BIOMEMBRANES

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Preface

Both science and religion are aspects of human endeavor that do not observe political constraints. It is therefore appropriate that contributions should come from many different countries for a series which attempts to chronicle developments in an interdisciplinary field such as membrane research. This volume is an excellent example of the diversity of thinking, background, and approach needed by the working scientist for his research planning.

From Canada comes a review by Silverman and Turner of the mechanisms by means of which the plasma membrane of the renal proximal tubule acts as a transport mediator. The two chapters that were written by American scientists are excellent examples of the comparative biochemical approach. Inouye feels he must apologize for being interested in the outer membrane of *E. coli*, but it is obvious, after a reading of his chapter, that no apology is required. On the contrary, we are grateful for his drawing our attention to this system and its unique properties. Holtzman, Gronowicz, Mercurio, and Masur are also on a consciousness-raising mission in summarizing for us a number of integrated functions of membranes using the toad bladder as an experimental system.

The other two chapters of this volume come from overseas. Northcote has again demonstrated his capacity to integrate a complex and difficult field. His chapter is an excellent summary of the cellular role of the Golgi apparatus and how it may function in glycoprotein and polysaccharide synthesis and secretion. The last chapter comes from the Soviet Union. It is a review by Ostroumov, Jasaitis, and Samuilov of the data describing the electrochemical proton gradient in phosphorylating bacteria with a special emphasis on the properties of bacteriorhodopsin.

It can be hoped that the advancement of science and human understanding may soon become a universal goal of all countries and peoples. Such is not the case today while this is being written. It is very difficult, if not impossible, for the basic scientist to concentrate only on his labo-

ratory research and be unaware of the world in which he lives. It seems almost utopian to look forward to a time when the privilege to carry on basic research for the advancement of civilization will be considered a fundamental human right.

Lionel A. Manson

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Chapter 1

The Renal Proximal Tubule

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I. INTRODUCTION

Methodologic as well as conceptual progress during the past decade has made it possible for renal physiologists to "peek" inside the epithelial "black box" of renal tubular function with a greater degree of confidence than ever before. Among the more important developments have been: (1) evolution of the fluid mosaic model of plasma membrane structure (Singer and Nicolson, 1972), (2) emergence of a role for the cytoskeleton as a regulator of membrane function (Nicolson and Poste, 1976), (3) maturation of the concept that electrochemical potential gradients can provide the driving force for transport systems, i.e., Na^+ gradient, chemiosmotic hypotheses (Crane, 1977; Mitchell, 1976) without being coupled directly to metabolic intermediates, (4) application of sophisticated electrophysiologic measurements to the elucidation of renal transport processes at opposing membrane surfaces (Gottschalk and Lassiter, 1973), (5) *in vitro* perfusion of isolated tubule segments (Burg and Orloff, 1973), (6) biochemical separation of epithelial cellular organelles, especially isolation and separation of clean membrane fractions from luminal (brush border) and antiluminal (basolateral) membrane surfaces (Heidrich *et al.*, 1972), and (7) *in vivo* characterization of substrate interactions with luminal as distinct from antiluminal nephron surfaces (Silverman *et al.*, 1970*a*,*b*).

In the light of these advances, it now seems appropriate to take a

fresh look at the renal proximal tubule. We shall emphasize particularly the molecular aspects of epithelial membrane function. In so doing, our purpose will be to highlight specific membrane processes and, whenever possible, to relate such mechanisms to the intact tubule in health and disease.

To help define the limits of the discussion and orient those readers who may not be overly familiar with the kidney, it is worth making a few brief introductory remarks.

In the human kidney there are approximately 1.3×10^6 functioning units called nephrons. Urine formation is initiated by transcapillary movement of solutes and water in accordance with convective and diffusive forces across a highly specialized glomerular filtration barrier. The resulting filtrate is collected in a blind loop of the nephron, called Bowman's capsule. Under normal conditions, urine is free of blood-cellular elements. The selectivity of the filtration barrier to passage of plasma constituents is determined largely by molecular size and charge discrimination capability (Maddox and Brenner, 1977). Approximately 1.5 nl/sec are filtered per nephron in the human. On a molar volume basis, the filtrate is made up almost entirely of water but includes such molecular species as sugars, amino acids, ions, and a variety of low-molecular-weight proteins. A monolayer of cells lining the tubular lumen "operates" on this plasma filtrate as it leaves Bowman's capsule, reabsorbing certain solutes and water and adding other components from blood by secretory mechanisms. Therefore, bidirectional molecular traffic between blood and urine exists continuously along the entire length of the nephron.

