Composition of the Ventral Gland-Pad Sebum from the Mongolian Gerbil, *Meriones unguiculatus*

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The chemical composition of the gland-pad sebum of gerbils of both sexes is analysed using gas-liquid chromatographical and mass spectroscopical techniques and compared with the lipids from distal skin areas. Cholesterol esters, with (ω-1)-methyl-substituted fatty acids as acidic constituents, were shown to occur in the gland-pad sebum of both sexes in high concentrations, whereas cholesta-3,5-diene could be detected in the males only.

Introduction

The Mongolian gerbil (*Meriones unguicalatus*), the natural occurrence of which is restricted to eastern Asia [1] is increasingly being used as an experimental animal, especially for cancer research [2]. It possesses a specific mid-ventral sebaceous gland-pad [3] which is present in both sexes. However, it is larger, and matures earlier, in males than in females. Its function seems to be connected with either reproductive behaviour [4] or territorial marking [3, 5, 6] and becomes active towards the onset of sexual maturity. Castration causes involution, while androgens, as well as estrogen, stimulate gland-pad growth [7, 8]. Treatment with estrogen and progesteron results in an increase in scent marking by ovariectomized females as shown by Owen and Thiessen [9].

The gland has a composite structure and is tightly packed with several hundred tubulo-alveolar holocrine gland units. Histologically, the large rounded inner cells of the gland alveoli are in all stages of fatty metamorphosis and contain sudanophilic materials and prominent paranuclear hyaline bodies. On maturity, the alveoli cells become larger until cellular breakdown occurs, setting the cell substance free. This sebum flows out of the ducts and onto the surface of the glandular pad.

The purpose of the present investigation is to analyse the chemical composition of the sebum by means of gas-liquid chromatography/mass spectrometry and to compare it with lipid material from other skin areas of the gerbil. Moreover, it was intended to examine possible sex-associated differences in the sebum.

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Material and Methods

The gland-pads and distal skin pads were excised from freshly killed male and female gerbils. Lipids were extracted with chloroform : methanol (2 : 1; v/v, 60 ml) and the mixture diluted with water (20 ml). The hypolayer was evaporated and yielded the crude lipids (δ_{gland}: 36 mg; φ_{gland}: 20.1 mg; δ_{skin}: 113 mg; φ_{skin}: 53 mg). Lipids were separated into single classes by column chromatography on SiO_2 (5 g, Woelm, 14.5% water content). Hydrocarbons were eluted with cyclohexane (70 ml), cholesterol esters with cyclohexane/benzene (9 : 1; v/v; 100 ml), triglycerides with benzene (70 ml), free cholesterol with benzene/chloroform (1 : 1; v/v; 50 ml), free fatty acids with chloroform (50 ml), and more polar lipids with chloroform/methanol (2 : 1; v/v; 50 ml).

Triglycerides, cholesterol esters, and free fatty acids were reesterified with 5% methanolic HCl, and the products purified by SiO_2 column chromatography.

Gas-liquid chromatography (GLC) was performed on 10 m glass columns with 3% OV 101 on GasChrom Q, and on 3 m glass columns with 5% DEGS on Celite. Mass spectra were recorded with a Varian-MAT 111 (GNOM) at 80 eV, equipped with a glass connection between inlet and ion source, using the OV 101 column. Details of the methodology are published elsewhere [10].

The synthesis of 13-methyltetradecanoic acid as reference material has been published earlier [11]. Chloesta-3,5-diene was synthesized by treatment of

Lipid	% weight			
	gland ♂	gland ♀	skin ♂	skin ♀
Hydrocarbons	2.2	0.2	0.5	0.7
Cholesterol esters	8.9	7.5	1.6	2.8
Triglycerides	64.7	77.8	91.1	88.5
Cholesterol	9.4	5.5	0.9	2.0
Free fatty acids	3.7	2.0	2.0	3.2
Polar lipids	11.1	7.0	3.9	2.8
total lipids material (in mg)	(35.9)	(20.1)	—	—

Table I. Quantitative composition of the lipids extracted from the gland-pad and distal skin areas from male and female gerbils.

