63. Progesterone metabolism in healthy and inflamed female gingiva

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It is known that the inflamed gingiva metabolizes progesterone more actively than the healthy one. When incubating subcellular preparations of normal and inflamed female gingiva, several metabolites of 4-[^{14}C]-progesterone could be identified with column and thin-layer chromatography and radioautography. The homogenate preparations yielded 5α-pregnane-3,20-dione, 3β-hydroxy-5α-pregnan-20-one, 20α-hydroxy-5α-pregnan-3-one, 20β-hydroxy-4-pregnen-3-one, and 5α-pregnane-3α,20β-diol. The mitochondria were inactive, but the microsomes produced 5α-pregnane-3,20-dione. In the 100,000 g supernatant incubations, 5α-pregnane-3,20-dione, 5β-pregnane-3,20-dione, 3α-hydroxy-5β-pregnan-20-one, 20β-hydroxy-4-pregnen-3-one, and 5β-pregnane-3α,20α-diol were found. As elsewhere in the alimentary canal, the β forms of metabolites can be shown in the supernatant but not in the homogenate incubations. The metabolic activity of the gingiva correlated with the degree of inflammation.

64. Steroid sulphates in ovarian tissues: determination of sulphohydrolase activity and influence of gonadotrophins

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Estrogen sulphation in ovarian tissues has been shown by us to be stimulated by gonadotrophins. To investigate whether sulphohydrolase activities are also influenced by gonadotrophins, these enzymes have been partially characterized and measured in bovine and rat ovaries. In both tissues similar K_M values have been observed, being 1, 7 and 10×10^{-5} M for estrone sulphate (ES), pregnenolone sulphate (PS) and dehydroepiandrosterone sulphate (DS) respectively, thus demonstrating a high enzyme affinity towards ES. In immature rats treated with HCG and HMG for 3 days the gonadotrophins had no influence on the ovarian sulphohydrolase activity for DS and PS as compared with controls. However, the enzyme activity for ES was significantly decreased in treated groups, being 3.1 ± 0.9; 2.2 ± 0.8 and 1.5 ± 0.5 pmol/mg protein min in the control, HCG and HMG/HCG groups, respectively. Thus, an inverse relationship between arylsulphotransferase and aryl sulphohydrolase activity following gonadotrophin treatment was observed, suggesting that changes in both enzymes favour an increased ES formation, presumably for the regulation of hormone production.

65. Androgen metabolism in human endometrium and endometrial carcinoma

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Former investigations indicated alterations of androgen metabolism in women with endometrial carcinoma (EC). We investigated the metabolism of testosterone (T) and 5α-androstan-17β-ol-3-one (DHT) to study the enzymes in endometrium (E) and alterations of their activities in EC. The tissue was obtained by curettage or operation and incubated with 4×10^{-12} mol 1,2-[^{3}H]-T or 1,2-[^{3}H]-DHT (Krebs–Henseleit phosphate buffer, NADPH generating system, 2 h, 37°C). The steroids were separated by gas chromatography (1% XE60) after adsorption with Florisil, extraction with ethanol and conversion to TMSi ethers. In E and EC, T was metabolized to 4ene-androstene-3,17-dione (Δ^4-A), 5α-androstan-17β-ol-3-one (DHT), 5α-androstan-3α-ol-17-one (A) and 5α-androstane-3α,17β-diol (A-diol). Degradation of T and formation of Δ^4-A were diminished in EC compared with E. In contrast more DHT, A-diol and A, indicating the activity of 5α-reductase, were formed in EC. In E and EC, DHT was metabolised to 5α-androstane-3,17-dione (A-dione), A-diol, A and 5α-androstane-3β-17β-diol (3β-A-diol). The degradation of DHT and the formation of A-dione were significantly lower in EC than in E. In contrast, the formation of A-diol and of 3β-A-diol was significantly higher in EC compared with E.

