

found when the hypothalamic LH-RF was determined, which also showed a significant decrease ( $p < 0.05$ ) at the dose of 25  $\mu\text{g}/\text{rat}$ .

A more pronounced effect was seen when cyclofenil was used. This compound was injected in doses ranging from 100 to 600  $\mu\text{g}/\text{rat}$ . It was found that the injection

of increasing amounts of this compound resulted in a straight-lined fall (semi-log scale) of both pituitary LH and of hypothalamic LH-RF content. An analysis of variance revealed the decrease of both pituitary LH ( $P < 0.01$ ,  $N = 7$ ) and of hypothalamic LH-RF ( $P < 0.05$ ,  $N = 5$ ) to be significant.

The results of this study show unequivocally that both ovulation inducers tested affect pituitary LH and hypothalamic LH-RF in the experimental model used. It was also found that the actions of clomiphene and of cyclofenil upon LH and LH-RF differed considerably from the effects previously observed on FSH and FSH-RF<sup>7,9</sup>. This indicates clearly that the modes of action of these compounds in respect to LH-release are not identical when tested under the present experimental conditions.

The effect of clomiphene citrate and of cyclofenil upon hypothalamic LH-RF content in the oophorectomized, oestrogen-progesterone blocked rat

Treatment	Dose ( $\mu\text{g}/\text{rat}$ )	No. of rats	O.D. <sub>500</sub> (mean $\pm$ SEM)
Clomiphene citrate	0	5	0.144 $\pm$ 0.007
	10	5	0.132 $\pm$ 0.014
	25	5	0.159 $\pm$ 0.002*
	50	5	0.115 $\pm$ 0.006*
Cyclofenil	0	6	0.171 $\pm$ 0.011
	100	6	0.180 $\pm$ 0.016
	300	6	0.193 $\pm$ 0.013
	500	6	0.214 $\pm$ 0.007
Castrated, unblocked animals	0	6	0.230 $\pm$ 0.014

\* Significant against solvent control ( $p < 0.05$ ). LH was measured according to PARLOW<sup>11</sup>, LH-RF by the method of RAMIREZ et al.<sup>10</sup>. The pooled hypothalamic extracts were injected into 3 recipient rats/dose and their pituitary LH content was determined. A rise of O.D.<sub>500</sub> indicates a fall in hypothalamic LH-RF. For experimental details refer to text. An analysis of variance of the results obtained with cyclofenil revealed significance at the 5% level.

**Zusammenfassung.** Der Effekt von Clomifenhydrogen-citrat und von Cyclofenil auf den LH-Gehalt der Hypophyse und den LH-RF-Gehalt des Hypothalamus wurde an kastrierten, weiblichen Ratten untersucht, die mit Östradiol und Progesteron blockiert worden waren. Während die Injektion von Cyclofenil im Dosisbereich von 100 bis 600  $\mu\text{g}/\text{Tier}$  zu einem Abfall von sowohl LH als auch LH-RF führte, konnte bei Verabreichung von Clomifen nur bei der Gabe von 25  $\mu\text{g}/\text{Tier}$  eine signifikante Herabsetzung von LH und LH-RF beobachtet werden.

H.-D. TAUBERT, R. KESSLER,  
G. BUSCH and H.-J. WERNER

*Abteilung für gynäkologische Endokrinologie der  
Frauenklinik der Joh.-Wolfg.-v.-Goethe-Universität,  
D-6 Frankfurt a. M. (Germany), 22 August 1969.*

## Vitellogenesis in Bidderian Oocytes After Diethylstilbestrol Dipropionate Treatment on *Bufo bufo* Adult Males

The problems about sexuality of *Bufo* are complicated by the presence, near the normal gonad, of a characteristic structure: the Bidder's organ, which in early larval stages always develops as a rudimentary ovary, both in the male and female genotypes, and which persists in all the adult males and in some adult females.

Bidder's organ oogenesis has to be referred to as 'abortive' because the diplotenic oocytes, unable to accumulate yolk, degenerate at the end of the II growth period (previtellogenesis)<sup>1</sup>. Only after testis ablation, the Bidder's organ becomes an active ovary in the space of 2–7 years<sup>2</sup>.

