J. steroid Biochem. Vol. 36, Nos 1/2, pp. 1–6, 1990
Printed in Great Britain

0022-4731/90 $3.00 + 0.00
Pergamon Press plc

VARIABLE EXPRESSION OF THE UTEROGLOBIN GENE FOLLOWING THE ADMINISTRATION OF NORETHISTERONE AND ITS A-RING REDUCED METABOLITES*

MARCO A. CERBÓN, ANA M. PASAPERA, RUBÉN GUTIÉRREZ-SAGAL, GUSTAVO A. GARCÍA and GREGORIO PÉREZ-PALACIOS†

Department of Reproductive Biology, National Institute of Nutrition S. Zubirán, ENEP-Zaragoza and School of Chemistry, National University of Mexico, Mexico City, Mexico

(*Received* 11 *September* 1989)

Summary—Enzyme-mediated A-ring reduction of norethisterone (NET) results in the transformation of a molecule with potent intrinsic progestational activity into neutral derivatives with estrogen-like effects. To ascertain whether these structural modifications of NET are able to modify the uteroglobin (U) gene (G) expression, a series of experiments assessing the UG products after the administration of NET and its reduced A-ring metabolites were conducted in prepubertal female rabbits. Synthesis of endometrial uteroglobin and its specific mRNA were studied in animals following the administration of NET, 5α-dihydro NET,3β,5α-tetrahydro NET and progesterone. Animals treated with either estradiol or vehicle alone served as controls. The uteroglobin content in uterine flushings and cytosols was determined by immunodiffusion and polyacrilamide gel electrophoresis techniques and by a specific double-antibody radioimmunoassay, while the U mRNA synthesis was assessed by its molecular hybridization to [α^{32}P]d-ATP uteroglobin cDNA. NET induced a significant increase of the uterine content of uteroglobin similar to that observed with progesterone with a simultaneous increase on U mRNA synthesis. On the contrary, 5α-NET and 3β,5α-NET induced very little, if any uteroglobin synthesis with a concomitantly low U mRNA production as compared with NET; thus exhibiting a similar effect to that observed in estradiol-treated animals. The overall results were interpreted as demonstrating that the enzyme mediated structural changes of NET which occur at the target organs induce variable expression of the uteroglobin gene. The data indicate that the rabbit uteroglobin gene products are suitable molecular markers to evaluate the hormonal potency of contraceptive synthetic progestins and their derivatives.

INTRODUCTION

Uteroglobin or blastokinin is a hormone-sensitive endometrial secretory protein involved in blastocyst development and implantation [1–6], which was first discovered in the rabbit uterus during early pregnancy [2]. Although the specific function(s) of uteroglobin is not completely understood, it has been well documented that this low molecular weight endometrial protein binds to [7, 8] and is regulated by progesterone [9]. Indeed, data from a number of laboratories have demonstrated that progesterone and synthetic progestins specifically regulate uteroglobin synthesis at the endometrial level [10–12].

To assess the progestational potency of norethisterone (NET), a widely used synthetic contraceptive progestin, and its neutral metabolic conversion products, we felt it was of interest to study their direct effects on the rabbit uteroglobin gene expression, taking advantage of the fact that the uteroglobin mRNA has been well characterized and translated *in vitro* [13] and that its complementary and genomic DNAs have been cloned [14]. Accordingly, the capability of NET and two A-ring reduced NET metabolites to induce uterine uteroglobin and its specific mRNA synthesis was studied in prepubertal rabbits.

Further impetus for the conduction of this study was furnished by the recent demonstration in our laboratory [15–19] that the enzyme-mediated structural modifications of the NET molecule not only direct its specific interactions with the high affinity binding sites of the putative steroid receptors but also modulate the expression of its hormone-like biological activity.

*A portion of this paper was presented orally at the *21st Annual Meeting of the Society for the Study of Reproduction*, Seattle, Washington, August 1–4 (1988) (Abstr. No. 419).

†To whom correspondence should be addressed: Dr Gregorio Pérez-Palacios, National Institute of Nutrition S. Zubirán, Vasco de Quiroga N. 15, 14000 Mexico, D.F. Mexico [*Tel.* (252) 573-11-60; *Fax* (525) 655-98-59].

