in the urine (Croxatto *et al.*, 1977) and a single renin injection which elevates ALD is followed by a significant decrease of KAL in the urine (Croxatto *et al.*, 1978). These results make it doubtful that in these conditions ALD can be the major factor implicated in KAL increase. The effect of ALD (1, 2 and 5 μg per 100 g b.w.) in adrenalectomized and in normal Sprague-Dawley rats, either normally hydrated or overhydrated was investigated. In adrenalectomized rats, KAL excretion is significantly reduced and a daily dose of 5 μg almost restores KAL in the urine excreted within 8 h after injection. KAL activity: in controls, 1.260 $\pm$ 0.29; adrenex + ALD, 1.093 $\pm$ 0.24; adrenex 0.606 $\pm$ 0.010. In normal rats ALD, 2-5 μg injected twice in a period of 8 h did not change KAL excretory rate, although there was a significant decrease in Na excretion. Similar negative results were also obtained in rats which had had for several days a high intake of NaCl. In overhydrated rats aldosterone given i.p. simultaneously with gavage, did not induce significant changes in KAL excretion in the urine (collected for 3 h). These negative results were in contrast with the effects of other hormones such as oxytocin (10-20 mU) and vasopressin (5 mU) which in similar protocols increase KAL excretion. The data suggest that in these experimental conditions endogenous aldosterone has only a permissive role in KAL excretion.

73. Isolation and partial identification of several new polar metabolites of aldosterone synthesized in the liver of male rats

LATIF, S. A., TSAI, R., REINHOLD, V.* and MORRIS, D. J., Department of Laboratory Medicine, The Miriam Hospital and Brown University, Providence, Rhode Island, and *Harvard University Medical School, Boston, MA, U.S.A.

Following administration of a physiological dose of [^{3}H]-aldosterone, we have previously found large quantities of several polar metabolites of aldosterone *in vivo* in both the liver and kidney of rats during the latent period of aldosterone. The dose-dependent quantities of these aldosterone metabolites in the target tissue, kidney, correlate well with the magnitude of the physiological response of aldosterone in the kidney. Most of these metabolites of aldosterone appear to be synthesized in the liver and their synthesis has been suggested to be of major importance in the mechanism of action of aldosterone. With the use of Sephadex DEAP-LH-20 column chromatography, the majority of the radiometabolites in the liver cytosol fraction were eluted in the "neutral metabolite" Fraction. High pressure liquid chromatography (HPLC) using C18-μBondapak reverse phase column chromatography and 50% methanol as the eluent separated these "neutral metabolites" into three distinct peaks of polar metabolites of aldosterone. These three peaks of metabolites were also demonstrated to be present in the kidney cytosol of male rats. Larger quantities of each of these three peaks of polar metabolites of aldosterone have now been synthesized using *in vitro* liver microsomal preparations. GC-Mass Spec. analysis of one of these peaks of polar metabolites of aldosterone (after purification with HPLC) has shown that it consists principally of two mono-hydroxylated metabolites of aldosterone. Detailed experiments are being conducted to attempt to fully characterize the chemical structure of the two mono-hydroxylated metabolites of aldosterone. GC-Mass Spec. analysis of the polar metabolites of aldosterone present in the other two HPLC peaks is under current investigation.

5. STEROID–PROTEIN INTERACTION

74. Levonorgestrel and progesterone binding in human uterine cytosol and plasma

SRIVASTAVA, A. K., HABIB, F. K. and STITCH, S. R., Division of Steroid Endocrinology, The University of Leeds, 26–28, Hyde Terrace, Leeds LS2 9LN, England

Binding of levonorgestrel (D-norgestrel) and progesterone was studied in the human uterine cytosol and plasma. [^{3}H]-levonorgestrel demonstrated a high affinity binding to cytosol and plasma. In cytosol, competition studies with 100 fold molar excess of unlabelled steroids showed that the binding was inhibited by progesterone. On the contrary, progesterone failed to compete with levonorgestrel binding sites in plasma whereas dihydrotestosterone, testosterone and oestradiol-17β were strong competitors. Tritiated progesterone was also bound to cytosol and plasma. Competition studies (100 $\times$ excess) in cytosol revealed that levonorgestrel competed effectively. However, in plasma, cortisol was a strong competitor for progesterone binding sites whilst levonorgestrel did not compete at all. These results suggested that the binding proteins for levonorgestrel in cytosol and plasma are different. In plasma, levonorgestral binds to SHBG whereas progesterone binds to transcortin or CBG.

