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STEREOLOGICAL ANALYSIS OF TUBULAR CELLS IN NORMAL AND DILATED CHICK MESONEPHRIC NEPHRONS

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ABSTRACT

In order to elucidate the nature of the process of cystic dilatation spontaneously occurring in proximal tubules of 7-day-chick embryonic kidneys, a stereological analysis was performed. The kidney volume, the length density of proximal tubules in the kidney, the ratio of luminar volume to tubular wall volume, the mean tubular cell volume, and the cell number per unit length of tubule were estimated. The results of a preliminary study indicate that the development of proximal tubular dilatations is caused predominatly by the proliferation of cells around the periphery of the proximal tubules.

Key words: chick mesonephros, disector, orientator, proximal tubule dilatation, stereology.

INTRODUCTION

During the development of the chick embryonic kidney, mesonephros, spontaneous cystic dilatations of proximal tubules may appear (Friebová, 1972). Dilated tubules can be of varying size, from having a diameter twice as large as that of normal tubules up to giant cystic deformations. A passage through the dilated nephrons is always retained so that dilated parts communicate with the other segments of the nephron. The frequency of cystically dilated proximal tubules (CDT) increases during development from embryonic day, ED, 5, when they begin to appear, up until EDs 8 - 11, when the maximum is reached (Friebová, 1972). The incidence of CDT is obviously associated with the period of functional activity of the mesonephrons as well as with the sex of embryos and environmental influences related to seasonal changes. Experimentally, the CDT can be induced by a simultaneous occlusion of the terminal portion of the nephron and of the peritubular circulation by microclamping the collecting ducts and peritubular vessels (Friebová, 1973). The development of CDT is in this case rapid (45-65 min) and involves the whole proximal tubule. CDT are transitional here, having disappeared after reestablishing the peritubular blood flow.

The purpose of this study is to find out the structural

basis for the development of spontaneous CDT. Stereological methods enabling the evaluation of the proximal tubular cells lining the dilated lumen were chosen for this study. In order to elucidate the nature of the process of the formation of these CDT three hypotheses were tested (Fig.1):
1) simple distension of the tubule accompanied by lowering its epithelial lining; 2) hypertrophy of the epithelial cells on the periphery of the tubule, 3) hyperplasia of the epithelial cells without changes in their shape and volume.

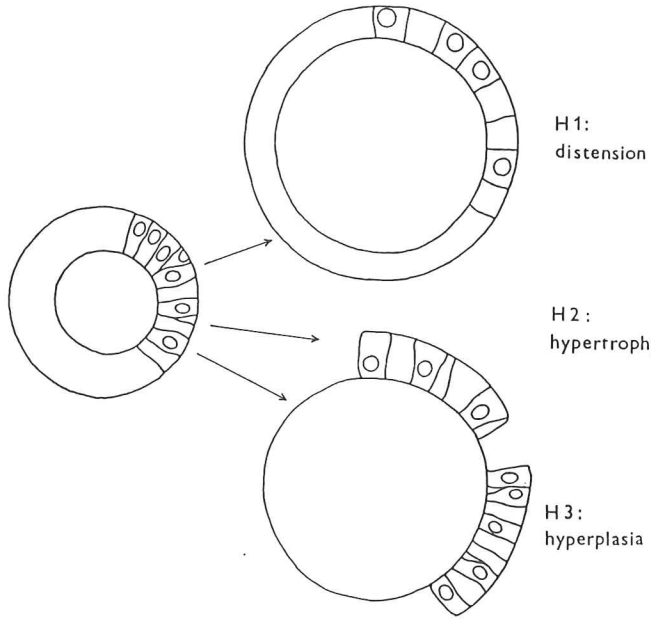

Fig.1. Diagram of hypotheses on the structural basis for the development of proximal tubular dilatations.

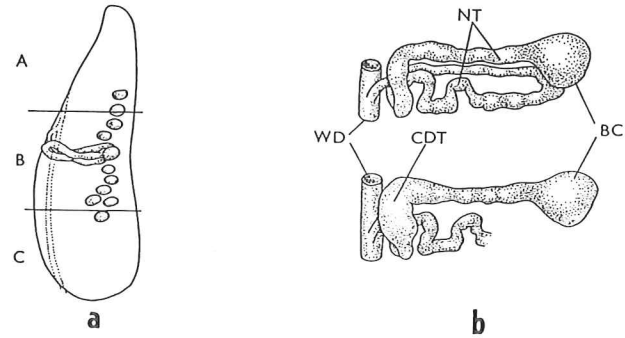

Fig.2. Schema of the 7-day chick mesonephros. a) Ventral view showing three portions of the kidney (A,B,C) and a course of one nephron; b) nephron with normal proximal tubule (NT) and the one with cystic dilatation (CDT). WD ... Wolffian duct, BC ... Bowman's capsule.

