Passing on the legacy: teaching capillary filtration and developing presentation skills using classic papers

J. Graham McGeown
Cell and Metabolic Signaling Group, School of Medicine and Dentistry, Queen's University of Belfast, Belfast, United Kingdom

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McGeown, J. Graham. Passing on the legacy: teaching capillary filtration and developing presentation skills using classic papers. Adv Physiol Educ 30: 108–112, 2006; doi:10.1152/advan.00032.2006.—Capillary filtration is a key area in the understanding of cardiovascular function and has both physiological and pathophysiological relevance in nearly every organ system. This article describes how classic papers in the Legacy collection of American Physiological Society publications can be used in a teaching symposium exploring the evidence supporting current concepts of capillary fluid exchange. Individual students are given papers to read, edit, and present to the class. The appropriate selection and sequencing of these papers allows the development of important physiological concepts to be tracked. A series of papers concerned with capillary filtration is suggested, and the contribution of each to the developing story is outlined. This approach allows students to develop critical and presentation skills and provides them with a case study of the scientific method as it is applied to physiology as well as establishing an appropriate knowledge base concerning the role of hydrostatic and oncotic forces in capillary fluid exchange. Relevant teaching points are explored further using questions based on a figure from one of the three classic papers used: “Microinjection studies of capillary permeability: II. The relationship between capillary pressure and the rate at which fluid passes through the walls of single capillaries,” by E. M. Landis (Am J Physiol 82: 217–238, 1927).

IN ADDITION to gaining relevant knowledge and understanding of physiological concepts, our students also need to develop a range of general skills that have wide applications within both science and the wider world. At the highest level, these skills include the ability to analyze and synthesize complex ideas, a critical approach to evidence, and the ability to communicate difficult ideas clearly, both orally and in writing. The approach outlined here describes how original research papers can be used to facilitate the development of these skills. Topics are explored through a series of oral presentations given by the students, each outlining an important study related to the theme. This approach emphasizes evidence over information while seeking to ensure that students gain a clear grasp of the basic concepts.

The class described here deals with capillary filtration and is taught within a module on advanced cardiovascular physiology. The teaching method could be applied to most areas of study and is particularly appropriate for postgraduate students. By the end of the module, each student has read, interpreted, edited, and presented 6 separate papers, 1 paper relating to each of 6 different topics in the module, as well as listened to and discussed nearly 50 other presentations by their fellow students. The selected papers should have made an important contribution to the field and should stand as good examples of clear scientific writing. In this regard, the classic papers identified in the American Physiological Society (APS) Legacy archive represent an excellent resource from which classic papers can be selected secure in the knowledge that their quality is not in doubt. This style of teaching has been a core element of teaching in our department since David Greenfield taught here in the early 1950s (2).

General Aspects of Class Preparation and Delivery

The main steps are summarized in Table 1. It is our practice to introduce each topic in the module through a lecture in which the range of the material to be considered can be outlined and issues that are likely to give students particular problems are addressed. It is often prudent to explain the principles underpinning the methods used in specific papers because these may not be easily sourced from monographs or other papers. At the end of this session, each student is assigned a paper from which they prepare a 10-min presentation with computer graphics. The presentations are delivered to the whole class as a teaching symposium, with 5 min set aside for questioning after each talk. Students are given early feedback based on presentation style, content, and their response to questions along with their mark for the session.

Application: Capillary Filtration

A proper grasp of the mechanisms determining capillary filtration is crucial if students are to understand how normal tissue hydration is maintained. It also provides a necessary foundation when considering microvascular specialization, e.g., to favor filtration in the kidney or absorption in the gastrointestinal tract and lungs. The significance of edema as a clinical marker of disease, a major contributor to the functional deficit that results from those diseases, and a therapeutic target in the relief of symptoms further underlines the importance of the ideas involved for students and researchers.

