A THREE-DIMENSIONAL MODEL OF EMBRYONIC DEVELOPMENT OF THE VASCULAR AND TUBULAR COMPONENTS OF THE OSMOREGULATORY APPARATUS OF THE KIDNEY

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UDC 612.46.014.462.1.014.2

KEY WORDS: kidney; spatial relations; osmoregulatory apparatus.

It is the physiological and physicochemical aspects of the filtration-reabsorption activity of the kidney which have received the most study [1, 2, 4-6, 8, 10, 11]. There are only extremely inadequate descriptions of the structural organization of the countercurrent system of the kidney and, in particular, of the principles governing the formation of its architectonics during embryonic development [2, 3]. Information on spatial relations between components of the osmoregulatory apparatus of the kidney, moreover, is contradictory [3, 7, 9].

It was therefore decided to study the structural organization of the tubular and vascular components of the concentration apparatus of the rabbit kidney and also to describe the spatial relations between vascular and tubular components during embryonic development.

EXPERIMENTAL METHOD

The most adequate method of judging the architectonics of the countercurrent system of the kidney during its embryonic development and the dynamics of morphogenesis and changes in spatial relations of the structures concerned is by preparing and studying a consecutive series of graphic and plastic reconstruction models. Data on the quantity of material studied and its distribution among different methods of study are given in Table 1.

EXPERIMENTAL RESULTS

The experiments showed that the diverticulum of the Wolffian duct, as it sinks into the mass of metanephrogenic cells, during each successive division gives rise not to two tubular structures of the new generation, as is generally considered [12, 13], but to three. This fact can be demonstrated only by the method of three-dimensional reproduction of spatial relations between test objects, namely by the method of reconstruction [14].

In the ampullary ends, which terminate blindly, of each of the three comparatively short newly formed collecting tubules (crown-rump length of the embryo 12.0 mm) the metanephrogenic cells are arranged as a more compact mass than the surrounding cells and they thus form a cell "conglomerate," from which the different elements of the nephron subsequently develop. The first signs of the anlage of the loop of Henle, the descending and ascending limbs of which belong to the tubular component of the countercurrent system of the kidney [3], appear in the 17.0-18.0 mm embryo, when one of the loops of the tubular part of the primitive nephron, lengthening a little, embeds itself in the thickness of the mesenchyme surrounding the collecting tubule, with which the anlage of the nephron now being described forms an anastomosis. Because the rates of growth of the derivatives of the diverticulum of the Wolffian duct are slower than those of the tubular part of the primitive nephron, the newly formed loop of Henle, as it lengthens, apparently "outstrips" the collecting tubule next to which it lies. In 22.0-24.0 mm embryos the newly formed loop of the nephron lies along the collecting tubule of the previous generation (Fig. 1). A similar picture also is observed during formation of the loops of two other nephrons of the corresponding population, belonging to the same collecting tubule. As a result of this, loops of nephrons of the corresponding population are arranged in threes along each of the collecting tubules. By means of a reconstruction model it is also possible to show that even in the anlage the ascending and descending limbs of the loop of Henle differ in their relations to the collecting tubule next to which they lie. For instance, in the 26.0 mm embryo a reconstruction model clearly shows that, first, the ascending limb lies nearer to the collecting tubule and, second, its course is more winding than that of the descending limb. The latter is straighter and it lies some distance away from the collecting tubule.

In embryos 24.0-26.0 mm long the anlage of the efferent arteriole and of its branches appears in the form of a slit-like space in the mesenchyme in the lumen of the hilus of the renal corpuscle. Since one of the loops of the anlage of the

Department of Human Anatomy, Chernovtsy Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 114-116, August, 1981. Original article submitted September 4, 1980.

0007-4888/81/9208-1133$07.50

TABLE 1. Distribution of Test Objects by Age and Method

Method of study	Crown-rump length of object, mm										Total
	11,0	12,0	14,5	17,0	18,0	22,0	24,0	26,0	28 0	33,0	
Microscopy	2	3	2	3	3	2	2	4	2	3	26
Graphic reconstruction	1	1	—	1	2	—	1	2	1	1	10
Plastic reconstruction	1	—	1	1	1	—	1	1	—	1	7

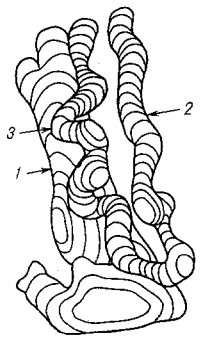

Fig. 1

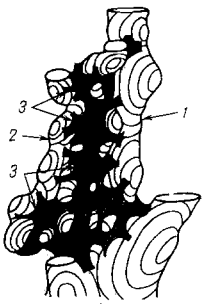

Fig. 2

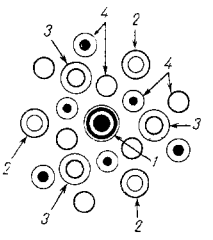

Fig. 3

Fig. 1. Graphic reconstruction of anlage of loop of nephron (24.0 mm). 1) Developing papillary duct; 2) descending limb of primitive loop of nephron; 3) ascending limb of primitive loop of nephron.

Fig. 2. Graphic reconstruction of anlage of branches of efferent arteriole (length of embryo 28.0 mm). 1) Anlage of papillary duct; 2) anlage of loop of nephron; 3) lacunar dilatations along course of branches of primitive efferent arteriole.

