Reaction of Glial and Phagocytic Cells under Wallerian Degeneration in the Pyramidal Tract of the Rhesus Monkey

By

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Summary. The reaction of glial elements in the pyramidal tract of rhesus monkeys under Wallerian degeneration was investigated with special reference to the response of phagocytes. On the non-affected side of the medullary pyramid, the glial elements were separated into astrocytes, oligodendroglia and microglia on the basis of criteria applied to the glial elements of rhesus monkey optic nerve. On the affected side of the pyramid, astrocytes and oligodendroglia did not reveal any essential changes in structure of the cytoplasm and nucleus, or any phagocytic reaction. Phagocytes occurring during Wallerian degeneration exhibited a diphasic reaction for engulfing degenerated axons and myelin sheaths. The phagocytes which engulfed and digested degenerated myelin fragments most intensively, and transformed them into lipid droplets, appeared predominantly at 9 weeks after operation on the affected pyramid. This type of phagocytes was so similar to the microglia observed on the undisturbed side of the pyramid of the same monkey as regards the features of the cytoplasmic matrix and nucleus, that it was impossible to distinguish between them when the phagocytes displayed no phagocytic action. On the other hand, the phagocytes which appeared in the early stage to invade the degenerated myelin tubes, differed from microglia in the features of their nucleus and the appearance of their cell organelles. They phagocytosed degenerated axons and myelin lamellae peeled off from the inside. However, they did not break them down into lipid droplets. After predominantly active phagocytosis, they disappeared from the myelin tubes probably as a result of autolytic breakdown. This type of phagocytic reaction was somewhat similar to that of hematogeneous mononuclear cells, such as the dust cells of the lung.

Introduction

We have previously described the disintegration and disappearance of nerve fibers under Wallerian degeneration in the pyramidal tract of the rhesus monkey (Inoue et al., 1978). During the degeneration process, the phagocytes revealed a diphasic response. That is to say, phagocytes appearing at the earlier stage dur-
ing degeneration, invaded the thick degenerated myelinated nerve fibers to engulf parts of the axon, peel off the lamellae of the myelin sheath simultaneously from the inside and engulf them in large quantities. This type of phagocytes rapidly disappeared from the degenerated tract, chiefly by autolytic breakdown and partly via the blood vessels. The phagocytes showing the next active phagocytic response, appeared at about 9 weeks after operation to engulf massive degenerated myelin sheaths and to break them down into lipid droplets. This type of phagocytes became transformed into so-called 'lipid-containing cells', and gradually disappeared from the degenerated region.

Regarding the source of the phagocytes, as discussed in a previous paper, much controversy has arisen due to the differences in animal species used, tracts selected for observation, etc. (Inoue et al., 1978). The present study aimed to elucidate in detail the reaction of phagocytic cells and glial cells under Wallerian degeneration, using the medullary pyramid of rhesus monkeys disturbed by cerebral injury.

### Materials and Methods

Since the injured monkeys were the same as those used in a previous paper (Inoue et al., 1978), detailed descriptions are not given here. In the present study, the medullary pyramids of rhesus monkey, in which the upper portions of the pyramidal tract, such as the motor area of the cerebral cortex, the internal capsule or cerebral peduncle had been destroyed, were used. The survival periods after operation were 3, 7, 19 (12 days after a second operation), 25, 33, 43, 76, 117, 761 and 787 days, as shown in Table 1.

Briefly, the rhesus monkeys were sacrificed by perfusion with a mixed solution of 4% paraformaldehyde and 0.25% glutaraldehyde in Millonig's buffered solution (adjusted to pH 7.2). After removal of the brain, tissue blocks of the pyramid were post-fixed with 2% osmic acid in the same buffered solution, followed by the usual embedding procedure in Epon 812. Ultra-thin sections were

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Injured regions</th>
<th>Survival period after operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 56</td>
<td>Ablation of the left motor cortex of the upper limb region</td>
<td>7 days</td>
</tr>
<tr>
<td></td>
<td>Ablation of the right motor cortex of the upper limb region</td>
<td>3 days</td>
</tr>
<tr>
<td>No. 45</td>
<td>Left pedunculotomy</td>
<td>239 days</td>
</tr>
<tr>
<td></td>
<td>Ablation of the right motor cortex of the upper limb region</td>
<td>19 days</td>
</tr>
<tr>
<td></td>
<td>Right pedunculotomy</td>
<td>12 days</td>
</tr>
<tr>
<td>No. 22</td>
<td>Left pedunculotomy</td>
<td>25 days</td>
</tr>
<tr>
<td>No. 51</td>
<td>Injury of the left internal capsule</td>
<td>33 days</td>
</tr>
<tr>
<td>No. 21</td>
<td>Left pedunculotomy</td>
<td>43 days</td>
</tr>
<tr>
<td>No. 59</td>
<td>Injury of the left internal capsule</td>
<td>76 days</td>
</tr>
<tr>
<td>No. 58</td>
<td>Injury of the left internal capsule</td>
<td>117 days</td>
</tr>
<tr>
<td>No. 17</td>
<td>Ablation of the left motor cortex of the lower limb region</td>
<td>787 days</td>
</tr>
<tr>
<td></td>
<td>Ablation of the left motor cortex of the lower limb region</td>
<td>652 days</td>
</tr>
<tr>
<td>No. 18</td>
<td>Ablation of the left motor cortex of the upper limb region</td>
<td>761 days</td>
</tr>
<tr>
<td></td>
<td>Left pedunculotomy</td>
<td>645 days</td>
</tr>
</tbody>
</table>
double-stained with 2% aqueous uranyl acetate solution and Sato’s mixed lead solution. In addition, semi-thin sections of 1 μm in thickness were stained with toluidine blue or paraphenylene-diamine; these were used for counting the number of glial cells and estimating their population under a light microscope.

