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**ANNUAL REPORT OF THE INSTITUTE FOR  
VIRUS RESEARCH KYOTO UNIVERSITY**

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**Editors:**

<b>C. HAMADA</b>	<b>M. HANAOKA</b>	<b>M. IMAI</b>
<b>Y.K. INOUE</b>	<b>S. MATSUMOTO</b>	<b>H. TANAKA</b>

**THE INSTITUTE FOR VIRUS RESEARCH  
KYOTO UNIVERSITY**

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	Haruko Komoda, B.H.E. (1968)

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Yoshiko Horita, M.D. (1966) Virol. Diagn. Center  
Masahiro Horiuchi, M.D. (1972) Dept. of Tumor Virus  
Masao Imai, M.D. (1968) Dept. of Serol. & Immunol.  
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Kazuo Kodama, (1966) Dept. of Prev. & Therap.  
Kensuke Miura, M.D. (1972) Dept. of Prev. & Therap.  
Takashi Mizumoto, M.D. (1970) Dept. of Pathol.  
Setsuko Nakajima, M.D. (1972) Dept. of Serol. & Immunol.  
Keizo Suzuki, D.Sc. (1972) Dept. of Biochem.  
Juji Takeuchi, M.D. (1968) Dept. of Biophys.  
Hiromichi Uehara, M.D. (1969) Virol. Diagn. Center  
Kazumasa Usami, M.D. (1972) Dept. of Pathology

#### **Graduate Students**

Hiroko Bessho, M. Nut. (1972) Dept. of Prev. & Therap.  
Takashi Horiuchi, B.Ag. (1971) Dept. of Genetics  
Toshihiko Ikeuchi, (1970) Dept. of Genetics  
Hidetoshi Inoko, (1970) Dept. of Biochem.  
Yoichiro Iwakura, (1970) Dept. of Genetics  
Makoto Kawai, B.Sc. (1969) Dept. of Genetics  
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Kazuo Tsukui, B.Ph. (1971) Dept. of Biophys.

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  - a. Immunocytology of lymphocytes and plasma cells
  - b. Immunochemistry of antibody formation
  - c. Pathology of lymphoid tissues in virus infection and other diseases
  - d. Biology of mouse mammary tumors and lymphomas
  - e. Immuno-electron microscopy of virus-infected cells
2. Department of Biophysics
  - a. Electron microscopic studies on the growth characteristics of animal viruses
  - b. Studies on the ultrastructure of virus and enzyme
  - c. Purification and chemical composition of animal viruses
  - d. Studies on interferon
  - e. Interaction of nucleic acid with animal cells-viral interference induced by RNA
3. Department of Biochemistry
  - a. Biochemical studies on the regulatory mechanism of RNA synthesis in *E. coli* and in the related phages
  - b. Genetic regulatory mechanisms in the expression of the tryptophan operon in *E. coli*
  - c. Studies on structure and function of DNA-dependent RNA polymerase
  - d. Mechanism of formation of DNA-dependent RNA polymerase
  - e. Interaction of RNA polymerase with DNA
4. Department of Serology and Immunology
  - a. Studies on biochemical events in cells destined to lysogeny after phage infection
  - b. Analysis of the process of integration of the phage genome into bacterial chromosome
  - c. Chemical structures of somatic antigens and phage receptors in bacterial strains
  - d. Genetic structures and recombination of conversion phages
  - e. Mechanism of viral tumorigenesis in mammalian cells
  - f. Mechanisms of immunity to virus infections
5. Department of Prevention and Therapeutics
  - a. Fundamental studies on live attenuated Japanese encephalitis vaccine and its application
  - b. Studies on variation and oncogenicity of adenovirus
  - c. Virological studies on S.M.O.N.
  - d. Protection mechanism against viral infection
    - (1) Local immunity on influenza virus infection
    - (2) Protection against vaccinia virus infection
    - (3) Cellular recognition system and viral infection
    - (4) Cellular receptor for Japanese encephalitis virus
  - e. Immunopathological electron microscopic studies on slow viral diseases
6. Department of Tumor Virus
  - a. Studies on the mechanism of viral leukemogenesis
  - b. In vitro control of growth and differentiation of blood cells
  - c. Studies on the biology of viral inclusion

