ABSTRACT  Slow flow was followed in unmyelinated olfactory axons, severed from their cell bodies, at 14°C, 21°C, and 31°C. Slow flow does not stop after axotomy but rather accelerates to a value 3.3 times faster than the rates measured in an intact nerve. These velocities are equivalent to the rates of slow flow characteristic of regenerating fibers. The injury appears to have an influence on the contralateral intact nerve, where slow flow velocity increases to severed nerve values for several days before reverting to intact nerve rates. It can be hypothesized that the increase in the rate of slow flow is triggered by a factor repressed in intact nerve but released into the blood stream following injury.

In this study, slow flow was investigated in the garfish olfactory nerve after removal of the mucosa containing the nerve cell bodies. The survival characteristics of the unmyelinated fibers make this preparation well suited for this type of investigation. In the garfish olfactory nerves at 21°C, <50% of the axons have totally degenerated 3 wk after the mucosa has been severed (6). Matsumoto and Scalia (1) have reported that at 20°C, 12% of the unmyelinated fibers of the frog optic nerve are still intact 10 wk after injury. Both studies indicate also that degeneration is initially more prominent in the proximal than in the distal area.

RESULTS
Electron micrographs taken 19 d after severance of the cell bodies indicate that the axons have totally disappeared from the proximal area of the nerve (Fig. 2a). In the distal part of the nerve, however, >50% of the original axonal population is still present and these axons are morphologically similar to intact fibers (Fig. 2b).

The nature of the labeled material present in the moving peak was assessed by gel electrophoresis 15 d after removal of the right mucosa. In both, the degenerating and the intact

MATERIALS AND METHODS
The experiments were performed at 14°C, 21°C, and 31°C. Garfish (Lepisosteus osseus) weighing 3-4 kg were kept in 1500-l tanks maintained at constant temperature (±0.1°C). The fish were acclimated to the temperature under investigation for 10 d before the beginning of the experiments. They were anesthetized with 140 mg/l of MS 222 (ethyl m-aminobenzoate methane sulfonate). Crescent Research Chemicals, Scottsdale, AZ). 600 μCi of [4,5-3H] L-leucine, specific activity 30-50 Ci/mrnole (ICN Radiochemicals, Irvine, CA) were concentrated to a final volume of 3-10 μl and applied bilaterally to the olfactory cavities as explained elsewhere (7).
contralateral nerve, the polypeptide distribution of slowly moving radioactivity (Fig. 3) was very similar to that determined previously in olfactory nerves of non-injured fish (8).

The size of the slow wave decreases very rapidly, and after about 30 d at 21°C and 17 at 31°C the moving peak can no longer be located. At 14°C the wave was not followed long enough to see it disappear completely. Representative profiles obtained at 21°C are presented in Fig. 4. They indicate that in detached axons large amounts of radioactivity remain behind the moving peak. It is only at 26 d that the labeling in the proximal segment of the nerve stump shows a significant decrease. From the distance-time functions (Fig. 5), slow flow rates of 1.6 ± 0.2 mm/d, 5.1 ± 0.5 mm/d, and 10.6 ± 0.7 mm/d were measured in isolated stumps at 14°C, 21°C, and 31°C, respectively. Because these functions can be extrapolated to zero, there is no significant period during which slow flow stops after removal of the perikarya.

As determined previously for slow flow in intact nerve (7), the rates increase linearly with temperature (Fig. 6) and the increase can be expressed by a slope of 0.53 ± 0.06 mm/d/°C. These accelerated velocities are identical to the rates of slow flow measured in axons regenerating after the olfactory nerve is crushed at 1.5 cm from the mucosa (10). In both cases, accelerated velocities are 3.3 times faster than the rates of slow flow characteristic of intact nerves.

Slow flow was also followed in the intact nerve contralateral to the isolated stump (Fig. 4). For 5–15 d after injury, depending on the temperature (Fig. 7), the rates of slow flow in the intact nerve are equivalent to the accelerated velocities measured in degenerated axons. Afterwards, the velocities decrease to 0.6

**Figure 1** Schematic of mucosa removal (see Materials and Methods). Once a peak of slowly transported radioactivity was established in both nerves, the right half of the tip of the rostrum containing the right olfactory mucosa was severed.

**Figure 2** Transmission electron micrographs of a degenerating olfactory nerve 19 d after severance of the cell bodies: (a) section taken 2 cm from the site of injury, no intact axons remain (× 6,200); (b) section taken 20 cm from the site of injury, a large number of apparently intact fibers have survived (× 13,900). Bars, 1 μm.
FIGURE 4 Peaks of slowly transported labeled proteins at 21°C at various times after removal of the right mucosa. Slow flow in isolated distal stumps (---). Slow flow in contralateral intact nerve (---). Slow flow in intact nerves (---).

FIGURE 5 Transport velocity of the leading edge of the peak front in detached nerves at 14°C, 21°C, and 31°C.

