



Effect of 17 p estradiol on prolactin secretion and thyroliberin responsiveness in two rat prolactin continuous cell lines. Definition of an experimental model

Nicole Brunet, Danièle Gourdj, Marie-Françoise Moreau, D. Grouselle, Françoise Bournaud, Andrée Tixier-Vidal, Nicole Baldet

► To cite this version:

Nicole Brunet, Danièle Gourdj, Marie-Françoise Moreau, D. Grouselle, Françoise Bournaud, et al.. Effect of 17 p estradiol on prolactin secretion and thyroliberin responsiveness in two rat prolactin continuous cell lines. Definition of an experimental model. *Annales de biologie animale, biochimie, biophysique*, 1977, 17 (3B), pp.413-424. hal-00897181

HAL Id: hal-00897181

<https://hal.science/hal-00897181>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Effect of 17 β estradiol on prolactin secretion
and thyroliberin responsiveness
in two rat prolactin continuous cell lines.
Definition of an experimental model**

par Nicole BRUNET, Danièle GOURDJI, Marie-Françoise MOREAU, D. GROUSELLE,
Françoise BOURNAUD, Andrée TIXIER-VIDAL

with the technical assistance of Nicole BALDET

Groupe de Neuroendocrinologie Cellulaire,
Chaire de Physiologie Cellulaire, Collège de France,
11, place Marcelin-Berthelot, 75231 Paris Cedex 05

Summary. The interaction of 17- β estradiol and thyroliberin (TRH) on prolactin secretion has been studied using two rat prolactin cell lines (GH3, SD1) as an experimental model. The estrogen level of the horse serum component of the normal culture medium is taken into account. For experiments, cells are grown either for 7 days or for several months on medium prepared from charcoal-dextran extracted horse serum (medium CD) supplemented or not with 17- β estradiol (9 ng/ml).

Cells grown for 7 days on medium CD are reduced in size, have a lower protein content, higher DNA/protein ratio (37-68 p. 100), lower basal prolactin secretion within 30 min, and fewer TRH binding sites (20 to 33 p. 100), but there is no significant modification of TRH-induced stimulation of prolactin release within 30 min. Addition of 17- β estradiol to medium CD for the same duration of treatment maintains GH3 cell morphology, partially maintains basal prolactin release (30 min), totally maintains or even increases the number of TRH binding sites, and potentiates the TRH-induced stimulation of prolactin release within 30 min.

Cells grown for several months on medium CD are smaller in size, have a much lower basal prolactin release within 30 min, show a sharp decrease (1/50-1/80) in the number of TRH binding sites accompanied by loss of TRH-induced stimulation of prolactin release within 30 min.

Introduction.

Rat prolactin continuous cell lines offer invaluable models for studying the action mechanism of thyroliberin (TRH, < Glu-His-Pro-NH₂) on prolactin secretion in an homogeneous population of target cells (Tixier-Vidal *et al.*, 1975). One of these cell

lines, GH3, is derived from a transplantable pituitary tumor, MtT/W5, induced by chronic estrogen treatment (Tashjian *et al.*, 1968, 1970). Its ability to develop tumor after *in vivo* transplantation to rats of the same strain (Wistar/Furth) depends on the estrogen blood level: it did not develop tumor in intact or castrated males, whereas it did in adult female rats (Sonnenchein *et al.*, 1973; Sorrentino *et al.*, 1976). These cells have been shown moreover to possess specific receptors for 17- β estradiol (Sonnenchein *et al.*, 1973; Mester *et al.*, 1973). According to Sorrentino *et al.* (1976) and Kirkland *et al.* (1976), no direct mitogenic stimulation of 17- β estradiol could be detected *in vitro* using various attempts to deplete estrogen level of the culture medium. From a functional point of view it has been shown that after a 3-day treatment, 17- β estradiol at low concentration increases prolactin production (Tashjian and Hoyt, 1972).

The other rat prolactin cell line, SD1, was isolated in our laboratory from a long-term primary culture of normal anterior pituitary cells of Sprague-Dawley male rats (Tixier-Vidal *et al.*, 1975). They probably occurred by a spontaneous transformation (origin unknown) of normal prolactin cells. Since they are phenotypically very close to GH3 cells, the possibility that they could result from a contamination by these cells cannot be definitely excluded so far. They differ nevertheless from GH3 cells by higher intracellular prolactin store (Morin *et al.*, 1975). After injection *in vivo* they develop tumor in both Sprague-Dawley and Wistar/Furth adult female rats.

The study of the interaction of estradiol on TRH regulation of prolactin secretion, by cells continuously grown *in vitro*, is hampered by the presence of 15 p. 100 horse serum and 2.5 p. 100 fetal calf serum in the culture medium; these sera are known to generally contain high levels of estrogens.

