the *trans* ester. The mass, infrared, and NMR spectra and the biological activity of the synthetic compounds were identical to those of the corresponding natural products.

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Changing Sensitivity of the Pubertal Gonadal Hypothalamic Feedback Mechanism in Man

Abstract. *Clomiphene citrate in doses which stimulate gonadotropin production in the adult suppresses urinary follicle stimulating hormone (FSH) excretion and plasma testosterone concentration in prepubertal children. Such results indicate that feedback between gonad and hypothalamus is operative and highly sensitive in prepubertal humans. Puberty in man, as in the rat, is accompanied by a decrease in the sensitivity of the feedback mechanism.*

Gonadotropins (*1*) and sex steroids (*2*, *3*) have been quantitatively determined in urine and blood of prepubertal children. The prepubertal gonad can be stimulated by gonadotropins (*4*), and the immature pituitary gland is responsive to hypothalamic gonadotropin-releasing factors (*5*). We now offer evidence for the existence of an operative feedback relation between the gonad and hypothalamus of the prepubertal child, the sensitivity of which changes during sexual maturation.

Clomiphene citrate, an antiestrogen, stimulates gonadotropin release in the adult, presumably by competing with steroids at hypothalamic receptor sites (*6*). The effect of this agent on gonadotropin secretion in the child is not known. Accordingly, doses of clomiphene which augment gonadotropins in the adult, approximately 1 to 100 mg/m^2 per day (*7*), were administered for 1 week to 16 children in various stages of sexual maturity. Clomiphene was administered to patients admitted for evaluation of short stature, delayed adolescence, or precocious puberty. Informed consent was obtained from the parents. Careful monitoring of hepatic and renal function was carried out before and after drug administration, as was close clinical follow-up. No untoward effects of clomiphene have been observed in any of these patients.

Urinary concentrates processed by the kaolin-acetone technique (*8*) were made from 30- to 60-ml portions of 24-hour urine specimens collected under hospital supervision. The concentrates were suspended in 2 ml of water, and samples of 50 to 200 μl were used for the radioimmunoassay of follicle-stimulating hormone (FSH) (*9*). Samples of concentrates from one or more urine specimens from each patient were parallel to samples of standard material, the 2nd International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP). The three serial dilutions used to show this parallelism were also appropriate for testing assay sensitivity with reference to the unknown material. Measurements of urinary luteinizing hormone (LH), and of plasma FSH and LH as well, were made. Serial dilutions could not be carried out since these unknowns fell at the lower limits of the standard curve; consequently, for technical reasons, suppression of urinary LH and plasma gonadotropins could not be demonstrated. Testosterone in plasma was measured by a competitive protein-binding method (*3*) on the first and last day of clomiphene treatment in a number of the male patients.

Figure 1a shows the effects of daily administration ration of 100 mg of clomiphene per square meter of body surface for 7 days to two normal adult males and three prepubertal children. After the drug course, both FSH and plasma testosterone increased in the adult males, but these hormones appeared to decrease in the children. Indirect evidence for the fact that suppression of FSH in the children was caused by the known intrinsic estrogen action (*10*) of clomiphene (despite its antiestrogen activity) was the appearance of a small nubbin of breast tissue after the 1-week clomiphene course in both boys whose data appear in Fig. 1a.

Figure 1b shows a second series of patients given clomiphene, seven children between the ages of 5 and 16 years, who received 10 mg/m^2 per day for 1 week. All were prepubertal except for one boy, deficient in growth hormone and ACTH, who was in the very early stages of sexual maturation. In six of the seven individuals receiving this lower dose, clomiphene suppressed either the FSH in the urine or testosterone in the plasma.

Figure 1c shows the effects of still further decreases in clomiphene dosage, namely to 1.0 mg/m^2 per day. Urinary FSH was suppressed in three boys on this very small amount of the drug. One of these three patients received 10 mg/m^2 per day for seven additional days immediately after the first week at the 1.0 mg dose; little further decrease in FSH excretion took place during the second week of drug administration.

Since clomiphene appeared to elicit contrary effects in the pre- versus the postpubertal patient, the transitional period of puberty was next examined. Three girls in the early stages of sexual maturation were given 100 mg/m^2 per day for 1 week. The results (Fig. 1d) reveal FSH stimulation in one girl, suppression in another, and no change in the third. Such variable responses might be expected during the period of change from child to adult.

