Table II. Changes in morphometric parameters of hypophysectomized rat adrenocortical cells, induced by corticosterone administration

Treatment of rats		Control	2 mg/kg × 4 days	4 mg/kg × 4 days
Volume of cells	μ^3	1603.4 ± 101.3	1353.7 ± 86.3	1195.2 ± 75.9
	-Δ		15.6	25.6
	P		<0.05	<0.05
Volume of nuclei	μ^3	136.9 ± 7.1	120.9 ± 6.4	115.8 ± 4.8
	-Δ		11.7	15.5
	P		<0.05	<0.05
Volume of mitochondria	μ^3	418.6 ± 30.5	397.4 ± 30.0	370.2 ± 24.6
	-Δ		5.1	11.7
	P		no significant	no significant
Surface of mitochondrial cristae	μ^2	2093.0 ± 155.5	2026.7 ± 153.6	1925.1 ± 127.9
	-Δ		3.2	8.1
	P		no significant	no significant
Membrane space	μ^3	877.0 ± 54.1	720.0 ± 44.1	619.1 ± 32.9
	-Δ		17.9	29.5
	P		<0.05	<0.05
Surface of ser	μ^2	9001.5 ± 601.2	7344.1 ± 497.6	6252.9 ± 351.3
	-Δ		18.4	30.6
	P		<0.05	<0.05
Volume of lipids	μ^3	156.0 ± 10.6	104.2 ± 7.0	85.4 ± 5.9
	-Δ		33.2	45.3
	P		<0.05	<0.01

is the effect of the physiological katabolism of the cellular membranes in presence of a deficit in the synthesis of new membranes.

The volume of mitochondrial fraction and the surface of mitochondrial cristae are found to be only slightly decreased after corticosterone administration (Table II). The decrement is not significant: this is consistent with our previous findings[13], and could be explained by assuming that either 1. the trophism of the mitochondrial fraction is exclusively or prevalently controlled by ACTH, or/and 2. the catabolic turnover of adrenocortical mitochondrial fraction is much slower than that of SER membranes. The first hypothesis may be sustained by considering the intrinsic differences in the regulation of the synthesis of mitochondrial and microsomal proteins[21]. Furthermore, ACTH was found to enhance the mitochondrial DNA-dependent protein synthesis[22–24]. However, some indirect evidence tends to support also the second possibility[20]. It must be pointed out that the two hypotheses do not necessarily conflict. To gain further insight into this problem, the effects of corticosteroids on adrenocortical mitochondrial protein synthesis are now being investigated in our laboratory.

In conclusion, our morphometric data, demonstrating a direct effect of paraphysiological doses of corticosterone on adrenocortical cells, lend credence to the view that, in the regulation of adrenocortical function, the peripheral component of the feed-back mechanism involved has a physiological significance[25].

Riassunto. Con metodi morfometrici è stato studiato l'effetto di dosi parafisiologiche di corticosterone sulle cellule corticosurrenaliche (zona fascicolata) di ratti ipofisectomizzati trattati con dosi di mantenimento di ACTH. I dati ottenuti indicano che tali dosi di ormone inibiscono direttamente la funzionalità delle cellule corticosurrenaliche. Questi risultati suggeriscono che la componente periferica dei meccanismi a feed-back che intervengono nella regolazione della funzione corticosurrenalica, giochi un ruolo fisiologico significativo.

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Identification and Biosynthesis of Steroid Hormones in the Gonads of *Ciona intestinalis*

Knowledge of the identification and biosynthesis of steroid hormones in vertebrates and invertebrates has been remarkably enriched in recent years. However, no information is available as regards the prothochordates.

In order to fill this gap, and to understand better the evolutionary factors which brought to light the pattern of steroid secretion in vertebrates, we have studied both the identification and biosynthesis of steroid hormones in the gonads of the urochordate, *Ciona intestinalis*. *Ciona* is a hermaphroditic animal, whose gonads consist of a separate ovary and testis. The pear-shaped ovary lies in the intestinal loop to the left of the stomach; the germinal epithelium is lined internally by a ciliated epithelium, while externally it is surrounded by vascular connective tissue. The testis is a diffuse structure composed of branching tubules spread over the intestine and the posterior part

of the stomach. The ends of the branches constitute the testicular follicles, whose cavity is filled with cells in all stages of spermatogenesis.

