solone and butyrate on $HeLa_{65}$ cell agglutinability were still observed with trypsin-treated cells (table, experiments 9 and 10).

Discussion. The quantiative spectrophotometric agglutination assay of Hwang et al.[19] revealed surface changes in $HeLa_{65}$ cells mediated by growth in prednisolone. Three parameters of agglutination kinetics were affected by prednisolone; the lag phase was decreased and both the rate of agglutination and total agglutination were increased. Cortisol has been reported to produce a variable increase in con A agglutination of 3T3 cells[20]. Growth of HeLa cells in medium containing prednisolone has been shown to increase agglutination by what germ agglutinin[21], to alter the phospholipids of membranes[22] and to render the cells more resistant to deoxycholate lysis[23].

Agglutination by plant lectins such as con A has been considered a marker for the level of dedifferentiation in neoplasia[11]. The failure of butyrate to stimulate con A-mediated agglutination may be an example of its activity as a naturally occurring reverse transformation agent[24,25]. Butyrate has been shown to increase cell surface fibronectin in CHO cells[26]. Some of the morphological changes of transformation (cell rounding, loss of cell alignment, increased microvilli and blebbing) may be caused by decreased adhesion secondary to the loss of fibronectin from the cell surface. Salvato et al.[21] hypothesized that increased agglutination of prednisolone-treated HeLa cells by wheat germ agglutinin is caused by increased sialopeptides at the cell surface. More studies will be needed to distinguish among alternative interpretations of these agglutination data. Characterization of the pleiotypic effects of differentially acting inducing agents as butyrate[27] and prednisolone may help to establish causal relationships between different biochemical events in gene expression in HeLa cells.

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Estimation of the odorous steroid, 5α-androst-16-en-3-one, in human saliva[1]

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Summary. The concentration of the urine-smelling steroid, 5α-androst-16-en-3-one, has been measured by radioimmunoassay in the saliva of 9 men and 4 women. The lower limit of detection was estimated to be 0.725 nmoles/l. In six of the men the range of concentrations of the odorous steroid was 0.8–1.8 nmoles/l saliva (3 men had less than the estimated lower limit of detection). In only one of the women studied could the 5α-androst-16-en-3-one be measured (0.83 nmoles/l) in the saliva.

Considerable interest has been aroused recently in the urine-smelling steroid, 5α-androst-16-en-3-one (5α-androstenone) and in the closely-related musk-smelling 5α-androst-16-en-3α-ol (3α-androstenol) because of the possibility of these being human pheromones. It is well-known that both compounds are formed in boar testis[4] and that they induce the female pig, in oestrus, to take up the 'mating stance'[5]. The odorous 3α-androstenol (as glucuronide) occurs in the urine of men and women[6] while 5α-androstenone has been found in axillary secretions of men but only to a limited extent in women[7,8]. In freshly-collected apocrine secretions, 5α-androstenone is either absent or present only in minute quantities[8,9] and this, together with the fact that washing the axillae with a bacteriocidal solution markedly reduces the 5α-androstenone content of 24-h collections[10], strongly suggests that the steroid may be formed on the skin surface from a precursor which is secreted from the apocrine glands and then modified by skin micro-organisms[11].

During the past few years, evidence has been provided to indicate that, when men and women are subjected to the smell of 3α-androstenol, their judgements may be altered[12,13]. Kirk-Smith and Booth[14] have further shown that the smell of 5α-androstenone may affect choice of location in others' presence. There has also been much discussion of sex difference in the ability to smell 5α-androstenone[15] and

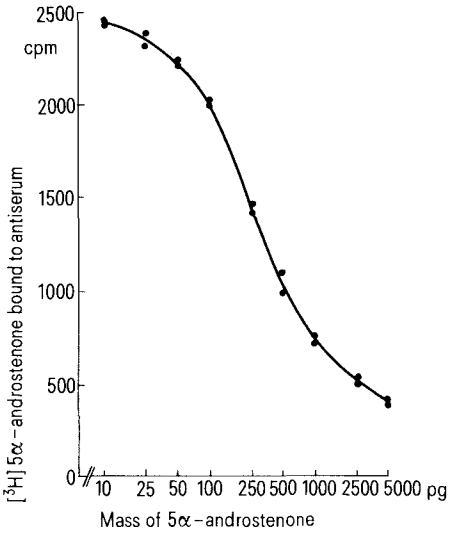

Figure 1. Standard curve for 5α-androst-16-en-3-one.