Abbreviations: norethisterone (NET): 17β-ethynyl-17β-hydroxy-4-estren-3-one; 5α-dihydro NET (5α-NET): 17β-ethynyl-17β-hydroxy-5α-estran-3-one; 3β,5α-NET: 17β-ethynyl-5α-estran-3β, 17β-diol.

The results obtained provide evidence that A-ring reduction of NET results in the formation of derivatives that lost their progestational intrinsic potency but acquire an estrogen-like activity as assessed in a rabbit uteroglobin gene model.

MATERIALS AND METHODS

Steroids

Authentic NET was kindly provided by Schering Mexicana, S.A. (Mexico City). 5α-dihydronorethisterone (5α-NET) was synthesized by lithium-ammonia reduction of NET as described by Bowers *et al.*[20] and the 3β,5α-tetrahydro derivative of NET (3β,5α-NET) was synthetized from 5α-NET by sodium borohydride reduction [18]. Chemical purity of NET and its derivatives was assessed by their melting points, high-performance liquid chromatographic behavior and H-nuclear magnetic resonance spectrometric analysis as has been previously published [19]. Progesterone and estradiol were purchased from Steraloids (Pauling, N.Y.).

Radioactive material

[α-^{32}P]d-ATP, (SA: 3000 Ci/mmol) and sodium [^{125}I]iodine (SA: 16.4 mCi/μg) were purchased from Amersham International (England). α-^{32}P was determined in a Packard Tri-Carb liquid scintillation spectrometer model 2660, using Instagel® (Packard, Downers Grove, Ill.) as the counting solution, while ^{125}I was determined in a Packard Auto Gamma 500. Counting efficiency was 45%.

Animals and treatments

Prepubertal New Zealand white rabbits weighing 1.0 ± 0.2 kg were used and they were fed with Purina Chow. Animals were distributed in 6 groups of 6 animals each. All animals received steroid treatments as follows: group I: NET (1.0 mg/day), group II: 5α-NET (1.0 mg/day), group III: 3β,5α-NET (0.5 mg/day), group IV: estradiol (0.01 mg/day), group V: progesterone (1.0 mg/day), and group VI: vehicle alone. All steroids were daily administered (s.c.) in 0.5 ml of corn oil–10% ethanol for five consecutive days. An additional group ($n = 6$) of adult rabbits in the fifth day of pregnancy served as positive control.

20 h after the last injection, animals were killed by cervical dislocation, bled and the uteri rapidly excised. For uterine flushings each uterine horn was rinsed with 1 ml of 0.15 M NaCl. Uteri were then homogenized with three 10-s bursts in a Polytron homogenizer (Brinckmann Instruments, Westbury, N.Y.). The cytosol fraction was obtained by centrifugation at 105,000 *g* for 1 h at 4°C in a SW 50.1 rotor (Beckman Instruments, Palo Alto, Calif.). Uterine washes and cytosols were kept frozen at −80°C until assayed.

Uteroglobin characterization and measurement

The uteroglobin content of uterine flushings and cytosols was determined by immunodiffusion, polyacrilamide gel electrophoresis (PAGE) and radioimmunoassay (RIA).

Specific anti-uteroglobin goat sera kindly supplied by Dr E. Milgrom was used throughout this study, its characteristics have been previously reported [21]. The immunoreactivity of the rabbit endometrial uteroglobin was assessed by the use of the classical Ouchterlony double diffusion technique using 1% agarose gels [22]. To determine the electrophoretic behavior of rabbit endometrial uteroglobin, uterine flushings and cytosol aliquots (30 μl) containing 10 μg of protein were applied to 10–20% sodium dodecyl sulfate (SDS)–polyacrilamide gradient gels as described by Laemmli[23]. Electrophoresis was carried out at a constant voltage (100 V) for 1 h in a mini-electrophoresis chamber (Mini-Protean II Bio-Rad, Richmont, Calif.). The gels were fixed in methanol–acetic acid–water (40:7:53) for 30 min stained with 0.25% (w/v) Coomassie brillant blue R-250 in 40% methanol and 10% acetic acid for 30 min and washed overnight in methanol–acetic acid–water (40:7:53). The molecular weight markers (Bio-Rad) employed were Phosphorilase B (98 K), BSA (68 K), Ovalbumin (43 K), Carbonic anhydrase (31 K), Soybean tripsin inhibitor (20 K), and lysozyme (14.3 K).

