ROLE OF GLYCOPROTEINS IN REGULATION OF ORGAN GROWTH IN EMBRYOGENESIS

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Glycoproteins isolated from the cock liver and spleen by the phenolic method, when injected into chick embryos, stimulate growth of the corresponding organs and lymphocytopoiesis in them. Relative organ specificity of action of cock liver and spleen glycoproteins has been established. Species specificity of action of the studied glycoproteins is observed.

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When studying systems regulating growth of embryonic tissues and organs, many investigators have discovered effects of tissue transplants, homogenates, and extracts on growth processes [6, 7, 8, 11]. Before an up-to-date theory of the regulation of growth processes can be formulated, the nature of substances responsible for these specific effects must be determined.

For various reasons [9] it is considered that stimulation of growth of the liver and spleen observed in the late stages of embryonic development is due to glycoproteins, which can be isolated from the tissues and organs of animals by Westphal's method [12]. The object of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on 3000 chick embryos of the Russian White breed. Preparations of glycoproteins were injected into the yolk sac or area vasculosa of embryos at the 8th-13th day of development. The embryos and their organs were weighed 1-7 days later. The effect of glycoproteins on growth of the embryonic organs was estimated from changes in the absolute and relative weight of the liver, heart, and spleen. Morphological investigation of the organs by the karometric method was also carried out. Intact embryos injected with physiological saline (0.85% NaCl solution) acted as controls. The glycoprotein fraction was isolated from the liver, spleen, and stomach of cocks by the method described above, reprecipitated from ethanol several times, and in some experiments purified by ultracentrifugation [12] and by treatment with ribonuclease [2]. The chemical composition of the liver and spleen glycoproteins is given in Table 1.

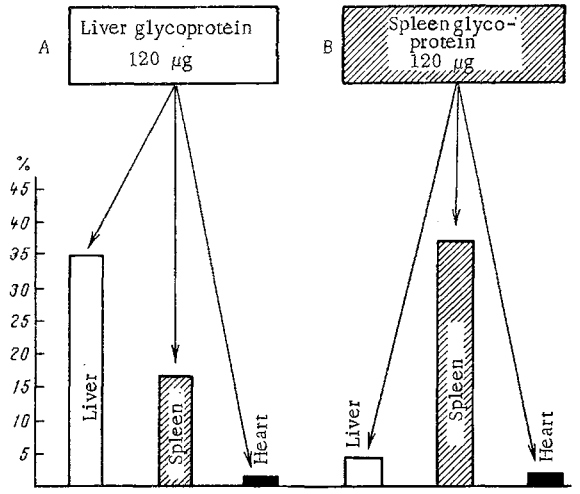

Fig. 1. Growth-stimulating action of glycoproteins (17-day chick embryos). Excess of relative weight of organs of experimental embryos over that of controls plotted along vertical axis. A, B) Relative organ specificity of glycoprotein action.

Monosaccharides (glycosamine, galactose, glucose, mannose, and fucose), and also the amino acids lysine, histidine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, valine, and phenylalanine, were identified in the liver and spleen glycoprotein preparations by chromatography on paper. Tryptophan, tyrosine, and cysteine were not found by qualitative reactions or

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TABLE 1. Chemical Composition (in %) of Maximally Purified Preparations of Cock Liver and Spleen Glycoproteins

Preparation	Carbohydrates	Polypeptide	RNA	Nitrogen	Hexosamines	Sialic acid	Sulfates	Uronic acids
Spleen glycoprotein	51.0	18.7	1.0	4.9	22.1	2.9	Absent	Absent
Liver glycoprotein	49.0	20.1	1.7	5.2	19.9	3.8	"	"

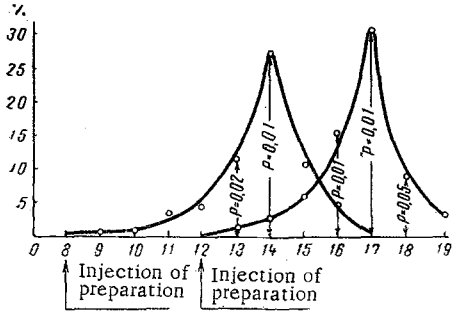

Fig. 2. Dynamics of changes in relative weight of liver of chick embryos after injection of cock liver glycoprotein. Abscissa, days of incubation; ordinate, excess of relative liver weight of experimental embryos over controls.

chromatography. The qualitative tests for lipids was negative. The numerical results were subjected to statistical analysis by Student's method and by dispersion and regression analysis [1, 5].

EXPERIMENTAL RESULTS

Injection of the glycoprotein fraction of liver and spleen into 11-12-day embryos in a dose of 120-250 μg caused an increase in absolute and relative weight of the corresponding organs on the 16th-17th day of incubation, showing relative organ specificity (Fig. 1A, B). As with the action of transplants and homogenates, the effect of stimulation of organ growth produced by glycoproteins appeared after a latent period of 3-5 days and reached a maximum by the 14th or 17th day of development, after which it diminished (Fig. 2). The existence of a latent period and a maximum of stimulation on the 14th or 17th day of development distinguished the action of glycoproteins from that of cock liver RNA [4] and plasma albumin [3], which stimulate organ growth after 24 h and at earlier stages of embryonic development.

