Presidential Address

ON THE

PRESENT POSITION OF THE NEURONE DOCTRINE IN RELATION TO NEURO-PATHOLOGY.

DELIVERED BEFORE THE SECTION OF PATHOLOGY OF THE ROYAL SOCIETY OF MEDICINE.

By F. W. MOTT, M.D., F.R.C.P., F.R.S.,

PATHOLOGIST, LONDON COUNTY COUNCIL ASYLUMS; PHYSICIAN TO CHARING CROSS HOSPITAL.

[WITH SPECIAL PLATES.]

GENTLEMEN,—Allow me first to thank you for the great honour in electing me to the chair of this Section.

I was recently informed by the Senior Secretary that I should be expected to open this session by an address, and I propose therefore to give a general survey of the present position of the neurone concept, a subject in which I have been especially interested, as it is fundamental in neuro-pathology.

During the last fifteen years it has given rise to much discussion, to many experiments and observations, and to new modes of improving our knowledge of the minute structure and development of the nervous system.

The neurone theory was at first accepted by most neurologists without hesitation, as it explained so many facts in pathology; but grave doubts were cast upon it, owing to observations and experiments made by those who disbelieved the interpretation put upon histological details by those who upheld the doctrine.

This controversy between scientific men seeking after truth was not without great advantages, for it led to the discovery of new methods of technique and new methods of embryological research, which now have finally established on a firm basis the *neurone concept*, which I may remind you was due to Forel

although reintroduced in the following terms by Waldeyer: A neurone is a nerve cell and all its processes, including the protoplasmic processes or dendrons and the single axis cylinder process with its cone of origin, its collaterals or sidebranches, and its terminal arborization. The *neurone theory* is that the nervous system consists of innumerable such anatomically independent nervous units in contiguity but not in continuity. There is interlacing of the processes but no network. The nervous units are genetically and trophically independent cellular organisms arranged in functionally correlated systems, communities, and constellations.

It is admitted by all that the nervous system consists of nerve fibres and nerve cells supported by neuroglia, and the main point of discussion has been the origin of the nerve fibres. Waller¹ more than fifty years ago showed experimentally that a nerve separated from its ganglion underwent degeneration, and every one has admitted there must be some intimate relation between the ganglion cells and the nerves. But it was asserted that

when regeneration occurred it was owing to new fibres being formed by the cells of the sheath of Schwann. The question has long been in dispute whether regeneration takes place by a new outgrowth from the cell of origin or whether it is effected by the sheath cells. Likewise, there have been two opposing theories with regard to the genesis of the nerve fibres, one being that the fibre is an outgrowth from the cell, the other that it is a product of the sheath cells. Consequently, the subject that I shall discuss first will be the genesis of the nerve fibre.

How is the nerve fibre formed? The answer given, first by Schwann and later by Kupfer, Balfour, Dohrn, and many others, and still more recently by Apathy, Bethe, and Schultze, is that the nerve fibre is the product of a chain of cells, which extends from the nerve cells to the peripheral termination; they assert that these sheath cells secrete the fibrillae within their protoplasm. Certainly such an interpretation of the histological appearances could not be refuted till new methods of staining and new methods of experimentation were adopted to disprove this statement.

His, by his embryological researches, on the other hand, claimed that the axis cylinder and the other processes of the nerve cell were the outgrowths of the cell protoplasm. Then came the chrome silver method of Golgi, which, in the hands of Ramon y Cajal, Kölliker, Retzius, Lenhossek, Van Gehuchten, and many others, apparently demonstrated the fact that the whole nervous system consisted essentially of independent anatomical units, and the cylinder axon was an outgrowth of the nerve cell.

Ramon y Cajal was able to follow the development of the neurone from the neuroblast in all its stages, from the

first appearance of the short nervous process up to the long outgrowth which formed the nerve fibre. His researches extended over the most varied motor and sensory regions of the central as well as of the sympathetic nervous system, and fully confirmed the researches of His. These results of the researches of Cajal were soon followed by important researches of Held, who was able to demonstrate that the nerve fibre is an outgrowth of the nerve cell. Moreover, he accurately followed the origin and further

development of the neuro-fibrils. They are situated, as already His, Besta, and Lenhossek had observed, in the interior of the neuroblastic protoplasm, first in the form of a loose skein, and then they grow out from the nerve cell body into the nerve process. Held, however, like Hensen, finds that the nerve processes are not free, but grow into preformed tracts, which Hensen calls intracellular bridges, and he further believes that instances may arise where the neuro-fibrils of one cell grow into another cell, and commingling with the fibres of that cell pass out of its axon process. As Verworn remarks, these facts are interesting histological details, but are completely indifferent for the neurone doctrine.

If we look at the figures [Plate I—1, 2, 3, 4] given by the neuronists and the antineuronists, we can understand why this different interpretation of the mode of development of fibres should occur. Ross Harrison truly remarks it is impossible to decide by microscopic examination of sections of embryos whether the spindle-shaped cells lying around the nerve fibres form the fibres, or whether they simply

Fig. 1.—A, Frog embryo 2.7 mm. long. The line a-b indicates the incision for the removal of the ganglion crest. B, C, Two double embryos, from each of which the ganglion crest has been removed—B, two days after operation; C, six days after. (After Ross Harrison.)

ensheath processes of the nerve cells. He states that his own observations upon the normal development of the salmon and frog had led him to a decided opinion in favour of the neurone conception, but the attitude of the opponents of the doctrine was such that the study of normal development would never prove convincing; consequently he set about finding some means of differential experiment.

