CHANGES IN THE NUCLEAR POPULATION OF THE POSTERIOR COLUMNS OF THE SPINAL CORD OF THE RABBIT DURING DEGENERATION.

Thesis submitted for the degree of

DOCTOR OF MEDICINE

by

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1. INTRODUCTION.

A. Object of thesis.

Detailed studies of the quantitative changes in nuclear population in degenerating peripheral nerve have already been published by various authors (Abercrombie & Johnson, 1946; Thomas, 1948; Joseph, 1947, 1948, 1950) and certain interesting conclusions were drawn from their work which is summarised in Table I (Joseph, 1950).

TABLE I.

Nerve studied (rabbit)	% age fibres >6 μ	Maximum nuclear increase	No. of nuclei per c.mm. Normal	Degenerated
Nerve to medial head of gastrocnemius (Thomas)	80	14x	27,000	450,000
Sciatic nerve (Abercrombie & Johnson)	33	8x	43,000	330,000
Sural nerve (Thomas)	25	5x	90,000	440,000
Greater splanchnic nerve (Joseph)	4	1¾x	448,000	470,000
Anterior mesenteric nerve (Joseph)	nil	nil	358,000	

The conclusions reached were

(1) that nerves of different fibre size showed different nuclear increases during degeneration;

(2) that the larger the fibres the greater was the maximum increase in the number of nuclei;

(3) that normal nerves consisting of fibres of large size contained fewer nuclei per c.mm. than those consisting of fibres of small size;

(4) that the nerves consisting of large fibres reached their maximum increase later than those consisting of small fibres;

(5) that the maximum number of nuclei in all nerves during degeneration, whatever their fibre size, was similar.

It was thought that similar studies in relation to the white matter of the spinal cord could give information regarding

(1) changes in nuclear population after different periods of degeneration;

(2) the behaviour of the different cells in the spinal cord during degeneration;

(3) differences between the fibres of peripheral nerves and of tracts in the spinal cord during degeneration;

(4) the problem of regeneration in the spinal cord.

B. Structure of posterior columns of spinal cord.

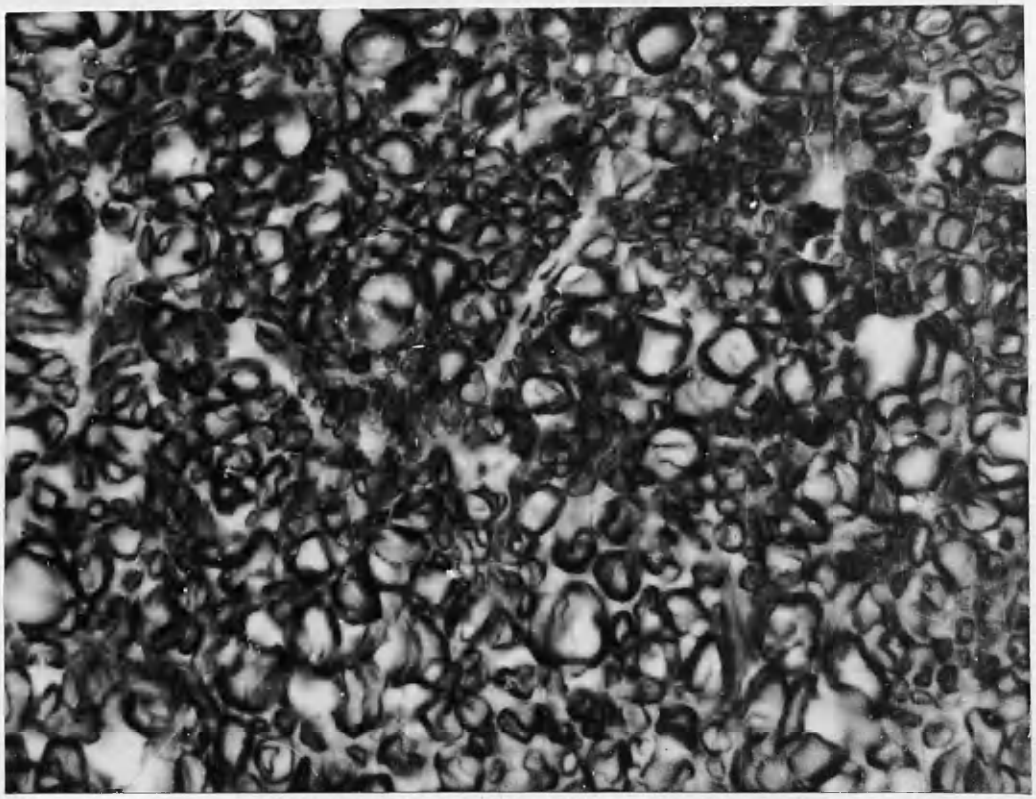
1. Nerve fibres.

It is accepted that the medium and large myelinated

nerve fibres in the posterior columns contain the central processes of some of the neurones in the posterior root ganglia and are mostly proprioceptive in function. In addition there are many smaller myelinated and unmyelinated fibres which are either ascending or descending and are intersegmental in type, that is, their cell bodies are in the grey matter of the spinal cord. The axon is surrounded by a myelin sheath which according to recent work possesses nodes (Feindel, Allison, & Weddell, 1948; Allison & Feindel, 1949; Hess & Young, 1952). These nerve fibres do not possess the distinct sheath known as the neurilemma which is found in myelinated peripheral nerves. Instead there is a network of glial fibres, both vertical and horizontal, in which the myelinated nerve fibres run.

In a typical myelinated peripheral nerve, the Schwann cell is found between the neurilemma and the myelin sheath. A comparable cell, the oligodendrocyte, is found next to the myelin sheath of the nerve fibres of the posterior columns. To what extent they are similar will be discussed in the next section.

Each nerve fibre in a typical peripheral nerve is surrounded by a connective tissue sheath called the endoneurium. There is no comparable sheath round the nerve fibres of the white matter of the



10 μ

Fig. 1.

Transverse section of cervical spinal cord stained Weigert-Pal showing myelin sheaths in posterior columns and indicating the size of the fibres in these columns.

spinal cord. Fibrous tissue septa are described, however, passing from the pia mater towards the grey matter, through the white matter.

In view of the different results obtained in various degenerating peripheral nerves, it is important to consider the size of the fibres in the posterior columns. These columns contain a considerable number of large fibres between 12 and 18 μ in diameter (see Fig. 1) and resemble a mixed peripheral nerve, such as the sciatic nerve of the rabbit which was used in the experiments of Abercrombie & Johnson (1946).

11. Cells and their processes.

The cells in the white matter can be classified as

(a) neuroglia (the majority of the cells);

(b) connective tissue;

(c) blood vessel (endothelial and smooth muscle).

(a) Neuroglia.

In spite of certain recent differences of opinion which will be referred to later, the Rio-Hortega classification of the neuroglia is still generally accepted. This is fully dealt with by Penfield (1932) and Rio-Hortega (1932) and

it is a brief version of their description which follows. Penfield (1932) separated the neuroglia into the neuroglia proper and the microglia and dealt with the neuroglia under the headings of astroglia and oligodendroglia. Until 1913, two elements were recognised in the central nervous system, nerve cells (neurones) and other cells (neuroglia). In that year Cajal differentiated in the neuroglia, one group of cells which he called astroglia consisting of astrocytes. He referred to the cells in the central nervous system which were neither neurones nor astrocytes as the "third element". It was Rio-Hortega who discovered that this "third element" consisted of two groups of cells, oligodendroglia, the cells of which he called oligodendrocytes, and microglia.

Astrocytes are subdivided into fibrous and protoplasmic, depending on whether there are fibres in the cell protoplasm. Fibrous astrocytes are found in the white matter of the central nervous system and protoplasmic occur in the grey matter. Both types have expansions passing in all directions and branching frequently. These expansions are attached to blood vessels. The expansions of the fibrous astrocytes tend to be straighter and larger than those of the protoplasmic astrocytes. Astrocytes

have numerous intracellular structures the details of which help to distinguish them, but they can be identified because of their large size and the ease with which their cytoplasm stains. They also have a larger, paler nucleus than the other neuroglial cells.

Held (1903) is quoted by Penfield as being the main supporter of the theory that the fibres produced by the astrocytes form a syncytium distinct from the astrocytes themselves, although Huber (1901) had already written about this. Hardesty (1904) agreed with this conception of an extracellular network in the central nervous system, and this theory has more recently been revived by Andrew & Ashworth (1944 a & b) who described two types of fibres, extracellular and neuroglial. They claimed that the extracellular fibres form investing covers for the blood vessels and are similar to the fibres of collagenous tissue. Bairati (1947) described a glial substance in the white matter of the central nervous system apart from the astrocyte elements. Most workers do not agree that the neuroglial network is separate from the cells which produce it.

Penfield (1932) described several types of astrocytes found in different parts of the nervous

system but for the purpose of this work the only important ones are the fibrous astrocytes.

Although Rio-Hortga differentiated oligodendroglia from microglia, it should be noted that Robertson (1900) described the same cells much earlier and called them mesoglia. The oligodendrocyte has a smaller body and nucleus than the astrocyte. Its prolongations do not end in relation to blood vessels. They have, however, expansions extending from the cell body. They are found closely applied to nerve cells and nerve fibres, and those associated with nerve fibres are called interfascicular oligodendrocytes. These are the important cells in this study. They form rows between the nerve fibres and are closely applied to the myelin sheath round which they form a loosely woven network. Rio-Hortega described four types of oligodendrocytes according to the differences in their expansions. The largest of these, which are found related to the largest type of nerve fibre in the central nervous system, bear a marked resemblance to the Schwann cells of peripheral nerves. Whether or not oligodendrocytes of this type are homologous with Schwann cells it is difficult to say. The evidence related to this may be considered here. They occupy the same position with regard to the myelin

sheath and their appearance suggests a similarity in structure. None of the authors who describe the nodes in the myelin sheaths in the central nervous system suggested that there is one oligodendrocyte for each node comparable with the single Schwann cell found associated with each node in a peripheral nerve.

Functionally both have been associated with the myelination of nerve fibres. Speidel (1932) correlated the movements of sheath (Schwann) cells with myelin sheath formation. More recently Peterson (1950) found that spinal ganglion sheath cells, which have the same origin as Schwann cells, develop myelin sheaths round axon sprouts in tissue cultures. The evidence linking up the oligodendrocytes with myelination is based to some extent on diseases associated with demyelination (Collier & Greenfield, 1924; Greenfield, 1933; Brain & Greenfield, 1950) in which it is claimed that the oligodendrocytes disappear before the degeneration of the myelin. Penfield (1924) made the statement that oligodendrocytes are associated with myelin formation because they first appear in large numbers at the time of myelination, they are not seen before myelination begins and at this period their protoplasmic granules are unusually

large and numerous. Lumsden (1950) suggested that in demyelinating diseases the oligodendrocyte is destroyed and releases an enzyme which destroys the myelin. Brain & Greenfield (1950) on the other hand put forward the theory that the abnormal myelin may affect the oligodendrocytes in these diseases.

Hörstadius (1950), summing up the evidence regarding the origin of the Schwann cells, concluded that there is general agreement that they come from the neural crest, although there is some doubt whether the rest of the spinal cord also makes a contribution (Detwiler, 1937; Raven, 1937; Jones, 1939; Detwiler & Kehoe, 1939). Oligodendrocytes have an ectodermal origin developing from the ectoderm which forms the neural tube and neural crest.

One would have expected that studies of the reactions of Schwann cells in degenerating peripheral nerve and of oligodendrocytes in degenerating fibre tracts in the white matter of the central nervous system would give information showing a possible similarity between these two cells. Unfortunately the interpretation of the experiments on the central nervous system varies so much that no conclusions can be drawn. Briefly it can stated that in degenerating myelinated peripheral nerves

Schwann cells multiply. That they are phagocytic is now generally accepted, although Weiss (1944,, who found that in in vitro experiments sheath (Schwann) cells became macrophages which were phagocytic, refused to commit himself with regard to whether Schwann cells in adult degenerating nerve behave in the same way. With regard to the reaction of oligodendrocytes in degenerating fibre tracts and elsewhere in the nervous system there are two completely different schools of thought. One states categorically that these cells neither multiply nor phagocytose, and the other that they do both. This will be considered in detail in the section devoted to former studies on the degeneration of the white matter and fibre tracts in the central nervous system.

Before leaving the description of astroglia and oligodendroglia it should be pointed out that some authors do not accept what may be termed the Penfield-Rio-Hortega classification. Andrew & Ashworth (1945) described two types of oligodendroglia, cells without processes which they call adendroglia and cells with processes. They claimed that Cajal described this adendroglia in 1920 and 1923. Bairati (1948 a & b; 1950) wanted to reclassify the neuroglia because there are no real structural differences between astrocytes and oligodendrocytes. He described three types of cells in neuroglia; the first includes protoplasmic

astrocytes and types three and four in Rio-Hortega's classification of oligodendrocytes; the second, types one and two in Rio-Hortega's classification and the third, fibrous astrocytes. It is interesting to note that even Rio-Hortega (1944) stated that oligodendrocytes can develop into an astrocytic type of cell, but added that this is found only in tumours where there are no nerve fibres. Bairati in his classification pointed out that the differences in neuroglial cells are due to the interstitial space between "nerve elements". D'Agata (1950) suggested that astroglia, oligodendroglia and microglia are all one type of cell with different morphological characters. Meanwhile he wanted to retain Rio-Hortega's classification and expressed disapproval of Bairati's.

