

Effects of castration and testosterone on *Fel d*I production by sebaceous glands of male cats:

II—morphometric assessment

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Summary

A morphometric study of cat sebaceous glands was performed to evaluate the effects of castration and testosterone treatments. Skin biopsies were taken in six cats before castration, after castration and after the testosterone injections administered after castration (total number of biopsies: 18). Ninety 8 μ m thick sections of each biopsy were assessed for image analysis processing (SAMBA 2005, ALCATEL TITN). The variations in glands and cells size were evaluated on digitized microscopic images by morphometric parameters included in the SAMBA software package. An original software was developed for the analysis of the spacial gland structure. The best morphometric parameters were selected in a first step of the study, and included the nuclear surface (NS), the cell surface (CS) and the nuclear/cellular surface ratio (N/C). These three parameters were then compared in each group of samples for the six cats. It was shown that after castration the N/C (21%) significantly increased compared with prior to castration (12.6%). This 59.8% increase was mainly due to cell cytoplasm shrinking reflecting a decrease of the cell activity. The testosterone administered after castration produced a reverse effect with a N/C ratio back to normal (11.4%) and a significant cell cytoplasm and gland enlargement as shown by the three dimension constructions. This morphometric data correlated with the measurement of sebum and *Fel d*I productions. The negative effects of castration and the positive effects of testosterone on the sebaceous cells and glands volume favour the hypothesis that cat sebaceous cells are subject to hormonal control this is also likely to apply to the *Fel d*I production.

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Introduction

In a previous study we demonstrated that *Fel d*I was immunocytochemically detectable within cell cytoplasm of sebaceous glands. In order to elucidate the *Fel d*I regulation, and the potential effect of hormones upon sebaceous cells secretion, we undertook a further experimental study. Using the image analysis system SAMBA, we measured the cellular changes of sebaceous glands in

skin biopsies of cats, which have been castrated then treated with testosterone. Microscopic images of sebaceous glands in skin biopsies were acquired with a high resolution camera, digitized and then processed using a morphometry software package (SAMBA).

Materials and methods

Materials

Material and study protocol were described in part I of

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this paper [2]. Referring to Table 1 of part I, skin biopsies were performed in cats from group II at D₀, D₂₈ (i.e. 4 weeks after castration) and D₅₆ (i.e. 4 weeks after the first injection of testosterone). Thus, a total of 18 biopsies in the six cats from group II were histologically investigated. The samples were fixed in Bouin's fluid and paraffin embedded.

Methods

Skin biopsies processing Ninety 8 µm thick sections were taken for each of the 18 biopsies. Sections were stained with hematoxylin phloxin saffron as currently assessed in our department.

Image analysis

Hardware Tissue sections were examined and evaluated through a Zeiss microscope (Axioplan), with a 6.25 objective. Images were acquired with a CCD Sony Camera increasing the magnification 10-fold. Images were processed with a SAMBA 2005 type (ALCATEL, TITN, Grenoble, France [3] in which images are digitized using a 512 × 512 array of grey-scaled pixels. Three-dimensional constructions were performed with a data tablet MM 1201.

Software The three-dimensional construction of the sebaceous glands arranged along the hair follicle were recorded from microphotographs of serial sections. The hair follicle and gland borders were recorded on the SAMBA processor using the data tablet. The constructions included several parameters depending upon the thickness of the sections, the space between each section and the hair follicle orientation.

The measurements of the variations in size of the sebaceous glands after specific treatments (castration, testosterone) were performed directly from the tissue sections. Images were acquired through the microscope and the CCD camera. During the first step of the morphometric study, the best parameters reflecting the gland size were determined. Because of the variations in shape of the sebaceous glands arranged along the hair, the glandular volume was difficult to calculate accurately. The following morphometric parameters were easier to assess: the cellular surface (CS), the nucleus surface (NS) and the nuclear vs cellular ratio (N/C) as used in previous studies [4–7]. In the untreated cats the reliability of the measurements was determined. For that purpose 10 sections were picked at random for each biopsy among the 90, for each cat. The three parameters (CS, NS, N/C) were evaluated. For each case, it was shown that these parameters remained unchanged whatever the hair follicle orienta-

tion within tissue section, longitudinal or transversal, or the level of the tissue sections with regard to the epidermis.

