Upsala J Med Sci 100: 19–32, 1995

# Charge Structure of the Glomerular Capillary Membrane

M. Wolgast
*Inst. of Physiology and Medical Biophysics, Uppsala, Sweden*

In 1935 Teorell (see Teorell, 1953, 1983) presented his theory about charged membranes; the title of the paper was "An attempt to formulate a quantitative theory of membrane permeability". The theory was based on the ion equilibria resulting from the presence of charged colloids suggested already in 1911 by Donnan - although this early work seems to have attracted little attention until it was summarized in 1922 by Loeb in the book "Proteins and the theory of colloidal behavior.

It should be emphasized, however, that this so called Donnan distribution refers to an equilibrium, whereas Teorell elaborated a set of equations also valid for steady state conditions as according to the Nernst-Planck equation.

Teorell also considered the electro-osmotic force resulting from the presence of an electric field in charged membranes, a consideration which later would form the basis of the so called *membrane oscillator* (Teorell, 1983). In this hypothesis it was inferred that fluid flow will lead to rearrangement of the ion distribution and thereby the electric field so as to reduce the fluid flow. Since this will tend to restore the ion distribution and the electric field, the fluid flow will again increase which, as before, will rearrange the ion distribution and the electric field with consequent reduction in the fluid flow and so forth.

**Fixed charge density of the glomerular capillary membrane**

Where the present article is concerned, the primary aim of our studies on the glomerular capillary membrane performed in the early 1980's was simply to determine its permeability in terms of the hydraulic permeability and its pore structure (Öjteg et al, 1987a, Öjteg et al., 1987b). However, at this time it was known, both from morphological (Rennke et al., Kanwar et al., 1980, Simionescu and Simionescu, 1984) and physiological studies (Chang et al., 1975, Deen et al., 1980, Deen and Bridges, 1982, Dworkin and Brenner, 1985), that the glomerular capillary barrier also possesses negative charges fixed to the membrane; their function was believed to hinder penetration of albumin into the membrane by simple Coulomb repulsion between the negative, fixed groups and the strongly negatively charged albumin molecule .

In order to quantify this repulsive force, experiments were undertaken to determine the membrane fixed charge density in terms of mEq/l. Due to the small dimensions of the glomerular capillary barrier, it was most certainly not possible to apply the technique of "slicing up" the membrane as made by Teorell on his relatively thick porous artificial membranes. Instead we chose to compare the transport of charged molecular probes with that of their neutral counterparts and where, as predicted by Teorell, the equilibrium distribution and hence the flux of all negative ions would be reduced.

A second obstacle arises from the fact that the transport of such charged molecules will be determined by so many forces, i.e. by diffusion, ion migration, hydraulic bulk flow and also by the so called streaming potential. Fortunately, however, this problem is solved by the present availability of powerful computers.

*The choice of molecular probes*

The choice of the probes does constitute a challenge. The most ideal probe would thus be a molecule nearly as small as sodium and chloride; unfortunately, however, the transport of such small ions would be virtually unrestricted and hence very difficult to assess. Very large ions on the other hand, will be affected by the charge-induced large electric field in the vicinity of the pore rim, so that the membrane would give the impression of having a very large average charge density.

Our choice of myoglobin was a compromise between these two extremes in the sense that its transport will be at least somewhat restricted, but that it is still small enough to occupy not more than a small fraction (11%) of the fluid of the pore. A further advantage arises from the fact that it is a relatively compact, spherical molecule and also from the relative ease of changing its valency without changing the molecular size; the latter was determined from filtration through Sephadex gels with about the same steric resolution as the glomerular capillary membrane.

Obviously, accurate determination of the valence of myoglobin, made polyanionic by a chemical procedure, is of key importance. For this purpose we therefore elaborated a method based on the determination of the electric potential difference that develops across a cellophane membrane, with on one side the probe molecule to be tested dissolved into 10 mM NaCl and on the other pure NaCl of the same ionic strength of 10 mM. Since the electric potential will be proportional to the concentration of charges in mEq/l, knowledge of the concentration of the test molecule in mM/l, enables its valence to be determined.

*Biological data*

In the biological experiments on rat kidneys, the glomerular membrane was found to

restrict the transport of the negatively charged myoglobin. More precisely, the fraction of the negative myoglobin in plasma filtered in the glomeruli was estimated at 15% as against 20% for its neutral counterpart, findings which would suggest a fixed charge density of about 35 mEq/l - a seemingly trivial finding.

