HUMORAL REGULATION OF REGENERATION IN THE LUNGS, KIDNEYS, AND LIVER

L. K. Romanova and I. A. Zhikhareva

UDC 612.215.3+612.46+612.35]:612.6.03

Either the left lung, the right kidney, or two-thirds of the liver was removed from pregnant noninbred rats. During compensatory hypertrophy of the lung and kidney and regeneration of the liver, substances capable of stimulating cell proliferation in the corresponding fetal organs appeared in the blood stream.

The possibility of specific humoral regulation of regeneration in the injured liver and other organs has been shown by results described previously [1, 3-7, 13]. However, many problems still remain unsolved. In particular, it is not clear where and when after trauma substances capable of activating cell division appear in the blood stream [10]. Some investigators [9, 12], indeed, deny that such regulation exists.

The investigation described below was undertaken to determine how universal is the manifestation of tissue humoral regulation and to discover at what period after injury to an organ growth-regulating substances appear in the blood stream.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 150-200 g were used in the experiment. Two females in a state of estrus were mated for 24 h with males. The day of mating was taken as the first day of pregnancy.

Three groups of experiments were performed. In group 1, on the 15th-20th day of pregnancy, the left lung was removed. The rat and its progeny were sacrificed 2, 3, 4, 5, and 7 days after unilateral pneumonectomy on the mother. In group 2, the right kidney was removed from 10 females on the 10th and 19th day of pregnancy. The rat and its progeny were sacrificed 48 h and 7 days after unilateral nephrectomy on the mother. In group 3, two-thirds of the liver was resected from 9 females on the 20th day of pregnancy by the method of Higgins and Andersen. The rat and its progeny were sacrificed 18 and 32 h after partial hepatectomy on the mother.

TABLE 1. Mitotic Index in Maternal and Fetal Lungs Five Days after Removal of Left Maternal Lung

Group of animals	№ Rat	MI in maternal lungs (‰)	MI in fetal organs (‰)	
			lungs	liver
Control (mock operation)	1	0,75	1,43	1,01
	2	0,75	1,16	—
	3	2,00	1,62	1,66
Experiments (removal of left lung from mother	1	2,75	5,05	0,70
	2	2,75	3,18	1,60
	3	3,00	6,25	—

*Mitoses were counted altogether in hepatocytes, blood cells, and Kupffer cells of the liver.

Mitotic activity was determined in the lungs, kidneys, and liver of the mother rats undergoing the operations, and also in the corresponding organs of 5 fetuses from each rat. The number of mitoses was counted in the lungs in 4000 nondividing alveolar epithelial cells, in the maternal kidney in 12,000 epithelial cells of the convoluted tubules in the cortex,

Laboratory of Growth and Development, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 1, pp. 84-87, January, 1972. Original article submitted June 28, 1971.

TABLE 2. Mitotic Index (in ‰) in Cells of Developing Convoluted Tubules of Kidneys, in Hepatocytes of Liver of Fetuses, and Epithelium of Convoluted Tubules in Maternal Kidneys at Various Times after Unilateral Nephrectomy on the Mother

Time after removal of maternal right kidney	№ Rat	Control (intact animals)			Control (mock operation)			Expt.		
		MI (‰)								
		maternal kidney	fetal liver	fetal kidney	maternal kidney	fetal liver	fetal kidney	maternal kidney	fetal liver	fetal kidney
48 h	1	0,0	0,20	7,80	0,33	11,00	0,50	1,91	14,20	1,80
	2	0,0	0,40	8,10	0,33	8,30	—	1,08	11,75	—
	3	0,0		8,70	0,33	9,00	0,25	2,33	20,10	1,30
	4				0,25	14,80	0,70	0,91	14,80	—
	5							1,33	10,80	—
	6							1,75	17,20	0,50
Mean . . .		0,00	0,30	8,20	0,31	10,77	0,48	1,55	14,80	1,20
7 days					0,16	9,20		1,00	7,00	
					0,08	6,80		0,25	10,50	
					0,16	10,20		0,58	8,70	
					0,00	9,10		0,41	10,87	
					0,00	9,20				
Mean . . .					0,08	8,90		0,50	9,26	