Hydrocarbon	ECL *	% of the GLC peak area			
		gland ♂	gland ♀	skin ♂	skin ♀
n-Alkanes (total)		(35.2)	(100.0)	(traces)	(traces)
n-C_{16}	16.00	—	33.5	tr.	tr.
n-C_{18}	18.00	—	43.1	tr.	tr.
n-C_{20}	20.00	—	11.9	tr.	tr.
n-C_{22}	22.00	35.2	11.5	tr.	tr.
Squalene	28.20	—	—	>99	>99
Cholesta-3,5-diene	29.20	64.8	—	—	—

Table II. Quantitative composition of the hydrocarbon fraction from the gland-pad and distal skin areas from male and female gerbils.

* Referred to octadecane = 18.00.

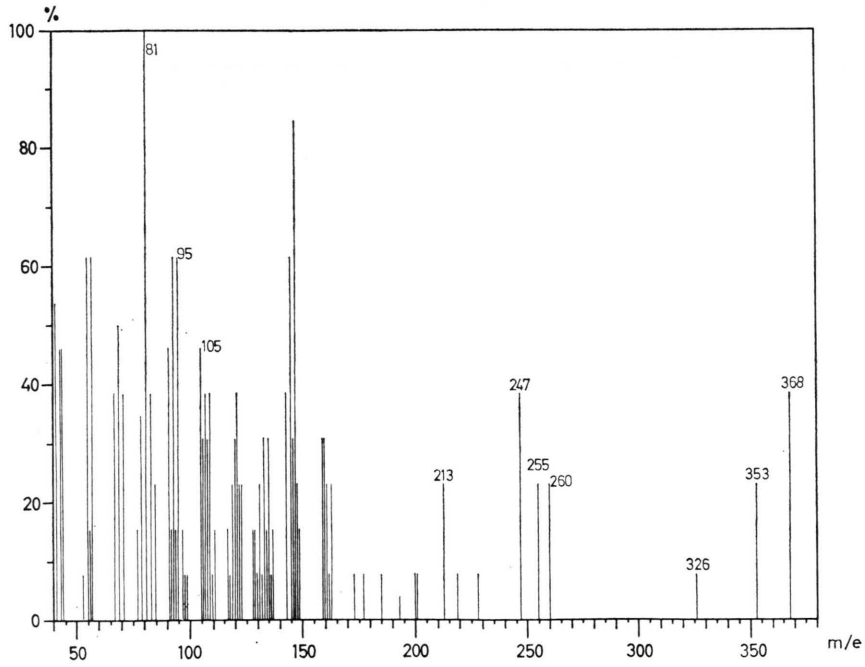

Fig. 1. Mass spectrum of the hydrocarbon with ECL = 29.2 (identical with cholesta-3,5-diene) from the gland-pad sebum of the male gerbil.

cholesterol with HCl in ethanol and subsequent column chromatography [12].

Results

Column chromatography on SiO_2 resulted in pure lipid fractions, (as determined by thin-layer chromatography) the percentages of which are given in Table I.

The total amounts of the gland lipids were significantly higher in the male. Among the single lipid classes, the amount of hydrocarbons was remarkably high in the male. The content of choles-

Table III. Quantitative composition of the cholesterol ester acids of the lipids from the gland-pad and distal skin areas from male and female gerbils.

