OBSERVATIONS ON THE BASEMENT MEMBRANES IN RAT KIDNEY*

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PLATE 91

Following is a summary of studies of several aspects of the basement membranes in rat kidney. The report includes observations on the fine structure of basement membranes, silver deposition in the kidneys after vital administration of silver nitrate, x-ray diffraction analyses of the silver precipitate, and histochemical techniques to demonstrate the presence of sulfhydryl and disulfide groups in the basement membranes.

Materials and Methods

Studies were made of rats that were untreated and 24 rats that were given 0.5 per cent silver nitrate solution as drinking water for 6 to 8 months. For electron microscopy, kidney tissue was fixed for 40 minutes in 1 per cent OsO_4, buffered to pH 7.6 with veronal-acetate buffer, dehydrated in methanol, and embedded in *n*-butyl methacrylate. Sections were made with the Servall cantilever microtome and were studied with a Philips electron microscope (EM 100A). With a Philips x-ray diffraction unit, silver-stained kidneys were analyzed in order to determine the form in which the silver was precipitated. Histochemical methods of Barrnett and Seligman (1954) were used to demonstrate the sulfhydryl and disulfide groups in the basement membranes; tissues were fixed in trichloracetic acid, embedded in paraffin, and sectioned at 5 μ before staining.

OBSERVATIONS AND DISCUSSION

In the glomerulus of the kidney, the basement membrane is the only constantly intact barrier between the blood and capsular space. The endothelium of the glomerular capillaries is fenestrated, the perforations apparently passing completely through the attenuated endothelium to the basement membrane; on the other side of this basement membrane are intermittent attachments of capsular epithelial pedicels (Dalton, 1951; Hall, 1953, 1955; Pease, 1955). Cells of the nephron tubules and peritubular capillaries rest on basement membranes, the most prominent of which enclose the convoluted tubules.

Fine Structure.—In our electron microscopic studies of rat kidney, the basement membranes were never seen to have a fibrillar or fibrous structure (Fig. 1). Rather, they appeared to be homogeneous in structure, being made up of an amorphous matrix. In a few random locations, convoluted tubule basement

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membranes seemed to be separated into irregular strata or lamellae, but still showed no evidence of a fibrillar structure.

Silver Deposition.—When 0.5 per cent silver nitrate solution was given to rats for periods of 6 to 8 months, granules of silver precipitate (2 to 200 mμ in diameter) appeared within the matrix of the basement membranes of the kidney, as well as in loose connective tissue and basement membranes elsewhere (van Breemen and Clemente, 1955; Dempsey and Wislocki, 1955 *a*, *b*). During argyria, silver remains in the blood for several weeks after administration of the metal has been stopped, circulating in the form of a silver albuminate (Minz, 1930; Blumberg and Carey, 1934). Since the glomerular basement membranes keep the plasma proteins from passing into the urine, they must also restrict the passage of the silver proteinate. One would not expect therefore to find the silver embedded in this form in the basement membrane of the glomerulus. The silver probably passes from the blood in the ionic form, diffusing into the basement membrane, where it is precipitated.

Silver appears in noticeable quantity in the rat kidneys only after several months of vital administration of 0.5 per cent silver nitrate solution. Apparently its rate of ionization is slow at "non-toxic" concentrations, so that most of the ions are precipitated in the basement membranes, not reaching the parenchymal cells in high enough concentration to cause irreparable damage. Nor does the silver reach the urine in large enough quantity to be readily identified except by spectrographic methods (Blumberg and Carey, 1934). At "toxic" concentrations of silver (several cases of which we have observed), the metal apparently saturates the basement membranes and passes into parenchymal cells, with subsequent degeneration of mitochondria and eventual degeneration of the involved cells.

Identification of Silver Precipitate.—Results of x-ray diffraction analyses of the silver-stained rat kidneys have indicated that the silver is combined with sulfur (van Breemen and Blackledge, unpublished preliminary studies). Though it is well known that silver and sulfur combine readily, this had not been expected in the basement membrane because the sulfur content of the basement membrane was unknown. The silver may be reduced by aldehydes in loose connective tissue, since here aldehydes are in higher concentration than sulfhydryl and disulfide; but its predominant form in the kidney is apparently some combination with sulfur.

