Histological and Polarization Studies of the Brush Border of the Proximal Convoluted Tubules of the Rat Kidney

By DOUGLAS B. BREWER

(From the Department of Experimental Pathology, University of Birmingham)

With one plate (fig. 1)

SUMMARY

The kidneys of rats were examined histologically after the intraperitoneal injection of haemoglobin and also after the intrarenal injection of a graphite suspension. During haemoglobin excretion spaces can be seen running across the brush border of the proximal convoluted tubules. These spaces are filled by the reabsorbed haemoglobin passing from the tubular lumen into the tubular cells. After intratubular injection of a graphite suspension, particles about 0.5 μ diameter cross the border and appear in the cell cytoplasm.

Examination with the polarizing microscope shows the brush border to have positive form and negative intrinsic birefringence with respect to the length of the striations.

These findings are considered in relation to the appearances found on electron microscopy. It is suggested that on present evidence it is most likely that the brush border consists of rodlets of the order of 300–600 Å diameter, with an orientated molecular structure at right angles to their length. These rodlets are gathered in tufts so that they diverge from one another. The spaces between the tufts at their bases is about 0.3–0.5 μ and at their tips they just touch one another.

MOST current textbooks of histology describe the brush border of the proximal convoluted tubules in a few lines. These brief descriptions vary somewhat, but most mention its striated appearance and compare it with the striated border of the small intestine. Von Mollendorff (1930) considered the matter in some detail. He suggested that it consists not of closely set rodlets as is commonly implied, but of holes with thin walls, resembling a honeycomb rather than a brush. He said that in longitudinal sections of the brush border he could, by focusing down, follow the walls of the holes through the thickness of the section. He also illustrated by a drawing a transverse section across the border showing a network of holes with thin, inter-connecting walls. Another view (Policard, 1944) is that the striations are produced by the passage of reabsorbed substances across the border.

It is well known that the mammalian proximal convoluted tubule can reabsorb haemoglobin (Rather, 1948) and certain other proteins (Oliver, 1948). Gérard (1935–6) has demonstrated that the open nephrons of amphibia can reabsorb much larger molecules and even minute particles of indian ink. Lison (1940) showed that the closed nephrons of the toad had this same
property. The lining cells took up particles of indian ink after micro-injection directly into the tubular lumen. Lambert (1947) found that the cells lining cysts in a polycystic kidney could also take up indian ink.

In general these findings suggest that there are holes in the brush border. They might be spaces surrounding rodlets or holes as in a honeycomb.

I examined numerous stained sections, and although occasionally oblique sections of the brush border produced appearances somewhat resembling that illustrated by von Mollendorff, the structures involved are just at the limits of resolution and nothing absolutely unequivocal was ever seen. It is often very difficult to identify positively a brush border that has been cut transversely. In fig.1, A, for example, the irregular masses in the lumen of the tubule show fine, closely set, pale areas producing a reticulated appearance resembling somewhat the 'honeycomb' of von Mollendorff. However, in this section the brush border is stained green whilst these masses are stained red, like the cytoplasm. The reticulated appearance is almost certainly due to fine vacuolation of cell cytoplasm.

The studies here described arose from the unsatisfactory results of the examination of ordinary stained sections. They consist of two parts. The first is a histological investigation of rat kidneys, after the intraperitoneal injection of haemoglobin or the intrarenal injection of a suspension of graphite particles. The second is a study of the polarization optics of the brush border.

**Histological Studies**

The work of Baker (1951) suggested to me the use of haemoglobin in an attempt to demonstrate spaces in the brush border. He has demonstrated, very beautifully, spaces in the striated border of the small intestine. He showed that during fat absorption the spaces become filled with fat which can be readily seen in frozen sections stained with sudan black. As this border has many structural and histochemical resemblances to the brush border of the renal tubules, it was hoped that in a similar fashion spaces in the brush border in the kidney could be filled with haemoglobin during its reabsorption.

**Experiment 1**

*Method.* Albino rats weighing about 300 g. were injected intraperitoneally once on one day and twice the next with a solution containing about 800 mg. of frozen-dried human haemoglobin, i.e. a total of about 2·4 g. They were killed 16 hours after the last injection when they were still excreting large amounts of haemoglobin. The kidneys were fixed in Helly's fluid, dehydrated, and embedded in ester wax. Sections were cut at 2 µ and stained by a modified Mallory technique using acid fuchsin and fast green FCF.

