Reprod. Nutr. Dévelop., 1987, **27** (2 B), 533-545.

Plasma somatotropin and somatomedin C concentrations following GRF or TRH injections in newborn calves

Véronique COXAM ([1]), Marie-Jeanne DAVICCO, F. A. OPMEER (*), J. P. RAVAULT (**), J.-P. BARLET

U.A. C.N.R.S., 04 1123, I.N.R.A., Theix, 63122 Ceyrat, France.
(*) *Wilhelmina Kinderziekenhuis, Postbus 18009, 3501 CA Utrecht, The Netherlands,*
(**) *Station de Physiologie de la Reproduction, I.N.R.A., Nouzilly, 37390 Monnaie.*

Summary. Plasma somatotropin (GH) and somatomedin C (IGF_1) concentrations were measured by radioimmunoassay in 3-day old and 10-day old calves intravenously injected with growth hormone releasing factor (GRF) 1-44, GRF 1-29 or thyrotropin-releasing hormone (TRH).

In 3 day old animals the increase in plasma GH concentration was GRF 1-44 dose-related (50, 100, 200 pmoles.kg^{-1} of body wt). In four 10-day old calves injected with the lowest dose, the increase in plasma GH concentration was not different from that observed in four 3-day old animals treated with 200 pmoles.kg^{-1} of body weight. However, the response observed in four 3-day old calves injected with GRF 1-29 (50 pmoles.kg^{-1} of body wt) was not different from that observed following the same treatment in four 10-day old calves.

In four 3-day old calves TRH (10 nmoles.kg^{-1} of body wt) induced a significant rise in plasma GH, prolactin (Prl), thyroxine (T_4) and triiodothyronine (T_3) concentrations. The same dose of TRH injected into four 10-day old calves elicited a similar rise in plasma T_3 and T_4 concentrations, but plasma GH and Prl increased less than in 3-day old animals.

In three 3-day old or 10-day old calves born spontaneously before term (258-260 days of gestation), the increase in plasma Prl and GH concentrations following TRH was not different from that observed in mature calves of the same postnatal age.

Neither GRF 1-44, GRF 1-29 nor TRH elicited any significant change in plasma IGF_1, insulin or glucose concentration in any group of calves.

Introduction.

We studied the regulation of somatotropin (GH) secretion in our work on the endocrine regulation of bone formation in calves during the perinatal period. Although GH may stimulate longitudinal bone growth in hypophysectomized rats (Isaksson, Jansson and Gause, 1982 ; Russel and Spencer, 1985), most of its effects on bone growth are mediated through somatomedin (IGF_1) (Canalis, 1983). This is a single-chain peptide, isolated from human and bovine plasma (Honneger and Humbel, 1986) and actively stimulating the growth of hypophysectomized rats (Schoenle *et al.*, 1982).

([1]) To whom correspondence should be addressed.

The availability of synthetic growth hormone-releasing factor (GRF) has opened a new era of investigation of the regulation of GH secretion (Wehrenberg *et al.*, 1985). Synthetic GRF and thyrotropin-releasing hormone (TRH) have been used to stimulate GH secretion in somatotrophs of various species both *in vitro* and *in vivo* (McGuffey, Thomas and Convey, 1977 ; Schanbacher, 1986). However, to our knowledge, nobody has studied the influence of GRF or TRH on both plasma GH and IGF_1 concentrations, especially during the postnatal period. This was the aim of the present work in newborn calves.

Material and methods.

Animals and treatments. — We used twenty-four 3-day old and sixteen 10-day old Holstein × Friesian male calves born spontaneously at term (278-280 days of gestation) and weighing 44 ± 2 kg at birth. Six premature twin calves of the same genotype, born spontaneously between days 258 and 260 of gestation (birthweight : 24 ± 3 kg), were also used 3 and 10 days after delivery. Each calf born at term or before was fed and managed as previously described (Richet *et al.*, 1985). The hormones were injected quickly through an indwelling catheter inserted into the right jugular vein on the day before the experiment. Serial blood samples were obtained through a catheter inserted into the left jugular vein. Blood was collected in EDTA-coated tubes containing a protease inhibitor (1.4 mg of EDTA and 10.000 KI units per ml of blood).

Synthetic human pancreatic GRF 1-44 was synthetized by the solid-phase method and purified using HPLC (Ohashi *et al.*, 1983). Synthetic GRF 1-29 (human amide) and TRH were purchased from Bachem (Saffron Walden, Essex, England). Five groups of four 3-day old or 10-day old calves, born spontaneously at term, were injected with GRF 1-44 (50, 100 or 200 pmoles.kg^{-1} of body weight), GRF 1-29 (50 pmoles.kg^{-1} of wt) or TRH (10 nmoles.kg^{-1} of wt). Premature twin calves were also injected in the same way with the same dose of TRH on days 3 and 10 after birth. Control animals were injected with the same volume (0.2 ml.kg^{-1} of body wt) of solvent (0.9 % NaCl containing 0.5 % bovine serum albumin). Each calf was fasted for 16 h before injection ; each injection was given at 9 a.m. to avoid any influence of injection time on animal response (Chihara *et al.*, 1983).

Assay. — Plasma GH concentration was determined using an heterologous radioimmunoassay (RIA). In our experimental conditions, the cross-reactivity of the antibody (NIADDK-anti oGH-2) with bovine GH (bGH) was 88 %. No significant cross-reaction was observed with highly purified bovine prolactin (NIH PB2), purified bovine thyroid stimulating hormone (NIAMDD-bTSH) or purified bovine chorionic somatomammotropin (gift of Professor J. F. Beckers, Brussels, Belgium). The standard and dilution curves were parallel. All values lower than 0.02 pmole were considered as not detectable. Within-assay variation was 5.5 % and interassay variation was 6.2 %. Each determination was made in triplicate.