Profiting by the knowledge of the transplantation experiments in tadpoles of Born, Ross Harrison planned a new and exact method of study—namely, the removal in turn of each of the two structures in the developing embryo—frogs. Embryological studies had already shown that the source of the sheath cells was the ganglion crest; accordingly he removed this structure in the embryo *Rana esculenta* before any differentiation of nerve cells and fibres had occurred. The dorsal portion of the medullary tube and the ganglion crest was removed, and the embryo was thus left with its nervous system as an open groove in its back, the walls of which contained the cells which would subsequently develop into the spinal motor cells. Two embryos so operated upon were joined back to back, so as to prevent regeneration processes vitiating the result. (Fig. 1.) These animals were allowed to live a certain time and grow; they were then killed and examined carefully afterwards by serial sections. No spinal ganglia or sensory nerves were found, and what was of great interest was the fact that the motor nerves were found as fine protoplasmic threads extending from the spinal cord with not a single sheath cell around them (Fig. 2, C). The nerve consisted simply of delicate fibrils. This experiment shows that the ganglion crest forms the posterior spinal ganglion sensory nerves and the sheath cells. The conclusion is that the ganglion cells can form the nerve fibres without the aid of the sheath cells.

The next question Harrison sought to answer—and it is complementary to the last—was this: Can sheath cells without ganglion cells form the nerve fibres? He made the same incision as before, but instead of removing the ganglion crest he lifted it up and replaced it after removing with a fine pointed glass pipette the remainder of the medullary tube. The embryo developed normally, but remained almost motionless; subsequent examination showed that the sensory fibres and the sheath cells developed, but no motor fibres; although normally the motor and sensory fibres run together, and this would allow the fibres to the muscles ample opportunities of developing from the sheath cells if they were really the source of the motor fibres. (Fig. 2, D.)

He found, moreover, that nerves separated from their cells of origin degenerate rapidly, and no signs of regeneration were observed.

The question may be asked, Does the ganglion cell

process extend as a nerve fibre to its peripheral termination? or is this process, as Hensen maintains, a differentiation of protoplasmic connexions already *in situ*? This view of Hensen, according to which protoplasmic bridges are supposed to be left between the dividing embryonic cells, has been supported by the difficulty of conceiving how it is possible for a nerve fibre to grow out a long distance (and always to arrive at the right place) in the case of regeneration of the nerves of the foot, after division of the sciatic nerve for example. Harrison sought to solve the problem thus: He cut out the medullary groove that will form the spinal cord of an embryo before there was any trace of cell differentiation, and placed a small piece of it in lymph removed from the lymph sac of a frog; the preparation was put on a cover-glass, and mounted on a hollow slide and sealed with paraffin. The lymph clots almost immediately and holds the trans-

planted tissue in place. He has kept preparations alive five weeks and watched the development of the neuroblast, and he has seen the axon develop and grow [see Plate II—3, 4, 6]. A somewhat similar structure to the cone of increase described by Cajal by his silver method of staining embryonic spinal cords [see Plate II—6] can be seen. Probably it is also similar to the terminal bulb of regenerating axons found in the peripheral stump of a divided nerve. Harrison has observed a growth of $20\ \mu$ in twenty-five minutes as shown in the figures. Control experiments with other tissues of the embryo gave no such results. These remarkable observations of Ross Harrison show beyond question that the nerve fibre begins as an outflow of hyaline protoplasm from cells situated within the central nervous system. This protoplasm is actively amoeboid; retaining its pseudopodia at its distal end, the protoplasm is drawn out into a thread which becomes

Fig. 2.—Diagrammatic views of the nerves in the abdominal walls of the tadpole. A, The body of a larva showing general arrangement of nerves. B, Arrangement in normal larva. C, Arrangement in larva from which ganglion crest has been removed, only the motor nerves showing. D, Arrangement in larva from which the ventral half of the spinal cord has been removed, showing only sensory nerves. (After Ross Harrison.)

the axis cylinder of a nerve fibre. Although Harrison has disproved the theory that the sheath cells form the essential portion of the nerve—namely, the axis cylinder—yet he fully admits, as all those who uphold the neurone doctrine do, that the cells of the sheath of Schwann play an important rôle in the nutrition and production of the nerve fibre. These experiments of Harrison place the outgrowth theory of His on the safest of all foundations—namely, that of direct observation. It is, moreover, interesting and important to note briefly that Marinesco, Goldstein, Minea, and Nageotte have observed in transplanted spinal and sympathetic ganglia of warm-blooded and cold-blooded animals an outgrowth of fibrils from the ganglion cells. As Verworin truly says, the embryological basis of the neurone doctrine has been established beyond dispute. The formation of connexions of one neurone with another by fibrils, the passage of fibrils from one nerve cell through another nerve cell are matters of detail which in no way shake the fundamental principle

of the neurone concept—namely, the cell and its processes form a nerve unit which has a genetic independence. According to Kölliker, all the nerve fibres arise from nerve cells of the central nervous system and the ganglia. The peripheral nerve fibres are surrounded by special cells, constituting in the adult the sheath of Schwann. The latter appear when the axis cylinder is developed, and constitute for it a superficial envelope. These sheath cells all arise from the mesoderm and multiply by karyokinesis. According to Froriep, however, the cells of the sheath of Schwann of the peripheral nerves are probably, like the neuroglia cells, of ectodermal origin. Certainly Harrison's experiments seem to support Froriep's views.