Since the turn of the 20th century, Starling’s basic model of capillary fluid exchange driven by hydrostatic and oncotic gradients across the capillary wall has been generally accepted. In mathematical form, this predicts that, for a single capillary, the fluid flux per unit surface area (Jc/A) should satisfy the relationship Jc/A = Lp[(Pc - Pp) - σ(πc - πi)] (Eq. 1), where Lp is a measure of capillary fluid permeability known as hydraulic conductivity; Pc and Pp are the hydrostatic pressures in the capillary and surrounding interstitium, respectively; πc and πi are the colloid osmotic (or oncotic) pressures exerted by protein in the plasma and interstitial fluid, respectively; and σ...
is the reflexion coefficient, a measure of how closely the capillary wall approximates to a perfect semipermeable membrane for protein. Early quantitative studies, including the classic papers by Landis and by Pappenheimer and Sota-Rivera (4, 5, 9), were consistent with this model if it was assumed that interstitial hydrostatic and oncotic pressures were small. Measurements of interstitial hydrostatic and oncotic forces, however, suggested that there should be a considerable filtration gradient along the entire length of most capillaries, even in tissues that are in fluid balance (6). This has led to proposed modifications of Starling’s original model, which retain his concept of hydrostatic and colloid osmotic pressures as the driving forces determining capillary fluid exchange but emphasize that it may be the values of these forces within very specific compartments of the extracapillary space that matter, e.g., just outside the endothelial glycocalyx within capillary pores (1, 8, 12). Guiding students through the key stages in the development, testing, and refinement of the hypothesis provides an excellent case study in the scientific method.

Papers and Teaching Points for Capillary Filtration

In any class such as this, the selection and sequence of the papers is crucial, and each should add something to the development of the story. For capillary filtration, the papers listed in Table 2 provide a useful set of landmarks. This list includes three classic papers from the APS Legacy collection.

Direct Measurements From Capillaries

It is useful to start with two papers by Landis. In the first paper (4), he describes the first reliable, direct measurements of capillary pressure in capillaries and microvessels. A micropipette filled with colored dye was either pushed across the wall of the vessel or inserted into a side branch of the capillary to be studied. A dye reservoir attached to the micropipette was moved vertically up and down using a micromanipulator, and the height at which dye was neither forced into the capillary nor back into the pipette was recorded as the intravascular pressure. Recordings made from the frog mesentery demonstrated that there was an average capillary pressure of 14.5 cmH2O, with an average pressure fall of 8–12 cmH2O along the length of the capillaries (see Figs. 7 and 8 in Ref. 4). Increased rates of blood flow through capillaries were correlated with both increased capillary pressures and increased pressure gradients between capillaries and venules. Landis was also careful to note that both pressure and flow in individual capillaries varied rapidly from “moment to moment” (Fig. 9 in Ref. 4).

The preceding paper (4) leads very naturally to a second landmark study (5) in which Landis combined measurements of capillary pressure and filtration in single capillaries. When capillaries were occluded so there was no luminal flow, transcapillary fluid exchange could be estimated from the rate of movement of red blood cells, either toward the point of occlusion in cases of filtration or away from it during absorption. By assuming cylindrical capillary geometry, Landis could calculate a volumetric rate of fluid exchange and normalize it to the available capillary surface area. He then measured capillary pressure in the same vessel, as described in the previous paper (4). Landis’ contribution to microvascular physiology has been highlighted in a recent essay celebrating his classic papers (10). Figure 10 from the second paper (5) is reproduced here (Fig. 1). Materials to be used with Fig. 1 as a tool for discovery learning are also included (Table 3 with teacher’s notes in Table 4).

The next paper (7), by Michel et al., extended Landis’ work to allow capillary pressure to be controlled by the experimenter. The micropipette was now used to perfuse a capillary at a given pressure and not just to measure the pressure. The capillary was then occluded, and the filtration or absorption rate was measured using the velocity of red blood cell movement. By repeating this for a series of different pressures, the relationship between capillary hydrostatic pressure and rates of fluid exchange could be determined for a single capillary (Fig. 3 in Ref. 7). This underlines an important point in terms of critical data interpretation. Landis’ results effectively showed a correlation between capillary pressure and the rate and direction of fluid movement across the capillary wall. It is useful to remind students that a correlation is not adequate proof of a

<table>
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<th>Authors</th>
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<td>Landis</td>
<td>The capillary pressure in frog mesentery as determined by micro-injection methods.</td>
<td>1926</td>
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<tr>
<td>Pappenheimer and Sota-Rivera</td>
<td>Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs.</td>
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<td>Guyton</td>
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<td>Capillary filtration-absorption balance reconsidered in light of dynamic extravascular factors.</td>
<td>1991</td>
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<td>Adamson et al.</td>
<td>Oncotic pressures opposing filtration across non-fenestrated rat microvessels.</td>
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*Classic papers from the American Physiological Society Legacy collection.
Table 3. Questions related to Fig. 1 for use in discovery learning

