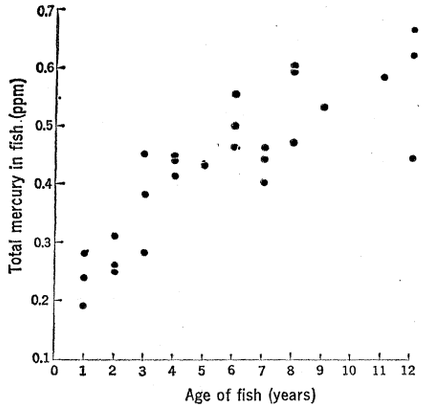

Fig. 1. Total mercury in Cayuga Lake trout as a function of age.

subsample was dried and ashed by Schoniger combustion (*3*). The total amount of mercury in the absorbing solution was determined by flameless atomic absorption spectrophotometry (*4*). This method is easily sensitive to 0.1 part per million (ppm) of mercury in fish. The accuracy of the method was checked by recovery studies in which mercury as mercuric chloride was added to the fish samples before drying, and the samples were then dried, combusted, and analyzed. The percent recoveries of 0.3 ppm of mercury added to four samples of lake trout of ages 1, 2, 3, and 5 years were, respectively, 77, 80, 93, and 83.

Figure 1 illustrates the relation between total mercury in lake trout and age. All mercury concentrations in Fig. 1 were corrected for the average percent recovery (83.25). The length of the fish varied from about 20 cm (for a 1-year-old fish) to about 76 cm (for a 12-year-old fish).

Residues of mercury in fish are often present largely as highly toxic methylmercuric salts (*5*). It was of interest therefore to determine the effect of age on the concentrations of this metabolite in the same fish samples. Westöö has reported a method for the extraction and isolation of methylmercury compounds from fish (*6*). In this method the sample is extracted with hydrochloric acid, the methylmercuric compounds are partitioned into benzene, the bonds linking mercury to sulfur are cleaved with mercuric chloride, the methylmercury is extracted as the hydroxide, and finally the mercury is reconverted to the chloride for gas chromatographic analysis. A microwave-powered helium plasma emission detector (*7*) was used to selectively monitor the emission line of atomic mercury at 2537 Å. The method is sensitive to 0.1 ppm of methylmercuric salts in fish. The percent recovery of 0.174 ppm of methylmercuric chloride added to one 2-year-old and three 3-year-old lake trout samples was, respectively, 56.3, 54.6, 56.3, and 54.6. Westöö found that there is approximately a 30 percent loss of methylmercuric salts in his procedure as a result of unfavorable partition coefficients.

Table 1 presents a list of concentrations of total mercury and methylmercury in fish by age and the percentage of total mercury that was present as methylmercury. The values for total mercury and methylmercury as listed are corrected for the average percent recoveries which were, respectively, 83.25 and 55.4. It is evident that the variation trend in the concentrations of methylmercuric salts with age in fish is, in general, the same as the variation trend of the total mercury with age, although the concentration of total mercury is consistently higher. This relation between total mercury (and methylmercuric salt concentration) and age may simply be a reflection of the time during which the fish have been exposed to their environment. It may also be significant that the total proportion of mercury as methylmercury appears to be smaller in the younger fish. Owing to the good reproducibility of analysis for both mercury and methylmercuric salts on several replicated fish samples, the higher total mercury concentrations may be significant and indicative of the presence of mercury in fish in a form or forms other than methylmercuric salts. Another possible metabolite is dimethylmercury, but there is at present no satisfactory method for the determination of this compound in fish. Concentrations of total mercury and methylmercury in the lake trout studied did not appear to be related to the sex of the fish.