The anatomical observations of Cajal and others I have named regarding the genetic independence of the neurone have thus been fully confirmed by the researches of Harrison. The next question is, Are the sheath cells of no further use than to enclose the axial core; in fact, is there a complete trophic independence of the nerve cell and its processes? Experimental and clinico-anatomical observations indicate that the sheath cells play an important part in regeneration after injury of the axon. To prove this statement we have only to consider the difference in the results on the extra-medullary and intra-medullary course of the axons of the posterior spinal ganglia after section of the posterior roots. Sherrington and many other observers have described fine medullated regenerated fibres around the cord—that is, central to the seat of injury—but only isolated observations have shown a few regenerated fibres in the exogenous systems of the posterior columns.* We may correlate these facts with the presence of sheath cells in the extra-medullary posterior roots and the absence of the same in their intra-medullary course. It was shown by Gudden that evulsion of the cranial nerve caused atrophy and disappearance of the nuclear cells of origin, and this method in his hands and that of his pupils, von Monakow and Forel, laid the foundation of much of our knowledge of the nuclear origin of the cranial nerves. Simple section of the nerves, as Nissl, Marinesco, Van Gehuchten, and others, have shown, produces chromolytic changes, but as a general rule the cells do not undergo atrophy. It may be assumed that evulsion was a more serious injury and destroyed not only all the sheath cells of the neuraxon up to the point of origin of the nerve from the central nervous system, but was also a much more serious injury of the neuraxon itself.

Before proceeding to describe the recent advances in our knowledge concerning the changes which take place in the central and peripheral ends of a divided nerve it will be well first to consider the changes in the nerve cells of origin as revealed by the Nissl method.

Changes in the Cells due to Lesions of Cranial and Peripheral Nerves.

Nissl first published (1892-1894) detailed accounts of the changes in the nerve cell (observed by his method of staining) produced by section of the nerves. Twenty-four hours after section of the facial nerve in the rabbit there is a reaction in the cells of the facial nucleus. The chromatic substances commence to disappear in limited regions of the cell body, and after two days instead of presenting the blocks of stainable substance, now known as the Nissl granules, there was observed a fine dust of coloured particles. By the third day the process had extended to the dendrons. At the end of five or six days the cell has changed its form, it has become rounder, the prolongations less visible and the protoplasm looks as if sprinkled with dust like particles of stainable substance. During this time the nucleus has changed its position and become eccentric. Nissl also studied the alterations in the cells of the posterior spinal ganglia produced by section. Marinesco repeated and confirmed Nissl's experiments, using the hypoglossal nerve. He was followed by Ballet et Dutil, Lugaro, Van Gehuchten, Flemming, and many others. The changes observed may be thus summarized: Swelling of the cell body with slight increase of volume of the nucleus and of the nucleolus, the cell becomes rounder and the prolongations in their turn swell; there is disintegration of the Nissl granules, a process which begins

in the central region of the cell near the axon-cone of origin. The first changes are observed twenty-four hours after section, and they progress until the whole perinuclear region exhibits this chromatolysis and the Nissl granules on the protoplasmic processes disappear. The nucleus becomes eccentric in proportion to the amount of chromatolysis. These changes are due to alterations of the osmotic conditions of the cell caused by the injury to its axon process, whereby fluid is absorbed from the ambient medium. Marinesco names this characteristic change "perinuclear chromatolysis"; a similar appearance of the nerve cells is seen in neuritis. I have observed it in all cases of alcoholic polyneuritis and in lead neuritis. This reaction of injury to the nerve cell when its axis cylinder process is injured is in direct confirmation of the correctness of the neurone doctrine, for if the sheath cells formed the axon it is much less likely that the nerve cell would show such changes, which may even proceed to their atrophy and disappearance. It is remarkable that when a nerve is divided only some motor spinal ganglion cells are so severely damaged that they do not recover but undergo atrophy and disappear, so it is found in polyneuritis some cells hardly show any change, while others are so injured as to be incapable of recovery and eventually atrophy and disappear. [Vide Plate I—6.] Again, in transverse lesions of the spinal cord, as shown by Pusateri, Marinesco, and Gordon Holmes, the Betz cells of the cortex undergo similar chromolytic changes which may proceed to atrophy, but the cells are affected very unequally, for we may observe two Betz cells lying side by side, the one seriously damaged and the other hardly affected at all. Drs. Sewell and Turnbull have investigated a case of transverse lesion of the spinal cord due to fracture of the spine at the level of the fifth cervical. The boy died at Charing Cross Hospital eight weeks after the injury. The Betz cells at the top of the ascending frontal convolution showed all degrees of chromatolysis, from cells with hardly perceptible changes to complete disappearance of the Nissl granules. I have observed also in experimental poisoning by botulin, absinthe, phosphorus, and other poisons, also in acute and chronic poisoning of human beings, that the neurones are not equally affected. Still more striking is the fact that, after experimental ligature of all four arteries, 2 carotids and 2 vertebrales, or 2 carotids, 1 vertebral, and 1 subclavian, whereby an experimental cortical anaemia in lower animals—for example, monkey, cat, and dog—was produced, the cells of the same type, for example, the Betz cells, were not equally affected; relatively only a few were so damaged as to undergo destruction. The conclusion is that the specific vital energy of some cells is greater than that of others, just the same as the individuals of a society are not all possessed of the same vital resistance to injury or poison; but as we know that the vital resistance of the individuals of a society is largely a matter of nutrition, fatigue, and powers of conservation of energy, so probably it is with the neurones. Those in which nutritional equilibrium is, either from inherent defect, fatigue, or other causes, in a low state, will suffer most.

