A MORPHOLOGICAL STUDY OF INTERNEURONAL CONNECTIONS

A MORPHOLOGICAL STUDY OF INTERNEURONAL CONNECTIONS

by

William Carleton Gibson, B.A.

A Thesis presented to

The Faculty of Graduate Studies and Research

of McGill University

In Partial Fulfilment of the Requirements for the Degree of

Master of Science

1935

From the Department of Neurology and Neurosurgery.

Acknowledgment is made of the generous assistance of Professor Wilder Penfield, the Director of the Montreal Neurological Institute, and of Dr. William Cone, the Director of Research. The staff of the Institute has co-operated in innumerable ways to make the work possible. The research here reported was carried out under the tenure of a Research Fellowship during the session 1934-1935.

TABLE OF CONTENTS

A MORPHOLOGICAL STUDY OF INTERNEURONAL CONNECTIONS

ABSTRACT.

Since the enunciation of the "neurone theory" by His and Forel (1886-1887) attention has been focussed on the nature of interneuronal connections. Max Schültze, the discoverer of neurofibrils (1871), surmised a discontinuity between the units of the nervous system. The subsequent discoveries of Cajal, Held, Auerbach, etc. have done much to explain on a structural basis many facts concerning the synapse long known to neurophysiologists. In the present work studies have been made of the "boutons terminaux" as they occur in synapses in the spinal cord, cerebellum, and cerebrum, both in the normal state and after axonal section. The early manifestations of degeneration have been carefully recorded, in order that the tracing of fibre tracts by the "bouton" method may be placed on a sound basis. Evidence is produced in favour of the conception of "contact" rather than "continuity" at interneuronal junctions, as implied in the "neurone theory".

INTRODUCTION.

The present study of interneuronal connections was undertaken primarily for the purpose of standardizing and expanding a technique developed in Sherrington's laboratory by E. C. Hoff for the exact determination of fibre tracts and connections in the nervous system. The possibilities which this technique has opened up in the fields of neuroanatomy and neurophysiology are so varied that a re-examination of the background of our knowledge relating to synapses has been thought advisable. To this end, the available information on the subject has been brought together under the headings of embryology, histology and physiology. Experimental methods and observations have been recorded and discussed, in an effort to point the way for future investigation of the nervous system, using the "bouton" technique. A list of"references" has been appended which encompasses much of the current literature on the modern conception of the "neurone theory", a theory which derives much of its support from our knowledge of the structure and function of the synapse.

(a) Embryology.

The embryological development of interneuronal connections is a fascinating chapter in the story of "form and function" in the animal body. The delicacy and constancy with which the minutiae of the nervous system link up the complex society of the body would indeed tax the imagination of Herbert Spencer. It is as if an unknown deposit of copper had suddenly awakened in the middle of a growing village, and set out to provide, according to a preconceived plan, a telephone system capable of serving a metropolis of ten million people.

Neurones are among the last cells in the body to mature. This may be attributed to the integrative function which they are called upon to perform, and also to the fact that in the economy of the body provision is made for but a single set of nervous equipment. The loss of regenerative power is the price which the nervous system has had to pay for its specialization in irritability and conduction.

The developing mammalian neuroblast is strikingly similar to the "neurosensory cell" and the "primary ganglion cell" of invertebrates. The "neurosensory cell" contains no chromidial substance. In the "primary ganglion cell" (as in coelenterates, etc.) we see the tigroid matter limited to the cell body (Wolff, 1904) lying close to the nuclear membrane. In the adult "neurone" of higher animals, Nissl substance is found both in the cell body and in its dendritic extensions.

It is of interest here to note that Apathy (1897) believes there is a neurofibrillar anastomosis between the dendritic processes of neighbouring "primary ganglion cells" - as seen in the intestine of "Pontobdella".