**Results**

1. **Glial cells in the undisturbed medullary pyramid**

Glial cells found in the undisturbed medullary pyramid were clearly separated into astrocytes, oligodendroglia and microglia (Fig. 1), on the basis of identification criteria previously applied to the glial cells of rhesus monkey optic nerve (Inoue et al., 1976).

   a) **Astrocytes**

   In the perikaryon and peripheral region of the soma of the astrocytes, glial filaments of about 7 μm in thickness which were characteristic of this type of cells, were found to be form bundles and to enclose groups of cell organelles (Figs. 1 and 2). The following formed conspicuous figures of the cell organelles: numerous mitochondria with a dark matrix; a well-developed Golgi complex consisting of saccules with a content of slightly electron-opaque material, and relatively large, light vacuoles around the saccules; granular endoplasmic reticulum which was barely arranged in layers and dispersed, and whose cisternae were sometime partially agranular, especially in the peripheral part; free ribosomes which were dispersed in the form of rosettes; frequent lysosomal dense bodis, etc. (Figs. 1 and 2). The nucleus of the astrocytes was oval in shape and frequently revealed invagination of the nuclear envelope. The nuclear envelope was penetrated by nuclear pores which were closed by a diaphragma, and its inner leaflet around the nuclear pores was covered by small masses of chromatin. The chromatin in the nucleus was finely granular and distributed diffusely, forming several small random aggregations. On the entire inner leaflet of the nuclear envelope the chromatin which occurred in very small amounts, was relatively densely attached, although the contrast between the chromatin associated with the nuclear envelope and nucleoplasm was more or less unclear. Ribosomes were attached to parts of the outer leaflet of the nuclear envelope. The nucleolus consisted of a nucleolonema which formed a coarse network with a thick band-like structure, and a pars amorpha which filled the network. Astrocytes were commonly found to be in contact with other astrocytes or sometimes other types of glial cells, and desmosome-like structures were formed between the plasma membranes concerned.

   b) **Oligodendroglia**

   The nucleus of the oligodendroglia, as a whole, showed a round or oval shape. However, small invaginations of the nuclear envelope were sometimes observed. Coarsely granular chromatin was distributed diffusely, giving the nucleoplasm a dark overall appearance. The chromatin was aggregated into clumps, although the density was not sufficiently compact as to produce a clear-cut contrast against the surrounding nucleoplasm (Fig. 1). The nucleolus consisted of large meshes of nucleolonema, without a clear-cut contrast against the surrounding nucleoplasm. The matrix of the cytoplasm had a granular appearance and showed a relatively high electron-density (Fig. 2). The granular endoplasmic reticulum was relatively well developed, and its cisternae were often piled up into several layers (Fig. 2, er). Free, ribosomes forming rosettes were rather abundant and were distributed diffusely throughout
the cytoplasm. The matrix of the mito-
chondria was as electron-dense as the
cytoplasmic matrix. The Golgi complex,
which was located around the perinuclear
region, was relatively well-developed.
Its saccules and vacuoles were frequently
distended and contained electron-lucent
material. Microtubules occurred most
frequently in the processes. Dense
bodies which were round or irregular-
oidal in shape and contained extremely
high electron-dense, homogeneous or
sometimes heterogeneous material, were
observed. However, their numbers were
relatively small.

c) Microglia

The microglia possessed nuclei with
variously shaped centours. Large num-
bers of very electron-dense clumps of
chromatin were associated with the nu-
clear envelope, giving a clear-cut border
against the finely granular nucleoplasm
(Fig. 1). The nucleolus was larger than
that of the astrocytes and oligodendro-
glia. It consisted of a nucleolonema,
which enclosed the pars amorpha. The
matrix of the cytoplasm was more or
less dark, but appeared lighter and more
finely granular than that of the oligoden-
droglia. Mitochondria were dispersed in
the perikaryon, the matrix of which was
relatively electron-dense, although their
cristae were rather clearly observed (Fig.
3). The cisternae of the granular endo-
plasmic reticulum, which was dispersed in
the perikaryon, were small and rarely
piled up into layers. Free ribosomes
forming rosettes were also distributed in
a disperse fashion, with fewer clusters
than in the case of the oligodendroglia.
When the nucleus was located eccentric-
ally located, in the polar region with a
large cytoplasmic volume (Fig. 3), the
Golgi complex was frequently observed
to be well-developed. Around the Golgi
complex, numerous vesicles with a round
or short rod-like shape were found to
contain electron-dense material (Fig. 3).
Heterogeneous dense bodies were fre-
quently observed to have a variable and
irregular shape. Microtubules and micro-
filaments were not seen.