I have also observed this chromatolysis of the Betz cells very marked in cases of chronic alcoholic and chronic lead polyneuritis; it was more marked to my mind than could be accounted for by the small amount of degeneration of the pyramidal tracts, and I cannot help thinking that it was the effect of the poison on the whole system of efferent neurones concerned with reflex and voluntary movements plus the parenchymatous and peripheral interstitial polyneuritis [Plate I—6]. All the neurones, however, do not undergo destructive atrophy; probably only those in which the nucleus is extruded are incapable of recovery. My experience has taught me that even in severe cases of polyneuritis recovery may result from continued passive movements of all the joints and massage of the muscles. The avoidance of pressure and weight of the bed-clothes in rendering a foot-drop permanent, the instruction of the patient to make voluntary effort by aid of attention and vision, and try to assist the operator making the passive movements; the placing the feet against a board while lying in bed, and the daily placing the feet on the ground in the effort to feel the weight of the body supported by the limbs, all tend, by favouring incoming and outgoing stimuli, to open up the old paths or to restore function by new paths. This

* Again, although Marinesco, Minea, and Fickler have seen attempts at formation of regenerating fibres after transverse lesions of the spinal cord, there is no restoration of function, because there is no re-establishment of connexion with the appropriate neurones.

is no mere hypothesis, for it is based upon the fact that Munk found that monkeys in which the motor area had been removed much more completely regained movements when placed in a cage large enough to enable them to spring about and take free exercise.

Upon this assumption, therefore, that stimulus exercised an important influence upon regeneration of nerves, Halliburton and I carried out a series of investigations to see if regeneration of the motor nerves was interfered with by cutting off, so far as we were able, stimuli from the spinal motor neurones of a limb. The following procedure was adopted: Unilateral section of the posterior roots from the third cervical to the third dorsal inclusive was performed, the animals used being monkeys. There was as a result a marked hypotonus of the limb, which hung useless as a flail, and the animal did not use it for refined voluntary purposive movements. Either the median or the ulnar nerves on the two sides were then divided and sutured. There was apparently no difference in the time taken for regeneration on the two sides, nor could I discover any more marked changes in the anterior horn cells of one side than of the other. Again, the motor area of the right side of the brain was removed in an animal in which those same posterior roots had been divided; the nerves were divided on both sides, and the result was the same as in the previous related experiment. A third experiment was performed: a unilateral section of the lumbo-sacral roots was combined with hemisection of the spinal cord in the mid-dorsal region and both sciatics were subsequently divided and sutured. Even in this experiment we could not claim that all sources of stimulus to the spinal motor neurones of the side of the double lesion had been removed. Still, the greater part of the paths exercising any influence upon the discharge from the spinal motor neurones had been removed, and if stimulus really did play an important part in the nutritional activity necessary for regeneration of the motor nerves, after section of the axons of the motor neurones, it might be certainly expected that regeneration would be delayed on that side; but it was not, and this accords with the fact that the characteristic changes in the ganglion cells were not more apparent on the side of the double lesion than on the opposite side. A careful examination of sections of the spinal cord failed to show more ganglion cells presenting chromolytic changes on one side than the other. In order to avoid any personal bias, I tried to see if I could ascertain on which side the double lesion had been made by an examination of the anterior horns; but I failed entirely, although when I shifted the specimen to the posterior cornua it was quite evident from the atrophy of the fibres and changes in the cells. Plate I, 6, shows two degenerated cells on the side opposite to the spinal and root lesions.

The trophic activity of the spinal motor neurones is therefore in all probability independent of stimulus arriving from other neurones. They possess, indeed,

a trophic autonomy. The observations of Harrison support this idea; moreover, this accords with the neurone doctrine of genetic and trophic independence. Direct injury of any part of the neurone will cause changes but not injury of neurones with which it is functionally but not nutritionally correlated.

Fig. 3.—Wallerian degeneration of regenerated nerve fibres in a cat. Marchi method of staining. (600 diameters.)

sequently may be [vide Plate II—5] assumed to act as the conductile element of the neurone.

The appearances presented by fibres undergoing degeneration when stained by the Marchi method are well known. I may call your attention to Fig. 3, which is of considerable interest for another reason to which I will briefly refer. The following experiment was performed by Halliburton and myself.

A small piece was resected from a nerve in which regeneration had taken place, and histologically the nerve was shown to possess many fine myelinated fibres. Ten days later the animal was killed, and the portion of the nerve below the resection was examined; no myelinated fibres were found, but a number showing Wallerian degeneration. If the sheath cells had been mainly instrumental in the formation of the new regenerated fibres, resection of the piece of nerve should have had no influence upon the nerve which had developed from the sheath cells.

The changes which occur in the central end of the cut nerve were very incompletely known before Cajal introduced his silver method, and the histological observations of Peroncio, Cajal, and Marinesco by this method have thrown a new light upon the process of regeneration. Peroncio, three hours after section of the nerve, has found in the central end of the nerve very fine

collateral ramifications of the axon, terminating after a short course, in very delicate filaments. After eighteen hours he has observed in the central end most of the anatomical conditions indicative of regeneration, which, previous to his researches, had been looked upon as late productions of the axis cylinder—namely, the plaquette, the ring, the button, and the helicoidal formation. [Plate II—1.] These

Fig. 4.—Section of the sciatic nerve of a dog five and a half days. Details of degeneration in the central end. A, Axis cylinder with a nearly normal appearance in its upper part, whilst in its lower part it is considerably swollen, and the neuro-fibrils are very apparent, and like a tress of hair. In reality it is a reticulated structure made evident by an increase of the interfibrillary and perifibrillary substance. B, Axis cylinder swollen and with the neuro-fibrils dissociated at the upper part, and reunited in diverging cords towards the upper third. Some of these end in a terminal bulk. C, The axis cylinder swollen in a still more marked manner, and consisting above of tresses of neuro-fibrils. These reunite into cords below, which surround more or less the debris of a degenerated axis cylinder. (After Marinesco).