Tubular cells are differentiated into specialized segments depending upon their location with respect to the glomerular filter. The very first proximal portion, or tubule, begins abruptly as a continuation of the parietal epithelium of Bowman's capsule and is lined by columnar cells with a well-developed microvillar surface. This segment traces a tortuous path several millimeters long through the superficial cortical region of the kidney and is referred to as the pars convoluta. Before dipping into the medulla, the tubule "straightens out" and is then referred to as the pars recta. There are clearly identifiable morphologic differences between cells of the convoluted and straight portions of the proximal tubule. Not surprisingly, these structural variations are reflected at the functional level. Moreover, these differences are species dependent. For the purpose of this review we shall restrict our attention almost exclusively to the pars convoluta. To a first approximation, this segment may be considered as a homogeneous cylindrical epithelial monolayer lying on a supporting basement membrane. The luminal or brush border membrane

(BBM) of the proximal tubule cell faces the urine, while its basal, or antiluminal membrane (ALM), is bathed by interstitial fluid (Fig. 1).

One of the constantly recurring themes in this chapter will be the asymmetric nature of the proximal tubular epithelium, resulting from differences in the opposing plasma membrane surfaces (luminal vs. antiluminal). It is of fundamental importance to understand the regulation of this asymmetry, since it is this property that ultimately determines the unique functional capabilities exhibited by all epithelia. In addition to morphologic considerations, we shall emphasize biochemical and physiologic manifestations of tubular asymmetry. Our aim will be to focus on the manner in which such asymmetries are reflected in global terms at the level of whole-organ function. We have in mind particularly such complex phenomena as integration of transport and metabolism, integration of hormone action and transport, and coupling of transport activities at opposing plasma membrane surfaces.

In the final sections we will consider models that rationalize certain

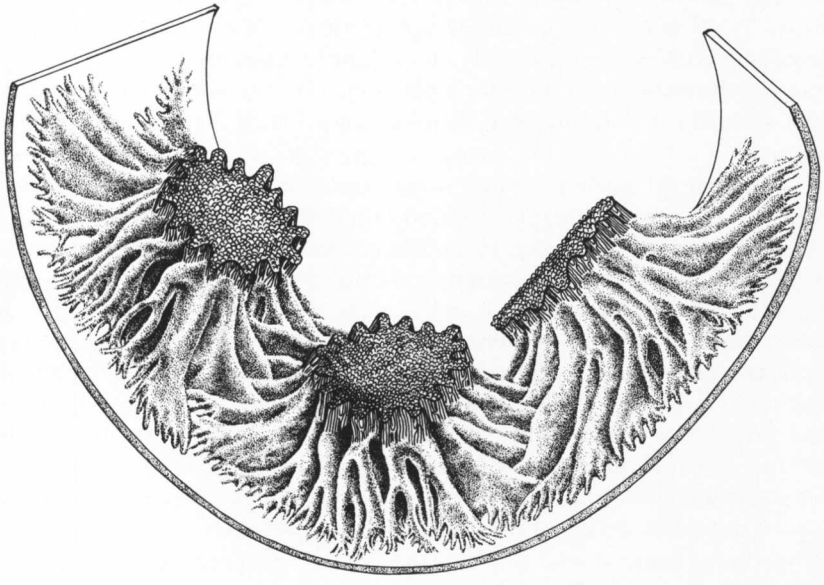

Fig. 1. Three-dimensional representation of a portion of the proximal tubular epithelium. Three cells are shown with their microvillar surfaces facing the lumen and their basal interdigitating processes resting on the tubular basement membrane. For clarity the tight junctions which couple proximal tubular cells in the apical region (see Fig. 3) have been omitted [reproduced with permission from Welling and Welling (1976)].

tubular molecular membrane defects, including those currently lumped under the clinical diagnostic umbrella of "Fanconi syndrome."