75. The influence of structural and steric alterations in the estradiol molecule on the, translocation of estrogen–receptor complex from cytoplasm to nucleus of the rabbit uterus

CHERNYAEV, G. A., BARKOVA, T. I., ANANCHENKO, S. N. and SOROKINA, I. B., Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences, Moscow, U.S.S.R.

The effect of structural and steric changes in the estradiol molecule upon the transfer of steroid–receptor complex from cytoplasm to nucleus has been studied by using an homogenate of rabbit uterus. The ability of unlabelled estradiol analogs to take part in the translocation has been determined by their capacities to inhibit the incorporation of labelled estradiol–receptor complexes into uterine nuclei. Previously we concluded that the alterations in the estradiol molecule resulted in a decrease of the estrogenic activity and affinity for uterine receptors, both cytosol and nuclear receptors having the same specificity. Now we have found that the inhibition of translocation by the estradiol analogs corresponds in general to their affinities to the receptors but this correspondence does not take place for some analogues. This shows that the translocation of the cytoplasmic receptor–estrogen complex to the nucleus (under conditions of the whole receptor system) is characterized by other features than the interaction of the steroid with receptors.

76. Protein binding of androgens in human placental cytosol

BARILE, G.,* MONTEMURRO, A.,† SCIRPA, P.† and MANGO, D.,†* TBM Laboratory, CNR, and †Department of Obstetrics and Gynecology, Catholic University, Rome, Italy

The binding of radioactive testosterone or 5α-dihydrotestosterone (DHT) to components of human placental cyto-

sol and nuclei was investigated. Kinetic analysis of cytosol revealed the presence of specific binding sites with a K_D of 11×10^{-9} M and a receptor site concentration of 0.32 pmol/ng protein. The complex was labile at a temperature of 45 C. The specificity was indicated in a competition study using unlabelled competing steroids at 10, 100, 1000-times the molar concentration of radioactive hormone. The inhibition was similar for both cold testosterone and DHT (80%), methyltestosterone (66%), androstenediol (64%), DHA (54%), androstenedione (42%), E_2 (40%), E_3 (3%). The specific binding protein has a sedimentation coefficient of 5S. Purified nuclei or whole tissue incubated with [^{3}H]-T or [^{3}H]-DHT at 35 C for 1 h gives a nuclear radioactivity that is completely inhibited by cold competitor and with approximately the same nuclear binding sites/DNA respectively for [^{3}H]-T and [^{3}H]-DHT. Our data support the presence of an androgen receptor in human placental cytosol the role of which needs further investigation.

77. Estrogen receptors in lactating mammary gland of the rat

MEHTA, R. G., PARK, D. C., BOYD, P. A. and WITTLIFF, J. L., Department of Biochemistry, University of Louisville Medical School, Louisville, KY, U.S.A.

Kinetic and molecular properties of estradiol receptor (ER) in cytosol were characterized by titration analyses, DNA-cellulose binding, ion-exchange chromatography and density gradient centrifugation. The rate constants for association and dissociation at 0 C were $2–3 \times 10^{-7}$ M^{-1} min^{-1} and $2–4 \times 10^{-3}$ min^{-1}, respectively. These data and those of Scatchard analyses indicated binding sites with high affinity ($K_D = 10^{-10}$ M). Only estrogenic compounds with $^{c}3$ and $^{c}17\beta$ hydroxyl functions, as well as unsaturation of A ring, were bound. The 8S ER complex chromatographed as 2 components on DEAE-cellulose columns. ER binding to DNA–cellulose was increased significantly if charged receptor complex was warmed at 28 C for 30 min; whereas the presence of 1 mM EDTA during activation reduced binding to DNA-cellulose by 40–50%, an effect that can be reversed by divalent cations. Activation of charged ER was a prerequisite for the translocation of ER into nuclei. Activated ER stimulated Mg^{2+} dependent RNA polymerase activity 3-fold without altering Mn^{2+} dependent activity. These results support the notion that activation of steroid receptors is essential for their translocation into nuclei and subsequent stimulation of nuclear synthetic activity.