However, as regards the latter number, it was calculated that the corresponding electroosmotic pressure would be about 50 mm Hg, i.e. about the same as the glomerular capillary pressure of 50-60 mm Hg. Obviously, this might merely be a coincidence, but since the charge-induced electroosmotic pressure in the peritubular capillaries (i.e. the network surrounding the tubules) also equaled their hydrostatic pressure of about 12 mm Hg, it would seem less coincidental.

A further highly questionable conclusion drawn from the ion equilibria refers to the prediction that, on the plasma side, the intramembranous hydrostatic pressure must be about 20 mm Hg lower than in plasma and thus that the membrane must be rigid, since only a rigid structure would allow such sub-pressures. This is in sharp contrast to both biochemical and morphological data, which suggest rather that the membrane is made up of flexible, gel compounds such as the glycosaminoglycans.

## The gel hypothesis

### *Construction of the model*

In the search for an alternative model, Teorell already in the early 1960's suggested that, in a charged membrane, the electric field rather than a hydrostatic pressure gradient may account for the net driving force for fluid transfer. He also predicted that in order to account for the fluid transport across the glomerular capillary the transmembranous electric potential difference need not to be larger than about 1 mVolt.

As to the question of the source of the electric field, he also pointed out that the intracapillary hydrostatic force, due to the action of the heart, might well be converted into such an intramembranous electric field.

As regards this "converting" mechanism, a closer examination of the electron-microscopical picture of the capillary membrane (see the below figure) indicates that it consists of a matrix of gel compounds, the glycosaminoglycans, supported by a relatively rigid basement membrane made up of collagen filaments (Curry, 1984, Simionescu et al., 1984).

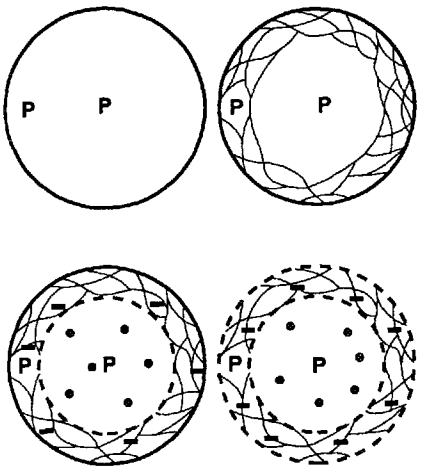


Fig. 1. Equality of pressure inside tubes surrounded by a rigid basement membrane. For further explanation see text.

In order to elucidate the characteristics of such a structure, we may first consider a (Fig. 1a) a capillary formed by a tight and rigid tubing and where the hydrostatic pressure, P, of e.g. 20 mm Hg will be the same in all parts of the tubing.

If (Fig. 1b) a network of flexible fibers is suspended along the wall of the tubing , the pressure both in the lumen and within the network is still uniform. The introduction of semipermeable barrier between the network and the lumen makes no difference, i.e. as

long as all the elements are flexible.

However, if (Fig. 1c) colloids exerting an osmotic pressure of 20 mm Hg, are added to the free fluid phase in the lumen, fluid will be dragged out of the gel with consequent shrinkage of the gel until it eventually collapses. This could be prevented by negative charges fixed to the fiber structure, which by attracting positive, mobile counter ions, may decrease the water activity to the same extent as the colloids in the free fluid phase in the lumen.

More precisely, the charges will lead to the formation of a Donnan equilibrium across the gel membrane-lumen interphase, where *the sum* of the positive and negative small ions in the gel will be larger than that in the free fluid space. As an example, if the concentration of Na and Cl in the lumen is 150 mEq/l and the charge density of the membrane is 25 mEq/l, the intramembranous concentrations of Na and Cl will be 163 mEq/l and 138 mEq/l, respectively. Since the sum of Na and Cl in the membrane thus is 1 mEq/l or 1 mOsm/l higher than that in the lumen, the osmotic pressure, RTC, resulting from the charges will be about 20 mm Hg (19.33 mm Hg at 37°C), a force which is able to balance the colloid osmotic pressure.

