Time Course of Rat Kidney Cell Proliferation and Influence of Endogenous Inhibitors

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The existence of tissue-specific proliferation inhibitors in the liver and kidneys was established by Saetren in 1956 [11], and in 1960 Bullough and Iversen proposed the term chalones for such compounds [9]. Chalones exert presumably local effects [1, 4] by blocking cell division in the G_1 and G_2 phases, hence their name, G_1 and G_2 proliferation inhibitors. Their activity is not limited to modulation of the proliferation rate but bears a certain relation to the differentiation processes as well. The nontoxicity of chalones, together with their ability to block cell reproduction, make them promising for investigations concerned with the diagnosis and treatment of tumor diseases. Chalones probably exert their effect via the cAMP- and Ca^{+2}-dependent systems [1]. Among the rather large body of information on the regulation of cell proliferation there are very few publications concerning the effect of cell proliferation inhibitors depending on age [1, 5, 6]. The proliferative activity of tissues does not remain unchanged during ontogenesis, but varies depending on the particular tissue. The regulation by chalones must follow the changes in proliferative activity in order to maintain tissue homeostasis at any stage of ontogenesis [4]. Age-dependent variations in the concentration or activity of the factors regulating tissue homeostasis may play an important role in the development of neoplasms in animals and humans, leading to an increase of their incidence rate [5].

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In the present study the effect of the inhibitor on the dynamics of DNA synthesis and the reaction of kidney cells under conditions of compensatory hypertrophy were investigated using experimental animals of different age.

MATERIALS AND METHODS

The experiments were carried out on Wistar rats 1, 3, 12, and 24 months old. For a study of the dynamics of DNA synthesis in the kidney under conditions of compensatory hypertrophy, the unilaterally nephroectomized animals were killed from 20 to 52 h after the operation at 2-hour intervals. All animals were intraperitoneally injected with ^{3}H-thymidine (2 MBq/100g) 2 h before sacrifice. The DNA concentration [7] and its radioactivity in the kidney tissue were determined with a Beta 1 counter. Two fractions containing cell proliferation inhibitors were isolated from cattle kidneys as described previously [8] by sequential precipitation with 70% and 81% ethanol, for the first and second fraction, respectively, from 55% ethanol extract. The concentration of the inhibitors in the precipitates were determined as the protein content after Lowry [10]. The effect of the inhibitors on proliferative processes in the kidney was studied on 3 groups of animals: normal, control, and experimental. Each group included animals of 4 different ages. The control and experimental animals were unilaterally nephrectomized. The animals of the experimental group were given three intraperitoneal

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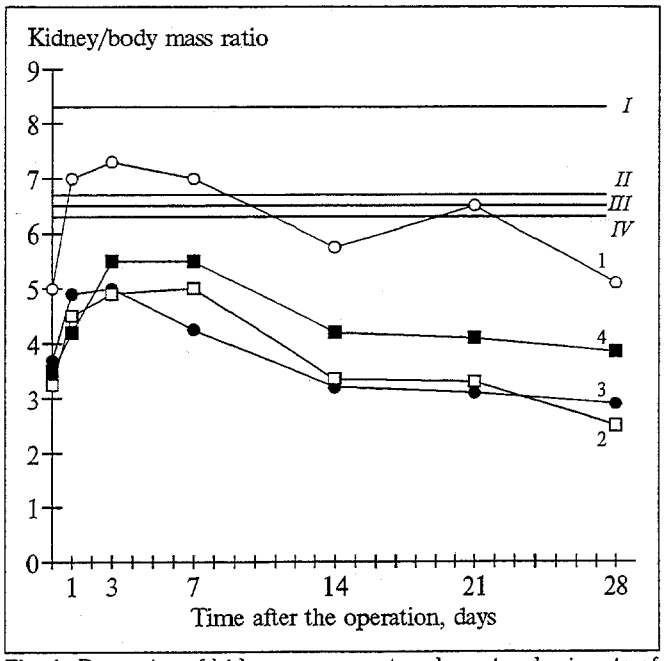

Fig. 1. Dynamics of kidney compensatory hypertrophy in rats of different age. *I, II, III, IV*) kidney/body weight ratio in normal animals 1, 3, 12, and 24 months old; 1, 2, 3, 4) kidney/body mass ratio in nephrectomized animals 1, 3, 12, and 24 months old.

injections of inhibitor fractions (15 mg protein/100 g body weight) in physiological saline 16, 28, and 40 h after the operation. The dose injected was chosen based on previous data [8,10]. The time points for the injection were estimated from the dynamics of DNA synthesis established earlier. The animals were killed 48 h after the operation. Radiolabeled thymidine was administered 2 h before sacrifice in the above dose. The concentration and radioactivity of DNA were assessed as described above. The data were statistically processed using the Student-Fisher method.