The purpose of the present work is to establish experimental conditions permitting the study of 17- β estradiol-TRH interaction in presence of known amounts of 17- β estradiol. Charcoal-dextran extracted serum was used, supplemented or not with 17- β estradiol. The effect of such treatments on cell morphology, cell growth, prolactin secretion and TRH responsiveness (TRH binding-PRL release within 30 min) has been studied as a function of time.

Material and methods.

Culture media.

In control conditions (*medium N*) GH3 cells and SD1 cells were grown as previously described (Gourdji *et al.*, 1973) on Ham F10 medium enriched with heat inactivated 15 p. 100 horse serum (Eurobio, Paris) and 2.5 p. 100 fetal calf serum (Eurobio, Paris) and with antibiotics (penicillin 50 mU/ml plus streptomycin 50 μ g/ml or gentamycin 40 μ g/ml). The present experiments were performed with a single pool of horse serum which contained 25 ng/ml of total estrogens, 3.1 ng/ml of progesterone and less than 0.2 ng/ml of testosterone. The estrogen concentration in fetal calf sera varied between 10-27 ng/ml. The maximum final concentration of total estrogens in the medium was 4.5 ng/ml.

A medium having very low estrogen concentration was prepared from the same horse-serum pool which was previously treated with charcoal-dextran, according to

Kirkland *et al.* (1976). The treatment was repeated twice and resulted in 99 p. 100 depletion of total estrogen concentration and in an undetectable level of progesterone. This medium was called *medium CD*. The maximum final estrogen concentration was 0.7 ng/ml. A medium supplemented with 17- β estradiol (*medium CDE*) was freshly prepared each week by introducing 9 ng/ml of 17- β estradiol (3.10^{-8} M) into medium CD.

All measurements of total estrogen and of estradiol serum concentrations were performed by Dr. Terqui (I.N.R.A., Nou zilly, France) using a specific radioimmunoassay.

Experimental schedules.

Short-term experiments : Short-term experiments were performed with a subclone of GH3, GH3/B6, and with SD 1 cells. Cells previously grown on medium N were collected by mild trypsinization (0.25 p. 100) and homogeneously distributed in small Falcon tissue culture Petri dishes (3.5 cm diameter) (2.5×10^5 cells/2 ml medium/dish) in three types of media-N, CD, CDE ; 8 to 12 dishes were prepared for each medium. The cells were grown in these conditions for 7 days, the medium being renewed every 2 or 3 days. At the end of this period, the cells were assayed for TRH responsiveness.

Long-term experiments : GH3 cells were previously grown for 6 months and SD1 cells for 18 months on medium CD. In view of studying their TRH responsiveness they were collected by mild trypsinisation and homogeneously distributed in small Falcon tissue culture dishes, as described for short-term experiments. They were then grown for 7 days in either medium CD or medium CDE (8 to 12 dishes per group).

Assay of TRH responsiveness.

On the day of experiment the 24-48-hour culture medium was separately collected from each dish, the cells were washed once with warmed (37 °C) F10 medium and then covered with 1 ml of warmed fresh complete culture medium (N, CD or CDE) supplemented with 10 ng/ml ^3H -TRH (60 Ci/mmol) prepared by Levine-Pinto (CEA Saclay), according to Pradelles *et al.* (1972). Control treatment consisted of 1 ml of fresh medium without ^3H -TRH. They were then placed in the usual incubator (Hot-pack, 5 p. 100 CO₂ in air) for 15 or 30 min. At the end of this period they were cooled down to ice temperature. The 15-30 min culture medium was separately collected for each dish. After 4 washings at ice temperature with F10 medium, the cells were scraped in 1 ml of distilled water and submitted to ultrasonic disruption (10 s 40 W Sonimasse apparatus T 50). One aliquote of the sonicate (0.5 ml) was used for radioactivity determination and another aliquote for protein and DNA determinations.

^3H -TRH uptake was determined by the measure of radioactivity bound to the cells, as previously described (Faivre-Bauman *et al.* 1975). It was expressed as fmole/mg cell proteins.

Cell proteins were measured by Folin's reagent according to Lowry *et al.* (1951). DNA was measured by the method of Karsten and Wollenberger (1972) slightly modified (Bournaud *et al.*, submitted for publication). *Prolactin medium content* was measured by radio-immunoassay using the rat PRL kit kindly provided by the NIAMDD rat Hormone Distribution Program.

Results.

Short-term treatment

A. Cell morphology and cell growth.

1° Effect of medium CD.

