

SANTIAGO RAMÓN Y CAJAL

The structure and connexions of neurons

Nobel Lecture, December 12, 1906

In accordance with the tradition followed by the illustrious orators honoured before me with the Nobel Prize, I am going to talk to you about the principal results of my scientific work in the realm of the histology and physiology of the nervous system.

From my researches as a whole, there derives a general conception which comprises the following propositions :

1. The nerve cells are morphological entities, neurons, to use the word brought into use by the authority of Professor Waldeyer. My celebrated colleague Professor Golgi has already demonstrated this property with respect to the dendritic or protoplasmic processes of the nerve cells; but at the beginning of our research there were only vague conjectures as regards the behaviour of the axon branches and collaterals. We applied Golgi's method, firstly in the cerebellum and then in the spinal cord, the cerebrum, the olfactory bulb, the optic lobe, the retina and so on of embryos and young animals, and our observations revealed, in my opinion, the terminal arrangement of the nerve fibres. These fibres, ramifying several times, always proceed towards the neuronal body, or towards the protoplasmic expansions around which arise plexuses or very tightly bound and rich nerve nests. The pericellular baskets and the climbing plexuses, and other morphological structures, whose form varies according to the nerve centres being studied, confirm that the nerve elements possess reciprocal relationships *in contiguity* but not *in continuity*. It is confirmed also that those more or less intimate contacts are always established, not between the nerve arborizations alone, but between these ramifications on the one hand, and the body and protoplasmic processes on the other. A granular cement, or special conducting substance would serve to keep the neuron surfaces very intimately in contact.

These facts, recognized in all the nerve centres with the aid of two very different methods (that of Golgi and that of Ehrlich), confirmed and notably developed by the research of Kölliker, von Lenhossék, Retzius, Van Gehuchten, Lugaro, Held, my brother, Athias, Edinger, and many others, imply three physiological postulates:

(a) As nature, in order to assure and amplify the contacts, has created complicated systems of pericellular ramifications (systems which become incomprehensible within the hypothesis of continuity), it must be admitted that the nerve currents are transmitted from one element to the other as a consequence of a sort of induction or influence from a distance.

(b) It must also be supposed that the cell bodies and the dendrites are, in the same way as the axis cylinders, conductive devices, as they represent the intermediary links between afferent nerve fibres and the afore-mentioned axons. This is what Bethe, Simarro, Donaggio, ourselves, etc. have confirmed quite recently by demonstrating, with the aid of neurofibrillar methods, a perfect structural concordance between the dendrites and the prolongation of the axon cylinder.

(c) The examination of the movement of nervous impulses in the sensory organs such as the retina, the olfactory bulb, the sensory ganglia and the spinal cord, etc. proves not only that the protoplasmic expansions play a conducting role but even more that nervous movement in these prolongations is *towards the cell or axon*, while it is *away from the cell* in the axons. This formula, called the *dynamic polarization of neurons*, originated a long time ago by Van Gehuchten and us as an induction from numerous morphological facts, is not in contradiction with the new research on the constitution of nerve protoplasm. Indeed we will see that the neurofibrillar framework constitutes a continuous reticulum from the dendrites and the cell body to the axon and its peripheral termination.

During twenty-five years of continued work on nearly all the organs of the nervous system and on a large number of zoological species, I have never met a single observed fact contrary to these assertions, and yet I have used in my research, in addition to the usual processes of coloration, the chosen methods of Golgi, Cox, Ehrlich and lastly the neurofibrillar methods. Let us add that the same doctrine also arises out of the assemblage of observations of Kölliker, von Lenhossék, Van Gehuchten, my brother, Edinger, Lugaro, etc., on the nervous system of the vertebrates and from those very important observations of Retzius on the nervous system of the invertebrates.

I ought now to review the principal observations on which the preceding deductions are based. This would also be the best way to show you the scope and the extent of my modest personal contribution to the knowledge of the nervous system. Unfortunately, it is absolutely impossible to cover in a few pages all the morphological facts whose description occupies a large number of brochures containing hundreds of drawings. However, in order not to

put too great a strain on your very kind attention, I will limit myself to choosing from all my works several striking examples of interneuronal connexion, which I have reproduced schematically in the pictures which follow:

First of all, let us look at the connexions of the sensory roots of the spinal cord. We know well, from the researches of Ranvier, Retzius and of von

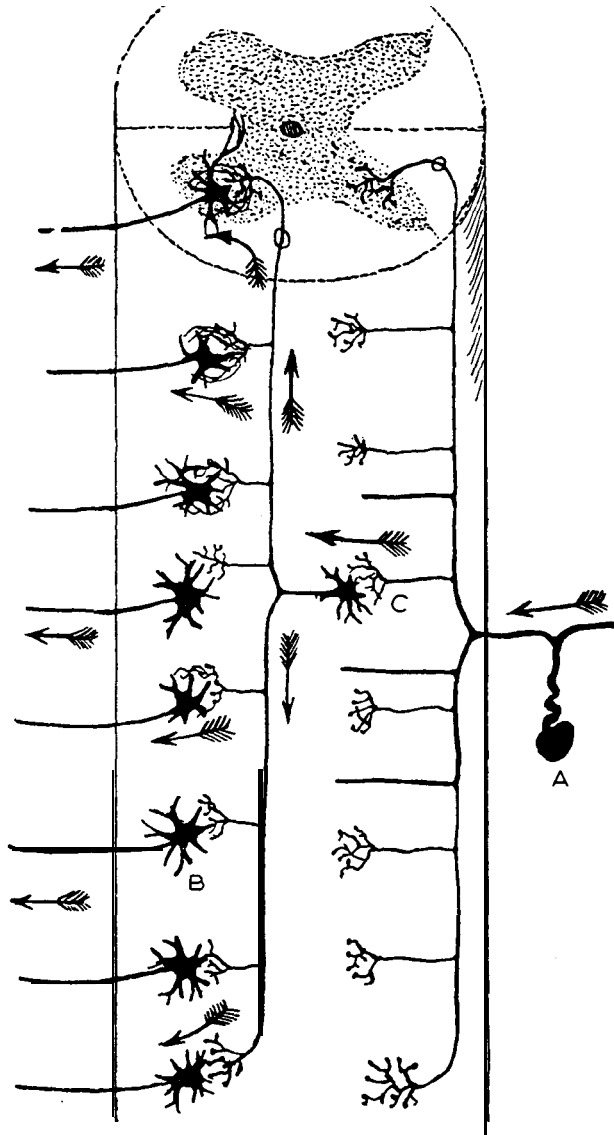


Fig. 1

Lenhossek, etc., that the single prolongation of the sensory corpuscles is divided into two branches: one external branch which leads to the periphery to end in the skin, or in the mucous membranes; another internal branch which penetrates the sensory or posterior root to end in the dorsal column of the spinal cord. This last branch, from my observation in birds, reptiles and mammals (confirmed by a large number of scientists such as Kölliker, von Lenhossék, Retzius, Van Gehuchten, Sala, Athias, etc.), does not penetrate the grey matter straight away, as some writers had supposed, but divides in the thickness of the posterior column in such a way as to give one ascending branch and another descending branch (A in Fig. 1). This bifurcation is in the form of a Y and the fibres arising from it run along the dorsal column for a considerable length to end finally within the grey substance by way of varicose, pericellular arborizations.

But in addition to these terminal arborizations, the sensory root fibres also give off at right angles a considerable number of collateral twigs, either from the main trunk or from the ascending and descending branches; thanks to these, the sensory root fibres connect up with all the neurons of the grey matter (a, b, c in Fig. 2 and C in Fig. 1). These collaterals can be divided into two principal varieties: the *long* or *reflexomotor* destined to come into contact with the motor neuron (C in Fig. 2) as our research, and above all that of Kölliker and of von Lenhossék, has indeed demonstrated; and the *short* destined to come into relation with the funicular neurons (homolateral and opposito-lateral) which lie in the two horns of the grey matter (a and c in Fig. 2).

The terminal arborizations of these fibres produce around the neurons and their dendrites very closely knit terminal plexuses, visible especially at the level of the motor corpuscles. Our researches, carried out firstly on embryos, and on animals a few days old, have drawn attention to terminal varicosities in these nests; just recently Held and Auerbach whose researches have been confirmed and completed by us, Van Gehuchten, Mahaim, Holmgren and many others with the help of our reduced silver method, have demonstrated that these varicosities are well developed in adult mammals, and contain a network or a neurofibrillar ring.*

*As regards the supposed anastomoses recently brought to notice by Held, Holmgren and Wolff between the neurofibrils of the terminal boutons and the neuronal soma, we consider them as anatomical hypotheses based on the observation of accidental images, and not sufficiently clear to justify such conception. This is also the opinion of Michotte, Van Gehuchten, Mahaim and Schiefferdecker.

