COMPARATIVE ENZYMIC ACTIVITY OF THE VARIOUS PARTS OF THE NEPHRON (HISTOCHEMICAL INVESTIGATION)

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The structural and functional specialization of the various subdivisions of the nephron remains incompletely studied despite the many investigations which have been made of this subject. The comparative study of the enzymic activity of the various structural units of the kidney is of great interest in this connection.

It is generally concluded that the enzymic activity is maximal in the proximal part and lower in the distal part of the nephron. The activity of the many enzymes is very slight or absent altogether in the glomerular structures, the thin segment of the loop of Henle, and the collecting tubules. However, the link between these observations and the functional specialization of the various parts of the nephron remains unexplained, and the purpose of the present investigation was to shed light on this problem.

EXPERIMENTAL METHOD

The proteins and oxidoreductases of the various parts of the nephron were studied in the kidneys of 10 male rats weighing 180-200 g. The following were estimated: tyrosine, tryptophan, histidine by Danielli's method, basic and acid proteins by Alfert's method, α-amino acids by the ninhydrin and PAS reaction, SH groups by the method of Barrnett and Seligman, RNA by Brachet's method, and polysaccharides by the PAS reaction and toluidine blue. The following enzymes of cell metabolism were studied: succinate dehydrogenase, α-glycerophosphate and glucose-6-phosphate dehydrogenase, lactate and malate dehydrogenase, alcohol dehydrogenase, NAD and NADP diaphorase, and nonspecific alkaline phosphatase. The material was also stained by the usual histological methods.

EXPERIMENTAL RESULTS

The results of the investigation are given in the Table 1.

The glomerular capillaries and the parietal layer of the capsule were clearly stained by the PAS reaction. This reaction was rather weaker in the cells of the glomerulus and, in particular, in the endothelium and the mesangial cells after treatment with amylase, indicating the presence of glycogen. Tyrosine, tryptophan, histidine, basic and acid proteins, and SH groups were detected in the membranes of the capillaries, the myo-epithelioid cells, the endothelium, the mesangial cells, and the nephrothelium of the capsule, although in only moderate amounts. The cells of the glomerulus, especially the mesangial cells, were weakly pyroninophilic, and orthochromatic with toluidine blue. Among the tissue respiration enzymes, only NAD and NADP diaphorase and lactate and malate dehydrogenase were found in the glomerular structures, and as a rule the reactions were weakly positive. No succinate dehydrogenase, glucose-6-phosphate dehydrogenases, or nonspecific alkaline phosphatase was found.

The low activity of the tissue respiration enzymes in the structures of the glomerulus reflected their small part in the filtration mechanism. However, the presence of glycogen, polysaccharides, reactive SH groups, and nucleic acides in the endothelium of the glomerular capillaries and, in particular, in the cells of the mesangium demonstrated the plasticity of these cells.

In the proximal tubules the brush border was clearly outlined by the PAS reaction, selectively revealing nonspecific alkaline phosphatase (Fig. 1). The cytoplasm of the epithelium was granular, strongly pyroninophilic, and orthochromatic with toluidine blue; it gave reactions of high intensity for tyrosine,

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TABLE 1. Distribution of Proteins and Enzymes in Various Parts of the Nephron of Healthy Rats

Proteins and enzymes	Glomerulus	Proximal tubules		thin segment	Distal tubules		Collecting tubules
		convoluted part	straight part – thick descending part of the loop of Henle		convoluted part	straight part – thick descending part of the loop of Henle	
Proteins by Danielli's method	+++	++++	++++	++	+++	+++	++
Basic proteins	++	++++	++++	++	++++	++++	++
Acid proteins	++	++++	++++	++	++++	++++	++
SH groups	++	++++	++++	+	++++	++++	++
RNA	++	++++	++++	++	++++	++++	++
Polysaccharides	++++	++++	++++	++	++++	++++	+++
Succinate dehydrogenase	–	++++	++++	++	++++	++++	–
α-Glycerophosphate dehydrogenase	–	++++	++++	+++	++++	++++	–
Glucose-6-phosphate dehydrogenase	+–	++	++	+–	++	++++	+–
Lactate dehydrogenase	+	++++	++++	++	++++	+++	+–
Malate dehydrogenase	+	++++	++++	+++	+++	++++	+
Alcohol dehydrogenase	+–	+–	+–	+–	+–	+–	+–
NAD diaphorase	+	++++	++++	+++	++++	++++	+
NADP diaphorase	+	++++	++++	+++	++++	++++	+
Alkaline phosphatase	–	++++	++++	–	+++	++++	–

