CONTRIBUTION OF AXONAL TRANSPORT TO THE RENEWAL OF MYELIN PHOSPHOLIPIDS IN PERIPHERAL NERVES. I. QUANTITATIVE RADIOAUTOGRAPHIC STUDY

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SUMMARY

Kinetics of phospholipid constituents transferred from the axon to the myelin sheath were studied in the oculomotor nerve (OMN) and the ciliary ganglion (CG) of chicken. Axons of the OMN were loaded with transported phospholipids after an intracerebral injection of [2-3H]glycerol or [3H]labeled choline. Quantitative electron microscope radioautography revealed that labeled lipids were transported in the axons mainly associated with the smooth endoplasmic reticulum. Simultaneously, the labeling of the myelin sheath was found in the Schmidt-Lanterman clefts and the inner myelin layers. The outer Schwann cell cytoplasm and the outer myelin layers contained some label with [methyl-3H]choline, but virtually none with [2-3H]glycerol. With time the radioactive lipids were redistributed throughout and along the whole myelin sheath.

Since [2-3H]glycerol incorporated into phospholipids is practically not re-utilized, the occurrence of label in myelin results from a translocation of entire phospholipid molecules and from their preferential insertion into Schmidt-Lanterman clefts. In this way, the axon–myelin transfer of phospholipid contributes rapidly to the renewal of a limited pool of phospholipids in the inner myelin layers.

When [methyl-3H]choline was used as precursor of phospholipids, the rapid appearance of the label in the inner myelin layers was interpreted also as an axon–myelin transfer of labeled phospholipids. However, the additional labeling of the outer Schwann cell cytoplasm adjacent to Schmidt-Lanterman clefts and of the outer myelin layers reflects a local re-incorporation of the base released from the axon.

By these two processes, the axon contributes to purvey the inner myelin layers with new phospholipids and the Schwann cells with new choline molecules.

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INTRODUCTION

The myelin sheath of peripheral nerves derives from the Schwann cells in which the plasma membrane is spirally rolled to form a compacted stack of lamellae. The role played by Schwann cells in the turnover of lipid and glycoprotein occurring in mature myelin sheaths has been clearly demonstrated by radioautography, but also to control maintenance of mature myelin sheath. After an axonal interruption, both axon and myelin sheath indeed degenerate in the distal segment separated from the nerve cell body. To account for the Wallerian degeneration process, Ramon y Cajal proposed the idea of a metabolic interaction between 'axon and myelin, which trophically depends on the nerve cell... This trophic action is perhaps exercised through some product of catabolism given out by the axon when the trophic current flows.

In this respect, axonal transport of radioactive phospholipids was found to give rise to a definite labeling of the myelin sheath in peripheral nerves. The appearance of radioactivity in the myelin sheath could be explained by a direct transfer of phospholipid from axon to myelin sheath and by a local incorporation of freed choline into myelin phospholipids. However, there was a need for further information on the kinetics of the labeled phospholipids in the substructures of the myelinated nerve fibres and on the classes of phospholipids which are axonally transported and eventually translocated to myelin.

Phospholipids were labeled with two different precursors: (1) [2-3H]glycerol, which is incorporated into newly formed phospholipids but scarcely recycled, and so allows to disclose the pathways followed by phospholipid molecules transferred from the axon to its myelin sheath; and (2) radioactive choline labeled with either 3H or 14C is also incorporated into phospholipids, but extensively recycled since labeled choline may be either re-utilized after lipolysis or exchanged with another base. Hence radioactive choline can visualize an axon–myelin passage of phospholipid as well as a local re-incorporation of the labeled base.

The choice of the oculomotor system was guided by the following advantages. Firstly, the injection into the cerebral aqueduct delivers the selected precursors in the immediate vicinity of the oculomotor neurons into which they are rapidly incorporated. Secondly, the transported labeled phospholipids reach the orbital portion of the oculomotor nerve (OMN) along which they are in transit and accumulate in the nerve endings of the ciliary ganglion (CG). Thirdly, comparison of radioautographic data in the right CG and of biochemical data in the left CG of the same chicken was expected to provide a deeper insight into the debated question of the contribution of axonal transport to myelin metabolism in peripheral nerves (Fig. 1).