As c in Fig. 2 shows, the nervous movement in the sensory roots is divided into two important currents: a direct or *direct reflexo-motor current* which is transmitted without an intermediary neuron from the posterior root to

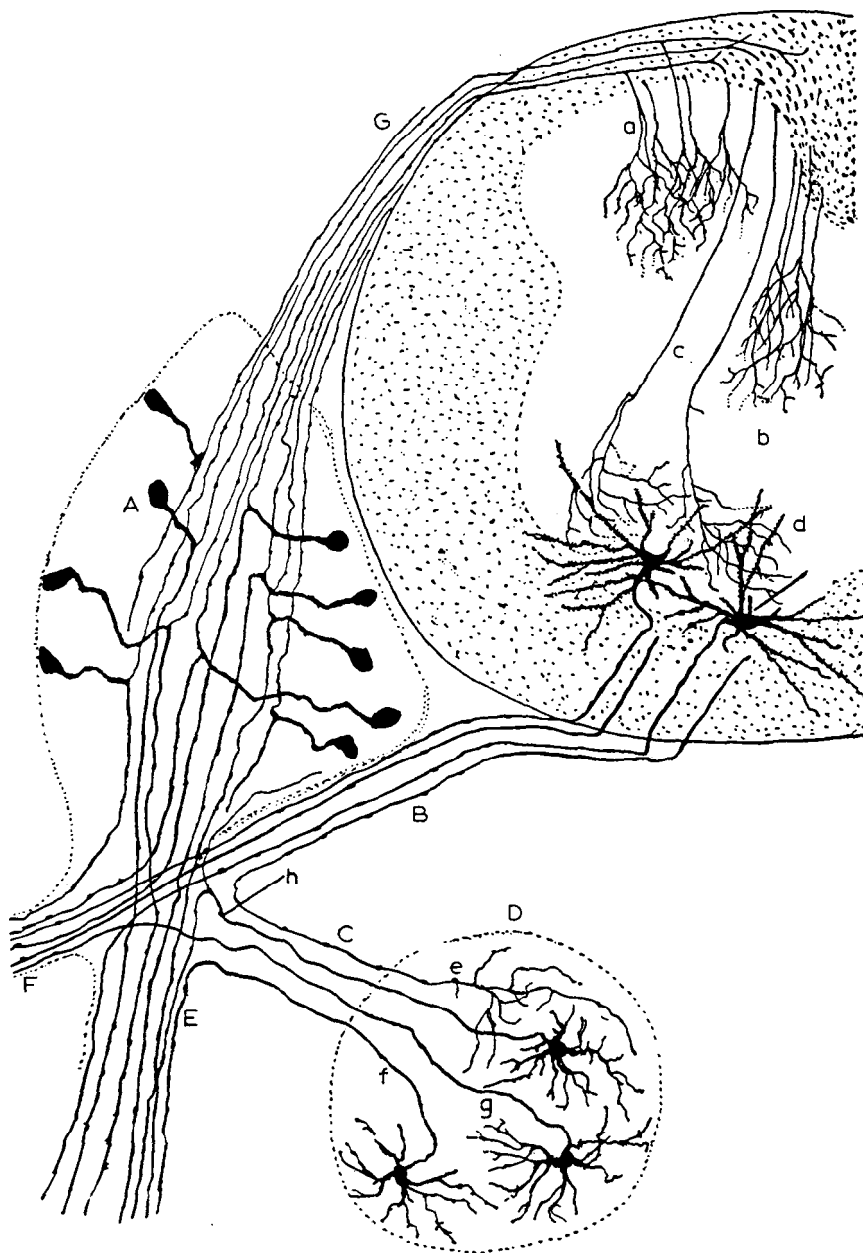


Fig.2

the motor neurons; and the *indirect* or *associated reflexo-motor current* which goes to very distant motor neurons having made a detour in passing an intermediary or associated nerve corpuscle on the way, that is to say, via the direct or commissural funicular neurons, the axons of which, from our observations, divide very often in the white matter giving an ascending and descending branch (C in Fig. 1).

In Fig. 3 I show the connexions of the visual fibres and the cells of the retina. The interneuronal relationships are shown with an admirable clarity and simplicity in this object of study. In spite of its great complication, the retina can be considered as a nerve ganglion formed by three rows of neurons or nerve corpuscles: the first row encloses the rods and cones with their descending prolongations forming the external granular layer (a and b in Fig. 3); the second is made up of the bipolar cells (c and d) and the third contains the ganglionic neurons (e); the three series of nerve corpuscles interconnect at the level of the said molecular or plexiform layers, internal and external.

Note that the external plexiform layer (C in Fig. 3) encloses a multiple connexion of which the elements are: externally, the terminal spheres of the rod fibres and the conical feet of the descending prolongations of the cones, equipped with filamentous attachments; internally, the external processes of the bipolar cells of which, as we have shown, there are two varieties: bipolar cells with flattened processes going to the cones (d in Fig. 3) and robust bipolar cells with ascending dendritic processes going to the rods (c in Fig. 3), and finally there are the protoplasmic branches and nerve arborizations of the horizontal cells of the internal granular layer.

The internal plexiform layer has even more complicated connexions which can be divided into three, or even many more, stages. The essential factors are represented, externally by the terminal processes of the descending prolongation of the bipolar cells and the terminal ramifications of the inferior expansions of the spongioblasts; internally by the flattened protoplasmic arborization of the neurons of the ganglionic layer.

In following the axons of the neurons of the ganglionic layer the length of the optic nerve, we will find in the *mid-brain* and the *intermediary brain* yet a third connexion brought to light, primarily by our researches on the optic lobe of birds and the mid-brain of mammals, and then by very interesting observations by my brother (*lateral geniculate body* of mammals, *optic lobe* of birds, reptiles and fishes), and by Van Gehuchten, Kölliker, Sala, Tello, etc. As we know, certain axis-cylinders of the optic tract go forward, ending by free, very complicated, ramifications into the depths of the lateral geniculate

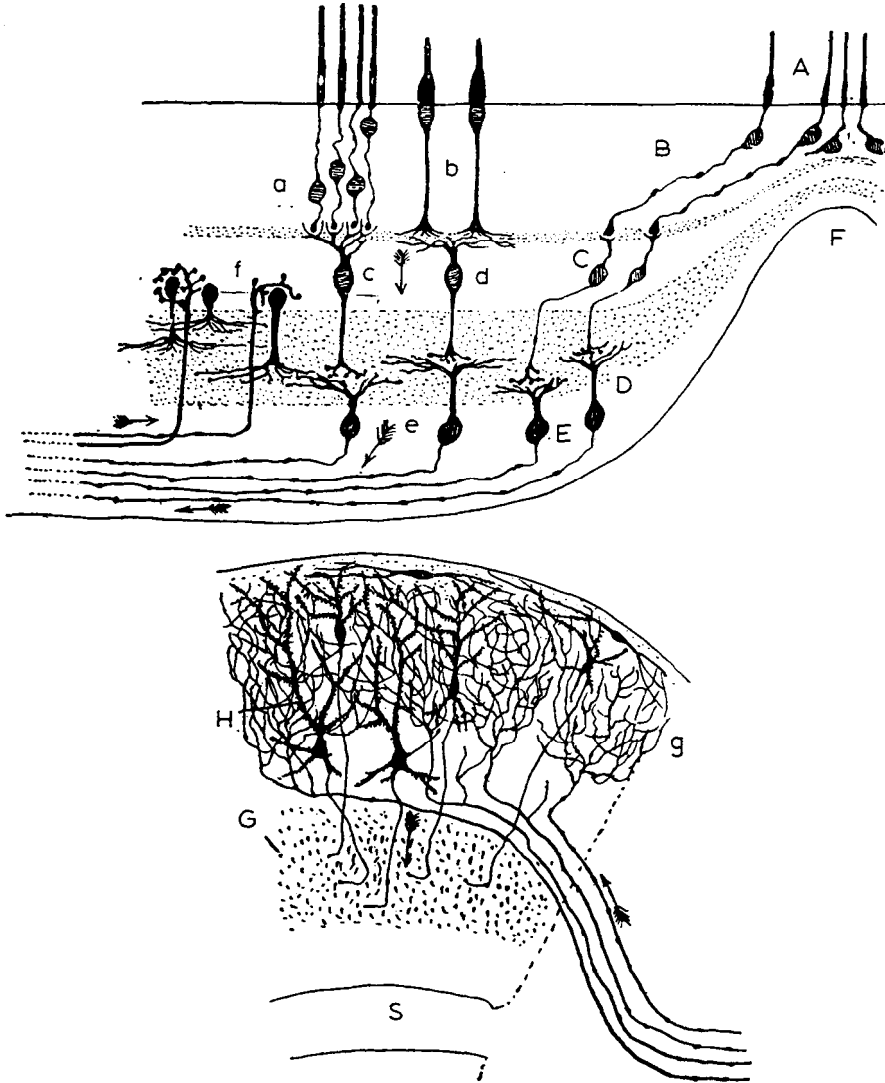


Fig.3

body; others, going backward, become large ascending arborizations in the cortex of the anterior quadrigeminal body. It is here that the visual *reflexo-motor* pathway originates (g in Fig. 3).

Finally, these observations in mammals and in children have demonstrated to us the fourth and last connexion of the optic conductors, that is to say, of the central optic pathway, the original neurons of which are in the lateral geniculate body. This interesting terminal connexion, verified by the im-

portant anatomico-pathological work of Henschen, is in the calcarine fissure at the level of the 4th and 5th cortical zone in which are found two very compact layers of astrocytes (g in Fig. 7).

Let us now look at Figs. 4 and 5, which are of the neurons, and of the connexions of cells and fibres in a cerebellar lamella. As we well know, a transverse section of these lamellae shows three concentric layers of neurons.

The first, or plexiform layer, is formed principally by small star-shaped cells (or *basket cells* according to some authors); the second, or intermediary, is made up of Purkinje cell bodies. The third and last is the result of granular reunion.

From my observations, confirmed by a large number of writers (Van Ge-

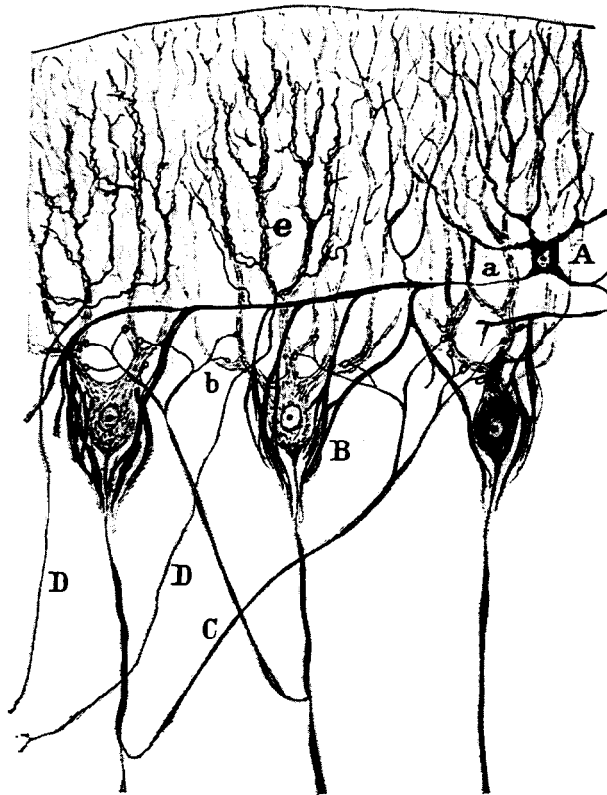


Fig. 4. Semi-schematic reproduction of the Purkinje cell connexions of the cerebellum. Reduced silver method. A, star-shaped cell of the molecular layer; a, initial narrowed portion of its axon; B, terminal baskets; C, recurrent collaterals; b, final fibrillae of these collaterals, terminating in rings leaning against the large trunks of the Purkinje cells.