The amino acid sequence of bovine IGF_1 is identical to that of human IGF_1 (Honneger and Humbel, 1986) ; therefore plasma IGF_1 concentrations were mea-

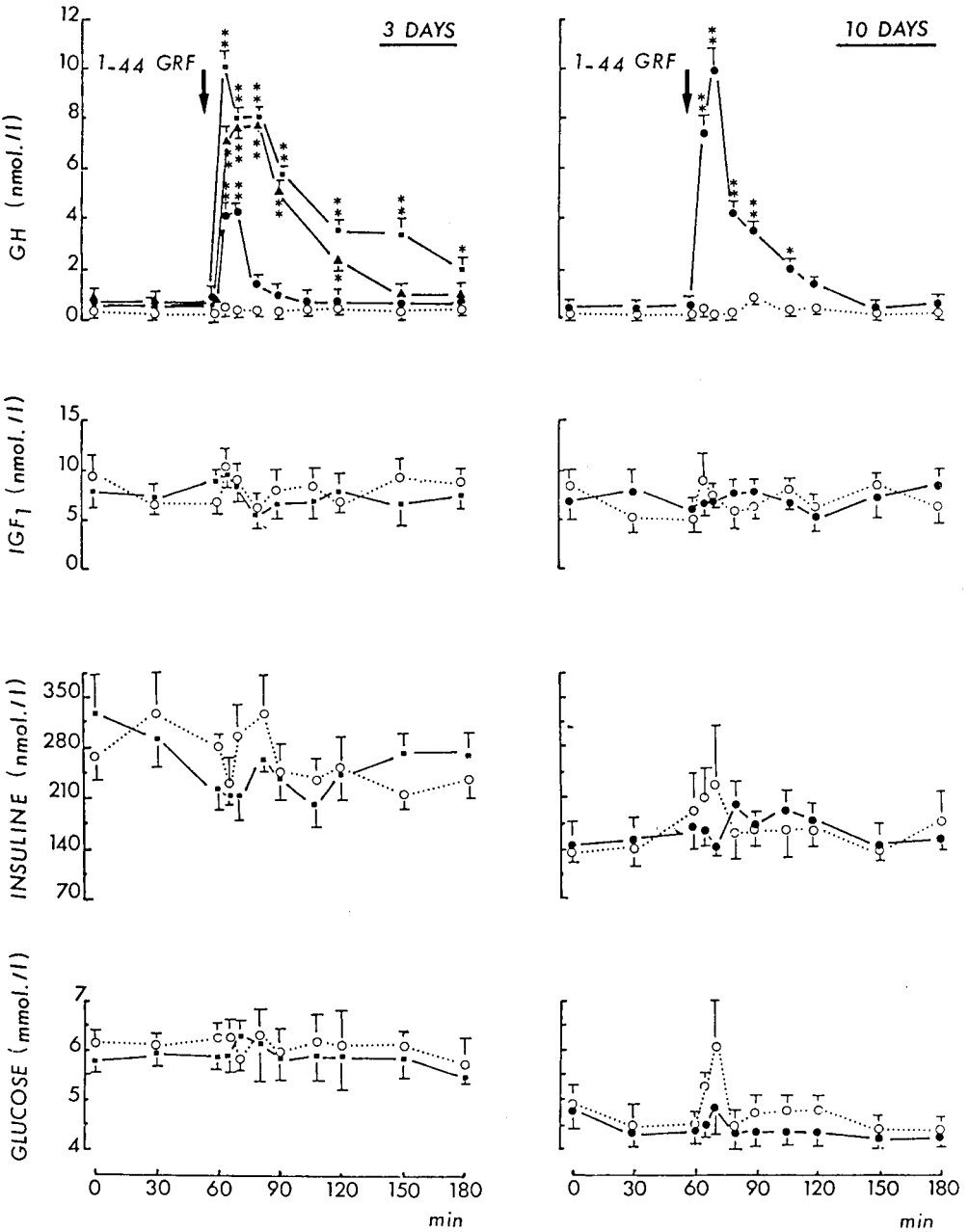


FIG. 1. — *Influence of 1-44 GRF* (●——●, 50 pmoles.kg^{-1}b wt ; ▲——▲, 100 pmoles.kg^{-1}b wt ; ■——■, 200 pmoles.kg^{-1}b wt) *on plasma growth hormone* (GH), *somatomedin C* (IGF_1), *insulin and glucose concentrations measured in 3-day-old and 10-day-old calves* [mean ± SEM, * $P < 0.05$, ** $P < 0.01$, comparison with control animals (○···○), time of injection is indicated by an arrow].

sured by RIA using a non-equilibrium double antibody system for human plasma (Zapf, Walter and Froesch, 1981). To dissociate and separate the IGF_1 from its carrier protein, calf plasma was mixed with equal volumes of 0.1 M glycyl-glycine HCl buffer and incubated in stoppered glass tubes for 48 h at a final pH of 3.8 and at 37 °C. After incubation, the samples were neutralized with small volumes (about 0.3 % of incubation volume) of 1 M NaOH (Underwood *et al.*, 1982) and assayed for IGF_1. In our conditions, within-assay variation was 8 % and interassay variation was 12 %. The minimum detectable amount was less than 2 nmoles.l^{-1}. Parallelism between standard and dilution curves was verified using synthetic IGF_1 (Ciba-Geigy, Basel, Switzerland).

Plasma prolactin (Prl) concentration was determined by double antibody RIA (Lacroix, Ravault and Pelletier, 1977). Plasma T_4 and T_3 concentrations were determined by RIA using T_3 (Trik) and total T_4 (Tetrak) RIA kits from the French Atomic Energy Commission (Davicco *et al.*, 1982). Plasma insulin concentration was measured by RIA (Richet *et al.*, 1985).

Plasma glucose concentration was estimated colorimetrically using the glucose oxidase method (Richet *et al.*, 1985).

The results were expressed as the mean ± SEM. The areas under the curves were calculated after subtracting the basal level before injection ; these levels were remarkably stable in each group of fasting animals (Coxam *et al.*, 1987). The Mann-Whitney U-test was used to compare between-group differences.

Results.