66. Inhibition of total corticosteroid secretion by metyrapone in man

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The purpose of the present study was to demonstrate an *in vivo* effect of metyrapone (M) on adrenal enzymes in the early steps of the biosynthetic pathway of corticosteroids. In four male subjects, serum concentrations of progesterone (P), 17-OH-progesterone (17-OH-P), 11-deoxycorticosterone (DOC), 11-deoxycortisol (S), corticosterone (B), 18-OH-11-deoxycorticosterone (18-OH-DOC), aldosterone (A) and cortisol (F) as well as plasma concentration of ACTH were measured before and after oral administration of 40 mg of M/kg at 8.15 h in short time intervals. *Results*: About one hour after drug administration plasma ACTH exhibited marked peaks. After a decrease, plasma ACTH started to increase, reaching maximum levels between 14.00 h and 16.00 h. Increase of serum DOC and S started 30–90 min after administration of M and peaked at about 10.00 h to 11.00 h. A second rise of DOC and S reached plateau values at 12.00 h to 14.00 h. Serum B, F, A and 18-OH-DOC fell during the first hour after administration of M. They increased from 14.00 h to 16.00 h. Serum P and 17-OH-P slightly increased or remained almost unaltered up to 12.00 h. They increased markedly from about 14.00 h. *Conclusions*: M inhibits adrenal 18-hydroxylase in addition to 11-hydroxylase. The relatively low serum levels of P and 17-OH-P during the first period after drug administration—although plasma ACTH is highly elevated at this time—indicate an inhibitory effect of M on an enzymatic step before corticosteroid biosynthesis.

67. The effect of hypophysectomy on the *in vivo* metabolism of testosterone in the skin and other tissues of the rat

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The responses to testosterone of the rat sebaceous glands, in contrast to those of the ventral prostate and seminal vesicles, are greatly diminished by hypophysectomy. On the other hand, the effects of 5α-dihydrotestosterone and 5α-androstane-3β,17β-diol have been shown to be less dependent on the presence of the pituitary. The role of the pituitary was therefore further investigated by studying the metabolism *in vivo* of 4-[^{14}C]-testosterone injected into castrated and hypophysectomized-castrated rats which were killed 1 h later. All the rats had been implanted with testosterone which had been removed 24 h previously. In the ventral prostate and seminal vesicles the testosterone was almost completely metabolized, with 5α-dihydrotestosterone and 5α-androstane-3α,17β-diol as the major products, irrespective of the presence of the pituitary. In

skin, hypophysectomy appeared to increase the amount of testosterone metabolized from 40 to 60%, but of the major identified metabolites, namely 3α-androstanediol, androstenedione, 5α-dihydrotestosterone and androsterone, only the last was produced in significantly greater amounts.

68. Steroid metabolism by cultured Sertoli cells

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The ability of cultured Sertoli cells, from testes of 80d rats, to metabolize [7(*n*)-^{3}H]-progesterone (P) (10 μCi/3.0 nmol) to testosterone (T), androstenedione (A), 5α-dihydrotestosterone (DHT), 17α-hydroxyprogesterone and 20α-dihydroprogesterone was demonstrated. Calculations from crystallization data indicated C-19 steroids (T, A & DHT) increased linearly between 0.5 to 3 h and 19.4 ng of androgen per 1×10^6 cells was formed (3 h) with the largest amount being DHT. Although the amount of C-19 steroids formed (19.4 ng) was relatively small (1.8% conversion) compared to T formation by isolated interstitial cells, it may be physiologically important for intratubular functions. Sertoli cells from adult and immature rat testes failed to aromatize T to estrogens even in the presence of FSH. Cultures of Sertoli cells from testes of 36d rats were incubated with 4-[^{14}C]-T ($1 \text{ Ci}/5 \times 10^{-7}$ M) with and without FSH (5 μg/ml NIH-FSH-S11). T (unconverted), A, 5α-androstan-3α,17β-diol and DHT were identified by crystallization, while ^{14}C activity behaving like estrone or estradiol did not crystallize to constant specific activity.

69. Metabolism of [1,2-^{3}H]-androstenedione in skin from hirsute women

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The metabolism of 4-[1,2-^{3}H]-androstene-3,17-dione was studied in skin from the axillary region and from the inner side of the upper arm of ten healthy, normally menstruating nonhirsute and thirty oligomenorrhoic amenorrhoic hirsute women between 17 and 39 years of age. All skin specimens metabolized 4-androstene-3,17-dione into 5α-androstane-3,17-dione, 3α-hydroxy-5α-androstan-17-one, 3β-hydroxy-5α-androstan-17-one and small amounts of 17β-hydroxy-4-androsten-3-one. Hirsute women were found to have a 30% lower ($P < 0.01$) 5α-reductase activity in axillary skin than normal women. Hirsute women also tended to have a lower 5α-reductase activity in skin from the inner side of the upper arm than healthy subjects. The hypothesis is suggested that the biologically active androgen in skin may be a 3-oxo-4ene-steroid (testosterone or 4-androstene-3,17-dione) rather than a 5α-reduced metabolite (e.g., 5α-dihydrotestosterone) and that the androgen hyperactivity in skin from hirsute women may be related to a relatively slower deactivation of the active androgen than in skin from non-hirsute subjects.