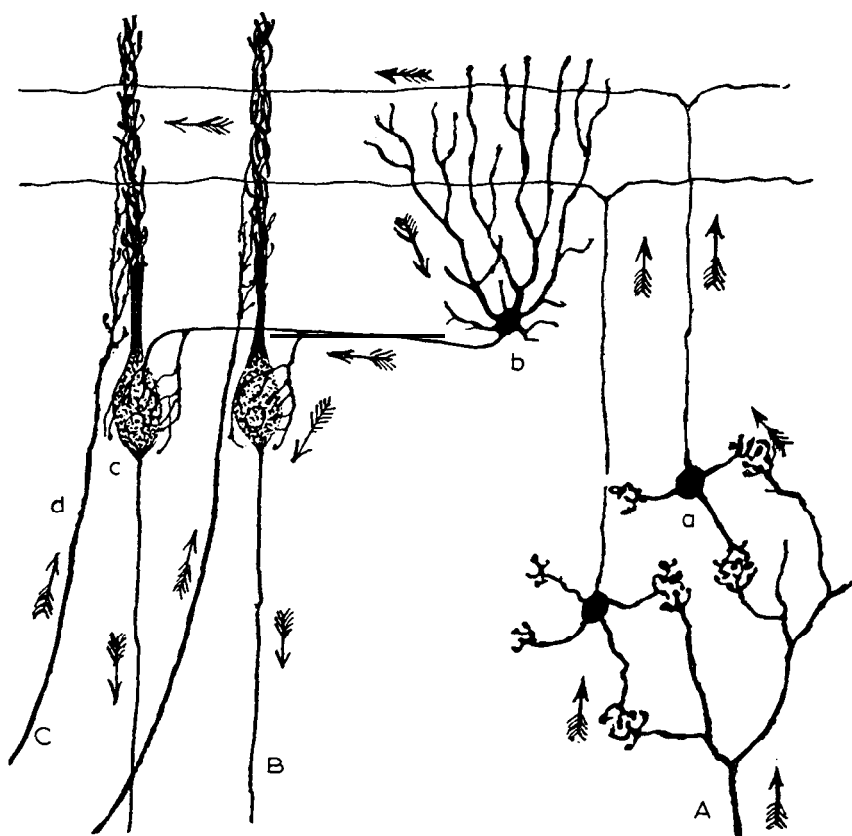


Fig. 5

huchten, Kölliker, Lugaro, Edinger, my brother, Falcone, Retzius, Azoulay, Held, etc.), it appears that all these elements have two sorts of connexions: intrinsic connexions, that is to say, established between the neurons of superimposed layers, and extrinsic connexions, taking place between the neurons of the cerebellum and nerve cells belonging to other central organs.

Let us examine firstly the intrinsic connexions and begin with those which take place between the Purkinje corpuscles and the axons of the small star-shaped elements of the plexiform layer.

These axons give off several collaterals and after a variable course, describe an arc to finish at the level of the bodies of the Purkinje neurons, with the help of a large number of successively thickened ramifications (A in Fig. 4). These terminal branches, in the same way as the descendant twigs of the descending collaterals, form around the Purkinje bodies a *nest* or tight plexus, which often terminates downwards in a pin-point (B in Fig. 4). The interest-

ing connexion by contact which is established in this way by two orders of neurons has been confirmed by Dogiel and by us, using the methylene-blue method. Recently we ourselves, Bielschowski, Wolff and others have succeeded in showing this admirably impregnated with the neurofibrillar methods.

Let us see now the connexions between the grains and the Purkinje cells. The grains are little nervous elements with several fine dendrites ending in a digitiform ramification, and having an extraordinarily delicate axon (a in Fig. 5). This nerve prolongation ascends to the plexiform layer, and bifurcating at different heights produces a very delicate so-called *parallel* fibre, because it is in a position parallel to the lamellae of the cerebellum. During its long longitudinal path this fibre makes contact with the spiny contours of the dendrite branches of the Purkinje cells. As each fibril runs the whole length of a cerebellar lamella, it follows that a single grain cell can affect a multitude of Purkinje cells.

Among these intrinsic connexions we should add those established between the recurrent collaterals of the Purkinje corpuscles, and the large dendritic branches of these last (b in Fig. 4). As we well know, the recurrent collaterals discovered by Golgi go to the molecular layer where, once again, they ramify repeatedly. For a long time we did not know the way these nerve branches end, and we thought up many suppositions concerning their connexions with the elements of the plexiform layer. Recently, in studying the cerebellum of dog and man, with the reduced silver nitrate method, we were lucky to discover that the final twigs of the said fibres, having become longitudinal, finish by way of a minute neurofibrillar ring on the surface of the dendritic stem of the Purkinje corpuscles (b in Fig. 4). This fact showed therefore, as we had admitted formerly, that the recurrent collaterals serve to associate in a dynamic ensemble the neurons of the same kind from the same area of the grey matter (C in Fig. 4).

The extrinsic connexions of the cells of the cerebellar cortex are only imperfectly understood. We know, as Golgi demonstrated, that the Purkinje cells give rise to long or motor nervous prolongations, of which the ending is unknown (probably it is in the olive or the roof ganglion). Inversely, we know how two sorts of afferent nerve fibres end in the cerebellum, the *man fibres* and the *climbing fibres* whose originating neurons are still puzzling.

On the other hand, the connexions established between these two types of conductors and the cells of the cerebellar cortex are very interesting theoretically. They have contributed greatly to persuading us of the truth of the

neuronal doctrine. We have confirmed the connexions even more plainly with the Ehrlich reduced silver nitrate method, than was revealed in the first place by the Golgi method.

You well know that the *moss fibres* are large medullary tubes which ramify and end in the granular layer, in contact with the digitiform branches of the grain cells, by means of their rosettes, or thick, varicose ramifications (A in Fig. 5). This very curious articulation, pointed out firstly by us (1894) and confirmed by Held, Berliner, Wolff, etc. takes place in certain anucleated parts of the granular area, which we call *cerebellar glomeruli* because of their resemblance to the *olfactory glomeruli*. According to our recent observations, confirmed by Bielschowsky and Wolff, who used the former's method, the final outgrowths of the moss fibres form a loose neurofibrillar reticulum, and even handle-like processes and terminal rings.

As regards the *climbing fibres*, they cross the granular layer and run the length of Purkinje cell bodies and envelop the ascending trunks and the principal secondary branches of these neurons with a wonderful terminal arborization which is stretched out and climbing, and may be compared with that of the motor fibres on the corpuscles of striated muscle (D in Fig. 4 and C in Fig. 5).

The result of what we have just shown is that the granule cells and Purkinje neurons can receive nerve impulses from other centres, probably from the ganglia of the protuberance, and from the ascending branch of the vestibular nerve; while the big, star-like elements of the granular zone seem to have no relation at all with the extrinsic fibres.

We have not the time to review all the very convincing examples of neuronal articulation in other nerve centres such as the olfactory bulb, the cerebral cortex, the optic thalamus, the sensory and sympathetic ganglia, etc., all centres which we have once studied very carefully. We will limit ourselves here to mentioning succinctly the existence of a special factor in the intercellular articulations - one whose physiological role is still vigorously debated and must be of great importance. We allude to the *centrifugal fibres*, shown a long time ago by us and by Dogiel in the retina, then found again in the olfactory bulb, the anterior quadrigeminal body, and, in especial abundance, in the optic thalamus.

As you can see in Fig. 6 (a) which is a schematic section of the retina of birds, centrifugal fibres arising from nerve centres not yet known cross directly the internal plexiform layer. As soon as they arrive at the spongioblast layer, they divide into a terminal arborization with short and varicose branch-

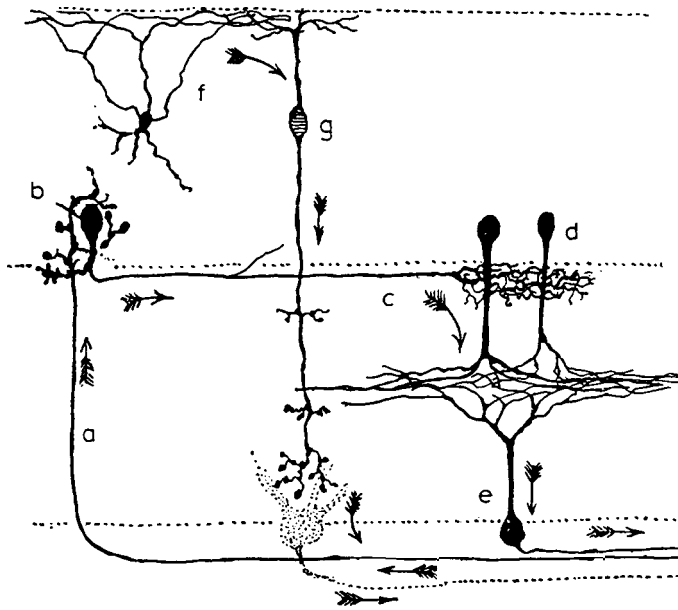


Fig.6

es which make contact with the soma and the descending stem of these last elements (b). In birds these terminal branches connect in a specific way with a particular type of neuron which we have called *association spongioblasts* (b). These special corpuscles which have a true axis-cylinder like ordinary neurons, receive the centrifugal nervous impulse through their bodies and their short dendrites propagate it horizontally, firstly via the axon to groups of very distant *amacrine* cells (d), and secondly via these last to the interneuronal articulations of the internal plexiform layer. These articulations are, as we know, made up of the descending plumes of the bipolar cells on the one hand, and of the dendrites of the ganglionic corpuscles on the other (e).

