EFFECT OF OPISTHORCHIASIS ON IMPLANTATION GROWTH OF THE HEPATIC EPITHELIUM

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Proliferation of the ductular epithelium, including the small bile ducts, is a favorable background for malignant transformation during opisthorchiasis [6, 12]. Proliferative activity of the epithelium is linked with permanent mechanical injury to the epithelial layer by opisthorchids [6]. However, changes in proliferative activity of the cells lining the small bile ducts, where parasites do not live, prevent this mechanism being regarded as the only one [4, 10, 15].

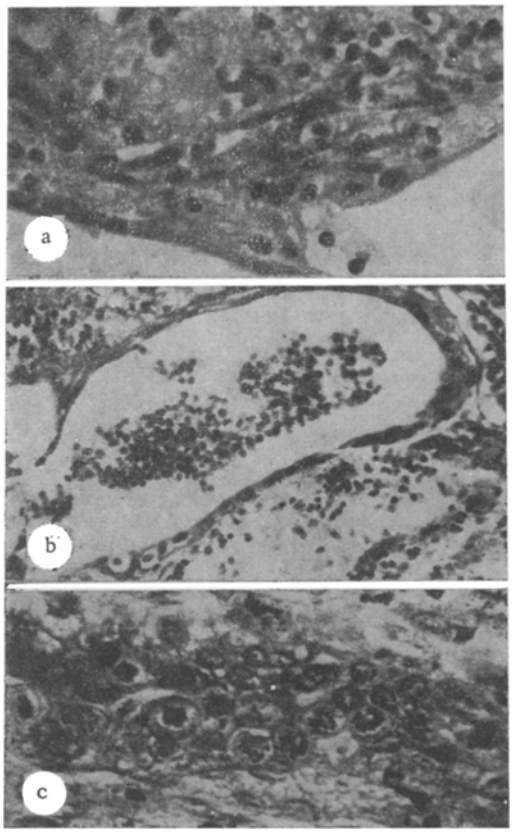

Fig. 1. Proliferation and growth of hepatic and ductular epithelium of liver affected by opisthorchiasis: a) single layer of ductular epithelium on surface of fibrin. Stage of growth 1 day, donor: golden hamster aged 3 months (time after infestation 30 days), recipient: intact animal of same age; b) growth of ductular epithelium on surface of implanted fragment, donor and recipient the same; c) proliferation of newly formed biliary canaliculus in intercellular connective-tissue band, donor and recipient the same. Stained with hematoxylin and eosin, 280 ×.

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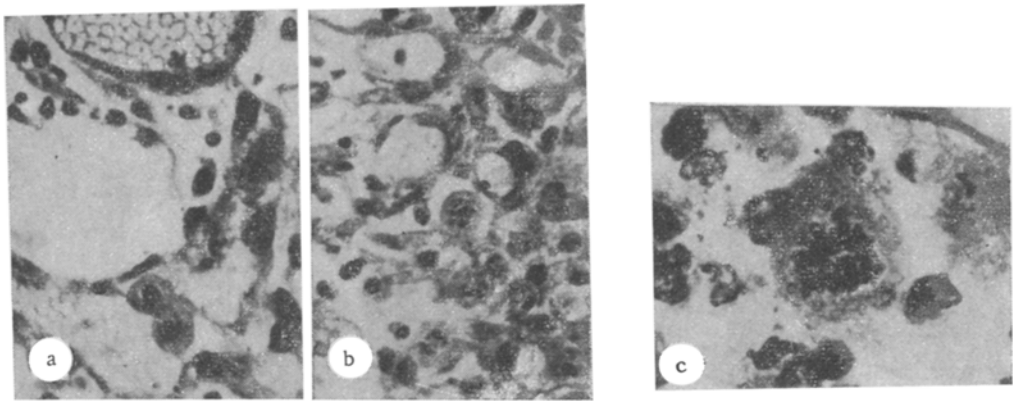

Fig. 2. Atypical proliferation of epithelial and stromal components of liver affected by opisthorchiasis in implants. a) Proliferation of fibroblasts and endothelial cells of recipients: donor — golden hamster aged 8 months, on 150th day of infestation, recipient — intact golden hamster aged 4 months (stage of growth 3 days); b) cancer-like growths of ductular epithelium in newly formed connective tissue of implant, donor and recipient the same; c) deletion of chromosomes in metaphase with fragmentation and scattering of chromosomes in proliferating focus of ductular epithelium, donor and recipient the same (stage of growth 6 days); d) complete metaphase in focus of proliferating ductular epithelium, donor and recipient the same (stage of growth 6 days). Stained with hematoxylin and eosin, 630 ×.