The quantitative determination of endometrial uteroglobin was done by a specific double antibody radioimmunoassay as described by Mayol and Longenecker[24] with minor modifications. [^{125}I]-uteroglobin was prepared by the lactoperoxidase method and rabbit anti-goat sera was used as the second antibody. The assay sensitivity was 0.5 ng/ml, while the intra- and inter-assay variation coefficients were 10 and 12% respectively.

Uteroglobin mRNA determinations

The endometrial content of specific uteroglobin mRNA was evaluated by its molecular hybridization to uteroglobin cDNA.

Total uterine RNA was extracted by the method of Cathala *et al.*[25]: in brief, uteri from steroid-treated and control animals were homogenized in a lysis buffer (5 M Guanidine isothiocyanate, 10 mM EDTA, 50 mM Tris–HCl, pH 7.5 and 8% (v/v) β-mercaptoethanol) and the homogenates submitted to direct RNA precipitation by the addition of 4 M LiCl.

Identical quantities of total extracted RNA (20 μg) from control and treated animals were submitted to Northern blot analysis which included its electrophoresis through 1.1% agarose/6% formaldehide gel [26], its transfer to an inmovilized nitrocellulose paper [27] and its hybridization to [α-^{32}P]d-ATP-uteroglobin cDNA. The nitrocellulose paper was washed [28], dryed, and exposed to an X-ray film. The

synthesis of the labeled uteroglobin c-DNA probe was done as follows: recombinant plasmid DNA (pUG12) was isolated and the uteroglobin cDNA insert was excised by using deoxyribonuclease *Pst I*, purified from 1% agarose gel and radiolabeled with [α-^{32}P]d-ATP by nick translation [29]. Final SA was 1–2 $\times$ 10^8 cpm/μg. Proteins were determined by the Bradford's dye binding method [30] using BSA as standard.

RESULTS

The s.c. administration of NET of prepubertal female rabbits was followed by an increase of the uteroglobin content in uterine flushings as tested by its reactivity with a specific antiserum in double immunodiffusion plates. The augmentation of the uterine uteroglobin content induced by NET was similar to that observed in the progesterone-treated rabbits, whereas the A-ring reduction of NET abolished the potency of uteroglobin induction; in fact 5α-NET, 3β-5α-NET and estradiol were almost ineffective.

When uterine flushings of control and steroid-treated animals were analyzed in SDS–PAGE, identical results to those observed using immunodiffusion were obtained as indicated in Fig. 1. Furthermore, to quantitatively assess the effects of NET and its metabolites upon the synthesis of uteroglobin, its immunoreactive content in uterine flushings and cytosols were determined. As shown in Fig. 2, the results obtained confirmed that: (1) NET exhibits a progestational activity similar to that of progesterone, (2) reduction at the 5 position and later at the 3 position of the NET molecule results in a significant diminution of its capability to induce uteroglobin synthesis and (3) estradiol-17β exhibits very little, if any, progestational potency.

To further investigate the mode of action of NET, the effect of this synthetic progestin and its metabolites upon the uterine content of specific uteroglobin mRNA was examined. Total uterine RNA from control and steroid-treated animals was submitted to molecular hybridization with [α-^{32}P]d-ATP uteroglobin cDNA. The identical quantity of total RNA used in the experiments was assessed by optical density, staining with ethidium bromide and methilen blue throughout Northern blot analysis. As shown in Fig. 3, NET administration induced a significant increase in the uteroglobin mRNA content as compared with the oil-treated animals. This increase, assessed by Northern blot analysis, was similar to that observed in progesterone-treated rabbits. A similar increase of uterine U mRNA content was noticed to a higher extent in pregnant rabbit uterus. On the contrary, administration of 5α-NET, 3β,5α-NET and estradiol-17β resulted in a limited increase of uterine uteroglobin mRNA as compared with the oil-treated animals (Fig. 3; tracks 4–7). Total RNA from untreated rabbit liver and human fibroblasts (tracks 8 and 9) did not hybridize at all with the labelled uteroglobin cDNA probe. Evidence that variations