As an example of centrifugal fibres of central organs we reproduce, in Fig. 7 (a and e), those which end in the sensory nucleus of the *thalamus*. Note firstly that this nucleus (*lateral nucleus* of Kölliker, *ventral nucleus* of Nissl) represents the relating station between the two encephalic sensory neurons, i.e. the *inferior neuron* formed by the axon bands of Reil, or the *internal lemniscus* (G in Fig. 7) and the *superior* or *thalamo-cortical neuron* (d in Fig. 7) whose body lies in the said nucleus while its nerve prolongation, having reached the striate body, ends by complicated ramifications in the sensory-motor areas of the cortex, making contact here with the pyramids from the third cerebral layer (b in Fig. 7). Well, my research carried out in the thalamus

of the mouse and the rabbit persuaded me that one of the absolutely constant factors in the articulation of the two neurons in the superior sensory pathway is the terminal ramification of the cortico-thalamic or descending fibres, whose origin is very probably in the sensory-motor areas of the cortex (a and e in Fig. 7).

What is the role of the centrifugal fibres? Are they, as Duval thinks, conductors destined to produce in the sensory pathway articulations a very intimate contact which would be indispensable for the satisfactory propagation of the nervous impulse? Or rather do they transport some form of energy from the brain, the rapid accumulation of which in the sensory stations is necessary for the passage of ascending nerve currents? Unfortunately, at this stage of science, it is impossible to give satisfactory and categorical answers to these questions.

The information and preceding conclusions relate preferably to the morphology of the nerve cell. In this sphere our idea, actually based on precise and exact revelations from plasma methods (those of Golgi and of Ehrlich) can be considered definitive. Once the research into the morphology of the reciprocal relations of neurons was finished or at least very advanced, it was

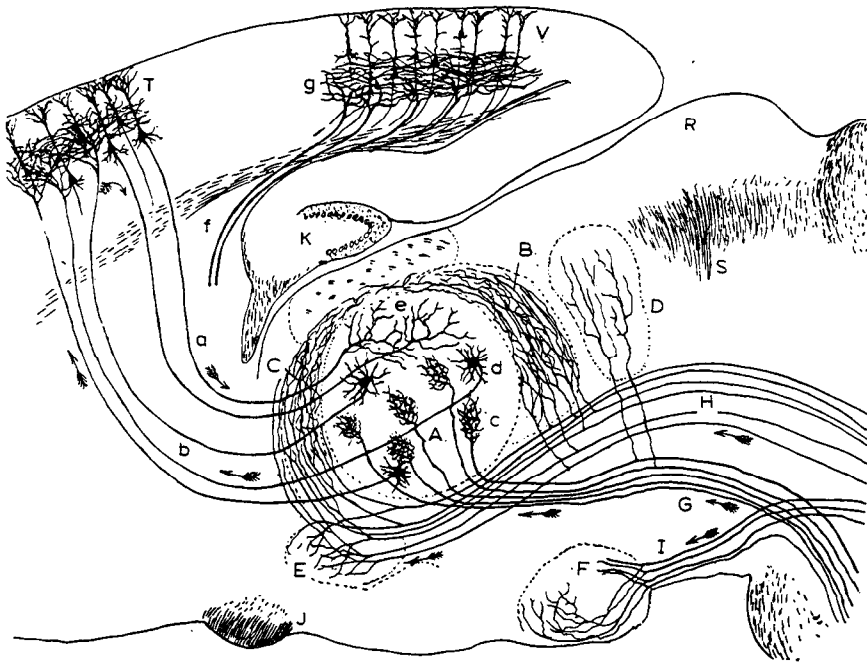


Fig.7

only natural that the neurologists should direct their attention to the very difficult and very important subject of the constitution of nerve protoplasm.

The work of Apathy and of Bethe has clarified this interesting subject, opened very fortunately by Nissl with the discovery of *basophilic bodies* of the protoplasm. The methods conceived by these scientists were unfortunately very inconstant and difficult, and very hard work has been performed to find other more perfect and feasible methods. This technical research has led to the neurofibrillar procedures of Simarro, ourselves and Bielschowsky, Donaggio, Lugaro, etc.

All these new research techniques have advantages in certain particular cases. Our reduced silver nitrate method, without being superior to those of Donaggio and of Bielschowsky with respect to the differentiation of the adult protoplasmic reticulum, does have certain advantages. It gives good results in human beings in normal and pathological states, it can be used easily in animals a few days old, and in nerve organs in the process of regeneration and degeneration, and lastly it is of particular use in morphological studies because of its efficacy on very thick and transparent sections.

Thanks to these properties of a method which has given brilliant results in the hands of Van Gehuchten, Michotte, G. Sala, Azoulay, Nageotte, Dogiel, Marinesco, Perroncito, Lugaro, Tello, etc. we have been able to make the following additions to our knowledge of the neuronal anatomy and physiology.

(a) The neurofibrillar framework of the neurons of vertebrates is not composed of the mixing up and intersection of many independent conductors, as Bethe thought, but on the contrary, of a continuous network in which certain long and thick trusses (*primary filaments*), and others, short, thin and pale (*secondary filaments*), are differentiated. This disposition has also recently been observed by Donaggio, Van Gehuchten, Marinesco, Retzius, Tello, von Lenhosstck, Dogiel and many others (a and b in Fig. 8).

(b) The same reticular disposition can be seen in the varicosities and enlargements of nerve branches in the motor-end plates (Ramón y Cajal, Tello), the sensory endings (Dogiel, Tello), the outgrowths of the moss fibres (Ramón y Cajal), and the tip of nerve fibres in process of regeneration (Ramón y Cajal, Perroncito, Marinesco, Nageotte, etc.). In Fig. 9 (C) look at the aspect of the neurofibrillar reticulum in the terminal branches of a motor-end plate, and notice that the finest twigs often contain a filamentous handle or a little knob formed by two or three links full of neuroplasm.

The importance of these easily controllable observations will escape no-

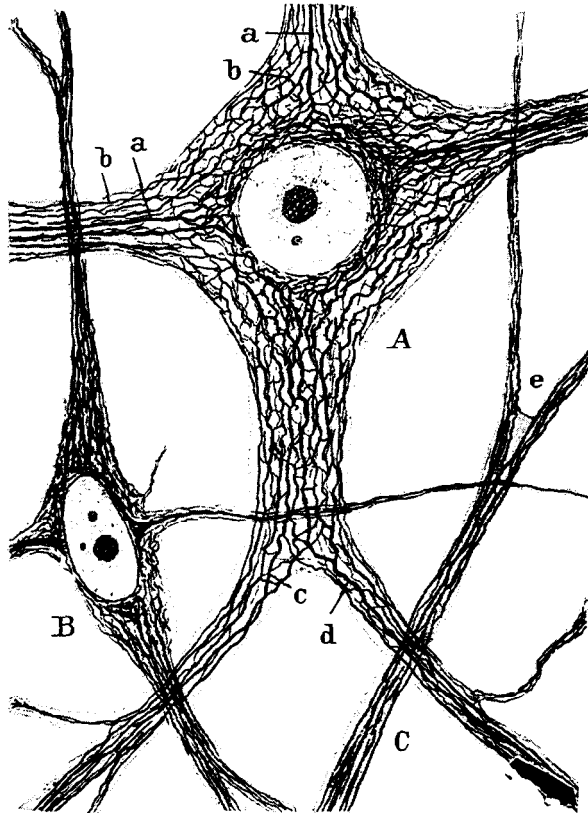


Fig. 8. Spinal cord cells of the several days old rabbit. Impregnation by the reduced silver nitrate method. A, large funicular corpuscle; B, small corpuscle; a, primary filament; b, secondary filaments; c, d, e, neurofibrillar anastomoses at the level of the dendritic divisions.

body; for if, as we think, in certain cases the neurofibril does not actually end in the free end of nerve branches, the opinion of Bethe and of Apithy, who consider these filaments as the sole conducting organs of nerve protoplasm, cannot be accepted.

(c) The neurofibrillar reticulum is not a fixed, stable, conducting system, but a framework capable of undergoing notable changes depending on the physiological state (the influence of heat and cold in the reptiles and young mammals, etc.) and under the influence of pathological conditions. Among the changes due to a pathological cause, let us mention those found by Garcia and us in rabid animals and confirmed by Marinesco and Franca (hypertrophy of neurofibrils, coalescence of neurofibrillar bundles, reabsorption of second-

ary trusses, etc.; those very curious ones observed in animals which have been cooled down (Ramón y Cajal, Tello, Marinesco, Donaggio), and, lastly, those in the reticulum of the terminal boutons of regenerating nerve fibres, and in the distal end of axons disconnected from their original neurons.

We show in Figs. 10, 11 and 12 (c) several examples of these peculiar changes in the protoplasmic framework. Note, in Fig. 10 (A) the profound changes in the motor neurons of the lizard following hibernation. In the large motor cells (A), the neurofibrils have bunched together into very tight bundles which, by fusion, progressively become completely homogeneous cords. Meanwhile, the transformation in the small cells is reduced to the accumulation of silver-staining matter in certain neurofibrillar areas (a in Fig. 10). Exactly similar phenomena are shown in Fig. 11 which shows the reticular alterations in the rabid dog (spinal cord), and in Fig. 12 (B and C) which shows the very interesting changes undergone by the funicular neurons in a 15-day-old rabbit exposed for six hours to a temperature of 10°.

Finally, Fig. 13 shows the transformations of the neurofibrils in the central end of a crushed nerve two days after the operation. The neurofibrils in process of regeneration can be seen, in B, penetrating the interior of the necrotic segment of the axon where they terminate in free handle-like projections; and in C a necrosed portion of the axis cylinder, invaded by branched neurofibrils, in the process of development. In E, these branched neurofibrils, budding from an axon irritated by the trauma, spiral around the axon which also shows unravelling, and longitudinal vacuoles. These strange phenomena,

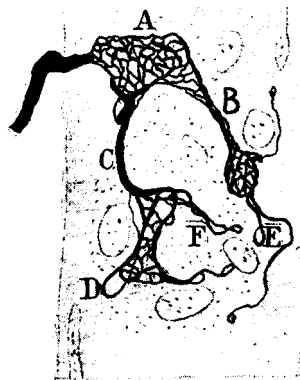


Fig. 9. Reticular disposition of the protoplasm of the nerve branches of a motor plate in the adult rabbit. A, network at the level of an enlargement; B, narrowed portion; D, F, neurofibrillar hinges; E, neurofibrillae terminating in rings.

