pregnadiene-12,20-dienone was postulated as one which fulfils all the requirements. The proposed structure can also explain the unusually deshielded chemical shift of the methine protone at C-17. Treatment of I with dilute alkali gave an intensely yellow colored solution, from which, after acetylation, II and an isomeric acetate, III, were isolated. III has the same UV-absorption and mass spectrum as II and was considered to be the isomer at C-17 or less possibly at C-9[5].

Although the unequivocal elucidation of the structure has to wait for correlational experiments which are now under way, the authors believe that fukujusonorone is the first example of a genuine 18-norsteroid in nature[6]. While many 19-norsteroids are known, an 18-norsteroid has not been found. As for the biogenesis of I, the authors present the following sequence which involves β-ketoacid decarboxylation (or similar reaction) followed by β-elimination. Co-existence of adonilide (IV) and some 12,14-oxygenated pregnanes in the same plant may support this hypothesis.

Zusammenfassung. Aus den Wurzeln des japanischen Adonisröschens (*Adonis amurensis* Regel et Radd) wurde Fukujusonoron, ein neues Aglykon, das sich von 18-nor-Pregnan ableitet, isoliert.

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[5] Failure of transformation to the conjugated ene-dione system shows the deconprigated system is more thermodynamically favoured.

[6] A few 'pseudo'-18-norsteroids, which have the 18-methyl group migrated to C-17, have been reported. J. TOMKO, A. VASSOVA, G. ADAM, K. SCHREIBER and E. HÖHNE, Tetrahedron Letters *1967*, 3907.

Morphogenetic Effects of Follicle Stimulating Hormone: Effects on the Synthesis of Nuclear RNA's by Chick Embryos

The processes of development, growth and maintenance of an organism are directed, in some phase or other, by hormones. Hormones are involved, for instance, in the processes of differentiation of germ cells[1] and mammary tissue[2,3], growth and moulting of insects[4,5], and metamorphosis in amphibia[6].

Follicle-stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. It is responsible for the growth and maturation of ovarian follicles. The possibility that it might get localized in the developing ovum and also play a role in organizing embryogenesis was suggested several years ago[7]. It was later shown to be able to induce differentiation of neural, notochordal, and somitic tissues in chick embryonic systems[8–10]. Cellular differentiation is a phenotypic expression of differential genetic activity. The morphogenetic property of the hormone probably reflects its ability to control genetic activity. If it were so it might produce specific effects on RNA synthesis by developing embryos.

Material and methods. Chick embryos at stage 4 (medium primitive-streak), stage 5 (head-process), stage 7 (neural-fold embryos with 1–2 pairs of somites), and stage 9 (embryos with 8–9 pairs of somites) were given H^3-uridine (25 μc/ml) in 15 min, 1 and 3 h pulses. Some embryos of each stage were treated with 0.5 mg/ml FSH (NIH-FSH-S4) for 1 h before the precursor was supplied. All of them were sectioned and autoradiographed after extraction with 5% TCA at 4 °C. The experiments were controlled using RNase. Nuclear grain counts were made in epiblast, mesoderm or their derivative tissues, viz. the neural tissue, notochord and somites respectively. The data are presented in Tables I and II. H^3-butyl-methacrylate (50 μc/g from Radiochemical Centre, Amersham) sections were used as standards and autoradiographic efficiency (ARE) was determined for each batch of autoradiographs[11].

Results and discussion. The object of these experiments was to see if the hormone produced any differential effect on the synthesis of nuclear RNA's (nRNA) by the different embryonic tissues and if they did, whether these effects could be correlated with morphogenetic events occurring at the time of treatment with the hormone. Differential effects were indeed noticed, and these were also found to be stage-specific.

Mesoderm cells appeared to have been stimulated by the hormone (see Table I) beginning with stage 5. The synthesis of nRNA by epiblast and mesoderm cells of stage 4 and epiblast cells of stage 5 was found to be inhibited by the hormone. But mesodermal cells of stage 5 embryos did not show such an inhibition. This may be related to the fact that this is roughly the stage of development when mesoderm enters into various inductive interactions. This is also the stage which is highly susceptible to anti-FSH action[9]. We do not know how an inhibition of nRNA synthesis in stage 4 cells might have

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Table I. Incorporation of H^3-uridine into nRNA by normal and FSH-treated chick embryonic tissues

