ROLE OF RIBONUCLEIC ACIDS IN REGULATION OF GROWTH OF TISSUES AND ORGANS

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Injection of RNA from cock liver into chick embryos stimulates growth of their liver: its weight and its mitotic activity increase. Messenger RNA (mRNA) has the greatest effect. Treatment of RNA with ribonuclease abolishes the observed effect. The action of RNA is seen only during the first 24 h after injection. Transfer RNA (tRNA) has no growth-stimulating effect.

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The impression has now been formed that all tissues of the body contain two types of substances capable of slowing or quickening growth processes [9]. Many investigators have shown that injection of extracts or homogenates of tissues or pieces of organs into the chorioallantois of chick embryos stimulates primarily the homonymous organs [2, 5, 6, 9]. However, in order to understand the mechanism of their action, it is necessary to know the chemical nature of substances participating in the growth-stimulating effect.

The writer has previously shown that injection of fractions of homologous RNAs into chick embryos causes organ-specific stimulation of growth [1].

The object of the present investigation was to study the effect of injected RNA fractions on the mitotic activity of parenchymatous cells of the embryonic liver, and also to examine the effect of treatment of the RNA preparations with various enzymes of their growth-stimulating activity.

EXPERIMENTAL METHOD

Experiments were carried out on 570 hen embryos of the Russian White breed, at the 12th day of incubation. Isolation of the RNA fractions, corresponding to messenger RNA (mRNA), ribosomal RNA (rRNA), and ribosomal RNA from the chromosome-nucleoulus system (RNA-CN) from the liver of cocks was carried out by the method of thermal phenolic fractionation [3], while transfer RNA (tRNA) was isolated by Titova's method [5]. Characteristics of the preparations are given in an earlier paper [1]. The RNA preparations were treated with ribonuclease, pronase, and trypsin by the known methods [4, 7, 9]. In the experiments to determine the mitotic index (MI), embryos were injected with RNA, and 18 h later the organs were weighed and transferred into Bouin's solution. Sections were cut to a thickness of 7 μ and stained with hematoxylin-eosin, and MI was determined in 15,000 cells.

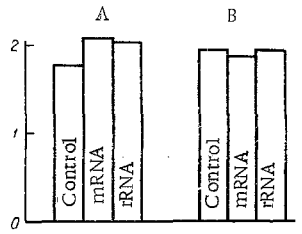

Fig. 1. Effect of injection of RNA fractions from 12th-day embryos on relative weight of liver: A) 13-day embryos; B) 14-day embryos.

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TABLE 1. Effects of Injection of RNA on Mitotic Activity and Relative Weight of Liver of Hen Embryos (injection on 12th day, results read on 13th day)

Control (0.85% NaCl solution)			mRNA			rRNA			rRNA-CN		
MI (in ‰)	relative weight	no. of nuclei in field of vision	MI (in ‰)	relative weight	no. of nuclei in field of vision	MI (in ‰)	relative weight	no. of nuclei in field of vision	MI (in ‰)	relative weight	no. of nuclei in field of vision
1,36±0,15	2,09±0,06	23,5±0,5	3,54±0,29	2,41±0,06	18,2±0,2	2,73±0,06	2,33±0,07	21,6±1,0	2,81±0,2	2,1±0,07	22,6±1,1
Percent excess over control			160	15,3	−22,55	102	11,5	−8,08	107	—	−3,8
P			0,0001	0,001	0,0001	0,0001	0,04	0,1	0,0001	—	0,5

Note. Preparations of all types of RNA were injected in a dose of 5 μg per embryo.

TABLE 2. Effect of Treatment of RNA with Various Enzymes on Its Growth-Stimulating Activity (preparations injected on 12th day, weighing on 13th day)

Preparation not treated with enzyme	Dose of RNA (in μg)	n	Relative weight of liver · 100	Δ%	*P*	Preparation treated with enzyme	Dose of RNA (in μg)	n	Relative weight of liver · 100	Δ%	*P*
Control	—	16	2,02±0,04	—	—	Control	—	13	1,97±0,07	—	—
mRNA	7	16	2,25±0,09	11,4	0,02	mRNA + trypsin	7	15	2,22±0,07	12,7	0,015
rRNA	7	15	2,19±0,06	8,4	0,03	rRNA + trypsin	7	14	2,21±0,06	12,2	0,020
Control	—	10	1,88±0,07	—	—	Control	—	10	1,80±0,09	—	—
mRNA	7	9	2,21±0,12	17,5	0,03	mRNA + pronase	7	9	2,10±0,05	16,6	0,012
rRNA	7	13	2,11±0,05	12,2	0,01	rRNA + pronase	7	11	2,11±0,05	17,2	0,005
Control	—	15	1,95±0,07	—	—	Control	—	11	1,98±0,13	—	—
mRNA	7	8	2,21±0,08	13,3	0,025	mRNA + ribonuclease	7	9	2,01±0,11	1	—
rRNA	7	13	2,25±0,09	15,4	0,03	rRNA + ribonuclease	7	11	1,94±0,07	−2	—

EXPERIMENTAL RESULTS

Mitotic activity in the embryonic liver after injection of RNA was much higher than in the control embryos (Table 1). The effect of mRNA was also accompanied by hypertrophy of the parenchymatous cells of the liver, as judged from the decrease in concentration of nuclei in the field of vision. The effect of rRNA was also manifested as an increase in MI, but in the period of the investigation this was less marked than the effect of mRNA and it was not accompanied by any significant decrease in the concentration of nuclei, i.e., it did not cause hypertrophy of the cells. The value of MI in the experiment with mRNA was higher than that of MI obtained in the experiments with rRNA with a level of significance of 0.05 calculated by Student's method, and 0.08 calculated by the median test, while the values of concentration of nuclei differed with a level of significance of 0.001.

Preliminary treatment of the RNA preparations with ribonuclease completely abolished the stimulant effect of RNA (Table 2), thus demonstrating that the observed effect is due to the action of RNA and also that small fragments of the nucleic acid molecule or individual nucleotides do not participate in growth stimulation. It also follows from Table 2 that RNA preparations, treated with pronase and trypsin, did not lose their activity, so that cytoplasmic and nuclear proteins present as impurities likewise do not participate in the studied effect.

Injection of the total tRNA fraction into hen embryos did not stimulate the increase in weight of the embryonic liver but, on the contrary, caused a decrease in both the absolute and relative weight of the liver when given in doses of between 7 and 180 μg (with a dose of 90 μg for instance, the relative weight of the liver fell by 26.6%; $P = 0.001$).

The results obtained demonstrated that exogenous macromolecular RNAs have a growth-stimulating effect. The action of all RNAs is short in duration (Fig. 1), for on the 2nd day after injection of RNA the weight of the liver returned to normal. It can thus be concluded that injection of homologous macromolecular RNAs into embryos stimulates their action in the organism as components of systems regulating the growth of organs.

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