QUANTITATIVE ASSESSMENT OF VIABILITY OF KIDNEY TISSUE TAKEN FROM DONORS SUBJECTED TO VARIOUS TREATMENTS

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The viability of mouse kidney tissues, assessed quantitatively by growth in a plasma-free medium, was shown to depend on the state of the donor. Growth of the cultures was inhibited after whole-body x-ray irradiation of the donors, prolonged starvation, or poisoning with cadmium chloride. Increased growth of the culture was observed if compensatory hypertrophy of the kidney had developed as a result of unilateral nephrectomy, and also after subcutaneous inflammation.

KEY WORDS: tissue cultures; kidney; transplantation.

The tissue culture method is used to assess the viability of organs destined for transplantation [6]. However, no attempt has been made to study how their viability depends on the state of the donor. Only the influence of the donor's age is known [3].

It was accordingly decided to make a quantitative assessment of the viability of kidney tissue taken from animals subjected to various extremal factors, and to use the method of plasma-free tissue culture for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on mice weighing from 2 to 33 g. Pieces of tissue measuring 0.3-0.6 mm^2 were cultured in tubes on the surface of the glass at 37°C in plasma-free medium No. 199 containing 10% bovine serum and antibiotics (penicillin and streptomycin, 100 units/ml of each) [7]. Control and experimental cultures of each series were set up at the same time in the same batch of medium No. 199 and serum. Sixty cultures, grown in 10 tubes, were taken from each animal. The viability of the tissue was assessed from the size of the cell colonies growing around the central explant after 72 h. For the morphological investigations pieces of tissue were cultured on coverslips in tubes. The cultures were fixed in Bouin's mixture and stained with hematoxylin–eosin. The number of cells was counted directly in all the colonies by means of an ocular grid [1]. Pathological mitoses were classified by Alov's method [2]. The results were subjected to statistical analysis by the nonparametric method using Wilcoxon's criterion [8].

EXPERIMENTAL RESULTS

The monolayer of a plasma-free tissue culture of kidneys from adult animals consists of cells of epitheliumlike type (wide and fusiform), similar to those described in monolayer cell cultures of kidney tissue [4, 5, 9]. The study of the dynamics of growth of the plasma-free cultures, their mitotic activity, and the number of pathological mitoses and binuclear cells without change of nutrient medium showed absence of degenerative changes in the cultures 3-5 days after the beginning of growth (Fig. 1). These times are evidently optimal for observations on cell viability.

The results of assessment of the viability of the kidney tissues by the plasma-free tissue culture method after various forms of treatment of the donor animals are shown in Table 1. If the donors had

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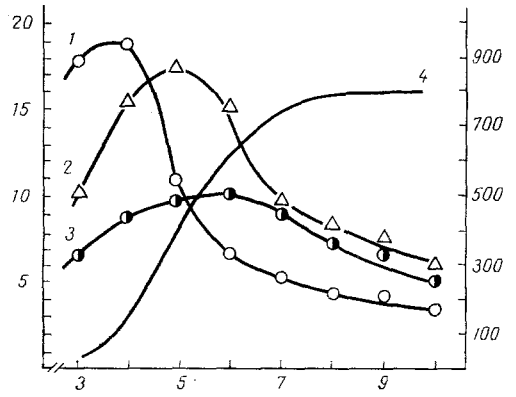

Fig. 1. Changes in mitotic activity of kidney cells (1), number of pathological mitoses (2), number of binucleate cells (3), and total number of cells in colony (4) at different times after beginning of culture of mouse kidney tissue. Ordinate: left, mitotic index (in ‰), number of pathological mitoses and binuclear cells (in %), right, mean number of cells in culture; abscissa, time from beginning of culture (in days).

TABLE 1. Size of Cell Colonies Growing After 72 h Around Explants of Kidney Tissue from Mice Receiving Various Treatments

Treatments and time of taking tissue	Control	Experiment	P
3 days after whole-body x-ray irradiation in a dose of 100 R (n = 16)	34,9	19,9	<0,001
6 days after whole-body x-ray irradiation in a dose of 1000 R (n = 9)	17,0	8,6	<0,001
1 h after administration of cadmium chloride in a dose of 0.05 mg/g (n = 14)	33,9	3,5	<0,001
1 h after administration of cadmium chloride in a dose of 0.005 mg/g (n = 23)	50,5	58,1	>0,1
After complete starvation for 2 days (n = 21)	15,6	12,7	>0,05
After complete starvation for 5 days (n = 15)	20,5	11,8	<0,05
3 days after beginning of subcutaneous inflamation (n = 14)	21,8	35,7	<0,05
5 days after beginning of subcutaneous inflammation (n = 20)	30,4	38,6	<0,05
3 days after unilateral nephrectomy (n = 19)	40,2	64,6	<0,01
6 days after unilateral nephrectomy (n = 34)	63,9	84,2	<0,01

subcutaneous inflammatory lesions or compensatory hypertrophy of the kidney, the kidney tissue fragments in culture showed increased viability. Conversely, after prolonged starvation of the donor animals, poisoning with cadmium chloride, or x-ray irradiation, growth of the cultures was inhibited.

The viability of organs in plasma-free tissue culture depends on the donor's age. For instance, growth of kidney tissue cultures from mice aged 1-1.5 months (weight 11.5 g) was 64% greater than that of cultures obtained from animals aged 1-1.5 years (weight 33 g).

On the basis of these results quantitative assessment of tissue growth by the plasma-free tissue culture method can be recommended for use when studying the viability of organs for transplantation.

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