Kappers (1932) believes that hand in hand with the increase in this cytoplasmic constituent goes the development of "polarization" in the nerve cell. The vital "vis a tergo" converges on the young nerve cell, with the result that a specialized outgrowth is produced on the opposite side. (See the neurosurgical experiments of Peterfi and Kapel, 1928.) This "axone" is a "stimulo-concurrent" growth (Bok, 1915) which leaves the cell body by a cone-shaped protrusion containing the diplosomes. Around the diplosomes the earliest neurofibrils develop, and extend into the axone as well as into the cytoplasm of the cell body (Held, 1909).

Correlated with the appearance of chromidial substance is the outgrowth of protoplasmic processes of the cell - the "dendrites". These arise in response to stimuli such as those which produced the axone, but they proceed in the opposite, or "stimulo-petal" direction (Kappers, 1907-1908). Dendrites can originate from any part of the cell, except the axone and its cone of outgrowth which do not contain Nissl granules. Dendrites branch at points known as "chromidial bifurcation cones", where this iron-containing matter is concentrated. In the "stimulo-petal tropism" of the dendrites the chromidial substance is of considerable significance. The presence of oxydase granules in the cytoplasm of the cell and

dendrites suggests a chemotactic function at work in directing dendritic growth. Child (1920) sees the dendritic pole of the neurone as a centre of increased metabolism - largely on the basis of the oxydase and iron-containing Nissl granules, which furnish the prerequisites for an oxygen-storing system. Coghill (1929) believes that the dendrites grow out into a gradient of higher metabolism. Kappers (1932) agrees that a metabolic force influences the dendrites, and may even play a part in the displacement of ganglion cells in a "stimulo-petal" direction.

To suggest that dendritic growth is largely directed by metabolic forces would be incorrect. By employing a galvanic current of the same strength as the action current in a stimulated nerve, Ingvar (1920) has proved that the direction of outgrowth of neuroblasts in a culture 'in vitro' is determined by the direction of flow of the electrical current. Peterfi (1934) has confirmed an earlier belief that dendritic growth was "cathodal". The work of Heringa (1920) and Grigorieff (1929-30) supports the statement that axonic growth is "anodal" in direction (Kappers, 1917). Kraus and Weil (1926) have made a study of changes in shape and size during the development of anterior horn cells in human embryos. Assuming the neurobiotactic theory to be true, they assert that during the fourth fetal month strong inter- and intra-segmental stimuli influence the form of anterior horn cells. "The afferents to cells determine their form just as according to the theory of neurobiotaxis of Kappers, afferents to nuclei determine their position".

More interesting than the "process" of growth of the nerve cells is the "purpose". The forces which determine the selectivity of connections between neurones are varied. Cajal (1893) introduced the conception that the production of repelling and attracting substances by two nerve cells was the reason for their relation and for the formation of dendrites. Lache (1906) made the speculation that cells predestined to a functional inter-relationship acquired structural inter-relationship in the synapse.

The property of "memory" inherent in all protoplasm is specially developed in growing neurones, with the result that cells which are stimulated simultaneously or in succession tend to function together. In this way Kappers sees the possibility of correlating Sherrington's "central excitatory state" with the neuroblastic law of the determination of the final course and ending of a stimulation, by a previously stimulated centre. Commenting on the effect of a focus of cell division on the course of growing axones, Kappers (1934) writes: "The summation necessary for establishing the final synapse in this case is given by the proliferative centres that act as a previously stimulated cell group in the physiological experiment".

Another property which may influence the selection of interneuronal connections is that of "resonance" described by Weiss (1926). He believes that a reflex started by an extensive skin stimulation can cause contraction of a <u>single</u> muscle, only if the stimulated cell is in "resonance" with the effector organ. Kappers asks if "resonance" is a determining element in the combining of nerve cells subserving a specific function.

However, in this connection Coghill (1924) reminds us that afferent and efferent systems are structurally ready to function some time before response to tactile (external) stimulation occurs. Windle (1930), working on the spinal cord of kittens, and Sepsenwol (1934) on the cranial ganglia of "Batrachians", have correlated neurofibrillar differentiation and nervous activity.

