Tight and leaky junctions of epithelia:

A perspective on kisses in the dark[1,2]

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Thomas Kuhn (30) has introduced the concept of scientific paradigms as the basis of a useful and profound analysis of how science progresses. According to Kuhn, a paradigm is a coherent tradition of scientific research in a particular field. The paradigm includes agreement on which problems are important and testable, and which are not; agreement on some basic facts and interpretations; shared experimental techniques; and shared faith in some unproven or unprovable assumptions and beliefs. Good research that falls outside of a paradigm is likely to be ignored, as illustrated by the fate of Mendel's work on genetics for several decades. Eventually a crisis arises when the inability of a paradigm to explain accepted facts becomes too obvious. The crisis may lead to a scientific revolution in which the old paradigm is replaced by a new one that does explain the puzzling facts, reinterprets and rejuvenates the scientific field, and redefines problems. For example, the transformation of geology following the widespread acceptance of continental drift constituted a major scientific revolution. Just as political and social revolutions must reinterpret political and social history, so scientific revolutions must reinterpret the history of science, stressing those past accomplishments that are relevant to the new paradigm. In this sense, when a scientist pretends to review the history of his field, he may really be creating a fable, selecting and reinterpreting those discoveries that helped or hindered the advance of the paradigm he espouses.

About 25 years ago epithelial biology went through a revolution and change of paradigm. Within the past decade studies on tight and leaky junctions have triggered a crisis, which at the moment is partly but not yet fully resolved. In this article I shall describe how the crisis came on, what puzzles have been clarified by recent discoveries about tight and leaky junctions, and what major problems remain unsolved. This account is frankly a personal point of view, especially as we do not yet have the wisdom and perspective (and selective memory) that solutions to the current crisis will eventually provide. Thus, what follows should perhaps be viewed as a fable rather than as an objective recounting of history.

Epithelia are the sheets of cells that line organs like the intestine, kidney, stomach, and exocrine glands. It had been known for a long time that epithelia absorb and secrete salts and water, and physiologists had been trying to understand the underlying mechanisms for a long time. In the 1950's the study of transport mechanisms in epithelia was transformed by a new paradigm introduced by the Danish physiologist Ussing (1, 27–29, 43, 46, 48). Ussing provided new methods, such as measurement of short-circuit currents and double-label tracer fluxes, for identifying actively transported ions in epithelia. He provided a new theory for the mechanism of ion transport in epithelia, based on differing and asymmetrical potassium and sodium permeabilities of the opposite cell membranes of an epithelium, combined with the same sort of potassium–sodium exchange pump that Glynn, Hodgkin, and Keynes were discovering in erythrocyte, muscle, and nerve around the same time. The beauty of this model arose not only from its simplicity, self-consistency, and success, but also from its explanation of epithelial transport in terms of known properties (exchange pumps and selective ion permeability) of single cells, thereby raising the prospect of a unified framework for understanding biological transport. Ussing's model made the tacit or explicit assumption that the cell membranes of epithelia were the rate-limiting barriers and routes of ion movement. His brilliant work provides a textbook example of a new paradigm in the sense of Thomas Kuhn, and it rejuvenated epithelial biology. Physiologists set out to short-circuit and measure the potassium/sodium permeability ratio of every epithelium in sight. Sometimes, as in toad urinary bladder, the results appeared to fit Ussing's pump theory well; in other cases, as in the kidney, the fit was not so good, but the new pump theory was nevertheless invoked and stretched. A book published in 1960 summarized and reinterpreted the transport physiology of most epithelia in terms of this paradigm (46). The paradigm appeared successful in accounting for other important phenomena, like the effects of antidiuretic hormone and aldosterone.

