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.

[1] J. FURTH and K. CLIFTON, in *The Pituitary* (Eds. G. W. HARRIS and B. T. DONOVAN; University of California Press, Los Angeles 1966), vol. 2, p. 460.

[2] L. M. BOOT, G. RÖPCKE and O. MÜHLBOCK, Proc. 2nd Internat. Congr. Endocrinol., London, 1964. Excerpta Medica Int. Congr. series No. 83.

[3] R. A. HUSEBY, in *Methods in Hormone Research* (Ed. R. I. DORFMAN; Academic Press, New York 1965), vol. 4, p. 123.

[4] C. W. WELSCH, J. A. CLEMENS and J. MEITES, J. natn. Cancer Inst. *41*, 465 (1968).

[5] C. W. WELSCH, T. W. JENKINS and J. MEITES, Cancer Res. *30*, 1024 (1970).

[6] S. KULLANDER, Cancer Res. *20*, 1079 (1960).

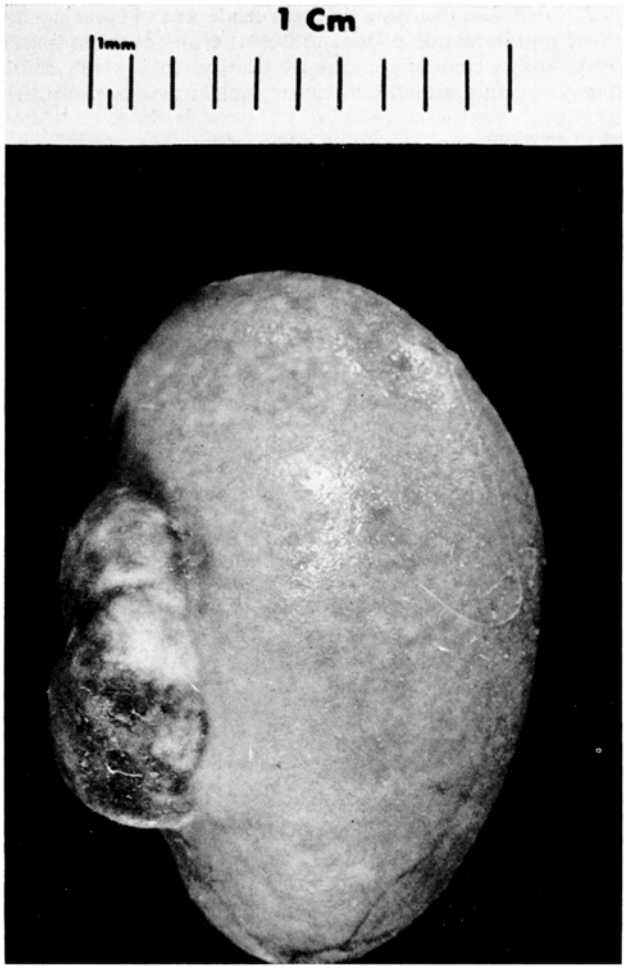

Fig. 1 and 2. A representative subcapsular tumorous pituitary homograft (Figure 1) and stimulated pituitary homograft (Figure 2) from female rats treated for 16 months with diethylstilbesterol.

Materials and methods. All animals used in this study were mature female Sprague-Dawley rats, obtained from Spartan Research Animals, Inc., Haslett, Michigan. They were housed in a temperature controlled room (75 ± 2° F) and fed a standard laboratory rat chow (Allied Mills, Inc., Chicago, Illinois). Seventeen 3-month-old rats were each grafted with 1 pituitary underneath the kidney capsule as previously described[5]. Pituitary donor rats were of similar age, sex and strain as the recipients. Immediately after pituitary grafting and every 3 months subsequently, each rat was implanted s.c. in the dorsum with a pellet containing 12 mg of DES. 16 months after the initial treatment with DES and grafting, the rats were sacrificed. The in situ and grafted pituitaries of each rat were excised and weighed. Pituitaries greater than 50 mg were arbitrarily designated as tumors.