observations of Peroncito show that active nutritive changes commence almost directly after the injury before the sheath cells divide. Marinesco has confirmed the observations of Peroncito, and has found that within twenty-four hours of section of the sciatic nerve very important and interesting phenomena of regeneration occur. These phenomena are the results of an increased nutritive activity associated with a swelling and hypertrophy of the large axis cylinders, whereby the constituent nerve fibrils which make up the axons become evident. The network or skein of fibrils are clearly seen, owing to a great increase of the interfibrillary substance, and a longitudinal dissociation of the bundle of fibrils takes place. These skeins of fibrils may run side by side or intercross one another. The various appearances of the fibrils resulting from this longitudinal dissociation are represented in Fig. 4, and are very different from one another. Between the tangled skeins of dissociated fibrils there may be offshoots of more delicate fibrils forming plexuses. Some of these delicate fibrils, as Fig. 5 shows, terminate in a little olive or cone of growth. This process of longitudinal

phase of very active growth, which assures the neurotization of the peripheral end of the divided nerve. After the stage of active ramification or dendro-amoeboidism numbers of terminal clubs of the young fibres are seen. These traverse paths rendered free to their passage between the sheath cells. Marinesco thinks that these terminal clubs only indicate a delay or retardation of chemiotropism of the sheath cells. The shape of these terminal bulbs, which are so striking a feature of the regenerating nerve fibrils, is variable; in structure they consist of a network or twisted skein of fibrils in the form of an olive or bulb and an interfibrillary amorphous substance (Plate II—2). It is difficult to decide whether these bulbous terminals represent cones of growth or a twisting up of a fibril on account of an obstacle to the growth or nutrition of the nerve fibril.

If sections be made of the junction of the central and peripheral ends at successive periods of time, three stages can be seen: (1) The sprouting of large numbers of new fibres from the central cut axon; (2) the penetration of the bridge of young vascular connective tissue joining the cut

Fig. 5.—Hypertrophied fibres with very obvious fibrils presenting an abundant lateral and terminal division. Some of the collateral and terminal ramifications are seen ending in a small or large bulb; others have a free extremity. The proliferated sheath cells are very obvious. (After Marinesco).

Fig. 6.—Longitudinal section of the sciatic nerve of a newborn dog eight days after section. A, Central end. B, Cicatrix. C, Peripheral end. (After Marinesco.)

Fig. 7.—Represents four fibres showing different degrees of exolysis. A, The axis cylinder no longer shows any neuro-fibrils, but consists of abundant fine granules disseminated in the interfibrillary substance. B, Formation of cavities and vacuoles in the interior of the axis cylinder, in consequence of resorption of the granules. C, The same condition more advanced. D, Fragmentation and breaking up into small pieces of the axis cylinder. (After Marinesco.)

fibrillary dissociation of the axon leads to the formation of a number of embryonic fibres, from which develop a number of young axis cylinders. These young fibres can be followed through the central cut end to the cicatrix. Some are seen to terminate in a button, olive, or club-shaped extremity, forming a cone of growth which penetrates between the more or less longitudinally-arranged sheath cells. [Plate II—7.] At the termination of the cut ends there is a cellular exudation and new-formed blood vessels; this undergoes organization, and at the end of six or seven days forms a bridge which unites the two cut ends for the passage of the young regenerating fibres. But in proportion as the axon fibres penetrate the cicatrix they diminish in numbers and their branches are less numerous. The new fibres may be seen to diverge, intercross, and form a felt work, as seen in Fig. 6. A certain number of them, however, can be seen taking a straight course and descending vertically in the peripheral stump. Cajal considers that the terminal bulbs correspond to the cone of growth of the developing axon in the embryo or pseudopodia in the advancing axon of the neuron observed by Harrison in his experiments. Probably Cajal is correct in maintaining that the terminals of the new-formed young fibres correspond to an amoeboid

ends by the young nerve fibres; and (3) the penetration and growth downwards of these young fibres between the proliferated sheath cells of the peripheral portion [vide Plate II—7]. There can be no doubt, then, that the new nerve fibres depend upon an outgrowth from the central cut end of the neuraxon.

What part, then, we may ask, do the sheath cells play in regeneration?

To answer this question it is well to consider some of the appearances presented by the peripheral fibres which are undergoing Wallerian degeneration when stained by the Cajal silver method. The lesions are the same in all animals, but the moment of their appearance depends upon the species, age of the animal, and severity of the injury which has caused the solution of continuity of the nerve. The changes appear first at the upper end and are propagated peripheral-wards through the whole extent of the nerve below the lesion. The degeneration does not affect all the fibres equally. The first change is a swelling of the axis cylinder whereby the fibrillary network is displayed. The neuro-fibrils are visible and appear thickened in their course. But very soon the axon appears only as a dark stained finely granular cord (Fig. 7, A). In place of neuro-fibrils there is a dark mass of fine granules.

