ORGAN CULTURE OF URINARY BLADDER TISSUE

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Urinary bladder tissue of young rats was explanted on Millipore filters, cellophane, or polyvinyl chloride film in Carrel's flasks. The nutrient medium consisted of Eagle's medium, human blood serum, and lactalbumin hydrolysate with the addition of glucose, insulin, and penicillin. When the Millipore filter and polyvinyl chloride film were used, under the conditions stipulated above the explants could be kept viable for 50 days, with preservation of the typical histological structure of the mucous membrane of the organ. The technique as described can be used to simulate various pathological processes in the bladder.

KEY WORDS: organ culture; urinary bladder.

During cultivation of the mucous membrane of the urinary bladder, during the first few hours much of the tissue degenerates and epithelial cells and fibroblasts migrate from the explant as a monolayer. Later, the two types of cells may survive concurrently or the cells of one type may die. In both cases a tissue culture without the characteristic typical histological features of the organ is obtained [1]. As yet no studies of the urinary bladder in organ culture have been undertaken. Such a model would be of great interest for the study of the morphological and biochemical properties of the mucous membrane and also of its so-called transitional epithelium under relatively normal conditions and during exposure to various factors.

It was accordingly decided to carry out the investigation described below with the aim of preserving organotypical structures of the urinary bladder mucous membrane for as long as possible during culture *in vitro*.

EXPERIMENTAL METHOD

Pieces of the urinary bladder of young rats aged 1 month were used. The animals were killed with ether and, under strictly aseptic conditions the urinary bladder was removed. The organ was washed in Hanks's solution and placed in a Petri dish with medium No. 199 for tissue culture. In this medium the bladder was cut with scissors into square pieces measuring 1-2 mm. The bladder was minced and explants formed under the control of a binocular loupe. Cultures were set up in Carrel's flasks. A piece of Millipore filter (type BX3 "Synpor VUFS" with a pore size of 0.23 μ, Czechoslovakia), cellophane, or polyvinyl chloride film, previously softened in medium No. 199, were placed into the flask. The explants were applied to this fragment (6 to 10 per flask), after 20 or 30 drops of nutrient medium of the following composition were added: Eagle's medium 79%, 40% glucose solution 1%, human blood serum (group AB/IV) 10%, lactalbumin hydrolysate 10%, insulin 0.08 unit/ml, and penicillin 100 units/ml.

The nutrient mixture covered the piece of filter or other material mentioned above in a very thin layer, so that the top part of the bladder explants was in contact with air. The flasks, sealed with rubber caps, were incubated at 37°C. The acidity of the medium was

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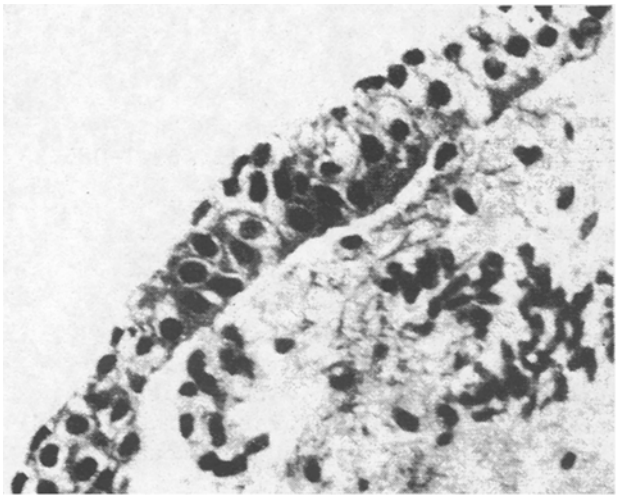

Fig. 1

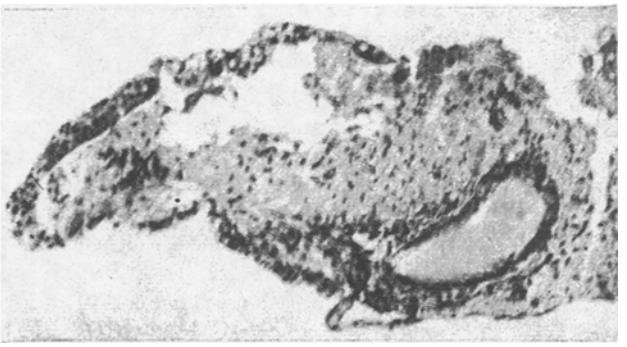

Fig. 2

Fig. 1. Epithelium of urinary bladder after 50 days in organ culture: Epithelium consists of two or three rows of cells. Hematoxylin–eosin, objective 40, Homal VI (900×).

