

with the A/J antigens [on several occasions, such experiments were unsuccessful (6, 9)]. To establish objectively the validity of these findings, we performed double-blind experiments (10); the results of these experiments demonstrated unambiguously that (i) on the basis of the activity transferred it was possible to correctly determine the identity of unknown RNA extracts and (ii) immune RNA extracts were capable of transferring migration inhibitory capacity to nonimmune cells (Table 2). The precise chemical nature of the "transfer" factor or factors (11) present in immune RNA extracts remains to be elucidated.

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  5. Lymph nodes from A/J or CBA mice were homogenized at room temperature in distilled water with a Tri R glass homogenizer, (50 ml of water for the lymph nodes of ten mice). The homogenate was centrifuged at 15,000 rev/min for 30 minutes, and the supernatant was dialyzed against distilled water (two changes of 6 liters) at 4°C for 24 hours. The nondialyzable portion was clarified by centrifugation, lyophilized, and stored at -20°C.
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controlling pineal activity in immature animals (2). Previous investigations of the mammalian Harderian glands showed two major cell types, the secretory cells and the myoepithelial cells. The structure of these cell types is well documented (3-7). However, to our knowledge there are no reports on the innervation of the Harderian gland at the ultrastructural level. Our observations of the innervation of the Harderian gland are of twofold significance: (i) to our knowledge, it has never been reported before in any of the ultrastructural investigations of the Harderian gland and (ii) it may help to explain how the Harderian gland is regulated.

Four-week-old hamsters (*Mesocricetus auratus* Waterhouse) of both sexes were killed by cervical dislocation. The Harderian glands were immediately removed, cut into small pieces, and fixed in 5 percent phosphate-buffered glutaraldehyde (pH 7.3) for 4 hours, followed by fixation in 1 percent phosphate-buffered osmium tetroxide (pH 7.3) and 1 percent aqueous uranyl acetate for 1 hour each. The fixations were carried out at 4°C. The samples were then dehydrated in a graded series of ethanol, followed by propylene oxide, and embedded in Epon 812. Thin sections in the range of 600 to 800 Å were cut with a diamond knife on a Reichert ultramicrotome. The sections were stained with uranyl acetate and lead citrate. They were then examined in a Hitachi HU-11A electron microscope operating at 50 kv.

Unmyelinated nerve fibers follow the blood vessels in the interlobular area of the Harderian gland. Nerve endings with characteristic dense-cored vesicles are observed in the connective tissue adjacent to blood vessels (Fig. 1). Nerve endings are also present in ap-

## Innervation of the Hamster Harderian Gland

**Abstract.** *Harderian glands of male and female hamsters have nerve endings associated with the secretory cells, myoepithelial cells, and the blood vessels. The nerve endings adjacent to the myoepithelial cells also have myoneural junctions.*

The Harderian gland is a compound tubuloalveolar gland, located on the posterior aspect of the eyeball of animals possessing a third eyelid. The ducts of the gland open on the deep

surface of the nictitating membrane (1). The function of the Harderian gland is not too well understood. At present the Harderian gland is thought to function in some unknown way in

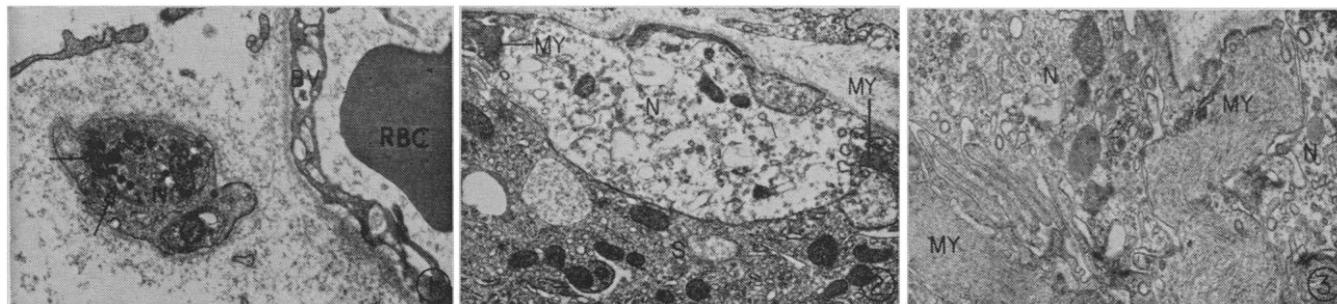


Fig. 1. Section of the hamster Harderian gland showing nerve ending (N) adjacent to a blood vessel (BV) in the interlobular connective tissue. Note dense-cored vesicles (arrows) in the nerve; RBC, red blood cell ( $\times 16,300$ ). Fig. 2. Section through a male hamster Harderian gland showing nerve ending (N) in close association with a secretory cell (S) and cytoplasmic extensions of the myoepithelial cells (MY) ( $\times 9,400$ ). Fig. 3. Section of a female hamster Harderian gland showing close association between nerve endings (N) and myoepithelial cells (MY) ( $\times 11,400$ ).

position to both the cytoplasmic extensions of the myoepithelial cells and the secretory cells (Figs. 2 and 3). In addition, membrane evaginations of the myoneural junctions are also seen (Figs. 2 and 3). There appear to be fewer dense-cored vesicles in the nerve endings associated with the myoepithelial and secretory cells than in the nerve endings found in the connective tissue. The innervation of the Harderian gland we observed is similar in both male and female hamsters.