The only clear description of developing synapses is that by Coghill (1924). "The synapse in its earlier functional condition occurs between loose brush-like endings of fine spiral pointed terminals that are structurally adapted to motility..... Fusion does not occur between neurones in the field of synapse". Practically nothing is known about the development of the synaptic end-organs such as the "boutons terminaux". Karl Bauer (1932) has demonstrated "Heldschen Endfüsse" in tissue cultures of chick embryos. The recent publications of Speidel (1933) contain figures which suggest the possibility that the boutons terminaux are the end products of the "growth cones" of developing nerves - perhaps of individual neurofibrils. Speidel's work on the tails of living frog tadpoles contains many observations of interest in the study of nerve cell growth and functional differentiation. Relevant to the question of factors influencing direction of growth, he states: "A 'hydrodynamic factor' may be considered of some importance in the orientation of nerve sprouts. Ample confirmation is presented of the principle of stereotropism (tactile adhesion)". While the following statements do not agree with Kappers' conception, they do not

necessarily invalidate his major thesis of the biophysical forces at work in the embryonic nervous system. "Subjection of the entire animal (tadpole) to electrical stimulation causes no appreciable effect, either on the rate or direction of growth of active cones. Growth cones may migrate in opposite directions along the same path at the same time."

From the literature then, it may be fair to conclude that the "integrative action" of the synapse has developed under the following influences: simultaneity of stimulation, succession of stimulation, metabolism, cell proliferation, and possibly "resonance", "tactile adhesion", and a "hydrodynamic factor".

The theory of neurobiotaxis offers the most adequate explanation of the facts known about developing interneuronal connections. Kappers' simple summary states the crux of the situation very clearly: "Neurobiotactic polarization implies that the normal interneuronal transmission of an impulse favours the approach of two neurones in the synapse".

With this background we are better prepared to approach the conceptions which will follow, of the irreversibility of the synapse, the "central excitatory state", and the vexed question of "contact or continuity" in the nervous system.

(b) Histology.

The growth of our knowledge of the structure of the synapse has closely paralleled the advances made in histological staining and technique during the last fifty years. Golgi's publication in 1885 of a method for staining chrome-hardened nervous tissue with silver solutions ushered in the Golden Age of neurohistology. A struggling young professor of histology from Barcelona, Ramon y Cajal, chanced to visit a psychiatrist in Madrid, Dr. Luis Simarra, who told him of the 'capricious'method introduced by Camillo Golgi. Taking up the method immediately, Cajal set out to fulfill his avowed intention (Valencia 1882) of achieving international recognition in ten years, "since it is a disgrace that among so many thousands of discoveries in anatomy there is not one to which the name of a Spaniard is attached".

By 1888 he was able to publish the first description of the termination of adult axones, taken from his work on humans, monkeys, dogs, and birds: "Il ne nous a jamais été donné de voir une anastomose entre des ramifications de deux prolongements protoplasmiques différents, ni non plus entre les filamente émanés d'une même expansion de Deiters; les fibres s'entrelaçaient d'une manière fort compliquée, engendrant un plexus enchevêtre et serré, mais jamais un réseau. Les observations que nous venons d'exposer sur la structure du cervelet des oiseaux viennent aussi à l'appui de cette manière de voir; on dirait que chaque élément est un canton physiologique absolument autonome".

Cajal's discovery that the collaterals in the spinal cord ended "sur la cellule au moyen d'une varicosité en contact avec la membrane" was confirmed by Kolliker in 1890, and later by Van Gehuchten, von Lenhossek and Retzius (1893).

In 1891 Cajal wrote: "Les ramilles protoplasmiques terminales des pyramides ne sont pas lissés de contours, comme les auteurs semblent les représenter; elles sont herissées de dents naissant à angle droit ou presque droit et terminées par un bout rond et un peu epaissi."

In the same year, Held, using the methods of Golgi and Nissl, discovered the 'calices' or synapses of the nucleus of trapezoid body (fig. 1.), and some very similar endings in the ventral acoustic ganglion.