In the second step of the morphometric study, CS, NC and N/C were evaluated in tissue sections from biopsies performed after specific treatments. The morphometric analysis consisted first in delineating with the mouse on the computer screen, the cells and nuclei of sebaceous cells outlines, on digitized microscopic images and then, the measurements of the respective surfaces of cells and nuclei were automatically assessed.

Results

Microscopic findings

On the tissue sections, the patterns of sebaceous glands around the hair follicle appeared very heterogeneous. This heterogeneity was related to the spiral arrangement of the glands around the hair follicle and the orientation of the latter within tissue sections, longitudinal or transversal (Fig. 1). Variations in glands and cells size were barely visible even on high magnification and impossible to evaluate by simple semi-quantitative evaluation.

Glands spacial construction

The variations in gland volume were more discernable when the glands were reconstructed from data provided by serial sections within a given biopsy. A decrease of gland size was visible when constructions from biopsies before and after castration were compared (Fig. 2). However, the degree and the significance of the reduction of the sebaceous glands volume were difficult to compute. Moreover, the procedure was time-consuming and tedious. Therefore, SAMBA automatic measurements on cell and nuclei surfaces appeared more accurate and easier.

Morphometric findings

Castration effects Morphometric data are shown in Fig. 3. After castration the nuclear/cellular surface ratio (21%) significantly ($P < 0.01$) increased (59.8%) compared with prior to castration (12.6%) (Fig. 3a). The increase essentially resulted from a major cytoplasmic surface decrease since, despite of a paradoxical nuclear enlargement (23%) (Fig. 3b), the overall cell surface decreased (–20%) (Fig. 3c). Mean nuclear surface was equal to 1231 ± 191 pixels before castration, 1587 ± 211 after castration, and 1378 ± 175 after testosterone. Corresponding figures for cellular surface was equal to

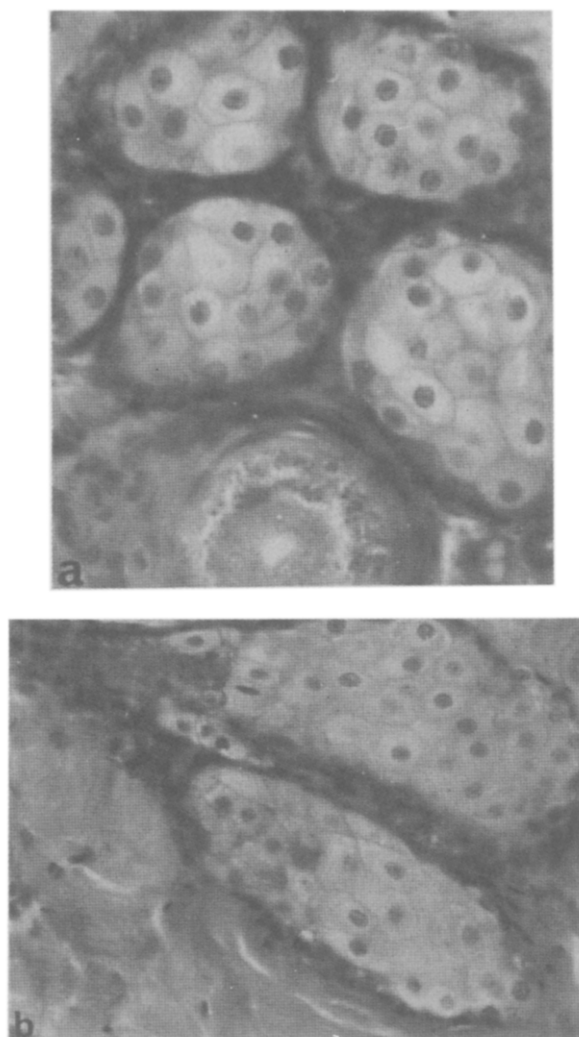


Fig. 1. Microscopic pattern of normal cat sebaceous glands around the hair follicle: (a) transverse section of the hair follicle, (b) longitudinal section ($\times 750$).

