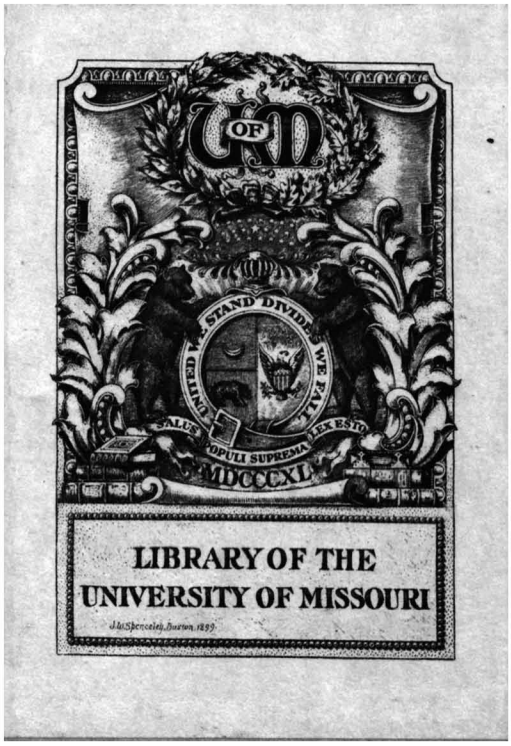

THE SIGNIFICANCE OF THE NEUROCYTOLOGICAL CHANGES FOLLOWING SECTION OF AXONES

by

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INTRODUCTION.

That a relation exists between the structure of a nerve cell and the integrity of its axone is shown, as Warrington points out, by evidence of three kinds. (1) Gudden showed that section of a nerve trunk gives rise to a complete disappearance or perhaps an arrest of development of the cells of origin, in newly born animals. (2) In adult animals similar conditions occur if a long time has elapsed since the occurrence of the lesion. (3) An alteration in structure, as noted by Nissl, occurs in the cells in so short a period as twenty-four hours after division of the axone.

Thus there has been a considerable amount of work done on the effect of an interruption of the continuity of nerve axones. But most of the work was done from an anatomical or from a pathological instead of from a physiological point of view. I have observed the changes which occur after the section of an axone and shall attempt to correlate these anatomical changes with those changes observed in depression.

First the nature of depression and its relation to functional activity will be considered. There are but two functional possibilities in the nerve cell, namely, activity and depression. Physiologically, activity represents an increase while depression repre-

sents a decrease in the intensity of vital phenomena. Verworn (General Physiology) says that excitation and depression are merely quantitative opposites. He says that the two are "merely different degrees of one and the same phenomena, namely, life, excitation being an increase, depression a decrease, of the normal intensity of vital phenomena". All nerve cells either work or are inhibited from working and into one or the other of these categories or into a combination of the two all things relating to function must fall (Dolley 1913). Thus according to Dolley the nerve cell exists only to function. "It can be excited or it can be depressed. There are no other possibilities. It is specialized for irritability and conduction".

The mode of response of all cells is the same whether from mechanical, chemical, tropic, thermal, or photic stimuli. The nerve cell is a specialized cell which is developed to react only in one particular way. Therefore when the cell is stimulated by any form of stimulation the mode of response is common and unvarying. The original statement of this, as cited by Dolley, was by Hering (1884-1888) in his doctrine of the specific energies of living substance.

Verworn especially noted that the different varieties of stimuli produce wholly similar reactions in the same object. He cites an example in the amoeba, which may be made to retract its pseudopodia and assume a spherical form by chemical, mechanical, thermal and galvanic stimuli. The cells of a ciliated epithelium respond by an acceleration of their ciliary motion to chemical, mechanical, thermal and galvanic stimulation. By all these agencies the production of light can be induced in Noctiluca. This important fact, he says, shows that in every form of living substance there must exist an extraordinary inclination toward a specific sequence of processes. This sequence is continually present in a slight degree and finds its expression in the spontaneous vital phenomena; but the slightest stimuli of all kinds augment the discharge of the processes always in the same characteristic sequence for each specific variety of living substance.

The final proof of the above doctrine rests upon an anatomical basis, and we find that the anatomical changes are the same in nature for representative types of cells whether produced by chemical, electrical or any other form of stimuli. Dolley found that not only the higher and more differentiated cells of the cerebrum

or cerebellum but also the lower primitive cells of the spinal and sympathetic ganglion as well go through the same sequence of changes, differing only in the degree of reaction. The less differentiated cells usually stop short of going as far as the more highly differentiated cells, but they can be driven to exhaustion, and if so driven, they pass through the same anitomical changes as do the specialized cells. Thus one can say that the higher cells run their courses sooner, i.e., they are more easily exhausted, but the mechanism is preserved throughout the ontogenetic scale.

