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IMPLANTATION GROWTH OF NEPHROGENIC TISSUE AND TUBULAR EPITHELIUM OF THE RENAL NEPHRON CULTURED *in vivo*

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Implantation growth of nephrogenic tissue of a 17-day rat embryo and of the epithelium from the nephron of animals aged 1 month was compared. Nephrogenic tissue in implants *in vivo* showed appearances characteristic of its histogenesis. The tubular epithelium from the nephron of month-old animals showed manifestations of tissue growth and formed atypical kidney structures, reflecting its ability to undergo tissue and organotypical differentiation. It is concluded that the epithelium of the renal nephron has a wide range of reactive and plastic properties and is capable of organotypical determination.

KEY WORDS: *nephrogenic tissue; nephron; implantation growth.*

Organotypical growth of epithelia of different origin cultured *in vivo* by Lazarenko's method [7] has been interpreted by some workers as evidence of the existence of the organ-specific determination of these epithelial tissues. However, there is as yet no general agreement as to how the epithelial growths observed in implants should be interpreted: as ordinary tissue differentiation (histoblastic growth) or as the result of realization of the organ-specific determination of epithelia of different organs. To obtain one of the possible alternative answers to this question, it was decided to compare implantation growth of the epithelium of formed organ structures and the implantation growth of the epithelium of their anlagen.

EXPERIMENTAL METHOD

Implantation growth of the epithelium of the renal nephron of rats aged 1 month and the nephrogenic tissue from the kidneys of 17-day rat embryos was studied. Homoimplantation was carried out by Lazarenko's classic technique [7]. Between the first and the 60th days of the experiment the implants were extirpated, fixed in Carnoy's fluid, and embedded in paraffin wax. Histological sections were stained with Mayer's hematoxylin and eosin. Nucleic acids (after Brachet and Feulgen), acid and neutral mucopolysaccharides (after McManus and Hale), and total protein (after Danielli and Pearse) were detected histochemically, with appropriate controls. In all series of experiments the recipients were male rats aged 3 months. Altogether 60 implants were studied. The choice of donors was determined by the fact that nephrogenic tissue predominates considerably in the kidneys of 17-day rat embryos, whereas in the kidneys of normal month-old rats no undifferentiated aggregates of cells of the nephrogenic tissue are found [1, 3, 7, 10].

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EXPERIMENTAL RESULTS

The study of implantation growth in these two series of experiments reveals a number of general principles of tissue conversion. Definite stages of transformation of the implanted tissues were identified: depression, destructive-progressive changes, proliferation and growth, tissue differentiation, organogenesis, and regression of the grafted and newly formed structures. The stages were identified in accordance with the predominance of the particular processes in the implanted tissues at definite stages of the experiments. During the first day of the experiments implanted tissue in a focus of aseptic inflammation emerged from the depressive state and embarked upon a phase of destructive-progressive changes. At this period growth of the nephrogenic tissue of the embryos and of the epithelium of the renal nephrons of the month-old animals showed certain distinguishing features. For instance, in implants of the kidneys of month-old rats marked destructive changes were found, and were particularly clear in the central areas of the implants, where conditions for nutrition were much inferior to those in peripheral zones. Destruction of cells in the implants of nephrogenic tissue was less intensive, evidently because the undifferentiated structures were less sensitive to a disturbance of nutrition. In the epithelium of the nephron sensitivity of different parts to the noxious factor varied. Cells in the proximal parts and ascending parts of the loop of the nephron underwent the greatest destruction, whereas the glomerular apparatus with the epithelium of the capsule and descending part of the loop of the nephron were most resistant to nutritional disturbances.

During the first 3 days of the experiment growth was observed on the surface of the fibrin in the form of monolayers in the implants of the kidneys from month-old rats, on account of the epithelium of the nephrons (Fig. 1a). After implantation of nephrogenic tissue, undifferentiated cells emerged from their depressive state and were transformed into epithelial bands and tubes, in accordance with the principles of embryonic histogenesis of this organ [5] (Fig. 2).

