

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 [^3H]-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 desensitized 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 desensitized 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 \times 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 immuno-enzymic 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 16 α -bromo-4-androstene-3,6,17-trione > 6 α -bromoandrostenedione (6 α BrA) > 6 β BrA \approx 6 α -bromotestosterone acetate (6 α BrTAc). Treatment with 16 α BrA, androstenedione, 16 α OHT, 16 β OHT, 16 α OH-DHEA, oestriol, progesterone and cortisol showed no inactivation. The inactivation by the 16 α -Br-trione and 6 α -BrA was linear to the logarithm of inactivator concentrations. The 16 α -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-monooxygenations 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-3 β -hydroxysteroid dehydrogenase (+isomerase), 17 α -hydroxylase, C $_{17}$ -C $_{20}$ lyase, 20 α -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-3 β -hydroxysteroid dehydrogenase activity per gland. (2) Drastic decrease in the activities of 17 α -hydroxylase and C $_{17}$ -C $_{20}$ lyase. (3) Marked increase in 20 α -hydroxysteroid dehydrogenase activity. (4) Increase in aromatizing enzyme activity. From these

results, it is concluded that production of progesterone and 20 α -dihydroprogesterone at the luteal phase would be enhanced not only by an increase in the activities of 5 α -3 β -hydroxysteroid dehydrogenase and 20 α -hydroxysteroid dehydrogenase, but also by a decrease in the enzyme activities related to C₁₉ steroid formation from progesterone.

40. Testosterone production by testicular tissue of the camel (*Camelus dromedarius*) during the breeding season

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Environmental factors including temperature and humidity affect the temporal pattern of reproduction in the camel. We found increased development of two histologically distinct populations of Leydig cells during the breeding season. During the non-breeding season spermatogenesis was endured, but only one population of Leydig cells was well represented whereas the second population was reduced. The objective of this investigation was to determine the potential of the testis to synthesize testosterone *in vitro* during the peak of the breeding season. Incubation of testicular homogenate with radioactive substrates indicated the following: (1) testosterone was synthesized at a very slow rate, primarily via the 4-ene pathway; (2) the activity of the converting enzymes (3 β -hydroxysteroid dehydrogenase and 5-ene isomerase), 17 α -hydroxylase, 17-20 lyase and 17 β -hydroxysteroid dehydrogenase was relatively low and (3) high activity of enzymes not associated with testosterone production via the 4-ene or 5-ene routes was observed.

41. The effects of ACTH and dexamethasone administration on testicular function

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The effects of ACTH simulated stress on plasma and testicular levels of 9 steroids in the biosynthetic pathway of testosterone, and the levels of 6 unconjugated steroids in plasma were determined to evaluate the effects of stress on testicular function. In addition, these levels were compared to the levels determined after dexamethasone suppression of the adrenal. ACTH treatment resulted in a sig-

nificant fall in testicular levels of all the steroids, and a fall in plasma unconjugated and conjugated testosterone, dihydrotestosterone, and plasma conjugated pregnenolone levels. In contrast, levels of plasma unconjugated pregnenolone, progesterone, and 20 α -dihydroprogesterone rose significantly. Plasma unconjugated progesterone, 17-hydroxyprogesterone, 17-hydroxypregnenolone, testosterone, dihydrotestosterone, and 20 α -dihydroprogesterone, and plasma conjugated testosterone, dihydrotestosterone, and pregnenolone levels also fell significantly. It is concluded that both ACTH and dexamethasone exert a strong suppressive effect on testicular function.

42. Influence of various factors on embryonic chick gonad steroid hormone production during organ culture

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Gonads from chick embryos of 7½–18 days incubation were cultured on synthetic medium during 24 and 48 h. Radioimmunoassay, after ether extraction of the media and of the homogenized organs, allowed us to determine the embryonic gonads steroid production and secretion. There were significant differences according to sex, stage of development and size of gonads but several factors seem to modify the *in vitro* production rates. Medium renewal at 24 h increased, chiefly in male, total steroid production during the second day of culture. Addition of precursors of sex hormones such as pregnenolone, DHA and 4 α -androstenedione to the culture media, increased significantly the sex steroid production but respected the sexual differences observed in control gonads. These experiments gave information on steroidogenic activity during culture and on the sex steroid biosynthetic pathways used by bird embryonic gonads. Addition of HCG increased significantly the total steroid production (chiefly DHA, estrogens and testosterone) and also permitted several conclusions on the biosynthetic pathways. Furthermore, these experiments show that the gonads had to be stimulated immediately by a trophic hormone at the beginning of explantation to be responsive 24 h later. Finally, we have shown a seasonal influence on steroid production. Gonads, removed from embryos incubated between the 15th of March and the 15th of September 'Summer gonads' were much more active than 'Winter' ones (15th September–15th March).

4. METABOLISM

43. The mineralocorticoid activity of reduced metabolites of aldosterone in rats

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From recent experiments in our laboratories, the hepatic metabolism of aldosterone to its polar and to its reduced metabolites has been suggested to be of major importance in the mechanism of action of this hormone in the kidney. We have shown that the dose dependent quantities of both the polar aldosterone metabolites and tetrahydroaldosterone in the kidney during the latent period of the hormone correlate well with the magnitude of the physiological response to aldosterone in the kidney. The biological activity

of some of the reduced metabolites of aldosterone 5 α -dihydroaldosterone and three of the isomers of tetrahydroaldosterone, 3 α ,5 β -tetrahydroaldosterone, 3 β ,5 β -tetrahydroaldosterone and 3 β ,5 α -tetrahydroaldosterone were examined. The mineralocorticoid activity of these steroids was determined in adrenalectomized male rats by measuring the changes in urinary NA⁺/K⁺ ratios following their s.c. administration. All these reduced metabolites of aldosterone were found to possess mineralocorticoid activity, ranging from 1/30th to 1/500th of that of aldosterone. Of major interest, both 5 α -dihydroaldosterone and 3 α ,5 β -tetrahydroaldosterone were shown to possess considerable mineralocorticoid activity, 1/30 and 1/50 respectively, that of the activity of aldosterone. 5 α -dihydroaldosterone possesses approximately twice the biological activity of 3 α ,5 β -tetrahydroaldosterone. Both 3 β ,5 β -, and 3 β ,5 α -