2. Glial cells in the injured medullary
pyramid

a) Astrocytes and oligodendroglia

The perikarya and processes of the
astrocytes were hypertrophied and had
an increased volume of cytoplasm. Parti-
cularly in severely injured cases, whose
medullary pyramids had become occupied
by numerous degenerated nerve fibers,
the hypertrophy and increase in the cyto-
plasmic element were so strong that
space formed by the disappearance of
degenerated nerve fibers, were filled up
(Fig. 4). However, during Wallerian de-
genation the structure of the nucleus
remained almost unchanged, although
the cytoplasm contained predominantly
increased glial filaments and well-develop-
ed cell organelles among the bundles of
glial filaments. The granular endoplasmic
reticulum which was dispersed in the
perikaryon contained distended cisternae.
Free ribosomes and mitochondria were
present in increased amounts, and the
Golgi complex became particularly well-
developed. Glial filaments were densely
distributed in the hypertrophied processes,
and slenderly elongated cylindrical mito-
chondria were rich among them. In cases
with numerous degenerated nerve fibers,
degenerated myelin fragments were some-
times found in the cytoplasm of the as-
trocytes, but they were never in the pro-
cess of disintegration.

Oligodendroglia, whose cytoplasm tends
to change in amount according to the
extent of brain injury and stage of the
degeneration process, did not exhibit any
apparent alteration in the fine structure
of their nucleus and cytoplasm (Fig. 5).
No phagocytic reaction was observed in
the oligodendroglia.
b) Microglia and phagocytes

As described previously (Inoue et al., 1978), the nuclear structure of the phagocytes resembled that of the microglia, especially on light microscopy, and it was difficult to differentiate between phagocytes and microglia. The glial elements in the medullary pyramid were thus grouped for convenience into astrocytes, oligodendroglia and a third category which included both microglia and phagocytes. These groups were used to estimate the numbers and population of glial elements by light microscopy and to investigate the tendencies towards reaction or movement (especially in the third category) during Wallerian degeneration.

Semi-thin sections of 1 μm in thickness were observed by light microscopy, using an oil immersion lens and an ocular micrometer. Thirty fields (each 100 μm square) were selected as objects for measurement, and the total glial cells observed were grouped into three based on the nuclear figures and their respective numbers were counted. In the light of the intimate relationship which existed between the number of phagocytes occurring and the amount of degenerated nerve fibers, the numbers of thick normal nerve fibers above 7 μm in outer diameter in both the injured left and normal right pyramids were estimated and compared to assess the degree of brain injury. The ratio of cells of the third category to the total glial cell numbers shown in Table 2. Injury in Cases 56 and 45 involved destruction on both sides of the brain, so that control data could not be given.

The third category of cells on the undisturbed side (namely microglia) was estimated to constitute 13 to 21% of the total glial elements. On the other hand, since the ratio of this cell type was greater on the injured side, it was considered that there was an apparent increase in number of the third category cells. In Case 51, whose tract was injured

Table 2. Population of glial cells, including phagocytes, in the medullary pyramid.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Survival period (days)</th>
<th>Ratio of degeneration</th>
<th>Left Astrocytes</th>
<th>Oligodendroglia</th>
<th>M-cells</th>
<th>M-ratio</th>
<th>Right Astrocytes</th>
<th>Oligodendroglia</th>
<th>M-cells</th>
<th>M-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 56</td>
<td>3</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>26</td>
<td>73</td>
<td>32</td>
<td>0.24</td>
</tr>
<tr>
<td>No. 56</td>
<td>7</td>
<td>*</td>
<td>40</td>
<td>87</td>
<td>48</td>
<td>0.27</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>No. 45</td>
<td>19(12)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>15</td>
<td>53</td>
<td>59</td>
<td>0.46</td>
</tr>
<tr>
<td>No. 22</td>
<td>25</td>
<td>99%</td>
<td>49</td>
<td>90</td>
<td>115</td>
<td>0.45</td>
<td>24</td>
<td>71</td>
<td>16</td>
<td>0.14</td>
</tr>
<tr>
<td>No. 51</td>
<td>33</td>
<td>10%</td>
<td>22</td>
<td>133</td>
<td>41</td>
<td>0.21</td>
<td>27</td>
<td>59</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td>No. 51</td>
<td>43</td>
<td>53%</td>
<td>32</td>
<td>68</td>
<td>87</td>
<td>0.47</td>
<td>13</td>
<td>82</td>
<td>26</td>
<td>0.21</td>
</tr>
<tr>
<td>No. 59</td>
<td>76</td>
<td>47%</td>
<td>25</td>
<td>62</td>
<td>154</td>
<td>0.64</td>
<td>37</td>
<td>72</td>
<td>29</td>
<td>0.21</td>
</tr>
<tr>
<td>No. 58</td>
<td>117</td>
<td>98%</td>
<td>47</td>
<td>115</td>
<td>202</td>
<td>0.55</td>
<td>27</td>
<td>76</td>
<td>22</td>
<td>0.18</td>
</tr>
<tr>
<td>No. 45</td>
<td>293</td>
<td>*</td>
<td>21</td>
<td>77</td>
<td>71</td>
<td>0.42</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>No. 18</td>
<td>761</td>
<td>96%</td>
<td>103</td>
<td>132</td>
<td>71</td>
<td>0.23</td>
<td>23</td>
<td>72</td>
<td>15</td>
<td>0.14</td>
</tr>
<tr>
<td>No. 17</td>
<td>787</td>
<td>41%</td>
<td>23</td>
<td>102</td>
<td>27</td>
<td>0.18</td>
<td>18</td>
<td>81</td>
<td>15</td>
<td>0.13</td>
</tr>
</tbody>
</table>

