Acta Paediatr Belg **32** : 105-111, 1979

USEFULNESS AND LIMITS OF GnRH TEST IN BOYS WITH LACK OF SEXUAL MATURATION*

Chr. ERNOULD**, J.P. BOURGUIGNON** *** and P. FRANCHIMONT***

*Department of Paediatrics, Endocrine Unit** and Radioimmunoassay Laboratory***, Department of Internal Medicine, University of Liège*

Abstract

In 39 boys between 14.0 and 22.6 years of age with lack of sexual maturation, the response of the serum gonadotrophins to a single intravenous administration of GnRH was studied and the clinical evolution was followed. In 30 patients LH secretion after GnRH showed a pubertal pattern. Subsequently they all had spontaneous puberty. In 9 patients, the LH response to GnRH was prepubertal. Four of them subsequently showed spontaneous pubertal maturation. In the 5 others, testicular size remained prepubertal, suggesting the diagnosis of isolated gonadotrophin deficiency.

Thus 34/39 patients had constitutionally delayed puberty. In 30 of the former biological evidence of hypothalamopituitary maturation preceded the appearance of physical puberty. Consequently, normal LH response allows to reassure the patients and their family as to the prognosis. If prepubertal in pattern, the gonadotrophin response to GnRH should be interpreted cautiously and the evolution awaited. (*Acta Paediatr Belg* **32** : 105-111, 1979).

The differential diagnosis between constitutionally delayed puberty (CDP), isolated gonadotrophin deficiency (IGD) and the other forms of retarded sexual maturation is often difficult (7, 9, 13, 25, 30, 32, 35, 38, 39, 42, 49). Exact diagnosis may require a prolonged follow-up.

Isolated determinations of circulating gonadotrophins were found to be of limited value, except in some cases of hypergonadotrophic hypogonadism (43, 48). Dynamic tests using gonadotrophin releasing hormone (GnRH) proved to be more useful since gonadotrophin response to a single intravenous administration of GnRH changes both quantitatively and qualitatively with puberty (1, 6, 10, 16-18, 27, 30, 37, 40, 47). Recently, the pituitary response to GnRH has been investigated using timed urinary gonadotrophin measurements (33), prolonged intravenous infusion of GnRH (36) or previous administration of clomiphene (44). These different GnRH tests were applied to patients with lack of sexual maturation and many data have been reported (3, 13, 21-24, 28, 31).

* Presented in part at the XVIth International meeting of the European Society for Paediatric Endocrinology, (ESPE), September 1977, Cambridge, UK.

The purpose of this work is : [1] to study further the diagnostic value of gonadotrophin response to GnRH in CDP and IGD, [2] to show that changes of pituitary responsiveness to GnRH precede the appearance of any sign of puberty in most patients with delayed puberty, and [3] to point out the limits of the results in our GnRH test conditions.

Subjects and methods

Subjects

Thirty-nine boys with lack of sexual development were studied. They were between 14.0 to 22.6 years of age at the time of the endocrine investigation. Testicular size was measured using Prader's orchidometer (50). Testicular volume was found to be prepubertal ($\leqslant$ 4 ml) in all patients. Pubic hair was absent in 32 patients, estimated at stage P_2 in 6 patients and at stage P_3 in 1 patient according to Tanner's scale (34, 45). Statural ages of the patients were between 8 10/12 and 15 5/12 years according to the growth charts of Geubelle *et al.* (19). Bone-ages were determined in 31/39 patients according to Greulich and Pyle (20) or Tanner *et al.* (46) and found to be between 11.0 and 15.0 years. Four patients had previously been given gonadotrophin therapy. None of the others had been treated with anabolic, androgenic or gonadotrophic hormones during the 3 months before the GnRH test or during the subsequent observation period. No patients had any sign of cryptorchidism, gynaecomastia, anosmia, hypoglycaemia, or hypothyroidism. In patients with short stature and low growth velocity, growth hormone secretion was evaluated and proved to be normal. After GnRH testing, all patients were regularly followed and their bodily growth and sexual maturation were noted. This allowed to recognize 2 groups of patients : 34 had a spontaneous pubertal development after a follow-up of 2 to 20 months. They were diagnosed as having CPD. The 5 remaining patients had IGD. They remained prepubertal and gonadotrophin response to GnRH was retested in 4 of these 5 patients.

