THE POLARITY OF THE PROXIMAL TUBULE CELL IN RAT KIDNEY

Different Surface Charges for the Brush-Border Microvilli and Plasma Membranes from the Basal Infoldings

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ABSTRACT

Two different membrane fractions were obtained from a brush-border fraction of rat kidney cortex by using their different electrical surface charges in preparative free-flow electrophoresis. One membrane fraction contained only morphologically intact microvilli and was characterized by a high specific activity of alkaline phosphatase. The other fraction morphologically resembled classical plasma membranes by possessing junctional complexes and a high Na-K-ATPase activity. The contamination of the isolated membrane fractions by other cell organelles was extremely low. These two fractions represent the apical (luminal) and the basal (interstitial) area of the renal proximal tubule cell membrane and clearly demonstrate the polarity of this cell.

INTRODUCTION

The cells of duct systems which perform a trans-epithelial transport of solutes are characterized by a morphological polarity of the membranes surrounding the lumen of the duct on one side, and by a contact with the interstitium on the other side. Thus, in the proximal tubule of kidney, the luminal surface of the cell is coated with microvilli (the so-called brush border), whereas, on the contraluminal side, plasma membranes form the basal infoldings of the cell.

In order to clearly determine the molecular basis for transepithelial transport, it is essential to isolate and characterize both membranes in pure homogeneous form. The fact that there are differences in the cell envelope due to the polarity of the cell has been overlooked, particularly for the kidney, and plasma membrane fractions have been isolated which did not allow a localization of biochemical processes in one special region of the cell membrane. Thuneberg and Rostgaard (1) and Kinne and Kinne-Saffran (2) tried to characterize the luminal membrane by isolating membrane fragments which are enriched in brush-border fragments. This approach was also followed by Berger and Sacktor (3), Willong and Neville (4), and Binkley et al. (5). In all of these membrane fractions, the alkaline phosphatase, a marker enzyme for microvilli, is enriched. There is also an enrichment of Na-K-ATPase activity in these preparations as compared with the starting material (6). Since this enzyme is present in the basal part of the cell, the activity of Na-K-ATPase in these preparations could be explained as very likely being due to the presence of plasma membranes from the basal infoldings. In accordance with this assumption, electron micrographs of those mem-

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brane fractions show that, in addition to brush-border fragments, vesicular structures are present which are similar in morphology to classical plasma membranes (7).

In this paper, the isolation and separation of microvilli (from the apical part) and of plasma membranes (from the basal part) from the proximal tubule cell of rat kidney cortex is described. After several centrifugation steps, a fraction rich in alkaline phosphatase and Na-K-ATPase (crude brush-border fraction) is obtained from the homogenate which is electrophoresed in the preparative free-flow electrophoresis apparatus developed by Hannig (8). On the basis of their different electrical surface charges, and the comparison with the activities in the crude brush-border fraction, the following is obtained. In the fraction closest to the anode, the Na-K-ATPase activity is enriched by a factor of 16 whereas the alkaline phosphatase is markedly reduced. In the membrane fraction closest to the cathode, the alkaline phosphatase activity is enriched by a factor of 14, whereas the Na-K-ATPase is reduced. Electron microscopy shows that the fraction rich in alkaline phosphatase contains nearly pure microvilli. In the fraction with higher Na-K-ATPase activity, typical plasma membranes with characteristic junctional complexes predominate. These results support the utility of alkaline phosphatase as a marker enzyme for microvilli, and of Na-K-ATPase for membranes derived from the basal infoldings of the proximal tubule cell from rat kidney.

**Materials and Methods**

**Preparation of Biological Material**

All the experiments were carried out between 0° and 4°C. Male Wistar rats, 180 g in weight, were sacrificed by a blow on the neck and the kidneys were removed immediately. The renal cortex (4–5 g from 10 rats) was removed with a razor blade. Membranes were then prepared in a refrigerated Sorvall RC2-B centrifuge according to the isolation diagram shown in Fig. 1. The fraction thus obtained, called crude brush-border fraction, was used for the separation in the FF4. A buffer which contained $1 \times 10^{-8} \text{M}$ triethanolamine-HCl and $2.5 \times 10^{-4} \text{M}$ sucrose, pH 7.6 ($1 \text{n NaOH}$) was used as an isolation medium.