As shown on figure 1, GH3 cells grown for 7 days on medium CD display a reduction in size as compared to control cells. This is true also for SD1 cells. The total cell protein content per dish was also significantly lower (57 p. 100 to 31 p. 100) than for control cells seeded at the same density. The DNA content per dish was only slightly reduced leading to a significant increase of the DNA/protein ratio (from 37 p. 100 to 68 p. 100 depending on the experiment) (table 1). Cell count performed on separate dishes of GH3 cells gave a final number of 1.5×10^6 cells/dish for cells grown on medium CD, and 2×10^6 cells/dish for cells grown on medium N.

TABLE 1

Short-term experiments. Effect on cell protein and DNA/protein ratio

GH3 and SD1 cells previously grown in control conditions (medium N) were grown for 7 days in normal serum supplemented medium (N), or charcoal-dextran serum supplemented medium (CD), or medium CD supplemented with 9 ng/ml 17- β estradiol. Each value represents the mean of 4 dishes \pm standard error.

Exp. N°	Medium	Cell protein $\mu\text{g}/\text{dish}$	$\frac{\mu\text{g DNA}}{\mu\text{g prot.}} \times 10^3$ per dish
SD1 340	N	358.7 ± 32	79 ± 6.5
	CD	$205.0 \pm 12^{**}$	$116 \pm 5^{**}$
	CDE	$235.0 \pm 15^{**}$	$90 \pm 6.5^{++}$
SD1 349	N	524.5 ± 24	61 ± 4
	CD	$255.0 \pm 2^{**}$	$86 \pm 1.5^{**}$
	CDE	$205.0 \pm 19^{**}$	$88 \pm 7^{**}$
GH3/B6 1160	N	487.5 ± 19	79 ± 9
	CD	$155.0 \pm 15^{**}$	$109 \pm 10^*$
	CDE	$185.7 \pm 8^{**}$	$80 \pm 9^+$
GH3/B6 1304	N	308.7 ± 20	79.9 ± 8
	CD	$147.2 \pm 13^{**}$	$135.0 \pm 11.7^{**}$
	CDE	$285.0 \pm 17^{++}$	$98.5 \pm 8^+$

Statistical comparisons using the F-test.

$^{**} P : 0.01$ } N vs CD
 $^* P : 0.05$ } N vs CDE
 $^{++} P : 0.01$ } CD vs CDE
 $^+ P : 0.05$ }

