hour in the case of coarctation of aorta; in kidney from Farber's tissue these two enzyme activities were similar to control levels, 13.6 and 0.93 nmole per milligram of protein per hour. In preliminary studies, which we reported previously, the activity of ceramidase in the liver of this same patient had appeared to be normal (*3*). However, these assays were performed at *p*H 7.0, which was later shown to be far from the optimum *p*H, and the assay included a technique that failed to separate the fatty acids completely from the remaining uncleaved substrate.

A specific inhibitor of ceramidase is absent in Farber's tissue (Fig. 1). In addition, it can be calculated from the data in (*3*) that dilution of the substrate or inhibition of enzyme activity by tissue ceramide could not be responsible for the observed low ceramidase activity in the case of Farber's disease, since the assay samples of Farber's tissue contained, at most, 6 percent of the amount of ceramide added as substrate. Also, there is a stimulation of activity caused by factors present in the added homogenate, whether it had been boiled or not, and by added bovine serum albumin itself, at lower protein concentrations.

The demonstrated accumulation of ceramide in two patients with Farber's disease, coupled with the deficiency of ceramidase in our patient, suggests that a genetically determined defect in ceramide degradation forms the biochemical basis of this disorder. Ceramide levels, however, have been reported in only two patients with this disease (*3, 4*), and to our knowledge assays of ceramidase activity have been carried out only in the patient reported here. A block in ceramide degradation could, secondarily, lead to the ganglioside and glycolipid accumulation which has been reported in this patient and in others (*1, 3, 14*). There is still no explanation for the glycosaminoglycan accumulation reported in two patients with Farber's disease (*15*). Yavin and Gatt in their studies with rat brain concluded that the same enzyme catalyzed the synthesis and the degradation of ceramide (*7*).

We have observed that ceramide synthetase of human kidney—assayed by the method of Yavin and Gatt (*7*) at *p*H 4.5, with 1.03 m*M* sphingosine and 4.13 m*M* [1-^{14}C]oleic acid as substrates—has a specific activity of 1.44 to 2.58 nmole of ceramide synthesized per milligram of protein per hour in six patients without Farber's disease, whereas in the kidney of the patient with Farber's disease the specific activity was found to be less than 0.02 nmole per milligram of protein per hour. This finding raises anew the question of what pathways are utilized for biosynthesis of ceramide (*18*).

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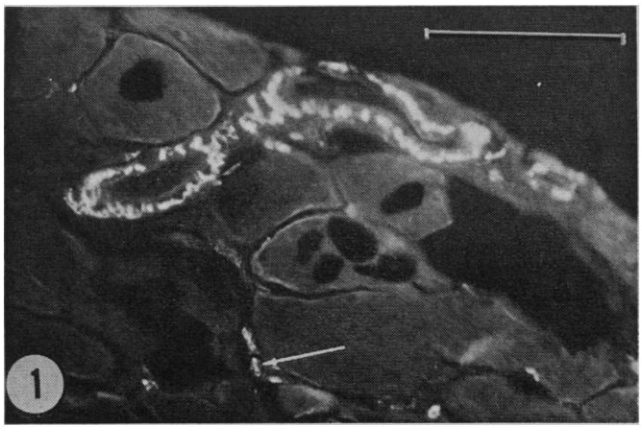
Adrenergic and Cholinergic Innervation of the Hamster Harderian Gland

Abstract. *Examination of Harderian glands of adult male and female golden hamsters by appropriate histochemical techniques reveals that adrenergic nerves are associated only with the blood vessels. Acetylcholinesterase-positive fibers are present in the connective tissue surrounding the gland, along the ducts, and among the acini.*

The gland of Harder, present in the orbits of many vertebrates that possess a nictitating membrane, is a compound tubuloalveolar gland that is separate from, but drains into, the bulbar surface of the nictitating membrane (*1*). In addition to numerous lipid droplets, this gland in several species of mammals is rich in porphyrins (*2*). The function of this gland is uncertain, but several investigations suggest that in rats and hamsters it may be a component of a system involving light, the pineal gland, and the reproductive organs. Wetterberg and his colleagues reported a regulatory effect of the Harderian gland on pineal serotonin and hydroxyindole-*O*-methyltransferase (HIOMT) in blinded suckling rats (*3*). Data from our studies of pineal–Harderian gland interaction in the hamster indicate that the pineal, which has a pronounced antigonadotropic action in the light-deprived hamster (*4*), has a regulatory effect on cell type and porphyrin metabolism in the Harderian glands of blinded male and female animals (*5, 6*). However, in an earlier study similar to ours, Hoffman (*7*) concluded that blinding alters Harderian gland metabolism but does so by a pathway exclusive of the pineal gland. Reiter and Klein (*8*) found that Harderian glands of adult female rats regress after exposure to constant light for 9½ weeks and that removal of the Harderian glands from rats on a cyclic lighting regimen leads to slight uterine enlargement; however, they observed no relation between the Harderian gland and pineal HIOMT or *N*-acetyltransferase activity. Since much is still unknown about the Harderian gland, knowledge of its innervation should be useful in determining how these various interactions may be mediated.