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29 January 1971

Garfish Olfactory Nerve: Easily Accessible Source of Numerous Long, Homogeneous, Nonmyelinated Axons

Abstract. *The olfactory nerve of the garfish,* Lepisosteus, *is about 1 millimeter in diameter and about 20 centimeters long, depending on the size of the fish; it is easily prepared by breaking off successive scored segments of the rostrum. It consists of a relatively homogeneous population of about 10^7 nonmyelineated nerve fibers, each about 0.24 micrometer in diameter. In most other nerves each fiber is separated from all others by an enfolding Schwann cell, but in the olfactory nerve the fibers are directly in contact with one another in groups of several hundred fibers. The Schwann cell, not directly concerned with propagation of the nerve impulse, forms a thin layer at the periphery of the group and makes up a small proportion of the total cellular material. The volume of axon cytoplasm is about five times greater than that of Schwann cell cytoplasm, and the axon surface is about 30 times the Schwann cell surface. The ratio of surface to volume for axons of a typical olfactory nerve is about 5400 times that for the squid axon of the same diameter. The large proportion of axonal membrane recommends this nerve for use in chemical and physical studies of properties of axon membranes.*

The nerve impulse is an event related to the axon surface: the electrical characteristics of the nerve fiber membrane at that surface have been most satisfactorily described for various giant fibers as large as 1 mm and more in diameter (*1*). The squid giant axon is admirably suited for the study of the

electrical attributes of the membrane, but the chemical events may require for their elucidation a greater mass of excitable membrane material than that axon can conveniently provide (*2*). The special helpfulness of large numbers of small fibers of uniform size and diameter for studies of changes in birefringence and fluorescence in nerve has been recognized (*3*).

The small nonmyelinated fibers of the olfactory nerve of vertebrates are electrically and anatomically uniform (*4, 5*). Not only is the ratio of surface to volume large in the fibers of this nerve, but the ratios of axon volume and membrane area to Schwann cell volume and area are also large in comparison to others. In the long-nosed garfish (*Lepisosteus osseus*) the olfactory nerve is, in addition, exceedingly long and easily accessible (*6*).

Work on mammals confirms that the fine structure of the olfactory nerve is probably similar throughout the vertebrates, but there appears to be no other animal in which the nerves are as large and long as they are in the garfish. In most vertebrates, the olfactory epithelium (wherein arise the axons from the olfactory cell bodies) is near to the olfactory bulb as well as to the brain. In the gar, the axons of the olfactory nerve traverse the entire length of the rostrum, which may be 20 cm or more in a large fish. The garfish olfactory nerve has been the subject of several preliminary investigations concerning some of its electrical (*6*), chemical (*7*), and physical (*8*) characteristics. This report describes some of the histological features that make this nerve an outstanding subject for the study of excitable membrane.

Most of these observations were made on olfactory nerve of *Lepisosteus osseus* (Linnaeus), the long-nosed garfish, but the nerves of *L. platyrhincus* and *L. ocellatus*, the short-nosed and the spotted gars, although shorter, were otherwise similar. The fish was decapitated by means of a large guillotine; the lower jaw was removed, and the two halves of the maxillae were separated from the rostrum. The terminal 1 to 2 cm of the rostrum, containing the olfactory epithelium, was cut off and discarded, and transverse, shallow cuts were made every 1 to 2 cm along the rostrum. The resulting sections were carefully snapped and pulled off the distal end, leaving the nerve attached proximally.

On each side of the rostrum, an olfactory nerve lies in a bony canal, together with branches of a myelinated nerve, the trigeminal, and blood vessels. All these structures are easily withdrawn from the snout of the fish. The nerve sheath, consisting of several overlapping cellular and collagenous layers, is easily stripped from the nerve, along with the myelinated nerve and the blood vessels (*9*). The diameter of the olfactory nerve averages about 1 mm, but it is generally somewhat larger in the larger fish, due to an increase in connective tissue. Nerves can be used at once for electrophysiological recording or stored in the refrigerator for use the next day (*10*).

The action potential of the olfactory nerve is a single elevation, consistent with the rather uniform fiber diameter. The impulse is only 1 to 2 mm long in this nerve, and the two phases of the diphasic recording of the action potential may therefore be completely separated during bipolar recording (*4, 6*).