Dolley noted the effect produced by special forms of stimulation. He found that trophic excitation: heat exhaustion with its thermal excitation; traumatic shock with its mechanical excitation; and certain infectious diseases, with the chemical excitation of bacterial toxins, broadly typified by the actual study of infections of B. diphtheriae, B.tetani, B. pyocyaneous, and certain pyogenic micrococci, all produced up to the time the cells pass into depression and degeneration anatomical changes of excessive normal functional activity.

On the other hand there is the possibility of depression. Separate and distinct changes in form and structure underlie the physiological conditions of

depression as well as those of activity. These cytological changes will be described elsewhere. A depressant stimulation may intervene at any stage of activity, block the mechanism in a uniform and constant way, and cause activity to come to a standstill. Thus far depression has been identified in the nerve cell after heat stimulation, rabies, and poisoning bromides, caffeine, beta-oxy-butyric acid, sarcolactic acid (Dolley 1913), and, as a final effect, after infections of diphtheria, staphlococcus, and tuberculosis (Simmons, 1914 Thesis).

Granting Verworn's theory, that activity and depression are merely quantitative opposites, and granting that the absence of activity is depression, would not the cutting of an axone produce a condition of depression? For it is a physiological fact that nerve cells are in a state of more or less continuous activity which is designated as tonic activity or tonus. If the axone is cut this continuous activity is broken, the tonus is lost, and degeneration results. The writer attempted to find out if this degeneration was not really to a certain degree a matter of depression.

THE SOURCE OF MATERIAL.

This study is based on material obtained from dogs. Normal dogs were selected for operation. First, a piece of the skull was removed so as to expose the cerebellum. The cerebellum was then stabbed, in a way to cut the axones of the Purkinje cells. A very thin double edged and pointed knife was used. The first stab was made parallel to the surface of the worm and at about 4 m/m. depth. Usually a second stab in the lateral lobe was made. The operations were done under aseptic conditions, and in most cases aseptic healing took place.

The operation was a very difficult one, as there was always considerable hemorrhage from the sinuses and from the bone. This was unavoidable on account of the place of the craniotomy. Eight dogs and one rabbit either died during or immediately after the operation. There was seven successful operations as follows:

Experiment 1 (9 days). Male Mongrel, weight 8.182 Kg. Operated Dec. 3, 9 A.M. Killed Dec. 12, 2:30 P.M. There was no signs of infection, and granulation tissue was abundant.

Experiment 2 (7 days). Black male Mongrel, weight 6.800 Kg. Operated Dec. 3, 10 A.M. Much hemorrhage

from sinus caused dog to be very weak. Killed Dec. 10, 2:30 P.M. The wound was slightly infected after operation but the character of the granulation tissues showed recovery at time of death. There was no apparent infection in the brain.

Experiment 4 (27 days). Male Mongrel, weight 13.240 Kg. Operated Dec. 12, 3:50 P.M. Killed Jan. 8, 4:15 P.M. The tissue showed perfect recovery without infection and there was no gross trace of the stabs.

Experiment 5 (2 days). Young male Mongrel, weight 11.540 Kg. Operated Dec. 10, 4:30 P.M. Made two deep stabs in cerebellum. There was considerable hemorrhage. Killed Dec. 12, 4:30 P.M. There was a large cavity filled with a clear fluid just outside of the cut through the skull, evidently a collection of serum in an empty space. Some blood clots were found in one of the cuts made in the cerebellum.

Experiment 6 (2 days). Operated March 3, 10 A.M. There was considerable hemorrhage and the wound was packed with cotton to stop the hemorrhage. On Dec. 5, the dog's head was found swollen,indicating infection. There was opisthotonos and jerking as if from pressure on the brain. Killed Dec. 5, 11 A.M. A bad infection was found with a was of cotton pressing on cerebrum, making a slight pit.

Experiment 8 (4 days). Young Male dog, not weighed. March 13, 4 P.M. Operation as before but the brain cavity was entered by means of a chisel instead of by the trephine. Recovery good. Killed March 17, 4 P.M.