As has already been pointed out it was Rio-Hortega in 1921 who distinguished in Cajal's third element, two types of cells which had different morphological appearances and differed functionally and embryologically. He called these cells dendroglia and microglia. He stated that microglia is of mesodermal origin and can migrate and become phagocytic. It is found in both grey and white matter, but more especially in the former. Rio-Hortega (1932) summarised his concept of the microglia in the following way. Morphologically it consists of cells with scanty cytoplasm which has a few branched

processes ending freely. The nucleus is small and dark. The cells lie in the framework of fibres formed by the astroglia and oligodendroglia. Functionally he regarded these cells as part of the reticulo-endothelial system and maintained that they become actively phagocytic when necessary, for example, during degeneration of nerve cells or nerve fibres. Consequently they become active in disease and he claimed that they are the only source of such phagocytic cells. Lastly they arise from the meninges at a late stage in foetal life, that is, they are mesodermal in origin, unlike the astroglia and oligodendroglia which are ectodermal in origin. Since more recent work on the origin of the pia mater claims that it is derived from the ectoderm forming the neural crest, Rio-Hortega's theory regarding the origin of the microglia must be reconsidered. Although Rio-Hortega himself listed the authors who agree and disagree with him regarding the origin of the microglia, it appears from the literature that the main disagreement is not regarding its origin but rather regarding its function in relation to degeneration in the central nervous system. Associated with this disagreement is the problem of the functions of the astrocytes and oligodendrocytes in the same circumstances.

Rio-Hortega pointed out that the microglia in the tracts in the spinal cord is relatively scarce as compared with that in the cerebrum and cerebellum. In the tracts the cells tend to lie in the direction of the nerve fibres with prolongations at right angles to the fibres.

Penfield & Cone (1950) gave a table with helps to classify the neuroglia. This table is partly reproduced in Table II.

TABLE II.

Classification of the interstitial cells of the central nervous system

Normal forms

Neuroglia	Astrocytes	(a) protoplasmic (grey matter)
		(b) fibrous (white matter)
	Oligodendroglia	(a) perineuronal satellites
		(a) perineuronal
		(b) interfascicular (white matter)
Microglia	Ubiquitous	

(b) Connective tissue cells.

Throughout the spinal cord there are fibrous tissue septa passing from the meninges on the surface of the cord towards the grey matter. These septa

contain fibrocytes and macrophages and may well constitute an important source of macrophages in conditions in which degeneration of the fibre tracts takes place. The most obvious septum is the postero-median between the two posterior columns.

(c) Blood vessels.

In the walls of blood vessels are found endothelial and smooth muscle cells. It is possible that the number of blood vessels may increase during degeneration and thus they must be considered as a possible source of any increase in nuclei in a degenerated area. Blood vessels however are few in the white matter.

To summarise, the cells found in normal posterior columns of the spinal cord are

(a) fibrous astrocytes;

(b) oligodendrocytes;

(c) microglial cells;

(d) connective tissue cells - fibrocytes and macrophages;

(e) blood vessel cells - endothelial and smooth muscle cells.

C. Changes in the white matter of the central nervous system due to its degeneration.

Degenerative changes have been produced by many different experimental methods and many workers have

drawn conclusions from one type of experiment and applied them to all kinds of degeneration without sufficient justification. The following are the methods used:

(1) Experiments in which the spinal cord has been cut and the resulting degeneration of fibre tracts studied at different time intervals (included in these is the experiment of removing half the cerebral cortex and studying the changes in the affected pyramidal tract).

(2) Experiments in which some part of the central nervous system is injured and the reaction of the surrounding tissues is studied at different time intervals. In these experiments it is usually the grey matter which is studied although sometimes the white matter is observed. Since similar cells are found in both grey and white matter, for example, oligodendrocytes, it is assumed by the authors that these cells react in the same way in both types of nervous tissue. There is also some doubt whether the first experiments, that is, cutting the spinal cord, are comparable with the experiments referred to in this section.

(3) Studies of diseases, sometimes experimentally produced, including tumours of the central nervous system. In these conditions the demyelinating

diseases, in which fibre tracts degenerate and disappear, are the obvious ones to be considered. Again one wonders whether the pathology of these diseases can be compared with that of degeneration of fibre tracts caused by cutting the cord. In these diseases continuity of the axons is maintained with neurones for a long time, the process of demyelination is slow and is the cause of the ultimate disappearance of the axon and the central nervous system is studied many years after the onset of the disease.

1. Experiments causing degeneration of fibre tracts.

If a peripheral nerve is interrupted, there takes place in the peripheral stump a process which is known as Wallerian degeneration. Briefly, this consists of a disappearance of the axons and myelin sheaths and a multiplication of the cells in the stump. Similarly if the fibres of a tract in the spinal cord are severed from their neurones there takes place a degeneration of the axons and myelin sheaths. What happens with regard to the number of the cells is not fully known. The only experiment of this nature was that of Lassek & Shapiro (1951) who studied the quantitative changes in the neuroglial cells of the pyramidal tract following its degeneration by removal of one half of the opposite cerebral cortex. They found that an increase occurs after 6 days; that

the increase is very variable at different times and averages 27% and that the increase may be only apparent because the affected area shrunk. Most authors, although they do not actually count the cells, refer to their increase in the course of degeneration although Halliburton (1907) specifically mentioned that the cells do not increase and maintained that regeneration does not occur in the spinal cord for this reason.

Qualitative studies of the cell changes on the other hand have been made by other workers. Jakob (1914) experimented on rabbits in which he sectioned the spinal cord and studied the changes in the white matter at different levels after varying intervals. His qualitative experiments have been repeated chiefly because of the controversy regarding the source of the macrophages which appear during degeneration. Jakob maintained that the oligodendrocytes could become phagocytic as well as the microglia. In fact he attributed a similar role to the astrocytes. Robertson (1900) suggested that what he termed mesoglia, now recognised as the same as the oligodendroglia, became phagocytic in the degenerating white matter. He has been mentioned in this section because he has one of the earliest references to this problem.

Jakob described five types of cell which appeared in the course of degeneration of fibre tracts, the myeloclast which breaks down the axon and myelin, the myelophage which acts on the products of the first stage and the "gitter" cells, which represent three stages in the changes in the fat in the myelophages before the broken down products of the myelin are transferred to the blood vessels. According to Jakob these "gitter" cells arise from all the cells in the neuroglia, including astrocytes and oligodendrocytes.

The next reference to degeneration in the fibre tracts of the spinal cord was made by Cajal (1928) who dealt chiefly with the changes in the vicinity of the wound. He maintained that both the microglia and the "satellite" cells, that is, the oligodendroglia, are phagocytic and compared the action of these latter cells with thst of the Schwann cells in degenerating peripheral nerves. He stated that the largest tubes degenerate first and that the neuroglia proliferates in columns. These studies were confined to the earliest stages of degeneration (a few days) following section of the spinal cord in young dogs, cats and rabbits.

Cramer & Alpers (1932) hemisectioned the spinal cord of rabbits and examined the spinal cords on the

3rd to the 8th day after section and also on the 10th, 12th, 24th, 35th, 52nd, and 78th days. They claimed that in the 3rd to the 5th days the oligodendrocytes next to the degenerating axis cylinder degenerate but those further away proliferate. At this early stage the cells of the microglia remain few but become active. The astrocytes hypertrophy. From the 5th to the 10th days the oligodendrocytes and the microglia, in the area where the nerve fibres are degenerating, also degenerate and the microglia outside invades this area. During this stage the astrocytes encircle the degenerating cells. From the 12th to the 35th days both the oligodendrocytes and microglia phagocytose the degenerating cells. From the 5th to the 8th week "gitter" cells are formed by both the oligodendrocytes and the microglia. They are found in groups and are swollen with fat. At this stage the fat is passed to the astrocytes. Finally the astrocytes form a cicatrix in the areas of degeneration.

Penfield (1932) dealt with many aspects of the neuroglial changes in white matter during degeneration but it is important to sort out the different conditions in which this takes place. He made little or no reference to the type of degeneration which is being

considered in this section, that is the degeneration which is comparable to the changes which take place in the peripheral stump of a cut nerve. There is a reference to gliosis, resulting from myelin degeneration such as is found in tabes dorsalis or a patch of disseminated sclerosis, but these diseases are hardly comparable with the demyelination of the posterior columns following section of the cord. Similarly he did not discuss the reaction of the oligodendrocytes to what may be termed acute tract degeneration in the spinal cord. His only reference to these cells in relation to demyelination was to point out that the disappearance of myelin results in a decrease of oligodendrocytes or the transformation of oligodendrocytes into astrocytes.

Rio-Hortega (1932) emphasised the phagocytic nature of the microglia and denied that astrocytes and oligodendrocytes perform this function. Again it should be understood that the various pathological conditions to which he referred do not include the type of degeneration which is dealt with in this thesis.

It can thus be seen that only Jakob, Cajal, Cramer & Alpers, and Lassek & Shapiro have considered the changes which take place in fibre tracts separated from their cell bodies for varying periods up to 100 days.

11. Experiments causing destruction of white matter.

Many workers have studied the early and late effects of direct injury to the brain, and sometimes the spinal cord, and described in detail the cell changes which take place in and around the wound. It is obvious from the literature that there are two schools of thought; those who maintain that in the early stages phagocytes are derived from microglia only and those who claim that oligodendrocytes and perhaps astrocytes can also become phagocytes.

Rio-Hortega & Penfield (1927) described the effects of a stab wound in rabbits' brains. The astrocytes near the wound show clasmato-dendrosis, that is, their processes disappear as a preliminary to their ultimate destruction. Somewhat further away from the wound these cells swell and multiply by amitotic division. Finally these cells are responsible for the production of the glial scar in the area of the wound. Early on, in the wound itself and at its margins, there is a microglial reaction in which both amoeboid types of cells and compound granular corpuscles are seen. These cells are phagocytic, acting as scavengers of the destroyed cells and fibres.

Penfield & Buckley (1928) carried out experiments

in which they produce puncture wounds of the brain with a hollow and a solid needle and compared the resulting scars. Again it was emphasised that phagocytosis is carried out by the microglia.

Ferraro & Davidoff (1928) on the other hand, using cats, found that trauma of the cerebral cortex produces swelling, hypertrophy and the formation of clusters of oligodendrocytes which form compound granular corpuscles, that is, phagocytic cells.

Cajal (1928) described several kinds of phagocytic cells some of which he suggested come from neuroglia, for example, the rod like cells and the amoeboid cells described by various authors.

Linell (1929) made no reference to oligodendrocytes in his description of the changes following cerebral trauma. He showed that within three days the microglia forms compound granular corpuscles reaching a maximum in six days. They phagocytose the myelin. The astrocytes show slight hypertrophy after three days but their maximal reaction is after three weeks, when they develop processes at right angles to the injury. After four weeks there is a felting of the injured area which develops the appearance of a felted mat after two months. This finally forms a scar.

to react to experimental brain wounds in adult animals are lymphocytes which become macrophages. His paper dealt largely with experiments which showed that it is the microglia which ingests lipids, Indian ink and trypan blue and his conclusions support the theory that phagocytes are derived from microglia and not from oligodendroglia.

Hicks (1947) maintained that microglia produces the phagocytes which are most active during the first week following cerebral injury. They persist for a long time. About the third week, astrocytes proliferate and then lay down fibrils. In demyelinating diseases he suggested that myelin is phagocytosed in the first and second week by microglia, and in the fourth week gliosis takes place by the action of the fibrous astrocytes. Kuhlenbeck (1952) stated that compound granular corpuscles appear after the fourth day following injury to the brain. They come from both microglia and the blood vessel walls. He thought that oligodendrocytes may also form these cells.

Summarising this literature it may be said that in a brain wound, astrocytes not immediately destroyed at the site of the injury undergo clasmatodendrosis as a preliminary to disappearing. The astrocytes near the lesion multiply and form processes which produce a glial scar. The tissue destroyed by the trauma is phagocytosed very quickly as a preliminary

to the formation of the scar. Most authors agree that the microglia performs this function aided by an invasion of similar cells from blood vessels nearby. Some suggest that oligodendrocytes can become phagocytic.

111. Diseases associated with degeneration of fibre tracts.

The first group to be considered are those which affect the posterior columns of the spinal cord, namely tabes dorsalis, subacute combined degeneration of the cord and Friedreich's ataxia. In tabes dorsalis only the posterior columns are involved. In the other two the lateral columns are also involved. Unfortunately the spinal cords of patients suffering from these conditions are usually seen after the demyelination has been progressing for years, and the literature does not make reference to the pathological changes found in the early stages.