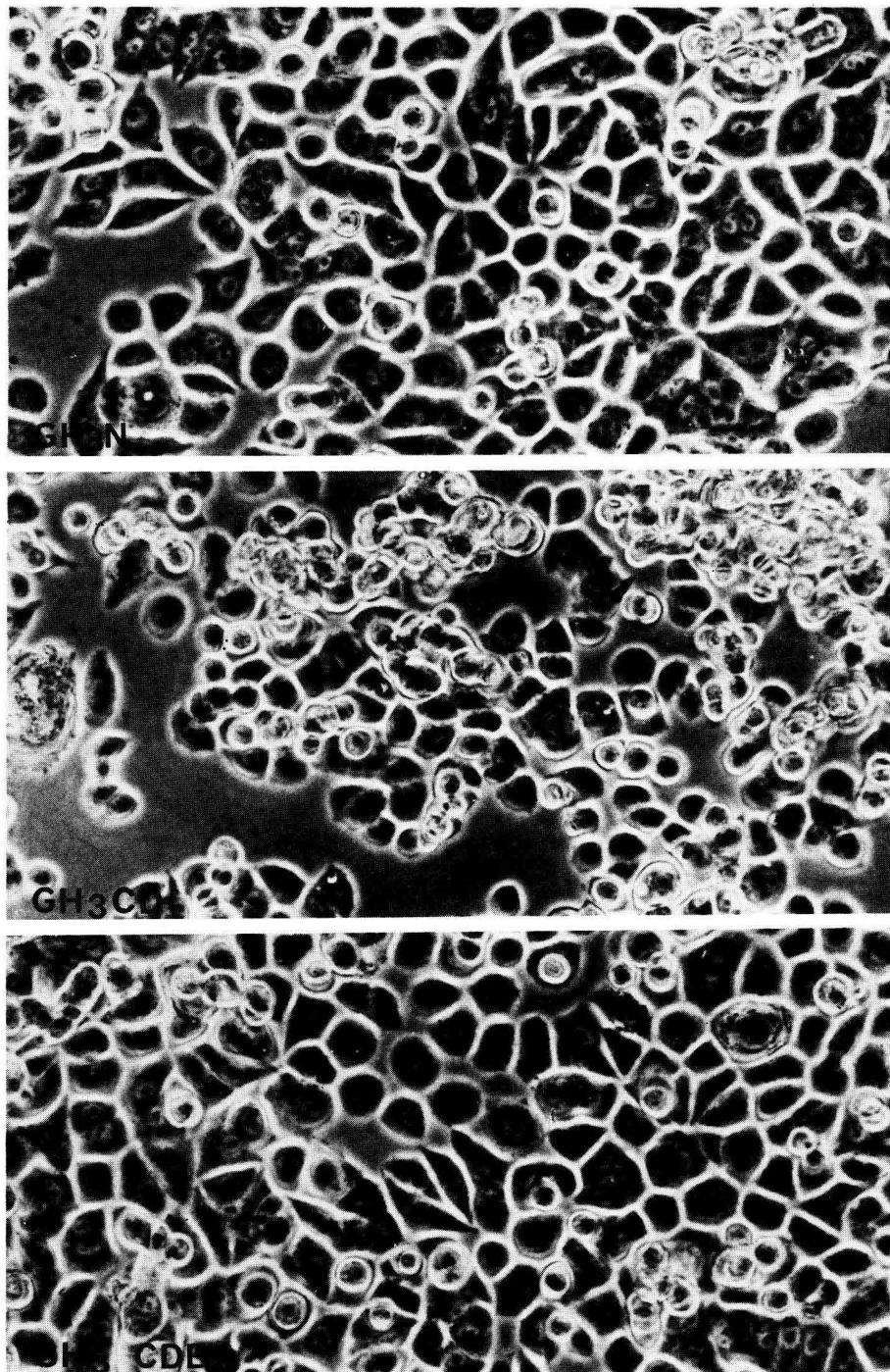


FIG. 1. — Comparative morphology of GH3 cells grown for 7 days in three different media : normal medium (15 p. 100 horse serum, 2.5 p. 100 fetal calf serum) (GH3 N), medium prepared from charcoal-dextran extracted horse serum (GH3 CD) and medium CD supplemented with 9 ng/ml of 17- β estradiol (GH3 CDE). Phase contrast microscope. $\times 300$.

2° Effect of medium CD supplemented with 17- β estradiol.

When grown on medium CD supplemented with 17- β estradiol GH3 cells, but not SD1 cells, retained their normal morphology (fig. 1). This was not accompanied by a normal value of the protein cell content, but the DNA/protein ratio was significantly higher (22-27 p. 100) than for cells grown on medium CD and tended to the value observed for control cells. This was constantly observed for GH3 cells, but not for SD1 cells (table 1). Cell count performed on separate dishes gave a final number of 1.2×10^6 cells/dish for GH3 cells.

B. Prolactin secretion (table 2).

In 3 experiments out of 4 the amount of prolactin released within 30 min at 37 °C was significantly reduced in GH3 as well as in SD1 cells previously grown for 7 days in medium CD (table 2). When medium CD was supplemented with 17- β estradiol the amount of PRL released within 30 min increased in 2 experiments out of 4 but never reached the value observed for control cells.

TABLE 2

*Short term experiments. Effect on prolactin release.
³H-TRH binding and TRH-induced stimulation of prolactin release*

For culture conditions see table 1. Cells were exposed to ³H-TRH (10 ng/ml) for 30 min at 37 °C. Each value represents the mean of 4 dishes \pm standard error.

Exp. N°	Medium	PRL release within 30 min ng/mg cell protein	³ H-TRH fmole/mg cell protein	p. 100 increase of PRL release under TRH
SD1 340	N	754 \pm 62	869 \pm 37	192
	CD	458 \pm 31 **	551 \pm 38 **	0
	CDE	372 \pm 46 **	530 \pm 36 **	239
SD1 349	N	1 256 \pm 54	573 \pm 96	131
	CD	484 \pm 42 **	451 \pm 37	164
	CDE	797 \pm 58 ***	750 \pm 21 **	172
GH3/B6 1160	N	972 \pm 60	1 974 \pm 76	199
	CD	353 \pm 36 **	1 503 \pm 55 **	217
	CDE	609 \pm 23 ***	2 263 \pm 75 ***	392
GH3/B6 * 1304	N	1 032 \pm 16.5	618 \pm 29	160
	CD	935 \pm 122 ns	475 \pm 22 **	144
	CDE	868 \pm 20 ns	689 \pm 39 ++	176

* Experiment 1304 have been performed on GH3/B6 cells which were at their 12th passage and in the way of loosing partially their TRH responsiveness.

Statistical comparisons using the F test.

** P : 0.01 } N vs CD
 * P : 0.05 } N vs CDE
 ++ P : 0.01 } CD vs CDE
 + P : 0.05 }

C. TRH responsiveness.

The number of ^3H -TRH molecules bound per mg cell protein was decreased significantly (20 to 33 p. 100) (excepting SD1 349) when GH3 and SD1 cells were previously grown for 7 days on medium CD. The p. 100 increase of PRL release in response to TRH was not significantly modified except in one experiment with SD1 where the effect of TRH was completely inhibited (table 2).