Fig. 2. Explant of urinary bladder tissue after 50 days in culture (general appearance). Hematoxylin–eosin, objective 10, Homal VI (230×).

TABLE 1. Composition of Explants as Revealed in Sections

Character of tissue	Number of explants	
	1–6 weeks	7 weeks
Epithelium and connective tissue	12*	38
Connective tissue	9	10
Necrosis	2	4

*Some explants fixed during the first 4 weeks also contained muscle fibers.

kept at the 7.0 level, with fluctuations of pH from 6.6 to 7.4.

The medium was changed every 3-4 days. All manipulations during feeding and changing the medium were carried out with strict aseptic and antiseptic precautions. The Millipore filters and cellophane were sterilized in a current of steam at 100°C.

Under the microscope round epithelial cells were clearly visible around the edge of the tissue. Some of them had migrated from the explant over the film. However, the characteristic monolayer and zones of growth (either epithelial cells or fibroblasts) characteristic of cell cultures were never observed. Possibly this was because of the poor adhesion of the explant and its individual cells to the base (the film or Millipore filter). Intravital microscopy showed degenerative changes in the center of the explants and in individual cells at the edge of the fragment. These changes were expressed as a highly granular cytoplasm of the cells and the formation of a certain amount of debris in the center.

Of 100 initial explants 23 were fixed in the first 6 weeks, 52 after 7 days, and 25 died in the flasks as a result of aseptic necrosis.

The results of investigation of the stained sections are given in Table 1.

During the first week in culture the epithelial layer of the explant underwent degenerative changes and was partly destroyed. The mesothelium and muscle fibers usually died. By the 10th day after seeding definite predominance of connective tissue was observed, although it showed not the slightest tendency toward forming a monolayer and retained the characteristic histological structure of the mucosa and submucosa. In the course of the following weeks the epithelium gradually regenerated and assumed the arrangement in two or three rows characteristic of the mucous membrane of the urinary bladder (Fig. 1). The polyvinyl chloride film was disinfected by boiling in 96° ethanol for 30 min.

Altogether 100 urinary bladder explants from seven rats were used in the experiment.

Once a week from three to five explants (from one Carrel's flask' were fixed in neutral formalin. After fixation the material was treated histologically in the usual way, but

restricting the time in the dehydrating fluids to 3 h in each case because of the small size of the fragments. Sections were cut and stained with hematoxylin–eosin and by Van Gieson's method. Stained sections were examined under the microscope and, in the case of transparent films (cellophane, polyvinyl chloride film) the explants were examined daily without removing them from the flasks, under a magnification of up to 400 ×.

EXPERIMENTAL RESULTS

The first seedings were made on Millipore filters. The explants did not adhere firmly to the filter, but as subsequent histological investigation showed, they remained viable for up to 7 weeks. No significant differences in development of the tissues were found whether the fragments were applied with the mucous membrane underneath or with the surface covered with mesothelium underneath. To begin with the pieces of urinary bladder were placed on cellophane, but they did not adhere to it at all. Later the cellophane was replaced by polyvinyl chloride film, and this choice proved quite successful. The film swelled adequately in the nutrient medium and was permeated with it. The explants adhered to the film just as insecurely as to the Millipore filter, although this did not prevent the tissues from remaining viable for an equally long time (up to 7 weeks).

The connective tissue showed definite degenerative changes in the thickness of the explant. Everywhere, however, the connective tissue retained as a whole the characteristic structure of the mucous membrane. Even the blood vessels in the explant were not destroyed on the 50th day in culture. The muscular and mesothelial layers, however, did not regenerate. After a stay of 7 days *in vitro*, the flask thus still contained a fragment of mucous membrane of the bladder, admittedly with some features of degeneration, but nevertheless preserving the typical histological features characteristic of that organ (Fig. 2).

LITERATURE CITED

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