70. Androgen levels in the homogenate, cytosolic and nuclear fractions of rat prostate (PR), skeletal muscle (SM), heart muscle (HM) and bulbocavernosus-levator ani muscle (BCLA)

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Besides typical androgen target organs such as the prostate, different muscle types have also been shown to possess a specific androgen receptor for testosterone (T)/5α-dihydrotestosterone (DHT) in the cytosol. To see whether cytosolic androgens in muscles are also able to be translocated into the nuclei, we measured the distribution of endogenous T and DHT between cytosolic and nuclear fractions in prostate and three types of muscle of male rats. The excised tissues of three animals (approx. 350 g) were pooled for each experiment, washed with buffer (0 C) and pulverized at −180 C to yield the homogenate (HOM). To one weight part was added 3 vol. of buffer and the mixture was centrifuged at 800 *g*. The pellet was washed 3× with buffer containing 0.1% Triton X-100, 1× with buffer and was regarded as the nuclear fraction (NUC). The 800 *g* supernatant was recentrifuged at 100,000 *g* to yield the cytosol (CYT). Aliquots of HOM, CYT and NUC were extracted with ether, T and DHT were separated by celite chromatography and measured by RIA. The main results are: (1) DHT is mostly accumulated in prostate (HOM: 11.1 ± 2.6 ng/g wt. weight, CYT: 1.23 ± 0.64 ng/ml of dil. cytosol, NUC: 2.35 ± 0.33 ng/g wt. weight, $\bar{x} \pm$ S.D.), considerably lower values were found for T (0.70 ± 0.40, <0.1, 0.38 ± 0.10, respectively). (2) In the three types of muscle, T is the predominant androgen present (SM: 1.3 ± 0.5, 0.17 ± 0.06, 0.30 ± 0.13, HM: 2.8 ± 0.8, 0.33 ± 0.08, 0.99 ± 0.47 and BCLA: 1.4 ± 0.2, 0.13 ± 0.04, 0.51 ± 0.27). (3) T is distinctly more accumulated in all fractions of HM compared to BCLA and SM. (4) Considerable amounts of the androgens found in the homogenate are located in the nuclear fraction (ca. 20–40%) in all organs. In conclusion: As in prostate, the three types of muscle also possess a mechanism that enables them to concentrate T and/or DHT in the nuclei. This might be related to the above mentioned cytosolic receptor proteins. (Supported by the DFG, Sonderforschungsbereich 34 (Endokrinologie).)

71. Thyroxine (T_4) and triiodothyronine (T_3) kinetics during prolonged estrogen administration

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Circulating levels of total thyroxine (TT_4), total triiodothyronine (TT_3), T_4-binding globulin (TBG) and T_4 and T_3 kinetics were studied before, during and after estradiol monobenzoate (E_2B, 50 μg/kg b.wt/day subcutaneously for 110 days) treatment. The mean ± S.E. plasma levels of TT_4, TT_3 and TBG prior to E_2B therapy were 7.5 ± 0.24 μg/dl, 117 ± 30 ng/dl and 1.52 ± 0.2 mg/dl respectively. A significant increase ($P < 0.01$) over the basal levels in plasma TT_4, TT_3 and TBG was recorded on day 6 of E_2B and continued to rise progressively up to day 19 and plateaued thereafter. Prolonged E_2B therapy significantly decreased ($P < 0.01$) distribution space (DS), metabolic clearance rate (MCR) and daily production rate of both T_4 and T_3. These data suggested that elevated hormone levels following E_2B were mainly due to decreased DS and MCR of the hormones, and not due to increased production by the thyroid.

72. Effect of aldosterone upon urinary kallikrein excretion in rats

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Aldosterone (ALD) would be one of the main factors which increase kallikrein (KAL) excretion in the urine (Margolius *et al.*, 1972–76). However, acute NaCl overloading, which inhibits aldosterone release, increases considerably KAL