FIGURE 6 Velocity-temperature functions of the leading edge of the peak front. In isolated nerve stumps (O). In intact nerves from intact animals (Δ), values for 14°C, and 21°C have been published previously (7).

FIGURE 7 Transport velocities of the leading edge of the peak front in intact contralateral nerves at 14°C, 21°C, and 31°C.

DISCUSSION

A persistence of slow flow in the goldfish visual system for at least one day after injury has been reported by Alpert et al. (5). Because the goldfish optic nerve is very short, it is possible that the early degeneration, which has been reported to occur in the preterminal nerve area (for review see reference 11), invades a sufficiently large proportion of the optic system to impair the slow flow mechanism in most fibers within 24 h. On the contrary, Frizell et al. (4) have shown that in the rabbit vagus nerve a phase of transported molecules moving at 25 mm/d stops immediately after axotomy. However, this velocity is so high that it might be difficult to unequivocally associate this material with slow flow.

Studies on the frog optic system (1) or on the garfish olfactory nerve (6) have revealed that in unmyelinated nerves the isolated distal stump does not degenerate simultaneously over its entire length after being severed from the cell bodies. Degeneration progresses proximodistally, starting at the site of injury and spreading toward the synaptic area. As revealed by electron microscopy, a large proportion of the axons remain intact in the distal segment of the nerve for an extended period of time (Fig. 2b). Masumoto and Scalia (1) have shown that in the frog, at 20°C, such distal segments of unmyelinated optic nerve axons are able to rapidly transport horseradish peroxidase up to 69 d after removal of the retina. The present study has revealed that in the goldfish olfactory nerve slow flow persists for up to 30 d after the cell bodies are severed, at ambient temperature (21°C). This peak of slow-moving radioactivity is not a simple artifact induced by degeneration because its polypeptide composition is very similar to the composition of slow flow characterized previously in intact nerves (8). The morphology of surviving isolated axons does not appear to be affected by the absence of cell bodies; it is therefore not surprising that the composition of the slow-moving material remains constant. Schlaepfer and Micko (12) have shown that in the transected rat sciatic nerve when >90% of the fibers have a degenerated axoplasm, changes in the polypeptide composition of the nerve are characterized by a decrease of only three polypeptides associated with microfilaments. The continued delivery of slow-moving material to the distal segment of the nerve is probably responsible for the long survival of the unmyelinated fibers. Conversely, the rapid disappearance of the axons in the proximal nerve area may be induced by a lack of slow-moving components, because new molecules are no longer delivered.
Not only does slow flow not stop in the isolated distal stump but, on the contrary, it accelerates to a value that we have seen previously to be characteristic of regenerating nerves (10). Degenerating and regenerating axons probably exhibit extremely different metabolic properties. One similarity is that, in both cases, the continuity of the neuron from the cell body to the synapses is no longer preserved. It can be hypothesized that the interruption of the neuronal continuity induces an increase in the rate of slow flow.

However, accelerated rates were also measured for several days in the intact contralateral nerve before they reverted to values characteristic of intact nerve. This appears to contradict the hypothesis that the accelerated rate is specific for interrupted neurones. It is possible that a factor maintaining slow flow at an accelerated velocity is repressed in intact neurones. We have seen previously (9) that the rate of regeneration of a discrete population of growing axons decreases at a time corresponding to the arrival of a faster-growing population of fibers in the synaptic area. It is not known, however, whether this phenomenon is linked to a decrease in transport velocity. The release of such a factor into the blood stream immediately after injury might be responsible for the transient increase in the slow flow rate in the intact contralateral nerve. This phenomenon may represent a general response of the nervous system to an injury. An increase in the rate of fast transport in intact goldfish optic nerves has been reported by McQuarrie and Grafstein (13) not only after a lesion of the contralateral nerve but also as a result of the removal of the cerebral hemispheres.

The profiles determined by the distribution of slowly transported radioactivity along the separated axons indicate that a large amount of labeled material remains in the proximal area of the nerve, whereas the size of the peak decreases very rapidly. It is not clear whether the disappearance of the peak is due to a loss of radioactivity through deposition or to a failure of the transport mechanism in an increasing number of detached fibers. Analysis of the polypeptide composition of the labeled material in the proximal area of the nerve as well as a careful determination of the decrease in the number of fibers with time should allow us to clarify this point.

The radioactivity accumulated behind the moving peak remains high for a long time, even after the complete disappearance of the axons from that area of the nerve (Fig. 2a). The nature of this material has not been determined. Schlaepfer and Micko (12) have shown that extensive chemical changes accompany the structural transformation of the transected rat sciatic nerve, reflecting mainly the proliferation of the Schwann cells and the deposition of collagen. It is only after about 26 d that the proximal radioactivity decreases significantly (Fig. 2). Studies on degeneration of unmyelinated axons have shown that axonal debris is ultimately removed by macrophages and Schwann cells (14). It can be expected that a large number of labeled axonal molecules are reutilized in situ by other nonneuronal cells after the elimination of the axons.

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