Blackwood, Dodds & Sommerville (1949) mentioned the gliosis in each of these conditions, pointing out the differences between them. In referring to subacute combined degeneration of the cord they stated that the myelin sheaths show great swelling and subsequent degeneration together with degeneration of the axons. The myelin is absorbed by phagocytes and large clear spaces are left. For some unknown reason there is very little reparative gliosis in this disease, unlike tabes dorsalis and Friedreich's ataxia in which there is dense gliosis.

Hassin's (1948) description of the fibre tracts in subacute combined degeneration suggested that the degeneration is rapid and consequently the cell changes in the tracts are very marked. He described myelophages and different types of "gitter" cells (Jakob's name for phagocytes or compound granular corpuscles) taking part in the phagocytosis of the degenerating myelin. Biggart (1949) compared the absence of glial proliferation in this disease with the absence of proliferation of Schwann cells in peripheral neuritis associated with vitamin B_1 deficiency. This however suggests that the cells forming glia, the astrocytes, are comparable with the Schwann cells, and this is unlikely.

There are many other diseases in which fibre tracts are affected, for example, disseminated sclerosis, optic neuromyelitis, diffuse cerebral sclerosis (this disease has several other names according to Wilson, 1947) and it was hoped that description of the pathology of these diseases could be correlated with the changes found in the posterior columns which degenerated after cutting the cord. Hassin (1948) gave details of the cell changes in some of these conditions but his most important contribution was the suggestion that the phagocytes vary in their origin according to the type of disease, in vascular

disturbances the phagocytes are microglial, in "secondary degeneration" they are neuroglial, and in "degenerative softening" they arise from both sources.

IV. Summary of section C.

In all conditions involving degeneration of the axons and myelin sheaths of nerve fibres in the central nervous system, two processes to a greater or less extent take place (a) phagocytosis of degenerated material (b) formation of a glial scar. During phagocytosis the cells are called compound granular corpuscles or "gitter" cells ("rod" cells are a variation of these cells).

What is the source of these cells? One school maintains that only microglia can produce phagocytes and the other that oligodendroglia can also do this. One gets the impression from the literature that some of the differences of opinion arise because the various workers were studying different conditions. Is a brain wound comparable with the degeneration of fibre tracts after cutting the cord? Is fibre tract degeneration after cutting the cord comparable with the demyelinating diseases? Are all the diseases in which fibre tract degeneration occurs comparable with one another? Is degeneration following vascular changes comparable with a toxic change? Are acute vascular changes comparable with chronic

vascular changes? When the questions are put in this way one realises that Rio-Hortega (1939) may have been too emphatic about the utter impossibility of any cell other than one derived from microglia causing phagocytosis and that Andrew (1941) for example may be right in claiming that in certain specific circumstances perineuronal oligodendrocytes may destroy neurones.

There is no controversy about the formation of the glial scar. This is brought about by the fibrous astrocytes. They multiply and send out processes which form a felting which replaces the destroyed nerve fibres.

2. MATERIAL AND METHODS.

A. Material.

Adult rabbits of mixed stock were used. The rabbit was anaethetised with intravenous nembutal (25mg. per kg.) and ether was administered, as required, by inhalation. A vertical midline incision was made in the back centred over the most prominent vertebral spine. This was estimated to be in the region of the last cervical and first thoracic vertebrae. The vertebral muscles were separated from the spinous processes and laminae for a distance of about 2.5 cm. The spinous process and laminae of the most prominent vertebra were removed and the cord with its meninges exposed. The meninges were cut traversely and the cerebrospinal fluid allowed to escape. The midline of the cord was easily identified by a vertically running blood vessel and a transverse incision was made with a Graafe knife on one or other side of the midline through what was estimated to be one posterior column. This incision was usually about 2.5 mm. wide and 2 mm. deep. The lips of the wound separated for about 1.5 mm. The muscles were brought together over the defect in the vertebrae and the incision in the skin closed.

Under anaesthesia a biopsy was performed on the rabbits 10, 20, 50, and 100 days after the

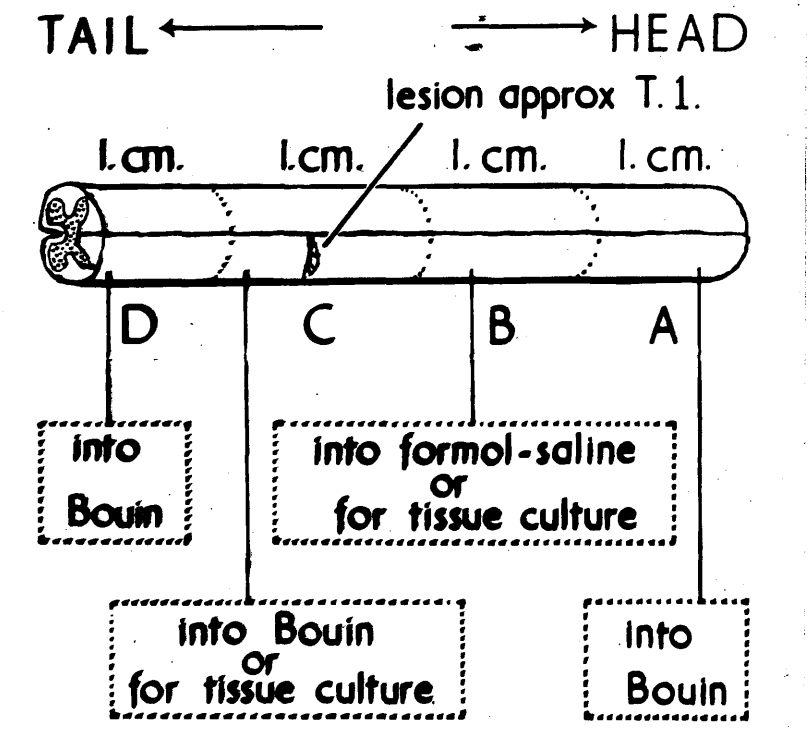


Fig. 2.

Diagram showing site of operation and way in which the spinal cord was divided and fixed.

operation. 4 cm. of the spinal cord, so that there were 2.5 cm. above the site of the lesion, were taken out and the meninges carefully removed with scissors. The cord was divided into 4 pieces each 1 cm. long. The most rostral piece (A) was fixed in Bouin. In at least two of each group of rabbits 1 cm. of the spinal cord immediately caudal to the first piece (B) was fixed in formal saline. In the others it was used for tissue culture. 1 cm. of the spinal cord containing the site of the lesion (C) was taken either for tissue culture or fixed in Bouin and 1 cm. caudal to this (D) was fixed in Bouin (see Fig. 2). A considerable number of normal cervical spinal cords were obtained and divided into four pieces (A, B, C, and D) each 1 cm. long. These corresponded to the pieces taken from the operated animals and were fixed or used for tissue culture in the same way.

B. Histology.

Segment A was embedded in paraffin wax and transverse sections were cut at 5μ from each end. At least one segment A from each group of animals was sectioned longitudinally at 7μ. One slide of transverse sections was stained with haematoxylin and eosin and used for counting nuclei and one by Bodian's technique for axon staining. One slide of longitudinal sections was stained with haematoxylin and eosin. The Bodian

stained sections were used for confirming the degeneration of the axons.

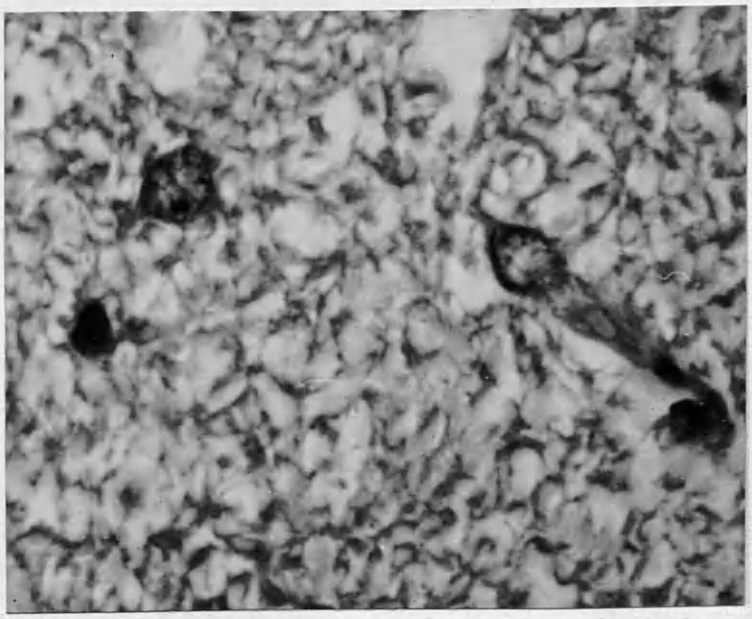
Segment B was used for the demonstration of myelin by Weigert-Pal's method. Transverse sections at 10μ were cut and then longitudinal sections at 10μ. These were used to demonstrate the degeneration of the myelin of some part of the posterior columns.

C. Counting of nuclei.

A squared grid was placed in the microscopic eyepiece and the $\frac{1}{6}$" objective used. The number of nuclei seen in a field was counted. In the normal spinal cords at least 10 fields were taken and the mean number of nuclei per field calculated, since in the posterior columns different areas contain different numbers of nuclei. In the degenerated spinal cords the nuclei in as many fields as possible in both the normal and degenerated areas were counted and the mean number of nuclei per field was found. This was done at two levels of each spinal cord.

D. Area of posterior columns and length of nuclei.

The area of the posterior columns was measured by means of the grid and a 1" objective. The unit used was the single square in the grid. This was done because the number of nuclei per field can be



10 μ

Fig. 3.

Transverse section of cervical spinal cord, stained haematoxylin and eosin, showing typical large pale nucleus of two astrocytes. The nucleus of one has a well marked nucleolus and the other has a considerable amount of cytoplasm.

changed by swelling or contraction of the posterior columns, without any actual change in the number of nuclei. The length of the nuclei, as was shown by Abercrombie (1946), also influences their number in a transverse section. Using longitudinal sections stained with haematoxylin and eosin samples of the length of the nuclei in the normal and degenerated areas were taken and compared.

E. Differential counting of nuclei.

In the sections stained with haematoxylin and eosin two types of cells were easily distinguished, large cells with a large pale nucleus and a nucleolus (these cells frequently had well stained cytoplasm) and smaller cells with a smaller, more darkly staining nucleus, no nucleolus and practically no stained cytoplasm (see Fig. 3). These two types of cells were differentiated in all sections and counted. The percentage of each type of cell in both the normal and degenerated areas was calculated.

Although both connective tissue and blood vessel cells contribute to the number of nuclei counted, careful examination of the sections showed that blood vessels were seldom seen in the normal and degenerated areas of the posterior columns and there was little evidence that connective tissue cells occurred in significant numbers. It was therefore assumed that

the nuclei counted belonged almost entirely to neuroglial or microglial cells.

3. RESULTS.

A. Number of nuclei per field in normal posterior columns.

Table 111 shows the number of nuclei per field at 2 levels in 15 animals and the total area of the posterior columns at each level.

TABLE 111.

Rabbit number	Level	No.of nuclei per field	Area of posterior columns (in arbitary units).
1029	1	25.00	25
	2	28.00	20
1054	1	20.50	21
	2	24.00	20
1062	1	22.58	22
	2	27.80	28
1069	1	20.73	25
	2	16.91	21
1453	1	20.40	22
	2	25.20	20
1456	1	28.33	22
	2	26.33	20
1457	1	24.63	21
	2	25.30	24
1498	1	30.70	20
	2	23.63	21
1502	1	18.20	27
	2	13.50	33
1560	1	16.00	22
	2	16.67	26
1598	1	21.75	27
	2	15.43	33
1651	1	24.14	22
	2	21.91	24

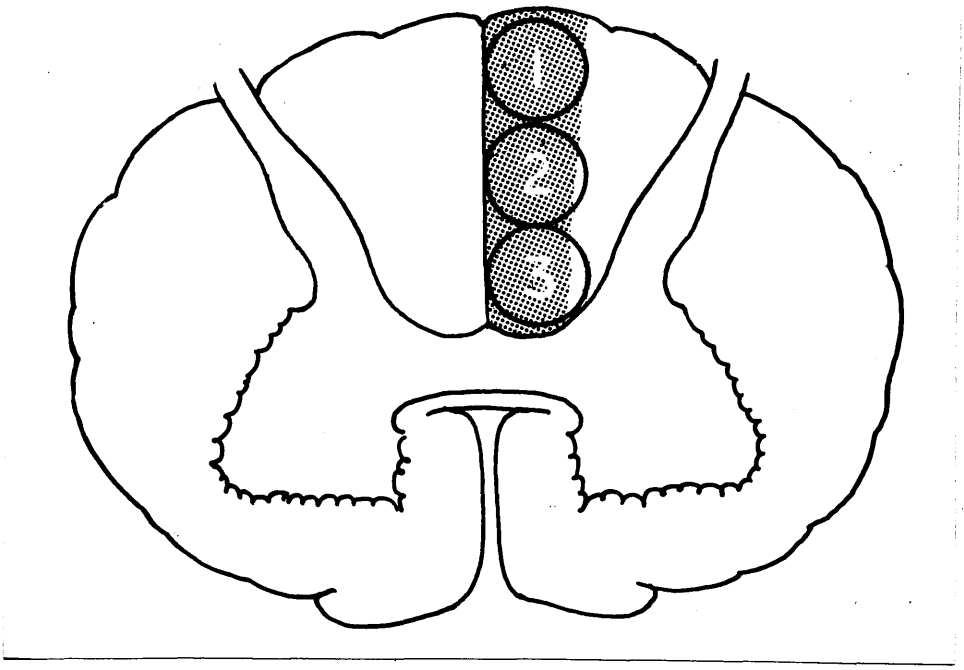


Fig. 4.