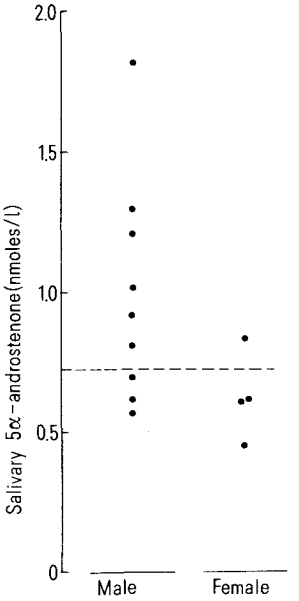

Figure 2. Salivary levels of 5α-androst-16-en-3-one in healthy men and women. ---- denotes lower limit of sensitivity of assay.

of differences in axillary odour between male and female subjects[16].

In view of the occurrence of odorous 16-androstenes in boar saliva and because human saliva is known to contain numerous steroids, including corticosteroids[17], progestagens[18] and androgens[19], we have attempted to measure the concentration of 5α-androstenone in the saliva of 9 men and 4 women by radioimmunoassay.

Unstimulated saliva was collected into large Pyrex glass tubes (150×25 mm) 10 min after rinsing the mouth with distilled water. None of the subjects experienced any difficulty in producing sufficient saliva. This was centrifuged at 2000 rpm for 5 min to remove particulate matter and 1 ml supernatant taken for analysis. [5α, 6α-^{3}H] 5α-Androstenone (1435 dpm) was added to each for an estimation of recovery, mixed well and incubated for 15 min at room temperature. [^{3}H]5α-Androstenone was purchased from Isocommerz, Kontor, Dresden, DDR-8051 and had a specific radioactivity at the time of use of 21 Ci/mmole. Extraction was performed with ethyl acetate (2×3 ml), centrifuging briefly each time to separate the ethyl acetate and aqueous phases. The organic phase was removed and evaporated to dryness under water-pump vacuum (Büchi Rotovapor), taking care that the water-bath temperature was not higher than 40 °C, so as to minimize losses of the relatively volatile 5α-androstenone. The residue was dissolved in ethanol and duplicate aliquots (0.1 ml) were subjected to radioimmunoassay, while a further portion (0.2 ml) was counted for estimation of tritium for recovery purposes.

Duplicate unknown or standard amounts of 5α-androstenone in ethanol were added to glass tubes containing [^{3}H] 5α-androstenone (2×10^4 dpm, 0.6 pmoles). After evaporation of the solvent, the residue was incubated with the antiserum dilution (1 in 3289) (300 µl), kindly provided by Dr Ø. Andresen[20], for 1 h at 37 °C, followed by 18 h at 4 °C. Separation of bound and free steroid was achieved by the addition of dextran-coated charcoal[8] solution (300 µl) to each tube. After mixing and incubating for 15 min at 4 °C, tubes were centrifuged at 2000×g for 10 min and aliquots (400 µl) of the supernatants, containing bound-steroid, were taken for measurement of radioactivity by scintillation counting (Beckman Scintillation Spectrometer, model 1650). A typical standard curve for 5α-androstenone is shown in figure 1. In addition, 4 distilled water blanks were analyzed and the lower limit of sensitivity for the assay, calculated from these values, was 0.725 nmoles/l.

The salivary 5α-androstenone levels of men (fig. 2) were all below 2 nmoles/l but were nevertheless much higher than those of the 4 women studied, only one of the latter having a level higher than our estimated lower limit of detection. Our results are in keeping with earlier work when little or no radioactivity was found in saliva after i.v. administration of radiolabeled 16-androstenes to human subjects[21]. Current knowledge of salivary steroids suggests that they are related to the free steroid fraction of plasma which in the case of 5α-androstenone in men, is quite low (8–16 nmoles/l)[7]. Although 4,16-androstadien-3-one may also have contributed to our 5α-androstenone results since the antiserum is not entirely specific[20], the sex-difference may be significant. A similar marked sex-difference has been reported with respect to the content of 5α-androstenone in human axillary collections[8]. Further research may help to establish whether or not salivary 5α-androstenone at such low concentrations may make a contribution in human social communication.

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Autoradiographic analysis of FSH binding during follicular atresia

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Summary. Binding of FSH to ovarian cells was studied in PMSG primed immature Swiss mice. 48 h after PMSG treatment, FSH-binding was higher in the periphery than in the cumulus cells of the antral follicles. Binding of FSH to granulosa cells of normal follicles was observed to be specific, 48 h after PMSG injection. No localization in the atretic follicles could be seen by autoradiography 72 h after priming.

