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In vitro studies of enzymatic activities in human adrenal tumours.

In order to correlate in vitro enzyme activity with clinical data, several enzymes involved in adrenal steroidogenesis were determined in human adrenal tissue, obtained from patients undergoing adrenalectomy.
A) Adrenal hyperplasia due to pituitary dependent Cushing. B) Adrenal adenoma in a patient with 11β-hydroxylase deficiency. C) Normal tissue of the same patient. D) Feminizing adrenal carcinoma. E) Cortisol producing adrenal adenoma.
Bovine adrenal tissue and human adrenal tissue obtained at autopsy were used as reference materials.
3β-OH-steroiddehydrogenase (3β), 11β-hydroxylase (11β), 21-hydroxylase (21), aromatase (Ar) and C_{17-20} lyase (Ly) were measured, using HPLC-techniques for substrate and product determination. This allowed direct and accurate quantitation of the activity of these steroidogenic enzymes. The results showed a good correlation between in vivo and in vitro data.
Among the most interesting findings were: 1) Very low 3β activity in D. 2) Virtually absent 11β activity in B, C and D. 3) Increased 21 and Ar activity in D. 4) Undetectable Ar activity in A, B, C and E. 5) High Ly activity in A.

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Effect of adrenal androgens on bone rudiments in organ culture.

In man, a temporal relationship exists between rising plasma adrenal androgen concentrations and skeletal maturation. To restudy and extend the in vitro work of Puche & Romano & Howard, we assessed the direct growth-promoting potential of dehydroepiandrosterone (DHA), its sulfate (DHAS), testosterone (T) and dihydroT (DHT) upon hindleg rudiments of chick embryos and newborn mice grown in organ culture. Our experiments utilized chemically defined and variously enriched media; contralateral limbs in medium alone were controls for those incubated with added steroid. Growth was measured by: wet weight, protein content, alkaline phosphatase activity (AP) and 3H-thymidine incorporation. Added steroid did not accelerate growth of tibiae and femora from 11 and 12-day-old chick embryos cultured for up to 7 days. Pulse-labeling experiments and those using chick frontal bones were also negative. In 4-day-old mice only DHA promoted increased protein content (33.7±0.84 {SE} vs 30.2±3.33 µg/mg, $p<0.05$) and AP (43.3±3.47 vs 35.6±3.85 µg/mg, $p<0.05$). In 2-day-old mice DHT increased AP (54.5±7.01 vs 45.3±6.35 µg/mg, $p<0.05$). Conclusions: (1) No direct effect of added adrenal or gonadal steroid on in vitro growth of avian bone rudiments was demonstrable; (2) in a mammal model, only minimal DHA and DHT effects were demonstrable; (3) thus, androgen-stimulated bone maturation in man may relate to "late" development of androgen receptors or depend upon in vivo intermediary growth factors.

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Plasma renin activity (PRA), cryorenin (CR) and total renin (TR) in normotensive children (NTC).

TR (measured after 4 days of cryoactivation at -4°C) and PRA were determined by RIA, in the upright position, in 40 NTC (age 7 mo. - 15 yrs) on an "ad libitum" Na diet; 16 of them were obese (overweight > 30%). CR was calculated as TR minus PRA. Applied statistical analysis: non parametric for comparison between samples and parametric for linear first order regression.
Results: 1) TR, CR and PRA values were statistically not different in obese and normal children. 2) There was a statistically significant positive correlation between TR and PRA ($p < 0.001$) and between TR and CR ($p < 0.001$); however, no correlation was found between PRA and CR. 3). Correlation with age was not significant for TR, CR and PRA.
In conclusion, in our experience, PRA is a valuable index of TR in normotensive children, normal as well as obese. The importance of CR in different pathological situations is under investigation.

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Effects of androgen overproduction on skin target organs in children with congenital adrenal hyperplasia.

Two female sibs aged 2, resp. 3 years were studied with clinically and biochemically proven congenital adrenal hyperplasia (CAH) due to a 21-hydroxylase deficiency. Both of them showed clitoris hypertrophy and abundant growth of pubic hair.
Axillary sweat was sampled over a period of 24 hours from these children and from normal prepubertal controls. Analysis showed that prepubertal children excreted no detectable amounts of steroids, contrasting with the two CAH patients. In axillary swaet from the latter, significant levels of steroid sulphates were found.
The steroid moieties of these sulphated were mainly dehydroepiandrosterone, androsterone, testosterone and 5-androstene-3β,17β-diol.
The excretion of steroid sulphates and the clitoris hypertrophy observed in both sibs are clearly a consequence of the androgen overproduction in CAH.
Our results also suggest that the presence of functioning apocrine sweat glands and pubic hair follicles at this early age is due to the effect of circulating androgens.

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Evidence for involvement of arachidonic acid in the teratogenic action of glucocorticoids.

In addition to inhibiting shelf elevation glucocorticoids have been shown by us to inhibit breakdown of the medial edge epithelium of fetal palatal shelves from steroid-sensitive mouse strains in vivo and in a single shelf culture model in vitro. Glucocorticoids do this by reducing in the medial edge epithelial population the synthesis and/or release of lysosomal enzymes. We have also shown in rats in vivo that the clefting action of glucocorticoids, like their anti-inflammatory action, is prevented by arachidonic acid, a precursor of prostaglandins and thromboxanes. We now report that arachidonic acid is also effective against the teratogenic action of glucocorticoids in palatal shelves from steroid-sensitive mouse strains in vivo and in vitro. In vivo, arachidonic acid (200mg/kg) significantly lowers palatal clefting induced by cortisone in CD-1 (65.9% to 44.9%) and A/J (75% to 32.6%) mice. In vitro, arachidonic acid (0.001µg/ml to 1µg/ml) reverses the inhibition of medial edge epithelial breakdown by cortisol in single palatal shelves from CD-1 (10 reversals in 10 tests) and A/J (7 reversals in 8 tests) mice. Thus, arachidonic acid reverses the teratogenic action of glucocorticoids in both the fetus and our shelf culture model and evidence is provided for the possible biochemical pathway of steroid-induced palatal teratogenesis.

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Simultaneous plasma and saliva steroid measurements as an index of control in congenital adrenal hyperplasia (CAH): A longitudinal study.

Plasma concentrations of 17OH-progesterone (17P), testosterone (T) and saliva concentrations of 17P have been determined serially for 3 yr in 16 treated CAH patients with 21-hydroxylase deficiency (present age 4.0-16.5 yr). Analysis of 71 paired samples showed significant correlations between plasma 17P and T ($r=0.70, p<0.001$) plasma and saliva 17P ($r=0.85$, $p<0.001$) and plasma T and saliva 17P ($r=0.77, p<0.001$). These 3 biochemical indices of control were further analysed in 9 prepubertal patients in relation to linear growth over 2-3 yr expressed as height velocity SD scores (ht.vel SDS). Correlation co-efficients ($r=0.71, 0.77, 0.78$) were significant in each instance ($p<0.01$). There was no correlation between ht. vel. SDS and glucocorticoid dose expressed in mg cortisol/m^2/day for the whole group ($r=0.28, p>0.1$), presumably because of the marked individual variation in the adrenal suppressive effect of glucocorticoid. The ease of saliva collection permitted 24-hour home 17P profiles in treated patients. All demonstrated a marked diurnal rhythm, but well-controlled patients showed normal 17P levels during the afternoon. Frequent serial measurements of 17P through saliva collection at home is an additional simple and useful parameter for "fine-tuning" control in CAH patients.