When cells previously grown on control medium were exposed to ^3H -TRH in medium CD, there was no modification of the amount of bound ^3H -TRH or of the percentage increase of PRL release as compared to cells binding TRH in normal medium (data not given).

In 3 experiments out of 4, when GH3 and SD1 cells were previously grown on medium CD supplemented with $17\text{-}\beta$ estradiol, they bound significantly more TRH molecules than when grown on medium CD. The number of bound ^3H -TRH molecules per mg cell protein was equal to or even higher (SD1 349-GH3 1160) than that observed for cells grown in control medium. This was consistently accompanied by an increase in the p. 100 of prolactin release stimulation induced by TRH, higher than in control cells (table 2).

Long-term culture on medium CD

A. Cell morphology and cell growth.

GH3 and SD1 cells, grown for several months on medium CD, displayed striking morphological modification (fig. 2). They formed discontinuous colonies of very

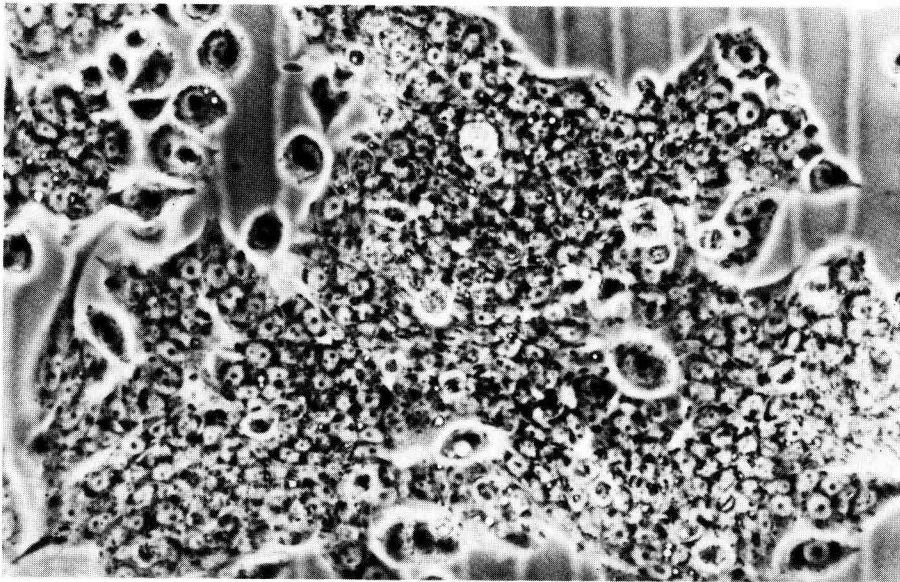


FIG. 2. — Morphology of GH3 cells grown for 10 months on medium CD, prepared from charcoal-dextran extracted serum. Compare to fig. 1. GH3 N. Phase contrast microscopy. $\times 300$

packed small cells with very few larger cells. Preliminary electron microscope examination revealed that these colonies did not represent syncytium. Each cell possessed a well-defined cell membrane and retained features of glandular cells, as attested to by the organization of the Golgi zone and the presence of a few small secretory granules. Such cells could be frozen and then put back into culture while retaining their particular features. They were called GH3 CDL or SD1 CDL.

The DNA/protein ratio of CDL cells did not clearly differ from that of normal cells, except in one experiment where they were seeded at a lower density (table 3).

TABLE 3

*Long-term experiment. Effect on DNA/protein ratio ;
prolactin release, ^3H -TRH binding and TRH induced stimulation of prolactin release*

GH3 CDL cells or SD1 CDL cells were previously grown for 6 months (GH3 cells) or 18 months (SD1 cells) on medium CD. They were then grown for 7 days on medium CD supplemented or not with 9 ng/ml of 17- β estradiol. They were exposed to ^3H -TRH as described in table 2. Each value represents the mean of 6 dishes \pm standard error.