Held's next contribution was in 1897, in the report of his 'Endfüsse' (fig. 2.) confirmed by Auerbach (1899) who named them 'boutons terminaux'. These were assumed to be the pericellular terminations of axis cylinders, but it was not until 1903 that their connections with the neurofibrils of the axone were demonstrated by Cajal's new reduced silver method. Auerbach at first believed there was "eine haarscharfe Linie als Grenze zwischen marklosen Nervenfaser und Ganglienzelle", but he later accepted the conception of Held (1904, 1905) of continuity between the Endfüsse and the reticulum of the neurone. Auerbach and Held saw the Endfüsse embedded in the interstices of the 'pericellular nerve net'. Held believed there was continuity with the inner reticulum also, but Auerbach thought that a transparent cuticle intervened. Thus

began the controversy of 'contact or continuity' in the nervous system.

Among the early proponents of the 'neurone theory' were Joris, Van Gehuchten, Marinesco, etc. In a masterly contribution, Joris (1903) summoned the researches of His in embryological and of Forel in the pathological changes of the nerve cell, to the defense of 'contiguity' in the nervous system. The anastomosing nets depicted by the gold chloride method in vogue before Golgi's work were still looked upon by many as the final 'nexus' between neurones.

Thus the first decade of this century was filled with the struggle between 'reticularists' and 'neuronists'. The Italian school of Donaggio defended the theory of seemingly innumerable reticula in the neurone. He himself wrote (1905): "All this uncertainty is due to the imperfection of Cajal's stain". Fragnito (1905) claimed that Donaggio's method could demonstrate the pluricellular origin of axis cylinders! Turner (1905) believed in the continuity of the prolongations of nerve cells by means of a pericellular 'réseau'. It would be fruitless to pursue the history of the controversy on 'reticularism' any further. Suffice it to say that it was started by Golgi's discovery in 1893 of a pericellular net. Since that time Bethe, Apathy, Nissl, Bielschowski, and others have reopened the question periodically, but all have been outdone in this by Held (1929) whose recent 'Grundnetz' theory envisions connections between all the processes of all the nerve cells and all the glial cells. It is little wonder that

Cajal called these "des speculations presque metaphysiques des partisans de la continuité protoplasmique".

To return to the two chief types of synapses already mentioned -- the calices of Held and the boutons terminaux Fig.1 Fig.17 we shall examine the literature for further information about them. Tricomi-Allegra (1904) could not accept Cajal's description of the calices as 'arborizaciones pericellulares finas', but preferred to consider them as part of the 'interstitial plexus'. Cajal, and Windle (1928) have found that Held's calices can be demonstrated in a young animal earlier than can the boutons. For this reason and for reasons of size and shape, some doubted the nervous nature of the calices. However, Cajal (1934) reports that De Castro and De Juan, in an unpublished work, have recently seen in the calices the swelling and granular degeneration typical of nerve endings whose axis cylinder has been cut.

The more important interneuronal connection in this study is the terminal bouton. The recent interest of Sherrington and the Oxford group in the boutons terminaux reminds us that Sherrington along with Michael Foster introduced the term 'synapse' in 1897 to indicate the "nexus between neurone and neurone in the central nervous system".

Cajal compared the boutons to spots on a tiger's skin. Marinesco thought they resembled spermatozoa. The suggestion has been made in the preceding chapter that they are derived from the 'growth cones' of developing axones. Karl Bauer (1932) has demonstrated 'Endfusse' in tissue

cultures, but puts Held's interpretation of 'continuity' (Fig.4) upon them. Cajal was unable to show boutons in the nervous system of an eight-day old kitten. Even in cats of one month, their occurrence was 'sporadic'. In a series of cats ranging in age from one day to two months, Hoff (1932c) stained the earliest boutons at twenty-one days. This finding bears out Windle's work in 1930 on behaviour reactions in kittens. However, it might be well to recall Coghill's (1924) warning that many writers have reported early neurofibrillar structures to be heavier in outline than adult ones, when actually their silver technique could be accused of matting neurofibrils together. Held maintained that the independence of the ramifications of axis cylinders in newborn or very young animals was only temporary. He demonstrated Endfüsse in cats twenty days old, which he said were taking part in the anastomosis around the cell.

