

and immature animals were prepared. Equilibrium dialysis, dextran-coated charcoal adsorption, gel electrophoresis, hydroxyl apatite chromatography, and gel filtration were employed to separate free from bound steroids. Our principal finding is the demonstration of specific protein binding of progesterone, in addition to binding of testosterone, and dihydrotestosterone by nonflagellate germ cells. The progesterone binding receptor was absent from extracts of epididymal spermatozoa in which some binding capacity for androgen was retained. (Supported by a grant from the Population Council.)

8. *Boar and Human Acrosin and Acrosin Inhibitors: Isolation and Biochemical Characterization.* H. Fritz,* H. Schiessler, W. Schleuning, University of Munich, D-8 Munich 2, E. Fink,* Dept. Biology, Brookhaven National Lab., Upton, N.Y. 11973, and H. Tschesche, O. Lengel, Technical University of Munich, D-8 Munich 2.

Acrosomal proteinases (acrosins) and acrosin inhibitors have important functions in fertilization. Therefore, boar and human acrosins were isolated by (1) extraction of washed ejaculated spermatozoa with 2% (w/w) acetic acid, (2) separation of acrosin and acrosin inhibitors (present in about equimolar amounts) by gel filtration, (3) affinity chromatography using benzamidine cellulose as acrosin-specific adsorbent, and (4) desalting and lyophilisation of the acrosin solutions in the presence of stabilizing agents (saccharose, etc.). The specific activities of the electrophoretically homogeneous acrosins thus obtained amounted up to 15 U (substrate: BAPA)/mg.

A combination of ion exchange chromatography, gel filtration, and affinity chromatography (with trypsin cellulose as inhibitor-specific adsorbent) was employed for the isolation of the acrosin inhibitors from boar and human sperm plasma. Separation of the result and inhibitor mixtures into the homogeneous (established by amino acid and end group analysis) iso-inhibitors was achieved by gradient and equilibrium chromatography. From human sperm plasma we obtained eight acrosin-trypsin iso-inhibitors (mol wt near 8,000) and several trypsin-chymotrypsin-acrosin(?) iso-inhibitors (mol wt near 13,000), from boar sperm plasma five acrosin-trypsin-plasmin iso-inhibitors (mol wt near 8,000). No structural homology was found between pancreatic secretory trypsin-(acrosin) inhibitors and sperm plasma acrosin-(trypsin) inhibitors.

Kinetic measurements of the acrosin activities applying synthetic trypsin substrates (BAEE, BAPA, BLNA) and inhibitors (TLCK, DFP) and the high affinity ($K_i < 10^{-9}$ mole) of trypsin inhibitors from natural sources (e.g., pancreas, seminal plasma, serum) indicate a great similarity between the enzymatic reaction mechanisms of pancreatic trypsin and sperm acrosin. The kallikrein-like activity (kinin liberation) of the acrosins may be important for the passive transport of the sperms in uterus and oviduct.

9. *Accumulation and Retention of the Estrogen Receptor in the Nucleus of Hypothalamic and Pituitary Cells.* J. H. Clark,* J. N. Anderson, and E. J. Peck, Jr., Department of Cell Biology, Baylor College of Medicine, Houston, Texas.

The effect of estradiol injection on the quantity of estrogen receptors in the nuclear fraction of the hypothalamus and the anterior pituitary was examined by the [³H]estradiol exchange assay. The accumulation of the receptor-estradiol (R·E) complex in the nuclear fraction of both tissues in the female rat reached a maximum one hour after an injection of 25 µg of estradiol. The quantity of receptor in the pituitary is several fold greater than the quantity in the hypothalamus. However, hypothalamic receptors are confined to the basal medial region, and the concentration in this region (1.1 fmole/µg DNA) is similar to the concentration in the pituitary (1.3 fmole/µg DNA). The increases in nuclear R·E in both tissues are equally dependent on the quantity of estrogen administered. One hour after an injection of a physiological dose (0.5 µg) of estradiol the amount of receptor in the nuclei in both tissues is ~50-60% of maximum. Between the first and 12th hours after treatment, parallel declines in hypothalamic and hypophyseal receptor levels are observed. Estradiol injection also increases the quantity of nuclear R·E in the hypothalamus and pituitary of 21 day old male rats. Further, the accumulation of nuclear R·E in male and female rats is equally dependent on estradiol dose. The results of this investigation are consistent with the proposal that the presence of the estrogen receptor in the nucleus of hypothalamic and pituitary cells is important in the regulation of reproductive processes.

10. *Catecholaminergic and Serotonergic Control of Progesterone-Induced Ovulation in Immature Rats.* A. J. Zolovick* and A. P.