Exp. N°	Medium	Cell protein $\mu\text{g}/\text{dish}$	$\frac{\mu\text{g}/\text{DNA}}{\mu\text{g}/\text{prot.}} \times 10^3$ per dish	PRL release within 30 min ng/ml cell protein	^3H -TRH fmole/mg cell protein	p. 100 increase of PRL release with TRH
SD1 CDL 235	CD CDE	433 \pm 19 368 \pm 10 *	81 \pm 2.5 61 \pm 1.2 **	< 2 ng 5.6 \pm 0	11 \pm 3 13 \pm 1 ns	0 0
SD1 CDL 334	CD CDE	276 \pm 9 222 \pm 13 **	102 \pm 6 90 \pm 3 ns	< 2 ng \leq 2 ng	11 \pm 2 24 \pm 0.5 **	0 0
GH3 CDL 1126	CD CDE	530 \pm 23 593 \pm 27 ns	62 \pm 2 48 \pm 1.2 **	3.5 \pm 0.14 4.4 \pm 1.2	25.5 \pm 2.7 37 \pm 3.2 *	0 0
GH3 CDL 1141	CD CDE	568 \pm 14 569 \pm 11 ns	76 \pm 3.2 68 \pm 2.5 ns	1.7 0.6	26 \pm 1 47 \pm 3 **	0 0
GH3 CDL 1221	CD CDE	163 \pm 7 142 \pm 12 ns	143 \pm 4 141 \pm 12 ns	< 1 ng	156 \pm 8.6 261 \pm 20 **	0 0

Statistical comparisons using the F test.

** P : 0,01.

* P : 0.05.

B. Prolactin secretion and TRH responsiveness.

The GH3 and SD1 CDL cells spontaneously released very low quantities of PRL within 30 min, i.e. considerably less than did normal cells (compare table 3 and table 2). They also bound considerably fewer TRH molecules than did normal cells : 50 to 80 times less. No stimulation of PRL release was observed after exposure to TRH.

C. Effect of 17- β estradiol.

When SD1 CDL cells were grown for 7 days on medium CD supplemented with 17- β estradiol, they were not morphologically altered. In the same conditions,

some GH3 cells showed an enlargement ; this reaction however decreased after several passages, even in the continuous presence of the added 17- β estradiol. In both CDL cell lines exposure to 17- β estradiol tended to decrease the DNA/protein ratio, although the decrease was highly significant ($P : 0.01$) in 2 experiments out of 5. This effect was lower than that observed in short-term experiments. In both CDL cell lines, exposure to 17- β estradiol significantly increased the amount of bound TRH per mg protein by 45 to 118 p. 100, depending on the experiment (except in SD1 235). Nevertheless, this increase was not accompanied by stimulation of PRL release within 30 min of exposure to TRH (table 3).

Discussion.

The importance of estrogens in the control of prolactin secretion at the pituitary level has been established for a long time, although its mechanism is still completely unknown. As with many other of their target cells, estrogens are known to also act on prolactin cell division and may even induce transplantable tumors. The exact link between these two aspects of estrogen action is not understood in spite of evidence for the presence on those cells of cytoplasmic and nuclear receptors for 17- β estradiol (see Introduction). Prolactin continuous cell lines, which by definition are continuously dividing, are grown on serum-supplemented medium, containing rather large amounts of steroids. This must be taken into account when using such cell lines to study the action mechanism of an hypothalamic peptide, TRH.

The present work represents preliminary investigations to establish an experimental model for analyzing the molecular mechanism of estradiol-TRH interaction in the control of prolactin secretion. The results will be discussed from two points of view : 1) as a valuable model for the initial purpose mentioned above, 2) as providing new insight into the complexity of the cellular events induced by changes in the medium composition resulting from charcoal-dextran treatment of serum followed or not by the addition of 17- β estradiol.

1) *A model for the study of 17- β and TRH interaction on prolactin secretion.*

From the present results, it appears that short-term experiments with GH3 cells using three types of media — N, CD, CDE — offer a valuable model for further studies on the mechanism of 17- β estradiol-TRH interaction. Indeed, a comparison of the three experimental groups shows that 17- β estradiol alone is able to : 1) maintain cell morphology, 2) partially maintain basal PRL secretion close to normal without acting significantly on total cell protein, 3) at least maintain the normal titer of TRH receptors, 4) potentiate the action of TRH on prolactin release. The effects of media CD and CDE were observed only after pretreatment of the cells before exposure to TRH. This suggests that the interaction of 17- β estradiol with TRH is mediated through a complex chain of events. SD1 cells have fewer TRH receptors than GH3 cells and seem less dependent on 17- β estradiol, at least from their morphological reaction. Our further studies will therefore be concentrated on GH3 cells only.

The GH3 cells, cultivated long-term on medium CD (GH3 CDL), also offer a very useful tool for studying the mechanism of TRH action. Indeed, they represent a stable

variant of the GH3 cell line which has lost its responsiveness to TRH, and permit definition of the component of TRH binding which is not related to its biological action. This is of particular importance when working with subcellular fractions (Bournaud *et al.*, submitted for publication). Whether the loss of TRH receptors results or not from the total or partial disappearance of 17- β estradiol receptors, remains to be examined. The fact that pretreatment of the GH3 CDL cells with 17- β estradiol does not restore the TRH-induced prolactin release, although increasing the number of bound TRH molecules, suggests that the synthesis of TRH receptors remains under the control of 17- β estradiol in those cells.

