER USED TO DETERMINE $A/\Delta X$

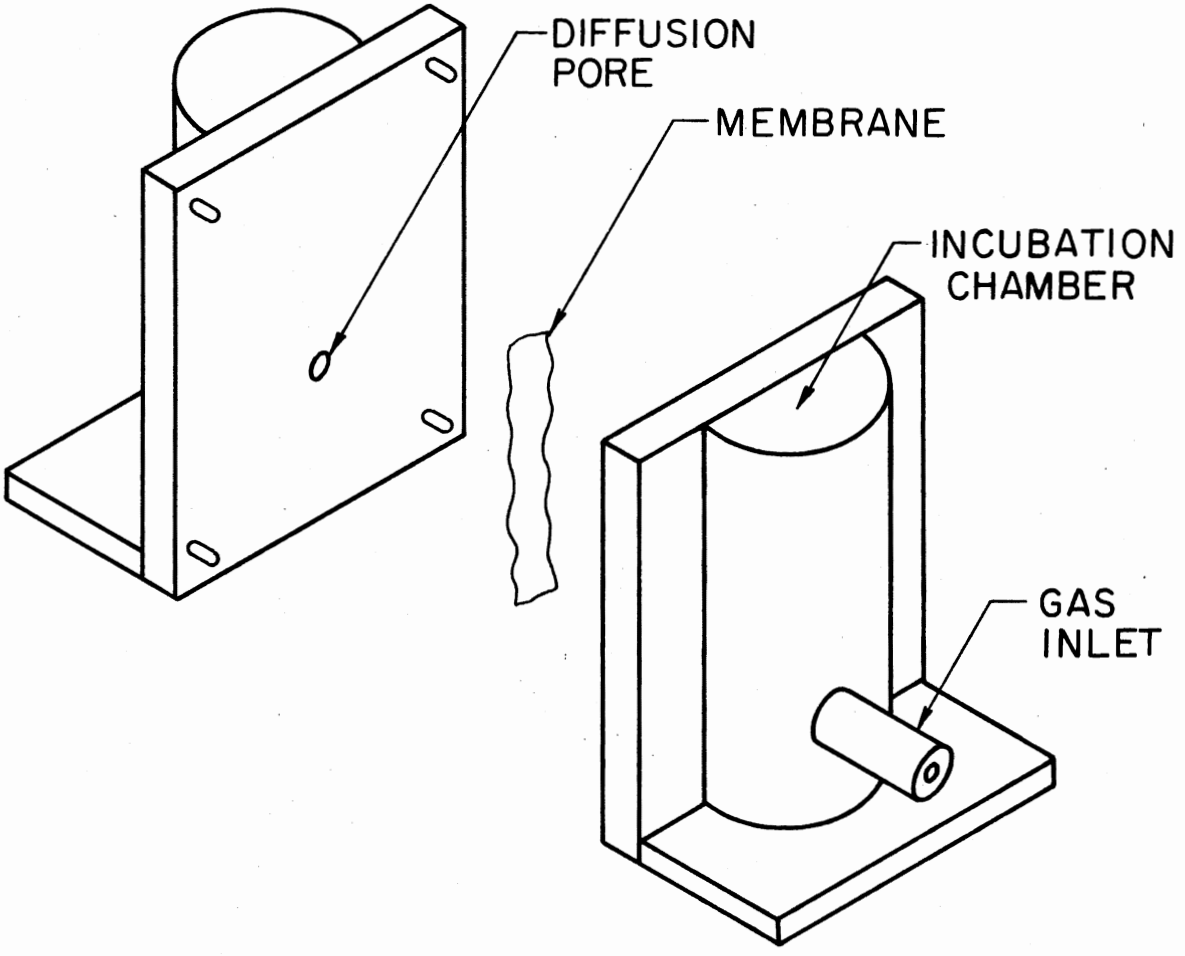

Figure 1. Ussing Chamber

Non-Electrolytes

A/Δx Measurements

Standardization of the Apparatus. Tritiated water, or ^{14}C labelled urea, glucose, sucrose, or inulin are added to one side of the diffusion chamber. Diffusion through visking cellulose (dialysis tubing) is determined by taking a sample aliquote from the opposite chamber and determining total radioactivity. A/Δx, the relative geometric pore area per unit path length is determined by the formula

$$\frac{A}{\Delta x} = \frac{v_1 v_2}{v_1 + v_2} \frac{2.3}{D \cdot t} \log \frac{C_0}{C_0 - C_1\left(1 + \frac{v_2}{v_1}\right)}$$

(for derivation and symbol meanings see Literature Review, Equation 32).

These A/Δx values are compared with those in the literature for water, urea, glucose, sucrose, and inulin to establish that the diffusion chamber gives comparable results for diffusion through cellulose as has been previously reported.

A/Δx Measurements for the Basement Membranes of Ascaris. The intestinal basement membrane of Ascaris can be isolated from the epithelial cells by three different methods: first by sonication, using ultrasonic sound to dismembrate the cells, secondly by increasing the sodium chloride concentration in the holding medium and then taking a camel hair brush and brushing off the epithelial cells, and thirdly by shaking ribbons of the intestine in a suspension of glass beads. Basement membranes that are prepared by one of these techniques can be clamped individually into the diffusing chamber and the diffusion of

water or urea, glucose, sucrose, or inulin determined as described previously for visking cellulose.

Filtration Coefficient Determinations Measured Using Hydrostatic and Osmotic Pressures

Standardization of the Apparatus--Osmotic Pressure. Following the method that is described by Vargas (58), the osmotic pressure on one side of the diffusing chamber is increased by increasing the sucrose concentration in the bathing medium. At pressures below 6×10^6 dyne/cm^2 (58), the relationship between volume flux and pressure is linear. Measurements of the volume flux of the tritiated water through visking cellulose is linear and the slope of the line is defined as the filtration coefficient (L_p) of the membrane. This filtration coefficient compares favorably with previously published results of the filtration coefficient of visking cellulose.

Standardization of the Apparatus--Hydrostatic Pressure. The diffusing chamber can be modified by the addition of a water column with a capillary manometer to establish and measure the hydrostatic pressure gradient. Again the volume flux of tritiated water through visking cellulose is determined. It can be compared with the filtration coefficient determined previously using a hydrostatic pressure gradient to determine a filtration coefficient for visking cellulose.

Basement Membrane. The basement membrane of Ascaris suum prepared by sonication is clamped into the diffusing chamber and the volume flux of tritiated water is measured under first an osmotic and then a hydrostatic gradient.

Reflection Coefficient

The reflection coefficient is measured by comparing the $A/\Delta x$ values of penetrating molecules of increasing molecular radius with the $A/\Delta x$ values for water.

$$\frac{\frac{A_{sf}}{\Delta x}}{\frac{A_{wf}}{\Delta x}} = \frac{A_{sf}}{A_{wf}}$$

$$\sigma = 1 - \frac{A_{sf}}{A_{wf}} \qquad \text{or} \qquad \frac{A_{sf}}{A_{wf}} = 1 - \sigma$$

Equivalent Pore Radius Determinations

Pappenheimer's Method. Pappenheimer estimates the average pore radius of membranes by direct measurement of the $A_0/\Delta x$ of the membrane; given the following equation,

$$r_p = \sqrt{\frac{2L_p\eta}{\frac{A_0}{\Delta x}}}$$

where L_p is the filtration coefficient, η the viscosity of water, and $A_0/\Delta x$ the actual gemoetric pore area per unit path length.

Goldstein and Solomon Method. Goldstein and Solomon (18) plotted one minus the reflection coefficient $(1 - \sigma)$ as a function of the radius of the test molecule for various equivalent pore radii. A family of theoretical curves is obtained (see Figure VI) with the equivalent pore radius as the parameter. The experimental values of the reflection coefficient (σ) are then subtracted from one and plotted against the increasing molecular radius of the permeating molecules. The best fit

curve for the experimental values of the reflection coefficient is the pore radius.

Permeability Coefficient Determinations

Solute permeability coefficients are determined for a series of non-ionic molecules of graded molecular size. Tritiated water or ^{14}C labelled urea, glucose, sucrose, or inulin are added to one side of the chamber and at various time intervals sample aliquotes are taken from the opposite chamber. The volume flux of the permeating molecule is plotted against time to determine the permeability coefficients (Ps).

Electrolytes

Solute Permeability Coefficients

Solute permeability coefficients are determined for sodium, potassium and chloride ions. The radioactive ions are added to one side of the diffusing chamber and at various time intervals sample aliquotes are taken from the opposite chamber. The volume flux of the permeating molecule are plotted against time to determine the permeability coefficient (Ps).

Ion Selectivity

pH Changes. Solute permeability coefficients are determined for sodium, potassium, and chloride ions in basal saline (21) at a physiological pH of 7.2. To determine the net charge on the basement membrane, the pH of the bathing medium is lowered to 2.3 using a 1.6 mM potassium biphtahalate and hydrochloric acid (HCl) buffer. At this lower pH the permeability coefficient for the various ions is again determined.

The pH of the bathing medium is raised to 10.5 using 1.6 mM glycine and sodium hydroxide (NaOH) buffer solution and the permeability coefficients for the ions is determined and compared with the permeability coefficients that are obtained at neutral and low pH's.

Blocking Positive or Negative Sites in the Membrane. Solutions containing 1,5 difluro-2,4 dinitrobenzene (FFDNB) are prepared by dissolving 100 mg of FFDNB in one ml of absolute methanol. The methanol solution is added drop-wise to 160 ml of the basal saline with vigorous stirring. Since FFDNB undergoes slow hydrolysis in water with the liberation of hydrogen ions, solutions of it are prepared immediately prior to there use in experiment. The pH of the bathing medium containing FFDNB is measured immediately before and after the experiment, and the pH drop is never found to be below pH 6.5. The solution containing the FFDNB dissolved in the basal saline is used as a bathing medium for the basement membrane. The FFDNB solution should block the positive sites in the membrane. The radioactively labelled ions are again added and the permeability coefficient is again determined.

A 5.25 mM solution of calcium ions is added to the basal saline. This solution then blocks the negative sites on the membrane. The radioactive ions are again added and the permeability coefficients are determined and compared with those measured earlier.

Radioactive Counting

1. All of the radioactive material used in these experiments were purchased from New England Nuclear Company in Boston, Mass.

2. All radioactive counting was done with a Packard Tri-Carb scintillation counter Model 3993. Standard techniques were followed.

3. The scintillation fluid used was as follows:

   a. in the experiments using non-electrolytes, Bray's solution was used;

   b. in the experiments using electrolytes, scintiverse was used.

CHAPTER IV

RESULTS

Standardization of the Apparatus

In the characterization of the intestinal basement membrane of Ascaris as a diffusion barrier, measurements of the average pore radius were first made. A modified Ussing chamber, Figure 1, had been designed for this purpose. Before determining the average pore radius for the intestinal basement membrane however, the equivalent pore radius for visking cellulose was measured in order to demonstrate that the results from this chamber are comparable to the pore radii for cellulose previously determined and reported in the literature. This allowed for standardization of the chamber.

The cellulose tubing, after having been soaked in distilled water for several minutes, was clamped into the diffusing cell and sequentially, radioactive molecules of graded molecular size were added. The $A/\Delta x$, the relative geometric pore area per unit path length, was determined for each molecule and compared with those values determined by Renkin (48); the results are given in Table I.