For electron microscopic work, the fish were perfused with fixative via the ventral aorta before decapitation. The fixative consisted of garfish Ringer solution to which glutaraldehyde and phosphate buffer had been added. After removal from the animal, the tissue was treated with OsO_4, imbedded in Araldite, sectioned, mounted, and observed in a Philips 200 microscope. Figure 1A shows the entire nerve in relation to other structures associated with it (*11*).

Most nerve trunks have many kinds and sizes of fibers, each of which is usually surrounded separately by Schwann cell cytoplasm. However, the fibers of the olfactory nerve are in contact with one another and are assembled into groups, each of which contains several hundred axons (Fig. 1B). Fibers of this type are also found in other nerves of vertebrates and invertebrates (*12*), but they are usually present only in small bundles mixed with other types of fibers.

Each group of fibers of the olfactory nerve is enfolded once by a Schwann

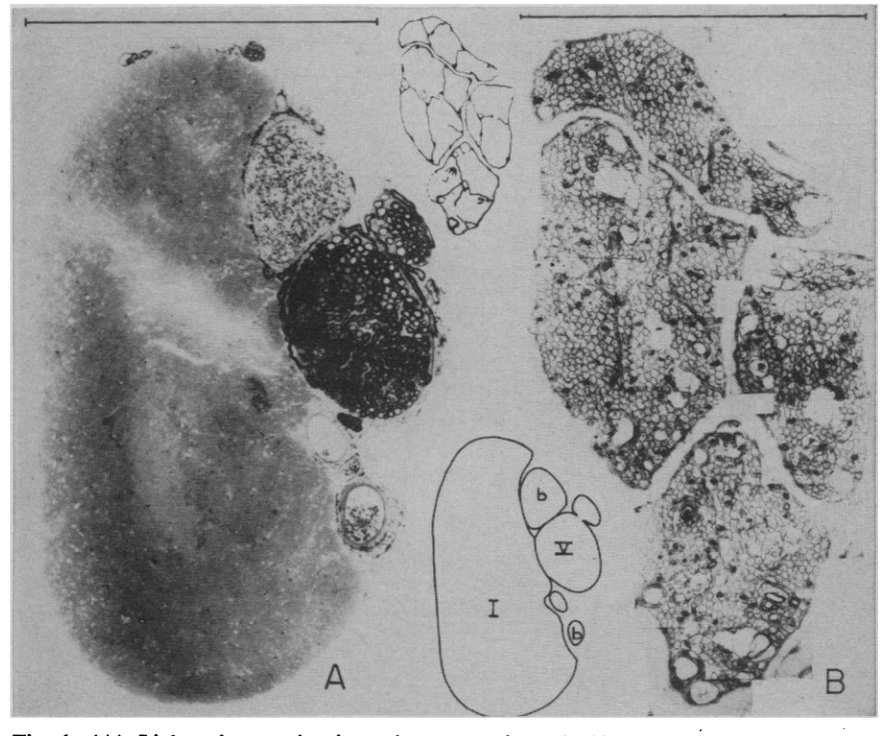

Fig. 1. (A) Light microscopic view of cross section of olfactory nerve and adherent structures identified in outline drawing in the lower center. The large gray area (*I*) is olfactory nerve. The smaller dark area is a branch of the trigeminal nerve (*V*). The remaining smaller circles are blood vessels (*b*). Scale, 1 mm. (B) Electron micrograph of small field from a cross section of olfactory nerve. Scale, 10 μm. The photograph is of a montage constructed at a size to permit measurements to be made of the olfactory nerve fibers; for the montage the scale equals 0.6 m. Four major Schwann cell domains are shown outlined in the inset upper center which indicates subdivisions in each major domain. The lines of the inset show the entire mass of Schwann cell material, whereas the enclosed blank areas are the groups of axons. The smaller circles in the photomicrograph are cross sections of axons packed closely in contact with one another. Each dark round spot is a mitochondrion, which fills almost the entire cross section of the axon.