Fourman and Ballantyne (8) showed by histochemical studies that nerve fibers are associated with blood vessels of the Harderian gland of the Aylesbury duck. They also suggested that the secretory activity of the gland is influenced by blood flow to the gland. Our observations of the presence of nerve fibers associated with blood vessels of the interlobular connective tissue support Fourman and Ballantyne's suggestion. Cohn (3) showed that in mice numerous blood vessels are present in the lobar and interlobular connective tissue of the Harderian gland, but he did not report any innervation of the gland.

Tashiro *et al.* (9) reported that injection of rats with the neurotransmitter acetylcholine will produce, in a matter of minutes, a copious secretion of "bloody tears" from the Harderian gland. Chiquoine (10) suggested that myoepithelial cells may respond to acetylcholine by contraction, thus squeezing out the contents from the secretory cells. This is analogous to the contractile response of the myoepithelial cells of the mammary gland to oxytocin that is responsible for the phenomenon of "milk let down." The presence of nerve endings in apposition to the myoepithelial cells and the myoneural junctional folds (Fig. 3) indicates that the neurotransmitter passes a myoneural junction instead of diffusing through the connective tissue. This may also explain the fast response of the Harderian gland to the injection of acetylcholine.

It has been observed that the nature of the secretion of the Harderian gland is intermediate between the apocrine and holocrine types, with a greater tendency toward being apocrine (5). It is difficult at present to explain the significance of the relationship between the nerve fibers and the secretory cells which we observed. However, it appears that the process of secretion is affected directly by

nervous stimulation of the myoepithelial cells.

Derrien and Turchini (11) and Strong (12) reported the appearance of high concentrations of porphyrin compounds in the Harderian glands of mice and rats soon after their birth. A recent study by Wetterberg, Geller, and Yuwiler (2) suggested that the Harderian gland might serve as an extraretinal photoreceptor in young rats. Our observations do not provide any direct evidence to support their suggestion.

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## High-Resolution Proton Magnetic Resonance Spectra of a Rabbit Sciatic Nerve

**Abstract.** Proton magnetic resonance spectra (220-megahertz field) of an isolated rabbit sciatic nerve in its native state have been observed and assigned to the extracellular water, intracellular water, and phospholipids of the nerve. This study indicates that the nerve fibers contain fluid-like hydrophobic regions, in agreement with the results of recent electron spin resonance spin-labeled studies of excitable membranes of nerve and muscle.

Most mammalian nerve fibers are surrounded by a sheath of insulating material known as myelin, which consists of multilayers of membranes of the Schwann cells. Because of its lamellar structure, the myelin sheath has served as an important model for the lamellar membrane and has been the subject of numerous investigations, including extensive histological examinations and electron microscopy and x-ray diffraction studies (1). As a result of these efforts, the structure of the myelin

sheath is perhaps better understood than that of any other membrane system.

In recent years, magnetic resonance spectroscopy has also been applied to the study of nerve tissues. Most of these studies, however, have been concerned with the state of intracellular water in the nerve. Fritz and Swift (2) have studied the state of intracellular water in frog sciatic nerves coated with mineral oil and have suggested that this intracellular water can exist in two states, depending on whether the nerve

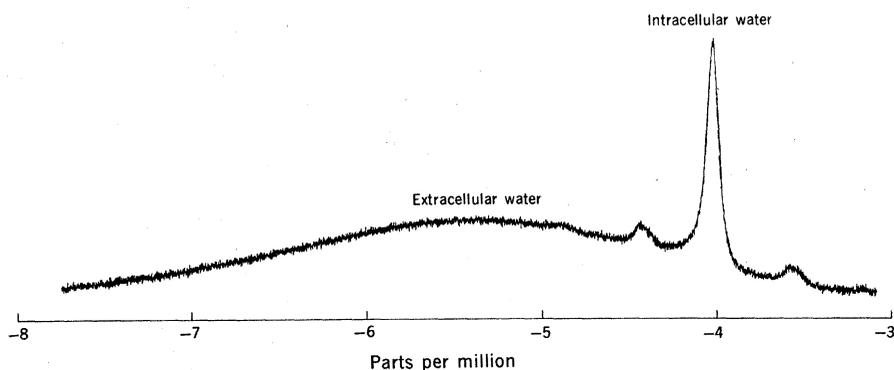


Fig. 1. The 220-Mhz PMR spectrum of intracellular and extracellular water for a sciatic nerve trunk of rabbit immersed in a bathing solution doped with 0.002M  $Mn^{2+}$ . The two smaller peaks adjacent to the intracellular water resonance are spinning side bands.