Studies on VERTEBRATE NEUROGENESIS

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AUTHOR'S ACKNOWLEDGMENT

THIS COLLECTION of my neurogenetic studies, of which some are quite old, has been compiled by a group of scientists from Montevideo. Inspired by love of science, they have nobly and generously desired to pay a magnificent tribute of affection and veneration to an old investigator. Would that all those who have contributed to this spiritual endeavor will accept this statement of my most profound and cordial gratitude; I shall be eternally grateful. At the risk of offending their modesty, I wish to cite the most renowned of the editors and offer special thanks to Dr. Alfredo Navarro, Dean of the Faculty of Medicine and to his worthy colleagues, Dr. Pou y Orfila, Manuel Quintela, Lasmier, Isola, Scaltritti, and Poney. My sincere thanks also to Dr. Vincent Arcelus, President of the *Institución Cultural Española,* to the sympathetic students of the *Instituto de Estudios Científicos* who have brought the zest and warmth of youthful enthusiasm to this undertaking, and finally to my dear and learned friend Dr. Estable, President of the *Comité d'Hommage.*

Madrid, May 20, 1929 S. R. CAJAL

PREFACE

IN THIS VOLUME we have collected some monographs which are barely or not at all known because they were published a long time ago in Spanish. These results have been especially neglected following the death of the master neurologists such as His, Kölliker, Waldeyer, Golgi, van Gehuchten, Retzius, Edinger, and others who had the kindness to confirm our findings. For the convenience of those who are interested in the question of neuronal development, we have added some other works which were published a long time ago in journals that are either infrequently consulted today or that have ceased publication and are consequently difficult to obtain.

All the studies are faithfully reproduced here, insofar as the text and illustrations are concerned. We have only permitted two modifications, for the benefit of the reader. We have omitted certain polemical arguments which no longer are of interest and which would have excessively lengthened this book. We have added several footnotes describing our current views when, by virtue of subsequent investigation by ourselves or others, our present opinions differ from our old interpretations. These footnotes terminate with the statement: (*Note for the French edition.*).

We hope that this compilation of more or less old, but scrupulously objective, neurogenetic facts will be useful to modern neurologists. Understanding of these facts will allow the neurologist to trace the history of each discovery and to impartially evaluate the contributions of all those who, like ourselves, have devoted themselves to the study of the difficult but enticing problem of the ontogeny of the central nervous system.

Madrid, May, 1929

TRANSLATOR'S FOREWORD

IT IS UNFORTUNATE that no comprehensive and systematic treatise on vertebrate neurogenesis exists today. Publication of the present volume partially fills this gap by making available to the English-speaking scientist the studies of Ramón y Cajal dealing with neuronal histogenesis in the spinal cord, cerebellum, cerebral cortex, retina, and peripheral nervous system.

In addition to its obvious value to neurologists and embryologists, this book should serve the biology student well, for these studies reveal how the static techniques of histology can be employed in solving the important dynamic problems of development.

I have attempted to render Cajal's text into current scientific English. The style of the French text is often awkward; therefore any attempt to retain the tone of the original would do disservice to the reader. However, in the purely polemical sections of the book (portions which are more valuable for their historical and psychological aspects than for their scientific merit) I have attempted to maintain the atmosphere of the original text.

I have rearranged some chapters and some chapter parts, hoping to improve the continuity of the book and more clearly outline the subject matter. For the sake of consistency some illustrations have been inverted. No portion of the text or figures has been eliminated. I have also added an author and a subject index as they were not included in the French edition.

I wish to thank Dr. J. G. Frontera for the photograph of Cajal that serves as frontispiece. This photograph was made in 1914 and appeared shortly thereafter in *La Esfera.* I am grateful to Professor F. DeCastro for having given me the copy of this little-known book from which I prepared the translation, and I sincerely acknowledge the encouragement and assistance of Dr. W. F. Windle in the preparation of the manuscript.