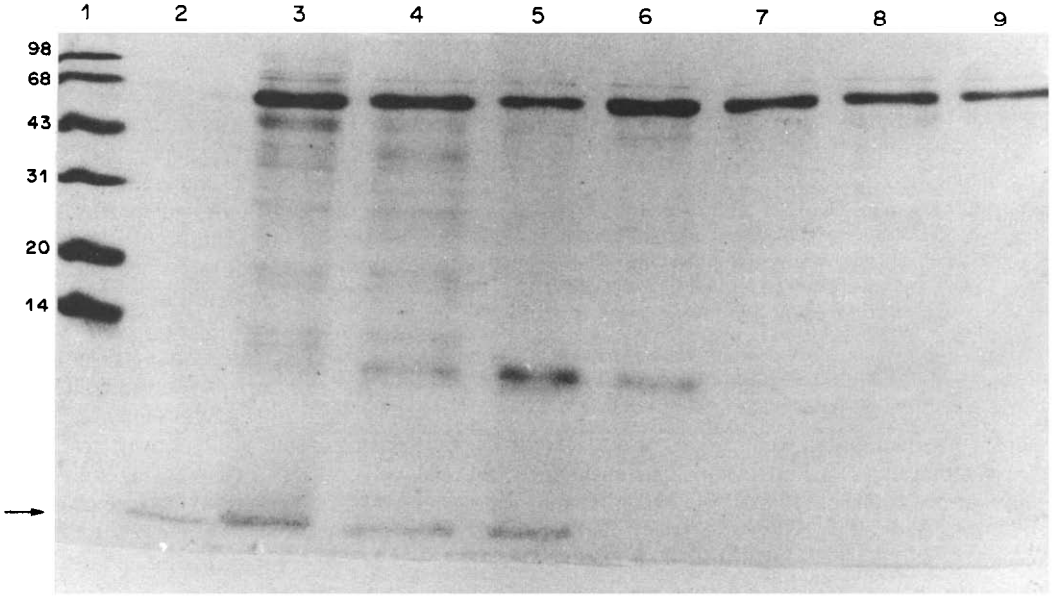

Fig. 1. Uteroglobin assessment in uterine flushings from control and steroid-treated rabbits by SDS–polyacrilamide gel electrophoresis (PAGE). Samples were analyzed in gradient gels (10–20%). Marker proteins are on track 1 and their mol. wt is indicated on the left (in 1000 Da). Uteroglobin standard is on track 2. Uterine flushing proteins from 5-days pregnant animals are on track 3. Samples from treated prepubertal rabbits are located as follows: progesterone (track 4), norethisterone (track 5), 5α-NET (track 6), 3β,5α-NET (track 7), estradiol-17β (track 8) and vehicle alone (track 9). The uteroglobin band, which is indicated by the arrow, was only noticed in tracks 3, 4 and 5. For details see the text.