1. List the main factors controlling capillary filtration in Starling’s model.
2. Explain the units used in the measurement of the rate of capillary filtration? Why is the term “square micron” important?
3. In what two ways are the data in Fig. 1 consistent with Starling’s model?
4. What inference can be drawn from these results concerning the interstitium?
5. What would you expect to happen to the data points in Fig. 1 if
   A. Intercellular hydrostatic pressure increased?
   B. Intercellular hydrostatic pressure decreased?
   C. Intercellular protein concentration increased?
   D. Plasma protein concentration increased?
6. What does the slope of the line fitted to these points measure?
7. What effect(s) would inflammation have on the results obtained in such an experiment?
8. The data in Fig. 1 show a positive correlation between capillary pressure and filtration rate. Correlations like this are regarded as “necessary but not adequate” proof of a causal relationship between two variables. Explain what this means and why it is so?
9. Suggest, in principle, how these experiments might be adapted to provide stronger evidence that capillary pressure affects filtration.
10. Write out an equation summarizing the factors responsible for capillary filtration. Relate as many of the terms in this equation as possible to the data in Fig. 1. Which elements remain unknown in Landis’ study?

Table 4. Teacher’s notes related to the questions in Table 3

1. Capillary and interstitial hydrostatic and oncotic pressures, capillary permeability, and available surface areas (this should be drawn out in question 2).
2. Volume of fluid filtered or absorbed/capillary surface area/time.
3. Linear relationship between capillary pressure and filtration rate; no net fluid movement when capillary hydrostatic pressure is close to plasma oncotic pressure.
4. Intercellular forces appear to be negligible; balance between hydrostatic and oncotic forces inside the capillary can explain the equilibrium point for fluid exchange.
5. Regarding changes to the data points in Fig. 1:
   A. Shifts data points to the right (favors absorption).
   B. Shifts data points to the left (favors filtration).
   C. Shifts data points to the left (favors filtration).
   D. Shifts data points to the right (favors absorption).
   There is no reason why the slope should change with these interventions, as they do not affect the capillary wall itself.
6. The case with which fluid moves across the capillary wall (hydraulic conductivity or filtration coefficient). (This is actually an average value for all the capillaries studied, not the value for any one capillary.)
7. Increase the slope of the line (increased hydraulic conductivity) and shifts line to the left (due to increased permeability to protein). Reduced reflexion coefficient and increased interstitial [protein]. See Fig. 12 in Landis’ paper (5).
8. If there is a casual relationship, then the variables should be correlated, “necessary.” Both variables may, however, be causally dependent on a third, unmeasured variable, “not adequate.”
9. It is necessary to experimentally control the proposed causal variable experimentally, not just to measure it. See the development of Landis’ technique by Michel et al. (7).
10. See Eq. 1. Interstitial forces and reflexion coefficient are unknown.
light of fluid exchange measurements in whole organs, for which more indirect techniques have to be applied. Many such studies use concepts first presented in another classic paper (9) from the APS legacy archive, which reports findings from isolated hindlimb preparations. Pappenheimer and Sota-Rivera (9) perfused the tissue, controlling the arterial and venous pressures. Both blood flow and tissue mass were measured throughout each experiment. They demonstrated that, for any set arterial pressure, a corresponding venous pressure could always be found at which tissue mass remained constant (the isogravimetric state: Fig. 4 in Ref. 9). Under such conditions, it was assumed that there was no net filtration or reabsorption within the tissue. They showed that the relationship between blood flow and venous pressure for the isogravimetric state was linear (Fig. 5 in Ref. 9). This result is central to the paper and allowed two important variables to be estimated. By extrapolating this relationship to zero flow, where the pressure in all elements of the vasculature must be equal, they were able to estimate the capillary pressure under isogravimetric conditions. They showed that this pressure increased as they increased the protein concentration of the perfusate, and the data could be estimated to the capillary pressure under isogravimetric conditions. They showed that this pressure increased as they increased the protein concentration of the perfusate, and the data could be explained if it was assumed that the plasma protein exerted a colloid osmotic pressure = 0.95 × the colloid osmotic pressure they would exert at an ideal semipermeable membrane (Fig. 8 in Ref. 9).

