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SPATIAL ARANGEMENT OF PERITUBULAR VASCULAR BED IN CHICK MESONEPHROS

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ABSTRACT

Changes of spatial arrangement of peritubular vessels of chick mesonephros at embryonic days (ED) 5 and 7 were studied by 3-D reconstruction of images captured by a confocal laser scanning microscope. Specimens were fixed, embedded and cut into transversal physical sections 30-40 μm thick. Serial optical sections were obtained at objective magnification 20×. Pictures of mesonephros on ED 5 were collected from one field of view, in more voluminous mesonephros on ED 7 it was necessary to take the pictures from at least three fields. Digitised contours of mesonephric surface, vessels and other structural components were converted into 3-D greyscale images. The images of parts of mesonephros were interactively registered and put together. Triangulated surfaces representing mesonephros components were obtained by isosurface detection. The 3-D reconstruction of vascular bed on ED 5 demonstrated clearly the regular segmental arrangement of marginal blood sinuses interconnected by short longitudinally oriented vessels. Extensive development of mesonephros between ED 5 and 7 is manifested by rapid growth of tubules and peritubular vascular bed. Strictly segmental arrangement of vessels typical on ED 5 is no more distinct on ED 7. Dense capillary network occupies peritubular spaces and only remnants of marginal blood sinuses are observable, namely in ventromedial part of mesonephros and in close proximity of Wolffian duct.

Keywords: confocal microscopy, mesonephros, peritubular vessels, 3-D.

INTRODUCTION

The knowledge of mutual spatial relationships of developing structural components is necessary for understanding the structural and functional development of the whole organ, however obtaining a reliable 3-D representation of the object is not a trivial problem. The majority of classic methods of three-dimensional visualisation in embryology is based on series of thin physical (histological) sections. This approach is extraordinarily time- consuming and laborious and generates technical difficulties, e.g. accurate matching of sections deformed during sectioning. The confocal laser scanning microscope allows to record series of perfectly registered optical sections in a thick physical section and thus to obtain valuable initial data for 3-D visualisation (Cossmann *et al*., 1997; Camp *et al*., 1997; Jirkovská *et al*., 1998; Hanley *et al*., 1999). In order to obtain a microscopical picture of tissues with an optimal resolution, it is often necessary to collect serial optical sections by using higher objective magnification. If it is not possible to capture the image of the whole organ in one microscopical field, more series of optical sections have to be taken and then put together. We tried to solve this task in a study of 3-D arrangement of chick embryonic kidney (mesonephros). The spatial arrangement of peritubular vessels in mesonephros in two developmental stages, in embryonic days (ED) 5 and 7 was studied using 3-D reconstruction of internal structure of mesonephros (Janáček and Kubínová, 1998).

MATERIAL AND METHODS

Five White Leghorn embryos on 5 and 7 ED were used for this study. Samples of mesonephros were fixed by perfusion via umbilical vein or intracardially in both stages, in 7 ED embryos perfusion via left or right iliac vein was used, too. The fixative was composed of 8 ml 25% glutardialdehyde, 20 ml 36% formaldehyde, 2.62 g dried sodium phosphate, 1 g yellowish eosin, 170 ml distilled water, pH adjusted at 7.2. After perfusion, dorsal parts of embryos were immersed in the same fixative for 24 hours. After dehydration by graded ethanol, specimens were embedded in paraffin wax or glycol methacrylate resin. Transversal serial physical sections were cut at the thickness of 30 - 40 μm. As the postinduction organization of mesonephric nephrons takes place between 3 and 6 ED in craniocaudal gradient (Friebová, 1975), the sections from the middle thirds of mesonephros were studied.

Serial optical sections, 1.95 μm apart, were captured by confocal laser scanning microscope BioRad MRC 600 (excitation wavelength of 567 nm) at objective magnification 20× (N.A. = 0.4). In mesonephros on 5 ED, it was possible to collect stack of images from one field, on the 7 ED stage it was necessary to record images at least from three fields. Serial optical sections from about 30 μm thick stacks were printed. Contours of mesonephric surface, vessels, renal corpuscles, mesonephric tubules and Wolffian duct were digitised using digitalisation tablet and CutView programme (Jirák *et al*., 1997).