Adult golden hamsters of both sexes (100 to 120 g) were decapitated after



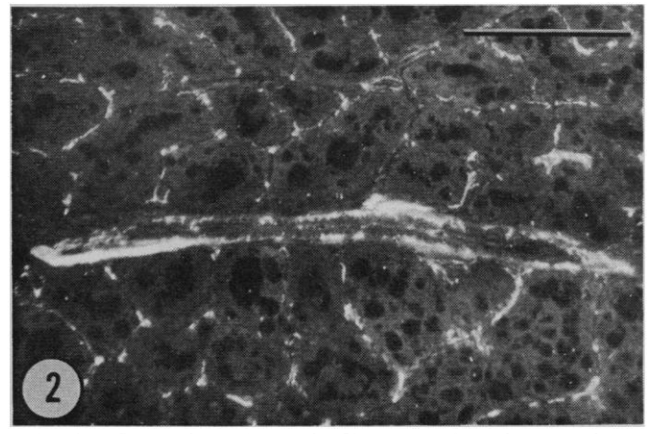


Fig. 1. Section through a Harderian gland of a female hamster showing fluorescing adrenergic fibers coursing along an interlobular blood vessel. Note the absence of fibers around acini of the gland and the presence of mast cells (arrow). Fig. 2. Dark-field (*17*) photomicrograph of a Harderian gland from a male hamster showing AChE-positive nerve fibers along an interlobular duct and in the connective tissue surrounding the acini (scale lines, 200 μm).

sodium pentobarbital anesthesia (0.12 ml/100 g of body weight); the Harderian glands were quickly removed and were frozen in 2-methylbutane at −70°C. The distribution of adrenergic nerves was studied by the fluorescent technique of Falck and co-workers (*9*) and of acetylcholinesterase positive fibers, by the thiocholine method of Karnovsky and Roots (*10*), as modified by El-Badawi and Schenk (*11*).

Fluorescing adrenergic nerves course through the adventitia of the interlobular blood vessels and their branches (Fig. 1). These fibers form a plexus immediately surrounding the tunica media of the vessels. No fluorescing fibers are associated with the acini or ducts of the gland. We noted no differences in the adrenergic innervation between male and female specimens; however, we observed abundant fluorescing mast cells only in the Harderian gland of females (Fig. 1).

Tenuous cytoplasmic processes, positive for acetylcholinesterase (AChE), are present throughout the interstitial tissue of the Harderian gland. Although the distribution of these fibers is similar in male and female hamsters, they were more prevalent in the males (Fig. 2). At the resolution of the light microscope, these fibers often appear to terminate in small bulbous endings adjacent to the myoepithelial cells that surround the acini. Small bundles of nerves lie along the ducts, which frequently contain cellular debris and, in the female, porphyrin. Larger bundles of nerves are present in the connective tissue surrounding the gland, especially in the vicinity of the hilum.

In an ultrastructural study of the hamster Harderian gland Bucana and Nadakavukaren (*12*) reported the presence of nerve fibers along blood vessels and nerve endings adjacent to blood vessels, myoepithelial cells, and secretory (acinar) cells. According to these authors, all of the nerve terminals contain dense-cored vesicles; however, they were less abundant in the nerves associated with myoepithelial and secretory cells. These vesicles resemble dense-cored vesicles that characteristically are associated with adrenergic nerves and are thought to contain norepinephrine (*13*). Since in our study we detected adrenergic nerves only along blood vessels, the significance of dense-cored vesicles in nerves that correspond in location to our AChE-positive fibers is not known. Although it is possible that the AChE represents AChE-containing adrenergic nerves, the fact that we observed no AChE-positive reaction along the blood vessels, even after 5 hours of incubation, strongly suggests that this is not the case and that the AChE-positive fibers are cholinergic. The adrenergic nerves probably serve a vasomotor function. The AChE-positive fibers, which ramify among the acini, may be involved in secretion. As pointed out by Bucana and Nadakavukaren, injection of rats with acetylcholine results in secretion of "bloody tears" (porphyrin pigments) from the Harderian gland (*14*), and this response supports our conclusion that nerves in this region are cholinergic.

The Harderian gland of the hamster exhibits a sexual dimorphism in that porphyrin is normally present only in the female (*15*). Also, the acinar cells of the female contain numerous minute droplets of uniform size; those of the male contain droplets of various sizes (*5, 7, 16*). The slight difference in the abundance of AChE-positive fibers in the two sexes may be related to this sexual dimorphism. Since the Harderian glands of castrated males assume the appearance of those of normal females (*5, 7, 16*), a corresponding change may occur in the AChE reactivity.

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