The gradient of the isogravimetric, venous pressure-flow relationship was also used as a measure of the vascular resistance between the capillaries and veins, often referred to as the postcapillary resistance. This allowed them to estimate the mean capillary pressure from the recorded venous pressure and blood flow rate for any set of conditions. They showed that, under nonisogravimetric conditions, the net rate of filtration or absorption was proportional to the difference between the calculated mean capillary pressure and isogravimetric capillary pressure (Fig. 9 in Ref. 9). Such measurements also allowed them to estimate the tissue filtration coefficient, which reflects the average hydraulic conductivity of the capillaries and the total capillary surface area available for fluid exchange in the tissue (Table 3 in Ref. 9). This concept should be linked to a discussion of the fact that, in contrast with direct observations on single capillaries, the results obtained in whole tissues reflect average behavior for the entire capillary bed. Thus, in the isogravimetric state, some capillaries may show net filtration, whereas others demonstrate net absorption, providing these effects balance out across the tissue. The possible influence of vasomotion, which will alter fluid exchange within individual capillaries over time, can also be usefully introduced at this point.

This paper (9) also provides an excellent introduction to pressure-flow relationships in the vasculature and the concepts of pre- and postcapillary resistance. Pre- and postcapillary resistances could be determined for a single preparation for the first time, an important step forward because the ratio of these resistances determines the mean capillary pressure for any combination of arterial and venous pressures (see DISCUSSION in Ref. 9). Experiments were also carried out confirming that postcapillary resistance is independent of flow rate, an important assumption in applying the resistance determined under isogravimetric conditions to the nonsogravimetric state (Fig. 6 in Ref. 9). In contrast to this, precapillary resistance increased rapidly as blood flow decreased (Fig. 6 in Ref. 9). This effect probably reflected flow-dependent changes in blood viscosity, because it was greatly reduced when physiological salt solution was used as the perfusate instead of blood. The phenomenon helps introduce students to the complexity of vascular rheology as well as emphasizing the need to test assumptions experimentally. It is not intuitively obvious (to me, at least) that pre- and postcapillary resistances would be expected to differ in this way.

Measurement of Interstitial Forces

The evidence presented up to this point provides strong evidence for the Starling model, showing that filtration is linearly related to capillary hydrostatic pressure and plasma oncotic pressure. Steady-state conditions, i.e., those in which there was no net filtration or absorption, were achieved when capillary hydrostatic pressure was 1–2 mmHg less than plasma oncotic pressure (5, 9). This is consistent with the formulation in Eq. 1, but only if one assumes that the contribution from interstitial forces is relatively small. None of the papers described above measured these forces, and so the next two papers were chosen as landmark papers addressing this issue.

One of the major difficulties in making interstitial measurements is that the interstitial fluid is normally restricted to microscopic channels within the connective tissue gel. Simply inserting a probe connected to a pressure transducer is likely to give a measure of the forces transmitted through the solid elements of the tissue rather than the interstitial fluid itself. This may be compounded by inflammation induced by the trauma of inserting a needle or micropipette into the tissue, especially if blood vessels are damaged. In an attempt to combat these difficulties, Guyton (3) developed a technique using chronically implanted, perforated capsules. Fluid gathered in the core of these capsules is in direct contact, and presumably hydrostatic equilibrium, with the surrounding interstitium. Pressure recordings were made after several weeks, when postsurgical inflammation had passed, by inserting a needle through the capsule perforations into the fluid-filled core. This produced negative (i.e., subatmospheric) values of the order of −5 mmHg for the interstitial hydrostatic pressure in subcutaneous tissue and muscle (Table 1 in Ref. 3).

