

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 -

tetrahydroaldosterone possess approximately 1/500 the mineralocorticoid activity of aldosterone. These findings indicate that both of the reduced metabolites of aldosterone, 5 α -dihydroaldosterone and 3 α ,5 β -tetrahydroaldosterone, may well be important mineralocorticoids. The potential mineralocorticoid properties of these reduced metabolites of aldosterone may, however, be underestimated at this time since these compounds may well be cleared from the plasma and target tissue, the kidney, at different rates from native aldosterone, thus altering their bioavailability. We believe that the findings presented in this report are of considerable interest and lend support to the concept that some of the metabolites of aldosterone synthesized in the liver may possess significant biological relevance.

- 44. Progesterone-6,7- ^3H]: fate in proliferative and secretory endometria in presence of unlabelled progesterone**
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Endometrial capability to biotransform 1 μCi of progesterone-6,7- ^3H (P- ^3H), in the absence as well as in the presence of 10 and 100 $\mu\text{g/ml}$ of unlabelled progesterone (P), was assayed *in vitro*. Metabolite formation was studied at 6, 24, 48 and 72 h incubation intervals. Also, total characterization of previously undescribed P- ^3H metabolites was performed in extracts from endometria incubated with 4.4 μCi of P- ^3H for a 72 h period. P- ^3H derivatives reduced at C-5 and C-20 were found in lower proportions than polar unconjugated and water soluble conjugates in both, proliferative and secretory endometria. Higher concentrations of P inhibited metabolite formation. The newly identified P- ^3H derivatives are: 4-pregnene-3,11,20-trione, 17 α -hydroxy-4-pregnene-3,20-dione and the glucuronide of 3 β -hydroxy-5 α -pregnane-20-one. It is thought that similar events might occur in endometria continuously exposed to P released from intrauterine devices. Also, conjugate formation might play a role in local regulatory processes.

- 45. Peculiarities of steroid compound transformation by microorganisms trapped in polyacrylamide gel**
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The ability of various microbial cells trapped in polyacrylamide gel to perform 1,2-dehydrogenation and 1,2-hydrogenation, 20 α - and 20 β -reduction and 17 β -reduction has been investigated. 1,2-dehydrogenase activity of immobilized and free *Mycobact. globiforme* cells is the same (1.2g prednisolone/g cell/h). 1,2-hydrogenase and 20 β -hydroxysteroiddehydrogenase activities (20-OSD) of immobilized *M. globiforme* cells are higher than those of free cells, but less stable than 1,2-dehydrogenase activity of immobilized cells. 20 α - and 20 β -OSD activity of *Bac. megatherium* cells and 17 β -OSD activity of *Sacch. cerevisiae* cells trapped in gel were shown to be unstable and lower than those of free cells, which is assigned to rapid autolysis of these cells in gel. The rise of dehydrogenase activity and its stability has been observed after periodic incubation of immobilized cells of *M. globiforme* and *S. cerevisiae* in nutrient aerated medium with inducer. These changes of enzymic activity were due to the increase in the amount of intact cells on the surface of the gel and the stability of the cell ultrastructure inside the gel.

- 46. Effects of age and FSH on capacity of Sertoli cells from immature rats to convert progesterone (P) to 20 α -hydroxy-pregn-4-en-3-one (20-HP), 3 α -hydroxy-5 α -pregnan-2 α -one (3-HP) and 5 α -pregnan-3,20-dione (P-DIONE)**

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Sertoli cells isolated from 6, 10, 17, 32 and 65 day old rats, were incubated with [^{14}C]-P for periods of 1, 3, 6, 20 and 48 h. In addition, Sertoli cells isolated from 6, 10, 17 and 25 day old rats were incubated with FSH and [^3H]-P. The extracted radioactive products were identified by autoradiography, thin layer and gas chromatography, derivative formation and crystallization with authentic steroids. Conversion of P to 20-HP, 3-HP and P-DIONE was age dependent. Maximum conversion to 20-HP (15.2%: 1370 ng/mg protein), 3-HP (3.8%: 317 ng/mg) and P-DIONE (1.2%: 193 ng/mg) occurred in cells from 10 day old rats; cells from 65 day old rats produced no detectable amounts of 20-HP and conversion to 3-HP and P-DIONE was greatly reduced. Sertoli cells from 10 day old rats responded to FSH with significant (2 to 2.7 fold) increases in conversion of P to 3-HP and P-DIONE but the FSH response was greatly reduced or absent in 25 day old rats. 20-HP showed no significant increases due to FSH treatment. The peak steroidogenic activity and FSH sensitivity of Sertoli cells may be related to the onset of gametogenesis.

- 47. *In vitro* metabolism of [^3H]-androstenedione in the rat epididymis**

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The object of the present study was to examine *in vitro* the metabolic fate of [^3H]-androstenedione (4-androsten-3,17-dione) (A) in the epididymis and vas deferens (V) of the rat. Tissue homogenates of caput (Ca) and cauda (Cd) epididymides and V were extracted with diethyl-ether and analysed by gas-liquid chromatography interfaced with a radio-gas detector system. Incubation of slices of Ca for 2 h at 34°C metabolised 90% A. Similar incubations of tissue samples from Cd and V metabolised 60 and 25% of A, respectively. The major metabolites formed in the epididymis were 5 α -androstan-3,17-dione (5 α -androstanedione: Ca: 48%; Cd: 33%) and 3 α -hydroxy-5 α -androstan-17-one (androsterone: Ca: 35%; Cd: 13%). These metabolites appeared at a much lower concentration in the incubations with V (about 8% each). In general, conversion to testosterone (17 β -hydroxy-4-androstene-3-one) and dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one) was very low (2-4%) in all three organs examined. Castration did not significantly alter the metabolic pattern in the Ca epididymis and V but promoted the formation of androsterone (38%) in the Cd epididymis. Androsterone appears to be one of the important androgenic metabolites formed in the epididymis of rat.

- 48. Different metabolism of testosterone in human and rat liver**

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The metabolism of [^3H]-testosterone (T) to hydrogenated and glucuronic metabolites in tissue slices and subcellular fractions from human and rat liver was studied. Testosterone (T) and metabolites were separated and determined