17β-o1-3-one, 5(10)-estren-17β-01-3-one, an isomer each of 5-estrane-3,17-diol and estradiene-3,17-diol and two isomers each of 5(10)-estren-3-01-17-one and 5(10)-estren-3,17-diol were identified. None of these steroids was detected in the blank incubated samples. In the blank, phenolic fractions traces of oestrone and oestradiol-17β were present. In the post-incubation extracts a definite increase in a proportion of individuals, in the presence of human endometrium has also been reported [9]. This forms the 4-estrene-3,17-dione found in stallion testes and urine. 

Oxygenase hydroxylation and subsequent dehydration. If as previously reported for other 19-nor steroids, this metabolite in vitro has a 3-one configuration. Further reduction of 19-nortestosterone to 5-estrane-3,17-diol demonstrates the presence of 4-en-3-one reductases in stallion testes.

The mass spectrum of estradiene-3,17-diol identified in the blank incubated samples. In the blank, phenolic fractions traces of oestrone and oestradiol-17β were present. In the post-incubation extracts a definite increase in a proportion of individuals, in the presence of human endometrium has also been reported [9]. This forms the 4-estrene-3,17-dione found in stallion testes and urine. 

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Phenoyin stimulation of testosterone metabolism in inflamed human gingival fibroblasts

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Usage of the anti-convulsant phenytoin causes gingival enlargement in a proportion of individuals, in the presence of plaque-associated gingival inflammation. Since androgens such as 5α-dihydrotestosterone (5α-DHT) have been implicated in stimulating fibroblast proliferation and collagen synthesis, it was pertinent to study the extent of androgen metabolism in inflamed tissue fibroblast preparations in the presence of phenytoin. Androgens are metabolized actively in gingival tissue, largely to ring A-reduced compounds [1]. Similar conclusions were arrived at by Rappaport et al. [2], who found that testosterone metabolism occurred to a greater extent in healthy gingivae of men than women, due mainly to increased formation of 5α-DHT. Ojanok et al. [3] found that subcellular fractions of inflamed gingival tissue metabolized testosterone to a greater extent than those of non-inflamed tissue; healthy female preparations showed hardly any metabolic conversion. In inflamed tissue preparations, metabolism of testosterone increased to about the same magnitude.


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were suspended in PBS (1 ml) and transferred to centrifuge tubes. They were spun at 1500 rev./min (180 g) in a bench centrifuge (Centra 3RS, Damon/1EC, Dunstable, U.K. Ltd).

The 180 g supernatants of the control and inflamed fibroblast preparations were incubated in duplicate with radio-labelled testosterone and phenytoin (0.01-25 µg/ml) for 24 h. At the end of the incubation period, the medium was extracted with ethyl acetate, after the addition of carrier steroids. T.l.c. was then performed, and the metabolites were quantified by scintillation counting. G.l.c. was carried out to correct for analytical losses of metabolites incurred [4].

Basal rates of 5α-DHT and 4-androstenedione production were significantly elevated in the inflamed cell supernatants, compared with those of normal cells. There was a 15-fold increase in 5α-DHT production and a 12-fold increase in 4-androstenedione formation.

The inflamed cell sample showed stimulation of 4-androstenedione synthesis at 0.01 and 0.1 µg of phenytoin/ml, with maximum synthesis (495 fmol/ml) at a concentration of 1 µg/ml. Decreased synthesis occurred at 10 and 25 µg/ml. Similarly, increased 5α-DHT synthesis occurred at phenytoin concentrations of 0.01, 0.1 and 1 µg/ml, but rose to a maximum value (110 fmol/ml) at 10 µg/ml, and decreased almost to baseline values (70 fmol/ml) at 25 µg/ml. With the non-inflamed female sample, inhibition of 5α-DHT synthesis occurred at a phenytoin concentration of 0.01 µg/ml (from 3.8 to 3.0 fmol) and a further inhibition at 10 µg/ml to 0.7 fmol/ml. Synthesis of 4-androstenedione was again inhibited from 3.8 to 2.8 fmol at a phenytoin concentration of 0.01 µg/ml with further inhibition at 0.1, 1 and 10 µg/ml to values of 2.5, 0.55 and 0.2 fmol, respectively.