Much of Guyton’s paper (3) concentrates on validating the technique and testing it against the conventional tissue needle technique for recording interstitial pressures. In simultaneous recordings using both techniques, the capular readings were negative, whereas the needle gave positive pressures. The capsule pressure started to rise after an increase in venous pressure, but there was no increase in the needle pressure until frank edema developed (Fig. 3, A and B, in Ref. 3). Both techniques gave identical results beyond this time point, indicating that the differences were not due to a fixed pressure error in the capsular values. The fact that the capsular pressure rose immediately after a step increase in venous pressure increased capillary filtration, whereas tissue needle pressure did not rise for some 6 h, was interpreted as evidence that the former more truly reflected interstitial conditions. Even more persuasively, vascular infusion with 20% dextran, which increases the colloid osmotic pressure within the capillaries and should therefore increase absorption, led to the expected decrease in capsular pressure but was associated with a small increase in the tissue needle pressure (Fig. 4 in Ref. 3). Such studies have led to the widespread acceptance that the interstitial pressure in
many normally hydrated tissues is negative. This would increase the hydrostatic gradient favoring filtration above the measured capillary pressure, although currently accepted values are considerably less negative than Guyton’s early results (see the large pore osmometer results in Ref. 11 and the review of many such studies in Table 1 in Ref. 6).

Direct measurements of interstitial oncotic pressure have also been carried out in vivo using implantable osmometers (11). Recordings from subcutaneous tissue in rabbits gave average values of around 10 mmHg (Table 1 in Ref. 11). Control recordings using osmometers with a pore size large enough to allow free diffusion of proteins, which effectively record hydrostatic pressures, gave an average value of −1.2 mmHg, confirming the negative values obtained by Guyton (3) (although with considerably reduced magnitude) and establishing that the positive values recorded with the small-pore osmometers were indeed due to large tissue solutes, presumably proteins. Volume loading the circulation by infusing Ringer solution decreased the interstitial colloid osmotic pressures, as expected if tissue protein were diluted by increased capillary filtration (Fig. 2 in Ref. 11). Dehydrating the animals by using a diuretic increased these pressures, as expected if capillary fluid absorption led to concentration of tissue protein around the body (Fig. 2 in Ref. 11).

**Apparent Imbalance in Starling Forces**

The combined effects of the measured interstitial forces would be to increase the net filtration force by approximately 12–16 mmHg over and above values based solely on capillary hydrostatic and oncotic pressures. This contrasts with the results in earlier studies (5, 9) in which capillary hydrostatic pressure and plasma oncotic pressure were closely matched in the steady state. Furthermore, combining the available measurements for capillary and interstitial pressures predicts that there should be filtration along the whole length of most capillaries in most tissues, yet steady-state lymph flow is usually low. Because few, if any, studies have been carried out in which all relevant parameters are measured simultaneously, these concepts are most easily introduced by having one of the students (or the teacher) present a relevant review (6). This provides a useful summary of much of the material covered so far, along with many supporting references.

Despite the fact that they cast doubt on the actual values to be used in the Starling equation (Eq. 1), it is worth drawing students’ attention to the fact that the original papers (3, 11) dealing with measurements of interstitial fluid forces actually make use of assumptions based on the Starling model. In both cases, interventions were used that, on the basis of the expected changes in the intracapillary forces, were predicted to increase or decrease fluid filtration. The observed changes in the experimental recordings (increased or decreased interstitial hydrostatic or oncotic pressures) were then validated against these predictions. So the qualitative predictions of the Starling model hold true, but the measured values of the interstitial forces (particularly oncotic forces) are difficult to reconcile with some of the evidence.

**Reformulation of the Starling Model**

It is satisfying to be able to point students toward a possible resolution of this dilemma. The last paper (1) in my selection reports a study investigating the effects of experimentally induced changes in interstitial protein concentration on filtration. The interstitium was superfused with a solution containing protein at the same concentration as the vascular perfusate. The time course of resulting changes in interstitial protein concentration was confirmed using fluorescently labelled albumin (Fig. 2 in Ref. 1). The key finding was that increasing the interstitial protein concentration from zero to that of plasma had relatively little effect on the effective oncotic force opposing filtration, even though the oncotic gradient between plasma and the bulk interstitium was decreased from 27 to 0 cmH2O (Fig. 4 in Ref. 1). These results are discussed as possible evidence for a model of capillary filtration proposed independently by both Michel and Weinbaum (8, 12), in which the interstitium is regarded as the filtration barrier rather than the capillary wall itself. The mathematical details of this model are not crucial to the learning objectives. Crucially, however, it allows for the possibility that a large fraction of the plasma oncotic pressure may be effective in opposing filtration forces regardless of the protein concentration in the interstitium itself. This could explain how filtration and absorption can be more closely in balance than would be predicted from the unmodified Starling model. This also provides an excellent opportunity to explore with students what sort of experiments might be needed to test such models further.

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**REFERENCES**