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which contained antibiotics, HEPES and TES, and inoculated into plastic dishes. After 2 or 3 passages, cloning was done using micro plates. Anti-prolactin rabbit serum was labelled with FITC. The cultured cells on the micro plates were incubated with prolactin and washed with PBS. Then they were incubated with FITC-labelled anti-prolactin. Immediately after removal of excess antibody by washing with PBS immunofluorescence was observed by fluorescence microscopy. Prolactin receptors could be observed in the cultured carcinoma cells.

## PROSTATE CANCER

44. Characterization of a transplantable androgen-dependent human prostatic carcinoma (PC 82), J.C. ROMIJN, G.J. v. STEEN-BRUGGE, K. OISHI, J. BOLT-DE-VRIES, W. HÖHN and F.H. SCHRÖDER, Erasmus University, Rotterdam, Dr. Molewaterplein 40, Rotterdam, The Netherlands

Recently a human prostatic tumor line (PC 82), growing on athymic (nude) mice by serial transplantation, has been established in our laboratory. Characterization of this tumor line was carried out to establish whether the PC 82 line might serve as an appropriate model system for the investigation of human prostatic cancer.

The histological characteristics of the PC 82 tumor closely resemble those of the original tumor, a moderately differentiated adenocarcinoma, even after two years of growth in nude mice. Doubling times were estimated from a large number of growth curves; in the exponential phase of growth a doubling time of 2-3 weeks was measured. The presence of large amounts of (human) prostatic acid phosphatase, in the original tumor as well as in the PC 82 line, was demonstrated by means of an immunohistochemical technique. The PC 82 tumor did not grow on female nude mice, whereas in male nude mice the take rate was around 75%. Furthermore, castration of a tumor-bearing male resulted in regression of the tumor. The presence of an androgen receptor was demonstrated.

The results mentioned above (maintenance of original histology, relatively slow growth rate, presence of acid phosphatase, androgen dependence) suggest that PC 82 might be considered as a useful system for the study of human prostatic cancer.

45. In vitro metabolism of androgen by rat prostate, J.C. PLASSE<sup>1</sup>, A REVOL<sup>1</sup> and B.P. LISBOA<sup>2</sup>, <sup>1</sup>Hospices Civils de Lyon, Hôpital Jules Courmont-Sainte Eugenie, 69230 Saint Genis Laval, France, and <sup>2</sup>Universitäts-Frauenklinik Eppendorf, 2000 Hamburg 20, West Germany

In order to investigate the metabolism of some physiological androgen steroids by rat prostate, testosterone, androstenedione,  $5\alpha$ -

androstanedione,  $5\alpha$ -androstane- $3\beta$ ,  $17\beta$ -diol,  $5\alpha$ -androstane- $3\alpha$ ,  $17\beta$ -diol, epiandrosterone and androsterone were separately incubated with a ventral rat prostate homogenate in a medium supplemented with a NADPH regenerating system at 370C under air. All incubations were carried out simultaneously with a portion of the same rat prostate homogenate. Identification and evaluation of metabolites was done by liquid and radio-gas chromatography. In vitro metabolism of testosterone by rat prostate can follow two main pathways of similar quantitative intensity, through either 5a-dihydrotestosterone or 4-androstenedione. The relationship between both the 17-keto and the  $17\beta$ -hydroxy-pathway exists although the 176-hydroxysteroid oxidoreductase activity is weak towards 17-ketosteroids. Both 17-keto and 17β-hydroxysteroid are good substrates for 3a-hydroxysteroid oxidoreductase and thus an equilibrium is reached between 3a-hydroxy and 3-keto-metabolites. On the contrary equilibrium between 3-keto and 3B-hydroxy-metabolites is in favour of the 3β-hydroxysteroid formation. Some hydroxylated metabolites such as  $6\alpha$ - and  $6\beta$ -hydroxysteroid were identified.

46. Characterization of androgen receptors in a rat prostate adenocarcinoma, O.A. LEA and F.S. FRENCH, Hormone Laboratory, University of Bergen, Norway, and Department of Pediatrics, University of North Carolina, Chapel Hill NC 27514, U.S.A.

Androgen receptors present in cytosols prepared from transplants of the Dunning H 3327 adenocarcinoma were characterized following labelling in vitro with <sup>3</sup>H-dihydrotestoster-one and <sup>3</sup>H-testosterone. Apparent dissociation constants measured at 0°C were 3·10-9 M for both steroids. Dissociation halflives were 87 h and > 200 for testosterone and DHT, respectively. In buffers of low ionic strength the receptor showed a sedimentation coefficient of 85. A shift to approximately 5 S was observed when ionic strength was increased to 0.5. Two forms of androgen receptor were recovered when cytosol was fractionated on phosphocellulose columns. A predominant form eluted from the cation exchanger at 0.15-0.20 M KCl. Further characterization in the presence of 0.5 M KCl revealed a sedimentation coefficient of 5.0 S, a gel filtration radius of 53 Å, a molecular weight of 110-120,000 Daltons and a frictional ratio of 1.7. A smaller form of the androgen receptor was eluted from the phosphocellulose columns at a higher salt concentration. It has a sedimentation coefficient of 3.6, a gel filtration radius of 35 Å, a molecular weight of 55-60,000 Daltons and a frictional ratio of 1,4. Heating of tumor cytosol at 25-37°C for periods up to 30 min did not produce an inter-conversion of the two receptor forms. Androgen receptors with similar size, charge and symmetry were present in cytosols derived from testis; epididymis, ventral and dorsal prostate and seminal vesicles.