Table 1. Relationships of axon and Schwann cell surfaces and volumes in olfactory nerve. Measurements for the filament are based on Fig. 1B. Measurements for whole nerve are calculated from filament measures, an entire cross section as in Fig. 1A being assumed.

Measurement	Filament	Whole nerve
Number of fibers	1577	1.3×10^7
Cross-sectional area	188	0.0154 cm^2
Axon surface area	1219 $\mu m^2/\mu m$	1000 cm^2/cm
Axon volume	80 $\mu m^3/\mu m$	0.0065 cm^3/cm
Schwann surface area	388 $\mu m^2/\mu m$	320 cm^2/cm
Schwann volume	16 $\mu m^3/\mu m$	0.0013 cm^3/cm
Domain volume excluding collagen	122 $\mu m^3/\mu m$	0.1 cm^3/cm

cell layer, in contact with only the outermost axons of the group. Each such Schwann cell domain may be further subdivided by occasional folds of Schwann cell cytoplasm insinuated among the fibers. Schwann cell domains are separated from one another by fibrils of collagen. Compared with other neural tissue, the olfactory epithelium, where axons of the olfactory nerve arise from olfactory cell bodies, is in a particularly vulnerable situation, easily accessible to infection and to destruction by toxic substances present in polluted waters. Empty areas such as are seen in Fig. 1B perhaps reflect axon degeneration associated with destruction of olfactory neuron soma, rather than fixation artifact, for such areas are more common in older specimens.

The total cross-sectional area of the small filament of nerve containing the four Schwann domains shown in Fig. 1B is about 188 μm^2; the distribution of fiber diameters for the 1577 fibers in this filament is shown in Fig. 2A. Occasionally, one may find fiber cross sections up to 1 μm in diameter. Longitudinal sections appear to show that many, if not all, of such large fibers are distended zones of the ordinary 0.2 to 0.3 μm fibers. Inasmuch as these zones, several micrometers in length, are seen in nerves prepared by perfusion of the animal's circulation with fixative, they are probably not artifacts. They may be localized swellings resulting from topical osmotic differences, or frozen microperistaltic waves, like those said to occur in myelinated fibers (*13*). The total cross-sectional area of the entire olfactory nerve shown in Fig. 2A is about 1.54 mm^2. Assuming that the fiber density in Fig. 1A is the same as that in Fig. 1B, I estimate there are about 1.3×10^7 fibers in the entire nerve cross section in Fig. 1A.

The relative amounts of membrane and the volumes of the axons were estimated from measurements of the greater and lesser axes of each axon cross section. For each axon, the average of these two measurements was taken as the diameter of a circle. The cross-sectional areas and the circumferences of the axons were calculated from these data and were suitably summed to provide the cumulative volume and membrane surface area per unit length of nerve. The areas of Schwann cell cross sections were determined by planimeter, as was the total cross-sectional area of each Schwann cell domain, including the axons. The total amount of Schwann cell membrane was determined with the help of a wheel for measuring map distances. Measurements from the filament illustrated in Fig. 1A are compiled in Table 1.

The fibers of the olfactory nerve show an astonishing uniformity and small size in several species, and they are very numerous compared with the fibers of any other nerves about which information is available. In the pike, there are about 6.6×10^6 fibers in each olfactory nerve near its exit from the epithelium (*4*). In the burbot (*Lota lota*) there are reported to be 36 fibers per square micrometer, with a mean diameter of 0.11 μm, and 10^7 fibers per nerve (*14*). When the different situations, the different relative amounts of connective tissue, and other factors likely to affect the estimates are considered, comparisons with my figure of 1.3×10^7 per nerve are not unfavorable.

There are about 10^5 nonmyelinated C fibers in the rabbit vagus nerve which also has, however, about 3000 myelinated fibers. The distribution curve of the nonmyelinated fibers of the vagus shows an average diameter of 0.75 μm, three times the diameter of the fibers of the olfactory nerve, which has no myelinated fibers.