Experiment 9 (12 days). Young male dog. Operated March 13, 4 P.M. the brain cavity being entered by means of chiseling. Hemorrhage was slight and the dog recovered in good condition. March 18, wound found infected. The pus was removed and the wound drained. March 23, dog appeared normal with complete recovery from the infection. Killed March 25, 4 P.M. The stabs were so healed that they could not be made out in gross sections.

MICROSCOPICAL TECHNIC

The material obtained for the study of the cells was placed in the following fixative.

Saturated corrosive sublimate.....95 cc.

40% Formaldehyde solution..... 5 cc.

Much depends upon the shape of the cells in determining nerve cell activity and depression. Mann (1894), Flemming (1895), v. Lenhossek (1897), and Dolley (1911) have all agreed that saturated aqueous sublimate is the most satisfactory fixing fluid for nerve cells, and that undue distortion of shape is absent. Therefore it is immeasurably superior to other fixatives employed, particularly strong alcohol. Dolley (1911) found that his results were essentially constant whatever the fixation.

After fixation for 24 hours, the blocks were run up through the graded alcohols to 70%. They were then placed in 70 % iodized alcohol for 24 hours and then in 80% iodized alcohol until saturated, thence to 95 % alcohol, absolute alcohol, alcohol-zylol, zylol, and zylol-paraffin solutions. They were finally imbedded in paraffin and routine sections cut at five micra thickness.

Following Mann, v.Lenhossek and Dolley the sections were stained in saturated aqueous toluidin blue. Erythrosin was used as a counterstain in the solution recommended by Held, (i.e., one gram to one hundred and fifty cubic centimeters of distilled water , to which is added two drops of strong acetic acid).

The technic is extremely simple. The paraffin was removed by placing the slides in zylol for 5 minutes and the sections were run down through the alcohols to water. They were then stained for a minute and a half in erythrosin solution warmed to 42° C. After thoroughly washing in water, they were stained in toluidin blue for 7 minutes, again washed, and differentiated in 80% and 95% alcohol. The process was followed under the low power and stopped when the internal cell markings were clear cut. After absolute alcohol the sections were cleared in zylol and mounted in Canada balsam.

DESCRIPTION OF THE CHANGES IN CORRELATION WITH DEPRESSION.

Activity is a continuous process. A nerve cell passing from a resting stage to a stage of exhaustion passes through continuous anatomical changes. These stages have been analyzed by Dolley and divided into distinct stages.

Depression may interrupt the progress of activity at any one of these stages, that is, depression may occur at any time during activity. The normal chromatin content varies widely for cells in different stages of activity. But fortunately the characteristic changes in depression are not of such a nature as to preclude the accurate diagnosis of the degree of normal progressive activity.

A deficiency of extra-nuclear chromatin is one of the most marked and earliest of the alterations noted in depression. In the early stages of normal activity the cells show an excess of extra-nuclear chromatin while these same cells in depression will range from an appreciable lessening to an absolute disappearance of chroma-

tin. Discrete formed granules become less and less apparent. The acid red staining element of the cytoplasm becomes more and more evident, though before the basic staining element disappears it is evenly tinged or mottled irregularly in patches by a bluish color (Dolley 1913). This is thought by Dolley to be chromatin in solution. He says that in more advanced depression the cytoplasm assumes a murky turbid appearance with finer granular or somewhat flocculent structures. This corresponds to the usual description that what is left of the stainable substance appears as fine dust-like particles or as a diffuse blue stain. Finally a cell in such advanced depression comes to homogeneity of its cytoplasm with a red staining hyaline appearance.

The nuclear content of a cell in depression is maintained or is actually increased in amount. The elements in the nucleus are chromatin and nucleolar substance (plastin).

In activity the chromatin is always present in the karyosome, but outside of that it is only present in hyper-chromatic stages. In depression, however, basichromatin appears in the nucleus at any and all stages.

The chromatin which is consumed during activity, is thus seen to be stored in the nucleus in depression. Therefore there is an extra-nuclear deficiency of chromatin and an intra-nuclear hyperchromatism in depression.

The nucleolar substance is also increased. This is especially marked in cells of later activity where there is normally a deficiency of this substance. Instead of the normal loose reticulum and vesicular structure , the nucleus is dense and matted, the acid-staining plastin frequently forming definite masses or true nucleoli.

To summarize the changes: First there is a relative and an absolute increase of the intra-nuclear chromatin and an increase of the nucleolar substance. Second, there is a deficiency or loss of the extra-nuclear chromatin. Expressed in terms of the nucleus-plasma relation, the nucleus-plasma balance is disturbed in favor of the nucleus. The chromatin does not pass out of the nucleus, either in formed shape or in modified way in depression.