M-cell; Microglia or/and phagocytes
M-ratio; M-cells/Total glial cells
Counted area; (100 μm x 100 μm) x 30 fields (0.3 mm²)
to an extent of approximately 10% as estimated from the amount of normal nerve fibers remaining, the proportion of the third category was greater than that in the control (right side pyramid), whereas the degree of increase was smaller than that in other cases whose tracts were more severely. However, the degree of occurrence of phagocytes was related not only to the amount of degenerated nerve fibers but also to the stage of the degeneration process. In Cases 22 and 45 (on the right side), even though almost all the nerve fibers were degenerated, the degree of occurrence was lower or as high as that in Cases 21 and 59, in which the remaining normal fibers far exceeded those in Cases 22 and 45. Comparison of Case 21 with Case 59 revealed a higher degree of degeneration in the former than the latter, but the degree of appearance of third category cells was higher in the latter. In the course of time after Case 58, the degree of appearance became lower.

The characteristics of the ultra-fine structure of the third category of cells at each stage during Wallerian degeneration can be summarized as follows. At 3 days after operation (Case 56, right pyramid), degeneration of myelinated nerve fibers was not predominant, and third category cells were very rarely found to invade the thick degenerated myelin sheaths. Among the nerve fibers typical microglia (Fig. 6), cells which resembled phagocytes observed invading the myelin sheaths commonly found at 7 days after operation (Case 56, left pyramid) existed (Fig. 7). The cells were more or less different from the microglia in the uninjured pyramids, in that the chromatin granules associated with the nuclear envelope were more coarsely aggregated to form various clumps, and the nucleoplasm was lighter, containing dispersed chromatin granules. The cytoplasmic matrix was finely granulated, resembling that of microglia (Fig. 7), but the periphery of the cell body extended irregularly, revealing very uneven contours. The extended periphery of the cytoplasm became even lighter (Fig. 7, arrow). The cytoplasm contained well-developed Golgi complex, numerous mitochondria and granular endoplasmic reticulum with more distended and sometimes piled-up cisternae (Fig. 8). The cisternae contained electron-dense material and their peripheries were partially distended so as to be budded off (Fig. 8, arrow-heads). Furthermore, around these cisternae numerous round or short rod-shaped vesicles containing electron-dense material like that in the buds of the cisternae were observed. It appeared probable therefore that the budding of the granular endoplasmic reticulum was intimately related to these vesicles. In addition, laminated inclusions or lysosome-like bodies containing heterogeneous material, were found. This type of cells and microglia were apparently identical to the third category of glial cells observed by light microscopy.

At 7 days after operation (Case 56, left pyramid), besides typical microglia (Fig. 9), cells similar to the type described above were observed either among the nerve fibers or within degenerated myelin sheaths (Fig. 10). Such third category cells invaded degenerated myelin sheaths and engulfed degenerated axon fragments (Figs. 10 and 11) and myelin lamellae, apparently demonstrating phagocytic properties as reported previously in detail by Inoue et al. (1978). This type of cells showed and identical nuclear structure to that of the cells found among nerve fibers at 3 or 7 days after operation. However, there were certain differences in the cell organelles; that is to say, the granular endoplasmic reticulum became
remarkably well-developed (Fig. 10, 11 and 12). The cisternae were piled up into a few layers, and vacuoles of 300 to 500 nm in diameter containing electron-dense material, were frequently attached to or slightly apart from their ends (Fig. 12). Round or short rod-shaped vesicles of about 50 nm in diameter containing electron-dense material, were also found in the neighbourhood of the granular endoplasmic reticulum (Fig. 11). These vesicles were more predominantly developed in phagocytes than in microglia. Free ribosomes were more abundant in phagocytes.

At 19 days after operation (12 days after a second operation), phagocytes which engulfed large amounts of myelin fragments, were still observed within the myelin sheaths (Fig. 13). At this stage, the phagocytes again possessed the Golgi complex, but the granular endoplasmic reticulum was less developed. It was common at the periphery of the perikaryon for many vacuoles to appear and filamentous structures to be found in the cytoplasm, forming dispersed bundles (Fig. 12). In addition, the nucleus became pyknotic. These cells within the degenerated myelin sheaths had thus probably commenced autolytic breakdown and begun to disappear. From this time on, phagocytes rarely occurred within the myelin sheaths, although they appeared among the nerve fibers. Furthermore, many myelin fragments engulfed at this early stage were characterized by not showing disintegration or digestion of the myelin lamellae, as was found in later phagocytes, but by showing only disturbance or loosening of the myelin lamellae (Fig. 13).

The structure of the phagocytes occurring among nerve fibers subsequently resembled that of the microglia on the uninjured side of the pyramid, although the cell organelles of the phagocytes were modified to various degrees according to the phagocytic reaction. Thus, phagocytes could be identified only from the finding of engulfment of degenerated myelin fragments.