The question has always arisen as to whether Held's 'Endfüsse' were identified with Auerbach's 'boutons'. Van Gehuchten (1904) has pointed out that Held's preparations stained only the granular 'neurosomes' at the axone terminations, while Auerbach pictured the oval or annular form of ending, which was smooth and regular in outline. These always outnumbered Held's structures, and were more evenly spaced. Bethe (1903) was satisfied that the Endfüsse were fragments of the Golgi net which had been broken up by fixatives. On the other hand, he asked if the filaments on Auerbach's boutons might not be those of neuroglial cells.

Golgi (1906) recognized the boutons as the protoplasmic ends of ramifying fibres. He found them, he said, on meningeal vessels (!), from which he deduced their supposed nutritive function.

Lache (1906) credits Auerbach with the discovery of the boutons in 1896, and discounts the statements of Held, Bethe, Bielschowsky, Holmgren, etc. concerning filamentous projections arising from the bouton and penetrating the adjacent cell membrane. Lache emphasized particularly the facts that boutons occurred around Purkinje cells, that there were variations in size, and that they were very numerous on the dendrites.. Mahaim (1905) has criticized Held for his designation 'Endfusse', which he describes as "ce triste nom de 'pieds terminaux' qui, en français, est intolérable".

From the first, workers have alluded to the different types of boutons found, and also to their occurrence in the various parts of the nervous system. Van Gehuchten (1904) showed a fibril ending in a large bouton which, on its distal surface, was attached by a thin fibril to a very small ring-like structure. He has also noted the diverse directions from which adjacent boutons on a cell body or dendrite draw their respective neurofibrils. No two fibrils have ever been seen to end on the same bouton.

Marinesco and Cajal have written of the extreme difficulty of staining boutons on the pyramidal cells in the cortex. This may be due to the dense vascular and glial anastamosis about these cells.

An interesting anomaly is to be found in Wolff's paper (1905) in which he adheres to the idea of 'continuity' in the synapse, based on his illustrations which in one case at least (fig. 5) show boutons having no semblance of continuity with other structures. Molhant (1911-12) states: "Les boutons terminaux que nous avons observés sont indépendants les uns des autres et indépendants également du réseau endocellulaire". Van Gehuchten (1911) draws attention to the striking fact that the boutons are only rarely seen on the cone of origin of the axone. In this connection, Stefanowska (1897) reported a lack of these 'appendices piriformes' on the axone and its adjacent segment of the cell body.

The second decade of the twentieth century was particularly free from the controversies which had previously saturated the subject of interneuronal connections. The vitriol poured out by Cajal upon Apathy in 1908, in a polemic in defense of the neurone theory, has never been equalled. Bartelmez (1915, 1920) and Marui (1918, 1919) did cross swords over the vestibular club endings on the Mauthner cells of Ameiurus, but Bartelmez's most recent work (1933) has conclusively cleared the issue. Turning to the work of recent years, we read of the reassuring opinion of Ballantyne (1925) that boutons and pericellular networks are optical illusions due to the use of monocular microscopes.

After fifty years of controversy as to the existence of neurofibrils in the living nerve cell, Bozler (1927) 'turned the corner'. He was able to demonstrate living neurofibrils in the bipolar ganglion cells of a species of Coelenterates, Rhizostoma. For the first time, too, a description of the

living synapse was given: "Die Fortsätze der Bipolaren sind gerade und unverzweigt. An ihrem äussern Ende befindet sich eine kleine anschwellung, von der ausserst feine Fasern ausgehen, die sich an die Nervenfortsätze anderer Bipolaren anlegen und so offenbar eine Erregungsleitung von Zelle zu Zelle ermöglichen." "Die Fortsätze der Bipolaren bilden keine Anastomosen untereinander." "Das Nervensystem ist demnach kein Nervennetz, sondern besteht aus einzelnen Neuronen, die durch innige Kontakte miteinander physiologisch verknüpft sind. Die angaben in der Literatur über das Vorkommen von Nervennetzen bei Coelenteraten sind nicht beweisend."