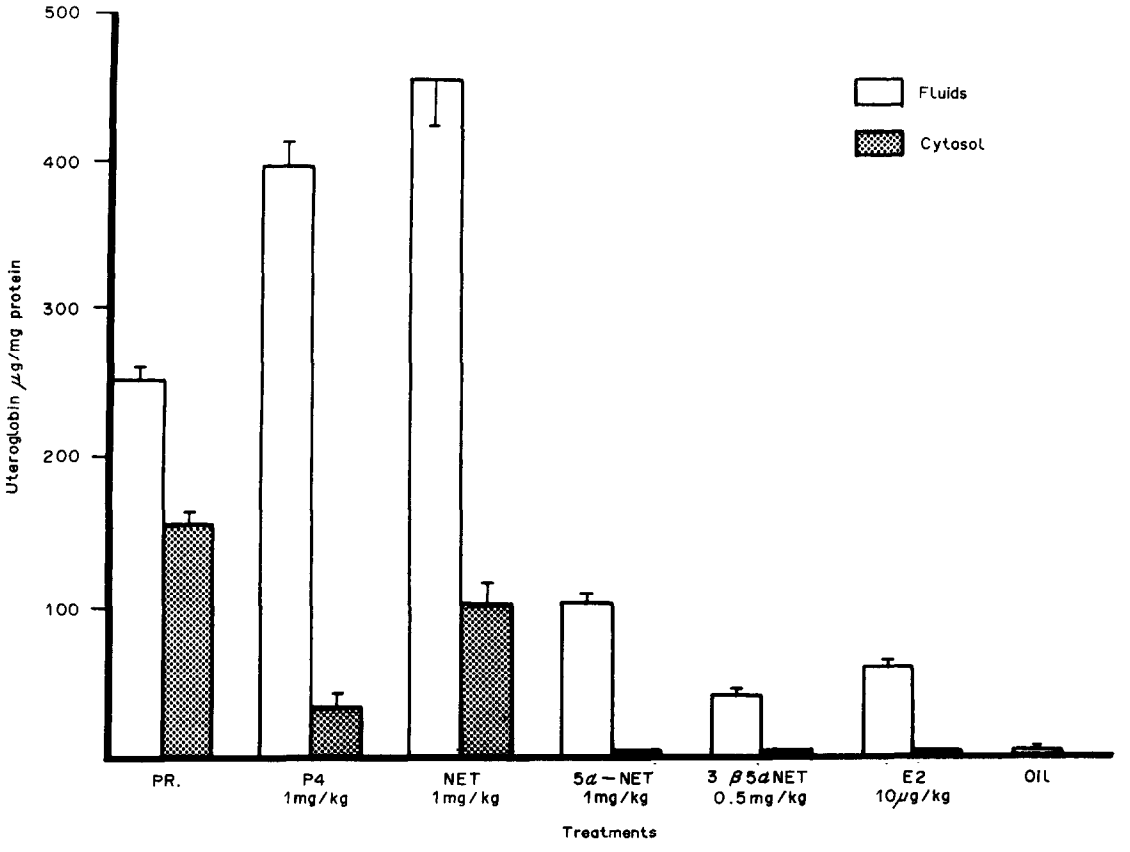

Fig. 2. Immunoreactive concentrations of uteroglobin in uterine flushings (fluid) and cytosols of prepubertal rabbits treated with natural and synthetic steroids. Samples were analyzed by a specific uteroglobin double antibody radioimmunoassay. Groups of animals ($n = 6$) were daily treated for five consecutive days with steroids as indicated and samples were obtained 20 h after last injection. The results are expressed as μg of uteroglobin/mg protein (mean $\pm$ SD). Samples from pregnant animals (PR) on the 5th day and oil-treated prepubertal rabbits were used as the control groups.

on the endometrial U mRNA content in the experimental and control groups are indeed reflecting different progestational potencies of the steroids tested was obtained by lack of variations on the H_4-histone mRNA content after hybridization with the corresponding non-hormone regulated cDNA probe (data not shown).

DISCUSSION

The results presented herein give evidence that the administration of NET induces a significant increase of the uteroglobin gene products in the prepubertal rabbit uterus in a manner similar to that observed with progesterone. Indeed, the observation that NET-induced uteroglobin content increases in uterine cytosol and flushings occurred in parallel with a significant rise in specific U mRNA content demonstrated that both NET and progesterone share a common mode of action. Whether the increased endometrial content of U mRNA observed after NET administration was due to an increase of gene transcription rate or to a messenger stabilization effect cannot be drawn from this study. However, since the natural hormonal action of progesterone is exerted at the transcription level [12, 21], it seems plausible that NET may act through a similar mechanism. The progestational potency of NET, as assessed by its capability to increase the immunoreactive content of uterine uteroglobin, was found to be quantitatively similar to that of progesterone. These data rule out early observations which had suggested that NET was unable to induce uteroglobin synthesis in the rabbit uterus [12].

The fact that NET, a derivative of 19-nor testosterone, is used in a number of contraceptive formulations [31, 32] coupled with the observation that this synthetic progestin is extensively converted to neutral metabolites [33] with potent biological effects at the target organs [17, 19], prompted us to evaluate two of the most important A-ring derivatives of NET: 5α-NET and 3β-NET, for their capability to modify the uteroglobin gene expression.