II. MORPHOLOGIC ASYMMETRY

The asymmetry of the epithelium of the proximal tubule is immediately evident on inspection, since the microvillar structure of the luminal surface stands out in marked contrast to the relatively smooth infoldings at the basal side (Fig. 1). A typical proximal tubule cell is ~15 μm in height and 20 μm in diameter. There are about 9.2×10^5 microvilli per mm of proximal tubular length, or roughly 2000–6000 per proximal tubule cell. The average height of an individual microvillus is 2.8 μm, with a diameter of ~0.5–1.0 μm.

Application of morphologic techniques (Welling and Welling, 1976) to isolated perfused proximal tubules from rabbits has shown that the luminal and lateral surfaces of the tubule cell are roughly equal in area. In the proximal convoluted tubule, each surface is approximately 2.9×10^6 μm^2/mm length. The microvillar brush border enlarges the apical surface some 36-fold in the pars convoluta and about 15-fold in the pars recta. In the pars convoluta the luminal and lateral cell surfaces each are approximately 20-fold greater than the area of the basal surface of the cell.

At the basal surface it is generally accepted that the infoldings have no particular regularity and contain no obvious fine structure. But to anyone concerned with structure–function correlations, two features are immediately striking: (1) the high degree of interdigitation between basal infoldings from adjacent cells, and (2) the close proximity of the mitochondria to the antiluminal plasma membrane. This latter characteristic suggests that some ALM functions (e.g., transport) may be highly dependent upon oxidative phosphorylation. Moreover, depending upon relative membrane permeability characteristics, certain substrates may find it just as convenient to traverse the epithelium via short-circuits between neighboring cells as to cross the cytoplasm of the same cell. Also of interest are some recent morphologic observations of Evan *et al.* (1977). Using both scanning and transmission electron-microscope techniques, these authors have obtained evidence that the basal surface possesses numerous tiny, short, nipple-like extensions devoid of organelles. To our knowledge, this is a new observation that has not been apparent from standard published transmission electron micrographs. Its significance remains to be evaluated.

In contrast to the basal infoldings at the antiluminal nephron surface,

brush border microvilli contain a well-defined cytoskeletal substructure consisting of contractile core material representing ~12% of the total microvillar protein content (Booth and Kenny, 1976). Figure 2 is a detailed schematic representation of a microvillus with its associated cytoskeletal framework. The diagrammatic features are drawn to scale and