Distribution curves for the vagus and the olfactory nerve fibers have been overlaid in Fig. 2B. Fiber groups of the olfactory nerve shown in Fig. 2A have been recast into diameter increments of 0.05 μm for a total of 400 fibers to correspond to the distribution

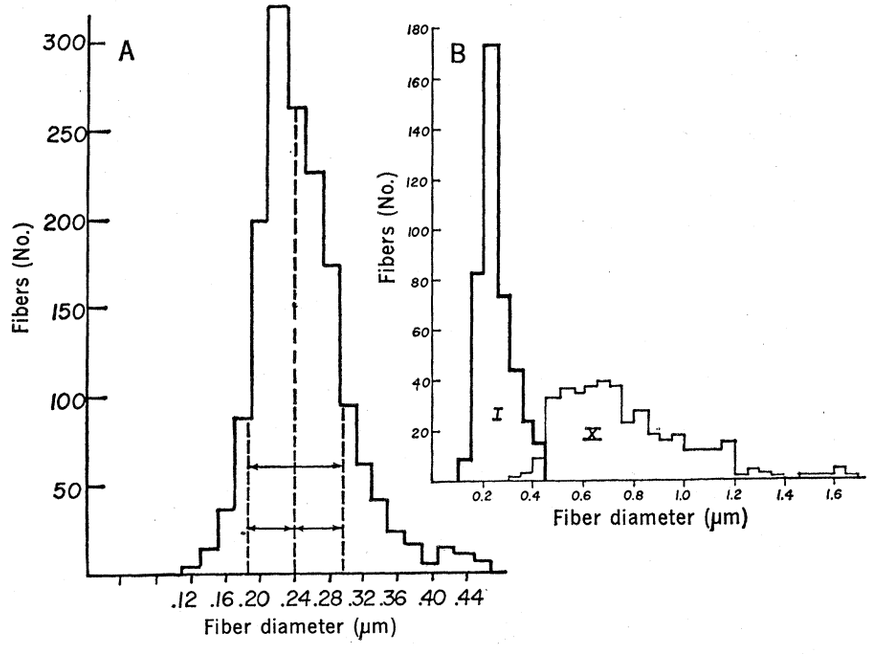

Fig. 2. (A) Distribution curve of fiber diameters in the olfactory nerve of *Lepisosteus osseus*. The olfactory nerve axons of the four domains of Fig. 1B were measured, and the distribution of diameters of fibers in each domain was plotted. The four domains contain a total of 1577 fibers in 188 μm^2; 79 percent of the fibers have diameters falling within 1 standard deviation of the mean (indicated by the arrows on either side of the median dashed line). (B) The distribution of fiber diameters in the garfish olfactory nerve compared with a corresponding distribution for the nonmyelinated fibers of the rabbit vagus nerve. (*I*) Data of Fig. 2A are replotted for 400 fibers and recast in incremental classes of 0.05 μm (as in *X*). (*X*) Distribution of diameters of 401 C fibers of the rabbit vagus nerve (*2*).

curve for the rabbit vagus reported by Keynes and Ritchie (*2*). Nonmyelinated nerve fibers that arise in the dorsal root ganglia and travel in the saphenous nerve of the cat have a distribution of diameters essentially the same as that for the vagus (*15*).

The small filament shown in Fig. 1B, occupied by 1577 axons, together with Schwann cells, empty spaces, and collagen, is equivalent in cross-sectional area to one large myelinated fiber about 15 μm in diameter. Assuming, in such a fiber, 100 Schwann cell layers totaling 1 μm thick, we may compare the two kinds of fibers. In the olfactory nerve, the relative amount of axon surface is more than three times the Schwann cell surface, whereas in the myelinated fibers, conversely, the area of Schwann cell membranes is at least 100 times the axon surface, but axon volume is five times the Schwann cell volume. The ratio of axon membrane surface to Schwann cell membrane surface is over 400 times as great in the olfactory nerve filament as it is in the myelinated fiber. The axons provide 80 percent of the cell membranes in the olfactory nerve, but only 1 percent in the hypothetical A fiber chosen as an example. The ratio of surface to volume for the Schwann cells is nearly ten times as great in the myelinated fiber as in the olfactory nerve filament, but the corresponding ratio for the axons is 50 times greater, in favor of the nonmyelinated filament. The entire olfactory nerve has about the same cross-sectional area (about 1.54 mm^2, Fig. 1A) as a squid giant axon. The ratio of axon membrane to axon volume in the 10^7 fibers of the garfish olfactory nerve is about 5400 times as great as that in the single giant axon.