Marinesco terms this condition "axolysis." He believes that the fragmentation of the myelin sheath and axis cylinder constitute connected phenomena dependent upon the same cause, namely, the formation of a digestive ferment on the inner surface of the sheath of Schwann. He moreover considers the ferments formed are analogous to the proteolytic and lipolytic ferments of the pancreatic juice, for Bokay has found that the pancreatic juice splits lecithin up into cholin glycerophosphoric acid and fatty acid. It is the oleic acid which stains black with the Marchi reaction. Resorption occurs in this softened and swollen axon, causing vacuolation. The vacuolation becomes more marked as the process of resorption continues, and this results in fragmentation of the axon, and finally the fragmented axon is completely destroyed and absorbed (Fig. 7, B, C, D). The sheath cells undergo rapid proliferation and exercise a phagocytic action upon the products of degeneration, while others arrange themselves in a longitudinal direction to receive and attract the rapidly growing young fibres which have come from the central cut end. The experiments of Forssman appear to show that the products of degeneration have a chemiotropic influence upon the developing fibrils of the cut central end of the nerve.

Forssman cut the tibial nerve and brought the central cut end into relation with the divided peroneal and tibial nerves which he placed in a celloidin tube; two months later he found the same number of regenerated fibres in each of the nerves. This may support the view that the sheath fibres exercise a chemiotactic influence, but Forssman made another series of experiments showing that this chemiotropism depends upon chemical attraction of the degenerated myelin. His experiments were conducted thus: He divided the central end of a nerve and placed each half into two separate celloidin tubes; one was filled with emulsion of liver and the other with brain emulsion; the fibres all grew in a direction towards the tube containing the brain emulsion. It may be concluded from this experiment that the degenerated myelin of the peripheral portion of the nerve attracts the young fibres and the sheath cells ensheath them.

Forssman also showed that the fibres of the central end do not take the line of least resistance, but the direction to which chemiotropism attracts them. Cajal, however, asserts that it is the attraction of the protoplasmic strands formed by the sheath cells which are arranged in dense fusiform bundles, and Cajal and Marinesco believe that these neurilemmal cells secrete a chemiotropic substance capable of exciting amoeboid movement in the pseudopodia of the young regenerating axons, and having attracted, direct and nourish them. Marinesco claims to have proved this by the following experiments. He took a piece of nerve in which the sheath cells are dead; it was obtained either from a dead animal or by resection of a piece of nerve from a living animal which was allowed to remain a sufficient time in serum for death of the sheath cells to have taken place. This was connected to the central cut end of the sciatic nerve of an animal. To the central end of the other divided sciatic nerve he connected a piece of nerve kept alive in Locke's oxygenated serum. In the

former case, when the sheath cells are dead there is no chemiotropic influence upon the fibres of the central cut end, whereas in the latter, if the resected nerve is from the same species, the fibres of the central end grow in. As the cell processes grow down into the new sheath an interaction takes place between the new axis cylinder and its sheath, leading to the formation of intervening myelin. Moreover, Halliburton and I found, in regenerating nerves, a much larger number of myelinated fibres and the myelin sheath of the fibres thicker near the point of section than in a remote portion of the nerve [vide Plate II-5]. This was an indirect support to the outgrowth theory, and has also been observed by Langley and Anderson.

It may be assumed, therefore, from what I have said, that the trophic and genetic independence of the neurone has been established. A question still remains, and that is, How are these nervous units connected with one another—if they are independent, how are they functionally correlated? This has, from the foundation of the neurone concept, been a point in dispute. Although many valuable researches have been published, still we

are far from understanding the problem. The Golgi method appeared to show that the neurones are arranged in systems and communities in contiguity but not continuity, and numbers of experiments and observations were made to show that the dendrites exercised amoeboid functions, and little buds or gemmules on these processes of the cells were supposed to appear and disappear owing to the contractility of the protoplasm, thus leading to varying degrees of contact influence of one neurone upon another. But there is no proof that these gemmules exist in the living cells, although the amoeboidism of the branching processes of the neurones does seem possible as an explanation, when Harrison's experiments combined with Peroncito's observations are considered. But, as Verworn justly remarks, we must be careful not to generalize upon insufficient data, for the physiological connexions in the nervous system of the lowest types of animals as compared with the higher vertebrates are different; take, for example, the

simple reflex processes in the lower invertebrates and the complicated processes of association in the human brain. In the invertebrates there can be no question that Apathy and Bethe are right in asserting that there is a fibrillary continuum through several ganglion cells.

But does a continuity exist in the vertebrates? In my Croonian lectures on the Degeneration of the Neurone, I referred in the following words to some important researches of Held which had been put forward to disprove the neurone theory. "In my opinion, however, one can still accept the neurone theory and admit the truth of Held's observations—namely, that the terminal arborization of the axis cylinder process of one neurone forms protoplasmic concrescences by fusion with the cell body and dendrons of another. This, of course, implies continuity of the protoplasm of one neurone with another, but trophically and genetically the two are independent, and it is merely a question of degree of contact of the protoplasm of one with the other. Held agrees with other investigators that in embryonic tissues, and even

DESCRIPTION OF PLATE I.

1.—Section through the spinal cord of a chick embryo of three days. A, Motor root. B, spinal ganglion; a, motor neuroblast; b, c, commissural neuroblasts. (After Ramon y Cajal.)

2.—Cross section through a chick embryo of seventy-three hours, to show the beginning of a spinal nerve (*sp. n.*); *m. c.*, medullary cord. (After Bethe.)

3.—Cross-section through the medullary cord of a salmon embryo, to show neuroblasts and motor nerve fibres (*m. n.*). (After His.)

4.—Semi-diagrammatic section through the spinal cord and adjacent organs of an axolotl embryo. The peripheral nerve fibre is seen arising from the ganglion cell; it does not grow out free into spaces between the cells, but into protoplasmic bridges or plasmodesmata which have been already formed by other cells. According to this theory the nerve paths arise through the transformation of these plasmodesmata into neurodesmata. (After Held.)