9874 ± 1629 , 7953 ± 2330 and 12441 ± 3295 , respectively.

Testosterone effects Testosterone produced reverse effects on sebaceous cells. The nuclear/cellular surface ratio came back to normal, and even a bit lower (11.4%) (Fig. 3a). The reverse effects resulted from a minor nuclei changes whose size remained intermediate between control and after castration (Fig. 3b) and major cytoplasmic enlargement with an overall cell surface increase (Fig. 3c).

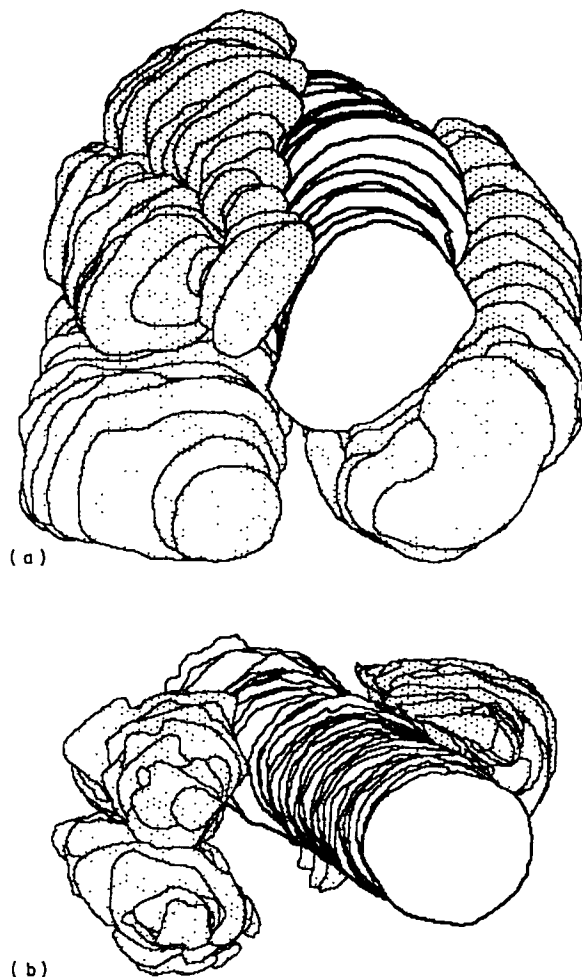


Fig. 2. Three dimensional constructions of sebaceous glands from serial tissue sections of skin biopsies in cats. (a) before castration; (b) after castration. Note a macroscopically discernible decrease of the sebaceous glands volume (centre: hair transversal section; external shell shaded: glands volume).

Discussion

Sebaceous cells are known to have a prominent large vacuolated cytoplasm which is anti-*Fel d 1* monoclonal antibody reactive [1]. The nucleus of this cell is small. The large cytoplasm of sebaceous cells results from the intracytoplasmic storage reflected by clusters of lipid rich vacuoles. In this type of cells the cytoplasm volume reflects the cell activity. The amount of cytoplasmic vacuoles increases with a marked metabolic activity and storage of the products of cell synthesis. Conversely, a small amount of cytoplasm vacuoles reflects a mild cellular