Diagram of cervical spinal cord showing degenerated area (shaded); 1, 2, 3 are degenerated fields; note that fields 2, 3 include a small normal area.

1670	1	25.33	23
	2	25.75	24
1723	1	21.83	23
	2	17.17	32
1761	1	19.60	24
	2	25.90	23
Total 15 rabbits	Total 30 levels	Mean 22.47 S.D. 4.73	Mean 23.83 S.D. 3.67

It can be seen from Table 111 that the number of nuclei per field does not vary to any extent with the level of the spinal cord. Furthermore there is very little difference between rabbits.

B. Number of nuclei per field after 10 days' degeneration.

The area of degeneration in the posterior columns varied considerably. It was usually to one side of the midline and did not extend over the whole of one posterior column. It was not sharply marked off from the surrounding normal area although it was easy to see where the degenerated area changed to the normal area. The Bodian stained section was used to check the area of degeneration. Frequently however, a field, which was regarded as degenerated, contained a small area of normal fibres (see Fig. 4). This applies to all the cords after the different periods of degeneration.

Table 1V shows the number of nuclei per field at two levels in the normal and degenerated areas of the

100μ

Fig. 5.

Transverse section of cervical spinal cord, stained haematoxylin and eosin, showing nuclear changes after 10 days' degeneration.

posterior columns of 9 rabbits after 10 days' degeneration and also the total area of the posterior columns at each level.

TABLE IV.

Rabbit number	Level	Nuclei per field Normal	Nuclei per field Degenerated	Area of posterior columns (in arbitrary units)
1382	1	33.20	46.38	20
	2	33.14	51.80	19
1473	1	24.00	55.50	28
	2	25.50	65.25	19
1497	1	27.17	60.20	19
	2	28.17	61.50	17
1574	1	13.80	33.50	28
	2	20.33	43.00	25
1575	1	17.00	28.33	32
	2	12.17	25.33	30
1620	1	11.38	29.33	27
	2	22.00	36.33	16
1729	1	11.20	25.33	38
	2	14.75	25.75	36
1730	1	12.20	23.75	40
	2	13.00	29.25	35
1732	1	20.67	35.25	29
	2	18.50	38.00	27
Total 9 rabbits	Total 18 levels	Mean 20.50 S.D. 7.04	Mean 38.06 S.D. 14.45	Mean 26.94 S.D. 7.60

After 10 days' degeneration if one compares the number of nuclei per field in the degenerated area with the number in the normal area, it can be concluded that there is an increase. This increase is of the order of 1.8 times the normal number (see Fig. 5) although it varies in different rabbits (from 1.5 to 2.7 times

the normal).

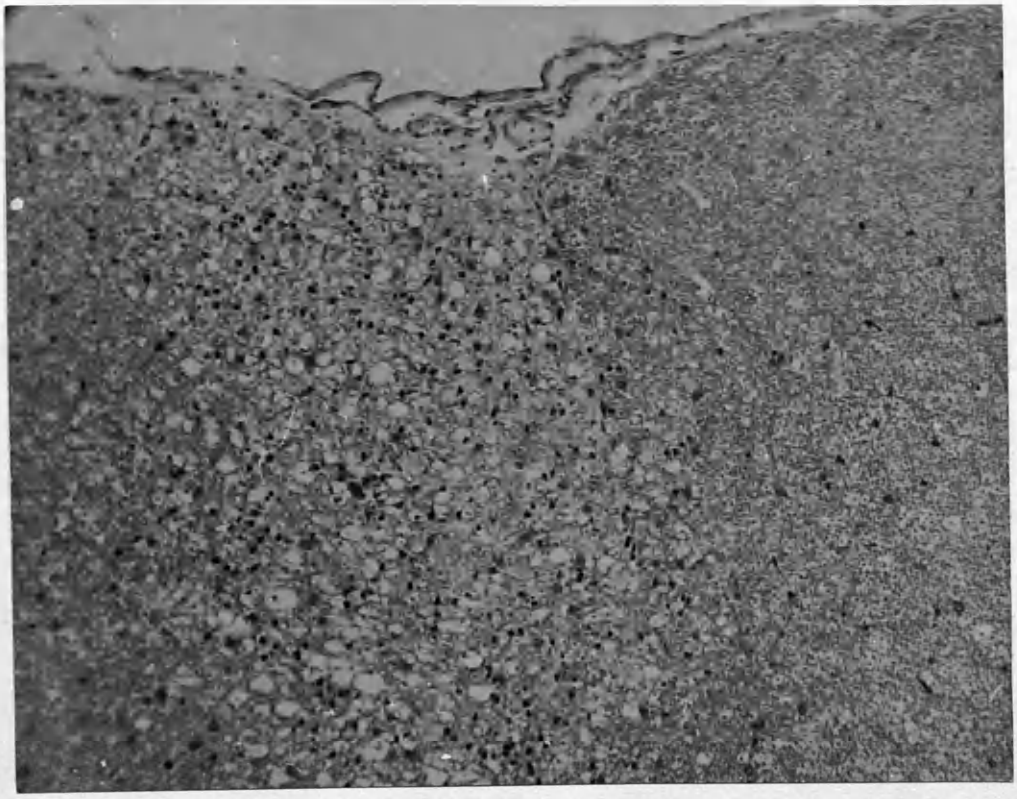
There is another way of assessing this difference. The mean number of nuclei per field in the normal areas is 20.50 and in the degenerated areas is 38.06. By using the t test, these means are compared and their difference is found to be highly significant ($p < 0.001$).

C. Number of nuclei per field after 20 days' degeneration.

Table V shows the number of nuclei per field at two levels in the normal and degenerated areas of the posterior columns of 10 rabbits after 20 days' degeneration and also the total area of the posterior columns at each level.

TABLE V.

Rabbit number	Level	Nuclei per field		Area of posterior columns (in arbitrary units)
		Normal	Degenerated	
1052	1	23.00	53.50	24
	2	22.80	55.00	24
1053	1	19.11	49.00	24
	2	22.80	69.20	27
1074	1	27.14	109.50	19
	2	25.50	110.50	20
1075	1	23.00	92.60	24
	2	35.00	136.00	17
1383	1	19.17	52.50	23
	2	24.29	57.25	21
1384	1	24.33	85.73	23
	2	23.80	76.17	22
1392	1	23.80	60.40	20
	2	24.57	55.00	19
1435	1	20.00	56.00	20
	2	25.00	61.00	20



100 μ

Fig. 6.

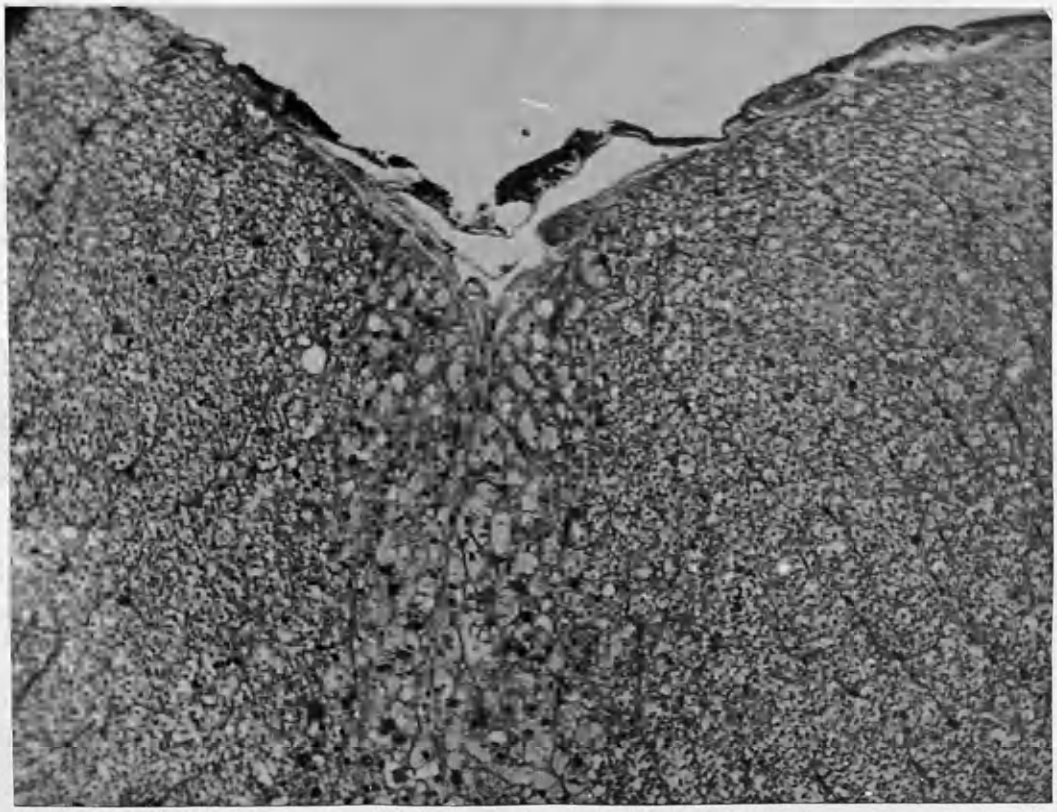
Transverse section of cervical spinal cord, stained haematoxylin and eosin, showing nuclear changes after 20 days' degeneration.

1436	1	16.00	50.66	25
	2	14.86	38.66	21
1471	1	19.33	51.25	22
	2	18.00	48.00	24
Total 10 rabbits	Total 20 levels	Mean 22.75 S.D. 4.50	Mean 68.40 S.D. 29.17	Mean 21.95 S.D. 2.38

It can be seen from Table V that after 20 days' degeneration there is an increase in the number of nuclei in the degenerated area as compared with the number of nuclei in the normal areas of the posterior columns. This increase is of the order of 3 times the normal (see Fig. 6) varying from 2.5 to 4.5 times the normal. By using the t test, the mean number of nuclei per field in the normal areas (22.75) can be compared with the mean in the degenerated areas (68.40). The difference between these means is highly significant ($p < 0.001$).

D. Number of nuclei per field after 50 days' degeneration.

Table VI shows the number of nuclei per field at two levels in the normal and degenerated areas of the posterior columns of five rabbits after 50 days' degeneration and also the total area of the posterior columns at each level.



100 μ

Fig. 7.

Transverse section of cervical spinal cord, stained haematoxylin and eosin, showing nuclear changes after 50 days' degeneration.

TABLE V1.