Relationships of this sort may be helpful in allocating to specific compartments substances determined to be present in nerve. Of particular interest may be the comparison of materials likely to be found mainly in axon membrane where they may relate to the process of impulse conduction, in contrast to Schwann cell membrane where they will have less direct relevance to the molecular mechanism of nerve excitation. For some problems in axonology, therefore, the olfactory nerve of the garfish may be even more useful than the giant axon of the squid. The elucidation of metabolic phenomena and their correlation with electrophysiological events may perhaps be made more easily with the help of this large nerve, composed of a homogeneous population of small fibers having slow conduction velocity, short-length impulses, and extensive surface relative to volume.

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10. The nerve functioned satisfactorily for several hours in a physiological solution containing in final concentration: 125 m*M* NaCl, 3.5 m*M* KCl, 3.5 m*M* $CaCl_2$. Survival during stimulation for a day or more was favored by addition of sucrose (60 mmole/liter) to increase osmotic pressure and prevent swelling during prolonged experiments, of glucose (24 mmole/liter) as energy source, of phosphate buffer (1 mmole/liter), and, when 95 percent O_2 and 5 percent CO_2 was used as the gas phase, of $NaHCO_3$ (10 mmole/liter).
11. Because the perfusion fluid was delivered by way of the circulation, diffusion time was greatly reduced, and presumably fixation artifacts were minimized. See (*6*) for further description of the fixation procedure and a photomicrograph showing individual axons of the garfish olfactory nerve.
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DDE Residues and Eggshell Changes in Alaskan Falcons and Hawks

Abstract. *Eggshell thickness after exposure to DDT was reduced by 21.7 percent in Alaskan tundra peregrines, by 16.8 percent in taiga peregrines, by 7.5 percent in Aleutian peregrines, by 3.3 percent in rough-legged hawks, and not at all in gyrfalcons. Tundra peregrine eggs contain an average of 889 parts of DDE per million (lipid basis); taiga peregrine eggs contain 673 parts per million; Aleutian peregrine eggs contain 167 parts per million; rough-legged hawk eggs contain 22.5 parts per million; and gyrfalcon eggs contain 3.88 parts per million. These changes in eggshell thickness and the pesticide residues reflect different degrees of exposure to contamination. There is a highly significant negative correlation between shell thickness and DDE content in peregrine eggs. Tundra and taiga peregrines have fledged progressively fewer young each year since 1966.*

During 1967 to 1970 we studied organochlorine contamination in Alaskan breeding populations of raptors, especially the peregrine falcon *Falco peregrinus*, which has experienced serious population declines on two continents since the late 1940's (*1*). Work along the Yukon River in 1966 revealed that the peregrines there were already heavily contaminated with organochlorine residues, mostly 2,2-bis(*p*-chlorophenyl) 1,1-dichloroethylene (DDE), although the number of breeding pairs and reproductive output remained within historically predictable limits (*2*).

In 1967, Ratcliffe demonstrated a close correspondence in time and space between sudden, dramatic changes in eggshell weight and exposure to persistent organochlorine pesticides in three declining species of British raptors (*3*). The DDE has become increasingly implicated as the main organochlorine compound causing shell thinning and population decline (*4, 5*), but the polychlorinated biphenyls may also be involved (*6*).

We now report data on eggs from three geographic populations of *Falco peregrinus*: (i) on Amchitka Island in the Aleutians, (ii) along the Yukon River in the interior taiga, and (iii) along the Colville River in the foothill tundra of the Arctic Slope. For com-