Bethesda, 1959 LLOYD GUTH

Chapter 1

DEVELOPMENT OF EMBRYONIC NERVE FIBERS AND OBSERVATIONS CONTRARY TO THE CATENARY THEORY [179]

INTRODUCTION

IN RECENT YEARS there has been propagated a concept of nerve development which is in frank opposition to His's and Kupffer's classical monogenetic view. This erroneous *catenary theory,* revived by Beard, Dohrn, Balfour and their enthusiastic disciples, has been refuted a thousand times by scientific observation. But it springs up again ceaselessly, cultivated by scholars in whom the spirit of paradox and the thirst for novelty leads them to the denial of the best-demonstrated facts, and to the sterile and naive endeavor of attempting to rediscover that which has already been discovered.

This conception consists of the hypothesis that the axon is not the result of the growth of the principal process of the neuroblast or primordial nerve cell, but that it represents the common product of the activity of a great many embryonic cells of ectodermal origin. The primitive cellular series, whose supposed fusion gives rise to the axis-cylinder, are said to be in the form of a chain. A conductive portion, in secondary continuity with nerve cells, and an adventitial part, formed by the Schwann cells, supposedly differentiate subsequently within this communal protoplasmic mass. Several nerve fibers could arise within the protoplasm of each chain.

Such is the simplest form of the rash *catenary hypothesis.* It has now been supported for a long time by Beard,[6,7] Dohrn,[30] and Balfour [5] for peripheral nerves and by Paladino [136] for central nerves.

It is in vain that this opinion, based on the questionable disclosures of unselective methods, was refuted by His, Lenhossék, Retzius, Kölliker, Harrison, ourselves and all those completely impartial and objective scholars who have scrupulously studied the development of nerve cells and fibers in the embryonic vertebrate spinal cord. The error is repeated daily and one can even say that it has been gradually transformed in recent times, while countenancing the most bizarre fantasies. And, as it is with all concepts that lack objective reality, each of the supporters of this theory professes a particular belief which is at least partially irreconcilable with the view of his fellow-believers. It is really very distressing to see how these research romanticists, instead of occupying their talents by expanding and consolidating the biological edifice, have no other care than to bring it down under the pretext of rebuilding its foundation!

Proof that our judgment does not err by intemperance is found in the opinions recently expressed by the partisans of the catenary theory, among whom Sedgwick, Bethe, Capobianco and Fragnito, Joris, Besta, and Pighione merit especial mention.

According to Sedgwick,[214] the nerve elements lack individuality at the outset; the gray matter is merely represented by a group of nuclei disseminated throughout a common protoplasmic mass. The nervous and protoplasmic processes later differentiate within this intercellular matrix. The neuroblast of His and the formation of cellular processes by growth and ramification would therefore be purely illusory. Such a neuroblastema would form the primitive nervous framework, and the axons would be created in the midst of this amorphous matter.

There is no positive proof for such a supposition. The hypothesis in question results from the application of methods that are incapable of revealing cell contours and from an excessively literal interpretation of the appearance of badly-stained fine funiculi. Is it not true that by failing to utilize all the advances of histological technique and critique we have retraced our steps towards the unsophisticated age of the blastema of Schleiden and Robin?

Nevertheless, this fantasy of Sedgwick is warmly welcomed by Bethe,[14] an eminent investigator whose scientific labor, although remarkable in many respects, tends excessively towards negative criticism. The Strasbourg physiologist begins by reporting that although the works of Kupffer, His, Lenhossék, Harrison, Grunsberg, and ourselves do show that the neuroblastic expansions emerge from the spinal cord during the first phases of embryonic development, these studies do not prove that the extramedullary portion is the result of the stretching of these neuroblastic prolongations. On the contrary, it seems more probable to him that axis-cylinders result from the fusion and differentiation of a chain of peripheral neuroblasts, in accord with the opinion of Balfour, Götte, Beard, Dohrn and others.