MATERIALS AND METHODS

Labeled precursors

In a first series (Experiment I), two-week-old chickens (Leghorn, I.N.R.A., Nouzilly) were anesthetized with chloroform and held in a stereotaxic apparatus. Each
animal was injected in the cerebral aqueduct of the brain with 45 µl of saline containing 900 µCi of [2-3H]glycerol (spec. act. 6.6 Ci/mmol, N.E.N.) or [methyl-3H]choline (spec. act. 10.1 Ci/mmol, Radiochemical Center).

In a second series (Experiment II), 6 chickens were intracerebrally injected with 400 µCi of L-[1-3H]fucose (spec. act. 10.8 Ci/mmol, C.E.A., Saclay) or L-[4-3H]lysine (spec. act. 25 Ci/mmol, C.E.A., Saclay).

In a third series (Experiment III), ciliary ganglia were incubated in vitro in an oxygenated Tyrode solution at 38 °C with 200 µCi/ml of [2-3H]glycerol or [methyl-3H]choline for 15–60 min.

In a fourth series (Experiment IV), 900 µCi of myo-[2-3H]inositol (spec. act. 5.0 Ci/mmol, N.E.N.) was intracerebrally injected.

**Experiment I**

Three chickens were sacrificed at each time interval ranging from 45 min to 21 days after the intracerebral injection of [2-3H]glycerol or [methyl-3H]choline. The periaqueductal region of the midbrain and the left ciliary ganglion were excised to measure the radioactivity in water-soluble fractions and in the chloroform–methanol extract.
Radioactivity assays. Slices of the periaqueductal region weighing 5–10 mg and the left ciliary ganglion were separately homogenized in 1 ml of 0.05 M phosphate buffer (pH 7.3) before adding an equal volume of 20% TCA. After centrifugation at 4000 g, the supernatant was collected and radioactivity, measured in an Intertechnique scintillation counter, was referred to as radioactivity of the water-soluble fraction. The sediment was resuspended in 1 ml of chloroform–methanol (1:1), then centrifuged again and rinsed with chloroform–methanol. All the supernatants were pooled and the radioactivity was measured in glass scintillation vials. Efficiency was evaluated by spiking 5 vials of each solution and 2 background vials of each set. The amount of protein contained in the chloroform–methanol precipitate was determined. Light and electron microscope radioautography. The right ciliary ganglion was fixed by immersion for 1 h in 0.1 M phosphate buffer (pH 7.2) containing 3.6% glutaraldehyde and CaCl₂. After rinse in 0.1 M phosphate buffer and post-fixation in 2% OsO₄ for 2 h, the ganglia were dehydrated through acetone at −10 °C, transferred into a mixture of acetone and Epon (1:1) and directly embedded in Epon by omitting the propylene oxide step. Under these conditions, phospholipids labeled with radioactive choline or glycerol are safely preserved in embedded tissue at the same sites as in radioautographed sections obtained by cryo-ultramicrotomy. One-μm thick sections were prepared for light microscope radioautography. At each time interval, the grain density (number of silver grains per 100 sq. μm) was measured in 30–60 preganglionic axons (diameter > 8 μm), myelin sheaths and Schwann cell cytoplasmics by means of an ocular grid. Thin sections were prepared for electron microscope radioautography. The radioautographs were developed with phenidon after a 2-month exposure. In each examined ganglion a quantitative estimation of the label in the substructure was determined as follows: 700–1300 silver grains were counted and their distribution was analyzed in axon, myelin sheath and outer Schwann cell cytoplasmics at a uniform magnification of × 4000 by direct scoring. Each silver grain seen over a preganglionic axon was ascribed to the structure located underneath the center of the grain. Over the myelin sheath, the silver grains were ascribed to 3 concentric bands of 180 nm width, referred to an inner, medial and outer myelin layers. Each band was slightly greater than one ‘half-distance’ (HD). The HD corresponds to the radius of an area around a developed silver grain with a 50% probability of containing a radioactive source. All the grain count were expressed in per cent of label content in the preganglionic nerve fibres. The relative volume occupied by each substructure of the nerve fibres was measured by the hit point method. Finally the label concentration in each substructure at any time was calculated by combining the radioautographic data obtained from electron microscopy (per cent of label and relative volume of the substructure) and light microscopy (grain density). In Tables I–III, the relative label concentration corresponds to:

