The Effects of Follicle-Stimulating Hormone on the Uptake of Labelled Glycine by the Chick Embryo

It appears from our recent studies that follicle-stimulating hormone (FSH) has definite morphogenetic properties. Experiments using the chick embryonic system have shown that the hormone is capable of inducing differentiation of neural tissue, notochord-like tissue and somites[1]. In this paper the effects of FSH on the incorporation of glycine-1-C^{14} by chick embryos have been examined.

Material and methods. The experiments were performed in 2 series. In 1, glycine-1-C^{14} (5 μc in 0.2 ml Pannett-Compton saline/egg) was injected into the albumen of unincubated eggs as close as possible to the embryonic disc. In the corresponding experimental embryos in addition to glycine 1 mg of FSH (NIH-FSH-S2) per embryo was injected. The eggs were left in the same position overnight and then incubated for 48 h at 37 °C and opened in saline. The embryos were washed thoroughly and fixed in Bouin's fluid. In the second series of experiments eggs were incubated for about 18 h to obtain primitive-streak embryos which were explanted in vitro according to NEW's method[2]. On the embryos 0.2 ml Pannett-Compton saline containing 2 μc glycine-1-C^{14} was added. Again in the corresponding experimental embryos of the series along with glycine 0.5 mg FSH was added. The embryos of this series were incubated for 24 h at the end of which they were washed thoroughly and fixed in Bouin's fluid. The embryos of both series were sectioned at 6 μ, coated with Ilford K-5 nuclear research emulsion and exposed for 4 days, developed in Kodak D19b and fixed with Amfix (M & B) high speed fixer. The autoradiographs were lightly stained with hematoxylin after developing.

Results and discussion. The aim of the present studies has been to compare the incorporation of labelled glycine by the neural tissue, notochord and the somitic tissue and to see if FSH changed the pattern of distribution of the label between these tissues.

The embryos differed considerably in their uptake of labelled glycine irrespective of the group (control or experimental) or series to which they belonged. Further, the label had been supplied in 2 different ways which bring either the epiblast or hypoblast closer to the C^{14}-source. Therefore, grain counts corresponding to the neural tissue, notochord and somites were made from 3 embryos of the first series and 3 of the second series belonging to the control group. In all the 6 embryos the incorporation was the highest in the neural tissue, followed by the notochord and the somites. A statistical analysis of the distribution of the label between the neural tissue, notochord and somites showed that the pattern of distribution was very consistent ($P > 0.3$).

From the experimental group (C^{14}-glycine + FSH) grain counts were made from 8 embryos, 4 embryos each from series I and II. Six of these embryos showed highest incorporation of the label in the notochord, followed by somites and the neural tissue. In 1 embryo the notochord incorporated more label than the somites. In another the neural tissue incorporated less glycine than the somite but more than the notochord. Since 6 embryos had been taken for analysis in the control group, only 6 were taken for analysis from the experimental group and these included the 2 embryos which showed slight variation in the pattern. The statistical analysis showed that the pattern of incorporation observed in the experimental group was not fortuitous ($P > 0.5$). The difference in the pattern of distribution of label between the control and experimental groups of embryos was also statistically significant ($P < 0.001$). It is therefore possible to say that FSH has changed the pattern of incorporation of glycine by the 3 tissues concerned and that the hormone seems to preferentially stimulate incorporation of the amino acid by the notochord and the somites. This point is further clarified by the data presented in Table II. The notochord and somites of an embryo which is not treated with FSH incorporate on an average 64.7 and 53.8% respectively of C^{14}-glycine incorporated by the neural tissue. In the presence of FSH a remarkable increase in the incorporation by notochord and the somites occurs: the said tissues incorporating 140.2 and 159.2% of the incorporation by the neural tissue.

The effects exerted by FSH are probably morphogenetic. The fact that the hormone causes preferential

Table I. Effects of follicle-stimulating hormone on the incorporation of glycine-1-C^{14} by the chick embryo

	Embryos	Mean grain counts in 130 μ^2 Neural tube	Notochord	Somites	
Glycine-1-C^{14} (control)	1	12.6 (16)	10.8 (16)	7.8 (16)	
	2	102.9 (6)	87.3 (6)	76.8 (6)	
	3	76.8 (8)	63.0 (8)	59.0 (8)	
	4	84.0 (6)	60.3 (6)	84.0 (8)	$P > 0.3$
	5	27.0 (9)	18.0 (9)	18.0 (9)	
	6	33.0 (8)	24.0 (8)	18.8 (8)	
					$P > 0.001$
Glycine-1-C^{14} + FSH (experimental)	1	52.2 (8)	60.0 (8)	69.0 (8)	
	2	57.0 (6)	81.0 (8)	67.5 (8)	
	3	43.8 (10)	63.9 (10)	60.0 (10)	$P > 0.5$
	4	51.0 (6)	57.0 (6)	68.0 (6)	
	5	129.0 (5)	183.0 (5)	189.0 (5)	
	6	22.1 (19)	16.8 (19)	25.5 (19)	

Figures in brackets refer to number of graticule squares counted. Average grain counts for 3 graticule square areas has been presented in the Table. Calculation of values of P was made for sets of values of grain counts before they were pooled together. The probability therefore takes into account variations occurring in the different graticule square counts. For this reason standard deviation has not been given for the averages. This statistical analysis also takes into account variations occurring due to factors such as differences in application of the label and its diffusion when injected into the albumen and also site of injection.