The work of Tiegs (1926 etc. fig.6)

revived the question of continuity at the synapse. This author has stated quite definitely that there are no discontinuous interneuronal connections in the spinal cord, and that the work of Stohr (1927) has given evidence strongly in favor of a syncitial arrangement of the neurofibrils in the sympathetic ganglia. In an effort to do justice to Cajal, Parker, and Boeke, etc., he concludes (1927c): "The collaterals which have arisen from the longitudinal axons of the white matter by separation out of individual neurofibrils, traverse the grey matter of the cord, and after undergoing considerable branching penetrate the dendrites of the nerve cells; they pass as neurofibrils along the dendrites to the middle of the cell, where they form a perinuclear network, which Cajal himself observed, and from this network a relatively small number of neurofibrils enter the axon".

The evidence upon which Tiegs bases his remarks is chiefly negative. His inability to stain boutons in the spinal cords of newborn and young cats, guinea pigs, and rabbits, has led him to conclude that such structures do not exist. He credits the microtome knife with the creation of the boutons. His statement that incomplete staining has given rise to the idea of 'contact' is interesting in view of the fact that many of his most conclusive preparations have been stained by Bielschowski's method, which is listed among those methods characterized by Bartelmez (1933) as 'barbaric'. Beccari (1918) is cited as an advocate of 'continuity'.

Windle and Clark (1928) have given an excellent criticism of Tiegs' work, and have produced slides from Ranson's collection to support the theory of discontinuity, and the fact of the existence of boutons in the cord and cerebellum.

Boeke (1929) gives a fair appraisal of the physiological evidence in favor of the independence of the individual neurone, but he announces that the term 'synapse' has now come to include peripheral nerve endings, as well as interneuronal connections. Proceeding to describe the facts of degeneration and regeneration in 'end-plates', etc., he attempts to draw a parallel between peripheral nerve endings which seem to show intraprotoplasmic penetration and continuity, and central nerve junctions which should show the same properties. Rodriguez Perez (1934) working on fish has refuted the ideas of continuity and penetration at the nerve-muscle junction.

Cajal has cited the work of Estable (1931) on 'Friedreichen Krankheit', in which the boutons are shown detached from the cell-body with which they were once in contact. In no case could fragments of filaments be seen adherent either to the bouton or to the periphery of the denuded cell, a finding suggestive of 'contiguity' at the junction.

The considered opinion of Cowdry (1932) is that "the burden of proof must rest with those who assert that protoplasmic continuity exists between nerve cells".

Beginning in 1932, Hoff/fig.7, has made a number of very valuable contributions on the histology of the synapse. He has confirmed the work of Cajal/fig.17, and others on the existence of boutons terminaux on the surface of nerve cell bodies, and of "boutons de passage" " in tandem". The only differentiating factor structurally is that the "boutons de passage" are often smaller. Using an improved method of fixation by the injection of chloral hydrate, Hoff has demonstrated boutons in the spinal cord, cerebellum and cerebrum of cats, and in the spinal cord of humans and monkeys. It is computed that there are at least three hundred boutons on the surface of a ventral horn cell in the spinal cord of the cat. This author has revived the bouton degeneration method for the ascribing of fibre tracts in the nervous system. His findings on degenerative manifestations of the boutons will be referred to later. In the present section, it is sufficient to note that he considers the boutons to be the characteristic synapse stations of the nervous system, in no way continuous with the contents of the adjoining cell. They are said to increase with the age and

reflex behavior of a developing animal.

In the adult cat, Hoff found the boutons on ventral horn cells measuring from 2.2μ by 1.1μ to 1.1μ by 0.5μ. In the adult monkey the terminations on Clarke's Column cells were of the order of 3μ by 2.3μ etc., while in monkeys eighteen months old, boutons of 1μ in diameter were seen. More recently, boutons have been studied in the rabbit, baboon and chimpanzee.

Sereni and Young (1932) have reported bouton-like structures in the nervous system of cephalopods, fig. 8, which system was once assumed to be an example of fibrillar continuity and reversible conduction. Examination of the normal and pathological structure of these synapses has convinced them of the discontinuity in the nervous system of one of the higher forms of invertebrates. (These authors have reported a connective tissue reticulum, which is rather suggestive of the 'nervous' syncitium of the early workers.) Foerster, Gagel and Sheehan (1933) have confirmed Hoff's 'Endösen' in the spinal cord of the monkey, and have used the term 'zwischenöse' to describe a bouton-like structure with a neurofibril passing through it on its way to a more distal termination (fig. 9 a & b).