In six 3-day old calves, GRF 1-44 (50 pmoles.kg^{-1} of body wt) increased plasma GH concentration (nmoles.l^{-1}) from 0.5 ± 0.08 at time 0 to 3.5 ± 0.9 ($P < 0.01$) five min after injection. Plasma GH concentrations increased progressively ($P < 0.01$) with doses of 50, 100 or 200 pmoles.kg^{-1} of body weight to reach peak concentrations of 3.9 ± 0.9, 7.2 ± 0.6 and 10.7 ± 1.0, respectively, 10 min after injection (fig. 1). Similarly, the area under the GH curve increased progressively ($P < 0.01$) with doses of GRF 1-44. In six 3-day old calves injected with GRF 1-29 (50 pmoles.kg^{-1} of body wt) the highest plasma GH concentration at 10 min post injection (3.5 ± 0.5) was not different from that observed in 3-day old calves injected with the same dose of GRF 1-44. The time required to return to basal GH level after injection was 90 and 105 min in calves injected with GRF 1-44 and GRF 1-29, respectively. However, maximal GH response to the same dose of GRF 1-44 or GRF 1-29 (50 pmoles.kg^{-1} of body wt) in 10-day old calves was 2.2 ± 0.2 nmoles.l^{-1} or 9.9 ± 2.6 nmoles.l^{-1} ($P < 0.01$), respectively (figs. 1, 2). Neither GRF 1-44 nor GRF 1-29 had any significant effect on plasma IGF_1 (mean value for 3 h : 7.9 ± 0.5 or 9.5 ± 0.6 nmoles.l^{-1}), insulin (157.4 ± 80 or 131.5 ± 6.3 nmoles.l^{-1}) or glucose concentration.

In four 10-day old calves injected with GRF 1-44 (50 pmoles.kg^{-1} of body wt), the highest value measured for plasma GH concentration (9.9 ± 2.6) was not different from that (9.8 ± 1.0) observed in 3-day old calves injected with a fourfold dose (200 pmoles.kg^{-1} of body wt). In these 10-day old animals, GRF 1-44 had no significant effect on plasma IGF_1 (6.9 ± 0.3 and 7.2 ± 0.5 nmoles.l^{-1},

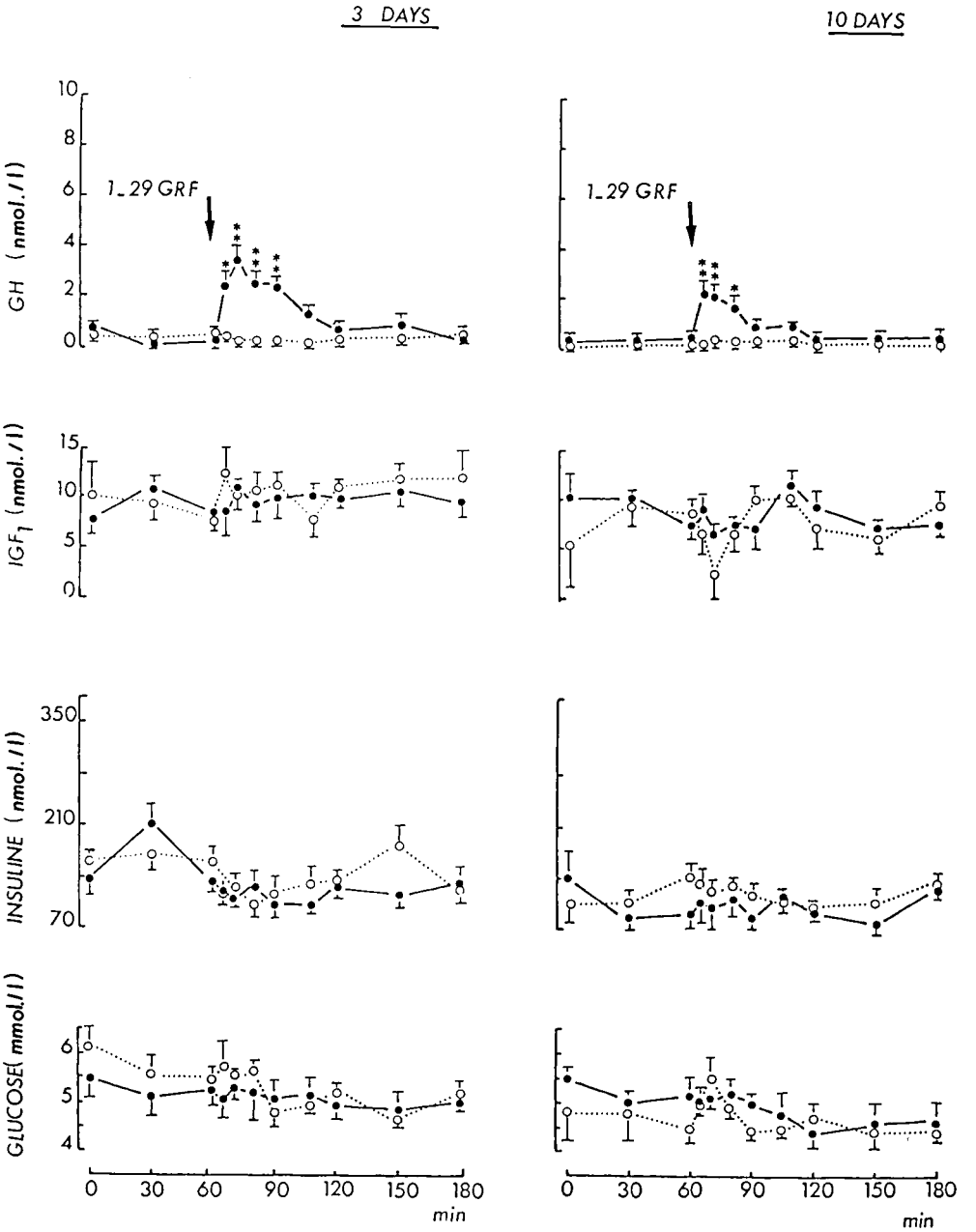


FIG. 2. — *Influence of 1-29 GRF* (●——●, 50 pmoles.kg^{-1}b wt) *on plasma growth hormone* (GH), *somatomedin C* (IGF_1), *insulin and glucose concentrations measured in 3-day-old and 10-day-old calves* [mean ± SEM ; * $P < 0.05$, ** $P < 0.01$, comparison with control animals (○···○), time of injection is indicated by an arrow].

respectively), insulin (159.2 ± 5.5 and 112.1 ± 6.1 nmoles.l^{-1}, respectively) or glucose (4.6 ± 0.1 and 4.8 ± 0.1 nmoles.l^{-1}, respectively) concentration.