The glycoprotein preparations contained RNA as an impurity (Table 1), but treatment with ribonuclease [2] did not remove their growth-stimulating activity, as demonstrated by coincidence of the regression lines of liver weight of embryos after injection of liver glycoprotein, both treated with ribonuclease and untreated (Fig. 3), and the result of dispersion analysis (Table 2). Treatment of the glycoprotein preparations with trypsin [2] likewise did not remove their growth-stimulating activity, i.e., the peptide bond in carboxyl residues of the diamino acids does not particpate in the growth-stimulating effect.

After injection of liver glycoprotein, islets consisting of cells of the reticulo-endothelial system (RES) were formed in the liver parenchyma along the course of the blood vessels. These cells were closely related to the adventitia of the blood vessels and isolated them from the liver parenchyma. The ring formed by the RES cells was usually irregular in shape and its area was independent of the diameter of the vessel. Small, round islets of RES cells, unconnected with blood vessels, were also seen in the liver. In the control experiments, no islets of RES cells developed in the liver parenchyma of 17-day embryos.

Injection of spleen glycoprotein into 12-day embryos accelerated the development and formation of the splenic pulp: in 16- and 17-day embryos the number of developing and differentiating follicles was greater than in the control embryos. Lymphocytes were present in the stroma of the developed splenic follicles. The identical character of variance curves of the dimensions of the nuclei of lymphocytes formed in the liver and spleen after injection of liver glycoprotein was demonstrated karyometrically. The curves were unimodal. The mean diameter of the lymphocyte nuclei in the liver was 3.4±0.06 μ and in the spleen 3.29 ± 0.07 μ (P = 0.2).

Stomach glycoprotein, when injected into chick embryos in doses of between 1 and 7 μg (in doses of between 20 and 500 μg it gave a definite toxic effect) produced changes neither in the weight of the embryos nor in the absolute or relative weight of their organs.

Injection of glycoproteins from rabbit liver, human liver and stomach, mouse spleen and liver, and monkey stomach into 11- and 12-day chick embryos in doses of between 30 and 2000 μg, and injection of pyrogenal (bacterial lipopolysaccharide) in doses of between 5 and 100 μg caused no changes in the weight

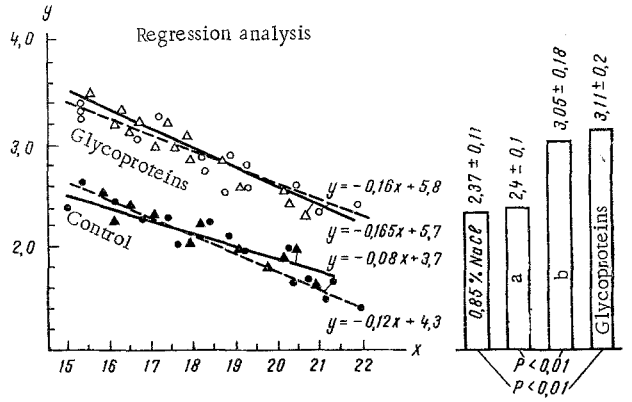

Fig. 3. Effect of liver glycoprotein, treated with ribonuclease, on relative weight of liver of 17-day chick embryos. Abscissa, weight of embryos (in g); ordinate, relative weight of liver ($\times 10^2$). Δ Glycoprotein untreated with ribonuclease; O– – – – glycoprotein treated with ribonuclease; Δ 0.85% NaCl; ●– – – – – 0.85% NaCl + ribonuclease. a) 0.85% NaCl + RNAase; b) Glycoprotein + RNAase.

TABLE 2. Dispersion Analysis of Action of Liver Glycoprotein before and after Ribonuclease Treatment

Preparation	Degree of effect η^2	Significance of effect F
Glycoprotein before treatment	0.45	57.0 ≡
Glycoprotein treated with ribonuclease	0.46	63.3 ≡

Note. Limiting values for $P \leq 0.001$, $F \geq 14$.

of the liver, heart, and spleen of embryos between the 16th and 18th day of development. Disaccharide (sucrose) and monosaccharide (glucose, galactose, glycosamine), as was to be expected, in analogous doses had no effect on the weight of the embryonic organs.

It can be concluded from the results described above that glycoprotein fractions of the liver and spleen, which have been called "tissue mucopolysaccharides" [10], if injected into chick embryos stimulate growth of the corresponding organs. The action of these glycoproteins shows relative organ-specificity. Growth stimulation is to some extent linked with stimulation of lymphocytopoiesis. However, it is difficult to imagine that increased lymphocytopoiesis lies at the basis of the relative organ-specificity of the effect, and for this reason the effect of glycoproteins on growth of the liver parenchyma is being investigated.

Species-specificity of action of the investigated glycoproteins is noteworthy.

The results of these experiments suggest that the tissue glycoproteins are one of the links in the general system regulating growth of organs during embryogenesis.

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