To determine the average pore radius of the cellulose using the Pappenheimer method, measurement of the filtration coefficient is also necessary. The filtration coefficient for visking cellulose was determined by measuring the radioactive volume flux of water through the membrane. This volume flux was measured at varying sucrose osmotic pressure

TABLE I

STANDARDIZATION OF THE APPARATUS-VISKING CELLULOSE

Substance	Molecular[1] Radius Used Å	Renkins[2] Results	Visking[3] Cellulose A/Δx CM
Water	1.97	19.0	15.00
Urea	2.70	17.2	13.60
Glucose	3.57	9.6	8.08
Sucrose	4.40	6.6	6.90
Inulin	12.00	----	2.09

[1]Principle of polography (22).

[2]Renkin (48).

[3]Experimental determined values.

concentrations in the membrane. Table II gives the values of the sucrose osmotic gradient and the radioactive volume flux they created. Figure 2 is a graphic presentation of this volume flux of water versus increasing sucrose osmotic pressure for the cellulose membrane. A linear regression of the graph renders analysis of the slope of the line which represents, by definition, the filtration coefficient.

This information allows for calculation of the average pore radius of visking cellulose determined by the modified Ussing chamber in this experiment and substituted into the equation derived by Pappenheimer (for derivation see Chapter II). The results of this substitution measure the average pore radius of the visking cellulose as 16.3 Å. The results of previous determinations of pore radii for visking cellulose are 18.9 Å (48).

The apparatus gave results with cellulose that are comparable with those previously published in the literature, and suggested that one could use the method to calculate the average pore radius of the intestinal basement membrane of Ascaris suum using this modified Ussing chamber.

Non-Electrolytes

A/Δx Determinations

Preparation of the intestinal basement membrane of Asacris suum was done by three different methods, as described in previous paragraphs. Plates 1, 2, and 3 are electron photomicrographs of the various preparations of the basement membrane. Plate 4 is an enlargement of Plate 3 showing the remains of part of the epithelial cells which were not removed by the process. The typical bimolecular leaflet is apparent.

TABLE II

DETERMINATION OF FILTRATION COEFFICIENT OF CELLULOSE MEASURED USING OSMOTIC PRESSURE[1]

Molarity	π	Dyne/CM^2 x 10^6	Vol. Flux. CM/Sec x 10^6
0	0	0	0
0.05	1.22	1.22	0.93 ± 0.03
0.10	2.44	1.44	1 90 ± 0.07
0.15	3.66	3.66	2.70 ± 0.10
0.20	4.88	4.88	3.67 ± 0.10

[1]All figures are for 1.0 CM^2 membranes at 25° C.