TABLE 3. Mitotic Index in Hepatocytes of the Fetal and Maternal Liver 18 and 32 h after Partial Hepatectomy on the Mother

Time after removal of two-thirds of maternal liver	№ Rat	Expt.		Control	
		MI in liver (‰)			
		maternal	fetal	maternal	fetal
18 h	1	0	0,48	0	0,29
	2	0	0,46	0	0,31
	3	0	0,32	—	0,20
	4	0	0,39	—	0,40
	5	0	0,75		
	6	—	1,10		
Mean		0	0,58		0,30
32 h	1	0,5	0,99	0	0,18
	2	10,2	1,32	0	0,36
Mean		5,3	1,15		0,27

and in the fetal kidney in 2000 epithelial cells of the convoluted tubules. The number of mitoses in the liver was counted in 4000 hepatocytes. Pregnant rats undergoing a corresponding mock operation and intact pregnant rats with their progeny were used as the control.

EXPERIMENTAL RESULTS

Left-sided pneumonectomy and removal of two-thirds of the liver in rats in the last third of pregnancy adversely affected the viability of the progeny: in 3 of the 15 rats with a solitary lung there were 1-3 dead fetuses 6 of the 13 fetuses of one rat after resection of the liver were dead, and another rat had only traces of an absorbed fetus.

Compensatory hypertrophy of the lung and kidney and regeneration of the liver in the pregnant rats were apparent in the early stages after the operation as an increase in weight and also as an increase in the level of proliferation in the residual organ.

The effect of maternal pneumonectomy on the state of the fetal lung was most clearly revealed on the 5th day after the operation, when compensatory hypertrophy of the lung in the mother was well marked and the mitotic index of the cells was at its highest (MI = 2.75-3.00 ‰). The lungs of fetuses belonging to the pneumonectomized mothers sacrificed at this time were as a rule 20-40% larger in relative weight than the lungs of fetuses belonging to rats undergoing the mock operation ($P = 0.02$). The level of proliferation in the lungs of fetuses belonging to rats with many mitoses in their hypertrophied lungs was 3-4 times higher than in the lungs of the control fetuses (Table 1). No stimulation of cell division was found in the liver of these animals.

After removal of the right kidney on the 19th day of pregnancy (rats sacrificed 48 h after the operation) the weight of the kidneys, liver, and lungs of fetuses belonging to the experimental and control rats was almost the same. Kidneys of fetuses belonging to mothers sacrificed 7 days after unilateral nephrec-

tomy were slightly smaller in weight than in the control, although the differences were not significant ($P = 0.368$). At the same time, the weight of the liver and lungs of the fetuses in the experimental group was significantly smaller than in the control group ($P = 0.01$).

The level of proliferation in the kidneys of fetuses belonging to unilaterally nephrectomized mothers 48 h after the operation was 1.8 times higher than in the kidneys of fetuses belonging to intact rats ($P = 0.001$). Mitotic activity in the kidney of the fetuses belonging to mothers undergoing the mock operation was higher than in intact rats but lower than in the experimental group ($P = 0.08$).

Mechanical injury to the kidney has been shown [8] to stimulate division of the kidney cells. Evidently the mock operation in the present experiments must also have affected the mitotic activity of the maternal kidneys and led to proliferation of the kidneys of the fetuses belonging to these mother rats.

It thus follows that 48 h after unilateral nephrectomy, a humoral growth-stimulating factor was detected in the maternal body which stimulated proliferation in the corresponding fetal organ. The action of this factor was not strictly organ-specific because an increase in the number of mitoses was also observed in the hepatocytes in the liver of these fetuses (Table 2).