\[
\text{relative label concentration} = \frac{\text{per cent of label content} \times \text{grain density}}{\text{relative volume of the substructure}}
\]
Experiment II
The 3 chickens intracerebrally injected with L-[1-\(^{3}\)H]fucose were sacrificed 18 h later. The other 3 injected with L-[4-\(^{3}\)H]lysine were killed after 6 days. The CG were prepared for light and electron microscope radioautography.

Experiment III
The CG, which were incubated for 15–60 min with [2-\(^{3}\)H]glycerol or [methyl-\(^{3}\)H]choline, were fixed in glutaraldehyde containing an excess of non-radioactive glycerol or choline and prepared for light and electron microscope radioautography.

Experiment IV
A series of 3 chickens were intracerebrally injected with myo-[2-\(^{3}\)H]inositol and sacrificed at different time intervals between 45 min and 14 days. The midbrain and the CG were prepared for radioactive assays and radioautography according to the procedure of Experiment I. Electron microscope radioautographs were exposed for 8 months.

RESULTS

Experiment I
[2-\(^{3}\)H]glycerol. After the intracerebral injection of the tracer, the radioactivity in the water-soluble fraction, containing the labeled precursor and its metabolites, fell rapidly in the midbrain while the radioactivity in lipids showed a sudden rise followed by a slow decrease (Fig. 2). In the ciliary ganglion, the lipid extract was still devoid of label at 45 min; its radioactivity increased after a 3-h delay and reached a maximum around 40 h. During this period, the water-soluble radioactivity in the ciliary ganglion remained at a very low level (Fig. 2); hence the [2-\(^{3}\)H]glycerol-labeled lipids of the ciliary ganglion could hardly be contributed by local synthesis. This fact is corroborated by radioautography which reveals a low grain density over ganglion cell bodies and Schwann cells (Figs. 2 and 5b) though they are actively able to incorporate [2-\(^{3}\)H]glycerol into phospholipids (Fig. 15). In contrast, a high grain density was observed over the preganglionic axons and their caliciform nerve endings (Figs. 2, and 5a and b). In electron microscope radioautographs of preganglionic axons, the label was first concentrated in areas containing narrow membrane profiles of the smooth endoplasmic reticulum (SER), especially at the axonal periphery, then to a lesser extent, the axon–myelin interface (Fig. 7; Table I). By 72 h, a large part of the label was associated with mitochondria, which are known to contain a fair amount of diphosphatidylglycerol, and with membranous bodies. At any time, the radioactivity of the axoplasm was low (Table I).

The myelin sheath showed a gradual increase of label (Fig. 2). The silver grains predominated over the inner myelin leaflets (Figs. 6 and 7) and reached a maximal density by 40–72 h (Table I). Sections parallel to the longitudinal axis of the nerve fibres, showed an uneven distribution of silver grains along the myelin sheath. Between 6 and 40 h, many Schmidt-Lanterman clefts were indeed 5–8 times more labeled than
the rest of the myelin sheath (Figs. 5b and 7). At 3 and 7 days, the label was redistributed outwardly (Fig. 6; Table I). At that time, the paranodal loops of myelin, which were almost unlabeled at early time intervals, exhibited a slight increase of the grain density.