In support of such a supposition he makes use of 2 to 3 day chick embryos fixed in alcohol and stained with hematin or aniline acids. He finds that in their first phases, the ventral roots are composed only of cellular chains which are united at their poles and have apparently emigrated from the spinal cord. He mentions some elements of this class which show half of the soma within the spinal cord and half outside. But strangely enough, and contradictory to the catenary doctrine, among the constituents of the root he also detects some processes arising from spinal nerve cells (cells not neuroblastic, although of ectodermal origin).

Thus, the peripheral nerves would supposedly be engendered at a distance from the central nervous system by the differentiation of cellular, neuroblastic chains. He regards the origin of these chains as uncertain, in spite of his agreement that those chains which compose the ventral root originate from the cord. From the second to the fourth day of incubation in the chick the nerves change in appearance, and in the depths of the cellular chains appear some brilliant axons which are perfectly detached from the common protoplasm. Their independent appearance, in his opinion, is the cause of the error of the monogenesists.

Finally, the nerve cells of the spinal cord supposedly form very prematurely. Contrary to Lenhossék's and our assertions, the dendrons appear after the second day of incubation. These processes do not arise by growth of neuroblastic protoplasm but by apposition and condensation of an intercalary plasma (the plasma of Sedgwick) in the midst of which the embryonic nerve cells reside. Both the growth of nerves and the multiplication of their axons takes place by successive solidification and apposition of this blastema. The Schwann nuclei multiply by mitosis.

Although Bethe's praiseworthy aim was to reconcile the catenary theory with the positive facts of neurogenesis, we see that the vague assertions and complexities of his doctrine present every appearance of a scientific preconception. In his great desire to solidify the theory of discontinuity, this author zealously gathers every indication that seems favorable in this regard, and he omits citing (and consequently refuting) the contrary facts which are provided by the clear and conclusive disclosures of the Golgi method. Nevertheless, in spite of himself, the truth frequently slips from his pen, appearing in the objectivity of his descriptions and in the realistic accuracy of his figures. His valuable confession that the ventral roots represent processes from cells of the gray matter; his declaration that many axons lie intercatenarily or intercellularly (although they were previously intracatenary); the recognition that nuclei are plentiful in nerves and that the number of nuclei increases as the nerves are more advanced in development; the figures in which he draws fascicles of fine, independent and continuous axons, located between a series of cells, just as the partisans of the monogenetic doctrine have described them, are all further proof that the Strasbourg physiologist has come very near to the truth. He only lacked the necessary independence and serenity of spirit to recognize and proclaim it.

Other observers imbued with the same prejudice as Bethe, including Capobianco, Fragnito, Besta, Pugnat, Joris, Olmer, Pighini and Schültze* have studied the subject of nerve development.

To attack the neurogenetic problem, Schültze [211,212] has chosen to study a very specific subject: the peripheral sensory nerve terminations of newt and salamander larvae. He fixed the larvae in an osmium bichromate bath, and stained the skin *en masse* afterwards in an alcoholic solution of hematoxylin. In preparations of this kind he recognizes numerous fusiform or stellate neuroblasts in the depths of the subcutaneous connective tissue. These neuroblasts are joined to each other by their ends and are laid out in a complex network having narrow polygonal meshes. The ensemble forms a true multinucleated reticular syncytium of protoplasm, in the midst of

*In this French translation we exclude discussion of these scholars' opinions because they have lost all interest since the modern investigations by Lenhossék, Kölliker, Held, Tello and us. We only retain the criticism of Schültze's opinion with a view to demonstrating the difficulties faced by those who attempt to resolve the problem of neurogenesis by means of routine histological procedures which are incapable of clearly revealing axons and their ramifications. (*Note for the French edition.*)

which the future axons will be differentiated. As several other authors have recognized, these nuclei multiply by mitosis.