Lorente de Nó (1934) has used the Cox modification of Golgi's stain to demonstrate the synapses in the cerebral cortex. The author reports as many as forty fibrils ending in boutons terminaux on pyramidal cells (fig. 10). Each dendrite, soon after leaving the cell body, acquires several

hundred collateral 'thorns' (fig.10b) which are thought to be a second type of synaptic structure.

The outstanding contribution of recent years to the solution of the structural enigma of the synapse has been made by Bartelmez and Hoerr (1933). They have returned to the 'bullhead' Ameiurus, in which the vestibular endings on the lateral dendrite of the giant Mauthner cell offer excellent material for study (fig.11). Marui's negative findings (1918) seem the more remarkable now, when with improved technique Bartelmez has been able to portray such clear-cut limiting membranes in the synapse. After perfusion and fixation, the tissue is mordanted in preparation for Bensley's mitochondrial technique, which is augmented with differentiating stains. The end-feet and the neuropil appear scarlet, and the coarse overlying Golgi net is bright blue. In no case can this pericellular net be seen intervening between the end-foot and the surface of the large dendrite. The mitochondria are seen to palisade at the membrane of the club ending, a fact reminiscent of the concentration of the 'neurosomes' of Held (1897) at synaptic interfaces. It is generally believed that the neurosomes which Held stained by Altman's method are what we today call mitochondria. The interface between the club ending and the surface of the dendrite is clear-cut and there is no suggestion of filaments crossing between the two or penetrating the adjacent cytoplasm.

Bartelmez has conducted experiments with the Bielschowski technique and has come to the following conclusions:

The block method of impregnation is unreliable, and is definitely inferior to the individual section method; the time allowed for adsorption of the silver solutions is the most important variable; ammoniacal alcohol used before mordanting in silver prepares the whole tissue surface for an overlay of silver which will give untrue pictures. The interesting statement is made that the neurofibrils of the club endings are uniformly darker than those of the axis cylinder. This is perhaps to be accounted for by the lack of protective myelin on the distal part of the club ending. Bartelmez's perseverance in the use and refinement of the Bensley technique has brought us irrefutable information on the synapse, and incidentally has indicated the necessity for a reconsideration of technical methods in neurohistology. A propos of this suggestion, Bartelmez remarks: "It is not to be wondered at that Cajal and Held came away from their celebrated conference, each certain that his own view was correct, although each had studied the other's most convincing preparation".

In a subject such as histology, which depends so much on the empirical use of dyes and stains, we are bound to find the greatest variance in the interpretation of results. According to Bartelmez, formaldehyde is only to be considered as a fixative, and must be followed by a mordant if gross shrinkage is to be avoided. He refers to the secondary swelling produced by the Bielschowsky technique, and suggests that the use of myelin solvents have given rise to the so-called 'cement unitiv' between end-feet and cell body, etc.

That many supposed anastomoses in the nervous system are produced by our technical methods is generally recognized. The following statement by Karl Bauer (1932) is interesting from this angle: "Bei anwendung geeigneter Fixierung (Zenkersche Lösung) und Farbungsmethoden (Molybdän-hämatoxylinfärbung nach Held) ist es relativ leicht, sichere anastomosen und Netze in gut gewachsenen Kulturen zu finden". Bielschowsky's defense of anastomosing structures has always been a statement that techniques which show boutons and no more are 'incomplete'. Tiegs has followed the same line of defense.