The control group consisted of 16 prepubertal boys aged 7.0 to 10.0 years (stage G_1) and 21 pubertal boys aged 11.0 to 16.5 years [12 at stage G_2 and 9 at stage G_3 according to Tanner's classification (45)].

GnRH test and gonadotrophin radioimmunoassay

All the subjects underwent a GnRH test between 8.00 a.m. and 11.00 a.m. A single intravenous dose of 25 µg GnRH (LHRH — Hoechst A.G.) per square meter body surface was administered. Blood samples for follicle stimulating hormone (FSH) and luteinizing hormone (LH) radioimmunoassay (14, 15) were taken 15 and 1 minutes before the GnRH injection and at 5, 10, 15, 20, 30, 45, 60 and 90 minutes afterwards. The peak-response was defined by the maximum increment, i.e. the difference between the mean basal gonadotrophin level and the highest value observed following GnRH injection.

The standards used for the radioimmunoassay were MRC 68/39 for FSH and 68/40 for LH. The limit of sensitivity was 0.3 mIU/ml serum for both gonadotrophins.

Results

The FSH and LH-responses to GnRH observed in the 39 patients are shown in figures 1 and 2. These individual values are compared to the responses found in the control boys. The FSH peak-response found in the patients and in the 3 control groups were similar (fig. 1). On the contrary, in normal boys the LH peak-response showed a highly significant increase according to the different pubertal stages, as has previously been described (16, 23, 40).

In 30 patients, the LH-response to GnRH was in or even in excess of the range of that

of normal pubertal boys (fig. 2). Follow-up confirmed the tentative diagnosis of CDP. Clear-cut signs of puberty appeared 2-10 months after testing. There was no relationship between the time elapsed before the appearance of pubertal signs and the magnitude of the LH peak-response to GnRH.

Two CDP patients with low LH-response and 4 IGD patients were retested (fig. 3). In the former, a pubertal pattern of LH-response was observed after 8 months in one boy and after 17 months in the other, together with a pubertal increase in testicular size; in the latter, the LH-response to GnRH and the testicular size remained prepubertal.

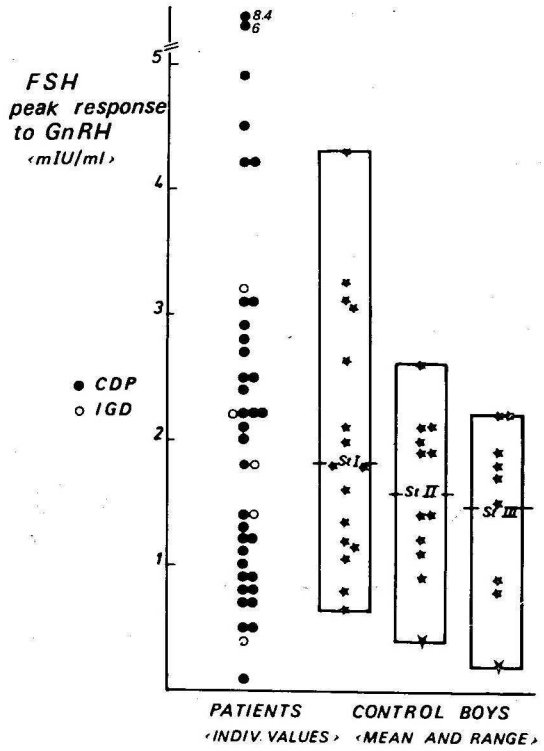

FIG. 1. — Maximal increments of FSH following intravenous injection of 25 µg/m² GnRH. Individual values observed in boys with constitutionally delayed puberty (CDP) and isolated gonadotrophin deficiency (IGD) are compared with the mean and range of values found in normal boys at pubertal stage G_1 (n = 16), G_2 (n = 12) and G_3 (n = 9) according to Tanner's standards.