At 25 days after operation, phagocytes engulfing degenerated nerve fibers were very rarely found, and if present, the amount of myelin fragments engulfed was very small, even though in this case (Case 22) most of the nerve fibers in the medullary pyramid has begun Wallerian degeneration (Fig. 14). Some of the third category cells, although resembling microglia, possessed a more well-developed Golgi complex and greater numbers of mitochondria than the microglia in the uninjured pyramid (Fig. 14, arrow). Around the Golgi complex, numerous vesicles of about 50 μm in diameter containing electron-dense material, were observed. The granular endoplasmic reticulum was less well-developed than that in the phagocytes at 3 to 7 days after operation, consisting of small cisternae with a disperse distribution. Lysosomal bodies were frequently found, containing heterogeneous materials. Cells engulfing small fragments of myelin sheath commonly contained a number of lipid droplets.

At 33 days after operation, few degenerated nerve fibers apparent in the left pyramid, and phagocytes engulfing myelin fragments were rarely found. Third category cells were identical in fine structure to the microglia of the normal right pyramid.

At 43 days after operation, phagocytes engulfing myelin fragments, although very small in amount, were more frequently present in the injured pyramid, where more than half of the nerve fibers were subject to degeneration (Fig. 15). The structural characteristics of these cells were almost the same as those of the third category cells of Case 22 at 25
days after operation. Although degenerated myelin sheaths displayed loosening or disturbance of the myelin lamellae before they were phagocytozed, they maintained a laminated structure which was composed of periodic and intraperiodic lines (Inoue et al., 1978). However, after myelin fragments had been engulfed by phagocytes, the laminated structure of so-called compact myelin disappeared from the peripheral part and became a low electron-dense membranous structure, in which limiting membrane-like structures of about 10 nm in thickness were piled up concentrically and enclosed myelin masses remaining undigested to various degrees at the center (Fig. 15, asterisk). After all or most of the engulfed degenerated myelin fragments had been altered to such membranous structures, the spaces between the membrane laminae or the control lacuna came to be occupied by their own phagocytic cytoplasm (Fig. 15, arrow-head). Around the engulfed and digested myelin fragments small vesicles containing electron-dense materials were very abundant (Fig. 15). As the engulfed myelin fragments began to be digested, it was very common to find that the cisternae of the granular endoplasmic reticulum extended partly and revealed an agranular surface related to the small electron-dense vesicles. Laminated inclusions were commonly found. Lipid droplets of variable electron-density from relatively high to near-transparency, probably related to the degree of digestion of the myelin fragments, appeared in the phagocytic cytoplasm, although they were small in amount at 43 days after operation.

The case at 76 days after operation showed a smaller degree of brain injury than that at 43 days after operation, and phagocytes engulfing greater amounts of degenerated myelin were more commonly observed (Fig. 16). The appearance of the cell organelles and nuclear chromatin of the phagocytes was identical to that of microglia from the opposite, healthy pyramid, although extremely active phagocytosis gave rise to alteration of the contours of the nucleus. Myelin fragments which were engulfed lost the structure of compact myelin from the peripheral to central regions and formed the same loose, low electron-dense laminate structure as that described above (Figs. 16 and 17). Considering the arrangement and continuity between the newly formed laminated membranous structure and compact myelin, the paired dense layers in the former may represent a transformation of the periodic dense line of the latter by splitting into two layers (Fig. 17, arrow-head). After being completely transformed, these laminated structures were disrupted by the phagocytic cytoplasm invading them, resulting in an alteration to laminated inclusions of various shapes and sizes, with a remarkable increase in the number of lipid droplets. However, phagocytes containing numerous lipid droplets were less abundant at this stage, as in the next case at 117 days after operation.

At 117 days after operation, there were few phagocytes engulfing degenerated myelin fragments, even though degenerated nerve fibers still remained. However, a number of phagocytes were observed to contain so many lipid droplets that the cytoplasm occupied only a narrow space between the droplets (Fig. 18, arrow-heads), whereas the structure of the cells was similar to that of the phagocytes described above. As described previously (Inoue et al., 1978), needle-shaped or board-like laminated inclusions, which might have developed from digested, phagocytozed myelin fragments, were present in the narrow space between the lipid droplets. At this stage, filamentous structures were sometimes observed.
Subsequently, at 293 days after operation, cells containing lipid droplets and inclusion as described above remained numerous.

At 761 and 787 days after operation, however, no cells containing lipid droplets were found, and it was considered from histograms of the glial elements that phagocytes might have disappeared from the degenerated pyramid.

**Discussion**

When discussing the reactions of phagocytes after injury of the central nervous system, consideration should first be given to whether the region of the investigated subject represents a directly damaged area or a region remote from the injured area as in cases of Wallerian degeneration or retrograde degeneration. At sites of the former type, vascular disturbances such as inflammation and bleeding occur, and the surrounding areas also probably fall into an ischemic condition (Rose et al., 1960; Cook and Wisniewski, 1973). It has been widely accepted by many workers that in the situation of mechanical injury such as stab wounds, degeneration by local treatment with chemical substances or experimental allergic encephalitis, reactive phagocytes might originate from blood cells (Konigsmark and Sidman, 1963; Gonatas et al., 1964; Huntington and Terry, 1966; Lampert and Cressman, 1966; Howell and Kidd, 1969; Kitamura et al., 1972; Adrian and Williams, 1973). However, the possibility that microglia might participate to some extent in the phagocytic reaction has not been completely excluded (Konigsmark and Sidman, 1963; Adrian and Williams, 1973). On the other hand, various reports have appeared on the reaction of phagocytes against necrotic tissue occurring in regions remote from the injured site. Phagocytes observed during Wallerian degeneration in the central nervous system varied as regards their time of appearance and origin, according to the animal species used and nerve tracts investigated, as described by Inoue et al. (1978) and Fernando (1973a). The age of the animal species was also found to influence the degenerating process (Cook et al., 1974). However, there was general agreement that it was at a later time that phagocytes actively engulfed degenerated myelin sheaths.

