Histological sections of the *O. cultripes* males × *O. americanus* females hybrids, up to 7 months after hatching, were analysed. The specimens apparently do not show abnormalities in their somatic development.

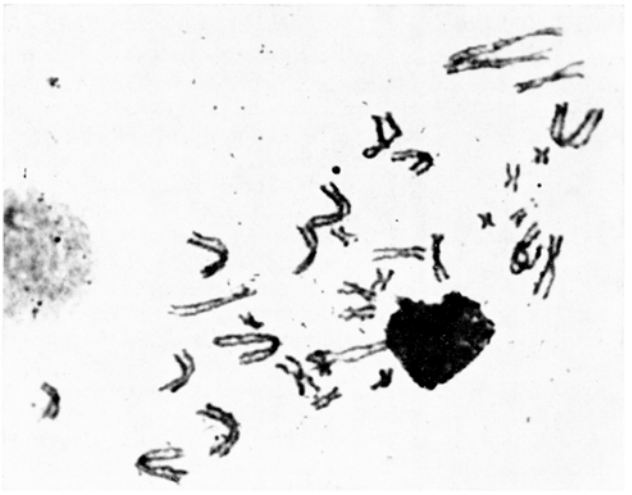

Fig. 3b. Somatic metaphase of the triploid hybrid *O. americanus* ♂ × *O. cultripes* ♀ showing 33 chromosomes. × 1350.

Development of gonads has been followed; the results will be published elsewhere, together with the results of sex-ratio analysis in hybrids[5].

Résumé. Des hybrides triploïdes ont été obtenus par croisement de *Odontophrynus cultripes*, amphibien diploïde, avec *O. americanus*, tétraploide. Les hybrides interspécifiques présentent normalement 33 chromosomes dans les cellules somatiques. Les hybrides *O. cultripes* ♂ × *O. americanus* ♀ sont plus viables que les *O. americanus* ♂ × *O. cultripes* ♀.

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[4] M. L. BEÇAK, *Cariótipos e evolução cromossômica em Amphibia Anura* Thesis, São Paulo (1967).

[5] This work was supported by Public Health Service Research Grant No. GM-14577-02 from the National Institute of General Medical Sciences and by Fundo de Pesquisas do Instituto Butantan.

Abnormal Response of Mice and Rats to Low Doses of Follicle-Stimulating Hormone

An examination of the response of mice to low doses of follicle-stimulating hormone (FSH) by the method we described previously[1], led to the surprising result that, when the dose was reduced below a certain level, a significant lowering of intravaginal triphenyltetrazolium chloride (TTC) reduction response was observed as compared with controls treated with 20 IU HCG only.

Below 1 μg of FSH (lyophilized pig FSH, Mann Research Laboratories, New York) this fall reaches a maximum at 0.25 μg (approximately 25%, $P < 0.01$) (Figure 1).

The same phenomenon occurs with another sample of FSH (Ormonoterapia Richter, Milan; sample 223 MMP), of high content (2–2.5 times higher than the first). In this case, the response lowering range is below 0.5 μg of FSH, with a maximum fall value at 0.125 μg, as expected, in view of the greater specific activity of this sample, equivalent to 23% ($P < 0.01$) (Figure 1).

This anomaly is encountered also in the classic STEELMAN and POHLEY test[2], where a decrease of the ovarian weight can be noticed, compared with controls treated with HCG alone (40 IU).

When using sheep NIH-FSH-S2, such a decrease is significantly below 22.5 μg of FSH. The same result can be obtained with other samples of FSH (Ormonoterapia Richter, sample 223 MMP; lyophilized pig FSH, Mann Research Laboratories); in the latter case the decrease is reported below 5 μg of FSH (Figure 2).

The result may be considered as being evidence of an interaction between exogenous hormone administered and the endogenous follicle-stimulating system. This interaction indicates the presence of a direct (or indirect, acting at hypothalamic level instead of directly on the hypophysis) feed-back system controlling the secretion of pituitary gonadotropins in the rat and in the mouse.

If we now interpret the dose-response curves in the light of this hypothesis, we can see that each is the sum of 2 curves which represent the endogenous and the exogenous contribution to the circulating level of follicle-stimulating activity.

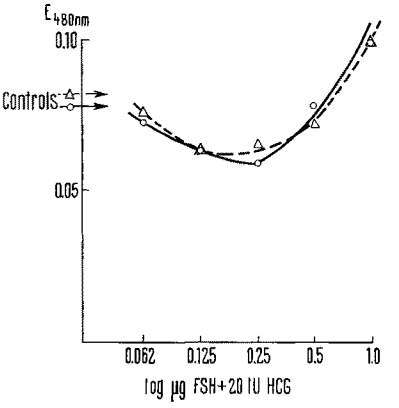

Fig. 1. Evaluation of FSH activity by intravaginal TTC reduction[1]. ○——○ Pig FSH (Mann Res. Lab.); △——△ FSH (Ormonoterapia Richter).

[1] M. M. CASELLATO, G. LUGARO and K. WEYDANZ, Experientia *23*, *1078* (1967).