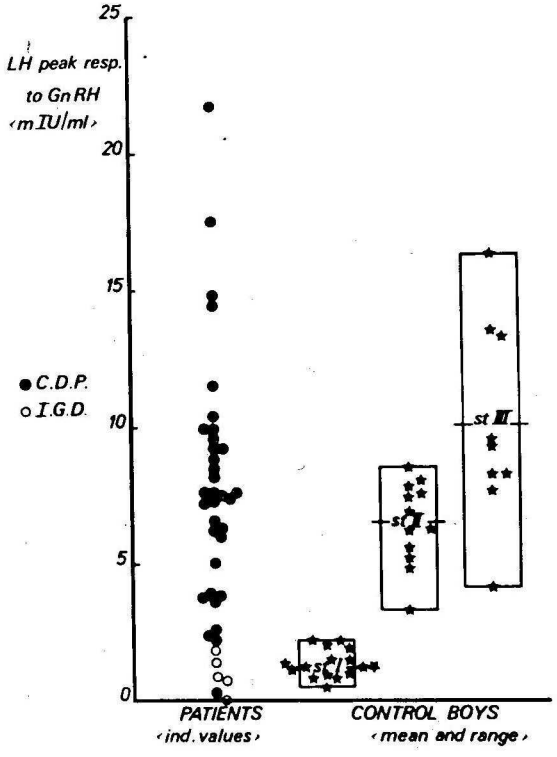

FIG. 2. — Maximal increments of LH following intravenous injection of 25 µg/m² GnRH. Individual values observed in boys with constitutionally delayed puberty (CDP) and isolated gonadotrophin deficiency (IGD) are compared with the mean and range of values found in normal boys at pubertal stage G_1 (n = 16), G_2 (n = 12) and G_3 (n = 9) according to Tanner's standards.

Nine patients had a low LH-response, below the range of the control pubertal boys. Nevertheless, puberty occurred spontaneously in 4 of them showing that they also had CDP.

In the 5 remaining patients with low LH-response to GnRH no signs of puberty appeared. They were considered as having IGD (fig. 2).

Discussion

Several authors have used the GnRH test in delayed sexual maturation, a condition common in boys, in order to distinguish between CDP and hypogonadotrophic hypogonadism, including IGD (3, 13, 22-24, 28, 44).

Since the onset of puberty is characterized by a sharp rise of LH-response to GnRH (16,

23, 40), the usefulness of GnRH test in delayed puberty was stressed by many investigators (3, 13, 22, 24, 28, 44).

However, others considered it of limited value for two reasons. On the one hand, the wide ranges of response to GnRH in normal prepubertal and pubertal boys were found to overlap (25, 31). This, however, could be due

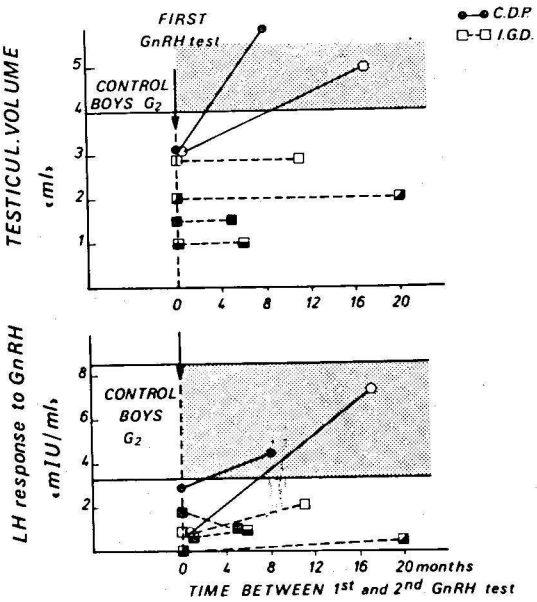

FIG. 3. — In two boys with constitutionally delayed puberty (CDP) and 4 boys with isolated gonadotrophin deficiency (IGD) testicular volume and LH response to 25 μg/m² GnRH were estimated twice. These values are compared with the normal ranges of testicular volumes and LH response (mIU/ml) to GnRH observed after the onset of puberty (stage G_2 according to Tanner's classification). Boys with CDP are aged 16.3 and 16.9 years. Boys with IGD are aged 17.9, 22.5, 15.4 and 14.0 years at the first GnRH test.

to the dose of GnRH used. Kastin *et al.* (29) reported the same response in prepubertal and adult subjects receiving 300 μg GnRH. Job *et al.* (25) found 2 to 3-fold pubertal increase in LH-response using a lower dose of 100 μg GnRH per square meter body surface. Following the injection of only 50 μg GnRH per square meter, Dickerman *et al.* (10) observed an approximatively 5-fold increase in LH-response between stages I and II of puberty. Using only 25 μg GnRH per square meter similar results were obtained in this study.