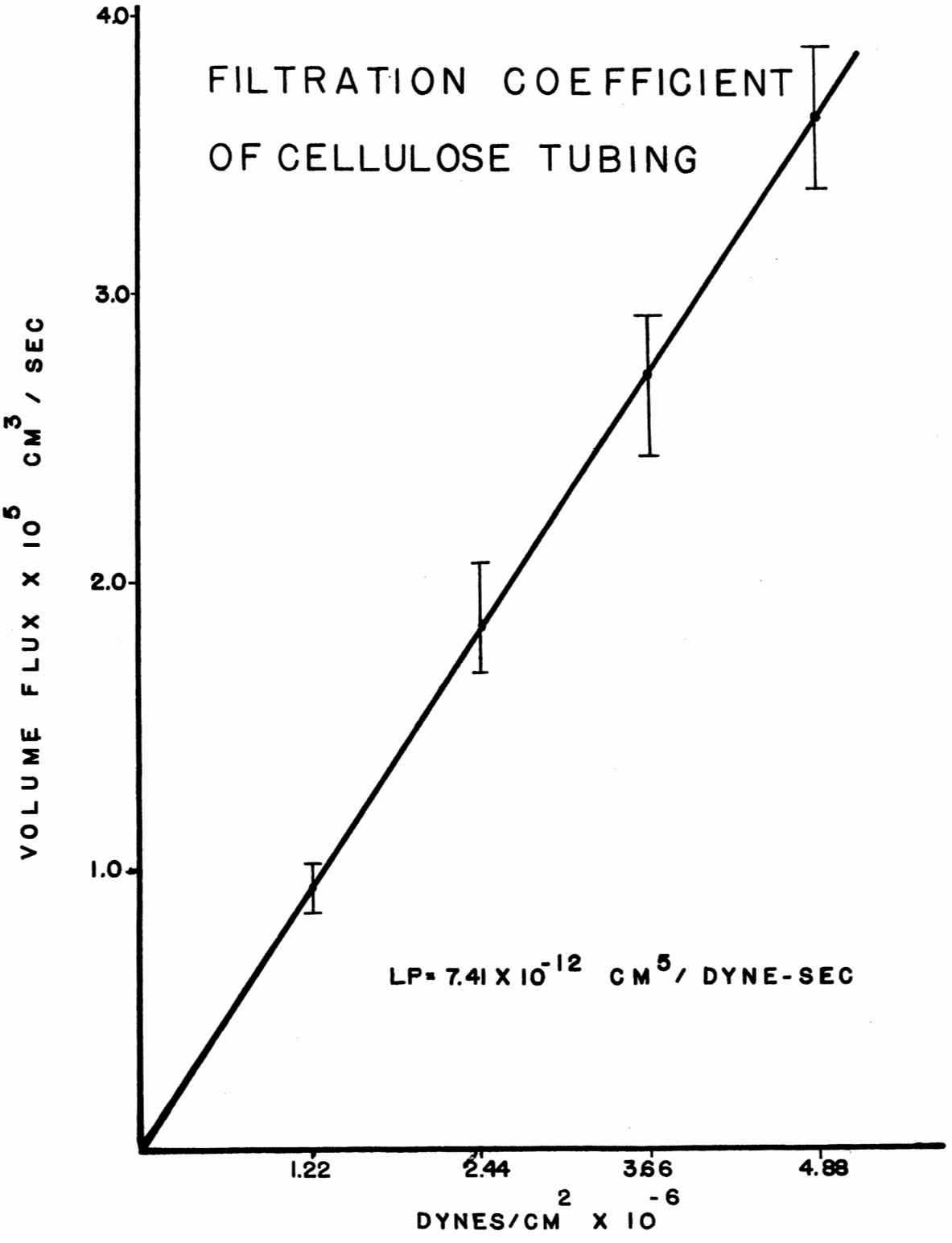

Figure 2. L_p of Cellulose Determined With an Osmotic Pressure Gradient

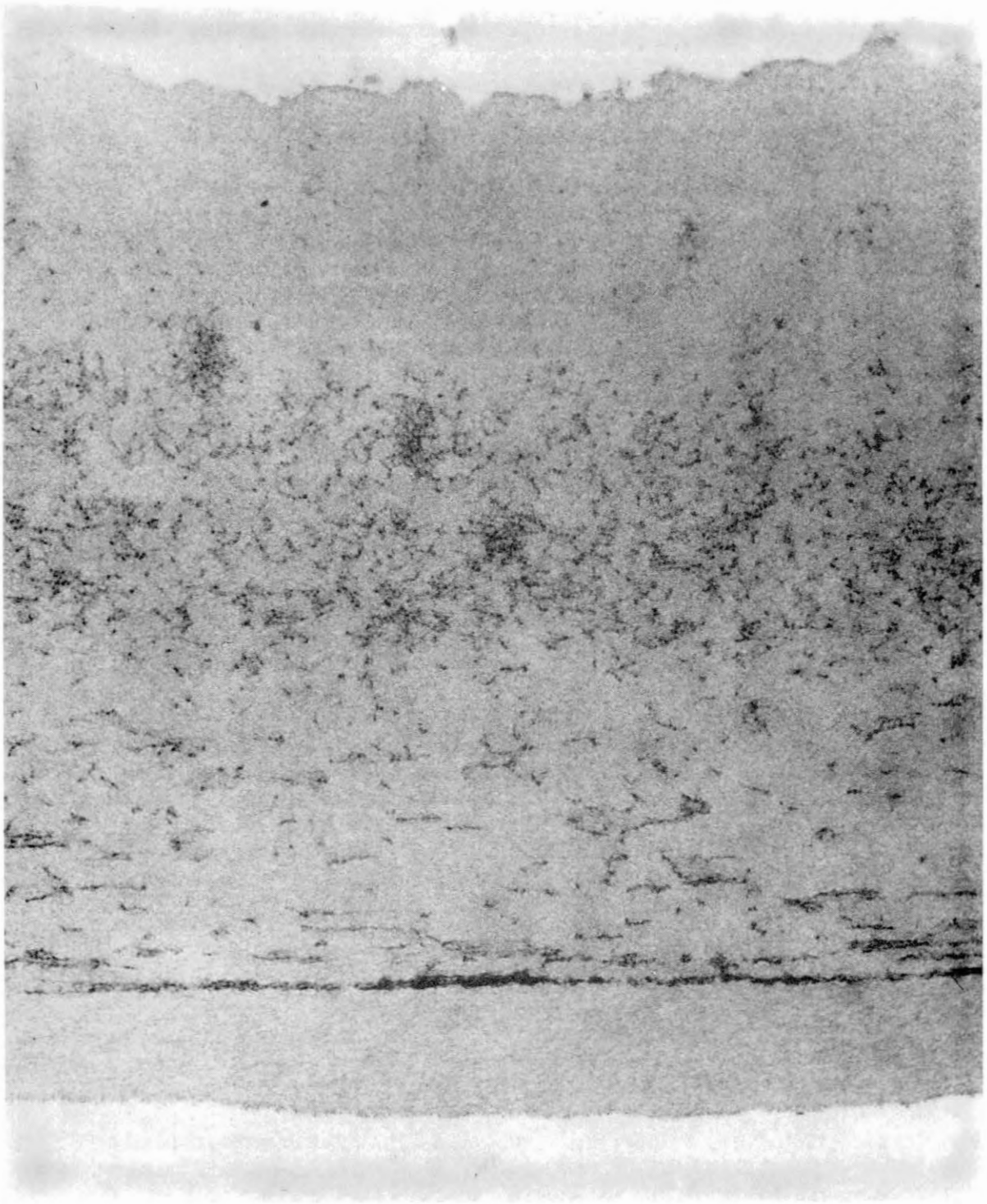

Plate 1. Sonication Preparation of the Basement Membrane

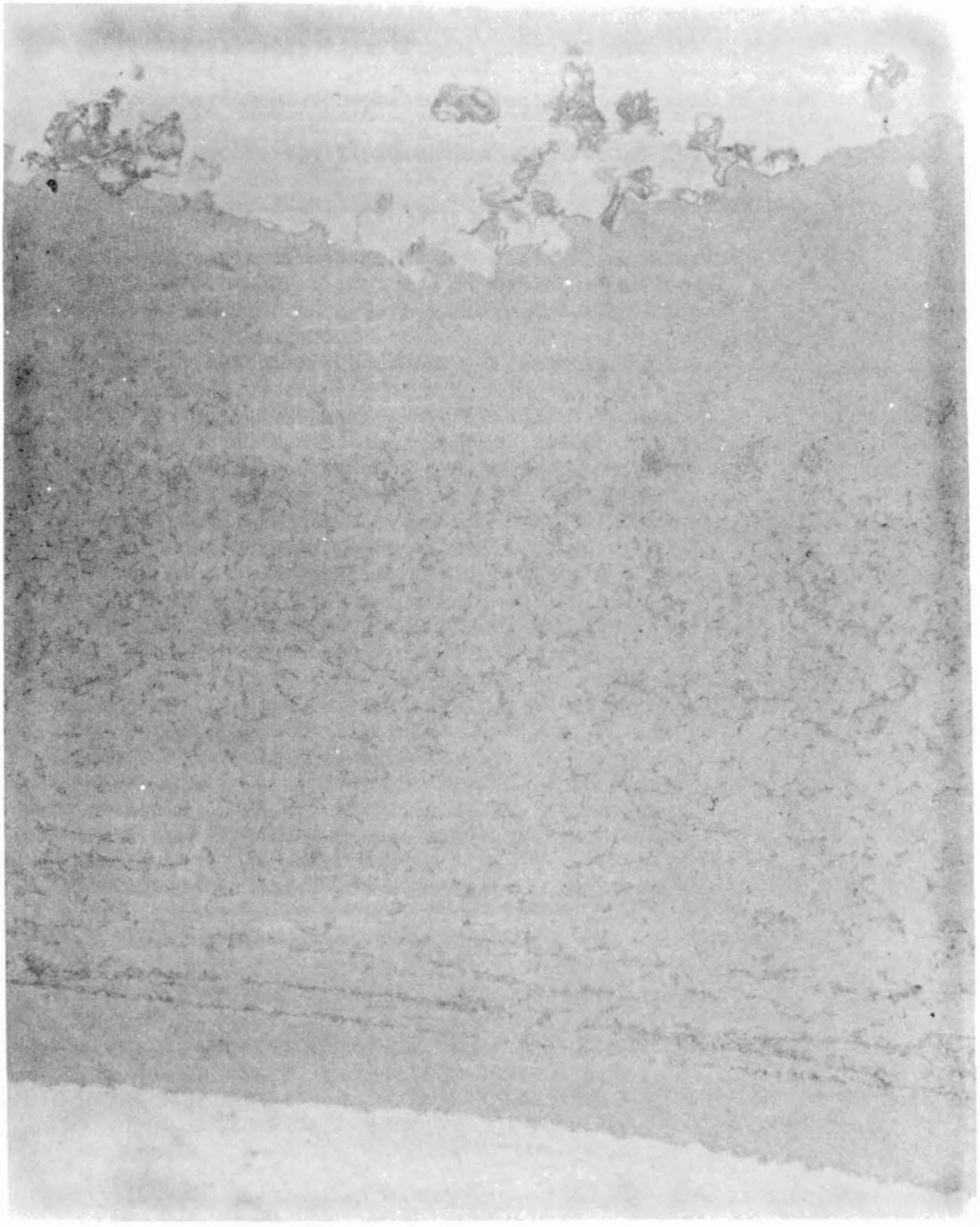

Plate 2. Sodium Chloride Preparation of the Basement Membrane

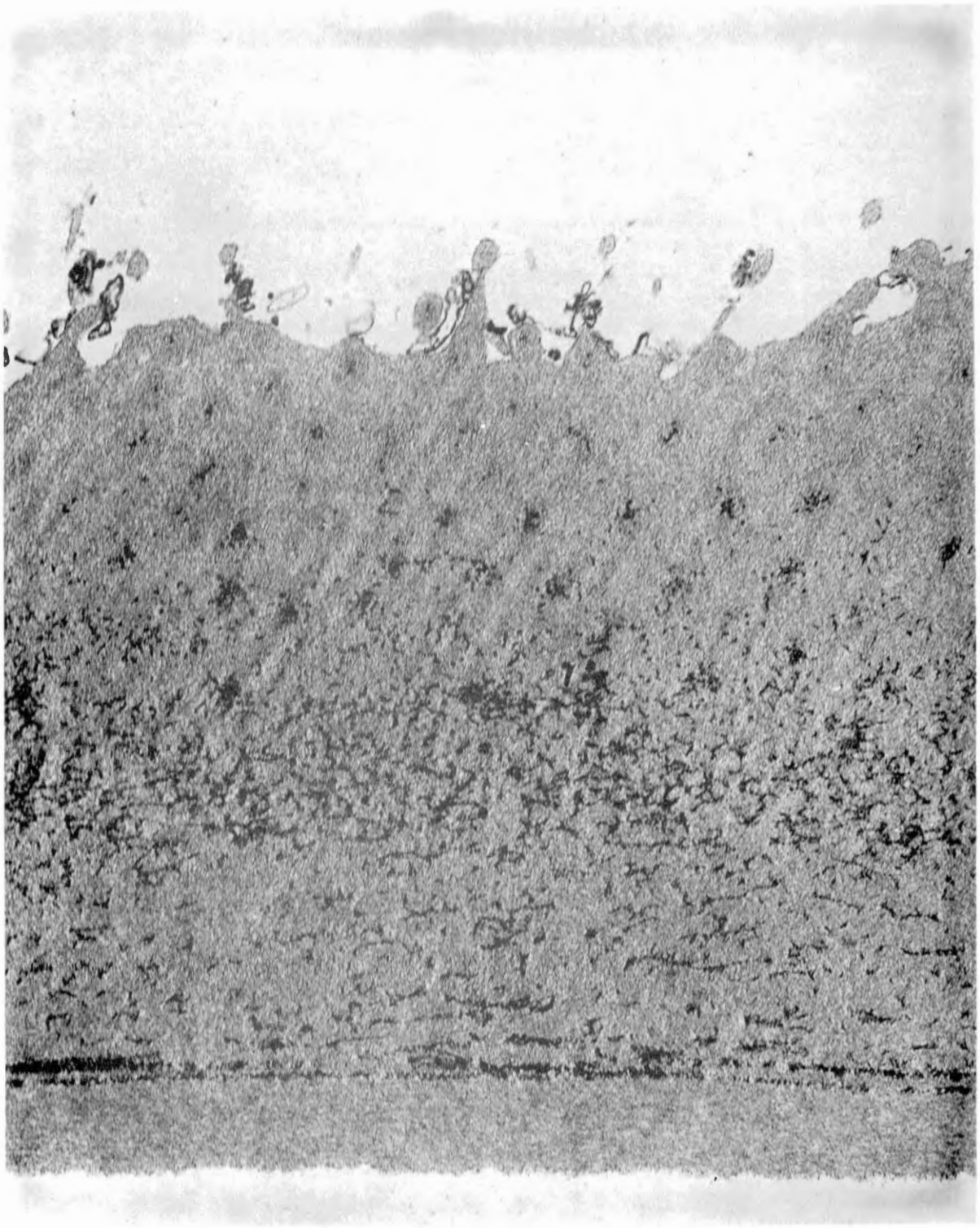

Plate 3. Glass Bead Preparation of the Basement Membrane

Plate 4. Bimolecular Leaflet

These isolated basement membranes were subsequently clamped into the diffusing cell, and sequentially, radioactive molecules of graded molecular size were added. The $A/\Delta x$ values were determined for each series of permeating molecules, i.e., water, urea, glucose, sucrose, and inulin through the basement membrane prepared by the three methods. These results are shown in Table III.

$A_0/\Delta x$ Determinations

Measurements of the diffusion of radioactive molecules through the basement membrane allows for experimental calculation of the $A/\Delta x$ values. the relative geometric pore area per unit path length. In Pappenhiemer's equation for calculation of the average pore radius the measurement used is the $A_0/\Delta x$, the actual geometric pore area per unit path length. The $A_0/\Delta x$ value is determined graphically by plotting the $A/\Delta x$ values experimentally determined against the increasing molecular radii of the probing molecules. Extrapolation of the points to a zero molecular radius molecule gives an $A/\Delta x$ value. This $A/\Delta x$ value for a molecule of zero molecular radius and no net electrical charge is the $A_0/\Delta x$ value. Figure 3 is a graphic representation of the experimentally determined $A/\Delta x$ values of the diffusion in the basal lamella against molecules of increasing molecular radii. Since the basement membrane can be isolated by three different methods, three graphs of $A/\Delta x$ values versus molecular radii are represented. The $A_0/\Delta x$ values for the basement membrane are 22, 21 and 26 cm for the sonication, sodium chloride, and glass bead preparations, respectively (see Table IV).

TABLE III

A/Δx MEASUREMENTS OF SONICATION, SODIUM CHLORIDE AND GLASS BEADS PREPARATION OF THE BASEMENT MEMBRANE

Substance	Sonication A/Δx CM	Sodium Chloride A/Δx CM	Glass Beads A/Δx CM
Water	12.6 ± 0.70	16.0 ± 0.50	18.36 ± 0.90
Urea	10.8 ± 0.60	15.2 ± 0.10	12.90 ± 0.50
Glucose	4.9 ± 0.65	13.67 ± 0.23	11.71 ± 0.17
Sucrose	3.4 ± 0.76	12.80 ± 0.16	12.17 ± 0.50
Inulin	2.3 ± 0.90	8.20 ± 0.80	3.12 ± 0.70