[Methyl-\(^3\)H]choline. The water-soluble radioactivity recorded in the midbrain after the intracerebral injection of the tracer dropped to a low value within 40 h (Fig. 3). After an early peak, the [methyl-\(^3\)H]choline-labeled lipids declined steadily, at a slower rate than the [\(^2\)H]glycerol-labeled lipids. In the ciliary ganglion, the radioactivity of lipid extracts started rising after 3 h and reached a maximum by 72 h. Meanwhile the water-soluble radioactivity showed a slight enhancement at 6–18 h before returning slowly to a lower level. Thus in contrast with [\(^2\)H]glycerol, the appearance and persistence of free labeled choline and metabolites make possible a local

Figs. 2 and 3. Time curves of radioactivity in the midbrain, ciliary ganglion and elements of the preganglionic nerve fibres after an intracerebral injection of [\(^2\)H]glycerol (Fig. 2) or [methyl-\(^2\)H]-choline (Fig. 3). dpm per mg of protein (in the midbrain) and dpm per ganglion (in the CG) refers to the radioactivity measured in chloroform–methanol extracts (●) and in water-soluble fractions (○). Grain density was recorded in elements of the preganglionic nerve fibers (axon, myelin sheath and outer Schwann cell cytoplasm) by counting silver grains per 100 sq. \(\mu\)m in light microscope radioautographs of the CG. Note the exceedingly low level of water-soluble radioactivity in the CG and the virtual lack of label in the Schwann cells after injection of [\(^2\)H]glycerol as compared with [methyl-\(^2\)H]choline.
### TABLE I

*Time sequence of relative label concentration measured in constituents of nerve fibres after the intracerebral injection of $[^2\text{H}]$glycerol*

The relative volume occupied by axonal substructures is: $83.9\% \pm 4.1$ for axoplasm, $2.2\% \pm 0.9$ for intra-axonal membranous profiles which include SER and membranous bodies, $8.5\% \pm 4.1$ for mitochondria and $5.3\% \pm 1.5$ for the axolemma. The axon–myelin includes the axolemma and the space between axolemma and inner plasma membrane of Schwann cells. The inner layers of the myelin include adaxonal Schwann cell cytoplasm, while the outer layers include junction between outer myelin membrane and Schwann cell cytoplasm.

<table>
<thead>
<tr>
<th></th>
<th>6 h</th>
<th>18 h</th>
<th>40 h</th>
<th>72 h</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Axon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>axoplasm</td>
<td>0.03 ± 0.02</td>
<td>0.09 ± 0.03</td>
<td>0.13 ± 0.03</td>
<td>0.47 ± 0.09</td>
<td>0.11 ± 0.04</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>intraaxonal membranous profiles</td>
<td>2.08 ± 1.73</td>
<td>4.65 ± 2.18</td>
<td>9.36 ± 4.02</td>
<td>12.51 ± 5.61</td>
<td>4.47 ± 2.44</td>
<td>0.71 ± 0.34</td>
</tr>
<tr>
<td>mitochondria</td>
<td>0.22 ± 0.19</td>
<td>0.46 ± 0.27</td>
<td>0.47 ± 0.28</td>
<td>4.21 ± 2.19</td>
<td>1.74 ± 1.04</td>
<td>0.49 ± 0.25</td>
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<tr>
<td>Axon–myelin interface</td>
<td>0.25 ± 0.17</td>
<td>0.90 ± 0.10</td>
<td>0.92 ± 0.30</td>
<td>4.32 ± 1.10</td>
<td>1.46 ± 0.25</td>
<td>0.83 ± 0.12</td>
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<td><strong>Myelin sheath</strong></td>
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<tr>
<td>inner layers</td>
<td>0.91 ± 0.18</td>
<td>2.83 ± 0.79</td>
<td>4.09 ± 0.95</td>
<td>3.63 ± 0.65</td>
<td>2.19 ± 0.37</td>
<td>0.54 ± 0.52</td>
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<tr>
<td>medial layers</td>
<td>0.40 ± 0.10</td>
<td>1.58 ± 0.47</td>
<td>2.26 ± 0.68</td>
<td>2.82 ± 0.52</td>
<td>1.87 ± 0.32</td>
<td>0.57 ± 0.54</td>
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<tr>
<td>outer layers</td>
<td>0.18 ± 0.05</td>
<td>1.36 ± 0.42</td>
<td>1.48 ± 0.49</td>
<td>1.51 ± 0.44</td>
<td>0.97 ± 0.21</td>
<td>0.51 ± 0.49</td>
</tr>
<tr>
<td>Outer Schwann cell cytoplasm</td>
<td>0.08 ± 0.02</td>
<td>0.18 ± 0.08</td>
<td>0.14 ± 0.05</td>
<td>0.13 ± 0.03</td>
<td>0.07 ± 0.03</td>
<td>0.03 ± 0.02</td>
</tr>
</tbody>
</table>