**Preparative Free-Flow Electrophoresis**

Preparative free-flow electrophoresis was carried out in the FF4 Electrophoresis Apparatus developed by Hannig (8) (Desaga, Heidelberg, Germany, and Brinkmann Instruments Inc., Westbury, N. Y.) The electrophoresis buffer (buffer in the separation chamber) was prepared from $1 \times 10^{-2} \text{M}$ triethanolamine, $1 \times 10^{-2} \text{M}$ acetic acid, and $3.3 \times 10^{-1} \text{M}$ sucrose, pH 7.4 ($2 \text{n NaOH}$). The conductivity was 5.0–5.2 $\mu$hmhos. The buffer for the electrode vessels consisted of $1 \times 10^{-1} \text{M}$ triethanolamine and $1 \times 10^{-1} \text{M}$ acetic acid, pH 7.4 ($2 \text{n NaOH}$). The conditions of the runs were as follows: 90 ± 10% $\text{v/cm}$, 110 $\text{mA}$, temperature 5°C, electrophoresis buffer flow 2 $\text{ml/hr}$ and fraction. All the samples were spun down for 10 min at 300 $\text{g}$ before the separation in order to remove aggregates. The protein content of the super-

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**Figure 1** Isolation scheme for the preparation of a crude brush-border fraction of rat kidney cortex.
natant thus obtained was brought to about 10 mg per ml with electrophoresis buffer, and this sample was then injected into the FF4 apparatus above fraction 70. Sample injection was 2 ml per hr. 92 fractions were collected at 0°C.

Quantitative Protein and Enzyme Assay

Protein was quantitatively determined by using a modified Technicon Peptide AutoAnalyzer System (9) (Technicon Corporation, Ardsley, N. Y.). The system was calibrated with test solutions of bovine serum albumin.

Commonly used tests were applied for determining the enzymatic activities. Alkaline phosphatase (E.C.3.1.3.1) and the ATPases (E.C.3.6.1.3) were measured according to Kinne et al. (10) with deoxycholate (DOC) as activator, as was acid phosphatase (E.C 3.1.2) and glucose-6-phosphatase (E.C.3.1.3.9). Monoamino oxidase (E.C.1.4.3.4) and succinate dehydrogenase (E.C.1.3.99.1) activities were determined as previously described (11).

Electron Microscope

For preparing the thin sections for the microscope, FF4 fractions containing the membranes to be characterized were spun at 37,000 g for 10 min at 4°C. The pellets were fixed with glutaraldehyde and OsO₄, dehydrated, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. For negative staining, samples from the FF4 fractions were directly stained at 0°C either with 2% phosphotungstate, pH 7.2, or with 2.5% ammonium molybdate, pH 7.4 (droplet method). A JEM T7 electron microscope was used for viewing the specimens (60 kv).

RESULTS

Enzymatic Characterization of the Membranes

STARTING MATERIAL FOR THE FF4 EXPERIMENTS: In order to preserve the structural and morphological integrity of the membranes, an isotonic isolation and separation medium was used. Particularly, ethylenediaminetetraacetate (EDTA) was omitted from the buffer, since it was found in pilot experiments that EDTA-treated membranes have a much lower electrophoretic mobility than untreated ones. This agrees with similar findings on isolated mitochondrial membranes1. In the crude brush-border fraction prepared with this buffer and according to the isolation scheme (Fig 1), Na-K-ATPase is already enriched by a factor of 5, and alkaline phosphatase by a factor of 8, when compared with the starting cortex homogenate (12). This indicates that in this fraction, brush borders as well as plasma membranes must be enriched. Almost all of the brush-border fragments described earlier (1), (3), (4), (13), (14), (15), (16), and almost all of the mitochondria were excluded by the centrifugation procedure. A very important condition for a reliable separation in the FF4 is the removal of adhesive, masking proteins from the material to be separated. This was achieved by several washings during the preparation. Finally, before electrophoresis, the crude brush-border suspension was centrifuged at low speed in order to remove aggregates formed during the isolation of the membrane fraction. The preparation thus obtained was suitable for an electrophoretic separation.