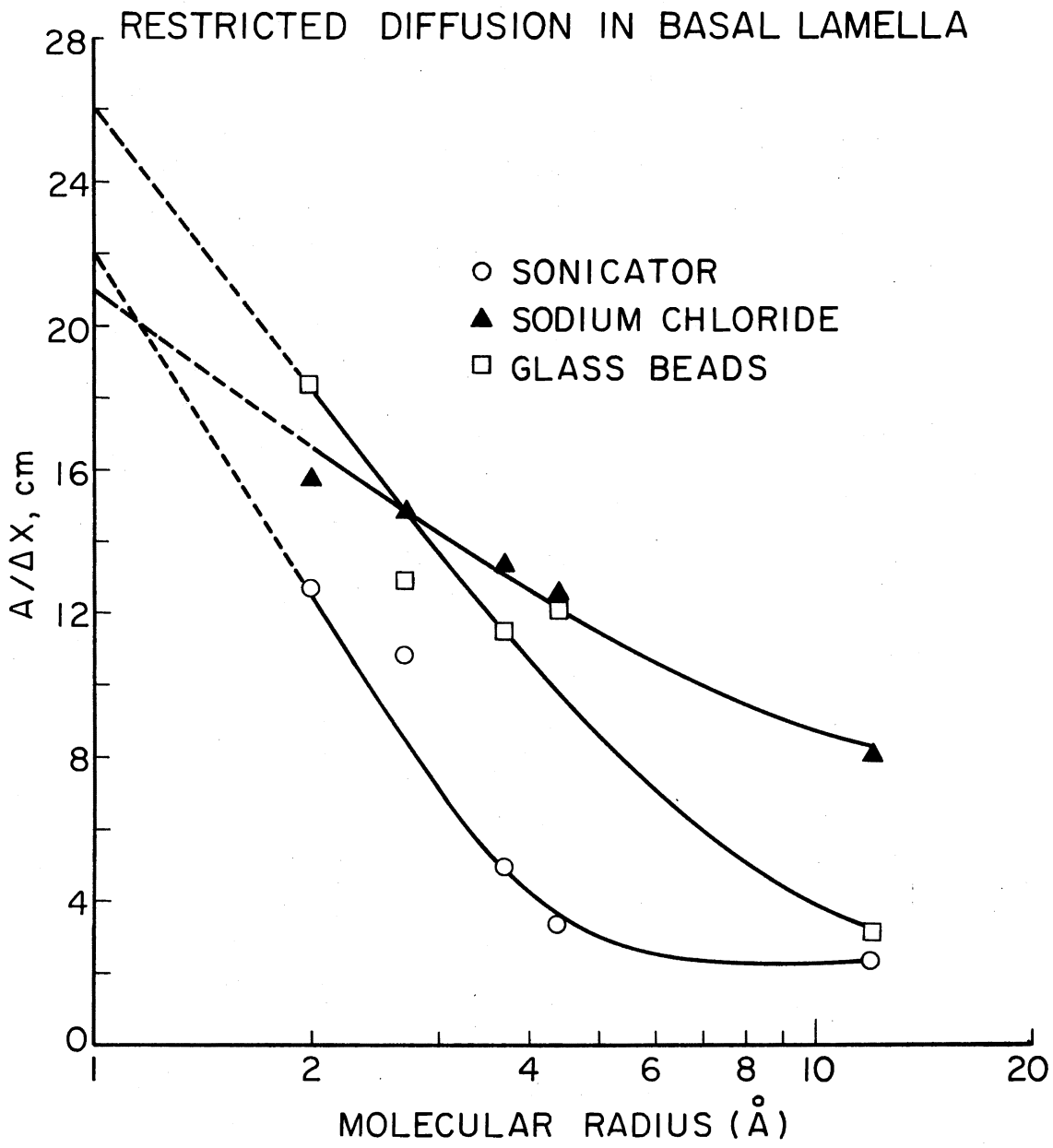

Figure 3. A/Δx Versus the Molecular Radius for the Basement Membrane

TABLE IV

$A_0/\Delta x$ VALUES-DIFFUSION COEFFICIENTS AND REFLECTION COEFFICIENT MEASUREMENTS

Method		Sonicator		Sodium Chloride		Glass Beads	
$A_0/\Delta x$ CM		22		21		26	
Calculated Pore Radius-Å		24.3		24.8		22.3	
Substance	D[1]	σ[2]	1-σ	σ	1-σ	σ	1-σ
Water	2.36	-	-	-	-	-	-
Urea	1.45	0.15	0.85	0.05	0.95	0.29	0.71
Glucose	0.68	0.61	0.39	0.14	0.86	0.36	0.64
Sucrose	0.55	0.73	0.27	0.20	0.80	0.33	0.67
Inulin	0.16	0.81	0.19	0.48	0.52	0.83	0.17

[1]Diffusion coefficient $CM^2/Sec \times 10^5$.

[2]Reflextion factor.

Filtration Coefficient Determinations

Osmotic Pressure Gradient. The filtration coefficient (L_p) was measured by increasing the osmotic pressure on the intestinal basement membrane. The relationship between volume flux and osmotic pressure is linear below pressures of 6×10^6 dynes/cm^2 (48) and the slope of the line is defined as the filtration coefficient. Table V gives the values of the sucrose osmotic gradient and the radioactive volume flux they created through the basement membrane of Ascaris suum. The filtration coefficient as determined for the basement membrane of Ascaris is 18.1×10^{-12} cm^5/dyne-sec (see Figure 4).

Hydrostatic Pressure Gradient. The diffusion chamber was modified by the addition of a water column with a capillary mamometer to establish a hydrostatic pressure gradient. Again, the radioactive volume flux of tritiated water through the basement membrane of Ascaris prepared by sonication was determined. Table VI gives the values of the hydrostatic pressure created and the radioactive volume flux through the membrane. The filtration coefficient for the basement membrane is 22.2×10^{-10} cm^5/dyne-sec (see Figure 5).

Reflection Coefficient Determinations

The reflection coefficient or Staverman coefficient, σ, is a measure of the leakiness of a membrane. A reflection coefficient of one indicates that the membrane is impermeable to the solute while a reflection coefficient of zero indicates that the solute is freely permeable in the membrane. Table IV lists the reflection coefficients for the basement membrane prepared by the three different methods, as discussed previously.

TABLE V

DETERMINATION OF FILTRATION COEFFICIENT OF THE BASEMENT MEMBRANE OF ASCARIS MEASURED USING OSMOTIC PRESSURE[1]

Molarity	π	Dyne/CM^2 x 10^2	Vol. Flux. CM/Sec x 10^6
0	0	0	0
0.05	1.22	1.22	2.26 ± 0.24
0.10	2.44	2.44	5.05 ± 0.23
0.15	3.66	3.66	7.00 ± 0.26
0.70	4.88	4.88	8.75 ± 0.39

[1]All figures are for 1.0 CM^2 membranes at 25° C.

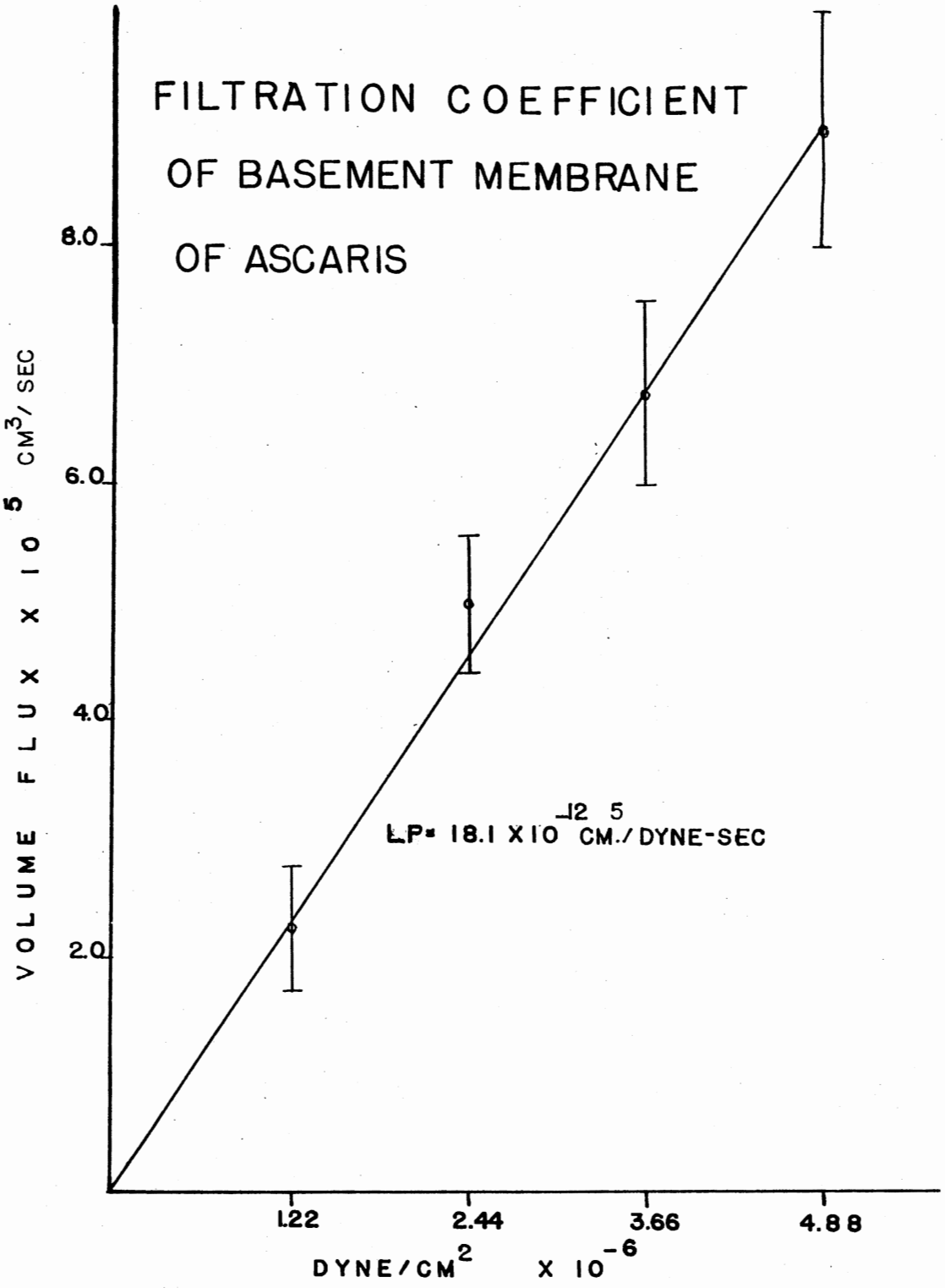

Figure 4. L_p of the Basement Membrane Determined With an Osmotic Pressure Gradient

TABLE VI

FILTRATION COEFFICIENT DETERMINATIONS USING HYDROSTATIC PRESSURE GRADIENT ON THE BASEMENT MEMBRANE

Hydrostatic Pressure CM of H_2O	Vol. Flux x 10^5	Hydrostatic Pressure in Dyne/CM^2 x 10^{-3}
3.5	1.6	3.4
3.8	2.1	3.7
4.1	2.2	4.0
5.0	2.4	4.9
5.8	2.5	5.6
9.2	3.1	9.0

