tein from more than one virus (*3*). Another distinctive feature of this system is that mixing has been detected only in one direction.

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Metabolic Dependence of Fast Axoplasmic Transport in Nerve

Abstract. *Fast axoplasmic transport, shown in cat sciatic nerves by a crest of labeled activity after injection of the L7 ganglion with [³H]-leucine or [³H]-lysine, was stopped within 15 minutes after death of the animals by bleeding. If the sciatic nerves were removed from the animals and placed in a chamber supplied with oxygen at 38 degrees centigrade, fast transport was sustained. Transport was rapidly blocked in similar in vitro preparations when the nerves were kept in a nitrogen environment. Fast axoplasmic transport is closely dependent upon oxidative metabolism.*

A fast transport system moving materials outward in nerve fibers at the rate of 410 mm/day was shown by the distal displacement of a crest of labeled activity in cat sciatic nerves after injection of the lumbar seventh (L7) ganglia with the precursor [³H]-leucine (*1*). In a more extensive series of experiments the rate was 401 ± 35 mm/day (*2*). The cell bodies are likely to be the major if not the sole, site of incorporation of precursor into labeled soluble proteins, polypeptides, and small particulates carried down inside the nerve fibers (*2, 3*).

Transport is not brought about by an active force exerted from the cell bodies or by diffusion from the somas. This was shown by eliminating the contribution of the cell bodies either by a ligature placed just distal to the ganglia or by its destruction after the ganglia were injected with [³H]-leucine. In such experiments a peak of labeled materials moved down the nerve fibers at the same rate as that present in control nerves (*2*).

If, as indicated by such results, fast axoplasmic transport is an active process present locally in the fibers, we would expect it to depend on metabolism. A similar consideration would apply to the implication of neurofilaments or neurotubules, or both, in axonal transport (*4*). To investigate this possibility the L7 ganglia on each side of a series of four animals were injected with 5 to 10 μl of either [³H]-leucine or [³H]-lysine in saline solution at a concentration of 5 mc/ml (*1, 2*). The animals were subsequently killed. The nerve, ganglion, and dorsal root of one side was removed for determination of the distribution of activity. The nerve on the opposite side was left *in situ* in the dead animal

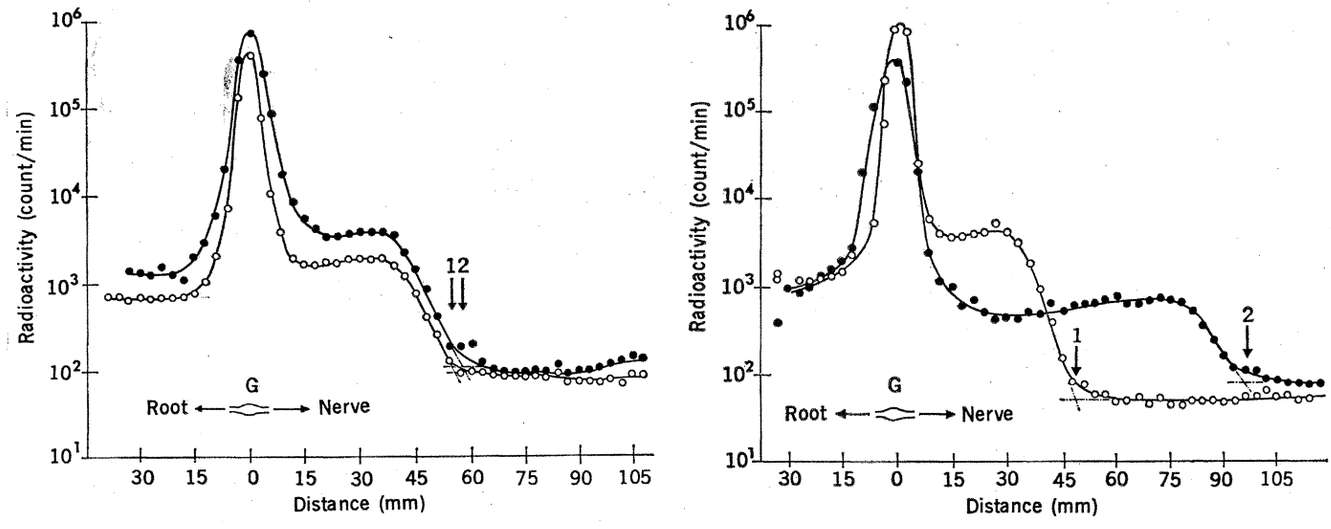

Fig. 1 (left). The two L7 ganglia were each injected with 7 μl of [³H]-leucine in saline solution (5 mc/ml). Three hours later the sciatic nerve, ganglion and dorsal root, from the left (○) side was removed from the animal and sectioned into 3-mm segments at the points represented along the abscissa. The radioactivity (counts/min) present in these segments is given by the log scale along the ordinate. The extent of transport is determined by the intersection of the slope of the forward edge of the crest with the base-line activity, shown at the foot of the crest by the dashed lines and arrow 1. The nerve on the right side (●) was left in situ in the dead animal for an additional 3 hours, and the small additional extent of the crest movement is shown by arrow 2. Fig. 2 (right). The L7 ganglia were injected with [³H]-lysine and the animal was killed 3 hours later. The control nerve, L7 ganglion and dorsal root, removed from the right side (○) shows a crest with the distal displacement shown by arrow 1 as expected of a fast transport lasting 3 hours. The opposite nerve (●) was placed in a chamber containing oxygen and kept moist with Ringer solution at 38°C for an additional 3 hours. The displacement of the crest in this preparation shown by arrow 2 is in accord with a fast axoplasmic transport for a period of 6 hours.

which was kept at 38°C for an additional period before it was similarly removed and we determined the distribution of activity in it.