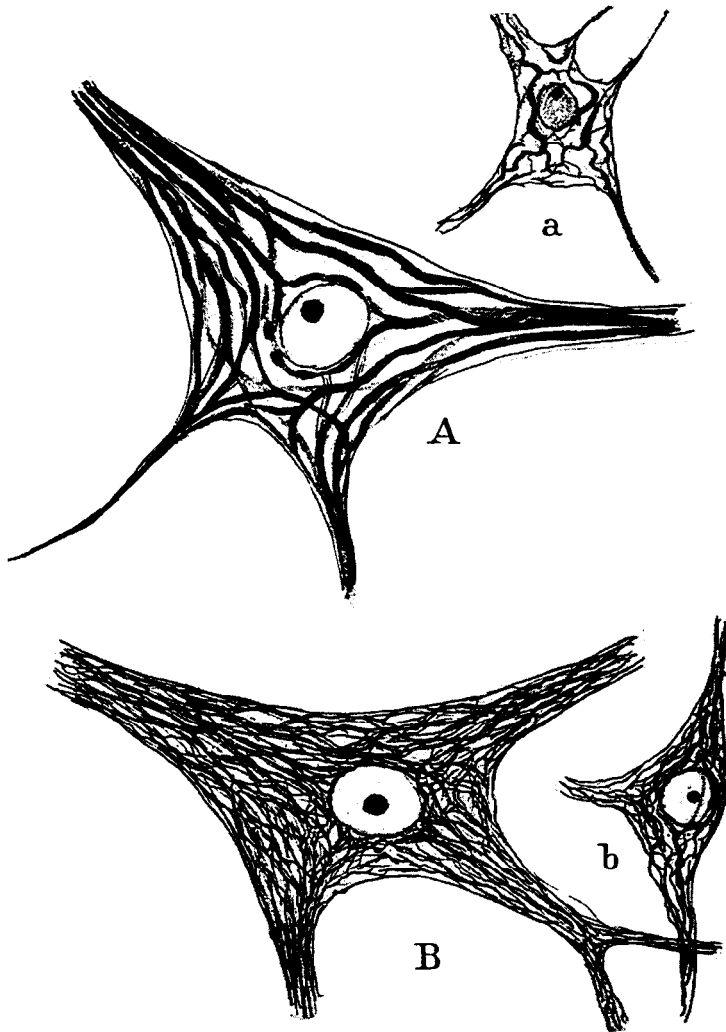


Fig. 10. A, spinal-cord motor cell of the hibernating lizard; a, another funicular cell; B, b, the same spinal-cord cells of the lizard after several hours at a temperature of 30° C.

as well as attesting a certain autonomy of the neurofibrils, also show the great capacity they have to change, forming structures remarkable in their variety and in their variability.

Our recent research with the reduced silver method has revealed something of the morphological order:

(a) The existence in the neurons of the human sympathetic chain of a particular type of dendrites. These are the *short dendrites*, characterized in ad-

dition to their thinness by the fact that they are found only in the subcapsular space (b in Fig. 14). It is the terminal ramifications of these which make contact with the nerve nests which in man are extraordinarily complicated, as can be seen in Fig. 15.

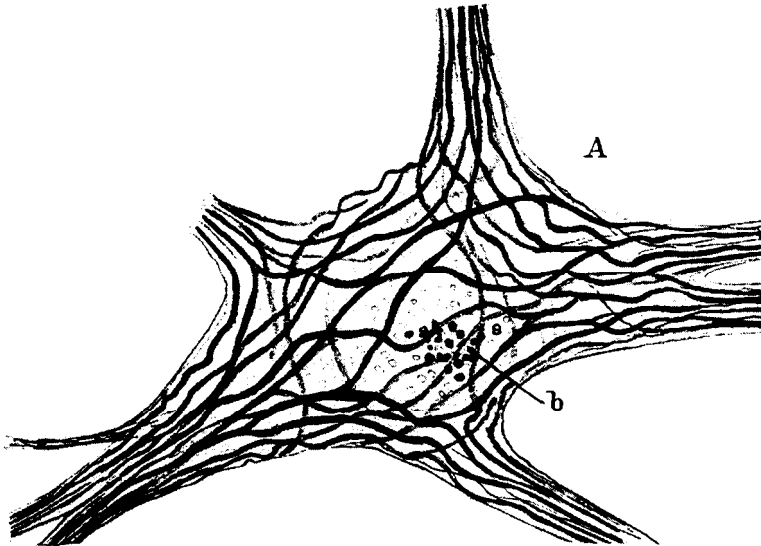


Fig. 11. Spinal-cord cell of a rabid dog - alteration of the reticulum.

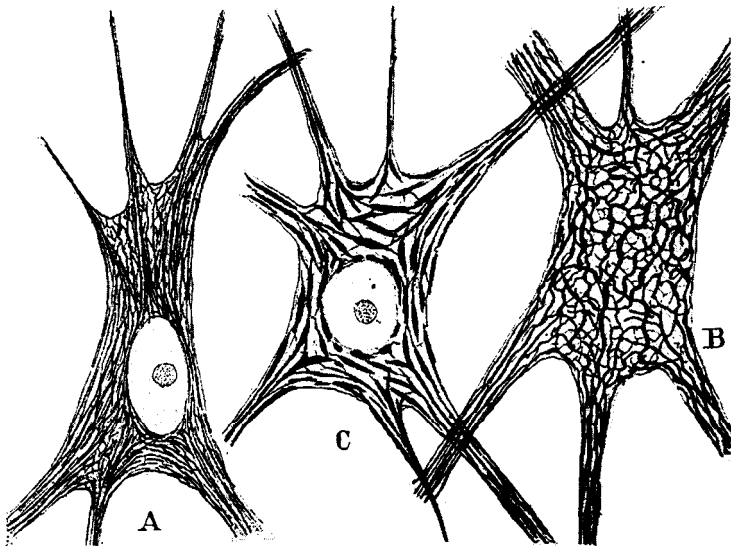


Fig. 12. B, C, changes occurring in the funicular neurones of a rabbit, aged 15 days, after 6 hours' exposure to a temperature of 10° C.

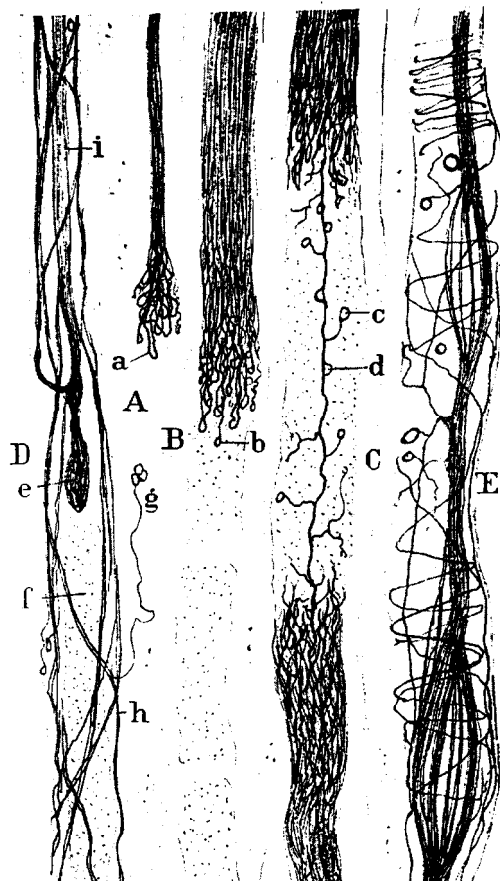


Fig. 13. Crushed portion of a nerve, taken between the tongs. (Cat, killed 52 hours after the operation.) A, B, the extremity of the living portion of the axon; C, E, necrotic tubular segments with neurofibrillae in the process of neof ormation; D, necrotic piece of an axon, invaded by branches, springing up above the terminal bouton.

(b) The presence in these same sympathetic ganglia in man of *glomeruli* or *connexion plates*. That is to say, special plexuses, formed by the concurrence of a large number of dendrites belonging to different neurons, and in which afferent nerve fibres come to arborize and end.

(c) The discovery, in the sensory ganglia, of cells of which the protoplasm is partially disposed in a system of anastomotic cords (window cells) (Fig. 16).

(d) The presence of special appendices ending in capsulated balls or buttons, in certain sensory, sympathetic and even cerebellar neurons (a and b in Fig. 17). Later we will come across the interpretation of these bizarre for-

mations, which demonstrate the capacity of the neurons to produce new expansions, under normal and pathological conditions.

(e) Lastly, the existence in the final bud in process of development of embryonic axons, as in the case of adult axis cylinders during regeneration, of buttons or spheres possessing a neurofibrillar reticular frame work, etc. etc.