From 1000 sexually ripe *Ciona intestinalis*, collected in the Gulf of Naples during May-June, 185 g of testis and 95 g of ovaries were obtained. Sexual maturity was established by the presence of semen and eggs in the genital ducts. Testis weight also includes the intestinal epithelium which is composed of columnar epithelium separated from the testicular substance by loose connective tissue; therefore, the instestinal component constitutes not more than 1/5 of the testis weight. After homogenization, the tissues were extracted with organic solvents to obtain the free steroids. The conjugated steroids were extracted following acid hydrolysis with HCl N and dioxane 1:1 for 24 h at room temperature. Both free and conjugated steroids were separated into neutral and phenolic fractions by extraction with NaOH N. The residues were purified by column chromatography and thin-layer chromatography. The identification of steroids was done by UV-spectra, gas-liquid chromatography and conversion to derivatives. For corticoids the method of DIAB and GOMOLL[1] was used. The latter method is based on the characterization of corticoids on thin layer plates sprayed with 25% sulfuric acid and immediately heated in an oven at 175°C. Although the color developed by the corticoids is considered specific by the authors, it has not been possible to proceed towards further identification owing to the low amounts of steroid extracted (< 2 µg).

standard preparation on thin layer chromatogram developed with the different solvent systems; 2. chromatographic behavior identical with that of the standard preparation through chemical reactions such as reduction and acetylation; 3. constant specific activity after repeated crystallization of the radioactive metabolites with the corresponding authentic preparations.

The radioactivity was counted by a Packard spectrometer liquid scintillator Mod. 3320, using the quench correction by automatic external standardization.

In incubations with ovarian tissues, the following metabolites were identified: 17α-hydroxypregnenolone, dehydroepiandrosterone, androstenedione, testosterone, deoxycorticosterone and cortisone. In incubations with testicular tissue, dehydroepiandrosterone, testosterone and deoxycorticosterone were identified. The results are summarized in the Tables I and II.

It seems that in gonadal tissues of *Ciona intestinalis* the biosynthetic pathway proceeds via 17α-hydroxypregnenolone, dehydroepiandrosterone and androstenedione, since all of these steps are present, especially in the ovarian tissue. The formation of cortisone with a ratio $H^3/C^{14} \gg 1$ can also be explained as suggested by SANDOR et al.[2] for the adrenocortical tissue of *Anguilla anguilla*, where cortisol formed from pregnenolone is synthsized via 17α-hydroxypregnenolone, not through progesterone.

Some ovaries and testes were also sectioned with a cryostat for the histochemical demonstration of 3β-hydroxysteroid dehydrogenase, using pregnenolone and dehydroepiandrosterone as substrates. No specific reaction was observed.

Table I. Transformation of pregnenolone-7-H^3 and progesterone-4-C^{14} by ovary of *Ciona intestinalis*

Cofactor	Time of incubation (h)	No. of crystallization	Steroids (H^3/C^{14}) 17α-OH pregnenol. ac.	Dehydroepi. ac.	Androstenedione	Testosterone ac.	Deoxycorticos. ac.	Cortisone
NAD	2	1	5.2	18.8	–	–	–	–
		2	4.3	13.3				
		3	3.3	13.3				
	4	1	9.8	11.5	–	20.7	3.6	–
		2	7.0	11.3		17.5	3.0	
		3	6.5	11.3		17.0	3.0	
NADP	2	1	1.1	1.7	1.2	–	–	–
		2	1	1.2	1.1			
		3	1	1.2	1.1			
	4	1	1.7	10.4	2.3	9.8		9.9
		2	1.7	9.6	2.3	10.7		8.6
		3	1.5	9.5	2.3	10.0		8.3

In the neutral free fraction of testes, only dehydroepiandrosterone (0.2 µg/g of fresh tissue) was found, whereas in the conjugated fraction only cortisone and cortisol (presumably) were identified. In the conjugated fraction of the ovary, only cortisone (presumably) was found.

