quantitatively the concentration of progesterone in the insect at 2 stages of development: 12–13-day-old larvae and 8–9-day-old adults and in their diet.

The starting material was 60 g of larvae, 49 g of adults and 100 g of diet. At the beginning of the extraction procedures, a tracer amount of progesterone-7-^{3}H was added to determine the recovery. The fresh tissues and the diet were extracted with chloroform:methanol (2:1)[9]. After evaporation of the solvents, the residue was partitioned between hexane and 90% methanol. The residue of the aqueous methanol phase was further purified by column chromatography, thin-layer chromatography, acetylation and thin-layer chromatography again. The identification of progesterone was done by its mobility on thin-layer chromatography, before and after acetylation and by gas-liquid chromatography.

Progesterone appeared to be present in the larvae and in the adults of *T. confusum* and also in their diet. The concentration of progesterone determined from the gas-liquid chromatographic areas after correction for the recovery was as follows: larvae 23 µg/100 g; adults 17 µg/100 g; diet 9 µg/100 g.

If we compare the concentration of progesterone found in the wheat flour with that earlier reported in apple seeds[10], we notice that the latter contains relatively much higher concentration (approximately 6 times) of this steroid.

The present study has shown that the steroids, particularly progesterone, in *T. confusum* might indeed come from its dietary source since the concentration of this steroid in the insects and in their diet is of the same order of magnitude. The relatively higher concentration in the insects could be explained by the accumulation of progesterone in the tissues particularly during the most active feeding period of larval growth. The fact that RITTER and MEIJER[11] have been unable to detect steroids in *T. confusum* might possibly be explained by a different source of wheat flour used for rearing this insect.

Résumé. La progestérone présente dans les tissus de *Tribolium confusum* (coléoptère) tire probablement son origine de la ration alimentaire puisque la concentration de ce stéroïde est du même ordre de grandeur dans l'insecte et dans la ration alimentaire.

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Compensatory Hypertrophy of the Contralateral Testis after Unilateral Castration in the Toad, *Bufo melanostictus* (Schn)

Studies on compensatory hypertrophy of the contralateral gonad after unilateral castration in lower vertebrates are sporadic and meagre. These studies involve the female sex only[1–4]. In mammals extensive studies have been made[5–8], but as to its occurrence in the male there is much controversy[7]. Thus, realizing the need for research on compensatory hypertrophy on the male side, this study was undertaken in the male toads.

Gravid male toads weighing 25–40 g, caught in Mysore City (India), were maintained in semi-moist cages. They were unilaterally castrated under light ether anaesthesia and their right testis removed. These operated toads were force-fed with minced meat once a day. On the 10th day they were autopsied and the remaining testis were dissected out, weighed on a torsion balance, fixed in Bouin's fluid, paraffin sections cut at 8 µ thick and stained in iron haematoxylin. The sections were observed and measured for changes and calculated results are shown in the Table. These studies were made in August and September, and a total of 25 toads were used for these studies.

There is an increase in the weight of the remaining left intact testis (55.6 ± 5.5), 10 days after unilateral castra-

Effect of hemicastration on the intact contralateral testis

Testis	Wt. of the testis/100 g body wt. (mg)	Average diamter of the testis (µ)	Average diameter of the seminiferous tubules (µ)	Average No. of sperm bundles per seminiferous tubule	Average No. of cell nests per seminiferous tubule	Interstitial cells
Testis removed at operation (10)	107.2 ± 8.2	1191.6 ± 55.3[a]	175.2 ± 2.9	5.4 ± 0.90	5.9 ± 0.75	+++
Contralateral testis removed after 10 days (10)	163.3 ± 12.6[c]	1694.9 ± 63.8[b]	328.7 ± 11.9[b]	6.6 ± 0.93	8.6 ± 0.59[d]	++++

[a] Standard error; [b] probability: > 0.001; [c] < 0.001; [d] < 0.01. Number of toads in parentheses. Normal average difference between right and left testes of an animal = 12.8 ± 1.6.

tion. This indicated that there is compensatory hypertrophy of the contralateral testis. There is an increase in the average diameter of the testis and the seminiferous tubules as observed and measured in the sections. There is also a significant increase in the number of sperm bundles and cell nests per seminiferous tubule. There is an increase in the amount of interstitial cells (Table).