The aim of this paper is to present preliminary results of research work in which we have approached the problem of endocrine factors concerning the abortive bidderian oogenesis.

**Material and methods.** In March 1968 we administered a synthetic oestrogen, the diethylstilbestrol dipropionate (DSD), on *Bufo bufo* adult males in form of subcutaneous implant of a 25 mg solid tablet. We have chosen this way of administration of DSD because the tablet is very slowly adsorbed in the period of several months.

According to the indications on the bidderian oogenesis obtained in our precedent research work<sup>3</sup>, we have killed some specimens of control and treated groups, 1, 5, 9 months after the administration of synthetic oestrogen.

**Results and discussion.** The histological study of testes proves that, until the end of the experiment, both in the control and in the DSD treated animals, the spermatogenesis goes on normally, since we have always found all the stages of spermatogenesis (spermatogonia, spermatocytes, spermatides, spermatozoa).

On the contrary, the histological study of Bidder's organs has given some interesting differences between the control animals and the treated ones.

In spring, Bidder's organs, both in the control and in the treated group, show a lot of diplotenic oocytes, provided with lampbrush chromosomes, engaged in the previtellogenetic II growth period; more increased oocytes, unable to accumulate yolk, as usually, degenerate.

In summer, the Bidder's organs of control animals show, as in spring, diplotenic oocytes at different stages of the II growth period and several degenerating oocytes. On the contrary, the Bidder's organs of DSD treated animals are

<sup>1</sup> N. BECCARI, *Archo. ital. Anat. Embriol.* 26, 273 (1929).

<sup>2</sup> K. PONSE, in *La différenciation du sexe et l'intersexualité chez les Vertébrés* (Ed. F. ROUGE; Librairie de l'Université, Lausanne 1949).

<sup>3</sup> F. ZACCANTI e G. GARGENHI, *Boll. Zool.* 35, 165 (1968).

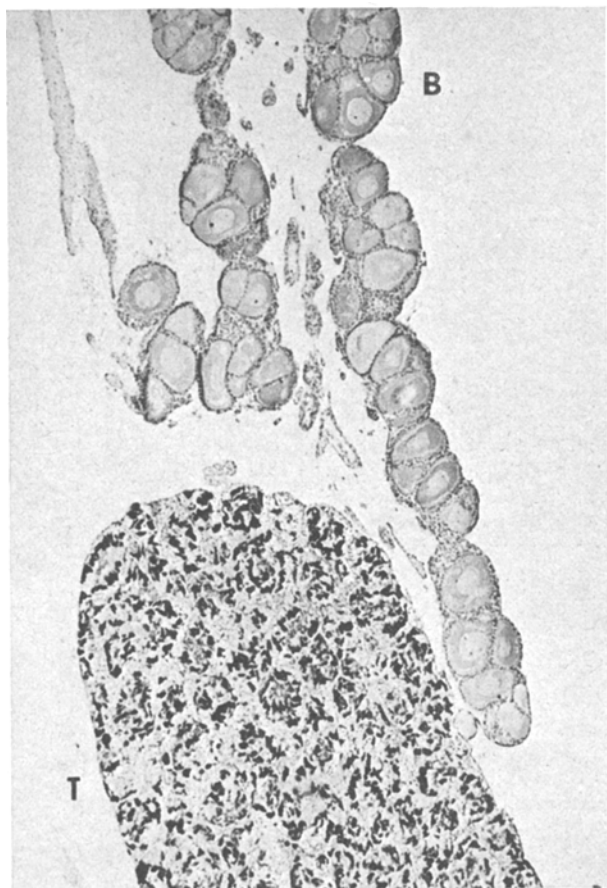


Fig. 1. Sagittal section through genital body of *Bufo bufo* adult male of the control group, killed in autumn. (B) Bidder's organ with diplotenic oocytes in the II growth period (previtellogenesis). (T) Testis.  $\times 37.5$ .