From the whole of these facts, the neuronal doctrine of His and of Forel, accepted by many neurologists and physiologists, is derived as an inevitable postulate. However, it must be said that some of the physiological inferences drawn from observations made by the elective methods of these last twenty-five years have been contended, and naturally cannot be considered as unimpeachable dogmas. Present-day science, in spite of its well-founded conclusions, has not the right to foretell the future. Our assertion can go no further than the revelations of contemporary methods. Perhaps, with time, technique will discover some coloration process capable of revealing new and more intimate connexions between neurons thought to be in contact. We cannot reject, a priori, the possibility that the inextricable forest of the brain, the last branches and leaves of which we imagine ourselves to have

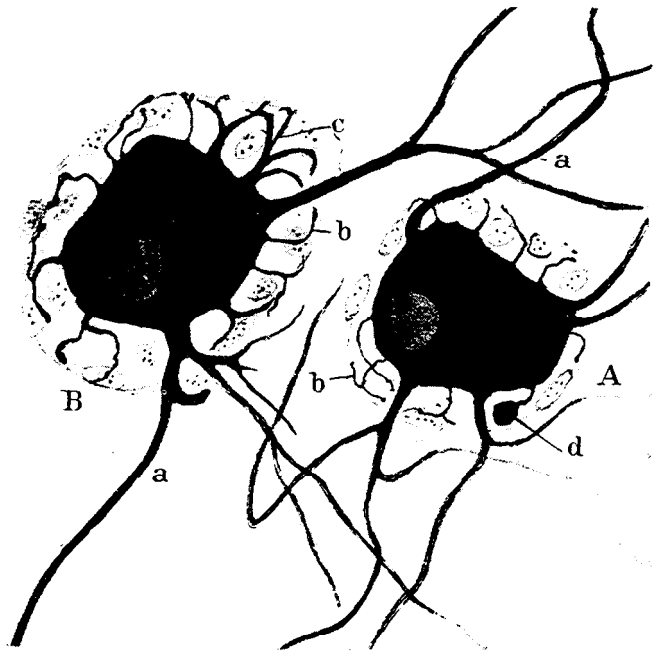


Fig. 14. A, B, star-shaped cells of the sympathetic chain in adult man; a, axon; b, short dendrites or sub-capsular appendages; d, appendage, ending in a ball.

discerned, does not still possess some enigmatic system of filaments binding the neuronal whole, as creepers attach the trees of tropical forests. This is an idea which, appearing to us with the prestige of unity and of simplicity, has exerted and still exerts, a powerful attraction for even the most serene of spirits. True, it would be very convenient and very economical from the point of view of analytical effort if all the nerve centres were made up of a continuous intermediary network between the motor nerves and the sensitive and sensory nerves. Unfortunately, nature seems unaware of our intellectual need for convenience and unity, and very often takes delight in complication and diversity.

Besides, we believe that we have no reason for scepticism. While awaiting the work of the future, let us be calm and confident in the future of our work. Let us recall that these terminal dispositions, which modern neurology has discovered in the axons, have been established by the concordant revelations

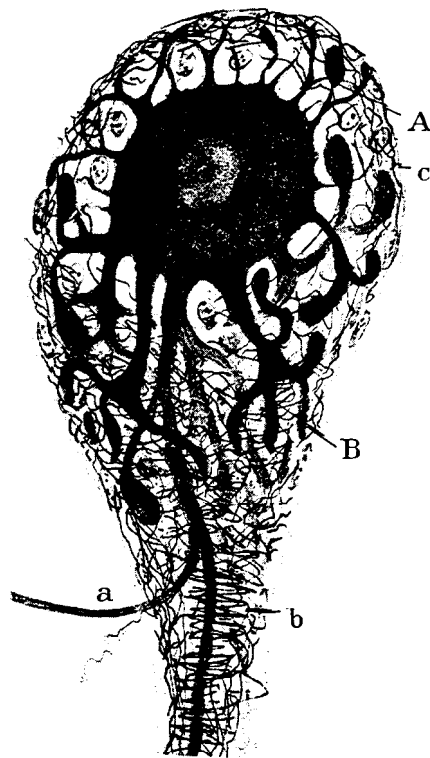


Fig. 15. Terminal nerve apparatus of a sympathetic corpuscle in man. A, subcapsular dendrites; B, thick dendrites forming a complicated glomerulus; a, axon; b, afferent nerve fibrillae.

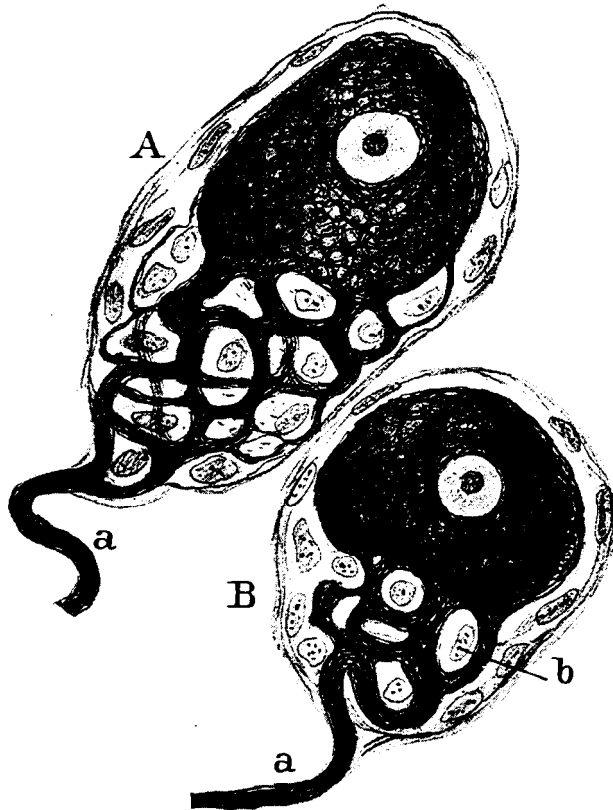


Fig. 16. *A, B*, window cells of the plexiform ganglion of the vagus nerve in an adult dog; *a*, axon; *b*, satellite cells.

of several methods. If future science reserves big surprises and wonderful conquests for us, it must be supposed that she will complete and develop our knowledge indefinitely, while still starting from the present facts.

The irresistible suggestion of the reticular conception, of which I have spoken to you (and the form of which changes every five or six years) has led several physiologists and zoologists to object to the doctrine of the propagation of nerve currents by contact or at a distance. All their allegations are based on the findings by incomplete methods showing far less than those which have served to build the imposing edifice of the neuronal conception. Some of these arguments belong to the morphological order, and others to the histogenic order.

With regard to the morphological objections (of which we talk less now than before, after the discovery of Donaggio's method and ours) I will only

say that in spite of the pains I have taken to perceive the supposed intercellular anastomoses in preparations made with diverse coloration processes (those of Bethe, Simarro, Donaggio, Ramón y Cajal, Bielschowsky, etc.), I have never succeeded in finding any definite ones (that is to say, showing themselves as clearly and sharply as the free endings). I have seen none in the pericellular nerve plexuses, nor in the boutons of Held-Auerbach, nor between the neurofibrils belonging to different neurons. Neurologists as wise and expert as His, Kölliker, Retzius, von Lenhossék, Duval, Van Gehuchten, Lugaro, Schiefferdecker, Dejerine, etc. etc. are of the same opinion. If the said intercellular unions are not the result of an illusion, they represent accidental dispositions, perhaps deformities whose value would be almost nil in the face of the nearly infinite quantity of the perfectly observed facts of free ending.

As for the histogenic arguments, on which the opponents of the neuronal doctrine have lately much insisted, considering them to be the most weighty and decisive with which one could oppose the neuronal conception, I would reply that my recent researches, as those of Perroncito, Marinesco, Lugaro and Nageotte, done with a more revealing process than those used by the anti-neuronists, proves in the most incontrovertible fashion the lack of foundation for the hypothesis of the discontinuous development of nerve fibres.

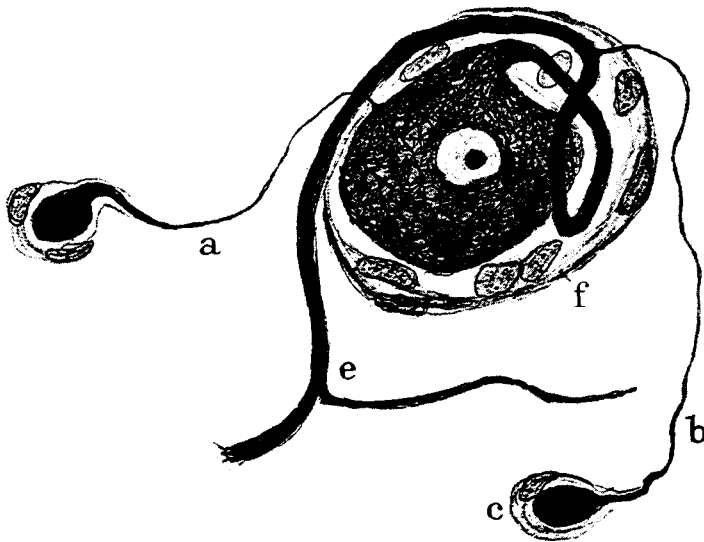


Fig. 17. Cell provided with appendages terminating in boutons of growth. Man aged 65 years. *a*, appendages; *c*, denuded capsule; *f*, cell capsule.

Purpura, a pupil of my illustrious colleague C. Golgi, in analysing the regenerative process of sectioned nerves with the silver chromate method, and very recently Krassin, of St. Petersburg, using the Ehrlich method with the same object in view, have arrived at the same conclusions.

Allow me to insist a little on this point of the regeneration and normal histogenesis of nerves, because it is a question of immediate interest to which we have devoted two years of work. Besides, the conclusions deriving from our observations - apart from their critical importance - reveal very curious phenomena on the physiology of the reticulum.

Proofs of the neurogenetic doctrine of Kupffer and His

You are aware that recently the former and nearly forgotten conjecture of Beard and Dohm of the histogenic mechanism of the nerve cords of the embryo has been resuscitated in the *link theory* (catenary theory) which states that the nerve axons, instead of being the result of the development of the primordial expansion of the neuroblast of His, are formed following the fusion of a large number of ectodermal corpuscles which have emigrated towards the periphery. These elements, bound in a chain, would be the site of a fibrillar differentiation at first discontinuous, then continuous, ending in the construction of a large number of axons fused ulteriorly with the medullary neurons. As regards the nuclei and the rest of the non-transformed protoplasm they would become, in the adult, the Schwann cells.

That is the conception which, with variations and even contradictions which we have not the time to set forth in detail, has been recently defended by Sedgwick, Bethe, Joris, Capobianco and Fragnito, Besta, Pighione, etc. following the observations on nerve histogenesis in the embryo, and by Büngner, Ballance, Bethe, Levi, Durante, Van Gehuchten, etc. based on experiments concerning nerve regeneration.

Like many scientific errors professed in good faith by distinguished scientists the link theory is the result of two conditions: one subjective, and the other objective. The first is the regrettable but inevitable tendency of certain impatient minds, to reject the use of elective methods, such as those of Golgi and of Ehrlich which do not lend themselves easily to improvisation; the second is the exclusive application of processes simple and convenient, but without a specific action on axons, and as a consequence incapable of presenting clearly the neuronal expansions and their peripheral ramifications.