5.—(a) Regenerating motor bundle of a cat's sciatic nerve ninety-one days after the nerve was divided and sutured. Marchi's method of staining. The transverse section shows well marked myelination in the majority of the fibres; it was taken from the upper end of the nerve. (Magnified 220 diameters.) (b) The same bundle 3 in. lower down. Myelination much less advanced. (Magnification 220 diameters.)

6.—(1) Group of anterior horn cells from a monkey's spinal cord on the side in which section of the sciatic nerve had been performed. On the opposite side hemisection and section of all the lumbo-sacral roots had been performed, in addition to section of the sciatic nerve. Yet there were as many altered spinal motor neurones on the one side as the other, thus showing that the cause of this alteration was the section of the nerves, and that interference with the passage of stimulus has had little to do with the trophic condition of the cell. It will be observed that some cells have the normal Nissl pattern. (2) Cells of the cortex cerebri in phosphorus poisoning of the rabbit. It will be observed that one cell shows considerably more chromolytic change than the other. (3) Cells of the anterior horn; cervical enlargement in a case of peripheral neuritis caused by chronic lead poisoning. The perinuclear chromatolysis and eccentric condition of the nucleus is very evident, and the appearance presented is the same as by the large cells in Fig. 1. (4) A large Betz cell of the cerebral cortex with eccentric nucleus and chromatolysis specially marked around the axon cone of origin; from the same case of lead neuritis. (5) Five cells showing various degrees of eccentricity of the nucleus; chromatolysis from the posterior spinal ganglion groin; a case of alcoholic neuritis.

PLATE I.

1.

2.

3.

4.

5.

6.

PLATE II.

1.

2.

3.

4.

7.

5.

6.

in early life, the neurones are entirely independent of one another. This independence he can determine by a line of demarcation at the points of contact due to a difference in refraction. This refractile limiting line is, however, not demonstrable in the adult, and he comes to the conclusion that during the process of growth the protoplasm of related neurones fuses." Verworn states that the pictures which Held publishes are not clear enough to satisfy him. On the other hand, the possibility is undeniable that where the axon of one neurone is in contact with the dendrites of another there is a "receptive substance," which Sherrington has termed the synapse; this substance may possess certain physiological properties which have a similarity with those of the end organ in striped muscle—for example, easy fatigability, great summation, capacity for excitations, and specific reactions for certain poisons, etc. "But it remains undecided whether the acceptance of such a synapse having the above related physiological properties is necessary for the explanation of the physiological processes. In any case great difficulties exist both for the acceptance of the synapse as for the acceptance of a continuous path of conduction. It is a fact that conduction of the stimulus through the reflex arcs only occurs in the physiological and not in the opposite direction. Yet it can be demonstrated that the nerve possesses conductivity in both directions; therefore the necessary conditions for this difference of conductivity must be in the centre. Where the conditions are situated and how they are affected, the future must decide." It is admitted that the lower we descend in the zoological scale the more obvious is the fibrillary continuum, and the simpler and less varied are the motor adaptations to environment. We may assume, therefore, that in the upward development of the animal series with the complexity and refinement of motor adaptation there co-exists a neuronie independence. We may ask the question, What would be the advantage in the evolution of the nervous system of neuronie independence? In the attempt to answer such a question it is desirable to look at it from a broad biological standpoint. What are the properties of nerve tissue, and how does it differ from other cell protoplasm? We may say it is irritable and conductile, but we know that the protoplasm of the carnivorous plants—the sundews and Venus flytrap—is irritable, conductile, contractile, and secretory, and these plants are capable of exhibiting reflex action without a nervous system when the appropriate stimulus is applied. This reflex action, however, differs from that of the plant-like animals with their diffuse network of neuro-muscular cells in speed of reaction.

Consider, moreover, the advantage of speed of voluntary action initiated in the brain by the impulse having to pass through the conductile fibrils of only two nervous cells in the production of motor reactions of the arm, leg, face, and tongue. But if there existed a fibrillary continuum from the cerebral neurone through the anterior spinal neurone, how could the anterior motor spinal neurones form the "final common path" for a multitude of different muscular activities?

The idea that the neuraxon of a Betz psycho-motor cell is connected by its conductile fibrils with one anterior spinal motor neurone is a textbook myth, which instead of making the nature of voluntary movements to the thoughtful student clear, only makes him ask the question, Are the number of fibres in the pyramids of the medulla equal to the sum of all the fibres in the anterior roots? Clearly the fibrils, then, of the Betz cells must be connected with a

number of spinal motor neurones forming the final common path for a multitude of different voluntary muscular activities. Moreover, we know that there is not a direct connexion between the upper cerebral neurones and the lower spinal motor neurones, for intercalary neurones with axons which do not leave the grey matter intervene and the terminal arborization of sensory afferent neurones, cerebral, mesencephalic, and bulbar efferent neurones, combine with spinal association neurones through the intercalary neurones in augmenting and inhibiting the outflow of energy of the spinal motor neurones, whereby the most varied, refined, and complex muscular reactions in adaptation to environment are effected. The nervous energy is expended in the grey matter of the brain and spinal cord.

There are many other interesting points concerning the neurone which I might, had time permitted, have spoken about—for example, the significance of the nodes of Ranvier and the perifibrillary protoplasm; the theories in respect to the functions of the interfibrillary chromophilous substance of the ganglion cell and the dendrons. Is this chromophilous substance the source of nerve energy, and if so, is it used at the seat of its formation, the nerve cell, or is this substance, which in the dead cell corresponds to the Nissl granules, a store of energy-producing substance in the latent state analogous to

the proferment of secretory glands—a substance which is transformed into the active energy-producing substance at the terminal arborizations of the neurone? I must, however, rest content if the facts and arguments which I have related have satisfied you that the neurone doctrine, pronounced dead only a few years ago, now rests on a surer basis than ever, this satisfactory result being due especially to the introduction of new methods of investigation by Ramon y Cajal and Harrison.

