Relationship of sulfatase activity to 
radiosulfate uptake in guinea pig 
orbital tissues: Effect of TSH

Heskel M. Haddad*

The sulfating effect of TSH on guinea pig Harderian and ventral lacrimal glands was investi-
gated by differential centrifugation and by studies on the two glands' sulfatase activity. Radio-
sulfate incorporation in the Harderian as compared to that in the ventral lacrimal gland, was 1:10. The particulate elements of the two glands incorporated radiosulfate differently—58 per cent of the Harderian's and 11 per cent of the ventral lacrimal's. Almost all the sulfating effect of TSH on the Harderian was accounted for by the increase in the radiosulfate of the gland's particulate elements. Sulfatase activity in the Harderian, as compared to that in the ventral lacrimal, was 5:1 and the specific activity ratio was 20:1. The recovery of sulfatase activity in the particulate elements was 43 per cent in the Harderian and 80 per cent in the ventral lacrimal. TSH did not significantly affect total sulfatase in either gland, but particulate sulfatase under the effect of TSH constituted 85 per cent of the Harderian's sulfatase and 94 per cent of the ventral lacrimal's. The relationship of these findings to experimental eno-
docrine exophthalmos is discussed.

The 24 hour uptake of $^{35}$S-labeled sulfate by guinea pig orbital tissues, particularly by the Harderian and ventral lacrimal glands, is enhanced by the administration of thyrotrophic hormone (TSH). Even though the uptake by the Harderian gland is about one tenth that of the ventral lacrimal gland, the former appears to be the one affected most by TSH. This response of the Harderian gland to TSH becomes more marked and the difference between the two orbital glands in sulfate uptake and TSH response becomes magnified with 72 hour, rather than 24 hour, sulfate incorporation.

In order to assess the factors related to the dissociated behavior of these two glands in terms of both radiosulfate incorporation and response to TSH, the cellular qualities of each gland were investigated by differential centrifugation and by the assay of sulfatase activity in the cellular fractions, with and without the effect of TSH.

Material and methods

Young female guinea pigs (in a group of six) were injected intraperitoneally with
Table I. Sulfatase activity in the homogenate and particulate fractions of the Harderian and ventral lacrimal glands and its specific activity are shown

<table>
<thead>
<tr>
<th></th>
<th>Homogenate (U./mg. ± SE)</th>
<th>Particulate (U./mg. ± SE)</th>
<th>Particulate Homogenate (%)</th>
<th>Protein (mg./Gm. ± SE)</th>
<th>Specific activity (U./mg. protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harder’s</td>
<td>59.0 ± 4.0</td>
<td>25.5 ± 2.3</td>
<td>43.2</td>
<td>29.0 ± 3.2</td>
<td>20.10</td>
</tr>
<tr>
<td>Ventral lacrimal’s</td>
<td>12.5 ± 1.4</td>
<td>10.0 ± 1.1</td>
<td>80.0</td>
<td>55.0 ± 4.8</td>
<td>2.27</td>
</tr>
</tbody>
</table>

TSH (Thyropar*) suspended in normal saline daily for 3 days. A control group of another six guinea pigs were similarly injected with normal saline alone. On the first day, with the initial injection of normal saline or TSH, 50 to 500 μc of carrier-free 35S-labeled sodium sulfate† was also injected. On the third day, the animals were anesthetized by ether and exsanguinated. The ventral lacrimal and Harderian glands were dissected, cleaned of adventitia, weighed, and separately homogenized with normal saline in the cold, with the use of a glass homogenizer. Each homogenate was centrifuged at 1,500 x g for 10 minutes. The supernatant fluid was further centrifuged at 10,000 x g for 30 minutes. The precipitates obtained (the particulate fraction) were resuspended to original volume. Radioactivity and sulfatase activity were assayed in the homogenate and in the particulate. The preparation for radioassay has been described previously. The results were expressed in terms of counts per minute per milligram of original wet tissue.

Sulfatase was assayed with the use of p-nitrophenol sulfate as substrate and the results were expressed in units per milligram of original wet tissue.

Protein determination was made by the Lowry method and was expressed in milligrams of protein per gram of original wet tissue.

Results

Radiosulfate incorporation (Fig. 1). In the control animal, i.e., without the effect of TSH, the 72 hour incorporation of 35S-labeled sulfate was 73.1 c.p.m. per milligram (SE ± 4.1) in the Harderian gland and 697.6 c.p.m. per milligram (SE ± 15.9) in the ventral lacrimal gland. With minor variations, this ratio was maintained in almost all experiments.