When 5α-NET was administered to prepubertal female rabbits the uterine content of uteroglobin (flushings and cytosols) increased very little as compared with the oil-treated animals; thus indicating that formation of the 5α (*trans* A/B ring junction) NET derivative by hydrogenation of the double bond results in a significant diminution of its

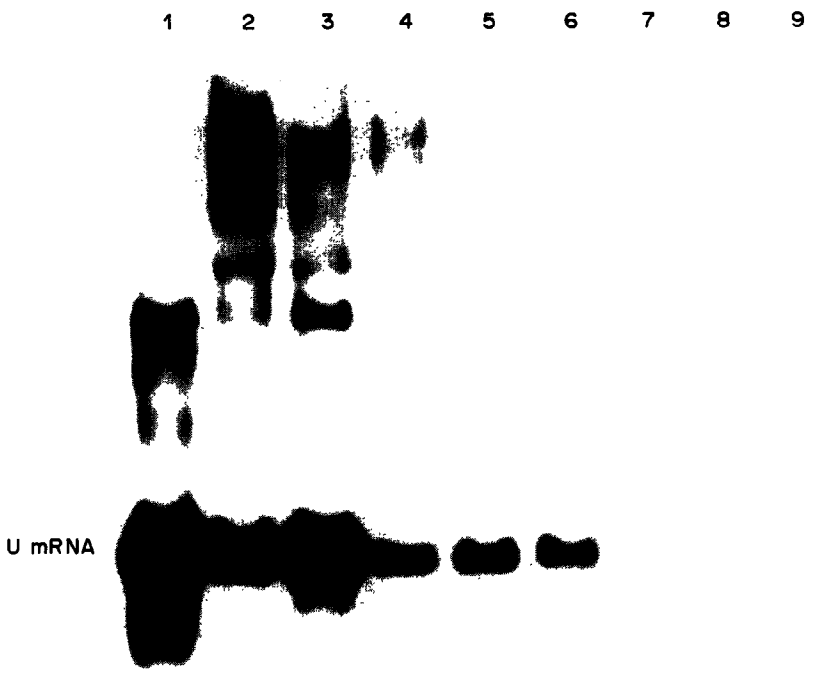

Fig. 3. Effect of natural and synthetic steroids upon the uteroglobin mRNA content in the uterus of prepubertal animals as assessed by Northern blot analysis. Total uterine RNA was extracted from an uterine horn of treated animals with: progesterone (track 2), norethisterone (track 3), 5α-NET (track 4), 3β,5α-NET (track 5), and estradiol-17β (track 6). Samples from pregnant (track 1), and prepubertal oil treated rabbits (track 7) were used as controls. Samples from rabbit liver and human fibroblasts were also included. All samples were submitted to Northern blot analysis in duplicate and the experiments were run in triplicate. The figure shows a typical autoradiography.

progestational potency. This finding was not in parallel with the previous observation that 5α-NET binds to the progesterone receptor with an adequate relative affinity [18]. Whether the 5α-reduced metabolite of NET, which is depleted of progestational activity, may exert a synergystic or even an antagonistic hormonal effect cannot be elucidated from these experiments and deserves further studies. The decrease of progestational activity of the 5α-NET derivative was not only observed in terms of uteroglobin synthesis but it was also accompanied by a significant diminution in the uterine content of specific uteroglobin mRNA, an observation which gave additional support to the concept that NET hormonal actions are exerted at the transcriptional level.

Further reduction of the 3 keto group of 5α-NET to the 3β configuration diminished even more its progestational activity as was evidenced by the decrease of uteroglobin in those animals treated with 3β,5α-NET. Indeed, the effect of this tetrahydro NET derivative was similar to that noticed in the prepubertal rabbits treated with estradiol-17β. Interestingly, this finding fits well with the previous demonstration that 3β,5α-NET specifically binds to the estradiol receptor [18] and it initiates cellular events that are considered to be estrogen-dependent [19]. These data confirm and extend a previous observation of Loosfelt *et al.*[34] that the estrogen-induced increase of endometrial uteroglobin in the prepubertal rabbit is very limited and significantly lower than that observed with progesterone.