Pregnant mares' serum gonadotropin (PMSG) is known to induce follicular maturation in rodents. Human chorionic gonadotropin (HCG) injected 48 h later can stimulate these follicles to ovulate, but in the absence of HCG these follicles do not ovulate and undergo degeneration[2,3]. Based on this observation, a model has been developed[3] by which 90% atretic follicles could be obtained 72 h after PMSG injection. In the present study, quantitative analysis of follicle stimulating hormone (FSH) binding sites during atresia were studied, using this model.

Materials and methods. Immature Swiss mice, weighing about 8–10 g, housed in controlled environment (24 °C, 60% humidity) were injected s.c. with 5 IU of PMSG (1790 IU/mg) at 16.00 h on day 21 of age and were divided into 4 groups of 5 animals each. Mice were autopsied 48 h or 72 h after PMSG injection. 2 h before autopsy, groups I (48 h-PMSG) and II (72 h-PMSG) received 5 µg per 100 g b.wt of labeled ovine FSH (L.E. Reichert, Jr), and group III (48 h-PMSG) received 0.0597 mCi/ml (volume was adjusted on the basis of radioactive counts), administration was by i.v. injection through the tail vein. Specific activity of the labeled FSH was 69 µCi/µg, iodination being performed by the Chloramine-T method[4]. To a 48 h-PMSG injected group IV the unlabeled FSH (NIH-FSH-S 11, 0.5 mg/100 g b.wt) was administered i.v. 1 h prior to the labeled material.

The animals were sacrificed, and the ovaries were removed, fixed in Bouin's fluid, embedded in paraffin and sectioned. The sections were washed, air-dried, dipped in NTB-3 emulsion and exposed at 4 °C. Three weeks later these sections were developed in Kodak-D-19 and stained with hematoxylin and eosin. For a quantitative estimation of FSH binding, grain counts were made on stained sections. The number of grains per cell of 4 antral follicles in each ovary was counted. Grain counts within the emulsion (background) adjacent to ovarian sections were also made. FSH binding was expressed as the difference between total grain counts over the granulosa and the background counts within the emulsion.

Results. Ovary 48 h after PMSG: The distribution of reduced silver grains, corresponding to bound 125_I-FSH, in the mouse ovary 48 h after PMSG in group I, was very selectively localized in the granulosa cells of antral follicles (fig. 1). The density of the grains was highest in the outer peripheral cells and lowest in the inner cumulus (table). The theca and interstitial tissue did not show localization of grains. The quantitative analysis of grain distribution in the various ovarian compartments demonstrated that the grain density in the granulosa cells was about 3–5 times higher than in the theca and interstitial compartments, in which it corresponded closely to the nonspecific value (table).

Ovary 72 h after PMSG: The binding of 125_I-FSH to granulosa cells was low in the antral follicles of mouse ovary, 72 h after PMSG injection in group II. The distribution of the silver grains was similar in the granulosa and theca cells, and was not specific (fig. 2). Quantitative analysis of the grain distribution over the various ovarian compartments demonstrated that none of the cell types showed more than 2 grains per cell (table).

When an excess of unlabeled ovine FSH (100 times more) was injected 1 h before the 125_I-FSH in group IV no specific uptake was observed in any of the cell types. Injection of labeled (free) iodide in group III did not give rise to localized silver grains in the ovary either.

Discussion. Exclusive binding of 125_I-FSH to the granulosa cells of antral ovarian follicles of immature mice 48 h after priming with PMSG, has been demonstrated in this study. Specificity of the binding of FSH to granulosa cells of antral follicles was further confirmed by its inhibition with excess of unlabeled FSH in the competitive binding test.

Distribution of radioactivity in antral mouse ovarian follicles 2 h after labeled FSH and iodide

Tissue	No. of reduced grains/cell body (mean ± SE)		
	Labeled FSH 5 µg		Unlabeled FSH 0.5 mg + labeled FSH 5 µg
	48 h	72 h	
Theca and interstitial	1.45 ± 0.24^a	0.88 ± 0.09	0.02 ± 0.01
Granulosa central (cumulus)	5.30 ± 0.42^b	1.22 ± 0.27^c	0.04 ± 0.02
Granulosa peripheral	6.65 ± 0.68^b	1.17 ± 0.20^c	0.09 ± 0.01

[b] compared to a $p < 0.01$; [c] compared to b $p < 0.001$.