In four 10-day old calves plasma GH and IGF_1 response to GRF 1-29 (50 pmoles.kg^{-1} of body wt) was not different from that observed in 3-day old animals receiving the same dose of this peptide (fig. 2).

In six 3-day old calves born spontaneously at term and injected with TRH (10 nmoles.kg^{-1} of body wt), plasma GH concentration increased from 0.4 ± 0.1 to 1.7 ± 0.3 nmoles.l^{-1} five min after injection ($P < 0.01$) and remained stable for at least 2 h. In these animals, plasma Prl increased from 0.5 ± 0.1 to 62 ± 14 nmoles.l^{-1} ($P < 0.01$) in the same way. Plasma T_3 and T_4 levels increased significantly only at 105 and 120 min post injection, respectively. In these animals, TRH had no significant effect on plasma IGF_1 (9.8 ± 0.6 nmoles.l^{-1}) or insulin (131.7 ± 7.2 nmoles.l^{-1}) concentration.

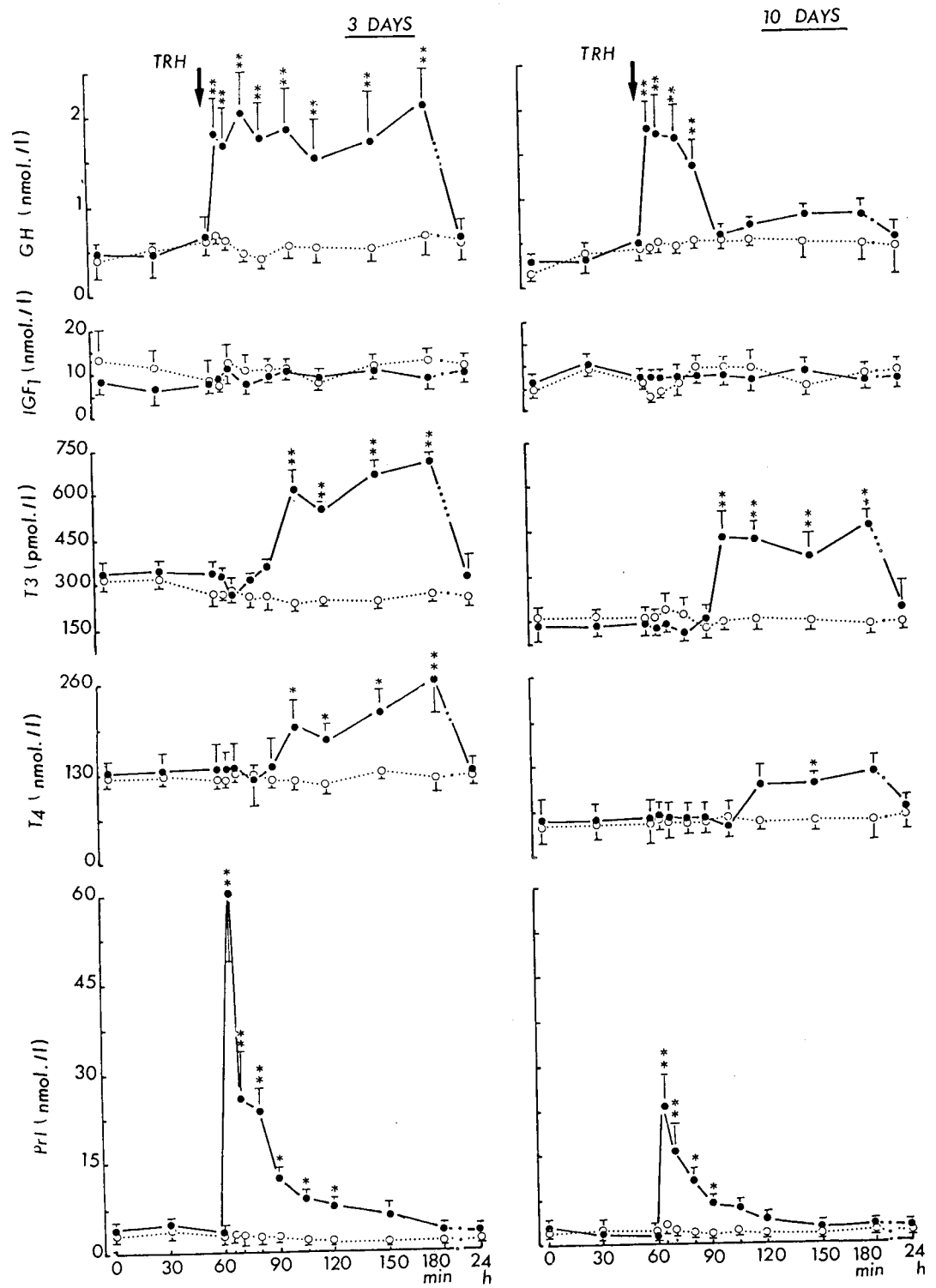
In 10-day old calves born spontaneously at term, the increase in plasma GH, Prl, T_3 and T_4 concentrations after TRH was less intense ($P < 0.05$) and shorter ($P < 0.05$) than in 3-day old calves injected with the same dose of TRH. In both groups of calves, plasma IGF_1 concentration was unchanged after TRH (fig. 3).