In summary, the results demonstrate that the rabbit uteroglobin gene model is suitable to be used in screening progestational effects, at the molecular level, of synthetic contraceptive steroids. The data support the concept that A-ring reduction of NET precluded its effects upon uteroglobin gene expression.

Acknowledgements—We thank Professor E. Milgrom from the Groupe de Recherches sur la Biochimie Endocrinienne et la Reproduction, Faculté de Medicine de Bicetre, France for the generous supply of uteroglobin standard, antibodies, and uteroglobin cDNA. The expert assistance of Drs G. Nava, R. Ocadiz, V. Ortega and G. Alfaro from the National Institute of Oncology (Mexico City) is acknowledged. We also thank to L. Salazar for typing the manuscript. This work was supported in part by the National

Council of Science and Technology, PUIS-UNAM (Mexico City), the Rockefeller Foundation (New York), and the Special Programme in Human Reproduction, W.H.O. (Geneva).

REFERENCES

1. Beier H. M.: Uteroglobin: a hormone-sensitive endometrial protein involved in blastocyst development. *Biochim. Biophys. Acta* **160** (1968) 289–291.
2. Krishnan R. S. and Daniel J. C., Jr: "Blastokinin": inducer and regulator of blastocyst development in the rabbit uterus. *Science* **158** (1967) 490–492.
3. Kirchner C.: Uteroglobin in the rabbit. *Cell Tiss. Res.* **170** (1976) 415–424.
4. Clauberg C.: Untersuchungen in uterus spezifischen glycoprotein während der frünen gravidität des kanichens (*Orycotolagus cuniculus*) Willrelum Ranx Arch. *Entw. Smech. Org.* **164** (1969) 97–133.
5. Nieto M. and Beato M.: Synthesis and secretion of uteroglobin in rabbit endometrial explants cultured *in vitro*. *Molec. Cell. Endocr.* **17** (1980) 25–39.
6. Mukhergee A. B., Ulane R. S. E. and Agrawal A. K.: Role of uteroglobin and tranglutaminase in masking the antigenicity of implanting rabbit embryos. *Am. J. Reprod. Immun.* **2** (1982) 135–141.
7. Arthur A. T., Cowan B. D. and Daniel J. C. Jr: Steroid binding to blastokinin. *Fert. Steril.* **23** (1972) 85–92.
8. Fridlansky F. and Milgrom E.: Interaction of uteroglobin with progesterone, 5-pregnane 3,20 dione and estrogens. *Endocrinology* **99** (1976) 1244–1251.
9. Savouret J. F. and Milgrom E.: Uteroglobin: a model for the study of progesterone action in mammals. *DNA* **2** (1983) 99–104.
10. Isomaa V.: *In vitro* binding to and *in vivo* effect on the cytosol and nuclear progesterone receptors of various progestins, and their relation to synthesis of uteroglobin in rabbit uterus. *Biochim. Biophys. Acta* **675** (1981) 9–16.
11. Kontula K., Jänne O., Vihko R., de Jager J., de Visser J. and Zeclen F.: Progesterone binding proteins: *in vitro* binding and biological activity of different steroidal ligands. *Acta Endocr. (Copenh.)* **78** (1975) 574–592.
12. Arthur A. T. and Chang M. C.: Induction of blastokinin by oral contraceptive steroids: implications for fertility control *Fert. Steril.* **25** (1974) 217–221.
13. Bullock D. W., Woo S. L. C. and O'Malley B. W.: Uteroglobin messenger RNA: translation *in vitro*. *Biol. Reprod.* **15** (1976) 435–443.
14. Bailly A., Atger M., Atger P., Cerbon M. A., Alison M., Hai M. T. V., Logeat F. and Milgrom E.: The rabbit uteroglobin gene: structure and interaction with the progesterone receptor. *J. Biol. Chem.* **258** (1983) 10384–10389.
15. Pérez-Palacios G., Fernandez-Aparicio M. A., Medina M., Zacarias-Villareal J. and Ulloa-Aguirre A.: On the mechanisms of action of progestins. *Acta Endocr. (Copenh.)* **97** (1981) 320–328.