Legend: a) for proteins and polysaccharides: ++++ intensive staining of cytoplasm, +++ less intensive, ++ weak; b) for enzymes: ++++ intensive enzymic reaction, +++ moderately intensive, ++ much weaker, + weak, +– traces of enzyme activity, – absent.

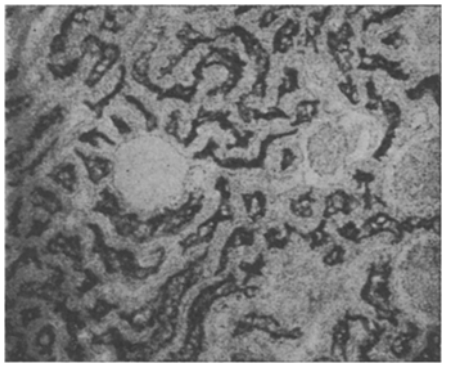

Fig. 1. Alkaline phosphatase in the brush border of the proximal tubules. Gomori's reaction. 100×.

tryptophan, histidine, basic and acid proteins, and active SH groups. Very high succinate dehydrogenase, NAD and NADP diaphorase (Fig. 2), lactate and malate dehydrogenase, and α-glycerophosphate dehydrogenase activities were found in the epithelium. The glucose-6-phosphate dehydrogenase activity was slightly lower by comparison with the other enzymes. The reaction for alcohol dehydrogenase was very weak in the proximal tubules, as in the other parts of the nephron.

These results demonstrated the complex and differentiated function of the proximal tubules, with a high intensity of all forms of metabolism, as reflected by the high NAD and NADP diaphorase and dehydrogenase activities. However, the diaphorase and dehydrogenase activities in the proximal tubule were mainly directed toward the reabsorption of protein. The selective reabsorption of protein in the proximal tubule was demonstrated not only by the enzyme activity and the high concentration of amino acids, proteins, nucleic acides, and active SH groups in its epithelium, but also by the characteristic morphology of this reabsorption. The cytoplasm of the epithelium of the proximal tubule constantly contained

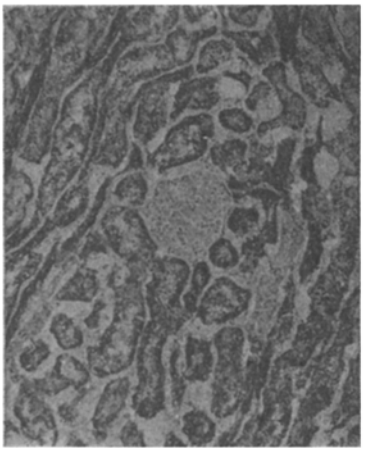

Fig. 2. NADP diaphorase. High concentration of the enzyme in the epithelium of the proximal and distal tubules, and traces in the glomeruli. Pearse's reaction. 100×.

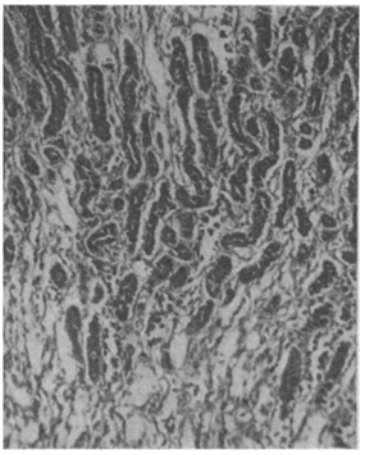

Fig. 3. High concentration of SH groups in the ascending, thick part of the distal tubules and a lower concentration in the segment and the collecting tubules. Barrnett and Seligman's reaction. 100×.

protein granules, which have been shown by electronmicroscopic investigation to enter the cell through small tubules commencing at the base of the processes of the brush border and ending in the vacuoles of the apical part of the cell. The protein accumulates there, reacts with the mitochondria, and is transformed by enzyme action into polypeptides and amino acids.