Bignami and Ralston (1969) reported that in cat spinal cord and thalamus, degenerated myelin sheaths were phagocytosed by mononuclear cells at about 60 days after operation, and further, at the later stages of degeneration, microglia displayed a phagocytic ability. According to Lampert and Cressman (1966), phagocytosis and digestion of degenerated myelin sheaths by macrophages was predominant at the injured region of the posterior funiculus of rat spinal cord from 1 week to 52 days after operation (the longest survival in their experiments), while in the remote areas where Wallerian degeneration appeared, macrophages probably originating from resting microglia did not engulf the degenerated myelin fragments until several weeks after the operation. Fernando (1973a) noted that in rat pyramidal tract subject to Wallerian degeneration, degenerated nerve fibers were engulfed at 2 to 3 weeks after operation, by astroblasts. Cook and Wisniewski (1973) observed processes of oligodendroglia engulfing degenerated myelin sheaths in adult cat optic nerve at 56 days after operation. Vaughn et al. (1970) reported that in the Wallerian degeneration of rat optic nerve after enucleation, multipotential cells (as named by them) frequently engulfed degenerated myelin sheaths at 14 to 42 days after operation. Gray and Hamlyn (1962) examined the avian optic tectum after enu-
cleation and suggested that since degenerated myelin fragments were found to be engulfed by phagocytic glial cells only in a case at 30 days after operation, which represented the longest survival time in their experiments, they might be phagocytozed and finally digested over the course of a more prolonged period.

Wallerian degeneration of unmyelinated nerve fibers or axonal terminals in the nuclei has revealed different features from those described above. For example, Ghetti et al. (1972) reported that degenerated axonal terminals in the lateral geniculate body of the rhesus monkey after enucleation were phagocytozed by microglia or astrocytes from 4 days after operation. Many studies have indicated that axonal terminals disappeared in the early period by phagocytosis or self-disintegration, and that phagocytes might originate from glial cells in situ (Gray and Hamly, 1962; Colonnier, 1964; Smith et al., 1966; Saavedra et al., 1969; Wong-Riley, 1972).

Based on the examples described above, the various reactions of phagocytes during Wallerian degeneration in the central nervous system should be discussed separately according to two different categories. One is the reaction towards axons and axonal terminals which formed parts of the cytoplasmic processes of nerve cells. The other is the reaction towards myelin sheaths which represented accumulations of the plasma membrane of glial cells or non-neuronal elements.

Some reports have indicated that degenerated axons of myelinated nerve fibers were engulfed by phagocytes which entered the tubes of myelin sheaths, as observed in the present study (Lampert and Cressman 1966; Cook and Wisniewski, 1973; Cook, 1974). However, it is also possible that certain degenerated axons were disintegrated autolytically by enzymes in the nerve cells themselves or extracellular space (Inoue et al., 1978).

On the other hand, the myelin sheaths in the central nervous system were formed at the tips of thin processes of oligodendroglia as a compact accumulation of their plasma membrane around an axon, and primary axonal degeneration resulted in such changes of the myelin sheaths as shortening of the internodal segments by invagination of myelin sheaths into their own lumen, etc. (Inoue et al., 1978). It seemed unlikely, however, that oligodendroglia as myelin-forming cells might respond to degenerated myelin sheaths in order to remove them, since single oligodendroglia extended processes to many surrounding axons at random to form myelin sheaths (Inoue et al., 1973, Sternberger et al., 1978), and the cytoplasm around the nerve fibers was insufficient to engulf and digest them. In addition, they did not undergo any essential changes in cytoplasmic and nuclear structure, as demonstrated in the present study and by other workers (Lampert and Cressman, 1966; Bignami and Ralston, 1969; Vaughn and Pease, 1970).

Thus, under Wallerian degeneration in the central nervous system, the degenerated myelin sheaths might become detached from the processes of myelin-forming oligodendroglia to remain as endogenous foreign bodies, most of which would be removed by phagocytosis. Electron microscopy has previously provided no evidence that phagocytes invade myelin tubes and engulf degenerated myelin lamellae from the inside at the early stage, until the present study. Only by light microscopy, had Jakob (1913) and Daniel and Strich (1969) described this type of phagocytosis.

