INTRODUCTION

The neuron is a specialized cell, which has one or more long processes. One of these, the axon, may assume an impressive length (up to 1 m) relative to the perikaryon (~100 μm). The perikaryon is the metabolic center of the cell, where macromolecules and organelles are manufactured, products that are exported into the axon, to undergo axonal transport towards the nerve ending. Retrograde axonal transport also occurs, and this bidirectional phenomenon, intra-axonal transport, is of vital importance for the function of the neuron (cf. Grafstein and Forman 1980, Dahlström 1983). It has therefore been the subject of a number of investigations that have dealt with e.g. the influence of various experimental procedures on amount of transported material or rate of transport.

If an endogenous substance is to be studied, a technique must be applied which results in arrest of axonal transport, e.g. a cold block or ligature. The transported organelles then accumulate on either side of the crush region in a manner depending on amount, rate and direction of transport.

Using microscopical techniques the number of axons with accumulated material, the distance the accumulations reach from the crush as well as the character of the axon type involved can be registered. However, in order to obtain quantitative data biochemical estimation methods must be employed, using homogenates of nerve.

The situation is examplified in Fig. 1, symbolizing two consecutive sections from a ligated peripheral nerve, treated for indirect immunofluorescence. One section was incubated with an antiserum to cholinergic vesicles, and the immunoreactive material has accumulated in thick myelinated axons reaching a
considerable distance (5 mm) on either side of the crush region. The lower section was incubated with an antiserum to substance P (SP), and very thin axons with material accumulated over 0.5-1 mm proximal to the crush are demonstrated. This can be clearly discerned in the fluorescence microscope. However, in order to get a sufficiently good quantitative resolution of these two accumulation patterns, the nerve would need to be chopped up in very short segments before assay, and probably many nerves would have to be pooled.

We have therefore developed a method, based on morphological techniques, which can supply quantitative data as well as morphological details.

METHODOLOGY

Equipment

The technique is in its present form based on fluorescence microscopy. Any substance that is fluorescent, or can be made to fluoresce, can be studied. With the modern immunofluorescence technique any substance that can be identified by appropriate antisera can thus be measured.

The basic instrument in our equipment is a Leitz MPV II fluorescence microscope with incident light, equipped with a scanning system (Fig. 2). The fluorescent light passes via a narrow slit (measuring diaphragm) into a PM-tube, and the emitted light is amplified in a Leitz amplifier and fed into a recorder with integrator (see Fig. 3). The recorded curve shows the

Fig. 1. Schematic illustration of the different patterns of accumulation between cholinergic vesicle-like material in thick myelinated axons (top) and SP-like material in thin axons (bottom) as viewed in a fluorescence microscope. As indicated by the hatched lines 0.5 mm sections would have to be assayed to give accumulation profiles with sufficient resolution.