- d. Morphological studies of rabies virus multiplication
- 7. Department of Genetics
  - a. Genetics of DNA-dependent RNA polymerase of *E. coli*
  - b. Control mechanisms in bacteriophage growth
  - c. Regulation of chromosomal and episomal replication
- 8. Virological Diagnosis Center
  - a. Studies on the antigenic differences between influenza type A group virus strains
  - b. Virological and serological studies on the specimens collected in Burma
  - c. Studies on the agent of Herpangina
  - d. Studies on the epidemics of ECHO virus
  - e. Studies on the agent of acute hemorrhagic conjunctivitis

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## **Ultrastructure Studies on the Glomerular Barrier in Physiologic and Pathologic Condition**

Michal WALSKI\*

### **Introduction**

The problem of the integration and function of a glomerular barrier in kidney falls within the scope of the ultrastructure research. Early experiments, using Thorotrast (10) and ferretin (2) as markers, have shown the central part of the basement membrane acting as a fine filter in kidney secretion. Its surmised structure of a loose gel stops molecules larger than 100–120 Å in diameter (11). The most recent experiments at the ultrastructural level have revealed a finer filter on the surface of the cell membrane of the foot processes of the podocytes and the slit membrane. This barrier, as reported by Latta (11), accounts for restraint of the protein molecules in the range of 64–95 Å in diameter. Groniowski (3) has described the morphological structure of the peripheral surface coat in a variety of the animal cell-types in physiological condition and has, simultaneously, given an outline of the formation mechanism of that structure. The surface coat of the nephron has been visualized and described with the use of the heavy metal techniques (4). Jones (8) has visualized the mucopolysaccharide complex on the surface of the renal glomerular cells using the colloidal iron method, whereas Cossel (1) using the ruthenium red procedures has illustrated thoroughly the mucopolysaccharide lining of the outlying sectors of the nephron.

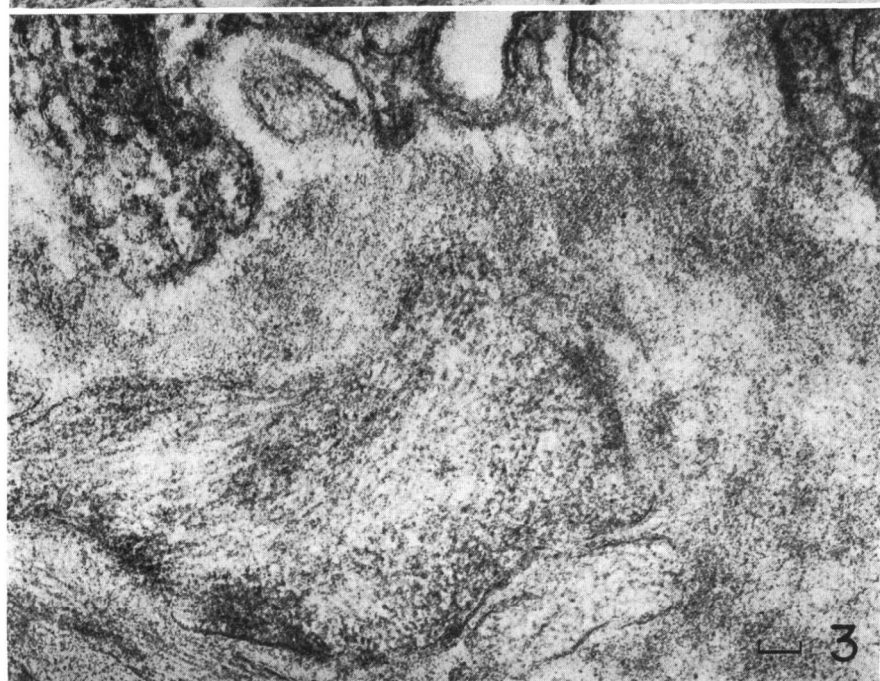
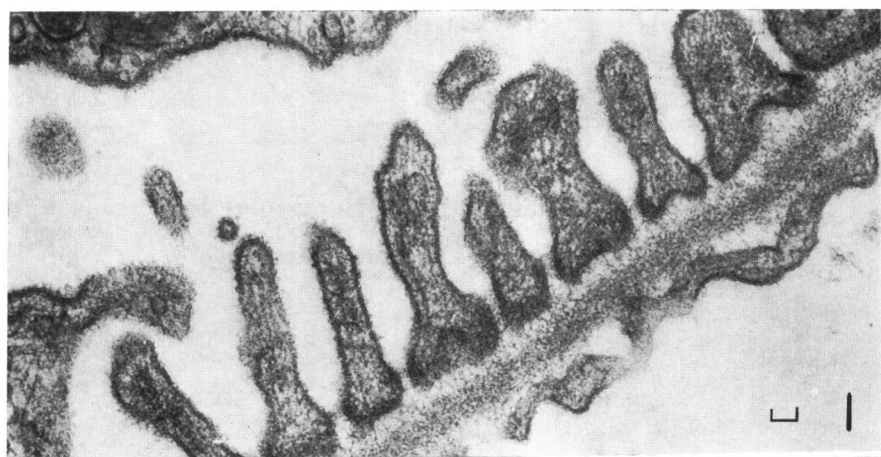
The present study was undertaken to illustrate the role of the diffusion barrier in kidney in a physiological condition and to make a thorough analysis of that structure in the pathological processes. The interpretation of results obtained from this analysis will concern a precise localization of pathologic changes in the biological barrier structures with the use of histochemical markers.