In severe depression there is a disintegration of the karyosome. The chromatin which covers the nucleolus gardually breaks up and passes out toward the nuclear membrane as definite irregular granules. These chromatin

granules adhere to the inner surface of the nuclear membrane. They have never been found outside of this membrane which indicates that they do not pass out into the cytoplasm in depression. After the chromatin disappears and the cytoplasm is reduced to a homogeneous, eosinophilic and hyaline material, the nucleus becomes very shrunken and finally goes into solution. The cell then appears as a homogeneous or vacuolated, red-staining, hyaline-like mass, devoid of a nucleus. Thus profound depression merges into degeneration, necrosis follows and finally a disappearance of the cell is the end result of a profound depression. All degrees of these degenerative changes were found in the areas most directly affected by the stab wound. Such cells as just described could not recover. According to Dolley's work on recuperation after normal activity (1911), a certain amount of recovery would be expected if any of the nuclear material were left still organized.

The intracellular deposition of yolk material in excess and of glycogen is also characteristic of depression. The yolk material, in the sections studied, appeared as definite round and reddish staining globules. No special stain for this material was needed.

The changes in the cerebellum where the stab was made have been found to correspond to the changes just

described for depression and depression with degeneration in all types of cells. The control sections which were taken from the normal side of the cerebellum showed normal activity but no depression. Depression was limited to the part supplied by the cut axones. This was to be expected for the result of cutting the axones was the only depressant factor. The anesthetic, the operation, and the cut itself were excitant stimuli, causing increased activity.

The experiments will now be discussed briefly to bring out their essential changes and to show the sequence at different periods of time.

Experiment 6 (2 days). In the convolutions in which it is reasonably certain that all of the axones have been cut, depression is already marked, particularly adjoining the cut. Here some cells have probably disappeared and mere traces of completely degenerated cells are found. Further away from the cut the cells show depression rather than degeneration and there are a few cells which are but little affected.

The convolutions adjoining the cut but definitely not involved in it show in general no depression or only a minimal amount in an occasional cell. It is possible that some of the axones in these convolutions were affected by the cut.

Experiment 5 (2 days). This material shows the same changes as above but to a more marked degree. In the convolutions where the fibres have all been cut there is a marked depression of all the cells in the convolution except in the lower or deeper part, which is next to the cut. Here all the cells have entirely disappeared.

Experiment 8 (4 days). The convolutions most involved show advanced degeneration of such cells as are left and obvious disappearance of the majority of cells. There are, however, a small number of hyperchromatic cells still diagnosticable which show a marked degree of depression. The convolutions adjacent to the cut but apparently not involved in it show no appreciable alteration. In one convolution where the stab apparently cuts only a part of the axones, a few practically unchanged cells appear which doubtless correspond to the uncut fibres, while the rest of the cells show marked depression and degeneration.

Experiment 1 (9 days). The convolutions evidently involved and closest to the cut show complete absence of all cells. In other convolutions there is a disappearance of cells but more unaffected cells also appear. While these yet show indications of depression their appearance suggests recovery chiefly in the point of restoration of extra-nuclear chromatin.

Experiment 2 (7 days). In convolutions completely cut, the seven day dog exhibits an appearance partaking of the nature of both the four day and the nine day dog. Corresponding to the four day dog there are cells in complete degeneration, hyaline and without nuclei. A certain number of cells have evidently disappeared and others show advanced degeneration. On the other hand corresponding to the nine day dog there are cells which are slightly in depression but which show an exceptional amount of chromatin to be located in a complete cut as compared with the four day dog. This is interpretated as beginning recovery with restoration of chromatin.

Experiment 9 (12 days). In the twelve day dog a large area is involved. In the blocks taken the cut goes rather deep and does not involve the whole axone supply to the area studied. Still in the parts most certainly affected, the cells show very little alteration. Closer to the cut there is a moderate amount of depression and some degeneration, and an apparent loss of cells. On the whole though, from the evident completeness of the cut, the picture is one of general recovery. If it were not recovering there would be a more widespread degeneration, as in the shorter time animals. In another block,

in convolutions closer to the cut, the depression is more uniform and general.

Experiment 4 (27 days). In the twenty-seven day dog there is no depression whatever in the cells supplied by the cut fibres. However, there is undoubtedly a complete disappearance of a certain number of cells. Occasionally areas occur, as wide as the diameter of the low power field, in which the line of Purkinje cells is entirely absent, and frequently there are shorter stretches in which the number of cells is less than usual.