This investigation was devoted to an analysis of proliferative and organogenetic potentials of the hepatic and ductular epithelium of the liver with and without infestation by parasites.

EXPERIMENTAL METHOD

Experiments were carried out by Lazarenko's method, described in [11], and 100 male golden hamsters. There were six series of experiments: I) donor and recipient were intact animals aged 3 months, II and III) implantation of the liver, infested with opisthorchids (II — on the 30th day, III — On the 150th day of infestation) into uninfested recipients of the same age; IV and V) both donors and recipients were infested golden hamsters (on the 30th and 150th days of infestation respectively); VI) implantation of the liver of intact donors into infested recipients.

The technique of infestation of the animals with opisthorchids was described previously [3].

Altogether 200 implants were studied at stages of growth from 1 to 45 days. Material was fixed in Carnoy's fluid, embedded in paraffin wax, and stained with hematoxylin and eosin and by the PAS reaction according to McManus.

EXPERIMENTAL RESULTS

Implantation growth of the liver, both intact and infested with opisthorchids at all stages studied was indistinguishable in principle from the corresponding picture for other implanted epithelial tissues [2, 11], but the stages of conversion of ductular and hepatic epithelium in intact animals were fully comparable with the results described previously [11]. The main differences were found in experiments of series II-VI. Irrespective of the length of time spent by the parasites in the host (30-150 days) implantation growth of the epithelial structures was identical in type. On the 1st-3rd days active proliferation took place on the ductular epithelium over the surface of a fibrin clot (Fig. 1a) and of an implanted fragment (Fig. 1b) in the form of a single layer of cells in the peripheral zone of the implant. Hepatocytes of the pericentral and interstitial zones of the lobule also were activated and proliferated in this same region. Pre-existing and newly formed biliary canaliculi grew into the newly formed connective tissue in the form of an epithelial band (Fig. 1c).

In the early stages of implantation (1-5 days) the implanted fragments induced an active fibroblastic reaction in the recipient's subcutaneous connective-tissue bed, in which endo-