2) Complexity of cellular events induced by various media.

A medium prepared from charcoal-dextran extracted serum strongly modifies the growth pattern of GH3 and SD1 cells as revealed by reduced cell size, decrease in total cell proteins, increase of DNA/protein ratio and lower basal prolactin secretion. These effects are amplified by long-term culture on the same medium, particularly as concerns cell size and decrease of the basal prolactin secretion. Addition of 17- β estradiol to medium CD at the beginning of treatment does not completely maintain normal growth pattern : cell morphology and DNA/protein ratio are close to those of normal cells but total cell protein and, to some extent, basal prolactin release in 30 min, remain below normal values. Studies on total prolactin and growth hormone production in the same conditions are in progress.

These results are in apparent contradiction with those of Sorrentino *et al.* and Kirkland *et al.* (1976), working on another subclone (C14) of GH3 strain. They observed no alteration of cell growth on medium prepared from charcoal-dextran extracted serum, supplemented or not with estrogens. Nevertheless, those authors considered only the mitogenic aspect of cell growth. In our experiments, we observed important modifications of several functional parameters. Although we did not do a kinetic study of the cell number evolution, we found that the final cell number was lower on medium CD as compared to medium N, and that 17- β estradiol did not impede this decrease. This difference with the above-mentioned results can be explained by the fact that we did not take into account the loosely attached or floating cells, eliminated at each medium renewal. The number of such cells was clearly greater on medium CD than on medium N. This observation indicates that even if the total cell number was not modified according to Sorrentino *et al.* (1976) and to Kirkland *et al.* (1976), the « quality » of the cells was indeed changed.

The incomplete ability of 17- β estradiol to compensate the effects of serum charcoal-dextran extraction is to be related to the fact that such a treatment is known to eliminate not only sex steroids, but also glucocorticoids, thyroid hormones and small peptides. Triiodothyronine has been shown to regulate the growth hormone secretion and cell growth of another cell line, GH1, derived from the same tumor as GH3 cells (Samuels *et al.*, 1973 ; Samuels and Tsai, 1973). A direct mitogenic effect of thyroid hormones not synergized by estrogens was also recently reported on GH3/C14 (Kirkland *et al.*, 1976). Other still unknown serum components may also participate in the growth regulation of cell lines. An ideal way to eliminate such unknown serum factors would be to compose a synthetic medium. Such an attempt has been recently

proposed by Hayashi and Sato (1976) for GH3 cells as well as for other cell lines. They found it possible to eliminate serum if the F12 synthetic medium was supplemented with triiodothyronine, TRH, transferrin, parathyroid hormone and a partially purified somatomedine preparation. With a 10-day treatment, this hormone « cocktail » was found able to maintain cell growth at the level obtained with serum-supplemented medium. No data on hormonal secretion was reported in these conditions. Increased PRL production was nevertheless observed when GH3 cells were grown in a similar synthetic medium supplemented with fibroblast growth factor and insulin in addition to the above-mentioned substances (Sato, personal communication). In any case, such a cocktail would not be suitable for studying the action mechanism of TRH.

8^e Colloque de Neuroendocrinologie. — Table ronde Prolactine.
Tours, 7 septembre 1976.

Acknowledgments. This work was supported by a grant from INSERM (AT 24 75 47).

Résumé. Dans le but d'étudier l'interaction de l'œstradiol 17- β et de la thyrolibérine (TRH) sur la sécrétion de prolactine par deux lignées continues de cellules à prolactine de rat (GH3, SD1), un modèle expérimental a été établi, tenant compte de la teneur en œstrogènes du sérum de cheval participant à la composition des milieux de culture. Pour cela les cellules ont été cultivées soit à court terme (7 jours), soit à long terme (plusieurs mois) sur un milieu préparé à partir de sérum traité au charbon dextran (milieu CD). Celui-ci a été supplémenté, ou non, par de l'œstradiol 17- β (9 ng/ml).

La culture pendant 7 jours sur milieu CD réduit la taille des cellules ainsi que leur teneur en protéines, augmente le rapport DNA/protéines, diminue le taux de sécrétion basale de la prolactine (pendant 30 mn) ainsi que le nombre de sites de liaison du TRH, sans toutefois modifier l'amplitude de la stimulation induite en 30 mn sur la libération de prolactine. L'addition d'œstradiol 17- β au milieu CD et pour une même durée, maintient la morphologie normale des cellules GH3, maintient partiellement la sécrétion basale de la prolactine (30 mn), maintient totalement ou accroît le nombre de sites de liaison au TRH et potentialise la stimulation induite en 30 mn sur la libération de prolactine.