### TABLE II

*Time sequence of relative label concentration measured in constituents of nerve fibres after the intracerebral injection of $[^3\text{H}]$choline*

<table>
<thead>
<tr>
<th></th>
<th>6 h</th>
<th>18 h</th>
<th>40 h</th>
<th>72 h</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Axon</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>axoplasm</td>
<td>0.07 ± 0.02</td>
<td>0.27 ± 0.13</td>
<td>0.47 ± 0.22</td>
<td>0.57 ± 0.12</td>
<td>0.35 ± 0.23</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>intraaxonal membranous profiles</td>
<td>5.03 ± 2.07</td>
<td>11.85 ± 7.11</td>
<td>14.42 ± 5.05</td>
<td>17.18 ± 7.68</td>
<td>3.55 ± 2.87</td>
<td>1.76 ± 1.03</td>
</tr>
<tr>
<td>mitochondria</td>
<td>0.27 ± 0.15</td>
<td>1.31 ± 0.91</td>
<td>3.13 ± 2.62</td>
<td>3.68 ± 1.98</td>
<td>1.26 ± 1.06</td>
<td>0.25 ± 0.17</td>
</tr>
<tr>
<td>Axon–myelin interface</td>
<td>0.31 ± 0.15</td>
<td>1.57 ± 0.95</td>
<td>3.06 ± 1.70</td>
<td>6.46 ± 2.24</td>
<td>2.01 ± 1.45</td>
<td>0.59 ± 0.31</td>
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<tr>
<td><strong>Myelin sheath</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inner layers</td>
<td>3.38 ± 0.58</td>
<td>3.12 ± 0.61</td>
<td>3.24 ± 0.74</td>
<td>3.81 ± 0.99</td>
<td>4.40 ± 1.57</td>
<td>2.53 ± 1.21</td>
</tr>
<tr>
<td>medial layers</td>
<td>1.69 ± 0.34</td>
<td>3.69 ± 0.68</td>
<td>3.59 ± 0.81</td>
<td>8.31 ± 1.37</td>
<td>6.62 ± 2.32</td>
<td>3.84 ± 2.13</td>
</tr>
<tr>
<td>outer layers</td>
<td>0.34 ± 0.17</td>
<td>4.35 ± 0.67</td>
<td>5.38 ± 1.21</td>
<td>7.98 ± 1.54</td>
<td>4.24 ± 2.10</td>
<td>2.97 ± 1.67</td>
</tr>
<tr>
<td>Outer Schwann cell cytoplasm</td>
<td>0.90 ± 0.21</td>
<td>1.22 ± 0.41</td>
<td>3.25 ± 0.82</td>
<td>1.81 ± 0.68</td>
<td>0.62 ± 0.08</td>
<td>0.27 ± 0.11</td>
</tr>
</tbody>
</table>
Fig. 4. Time curves of radioactivity in the midbrain, ciliary ganglion and elements of the preganglionic nerve fibres after an intracerebral injection of myo-[2-3H]inositol (Experiment IV). Note the high level of water-soluble radioactivity in the midbrain and the CG as compared with the low proportion of precursor incorporated into lipids.

incorporation of the tracer which co-exists with the arrival of axonally transported [methyl-3H]choline-labeled lipids. Evidence that [methyl-3H]choline-labeled lipids of the ciliary ganglion could originate from both axonal transport and local biosynthesis was reinforced by examining the radioautographs. The preganglionic axons and their presynaptic endings were indeed progressively invaded by large amounts of labeled lipids after a 3-h delay (Figs. 3 and 8). In the preganglionic axons, the label concentration increased first in areas containing SER profiles, then later in the axon–myelin interfaces and mitochondria (Table II), as observed after injection of [2-3H]glycerol. However, distinct from [2-3H]glycerol labeling (Figs. 5–7), the [methyl-3H]choline-labeled phospholipids were also found in the outer Schwann cell cytoplasm, satellite cells and perikarya of the ganglion cells (Figs. 3, 8–12).