78. Androgen and progesterone binding in human testis cytosol

STOA, K. F., DAHL, O., HEKIM, N. and HOISAETER, P. Å., Hormone Laboratory and Departments of Oncology and Surgery, University of Bergen School of Medicine, Bergen, Norway

The present report describes an attempt to detect, characterize and measure in human testis cytosol proteins corresponding to the androgen receptor which has been found to be present in the testis of hypophysectomized rats. The methods used have been exchange assay with [^{3}H]-methyltrienolone ([^{3}H]-R 1881), studies of dissociation rates and heat sensitivity, as well as sucrose gradient centrifugation. In normal human testis cytosol, a concentration of 119 ± 59 fmol/mg protein receptor-like androgen-binding activity, was found. In only two out of five tumour specimens (seminomas) a relatively low (23–33 fmol/mg protein) exchange activity could be detected. Receptor affinities for R 1881 and testosterone were found to be about equally high, whereas the affinity for progesterone was slightly lower. There was only negligible binding inhibition by oestradiol. Gradient centrifugation revealed two peaks, corresponding to 3S and 4S, respectively. Progesterone affinity could be demonstrated solely for the 4S peak. The results indicate that two types of high affinity binding proteins are present in human testicular cytosol, one with affinity for both androgens and progestins, and the other with specific androgen-binding activity.

79. Estrogen induction and functional importance of carrier proteins for riboflavin and thiamine in the rat during gestation

ADIGA, P. R. and MUNIYAPPA, K., Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

During gestation in higher animals thiamine (B_1) and riboflavin (B_2) are preferentially transported across the placental barrier and concentrated by the foetus. The mechanisms of such facilitated transport are unknown. We have shown in the hen that vitamin transport for the embryonic development is mediated through B_1 and B_2 carrier proteins. We report here that in the pregnant rat (but not in the male or immature female) similar proteins with immunological cross reactivities to chicken B_1 and B_2 carrier proteins do exist. The hormone responsible for induction of these proteins was estrogen (E) since (1) specific induction of these proteins in the male and in ovariectomized adult female rats could be elicited by E (2) the blood levels of these proteins alter in concert with the changing E in cycling female and pregnant rats. Passive immunization of pregnant rats (4–16 d) with antibodies to chicken vitamin carrier proteins (but not to ovalbumin) resulted in foetal resorption/abortion showing functional importance of carrier proteins for embryonic development and survival.

80. Influence of prolactin on testosterone production and action in the male rat

PRASAD, M. S. K., Department of Biochemistry, Indian Institute of Science, Bangalore, India

Prolactin (PRL) facilitates testosterone (T) action on male accessory sex organs of rodents, but the mechanism is not clear. A temporal relationship between circulatory levels of PRL and T in developing rats was observed. In 90-day old rats, neutralization (for 5 days) of PRL by specific antiserum (A/S) significantly reduced the serum T and weights of ventral prostate and seminal vesicles, whereas injection of PRL (1 mg/100 g.b.wt. × 5d) to such rats significantly enhanced serum T levels and the weights of the accessory organs. However, their testes responded to saturating levels of LH to a similar extent in terms of T production *in vitro*. Injection of PRL enhanced the ability of the prostate and seminal vesicles to concentrate [^{3}H]-T *in vitro*, whilst A/S treatment had no significant influence. PRL treatment enhanced the binding of [^{125}I]-LH to testicular membrane preparations whilst A/S was without any effect. Neither PRL nor A/S modulated the binding of [^{125}I]-PRL to membranes.

81. Protein induction and estrogenic potency

VON SCHOULTZ, B., DAMBER, M.-G., SANDSTRÖM, B. and STIGBRAND, T., Department of Obstetrics and Gynecology and Physiological Chemistry, University of Umeå, S-901 87 Umeå, Sweden

Estrogen treatment has become more frequent during the last years. There are few methods to quantify and compare the estrogenic effect of various preparations. Animal