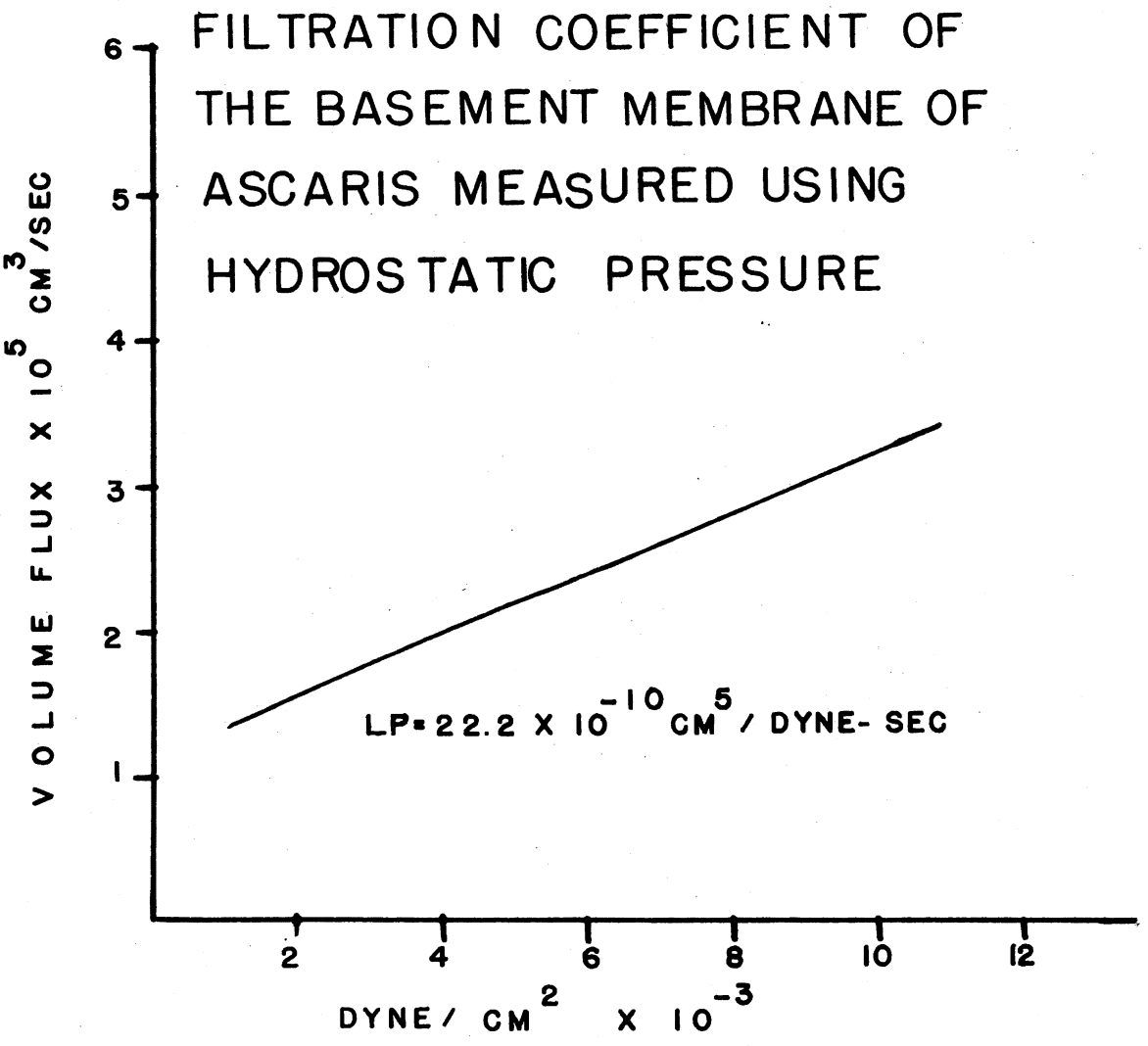

Figure 5. L_p of the Basement Membrane Determined With a Hydrostatic Pressure Gradient

Equivalent Pore Size Determinations

Pappenheimer's Method. Determinations of the average pore size of the intestinal basement membrane of Ascaris using Pappenheimer's equation,

$$r_p = 2\sqrt{\frac{2L_p\eta}{\frac{A_0}{\Delta x}}} \quad ,$$

allows for calculation of the pore radius and the results are given in Table IV.

Pappenheimer's method used the filtration coefficient in estimating the average pore radius. As has been previously established the filtration coefficient can be determined by creating either an osmotic or a hydrostatic pressure gradient across the membrane. For an osmotic pressure gradient, a filtration coefficient of 18.1×10^{-12} cm^5/dyne-sec is measured. If a hydrostatic pressure gradient is established, a filtration coefficient two orders of magnitude higher, 22.2×10^{-10} cm^5/dyne-sec, is measured. When these filtration coefficients are subsequently used in the Pappenheimer equation for calculation of the average pore radius, drastic differences in the pore size are seen. Table VII is a comparison of the average pore size of the intestinal basement membrane of Ascaris suum as measured by using hydrostatic and osmotic pressure gradients to determine the filtration coefficient. With the hydrostatic pressure measurements for determinations of the filtration coefficient, the average pore radius is ten times that of the previous method.

Goldstein and Solomon Method. Using Renkin's equation for

TABLE VII

COMPARISON OF THE AVERAGE PORE SIZE OF THE BASEMENT MEMBRANE OF ASCARIS AS MEASURED USING HYDROSTATIC AND OSMOTIC PRESSURES

	Sonicator	Sodium Chloride	Glass Beads
Osmotic Pressure	24.3 Å	24.8 Å	22.3 Å
Hydrostatic Pressure	268.0 Å	275.0 Å	247.0 Å

filtration, Goldstein and Solomon (18) expressed the ratio of the apparent area for filtration of the solute (A_{sf}) to the apparent are for filtration of water (A_{wf})

$$\frac{A_{sf}}{A_{wf}} = \frac{[2(1 - \frac{a}{r})^2 - (1 - \frac{a}{r})^4][1 - 2.104\,\frac{a}{r} + 2.09\,(\frac{a}{r})^3 - 0.95\,(\frac{a}{r})^5]}{[2(1 - \frac{A_w}{r})^2 - (1 - \frac{A_w}{r})^4][1 - 2.104\,\frac{A_w}{r} + 2.09\,(\frac{A_w}{r})^3 - 0.95\,(\frac{A_w}{r})^5]}$$

where a is the molecular radius of the probing molecule, a_w is the molecular radius of water and r is the equivalent pore radius, knowing that

$$1 - \sigma = \frac{A_{sf}}{A_{wf}} \quad .$$

Rewriting these two equations, they become:

$$1 - \sigma = \frac{[2(1 - \frac{a}{r})^2 - (1 - \frac{a}{r})^4][1 - 2.104\,\frac{a}{r} + 2.09\,(\frac{a}{r})^3 - 0.95\,(\frac{a}{r})^5]}{[2(1 - \frac{A_w}{r})^2 - (1 - \frac{A_w}{r})^4][1 - 2.104\,\frac{A_w}{r} + 2.09\,(\frac{A_w}{r})^3 - 0.95\,(\frac{A_w}{r})^5]}.$$

Goldstein and Solomon plotted $(1 - \sigma)$ as a function of the radius of the test molecule for various equivalent pore radii. A family of theoretical curves was obtained with the equivalent pore radius as the parameter (see Figure 6). The experimentally determined values of $1 - \sigma$ for the permeating molecules through the intestinal basement membrane are given in Table IV. The best fit curve for these values for the pore radius is 16.88 Å and thus is the equivalent pore radius for the basement membrane (see Figure 7).

Permeability Coefficients

The permeability coefficients (P_s) were determined for these same permeating molecules. The radioactive volume flux was plotted against

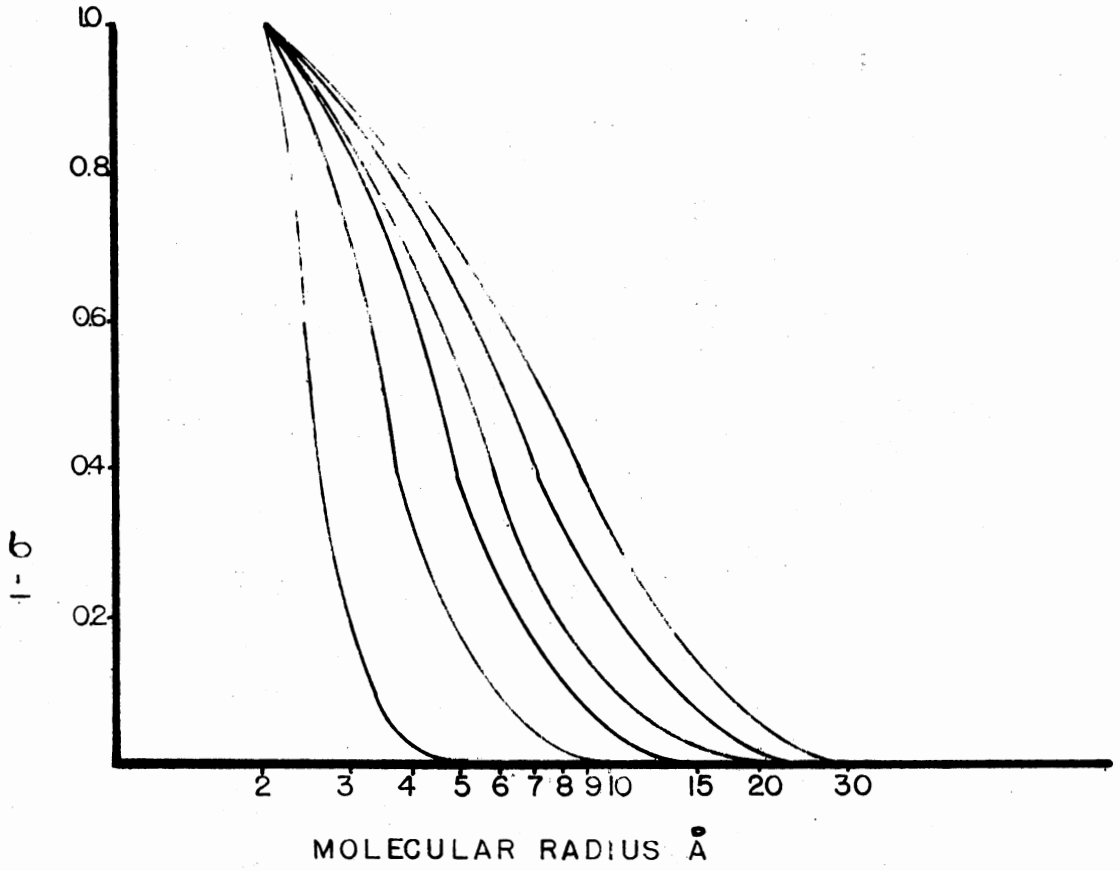

Figure 6. Theoretical Curve of 1 - σ Versus Molecular Radius

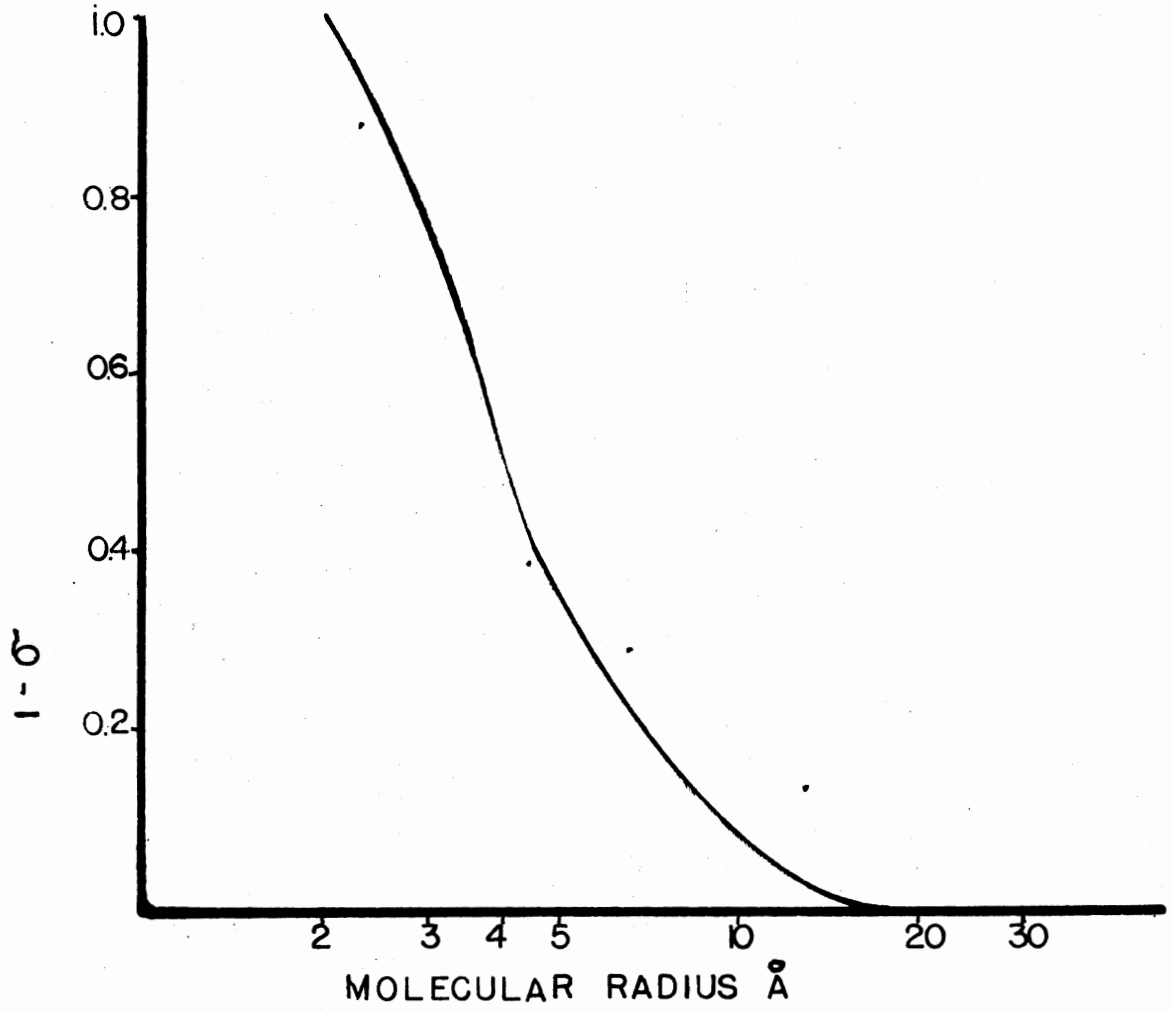

Figure 7. Plot of 1 - σ Versus Molecular Radius for Basement Membrane

time and a linear regression was used to determine the slope of the line which is the classical permeability coefficient (see Table VIII).