In order to avoid regrettable miscalculations into which so many talented observers have fallen, we have chosen (as have Medea, Perroncito, Marinesco and Lugaro) the reduced silver method. This has the property of staining, with transparent colouring, the medullated fibres, as well as those without myelin and in the process of formation.

The results obtained demonstrate almost beyond doubt that at no moment of evolution can the axons be taken as cellular chains, or as discontinued axon cylinders as is supposed by the anti-neuronists: on the contrary, and agreeing with the doctrine of His and of Kölliker, the new fibres are produced following the budding of the axons, and are in perfect continuation with the motor or sensitive neurons, in the embryo as well as in regenerating nerves. The so-called *peripheral neuroblasts* forming a chain, represent late formations, always separated from the protoplasm of the axon cylinders; they probably belong to the mesoderm. It is perhaps as the result of an attraction exerted by the axons emigrating towards the periphery that these elements at first indifferent, approach the fibres and become the Schwann cells.

The proofs of this doctrine on the histogenesis of nerves are many, in the history of neurogenesis as well as in that of nerves regenerating after section. Let us mention a few here.

Proofs derived from the regenerative mechanism of nerves

1. When the sciatic nerve of a mammal (rabbit, dog or cat, aged several weeks) is cut, and the animal is killed after three days, it can be verified, in preparations made by our process, that a very active phenomenon of budding takes place in a large number of nerve fibres of the central end. In following the fibres towards their origin it can be seen that each among them presents two continuous and well-differentiated portions (Fig. 18): the old segment, completely normal and easily recognizable by its myelin sheath (F), and the thinner and paler, newly formed segment without myelin (B). This last portion of the axon, which resembles the fibres of Remak, penetrates the thickness of the scar, or the middle of the inflammatory exudate, divides at an acute angle very often and its branches (or the trunk in non-divided axons) finish by means of a large excrescence often in the form of a button (C in Fig. 18). This terminal ball sometimes very large and irregular, appears bare at first, but in the days following (from the fourth to the sixth) it becomes surrounded with a conjunctival capsule interspersed with nuclei. Several

branches of the bifurcation, situated in the neighbourhood of the scar tissue, give off arched nervous filaments which move into the interior of the central end in a retrograde direction (e in Fig. 18).

We observed these budding phenomena of the central end very well from the fifth day of the operation. But Perroncito, using the reduced silver meth-

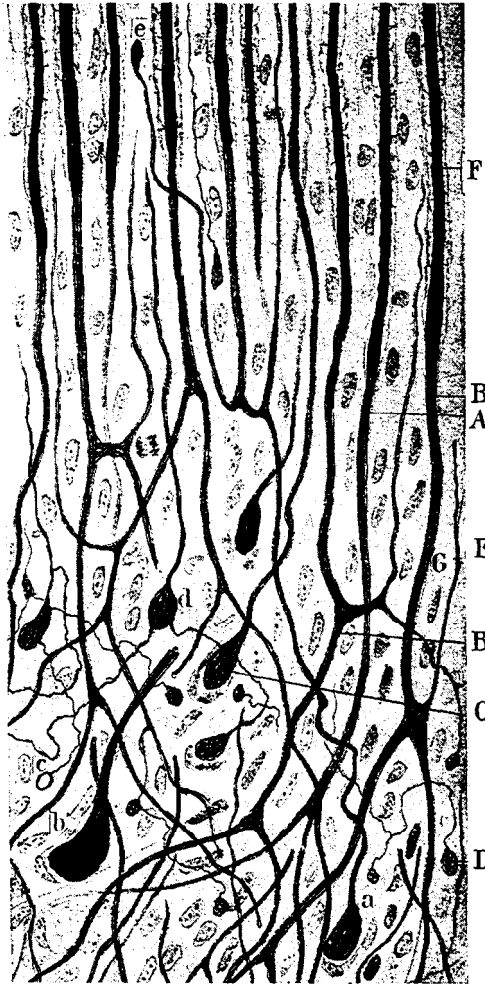


Fig. 18. Portion of the central end of the scar of a cut sciatic nerve, of a week-old cat killed three days after the operation. *A, B*, non-medullated portion of nerve tubes in the process of development; *F*, old or medullated portion of these tubes; *C*, development-bouton; *D*, small terminal bouton; *G*, fibre emitting retrograde branches; *a, b*, boutons working through the scar; *c*, free neurofibril ending in a ring; *e*, retrograde bouton; *d*, bouton, from which emerge fine appendages ending in little boutons.

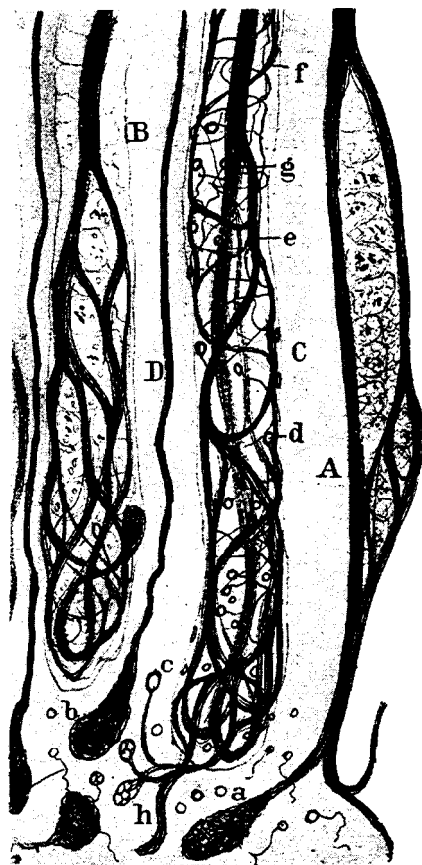


Fig. 19. Central end of the sciatic nerve of a cat killed 52 hours after the operation. *A*, ravelling-out and vacuolization of the axon; *B*, *C*, apparatus of Perroncito; *D*, fine medullary fibre; *a*, *b*, terminal masses; *f*, neoformed fibrils; *c*, fibres terminating in rings.

od, had the good fortune to demonstrate that this regeneration process begins very early, appearing from the beginning of the second day. In our preparations, however, the formation of the buttons and the penetration of these in the intercalary conjunctive tissue seemed to begin only at the end of the second day, or the beginning of the third. We also stated the reality of a very curious phenomenon of ravelling out, and of dispersion of the axon neurofibrils. This phenomenon leads on to the early creation of a fasciculus of young nerve fibres, of which some, ramifying and developing very actively, give rise to a system of spiral filaments which surround the rectilinear neurofibrillar and axial fascicula without leaving the space limited by the Schwann

sheath. These odd fibrillar apparatuses, which we call the *organs of Perroncito* are often missing in young animals (B and C in Fig. 19). However, in studying the regenerative phenomenon in the adult cat and dog, one can often see them very abundantly, particularly in the large axons which have undergone bruising. They can never be seen in the fibres of Remak or in the thin medullary tubes. This is why we are inclined to consider the apparatuses of Perroncito as a pathological neoformation, which would sometimes lead to the formation of nerve fibres going to the scar, but would more frequently lead to the

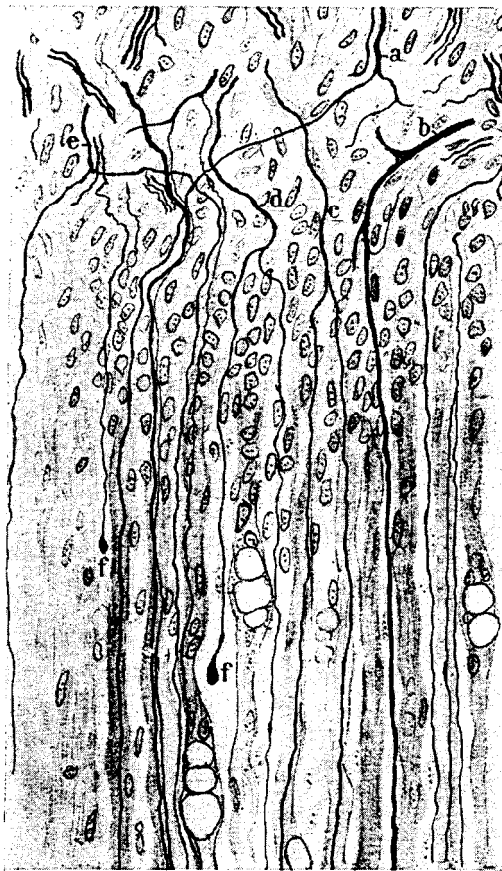


Fig. 20. Hilus of the peripheral end of a nerve cut and prepared 72 hours after the operation. (Cat several weeks old.) *a, b*, large fibres working through the scar, and coming from the central end; *d, e*, fibres divided at an acute angle; *f*; developing-boutons lying between the bands of Büngner; *e*, fibre of which the two branches are leading towards different bands.

construction of clusters and barren spiral dispositions (spiral apparatuses of the central end, already seen by the ancient authors such as Ranvier, Vanlair, Stroebe, etc.).

It is easy to understand that the phenomenon of Perroncito as well as the early budding of the nervous fibres of the central end, and above all the presence of balls of development in the extremities of axons penetrating the scar, are facts of great value in favour of the neurogenetic doctrine of His, because all these dispositions are there before the Schwann cell multiplication phase and, as a consequence, earlier than the differentiation process of the bands of Büngner.

2. When, repeating the experiment of Vulpian, Brown-Séguard, Bethe and Van Gehuchten, after cutting the nerve, obstacles are put between the two fragments in order to stop immediate reunion, a very advanced regeneration of the peripheral segment can often (but not always) be observed three or four months after the operation. In these circumstances it follows now and then that there must be a continuous cord, not visible macroscopically, uniting the two ends of the interrupted nerve. This then is the big histological argument of the catenary school which would become irrefutable if the anti-neuronists had submitted the intermediary scar to a thorough micrographic analysis. Unfortunately this exploration has only been done very incompletely by methods incapable of staining unmyelinated nerve fibres. Now, this intercalary tissue, supposed to be void of newly formed fibres, contains a considerable number of them, as Marinesco and I have demonstrated, yet the nerve fibres hardly form a cord, but a very free plexus scattered through the muscular and aponeurotic interstices, and uniting the central end with the peripheral end across all the obstacles, and often after very large detours.