### **Materials and Methods**

Young rats were used in all experiments. They had been previously subjected to an analysis for protein content with the use of colorimetric techniques (12). Some of the animals under examination were treated for two days by the

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\* Department of Biophysics, Institute for Virus Research, Kyoto University, Kyoto  
Present address: Institute of Biostructure, Str. Chalubinskiego 5, Warsaw, Poland



intraperitoneal injection of 24 hr/10 mg/1000 g body weight of Daunomycin (C-Calbiochem, Los Angeles, California). Protein content in the urine was checked regularly, then nine days after the first injection the samples of material were collected. Fixation of the material obtained from control and experimental animals was by arterial perfusion of the kidney with paraformaldehyde fixative using Karnovsky method (9). Perfusion fixation of kidney with paraformaldehyde was also performed with simultaneous exposure to either rhutenium red or lanthanum nitrate. For rhutenium red staining Luft method (13) was used. The final pH of lanthanum nitrate used in the experiment was pH 7.6–7.8 as described by Ravel and Karnovsky (14). Fixation of material was followed by postfixation in osmium tetroxide with simultaneous addition of either rhutenium red or lanthanum nitrate. After dehydration, material was embedded in Epon 812 and examined in an JEM-6C electron microscope.

### Results

Glomerular barrier in physiologic condition has three layers. The endothelial cells along the capillary lumen are perforated by fenestrations and apposed diametrically at a distance of about 1000 Å from one another. They adhere tightly to the basement membrane. The basement membrane shows a distinct differentiation in the electron-optical density of the layers. The central layer has a fine fibrillar structure. The foot processes of the epithelial cells are embedded in the outer layer of the basement membrane (Fig. 1). There is a distance of about 800 Å wide between those processes and within the spaces separating them the slit pores of a very fine structure are distinctly visible. By using histochemical markers such as rhutenium red and lanthanum nitrate, mucopolysaccharide areolas were visualized on the surface of the cell membrane. Endothelial and epithelial cells together with their foot processes were covered by a regular surface coat. The slit pores of glomeruli were also covered by a coat continuous with the surface coat of the adjacent foot processes. According to the measurements, the surface coat together with the external lamina of the cell membrane was 100–150 Å thick (Fig. 2).