Simple inspection of the prints attached to Schültze's monograph is sufficient to demonstrate that his error is the result of the method employed. Hematoxylin or basic aniline dyes do not differentiate the protoplasm of the embryonic Schwann cells from that of the axon; this method uniformly stains them equally, and demonstrates them as continuous and fused. Because of so grave a technical defect, the nuclei of the connective or adventitial elements seem enclosed within the substance of the primordial axis-cylinder while in reality they lie outside the latter. In some figures Schültze himself pictured arrangements which suggest this interpretation, e.g., alleged fusiform neuroblasts with tangential nucleus and, still more significantly, angulated vacuoles instead of interfibrillary spaces where the processes of diverse elements converge. In reality, what this author takes for interneuroblastic anastomoses are nothing more than loci of growth and ramification of fine independent nerve fibers. These fibers are surrounded by a common sheath formed by the protoplasm of connective satellite cells.

Proof that such is the case can be found by examining reduced silver nitrate preparations of the subcutaneous nerve plexus and the fine intermuscular plexus of frog larvae. Figure 1 is a composite illustration of the plexus as observed by several techniques (hematoxylin, methylene blue and silver nitrate.) The hematoxylin method alone (Schültze's technique) only visualizes the continuous protoplasmic branches without revealing appreciable axonal differentiation. Nevertheless, one notes how each trabecula, although massive in appearance with routine methods, actually surrounds several very fine nerve branches which disperse, ramify or come together at the level of the anastomotic bridges. These packets of extremely delicate filaments are surrounded by the Schwann cell protoplasm. At their thin ends, the Schwann cells appear anastomosed or in intimate contact. The isolated (probably terminal) nerve filaments lack nuclei and marginal protoplasm (Fig. 1, d) and dichotomize. Finally one occasionally notes some connective tissue expansions abutting against Schwann cell protoplasm and perhaps fused with it.

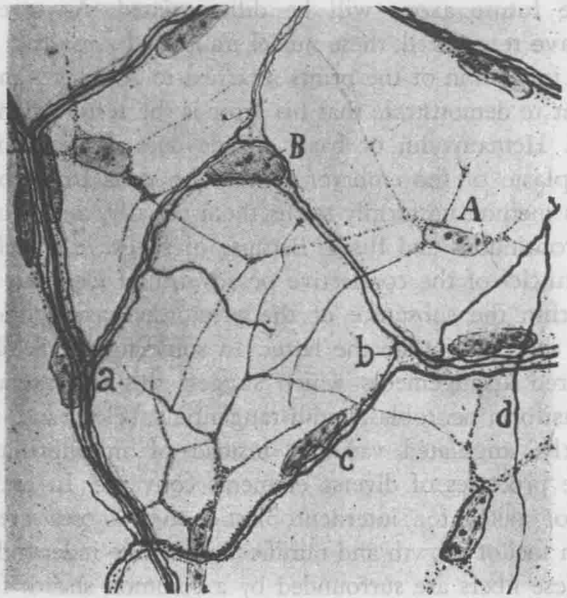

Fig. 1. Sensory nerve plexus of the tadpole tail. Reduced silver nitrate method. A) Connective tissue cell; B) Schwann cell; a, b) region of bifurcation of fine, unmyelinated nerve fibers.

From what we have just described it is seen that we no longer accept Kölliker's interpretation [84] of Schültze's nets. According to the Würzburg scholar, these nets are not an early phenomenon and are not composed of neuroblasts, but are the result of fusions or secondary anastomoses occurring in nerve fibers which have developed and grown from the central nervous system.

Such are the theories defended by the principal adversaries of the doctrine of His. It is unnecessary to add that to support them it was first necessary to omit all the precise observations of the esteemed Leipzig embryologist and, furthermore, by a retreat of more than thirty years, to systematically set aside the marvelous, precise and definitive disclosures of silver chromate.

But the refutation of the catenary theory is not based only on the results of staining techniques which are selective for nerve protoplasm. Do not forget that the renowned His, founder of the neu-