The permeability coefficient is related to the apparent solute permeability coefficient (ω) by the equation

$$P_s = \omega' RT$$

where R is the gas constant 8.317 erg/deg/mole and T is the absolute temperature.

To ascertain the absolute solute permeability coefficient (ω), the magnitude of the unstirred layer must be defined. Unstirred layers are regions of slow laminar flow parallel to the membrane (9). According to Dainty (9) the thickness of the unstirred layer can vary between 20 and 500 μ, depending on the rigorousness of the stirring.

In our system the permeability coefficient was found to be dependent on the air lift apparatus at slow bubbling speeds. At rates below three bubbles per second the unstirred layer is considerable and the permeability of water to the membrane was decreased. At very fast rates of the air lift, the unstirred layer was reduced to a minimum and the permeability of the membrane to water remained constant (see Figure 8).

To calculate the unstirred layer measurements of the diffusion of tritiated water across the basement membrane, the permeability coefficient was compared with the filtration coefficient of the membrane as determined earlier under an osmotic pressure gradient. Cass and Finkelstein (13) have shown that in the case of zero unstirred layer, these two coefficients (P_s and L_p) are equal. The ratio of the two permeability coefficients may be used to estimate the thickness of the unstirred layer. I use the equation derived by Milgram (45),

TABLE VIII

PERMEABILITY COEFFICIENTS

Substance	T	$P_s \times 10^4$ CM/Sec	$\omega' \times 10^{14}$ Mol/Dyne/Sec	$\omega^0 \times 10^{14}$ Mol/Dyne/Sec
Water	25	9.74	3.93	4.58
Urea	25	8.34	3.37	5.23
Glucose	25	6.43	2.59	6.22
Sucrose	25	6.26	2.53	8.56
Inulin	25	2.53	1.02	42.74

[1] $R = 8.314 \times 10^7$ ERG/DEG/MOLE

[2] $T = 298$ K.

[3] $P_s = \omega' RT$

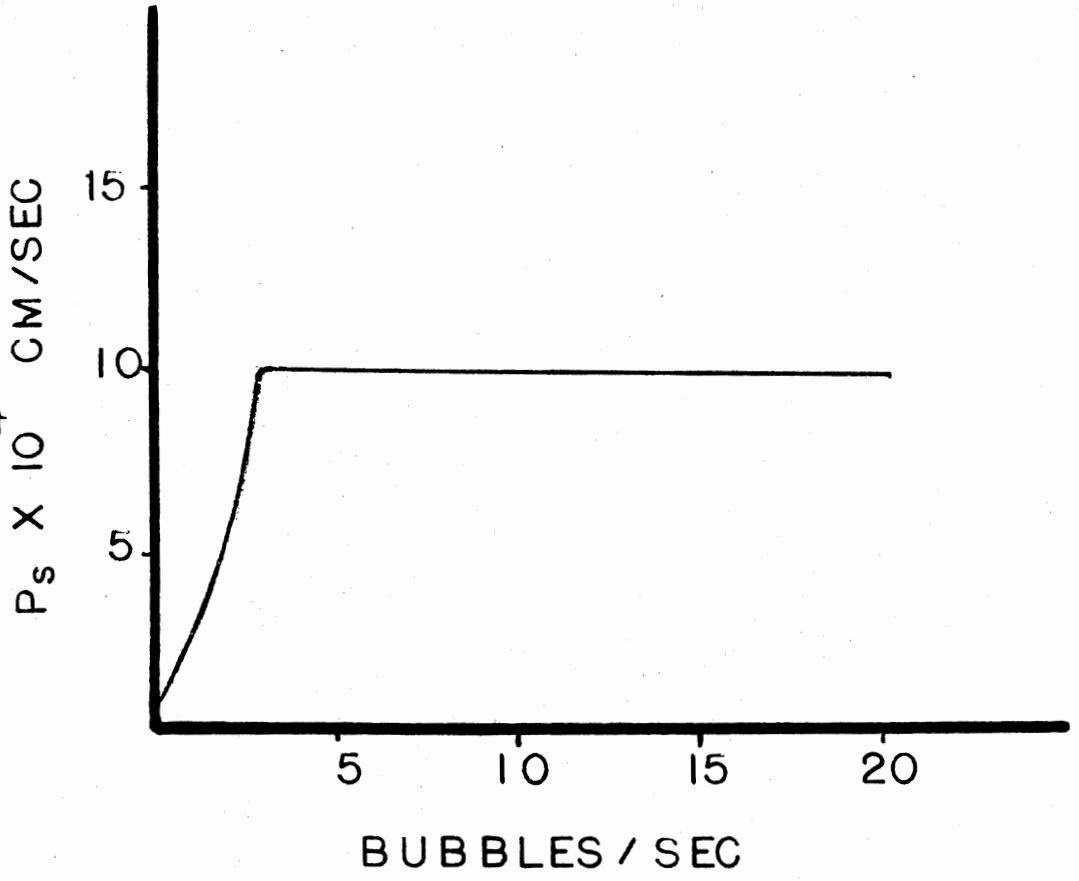

Figure 8. Unstirred Layer Graph

$$\frac{1}{P_{s(TRUE)}} = \frac{1}{P_{s(OBS)}} - 2\frac{t}{D}$$

where $P_{s(true)}$ is the filtration coefficient (L_p which is equal to $P_s = LP(RT/\bar{v}_\omega)$ where $\bar{v}_\omega$ is the molar volume of water), $P_{s(obs)}$ is the tritiated water permeability coefficient, D is the diffusion coefficient, and t is the thickness of the unstirred layer on both sides of the basement membrane. The total unstirred layer is thus equal to a t value of 61.8 μm for the basement membrane of Ascaris suum using our air lift system. The unstirred layer on one side of the membrane thus is equal to half this value or 30.9 μm.

The absolute permeability coefficient can be determined from the equation (13)

$$\frac{1}{\omega'} = \frac{1}{\omega^0} + \frac{2RT}{D}\delta$$

where ω^0 is the absolute solute permeability coefficient and is given in Table VIII.

Electrolytes

The permeability coefficients (P_s) were determined for sodium, potassium and chloride ions to try and determine the net charge on the intestinal basement membrane. Sodium and potassium ions each have a permeability coefficient of 4.0×10^{-4} cm/sec while chloride ions have a permeability coefficient of 7.0×10^{-4} cm/sec. Since the chloride ions diffuse more readily in the membrane there is a net positive charge on the basement membrane. To confirm this finding, that the net charge on the membrane was positive, the pH of the bathing solution was raised to a value of 10.5, thus effectively blocking the net positive charge. The

permeability coefficient for sodium, potassium and chloride ions were again measured and the results are given in Table IX. The membrane was now found to be more permeable to sodium and potassium ions. If the pH is lowered to 2.5, no change in ion selectivity is observed. To confirm our findings, the bathing medium was kept at a pH of 7.2 and the positive charges on the membrane blocked by the addition of FFDNB to the bathing medium. The membrane increased its permeability for sodium and potassium ions. If the negative sites in the membrane are blocked by the addition of a 5.25 mM calcium ion solution, the membrane is then more permeable to the chloride ions. Collectively the evidence indicates that at a physiological pH the net charge on the basement membrane of the intestine of Ascaris suum is positive. Figures 9 and 10 are diagrams of the effects of blocking the charges on the membrane and observing the subsequent changes in permeability of the membrane to the permeating molecules.

TABLE IX

P_s AT DIFFERENT pH'S AND WITH VARIOUS BLOCKING AGENTS

Substance	Molecular Radius	$P_s \times 10^4$ CM/Sec pH 7.2	$P_s \times 10^4$ CM/Sec pH 2.3	$P_s \times 10^4$ CM/Sec pH 10.5
Cl^-	2.9	7.0	8.2	7.0
Na^+	4.5	4.0	4.0	8.2
K^+	2.9	4.0	4.0	8.0
			FFDNB	$CaCl_2$
Cl^-	2.9	7.0	6.0	28.0
Na^+	4.5	4.0	11.5	5.0
K^+	2.9	4.0	12.0	5.4