The distribution of the radiosulfate following differential centrifugation showed a concentration of 58 per cent of the radioactive activity in the particulate elements of the Harderian gland and only 11 per cent in those of the ventral lacrimal gland.

Sulfatase activity (Table I). There was a significant difference in the activity of sulfatase in the two glands. Whereas, in their homogenate, the ratio between the Harderian gland’s sulfatase and that of the ventral lacrimal gland was 5:1, the specific activity of the enzyme in the Harderian gland was 20.10 U. per milligram protein, as compared to only 2.27 U. per milligram protein in the ventral lacrimal gland. The protein concentration of the two glands was 29.0 mg. per gram of the Harderian gland’s wet weight and 55.0 mg. per gram of the ventral lacrimal gland’s.

The sulfatase activity of the ventral lacrimal gland was concentrated mostly in the particulate elements, where about 80 per cent of the total gland’s sulfatase was recovered. In the Harderian gland, on the other hand, only 43 per cent of the sulfatase activity was recovered in the particulate fractions after differential centrifugation.

Effect of TSH

Radiosulfate incorporation (Fig. 1). After 3 daily 1 U. TSH administrations, there

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*Purchased from Armour Laboratories, Kankakee, Ill.
†Purchased from Abbott Laboratories, Oak Ridge, Tenn.
Fig. 1. The 72 hour incorporation of radiosulfate by the Harderian and ventral lacrimal glands and its response to TSH, both in total homogenate and in particulate fractions, is graphically demonstrated.

was an average increase in the glands' radioactivity of 51 per cent in the Harderian and 18 per cent in the ventral lacrimal. The distribution of the radioactivity in the particulate elements of the ventral lacrimal gland appeared to be unaffected by TSH. In the Harderian gland, on the other hand, 71 per cent of the radioactivity of the gland was recovered in the particulate elements after treatment with TSH, or, in terms of counts per minute per milligram of wet weight, the particulate elements showed an average increase of 36.3 c.p.m. per milligram of original tissue as compared to an average increase of 37.0 c.p.m. per milligram in the gland's homogenate under TSH stimulation.

Sulfatase activity (Fig. 2). Under TSH stimulation, there was no appreciable change in the concentration (either in terms of units per milligram of tissue or in specific activity) of the sulfatase activity in either gland. There was a very insignificant increase in the total activity of the two glands, amounting to 15 per cent in the Harderian and 4 per cent in the ventral lacrimal.

The significant effect of TSH, however, was noted in the distribution of this enzyme in the particulate elements, especially in the Harderian gland. There seemed to be a stimulation of the sulfatase activity of the particulate elements at the expense of the soluble enzyme fraction in both glands, but it was more noticeable in the Harderian than in the ventral lacrimal. In the Harderian gland, the sulfatase activity was 71 per cent in the particulate elements in the control group. Under TSH stimulation, however, the concentration of the enzyme recovered in the particulate elements was 85 per cent of the total gland's enzyme activity, which was only slightly increased. In the ventral lacrimal gland, almost all the sulfatase activity was recovered in the particulate elements after stimulation with TSH (12.5 U. as compared to the total of 13.0 U. per milligram).
Discussion

In the guinea pig, there seems to be a dissociation between two orbital glands—the ventral lacrimal and the Harderian—in their incorporation of radiosulfate and their response to TSH. That the ventral lacrimal gland has more avidity for radiosulfate than the Harderian gland has is evident from our work and from the work of others. Yet it is the Harderian gland that responds to TSH stimulation by an increase in radiosulfate incorporation. Thus, this response to TSH by the Harderian gland must bear a relationship to local characteristics which differentiate these two orbital glands in the guinea pig. Radiosulfate in both glands is incorporated organically in the 24 hour as well as the 72 hour uptake studies (Figs. 3 and 4), the only difference being that at 24 hours there is more inorganic unincorporated radiosulfate floating in the tissue. At 72 hours, the animal has been allowed at least a biological half-time to dispose of this inorganic unincorporated radio-
sulfate and thus its level is diminished in the tissues. For this reason, especially if the TSH effect is on the organically bound radiosulfate, a better response at 72 hours than at 24 hours would be expected. Furthermore, hydrolysis studies showed that most of the radiosulfate incorporated in the gland was in the form of sulfated oligosaccharides, of which one was probably a chondroitin sulfate–like compound (Fig. 4). Radioscans of chromatograms of enzymatic hydrolysates of sulfated Harderian glands (Fig. 5) showed that such oligosaccharides are less evident when proteolytic enzyme (trypsin) or elastase enzyme is used. However, when sulfatase and/or hyaluronidase are used, the organic sulfate in the Harderian gland is hydrolyzed into three components equal in \( R_f \) characteristics. The largest of these has an \( R_f \) value equal to that of chondroitin sulfate, which also appears on the chromatogram of trypsin or elastase hydrolysates. The hydrolysis into inorganic radiosulfate is
minimized under the effect of hyaluronidase and sulfatase but is almost complete under the effect of sodium hydroxide.