Thus, low doses of GnRH seem to discriminate better prepubertal from early pubertal boys. The LH-response might also already be increasing at the end of the prepubertal period as shown by Grumbach *et al.* (21). When such subjects are included in a prepubertal control group, their responses lead to a wide scatter which may overlap the pubertal range.

On the other hand, Illig *et al.* (22), Job *et al.* (24) and Dickermann *et al.* (11), showed that patients investigated for delayed puberty generally presented with physical signs of incipient puberty. Finally, the biological signs of hypothalamo-pituitary puberty were found to be inconstant and unreliable in some cases, despite evidence of physical puberty.

The particular features of our survey were the use of a low GnRH dose in physically prepubertal patients and the finding of a pubertal pattern of pituitary responsiveness in CDP, generally preceding the physical onset of adolescence.

The time lag between the test and the onset of puberty was highly variable but generally about 3 months. This pituitary responsiveness in CDP might be a sign of neuroendocrine sexual maturation preceding the gonadal stimulation and the appearance of physical pubertal maturation, as described by Grumbach *et al.* in some normal late prepubertal children (21). A prepubertal hypothalamo-pituitary maturation is also supported by the observation of a prepubertal rise of urinary excretion of GnRH-like material before the increase in gonadotrophin excretion (4).

Furthermore, other findings point to endocrine signs of hypothalamo-pituitary maturation preceding the onset of physical puberty : the fluctuations of basal levels of LH (26), the response to GnRH, to clomiphene administration (44) or to prolonged infusion of GnRH (36). In our experience, the pituitary response to a single low dose of GnRH proved

to be of diagnostic and prognostic value. Still, the fact that a prepubertal LH-response was found in 4 out of 34 patients with CDP demonstrates the limits of our test procedure. Indeed, only the follow-up allowed to distinguish these 4 patients with CDP from patients with gonadotrophin deficiency. Chaussain *et al.* (7) showed the usefulness of GnRH test with a higher dose of 100 $\mu g/m^2$ in hypogonadotrophic hypogonadism. This diagnosis remains, however, particularly difficult in cases of isolated gonadotrophin deficiency without obvious clinical or functional signs of hypothalamo-pituitary failure : the heterogeneity of the response to GnRH observed in hypogonadotrophic eunuchoidism has been emphasized by several authors (2, 5, 8, 41). The diagnosis of IGD was made in the 5 patients with low LH-response to GnRH who remained clinically prepubertal during follow-up. But this diagnosis should remain provisional in boys aged less than 17 years.

When the diagnosis of CDP is suspected on a clinical basis, the finding of a pubertal pattern of LH-response to GnRH allows to reassure the patient and his family. In our patients no hormonal therapy was needed and puberty occurred spontaneously within the following months.

A more awkward situation is that of boys with delayed puberty showing a prepubertal response of LH to GnRH. Although these patients are generally younger than those seen for hypogonadotrophic hypogonadism, diagnosis is difficult and prognosis uncertain. In such cases, retesting with GnRH after six months could be useful. The need for treatment with androgens is to be considered taking into account psychological factors (12, 35) and the possible unfavourable influence on final height.

In conclusion, GnRH testing with a single shot, 25 $\mu g/m^2$ dose, together with the clinical data allow to recognize most cases of CDP. Patients with prepubertal response to GnRH should be carefully followed and further retested before being diagnosed as IGD. Further detailed studies of the time elapsing between hypothalamo-pituitary maturation and physical signs of puberty will help to a better understanding of the pathophysiology of puberty.

*
* *

Acknowledgements. — We are grateful to the paediatricians and physicians who referred their patients to us. We thank Drs R.J. Santen and J.J. Legros for their helpful comments. We have appreciated the expert criticism of the paediatric endocrinologists of the Belgian national committee for the treatment of pituitary dwarfism.

We are indebted to Fr. Henuzet for her excellent technical assistance. We thank Mrs M. Beyer and I. Damseaux for drawing the illustrations and Mrs Y. Vos de Wael for typing the manuscript.