ENZYMATIC DATA AFTER ELECTROPHORESIS: After electrophoresis of the crude brush-border fraction, a protein distribution as is illustrated in Fig 2 a was obtained. A maximum can be seen in fraction 32 with a shoulder towards the anode. Both marker enzymes, Na-K-ATPase and alkaline phosphatase, show a completely different distribution in the fractions collected (Fig. 2 b) The maximum for the Na-K-ATPase is in fraction 24, and for the alkaline phosphatase in fraction 32. The maxima are clearly separated from each other. The lack of a protein maximum for the Na-K-ATPase-carrying membranes in fraction 24 is due to the relatively low amount of this type of membranous material in comparison to the membranes in fraction 32. In Fig. 2 c the distribution of Mg-ATPase is illustrated. This enzyme is present in both types of membranes. Fractions 20-28 and 31-38 were pooled separately and reelectrophorized. Fig. 3 shows the protein and enzyme distribution after the run. It can clearly be seen that this step leads to a further purification of both types of membranes, as is indicated by the broad protein distribution in comparison with the sharp peaks of the enzymes. The slight shift of both enzyme maxima is due to the fact that for the reelectrophoresis run purer fractions were injected into the apparatus. As a rule, this regularly influences the electrophoretic mobility. After this second run the left membrane fraction was obtained with a Na-K-ATPase activity enriched by a factor of 16, whereas the specific activity of the alkaline phosphatase is only two-thirds of that in the starting suspension.

1 Heidrich, H.-G. Unpublished results.
the other hand, the alkaline phosphatase in fraction 33 is 15 times that in the starting material, and the Na-K-ATPase is reduced (Fig. 4).

Contamination by other cellular components was generally very low, even in the crude brush-border fraction itself before electrophoresis (12). Table I shows that after this purification step, the amount of mitochondria and endoplasmatic reticulum is reduced even more. The acid phosphatase, however, was not significantly changed by electrophoresis, but this might be due to the reasons discussed below.

**Electron Microscope**

Starting material for the FF4 experiments: Figs. 5a and 5b show the micro-
graphs of the starting suspension injected into the FF4 apparatus. Large amounts of typical microvilli can be seen, particularly with the negative staining technique. They have a length of 1 μ and a width of about 0.07–0.08 μ. Some of the microvilli contain apical or basal vacuoles. In thin sections the characteristic external surface coat can easily be detected. Another predominant group of membranes in the starting suspension can be characterized as large membrane sheets with a bright border line. These particles appear in thin sections as large open circles with a clear trilaminar membrane. Occasionally, junctional complexes can be detected, particularly relatively short tight junctions. These membranes are the classical plasma membranes. Mitochondria can rarely be seen in this material, as is the case with lysosomes. It should be emphasized that the suspension does not contain the normally described brush-border fragments with clusters of microvilli hanging together, but only single microvilli and plasma membranes.

**MICROVILLI FROM THE FF4:** Micrographs. (negative staining, Fig. 6 a, and thin sections, Fig. 6 b) of fraction 33 (from Fig. 2) illustrate that already after the first electrophoresis run, it is mainly microvilli which are present in this fraction. In the negatively stained specimens, the external coat can clearly be seen and protuberances are quite frequent. The tops of the microvilli are closed, but the other ends are open and quite often form trumpet-like structures, the membrane pieces of which may stem from the intervillous space of the luminal surface in which pinocytotic processes occur. No filaments can be found, as are described for brush borders of rabbit, but longitudinal struc-

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2 Rostgaard, J. April 1971. Personal communication.
FIGURE 4  Enrichment of Na-K-ATPase □ and alkaline phosphatase □ in the FF4 fractions 33 and 35 of the reelectrophoresis run shown in Fig. 3.

TABLE I  Specific Activities of Contaminations

| Fraction                  | Enzyme Activities mU/mg protein per mm |
|---------------------------|----------------------------------------|
|                           | Total (mitochondria)                   |
| Crude brush-border fraction| 0.0032 0.08 0.199                       |
| 23 (from Fig 3)           | 0.00025 0.049 0.034                    |
| 35 (from Fig 3)           | 0.00016 0.065 0.027                    |

The contamination of the purified membrane fraction with other cellular particles is extremely low, as is indicated by the specific activities.

cules can be seen at higher magnification. Whether or not these structures resemble the filaments of the inner side of the microvilli described by Mukherjee and Staehelin for the rat intestine (17) has yet to be clarified. Freeze-etching experiments are presently under way in an effort to determine the character of these structures. In thin sections, the limiting membrane can only occasionally be seen as a trilaminar membrane, but not very clearly nor conclusively. The external fuzzy coat is always preserved. Only very few vesicles from the endoplasmic reticulum are present as contamination.