In the next step (Fig. 1d) also the barrier facing the interstitium is made permeable. Since the pressure within the gel membrane is still 20 mm Hg, fluid will leak out with consequent shrinkage of the gel eventually leading to collapse of the structure. Again, this could be prevented by introducing 25 mEq/l of negative charges fixed to the matrix at this side of the membrane.

Lastly if, as in the glomerular capillary membrane, the hydrostatic pressure drop is higher than the colloid osmotic force, the charge density on the Bowmans space side has to be larger that on the luminal side (Table I). The consequent larger osmotic force on

this side of the membrane will then drag fluid from lumen through the membrane and out into Bowmans space. The driving force is thus an electro-osmotic pressure gradient rather than a hydrostatic pressure gradient.

By estimating the hydrostatic forces across to the boundaries of the glomerular capillary membrane (Källskog et al., 1975), we are thus able to predict the intra-membranous charge densities at the plasma and Bowmans space side of the membrane, respectively and thence also the respective Donnan equilibria (Wolgast and Öjteg, 1988). This is illustrated in Table I, which also demonstrates that the difference in the ion equilibria results in a transmembranous electric potential gradient within the membrane, a gradient which will account for the fluid flow.

TABLE I.
Ion distribution, osmotic and hydrostatic pressures and electric potentials across the glomerular capillary membrane as predicted by the gel hypothesis. The concentration of the two myoglobins, $C_{Bow}$, in Bowmans space were considered to be unknown.

| Parameter | Plasma | Membrane | | Bowmans space |
|---|---|---|---|---|
| | | Plasma side | Bowm. sp. side | |
| Fixed charge density (mEq/l) | - | 26.49 | 36.86 | - |
| Albumin (mEq/l) | 7.87 | 0.0 | 0.0 | 0.0 |
| Na (mEq/l) | 150.00 | 159.50 | 165.58 | 146.01 |
| Cl (mEq/l) | 142.13 | 133.66 | 128.76 | 146.01 |
| (Na+Cl) mOsm/kg | 292.13 | 293.16 | 294.33 | 292.02 |
| Osmotic pressure (mm Hg) | 20.0 | 20.0 | 44.7 | 0.0 |
| Hydrostatic pressure | 56.9 | 56.9 | 56.9 | 12.2 |
| Electric potential (mV) | -0.72 | -2.36 | -3.36 | 0.0 |
| Myoglobin (0) | 1.041 | 1.041 | $C_{Bow}$ | $C_{Bow}$ |
| Myoglobin (-6) | 1.065 | 0.737 | $C_{Bow} \cdot 0.43$ | $C_{Bow}$ |

Single nephron filtration rate, SNGFR 40 nl/min
Net driving electroosmotic pressure difference 1% below 22.7 mm Hg

*Validification of the gel hypothesis*

Knowing the above-mentioned ion equilibria, we may obviously also predict the intra-membranous concentration of a polyanion such as our probe molecule, myoglobin, and thence, knowing also the bulk flow and the electric potential gradient, its net transport. A comparison between this predicted transport with that found experimentally, then showed an excellent agreement, i.e. in support of the theory; it should be emphasized, however, that it does not constitute a proof.

*Functional ultrastructure of the glomerular capillary membrane*

In the next step the glomerular capillary membrane is considered to be made up of a network of fibers (Fig. 2). Utilizing neutral probe molecules such as inulin, native myoglobin and native horse radish peroxidase the size of the pores (or rather meshes in a network) could be determined; the results showing a so called "equivalent" pore radius of 40 Å. This would then suggest that the width of the meshes in the network is about 80 Å. On this fibers we may then distribute the fixed charges, where a charge density of 35 mEq/l suggests an average distance between the charges of 40 Å.

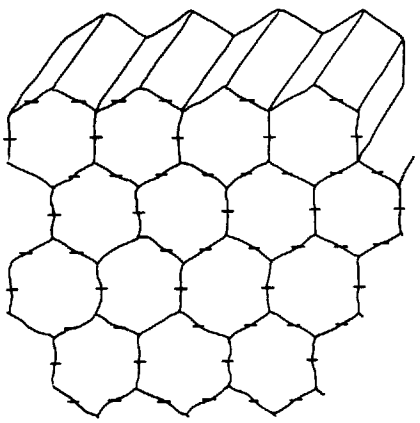

Fig. 2. Network of fibers assumed to constitute the capillary membrane.