Fig. 3. Diagram of topographic-anatomical relations of vascular and tubular structures during embryonic development of concentration apparatus of rabbit kidney: 1) collecting tubule; 2) descending limb of loop of nephron; 3) ascending limb of loop of nephron; 4) arterial vessels.

convoluted distal segment of the nephron is also present here, the subsequent formation of the efferent arteriole and its branches takes place along the course of this tubule, and it runs in both proximal and distal directions. From the time of definite identification of the anlage of the loop of the nephron (embryos 26.0-28.0 mm long) a chain of lacunar dilatations connecting the slitlike spaces, both with each other and with the lumen of the anlage of the efferent arteriole, forms in the substance of the surrounding mesenchyme. Similar formations also can be seen between the loops of the proximal and distal convoluted segments of the anlage of the tubular part of the nephron (Fig. 2). Characteristically these lacunar dilatations, which are essentially a continuation of the lumen of the primitive efferent arteriole, fill the spaces between the loops of the convoluted segment and also between the descending and ascending limbs of the loop of Henle and the collecting tubule, next to which this loop lies.

On the basis of data obtained by the description of reconstruction models of the newly formed tubular part of the nephron it can be concluded that the distinguishing features of topographic-anatomical relations between the tubular component of the countercurrent system of the kidney during the period of its embryonic development are that along each collecting tubule of the juxtamedullary nephrons lie loops of Henle of three nephrons of a homogeneous population, connected with this same tubule. The collecting tubule occupies the central position in this system with the ascending portion of the loop of Henle next to it, and the descending portion rather more peripherally (Fig. 3). Branches of efferent arterioles of these same three nephrons, belonging to the vascular component, during the period of their appearance in embryonic development consist of interconnected lacunar dilatations, accompanying the tubular component.

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METHOD OF OBTAINING A PRIMARY MONOLAYER CULTURE OF RAT HEPATOCYTES AND ESTIMATING ITS FUNCTIONAL ACTIVITY

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UDC 612.35-085.23

KEY WORDS: hepatocyte culture; growth hormone; synthesis and secretion of labeled albumin.

Many workers have shown that the action of different hormones on synthesis and secretion of serum proteins and the activity of certain enzyme systems in the liver *in vitro* are most satisfactorily studied in primary monolayer cultures of hepatocytes [3, 4, 7-9, 12]. In the last 10 years hepatocytes have been isolated mainly by perfusion of the liver with a saline solution of collagenase. The use of trypsin as dispersing agent gives a much lower percentage yield of viable hepatocytes than collagenase [6]. Nevertheless, regardless of the method of their isolation, when cultured the cells become completely adapted to their new conditions after 24-48 h and they restore their receptor apparatus, when damaged to some degree or other in the course of enzymic disintegration [10]. It has been shown that hepatocytes, whether obtained with the aid of trypsin or of collagenase, are functionally equally active during the first 4 days of culture, after which they gradually undergo degradation [3, 5].

Grisham et al. [6] found that the cell ultrastructure is virtually unchanged as a result of the relatively strong but reversible action of trypsin on membrane receptors.

It was accordingly decided to attempt to obtain viable and functionally active rat hepatocytes by a simpler method than perfusion of the liver. Techniques widely used and described in the literature were chosen as the basis. The more readily available trypsin was used as dispersing agent. The functional activity of the cells in culture was estimated by their ability to synthesize and secrete serum albumin. To assess the hormonal sensitivity of the hepatocytes in culture, the action of growth hormone on albumin synthesis was determined.

EXPERIMENTAL METHOD

To obtain cultures of hepatocytes the liver of female Wistar rats aged 1-2 months and weighing under 100 g was used. According to Ricca et al. [11], it is at this age that the maximal incorporation of labeled precursor into albumin is observed in rats. The liver was removed with sterile precautions, washed in 0.02% Versene solution (to remove ions of heavy metals), containing 50 i.u./ml each of penicillin and streptomycin, and about 500 mg of tissue (always the same region) was taken from the middle of greater lobe and transferred to a sterile penicillin flask. In the flask the tissue was carefully cut into small pieces with scissors, after which it was washed 3 or 4 times with 10 ml of 0.02% Versene solution to remove blood. The minced and washed tissue was covered with 7 ml of 0.25% trypsin solution and incubated at 37°C for 5-8 min. The trypsin was removed, 7 ml of medium No. 199 with 10% embryonic calf serum was added, and the tissue was dispersed by pipeting 8-10 times. The resulting primary suspension was allowed to stand at room temperature to sediment the cells. A white, light residue of hepatocytes appeared after 15-20 min at the bottom of the flask. The supernatant was removed with a syringe and the residue diluted with fresh medium No. 199 with 10% embryonic calf serum to obtain the final suspension. The suspension containing 5×10^6 cells/ml was poured in a volume of 1 ml into ordinary sterile glass tubes. The medium was changed every 24 h for 2 days. The cells were cultured at 37°C in an atmosphere with 95% air and 5% CO_2. A 48-h

Laboratory of Biological Standardization of Hormones, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 116-119, August, 1981. Original article submitted February 24, 1981.

0007-4888/81/9208-1135$07.50