In order to establish the optimal conditions for negative staining, experiments were conducted with different reagents. Microvilli could be contrasted much better with 2.5% ammonium molybdate, pH 7.4, than with the normally used phosphotungstate. With this stain the subunits of the external coat of the microvilli could be recognized as knobs of about 60 Å, whereas with phosphotungstate the coat was seen only as a cloudy layer (13). The electrophoretic mobility of the microvilli appears to be determined by a relation between the length of the microvillus and the amount of adhering membrane base. The "cleaner" the microvilli, the less they move toward the anode. The more dominant the base in relation to the length of the villus, the more this particle moves toward the anode.

PLASMA MEMBRANES IN THE FF4: Figs 7 a and 7 b show the membranes collected in FF4 fraction 23 (from Fig. 2). This fraction was shown to contain a high specific activity of Na-K-ATPase, which is a typical plasma membrane marker. The electron micrographs of the negatively stained specimen showed, almost exclusively, large membranous sheets with bright borders. In some specimens a regular pattern on the surface could vaguely be discerned, and on rare occasions, regularly arranged knobs on the limiting border. In contrast to the microvilli, the membranes from this fraction could be stained much better with 2% phosphotungstate, pH 7.2, than with ammonium molybdate. Staining experiments at different temperatures have not been conducted with this membrane (18). Micrographs of thin sections of this membrane also presented the typical morphology which is known for plasma membranes i.e. large open unilamellar membranes with occasional granular content and with junctional complexes. Relatively short tight junctions occurred quite often, but not desmosomes. Again, no other cell particles could be found, only very few microvilli were
Figure 5  Electron micrographs of a crude brush-border fraction prepared according to the isolation scheme of Fig. 1 (starting material for the FF4 experiments). Microvilli (mv), membranes of the basal infoldings (mbi), mitochondria (mi), and vesicles from the endoplasmic reticulum (rer) can be recognized. rer, rough endoplasmic reticulum. Fig. 5 a, thin section. X 31,000. Fig. 5 b, negative staining. X 31,000.
Figure 6  Microvilli from rat kidney brush border purified in the FF4 fraction 31-88 from Fig. 6). Some endoplasmic reticulum vesicles are still present but they can be removed by reelectrophoresis. The insert in Fig. 6 b shows the base of a microvillus, the surface coat is clearly to be recognized. Fig. 6 a, thin section $\times$ 31,000. Fig 6 b, negative staining. $\times$ 43,000; insert, $\times$ 70,000.
Figure 7 Membranes of the basal infoldings of the proximal tubule cell purified by preparative free-flow electrophoresis (fraction 20-28 from Fig. 2). The insert in Fig. 7 a shows a relatively short tight junction. Fig. 7 a, thin section. × 48,000; insert, × 70,000. Fig. 7 b, negative staining × 30,000.
Figure 8  Comparison of the two acid phosphatase-positive particles from rat kidney cortex as isolated by preparative free-flow electrophoresis. The diagram represents the distribution of acid phosphatase activity in a normal run in which only a very few of the unidentified vesicles could be found ——, and the distribution in a run with a larger amount of vesicles —— (for explanation see text). The electron micrographs show rat kidney lysosomes as can be found in fractions 25–38 of the run (A), (B). The different locations of the coats in these vesicles and in the microvilli can clearly be seen (arrows). × 30,000.

present, and only those with relatively large base membrane pieces in comparison to their length

**UNIDENTIFIED VESICLES:** In some experiments, large empty vesicles could be detected in FF4 fractions 35–38 (Fig 8). These vesicles were similar in size to lysosomes (19). No external surface layer could be detected, but at the inside of the membrane, a coat was present which varied in thickness. In these “vesicle positive” experiments, acid phosphatase activity was positive not only in those fractions in which lysosomes were present but also in fractions 35–38 (Fig 8). However, histochemical experiments which could possibly demonstrate the acid phosphatase activity within the vesicles have not yet been conducted. The image of these vesicles resembled that of outer mitochondrial membranes which could have been formed during the preparation of the crude brush-border fraction. This possibility can be excluded since the ratio of the specific activities for succinate dehydrogenase/monoamine oxidase was $4 \times 10^{-4}$. This is the same ratio found in intact mitochondria, but the activities were, of course, extremely low. In addition, outer mitochondrial membranes never show any kind of coats, either on their outer or on their inner side.