In the Harderian gland, both with and without the effect of TSH, the incorporation of radiosulfate appears to be most pronounced in the particulate elements, suggesting an active and possibly specific sulfation process. That the TSH-induced radiosulfate incorporation in the gland affects only the particulate fraction and not the homogenate as a whole (Fig. 1) needs further evaluation but is further corroborated by the study of the sulfatase in the gland.

Not only is there a significantly higher level of sulfatase activity in the Harderian gland than there is in the ventral lacrimal gland, but the difference between the two glands is most noticeable in the concentration of the enzyme in the particulate fractions where most of the TSH stimulation of the sulfatase activity is recovered.

It is indeed intriguing to think that endocrine exophthalmos is related to a humoral factor of close relationship to TSH, possibly in the form of an exophthalmos-producing substance (EPS)\textsuperscript{11-12} or a late-or long-acting thyroid stimulator (LATS).\textsuperscript{16-18} Yet, how these factors affect orbital tis-

**Fig. 3.** Radioautograph of direct tissue chromatograms of Harderian and ventral lacrimal glands, showing 24 hour radiosulfate incorporation in the control and the TSH-treated animals. Small amounts of oligosaccharides are seen (in the ventral lacrimal, could be confused with radioactive trailing). Over 90 per cent of the radioactivity in each gland was inorganic radiosulfate following 4 hour hydrolysis with 1N NaOH.

**Fig. 4.** Radioautograph of Harderian gland homogenate, showing 72 hour radiosulfate incorporation and the effect of 4 hour 1N NaOH hydrolysis. A chondroitin sulfate-like component appeared in addition to the inorganic radiosulfate spot seen at the bottom of the chromatogram.
Fig. 5. Autoscan recordings of chromatograms of 72 hour sulfated Harderian gland homogenates—treated for 24 hours with trypsin, elastase, hyaluronidase, sulfatase, or NaOH. The numbers under the peaks indicate their percentages of the total paper radioactivity.

The number of patients with progressive endocrine exophthalmos. Because of the difference in the effect of TSH in vitro and in vivo, and because of the difference in the response of the various guinea pig orbital tissues, mostly the Harderian and the ventral lacrimal glands, local factors in these orbital tissues must be considered for the understanding of the effect of TSH and the humoral factors that are possibly related to endocrine exophthalmos.

Sulfated mucopolysaccharides are increased in the orbital tissues in experimental endocrine exophthalmos in the guinea pig and in patients with endocrine exophthalmos as well. This is in accordance with the increase in radiosulfate incorporation under the influence of TSH and with the presence of mast cells in fish tissues treated with TSH or EPS.27, 28

Our interest in the investigation of the sulfatase system in the orbital tissues of the guinea pig was prompted by the finding that the in vitro incubation of the Harderian gland with TSH, EPS, or sera of patients with progressive endocrine exophthalmos induced not a stimulation but rather an inhibition of the sulfation process. Actually, the inhibition by TSH was of a competitive nature. Such an in vitro inhibition, as compared to the stimulation of in vivo sulfation, suggests the involvement of at least two factors in the modus operandi of TSH: one locally operating, possibly related to a sulfatase enzyme system (in vitro) and the other systemically or humorally active, specifically on the sulfation of the orbital tissues (in vivo). It is possible that the effect of one factor counterbalances the effect of the other in the breakdown and synthesis of sulfated mucopolysaccharides in the normal and that the balance is upset by TSH stimulation in favor of excess in vivo sulfation and decreased in vitro sulfation.

The studies of sulfatase activity in the Harderian and ventral lacrimal glands, its distribution in the particulate fractions of each gland, and its stimulation by TSH in the Harderian gland favor this hypothesis. The effect of TSH on sulfatase activity in vitro and the nature of the other sulfating factors, which are possibly in the form of active sulfate compounds, require further investigation.

REFERENCES
3. Wegelius, O., and Lamberg, B. A.: 35S-Autoradiography of the retrobulbar connective tis-
sulfatase activity and radiosulfate uptake 87