Acid	% of the GLC peak area			
	gland ♂	gland ♀	skin ♂	skin ♀
unbranched (total)	(16.6)	(19.6)	(45.0)	(53.0)
n-C_{12}	0.1	0.3	0.9	0.8
n-C_{13}	0.4	1.0	0.1	0.8
n-C_{14}	4.9	5.2	8.9	6.5
n-C_{15}	1.2	1.4	0.8	1.4
n-C_{16}	5.8	6.0	26.7	26.9
n-C_{17}	0.8	1.5	tr.	tr.
n-C_{18}	3.4	4.2	7.6	16.6
branched (total)	(73.4)	(65.1)	(21.7)	(8.7)
11−C_{12}	0.7	1.5	—	—
12−C_{13}	0.5	1.2	0.3	—
13−C_{14}	61.6	52.8	15.0	5.3
14−C_{15}	tr.	tr.	0.4	0.6
15−C_{16}	5.0	4.3	0.8	0.4
17−C_{18}	5.6	5.3	5.2	2.4
unsaturated (total)	(10.0)	(15.3)	(33.3)	(38.3)
n-$C_{16:1}$	0.8	1.9	5.1	3.6
n-$C_{18:1}$	6.2	7.9	20.0	27.2
n-$C_{18:2}$	3.0	5.5	8.2	7.5

Table IV. Quantitative composition of the triglyceride acids of the lipids from the gland-pad and distal skin areas from male and female gerbils.

Acid	% of the GLC peak area			
	gland ♂	gland ♀	skin ♂	skin ♀
unbranched (total)	(25.4)	(30.5)	(22.5)	(24.5)
n-C_{12}	0.1	0.2	0.2	0.1
n-C_{13}	—	0.1	—	—
n-C_{14}	1.4	1.6	1.1	0.6
n-C_{15}	0.2	0.4	0.1	tr.
n-C_{16}	20.1	22.6	18.6	22.3
n-C_{17}	tr.	0.5	tr.	tr.
n-C_{18}	3.6	5.1	2.5	1.5
branched (total)	(0.1)	(0.9)	(—)	(—)
11−C_{12}	—	tr.	—	—
13−C_{14}	0.1	0.2	—	—
15−C_{16}	—	0.7	—	—
unsaturated (total)	(74.5)	(68.6)	(77.5)	(75.5)
n-$C_{16:1}$	5.9	5.3	5.4	3.5
n-$C_{18:1}$	45.6	41.8	47.8	44.5
n-$C_{18:2}$	23.0	21.5	24.3	27.5

terol, cholesterol esters and free fatty acids was slightly increased in the male whereas the percentage of triglycerides was reduced. The skin lipids were very similar in both sexes

The structure of the hydrocarbons were determined by comparison of the ECL (equivalent chain length) values from the GLC in a semi-log plot, and by mass spectrometry (Table II). The main hydrocarbon component of the male gland showed an indentical retention time and mass spectrum as cholesta-3,5-diene (Fig. 1). In addition to cholesta-3,5-diene, docosane was also detected in males. In contrast to this, the hydrocarbons from the female gland-pad were alkanes (C_{16}, C_{18}, C_{20}, C_{22}). The skin areas contained only low amounts of hydrocarbons, consisting of traces of alkanes and squalene.

The composition of the cholesterol esters contained in the gland-pad lipids was very unusual, in that $(\omega-1)$-methyl-substituted acids predominated as acidic constituents. The acids pattern of the cholesterol esters from the distal skin areas differed significantly and approximated to those of triglycerides in possessing large amounts of hexadecanoic, octadecenoic, and octadecadienoic acid. Details are summarized in Table III.

The composition of the triglycerides was very similar in the gland-pad and the skin lipids (Table IV); no or only traces of branched acids could be detected.

As can be seen from Table V, in the case of the gland-pad sebum the composition of the free fatty

Table V. Quantitative composition of the free fatty acids from the gland-pad and distal skin areas from male and female gerbils.

