cyte catalase from either source can be noticed. Normal catalase retained as much as 50% of its original activity after preincubation at 64 °C, whereas – under identical conditions – complete inactivation had occurred in the acatalasemic leukocyte preparation. In this instance, the 50% activity level was reached after incubation at 51 °C already. Immunological antigenic properties of normal leukocyte catalase have been compared with those of erythrocyte catalase in the double immunodiffusion test (Figure 2). A strong precipitin line showing complete identity is formed between the wells containing the preparations of normal leukocytes, normal erythrocytes and anti-catalase IgG (Figure 2, right side). This line exhibits strong peroxidatic activity (Figure 2, left side). A second, as yet unidentified weaker line exerting no peroxidatic activity is precipitated in the range of the leukocyte preparation only. Experiments of the same type performed with analogous preparations from A.B. suggest that there is also antigenic identity between normal and acatalasemic leukocyte catalase.

Discussion. Evidence is presented that leukocytes of an acatalasemic subject (homozygous for Swiss Type Acatalasemia) contain appreciable amounts of catalase. In A.B. the level corresponds to about $^1/_7$ of that of normal, which is distinctly higher than that found in the erythrocytes of the same individual (1–2% of normal). Like the mutant enzyme in the erythrocytes[1], its antigenically identical counterpart in leukocytes also exerts the same unusually low degree of thermostability. This observation is compatible with the assumption that, in an enzyme deficiency, two different types of structural gene mutations may lead to an apparent loss of enzymatic activity: 1. Enzyme variants of low specific activity, 2. variants with approximately normal specific activity, but of low stability. Eventually, both possibilities may lead to the same type of disorder. Examples are the different forms of glucose-6-phosphate-dehydrogenase-deficiency or of acatalasemia[1,10]. In the unstable mutant-type, the low level of residual activity in red blood cells must be considered as a poor indicator for the general situation, since in most other cells and tissues distinctly higher levels are found. In conclusion, there is a close analogy as to catalase stability and activity distribution pattern between acatalasemic mice and individuals homozygous for this particular type of acatalasemia[11,12].

Catalase activity in leukocytes and erythrocytes of normal and acatalasemic humans (Family B.)

Material analyzed		Catalase activity
Leukocytes	Normal ($N = 15$)	3.09 ± 0.73 μg/mg Protein
	Acatalasemic ($N = 2$)	0.41 (= 13% of normal)
Erythrocytes	Normal ($N = 15$)	2.38 ± 0.36 μg/mg Hb
	Acatalasemic ($N = 2$)	0.02 (~1% of normal)

Zusammenfassung. Beim Akatalasie-Fall A.B. ist die in den Leukozyten vorhandene Katalase-Restaktivität wesentlich höher (13%) als diejenige in den Erythrozyten (etwa 1% der Norm), wie dies beim Vorliegen einer instabilen Enzymvariante zu erwarten ist. Die Enzyme beider Zelltypen sind antigen-identisch und zeigen denselben Grad von Thermolabilität.

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The Detection of 5α-Androst-16-en-3α-ol in Human Male Axillary Sweat

The 16-dehydro C_{19} steroid 5α-androsten-3α-ol (androstenol) is excreted in the urine of adult human males in substantial quantities (of the order of 1 mg/24 h urine) and in lesser amounts in adult female urine[1]. The physiological function, if any, of the 16-dehydro C_{19} steroids in man is unknown, but androstenol secreted in the saliva of the boar acts as a releaser sex pheromone in eliciting the characteristic immobilization response of the oestrous sow to the advances of its mate[2–4].

There are, however, circumstantial grounds for believing that pheromones may play a part in human behaviour[5,6]. Androstenol, which has a musk-like odour detectable by human subjects at extremely low levels (1–5 ng on water at 20 °C, 10 cm from the nose; BROOKSBANK, unpublished) is a likely candidate as a pheromone in man, though various other steroids can be smelt at much higher concentration[7]. External secretion, onto the skin surface, would be the most probable way in which human pheromones are exhibited. In view of the evident capacity of axillary glands and hairs for preferential uptake and release of steroids[8,9] and the similarity of axillary apocrine glands in morphology and in androgen-dependence to apocrine glands specialized in lower mammals for pheromone secretion[10–12], we believed that axillary sweat might contain sufficient quantities of 16-dehydro C_{19} steroids to be detectable by gas chromatography-mass spectrometry (GC/MS). Previous efforts, using GC alone

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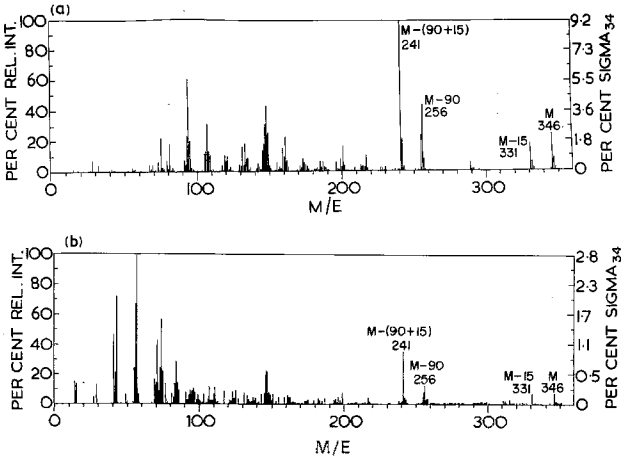
without internal checks on recovery (BROOKSBANK and CUNNINGHAM, unpublished), to detect these steroids in human axillary sweat had been unsuccessful, but GOWER and LLEWELLYN (see[4]) had detected 5α-androst-16-en-3-one (androstenone).