*Results.* The structure of the kidneys of normal control rats is well demonstrated by this method. The brush border stains green, the cytoplasm and its granules red, and the basal granules of the brush border dark reddish-purple. The basal granules are quite prominent, and although they are described as basal a small amount of green staining brush border can be seen below them
separating them from the cell cytoplasm (fig. 1, b). Under low power objectives they appear as a continuous line, but at higher magnification they can be resolved into distinct granules (fig. 1, a).

The appearances seen in the kidneys during the excretion and reabsorption of haemoglobin are very striking. Many of the proximal convoluted tubules are filled with haemoglobin which is stained bright red by the acid fuchsin. In the cytoplasm of many of the cells can be seen droplets of reabsorbed haemoglobin. The normal green-staining brush border is replaced by an alternation of green and bright red striations (fig. 1, c). The red colour is due to haemoglobin passing through and filling spaces in the brush border. In many of the tubules the haemoglobin-containing spaces do not appear to extend right up to the edge of the border. They are separated from the haemoglobin in the tubular lumen by a narrow band of green-staining brush border (fig. 1, c and d). The spaces are about 0.3–0.5 μ in diameter in fixed and stained sections.

From the size of the spaces it seemed possible that the cells of the rat nephron, like those of the closed nephrons of the toad, would be able to re-absorb minute particles introduced directly into the tubular lumen. The second experiment was undertaken in an attempt to demonstrate this.

Experiment 2

Method. Albino rats weighing about 300 g. were anaesthetized with ether. The left kidney was exposed through a loin incision. A suspension in water of graphite particles, 0.5–1.0 μ particle-diameter containing 0.39 per cent. graphite (kindly supplied by Acheson’s Colloids Ltd., Plymouth), was injected into the cortex of the kidney with a very fine needle. About 0.2 ml. was slowly injected into the subcapsular cortex as the needle was being withdrawn. The rats were killed after 6 hours. The kidneys were fixed in formalin, dehydrated, and embedded in paraffin wax. Sections were cut and stained with Mayer’s haemalum and eosin.

Results. The aim was to inject some of the graphite into the lumen of the tubules. This was achieved in one of three animals. Numerous particles could be seen in the lumen of a number of adjacent coils of proximal convoluted tubule. It was obviously the same tubule cut at different points along its length. In addition a number of particles had passed into the cell cytoplasm where they could be seen adjacent to cell nuclei and in the same plane of focus (fig. 1, e). These particles must have passed across the brush border, presumably in the spaces seen during haemoglobin reabsorption.

Polarization Microscopy

Schmidt (1943) found that the striated border of the small intestine of the tadpole shows positive form birefringence. It seemed likely in view of this and the reported findings on examining the brush border with the electron microscope that it too would show similar properties. An examination of the brush border with the polarizing microscope was therefore undertaken. It was
only when this was partly completed that I became familiar with the work of Sjöstrand (1945-6) and Hillarp and Olivecrona (1946-7). With the knowledge of their findings the examination was extended. Our results are largely in agreement.

Method. Frozen sections of fresh unfixed rat kidneys were examined mounted in normal saline and in glycerol, refractive index 1.467. These sections proved very difficult to handle and the bulk of the observations were made on frozen sections of formalin-fixed kidneys. Frozen sections of formalin-fixed material were cut at 30 μ. After being washed in water some were examined mounted in distilled water (R.I. 1.332), some in 50 per cent. human serum albumen (R.I. 1.405), some in propylene glycol (R.I. 1.432), and some in glycerol.

Other sections were dehydrated in alcohol and mounted in each of the following media: distilled water, tertiary butyl alcohol (R.I. 1.387), 50 per cent. serum albumen, chloroform (R.I. 1.446), glycerol, benzene (R.I. 1.500), oil of cloves (R.I. 1.534), methylene iodide 1 part + xylene 1 part (R.I. 1.606), methylene iodide 2 parts + xylene 1 part (R.I. 1.639), and pure methylene iodide (R.I. 1.75). The refractive indices were all measured with an Abbé refractometer except methylene iodide which has too high a refractive index for the instrument used.