- Fig. 1. Glomerular capillary wall. The endothelial fenestrations appear open. The central layer of the basement membrane is much darker than the inner and outer layers. Regular foot processes are embedded in the outer layer of the basement membrane.  $\times 35,000$
- Fig. 2. Continuous coat at the surface of a podocyte. The slit pores are covered by the material continuous with the surface coat of the adjacent foot processes. Rhutenium red procedure.  $\times 80,000$
- Fig. 3. Glomerular basement membrane in kidney nephrosis. Fine fibrillar substance is seen in the basement membrane and in the irregular subendothelial deposits.  $\times 52,000$

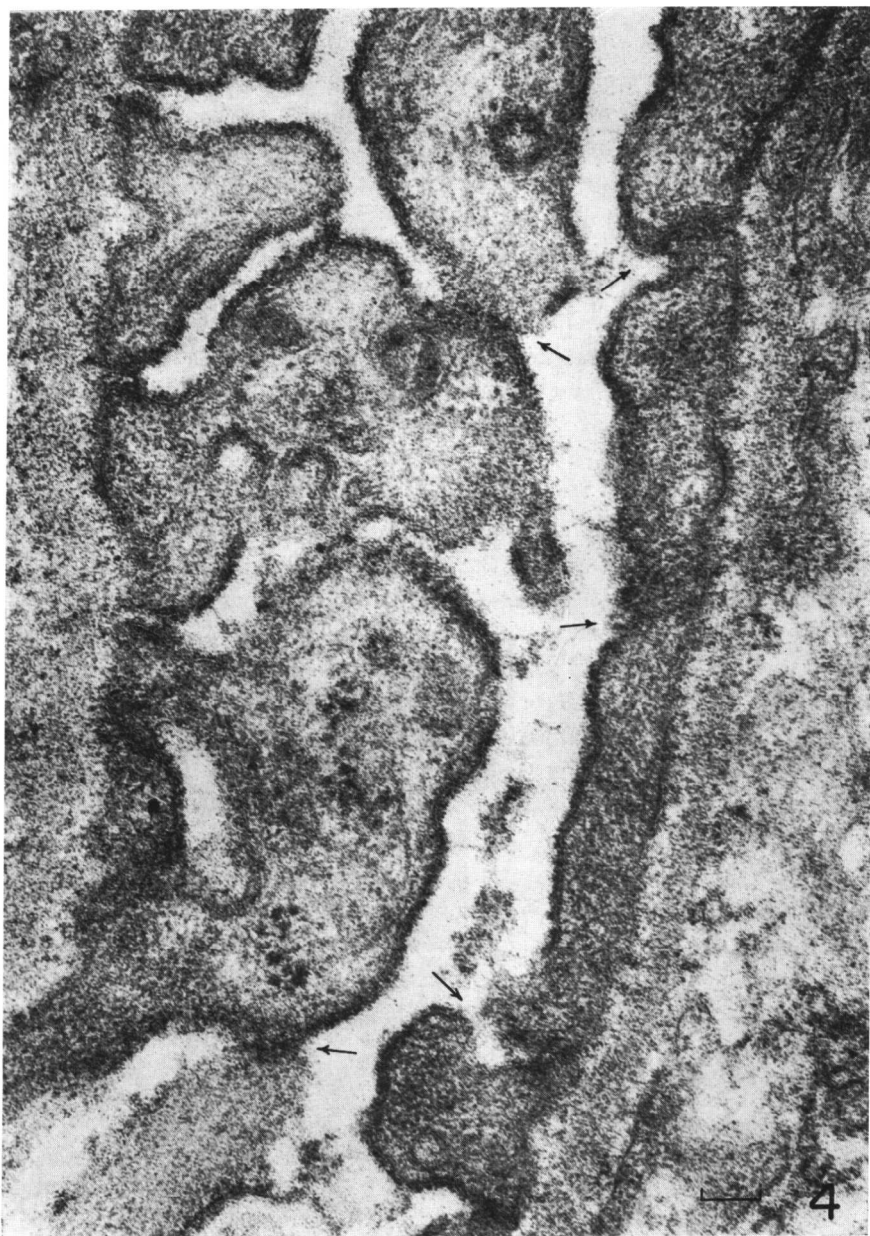


Fig. 4. Foot processes representing a stage in the process of fusion. Fusion of the foot processes is seen on a wide area simultaneously with effacement of the junction structures (arrows). Lanthanum nitrate procedure.  $\times 80,000$