The myelin sheath of the preganglionic axons showed a gradual rise of label. At 6 h, the label concentration was the highest in the inner myelin layers, including the thin adaxonal cytoplasm of Schwann cells (Table II), and displayed an outward decreasing gradient similar to that observed with [2-3H]glycerol. Then a reversal of the
gradient occurred at 18–40 h simultaneously with the increasing labeling of the outer Schwann cell cytoplasm (Table II). Sections parallel to the long axis of the nerve fibres revealed also a heterogeneous distribution of the silver grains. Schmidt-Lanterman clefts were frequently signalled by clumps of grains located over the outermost layers of myelin and adjacent portions of the outer Schwann cell cytoplasm (Fig. 10). Between 3 and 14 days, the label concentration in the myelin sheath diminished less rapidly in medial rather than in inner and outer myelin leaflets. During this period, paranodal myelin loops displayed more frequent clusters of silver grains (Figs. 13 and 14) than at earlier time intervals. Finally, a definite reaction persisted over the myelin sheath after 3 weeks whereas the outer Schwann cell cytoplasm was almost depleted of label (Fig. 3).

**Experiment II**

By 18 h after the injection of L-[1-3H]fucose into the cerebral ventricle, fast transported radioactive glycoproteins were concentrated in the SER profiles and the axolemma of the preganglionic nerve fibres. The myelin sheath and the Schwann cell cytoplasm were practically free of label (Table III).

After 6 days, large amounts of slowly transported proteins labeled with L-[4-3H]-lysine were found in the axoplasm and the mitochondria, but not in the myelin sheath and the Schwann cells (Table III).

**Experiment III**

After a 15-min incubation with [2-3H]glycerol or [methyl-3H]choline, the radioautographic reaction was confined to a thin zone at the periphery of the CG. By 1 h, the reaction extended almost evenly throughout the CG with [2-3H]glycerol (Fig. 15) but displayed a clear gradient of radioactivity decreasing from the periphery to the core of the CG with [methyl-3H]choline (Fig. 16). With both precursors, the silver grains were mainly concentrated over ganglion cell bodies and glial cells. Incorporation of [methyl-3H]choline took place in the outer Schwann cell cytoplasm without preferential localization.

**Experiment IV**

After the intracerebral injection of myo-[2-3H]inositol, the water-soluble radioactivity reached a high level and persisted for several days in the midbrain. In spite of the high concentration of the free tracer, only a minute part was incorporated into cerebral lipids (Fig. 4). In the CG, the water-soluble radioactivity was 4–20 times as high as the lipid labeling, a fact never observed with the previous precursors. In radioautographs, the preganglionic axons and their terminals became gradually but weakly labeled. The ganglion cell bodies, the Schwann cells and the myelin sheaths were moderately radioactive; no characteristic labeling of the Schmidt-Lanterman clefts was noted.
TABLE III
Relative label concentration in components of nerve fibres after the intracerebral injection of L-[1-3H]-fucose or L-[4-3H]lysine