On the 7th day after nephrectomy on the mother, no activation of the fetal kidney cells to mitotic division could be observed, although the level of proliferation in the maternal kidney still remained higher than in the control.

The results of the experiments of group 3 deserve particular attention. Despite the fact that 18 h after resection of the maternal liver mitoses were virtually absent in the regenerating remnant of the organ, the mitotic index of the fetal liver of these rats was 1.7 times higher than in the control ($P = 0.026$). Clearly defined stimulation of mitotic division of the hepatocytes of the fetal liver was observed 32 h after partial hepatectomy on the mother (Table 3).

It can be concluded from these results that during compensatory hypertrophy of the lung and kidney and regeneration of the liver, substances stimulating cell proliferation in the corresponding organ appear in the bloodstream.

The action of these humoral factors in the present experiment appeared mainly at the time of maximal nucleic acid synthesis (in the liver) and, in particular, at the time of increased cell proliferation in the regenerating organ (in the lungs and kidneys). Assuming that activation of cells of the fetal lungs, kidneys, and liver was synchronized with activation of the cells of the corresponding regenerating maternal organ, there is reason to suppose that the appearance of growth-stimulating substances in the humoral medium coincided with the early period of premitotic activation of nucleic acid synthesis.

Tissue humoral regulation of growth is most easily detected after extensive resection of the liver [5, 10] and less easily during regenerative growth of the kidney [2, 11]. This is evidently due to the morphological and physiological features distinguishing the fetal kidneys, in which rapid morphogenetic processes take place on the last days of development. On the 21st-22nd day of fetal development the mitotic index in the epithelium of the developing convoluted tubules of the kidneys averaged 8-10 ‰, compared with 1.4-1.6% in the alveolar epithelium of the lungs and 0.30 ‰ in the hepatocytes of the liver. It can accordingly be postulated that the ability of most cells of the rapidly growing and already functioning fetal kidneys to respond to an additional stimulus by proliferation is limited. This factor, in particular, can explain the negative results obtained by Goss [9] and by Screb et al. [12].

There is thus every reason to suppose that tissue humoral regulation of growth is a universal mechanism at the initial stages of regeneration of injured organs.

LITERATURE CITED

1. A. G. Babaeva, Regulation of Compensatory and Regeneration Hypertrophy. Author's abstract of doctoral dissertation, Moscow (1970).
2. I. A. Zhikhareva, Abstracts of Proceedings of a Conference of Junior Research Workers at the Institute of Experimental Biology, Academy of Medical Sciences of the USSR [in Russian], Moscow (1968).
3. A. I. Murasheva, Experimental Study of Humoral Connections between Corresponding Organs of the Rat Mother and Fetus. Author's abstract of candidate's dissertation, Moscow (1969).
4. L. K. Romanova, Physiological and Reparative Regeneration of Mammalian Lungs. Author's abstract of doctoral dissertation, Moscow (1968).

5. L. K. Romanova, in: Conditions of Regeneration of Organs in Mammals [in Russian], Moscow (1972), p. 179.
6. S. I. Telepneva, The Role of Humoral Factors in the Regulation of Growth and Regeneration of the Mammalian Liver. Author's abstract of candidate's dissertation, Moscow (1968).
7. G. D. Tumanishvili, Some Problems in the Regulation of Growth of Living Tissues [in Russian], Tbilisi (1965).
8. T. S. Argiris and M. E. Trimble, Anat. Rec., 50, No. 1, 1 (1964).
9. R. S. Goss, Nature, 198, 1108 (1963).
10. B. Fisher, P. Szuch, M. Levine, and E. R. Fisher, Science, 171, 575 (1971).
11. H. D. Rollason, in: Compensatory Renal Hypertrophy, New York (1969), p. 61.
12. N. Screb, L. J. Hobman, and G. Lukovi, Experientia, 21, No. 7, 412 (1965).
13. P. Weiss, Science, 115, 177 (1952).