An example of such a network is illustrated in Fig 2, showing quasihexagonal-shaped meshes with a width of about 80 Å as formed by fibers with binding sites located 40 Å apart and with one negative fixed charge in between two binding sites. Obviously the data do not permit the assessment of the true configuration of the network, but merely that the network shown in the figure is in accordance with the experimental data both with respect to the charge density and its steric structure.

It should be remarked in this context, that the distribution of the charges and the size of the meshes are coupled to each other. This is because the repulsion between the charges actually determines the final size of the meshes, i.e. since in the absence of these repulsive forces, the network would collapse (*vide supra*).

However, the electric field of the charges and hence the repulsive force, is also determined by the prevailing ionic strength; this is because the charges at least partially will be shielded by mobile, positive ions like sodium. The structure seen in Fig 2 is thus restricted to physiological ionic strength, whereas an e.g. higher ionic strength would thus result in a more complete shielding with consequent lower repulsion between the charges and hence in smaller meshes. Teorell also anticipated that biological membranes would be in a constant movement; because the electric field of a charge will be subjected to random fluctuations. It should thus be realized that the distance between the "neutralizing" sodium ion in a 150 mM saline solution is as much as 20 Å and hence that the occurrence of these ions in the vicinity of the charges will show a random fluctuation. If thus one sodium ion (in excess of a chloride ion) is located close to a charge, the electric field outside the charge will be reduced towards zero. If, on the other hand, no sodium ion is located nearby the charge, the extension of the electric field will be very large. In pure water, for instance, the extension of the electric field will be several hundred Ångströms.

This fluctuating electric repulsion between the charges will then also be followed by fluctuations of the size and form of the meshes; if there are few "neutralizing" sodium ions, the mesh tends to expand and vice versa - a "living membrane" in its true sense.

The model also anticipates that the *average* size and form of the meshes will change from one condition to the other. If, for instance, the plasma colloid osmotic pressure rises, the membrane has to shrink, i.e. since the charge- induced balancing electro-osmotic pressure has also to be increased. Such a shrinkage could be brought about either by a reduction in the size of the meshes or by a reduction of the pore length or, which is most probable, by both these changes.

*Formation of the glomerular capillary membrane*

The model also allows for a rational explanation as to the formation of the capillary barrier. It is thus difficult to imagine that the capillary endothelial cells are able to form a homogenous membrane penetrated by discrete pores. They may, however, synthesize fibers carrying regularly arranged "binding sites" and charged groups such that, after extrusion from the cells, they are able to form a network like that shown in Fig. 2.

Evidently, aging processes might damage the molecules forming the lattice structure, but this problem could be solved by a slow extrusion of damaged molecules and replacement by new molecules synthesized by the endothelium.

*The self-rinsing ability of the glomerular capillary membrane*

A charged membrane, positive or negative, might also be expected to possess a rinsing ability. This is illustrated in Fig 3, where the mesh in the upper left corner refers to an intact mesh, where the charges are partially shielded by 3 mobile, positive ions. Obviously this shielding will reduce the repulsive force such that the width of the mesh becomes 80 Å.

If then, by accident, a large molecule enters into a pore (the upper right corner), it will knock out these "neutralizing" ions thence leading to a rise in the repulsive force between the fixed charges. The mesh will then widen at the expense of the surrounding meshes (lower panel), whereby the large molecule is free to move in the downstream direction until it will be expelled in Bowman's space and subsequently into the urine.

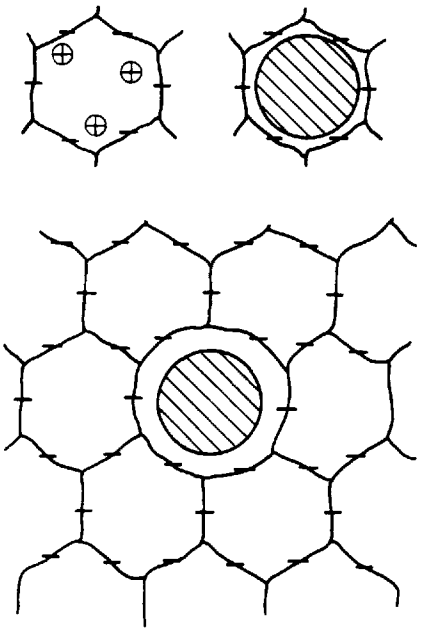

Fig. 3. Mechanism for the self rinsing ability of the capillary membrane. For explanation see text.