Table II. Incorporation of glycine-1-C^{14} by notochord and somites given as percentage of incorporation by the neural tissue

Medium	Notochord	Somites
Glycine-1-C^{14}	64.7	53.8
Glycine-1-C^{14} + FSH	140.2	159.2

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incorporation into the anterior (chordal) and posterior (somitic) mesoderm seems to support this idea. We would also like to focus attention on the fact that the axial mesoderm which plays an important role in the induction of the nervous system in the chick embryos appears to be the target tissue for FSH[3].

Résumé. On a étudié par autoradiographie l'incorporation de la glycine-1-C^{14} par l'embryon du poussin. L'incorporation était la plus élevée dans le tissu neural et puis dans le notochorde et les somites. Après traitement avec le FSH, une incorporation plus grande s'est produite dans les mésodermes chordal et somitique. On propose qu'il peut s'agir d'un effet morphogénétique. Il est particulièrement significatif que le mésoderme axial, qui joue un rôle si important dans l'induction du système nerveux, paraît être également le point d'action de l'hormone.

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A Contribution to the Study of Lens Regeneration Capacity in Chick Embryos

Several investigators[1–7] have reported lens regeneration from the retinal or pigment layers in the lenectomized eyes of chick embryos. However, not all these reports are easy to evaluate and, in fact, recognition of the possible existence of this capacity in the avian embryos by some of the recent reviewers[8–10] is not without reservations. MCKEEHAN[11] reported completely negative results and has also seriously questioned the affirmations of other authors. Although GENIS-GÁLVEZ[12] obtained a number of cases of positive lens regeneration, he was persuaded not to interpret them in this way; but he also did not deny the possible existence of this ability in the chick embryos. The question being still open, the present study was undertaken specifically to investigate if lenses would regenerate in lenectomized eyes of chick embryos.

Embryos of White Leghorn fowl of approximately 3, 4 and 6 days incubation ages were used in this study. The embryo, taken from the egg, was placed in warm saline and its extra-embryonic membranes were removed. The ectoderm was then peeled away from over the eye region and the lens removed with the help of fine forceps or glass or platinum needles. Special care was taken to remove the lens in one piece. The lenectomized eye was then isolated from the head and cleansed of the adhering mesenchyme as much as possible. It was then explanted on about 1.5 cm^3 of Spratt's saline-agar-albumen medium in a moist chamber and incubated for 48–100 h at 100 °F. The control eyes were explanted in an identical way, except that the lenses were not removed from them. All operations were performed under sterile conditions. The explants were fixed in Bouins', sectioned at 8 μ thickness and stained with hematoxylin-eosin.

The controls were kept to find out if the culture conditions were suitable to maintain explants in a healthy condition and support further differentiation to any extent. The results showed that it was so, except for the eyes donated by 6-day-old embryos in which necrosis and degeneration set in after 2 days of cultivation in vitro. This was also true for the lenectomized eyes. The explants frequently became flattened and neural retina developed evaginations into the vitreous chamber. Flattening and retinal evaginations were much more pronounced in the experimental eyes than in controls.

Thirty-one out of 42 lenectomized eyes from approximately 3-day-old embryos survived cultivation for 72 h. In several cases one or both the iris borders, as seen in the sections, were thickened and bent inwards into the cavity of the eye-cup. These swollen regions were often devoid of pigment and possessed columnar cells with elongated nuclei. Definite lens formation was found, however, only in 6 cases, in 3 of which the connection of these bodies with the pigment layer was quite certainly present. These 3 cases showed unmistakable evidence of lens regeneration from the iris margin. They, in fact, represented 3 different stages in lens regeneration.

In case No. E72-14, shown in Figure 1, the lens-like structure is still nothing more than the rather thickened edge of the iris in which the pigment has disappeared and some lens fibres have been secreted in the middle. It is a small, solid structure, slightly inclined towards the interior of the eye. Its cellular continuity with the pigment layer is quite obvious. Case No. E72-9 (Figure 2) shows a more advanced stage in which the regenerating lens has assumed a distinctly globular shape with a core of fibre secreting cells. This also is a solid body whose cellular continuity with the iris border of the pigment layer is very clear. Case No. E72-11 (Figure 3) shows a still more advanced stage in the regenerative development of the

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