The increase in plasma GH concentration observed in premature 3-day old or 10-day old calves injected with TRH (10 nmoles.kg^{-1} of body wt) was not different from that observed in mature calves of the same postnatal age. However, the increase in plasma Prl, T_3 and T_4 concentrations following TRH was less intense ($P < 0.01$; $P < 0.05$; $P < 0.05$, respectively) than in calves born spontaneously at term. In these premature calves, plasma IGF_1 concentrations were unchanged TRH at 3 days (6.0 ± 0.6 nmoles.l^{-1}) as well as at 10 days (10.3 ± 0.6 nmoles.l^{-1}) (figs. 3, 4).

Discussion.

GRF has been used to stimulate GH secretion in farm animals (Johke *et al.*, 1984 ; McCutcheon *et al.*, 1984 ; Moseley *et al.*, 1984 ; Hodate, Johke and Ohaschi, 1985) so as to increase milk yield in cows (Enright *et al.*, 1985 ; Lapierre *et al.*, 1985) and ewes (Hart *et al.*, 1985). Similarly, when GRF 1-29 was injected intravenously (0.2 nmoles.kg^{-1} of body wt) six times per day for 10 days, cow milk yield increased by 16.1 % (Petitclerc *et al.*, 1985). TRH increased plasma GH concentration in both 45-day old calves (McGuffey, Thomas and Convey, 1977) and lactating cows (Convey *et al.*, 1973). These results demonstrated that both TRH and GRF could stimulate GH secretion in cattle, but nobody had studied the influence of these peptides on GH and IGF_1 release during the postnatal period in calves. It has been demonstrated that plasma GH response to GRF 1-44 in bovi-

FIG. 3. — *Influence of TRH* (●——● 10 nmoles.kg^{-1}b wt) *on plasma growth hormone* (GH), *somatomedin* C (IGF_1), *triiodothyronine* (T3), *thyroxine* (T4) *and prolactin* (Prl) *concentrations in : 3-day-old and 10-day-old calves born spontaneously at term* [mean ± SEM, * $P < 0.05$, ** $P < 0.01$, comparison with control animals (○···○), time of injection is indicated by an arrow].


3 DAYS
10 DAYS
TRH
TRH
GH (nmol./l)
IGF1 (nmol./l)
T3 (pmol./l)
T4 (nmol./l)
Prl (nmol./l)
0
30
60
90
120
150
180
min
24
h

nes decreases with age : the response observed in 4-month old calves was more intense than that measured in heifers or cows (Johke *et al.,* 1984). In these animals as well as in our calves, GRF induced a marked rise in plasma GH concentration at all the doses studied (20 to 200 nmoles.kg^{-1} of body wt), and this elevation occurred within 10 min after injection (Johke *et al.,* 1984) (figs. 1, 2). The addition of GRF 1-44 at a concentration of 1 pmole.ml^{-1} to cultured somatotrophs from 28-day old rats causes a 3.2-fold increase in the GH content of the medium. The same dose of GRF added to cultured pituitary cells from adult rats results in only a 2.3-fold increase in GH content (Niimi *et al.,* 1985). Pituitary responsiveness to GRF 1-40 is higher in infant than in postweaning rats (Cella *et al.,* 1985). In the same way, somatotrophs from newborn rats are more sensitive to GRF 1-40 and dibutryl cyclic AMP than those from 2 to 3-month old rats (Szabo and Cuttler, 1986).

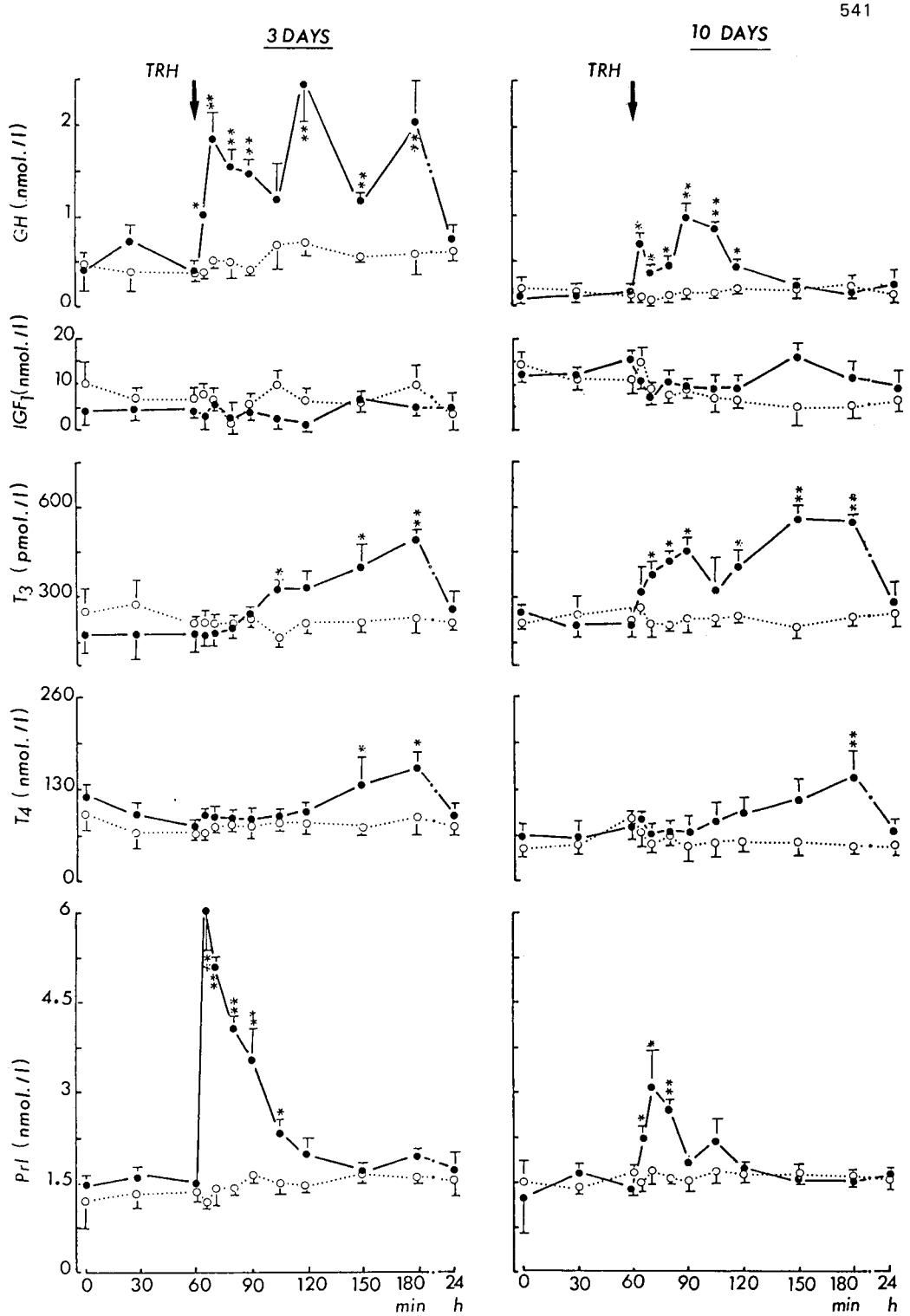
However, Shimano *et al.* (1985) observed no difference when comparing response to GRF in babies less than 7 days old to that in neonates older than 8 days. In our experimental conditions, the increase in plasma GH concentration following the same dose (50 pmoles.kg^{-1} of body wt) of GRF 1-44 was more intense in 10-day old calves than in 3-day old ones (fig. 1), indicating a difference between ovine and bovine somatotrophs : lamb response to GRF 1-44 falls with advancing gestation and is further reduced after birth (Gluckman, 1984a). Thus, the drop in plasma GH concentration observed during the perinatal period in lambs would partly result from the altered responsiveness of the anterior pituitary to GRF (Gluckman, 1984b). Such a decrease in plasma GH concentration was not observed in newborn calves but occurred between the second and tenth days before parturition in foetal calves chronically catheterized *in utero* (Coxam, unpublished data).