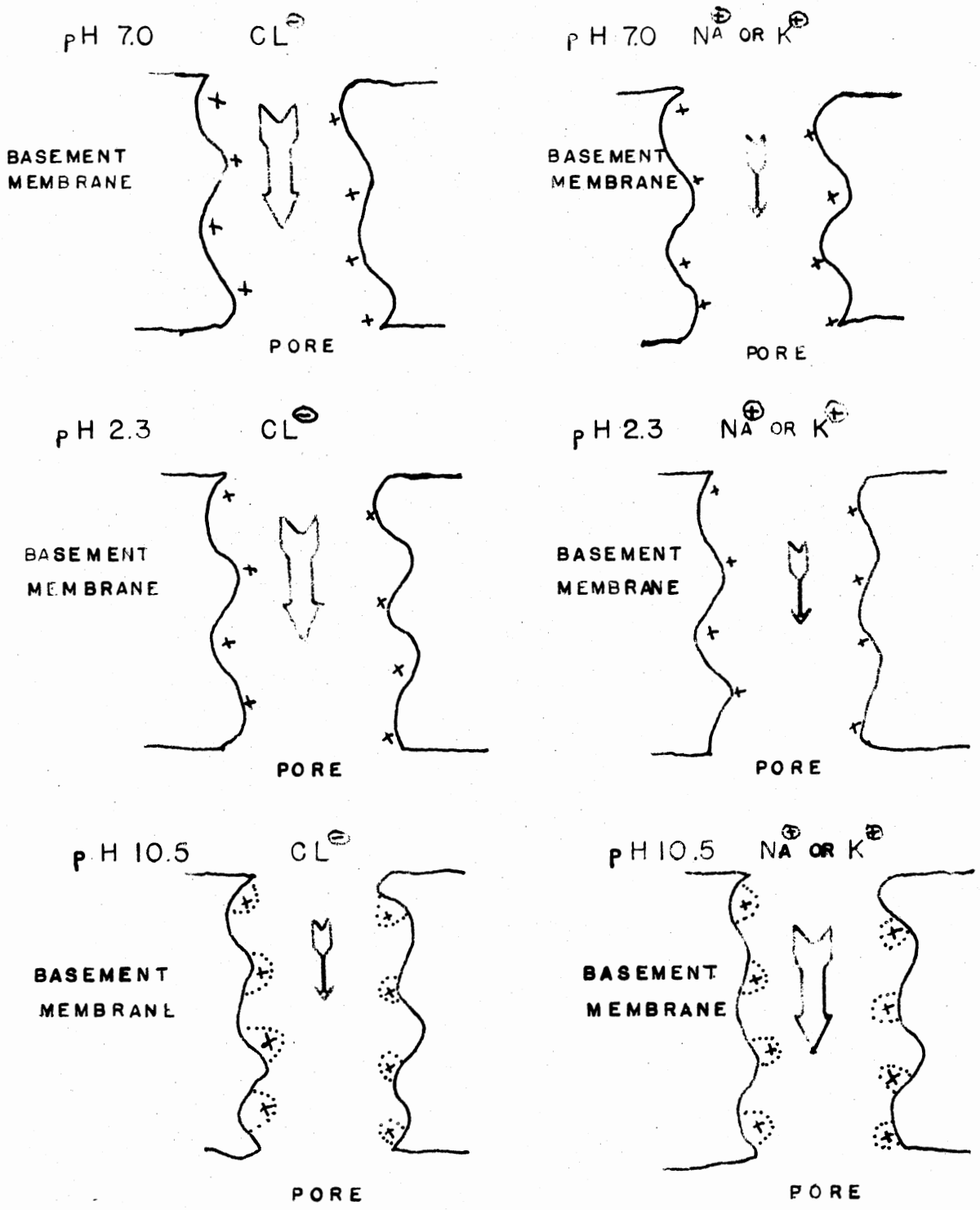

Figure 9. Diagram of the pH Effects on P_s of the Basement Membrane

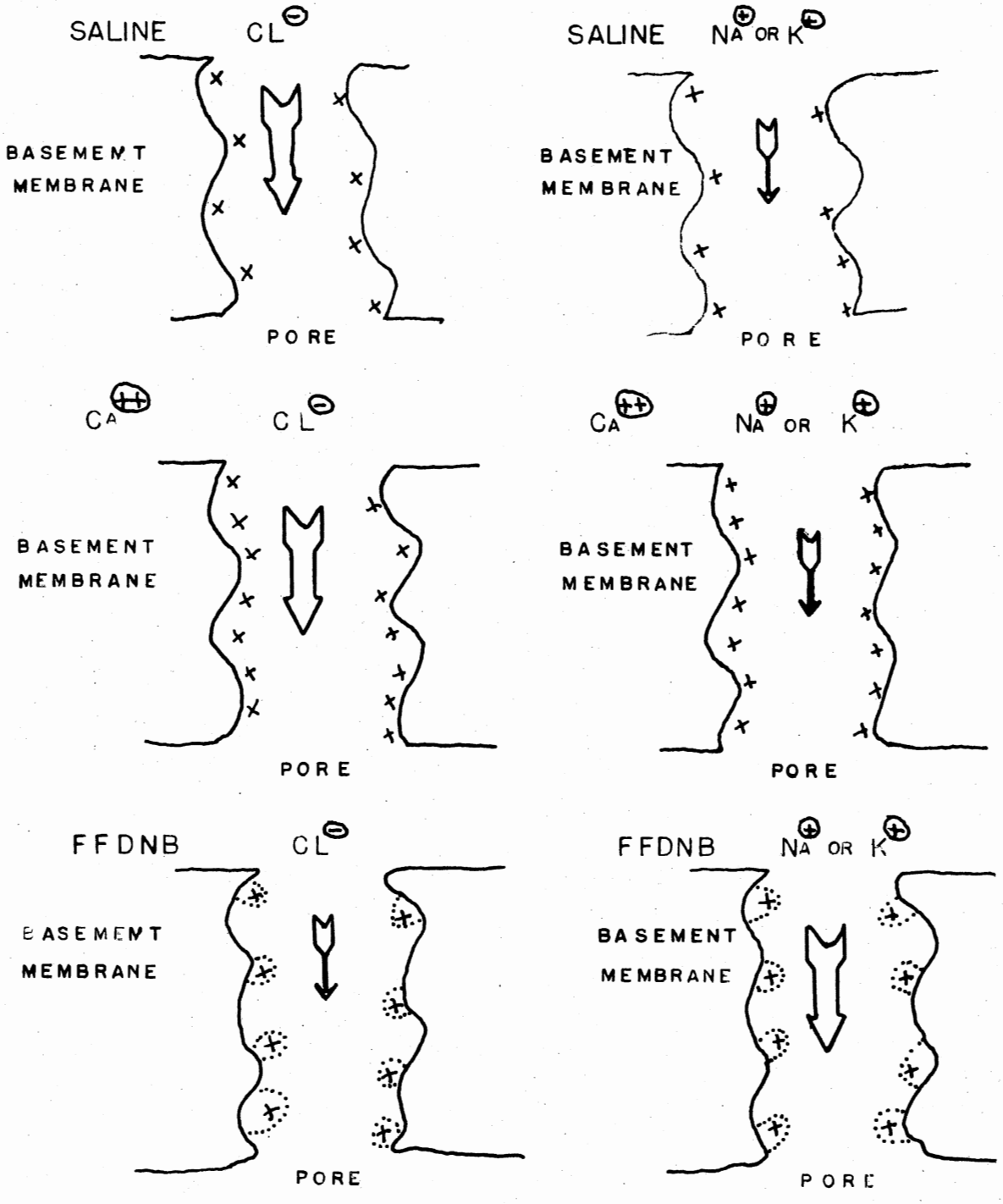

Figure 10. Diagram of the Blocking Compound Effects on P_s of the Basement Membrane

CHAPTER V

DISCUSSION

The intestinal basement membrane of Ascaris suum functions as a support structure upon which the overlying columnar epithelial cells rest. This basement membrane also functions as a diffusion barrier which can be seen by examining its permeability characteristics to a series of non-electrolytes of graded molecular size. The permeability characteristics which completely describe a membrane (28) are the pore radius, the filtration coefficient, the reflection coefficient, and the solute permeability coefficient. Collectively these characteristics establish the intestinal basement membrane of Ascaris suum as a barrier to diffusion.

Determining the permeability characteristics of the intestinal basement membrane to various hydrated ions, both anions and cations, allows for the further identification of this membrane as an ion selective barrier. The basement membrane is more permeable to negatively charged ions than to positively charged ions. This information coupled with the transmural potential work of Merz (35) and biochemical analysis of Peczon, et al. (43) showing a complete lack of sialic acid in the membrane, convincingly demonstrates that the net electrical charge in the basement membrane is positive.

Pore Size

We have used two separate techniques in the determination of the equivalent pore radius of the intestinal basement membrane of Ascaris suum. Pappenheimer's method is based on the direct measurements of the $A/\Delta x$, the relative geometric pore area per unit path length, in the membrane with isotopically labelled molecules of graded molecular size. It is independent of the fact that the pores are perpendicular to the membrane surface and that all the water in the membrane is free. Pappenheimer's method considers two factors restricting the diffusion of spherical molecules through pores; the first condition is that the permeating molecule must pass through the opening of the pore without striking the edge of the pore and secondly, viscous resistance inside the pore should be as if no pore were present. This method is further dependent upon the measurement of the filtration coefficient. The filtration coefficient is measured by determining the radioactive volume flux of water through the membrane created by a pressure gradient. Consequently, knowing the $A/\Delta x$ values and the filtration coefficient allows for calculation of the average pore radius of the basement membrane. Using the Pappenheimer method (40), values of 24.3 Å, 24.8 Å, and 22.3 Å were measured for the basement membrane prepared by sonication, increasing sodium chloride concentration in the holding medium, and shaking the intestine with glass beads, respectively.

A second method, the Goldstein and Solomon method, makes use of the reflection or Staverman coefficient, (σ). The osmotic pressure developed across the membrane by a substance to which the membrane is permeable is always smaller than that produced by a nonpermeating molecule (65). If the solute molecules permeate across the membrane through the same

channels as water, the movement of solute and water interact in the membrane and the observed osmotic pressure (π_{obs}) may be lower than the theoretical osmotic pressure (π_{theor}). The ratio of the two pressures is the reflection factor, .

$$\sigma = \frac{\pi_{OBS}}{\pi_{THEOR}}$$

It was stated by Durbin (13) and shown in detail later by Durbin (14) that

$$1 - \sigma = \frac{A_{sf}}{A_{wf}}$$

where A_{sf}/A_{wf} is the relative geometric pore area per unit path length ($A_{sf}/\Delta x$) of the solute divided by the relative geometric pore area per unit path length ($A_{wf}/\Delta x$) for water. Goldstein and Solomon plotted ($1 - \sigma$) as a function of the radius of the test molecule to determine the best fit curve for the pore radius. For the basement membrane of Ascaris a value of 16.88 $\pm$ 4.7 Å was measured. The main point of the criticism of this method is that $1 - \sigma$ is always different from zero for a permeating solute, even if there are no pores in the membrane (9, 10).

The average pore size of the basement membrane determined by these two methods is about 17 Å and 24 Å. Statistically they are from the same group. An average pore radius of 17-24 Å moves through this membrane very slowly. Most molecules, however, that would normally be passing into the pseudocoelomic cavity (molecules such as glycerol with a molecular radius of 2.74 Å, or glucose with a molecular radius of 4.40 Å, and small molecular weight amino acids, dipeptides or tripeptides), all are sufficiently small to pass through the basement membrane by simple diffusion, i.e., without an active carrier mediated process.

The obvious advantage of the Goldstein-Solomon method over the Pappenheimer method is the fact that it is not dependent on measurements made of the filtration coefficient, as is the Pappenheimer method. The reason that the filtration coefficient determination can be of concern will be discussed in the following section. The Pappenheimer method is independent of the fact that the pores are perpendicular in the membrane. This is of real advantage for characterization of a basement membrane. The basement membrane does not have pores in the classical sense, i.e., tubes which pass through the entire width of the membrane. Rather, the basement membrane is a felt-like or cheese-cloth type of membrane through which the solutes must "trickle". Measurement of the basement membrane as a barrier to diffusion is characterized by an "average" pore radius, or a theoretical pore which, if it did exist, would impede the free flow of solutes through it.

Determination of the Filtration Coefficients

The filtration coefficient is measured by determining the radioactive volume flux of water through the membrane created by a pressure gradient. This pressure gradient may be established either by increasing the sucrose concentration on one side of the membrane, an osmotic pressure gradient, or by the addition of a water column to one side of the membrane to create a hydrostatic pressure gradient. According to Onsager (37) the flux of water through the membrane is the same regardless of the type of pressure gradient established, i.e., osmotic or hydrostatic pressure. It is not obvious that a concentration difference across a membrane should have the same effect as a pressure difference and this leads to an osmotic bulk flow rather than a diffusion of water

through the pores in the membrane. The fact that the same coefficient, L_p, is used to describe flow of water through a semipermeable membrane under the action of either a hydrostatic or an osmotic pressure difference doesn't imply that measurements of the same coefficients, L_p, on a basement membrane using both a hydrostatic and osmotic pressure difference will yield comparable results.