Like any paradigm, the new epithelial paradigm suggested not only which problems might be profitable to study but also which problems should best be ignored. Around 1960, for example, a visitor to Ussing's laboratory in Copenhagen set out to study the gallbladder, an epithelium that had received little attention. To the visitor's alarm he found that the gallbladder he dissected had no open-circuit voltage or short-circuit current, so that the paradigmatic method for identifying actively transported ions failed. When, after several further dissections, he still got no short-circuit current, he concluded that these gallbladders must somehow have been damaged by the dissection, chucked them into the waste-bucket, and went on to short-circuit epithelia that were paradigmatically more

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promising. Around the same time, without knowing of these results, I was studying transport mechanisms in the gallbladder for my Ph.D. thesis at the University of Cambridge. I found that dissected gallbladders did continue to transport salt and were not damaged. Nevertheless, like the visitor to Copenhagen, I recorded no short-circuit current, and I also found that the potassium and sodium permeabilities were equal and symmetrical, so that the Ussing pump model clearly could not apply without modification. In the papers that I published in 1962 (11–13), I proposed the minimum modification of the Ussing model necessary to explain the gallbladder results, namely, sodium and chloride pumps coupled so as to be electrically silent. It did not even occur to me then to question the more basic, almost implicit assumption of the paradigm that cell membranes are the sole site of electrical phenomena and ion fluxes.

In the 1960's strains in the paradigm began to appear from at least four lines of work and to lead to a scientific crisis in the sense of Kuhn.

First, detailed tests of Ussing's pump model in frog skin, the epithelium for which it had initially been proposed, as well as in a physiologically very similar epithelium, toad urinary bladder, failed to confirm many predictions from the model (e.g., 8, 9, 18).

Second, epithelia were proving very diverse in their properties, and the existing paradigm did not provide a satisfying explanation for this diversity. The open-circuit voltages of epithelia ranged from 0, as in gallbladder (12), to 100 mV, as in frog skin (28). The electrical resistances ranged from 5 ohm-cm^2, as in renal proximal tubule (22), to 30,000 ohm-cm^2, as in mammalian urinary bladder (31). Some epithelia were asymmetrical in their permeability properties, as assumed in the Ussing model, others were symmetrical; some, like frog skin, supported very steep concentration gradients, others, like renal proximal tubule, only very shallow gradients; some, like avian salt gland, transported hypertonic fluids, others, like gallbladder, isotonic fluids. This recognition of diversity in epithelia, and this sense of a failure in the existing paradigm and search for a new paradigm, are depicted well by a comparative review of epithelia (26) written by Keynes in 1969, a few years before progress in understanding tight and leaky junctions in epithelia succeeded in providing a satisfying explanation for much of this diversity.

A third precipitant of the crisis was research on the ultrastructure of tight junctions. Epithelia do not just consist of cell membranes. Instead, an epithelium can be visualized as a six-pack of beer extended indefinitely in two dimensions. The barrel-shaped cells, represented by the beer cans, are held together at the luminal surface of the epithelium by specialized structures called tight junctions, represented by the plastic frame of the six-pack. In principle, solutes have two parallel routes available for traversing an epithelium: the so-called tight junctions, and the two epithelial cell membranes in series. Throughout the body, cells differ from each other enormously in their membrane properties. Thus, the beer-pack structure of epithelia hinted that, superimposed on variation due to the usual gamut of cell membrane properties, epithelia might exhibit additional variation associated with junctional properties. The anatomists who discovered the structures between epithelial cells assumed that they were tight, named the structures accordingly as tight junctions, and subsequent biologists accepted the implications of the name without examining the name's basis. The Ussing pump model reinforced this acceptance by concentrating attention on the supposedly contrasting permeability properties of the two cell membranes. Only after the first detailed electron-microscopic study of junctions by Farquhar and Palade (17) in 1963 did anatomists really begin to worry about whether the junctions are tight to solutes as small as inorganic ions. More recent anatomical work has shown that a junction consists of a series of barriers where cell membranes from adjacent cells come in very close proximity (7, 10, 23, 35, 37, 49, 50). These barriers are called punctate contacts or, more picturesquely, kisses. The kisses apparently represent fibrils as seen in cross section. While the structure of kisses is an active subject of study, we are still in the dark about high-resolution details of junctional structure.

