EFFECT OF ADMINISTRATION OF HOMOLOGOUS RNA ON CONTENT RENEWAL OF NUCLEIC ACIDS IN THE LIVER OF CHICK EMBRYOS

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Injection of RNAs isolated from chicken liver into chick embryos increases the weight of the liver and stimulates incorporation of P^{32} into the RNA of the embryonic liver but has no appreciable effect on the nucleic acid content in the liver.

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Present theories concerning the mechanisms controlling growth of tissues and organs in embryogenesis are largely based on experiments in which the effect of homogenates, organ extracts, and relatively unpurified fractions of these extracts on embryonic growth was investigated [11, 12, 19]. It is necessary at this stage to obtain concrete evidence of the chemical nature of the factors determining the effect of extracts [5] on growth of organs.

The object of this investigation was to study the effect of messenger RNA (mRNA) and ribosomal RNA (rRNA) from chicken liver on growth of the embryonic liver, on its nucleic acid content, and on incorporation of P^{32} into these nucleic acids.

EXPERIMENTAL METHOD

Experiments were carried out on 1560 chick embryos of the "Russian White" breed, at the 8th and 12th days of incubation. RNA fractions corresponding to mRNA, rRNA, and rRNA-CN (ribosomal RNA of the chromosomes and nucleoli) were isolated from chicken livers by Geogiev's method [2], and their characteristics are described elsewhere [1]. The RNA preparations were injected into the embryos on the chorioallantois. The rate of growth of the liver was estimated from changes in the relative weight of the organ, which is correlated [10] with changes in mitotic activity in the liver. The content of nucleic acids in the embryonic liver was investigated 3, 6, and 12 h after injection of RNA as described in [18] and by subsequent spectrophotometry [9]. In experiments in which the effect of RNA on incorporation of P^{32} into the nucleic acids was studied, 50-100 μCi P^{32} as $Na_2HP^{32}O_4$ was injected through the same hole in the shell 1 h after injection of the RNA fraction [13].

Incorporation of P^{32} into the nucleic acids and the specific activity of the acid-soluble phosphate were determined by the usual methods [8, 16]. Statistical treatment of the results was carried out by the usual methods of variants, dispersion, and regression analysis [6].

EXPERIMENTAL RESULTS

The weight of the liver was increased 24 h after injection of the tested RNA fractions into 12-day chick embryos. The fraction corresponding to mRNA caused organ-specific stimulation of liver growth starting with a dose of 1.3 μg per embryo, while rRNA had a stimulant action only from a dose of 5-7 μg (Fig. 1). Injection of large doses of RNA was accompanied by nonspecific stimulation of an increase in weight of the embryos against the background of which the increase in weight of the liver was not significant. The observed effect cannot be explained by accumulation of water in the tissues, because it was not accompanied by a decrease in the content of dry residue in the liver.

The stimulant effect of the RNA fractions studied was also observed when they were injected on the 8th day of incubation.

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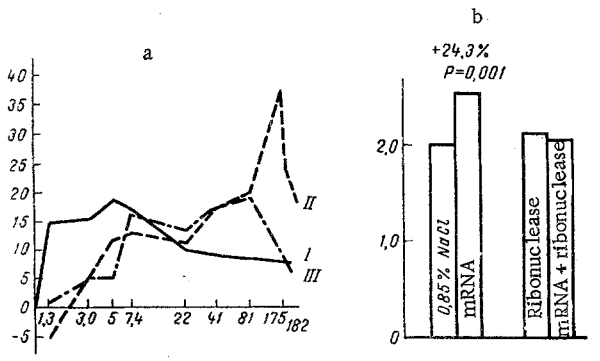

Fig. 1. Effect of injection of RNA fractions (in a dose of 7 μg) on relative weight of liver. a) Stimulant action of RNA as a function of dose. I) mRNA; II) rRNA; III) rRNA-CN. Abscissa: dose (in μg); ordinate: excess of experimental over control value (in %); b) effect of treatment with ribonuclease on stimulant activity of RNA. Relative weight of liver multiplied by 100 plotted along ordinate.