The general conclusions drawn from this series of experiments are as follows: Where the cut actually separates a convolution from all connections with the rest of the cerebellum, there is complete degeneration and disappearance of the Purkinje cells. In other cases where it is uncertain whether the cut involves all the cells of the convolution and in still others in which it is certain that the cut involves only part of the axones to the convolution there are varying degrees of depression and degeneration. Presumably complete degeneration corresponds to a complete cut and separation of all connections. Such degeneration as occurs reaches its maximum on or after the fourth day while recovery had its beginning on the seventh day and was definite on the ninth day.

The twelve day dog confirmed the progress of recovery and the twenty-seven day dog showed complete restoration of such cells as were not destroyed. Recovery then begins at an early stage. It does not seem possible that there could be complete regeneration of the axone within seven days. The average time for regeneration of the neurone is said to be much longer. Marinesco claims he found a difference in the cells whose axones had been cut sixty-three days after the operation. He cut the hypoglossal nerve on one side and then examined and compared the cells of the nuclei of both sides. At the end of sixty-three days he says the cells of the nucleus of the cut side were more voluminous than the cells of the opposite side; the cells of the cut side were more strongly colored not only because the fundamental substance was colored but also because the Nissl substance was more voluminous than the normal and this substance was colored more intense. This description, however, corresponds exactly to the hyperchromatic cell of the second stage of normal activity, as described by Dolley. This description therefore can be regarded as of little significance, as Marinesco probably mistook normal hyperchromatic cells for cells affected by the cut. He claims that at the end of one hundred and twenty five days there is no perceptible difference between the

cells of the two sides. Regeneration of the severed nerve fibres within the spinal cord and brain is, according to Howell, very much less complete than in peripheral regions. Nissl thinks that after the sectioning of a nerve, the majority of cells, perhaps through the formation of other unions, begin slowly to recover, so that by the fiftieth or sixtieth day it may be difficult for the inexperienced to distinguish them from entirely healthy cells. Warrington, however, claims that the alterations in the cells of the third nucleus are less marked after sixteen days than after eight, and in exact agreement with the present results found no changes after twenty-eight days. The latter experiment was performed on a monkey.

It must be remembered that the cut was made by a very thin knife and involved relatively only a few fibres completely. Only the cells of such fibres correspond to more complete segregation of cells which was accomplished by the experiments of Nissl and Marinesco in cutting a whole nerve. Of such cells some go to death, and in others the depression and degeneration is still persistent in our series even to the twelfth day which corresponds more to the results

of others in cutting the whole nerve supply to a definite part of the brain.

Without definite knowledge therefore as to how long it takes the axone of a Purkinje cell to regenerate and from the probability that it takes longer than seven days, it is probable that recovery here does not mean that the axone had regenerated but only indicates that sufficient connections remained established so that the cell, while depressed from the cutting off of the main connections, continued to receive impulses from the side. It is such cells that begin to recover at seven days. Cells more definitely segregated from all possibility of connections are more profoundly affected and agree more with the general results of others. From the amount of total disappearance of cells, it is suggested that in agreement with Howell's statement above, the specialized Purkinje cell may have little or no power of regeneration of its axone.

REVIEW OF LITERATURE.

The investigators who have experimented by cutting the nerve axone first studied the peripheral or Wallerian degeneration, then recognized the degeneration of the central part of the axone and later changes in the cell itself. In reviewing the work, the writer found that the cell changes were for the most part identical with those of his own work.

As early as 1839, Nasse and Valentin had proved that interruption of the connection of peripheral nerves with the central nervous system could lead to their degeneration. Waller (1850) made a thorough study of the subject and formulated the fundamental law of the physiology and pathology of the nervous system known by his name. By Wallerian degeneration, as cited by Barker, is understood the change which takes place in the distal end of a peripheral nerve after it has been cut through. This change is described as the coagulative breaking up of the myelin sheath and the dissolution of the axis cylinder, the neurilemma with its nuclei remaining for some time at least preserved. Waller claimed that the peripheral end degen-

erated and that the central end remained appraently intact except for degeneration as far as the first node of Ranvier, which was explained as the direct affect of trauma. Barker converted the Wallerian doctrine into terms of the neurone concept as follows: "Whenever it has suffered a solution of continuity with a severing of its connection with the cell body and dendrites of the neurone to which it belongs, the axone, together with the myelin sheath covering it, undergoes in the part distal to the lesion acute and complete degeneration. This degeneration includes not only the main axone, but also its terminals,together with the collaterals and their terminals connected with it". He therefore concluded and was supported by many others in the belief that what are called the"nerve cells" represent tropic centers for the nerve fibers in general. Ranvier, Homen, Howell, and Huber, Tooth, and von Notthaft have studied the histology of the degeneration of the nerve fibers after separation from their cells of origin.