Acid	% of the GLC peak area			
	gland ♂	gland ♀	skin ♂	skin ♀
unbranched (total)	(55.1)	(48.6)	(28.6)	(35.6)
n-C_{12}	1.3	—	0.3	0.3
n-C_{13}	0.3	—	—	—
n-C_{14}	3.2	2.0	1.2	1.4
n-C_{15}	2.9	1.9	0.3	0.2
n-C_{16}	20.4	24.0	18.0	25.3
n-C_{17}	6.3	3.9	0.2	0.2
n-C_{18}	16.4	16.8	8.6	8.2
n-C_{19}	1.4	—	—	—
n-C_{20}	2.9	—	—	—
branched (total)	(11.3)	(4.7)	(—)	(—)
11−C_{12}	0.1	—	—	—
13−C_{14}	2.6	1.3	—	—
15−C_{16}	6.1	3.4	—	—
17−C_{18}	2.5	—	—	—
unsaturated (total)	(33.6)	(46.7)	(71.4)	(64.4)
n-$C_{16:1}$	3.4	2.5	4.7	3.5
n-$C_{18:1}$	18.5	27.3	41.6	37.1
n-$C_{18:2}$	11.7	16,9	25.1	23.8

acids lies between those of the cholesterol esters and the triglycerides. Hence it may be concluded, at least for this skin area, that decomposition of the complex lipid classes occurs there.

The series of $(\omega-1)$-branched fatty acid methyl esters were identified by their ECL values resulting from the GLC retention times showing an increment of $+0.65$ for the $(\omega-1)$-methyl-substitution in accordance with previous published data [10]. Moreover all mass spectra recorded from this series showed the characteristic fragmentation pattern (M-15) $\xrightarrow{-CH_3OH}$ (M-47) $\xrightarrow{-H_2O}$ (M-65) indicating a methyl branch at the end of the carbon chain. It should, however, be mentioned that the GLC peaks showed a slight asymmetry and the occurrence of minor amounts of $(\omega-2)$-methyl-branched acids can consequently be assumed. The corresponding mass spectrometric fragments (M-29) $\xrightarrow{-CH_3OH}$ (M-61) $\xrightarrow{-H_2O}$ (M-79) could be detected even more clearly in the descent of the peaks.

Discussion

The sebum production of the gerbils gland-pad is much more active in males than in females; this corresponds to the observation that territorial marking is done more often and more intensely by males [6]. Although hitherto untested in our laboratory, the very unusual composition of the cholesterol esters of the ventral gland-pad in both sexes containing predominantly $(\omega-1)$-methyl-branched fatty acids supports the assumption that they are involved in territorial marking. Further investigations will show whether these substances identified in the gerbils gland-pad sebum have any effects on its behaviour. These acids have also been detected in free form in the gland-pad sebum.

A striking sex difference in the lipid composition was found in the hydrocarbon composition, in that males possessed a steroid that was identified as cholesta-3,5-diene; this was not detected in females. Although no experimental evidence for a comparable function can be given so far for the gerbil, it should be noted that steroids have been reported (3α-hydroxy-5α-androst-16-ene) as occurring in the submaxillary salivary gland of the boar as sexual pheromones [13].

N-methyl-N-nitrosourea (NMU) when intravenously injected in rats resulted in a high percentage of tumors of the peripheral and central nervous system [14]. In contrast, the same administration of NMU to gerbils induced neoplasms of the midventral sebaceous gland-pad and were more frequent in males than in females [15]. Therefore, it might be presumed that not only hormonal factors, but also substances of the sebum have an effect on the development of tumors of the gland-pad. A few preliminary investigations have confirmed that after castration, tumors of this gland could no longer be induced in the gerbil. Moreover, Hradec stated in a series of papers [16–19] that cholesterol 14-methyl-hexadecanoate (carcinolipin), which is chemically closely related to the predominant cholesterol ester occurring in the gland-pad sebum of the gerbil, (cholesterol 13-methyl-tetradecanoate), is carcinogenic and stimulates significantly *in vitro* protein synthesis.

It would appear, therefore, to be of interest to investigate the influence of $(\omega-1)$-methyl-branched fatty acids or their cholesterol esters on the growth of chemically induced tumors.

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