After 6-8 days the activated epithelium in the region of the mouth of the capsule of the renal corpuscle, the descending part of the loops of the nephron, and surviving cells from other parts of the nephron proliferated as bands in the connective tissue of the implanted fragment of organ, and as the connective-tissue framework of the implants was formed, they grew into the spaces enclosed by celloidin. Attention was concentrated on the dynamics of the epithelial growths in the newly-formed connective-tissue framework of the implant. The zone of contact between connective tissue of the grafted fragment and connective tissue of the implant stroma was identified in histological sections stained by the Danielli–Pearse method for total protein [2]. These growths were not surviving transplanted tissue, but tissue newly formed during implantation growth. This stage is characterized by differentiation of the newly formed structures: the formation of atypical proximal portions (Fig. 3a), of the loop of the nephron, and of atypical renal corpuscles. During the formation of the new renal corpuscles primitive–layered capsules of the renal corpuscle were formed and were lined with a simple squamous epithelium with a slit-like space between the epithelial layers (Fig. 3b). The renal corpuscle contained loose connective tissue with blood capillaries. However, no typical glomeruli of capillaries formed as in the renal corpuscles of functioning organs.

In foci of prolonged inflammation in the implants of kidneys from month-old animals the epithelial bands spread over the free surface of the compartments enclosed by celloidin and formed monolayers. In the implants at this stage of the experiments, superficial growths of the epithelium predominated over organotypical growth, when atypical nephrons and epithelial tubules similar to the initial portions of the urinary tract (collecting tubules) were formed. The epithelial monolayers could form bands of submerged growth into the underlying connective tissue (Fig. 1b), followed by their differentiation and the formation of epithelial tubules. Later, most of the tubules in the kidney implants from month-old animals were transformed into cyst-like structures of different sizes.

Starting from the 12th to 15th day of the experiment processes of immunological incompatibility were exhibited in implants of the renal nephron from month-old animals. The connective tissue was infiltrated by lymphocytes and regression of the epithelial structures, both transplanted and newly formed in the implants, began to occur. Considerable proliferation of the recipient's connective tissue was observed in the implants of nephrogenic tissue, so that the degree of differentiation of the structures of the connective-tissue bed did not correspond to that of the newly formed epithelial structures. This evidently prevented the nephrogenic tissue from exhibiting its capacity for specific tissue growth during culture in the adult recipients.

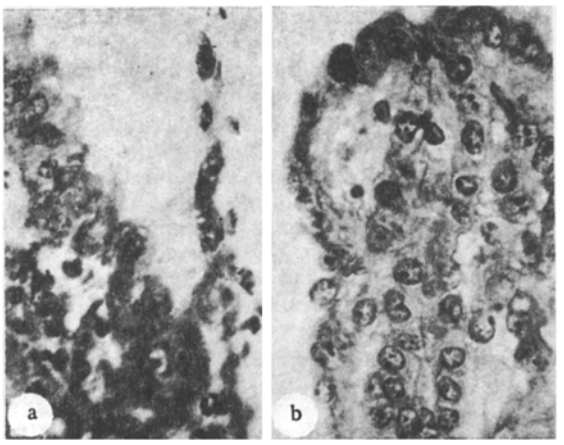

Fig. 1

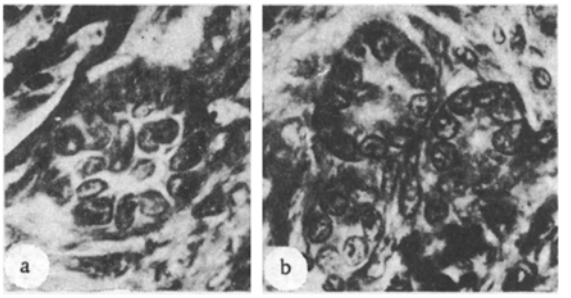

Fig. 2

Fig. 1. Tissue differentiation of epithelium of renal nephron from month-old animals in implants: a) growth along fibrin; formation of epithelial monolayers; b) submerged growth. Third day of experiment, Carnoy, PAS reaction after McManus, 630×.

