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References

Gender-Related Differences in the Morphology of the Lacrimal Gland
Ann H. Cornell-Bell, David A. Sullivan, and Mathea R. Allansmith

Previous research has demonstrated that distinct, gender-related differences exist in the morphology of the rat lacrimal gland. The purpose of the present study was to determine whether this sexual dimorphism is unique to the rat, or extends as well to other species. Lacrimal glands were collected from adult male and female rats, mice, guinea pigs, rabbits, and humans (biopsies). Tissues were processed for light microscopy and examined with a Zeiss Videoplan II image analysis system. For morphometric determinations, we measured the area of approximately 50 glandular acini per animal for a total count of greater than 244 acini per gender per species. Our results demonstrated that significant gender-related differences exist in lacrimal glands of rats, mice, guinea pigs, rabbits, and humans. In all species analyzed, acinar area in lacrimal glands of males was larger than that of females. These findings suggest that gender differences in lacrimal gland morphology may be a general phenomenon in a variety of species. Invest Ophthalmol Vis Sci 26:1170-1175, 1985

Recent studies from our laboratory have demonstrated that significant, gender-related differences exist in the ocular secretory immune system of the rat. \(^\text{1-3}\) Concentrations of both immunoglobulin A (IgA) and secretory component (SC) were much higher in tears of male rats than those of female rats. Similarly, lacrimal (exorbital) glands from males produced significantly more IgA and SC in vitro than did glands from females. Because lacrimal tissue is the primary source of tear IgA and SC, \(^\text{3,4}\) our results suggest that the lacrimal gland is responsible for the gender-associated differences in mucosal immunity in the eye.

This sexual dimorphism of the exorbital gland, though, does not appear to be limited to involvement with the secretory immune system. Over the past 40 yr, researchers have shown that distinct differences exist between lacrimal glands of male and female rats and these include variations in the following parameters: acinar size, membrane appearance, nuclear volume and morphology, DNA and RNA content, the quantity of nucleoli and vesicles, enzyme activity, glycoprotein and total protein levels, and connective tissue area\(^\text{5-8}\) (also see Sullivan et al, \(^\text{3}\) Cavalleri, \(^\text{2}\) and Paulini et al\(^\text{1}\)). Thus, gender has a significant impact on the morphology, histochemistry, biochemistry, immunology, and genetics of the rat lacrimal gland.

The purpose of the present study was to determine whether the sexual dimorphism in the lacrimal gland is unique to the rat, or extends as well to other species. As a means for experimental comparison, we have focused upon the size of acinar structures. Lacrimal acini in the rat are qualitatively much larger.
in males than in females. Our research includes analysis of lacrimal tissues from male and female rats, mice, guinea pigs, rabbits, and humans.

**Materials and Methods.** Lacrimal glands were obtained after sacrifice from adult male (n = 5/species) and female (n = 5/species) Sprague-Dawley rats, Swiss-Webster mice, Hartley guinea pigs and pigmented rabbits. Rats, mice and guinea pigs were 12, 8 and 7 wk old, respectively, and purchased from Charles River Breeding Laboratories (Wilmington, MA). Adult rabbits were obtained from Pine Acre Farm (Norton, MA). Treatment of animals conformed to the ARVO Resolution on the Use of Animals in Research. Human lacrimal tissues were obtained from biopsies of adult patients (n = 5/gender) at Beth Israel Hospital (Boston, MA). All human tissues utilized in these experiments had been judged normal by histologic analysis.

Lacrimal tissues were fixed in either 10% buffered formalin (humans, rabbits, guinea pigs, and mice) or ethanol:glacial acetic acid (19:1; rats) solutions. Samples were then dehydrated in an increasing ethanol series, embedded in paraffin, cut into four μ sections and deparaffinized. Sections were stained with hematoxylin and eosin and examined at ×160 magnification with a Zeiss Videoplan II image analysis system (Zeiss; West Germany). This system, which includes a computer-assisted stereology program, permits detection of morphologic differences that are below the level of discrimination by the human eye.

For morphometric determinations, we measured the area of approximately 50 glandular acini per animal for a total count of greater than 244 acini per gender per species. In each tissue section, at least two fields in the central portion of the section were evaluated and all acini in a given area were included for analysis. Measurements were made by two investigators, and comparative results were identical. In addition, area determinations were the same whether tissue sections were "masked" or "unmasked." Photographs were taken at ×160 magnification with Kodak plus-X film. Statistical analysis of the data was performed by using the Mann-Whitney U test.