Tooth in his Goulstonian Lectures on secondary degeneration of the spinal cord says that it is probable that in man degeneration is slower than in animals. He confirms Bouchards opinion that when a fibre is cut off

from its center every point in its length degenerates equally and at the same time as far as early chemical changes are concerned. He claims, however, that the actual histological changes pass along the nerve fibre at varying rate. Tooth devoted his entire time on the subject to the degeneration of the fibres and failed to notice the changes in the nerve cell.

While this belief of Waller held sway for more than forty years, there gradually arose an opinion that the nerve cell itself was extensively involved. Barker gives a review of the literature on both Wallerian degeneration and the degeneration of the central part of the nerve fibre and of the nerve cell. He says that as early as 1829 Berard had noticed at autopsy that there was distinct atrophy of the ventral roots in the spinal nerves supplying a limb amputated some time before. Marinesco convinced himself that after amputation of the limb or after section of a peripheral nerve, there occur in the central part, definite pathological changes, the intensity of which depends upon the species, upon the length of time intervening between the injury and death and especially upon the age of the animal. He claims that the younger the animal at the time of amputation the more marked are the alterations.

After reviewing the work of Vulpain, Cruveilhier, Hayem and Gilbert, Dickinson, Friedlaender and Krause, Homen, Vanlair, Grigorieff, and Marinesco, Barker concludes that the degeneration in the central stump of the divided nerve, although it appears later than in the distal portion, presents similar morphological appearances and is apparently an analogous process, although the central end still maintains its continuity with the "trophic centre". He claims that the fibres of the central stump of a motor nerve, after section, gradually diminish in number and that a large number of motor cells of the ventral horns dwindle in size and after a time may be actually lost.

Barker sites Bergman who established by the delicate method of Marchi that extensive and undoubted degenerative processes occur in the fibres.

Marinesco, Nissl, Foa and van Gehuchten claimed that if the ends of a sectioned nerve fibre are brought together, the axone will regenerate over its original tract and function will be restored. This is now a well recognized fact. But if the ends are not brought together the cell will atrophy and finally disappear. This is the way he explains the absence of regeneration of nerve cells in the anterior horn of the spinal cord and the disappearance of these cells in old amputa-

tions. He also uses this ~~this~~ same theory to explain the reaction in the cells of the anterior horn in subjects dead with polyneuritis, and in the case of recovery from polyneuritis where the cells regenerate.

Forel (1887) and others experimented by tearing spinal or cerebral nerves away from their connections with the central nervous system, especially in new born or very young animals. These animals were allowed to live for several months, when they were killed. The central portion of the nerve involved, together with the group of nerve cells corresponding to it, was studied microscopically. The histological examination revealed marked changes in the nucleus of origin. The cells present showed distinct atrophic alterations and many of them had entirely vanished, so that enumerations of the cells of the groups concerned revealed a decided discrepancy in the counts of the two sides. The nerve fibres in the central portion of the nerve had suffered degenerative changes, many of them having totally disappeared. The different stages of the cells in degeneration are not described. Forel obtained the same results by cutting the facial nerve in two guinea pigs. Darkschewitsch in 1892 also divided the facial and

hypoglossal nerves and found after six weeks that the cells were diminished in number, atrophied and shrunken.

Marinesco experimented by removing about three centimeters of the hypoglossal nerve and found that at the end of a certain time the cells atrophied and disappeared. Marinesco gives the factors capable of producing atrophy of nerve cells, other than the severing of the nerves, as follows: Intense traumatism, pulling out of a nerve, or the attempt at pulling out of a nerve. Secondary factors which Marinesco says have to do with the production of atrophy of the cells are the amount of nerve resected and the level at which the resection is made. He claims that if the repair is retarded or incomplete it is very rare that the chromatophile elements return to the same volume, the same density or the same form as was observed after the simple section of the hypoglossal nerve.

Even after simple section all the cells have not the same degree of resistance and a few succumb to the traumatism produced by the section. Another reason which he gives for the atrophy of the nerve cells after section of their axis cylinder is the suppression of the excitations from the higher centres which was the basis for the present work.