The strength of the force is in fact very large. As an example, if the large molecule occupies 75% of the space of the pore, the pressure in the space between the large molecule and the walls of the pore will be about 1/2 atmosphere.

*In conclusion*, the concept of charged membranes presented in 1935 by Teorell would seem to apply also to the glomerular capillary membrane as well as, probably, all other capillary membranes. More precisely the membrane is suggested as consisting of a hydrated gel rather than a rigid structure, the integrity of which is maintained by negative charges fixed to flexible fibers. As a consequence of this gel concept, the fluid transfer will be governed, not by the Starling forces, but rather by an electric field. The gel model would also seem attractive since it explains 1) the ability of the glomerular capillary membrane to change its permeability in response to external forces, 2) its rinsing ability and since 3) it gives a rational explanation for the formation of the membrane by filaments synthesized by the endothelial and epithelial cells on the two sides of the glomerular capillary membrane.

## REFERENCES

CHANG, R.L.S., W. M. DEEN, C. R. ROBERTSON and B. M. BRENNER. 1975. Permselectivity of the glomerular capillary wall. III. Restricted transport of polanions. *Kidney Int* **8**, 212-218.

CURRY, F. R. E. Mechanics and thermodynamics of transcapillary exchange. In: *Handbook of Physiology. The Cardiovascular system.* Bethesda, MD. Am Physiol. Soc., 1984, sect 2 , Vol IV., part 1, chapt. 8, p 309-374.

DEEN, W. M. and C. R. BRIDGES. 1982. Molecular charge of horse radish peroxidase. *Am J Physiol* **242**, F750-F762.

DEEN, W. M., B. SATVAT and J.M. JAMIESON. 1980. Theoretical model for glomerular filtration of charged solutes. *Am J Physiol* **238**, F126-F139.

DWORKIN, L. D. and B. M. BRENNER. Biophysical basis of glomerular filtration. In: *The Kidney: Physiology and Pathophysiology,*edited by D. W. Seldin and G. Giebisch. New York: Raven, 1985, p. 397-427.

KÄLLSKOG, Ö., L. O. LINDBOM, H. R. ULFENDAHL and M. WOLGAST. 1975. Kinetics of the glomerular filtration in the rat kidney. An experimental study. *Acta Physiol Scand* **95**, 295-300.

KANWAR, Y. S., A. LINKER and M. G. FARQUHAR. 1980. Increased permeability of the glomerular basement membrane to ferritin by removal of glycosaminoglycans (heparan sulphate) by enzyme digestion. *J Cell Biol* **86**, 688-693.

LOEB, J. Proteins and the theory of colloidal behavior. New York: McGraw-Hill, 1922.

ÖJTEG, G., K. NYGREN and M. WOLGAST. 1987. Permeability of renal capillaries. I. Preparation of neutral and charged molecular probes. *Acta Physiol Scand* **129**, 277-286.

ÖJTEG, G., K. NYGREN and M. WOLGAST. 1982. Permeability of renal capillaries. II. Transport of neutral and charged protein molecular probes. *Acta Physiol Scand* **129**, 287-294.

RENNKE, H.G., Y. PATEL and M. VENKATACHALAM. 1985. Glomerular filtration of proteins: clearance of anionic, neutral and cationic horseradish peroxidase in the rat. *Kidney Int* **13**, 324-328.

SIMIONESCU, M. and N. SIMIONESCU. Ultrastructure of the microvascular wall: functional correlations. In: *Handbook of Physiology. The Cardiovascular system. Microcirculation.* Bethesda, MD. Am Physiol. Soc., 1984, sect 2 , Vol IV., part 1, chapt 3, p 41-101.

TEORELL, T. History of the physical chemistry of charged membranes. In: *Structure and function in excitable cells.* New York: Plenum Publishing Co, 1983, p. 321-334.

TEORELL, T. 1953. Transport processes and electrical phenomena in ionic membranes. *Progress in Biophysics* **3**, 305-369.

WIEDEMANN, G. Die Lehre von der Elektrizität. Berlin: 1893, Fr Vieweg und Braunschweig.

WOLGAST, M. and G. ÖJTEG. 1988. Electrophysiology of the renal capillary membranes: gel concept applied and Starling model challenged. *Am J Physiol* **254**, F750-F762.