<table>
<thead>
<tr>
<th></th>
<th>L-[1-3H] fucose (18 h)</th>
<th>L-[4-3H] lysine (6 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>axoplasm</td>
<td>0.25 ± 0.15</td>
<td>2.60 ± 0.45</td>
</tr>
<tr>
<td>intraaxonal membranous profiles</td>
<td>4.45 ± 2.20</td>
<td>2.03 ± 1.15</td>
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<tr>
<td>mitochondria</td>
<td>0.61 ± 0.37</td>
<td>5.68 ± 1.68</td>
</tr>
<tr>
<td>Axon–myelin interface</td>
<td>3.47 ± 0.86</td>
<td>2.67 ± 1.56</td>
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<tr>
<td>Myelin sheath</td>
<td></td>
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<tr>
<td>inner layers</td>
<td>0.21 ± 0.15</td>
<td>0.22 ± 0.12</td>
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<tr>
<td>medial layers</td>
<td>0.07 ± 0.05</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td>outer layers</td>
<td>0.15 ± 0.09</td>
<td>0.57 ± 0.15</td>
</tr>
<tr>
<td>Outer Schwann cell cytoplasm</td>
<td>0.27 ± 0.08</td>
<td>0.45 ± 0.18</td>
</tr>
</tbody>
</table>

Figs. 5-7. Radioautographs of preganglionic nerve fibres after the intracerebral injection of [2-3H]-
glycerol. After 18 h, silver grains are seen over Schmidt-Lanterman clefts (SLC, ↓↑) of the myelin
sheath (My) in light (Fig. 5a) and electron microscope radioautographs (Fig. 7). In the axon (Ax), note
the close relationship between silver grains and profiles of the SER (→). By 3 days, numerous silver
grains are found over the myelin sheath of large (Fig. 5b) and thin (Fig. 6) nerve fibres. They are
mainly distributed over the inner layers of myelin (I = inner Schwann cell cytoplasm). In Fig. 5b,
note the accumulation of label in the presynaptic nerve ending (NE) which encompasses a
postsynaptic ganglion cell body (GC) almost devoid of label. Only stray silver grains are found over
Schwann and satellite cells at any time.
Figs. 8–14. Radioautographs of preganglionic nerve fibres, after the intracerebral injection of [methyl-\(^{3}H\)]choline. After 18 h, silver grains are found over the myelin sheath and the areas occupied by the outer Schwann cell cytoplasm (Fig. 8), frequently in the close vicinity of Schmidt-Lanterman clefts (Fig. 10, SLC). At 40 h (Fig. 11) and 3 days (Fig. 12), silver grains are redistributed radially throughout all the myelin layers and longitudinally to paranodal loops (Fig. 13) which are intensely labeled 7 days later (Fig. 14). By 10 days (Fig. 9), the labeling of the myelin sheaths persists at a high level whereas the grain density has declined over the areas occupied by strands of Schwann cell cytoplasm. In Figs. 8 and 12, note the density of label accumulated in presynaptic nerve endings (NE) and incorporated in postsynaptic ganglion cell bodies (GC) and satellite cells (Sat).
Figs. 15 and 16. Unstained radioautographs of CG incubated for 1 h with [2-3H]glycerol (Fig. 15) or with [methyl-3H]choline (Fig. 16) (Experiment III). From the periphery (upper part) toward the core of the CG (lower part), the decrease of the silver grain density reflects a lower diffusibility of [methyl-3H]choline than of [2-3H]glycerol. Note the intense incorporation of the precursors into ganglion cell bodies and Schwann cells.

DISCUSSION

The renewal of phospholipids in peripheral nerve fibres results from two distinct mechanisms: axonal transport\textsuperscript{1,10,14–16,28,30} and local synthesis of lipids\textsuperscript{11,14,15,18–20,25,27}.

Kinetics of the tracers and axonal transport

When myo-[2-3H]inositol is administered into the cerebral ventricle, the massive water-soluble radioactivity lasting in the midbrain constitutes a large pool for leakage of the tracer out of the brain and for diffusion along the OMN to the CG (Fig. 4). The high proportion of water-soluble radioactivity recovered in the CG suggests strongly an extra-axonal route. Such a contamination prevents us from considering myo-[2-3H]inositol as a suitable precursor to study the movement of axonally transported phospholipids in the OMN system.