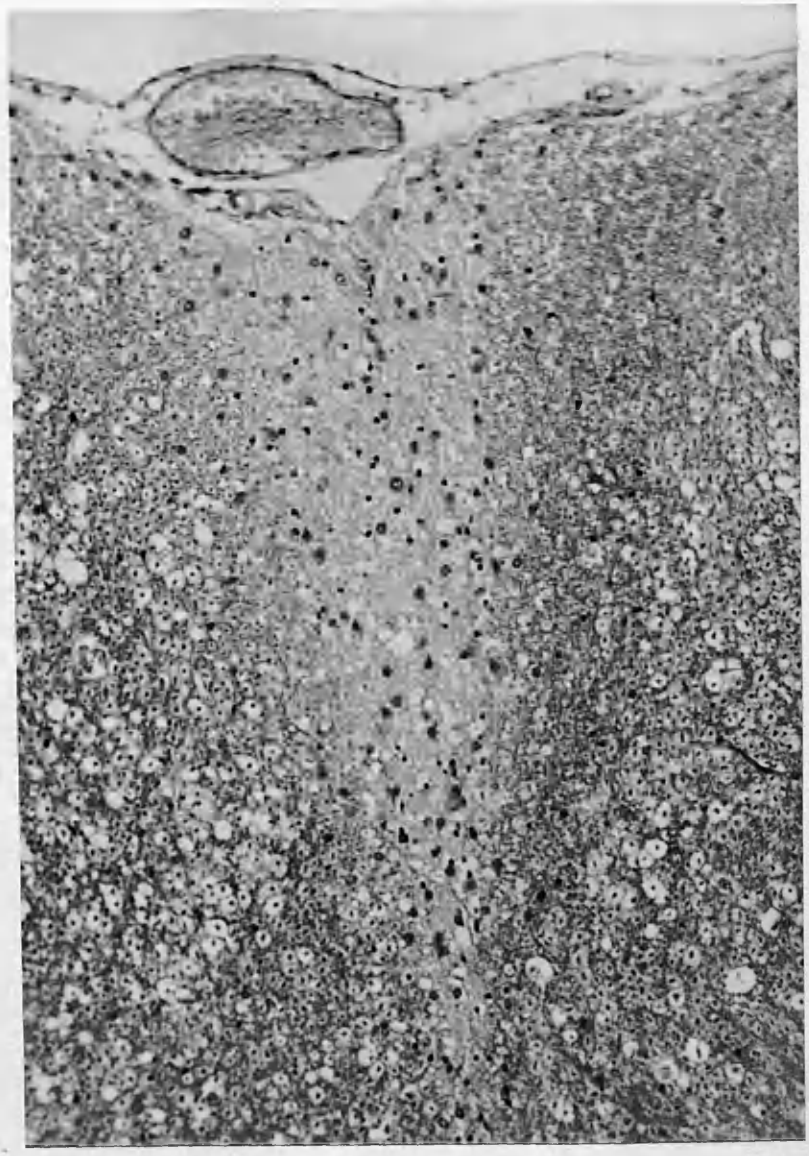
Rabbit number	Level	No. of nuclei per field Normal	Degenerated	Area of posterior columns (in arbitrary units)
1483	1	14.60	33.80	22
	2	18.60	41.00	29
1484	1	21.17	65.00	24
	2	16.67	64.75	24
1485	1	20.20	69.80	31
	2	15.37	79.60	31
1501	1	17.80	45.00	30
	2	14.20	31.75	30
1556	1	18.60	78.67	23
	2	24.20	98.67	24
Total 5 rabbits	Total 10 levels	Mean 18.14 S.D. 3.08	Mean 60.80 S.D. 19.23	Mean 26.80 S.D. 3.58

It can be seen from Table V1 that after 50 days' degeneration there is an increase in the number of nuclei in the degenerated areas as compared with the number in the normal areas. This increase is of the order of 3.5 times the normal (see Fig. 7) varying from 2.3 to 5.3 times the normal.

If the means of the normal and degenerated areases are compared by using the t test (18.14 and 60.80 respectively) the difference is found to be highly significant ($p < 0.001$).

E. Number of nuclei per field after 100 days' degeneration.

Table V11 shows the number of nuclei per field at two levels in the normal and degenerated areas of the posterior columns of 4 rabbits after 100 days' degeneration and also the total area of the posterior



100 μ

Fig. 8.

Transverse section of cervical spinal cord, stained haematoxylin and eosin, showing nuclear changes after 100 days' degeneration.

columns at each level.

TABLE V11.

Rabbit number	Level	No. of nuclei per field Normal	Degenerated	Area of posterior columns (in arbitrary units)
1443	1	11.86	72.50	28
	2	13.36	88.50	25
1444	1	20.50	53.33	25
	2	15.00	63.00	26
1452	1	15.38	72.67	26
	2	15.13	54.00	23
1463	1	18.63	81.75	22
	2	14.86	63.00	27
Total 4 rabbits	Total 8 levels	Mean 15.59 S.D. 2.62	Mean 68.59 S.D. 12.12	Mean 25.25 S.D. 1.87

It can be seen from Table V11 that after 100 days' degeneration there is an increase in the number of nuclei in the degenerated areas as compared with the number in the normal areas. This increase is of the order of five times the normal (see Fig. 8) varying from 2.6 to 6.5 times the normal.

If by using the t test the mean (15.59) of the normal areas is compared with the mean (68.59) of the degenerated areas, the difference between the means is found to be highly significant ($p < 0.001$).

F. Area of posterior columns of all spinal cords studied.

Table V111 shows the means and standard deviations of the total areas of the posterior columns of the spinal cords in both the normal animals and after different periods of degeneration. (The individual areas have been given in the last columns of Tables 111-V11).

TABLE V111.

Period of degeneration	Area of posterior columns (in arbitrary units) Mean	Standard deviation	Number of levels
nil	23.83	3.67	30
10 days	26.94	7.60	18
20 days	21.95	2.38	20
50 days	26.80	3.58	10
100 days	25.25	1.87	8

The means of the areas of the posterior columns after 10, 50 and 100 days' degeneration are not significantly different from one another. By means of the t test, it can be shown that the mean of the areas of the normal rabbits is probably signficantly different from the other means (p lies between 0.05 and 0.01) and that the mean of the areas of the 20 day rabbits is signficantly different from the means of the other areas (p = 0.01). These differences may be due to shrinkage but it seems unlikely that the columns swell up after 10 days' degeneration, shrink after 20 days', swell again after 50 days' and remain in that state after 100 days'. Slightly different levels of the cord could account for these differences. Even if shrinkage and swelling were the cause, the differences would not materially affect the results. In any case, comparisons in the nuclear counts have been made between the normal and degenerated areas within each cord or group of cords in estimating the increase of nuclei.

G. Length of nuclei in normal and degenerated areas.

Table IX shows the mean length of 150 nuclei in both the normal and degenerated areas of the different groups of rabbits.

TABLE IX.

Period of degeneration	Mean length of nucleus (in μ) Normal	Degenerated
nil	6.79	-
10 days	6.10	6.60
20 days	7.34	6.73
50 days	7.18	6.57
100 days	7.30	6.67

The small differences between the mean lengths of the nuclei in the normal and degenerated areas can be ignored when comparing the number of nuclei in the transverse sections of the different rabbits, since Abercrombie & Johnson (1946) showed that the correction factor for nuclear counts due to differences in nuclear length in sections of equal thickness is $\frac{a}{b}$ when a is the mean length of the nucleus in undegenerated nerves and b the mean length of the nucleus in degenerated nerves.

H. Number of nuclei in normal cords compared with number in normal areas of degenerated cords.

Table X shows the number of nuclei per field in the posterior columns of normal cords and in the normal

areas of these columns after different periods of degeneration.

TABLE X.

Period of degeneration	Number of nuclei per field (normal) Mean	Standard deviation	Number of levels
nil	22.47	4.73	30
10 days	20.50	7.04	18
20 days	22.75	4.50	20
50 days	18.14	3.08	10
100 days	15.59	2.62	8

There is no significant difference between the number of nuclei in normal posterior columns and the number in normal areas after 10 and 20 days' degeneration. By using the t test, it can be shown that after 50 and 100 days' degeneration, the number of nuclei per field in the normal areas of these cords is significantly different from the number in normal posterior columns. If the normal cords are compared with the 50 day cords, p is found to be < 0.002 and > 0.001. If the normal cords are compared with the 100 day cords p is < 0.001. Thus it can be concluded that the nuclei in the normal areas of the posterior columns of 50 and 100 day cords decrease in number as compared with the number found in normal cords. The possible explanation of this will be discussed later.

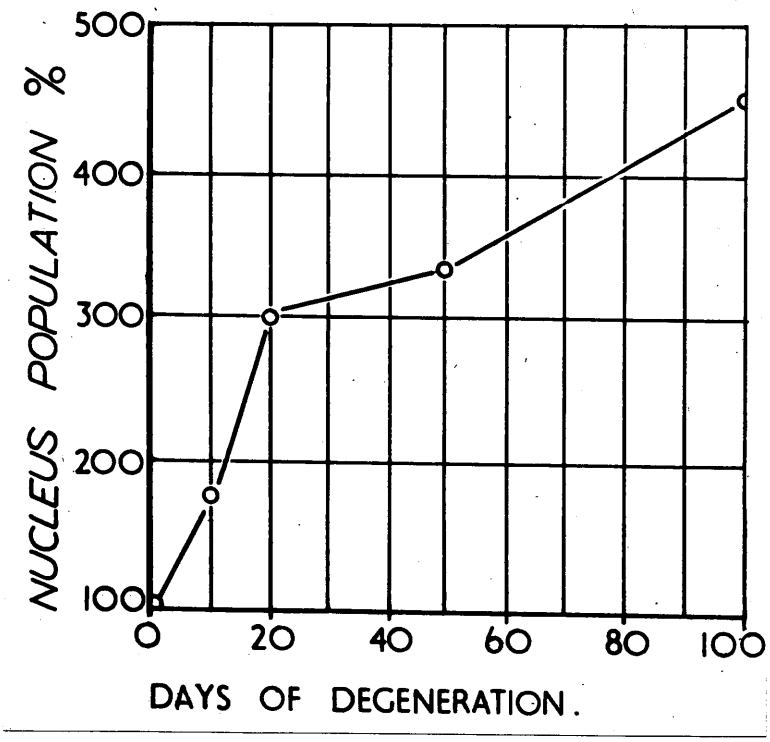


Fig. 9.

Graph to show mean nuclear populations per field after different periods' of degeneration expressed as percentage of that of undegenerated posterior columns.

I. Number of nuclei in normal areas compared with number in degenerated areas after different periods of degeneration.

Table XI shows the means and standard deviations of the number of nuclei per field in the normal and degenerated areas of the posterior columns after different periods of degeneration.

TABLE XI.

Period of degeneration	Number of nuclei per field Normal Mean	Normal Standard deviation	Degenerated Mean	Degenerated Standard deviation	Estimated increase after degeneration
Nil	22.47	4.73	-	-	-
10 days	20.50	7.04	38.06	14.45	1.8x
20 days	22.75	4.50	68.40	29.17	3x
50 days	18.14	3.08	60.80	19.23	3.4x
100 days	15.59	2.62	68.59	12.12	4.5x

It can be seen that up to 100 days, the number of nuclei is increasing, although the rate of increase slows down considerably (see Fig.9). In view of the significant decrease in the number of nuclei in the normal areas of the 50 and 100 day rabbits, the increase in nuclei after 20 days may be more apparant than real. There is certainly no evidence of a decrease. This can be contrasted with peripheral nerves in which a maximum increase of nuclei takes place within 30 days (or much sooner in nerves with a small fibre spectrum) and is followed by a decrease in the number of nuclei

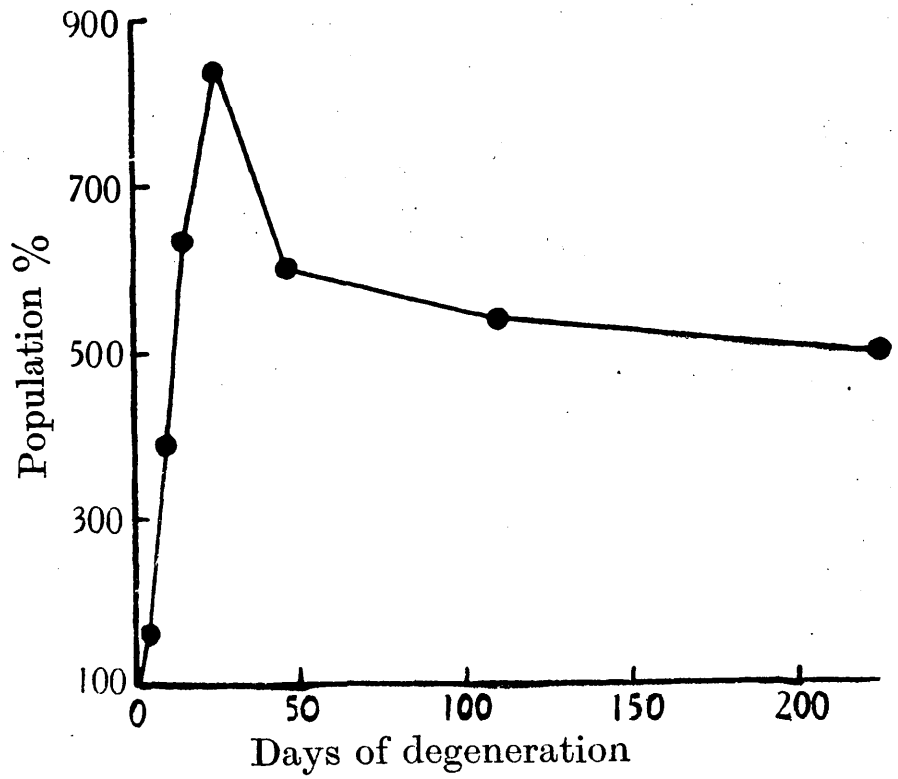


Fig. 10.

Graph to show mean total population of nuclei of nerves at different times of degeneration expressed as percentage of that of undegenerated nerve (from Abercrombie & Johnson, 1946).

(Abercrombie & Johnson, 1946; Thomas, 1948; Joseph, 1950). Fig. 10 is a reproduction of the graph presented by Abercrombie & Johnson in their paper on the nuclear population changes in the sciatic nerve of the rabbit.