Axillary sweat was collected on defatted cotton-wool pads worn in the armpits by 12 healthy young men over a period of 5–7 days. The pads were exhaustively extracted with acetone followed by methanol, in the presence of a tracer quantity (2.4 ng) of 4,16-[7α-^{3}H] androstadien-3-one (androstadienone) (4.0 Ci/mmole). The soluble residue from the extractions was partitioned between water and benzene to furnish an oily extract ('sebum'[13]; 620 mg) containing unconjugated steroids, and an extract ('sulphated steroids') containing steroids excreted as sulphate conjugates that were recovered by acid solvolysis in ethyl acetate. The 'free steroid' fraction was redissolved in the minimum volume of hot methanol, and the material that precipitated on refrigeration overnight was removed; this cold methanol precipitation step was repeated. The methanol-soluble residue was taken up in benzene and free fatty acids were removed by washing the benzene solution with M.NaOH and with water. The neutral lipid material remaining, and the neutral extract obtained directly from solvolysis of the 'sulphated steroid' extract, were then separately chromatographed on alumina columns, additional tracers of [7α-^{3}H]androstenone (0.6 ng) and of [4-^{14}C]cholesterol being added at this stage. Fractions containing ^{3}H-androstenone, ^{3}H-androstadienone (marker for androstenol) and ^{14}C-cholesterol (marker for 5α-androst-16-en-3β-ol (3β-androstenol) were obtained by elution (cf.[4]) with light petroleum-benzene (9:1) followed by light petroleum-benzene (1:1). The column fractions were subjected to thin-layer chromatography (TLC) on silica gel in toluene-ethyl acetate (19:1 for the androstenone fraction; 9:1 for the other fractions).

Examination by GC on 3% QF-1 at 200° ('androstenone' fractions run underivatized, other fractions after reaction with chloromethyldimethylsilyl (CMDMS) ether reagent[14]) of the fractions recovered from the TLC plates showed that some androstenone might be present in the free steroid extract but that further purification of the androstenol and 3β-androstenol fractions was necessary. This was carried out on alumina columns after hydrolysis of the CMDMS ethers (0.02 *M* HCl in 25% aqueous ethanol at 60° for 30 min), using the same solvent systems for elution as before. Analysis by GC on 3% QF-1 and 3% OV-1 columns at 195° and without formation of derivatives, showed that androstenol might be present in the appropriate fractions derived from the 'free steroid' extract. The small remainder of the purified fractions from the 'free steroid' and 'sulphate conjugate' extracts of sweat were therefore subjected to GC/MS, after reaction with trimethylsilyl (TMS) ether reagent, on a 1% SE-30 column incorporated in an LKB-9000 GC/MS system. Mass spectra, taken at 6 sec intervals, were analyzed in an IBM 1800 computer[15]. The presence of androstenol in the 'free steroid' extract of axillary sweat was shown by the occurrence in the 'androstenol' fraction of a peak with the relative retention time (t_R) (with respect to 5α-cholestane) of 5α-androst-16-en-3α-ol TMS ether (t_R = 0.16) with the fragmentation pattern characteristic of the authentic compound (Figure). No androstenol was detected in the corresponding fraction from the sweat 'sulphate conjugate' extract, nor was any androstenone detected in either extract.

The quantity of androstenol in the sweat 'free steroid' extract can only be estimated very roughly from the peak heights in the final GC chromatograms as no attempt at quantitative measurement was made. Allowing for the manipulation losses, assessed from the recovery of ^{3}H-androstadienone – which was not separated from androstenol except on GC –, there was about 4 μg of androstenol in the original 620 mg of sebum derived from the axillae of 12 adult men, that is from an area of approximately 300 cm^2 per day for about 6 days. Thus, androstenol, and probably also androstenone[4], do occur in axillary sweat from adult men. If they are significant as pheromones the excretion is almost certain to vary enormously between and within individuals and the amounts we have found are large enough to be consistent with a pheromonal function. However, the demonstration of a pheromonal function of any steroid in man remains to be made by other methods.



Mass spectra of authentic (a) and isolated (b) 5α-androst-16-en-3α-ol trimethylsilyl (TMS) ethers. The base peak in (b) (*m/e* 57) is formed from contaminating material with the same t_R as 5α-androst-16-en-3α-ol TMS ether; this explains the lower relative intensities of the peaks at *m/e* 346 (molecular ion, M), 331 (M–15), 256 (M–90) and 241 (M–(90 + 15)) in the mass spectrum of the isolated steroid when compared to that of the authentic compound.

Résumé. La présence du 5α-androstène-16-3α-ol d'odeur musquée dans la fraction contenant les stéroïdes libres de la sueur des aisselles, recueillie chez des hommes sains adultes, a été établie par chromatographie en gaz avec spectrométrie de masse. La quantité d'androsténol trouvée n'exclut pas la possibilité d'une fonction phéromonale de ce stéroïde.

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