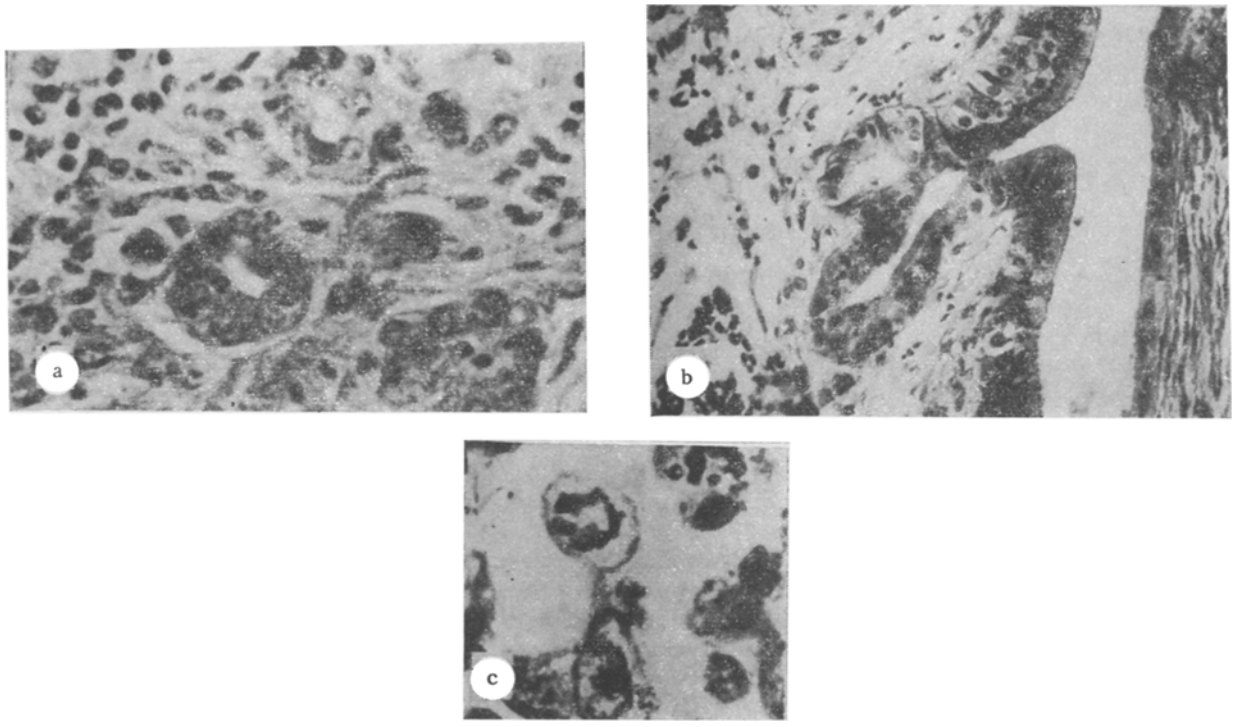

Fig. 3. Glandular differentiation of hepatic and ductular epithelium of liver affected with opisthorchiasis, in plants. a) Glandular differentiation of hepatic epithelium in newly formed connective tissue of implant, donor — golden hamster aged 8 months (150th day of infestation), recipient — intact hamster aged 4 months. Stained with hematoxylin and eosin, 280 ×; b) glandular differentiation of ductular epithelium within boundaries of implanted fragments, with differentiation of pale basal cells, donor and recipient the same. PAS reaction after McManus, 280 ×. [Letters for Figs. 2 and 3 as in Russian original; presumably Fig. 3c should be regarded as Fig. 2d — Publisher.]

thelial cells also were involved (Fig. 2a). This is the distinguishing feature compared with implants of the intact liver. Stimulation of the fibroblastic reaction could be connected with the action of soluble products of parasitic granulomas, induced by the eggs of the helminths [16].

In the process of proliferation (3-6 days) cancer-like growths were formed in the ductular epithelium, with marked dissociation of the cells, high mitotic activity, and many pathological mitoses (Fig. 2b-d).

The presence of the latter reflects injury both to chromosomes (fragmentation, deletion in metaphase) and to the mitotic apparatus (delay of mitosis in metaphase, complete metaphase, c-mitosis). The increase in the number of pathological mitoses is one of the mechanisms of the increase in genetic heterogeneity of cell populations [7], and the disturbance of intercellular interaction (marked cell dissociation in the foci of proliferation) reflects reconstruction of the cell surface [1]. Both these factors may be the potential basis for more frequent malignant metamorphosis of the ductular epithelium in opisthorchiasis. The primary factor is probably disturbance of the state of the cell surface, for a change takes place in the composition of the phospholipids of the liver [5] — one of the principal components of cell membranes, and modification of the cell surface regularly intensifies proliferative activity [9].

In addition, glandular differentiation of the epithelial regenerates also takes place in the implanted fragment *in situ* (Fig. 3a, b). The hepatic epithelium forms pseudotrabecular structures (Fig. 3a), and the ductular epithelium, differentiating in the composition of the epithelial layer, forms simple glandular complexes with differentiation of pale reserve basal cells (Fig. 2b). The newly formed glands have a merocrine type of secretion. The secretion contains neutral glycosaminoglycans.

In the final stages of implantation growth the glandular tubules are converted into epithelial cysts, lined with simple epithelium. The morpho-histochemical characteristics of the implants in the experiments of series VI were similar to changes in the implants in series II and V. Degradation of the newly formed epithelial structures was complete by the 20th-30th days of the experiment.

Glandular metamorphosis of the hepatic epithelium in implants of the liver affected by opisthorchiasis probably reflects a process of structural disinhibition [8], for in the fetus under normal conditions of intrauterine development, liver cells can be converted into epithelium of the bile ducts [14, 15]. In the experiments of series IV and V marked inhibition of growth of components of the liver takes place, destruction of the implanted fragments occurs, with marked depolymerization of glycosaminoglycans of the surrounding connective tissue, probably on account of allergic changes in the recipient and injury to implanted fragments by circulating immune complexes [13]. In the course of implantation growth high proliferative powers of the ductular epithelium with disturbance of the normal course of mitosis and of intercellular interaction are thus found, together with a unidirectional course of differentiation of both types of epithelium toward glandular metamorphosis.

Infestation by opisthorchids modifies the proliferative activity of the epithelial component of the liver, not by mechanical action of the parasites, but as a result of modification of the biological properties of the epithelium, probably at the genetic level.

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