References

1. AUGUST, G.P., GRUMBACH, M.M. and KAPLAN, S.L. Hormonal changes in puberty. III. Correlation of plasma testosterone, LH, FSH, testicular size and bone age with male pubertal development. *J Clin Endocrinol Metab* 34 : 319-326, 1972.
2. BELL, J., SPITZ, I., SLONIM, A., PERLMAN, A., SEGAL, S., PALTI, Z. and RABINOWITZ, D. Heterogeneity of gonadotrophin response to LH-RH in hypogonadotrophic hypogonadism. *J Clin Endocrinol Metab* 36 : 791-794, 1973.
3. BOURGUIGNON, J.P., ERNOULD, CH., BRICHANT, J. and FRANCHIMONT, P. Diagnostic and prognostic interest of LHRH test in delayed puberty in males with a supportive longitudinal clinical study, *in Convegno internazionale di Endocrinologia Pediatrica,* Pacini, Pisa, pp 73-89. 1976.
4. BOURGUIGNON, J.P., HOYOUX, C., REUTER, A. and FRANCHIMONT, P. Urinary excretion of immunoreactive luteinizing hormone releasing hormone-like material and gonadotropins at different stages of life. *J Clin Endocrinol Metab* 48 : 78-84, 1979.
5. BREMNER, W.J., FERNANDO, N.N. and PAULSEN, C.A. The effect of luteinizing hormone-releasing hormone in hypogonadotrophic eunuchoidism. *Acta Endocrinol (Kbh)* 86 : 1-14, 1977.
6. BURR, I.M., SIZONENKO, P.C., KAPLAN, S.L. and GRUMBACH, M.M. Hormonal changes in puberty. I. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size and bone age in normal boys. *Pediatr Res* 4 : 25-35, 1970.
7. CHAUSSAIN, J.L., GARNIER, P.E., BINET, E., VASSAL, J., SCHOLLER, R. and JOB, J.C. Effect of synthetic luteinizing hormone-releasing hormone (LH-RH) on the release of gonadotropins in hypophyso-gonadal disorders of children and adolescents. III. Hypopituitarism. *J Clin Endocrinol Metab* 38 : 58-63, 1974.