The increased response to GRF 1-44 observed in our calves between 3 and 10 days post partum (fig. 1) might result from an increased number and/or maturation of somatotropic receptors to GRF. However, the response to the lowest dose of GRF 1-44 (50 pmoles.kg^{-1} of body wt) in 10-day old calves was not different from that observed with 200 pmoles.kg^{-1} of body weight in 3-day old animals (fig. 1). Thus the number of receptors probably did not increase between 3 and 10 days of age since the GH response of 3-day old calves was proportional to the dose of GRF injected (fig. 1). Another possibility is that the increase in plasma protein concentration during the first postnatal week in calves (Brambell, 1958) augments GRF specific binding to bovine somatotrophs, as previously shown *in vitro* (Velicelebi, Santacroce and Harpold, 1985).

In our animals, TRH quickly increased (within 5 min) plasma GH concentration for at least 2 h in 3-day old calves born spontaneously at term (fig. 3) or before term (fig. 4). No significant difference in plasma GH response to TRH was

---

FIG. 4. — *Influence of TRH* (●——● 10 nmoles.kg^{-1}b wt) *on plasma growth hormone* (GH), *somatomedin C* (IGF_1), *triiodothyronine* (T3), *thyroxine* (T4), *and prolactin* (Prl) *concentrations in 3-day-old and 10-day-old premature calves* [mean ± SEM, * $P < 0.05$, ** $P < 0.01$, comparison with control animals (○···○), time of injection is indicated by an arrow].


3 DAYS
10 DAYS
TRH
TRH
GH (nmol./l)
IGF1 (nmol./l)
T3 (pmol./l)
T4 (nmol./l)
Prl (nmol./l)
0
30
60
90
120
150
180
24
min
h

found between these two groups (figs. 3, 4). In these TRH-injected calves, the increase in plasma T_3 and T_4 concentrations occurred after that observed in plasma GH and Prl, suggesting a direct effect of TRH on GH and Prl cells (figs. 3, 4). This hypothesis is strengthened by the fact that the increase in plasma T_3 and T_4 concentrations after TRH was lower in preterm than in full-term calves, while plasma GH response was similar in both groups (figs. 3, 4).This might be due to the shorter half-life of TRH in the last group ; TRH-degrading activity in rat plasma increases during the first days of postnatal life (Neary *et al.,* 1978) and thyrotropin response to TRH in lambs decreases during the first three weeks of life (Klein and Fisher, 1980). This decrease might also be explained by changes in pituitary somatotroph and lactotroph TRH receptor or post-receptor response ; the negative feedback system controlling thyrotropin release following TRH has been shown to be immature in ovine thyrotrophs during the perinatal period (Klein and Fisher, 1980).

In our experimental conditions, the increase in plasma GH concentration induced by GRF or TRH was never associated with significant changes in plasma IGF_1, glucose or insulin concentration (figs. 1, 2, 3, 4). Similar results for glucose and insulin have already been reported in goats injected with GRF 1-44 (Hart *et al.,* 1984). Hypophysectomy in foetal lambs reduced (Van Vliet *et al.,* 1982) plasma somatomedin C activity or had no effect (Gluckman and Butler, 1983). In these animals, the increase in plasma IGF_1 around birth is associated with a decrease in plasma GH concentration (Gluckman and Butler, 1983). Thus, even though somatomedin-like activity in the plasma of adult ruminants is related to growth rate and plasma GH (Davis, Hossner and Ohlson, 1984), plasma IGF_1 concentration in newborn calves seems to depend on factors other than plasma GH concentration alone.

In *conclusion,* our results demonstrate that GRF and TRH are potent secretagogues for GH in newborn calves. However, as demonstrated by the differences observed after treatment in 3-day old and 10-day old calves, the normal GH regulatory mechanisms do not seem fully developed during the first week of postnatal life. In these animals, short-term variations in plasma GH concentration do not affect plasma IGF.

12e Réunion du groupe Développement I.N.R.A., Montpellier, 28-30 mai 1986.

Acknowledgements. — The radioimmunoassay kit for bovine GH and the antibody against IGF_1 were supplied by NIADDK. The IGF_1 and the bovine chorionic somatomammotropin were gifts of Professor J. Nüesch (Ciba-Geigy, Basel) and Professor J. F. Beckers, respectively. The GRF 1-44 was a gift of Dr. T. Johke and Dr. S. Ohashi (Ibaraki, Japan).