The morphological substratum for the reabsorption of glucose in the proximal tubule is the brush border, for it is associated with the activity of nonspecific alkaline phosphatase. The reabsorption of sodium is associated with succinate dehydrogenase activity, as experimental physiological studies have shown.

The epithelium of the thin segment of the loop of Henle was found to be moderately PAS-positive and pyroninophilic, orthochromatic with toluidine blue, and it was distinguished by its small content of tyrosine, tryptophan, histidine, basic and acid proteins, and SH groups. Compared with the proximal tubules, this part of the nephron was characterized by extremely low enzyme activity (see Table) and by the absence of many of the enzymes tested for: nonspecific alkaline phosphatase, for example. The exceptions were NAD and NADP diaphorase, considerable amounts of which were found in the epithelium of the thin segment.

The histochemistry and enzyme chemistry of the thin segment, like the structural peculiarities of its epithelium (few mitochondria, cytoplasmic membranes and lamellae cross the cell body throughout its height, and so on), reflect the functional specialization of this poration of the nephron, which is a supplementary device for minimizing the filtration charge of the water, thus facilitating its passive resorption.

In the distal tubules the cytoplasm of the epithelium was moderately PAS positive, orthochromatic with toluidine blue, and highly pyroninophilic. It was rich in tyrosine, tryptophan, histidine, basic and acid proteins, and also in reactive SH groups (Fig. 3). The enzyme activity of the distal tubules was very high. High concentrations of succinate dehydrogenase, NAD and NADP diaphorase, lactate and malate dehydrogenase, and α-glycerophosphate dehydrogenase were found in both the convoluted and the straight portions. However, the diaphorase activity in the distal tubules was rather lower than in the proximal. Glucose-6-phosphate dehydrogenase and nonspecific alkaline phosphatase activity was found only in the straight, ascending portion of the distal tubules, where it was higher than in the proximal tubules; in the convoluted part it was lower.

The high activity of nonspecific alkaline phosphatase and glucose-6-phosphate dehydrogenase in the proximal and, in particular, in the distal tubules was probably associated with different ultrastructures of the cell, and reflected the different functions of these portions of the nephron. Glucose-6-phosphate dehydrogenase is known to be involved in the pentose cycle and to play an active part in carbohydrate

metabolism if oxidation takes place directly from the glucose-6-phosphate molecule and not via hexose diphosphate and trioses. This function evidently is performed to some extent by the glucose-6-phosphate dehydrogenase in the distal tubules, which is mainly concerned with the regulation of the acid-base balance of the urine. In the proximal tubules, where the reabsorption of glucose is effected principally by alkaline phosphatase, the function of glucose-6-phosphate dehydrogenase as a member of the pentose cycle is concerned with the formation of the raw materials for the synthesis of pentose nucleotides required for the formation of nucleotides and nucleic acids. It does not follow from this, of course, that this function of glucose-6-phosphate dehydrogenase is not performed also in the epithelium of the distal tubules.

The epithelium in the collecting tubules was PAS-positive, orthochromatic with toluidine blue, moderately pyroninophilic, and it reacted weakly for proteins and SH groups. The enzyme activity of the epithelium was extremely weak in the case of the diaphorases, lactate and malate dehydrogenase, and α-glycerophosphate and hexose-6-phosphate dehydrogenase; no succinate dehydrogenase or nonspecific alkaline phosphatase was detected. The basement membranes of the collecting tubules and the connective tissue of the papillae of the pyramids surrounding them were strongly metachromatic with toluidine blue. The metachromasia disappeared after treatment with testicular hyaluronidase and was reduced by bacterial hyaluronidase, demonstrating the presence of hyaluronic acid among the mucopolysaccharides. There results confirm A. G. Ginetsinskii's hypothesis that in mammals the hyalruonic structures have been displaced into the most distal parts of the tubules—into the collecting tubules. These structures, enveloping the basement membrances of the collecting tubules, are the point of application of the antidiuretic hormone of the pituitary. It can be taken as proven that the state of these structures (polymerization—depolymerization) determines the magnitude of the facultative reabsorption.

LITERATURE CITED

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