There was a 28-fold greater stimulation of 5α-DHT and 4-androstenedione synthesis by phenytoin in inflamed tissue fibroblasts, compared with inhibition by phenytoin in the normal cells. These findings are in keeping with those of other workers [2]. Healthy female gingival tissue showed very insignificant levels of testosterone metabolism. The presence of inflammation seems to alter the phenotype of the cell, giving a heightened basal metabolic rate and increased sensitivity to stimulation.

The above findings highlight the concept of responder cells being particularly sensitive to phenytoin stimulation. The healthy female gingival fibroblast is a good example of a non-responder cell in terms of testosterone metabolism. It is therefore interesting that the effect of phenytoin on such a cell type should be inhibitory rather than stimulatory, at the concentration used. Hence, clonal selection of a subpopulation of responder fibroblasts with respect to testosterone metabolism and sensitivity to phenytoin could well be instrumental in contributing to the gingival overgrowth phenomenon, given the milieu of inflammation.

Other workers have shown that microsomal preparations of inflamed gingival tissue metabolized testosterone mainly to 5α-DHT and the diols, whereas in non-inflamed tissue, the pathway of metabolism favoured the formation of 4-androstenedione and androstenedione, which are less potent androgens [5]. Most investigators have used gingival tissue to derive a cell-free system rather than homogenates of cultured fibroblasts. Hence, this study relates directly to metabolism by fibroblasts and precludes inflammatory and other cell types which may be present in the tissue.

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Species differences in specificity of hydrocarbon-inducible forms of cytochrome P-450

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The P450IA subfamily of the cytochrome P-450 superfamily [1] is currently known to contain two members, P450IA1 and P450IA2, in all species studied [2]. Treatment of rats or rabbits with polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene (3MC), induces both isoenzymes in the liver [3], with considerable toxicological implications.

Although the two isoenzymes show considerable amino acid sequence similarity (70% and 65% for the rat and rabbit, respectively) [1] and overlap in their specificity for some substrates, there is evidence for a high degree of specificity for a number of substrates of toxicological concern. Thus, activities which specifically reflect the content of these isoenzymes are keenly sought. Specificity is frequently assessed from the activity of the purified, reconstituted isoenzymes. However, the extent to which this reflects the situation within the microsomal membrane has yet to be established. This is particularly important when comparing orthologous isoenzymes between species. Some of these issues have been addressed in the present study.

Male Wistar rats (180-225 g) and New Zealand White rabbits (2.5-3.0 kg) were treated with an intraperitoneal injection of 3MC (80 mg/kg) in corn oil 48 h before killing. Hepatic microsomal fractions were isolated by the method of Boobis and co-workers [4]. Aryl hydrocarbon hydroxylase (AHH) activity was measured by the method of Nebert & Gelboin [5]. Phenacetin O-de-ethylase (POD) activity was determined by the method of Murray & Boobis [6] using a substrate concentration of 4 µM. The mutagenicity of 2-acetylaminofluorene (2-AAF) was assessed using 0.5 mg of microsomal protein in the preincubation (20 min, 37°C) mammalian microsomal mutagenicity assay described by Maron & Ames [7]. Monoclonal antibodies MAb107 [8] and MAb 12/3/2/9 [9] have been described previously.

Hepatic AHH activity is believed to be relatively specific for P450IA1 in the hydrocarbon-induced rat. This activity

Abbreviations used: 3MC, 3-methylcholanthrene; AHH, aryl hydrocarbon hydroxylase; POD, phenacetin O-de-ethylase; 2-AAF, 2-acetylaminofluorene.

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