conversion to T(2×) by granulosa cells. A to T in theca was not increased by LH or FSH. DHT accumulation from added A was detectable in both granulosa (1.1 ng) and theca (1.7 ng), with no further LH or FSH effect. DHT accumulation from exogenous T was approximately the same in both granulosa cell and theca (1.5 ng), with no LH or FSH effect. The results indicate the source of follicular A is the theca and that of T the granulosa cells. Both tissues have a small and comparable amount of 5α-reductase activity. LH appears to increase 17α-hydroxylase activity while FSH increases granulosa cell 17β-dehydrogenase.

30. Steroidogenesis in the accessory genital organs of adult male rats

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Synthesis and metabolism of testosterone in the caput and cauda regions of the epididymis and ductus deferens of adult male rats was investigated. In *in vitro* incubation studies with ^{14}C-labelled pregnenolone and acetate, caput epididymis synthesized more of testosterone than other tissues. Addition of LH to these tissues had no effect on steroidogenesis. Labelled testosterone was mainly converted to 5α-dihydrotestosterone(DHT) in caput epididymis and 4-androstene-3,17-dione in ductus deferens. The caput also had higher levels of progesterone, testosterone, DHT, 4-androstene-3,17-dione and dehydroepiandrosterone than other regions of the epididymis and ductus deferens. Unilateral ligation for 24 h decreased the levels of DHT in cauda epididymis and ductus deferens. Thus, both the epididymis and ductus deferens have all the enzymes necessary for the synthesis of testosterone. The biological actions of testosterone, however, may be manifested through different metabolites in different segments of the epididymis and ductus deferens and the caput may need testicular secretions for converting testosterone to DHT.

31. Serum testosterone, Leydig cell population and activities of marker enzymes during sexual maturation in the rat

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To define hormonal and morphological changes related to testicular function during sexual maturation, serum testosterone and LH were estimated by specific radioimmunoassays in male rats aged between 5 and 90 days. Simultaneously, the Leydig cell population in H & E stained testis sections and 5ene-3β hydroxy steroid dehydrogenase (5ene-3β-HSD) and alcohol dehydrogenase (ADH) were quantitated histochemically. Serum testosterone was 0.95 ± 0.04 (SE) ng/ml at day 5, decreased to a minimum (0.21 ± 0.05) by day 25 and thereafter increased progressively to the maximum level (2.94 ± 0.19) by day 90. No significant differences in serum LH were noted at any age. The relative proportion of Leydig cells per testis increased progressively throughout maturation. Concurrent to decrease in serum testosterone in early postnatal life, both 5ene-3β-HSD and ADH activities decreased. By day 30, the 5ene-3β-HSD activity was comparable to that in adults. The ADH activity progressively increased till day 50. Thus, in the absence of a parallel increase in serum LH the increasing testosterone levels during pubertal development could be due to increase in Leydig cell sensitivity.

32. Origin of oestrogen in pre-implantation rabbit blastocysts

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It has been hypothesized that pre-implantation embryos of rabbits, certain other rodents and pigs can synthesize oestrogen which plays an important role in implantation. The present study, carried out to investigate the oestrogen synthesizing potential of day-6 rabbit blastocysts, consisted of incubation of blastocysts in medium 199 containing ^{3}H-labelled steroid substrates (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione or testosterone) followed by separation of radiometabolites by thin layer and paper chromatography and their identification by coincidence of peaks of radioactivity with authentic steroids and recrystallization. Under these conditions blastocysts were unable to convert any of these substrates into oestrogen. There was a significant metabolism of all the substrates into other less potent neutral steroids suggesting detoxifying action of blastocysts. Using radioimmunoassay (RIA) we have also demonstrated the presence of oestrone and oestradiol in blastocysts and uterine fluid. [^{3}H]-Oestradiol administered (i.v.) to rabbits was localized in the blastocysts and uterine fluid. These studies reveal that rabbit blastocysts do not contain the enzymes necessary for oestrogen synthesis: the steroid present in them is of maternal origin.

33. On inhibition of testosterone synthesis by the rat Leydig cells following incubation with gonadotropin inhibiting material from human urine

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Gonadotropin inhibiting material (GIM) has been subjected to extensive studies in our laboratory to describe its presence in various patho-physiological states, and define its physico-chemical characterization. The present communication elucidates the mechanism of action of this anti-LH material at the cellular level. For this, Leydig cells were incubated with HCG both in the absence and presence of GIM and a graded decrease depending on the dose of GIM was observed in the HCG induced testosterone production. Our results also reveal that GIM inhibits, *in vitro*, the HCG induced conversion of [^{3}H]-ATP to cAMP. Further, when Leydig cells were preincubated with GIM, the binding of [^{125}I]-HCG to membrane receptors was prevented. This was further substantiated, using [^{125}I]-GIM as well as FITC-tagged GIM. GIM did not prevent the binding of prolactin to its Leydig cell receptors.

34. Estradiol receptors and steroid aromatization in the etiology of gynecomastia

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Pathogenic mechanisms for the development of gynecomastia in a large majority of young subjects remain obscure. Estradiol receptors (ER) were demonstrated in all the 17 gynecomastic tissues from males ranging from 17–48 years. Further, incubation of 12 breast tissues with [^{3}H]-androstenedione revealed formation of significant quantities of estrone (E_1) and estradiol (E_2) in addition to small amounts of testosterone (T) and 5α-dihydrotestosterone (DHT) in all the subjects irrespective of their genotypes. Furthermore, preoperative high urinary estrogen levels in these cases, dropped significantly following mas-

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