Contours of sections through the mesonephros components were converted into 3-D binary image with resolution $1 \times 1 \times 1.95$ μm by drawing the profiles in their corresponding planes and then to greyscale image with resolution $8 \times 8 \times 7.8$ μm using the feature voxel count to generate grey level in the image. The images of parts of mesonephros were interactively registered. Triangulated surfaces of the components were obtained by detection of isosurface of the grey level. Programming environment IRIS Explorer (SGI USA) with custom-made modules was employed for the image processing and reconstruction. The reconstructed surface model was converted to standard 3-D (VRML) format and rendered by free (Cosmo Player 2.0) PC software.

RESULTS

The 3-D reconstruction of the mesonephric vascular bed of each sample on ED 5 shows regular segmental arrangement with main channels of the mesonephric vascular network in the margin of mesonephros (Fig. 1). They are connected with wide primitive capillaries placed in peritubular spaces. Marginal blood channels are intersegmentally interconnected by short longitudinally oriented vessels contributing to formation of continuous vascular network of the organ (Fig. 2).

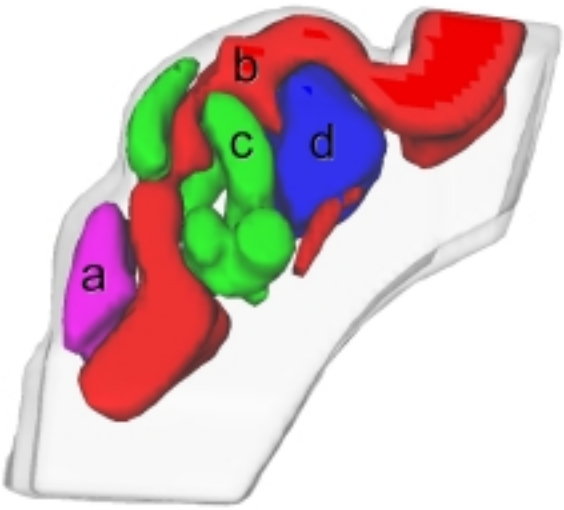

Fig. 1. *Chick mesonephros on 5 ED – a: Wolffian duct, b: marginal blood channel, c: tubules, d: renal corpuscle*.

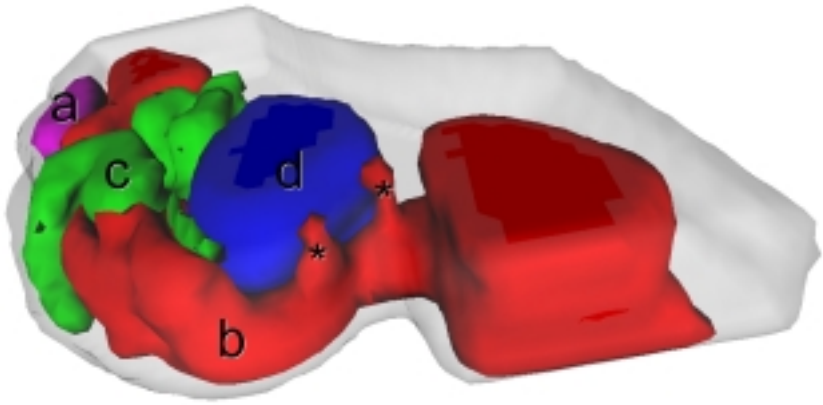

Fig. 2. *Chick mesonephros on 5 ED - a: Wolffian duct, b: marginal blood channel, c: tubules, d: renal corpuscle, *: connection between marginal blood channels.*

