tectomy, substantiating the findings of the *in vitro* biosynthetic pattern. To verify this finding that conversion of androgens to oestrogens at the breast tissue level is etiologically implicated, 3 patients were infused [^{3}H]-androgens (100 μCi) in normal saline containing 5% alcohol before and after mastectomy. The results on the levels of labelled urinary oestrogenic metabolites in both these situations confirm our hypothesis based on *in-vitro* biotransformation.

35. *In vitro* steroidal and cyclic AMP production pattern in adult rat testis under gonadotrophin induced desensitisation

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The steroidogenic and cyclic AMP producing capacity of desensitised rat testis induced by gonadotrophins either by HCG (100 i.u.) or O-LH (100 μg) were measured by radioimmunoassay after *in vitro* stimulation with HCG or $(Bu)_2$cAMP. This study was undertaken to elucidate how the modulation of LH/HCG receptor's function was expressed through target cell responses. The basal level of cyclic AMP remained unaltered while no stimulation of testosterone production either by HCG or $(Bu)_2$cAMP could be observed on two days after desensitisation. On the 6th to 8th day, the desensitised testis produced the testosterone pattern similar to that of the control, while the cyclic AMP pattern came to the normal level on the 8th day. These cellular responses were related to the continuous elevation of the receptor level at the plasma membrane. Ovine LH supplementation to adult rats (hypophysectomized) revealed good responsiveness to cAMP and testosterone production, although receptor level was not elevated.

36. Regulation of aldosterone biosynthesis: angiotensin II and K challenge following prolonged ACTH administration to normal subjects

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Our aims were: (i) to investigate why prolonged ACTH administration leads to adrenal refractoriness with respect to aldosterone (aldo) secretion: (ii) to determine whether this refractoriness can be overcome by the other major stimuli of aldo secretion: angiotensin II (angio) and potassium (K). Twelve healthy male volunteers on a regular diet received ACTH (40 IU, i.m.) twice daily for 5 days followed by 2 days of angio (5–13 ng/kg body wt/min for 60 min) or oral K citrate (30 mEq/h × 3). Initial ACTH stimulation increased aldo levels twofold, whereas sustained ACTH caused a drop in aldo values, an accumulation of the aldo precursors 11-deoxycorticosterone (DOC) and corticosterone (B) and a 2–50-fold rise in steroids of the cortisol pathway. Thus, prolonged ACTH results in selective inhibition localized in the conversion of B to aldo. A discordance in the aldo-stimulating and pressor responses of angio was unmasked with only the latter being positive following ACTH-induced refractoriness. In contrast to angio, K could enhance aldo secretion tenfold by overcoming the inhibition in B conversion to aldo, thus revealing a site of action of the cation at a late step of aldo biosynthesis.

37. Irreversible inactivation of aromatase in intact placental tissue with bromoandrogens

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In order to develop a radioautographic or immunoenzymic method to detect the distribution of estrogen synthetase in intact cell system, the ability for active-site-directed irreversible inactivation of androgen aromatase in human term placental tissue slices was studied on several bromoandrogens. Since the majority of aromatase activity in human placenta is in the 900 *g* precipitate fraction, the residual activity after inactivation of the slice at 25°C under air for 2 hr in TC MEDIUM 199 with 0.6 mM NADPH (pH 7.5) was analyzed using the washed 900 *g* pellet. The degree of inactivation at 60 μM was found 16α-bromo-4-androstene-3,6,17-trione > 6α-bromoandrostenedione (6αBrA) > 6βBrA ≒ 6α-bromotestosterone acetate (6αBrTAc). Treatment with 16αBrA, androstenedione, 16αOHT, 16βOHT, 16αOH-DHEA, oestriol, progesterone and cortisol showed no inactivation. The inactivation by the 16α-Br-trione and 6α-BrA was linear to the logarithm of inactivator concentrations. The 16α-Br-trione at 1, 5, 10 and 30 μM inactivated 30, 67, 78 and 93%. (Supported by USPHS Research Grants HD04945 and RR05716.)

38. Solubilized androgen aromatases; purification and characterization

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For a more definitive elucidation of mechanism of estrogen biosynthesis, apparently involving sequential multi-monoxygenations at one catalytic site and also having multicatalytic sites for different androgen substrates, isolation of purified aromatizing enzyme(s) is required. Detergent-free solubilized aromatizing system was prepared from lyophilized powder of the 900 *g* pellet of human term placenta by incubation in 0.5% deoxycholate buffer, centrifugation at 105,000 *g*, and passing the supernatant through Sephadex G25 column. Purification by DEAE-cellulose column gave two aromatase active forms, PII (major) and PIII (minor), each being an aromatase active single peak at 2×10^6 daltons in Bio-Gel A-15 m gel filtration. Relative total activity of major/minor showed 87/13, and specific activity of 235 and 152 pmol/min/mg protein, respectively. SDS-polyacrylamide gel showed that PII consists of two major bands, 82×10^3 and 47×10^3 daltons, and two minor bands, 68×10^3 and 25×10^3. PIII showed a relative abundance of the 68×10^3 dalton component. (Supported by USPHS Research Grants HD04945 and RR05716.)

39. Changes in enzyme activities related to steroidogenesis in rat ovaries in the luteal phase

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Immature female rats were treated with PMSG on the 21st day after birth, and subsequently with HCG on the 23rd day. On the 24th day, the ovaries rich in corpora lutea were examined for activities of 5ene-3β-hydroxysteroid dehydrogenase (+isomerase), 17α-hydroxylase, C_{17}-C_{20} lyase, 20α-hydroxysteroid dehydrogenase and aromatase on the basis of both unit weight of protein and a pair of ovaries. In comparison with these activities of the ovaries which were synchronously induced to estrous by the PMSG alone treatment, the following was observed: (1) Significant increase in 5ene-3β-hydroxysteroid dehydrogenase activity per gland. (2) Drastic decrease in the activities of 17α-hydroxylase and C_{17}-C_{20} lyase. (3) Marked increase in 20α-hydroxysteroid dehydrogenase activity. (4) Increase in aromatizing enzyme activity. From these