The phagocytes appearing at the early stage in the present study differed in the fine structure of their nucleus from microglia or those cells occurring at the later stage to phagocytoze degenerated myelin.
Reaction of Glial and Phagocytic Cells

fragments actively. Their reactive alteration of the cell organelles was also highly characteristic: when they were present among nerve fibers at 3 days after operation, the Golgi complex was well-developed and associated with the lysosomal dense vesicle around it. However, after they entered the myelin tubes of degenerated nerve fibers and began to engulf axon fragments, the Golgi complex became extremely rare and, conversely, the granular endoplasmic reticulum increased, with accompanying lysosomal dense vesicles around it as if they might be closely associated with the granular endoplasmic reticulum. It also produced large electron-dense vacuoles. When phagocytes came to engulf large amounts of myelin fragments, the Golgi complex reappeared and the granular endoplasmic reticulum became decreased in amount. Such changes in the Golgi complex and granular endoplasmic reticulum were considered to be related to the production of lytic enzymes required in the phagocytic reaction. Most of the phagocytes came to contain numerous empty vacuoles and bundles of filaments and finally disintegrated, leaving the engulfed myelin fragments since these were undigested. Such a phagocytic process was similar to that of the alveolar macrophages of dust cells, which are considered to originate from blood monocytes, being discharged together with the sputa after phagocytozing foreign bodies in the alveoli (Ham’s Histology, 1976). Gonatas et al., (1964) also reported that macrophages arising during experimental leukoneuropathy resembled the phagocytes in the lung.

On the other hand, the predominant phagocytic reaction observed from about 6 weeks after operation in the present study was essentially identical to the reaction of phagocytes which has been described in studies on Wallerian degeneration by other investigators. Phagocytes arising in this later stage differed from those at the early stage, possessing a nucleus and cytoplasm similar to microglia. Among the cell organelles, the Golgi complex became well-developed, and the granular endoplasmic reticulum was dispersed but contained more distended cisternae with a number of lysosome-like, small vesicles around them. The degree of development continuously ranged from dominantly reacted to almost unchanged as compared to the microglia on the normal side of the medullary pyramid. Thus if the phagocytes had not yet engulfed any myelin fragments, they could hardly be distinguished clearly from microglia. Many reports have indicated that during Wallerian degeneration, the chief cells engulfing myelin fragments were microglia or microglia-like cells (Lampert and Cressman, 1966; Bignami and Ralston, 1969; Adrian and Williams, 1973; Matthew, 1974; Matthew and Kruger, 1973a, b), although a few exceptions were noted (Cook and Wisniewski, 1973; Fernando, 1973a). Many investigators have also implicated microglia as the cells which engulf nerve cells which have fallen into retrograde degeneration (Fernando, 1973b; Torvik, 1972). In the present study, those cells which actively engulfed myelin fragments and digested them to give lipid droplets were entirely similar to the microglia observed on the normal side of the medullary pyramid of the subjects. However, the essential properties of microglia under normal conditions have not yet been fully elucidated (Peters et al., 1976). Also phagocytes which multiplied under such unusual conditions as encephalitis or vascular injury have been shown by an autoradiographic technique to have a hematogenous origin, although they were said to be similar to microglia (Peters et al., 1976; Blackwood, 1976). Thus, although cells other than astrocytes and oligodendroglia, which
were considered to include so-called resting microglia and phagocytic cells, were clearly recognized to be multiplied in number under Wallerian degeneration, probably without giving rise to any vascular damage or inflammatory reaction, it could not be ascertained whether they might multiply cell division of resting microglia themselves, or by the appearance of newly derived cells from blood cells or vascular cells independently of the resting microglia, to become phagocytic. Also, the possibility that resting microglia might react with endogenous foreign bodies arising by Wallerian degeneration or retrograde degeneration, cannot be completely excluded.

Throughout the entire process of degeneration in the present study, astrocytes and oligodendroglia could be clearly identified, and phagocytic ability could definitely be denied in them. Small pieces of myelin fragments, however, appeared to be engulfed within the cytoplasm of astrocytes, although very rarely, while the process of digestion of myelin fragments could not be demonstrated, as reported previously by Gonatas et al. (1964) and Lampert and Cressman (1966). It seemed reasonable to consider therefore that the extracellular myelin fragments were embedded within a depression in the hypertrophied cytoplasm, through which an ultra-thin section was cut for observation as described above. Fernando (1973a) found that during Wallerian degeneration of rat pyramidal tract the response of many astrocytes was intensive, so that they dedifferentiated into glioblasts: these further multiplied to become astroblasts and so acquired a phagocytic ability. He identified phagocytes as astroblasts due to the existence of filamentous structures in the cytoplasm, whereas the phagocytes observed in our study sometimes possessed filaments. Adrian and Williams (1973) noted that blood mono-nuclear cells appearing in injured areas of mouse spinal cord contained filaments so that they resembled astrocytes, and further carried out a phagocytic function. The basis used by Fernando (1973a) for identifying phagocytes as astroblasts may thus lack precision. Vaughn and Pease (1970) reported that during Wallerian degeneration of rat optic nerve, only degenerated myelin fragments isolated by hypertrophied processes of astrocytes were engulfed by the astrocytes themselves, since phagocytes might not approach them. It was thus difficult to accept that astrocytes in situ might positively take part in the phagocytic process under Wallerian degeneration of myelinated nerve fibers.