In vitro steroid biosynthesis was studied both in the ovary and testis using labelled precursors. Incubations were carried out at different times with 2 g of ovary and 3 g of testis in sterilized sea water with NAD and NADP as cofactors, using 0.75 µCi of progesterone-4-C^{14} (60 mCi/mM) and 20 µCi of pregnenolone-7-H^3 (14.7 Ci/mM) Amersham Center). The incubations were stopped with methylene chloride, extracted and purified by column and thin layer chromatography. Final identification of the metabolites obtained was based on the following criteria: 1. identical mobilities of the metabolites to the

It is of interst to note that sex hormones and corticoids can be synthesized also by sex glands of protochordates, which do not possess any structure homologous to the interrenal tissue of vertebrates. In addition, several investigators have shown the presence of sex hormones and steroid-transforming enzymes in the gonads of invertebrates (GOTTFRIED and LUSIS[3]; DROSDOWSKY et la.[4]).

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Table II. Transformation of pregnenolone-7-H^3 and progesterone-4-C^{14} by testis of *Ciona intestinalis*

Cofactor	Time of incubation (h)	No of crystallization	Steroids (H^3/C^{14})		
			Dehydroepiandrost. ac.	Testosterone ac.	Deoxycorticoster. ac.
NAD	1/2	1	7.1	–	7.1
		2	7.2		7.0
		3	7.1		7.0
	4	1	9.7	–	9.9
		2	9.9		8.5
		3	9.7		8.5
NADP	1/2	1	2.4	2.0	–
		2	1.9	2.0	
		3	1.9	2.0	
	2	1	10.5	–	7.5
		2	7.6		7.0
		3	7.6		7.0
	4	1	14	4.4	–
		2	11	3,7	
		3	11	3.7	

As far as corticosteroids are concerned, GOTTFRIED and LUSIS[3] have mentioned the presence (presumably) of cortisone in the eggs of the slug *Arion ater rufus*.

Therefore, it is possible to presume from these findings that chemical evolution of steroid hormone biosynthesis has arisen independently in different phyla of metazoa and that of protochordates has long preceded the pattern present in vertebrates. Whether these steroids possess any physiological significance in the animals other than vertebrates is yet to be investigated[5].

Riassunto. Da 185 g di tessuto testicolare di *Ciona intestinalis* sono stati estratti ed identificati i seguenti ormoni steroidi: deidroepiandrosterone, cortisone e cortisolo. Nel tessuto ovarico é presente il cortisone. Incubando il tessuto testicolare con pregnenolone-7-H^3 e progesterone-4-C^{14} sono stati isolati i seguenti metaboliti: deidroepiandrosterone, testosterone, e desossicorticosterone. Dalle incubazioni del tessuto ovarico con gli stessi precursori sono stati isolati i seguenti metaboliti: 17α-idrossipregnenolone, deidroepiandrosterone, androstenedione, testosterone, desossicorticosterone e cortisone.

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Tumorous Development of in situ and Grafted Anterior Pituitaries in Female Rats Treated with Diethylstilbesterol

Anterior pituitary tumors can be routinely induced in rats and mice by chronic treatment with estrogens[1]. In certain strains of mice, in situ pituitaries which rarely become tumorous spontaneously, do so readily when transplanted to sites distant from the hypothalamus[2]. Although transformation and growth of these transplanted pituitaries are not dependent on an exogenous supply of ovarian hormones, their growth can be markedly stimulated by the administration of these hormones[2]. By contrast, the rat pituitary, grafted to various sites distant from the hypothalamus, does not spontaneously become tumorous although in situ pituitary tumors are not uncommon to this species[3]. We have observed the homografts of well over 1000 rat pituitaries and not one has ever become a tumor; the weight of the graft rarely exceeding 1/3 that of the original graft[4,5]. KULLANDER[6] reported that pieces of rat anterior pituitary tissue became tumorous when grafted to the anterior chamber of the eye, provided ovarian grafts were grafted along side the pituitary graft. Pituitary grafts placed in the anterior chamber of the eye in the absence of ovarian grafts did not become tumorous. We believed, therefore, that the resistance of the rat pituitary graft to growth and tumorigenesis might be overcome by administration of estrogen. Thus, this communication describes the effects of chronic treatment of intact female rats bearing pituitary homografts with diethylstilbesterol (DES), and compares the growth and tumor development of these grafts with that of the in situ pituitary.

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