3. The nerve fibres, having arrived at their destination, that is to say at the approaches of the peripheral segment, divide over and over again producing very complicated nerve bouquets, already pointed out by the ancient anatomico-pathologists, and just recently by Purpura, Perroncito, Marinesco and Krassin. These secondary branches board the above segment, going there sometimes via the interior of the bands of Büngner, sometimes through their interstices, terminating at different levels by means of a developing bouton. Sometimes several fibres can be seen dividing again in the depths of the distal end, diverging at an acute angle, and orientating themselves in a centrifugal direction (Fig. 20).

The existence of all these ramifications from fibres issuing from the scar, and above all the presence at the tip of each branch of a growing-ball con-

stantly orientated towards the periphery (f in Fig. 20) are phenomena irreconcilable with the catenary hypothesis. Let us add to this that the more delicate fibres located in the bands of Büngner are never shown to be discontinuous, but in evident continuation with those coming from the scar; consequently they are independent of the Schwann cells and the adipose masses of the former nerve tubes.

4. The phenomenon of the balls (Fig. 17). Apart from the regenerative process of which we have just spoken, there are also processes of spontaneous nervous neoformation in the nerve centres of adult man which are very suitable for the analysis of the regenerative mechanism. As we have shown above, our recent researches into the structure of the nerve ganglia in the bigger mammals has shown the constant presence of a certain number of sensory and sympathetic corpuscles, whose body as well as the principal prolongation, give off nerve fibres which end at variable distances, sometimes under the capsule, sometimes in the very thickness of the white substance of the ganglion, by means of a developing-bouton surrounded by a nucleated envelope.

Before undertaking our research on nerve regeneration we thought that these curious ball-like appendages constituted stable arrangements belonging to some special category of sensory corpuscles; but now that we have met similar facts in the sympathetic system of old animals, in the cerebellum and ganglia of animals stricken with rabies and other infectious diseases, lastly in nerves undergoing regeneration; after Nageotte also, by his elegant studies on ganglia of tabetics, has revealed, with the reduced silver nitrate method, the existence of a large number of ball-shaped sensory neurons and neoformed nerve fibres, we do not doubt that the appendages terminated by encapsulated spheres represent quite simply the result of a very interesting process of nerve production. Thus it would be a physiological phenomenon within certain limits, which is exaggerated following toxic influences or other conditions.

We will not discuss here the interpretations which the above discovery suggests; we will say nothing, for example, of the ingenious hypothesis of *collateral regeneration* conceived by Nageotte; neither will we stop at present to determine whether the ball cells, when they produce nerve branches, obey an irritative process without finality or congruity, or if rather they attempt to re-establish conducting pathways, degenerated or seriously impaired following functional fatigue or toxic action. We will restrict ourselves to maintaining here that the ball corpuscles give a striking demonstration of the

regenerative autonomy of adult neurons, and their capacity to produce new fibres by simple budding, and without the help of the Schwann cells.

Proofs derived from embryonic neurogenesis

The following data, which fully confirm the neurogenetic conception of Kupffer, His and Kölliker, are also based on the revelations of the reduced silver nitrate methods which stain the nerve fibres of the chick embryo in the sixtieth hour of incubation quite constantly (fixation by alcohol). Here are several very significant facts.

1. The neuroblasts of His in the embryonic medulla of the chick (Fig. 21) take on, firstly at the beginning of the third day, the classical pear-shaped form, which eventually becomes fusiform with a large expansion growing

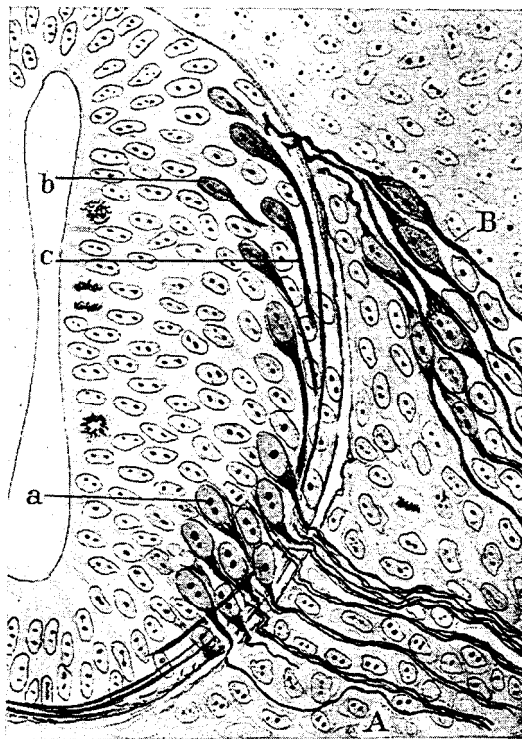


Fig. 21. Section of the spinal cord of the chick embryo at 3rd day of incubation. Reduced silver method. *A*, anterior root; *B*, sensory ganglion and posterior root; *a, a*, motor neuroblasts; *b, c*, commissural ends in axon neuroblasts whose a developing cone.

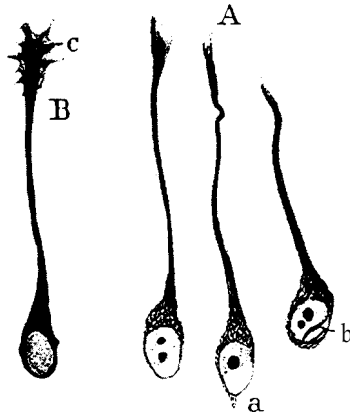


Fig. 22. Neuroblasts of the chick embryo at the 60th hour of incubation. *A*, developing-cone stained by the reduced silver nitrate method; *B*, cone impregnated by the Golgi method; *a*, primordial dendritic expansion.

out towards the medullary periphery, and a little soma formed almost exclusively by the nucleus. The neurofibrils, the only constituent factors of the cell attracting the silver deposit, begin in the cone of origin of the primordial prolongation, and extend under the fascicular form as far as the developing cone, in the interior of which they finish in a point like a paint brush. Thus these observations confirm the cone of development which was demonstrated a long time ago, using the silver chromate method by us, von Lenhossék and Retzius, but the comparison of sections stained by the two impregnation processes proves that this terminal mass contains, other than the said neurofibrillar brush point, an unstained plasma substance (Fig. 22) disposed in sheets and in diverging spines.* It is useless to point out that neuronal individuality seems certain in these preparations. In them one cannot discover a single trace of these cellular chains, or of these bizarre anastomosed neuroblastic columns, of which Sedgwick, Bethe, Fragnito, Joris, Besta, etc. speak.

2. It is also very easy to verify that, in their more embryonic phases (from the second day of incubation) the motor and sensory nerves are not made up of series of neuroblasts, as maintain Beard, Dohm, Balfour, Fragnito, Levi, etc. but of very thin continued prolongations issuing from the neurons; which go, sometimes independent, sometimes united to fascicles, across the intercellular spaces of the mesoderm. As for the marginal, or covering, cells (the

* We have not observed either the terminal cell recently indicated by H. Held who has made himself the defender of Hensen's old neurogenetic hypothesis. In our preparations the developing cone always appears extra-cellular.

lemmoplasts of Lenhossék) these will appear later (the fourth day) seated around the nervous fasciculae with which they have only contiguous relationships.

3. On examining the final branches of the motor and sensory roots in the depths of the mesodermal tissue, it can often be seen that each fibre ends freely by means of a developing-bouton, exactly identical to those seen in degenerating nerve tubes (a in Fig. 23). It is also very easy to see dichotomous divisions of young fibres, and even complicated ramifications (b). Let us note that in these very thin peripheral branches, and in consequence very recent ones, one can never find traces of cellular chains, or even of marginal or terminal corpuscles.

4. The motor nerve cords examined in the neighbourhood of centres (facial, hypoglossal, etc.) are completely devoid of interior nuclei, even in very advanced embryos (the rabbit embryo, 2.5 cm long). We will dwell no more on this particular fact, verified by Kölliker, His, Gurwitsch, von Lenhossék,

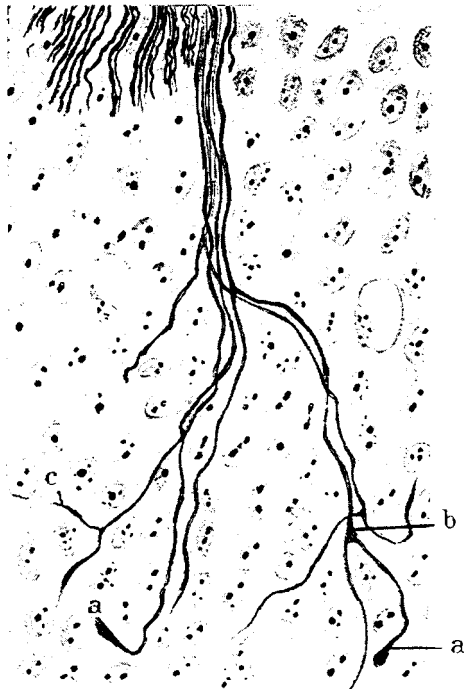


Fig. 23. Sensory axons in the process of development spreading across the embryonic conjunctive tissue (embryo of $3\frac{1}{2}$ days); *a*, terminal bouton; *b*, bifurcation of a fibre; *c*, branch ending in a point.

Harrison, etc. and never sufficiently explained by partisans of the catenary theory.

5. As we well know, all the central pathways are formed without the help of Schwann cells, and by virtue of a process of development continued from the axon of the association neurons. To this fact, brought out not only by the reduced silver nitrate method, but also by ordinary coloration methods, we will add that, in certain pathways, such as those of the cerebellum, a large number of developing young nerve fibres, provided with many terminal boutons, are often found, even in very much developed foetuses (dog, cat, rabbit).

To sum up: from the entirety of the observations which we have just shown, and from many others about which we have not the time to talk, the doctrine of neurogenesis of His is clearly revealed as an inevitable postulate. We mourn this scientist who, in the last years of a life so well filled, suffered the injustice of seeing a phalanx of young experimenters treat his most elegant and original discoveries as errors.

I finish by greeting most warmly and cordially this learned and sympathetic assembly, which I ought also to thank very much for their attention and kindness to me, during such a long and tedious lecture.