Résumé. *Influence d'une injection intraveineuse de GRF ou de TRH sur les concentrations plasmatiques de GH et d'IGF_1 chez le veau nouveau-né.*

Les concentrations plasmatiques de GH et d'IGF_1 ont été mesurées par radioimmunologie chez des groupes de 4 veaux mâles Holstein × Frisons, ayant reçu une injection intraveineuse de GRF 1-44, GRF 1-29 ou TRH, 3 jours ou 10 jours après la naissance.

Chez les veaux de 3 jours, l'augmentation de la concentration plasmatique de GH consécutive au GRF 1-44 est proportionnelle à la dose, pour des doses de 50, 100 et 200 pmoles.kg^{-1} PV. Chez 4 veaux de 10 jours l'élévation de la GH plasmatique consécutive à la plus faible de ces trois doses de GRF n'est pas différente de celle mesurée chez des veaux de 3 jours injectés avec la plus forte de ces trois doses.

Chez les veaux de 3 jours une injection de TRH (10 nmoles.kg^{-1} PV) induit une élévation significative des concentrations plasmatiques de GH, prolactine, T_4 et T_3. La même dose de TRH injectée à des veaux de 10 jours produit un effet identique sur la T_4 et la T_3, mais l'élévation de la somatotropinémie et de la prolactinémie est plus faible chez ceux-ci que chez les veaux de 3 jours.

Chez 6 veaux jumeaux de même génotype nés spontanément à 258-260 jours de gestation l'élévation des concentrations plasmatiques de GH et de prolactine consécutive à une injection de la même dose de TRH effectuée le 3e ou le 10e jour postnatal n'est pas différente de celle observée aux mêmes âges, dans les mêmes conditions, chez les veaux nés à terme.

Le GRF 1-44, le GRF 1-29, le TRH n'induisent aucune variation significative des concentrations plasmatiques de glucose, insuline et IGF_1 chez aucun lot de veaux.

References

BRAMBELL F. W. R., 1958. The passive immunity of the young mammal. *Biol. Rev.*, **33**, 488-531.

CANALIS E., 1983. The hormonal and local regulation of bone formation. *Endocrine Rev.*, **4**, 62-77.

CEDA G. P., HOFFMAN A. R., SILVERBERG G. D., WILSON D. M., ROSENFELD R. G., 1985. Regulation of growth hormone release from cultured pituitary adenomas by somatomedins and insulin. *J. clin. Endocrinol. Metab.*, **60**, 1204-1209.

CELLA S. G., LOCATELLI V., DE GENNARO V., PUGGIONI R., PINTO C., MULLER E. E., 1985. Human pancreatic growth hormone (GH)-releasing hormone stimulates GH synthesis and release in infant rats. An *in vivo* study. *Endocrinology*, **116**, 574-577.

CHIHARA K., MINAMITANI N., KAJI H., KODAMA H., KITA T., FUJITA T., 1983. Human pancreatic growth-hormone-releasing factor stimulates release of growth hormone in conscious unrestrained male rabbits. *Endocrinology*, **113**, 2081-2085.

CONVEY E. M., TUCKER H. A., SMITH V. G., ZOLMAN J., 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. *Endocrinology*, **92**, 471-476.

COXAM V., DAVICCO M. J., ROBELIN J., BARLET J. P., 1987. Growth hormone secretory pattern and somatomedin C plasma concentration in newborn calves. *J. dev. Physiol.*, (in press).

DAVICCO M.-J., VIGOUROUX E., DARDILLAT C., BARLET J. P., 1982. Thyroxine, thyriiodothyronine and iodide in different breeds of newborn calves. *Reprod. Nutr. Dévelop.*, **22**, 355-362.

DAVIS S. L., HOSSNER K. L., OHLSON D. L., 1984. Endocrine regulation of growth in ruminants, 151-183. In ROCHE J. F., O'CALLAGHAN D., *Manipulation of growth in farm animals*, Martinus Nisjhoff Publ., Amsterdam.

ENRIGHT W. J., CHAPLIN L. T., MOSELEY W. M., TUCKER H. A., 1985. Growth-hormone — releasing factor (GRF) stimulates milk production in Holstein cows. *Fed. Proc.*, **44**, 1358.

GLUCKMAN P. D., 1984a. Changing responsiveness to growth hormone releasing factor in the perinatal sheep. *J. develop. Physiol.*, **6**, 509-516.

GLUCKMAN P. D., 1984b. Functional maturation of the neuroendocrine axis in the perinatal period : studies of the somatotropic axis in the ovine fetus. *J. Develop. Physiol.*, **6**, 301-312.

GLUCKMAN P. D., BUTLER J. H., 1983. Parturition-related changes in insulin-like growth factors. I and II in the perinatal lamb. *J. Endocr.*, **99**, 223-232.

HART I. C., CHADWICK P. M. E., JAMES S., SIMMONDS A. D., 1985. Effect of intravenous bovine growth hormone or human pancreatic growth hormone-releasing factor on milk production and plasma hormones and metabolites in sheep. *J. Endocr.*, **105**, 189-196.

HART I. C., JAMES S., PERRY B. N., SIMMONDS A. D., 1984. Effect of intravenous administration of growth hormone-releasing factor (hp GRF-44) and Tyr-D-Trp-Ala-Trp-D-Phe-NH_2 on plasma hormones and metabolites in goats. *J. Endocr.*, **103**, 173-178.

HODATE K., JOHKE T., OHASHI S., 1985. Growth hormone, thyrotropin and prolactin responses to simultaneous administration of human growth hormone-releasing factor and thyrotropin releasing hormone in the bovine. *Endocrinol. japon.*, **32**, 375-383.

HONNEGER A. M., HUMBEL R. E., 1986. Insulin-like growth factors I and II in fetal and adult bovine serum. Purification, primary structures and immunological cross-reactivities. *J. biol. Chem.*, **261**, 569-575.