8. COSCIA, A.M., FLEISCHER, N., BESCH, P.K., BROWN, L.P. and DESIDERIO, P. The effect of synthetic luteinizing hormone-releasing factor on plasma LH levels in pituitary disease. *J Clin Endocrinol Metab* 38 : 83-88, 1974.
9. COPELAND, K.C., PAUNIER, L. and SIZONENKO, P.C. Secretion of adrenal androgens and growth patterns of patients with hypogonadotrophic hypogonadism and idiopathic delayed puberty. *J Pediatr* 91 : 935-940, 1977.
10. DICKERMAN, Z., PRAGER-LEWIN, R. and LARON, Z. Response of plasma LH and FSH to synthetic LH-RH in children at various pubertal stages. *Am J Dis Child* 130 : 634-638, 1976.
11. DICKERMAN, Z., BAR-HAIM, Y., PRAGER-LEWIN, R., KAUFMAN, H. and LARON, Z. Plasma LH and FSH response to LRH and plasma testosterone levels in boys with irregular puberty. *Acta Endocrinol (Kbh)* 85 : 456-464, 1977.
12. EHRHARDT, A.A. and MEYER-BAHLBURG, H.F.L. Psychological correlates of abnormal pubertal development. *J Clin Endocrinol Metab* 4 : 207-222, 1975.
13. ERNOULD, Chr., BOURGUIGNON, J.P. and FRANCHIMONT, P. Prognostic value of LHRH test in boys with delayed puberty (abstract). *Pediatr Res* 12 : 157, 1978.
14. FRANCHIMONT, P. *Le Dosage des Hormones hypophysaires somatotrope et gonadotropes et son Application en Clinique.* Arscia, Bruxelles, et Maloine, Paris, 1961.
15. FRANCHIMONT, P. Radioimmunoassay of gonadotrophic hormones, *in Protein in polypeptide Hormones,* Excerpta Medica, Amsterdam, p 99, 1968.
16. FRANCHIMONT, P., BECKER, H., ERNOULD, C., THYS, C., DEMOULIN, A., BOURGUIGNON, J.P., LEGROS, J.J. and VALCKE, J.C. Action de l'hormone hypothalamique libérant l'hormone lutéinisante (LH-RH) sur les gonadotrophines chez le sujet normal. *Ann Endocrinol* (Paris) 34 : 477-490, 1973.
17. FRANCHIMONT, P., BECKER, H., ERNOULD, C., THYS, C., DEMOULIN, A., BOURGUIGNON, J.P., LEGROS, J.J. and VALCKE, J.C. The effect of hypothalamic luteinizing hormone-releasing hormone (LH-RH) on plasma gonadotrophin levels in normal subjects. *Clin Endocrinol (Oxf)* 3 : 27-39, 1974.
18. GARNIER, P.E., CHAUSSAIN, J.L., BINET, E., SCHLUMBERGER, A. and JOB, J.C. Effect of synthetic luteinizing hormone-releasing hormone (LH-RH) on the release of gonadotrophins in children and adolescents. VI. Relations to age, sex and puberty. *Acta Endocrinol (Kbh)* 77 : 422-434, 1974.
19. GEUBELLE, F., LAMBRECHTS, L., SABATIER, J. and BALTIA, A. Poids et tailles des enfants et adolescents sains de la province de Liège. *Rev Méd Liège* 29 : 582-591, 1974.
20. GREULICH, W.W. and PYLE, S.I. *Radiographic Atlas of the skeletal Development of the Hand and Wrist,* 2nd ed., Stanford University Press, Stanford, 1959.
21. GRUMBACH, M.M., ROTH, J.C., KAPLAN, S.L. and KELCH, R.P. Hypothalamic-pituitary regulation of puberty in man. Evidence and concepts derived from clinical research, *in The Control of the Onset of* Puberty, (Grumbach, M.M., Grave, G.D. and Mayer, F.E. eds), Wiley, New York, pp 115-181, 1974.
22. ILLIG, R., PLUZNIK, S., WERNER, H. and PRADER, A. The effect of synthetic LHRH on plasma LH and FSH in 92 children with delayed, disturbed or deficient sexual maturation. *Horm Metab Res* 5 : 156-164, 1974.
23. JOB, J.C., GARNIER, P., CHAUSSAIN, J.L., BINET, E., RIVAILLE, P. and MILHAUD, G. Elevation of serum gonadotropin (LH and FSH) after releasing hormone (LH-RH) injection in normal children and in patients with disorders of puberty. *J Clin Endocrinol Metab* 35 : 473-476, 1972.
24. JOB, J.C., CHAUSSAIN, J.L., GARNIER, P.E. and TOUBLANC, J.E. Effect of synthetic lutein'zing hormone-releasing hormone on the release of gonadotropins in hypophysogonadal disorders of children and adolescents. VII. Constitutional delay of puberty in males. *J Pediatr* 88 : 494-498, 1976.
25. JOB, J.C., CHAUSSAIN, J.L. and GARNIER, P.E. The use of luteinizing hormone-releasing hormone in pediatric patients. *Hormone Res* 8 : 171-187, 1977.
26. JOHANSON, A. Fluctuations of gonadotropin levels in children. *J Clin Endocrinol Metab* 39 : 154-159, 1974.
27. KADRNKA LOVRENCIC, M., REINER BANOVAC, Z., OBERITER, V., SEKSO, M., PETEK, M., KSIVANEK SKRABALO, L. and RESETIC, J. Plasma gonadotropin (LH and FSH) concentrations in prepubertal and pubertal children upon stimulation by LH-releasing hormone. *Helv Paediatr Acta* 30 : 232-238, 1975.
28. KADRNKA LOVRENCIC, M., OBERITER, V., SEKSO, M., REINER BANOVAC, Z. and PETEK, M. Growth hormone and gonadotropins (LH and FSH) in children with constitutional delay in growth and maturation. *Helv Paediatr Acta* 33 : 105-116, 1978.
29. KASTIN, A.J., SCHALLY, A.V., SCHALCY, D.S., KORENMAN, S.G., MILLER, M.C., GUAL, C. and PEREZ-PASTEN, E. Characterization of the hormonal response to luteinizing hormone-releasing hormone (LH-RH) in prepubertal and adult subjects. *Pediatr Res* 6 : 481-486, 1972.
30. KELCH, R.P., CLEMENS, L.E., MARKOVS, M., WESTHOFF, M.H. and HAWKINS, D.W. Metabolism and effects of synthetic gonadotropin-releasing hormone (GnRH) in children and adults. *J Clin Endocrinol Metab* 40 : 53-61, 1975.
31. KELCH, R.P., MARKOVS, M. and HUSS, J. LH and FSH responsiveness to intravenous gonadotropin-releasing hormone (GnRH) in children with hypothalamic or pituitary disorders : lack of effect of replacement therapy with human growth hormone. *J Clin Endocrinol Metab* 42 : 1104-1122, 1976.
32. KULIN, H.E. and REITER, E.O. Gonadotropins during childhood and adolescence : A review. *Pediatrics* 51 : 260-271, 1973.
33. KULIN, H.E. and SANTNER, S.J. Timed urinary gonadotropin measurements in normal infants, children and adults, and in patients with disorders of sexual maturation. *J Pediatr* 90 : 760-765, 1977.
34. MARSHALL, W.A. and TANNER, J.M. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45 : 13-23, 1970.
35. PRADER, A. Delayed adolescence. *Clin Endocrinol Metab* 4 : 143-155, 1975.
36. REITER, E.O., ROOT, A.W. and DUCKETT, G.E. The response of pituitary gonadotropes to a constant infusion of luteinizing hormone-releasing hor-