DESCRIPTION OF PLATE II.

- 1.—Regenerating nerve fibres from the end of the central nerve stump of the sciatic nerve of a dog, taken from six to forty-eight hours after cutting the nerve. (After Perroncito.)
- 2.—Olive-shaped swellings at the end of nerve fibres, growing downwards from the central end twenty-one days after the nerve had been divided. Coiled-up fibrils can be seen in these swellings. (After Marinesco.)
- 3.—Portion of a horizontal longitudinal section through the spinal cord (*m.c.*) and portion of two muscle plates (*my.*) of a frog embryo. The cell (*m.*) with the branched process is a neuroblast, showing the first stage in the formation of the nerve fibre. (After Ross Harrison.)
- 4.—Isolated cell from a piece of embryonic spinal cord growing in a drop of clotted lymph. The cell body, which is fitted with yoke granules, is sending out a hyaline protoplasmic process which undergoes amoeboid movements. Drawn from a live specimen. (After Ross Harrison.)
- 5.—Two views, taken twenty-five minutes apart, of the same nerve fibre growing from a group of embryonic spinal cord cells into the lymph. (After Ross Harrison.)
- 6.—A, Neuroblasts stained with silver nitrate. B, Neuroblast impregnated by the Golgi method. Growth cone appears well marked at the top of B. (After Ramon y Cajal.)
- 7.—Regenerating nerve fibres of a cut nerve. The young fibres *a, b, c, d, e*, growing from the central end, can be seen passing through the cicatrix down into the peripheral portion between the proliferated sheath cells. Some of these are seen to bifurcate—for example *d, c, e*. One branch of *d* terminates in an olive-shaped bulb, *f*, the other proceeding onwards. It is possible that the former is arrested in its progress, and coils up to form the olive. (After Ramon y Cajal.)

BIBLIOGRAPHY.

- 1 Waller: Minute Structure of the Papillae and Nerves of the Tongue of the Frog and Toad. *Phil. Trans.* 1849; Experiments on the Glossopharyngeal and Hypoglossal Nerves of the Frog. *Phil. Trans.* 1850.
- 2 Verworn: Bemerkungen zum heutigen Stand der Neuronlehre. *Medizinischen Klinik*, 1908, Nr. 4.
- 3 Mott: The Croonian Lectures, The Degeneration of the Neurone. *BRITISH MEDICAL JOURNAL*, 1900.
- 4 Schultze, Oskar: Beiträge zur Histogenese des Nervensystems. Ueber die multizelluläre Entstehung der peripheren sensiblen Nervenfasern. *Arch. f. mikr. Anat.*, 1905, Bd. 66.
- 5 Ramon y Cajal: *Trabajos de Laboratorio de Investigaciones Biológicas de la Universidad de Madrid*, Tome iv. Madrid, 1906; Génesis de las Fibras Nerviosas del Embrión; *Anatomischer Anzeiger*, Bd. 5, 1890; Bd. 30, 1907; and Bd. 32, 1908.
- 6 Harrison, Ross Granville: Embryonic Transplantation and Development of the Nervous System, *Anatomical Record*, vol. ii, No. 9, December, 1903; Experiments on Transplanting Limbs, *Journal of Experimental Zoology*, Vol. iv, No. 2; Regeneration of Peripheral Nerves, *Anatomical Record* (No. 8) of the *American Journal of Anatomy*, vol. vii, No. 4; Observations on the Living Developing Nerve Fibre, *American Journal of Anatomy*, 1907, vol. vii.
- 7 Held: *Verhandlungen der anatomischen Gesellschaft*, Rostock, 1905; *Anatomischer Anzeiger*, Bd. 30, 1907.
- 8 Perroncito: *Zeigler's Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie*, Bd. 42, 1907; Sur la question de la régénération autogène des fibres nerveuses, *Archiv. Ital. de Biol.*, vol. xlv, 1905, No. 3.
- 9 Bethé: *Allgemeine Anatomie und Physiologie des Nervensystems*. Leipzig, 1903.
- 10 Hensen: *Die Entwicklungsmechanik der Nerventhaten im Embryo der Säugethiere*. Kiel und Leipzig, 1903.
- 11 Marinesco: *La cellule nerveuse*, Tome ii: Recherches sur la régénérescence autogène. *Rev. neurol.*, No. 23, 1905; Recherches sur la régénérescence des nerfs périphériques. *Rev. neurol.*, No. 5, 1906.
- 12 Marinesco et Goldstein: Recherches sur la transplantation des ganglions nerveux. *Comptes rendus de l'Académie des Sciences*, February 18th, 1907.
- 13 Marinesco et Minea: Nouvelles recherches sur la transplantation des ganglions nerveux. *Comptes rendus de l'Académie des Sciences*, February 25th, 1907.
- 14 Nageotte: Greffe des ganglions rachidiens, *Soc. de Biol.*, 19 janvier, 22 fév. et 9 mars, 1907.
- 15 Mott and Halliburton: *Proceedings of the Royal Society, B.*, vol. lxxviii, 1906, p. 259, in which is contained a number of references to the original work done on regeneration of nerves.
- 16 Forssman: Zur Kenntniss des Neurotropismus. *Zeigler's Beiträge zur path. Anatom. und zur allgem. Path.* Bd. 27, 1900.