Based on their observations of Wallerian degeneration in optic nerve of the cat, rhesus monkey and squirrel monkey, Cook and Wisniewski (1973) pointed out that the cells which phagocytosed myelin fragments and reduced them to lipid droplets were oligodendroglia. However, it has been shown that the glial elements in rhesus monkey optic nerve consist of astrocytes, oligodendroglia and microglia (Inoue et al., 1976). Since Cook and Wisniewski (1973) did not identify microglia in the optic nerve, doubt must be exist as to whether the phagocytes undergoing intensive reactive changes were all identical with oligodendroglia. As pointed out by Inoue et al. (1976), since the architectonics of the blood vessels in the optic nerve differ from those of the brain and spinal cord, it may not make sense to discuss and compare the degeneration of the optic nerve on a common basis with that of the medullary pyramid.

Lampert and Cressman (1966) examined the process of digestion of myelin fragment engulfed by macrophages during Wallerian degeneration in the rat spinal cord, and found that myelin lamellae were at first dissociated by hydration.
They were next changed into stacks having a uniformly layered structure within compartments containing acid phosphatase, and finally these layered structures disintegrated into globoid lipid bodies. Furthermore, they demonstrated a continuity between the myelin lamellae and layered structures. Bignami and Ralston (1969) reported that during Wallerian degeneration in cat spinal cord, the protein element of engulfed myelin fragments was at first digested to lose its lamellar structure and become electron-lucent, and that the proteolytic enzymes might depend upon dense bodies closely associated with the granular endoplasmic reticulum. Thus in the present study, it was considered that engulfed myelin fragments, of which the osmiophilic properties and laminated structure were preserved at the early stage, were gradually transformed into a low electron-dense layered structure from the peripheral part closely with the cytoplasm, probably by digestion of protein elements with the participation of small dense vesicles associated with the activated granular endoplasmic reticulum or Golgi complex, and that a bilayered membranous structure might be formed by splitting of the periodic dense line into two. This appears reasonable since small remaining periodic lines were often intercalated within the membranous structure. Furthermore, based on the fact that lipid droplets increased as these membranous structures were fragmented and consumed, the structures were considered to consist chiefly of lipid elements on the removal of proteins. These membranous structures, which thus apparently contain a high lipid element, may generally be disintegrated into numerous lipid droplets and crystalline layered structures.

References


31) Sternberger, N.H., Itoyama, Y., Kies, M.W. and Webster, H. deF. "Immunocytochemical method to identify basic protein in myelin-forming oligodendrocytes..."


Explanation of Figures

Plate I

Fig. 1. Three types of glial elements in the medullary pyramid in the normal rhesus monkey. as; astrocyte m; microglia ol; oligodendroglia

Fig. 2. Astrocytes (as) and oligodendroglia (ol) in the medullary pyramid in the normal rhesus monkey.
Plate II

Fig. 3. Microglia (m) in the normal medullary pyramid. asterisk; well-developed Golgi complex, associated with numerous small vesicles containing electron-dense material.

Fig. 4. Astrocytes (as) in the injured medullary pyramid at 761 days after operation.

Fig. 5. Oligodendroglia (ol) in the injured medullary pyramid at 43 day after operation. arrow-heads: degenerated nerve fibers.
Fig. 6. Typical microglia in the injured pyramid at 3 days after operation.
Fig. 7. The cell found among nerve fibers at 3 days after operation, which resembled phagocytes occurring within myelin tubes at 7 days after operation.
Fig. 8. High magnification of the cytoplasm of this type cell.
Plate IV

Fig. 9. Typical microglia (m) in the injured pyramid at 7 days after operation.
ol; oligodendroglia.

Fig. 10. The phagocyte, invading the myelin tube, engulfs a fragment of the degenerated axon (ax) at 7 days after operation.

Fig. 11. The phagocyte within the myelin tube engulfs a fragment of the degenerated axon (ax) at 7 days after operation.
arrow; well-developed granular endoplasmic reticulum, cisternae of which become partially agranular.
arro-head; numerous small vesicles around the granular endoplasmic reticulum.

Fig. 12. The cytoplasm of the phagocyte within the myelin tube.
arro-head; vacuoles of 300 to 500 nm in diameter, associated with layered granular endoplasmic reticulum (er).
Plate V

Fig. 13. The phagocyte within thin myelin tube which remained unengulfed (arrow-heads) at 19 days after the first operation.

my; a large amount of myelin mass in the cytoplasm.

Nu; the pyknotic nucleus.

f; bundles of cytoplasmic filaments.

Fig. 14. The phagocytes, occurring among nerve fibers in the injured pyramid at 25 days after operation.

arrow-head; a small amount of myelin fragment.

Fig. 15. The phagocyte occurring among nerve fibers in the injured pyramid at 43 days after operation.
Plate VI

Fig. 16. The phagocyte at 76 days after operation.
  my; engulfed myelin fragments.
  asterisk; an engulfed myelin fragment which loses the structure of characteristic
  lamination.
  arrow-heads; laminated inclusion.

Fig. 17. Higher magnification of two engulfed myelin fragments (my₁ and my₂) in the phagocyte
  in the injured pyramid at 76 days after operation.
  cy; the cytoplasm of the phagocyte.
  M; typical myelin lamellae remaining undigested.
  L; newly formed laminated structure composed of paired dense layers.
  arrow; typical myelin lamellae intercalating between newly formed layers.

Fig. 18. Phagocytes containing numerous lipid droplets (arrow-heads) at 117 days after
  operation.
  as; astrocyte.