Results. Tumors developed in 3 of 17 (18%) of the grafts as illustrated in Figure 1. Although tumors did not develop in 14 grafts, each graft was markedly enlarged (mean, 26.4 ± 3.2 mg) (Figure 2), approximately 3 times the size of the original graft. By contrast, the in situ pituitaries of these rats were even more enlarged than the grafted pituitaries. Mean in situ pituitary weight and percent of rats with in situ pituitary tumors were 63.5 ± 11.2 mg and 41% (7/17), respectively. No apparent relationship between the growth of the in situ pituitary and graft was observed, nor was there any evidence of metastases.

Discussion and conclusions. The results of this study clearly demonstrate that the transplanted rat pituitary is receptive to the tumorigenic stimulus of DES but to a considerably lesser degree than the in situ pituitary. The relatively greater growth and tumor incidence of the in situ pituitaries in response to chronic DES treatment suggest the existence of a DES-induced hypothalamic factor which is capable of promoting growth of pituitary tissue. Estrogen induced pituitary tumors secrete prolactin and this hormone (prolactin) has long been known to be under the predominantly inhibitory influence of the hypothalamus. Thus, the administration of estrogen decreases the hypothalamic content of prolactin inhibiting factor (PIF) and thereby increases pituitary prolactin secretion[7]. In addition, severance of the pituitary from the hypothalamus by median eminence lesions[8], stalk section[9] or pituitary grafting[10] also result in increased prolactin secretion. If pituitary growth and prolactin secretion were controlled solely by hypothalamic inhibi-

[7] A. Ratner and J. Meites, Endocrinology *75*, 377 (1964).
[8] C. W. Welsch, H. Nagasawa and J. Meites, Cancer Res. *30*, 2310 (1970).
[9] J. Meites and C. S. Nicoll, Ann. Rev. Physiol. *28*, 57 (1966).
[10] C. L. Chen, Y. Amenomori, K. H. Lu, J. L. Voogt and J. Meites, Neuroendocrinology *6*, 220 (1970).

tion (PIF), it would be expected that the transplanted pituitary would be more receptive to the DES-induced tumorigenic stimulus, a phenomenon which clearly did not occur in this study.

The existence of a hypothalamic prolactin releasing factor (PRF) in birds has been well established[11] and recent studies suggest that this factor may exist in mammals as well[12]. The results of the present study support this concept and provide evidence that this factor is influenced by estrogen administration. Estrogens have been reported to be actively concentrated by the hypothalamus as well as other areas of the limbic system[13] and to act directly on the pituitary tissue[9]. Although this study suggests that the hypothalamus plays an important role in pituitary tumorigenesis, it has not yet been determined whether the tumorigenic effects of DES are exerted primarily at the hypothalamic or pituitary level. The possibility cannot be ruled out that growth of the transplanted pituitary is not as vigorous as the in situ pituitary because of an inferior blood supply. This seems unlikely as all grafted pituitaries appeared to have a rich blood supply, as illustrated in Figure 2, and the subcapsular bed of the kidney is considered to be one of the best sites for effective revascularization of grafted tissue[15].

Résumé. Les résultats de cette étude démontrent nettement que la glande pituitaire du rat transplantée répond au stimulus tumeurigénique du diéthylstilboestrol, mais dans beaucoup plus faible mesure que la glande pituitaire in situ.

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Departments of Anatomy and Physiology, Michigan State University, East Lansing (Michigan 48823, USA), 8 April 1971.

[11] C. S. NICOLL and J. MEITES, Nature *195*, 606 (1962).
[12] C. S. NICOLL, R. P. FIORINDA, C. T. MCKENNEE and J. A. PARSONS, in *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry* (Ed. J. MEITES; Williams and Wilkins, Baltimore, 1970), p. 115.
[13] W. E. STUMPF and M. SAR, Proc. Soc. exp. Biol. Med. *136*, 102 (1971).
[14] L. M. BOOT, O. MÜHLBOCK, G. RÖPCKE and W. VAN EBBENHORST TENGBERGEN, Cancer Res. *22*, 713 (1962).
[15] Supported in part by NSF research grant No. GB-17034 and NIH research grants No. CA-10771 and No. AM-04784.

