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

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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

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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

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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

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