**Results.** The frequency distribution of acinar areas in lacrimal glands of male and female rats, mice, guinea pigs, rabbits and humans is shown in Figures 1 and 2, respectively. Statistical analysis of this data demonstrated that a distinct, gender-related dimorphism existed in glands of all species tested. The median area of acini was significantly larger (P < 0.0001) in lacrimal tissue of males as compared to that of females (Table 1). The extent of this difference varied among species. Median acinar areas in lacrimal glands were 71, 69, 63, 21, and 20% larger in male than female rats, mice, rabbits, humans, and guinea pigs, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Median acinar area (mm²)</th>
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<tbody>
<tr>
<td>Rat</td>
<td>2.74*</td>
</tr>
<tr>
<td>Mouse</td>
<td>2.11*</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3.73*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.02*</td>
</tr>
<tr>
<td>Human</td>
<td>2.18*</td>
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Lacrimal tissues (n = 5 per gender per species) were obtained from rats, mice, guinea pigs, rabbits, and humans and the area of lacrimal acini (n > 244 per gender) was determined by image analysis.

* Significantly (P < 0.0001) larger than "female" value.

**Discussion.** Our results demonstrate that a distinct, gender-related difference exists in the morphology of lacrimal glands. The median acinar area in lacrimal tissue of males was significantly larger than that of females in all species examined, including rats, mice, guinea pigs, rabbits, and humans. The extent of this difference varied between species. Nevertheless, given the uniform significance of the data, our findings suggest that sexual dimorphism of the lacrimal gland may be a general phenomenon.

In support of this hypothesis are the following observations: (1) In rats, numerous gender-related differences in lacrimal glands have been reported. These variations relate not only to morphologic characteristics, but also to biochemical, genetic, and immunologic parameters; (2) In mice, the levels of messenger RNA for specific proteins are approximately five times greater in lacrimal glands of males than those of females; and (3) In humans, significant, gender-associated differences have been found in the immunologic profile of the lacrimal gland, as well as one of its products, tear IgA.

The underlying cause of these gender-related differences in the lacrimal gland appears to be the influence of androgenic hormones. For example, castration of male rats results in a change of lacrimal gland morphology to that of the female pattern. Administration of androgens, but not other classes of steroid hormones, reverses the castration effect and reinstates the "intact male" characteristics of the lacrimal gland. Similarly, all other gender-associated differences in the lacrimal tissues have, when invest-
Fig. 1. Frequency distribution of acinar areas in lacrimal glands of males (n = 5). The total number of acini measured in rats, mice, guinea pigs, rabbits and humans were 273, 272, 271, 265, and 271.
Fig. 2. Frequency distribution of acinar areas in lacrimal glands of females (n = 5). The total number of acini measured in rats, mice, guinea pigs, rabbits and humans were 273, 272, 266, 245, and 273, respectively.
investigated, been shown to be due to the influence of androgens.\textsuperscript{1-3,6,9} In addition, testosterone is known to induce an enlarged and hyperactive lacrimal gland in the rabbit.\textsuperscript{12} In contrast to the effect of androgens, estrogen exposure does not appear to modulate lacrimal morphology or function.\textsuperscript{2,6} This finding is consistent with a recent report, which shows that estrogen receptors cannot be detected in the lacrimal gland.\textsuperscript{13}
The effect of androgens on the lacrimal gland may have clinical importance. For example, Sjogren’s syndrome, which is an immunologic disorder encountered almost exclusively in females, is characterized by a lymphocytic infiltration into the lacrimal gland, destruction of acinar and ductal tissues and generation of keratoconjunctivitis sicca (KCS). Overall, our findings and those of others indicate that the lacrimal gland is a sexually dimorphic tissue. Moreover, the gender-related differences in this gland appear to be due to the influence of androgens. Research is currently underway in our laboratory to further examine the interrelationship of the endocrine system with the lacrimal gland.

**References**


**Binding of Fluorescein Monogluguronide to Human Serum Albumin**

Shigeroshi Nagatani* and Isao Matsumoto†

The binding to human albumin of fluorescein monogluguronide, a fluorescent metabolite of fluorescein, was studied using two methods: pressure dialysis and fluorescence polarization. Both methods indicated that fluorescein monogluguronide binds to human albumin more loosely than fluorescein. The free fraction in human plasma estimated from the dissociation constant and the number of binding sites was in a range from 31 to 37%. Fluorescence of fluorescein was significantly quenched by the albumin binding, but fluorescence of fluorescein monogluguronide was not affected by albumin. The relative molar intensity of fluorescence between these fluorophores varied from 3.2 to 37.3, depending on the excitation wavelength. Invest Ophthalmol Vis Sci 26:1175-1178, 1985

Fluorescein is probably the most ideal tracer to measure the permeability of the human blood–ocular barriers, but a problem with its systemic use is that it is rapidly metabolized to a weakly fluorescent conjugate, fluorescein monogluguronide. Thus, the measurements of fluorescence in body fluids after systemic administration of fluorescein must be evaluated by characterizing the kinetics of this metabolite.