

ments and Sertoli cell aggregates. In the isolated germinal cells (spermatocytes and round spermatids) only a small amount of testosterone was bound to nuclear material. From the results it is concluded that the nuclear receptor for androgen, which is present in Sertoli cell preparations, is absent in spermatocytes and round spermatids.

Attempts have been made to correlate the presence of the oestradiol receptor in the nuclear pellet of Leydig cells with the inhibiting effect of oestradiol on LH stimulated testosterone production. Testosterone production in isolated Leydig cells from testes of immature and adult rats could be stimulated by addition of LH in a dose dependent way. The Leydig cell preparation from immature rats (containing 53%  $3\beta$ -hydroxysteroid dehydrogenase active cells) showed marked differences with Leydig cell preparations from adult rats. Although testosterone production in cells from immature rats could be stimulated by LH in a dose dependent way, the maximal amount of testosterone produced was 10 times lower than in cells from adult rats. Testosterone metabolism in cells from immature rats was higher due to conversion of testosterone to  $5\alpha$ -reduced metabolites.

Hypophysectomy of immature rats resulted after 5 days in a loss of LH responsiveness of Leydig cells. In contrast Leydig cells from hypophysectomized adult rats still responded to LH in the same way as cells from intact rats. LH responsiveness in cells from immature hypophysectomized rats could be partly restored by treatment with FSH for 5 days. This effect could be totally ascribed to FSH and not to contaminating LH present in the FSH preparation. When oestradiol was administered together with

FSH to hypophysectomized immature rats the induced LH responsiveness could not be observed.

The loss in LH responsiveness after hypophysectomy of immature rats in terms of testosterone production could not be explained by a change in the amount of Leydig cells present in the Leydig cell preparation or to a higher conversion of testosterone. The LH-stimulated cAMP production, however, was very low in cells from hypophysectomized rats as compared to cells from intact rats. There was no difference between cAMP production of Leydig cells from untreated, FSH-treated or FSH plus oestradiol benzoate treated hypophysectomized rats.

During the first 2 days after hypophysectomy LH responsiveness in untreated and FSH-treated rats declined in the same way. From day 2 after hypophysectomy LH responsiveness decreased further in cells from untreated rats but remained at a constant level in cells from rats treated with FSH. A single injection of oestradiol benzoate to FSH-treated hypophysectomized rats decreased LH responsiveness only when oestradiol was administered at that time after hypophysectomy, when a distinct effect of FSH on LH responsiveness was observed.

Three h after injection of oestradiol benzoate to FSH-treated hypophysectomized immature rats nearly all the receptor molecules were translocated to the nucleus. After 24 h part of the receptor molecules was still present in the nucleus, and also the inhibiting effect of oestradiol benzoate on LH-stimulated testosterone production in isolated Leydig cells could still be observed. These observations could reflect the possible involvement of the oestradiol receptor in this effect of oestradiol benzoate.

## POSTERS

### UTERINE SPECIFIC PROTEIN

- 1. Blastokinin: a utero-specific protein induced by the progesterone in the rabbit.** N. GARCEA, S. CAMPO, A. CARUSO, V. SCOTTO and P. SICCARDI, Dept. of Gynecology and Obstetrics, Catholic University, Rome, Italy

The AA have isolated from rabbit uterine secretions in the first days of pregnancy, a protein, Blastokinin (BKN), absent in the uterine secretion of the rabbits or in the serum during estrus. This protein, having an isoelectric point of 5.4, a  $R_f$  in polyacrylamide gel of 0.64 and a molecular weight of about 14.000, seems indispensable for the development of normal pregnancy. The AA obtain *in vivo* in different physiological or experimental conditions which mimic pregnancy (pseudopregnancy, administration of HCG or progesterone) the production of a protein having the same physico-chemical features as the BKN. The AA show that the protein synthesis is induced by progesterone. This has been confirmed *in vitro*. The AA incubate endometrium of rabbits, treated during 4 days with 3 mg/kg daily of progesterone with labelled aminoacids. The AA obtain the biosynthesis of a protein which because of its chromatographic and electrophoretic characteristics and linking capacity with steroids is recognized as BKN. Further confirmation of the utero-specificity of BKN has been obtained by the AA with immunological tests utilizing an anti-BKN antibody. This antibody reacts only with purified BKN or uterine secretion from a pregnant rabbit.

- 2. Blastokinin: endometrial protein for the blastocyst.** S. CAMPO, N. GARCEA, A. CARUSO, V. SCOTTO and P. SICCARDI, Dept. of Gynecology and Obstetrics, Catholic University, Rome, Italy

The AA have shown that rabbit endometrium in the first days of pregnancy produces a utero-specific protein: Blas-

tokinin (BKN). This synthesis seems to be induced by progesterone. BKN has a better linking capacity with progesterone than with estradiol. BKN-progesterone binding is influenced by the presence of estradiol, which interferes mainly during the stage of formation of binding, whereas it acts less on the binding already formed. The AA have shown *in vivo* and *in vitro* that BKN flows in the blastocelic fluid carrying progesterone. This protein is indispensable for the growth and implantation of blastocyst. In fact, administration during the first days of pregnancy in the rabbit of anti-BKN antibodies, which were obtained in the chicken, considerably reduced fetal survival.

- 3. Measurement of a specific estradiol-induced protein (IP) in rat tissues.** S. IACOBELLI, P. LONGO and F. RANELLETTI, Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica, 00168 Rome, Italy

Previous assays for IP have been based on changes in incorporation of isotopically-labelled amino acids into protein and subsequent electrophoretic separation of IP. In the present experiments, rat uterine IP has been purified by ammonium sulphate precipitation, ion-exchange chromatography and electrophoresis on cellulose acetate strips. Antibodies to IP have been prepared in rabbits and characterized by gel-diffusion and immunoelectrophoresis. A radioimmunoassay for IP has been developed using this antibody and [ $^{125}$ I]-labelled, purified IP. Using this assay, no IP was detected in rat serum, rat liver, or human endometrium but high amounts were detected in rat and mouse uterus and in rat brain of both sexes. Intermediate amounts of IP were found in adenohypophysis and kidney.

- 4. Differential regulation of the synthesis of rabbit blastokinin in uterine and extra-uterine tissues.** TUULA TORKKELI and OLLI JÄNNE, Departments of Biochemistry and Clinical Chemistry, University of Oulu, SF-90100 Oulu 10, Finland