J. Differential counting of nuclei.

The nuclei in the posterior columns of the normal cords and in the normal and degenerated areas of the cords in the experimental animals were differentiated as described on p. 31. Those which are large and pale are assumed to belong to astrocytes and the others to belong either to oligodendrocytes or microglial cells. The number of astrocytes was assessed as a percentage of the total cells counted in each section and Table XII gives the mean percentage of these cells in both the normal and degenerated areas of the cords.

TABLE XII.

Days of degeneration	Astrocytes % of total nuclei	
	Normal area	Degenerated area
Nil	21	-
10	20	19
20	17	12
50	17	16
100	17	27

It can be seen that the number of astrocytes in the normal areas is fairly constant. In the degenerated

areas it appears that all the cells have multiplied equally after 10 days, then the astrocytes lag behind so that after 20 days they form a smaller percentage of the total, then after 50 days they catch up on the other cells and finally after 100 days, they show a greater increase than previously. The possible significance of these changes will be considered later.

4. DISCUSSION.

It is proposed to deal with the discussion under the headings suggested on p. 2.

A. Changes in nuclear population after different periods of degeneration.

There can be no doubt that the nuclei of the posterior columns increase in number during degeneration of their fibres. The results show that this increase goes on fairly slowly at first, that is for a period of 10 days, and then more rapidly between 10 and 20 days. Apparently the increase is slowed after that, that is, between 20 and 50, and 50 and 100 days.

It is interesting to note the difference between these results and those of Lassek & Shapiro (1951). They removed most of the cerebral cortex of the left hemisphere including area 4 in cats and studied the quantitative response of the glial cells in the degenerated pyramidal tract in the medulla. They found a varied increase at different times, averaging 27%, they never found an increase of the order described in this thesis and even suggested that the increase may be an illusion due to shrinkage of the cord. Only one animal was studied after each period of degeneration which varied from 1 day to 365 days. The differences between their results and those found in the present investigation may be due to differences between the medulla and spinal cord neuroglia, to their use of

only one animal after each period of degeneration and to the differences between cats and rabbits with regard to neuroglial response. Furthermore it is possible that the whole of the pyramidal tract did not degenerate as a result of the operative procedure carried out. No attempt appears to have been made to check the extent of the degeneration following the operation.

The relationship between the multiplication of the cells in the peripheral stump of the degenerated nerve and regeneration was discussed by Joseph (1948) and it was pointed out in that paper that "it is unlikely that the proliferation of the Schwann cells in degenerating nerve fibres plays as important a role in regeneration as earlier writers suggest". That multiplication of cells occurs in degenerating fibre tracts of the spinal cord in which regeneration apparently does not occur appears to be confirmatory evidence of the fact that regeneration is not related to the proliferation of cells in the nervous system.

Other workers have not tried to assess the quantitative changes of the nuclei during degeneration of spinal cord tracts, although it is obvious from their work that many of them noticed an increase.

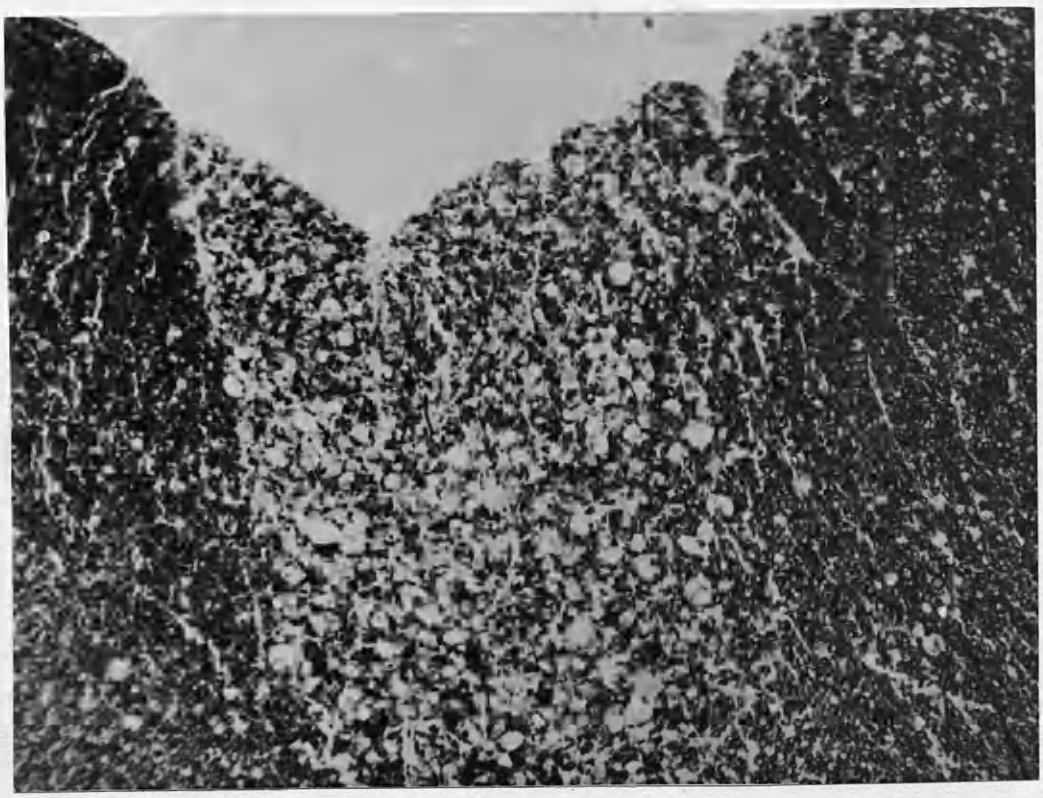
Cajal (1928) refers to the proliferation of the neuroglia in columns after 10 days' degeneration and an examination of the spinal cords after the same period of degeneration in the present study confirms this. It is of some interest, as will be shown later, that this columnar arrangement of the proliferating nuclei is no longer seen in the 20 days' degeneration specimens. The 50 and 100 day specimens similarly do not show a columnar arrangement of the multiplying cells (see Fig. 13a, b, c, d, e).

B. The behaviour of the different cells in the spinal cord tracts during degeneration.

It has been shown in the results that the degeneration of the posterior columns is accompanied by an increase in both the astrocytes and the rest of the cells. The ability to differentiate the cells is based on the properties of the nuclei of the different types of cells. It must be admitted that the classification of the neuroglial and microglial cells usually depends on special staining techniques and these have not been carried out. On the other hand a careful study of the literature indicates that astrocytes, oligodendrocytes and microglial cells can often be distinguished by their nuclei even after degeneration.

Astrocytes have large pale nuclei with a well marked nucleolus, oligodendrocytes have smaller nuclei which appear granular or reticular and microglial cells have small, darkly staining nuclei. In addition, the large pale nuclei are often distinguished from the others because they usually show cytoplasm round them. The rest of the nuclei have not been subdivided, although among them there appear to be very few small, darkly staining nuclei, that is there are apparently very few microglial cells. Examination of the 10 day degenerated specimens indicates that there are more of these nuclei than are found at other stages of degeneration. However the exact differentiation of nuclei which are not large and pale was not carried out because frequently it was difficult to distinguish one from the other with certainty. It should be pointed out that other authors who studied degeneration of spinal tracts following division of the spinal cord appear to be certain that the oligodendrocytes multiply early on (Jakob, 1914; Cajal, 1928; Cramer & Alpers, 1932). This is in agreement with the results in this experiment.

The figures showing the percentage of the two groups of cells suggest that during the period between 10 and 20 days' degeneration, the oligodendrocytes and the microglial cells multiply at a greater rate than



100 μ

Fig. 11 (a).

Transverse section of cervical spinal cord, stained Weigert-Pal, showing myelin present after 20 days' degeneration.

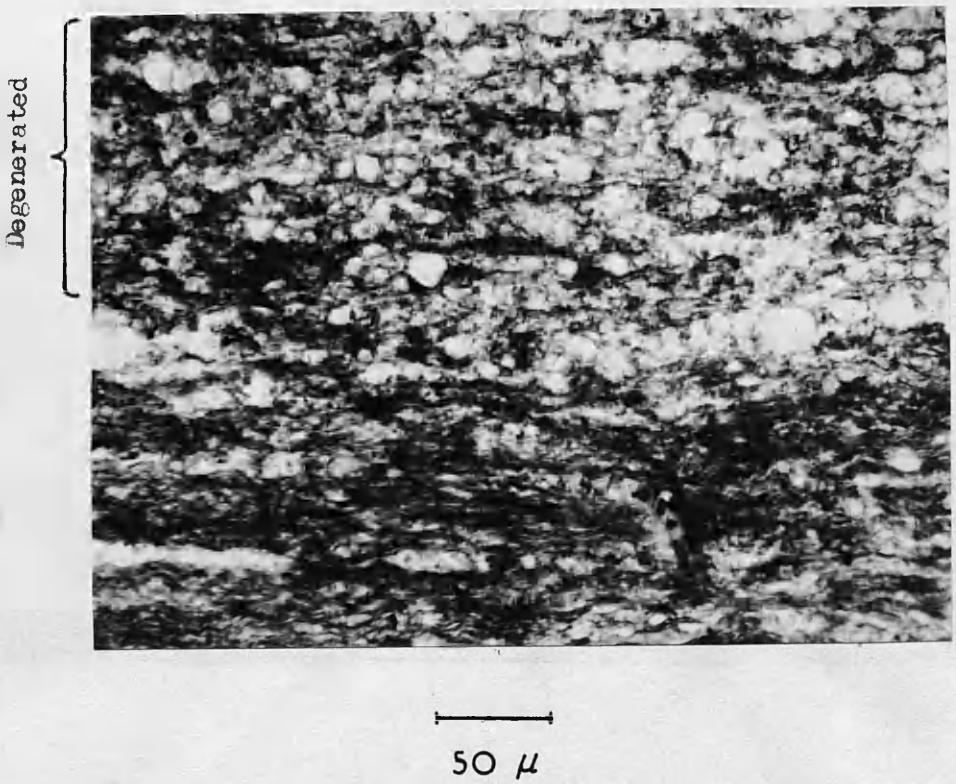
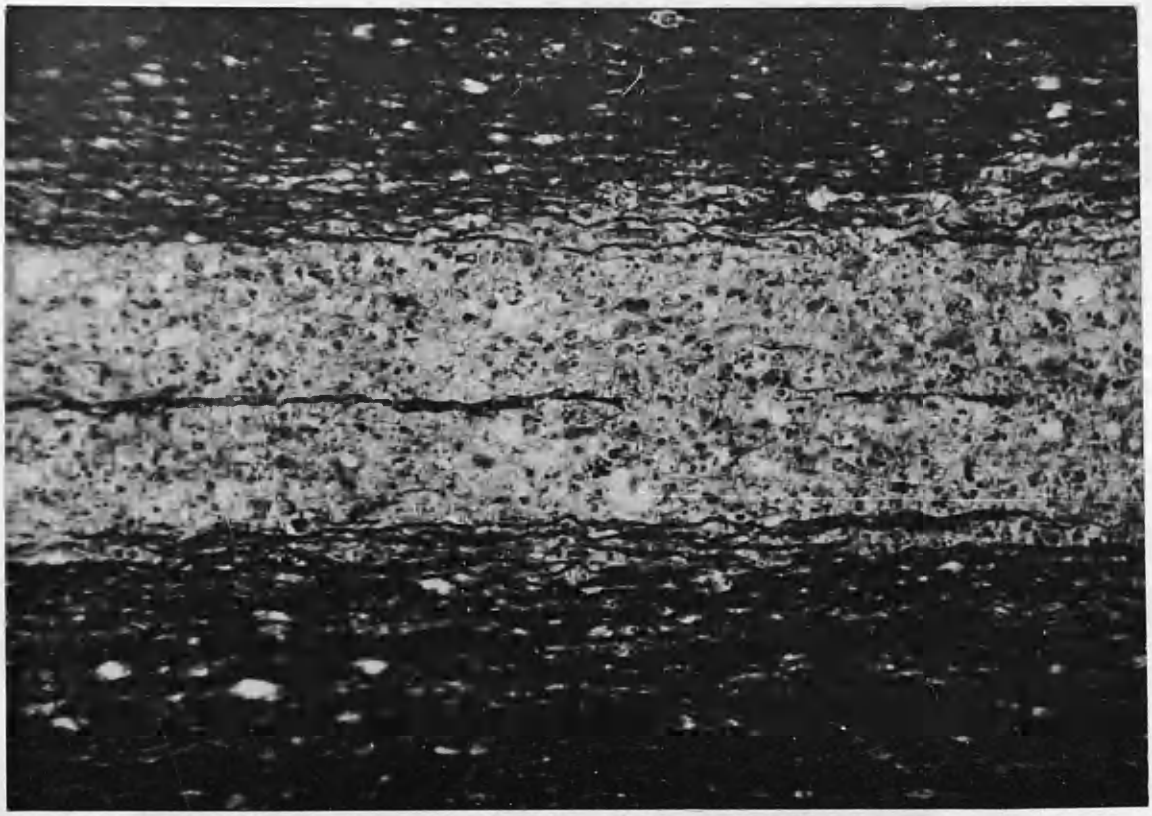


Fig. 11 (b).