In the animals injected with Daunomycin the presence of protein was stated in the urine. On the first and second day after the injection the protein content in the urine, collected during 24 hours, amounted to 60–80 mg. On the seventh and eighth day of the experiment the protein content in the urine from the sick animals has increased up to 200–220 mg. In the tissue samples obtained from those animals great ultrastructural changes were clearly visible. The endothelial cells were fused into bundles and the intercellular junctions were often seen. They had in their inner part a fine fibril-rich material. Now and then, the basement membrane had its original morphological structure completely blotted-out (Fig. 3). The greatest changes were observed in the structure of the foot processes of the podocytes. Distances between those processes became smaller and very often there were no more any free spaces left between them. Careful observations of the foot processes of the podocytes enabled to make a thorough study of the process of their fusion (Fig. 4). Results of the measurements have shown that the thickness of the mucopolysaccharide areolas on the surface of the podocytes together with the external lamina was about 300 Å. Fine fibrillar structures connecting the adjacent foot processes were visualized on the surface of the cell membranes of the podocytes by using ruthenium red and lanthanum staining. Those fibrillar structures were up to 700 Å long. On other epithelial cells fibrillar connections were not visible, whereas membranes of the adjacent cells formed tight junctions with preservation of the double cell membrane or sometimes a single one. The epithelial cells were also seen fused together on a wide area and forming as if a duplicate of the basement membrane.

### Discussion

The preliminary research on the ultrastructure of a glomerular barrier in physiologic condition were to serve as a point of support for studies on the pathomorphology of that barrier. Latta (11) in his morphological research on the glomerular capillary wall demonstrates the filtration barrier as a triple-layered metabolic filter. The present studies have shown a similar glomerular barrier pattern to that reported by Latta (11). The foot processes had a differentiated morphological structure, they could be seen lengthened up to 5000 Å or shortened to 2000 Å. Groniowski ascribes to that morphological differentiation a significant role in the mechanic transport of fluid to the filtration area. Assuming that the function of podocytes is rhythmic their movements may be described as resembling alternate squatting and straightening of the cells.

Ruthenium red and lanthanum nitrate markers used in the experiment have visualized the mucopolysaccharide areolas on the free surface of the renal barrier.



At the same time, the slit pores were marked out and, as it may be assumed, they constitute an integral part of a fine barrier formed by the surface coat of the podocytes. Results, obtained from measurements of the thickness of the surface coat of the foot process, agree with the data from the earlier studies (4). Latta (11) basing on his research infers that the surface coat of foot processes and the slit membrane make a finer filtration barrier to protein molecules between 64 and 95 Å, in greatest dimension. Each type of cell has a characteristic surface substance which integrates the structure and function of the cells in physiologic condition (3, 7).

Very significant changes in the morphological structure of the renal barrier were revealed by the application of a toxic dose of Daunomycin. The most interesting observations were morphological changes in the foot processes and functional changes ensuing. The obtained results indicate the thickening of the surface coat. Groniowski et al. (5) have demonstrated an increase in thickness of the surface coat of the podocytes in some pathological conditions of kidneys. The accretion of that substance was in close relation with a pathologic condition of the renal glomerular barrier. Those pathological conditions were associated with a serious proteinuria and a reverse transport of protein at the glomerular level could be expected in such cases. Sternberg (15) has described the process of fusion of the podocytes in his work entitled "Daunomycin Nephrosis", pointing out to the progressing by stages formation of, at first, the septate connections and then the intercellular junctions between two neighbouring foot processes.

The use of histochemical markers for demonstrating the mucopolysaccharide substances in the surface coat enabled a thorough examination of the process of fusion of the podocytes. The visualized fine fibrillar structures of the adjacent foot processes of the podocytes are stained with ruthenium red and lanthanum nitrate (Fig. 5). It may be inferred that those frail intercellular junctions are produced by the surface coat. They constitute a very important stage in the formation process of the lasting intercellular connections.

The use of the histochemical techniques has also revealed morphologic changes on the external areolas caused by the upset cell metabolism. The present experiments indicate that in a pathological condition the cell structure and functions affect the surface substance. Thus the ultrastructure research on the glomerular barrier carried out with the use of histochemical markers may contribute to an earlier and more effective recognition and identification of the cellular pathodynamics.