ISAKSSON O. G. P., JANSSON J. O., GAUSE I. A. M., 1982. Growth hormone stimulates longitudinal bone growth directly. *Science*, **216**, 1237-1239.

JOHKE T., HODATE K., OHASHI S., SHIRAKI M., SAWANO S., 1984. Growth hormone response to human pancreatic growth hormone releasing factor in cattle. *Endocrinol. japon.*, **31**, 55-61.

KLEIN A. H., FISHER D. A., 1980. Thyrotropin-releasing hormone stimulated pituitary gland responsiveness and 3,5,3′-triiodothyronine suppression in fetal and neonatal lambs. *Endocrinology*, **106**, 697-701.

LACROIX A., RAVAULT J. P., PELLETIER J., 1977. Plasma prolactin variations in the male calf in relation to age, season and breed. *Ann. Biol. anim. Bioch. Biophys.*, **17**, 1095-1099.

LAPIERRE L., PELLETIER G., PETITCLERC D., MORISSET J., COUTURE Y., BRAZEAU P., 1985. Effects of human growth-hormone-releasing factor 44-NH_2 (hGRF) on bovine growth hormone (bGH) release and milk production in lactating dairy cows. *Fed. Proc.*, **44**, 1358.

McCUTCHEON S. N., BAUMAN D. E., MURPHY W. A., LANCE V. A., COY D. H., 1984. Effect of synthetic human pancreatic growth-hormone-releasing factors on plasma growth hormone concentrations in lactating cows. *J. Dairy Sci.*, **67**, 2881-2886.

McGUFFEY R. K., THOMAS J. W., CONVEY E. M., 1977. Growth, serum growth hormone, thyroxine, prolactin and insulin in calves after thyrotropin releasing hormone or 3-methyl-thyrotropin releasing hormone. *J. anim. Sci.*, **44**, 422-430.

MOSELEY W. M., KRABILL L. F., FRIEDMAN A. R., OLSEN R. F., 1984. Administration of synthetic human pancreatic growth hormone-releasing factor for five days sustains raised serum concentration of growth hormone in steers. *J. Endocr.*, **104**, 433-439.

NEARY J. T., KIEFFER J. D., NAKAMURA C., MOVER H., SOODAK M., MALOOF F., 1978. The developmental pattern of thyrotropin-releasing hormone — degrading activity in the plasma of rats. *Endocrinology*, **103**, 1849-1854.

NIIMI M., TAKAHARA J., KAWANISHI K., IRINO S., 1985. Effects of age on GH responses to growth hormone-releasing factor in rats. *Endocrinol. japon*, **32**, 559-562.

OHASHI S., SHIRAKI M., SAWANO S., OZAKI S., MORI M., TAKAOKA S., 1983. Synthesis of a human pancreatic growth hormone releasing factor and its growth hormone releasing activity. *Folia endocrinol. japon*, **59**, 604.

PETITCLERC D., PELLETIER G., LAPIERRE H., GAUDREAU P., COUTURE Y., MORISSET J., BRAZEAU P., 1985. Use of human growth hormone-releasing factor end [1-29]-NH_2 analogue on growth hormone release and milk production in dairy cattle, 343-358. In JOYEAUX A., LEYGUE G., MORRE M., RONCUCCI R., SCHMELCK P. H. *Production d'agents thérapeutiques par génie génétique. Exemple de l'hormone de croissance et de son facteur de libération*, Medsi, Paris.

RICHET E., DAVICCO M. J., DALLE M., BARLET J. P., 1985. Réponses endocrines à l'insuline et âge conceptionnel chez le Veau. *Reprod. Nutr. Dévelop.*, **25**, 427-438.

RUSSEL S. M., SPENCER E. M., 1985. Local injections of human or rat growth hormone or of purified human somatomedin-C stimulate unilateral tibial epiphyseal growth in hypophysectomized rats. *Endocrinology*, **116**, 2563-2567.

SCHANBACHER B. D., 1986. Growth hormone releasing factor (GRF) : Physiological and immunological studies. In BUTTERY P., HAYNES N. B., *Manipulation of growth in farm animals*, Butterworths, London (In press).

SCHOENLE E., ZAPF J., HUMBEL R. E., FROESCH E. R., 1982. Insulin-like growth factor I stimulates growth in hypophysectomized rats. *Nature*, **296**, 252-254.

SHIMANO S., SUZUKI S., NAGASHIMA K., YAGI H., SAKAGUCHI M., KUROUME T., 1985. Growth hormone responses to growth hormone releasing factor in neonates. *Biol. Neonate*, **47**, 367-370.

SZABO M., CUTTLER L., 1986. Differential responsiveness of the somatotroph to growth hormone-releasing factor during early neonatal development in the rat. *Endocrinology*, **118**, 69-73.

UNDERWOOD L. E., D'ERCOLE A. J., COPELAND K. C., VAN WYK J. J., HURLEY T., HANDWERGER S., 1982. Development of a heterologous radioimmunoassay for somatomedin C in sheep blood. *J. Endocr.*, **93,** 31-39.

VAN VLIET G., STYNE D. M., KITTERMAN J. A., RUDOLPH A. M., KAPLAN S. L., GRUMBACH M. M., 1982. Plasma immunoreactive somatomedin C (IR-SMC) in the ovine fetus : ontogeny, role of the pituitary, liver and placenta. *Endocrinology,* **110,** 181A (Abstr.).

VELICELEBI G., SANTACROCE T. M., HARPOLD M. M., 1985. Specific binding of synthetic human pancreatic growth hormone releasing factor (1-40-OH) to bovine anterior pituitaries. *Biochem. biophys. Res. Commun.,* **126,** 33-39.

WEHRENBERG W. B., BAIRD A., ZEYTIN F., ESCH F., BOHLEN P., LING N., YING S. Y., GUILLEMIN R., 1985. Physiological studies with somatocrinin, a growth hormone-releasing factor. *Ann. Rev. Pharmacol. Toxicol.,* **25,** 463-483.

ZAPF J., WALTER H., FROESCH E. R., 1981. Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. *J. clin. Invest.,* **68,** 1321-1330.