The last major precipitant of the crisis was physiological evidence that some junctions are leaky to ions. Already in 1957, Lundberg (33) had tried to estimate junctional permeability in salivary gland. However, as with Mendel's work in genetics, Lundberg's work was too early and too different from an otherwise satisfactory paradigm to be fully appreciated. From the mid-1960's onwards, though, Ussing, Windhager, Boulpaep, Frömter, Sachs, and others began to accumulate and believe electrical evidence that "tight" junctions might not always be so tight after all (5, 6, 22, 40, 45, 47, 51).

For me the conversion to belief in the existence of leaky junctions began in the spring of 1969, when Peter Barry, Ernest Wright, and I were rushing to finish a series of papers on the cation permeation mechanism in gallbladder before I disappeared beyond reach of mail for several months on an expedition in New Guinea. At this time we still assumed cation permeation to be via the cell membranes. In our drafts of these papers we showed that most of our observations fitted a valinomycin-like permeation mechanism in the cell membranes, but we were having difficulty accounting for some recalcitrant details. Fortunately, we did not succeed in completing and sending off the papers in this form by the time I had to depart. When we resumed work on these papers after my return, we acquired a new piece of experimental evidence, a current-voltage relation linear up to 800 mV, that was in flagrant disagreement not only with our valinomycin-like model but with any mechanism in the cell membranes, and that was a strong indicator of cation permeation via tight junctions. The current-voltage relations of cell membranes and lipid bilayers encompass a varied and exotic spectrum of nonlinear forms, but are rarely or never simply linear for hundreds of millivolts. We then reexamined the possibility of a junctional permeation route and found that it could easily explain both the evidence that supposedly supported as well as the evidence that conflicted with a valinomycin-like model (2–4, 54).

Direct proof that cation permeation in gallbladder was via junctions came in 1971, when Eberhard Frömter visited my laboratory for half a year (20, 21). One approach to determining the ion permeation route was to measure with microelectrodes the sum of the resistances of the two epithelial cell membranes in series, for comparison with the total transepithelial resistance. The actual experiment is somewhat complicated because the junctions provide a low-resistance path for cell-to-cell current flow within the plane of the epithelium. To extract the answer, one must perform a cable analysis (6, 20, 21, 25, 32, 36, 40, 51). This consists of injecting current into a cell with one microelectrode, and measuring the voltage deflection in other cells with a second microelectrode as a function of distance from the current-injection site. The answer in *Necturus* gallbladder

proved to be that the cell membrane resistance is 22 times the transepithelial resistance, so that 21/22 of the transepithelial conductance must be in a shunt bypassing the cells. In order to locate this shunt, Frömter scanned the epithelial surface for current sinks by moving a microelectrode along the fringe of microvilli while passing a transepithelial current. To picture the problem posed by this experiment, imagine that you are annoyed by a gopher that is digging tunnels in your grass lawn and that you propose to locate the gopher with a heat-sensitive thermistor. The sensitivity of the thermistor is such that if you can hold it inside the grass and pass it over the gopher tunnels, you will be able to detect the gopher when the thermistor passes over him. Unfortunately, the grass is only 2 inches high, you are given the thermistor attached to the end of a rigid stick 4 miles long, and if the thermistor actually touches the ground, it will break. The task of holding the thermistor end of the stick inside the grass, to find the gopher, without scraping the ground, is similar in proportions to the task of keeping the microelectrode tip within the fringe of microvilli, to find the current sinks, without scraping the epithelial cell membrane. Frömeter did find the gopher. That is, whenever the microelectride tip moved across a cell boundary, he recorded a voltage deflection of the correct orientation for a current sink, showing that the shunts demonstrated by the cable analysis experiment are located in the cell junctions.