Longitudinal section of cervical spinal cord, stained Weigert-Pal, showing myelin present after 20 days' degeneration.

Fig. 12 (a).

Transverse section of cervical spinal cord, stained Weigert-Pal, showing myelin present after 50 days' degeneration.



100 μ

Fig. 12 (b).

Longitudinal section of cervical spinal cord, stained Weigert-Pal, showing myelin present after 50 days' degeneration.

the astrocytes and that subsequently the astrocytes multiply more rapidly. This can be related to two processes which are going on.. After division of the posterior columns, the fibres distal to the lesion degenerate. The first process consists of the breaking up of the myelin sheath and axon and their phagocytosis. This process takes much longer in the central nervous system than in peripheral nerves although one cannot offer any very good reason for this difference. Sections stained by the Weigert-Pal method confirm this. There is a considerable amount of myelin present after 20 days' degeneration, much more than one would expect when compared with a peripheral nerve (see Fig. 11a & b). Even after 50 days' degeneration there is still some myelin (see Fig. 12a & b). This phagocytosis is associated with the cell changes outlined above, namely the greater multiplication of the oligodendrocytes and microglial cells.

The second process takes place at a later stage and is indicated by the relative increase of the astrocytes at 50 days as compared with 20 days and their still greater increase after 100 days. It is during this period that the degenerated area becomes a glial scar. In addition the astrocytes seem to be more numerous at the periphery, as if they are walling off

the degenerated area. Many of the astrocytes at this stage of degeneration take on the appearance of what have been called Nissl's plump astrocytes (gemastate Zellen). They have been described by Penfield (1932) as having a large, oval nucleus with faintly staining nuclear chromatin and being actively fibre forming. He quotes Spielmeyer (1922) who pointed out that plump astrocytes are encountered where nervous tissue has gone through extreme but circumscribed degeneration.

The significant decrease in the number of nuclei found in the normal areas of the 50 and 100 day spinal cords (see Table X) may be related to this process of gliosis. There may be a migration of astrocytes towards the degenerated area resulting in fewer cells in the normal area.

Although there is no controversy regarding the cells which form a glial scar - there is complete agreement by all workers that astrocytes multiply to perform this function - the problem of which cells act as phagocytes earlier on has still to be solved. It should be made clear that Rio-Hortega, as far as can be ascertained, has never investigated the glial changes following interruption of fibre tracts in the spinal cord. In 1932, he listed seven different

pathological states in which microglial cells act as phagocytes but none of these conditions is truly comparable with degeneration of fibre tracts due to their separation from their cell bodies. It must be admitted that his evidence would suggest that only microglia is capable of phagocytosis and that the oligodendrocytes never participate in this process. On the other hand, all other workers who have studied degeneration of fibre tracts have been satisfied that oligodendrocytes multiply and act as phagocytes. The present work would support this view and it should be added that typical compound granular corpuscles ("gitter" cells), which are fairly easily distinguishable and are derived from microglia, are rarely seen after any of the periods of degeneration studied. Their small number after 10 and 20 days' degeneration is, in fact, rather surprising.

A further possibility has to be considered. If it is assumed that the astrocytes, oligodendrocytes and microglial cells can be distinguished from each other in the white columns of the normal spinal cord, may they not change their morphological characters during degeneration so that it becomes difficult to sort out the different types of cell? The more recent opinions of some of the Italian workers (Bairati, 1948 a & b, 1950; D'Agata, 1950) would support this view. They

claim that the neuroglia and microglia are really the same cells with different morphological properties due to the effects produced by the surrounding tissues. If these effects disappear during degeneration the distinct structural characters of the cells may be lost. Furthermore, it is common for differentiated cells to lose some of their characteristic features when they become active, for example, fibrocytes, when active in a wound, change their appearances. Consequently although the cells in the degenerated areas are assumed to be mostly astrocytes and oligodendrocytes the latter may include microglial cells whose nuclei have changed in their staining properties.

The early multiplication of the astrocytes is more difficult to account for, and it may be that Jakob is correct in maintaining that they are phagocytic. It is conceivable that they multiply early on due to the stimulus of some chemical agent released by the degenerating fibres. This will be considered in greater detail in the next section.

C. Differences between the fibres of peripheral nerves and of tracts in the spinal cord during degeneration.

The most striking difference between the nuclear population changes in degenerating peripheral nerve and fibre tracts in the spinal cord is that the peripheral nerves show a maximum nuclear population after a varying

period, which is never more than 30 days. This varies with the fibre spectrum of the nerve studied. On the other hand the nuclei in the posterior columns go on increasing up to 100 days. In the peripheral nerves, the larger the fibres the greater was the increase and the longer was the time taken to reach that increase, but the maximum was reached within 30 days even in the nerve with the largest fibres. Section of the normal posterior columns stained by the Weigert-Pal method show that from the point of view of fibre size these columns are best compared with a mixed nerve like the sciatic nerve of the rabbit, studied by Abercrombie & Johnson (1946). They estimated that the maximum nuclear increase occurs after about 25 days' degeneration, after which the nuclear population falls rapidly at first and then more slowly. At 50 days it has fallen considerably but at 100 days the additional decrease is not great (see Fig. 10). These authors suggest that the increase in cells is due to "a chemical stimulus coming by diffusion from the autolysing nerve fibres". They offer no explanation for the subsequent decrease in nuclei.

Joseph (1947, 1948) emphasised that the size of the nerve fibres may be an important factor in determining the nuclear population increase. In nerves with larger fibres the disappearance of the myelin sheath and axon

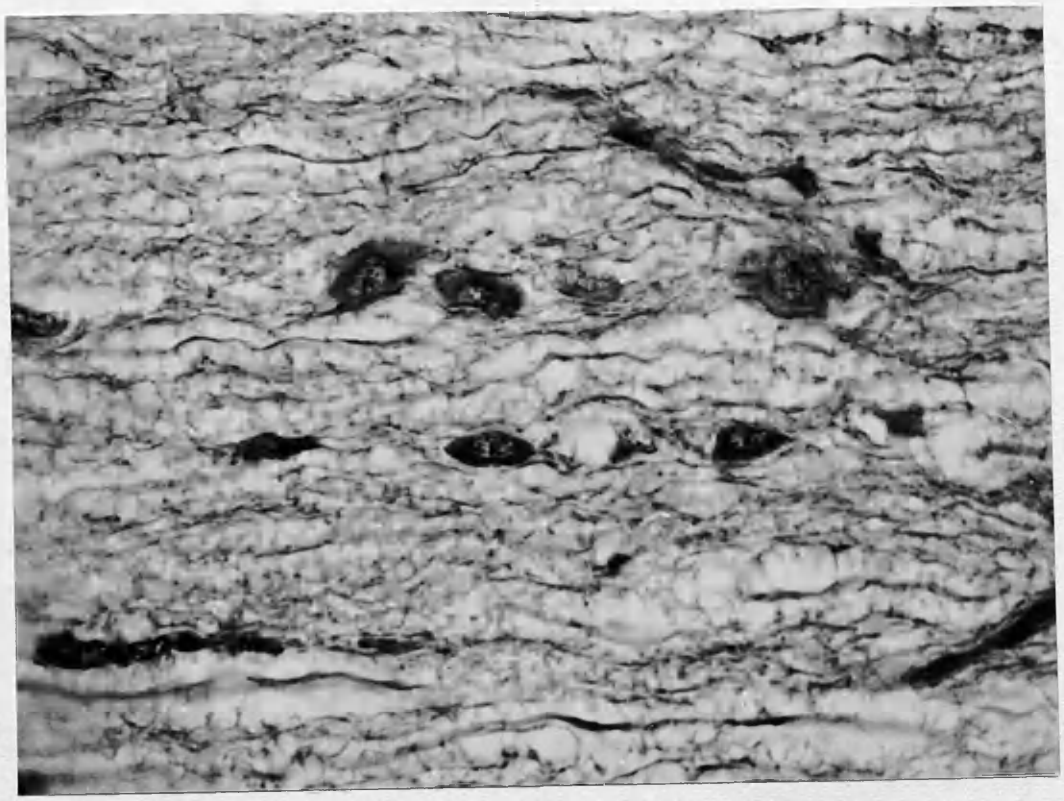
during degeneration leaves a large space which is filled by dividing cells. The nerves with smaller fibres have smaller spaces to fill and so reach their maximum increase earlier and the increase is smaller. Abercrombie & Johnson (1946) point out that the subsequent decrease in nuclei is associated with shrinkage of the tubes in the degenerated nerve together with a shrinkage of the whole nerve. They do not state that this shrinkage causes the decrease in the nuclei but it is suggested here that that may be the case.

In degenerating peripheral nerves, at least in the early stages, phagocytosis of the degenerating myelin sheath and axon takes place and the early multiplication of the cells must be related to some extent to this process. Macrophages from the endoneurium may be the main cells which remove the debris within the neurilemma and for this purpose these cells must enter the neurilemmal tube. Blood vessels in the nerve may be another source of these macrophages. Cajal (1928) stated that the multiplying Schwann cells inside the neurilemma contain fat droplets and called this process "fatty infiltration" rather than phagocytosis which, he maintained, is carried out by macrophages entering the tube after about 7 days. Abercrombie & Johnson (1946) do not discuss this problem.

When comparing degeneration in peripheral nerves

with that in the posterior columns of the spinal cord there is a similarity in that the myelin sheath and axon disappear in both cases. There is a difference however in that this process is much slower and takes correspondingly longer in the spinal cord. This difference may to some extent account for what appears to be a slower increase in the nuclear population of the degenerating posterior columns. On the other hand the nuclear increase in the posterior columns even after 100 days does not reach anywhere near the level of the increase after 25 days in what may be comparable peripheral nerves (4.5 times as compared with 8.4 times).

It is suggested that the main differences in nuclear population changes during degeneration are due to the differences in structure between peripheral nerve fibres and fibres in the spinal posterior columns. The important difference is the presence of a neurilemmal sheath and endoneurium in the former. Because of these structures, and it is difficult to determine which plays the more important part, the peripheral nerve after the break-up of the myelin sheath and axon consists of a large number of fairly rigid tubes containing at first multiplying cells. These tubes as they are fairly rapidly emptied of their debris are filled more and more with cells until cell division stops either because the chemical factor stimulating



10 μ

Fig. 13 (a).

Longitudinal section of cervical spinal cord, stained haematoxylin and eosin, showing normal cord with neuroglial cells in columns and longitudinal tubal structure of fibres.

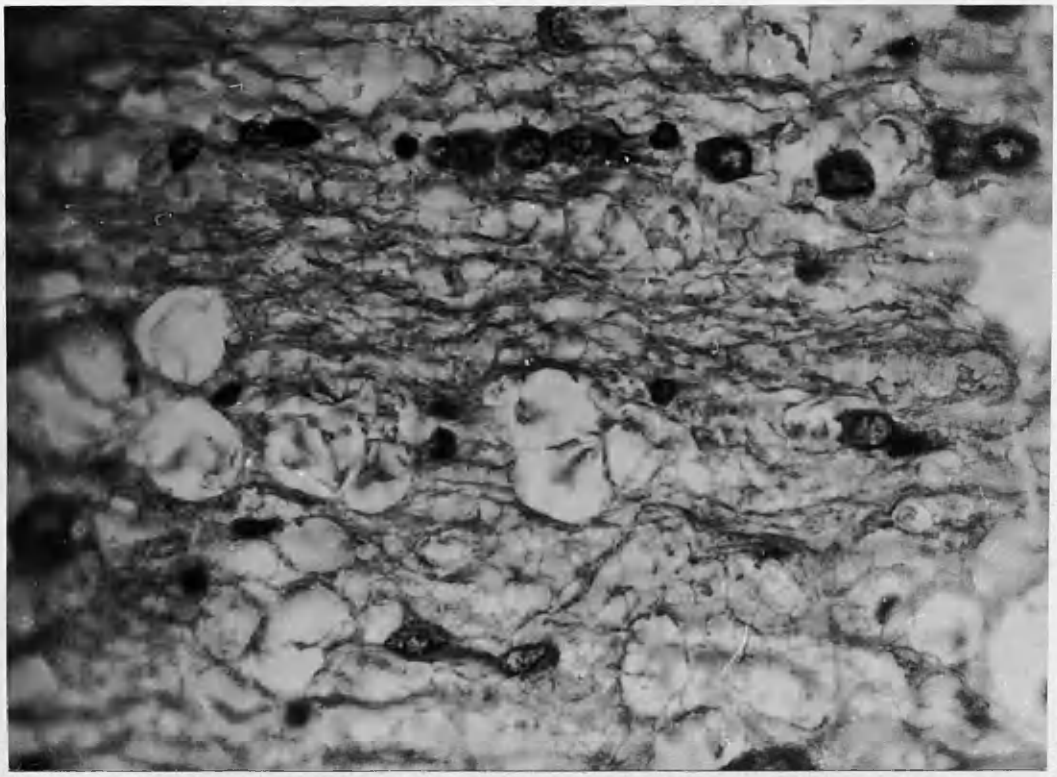


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Fig. 13 (b).