La culture pendant plusieurs mois sur milieu CD induit une diminution considérable de la sécrétion basale de prolactine, ainsi que du nombre de sites de liaison du TRH (50 à 80 fois moins) et entraîne une disparition de l'effet stimulant du TRH sur la sécrétion de prolactine en 30 mn.

References

- BOURNAUD F., GOURDJI D., MONGONGU S., TIXIER-VIDAL A. ³H-Thyrolibérine (TRH) binding by nuclei isolated from a pituitary clonal cell line (GH3). Submitted for publication.
- FAIVRE-BAUMAN A., GOURDJI D., GROUSELLE D., TIXIER-VIDAL A., 1975. Binding of thyrotropin releasing hormone and prolactin release by synchronized GH3 rat pituitary cell line. *Biochem. Biophys. Res. Commun.*, **67**, 50-57.
- GOURDJI D., TIXIER-VIDAL A., MORIN A., PRADELLES P., MORGAT J. L., FROMAGEOT P., KERDELHUE B., 1973. Binding of a tritiated thyrotropin releasing factor to a prolactin secreting clonal cell line (GH3). *Exp. Cell Res.*, **82**, 39-46.
- HAYASHI I., SATO G. H., 1976. Replacement of serum by hormones permits growth of cells in a defined medium. *Nature*, **259**, 132-134.
- KARSTEN U., WOLLENBERGER A., 1972. Determination of DNA and RNA in homogenized cells and tissues by surface fluorometry. *Anal. Biochem.*, **46**, 135-148.

- KIRKLAND W. L., SORRENTINO J. M., SIRBASKU D. A., 1976. Control of Cell Growth. III. Direct mitogenic effect of thyroid hormones on an estrogen-dependent rat pituitary tumor cell line. *J. nat. Cancer Inst.*, **56**, 1159-1164.
- LOWRY O. H., ROSENBROUGH N. J., FARR A. L., RANDALL R. J., 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- MESTER J., BRUNELLE R., JUNG I., SONNENCHEIN C., 1973. Hormone receptors in tumor and cells in culture. *Exp. Cell Res.*, **81**, 447.
- MORIN A., TIXIER-VIDAL A., GOURDJI D., KERDELHUÉ B., GROUSELLE D., 1975. Effect of thyrotrope-releasing hormone (TRH) on prolactin turnover in culture. *Molec. cell. Endocr.*, **3**, 351-373.
- PRADELLES P., MORGAT J. L., FROMAGEOT P., OLIVER C., JACQUET P., GOURDJI D., TIXIER-VIDAL A., 1972. Preparation of highly labelled ^3H -thyrotropin releasing hormone (PGA-His-Pro (NH₂)) by catalytic hydrogenolysis. *FEBS Letters*, **22**, 19-22.
- SAMUELS H. H., TSAI J. S., 1973. Thyroid hormone action in cell culture : demonstration of nuclear receptors in intact cells and isolated nuclei. *Proc. nat. Acad. Sci., USA*, **70**, 3488-3492.
- SAMUELS H. H., TSAI J. S., CINTRON R., 1973. Thyroid hormone action : a cell culture system responsive to physiological concentrations of thyroid hormones. *Science*, **181**, 1253-1256.
- SONNENCHEIN C., POSNER M., SAUDUDDIN S., KRASNAY M., 1973. Estrogen-sensitive cell lines. Uptake of labeled sex steroids and growth properties. *Exp. Cell Res.*, **78**, 41-46.
- SORRENTINO J. M., KIRKLAND W. L., SIRBASKU D. A., 1976. Control of cell growth. I. Estrogen-dependent growth *in vivo* of a rat pituitary tumor cell line. *J. nat. Cancer Inst.*, **56**, 1149-1153.
- TASHJIAN A. H. Jr., BANCROFT F. C., LEVINE L., 1970. Production of both prolactin and growth hormone by clonal strains of rat pituitary tumor cells. Differential effects of hydrocortisone and tissue extracts. *J. Cell Biol.*, **47**, 61-70.
- TASHJIAN A. H. Jr., HOYT R. F., Jr., 1972. Transient controls of organ-specific functions in pituitary cells in culture, 353-387. In M. SUSSMAN, *Molecular genetics and developmental biology*. Prentice Hall, Inc., Englewood Cliffs, New Jersey.
- TASHJIAN A. H. Jr., YASUMURA Y., LEVINE L., SATO G. H., PARKER M. L., 1968. Establishment of clonal strains of rat pituitary tumor cells that secrete growth hormone. *Endocrinology*, **82**, 342-352.
- TIXIER-VIDAL A., GOURDJI D., TOUGARD C., 1975. A cell culture approach to the study of anterior pituitary cells. *Int. Rev. Cytol.*, **41**, 173-239.
-