Lastly an attempt was made to study the effect on the intrinsic birefringence of extracting the fat found in the brush border. Sections were placed in alcohol, in a saturated solution of sodium taurocholate in water, and in a 20 per cent. solution of Teepol (a proprietary anionic synthetic detergent of the alkyl sulphate type) for varying times. Similarly treated sections were examined by the polarizing microscope and stained for fat by the 3:4 benzpyrene

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Fig. 1 (plate). A, proximal convoluted tubule showing brush border with well-defined basal granules. The faintly reticulated material in the lumen in the upper part of the field is cytoplasm from cells of the other wall of the tubule. Acid fuchsin and fast green FCF. Ilford microfilters 3 and 4.

B, proximal convoluted tubule showing brush border with basal granules appearing as a line and separated from the cell by a narrow band of brush border. Acid fuchsin and fast green FCF. Ilford microfilters 3 and 4.

c, proximal convoluted tubule during reabsorption of haemoglobin. The haemoglobin which fills the tubular lumen is also filling spaces in the brush border as it passes across it into the cells. Acid fuchsin and fast green FCF. Ilford microfilters 3 and 4.

D, proximal convoluted tubule during haemoglobin reabsorption. In this, as in the previous figure, the haemoglobin-filled spaces are separated from the lumen at some points by a narrow band of apparently intact brush border. Acid fuchsin and fast green FCF. Ilford microfilters 3 and 4.

E, longitudinal section of tubule showing particles of graphite in the lumen. In the cells on the left near the two adjacent nuclei can be seen a number of fine intracellular particles. Haemalum and eosin. Ilford microfilters 3 and 4.

F, unstained frozen section under crossed polaroid screens with full compensation in one axis. The brush border shows up brightly in the axis at right angles.

g, proximal convoluted tubule photographed in non-polarized light. In this section the lumen is lined by brush border sectioned along its long axis (in polarized light this shows marked birefringence) and filled by the brush border of the other wall of the tubule cut transversely (this shows no birefringence). Acid fuchsin and fast green FCF.
method of Berg (1951) and the propylene glycol sudan IV method (Pearse, 1953).

The polarizing microscope used was a Cooke, Troughton, and Sims advanced model polarizing microscope with a $1/30 \lambda$ rotating mica plate compensator.

**Results.** In fresh unfixed sections mounted in saline the brush border shows definite birefringence, positive in relation to the length of the striations. Fresh sections mounted in glycerol show a marked increase in birefringence and a striking change in its sign. It becomes negative along the length of the striations. The brush border in formalin-fixed frozen sections shows the same birefringence and the same change on immersion in glycerol. Immersion in propylene glycol and serum albumen also changes the sign of the birefringence from positive to negative.

In dehydrated sections subsequently mounted in water the birefringence is more marked than in undehydrated sections (fig. 1, f). It is again positive in character. It can readily be seen in transverse and longitudinal sections of the proximal convoluted tubules and is more striking in the inner cortex than in the subcapsular cortex.

The birefringence is readily detectable in butyl alcohol, slight but definite in 50 per cent. serum albumen and chloroform. It cannot be detected in glycerol, benzene, or oil of cloves, but reappears in a mixture of equal parts of methylene iodide and xylol. It is still stronger in the mixture of 2 parts methylene iodide and 1 part xylene, and in pure methylene iodide it is as strong as in distilled water.

Sections mounted in benzene were remounted in distilled water and the birefringence reappeared. When present in dehydrated sections the birefringence was always positive. Owing to the marked variation in retardation of the brush border from tubule to tubule in any one section, measurements of retardation are not given.

It is seen that in undehydrated sections the birefringence undergoes a change from positive to negative on immersion in media of high refractive index. This is an example of the so-called metatropic reaction (Bear and Schmitt, 1936–7). The birefringence of dehydrated sections shows the typical form birefringence curve on immersion in fluids of a wide range of refractive indices.

Sjöstrand (1945–6) found that the brush border shows form birefringence. Hillarp and Olivecrona (1946–7) confirmed this and also described the metatropic reaction of undehydrated sections, although they found that sections mounted in water were isotropic. The reaction they suggested was due to a combination of form and intrinsic birefringence, the intrinsic birefringence being due to orientated lipide.

Fat can be stained in the brush border both by the propylene glycol sudan IV technique and by the benzpyrene method. Five minutes' immersion in alcohol is sufficient to abolish almost completely the staining reaction for fat. The negative birefringence disappears after only a few seconds in alcohol.
Such sections show an undiminished staining for fat. Immersion in bile salt solution for up to 2 hours was without effect either on the staining reaction for fat or on the intrinsic birefringence. The 20 per cent. solution of Teepol abolished both the staining reaction for fat and the intrinsic birefringence in 5 minutes.