Marinesco divides the process into two stages: reaction, which is characterized by the breaking up of the chromatic substances, the nucleus retaining its central position and the cell its normal contour; degeneration, which is characterized by the occurence of a chromatolysis which begins in the neighborhood of the origin of the axis cylinder and spreads towards the dendrons. In this stage the nucleus assumes an eccentric position.

In speaking of the reaction of cells Marinesco has the following to say: The nerve cells are capable of reacting all the time after cutting an axis cylinder. If for example after the first section the animal is allowed to live until after the cells recover and then the nerve is sectioned the second time, the cells will again pass into the phase of reaction. He gives the following example: twenty-one days after the experiment of sectioning the hypoglossal nerve, he again sectioned the nerve and allowed the animal to live ten days. In this case the cells of this side of the section were very voluminous, and the nuclei of this same side were very eccentric. If the animal had been allowed to live for a long time after the second section the cells would have passed a second time from the phase of reaction to the phase of

repair. He gives another example where the hypoglossal nerve was cut twice. The second section was made twenty-six days after the first and the animal was then allowed to live eight days. He says the cells were increased in volume and were in a state of active repair. Some cells however, he said were hypochromatic around the nucleus and in the nucleus. In these cells the chromatin appeared different in the nucleus and in the periphery. The chromatin in the periphery of the cell body appeared pale and vacuolated while in the nucleus it appeared dense, strongly colored and consisted of compact blocks. This is exactly what was found in the two day dog, and is a definite description of a depressed cell. The repeated reaction of the nerve cells after cutting the axone agrees with the present results in showing that some cells remain capable of response.

Marinesco also describes variation in the distribution of chromatin, some cells particularly showing a perinuclear chromatolysis. He also states that the Nissl bodies may be pale and very voluminous when reformed. However, possible variations in the functional state of cells is neglected and in the opinion of the writer such differences as these in the Nissl substance much more probably belong to function. Certainly they are characteristic of various stages of function. It is likely

that the depression he describes was superimposed on such functional stages.

Nissl,(1894) with his methylene-blue and soap staining of alcohol tissues, found that he could actually demonstrate definite alterations within the nerve cells verysoon after the solution of continuity of their axones. He asserts that while the changes are most marked when the animals are killed after eight to fifteen days, to one acquainted with them alterations are recognizable within the cells as early as twenty-four hours after the operation. He describes the changes as follows: At the end of twenty-four hours the blue bodies undergo an alteration at one point in the cell which consists in a loss of their distinctive shape and arrangement and a dispersion over the body of the cell. In from two to three days this change spreads all over the cell. The Nissl bodies become paler and paler until they appear as minute specks of coloring matter. On the fourth day the whole cell is swollen and more globular and the processes are homogenous. On the sixth day the cells appear as if uniformly covered over with the finest colored particles and the processes have disappeared. The nucleus passes towards the periphery and finally disappears. He maintains that these changes take place with greater rapidity

in some cells than others. Nissl concludes from his observations that the cutting off of a nerve cell from its end organ calls forth regressive changes in the cell in fully grown animals. Van Gehuchten, Bach, Flatau, and others have confirmed Nissl's observations.

He further states that these changes can be made to appear simply by rendering the nerve temporarily incapable of functioning. This was done by the application of chemical substances to the trunk of the facial nerve, or by the applying of a temporary ligature to it. Barker, after repetition of Nissl's work, says that after the changes have reached a maximum (eighteen to twenty-two to thirty days) the appearances for a time do not alter materially, but Nissl thinks that later a majority of the cells, perhaps through the formation of other unions begin to recover slowly, so thay by the fiftieth or sixtieth day it may be difficult for the inexperienced to distinguish them from entirely healthy cells.

Sodovsky (1896) compressed a branch of the sciatic nerve of a rabbit between two half cylinders of wood. The rabbit was allowed to recover and live for forty-five days. Complete paralysis of that part of the foot supplied by this nerve followed. After killing the rabbit, he found that the nerve appeared almost normal to

granules

the naked eye at the level of the compression except that it was smaller. There was a swelling of the nerve for a distance of about one centimeter above and below the compressed area. He found upon microscopical examination that the peripheral end showed swelling and fragmentation of the myelin sheath, disappearance of the axis cylinder and proliferation of the nucleus of the sheath of Schwann. These changes were not noted in the central end of the nerve, but slight changes could be traced to the fibres of the cord. The spinal ganglion cells of the side operated upon were affected as follows: first, there was a concentration around the nucleus of thick formed rings of chromatophile substance which leaves the edges; second, there was a displacement of the nucleus toward the periphery; third, there was a concentration of chromatophile substance in the center. This is very vaguely stated and he possibly means that there is concentration within the nucleus. At any rate his description reads like one of depression.