MATERIAL AND METHODS

In the presented preliminary study, mesonephros (Fig.2a) of four freshly sacrificed 7-day chick embryos, exhibiting spontaneous cystic dilatations of proximal tubules, (CDT, defined as tubules with the diameter at least twice as large as in normal proximal tubules, see Fig. 2b, 3a,b), were fixed in Holland solution, dehydrated in ethanol and embedded in Histoplast S. The sections were stained with haematoxylin and eosin.

Taking into account that in the chick mesonephros, proximal tubules represent a non-homogeneous and anisotropic structure (Fig.2a), the following procedure using the orientator principle (Mattfeldt et al., 1990) was developed: The kidney was divided into three segments - A (cranial), B (middle), and C (caudal) - which were fixed separately. In each paraffin block containing one segment, an isotropic direction was generated by the orientator principle. Approximately half of the kidney segment was cut into serial sections (20μm) with this direction. The rest of it was cut into serial sections perpendicular to those in the first part of the segment. From each of the six serially sectioned parts of the kidney, the sections for further evaluation were sampled uniformly at random so that about 10 sections per kidney were examined (every tenth section was taken). These sections were used for the estimation of the following parameters:

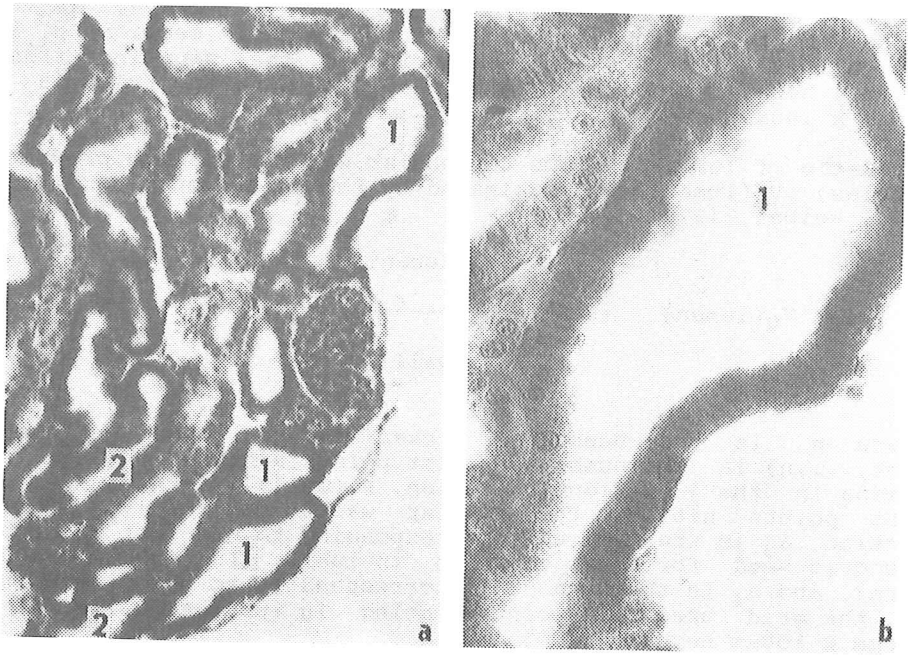

Fig.3. A thick section of 7-day mesonephros. a) Cystically dilated proximal tubule (1) and normal proximal tubule (2) at low magnification (x 360); b) dilated tubule from a) in detail (x 950).

(1) Kidney volume (V(kidney)) was estimated by Cavalieri's principle combined with the point-counting method (for its application in the case of stratified sampling see Michel and Cruz-Orive, 1988):

$$\text{est } V(\text{kidney}) = T \cdot a_1 \cdot \sum_{j=1}^{n} P_j(\text{kidney}), \qquad (1)$$

where n is the number of examined sections, a_1 is the area unit corresponding to one test point of the grid (a_1 = 100μm x 100μm here), T is the distance between examined sections (T = 200 μm here), and P_j(kidney) (j=1,...n) is the number of test points hitting the j-th kidney section.

(2) Length density of proximal tubules in the kidney (L_V(tub)) was estimated by the orientator principle (Mattfeldt et al., 1990) using the formula:

$$\text{est } L_V(\text{tub}) = 2 \cdot \frac{\sum_{j=1}^{n} Q_j(\text{lumen})}{\sum_{j=1}^{n} P_j(\text{kidney})} \cdot \frac{p_1}{a_2}, \qquad (2)$$

where n is the number of examined sections, Q_j(lumen) (j=1,...,n) is the number of luminar profiles sampled by unbiased sampling frames in the j-th section, P_j(kidney) is the number of test points hitting the j-th kidney section, p_1 is the number of points of the grid in the sampling frame (p_1 = 4 here), and a_2 is the area of the sampling frame (a_2 = 100μm x 100μm here).