Besides the smooth muscle of the blood-vessels and the perivascular collagen, the tubular basement membrane, the basement membrane of Bowman's capsule and of the glomeruli all show birefringence. In all it is positive circumferentially in transverse section and positive along the long axis of the wall in longitudinal sections. It is reduced in benzene and oil of cloves but it never completely disappears, so that it consists of both intrinsic and form birefringence.

**DISCUSSION**

The results of the histological part of the investigation show that in the rat there are spaces in the brush border of the proximal convoluted tubules that become filled with haemoglobin during its reabsorption. In whichever plane the individual tubule is cut the spaces always appear to be about 0.3–0.5 μ wide. Between the spaces are apparently solid portions of brush border of about the same width. When particles of graphite are introduced into the tubular lumen they can pass across the border into the cell cytoplasm.

Studies with the polarizing microscope yield some information about the possible nature of the walls between the spaces. The metatropic reaction, the change in sign of the birefringence on mounting undehydrated sections in media of high refractive index, is generally taken to indicate the presence of positive form and negative intrinsic birefringence (Mitchison, 1952). In water the positive form birefringence predominates. Media of higher refractive index reduce the form but do not affect the intrinsic birefringence and so the total effect is to change the sign from positive to negative. The combination of positive form with negative intrinsic birefringence indicates that the micellar axes are parallel to the striations of the border and the molecular axes at right angles.

The simplest explanation of the form birefringence is that the walls are made up of rodlets of a diameter small in relation to the wavelength of light (i.e. of the order of 500 Å).

Such an interpretation was put forward by Schmidt (1943) to explain his findings in the striated border of the small intestine. Flagella, cilia, and sperm tails also show positive form birefringence. Schmitt, Hall, and Jakus (1943) showed by electron microscopy that the basis for this in all these structures is the presence of long fibrils 300–600 Å wide running along their length.

However, there is another possible explanation of these findings. As was pointed out by Schmitt (1944), the finding of positive form birefringence need not mean that the submicroscopic particles are rodlets. In the case of the brush border all that is required is that the long axes of the particles are parallel to the visible striations. They could then be either rodlets or platelets.
However, platelets so arranged would appear anisotropic in one axis, e.g. in transverse section of the tubule, and isotropic in a direction at right angles. This is not so, but if it is assumed that the platelets are arranged around pores the parts of the platelets viewed tangentially would show positive form birefringence along the striations of the border, whilst those running across the optical axis of the microscope would be isotropic. Such an arrangement would show positive form birefringence in all axes at right angles to the striations. A similar effect, on a larger scale, is produced by the concentric protein layers of the myelinated axon sheath (Bear and Schmitt, 1936–7; Chin and Schmitt, 1936–7).

Theoretically it is possible to distinguish between these two possibilities by examining the brush border cut in transverse section. If it consists of rodlets it should appear isotropic, but if of lamellae arranged around pores each pore wall will be positively birefringent circumferentially as are the basement membrane of the tubules and the protein layers of the axon sheath. It is rather difficult to examine the brush border in transverse section. One has to be certain that it is in fact brush border and that it does not include cell cytoplasm. The section would have to be truly transverse, as oblique sections of rods would show some birefringence. Further, such sections of brush border might be too thin for their birefringence to be readily detectable.

An attempt was made to examine the brush border in transverse section. Paraffin sections cut at 2 μ and stained with acid fuchsin and fast green FCF were searched for suitable fields. When one was found (fig. 1, c), the cover slip was removed and the section taken down to water. Examination with the polarizing microscope shows marked birefringence in most of the brush borders. In the tubule showing the brush border cut transversely and longitudinally, that cut longitudinally was strongly birefringent, while that cut transversely filling the lumen showed no detectable birefringence with the 1/30 λ compensator. In making this observation it was unfortunately necessary to use paraffin-embedded material and to stain the sections so that the brush border could be identified. Nevertheless, the brush borders cut longitudinally still showed strong birefringence in sections cut at 2 μ. If it consists of concentric lamellae about pores one would have expected it to still show birefringence in transverse section. Despite these unavoidable difficulties this observation is evidence in favour of the view that the brush border consists of rodlets. Sjöstrand (1945–6) also examined the brush border in transverse section and found it isotropic.