RESULTS

The dynamics of the kidney/body mass ratio under conditions of compensatory hypertrophy was initially studied to ascertain the optimal time limits for exposure. As seen from the graphs (Fig. 1), the kidney proliferation processes were maximally expressed during the first 3 days with following normalization and completion by the 28th day. A certain decrease of this parameter in the later period may be explained by inhibition of body weight growth under conditions of compensatory hypertrophy in young animals and a loss of body weight in 12- and 24-mounth-old animals. At 28th days, after restoration of kidney function, the growth rate in young animals increases again, and the body weight is restored in 12- and 24-month-old animals, thus leading to a drop of the kidney/body mass ratio. In addition, it should be noted that the proliferation processes in the hypertrophied kidney are most intensive in 1-month-old animals.

Thus, within the 3-day period chosen for investigation of the dynamics of DNA synthesis, the incorporation of labeled thymidine into DNA was shown to start 18 h after the operation. At 32 h this parameter reached a plateau, and then remained unchanged until 52 h after the operation. This dynamics was typical for all ages. Accordingly, in further experiments we restricted the observation to a 48-h interval.

A single injection of the inhibitors 48 h after the operation had no effect on cell proliferation in the kidney, in contrast to the liver, where this effect was observed during the first 24 h following partial hepatectomy [11]. This may be ascribed to the entry of a portion of the cells into the cell cycle and their loss of sensitivity to inhibitors at the time of injection (40 h after unilateral nephroectomy) [1, 4]. So, to block cell proliferation, inhibitors were injected 16 h after the operation, the concentration being maintained at a constant level for the succeeding period by repeated injections.

It was found that the kidney cells in 1-month-old animals (Table 1) were insensitive to inhibitors in the given dose, while the proliferation level was half as high in 3- and 12-month-old animals and one third as high in 24-month-old animals, no significant differences being detected between the two inhibitor fractions. The reduction of the inhibition in 24-month-old animals was statistically inreliable. The lack of inhibition in the early stages of ontogenesis

Table 1. Inhibition of Kidney Cell Proliferation in Animals of Different Age by 70 and 81% Fractions of Inhibitor Preparations (cpm/mg DNA).

Experimental conditions	Age of animals, months			
	1	3	12	24
Norm	700±57	820±76	427±35	617±63
Control (NE)	2576±493	2024±273	2117±287	1305±102
NE+70% fraction	2959±578*	1070±124	1119±103	847±85
	(0)	(47.2)	(47.0)	(35.0)
NE+81% fraction	2268±270*	1136±130	974±40	871±150
	(0)	(43.9)	(54.0)	(34.0)

Note. Asterisk – statistically unreliable differences as compared with the control ($p>0.05$). In parentheses % of inhibition versus control. NE: nephroectomized.

may be explained by a predominance of factors stimulating cell proliferation and thus making the cells insensitive to the inhibitors.

From the results of the experiments it can be concluded that the sensitivity of kidney cells remain practically unchanged from pubescence to old age, in contrast to the liver cells and vaginal epithelium, which exhibit a distinct age dependence [5, 11]. The constancy of the kidney cells' sensitivity in mature and old animals allows the assumption to be made that at this regulatory level the organism controls the proliferation with a sufficient stability.

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MORPHOLOGY AND PATHOMORFOLOGY

Blood Serum Factors from Rabbits with Acute Pancreatitis as Stimulators of Regeneration of B-Cells of the Pancreatic Islets in Experimental Diabetes

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One of the most promising ways of regulating the regeneration processes is to utilize natural endogenous humoral modulators or to stimulate their synthesis in the organism. This aspect of the problem has been studied in most detail with regard to liver regeneration [1,2,5]. At the same time, the role of humoral factors in the initiation of reparative regeneration of the pancreatic endocrine tissue is poorly understood.

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