[2] S. L. STEELMAN and F. M. POHLEY, Endocrinology *53*, 604 (1953).

Assuming that the exogenous hormone is relatively more active in suppressing the hypophysis than stimulating the ovaries, then the reduction in the resultant level of follicle-stimulating activity is bound to follow when the dose is within a certain range.

As the dose increases, the initial decline in ovarian response is the result of the rate of increase in hypophysis, suppression being greater than the rate of increase in the level of follicle-stimulating activity of the exogenous hormone.

However, as the suppression reaches its maximum, the situation becomes reversed, and the ovarian weight increases to increasing doses of exogenous hormone.

Therefore the evidence presented leads to the conclusion that the injection of low doses of FSH can reduce the level of activity of the endogenous follicle-stimulating system, and that this property is not specific to a single preparation.

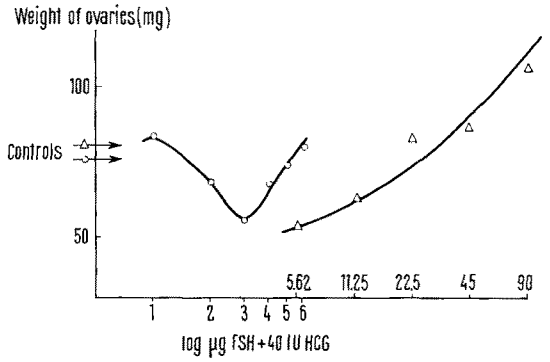

Fig. 2. FSH activity in the STEELMAN and POHLEY[2] test. Ovary weight after fixing in Bouin's liquid for 24 h. ○—○ Pig FSH (Mann Res. Lab.); △—△ NIH-FSH-S2 ovine.

Riassunto. Gli Autori riportano una interessante anomalia che si riscontra nel dosaggio di piccolissime quantità di FSH, sia nel test di STEELMAN e POHLEY quanto nel test di riduzione intravaginale del TTC. Tale anomalia si evidenzia in un netto e significativo abbassamento delle risposte rispetto ai controlli non trattati. Si fa l'ipotesi di una interazione tra l'ormone esogeno somministrato e la secrezione ipofisaria basale endogena dell'animale impubere, che si manifesterebbe in un feed-back a livello dell'ipofisi, diretto o indiretto tramite l'ipotalamo o centri ancora superiori. Tale effetto non è specifico di una singola preparazione.

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FSH-Releasing Effect of Clomiphene in the Female Rat

Clomiphene citrate (1-*p*-(β-diethylamino-ethoxy)-phenoxy-1, 2-diphenyl-2-chloroethylene), a derivate of the non-steroidal oestrogen chlorotrianisene, is a potent inducer of ovulation in man, but inhibits fertility in the rat. In the human its administration has been shown to increase the urinary excretion of total gonadotropines[1-3] and of LH[4]. In the rat a marked inhibition of pituitary gonadotropin secretion, probably due to blocking of hypothalamic receptor sites, was observed by HOLTKAMP[5]. To the contrary, IGARASHI et al.[6] found that clomiphene citrate (C.c.) caused a significant rise in plasma FSH- and LH-levels in the rat. As the implantation of C.c. crystals into the median eminence of the rat has been shown to decrease pituitary FSH content[6], we examined whether i.v. administered C.c. would affect the release of FSH in the ovariectomized, oestrogen-progesterone blocked rat.

Materials and methods. The FSH-releasing activity of C.c. was determined by a slight modification of the method of RAMIREZ et al.[7]. Wistar rats of approximately 180 g body weight which had been kept under standard condition were used. The experiments were performed 4 weeks after bilateral, dorsolateral ovariectomy. The certainty of anoestrous was assured by performing vaginal smears during the last week.

Pituitary blocking was achieved by injecting the animals s.c. with 50 µg oestradiol-17β and 25 mg progesterone on day 2 and 3 before administration of C.c.

C.c. was injected as a 0.03% aqueous solution into the tail vein. Each group contained 4 animals. In group I each animal received 300 µg and in group II 600 µg C.c. 15 min after the injection the animals were anaesthetized with ether and exsanguinated into heparinized test tubes. The blood of each group was pooled. Plasma was obtained by centrifugation at 3000 rpm for 15 min.

The plasma FSH-activity was assayed according to IGARASHI and MCCANN[8], utilizing NMRI mice. 7 animals were used per dose level. Each animal received 2 ml of a solution containing 1.5 ml pooled plasma and 0.5 ml HCG in normal saline. The HCG content of the final solution was 0.125 IU/ml. The injections were given in divided doses of 0.7 ml on the first and second day, and 0.6 ml on the third day. The mice were sacrificed on the day following the last injection. The uteri were removed immediately, and the wet weight was determined to the nearest 0.1 mg.

Since it was assumed that after 15 min circulation time plasma of C.c. treated animals still contained most of the substance, experiments were conducted to determine whether C.c. has a uterotropic effect in the mouse when administered together with HCG. 7 mice were used per dose level. Each animal received a total dose of 0.25 IU

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