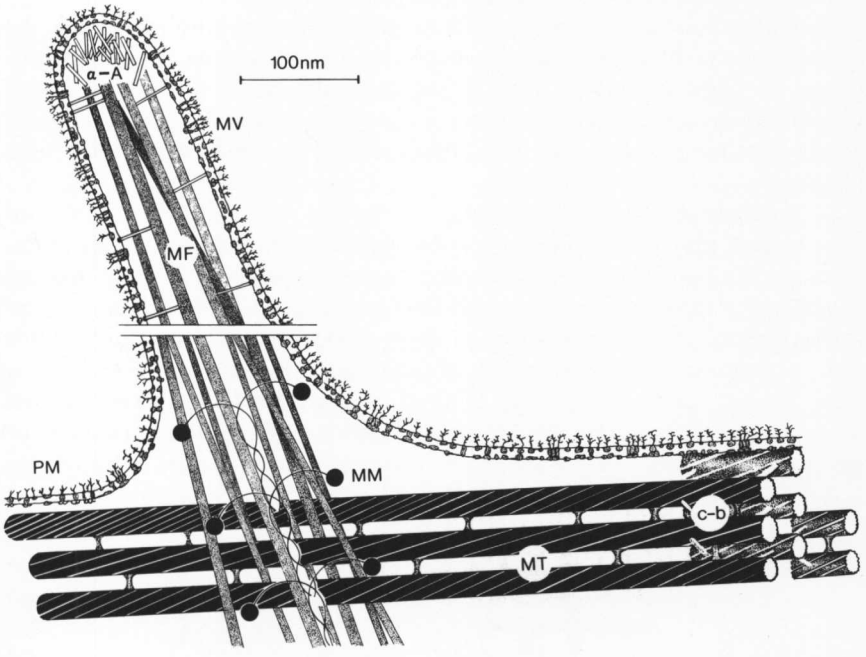

Fig. 2. Schematic representation of the brush border surface. Actual sizes are as follows: MV (microvillus), diameter ~100–200 nm with a variable length (some microprojections are several micrometers long); MT (microtubule), various lengths (up to several micrometers), ~24-nm outer diameter, ~15-nm inner diameter, made up of 13 adjacent protofilaments ~4–5 nm wide, c–b, cross-bridges with triphosphatase activity, ~2–5 nm thick and ~10–40 nm long; actin MF (microfilament) (made of two helically wound fibrous actin polymers) of varying length and ~5–8 nm thick; α–A (α-actinin), rods of ~2 × 30 nm which are concentrated in the MV tips and which determine the polarity of actin MF; although myosin filaments MM are not observed in nonmuscle cells, myosin can be detected associated with actin MF. Thus, these large molecules ~200 nm long are drawn randomly sited on top of actin MF; their light parts are arranged as to make a filament while their heavy parts (with the ATPase moiety, shown as black circles) are spread apart. The existence of a hinge between the light and heavy parts is still under discussion, and it is possible that myosin molecules are actually less flexible than represented here. For the PM (plasma membrane), the phospholipid bilayer of thickness ~4.0–4.5 nm; average cell membrane thickness ~7.5 nm. Shown are representative immunoglobulin molecules in a density of ~400 μm^{-2}. This is typical of a receptor species [with permission from Loor (1976)].

thus enable comparison of the relative sizes of plasma membrane constituents and the underlying microfilaments and microtubules. The functional role of this contractile cytoskeleton has not been clarified. However, it is worth pointing out that any process that regulates surface area could critically affect epithelial net transport activity. Microvillar contraction, such as has already been observed in the intestine (Mooseker, 1976; Rodewald *et al.*, 1976), may be one such mechanism.

What about points of contact between proximal tubule cells? An appealingly vivid approach is to begin with the "six-pack" model proposed by Diamond (1977) (Fig. 3). In this scheme, beer cans represent individual proximal tubule cells. The plastic collar (200–400 Å wide) encompassing the apex of the containers is the analogue of the tight junction.

At the level of tight junctions, connection is achieved by fusion of the external phospholipid head groups from adjacent cells (Pappas, 1975). This membrane fusion occurs in an interrupted fashion, and the overall functional tightness (i.e., permeability) is determined by the number of fusion points. When compared to other epithelial, the proximal tubule tight junction must be classified as relatively "loose" in the sense that only two or three regions of contact are revealed by freeze-fracture microscopy (Kühn and Reale, 1975). This contrasts with more distal nephron segments, where there are dozens of such contact strands be-

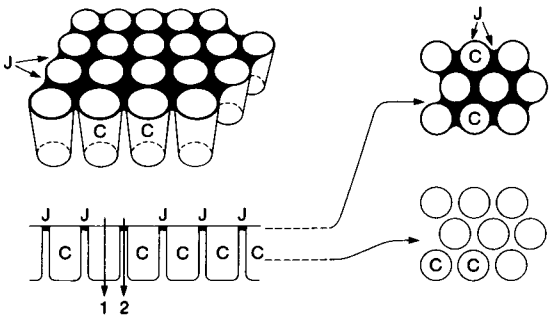

Fig. 3. Schematized conception of epithelial organization. An epithelium consists of cells (C) encircled and held together at one surface by junctions (J), and resembles a six-pack of beer cans extended indefinitely in two dimensions (upper left). Lower left is a section perpendicular to the epithelial sheet; upper right and lower right, sections in the plane of the sheet at the level of the junctions and lateral intercellular spaces, respectively. Alternative routes across the epithelium are via the cells or via the junctions (routes 1 and 2, lower left). The so-called apical membrane of each epithelia faces the top in the sketches at the lower and upper left, while the basolateral membrane faces the sides and bottom [with permission from Diamond (1977)].

tween neighboring cells (Kühn and Reale, 1975), making the epithelium of this nephron segment impermeable to charged and uncharged solutes.