Fig. 2. Manifestations of embryonic histogenesis of nephrogenic tissue in implants: a) band of cells in space between celloidin layers (6th day of experiment); b) structures of nephrogenic tissue in space between celloidin (8th day of experiment). Carnoy, PAS reaction after McManus, 630×.

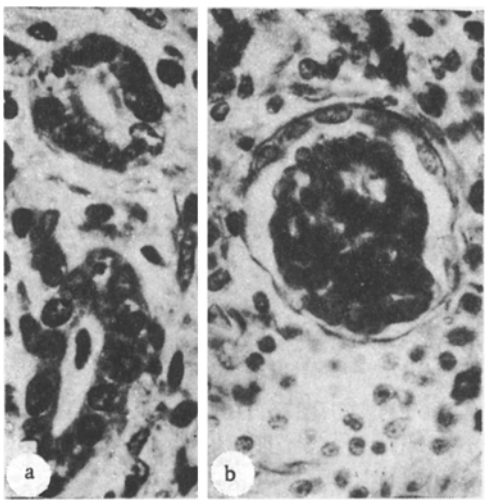

Fig. 3. Organotypical differentiation of epithelium of renal nephron of month-old animals in implants: a) atypical portions of nephron (8th day of experiment); b) atypical renal corpuscle (20th day of experiment). Carnoy, PAS reaction after McManus, 630×.

Lymphoid infiltration in cultures of nephrogenic tissue appeared on the 20th to 25th day of the experiment and was much less marked than in implants of epithelium of differentiated nephrons. This explains the longer existence of the epithelial growths of nephrogenic tissue (until the 30th day of the experiment) than implants of epithelium of the renal nephron of the month-old animals.

Analysis of the material showed that transformations in the implants of epithelium from the nephron of month-old animals and nephrogenic tissue of embryos differ considerably, despite some common features. Nephrogenic tissue, after implantation, was primarily capable

of manifesting embryonic histogenesis and was unable to form epithelial growths; this would characterize the nephrogenic tissue as definitive epithelial tissue of the kidney and not as a component of the embryonic anlagen of that organ. The nephrogenic tissue in the focus of aseptic inflammation underwent less severe destruction than the epithelium of the nephron of the definitive kidney. Surviving cells of nephrogenic tissue in a phase of depression did not undergo dedifferentiation, and when favorable nutritional conditions were established, they continued with the typical processes of late embryogenesis and the early stages of postnatal development of the rat kidney.

The epithelium of the renal nephrons of the month-old animals during implantation growth manifested processes of tissue and organotypical differentiation, with the formation of epithelial bands, single-layered tubules resembling different parts of the nephron, and atypical renal corpuscles. Manifestations of tissue growth (growth along fibrin strands, elimination of fragments of celloidin, submerged growth) and the absence of any similar transformations at these same stages of the experiment in implants of nephrogenic tissue are evidence of the tissue determination of the epithelial structures of the nephron. The discovery of atypical organ structures (tubules, cysts, atypical renal corpuscles), formed by dedifferentiation of the transplanted epithelial cells, in implants of epithelium of the nephron of the month-old animals is direct evidence of their ability to undergo not only tissue, but also organotypical differentiation.

It could be concluded from these results that the epithelium of the renal nephron possesses a wide range of reactive and plastic properties and the capacity for organotypical determination. Because of differences in the scale of the biological potential of the epithelium of different parts of the nephron under implantation conditions, differences in their sensitivity to nutritional disturbances can be determined. This opens the way for the scientific explanation of one of the causes of possible functional insufficiency of transplanted kidneys under both experimental and clinical conditions. The results can be used to assist with the solution of problems connected with assessment of methods of organ conservation and the period of viability of the functioning structures of the kidneys during transplantation.

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