In the example of Fig. 1, 3 hours were allowed after injection of the ganglion before the animal was killed and the control sciatic nerve was removed. The rate of transport was 424 mm/day. This falls within the expected rate distribution of 401 ± 35 mm/day. The nerve which had been left *in situ* in the dead animal for an additional 3 hours did not show much more of a distal displacement of the crest position. The displacement could represent no more than approximately 15 minutes more of downflow. Perhaps it could be accounted for by a variation in the estimation of the rate of transport. The shape of the crest was not altered to any great extent during the additional 3 hours except possibly for the foot of the crest. This finding further removes diffusion as a major factor in transport.

These experiments suggested that the loss of oxygen carried by the blood could have stopped fast axoplasmic transport. This was shown to be the case in a group of six experiments in vitro in which the L7 ganglia on both sides were injected with either [^{3}H]-leucine or [^{3}H]-lycine and the sciatic nerve on one side was removed as a control. After 3 hours the nerve from the opposite side was removed and placed in a chamber filled with oxygen. The distribution of activity in the control nerve (Fig. 2) moved 49 mm from the ganglion giving a rate of fast transport of 392 mm/day. This is within the range expected for a 3-hour downflow. The nerve placed in the chamber containing oxygen and kept moist with a lactate-Ringer solution at 38°C for an additional 3 hours showed continued fast transport in this in vitro preparation by the distal displacement of the crest. Activity had moved 96 mm from the ganglion giving a rate of fast transport for the total time of 6 hours of 384 mm/day in the oxygen-treated nerve. This rate falls within the range expected of a normal fast transport.

In other such experiments it was shown that the somas were not required for transport in vitro. Fast transport was maintained after the somas had been excluded by cutting the nerves just distal to the ganglion prior to placing them in the chamber.

In five similar experiments in vitro, nerves removed from the animals 3 hours after ganglion injection were exposed to nitrogen in the chamber for an additional 3 hours. In such cases there was no evidence of a further movement of the crest of activity beyond that seen in the initial 3-hour period. The two distributions of activity were particularly close, which shows that the failure of fast transport occurred well within 15 minutes in the anoxic nerves.

Thus, the mechanism underlying fast axoplasmic transport is closely dependent on oxidative metabolism. Mammalian nerves lose their excitability within 10 to 35 minutes after an interruption of their oxygen supply (*5*). This is the case also for single mammalian nerve fibers (*6*). The similarity of the two failure times suggests the exhaustion of a common metabolic pool supporting both the fast transport system and the mechanisms underlying excitability of the nerve membrane during anoxia. Alternatively, the metabolically driven fast transport system could maintain excitability by supplying some essential materials to the nerve membrane.

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Nucleotide Sequence of an RNA from Cells Infected with Adenovirus 2

Abstract. *The principal nucleotide sequence of an RNA in KB cells infected with adenovirus 2 has been determined. As isolated, the molecule shows some terminal and internal heterogeneity. The sequence permits extensive base pairing and contains prominent repeating sequences. A portion of the sequence resembles a sequence found in several transfer RNA's.*

After being infected with adenovirus 2, KB cells synthesize and accumulate in their cytoplasm a stable, low-molecular-weight RNA (*1*) species (VA RNA) whose primary structure is different from that of the principal RNA species found in the cytoplasm of uninfected cells (*2*). We have determined the nucleotide sequence of this RNA (Fig. 1). We prepared P^{32}-labeled VA RNA from infected cells and purified it by electrophoresis on an acrylamide gel. The methods for sequence determination have been described (*3*, *4*). In particular we obtained useful, large fragments by digestion of the RNA with ribonuclease T1 (1 unit per milligram of RNA) and by fractionation of the products on heated DEAE cellulose and DEAE Sephadex columns. Purified fragments were then extensively digested with ribonuclease T1, spleen acid ribonuclease (*5*), and pancreatic ribonuclease, and the products were fractionated and analyzed by two-dimensional electrophoresis (*4*).

The analyses were complicated by the existence of several points of heterogeneity in the molecule. Thus a portion of the molecule lacked one pU at the 3′-OH end. This type of hetero-

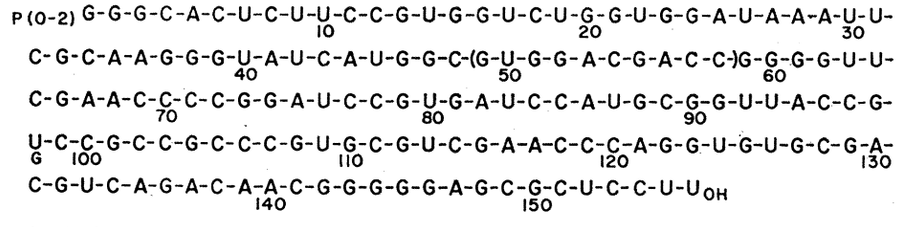

Fig. 1. A principal nucleotide sequence of adenovirus 2 VA RNA. Parentheses indicate a region of the molecule in which alternative sequences may also occur.