When Frömter and I reexamined the properties of other epithelia in the new frame of mind produced by these discoveries, it appeared to us that much of the variation among epithelia in physiological properties might arise from variation in the ratio of junctional conductance to cellular conductance (21). Besides gallbladder, epithelia in which the junctions are leaky—that is, the main route of ion permeation—probably include the small intestine, renal proximal tubule, and choroid plexus. On the other hand, frog skin, urinary bladder, stomach, and salivary duct probably exemplify epithelia in which the junctions are really tight to ions. The characteristics of low electrical resistances, high osmotic water permeabilities, isotonic transported fluids, and only shallow gradients built up by active solute transport all proved usually to be associated with the epithelia presumed to be leaky, while the reverse properties occurred in the presumably tight epithelia. These correlations are actually explanations, since the permeability of the junctions to ions and water directly determines each of these properties. Junctional permeability also appears to determine the differences in the magnitude and symmetry of potassium/sodium permeability ratios among epithelia. This bimodal classification of epithelia as "tight" or "leaky" is an oversimplification, as the ratio of junctional to cellular conductance can assume any value along a continuum from $\ll 1$ to $\gg 1$. Since the cell membranes in parallel with the junctions may also differ among epithelia, low electrical resistance in some epithelia may prove to be due to leaky cells, though this situation will probably prove exceptional.

Several other groups of workers have done cable analyses in other epithelia: Windhager, Boulpaep, and Giebisch; Hoshi and Sakai; and Boulpaep in renal proximal tubule (6, 25, 51); Sachs and colleagues in stomach (40); Lewis, Eaton, and Diamond in mammalian urinary bladder (32); and Reuss and Finn in amphibian urinary bladder (36). As a result of these studies, epithelial biology may be evolving toward a new paradigm that includes new techniques for epithelia, such as cable analysis and voltage scanning; new interpretations, such as those based on the ratio of junctional to cellular conductance; and new problems, some of which will be mentioned below. As in the case of any shift in paradigms, there is a transient phase during which new and old models overlap, facts are still being reinterpreted, and not everybody has been converted. For instance, studies of epithelial cell membrane properties with microelectrodes in leaky epithelia must be reinterpreted along lines indicated by Boulpaep and by Rose and Schultz (5, 39). It is not valid to continue to interpret voltages across a membrane solely in terms of salt concentration gradients across that membrane and the membrane's permeability properties, when there are also gradients and voltages across a parallel shunt. Reinterpretation of passive cation permeation through leaky epithelia has progressed substantially. Workers on all four tissues studied (gallbladder, intestine, proximal tubule, and choroid plexus) agree that the passive cation fluxes are almost entirely via junctions, and details of permeation in the four tissues are remarkably similar (3, 4, 19, 22, 52–54).

The recent studies of junctions have also raised a series of new problems that leave epithelial biology in a state of crisis. It remains to be seen whether the solution of these problems will provide exciting new tests, triumphs, and extensions of present epithelial concepts, or whether some of them will cause such strain as eventually to precipitate a change in paradigm.

One such problem concerns the route of water movement. If epithelia are classified as leaky or tight on the basis of junctional permeability to ions, measured values of osmotic water permeability turn out to be generally higher in leaky epithelia than in tight epithelia (21). Even the measured values may be gross underestimates because of a recently discovered unstirred-layer effect (58). If reflection coefficients (σ's)[3] for small solutes across the junctions of leaky epithelia are lower than σ's across the cell membranes, but the water permeabilities of junctions and membranes are similar, measured osmotic water permeabilities and σ's could be disproportionately influenced by the membranes, and flow measurements under hydraulic pressure heads may be needed to estimate junctional water permeability. Are junctions that are leaky to ions also leaky to water? How is the total osmotic water permeability of an epithelium divided between the cellular and the junctional pathways? Cell membranes vary greatly in their water permeability (e.g., as a function of cholesterol content or under the influence of antidiuretic hormone); do junctions vary equally greatly? At present we do not even have a good method for studying these questions.

A second problem similarly concerns the route of nonelectrolyte permeation. One might hazard a guess that the permeation of lipid-soluble nonelectrolytes is mainly via cell membranes, while small hydrophilic nonelectrolytes could go either via junctions (especially in leaky epithelia) or via pores in cell membranes (24, 56, 57). Actually, the nonelectrolyte permeability patterns of two leaky epithelia, the gallbladder and intestine, are very puzzling (41, 42, 57). Lipid-soluble nonelectrolytes behave as if they permeate these leaky epithelia via a route that does contain a lipid barrier but that is much