In determining the filtration coefficient on the intestinal basement membrane of Ascaris, a value of 18.1×10^{-12} cm^5/dyne-sec was obtained using an osmotic pressure gradient and a value of 22.2×10^{-10} cm^5/dyne-sec was obtained using a hydrostatic pressure gradient. Knowing from Onsager's relationship (see Chapter II) and from the work done with artificial membranes (33, 34) that the osmotic pressure is equivalent to hydrostatic pressure gradients, within the semipermeable membrane, the question arises as to why the apparent discrepancy in the filtration coefficient determinations.

Other workers in the field have observed and reported differences in the filtration coefficient using hydrostatic and osmotic pressures in the same living membrane. Coulter (57) determined the filtration coefficient of the blood-brain barrier in rabbits by applying a hydrostatic pressure. Fenstermaker and Johnson (57) measured a filtration coefficient across the same barrier by using an osmotic pressure gradient, and filtration coefficients two orders of magnitude lower than the one measured by the hydrostatic pressure method were seen. This was the same type of results found with the giant squid axon by Vargas (58) and with the alga cells (58) and also in our experiments with the intestinal basement membrane of Ascaris suum.

The explanation for the discrepancy in the filtration coefficients

measured using two different pressure gradients lies with the phrase "within the membrane". Mauro (34) has shown that for an artificial membrane the osmotic pressure is equivalent to the hydrostatic pressure measured within the membrane. The fact that these two pressures are equal within the membrane is due to the fact that the artificial membrane contains water-filled pores passing through the width of the entire membrane. Basement membranes, as was previously discussed, do not contain typical cylindrical-like pores which traverse the entire membrane. The glycoprotein structure of the membrane looks rather like felt-work. One or more channels of a larger diameter could permit a high flux of solute through the membrane when a hydrostatic pressure is applied, thus accounting for the increased filtration coefficient with the increased hydrostatic pressure. Another possibility is the expansion of the pores caused by a hydrostatic pressure increasing the permeability of the membrane to water and solutes. Again the effect is one of increasing the flow of water through the membrane, and hence the filtration coefficient.

Collectively these filtration coefficients are used in determining the average pore radius as given in Table VII. Note the vast difference between the average pore radius as determined using the two different filtration coefficients. This again shows the advantage of using the Goldstein-Solomon method of pore radius determinations, since it does not depend on the filtration coefficient measurements.

Two sources of error do occur in the calculations of the filtration coefficients by establishing an osmotic pressure gradient. One is that sucrose is not completely impermeable to the basement membrane, i.e., a reflection coefficient of 0.73 as determined by our experimentation, and as a result the calculated osmotic pressure is slightly greater than that

actually created across the membrane. However, the same solute, sucrose, was used in creating all of the osmotic pressure gradients and would effect the calculated osmotic pressure gradients at all concentrations to an equal and proportional degree. As such, it would not effect the slope of the line which is used in the determination of the actual filtration coefficients. Also, the time interval for the experiment was of short duration, allowing for the diffusion of only a very small amount of sucrose, and thus effecting the decrease in osmotic pressure on the side of the chamber only slightly.

The second error results from the "opposite" diffusion of water against either the osmotic or hydrostatic pressure gradient. The actual volume flux of water through the membrane would have been expected to have been smaller. This would be the diffusion of water driven by the osmotic or hydrostatic pressure minus the diffusion of water that occurred against the concentration gradient, i.e., the net volume flux of water. The reason this was not calculated was that the experiment was done during very short time intervals and the opposite diffusion then would be negligible.

Permeability Coefficients

In measuring the average pore radius of the basement membrane, three different methods for removing the epithelial cells were used. The comparison of the average pore radii as determined by these methods in conjunction with the electron photomicrographs indicates that both the glass bead and sodium chloride preparations fail to remove completely the epithelial cells from the basement membrane. Consequently, all the studies carried out on the permeability characteristics of the basement

membrane use the basement membrane prepared solely by the sonication method.

The classical permeability coefficient (P_s) has been determined for a series of non-ionic molecules of graded molecular size. The apparent solute permeability coefficient (ω') is derived from the equation $\omega' = P_s/RT$. In order to calculate the absolute solute permeability coefficient ω, the effect of the unstirred layer had to be determined.

The unstirred layers are regions of slow laminar flow parallel to the membrane. This means that in the modified Ussing chamber used in these experiments, with the air lift system reducing the unstirred layer to a minimum, the apparatus still had a calculated unstirred layer on one side of the chamber of 30.9 μm. Thus during permeability coefficient measurements the diffusing molecules were diffusing first through an unstirred layer of 30.9 μm, and subsequently through the membrane and finally through another unstirred layer on the opposite side of the chamber. This unstirred layer was kept to a minimum value of 30.9 μm only if the air-bubble rate was greater than three bubbles per second.

Uncorrected permeability constants higher than 10^{-4} cm/sec (31) are likely to be considerably affected by the presence of the unstirred layer for the permeability of the unstirred layer may be of the same order of magnitude as the membrane itself. Unlike the classical permeability coefficient P_s, the filtration coefficient L_p of the membrane is only very slightly affected by the unstirred layer (9), and so is not of concern in the differences seen between the hydrostatic and osmotic pressure measurements on the filtration coefficient (65).

Ion Selectivity

The transmural potential existing across the *in vitro* intestine of *Ascaris suum* is pseudocoelomic negative with respect to a positive luminal electrode. Merz (35) and Beames, *et al.* (3) have postulated that the reason for this positive luminal and pseudocoelomic negative potential difference is due to the basement membrane. Merz excogigated that the basement membrane is more permeable to chloride ions, allowing for faster diffusion through the membrane and thus a net negative pseudocoel. The tight junction between the columnar epithelial cells at the apex of these cells near the lumen appears to be a "leaky" tight junction allowing for a more rapid diffusion of positive ions back into the lumen of the intestine.

To determine if the basement membrane is an ion selective barrier, characterization of the membrane to various ions was investigated. The membrane is more permeable to chloride ions, 7×10^{-4} cm/sec, than it is to sodium or potassium ions, 4×10^{-4} cm/sec. Collectively this information suggests that the net electrical charge in the basement membrane is positive. At a high pH of 10.5 the positive charges are blocked and the basement membrane becomes more permeable to sodium and potassium ions. If the high pH fluid is replaced with our normal basal saline at a pH of 7.2, the permeability coefficient for sodium and potassium returns towards the normal value of 4.0×10^{-4} cm/sec, although it doesn't completely return to normal even after washing the tissue three times, presumably because of the remaining negative charges in the interstices of the basement membrane. At low pH values no permeability changes are noted because the net charge in the membrane is still positive.

When the bathing medium is kept at a neutral pH and the positive

charge in the membrane is blocked by the addition of 1,5 difluro, 2,4 dinitrobenzene (FFDNB) to the medium, the permeability of the sodium and potassium ions is increased. If the FFDNB is removed and replaced with our normal basal saline, the permeability characteristics for sodium and potassium return towards normal. When the negative sites in the membrane are blocked by the addition of a 5.25 mM calcium ion solution, the permeability of the sodium and potassium ions in the membrane remains unchanged.

Collectively the information demonstrates that the net charge in the intestinal basement membrane of Ascaris is positive, being more permeable to the negatively charge ions than to the positively charged ions. This gives credence to the hypothesis that the basement membrane is largely responsible for the negative transmural potential of the gut of Ascaris suum.

CHAPTER VI

SUMMARY AND CONCLUSION

The intestinal basement membrane of Ascaris suum functions as a support structure upon which the overlying epithelial cells rest. It functions also as a barrier to the free diffusion of electrolytes and non-electrolytes. To substantiate the latter, that the basement membrane functions as a barrier to simple diffusion, determinations were made as to the pore size, the filtration coefficient, the reflection coefficient and the solute permeability coefficient for a series of non-electrolytes of graded molecular size. This basement membrane functions further as an ion selective barrier, being more permeable to anions than to cations.

1. Pore size measurements made using Pappenheimer's method gave the results of 24 Å and the Goldstein and Solomon method gave a result of 17 Å.

2. Filtration coefficient measurements made using an osmotic pressure gradient gave an L_p of 18.1×10^{-12} cm^5/dyne-sec. A filtration coefficient measured using a hydrostatic pressure gradient gives an L_p of 22.2×10^{-10} cm^5/dyne-sec.

3. The reflection coefficient measurements made for the basement membrane prepared by sonication were 0.15, 0.61, 0.73 and 0.81 for urea, glucose, sucrose, and inulin, respectively.

4. Permeability coefficients were determined for water, urea, glucose, sucrose and inulin and the results are 9.74×10^{-4} cm/sec.,

8.34×10^{-4} cm/sec, 6.43×10^{-4} cm/sec, 6.26×10^{-4} cm/sec, and 2.53×10^{-4} cm/sec, respectively.

Collectively these results allow for characterization of the basement membrane as a barrier to diffusion.

This basement membrane also functions as an ion selective barrier. To demonstrate this the permeability coefficients were made for various ions. A permeability coefficient of 4.0×10^{-4} cm/sec was determined for sodium and potassium ions while chloride ions have a permeability coefficient of 7.0×10^{-4} cm/sec.

1. The P_s determined with FFDNB as a blocking agent of the positive charges in the membrane increased to sodium and potassium and remained constant for chloride ions.

2. At high pH's of 10.5, again the P_s was increased to sodium and potassium ions.

Collectively this indicates that the intestinal basement membrane of Ascaris suum is more permeable to anions than to cations. This supports the theory that the ionic selectivity of the basement membrane plays a major role in establishing the transmural potential across the gut of the worm.

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VITA[2]

Manus Joseph Donahue

Candidate for the Degree of

Doctor of Philosophy

Thesis: PERMEABILITY CHARACTERISTICS AND ION SELECTIVITY IN THE BASAL LAMELLA OF ASCARIS SUUM

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Yeadon, Pennsylvania, September 20, 1946, the son of Rose Teresa and Manus J. Donahue.

Education: Graduated from St. Bernadette Grammar School in Drexel Hill, Pa., in June, 1960. Graduated from Monsignor Bonner High School in Drexel Hill in June, 1964; received the Bachelor of Science degree in May, 1968, from Villanova University in Villanova, Pa. with a major in Biology; received the Master of Science degree in August, 1974, from North Texas State University, in Denton, Texas with a major in Biology; completed requirements for the Doctor of Philosophy degree in July, 1977, from Oklahoma State University, in Stillwater, Oklahoma with a major in Physiological Sciences.

Professional Experience: Taught biology and chemistry in high school in Kabala, Sierra Leone, West Africa as a Peace Corps Volunteer from Sept., 1968-Sept., 1972; Graduate Teaching Assistant at NTSU in Introductory Biology from Jan., 1973-July, 1974; Graduate Teaching Assistant at OSU in BiSci, Sept., 1974-June, 1975; Graduate Teaching Assistant in Introductory Physiology and Comparative Physiology, Sept., 1976-Dec., 1976; Cell Physiology and Metabolism, Jan., 1977-May, 1977.