Flatau (1896) cut the ocular-motor nerve of a cat and allowed the animal to live thirteen days. Upon examination he found that the cells were blurred in color and changed in form, being angular and drawn together. The cell body had undergone transformation and appeared granular. The ganglion cells appeared flabby

and dull as if covered with fine dust. The nucleus colored darker while the nucleolus showed no perceptable change. Other cells appeared homogenous except as regards the nucleolus. In a similar experiment he stained by the Marchi method and claimed that the fibres showed no clear degeneration.

Warrington observed the changes in the spinal cord after section of several posterior roots. He opened the dura mater and cut the roots in the region of the cauda equina about one-third of an inch from their entrance into the spinal cord. He stained the tissue by Held's method and described the following changes: The nucleus was markedly eccentric, and had a clearly defined membrane and well marked chromatin network. This is probably a definite statement of depression for the nucleus has normally no chramatin network except in hyperchromatic stages of activity. The nucleolus was well defined. The chromatin masses completely disappeared from the greater part of the cell, and localized round the nucleus. Here the Nissl bodies retained their form as discrete masses. He describes the red staining part of the cell as being distinctly striated, and in other cells he says the blue masses were broken down into the form of a fine powder. He also described a cell increased in volume without a vestige of the coloring matter, without a

nucleus and of a rather hyaline appearance. This cell was proabaly an exhausted cell in the last stage of depression. He claims that the changes which he noted were the result of the withdrawal of the afferent impulses which normally impinge upon the cells, in agreement with the assumption on which this work is based. He found that the changes which occurred after section of the posterior roots were of a very different type to those which followed division of an anterior root. The changes from cutting the posterior roots were much more intense, the process of chromatolysis advanced further and in many cases to such a degree that all traces of chromatic bodies were absent and the nucleus disappeared.

Of the anterior root experiments, the first animal was killed at the end of ten days, and the segment of the spinal cord examined. Very few apparently normal cells were met with and these were small. The most frequent change consisted in a loss of any definite arrangement of the blue colored Nissl bodies. Fine blue granules were observed throughout the cell bodies and the cells presented the appearance of being less deeply stained than normal. The nuclei of these cells were practically normal. In other cells he noted that the nucleus had lost its well defined margin, had become wavy in outline and was surrounded by a reddish zone from which chromaphitic bodies

were absent. The second animal was killed at the end of fourteen days. He describes the changes as having the same general appearance as above but in a more advanced degree. The nucleus of a typically altered cell lost its definite outline, and its membrane was not visible, the nuclear chromatin had not the ordinary net-like appearance but had the appearance of an irregular homogenous star-like body. The nucleolus was invisible but several dark blue stained bodies, he thought, might represent it. The periphery of the cell appeared as a diffuse blue mass. This is a very close description of a cell in depression passing into degeneration.

There is a complete agreement of all later investigators in regard to degeneration, strophy, and disappearance of cells following section of their axones. Many of the investigators definitely describe depression. The main points noted are the central massing of the chromatin and its increase within the nucleus, the chromatolysis of the peculiar sort that belongs to depression, the "powdery" chromatolysis, and the disintegration of the karyosome. The test of comparing my observations with those of other investigators is complicated by the fact as already stated that they recognize no changes of activity and so activity and depression are described together.

CONCLUSIONS.

1. When the axone of a nerve cell is cut, the continuous activity of the cell is broken, the tonus is lost, and the cell passes into depression.

2. If the cell is completely cut off from all kinds of stimuli, this depression passes into degeneration which finally results in a disappearance of the cell.

3. If, however, sufficient connections remain established, the depressed cell will recover. Such recovery in the Purkinje cell evidently begins in from seven to nine days, for it is at this period that a restoration of extra-nuclear chromatin is found. But a cell completely cut off from all kinds of stimuli and in profound depression can never recover. Such a cell will atrophy and disappear.

4. Complete recovery of the cells which were still present took place within twenty-seven days.

5. Complete degeneration is marked as early as two days and such degenerated cells disappear within twelve days.

6. By comparison with the results of others, the physiological effect resulting from the cutting of the axone is the same for less differentiated types of nerve cells as for the highly specialized type of the cerebellum.

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