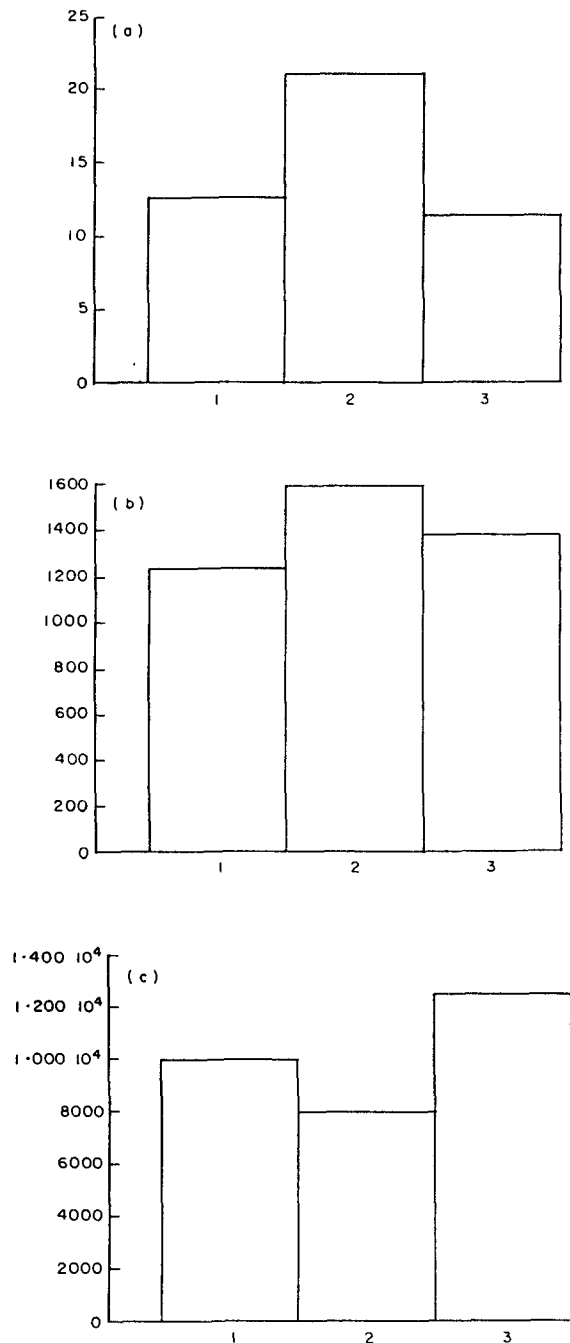


Fig. 3. Distribution of morphometric parameters in cats, (1) before, (2) after castration, and (3) after testosterone. (a) nuclear/cellular (%) surface ratio (SD = 1.3, SD = 5.1, SD = 1.6, respectively); (b) nuclear surface (pixels) (SD = 910, SD = 780, SD = 880 respectively); (c) cellular surface (pixels) (SD = 105, SD = 117, SD = 110 respectively).

activity. The variations in the overall cellular volume result essentially from variations in the volume of the cytoplasmic compartment. Therefore, the measurement of the variations in the nuclear/cellular surfaces ratio appears as an appropriate sensitive means to morphologically evaluate sebaceous cells metabolic activity.

In our study, we measured the variations in the morphometric parameters in sebaceous glands, to delineate the potential role of hormonal ambiance upon the sebaceous function.

We found that castration produced major cytoplasmic changes in the skin biopsies since the nuclear/cellular surface ratio significantly (59.8%) increased. Considering the lack of nuclear surface reduction and even an increase of the latter, the results show that the cell cytoplasm volume after castration is about twofold smaller than before castration. In contrast, testosterone was shown to have a reverse effect. These negative effects of castration on the cellular activity, enhance the hypothesis that hormones control sebaceous cells and their *FeldI* production. These findings correlate with measurements of sebum and *FeldI* under the same experimental conditions described in part I of this paper [2].

In conclusion, the morphometric analysis of sebaceous cells using a computerized system of image analysis showed that castration significantly reduced the cytoplasmic volume of sebaceous gland of cat skin reflecting a decrease of the metabolic activity, and that testosterone treatment has a significant effect reverse to castration.

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