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Exposure of bovine adrenal cortex microsomes to phospholipase A impairs the rate of the NAD-dependent  $3\beta$ -hydroxypregn-5-en-3-one dehydrogenation as well as the rates of the 5-androstene-3,17-dione and 5-pregnene-3,20-dione isomerization. None of the enzymatic activities is released into the supernatant upon digestion with phospholipase. Addition of Asolectin (a mixture of soybean phosphatides) to the treated membrane fraction does not restore the dehydrogenase activity whether the products of the phospholipase A action are present or not. In contrast to the  $3\beta$ -hydroxysteroid dehydrogenase the 3-oxosteroid- $\Delta 5$ -4-isomerase activities are restored to the original levels in the presence of Asolectin. The maximal activity which can be restored (minimum 70%) depends on the extent of the phospholipase A digestion. This methodology has not been able to show any significant difference whenever the 5-androstene-3,17-dione or the 5-pregnene-3,20-dione is used as substrate. The data suggest the phospholipid dependence of the isomerase(s). They would agree with the existence of only one  $C_{19}$  and  $C_{21}$  3-oxosteroid- $\Delta 5$ -4-isomerase. The results of our experiments indicate that the dehydrogenase and the isomerase behave differently towards phospholipids.

### 3B 2. Steroid biosynthesis: Adrenal Cortex—II

#### 63. Mammalian adrenal cells in monolayer culture: Biogenesis of steroid hormones

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Adrenocortical cells from different mammalian sources (beef, rabbit and human fetus) were dispersed by enzyme hydrolysis (trypsin 0.25%) and grown for short- and long-term periods under conditions of monolayer culture in Ham's F-10 supplemented with 10% fetal calf serum, in the presence or absence of ACTH. The biogenesis of corticosteroids was analyzed using labelled precursors, the products of which were purified and characterized by chromatography. Beef adrenal cells kept under such conditions were capable of responding to ACTH stimulation with accumulation of cyclic AMP (quantified by adrenal cytosol protein binding assay), increased production of corticosteroids (measured by CBG assay), and, in studies performed with either labelled progesterone or pregnenolone, changes in the specific activity of either cortisol or corticosterone. Responsiveness to ACTH was consistently observed for at least five days following implantation. From day five on there was an appreciable loss of  $11\beta$ -hydroxylase activity in both control and ACTH treated cells (100  $\mu$ U/ml), resulting in a sharp decrease in cortisol and corticosterone production, while 11-deoxycortisol and 11-deoxycorticosterone were formed in increasing amounts. In fetal rabbit adrenal cultures, using progesterone as precursor, a sequential appearance of steroid hydroxylases was observed as a function of gestational age. This was characterized by the exclusive formation of 11-deoxycorticosterone during early gestation, followed by that of corticosterone and finally of aldosterone near term. The presence of ACTH, while enhancing total corticosteroid production, did not affect enzyme ontogenesis. Similar cell preparations from midterm human fetuses actively metabolize pregnenolone to cortisol and corticosterone and to the sulfates of corticosterone and 11-deoxycorticosterone. Total corticoidogenesis was enhanced several fold in the presence of ACTH (500  $\mu$ U/ml).

#### 64. Isolation of subcellular fractions from the zona glomerulosa of the bovine adrenal cortex

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A method has been established to obtain uncontaminated mitochondrial or microsomal fraction from the zona glomerulosa of the bovine adrenal cortex with respect to the zona fasciculata-reticularis. Mitochondria thus obtained showed a different configuration from those of the mitochondria of the zona fasciculata-reticularis. They showed good controls in the presence of albumin; RCI = 7.8, ADP/O = 1.8, succinate as the oxidizable substrate. Distribution of cytochrome P-450 which might be concerned with steroid hydroxylase reactions in the zona glomerulosa was studied by CO-difference spectra using subcellular fractions; homogenates, 0.15, mitochondria, 0.70 and microsomes, 0.39 nmoles/mg protein. Amount of cytochrome P-450 in the microsomal fraction presented here is less than half that reported by others for the microsomal fraction of the zona fasciculata-reticularis. It was confirmed by electron microscopy that the microsomal fraction obtained in the present study was not contaminated with mitochondria. The microsomal fraction was further subfractionated into smooth- and rough-surfaced vesicles by sucrose gradient. Thus, it becomes possible to explore the roles of subcellular organelles in the biosynthesis of aldosterone in the zona glomerulosa of the adrenal cortex using uncontaminated materials.

#### 65. Interaction between the zona glomerulosa and the inner zones of the rat adrenal cortex incubated *in vitro*

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In conventional incubations of rat adrenal tissue, the rates of corticosterone (B) formation from endogenous precursors reach a maximum only after 60–90 min. This is at variance with results from superfusion or suspended cell preparations in which the maximum rate of steroid release occurs immediately, and also with the pattern of  $^3\text{H}$ -B formation from  $^3\text{H}$ -pregnenolone. It suggests that in conventional incubations unknown factors inhibit the early release of steroid from endogenous precursors. Further experiments show that inhibition only occurs when whole tissue is used; when capsule and inner zones are incubated separately, the rate of steroid formation is maximal immediately. When inner zone tissue was incubated (i) in capsule preincubation medium (ii) with a lipid extract of capsule preincubation medium (iii) with added aldosterone, significant inhibition of corticosterone was observed. This was related to aldosterone concentration, and could be observed with the addition of only 100 ng aldosterone per ml. B was affected specifically, and deoxycorticosterone and 18-hydroxycorticosterone were less affected: Also the conversion of  $^3\text{H}$ -pregnenolone to  $^3\text{H}$ -corticosterone was unaffected. The results are of special significance in relation to (i) consideration of the site of action of sodium depletion in the biosynthetic pathway for aldosterone production, since reduced corticosterone could indicate not increased 18-hydroxylase activity, but simply depressed corticosterone (ii) interpretation of the functional zonation of the rat adrenal cortex.

#### 66. Vinblastine-induced ultrastructural changes in the rat adrenal cortex

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