[3] The reflection coefficient σ is the ratio of the osmotic volume flow caused by a concentration gradient of some solute, to the flow caused by the same gradient of an impermeant solute. A σ value applies to a particular solute and a particular membrane. σ equals 1.0 for impermeant solutes, 0 for solutes as permeant as water, and may be negative for solutes more permeant than water. See ref 55 for discussion.

more watery than the barrier in some well-studied cell membranes, such as those of the algae *Nitella* and *Chara*. Can the permeability pattern of leaky epithelia be duplicated by the membranes of some single cells? Do leaky epithelia have distinctive cell membranes as well as leaky junctions? Or, is a leaky junction not just an aqueous channel, but does it include in parallel a peculiar lipid barrier?

A third problem concerns the function of leaky junctions. It is clear why epithelia like frog skin and urinary bladder have to have junctions that are tight: in order to maintain very steep solute concentration gradients (21). Why, though, should epithelia like gallbladder and proximal tubule have leaky junctions? One speculative possibility is that active salt transport into lateral spaces could pull water (by osmosis) and hence more salt (by solvent drag) across the junctions into the spaces if the junctions were leaky. This effect can be termed solute amplification[4] and will be maximal when σ for salt is approximately 0.5. Kidney physiologists especially have considered the possibility of solute amplification, but its reality and significance are still speculative.

The mechanism of active sodium transport in epithelia remains a fourth problem. The more we learn about the facts, the more complex and less aesthetically appealing they seem, compared to the elegant simplicity of the Ussing model. Although the model does not fit the facts well, there is no alternative model that is both simple and correct. As a result, it is often the beautiful Ussing model rather than the ugly facts that are taught to students. Here is a striking and instructive example of the practice of Eddington's precept that one should not put too much faith in facts until they are supported by theory.

A special problem in epithelial active sodium transport is still posed by the absence of a short-circuit current in some epithelia, that property that caused the gallbladder to be found paradigmatically unacceptable except as waste-bucket fodder. Leaky epithelia besides gallbladder that lack short-circuit currents include choroid plexus, renal proximal tubule, and small intestine under some conditions. The evidence for the former interpretation in terms of neutral coupled pumps has been undermined by the finding of junctional leakiness. It would thus have been simple if we could now have reasoned that the pumps in leaky epithelia were similar to sodium pumps of tight epithelia, differing only in the shorting out of a transepithelial voltage by junctional shunting. Yet this reinterpretation faces serious difficulties, which include anion transport against steep electrochemical gradients, and the anion dependence of small potentials attributed to a sodium pump (16, 34, 38).

Finally, much remains to be learned about the structure of junctions and their kisses, and about the origin of the differences between tight and leaky junctions. Are the differences morphological (10), such as that leaky junctions have fewer strands of wider gaps or fewer kisses? Or, will the differences be ones that are invisible in the electron microscope, such as differences in charge or molecular structure?

These are some of the main problems that epithelial biologists will soon be confronting in the dark, revolutionary, paradigmatic world of junctional kisses. EP

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[4] The rate of osmotic volume flow J_v across the tight junctions from the luminal solution to the lateral intercellular spaces, in response to local hypertonicity maintained within the lateral spaces by active salt transport, is

$$J_v = \sigma L_p \Delta C$$

where L_p is the osmotic water permeability of the tight junction, σ the reflection coefficient of the junction for salt, C the luminal concentration of salt in osmolar units, and $C + \Delta C$ the osmolar salt concentration in the lateral intercellular spaces. The rate of solute movement J_s into the lateral spaces arising from solvent drag across the tight junction (in addition to the rate of active solute transport J_s^* into the lateral spaces) is

$$J_s = J_v (1 - \sigma)(C + \Delta C/2)$$

$$= \sigma (1 - \sigma) L_p \Delta C (C + \Delta C/2)$$

This expression is maximal when $\sigma = 0.5$. More rigorously, the amplification factor $(J_s + J_s^*)/J_s^*$ depends on the rate of salt back-diffusion across the tight junction into the luminal solution (hence on the salt permeability of the tight junction) and also on all the factors that determine the steadystate value of ΔC (14, 15).

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