**Flow Diagram**

In addition to the investigations on the distribution of microvilli and plasma membranes in the FF4, the electrophoretic behavior of other cell organelles in kidney was studied on the basis of their marker enzymes and morphology. It should be emphasized that all the mitochondria, lysosomes, and vesicles from the endoplasmic reticulum demonstrated the same electrophoretic mobilities as those found for the rat liver organelles (20). Therefore the results on rat kidney are presented only schematically (Fig. 9). Although a strong
overlap of organelles and membranes can be recognized, it is apparent that a large part of the plasma membranes of the basal infoldings and a still larger part of the microvilli must be considered as being homogeneous. Also, the plasma membranes of rat liver have the same electrophoretic properties as the plasma membranes of the basal infoldings from the renal proximal tubule cell.

**DISCUSSION**

It has been shown in recent investigations (11), (20), (21), (22) that the technique of preparative free-flow electrophoresis is capable of solving problems which cannot be treated by other methods. One important condition for a successful application of this relatively new separation technique is a different electrical behavior of the materials to be purified. Therefore, in the separation of biological membranes, it is necessary that the membranes have different electrical surface charges. In most cases, this varying surface behavior appears to be connected with a functional difference.

For theoretical reasons, differences in the functional behavior have been postulated for the brush-border membrane and the membrane of the basal infoldings (23). Corresponding structural differences have been found by microdissection (24) and by histochemical (25) and radiouautographic (26) studies. However, satisfactory isolation and characterization of the functionally different areas of the cell membrane have not been achieved. Nearly all the preparations described previously and designated as brush-border fraction also contained membranes from the basal part of the cell.

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**Figure 9** Distribution of microvilli and membranes of the basal infoldings of the proximal tubule cell.

In addition, the distribution of mitochondria, lysosomes, and endoplasmic reticulum vesicles (microsomal vesicles) of a rat kidney cortex homogenate in an FF4 experiment is schematically illustrated in the flow diagram (see text).
In these preparations, the enrichment of alkaline phosphatase and Na-K-ATPase occurs at almost the same rate. However, indications of a possible separation of these two enzyme activities have recently been presented by Kinne et al. (10), using density gradient techniques, and also by Müller and Rosgaard.

In both investigations, clear but unsatisfactory separations of the two marker enzymes present in the brush-border fractions have been shown. The density difference of the membranes carrying the two enzymes is perhaps too small to accomplish a complete separation via density gradient techniques. With these considerations in mind, the preparation method described above was devised which met all the requirements for electrophoretic separation. The presence of larger particles, such as brush-border fragments, in the starting suspension was avoided as much as possible. Almost only single microvilli and free plasma membranes from the proximal tubule cell were used. Apparently, this kind of preparation is possible in rat kidney since a "lifting-off" from the apical and the basal part of the cell can occur quite easily in this organ (1) in comparison to the intestine. The surface charges of these microvilli and membranes from the basal infoldings appear to be sufficiently different to permit a separation via electrophoresis. Somehow these differences in the membranes can be already recognized in the electron microscope by previous staining of the membranes with ammonium molybdate. Microvilli are irregularly coated with particles. Similarly conspicuous particles cannot be detected with ammonium molybdate in the basal plasma membranes of the proximal tubule cell.

In addition, the marker enzymes for the renal brush border have been more completely specified in this work. Alkaline phosphatase and Na-K-ATPase are, in general, useful as markers for a brush-border fraction. The results presented here have clearly shown that two main groups of particulate material are obtained out of a brush-border fraction with electrophoresis. Since these two fractions correspond to two markedly different distributions, a specification of the enzyme markers can be made. Alkaline phosphatase and Na-K-ATPase have been established as markers for the different areas of the cell envelope of the renal proximal tubule cell. Alkaline phosphatase is retained only in the microvilli, whereas Na-K-ATPase is a constituent of the basal infoldings of this cell. The question whether it is useful to consider alkaline phosphatase as a marker for renal brush border in animals other than rat was not investigated. Berger and Sacktor (3), for example, have shown that, in rabbit kidney, trehalase is a better marker than alkaline phosphatase. This enzyme was not studied in the present paper.

The lack of alkaline phosphatase or Na-K-ATPase in one of the two fractions discussed here cannot be due to isolation methods which resulted in extraction of the enzymes. The homogenization methods, and particularly the isotonic isolation and electrophoresis medium, are considered to be gentle tools for cell fractionation and have been proven, in several previous studies (11), (20), (22) not to alter membranes. In the present work, this was verified by reelectrophoresis experiments, in which the resulting membranes were found to still have the same surface properties as in the first run. In addition, the morphological appearance, as seen in the electron microscope, was not altered by the separation methods. That is, the microvilli kept their coats, were closed at their upper ends, and were not broken or squeezed. Also, the membranes of the basal infoldings looked unaltered and represented the morphology which is described for the classical plasma membranes (7). The sharp triple-layered membrane of the large open membrane sheets, and especially the tight junction, are typical features of a plasma membrane well preserved during the purification procedure. Again, desmosomes and mixed junctions were not very often present in the material investigated here.