(3) Ratio of luminar volume to tubular wall volume (in proximal tubules) (V_V(lumen)) was estimated by the point-counting method (e.g. Weibel, 1979):

$$\text{est } V_V(\text{lumen}) = \frac{\sum_{j=1}^{n} P_j(\text{lumen})}{\sum_{j=1}^{n} P_j(\text{wall})} \cdot \frac{a_3}{a_4}, \qquad (3)$$

where n is the number of examined sections, P_j(lumen) (j=1,...,n) is the number of test points hitting the tubular lamina in the j-th kidney section, P_j(wall) is the number of test points hitting the tubular walls in the j-th kidney section, a_3 is the area unit corresponding to one test point of the grid used for point counting in lumina (a_3 = 50μm x 50μm here), and a_4 is the area unit corresponding to one test point of the grid used for point counting in tubular walls (a_4 = 100μm x 100μm here).

(4) Mean tubular cell volume (in proximal tubules) ($\bar{v}_N$(cell)) was estimated by the disector principle (Sterio, 1984) using the modified version of the optical disector: Firstly, cell nuclei, sampled by the disector frame with their largest

profile lying between the two disector planes, were counted. Secondly, the test points (placed in the frame) hitting the tubular walls were counted and the following formula was used:

$$\text{est } \bar{v}_N(\text{cell}) = \frac{\sum_{j=1}^{n} P_j'(\text{wall})}{\sum_{j=1}^{n} Q_j^-(\text{nucl})} \cdot \frac{a_5 \cdot h}{p_2}, \qquad (4)$$

where n is the number of examined sections, $P_j'(\text{wall})$ (j=1,...,n) is the number of test points hitting the tubular walls in the j-th kidney section, $Q_j^-(\text{nucl})$ is the number of cell nuclei sampled by the disectors in the j-th section, a_5 is the area of the disector sampling frame (a_5 = 50μm x 50μm here), h is the disector height (h = 10 μm here), and p_2 is the number of points of the grid in the sampling frame (p_2 = 4).

(5) Cell number per unit length of proximal tubule ($N_L(\text{tub})$ was estimated using the following relations:

$$\text{est } N_L(\text{tub}) = \frac{\text{est } N_V(\text{tub})}{\text{est } L_V(\text{tub})} = \frac{1}{\text{est } \bar{v}_N(\text{cell}) \cdot \text{est } L_V(\text{tub})}. \qquad (5)$$

$N_V(\text{tub})$ denotes cell number per unit volume of proximal tubule.

Differences in the parameters between CDT and NT were judged by paired Student's t-test.

Table 1. Embryo body mass, kidney volume and tubular characteristics in normal (NT) and cystically dilated proximal tubules (CDT) (mean ± SD).

	embryo	kidney	NT	CDT
body mass (mg)	943±85			
V(kidney) (mm^3)		1.32±.26		
$L_V(\text{tub})$ ($mm.mm^{-3}$)			82.1±25.9	7.75±4.79
$V_V(\text{lumen})$			.135±.016	.572±.037
$\bar{v}_N(\text{cell})$ (μm^3)			625±61	709±78
$N_L(\text{tub})$ (mm^{-1})			6250±2410	9783±5334

RESULTS

As shown in Tbl. 1, the body mass of embryos studied was in average 943 mg. The volume of a mesonephric kidney (V(kidney)) was 1.32 mm^3. The length density of proximal tubules ($L_V(\text{tub})$) varied from 0.055 to 0.101 mm/mm^3 and it was understandably lower in cystically dilated tubules (CDT) than in normal ones (NT). The ratio of luminar volume to tubular

wall volume (V_V(lumen)) was, as expected, larger in CDT than in NT (more than four times). In our preliminary study, the mean cell volume ($\bar{v}_N$(cell)) did not differ significantly in NT and CDT, although the cells of normal tubules were slightly larger. The cell number per unit length of proximal tubule (N_L(tub)) was higher in CDT than in NT.

DISCUSSION

The study on the nature of spontaneous cystic dilatations of the mesonephric proximal tubules involved measuring body weight and structural parameters. Body mass of embryo depends on the developmental stage of young embryos. It is used as a test of homogeneity of the group studied. The kidney volume gives the information about the mesonephros size. The length density of NT and CDT characterize the relative incidence of cystically dilated proximal tubules in single kidneys. The ratio of the volume of tubular lumina to tubular wall volume characterizes the size of the dilatations. The mean tubular cell volume was estimated in order to test the hypertrophy hypothesis on the structural basis of tubular dilatation (Fig.1) and it was not significantly larger in CDT. The cell number per unit length of proximal tubule was estimated to test the hyperplasia hypothesis (Fig.1) and it was found to be much greater in CDT than in NT.

Taking into account above results together with our previous findings that the length of cells in dilated tubules along the tubule does not decrease, it can be suggested that the tubular distension in CDT is caused rather by the increase in cell number around their periphery than by distension of the tubular wall or by tubular cell hypertrophy. The possible changes in tubular cell dimensions are under further study.

Paraffin sections were examined in this study and so the values of stereological estimates listed in Tbl.1 may be affected by tissue shrinkage, e.g., the kidney volume and the mean tubular cell volume are probably underestimated. However, it is assumed that the relative shrinkage of tubular cells is the same in CDT as in NT.

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