Though alternative hypotheses to explain the mechanisms of compensatory hypertrophy have been advanced, there is no doubt about the occurrence of compensatory hypertrophy of the contralateral ovary after unilateral ovariectomy in all vertebrates studied[1,3,4,6,9]. But as to its occurrence in the testis after unilateral castration, there is difference of opinion[7]. It is claimed that unilateral castration causes compensatory hypertrophy of the contralateral testis in young animals and not in mature males. Even this is described as accelerated growth and not compensatory hypertrophy[7]. Our studies show that there is 55.6% increase in the weight of the contralateral testis 10 days after unilateral castration (Table). It is statistically significant compared with the normal difference in weight between the right and left testis of the same animal (12.8). In addition to this increase in weight, there is also a significant increase in the diameter of testis, diameter of the individual seminiferous tubules, the number of sperm bundles and cell nests per seminiferous tubule and the number of interstitial cells in the intact testis (Table). All these facts indicate that there is an increase in the activity of the contralateral testis presumably under the influence of pituitary gonadotrophins[10].

Zusammenfassung. Nachweis kompensatorischen Wachstums des nach einseitiger Kastration verbleibenden Hodens bei indischer Krötenart (*Bufo melanostictus* (Schn)). Zunahme von Gewicht, Grösse und Spermienzahl.

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A Diploid Population of the Polyploid Amphibian *Odontophrynus americanus* and an Artificial Intraspecific Triploid Hybrid

Polyploidy has been well demonstrated in amphibians of the family Ceratophrydidae[1-4]. It has been demonstrated in other Anura too[5]. *Odontophrynus americanus* (Duméril and Bibron), 1882 was described as a tetraploid species. Somatic tissues and germ cells showed the same karyotype, with 44 chromosomes, arranged in 11 groups of homologues. Spermatocytes I formed mostly ring quadrivalents and metaphases II exhibited 22 dyads.

Comparative cytophotometric DNA measurements of Feulgen stained preparations as well as nuclear volume measurements of different tissues from the diploid species *Odontophrynus cultripes* ($2n = 22$), the tetraploid *O. americanus* ($4n = 44$) and the octoploid *Ceratophrys dorsata* ($8n = 104$) confirmed the expected 1:2:4 ratio[6].

In the State of São Paulo more than a hundred specimens of *O. americanus* collected between 45° W and 47° W meridians were karyotyped, confirming the tetraploid nature of that population. However, specimens collected in the surroundings of Botucatú, between the 48° W and 49° W meridians in the same State of São Paulo, are genetically different. Their karyotype, described in the present paper, has only 22 chromosomes and is characteristic of a diploid species.

Twenty-five specimens, comprising 21 males and 4 females, were analyzed. They were collected in the same area at a farm near the city of Botucatú. Compared with the tetraploid specimens they are apparently indistinguishable as to external characteristics and size (Figure 1).

The chromosomes for cytological study were obtained from spleen and gonads by the squash technique. Prior to sacrifice, the animals were treated for 2 h with a 0.5% solution of colchicine in the dosage of 0.2 ml/10 g body wt. The slides were stained with Giemsa, after hydrolysis in 1 *N* HCl at 60 °C for 10 min.

The karyotype consists of 22 chromosomes, sorted in 11 pairs of homologues. The pairs 1, 5, 6, 7, 10 and 11 consist of metacentrics; the pairs 2, 3, 8 and 9 consist of submetacentrics and the pair 4 of acrocentrics. Satellites

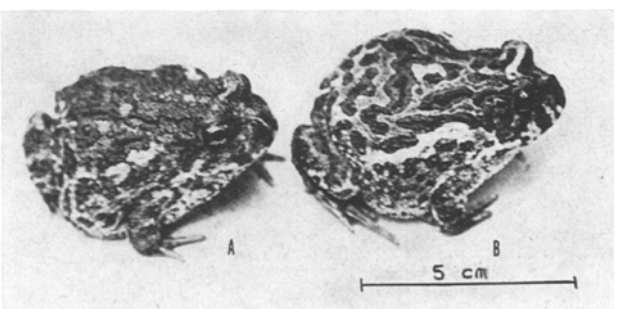

Fig. 1. *O. americanus*. (A) Tetraploid female ($4n = 44$). (B) Diploid female ($2n = 22$).