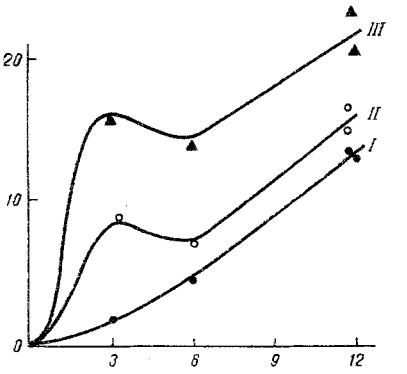

Fig. 2. Effect of injection of RNA fractions on incorporation of P^{32} into liver RNA of chick embryos. I) Control (injection of 0.85% NaCl solution; RSA values in control without injection were identical); II) rRNA; III) mRNA. Abscissa: time (in h); ordinate: relative specific activity of RNA phosphorus (in %).

The increase in weight of the liver caused by injection of RNA into 12-day embryos was transient. It was no longer found at the 18th day, distinguishing this effect from a reaction of the graft versus host type. The observed effect of RNA is analogous to the action of extracts on liver growth observed by other workers [19]. Treatment of the RNA preparations with ribonuclease abolished their action (Fig. 1). Injection of commercial RNA (Sigma), of RNA isolated from rat liver and rabbit brain, and also of bacterial RNA produced no stimulant effect.

Injection of RNA preparations isolated from chicken liver and producing an increase in weight in the embryos had no appreciable effect on the absolute content of nucleic acids in the liver tissue. The results indicate a difference between the process investigated and the increase in weight of the liver during regeneration hypertrophy [15] or under the action of DNP in the postnatal period [3], when an increase in the content of both RNA and DNA is observed in the liver. The stability of the nucleic acid content in the liver observed when growth of the liver is stimulated by injection of RNA may be associated with the higher rates of growth of the embryonic liver, so that the increase in RNA is compensated by an increase in weight, or with the fact that stimulation of growth of the embryonic liver is accompanied by the stimulation of differentiation to an extent marked by a gradual decrease in the content of nucleic acids in the liver of embryos between the 8th and 12th days of development [17]. The nucleic acid content is not therefore so reliable a criterion of changes in the rate of growth in the embryonic liver as in the postnatal period. Meanwhile, the increase in the velocity of nucleic acid synthesis in the embryonic liver would be adequate evidence of the stimulation of active growth of the organ because, despite a number of objections [7], many investigators [8] consider that the incorporation of labeled precursors into nucleic acids to some extent reflects the velocity of synthesis of these acids.

The results of investigation of the effect of the administered RNA on renewal of RNA in the embryonic liver are shown in Fig. 2. The relative specific activity (RSA) of the liver RNA was increase by the action of mRNA by 8.9 times after 3 h, by 3.1 times after 6 h ($P = 0.001$), and by 1.4 times after 12 h ($P = 0.04$); rRNA caused a smaller increase in RSA: by 4.6 ($P = 0.001$), 1.5, and 1.2 times (tendency toward increase only), respectively. The effect of both rRNAs was similar, for RSA of DNA following the administration of both mRNA and rRNA to the embryos exceeded the control value in all experiments by 200-250 and 40-50% respectively, although the radioactivity of the DNA phosphorus was low, especially 3 and 6 h after introduction of the label. Bearing in mind this fact and also the possibility of contamination of the DNA fraction by RNA, which has a higher RSA, the results indicating the effect of substances injected into the embryo on incorporation of P^{32} into DNA must be interpreted with caution. A final conclusion concerning an increase in RSA of DNA under the influence of the substances injected can therefore be drawn only after experiments with labeled thymidine.

It can thus be considered that injection of liver RNA fractions into embryos causes stimulation of growth of the liver, i.e., that their action is similar to that of liver extracts. This fact must be allowed for during discussion of hypotheses on growth regulators based on experiments with extracts. The increase in RNA renewal in the embryonic liver tissue under the influence of injected RNAs may have several explanations. It is possible that the injected RNAs, when they penetrate into the cell, stimulate protein synthesis, which leads to a secondary acceleration of nucleic acid synthesis. However, attempts by immunologic methods in vivo to detect a protein whose synthesis might be induced by injection of heterologous RNAs into the embryos were unsuccessful, although this has been demonstrated in experiments in vitro [4, 14]. A possible explanation could be that the RNAs injected, or the products of their mild breakdown, cause the formation (or liberation) of an unknown factor responsible for growth stimulation in the tissues.

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