16. Garza-Flores J., Vilchis F., García G. A., Menjivar M. and Pérez-Palacios, G.: A-ring reduction enhances the antigonadotropic potency of norethisterone. *Acta Endocr. (Copenh.)* **112** (1986) 278–283.
17. Larrea F., Vilchis F., Chavez B., Perez A. E., Garza-Flores J. and Pérez-Palacios G.: The metabolism of 19-Nor contraceptive progestins modulates their biological activity at the neuroendocrine level. *J. Steroid Biochem.* **27** (1987) 657–663.
18. Chavez B. A., Vilchis F., Perez A. E., Garcia G. A., Grillasca Y. and Perez-Palacios G.: Stereospecificity of the intracellular binding of norethisterone and its A-ring reduced metabolites. *J. Steroid Biochem.* **22** (1985) 121–126.
19. Vilchis F., Chavez B., Perez A. E., Garcia G. A., Angeles A. and Perez Palacios G.: Evidence that a non-aromatizable metabolite of norethisterone induces estrogen-dependent pituitary progestin receptors. *J. Steroid Biochem.* **24** (1986) 525–531.
20. Bowers A., Ringold H. J. and Denot, E.: Steroids, C1 19-Nordihydrotestosterone derivatives. *J. Am. Chem. Soc.* **80** (1958) 6115–6121.
21. Atger M. and Milgrom E.: Progesterone induced messenger RNA. Translation, purification and preliminary characterization of uteroglobin-mRNA. *J. Biol. Chem.* **252** (1977) 5412–5418.
22. Ouchterlony O.: *Handbook of Immunodiffusion and Immunoelectrophoresis*. Ann Arbor Science, Ann Arbor (1968).
23. Laemmli U.K.: Cleavage of structural proteins during the assembly of the head of Bacteriophate T_4. *Nature (Lond.)* **227** (1970) 680–685.
24. Mayol R. F. and Longenecker D. E.: Development of a radioimmunoassay for blastokinin. *Endocrinology* **95** (1974) 1534–1542.
25. Cathala G., Savouret J. F., Mendez B., West B. L., Karin M., Martial J. A. and Baxter J. D.: A method for isolation of intact, translationally active ribonucleic acid. *DNA* **2** (1983) 329–335.
26. Rave N., Crkvenjakov R. and Boedtker H.: Identification of procollagen mRNAs transferred to diazobenzil-oxymethyl paper from formaldehyde agarose gels. *Nucleic Acids Res.* **6** (1976) 3559–3567.
27. Thomas P. S.: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natn. Acad. Sci. U.S.A.* **77** (1980) 5201–5205.
28. Wahl G. M., Stern M. and Stark G. R.: Efficient transfer of large DNA fragments from agarose gels to diazobenzyl-oxymethyl paper and rapid hybridization by using dextran sulfate. *Proc. Natn. Acad. Sci. U.S.A.* **76** (1979) 3683–3687.
29. Rigby P. W. J., Dieckmann M., Rhodes C. and Berg P.: Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Molec. Biol.* **113** (1977) 237–251.
30. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
31. Johansson E. D. B.: Estrogen-progestogen combination. Past experience and future development. In *Research on the regulation of Human Fertility* (Edited by E. Diczfalusy and A. Diczfalusy). Scriptor, Copenhagen. Vol. 2 (1983) pp. 637–648.
32. Hall P. E. and Fraser I. S.: Monthly injectable contraceptives. In: *Advances in Human Fertility and Reproductive Endocrinology* (Edited by D. R. Mishell, Jr). Raven Press, New York (1983) pp. 65–88.
33. Pérez A. E., Hernandez A., Cervantes P., Vilchis F. and Chávez.: *In vitro* metabolism of ^{3}H-norethisterone in hypothalamus and pituitary from ovariectomized female rats. *Memories of the XXV Annual Meeting Mex. Endocr. Soc.*, Mazatlán, Mexico (1985) (Abstr. No. 50).
34. Loosfelt H., Fridlansky F., Savouret J. F., Atger M. and Milgrom E.: Mechanism of action of progesterone in the rabbit endometrium. Induction of uteroglobin and its messenger RNA. *J. Biol. Chem.* **256** (1981) 3465–3470.