Duration of pulse	Stage of development	Type	H^3-uridine in nRNA. Grain counts/45 μ^2 at 100 ARE		
			Epiblast	Mesoderm	
15 min	4	C	220.8 ± 8.0	187.2 ± 8.0	
		E	147.2 ± 6.2	113.6 ± 3.7	
	5	C	99.2 ± 8.6	68.4 ± 5.4	
		E	57.6 ± 2.7	62.4 ± 3.2	
			Neural tissue	Notochord	Somites
	7	C	86.4 ± 7.2	40.8 ± 1.8	64.0 ± 3.5
		E	76.8 ± 7.7	62.9 ± 9.9	70.4 ± 5.3
	9	C	40.0 ± 7.2	54.4 ± 9.6	72.0 ± 9.6
		E	51.2 ± 6.6	41.6 ± 1.8	78.4 ± 7.8
			Epiblast	Mesoderm	
3 h	4	C	242.0 ± 13.0	185.0 ± 9.2	
		E	133.0 ± 9.3	102.0 ± 7.5	
	5	C	108.0 ± 6.0	72.0 ± 3.0	
		E	68.0 ± 4.0	74.0 ± 3.0	
			Neural tissue	Notochord	Somites
	7	C	215.0 ± 13.1	117.0 ± 6.8	171.0 ± 8.5
		E	186.0 ± 13.8	147.0 ± 7.7	150.0 ± 9.0
	9	C	117.0 ± 6.0	89.0 ± 4.0	111.0 ± 5.5
		E	200.0 ± 9.3	155.0 ± 5.4	178.0 ± 7.3

C, Untreated embryos; E, FSH-treated embryos; ARE, Autoradiographic efficiency[11].

In stage 7 embryos, too, mesoderm cells (notochord) were specifically affected by the hormone treatment. It was noticed that the magnitude of increase in nRNA synthesis was of similar order in both 15 min and 3 h pulse labelling. This probably indicates that the species of RNA stimulated by the hormone in the notochordal cells of stage 7 embryos might have a high turn-over. It may also be noted here that an overt differentiation of the notochord occurs at this stage of development. It is therefore possible that there might be some connection between the stimulation of this particular RNA species and the differentiation of the notochord.

The synthesis of nRNA's in a 15 min pulse was not affected in stage 9 embryonic tissues. But in 1 h and 3 h pulse experiments a generalized increase of synthesis was noticed in all the tissues (see Table II for a summary of FSH effects). A differential effect of the hormone could again be noticed in the pattern of increase in nRNA synthesis that resulted from increasing the duration of pulse from 15 min to 3 h. The mesodermal derivative tissues of stage 9 embryos were found to be influenced by the hormone more than the neural tissue. The notochord cells were found to be affected the most. Presumably the nRNA's stimulated in stage 9 embryos in 3 h pulse labelling belong to a different species than those stimulated by the hormone in the 15 min pulse experiments[14].

Résumé. L'hormone de maturation folliculaire (FSH), qui possède des propriétés morphogénétiques nettes, produit des effets différentiels sur la synthèse des ARN nucléaires chez des embryons de poule dans les différentes étapes de leur développement. Les expériences indiquent que lors du traitement par l'hormone, celle-ci peut stimuler la synthèse de différentes classes d'ARN, selon l'étape du développement.

Table II. Summary of the effects of FSH on nuclear RNA synthesis by different cell types

Duration of pulse	Stage 4		Stage 5		Stage 7			Stage 9		
	Epiblast	Mesoderm	Epiblast	Mesoderm	Neural tissue	Notochord	Somites	Neural tissue	Notochord	Somites
0.25 h	−	−	−	±	±	+	±	±	±	±
1.0 h					±	+	±	+	+	±
3.0 h	−	−	−	±	±	+	±	+	+	+

−, statistically significant reduction in nuclear RNA; ±, no significant change; +, statistically significant increase. $P < 0.05$. (Statistical analysis by student distribution.)

been produced. It has been reported that gonadotropins can either inhibit or stimulate RNA synthesis depending upon the time lag between treatment with the hormone and the supply of the precursor to the cells[12]. But one would need to say more than this when the inhibition shows an obvious correlation with morphogenetic events.

The extent of contribution by embryonic genes to the complement of functional messengers of pregastrulation embryos is not precisely known. But CRIPPA and GROSS[13] have shown that in early developmental stages of *Xenopus laevis* maternal DNA-like RNAs are being utilized and used up by the time gastrulation commences. By this time embryonic messengers represent about 44% of the sequences translated. No data are available as regards these relative contributions in the chicken embryos. When such data become available they might have a bearing on the differences observed in the effects of the hormone on medium primitive-streak and head-process embryos.

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