Presence of Sulfur.—It is possible that free sulfur occurs in the basement membranes, but this was not demonstrated. Tissues from normal and silver-fed rats were subjected to histochemical techniques for the demonstration of sulfhydryl and disulfide groups. The positive results obtained in kidney sections are shown in Figs. 2 and 3. The glomerular and tubular basement membranes in normal and silver-stained rat kidneys showed sulfhydryl and disulfide activity. It is very likely that one or both of these are the agents that precipitated

the silver in the basement membranes. On this basis, the silver that reaches the cells would be expected to be precipitated in the mitochondria and other sites of sulfhydryl and disulfide activity and our findings indicate that this is the case. Mitochondria containing silver precipitate were reported by Dempsey and Wislocki (1955 *a*, *b*), and we have observed the same phenomenon.

SUMMARY AND CONCLUSIONS

Basement membranes in the kidney are made up of a homogeneous matrix. In argyria, silver passes from the blood in the ionic form and diffuses into the kidney basement membranes in which it is precipitated. X-ray diffraction studies of "silver-stained" rat kidneys show that most of the silver in the kidneys is combined with some form of sulfur. Histochemical staining for sulfhydryls and disulfides demonstrates the presence of these groups in basement membranes. It appears that silver ions combine with either or both the sulfhydryl or disulfide groups in the basement membranes and also in mitochondria (when the silver diffuses into a cell).

REFERENCES

Barrnett, R. J., and Seligman, A. M., *J. Nat. Cancer Inst.*, 1954, **14,** 769.

Blumberg, H., and Carey, T. N., *J. Am. Med. Assn.*, 1934, **103,** 1521.

Dalton, A. J., *J. Nat. Cancer Inst.*, 1951, **11,** 1163.

Dempsey, E. W., and Wislocki, G. B., *J. Biophysic. and Biochem. Cytol.*, 1955 *a*, **1,** 111; 1955 *b*, **1,** 245.

Hall, B. V., *Proc. 5th Ann. Conf. Nephrotic Syndrome*, New York, National Nephrosis Foundation, 1953, 1.

Hall, B. V., *Proc. 6th Ann. Conf. Nephrotic Syndrome*, New York, National Nephrosis Foundation, 1954, 1.

Minz, B., *Z. klin. Med.*, 1930, **114,** 623.

Pease, D. C., *J. Histochem. and Cytochem.*, 1955, **3,** 295.