Gap junctions provide specialized intercellular communication channels between adjacent cells (Pappas, 1975). This is true of all epithelia and in particular of the proximal tubule. Their presence permits "cross-talk" between cells by means of low-molecular-weight "messengers"—a mechanism that has potential for functional as well as structural coupling. In theory, therefore, the renal epithelium is capable of syncitial behavior similar to excitation-contraction prototypes. In the case of the mammalian renal proximal tubule, desmosomes are few in number and do not seem to have much structural or functional significance. We will therefore not give them further consideration.

With this brief introduction, we now turn our attention to the biochemical and functional polarization of the proximal tubule cell.

III. BIOCHEMICAL ASYMMETRY

In the renal proximal tubule, certain membrane-bound enzymes are preferentially localized to one or the other of the opposing cell surfaces. Although the functional significance of this distribution is not readily apparent in all cases, it at least provides research workers with useful markers during subcellular purification techniques. During the last seven years there has been a virtual explosion of data bearing on epithelial enzymatic activities. The underlying rationale for such investigations is that biochemical polarization is, in some way, a manifestation of functional polarization. Nevertheless, at present, we have only rudimentary concepts as to how this information can be assimilated into a holistic theory of epithelial physiology.

The methods employed to study membrane biochemistry range from histochemical procedures to the technique of free-flow electrophoresis. Regardless of methodology, interpretation of enzyme localization either to brush border or antiluminal membranes depends on criteria of purity. It is therefore not surprising that controversy still exists. In Table I we have attempted to summarize as far as possible the current situation for the renal proximal tubule.

The information provided in Table I should not be viewed as a static catalogue of epithelial membrane enzyme markers. Our approach is that these data are merely a first step in reconstructing the chemical structural mosaic of luminal and antiluminal plasma membrane surfaces for later application to nephron function.

Table I
Biochemical Polarization of Proximal Tubule[a]

Brush border	Antiluminal membrane
Disaccharides (maltase and trehalase)	Na^+-, K^+- dependent ATPase
5′-Nucleotidase	Ca^{2+}-, Mg^{2+}- dependent ATPase
Aminopeptidase(s), e.g., leucine aminopeptidase	Adenyl cyclase (PTH-stimulated)
Alkaline phosphatase	
γ-Glutamyl transferase	
cAMP-dependent protein kinase	
Carbonic anhydrase	
HCO_3-dependent ATPase	
Neutral endopeptidase	
Dipeptidyl peptidase IV	
Phosphodiesterase I	
Galactosyl (transferase)	

[a]Taken principally from George and Kenny (1973) and Kinne (1976).

The exact spatial arrangement of membrane proteins at each pole of the tubule cell may be as important a determinant of epithelial function as their localization to opposing surfaces. For example, it is possible that aggregates of membrane proteins might be regulated in their luminal distribution over microscopic distances (e.g., corresponding to the surface area of a single microvillus) by the underlying cytoskeleton. Such a mechanism would modulate the role of lipid fluidity in determining the lateral (in-plane) distribution of proteins of the bilayer. Intramembrane electrophoresis (Jaffe, 1977) of proteins may also contribute to the lateral in-plane distribution of membrane proteins at both or either of the luminal and antiluminal surfaces.

In the renal proximal tubule, disaccharidases exist as relatively superficial BBM proteins, with large polar fragments facing externally, i.e., toward the urine (Stevenson, 1972). By criteria of its accessibility to proteolytic digestion by papain, aminopeptidase may also be categorized as having a significant hydrophilic component (Thomas and Kinne, 1972). In addition, recent evidence suggests that this protein may actually transcend the entire membrane width (Maroux *et al.*, 1977). Another example of transmembrane asymmetry at the brush border is the existence of an externally oriented ConA receptor. Figure 4 documents the agglutination of isolated brush border fragments in the presence of this lectin.

Both cAMP-dependent protein kinase and HCO_3^--dependent ATPase must be suspected of having significant exposure at the cytoplasmic face of the BBM, although little direct experimental evidence is available.