An important question to be discussed is whether these plasma membranes were really the basal part of the proximal tubule cell or whether they might have been derived from other cells of the kidney. For the preparation of the membranes, only the renal cortex was used, and since this structure can easily be separated from the medullary parts of the organ, contamination with other parts of the kidney can be excluded. Consequently, in the cortex homogenate, only glomeruli and connective tissue with capillaries were present in addition to the tubular elements. Since the connective tissue and the glomeruli were not disintegrated by the homogenization methods used, all these structures are lost in the first centrifugation steps of the isolation procedure. The tubular elements generally consist of the proximal and the distal tubules, and the
proximal tubule is four times more abundant than the distal tubule (27). A similar ratio can be calculated on the basis of morphometric data. There can be no doubt therefore, that a major portion of the material consisted of proximal tubule cell elements.

The nature and function of the vesicles described above should also be considered. It is clear that these vesicles represent a type of membrane which is different from microvilli and membranes of the basal infoldings. They are most likely derived from the proximal tubule cells. Thoenes (28) has described particles of similar size and morphology in earlier studies and has designated them as pre-lysosomal vesicles, functioning in the pinocytotic process. These particles are positive in acid phosphatase activity, as were those found in the work reported here. The reason that these elements appear in larger amounts in some experiments and not others is still an unanswered question. There might be a chance of obtaining higher amounts of these vesicles via Ficoll after inducing the formation of these particles by an application of dextran to the animals (29). The other acid phosphatase-containing particles (the lysosomes) function as storage organelles and can also be found in the kidney, but to a much lesser extent than in other organs. However, those which have been isolated during this study have the same morphological appearance and electrophoretic mobility as those derived from rat liver. This means that they are completely different from the vesicles of interest in this investigation.

The specific localization of two different marker enzymes onto different plasma membrane regions of the same cell (the proximal tubule cell) leads to the consideration of the specialization and differentiation of this cell envelope. It has been described that the plasma membrane in mammalian cells can vary in thickness. Still more striking and of more importance for the function of the cell and its membrane must be the variation of the surface. Cell coats of a thickness between 100 and 5000 Å, especially in microvilli, have been reported (30). In the present study, a clear indication for the specialization within the cell envelope has been obtained, but no information is available concerning at what level of the cell development this occurs. The whole enveloping membrane contains Mg-ATPase. This may be considered as a hint for a common genotypic precursor of this membrane. This precursor must develop into a membrane of different phenotype, in which the apical part (microvilli) possesses alkaline phosphatase and the above mentioned coat, whereas the basal part (membranes of the basal infoldings) contains the Na-K-ATPase, a key enzyme for active transport (31), (32). The latter enzyme is being considered as a general marker and constituent of a mammalian plasma membrane. Almost nothing is known about the ontogenetic development of this enzyme in kidney. It was shown in a study by Beyth and Gutman (33) that during development of mice the renal cortex contains an almost constant level of Mg-ATPase activity, whereas the Na-K-ATPase is continuously increased and doubled at the end of the development. This is strong evidence for a specialization of the plasma membrane in this part of the organ. Whether Na-K-ATPase is synthesized in all the regions of the cell envelope, whether it has been possibly lost from the region of the microvilli during cell development, or whether it is still present in an inactive, repressed form should be clarified. As to the coat on the surface of the microvilli, this has been found to be created during cell development by the cell itself via the Golgi apparatus (34). However, since alkaline phosphatase has been shown to be contained not only in the coat but also in the membrane of the microvilli (35) as a real constituent, investigation should be carried out to determine if this enzyme is proliferated only into the apical part of the cell. Such differentiation processes of the membrane should be triggered, either by a direct enzymatic induction (repression or derepression of active protein molecules) or by a very complex heterotypic cellular interaction (with alterations in morphogenesis, proliferation, and protein synthesis as consequences). Upon those two phenomena a study of membrane differentiation should be focused. The present biological system in combination with the techniques used might make it possible to obtain answers on the embryologic (ontogenetic) development of the renal proximal tubule cell envelope (23).

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