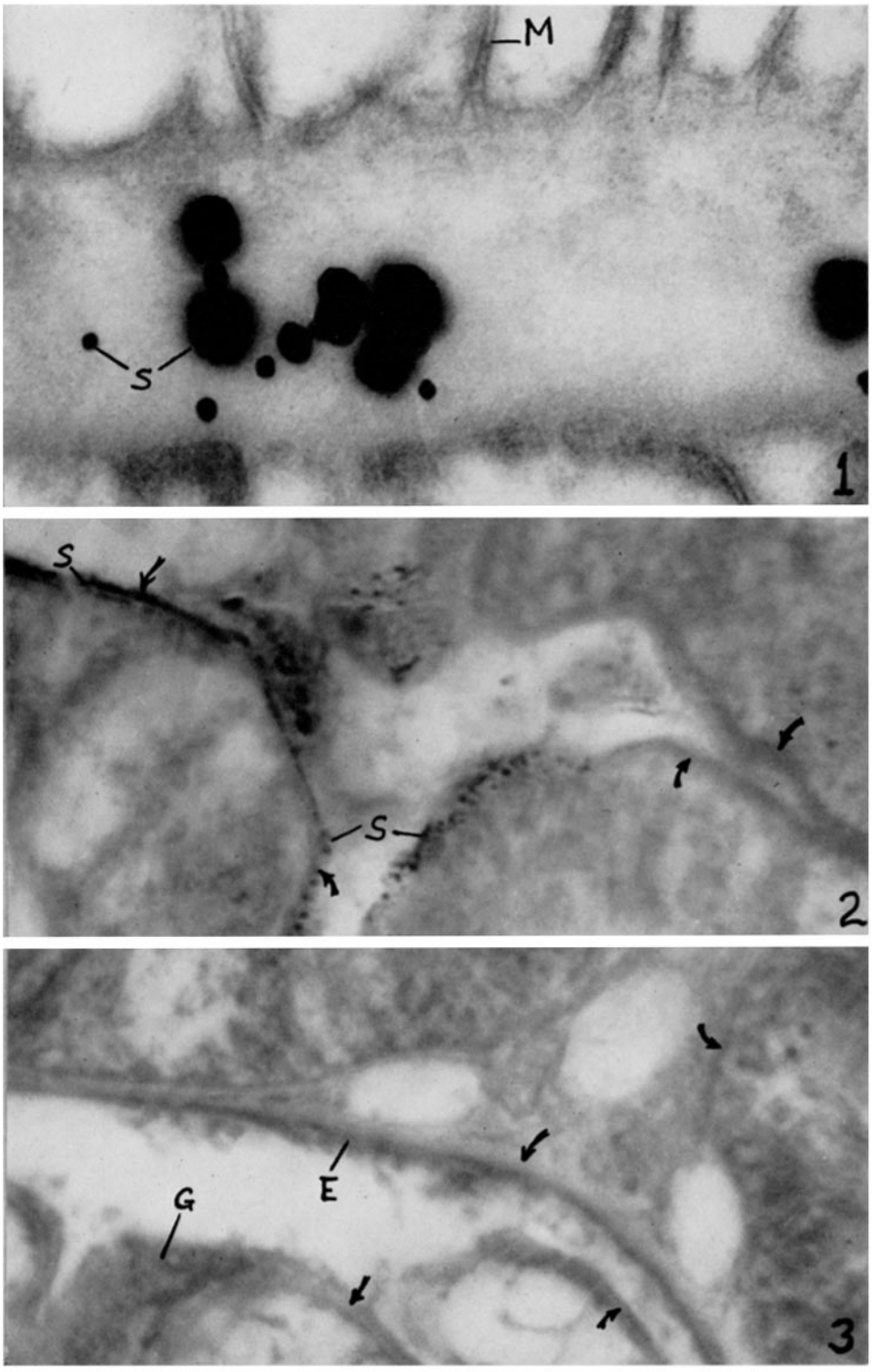
van Breemen, V. L., and Clemente, C. D., *J. Biophysic. and Biochem. Cytol.*, 1955. **1,** 161.

EXPLANATION OF PLATE 91

FIG. 1. Rat kidney vitally stained with silver. Figure shows portion of a basement membrane between cells of two different convoluted tubules. Infoldings of the cell membrane (*M*) of the distal convoluted tubule cell are seen on one side of the basement membrane. The basement membrane is made up of a homogeneous matrix, with no evidence of fibrils. Particles of silver precipitate (*S*) are embedded in the matrix. × 95,400.

FIG. 2. Rat kidney vitally stained with silver. It was fixed in trichloracetic acid, sectioned at 5 μ and stained for sulfhydryls and disulfides. Figure shows portions of distal convoluted tubules. Besides the intracellular reactive staining, the basement membranes (arrows) also demonstrate sulfhydryl and disulfide activity. Particles of silver precipitate (*S*) are also seen in some portions of these basement membranes. × 3,800.

FIG. 3. Rat kidney without vital silver stain but stained for sulfhydryl groups. Figure shows portions of distal convoluted tubules, parietal epithelium of Bowman's capsule (*E*), and portion of a glomerulus, with the glomerular epithelium (*G*) and basement membranes. Besides the intracellular reaction, the basement membranes (arrows) also show sulfhydryl activity. × 3,430.



(van Breemen *et al.*: Basement membranes in rat kidney)