The interpretation of our results may be based on data available from lower animals, particularly the rat. For many years it has been known that the amount of estrogen necessary to suppress gonadotropin production in the prepubertal rat is far less than that needed for stimulation of the acces-

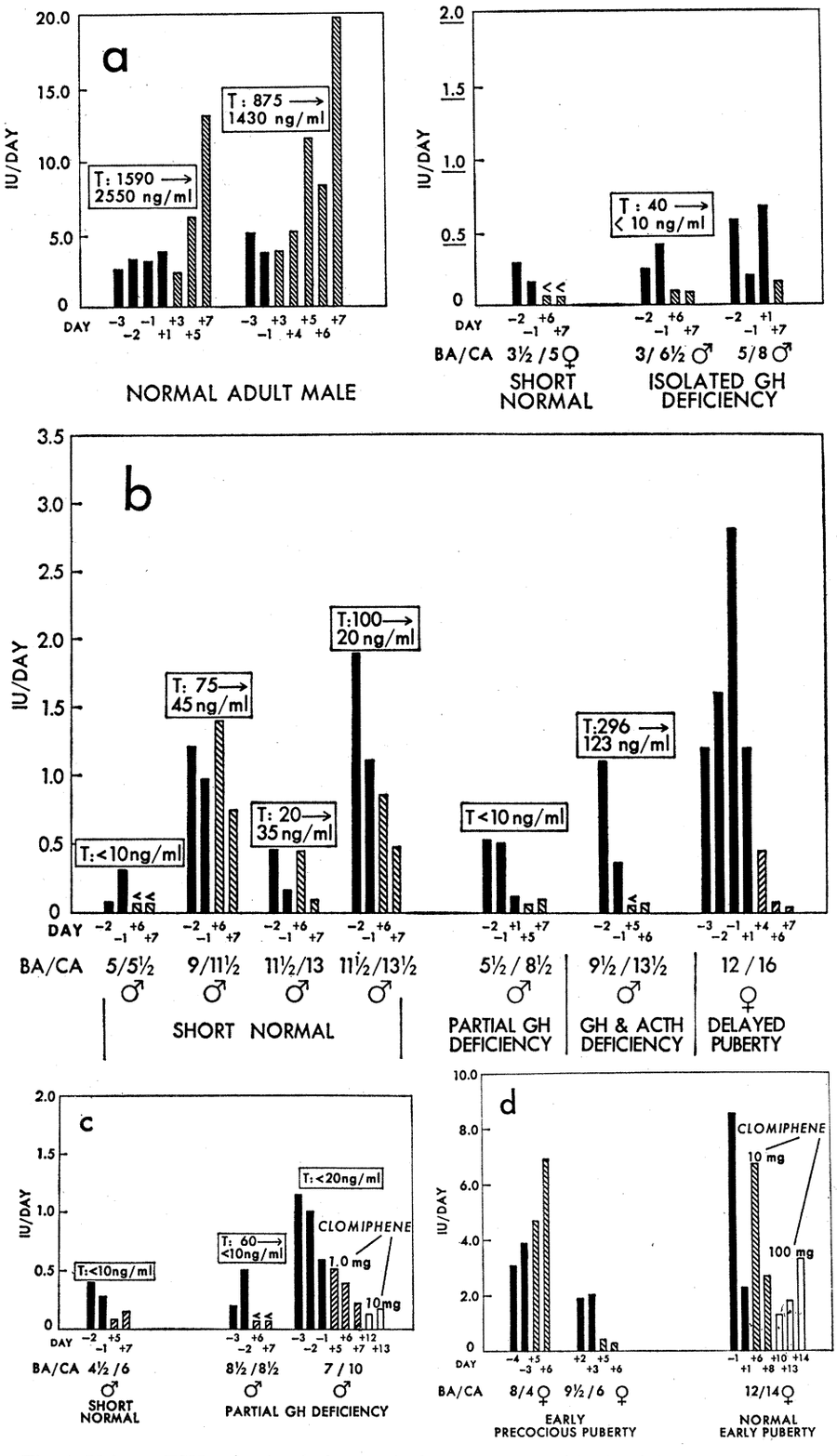

Fig. 1. Urinary FSH excretion before and after treatment with clomiphene: (a) 100 mg/m² daily; (b) 10 mg/m² daily; (c) 1 mg/m² daily; and (d) 100 mg/m² daily in early pubertal patients. The days before treatment are shown by solid bars and in some cases include the first few days of clomiphene administration. The days after treatment, shown by the hatched or open bars, include the last days of the drug course. The precise treatment days, with reference to administration of clomiphene, are indicated by the (—) and (+) numbers. The ordinate denotes FSH excretion in international units (*IU*) of the standard [2nd International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP)]. *T* in (a), (b) and (c) refer to the concentration of testosterone in the plasma on the first and last day of the clomiphene course. The ratio BA/CA indicates the ratio of bone age to chronological age. GH is gonadotropic hormone; ACTH is adrenocorticotropic hormone.

sory sex organs in the same animal (*11*). Some investigators have shown that the hypothalamic receptor sites in the prepubertal rat are much more sensitive to steroid feedback than these same sites in the adult (*12*). Current theories of puberty in the rat envisage a change in the sensitivity of a hypothalamic "gonadostat" as fundamental to the increase in gonadotropins and sex steroids which accompany the maturation process (*12*).

Our data lend support for a similar series of events taking place in man: Prepubertally, the known low levels of gonadotropins and sex steroids interact by means of a highly sensitive feedback mechanism. The initiation of puberty is accompanied by a decrease in the sensitivity of this feedback mechanism, such that the low prepubertal concentrations of steroid no longer effectively suppress FSH and LH secretion. Consequently, gonadotropin production increases and further gonadal stimulation entrains.

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