The nature of the probable molecular arrangement underlying the intrinsic birefringence is more doubtful. Until recently this combination of negative intrinsic with positive form birefringence in which the negative birefringence is readily abolished by exposure to alcohol had been interpreted as being due to an orientated micellar structure of protein with lipide molecules arranged at right angles, e.g. in the crustacean nerve axon sheath (Bear and Schmitt, 1936–7) and in the brush border (Hillarp and Olivecrona, 1946–7). However, this interpretation has recently been criticized (Mitchison, 1952). It depends...
on the demonstration of lipide in the structure concerned and on the assumption that exposure to alcohol only removes the lipide and leaves the protein micelles unaltered. As was pointed out by Mitchison, alcohol produces its effect very rapidly. In fact it was not found possible to remove sections from alcohol sufficiently quickly to leave unchanged the intrinsic birefringence. Because of this very rapid action of alcohol he suggests that it might produce its effect by denaturing or dehydrating the protein molecules. He proposes a structure of protein micelles made up of molecules folded like Chinese crackers so that the axes of molecules are at right angles to those of the micelles. Alternatively, he suggests the protein might be in piles of disks with the molecular chains parallel to the disk faces or in spirals. Alcohol could then abolish the intrinsic birefringence by disorganizing the alignment of the protein chains.

In the brush border the lipide is very readily removed both by alcohol and Teepol and at the same time the intrinsic birefringence disappears. However, alcohol of course dehydrates the protein structure and Teepol is likely to denature it (Putnam, 1948). The fact that the intrinsic birefringence disappears before the fat is extracted by alcohol might be due to the disarrangement of orientated lipide molecules. These findings then can be explained by the assumption of an orientated molecular structure of either lipide or protein and do not provide conclusive evidence in favour of either.

The reported studies of the brush border with the electron microscope have produced varying ideas as to its finer structure. Gautier and Bernhard (1950) say that the border is made up of complex branches and lamellae that join with one another to form very irregular pores quite unlike the very regular structures seen during haemoglobin reabsorption. In transverse sections they illustrate irregular pores surrounded by fine inter-connecting walls. The findings reported in two studies from America are quite different. Pease and Baker (1950) found a regular structure of fine rodlets which they illustrate very convincingly both in longitudinal and transverse section. These fine processes are only about 300 Å diameter and the spaces between them are about the same size. The findings of Dalton and others (1950–1) are similar. They also found rodlets, but they appeared to be gathered in little tufts each made up of numerous processes. In each tuft the rodlets diverge from one another so that the space between the tufts is gradually reduced. At the base there are spaces about 0.5 μ between each tuft, but at their distal ends the diverging processes almost touch.

In the most recent paper on this subject by Sjostrand and Rhodin (1953) the illustrations are technically of the highest standard yet published. They also found the brush border to be made up of rodlets. They described them as ducts and in their illustrations they have a thin dense wall and a lumen filled with a less dense substance. They are closed at each end by the wall so the description ‘duct’ is rather a misnomer. When cut transversely they appear circular in cross-section. According to Sjostrand and Rhodin the surface of the brush border adjoining the lumen is smooth and covered by a continuous
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membrane. This is difficult to make out from their illustrations as none of them includes identifiable tubular lumen and this is contrary to the finding that the tubular cells can take up particles of graphite.

Pease and Baker, Dalton and others, and Sjöstrand and Rhodin all agree in finding minute closely set roddlets in the brush border. They disagree regarding their dimensions. Dalton and others find them to be 700 Å in diameter, Sjöstrand and Rhodin 600 Å, and Pease and Baker 300 Å. However, any of these findings would produce form birefringence, but only Dalton and his colleagues found a structure of roddlets arranged in tufts consistent with the finding of spaces in the brush border during haemoglobin reabsorption.

From evidence at present available it seems most likely that the brush border consists of a complex arrangement of roddlets roughly 300–600 Å in diameter. At right angles to the long axis of the roddlets is an arrangement of parallel-orientated molecules of either lipide or protein. These roddlets are set in tufts each possibly arising in a basal granule. Between the tufts are spaces about 0.5 μ diameter, along which the reabsorbed glomerular filtrate passes and along which particles of graphite of about this diameter could also pass. The arrangement of roddlets in diverging tufts might account for the fact that in many tubules the haemoglobin-filled spaces are separated from the lumen by a narrow, apparently intact band of brush border.

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