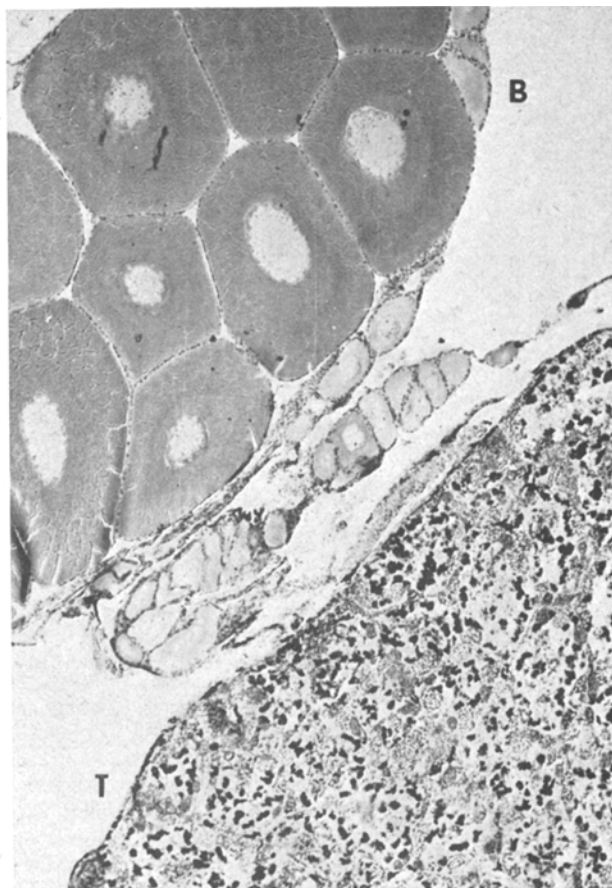


Fig. 2. Sagittal section through genital body of *Bufo bufo* adult male of the diethylstilbestrol dipropionate treated group, killed in autumn, after 9 months of treatment. (B) Bidder's organ with diplotenic oocytes in the II and in the III growth period (vitellogenesis). (T) Testis.  $\times 37.5$ .

decreased in volume and they contain only a few small oocytes in the II growth period.

Finally in autumn, 9 months after the beginning of the treatment, while the Bidder's organs of the control group show the usual oocytes composition, the Bidder's organs of the treated group show again many diplotenic oocytes and some of these have passed the II growth period and are in active vitellogenesis (diplotenic oocytes in the III growth period) (Figures 1 and 2).

In nature, as we have said, bidderian oogenesis stops at the previtellogenetic stages, and bidderian oocytes at the end of the II growth period do not accumulate yolk, but degenerate. After a treatment with DSD, the bidderian oocytes become able, on the contrary, to begin the vitellogenesis, as tested by the deposition of yolk at the boundary of cytoplasm.

Therefore genital glands are now composed of a cranial small ovary, whose diplotenic oocytes are at the beginning of vitellogenesis, and a caudal active testis.

On the whole, a treatment with 25 mg of synthetic oestrogen, the diethylstilbestrol dipropionate (DSD) in form of a s.c. implant of a solid tablet on *Bufo bufo* adult males, causes, at the beginning, a volumetric decrease of Bidder's organ with the destruction of middle and big dimension oocytes at the II growth period. Afterwards, the oestrogen seems to accelerate oogenesis to reach vitellogenetic stages of the III growth period in presence of

active testis, so that the animals are going to become authentic hermaphrodites.

Researches in progress aim to clarify in detail the results obtained in this first study on the effects of diethylstilbestrol dipropionate on bidderian oogenesis in *Bufo bufo* adult males.

**Riassunto.** Un trattamento con 25 mg di un estrogeno sintetico, il dietilstilbestrolo dipropionato, sottoforma di un cilindretto solido impiantato sottocute in maschi adulti di *Bufo bufo*, induce gli ovociti diplotenici dell'organo di Bidder, di norma abortivi, a raggiungere gli stadi vitellogenetici del III periodo di accrescimento ovocitario, così che gli animali si avviano a comportarsi da autentici ermafroditi.

F. ZACCANTI and G. GARDENGHI<sup>4</sup>

*Istituto di Zoologia dell'Università di Bologna, I-40126 Bologna (Italy), 25 July 1969.*

<sup>4</sup> This work was supported by grants from the Consiglio Nazionale delle Ricerche, Roma.