The kinetics of the tracer is completely different after the intracerebral injections of [2-3H]glycerol or radioactive choline. The rapid fall of the free tracers and their extensive incorporation into cerebral lipids (Figs. 2 and 3) indeed restrict the possibility of contamination by endoneural diffusion of labeled precursors. The delayed and large amounts of radioactive lipids appearing in the CG, while minor amounts of free tracers are detected (Figs. 2 and 3), follow kinetics similar to that of axonally transported phospholipids which accumulate in a target organ\textsuperscript{13,22,23,44}. With both precursors, invasion of the axons and nerve endings with label (Figs. 5–12) demonstrates transport of phospholipids within the axons. Analysis of the label concentration in the axonal substructures (Tables I and II) permits to specify the existence of an early arrival (6–18 h) of radioactive phospholipids, mainly associated
with the SER, and of later movements (40–72 h), mainly associated with mitochondria and retrogradely transported membranous bodies. Outside the axons, [2-3H]-glycerol, which nevertheless possesses a high diffusibility along nerves and through the CG (Fig. 15), does not produce a significant contribution of label to the ganglion cell bodies and Schwann cells (Figs. 2 and 5–7). Radioactive choline, which diffuses with difficulty in peripheral nerves and through the CG (Fig. 16), shows an extra-axonal incorporation which takes place preferentially in the outer Schwann cell cytoplasm adjacent to the Schmidt-Lanterman clefts (Fig. 10). Since this peculiar pattern of label incorporation is not reproduced after an in vitro incubation (Experiment I) or an injection of radioactive choline into the endoneural space, it is suggested that, in the present study, free radioactive choline has followed an intra-axonal rather than an extra-axonal route. Intra-axonal movement of labeled choline or acetylcholine could contribute in part to the delivery of free tracers in this cholinergic system. However, a major source of axonal choline seems to be provided by local catabolism of axonally transported phospholipids from which the labeled base is released and re-utilized (see below).

Transcellular transfer of the tracers

A mechanism by which the neuron can contribute to the renewal of myelin phospholipids in peripheral nerves consists of providing choline or derivative for myelin phospholipids made in the Schwann cells. The initial incorporation of radioactive choline in the outer Schwann cell cytoplasm close to the Schmidt-Lanterman incisures (Fig. 10) suggests that the cytoplasmic channels managed by Schmidt-Lanterman clefts would facilitate diffusion of choline or metabolites from the axon to the outer Schwann cell cytoplasm. [Methyl-3H]choline-labeled phospholipids made in the Schwann cells are then translocated to outermost myelin layers (Fig. 11) and eventually redistributed inwardly throughout the myelin sheath (Fig. 12). The inward movement of choline-phosphoglycerides (CPG) is also accompanied by a longitudinal redistribution of phospholipids along the myelin sheath up to paranodal junctional complexes (Figs. 13 and 14).

Another mechanism by which the neuron participates in the metabolism of myelin consists in a passage of entire phospholipid molecules from the axon to the myelinating Schwann cells. Such an exchange of phospholipids occurs directly between contiguous cells in culture. In the course of transport of phospholipids labeled with [2-3H]glycerol, more than 60% of the [3H]-labeled lipids recovered in myelin fractions are represented by CPG. Furthermore, the appearance of the radioautographic reaction over the innermost myelin layers (Figs. 5–7 and Table I) visualizes the passage of the [3H]-labeled phospholipids from the axon to the adjacent myelin sheath. As shown by [3H]-labeled glycoproteins and proteins highly concentrated in the axolemmal region, cross-fired radiations emitted from the axonal periphery do not produce a significant labeling of the myelin sheath (Table III). Thus, resolution of electron microscope radioautography and preservation of lipids in situ allow us to conclude that the labeling of the myelin is due to [3H]-labeled phospholipids translocated from the axon to the inner myelin layers. Moreover, the
initial and prominent labeling of Schmidt-Lanterman clefs (Figs. 5b and 7) indicates that the insertion of translocated phospholipids takes place preferentially in these structures rather than through the paranodal junctional complexes. When CPG are labeled with radioactive choline, the axon–myelin passage of lipid molecules is hidden in part by the local synthesis of myelin phospholipids labeled with choline (Table II and Figs. 10–12).

Finally, translocated myelin phospholipids are eventually redistributed throughout and along the myelin sheath (Table I).

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