In vitro sulfation of orbital tissues of the guinea pig

II. Relationship to endocrine exophthalmos

Heskel M. Haddad

The uptake of radiosulfate by the Harderian gland in vitro was inhibited by the addition of TSH or the sera from patients with endocrine exophthalmos.

The incorporation of S-35-labeled sulfate by slices of conjunctiva and ventral lacrimal and Harderian glands of the guinea pig was studied in an in vitro system. Sulfation of these tissues, particularly of the Harderian gland, resembled an enzymatic reaction in its temperature and pH characteristics and its dependence upon the viability of the tissues. Thyrotrophic hormone (TSH) added to the incubation medium decreased sulfation of these tissues, especially that of the Harderian gland.

Sera of patients with endocrine exophthalmos reduced the in vitro sulfation of the retroorbital tissues of the guinea pig. In the present study, this effect on the in vitro sulfation of the Harderian gland of the guinea pig was used for a qualitative assay of TSH-like activity in sera of 14 patients with endocrine exophthalmos.

Methods

Orbital tissues of the guinea pig were obtained after the animal was anesthetized with ether and exsanguinated. The tissues were cleaned of adventitia, sliced, and incubated immediately in Krebs phosphosaline (KPS) buffer pH 7.4 to which radiosulfate* and a penicillin-streptomycin mixture were added. Serum was diluted with KPS buffer in a 1:4 ratio and used as an incubation medium. Incubation was carried out aerobically for 21 to 24 hours in a constant temperature water bath at 37° C, with continuous shaking. At the end of the incubation, the slices were removed from the incubation medium, washed, blotted, and hydrolyzed for radiosulfate assay. The results were calculated in counts per minute per milligram (c.p.m./mg.) of wet weight of tissue.

Material

Sera of 9 euthyroid controls (18 to 63 years old) and of 14 exophthalmic patients (22 to 63 years old) were assayed for their effect on radiosulfate uptake by the Harderian gland of the guinea pig. At the time of the collection of the blood, 8 of these patients had progressive exophthalmos and 6 showed either no progression (5 patients) or

---

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis, Mo.

Supported by Grant A-5703, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service, Bethesda, Md.

*Special Fellow, National Institute of Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service, Bethesda, Md.

*Radiosulfate was purchased from Oak Ridge National Laboratories, Oak Ridge, Tenn.
regression (1 patient) of the proptosis. Eleven of these patients had idiopathic thyrotoxicosis, 2 were in a hypothyroid state, and the fourteenth patient was euthyroid clinically, but his serum protein-bound iodine and thyroid radioiodine uptake failed to be suppressed with triiodothyronine. At the time of the study 10 patients were euthyroid and 4 were hypothyroid. Only 2 patients had unilateral exophthalmos (progressive).

Results

Radiosulfate incorporation in orbital tissues of the guinea pig did not differ significantly when incubation was carried out in KPS or in normal serum-KPS medium. Whereas conjunctival and ventral lacrymal tissues showed a sulfation response to TSH and to sera of exophthalmic patients similar to that obtained in Harderian tissue, this presentation will be limited to the results obtained on Harderian gland sulfation only.

The uptake of radiosulfate by the Harderian gland, when incubated with normal serum-KPS, ranged between 20.7 and 34.5 c.p.m./mg (mean 26.09 S.E. ± 1.93); and when incubated with exophthalmic patient’s serum-KPS, it ranged between 4.9 and 21.5 c.p.m./mg. (mean 12.72, S.E. ± 1.63). There was no sex or age variation in the effect of normal sera on Harderian gland uptake of radiosulfate. Tissue boiling or freezing prior to incubation reduced radiosulfate uptake by the Harderian tissue to 13 to 26 per cent of the control. With 0.2 unit TSH an average reduction of 50 per cent of radiosulfate uptake resulted. The uptake of radiosulfate following incubation with sera of 7 patients with progressive exophthalmos and 3 patients with inactive exophthalmos was 26 to 45 per cent of the paired control serum. The sera of one patient with progressive exophthalmos* and of 3 patients with inactive exophthalmos failed to induce significant inhibition of sulfation (Fig. 1).

Discussion

Although TSH stimulates sulfation of the Harderian gland in the intact animal, it reduces sulfate uptake by the same tissue in vitro. Neither response is fully investigated and explained.

The response of the in vitro sulfation of Harderian tissue to exophthalmic sera may be a forerunner of a bioassay method for TSH-like activity in human serum. Despite a wide range of variability between different normal sera, there is a sharp and significant difference between the results of the sera of normal versus exophthalmic patients. This difference between the two groups of sera becomes more pronounced when the latter (exophthalmic cases) are divided into the increasingly progressive and the inactive or subsiding exophthalmos (Fig. 1).

The author wishes to thank Dr. Bernard Becker for his help and suggestions and Miss Aleene Schneider and Mr. Raymond Grant for their technical assistance throughout this study.

REFERENCES

1. Haddad, H. M.: In vitro sulfation studies of guinea pig orbital tissues, Read at the Mid-

*This patient had bilateral progressive exophthalmos following thyrotoxicosis which was treated with radioiodine. During decompression of the left eye, the biopsy was taken and was interpreted as pseudotumor.
In vitro sulfation of orbital tissues of guinea pig

Discussion

Dr. George K. Smelser, New York, N. Y. The material(s) contained in anterior pituitary extracts which causes exophthalmos has not been characterized even with respect to its biologic actions. There is a substance(s) in pituitary extracts which when injected causes, in some species, a protrusion of the globe. The affected structures in this case are the connective tissues, and Harder's gland. The latter is well developed in rodents and many other mammals, although not in carnivores or primates. Pituitary extracts which cause exophthalmos and hypertrophy of Harder's gland also cause hypertrophy and hyperplasia of other exocrine glands, mainly of the sebaceous type. The best evidence we have obtained for this has been provided by the perirectal and by the equivalent of the Meibomian glands in the guinea pig. Not only does this hypertrophy involve the connective tissue, but the secretory activity of these glands is greatly accelerated, which requires that they receive from the blood increased quantities of their ingredients of the secretory product, one of which is sulfate. Wegelius has shown that the increase in sulfate uptake in vivo by Harder's gland is a sensitive measure of the effect of the pituitary extracts on this process and showed that the sulfate uptake response was correlated with the presence of the exophthalmogenic material. He attempted to use this response as a basis for the assay of serum of exophthalmic patients but with variable results. Dr. Haddad has extended this work by investigating the sulfate uptake in vitro, which is a good idea for one may hope to gain sensitivity and constancy by such a procedure. The depression rather than stimulation of sulfate uptake caused by the sera of exophthalmic patients which he reports is puzzling and reminds one of the inhibitory effect of human sera on the exophthalmogenic effect of pituitary extracts when tested in fish.

It would be most interesting if the author would distinguish between free sulfate which may be adsorbed or held in tissue spaces from that which has become a part of the cell or its products. In addition, valuable information might be obtained by experiments in which the quantity of TSH or serum and the time of reaction were varied to determine if the depression noted was but one phase of the response.

REFERENCES


Dr. Haddad (closing). This study in effect confirms that there is a relationship between TSH and radiosulfate incorporation by the Harderian gland of the guinea pig. An effect similar to that of TSH could be demonstrated in the sera of exophthalmic patients. The decrease rather than the increase in the radiosulfate incorporation is difficult to explain. It raises the possibility, however, that TSH may stimulate the sulfation of the Harderian gland in vivo humorally, but activate a sulfatase system topically (in vitro). There is no evidence, biochemically and chromatographically, for an undue affinity between TSH and radiosulfate, nor is there any variation in TSH effect with the time of incubation.