Inactivation Studies of Angiotensin II by Purified Enzymes

This communication describes the effect of three highly purified, hydrolytic enzymes from pig brain on Ile^5-angiotensin II. The enzymes selected for this study belong to the following categories: intracellular acid proteinases, aminopeptidases and arylamidases. While the acid proteinase and aminopeptidase cause no significant inactivation of Ile^5-angiotensin II, arylamidase degrades this peptide as shown by bioassay as well as by quantitative determination of the end products by amino acid analysis.

Brain acid proteinase (or Cathepsin D)[1], aminopeptidase (determined by the specific cleavage of Leu from Leu-Gly-Gly)[2] and arylamidase (determined by the hydrolysis of Arg. βNA)[3] were prepared from pig brain and assayed as described. Ile^5-angiotensin II was obtained from Calbiochem., L.A., Calif. One aliquot of each hormone-enzyme incubate, together with appropriate controls, was subjected to bioassay on the isolated rat uterus according to the method of HOLTON[4] as modified by MUNSICK[5] with the use of Mg^{2+}-free van Dyke-Hastings solution as the bathing fluid. Another aliquot of the digest was directly applied to amino acid analysis on a Technicon analyzer. Amino acids released were identified and quantified according to the short-column procedure of CATRAVAS[6] with the following modification: a 0.6 × 60 cm column packed with Amminex A-4 (Biorad., Palo Alto, Calif.) was used and was eluted at 60 °C with a 9-chamber Varigrad containing sodium citrate buffers at pH 2.75, 2.88, 3.80 and 6.10[2]. Aminopeptidase and arylamidase were incubated with hormone for set periods of time at 37 °C. Enzyme-substrate ratios (on a molar basis) were 1:150 to 1:400 and 1:75 to 1:150, respectively, in a total volume of 0.4 to 0.6 ml of 40 *mM tris*-HCl (pH 7.6) containing 0.1 *mM* dithiothreitol. Acid proteinase-substrate mixtures (enzyme to substrate ratios 1:5 and 1:10) were incubated in a total volume of 0.4 to 0.6 ml of 50 *mM* citrate buffer, pH 3.2. Reaction mixtures not directly utilized were stored frozen at -20 °C.

Bioassay revealed full retention of the rat uterotonic activity of Ile^5-angiotensin II during a 24-h.-incubation with aminopeptidase or acid proteinase. Moreover, no free amino acids resulted from the incubation of Ile^5-angiotensin II with these 2 enzymes. Contrary, the incubation of 100–200 µg of substrate with purified arylamidase led to the rapid appearance of the first 5 N-terminal amino acids (Figure). Quantitative determination of the individual amino acids after a 20-min digestion showed approximately equimolar quantities of aspartic acid, arginine, valine, tyrosine and isoleucine. After a 24-h incubation period 35% of the hormone had been degraded. The existence of peptide intermediates in the breakdown of Ile^5-antiotensin II is indicated by an initially high ratio (amino acid released/aspartic acid released) for material eluting with histidine (at 20 min). Undegraded Ile^5-angiotensin II was retained on the column under these experimental conditions.

The vasoactive hormone angiotensin II, which appears to elicit its dipsogenic response via receptors located in certain regions of the brain[7], is known to be cleaved by a large number of exo- and endopeptidases[8]. Recently,

[1] N. MARKS and A. LAJTHA, Biochem. J. *97*, 74 (1965).
[2] N. MARKS and A. LAJTHA, in *Methods in Enzymology* (Eds. G. E. PERLAMNN and L. LORAND; Academic Press, New York 1970), vol. 19, p. 534.
[3] N. MARKS, R. K. DATTA and A. LAJTHA, J. Biol. Chem. *243*, 2882 (1968).
[4] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[5] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[6] G. N. CATRAVAS, in *Technicon Symposia* (Eds. L. T. SKEGGS; Mediad, New York 1966).
[7] A. N. EPSTEIN, J. T. FITZSIMMONS and B. J. ROLLS, J. Physiol., Lond. *210*, 457 (1970).
[8] N. MARKS and A. LAJTHA in *Handbook of Neurochemistry* (Ed. A. LAJTHA; Plenum Press, New York 1971), vol. 5, in press.