mone (LHRH) in normal prepubertal and pubertal children and in children with abnormalities of sexual development. *J Clin Endocrinol Metab* 43 : 400-411, 1976.

37. ROOT, A.W. Endocrinology of puberty. I. Normal sexual maturation. *J Pediatr* 83 : 1-19, 1973.
38. ROOT A.W. Endocrinology of puberty. II. Aberrations of sexual maturation. *J Pediatr* 83 : 187-200, 1973.
39. ROOT, A.W. and REITER, E.O. Evaluation and management of the child with delayed pubertal development. *Fertil Steril* 27 : 745-755, 1976.
40. ROTH, J.C., GRUMBACH, M.M. and KAPLAN, S.L. Effect of synthetic luteinizing hormone-releasing factor on serum testosterone and gonadotropins in prepubertal, pubertal and adult males. *J Clin Endocrinol Metab* 37 : 680-686, 1973.
41. SANTNER, S.J., KULIN, H.E. and SANTEN, R.J. Usefulness of urinary gonadotropin measurements to assess luteinizing hormone releasing factor (LRF) responsiveness in hypogonadotropic states. *J Clin Endocrinol Metab* 44 : 313-321, 1977.
42. SANTEN, R.J. and KULIN, H.E. Hypogonadotropic hypogonadism and delayed puberty, (in press).
43. SIZONENKO, P.C. Endocrine laboratory findings in pubertal disturbances. *Clin Endocrinol Metab* 4 : 173-206, 1975.
44. SNOEP, M.C., DE LANGE, W.E., SLUITER, W.J. and DOORENBOS, H. Differential response of serum LH in hypogonadotropic hypogonadism and delayed puberty to LH-RH stimulation before and after clomiphene citrate administration. *J Clin Endocrinol Metab* 44 : 603-606, 1977.
45. TANNER, J.M. *Growth at Adolescence,* 2nd ed., Blackwell, Oxford, 1962.
46. TANNER, J.M., WHITEHOUSE, R.H., MARSHALL, W.A., HEALEY, M.J.R. and GOLDSTEIN, H. *Assessment of skeletal Maturity and Prediction of adult Height* (TW2 Method), Academic Press, London, 1975.
47. WAALER, P.E., THORSEN, T., STOA, K.F. and AARSKOG, D. Studies in normal male puberty. *Acta Paediatr Scand* [Suppl] 249 : 1-36, 1974.
48. WINTER, J.S.D. and FAIMAN, C. Serum gonadotropin concentrations in agonadal children and adults. *J Clin Endocrinol Metab* 35 : 561-564, 1972.
49. WINTER, J.S.D. Analysis of clinical studies with LH-RH in children and adolescents. *Am J Dis Child* 130 : 590-592, 1976.
50. ZACHMANN, M., PRADER, A., KIND, H.P., HAFLIGER, H. and BUDLIGER, H. Testicular volume during adolescence. Cross-sectional and longitudinal studies. *Helv Paediatr Acta* 29 : 61-68, 1974.

Correspondence to Chr. Ernould
Clinique Pédiatrique
Hôpital de Bavière
B-4020 Liège (Belgium)