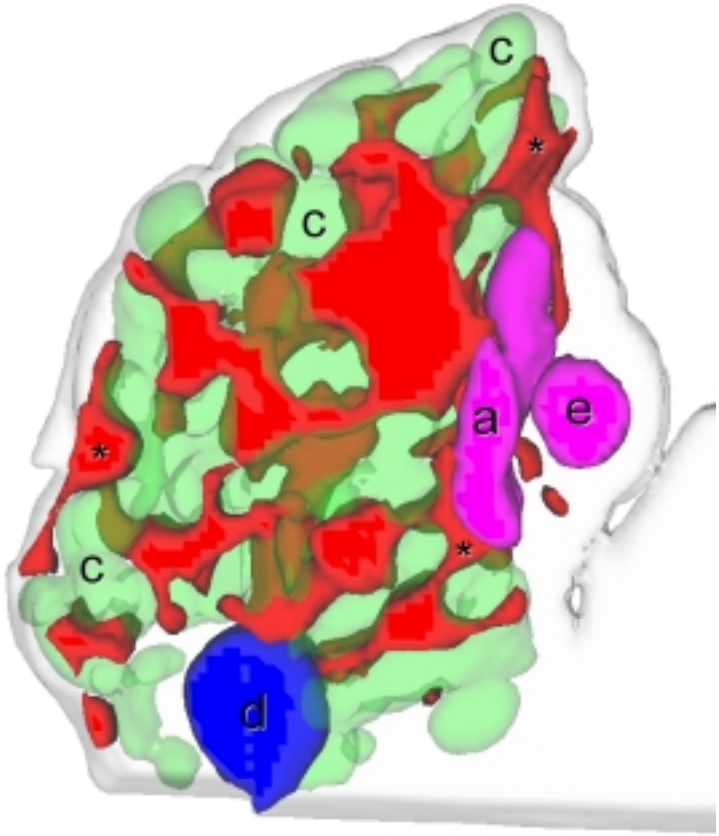

Fig. 3. *Chick mesonephros on 7 ED - a: Wolffian duct, c: tubules, d: renal corpuscle, e: Müllerian duct, *: remnants of marginal blood channel.*

Fig. 3 represents a typical reconstruction of mesonephros on ED 7. An extensive development of chick mesonephros between ED 5 and 7 is manifested mainly by rapid growth of tubules and expansion of peritubular vascular bed. Primary vascular architecture is changed by marked propagation of tubules to the lateral region of mesonephros. Previous strictly segmental arrangement of vessels is no more distinct. Capillaries form dense network closely attached to the tubular wall and completely occupy peritubular spaces. Marginal blood channels are no more continuous and their remnants are preserved in the ventromedial part of mesonephros. In the dorsal part a connection of marginal channel with v. cardinalis posterior persists in the proximity of Wolffian duct (Fig. 3).

DISCUSSION

The development of chick mesonephros between the embryonic days 5 and 7 is characterized by an increase of tubular length and an amplification of peritubular capillaries. Intensive growth of tubular component of mesonephros is accompanied by the development of new vascular segments and remodelation of preexisting vessels as an adaptation to growth of nephrons and increasing demands on blood supply. The microanatomy of blood supply of the segments of mesonephric nephrons in chick was studied using microinjections of Evans blue solution in combination with light microscopy (Friebová-Zemanová, 1980). This technique yielded basic information on mesonephric blood circulation; nevertheless, it did not allow examination of mutual spatial arrangement of all structures in mesonephros. The confocal microscopy of physical sections stained with eosin gives series of perfectly registered optical sections with well discernible vascular component, renal corpuscles, mesonephric tubules and Wolffian duct. In mesonephros on ED 7 the recording from several microscopic fields is needed. Registration of partial images of the object under study can be done using either original images or models resulting from reconstruction. Registration of the pre-processed image, using less data was used in this study, as tools for registration of original images was not available. The registration of original images is more robust to errors due to misinterpretation of data and is preferable.

3-D reconstruction provides detailed information on the mutual relationships of structural components of mesonephros and enables to compare the spatial organization in different developmental stages of the organ as well as to study an impaired development e.g. under experimental conditions.

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