Longitudinal section of cervical spinal cord, stained haematoxylin and eosin, showing, after 10 days' degeneration, neuroglial cells still in columns and still some evidence of longitudinal tubal structure of fibres.

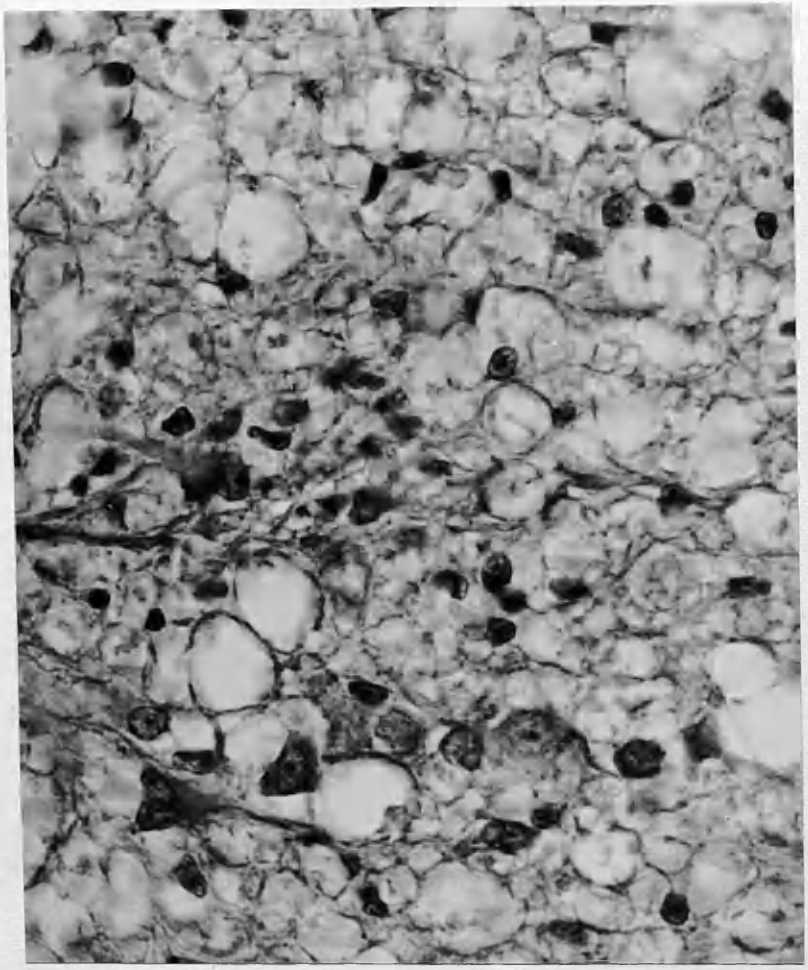

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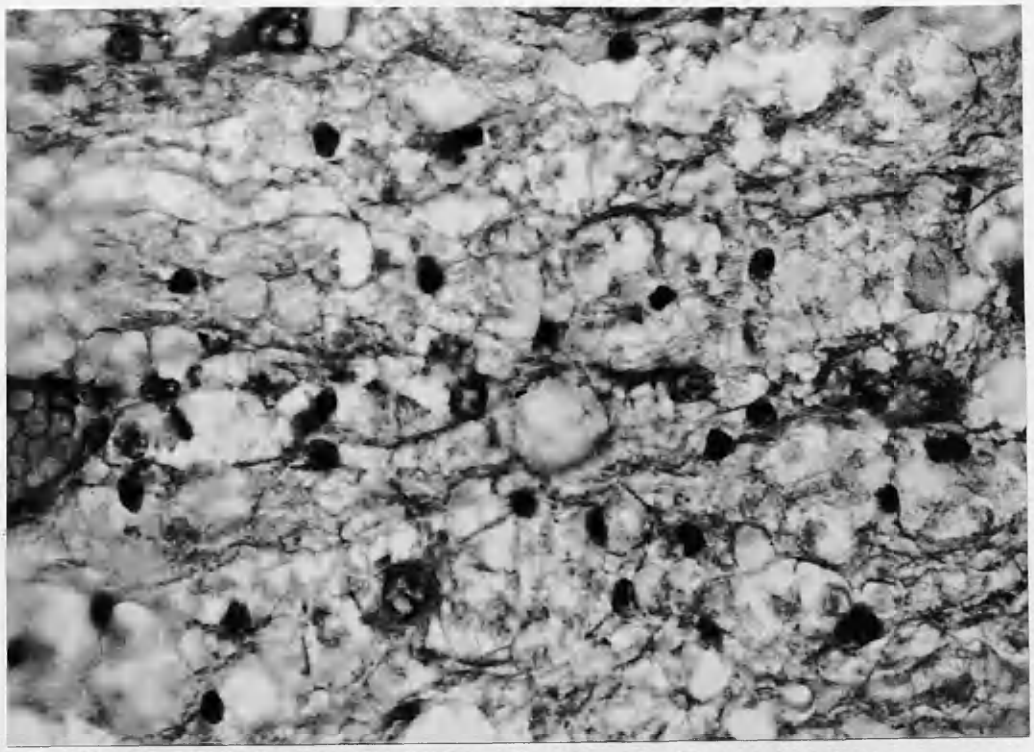
Fig. 13 (c).

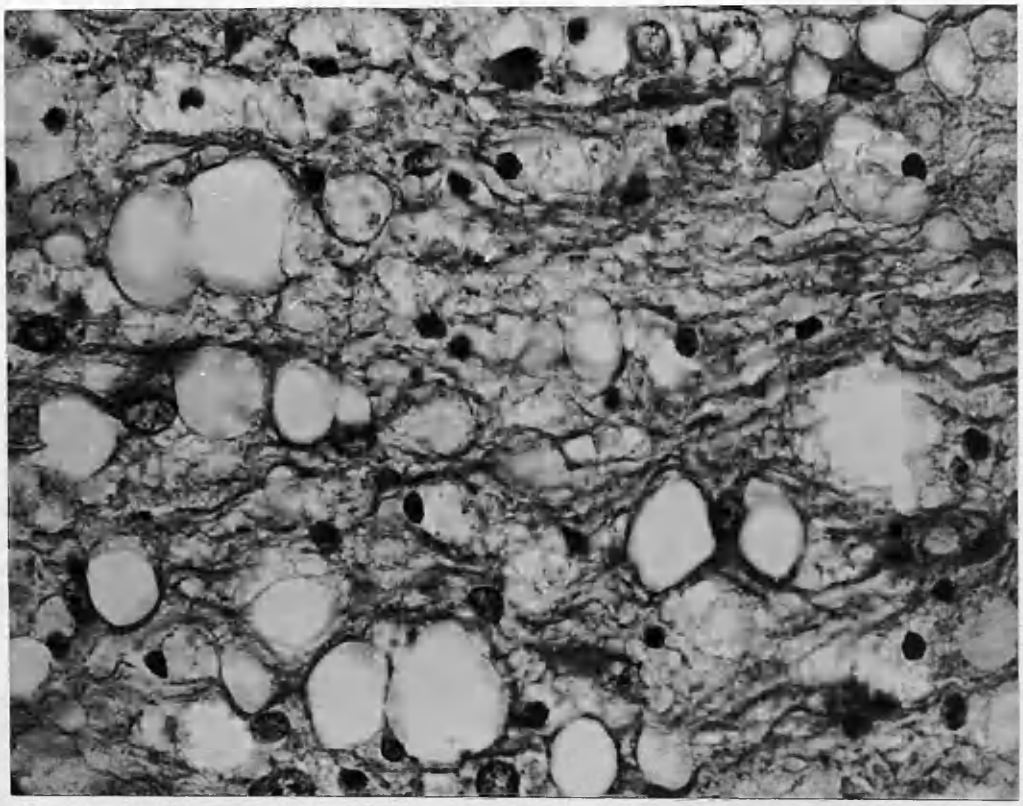
Longitudinal section of cervical spinal cord, stained haematoxylin and eosin, showing, after 20 days' degeneration neuroglial cells no longer in columns and loss of longitudinal tubal structure of fibres.



10 μ

Fig. 13 (d).

Longitudinal section of cervical spinal cord, stained haematoxylin and eosin, showing after 50 days' degeneration neuroglial cells no longer in columns and loss of longitudinal tubal structures in fibres.



10 μ

Fig. 13 (e).

Longitudinal section of cervical spinal cord, stained haematoxylin and eosin, showing, after 100 days' degeneration, neuroglial cells no longer in columns and loss of longitudinal tubal structures in fibres.

cell division ceases to have this effect or because there is no more room for further cells or because of both factors. Subsequently the tubes shrink and the cell population falls. In the spinal posterior columns the tubes, or rather the tubal structures, collapse and there are few tubal spaces waiting to be filled by multiplying cells. There is however an early multiplication of nuclei for phagocytosis. Subsequently the cells multiply to produce a glial scar. In support of this suggestion two histological findings are reported. In a section of a normal spinal cord stained for reticulin a posterior rootlet cut in tranverse section showed very clearly reticulin surrounding the fibres, whereas the fibre tracts of the spinal cord showed no evidence of reticulin in a similar site thus confirming the work of Laidlow (1930). Secondly longitudinal sections of the degenerated spinal cords after 20, 50 and 100 days' degeneration show that the tubal nature of the posterior columns is lost in the degenerated areas and the multiplying nuclei are no longer in columns. In addition the nuclei are round, unlike those in degenerating peripheral nerve in which they are markedly elongated, this elongation being due to the comparatively rigid tube in which the cells lie (see Fig. 13a, b, c, d, e).

Does this investigation help to elucidate the problem as to whether the oligodendrocyte and the Schwann cell are the same cell but in a different situation? Both cells multiply early on during degeneration and both cells show fat inclusions in their cell body derived from the myelin sheath. One may accept Cajal's suggestion that this is not phagocytosis. The continuing increase of the nuclei in the degenerating posterior columns is apparently due to the astrocytes and it may be assumed that the oligodendrocytes stop multiplying in the spinal cord as do the Schwann cells in peripheral nerves. It may be said therefore that the oligodendrocytes respond in a way comparable with the Schwann cell response.

D. The problem of regeneration in the spinal cord.

One of the most important prerequisites for regeneration in peripheral nerves is the existence of the tubes in the peripheral stump. From what has been already said in the discussion it can be seen that this prerequisite is missing in the spinal cord. The absence of these tubes probably plays a very important part in preventing complete regeneration of fibre tracts in the spinal cord. Windle & Chambers (1950) by means of pyrogens prevented the formation of a glial scar at the site of section of the spinal

cord in cats and dogs and as a result new axons grew across the site of the lesion. They did not obtain any fuctional regeneration, however, and that may have been due to the absence of tubal structures along which the fibres could grow. One may speculate on whether functional regeneration would be achieved with the continued use of pyrogens which may prevent glial scarring of the degenerated tracts.

It is suggested that this investigation leads one to believe that complete regeneration in the spinal cord is prevented to some extent by the collapse of the tubes and the formation of the glial scar due to the activity of the astrocytes in the degenerated area.

5. SUMMARY.

1. Changes in the nuclear population in the posterior columns of the spinal cord of the rabbit have been studied after 10, 20, 50 and 100 days' degeneration.

2. It has been found that there is a progressive increase in the nuclei up to 100 days. After 10 days the increase is about 1.8 times the normal, after 20 days 3 times, after 50 days 3.4 times and after 100 days 4.5 times.

3. There are some signficant changes in the area of the posterior columns. It is suggested that these changes are not important in considering the results. There is no signficant change in the length of the nuclei after all periods of degeneration studied.

4. The astrocytes have been differentiated from the other cells (oligodendrocytes and microglial cells) by reason of the appearance of their nuclei in haematoxylin and eosin stained sections. The astrocytes were found to increase early on equally with the other cells. Between 10 and 20 days they increase to a less extent. Subsequently they catch up with and increase to a greater extent than the other cells.

5. It is suggested that the increase in oligodendrocytes and microglial cells is for phagocytosis of the degen-

erating myelin sheaths and axons of the fibre tracts and the subsequent increase of the astrocytes is for the formation of a glial scar.

6. Comparison is made between the nuclear population changes in degenerating peripheral nerves and in the posterior columns of the spinal cord. Most of these differences are probably due to the difference in structure of the nerve fibres, the main one being the presence of a fairly rigid tube in the peripheral nerve due to the neurilemma and endoneurium. The consequent collapse of the tubes in the posterior columns is also responsible for preventing functional regeneration of fibre tracts in the spinal cord.

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