**Metabolism of testosterone by human gingival fibroblasts in culture**

The aim of the present study was to investigate the possibility of enhanced testosterone metabolism by human gingival fibroblasts in culture in the presence of phenytoin or its metabolites and hence to determine the role of biologically active androgens in stimulating fibroblast proliferation and collagen biosynthesis, which results in the clinically apparent phenytoin gingival hyperplasia.

The source of human gingival fibroblasts was connective tissue from the third molar region. The tissue was finely minced and distributed in angled neck flasks with Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (MEM + FCS). When the primary cell line was confluent, serial passaging was carried out as follows. The primary cell line was trypsinized and the cell aggregates were separated by passing them through finely drawn capillary tubes. They were redistributed in angled neck tissue culture flasks with MEM + FCS (4ml), and incubated in a CO₂ humidity incubator.

Monolayer cultures between the fifth and ninth passage were trypsinized and cell counts made in a haemocytometer. Appropriate dilutions were made with MEM + FCS and the cells were plated at a density of 50,000 cells/well in multiwell plates; incubation was for 24h. Unlabelled testosterone was added as substrate at concentrations of 10⁻⁸, 10⁻⁷ or 10⁻⁶mol/ml along each row. There were two control rows: one without cells and one with cells but with no substrate. The reaction was terminated by the addition of ethyl acetate (2ml). Unlabelled carrier steroids were added to each sample and extraction performed with ethyl acetate (3×2ml). Extracted metabolites and carrier steroids were quantified by scintillation counting. Gas-liquid chromatography was used to estimate the mass of carrier steroids, their recovery, and the ratio of radioactivity of the extracted metabolites to the added substrate. The metabolites were radiodinated by N-alpha-monomethylation at the 5α position of the A-ring. Monolayer cultures were counted for radioactivity using a scintillation counter.

The above methodology was used for the comparison of...
Steroid metabolism by human axillary bacteria

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The odorous boar pheromone 5α-androstenone has been quantified in axillary secretions of men and women (Claus & Alsing, 1976; Bird & Gower, 1981). Pure apocrine secretions are odourless until incubated with certain bacteria (Shehadeh & Kligman, 1963), and a coryneform-dominated axillary microflora is associated with pronounced axillary odour (Jackman & Noble, 1982). The possibility that 5α-androstenone is formed by microbial action was shown by Bird & Gower (1982), using a general germicidal agent, and it seems likely that an odourless precursor is secreted in apocrine sweat and then modified microbially.

In the present work we have studied the metabolism of some putative steroid precursors of 5α-androstenone by cultures of human axillary bacteria. Pregnenolone was tested first because of its role as a precursor of 16α-androstenediones in porcine testis (Cower, 1981). Pure cultures of 5α-androstenone were spotted on t.l.c. plates and run in benzene/acetone (85:15) followed by a second run in acetone/chloroform (1:3). Other 5α-androstenones and pregnenolone were spotted on t.l.c. plates and run in dichloromethane/ethyl acetate (95:5). The cultures (and controls) were extracted with redistilled ethyl acetate (3×5 ml) and the Na2SO4-dried extracts were evaporated in a Buchler Vortex-Evaporator at 20°C before spotting on t.l.c. plates (20 cm×20 cm, 0.2 mm thickness; Kieselgel 60, Merck, Darmstadt). Pregnenolone metabolites were identified by running twice in benzene/acetonitrile (8:1, v/v), while testosterone and 5α-DHT extracts were run once in benzene/methanol (9:1, v/v) followed by a second run in benzene/acetone (6:1, v/v). Radio-labelled metabolites were located by autoradiography and examined by capillary g.c. and g.c.-m.s. The former was performed using a Pye-Unicam 104 gas chromatograph with a SGE BPI capillary column (25 m×0.33 mm, 0.25 μm film thickness, FID detector). The cultures (and controls) were extracted with redistilled ethyl acetate (3×5 ml) and the Na2SO4-dried extracts were evaporated in a Buchler Vortex-Evaporator at 20°C before spotting on t.l.c. plates (20 cm×20 cm, 0.2 mm thickness; Kieselgel 60, Merck, Darmstadt). Pregnenolone metabolites were identified by running twice in benzene/acetonitrile (8:1, v/v), while testosterone and 5α-DHT extracts were run once in benzene/methanol (9:1, v/v) followed by a second run in benzene/acetone (6:1, v/v). Radio-labelled metabolites were located by autoradiography and examined by capillary g.c. and g.c.-m.s. The former was performed using a Pye-Unicam 104 gas chromatograph with a SGE BPI fused silica column (25 m×0.33 mm, with helium as carrier gas and argon as make up gas). G.c.-m.s. was performed using a V.G. Analytical Ltd Model 305 mass spectrometer-2025 data system (source conditions: Electron impact; 500 eV trap current, 40 eV ionization potential; 4 kV accelerating voltage). A Pye-Unicam 204 gas chromatograph, with a SE-30 quartz capillary column (25 m×0.32 mm, with helium as carrier gas and argon as make up gas). G.c.-m.s. was performed using a V.G. Analytical Ltd Model 305 mass spectrometer-2025 data system (source conditions: Electron impact; 500 eV trap current, 40 eV ionization potential; 4 kV accelerating voltage). A Pye-Unicam 204 gas chromatograph, with a SE-30 quartz capillary column (25 m×0.32 mm, with helium as carrier gas and argon as make up gas). G.c.-m.s. was performed using a V.G. Analytical Ltd Model 305 mass spectrometer-2025 data system (source conditions: Electron impact; 500 eV trap current, 40 eV ionization potential; 4 kV accelerating voltage). A Pye-Unicam 204 gas chromatograph, with a SE-30 quartz capillary column (25 m×0.32 mm, with helium as carrier gas and argon as make up gas). G.c.-m.s. was performed using a V.G. Analytical Ltd Model 305 mass spectrometer-2025 data system (source conditions: Electron impact; 500 eV trap current, 40 eV ionization potential; 4 kV accelerating voltage).

Radio-labelled metabolites were identified where possible by the gas chromatographic behaviour and mass spectra of their underivatized and MO-TMS derivatives compared with those of standards (obtained either commercially or as gifts from the MRC steroid reference collection).

5α-Androstenone was synthesized from 5β-androst-16-en-3α-ol by chronic acid oxidation (Bush, 1961). This and 5α-androstenone were spotted on t.l.c. plates and run in dichloromethane/ethanol acetate (95:5, v/v) (Bird & Gower, 1981).

So far there is evidence for two metabolites of pregnenolone. One of these was found in trace amounts, and so was not conclusively identified, but had a similar g.c. retention time and underivatized mass spectrum to 17α-hydroxy-pregnenolone. The second compound was more abundant, and had a g.c. retention time identical to that for 5α-DHT, both underivatized and as the MO-TMS derivative. The mass spectra were also consistent with this identification of the metabolite.