For the past fifty years, histologists have critically examined the staining techniques of their rivals, especially those interested primarily in the neurone theory. In Nissl's evaluation of Golgi's silver method we read: "Die Neuronenlehre verdankt ihr Dasein einer Methods, welche eine vorzügliche anatomische Method ist, mit der man aber in Ewigkeit nicht histologische Detals erschliessen kann", and later Nissl's last retort: "Ist doch die Neuronenlehre ein Kind der Golgischen Method?". Recently Cajal has described the work of Ramon Vinos, who has shown with silver stains nets surrounding nerve cells and their processes, and covering both the interior and exterior surfaces of blood vessels. These nets may be analogous to those stained by Donaggio (1905), and may be delicate strands of coagulated protein.

A study of the pathological histology of the boutons terminaux is of interest for two reasons: degeneration experiments have contributed a large amount of evidence in favour of the discontinuity of the nervous system; and, the appearance

of boutons degenerating after their central connections have been destroyed has given us a useful method for tracing fibre tracts to their exact destination.

Nikolajew in 1893 conceived a method for investigating the innervation of the frog's heart by cutting the vagus, and studying degeneration of the pericellular endings on the cardiac ganglion cells. The previous work of Smirnow (1890) had provided examples of normal nerve endings on the heart ganglia, and by comparison Nikolajew was able to decide what endings showed degeneration after vagotomy.

Marinesco (1904-1906) made a very comprehensive study of lesions of neurofibrils arising from a variety of causes. He described the successive phases of degeneration of the fibrils: hypertrophy, atrophy and granulation, fragmentation, and finally disappearance. In many clinical cases of myelitis, hemiplegia, etc., he found that apart from the variation of the onset and etiology the sequence of degeneration was fundamentally the same as above/ fig.12. Four and a half hours after permanent ligature of the abdominal aorta in a dog, Marinesco found the boutons shrunken, small and pale. At the same time he noticed that the intracellular neurofibrils were no longer intact. Scarpini has reported degeneration of the endocellular reticulum three hours after the circulation has been clamped off, but Tiberti, using Donaggio's method, denies any internal fibrillar change. Gozzano and Vizioli (1931) lay great emphasis on the resistance of the inner reticulum, both to toxins and pathological changes, and Gurewitsch (1908) asks how much of the recorded change is secondary to dehydration and vacuolization.

This is entirely a side issue, and throws little light on bouton degeneration. In 1906 Marinesco made further contributions from his clinical studies: "J'ai montré antérieurement la résistance des boutons terminaux envers les lesions consécutives à l'inflammation dans les cas d'anemie et envers les lesions cadavériques. Actuellement, je puis signaler un nouveau fait relatif à la pathologie des boutons terminaux; il consiste dans leur dégénérescence, leur atrophie partielle allant à leur disparition plus ou moins complète dans le tabes et l'hemiplégie, alors que le cytoplasma cellulaire est à peu près intact". Post mortem changes in neurofibrils were reported by Lache (1906). He found that from twelve to sixteen hours after death, marked changes occurred in humans, and much earlier in mice. Golgi (1906) returned to the question of degeneration of nerve endings with the information that in 'la dégénération calcaire' involving the Purkinje cells, the most distal ramifications of the cells were most affected.

The work of Achucarro (1909) on changes in the nervous system in rabies is outstanding for the meticulous technique which is shows (fig. 13). Cells were found in the olive which, although degènerating themselves, had normal 'endknopchen' on their surface.

Mott (1912) noted the extraordinary resistance to degeneration possessed by unmyelinated fibres. He discovered a biochemical change taking place in the peripheral part of a nerve process six hours after it had been severed from the cell body.

In recent years the bouton method has been revived, and Lawrentjew (1925) has applied it with excellent results to the sympathetic system. His preparations show maximal degeneration of the boutons in the superior cervical sympathetic ganglion five to six days after section of its pre-ganglionic fibres. In 1929 the same worker confirmed the discoveries of Nikolajew (1893) already referred to, and concurred that though the synaptic end-feet on the cardiac ganglion cells degenerated on section of the vagus, the cells themselves remained normal.

Cajal has referred to cases of general paralysis in which the Purkinje cells have disappeared without in any way affecting the 'corbeilles', or synapses, around them. Conversely, experiments have produced degeneration of the pericellular synaptic structures, without affecting the Purkinje cells. Reference has already been made to the degeneration experiments of De Castro and De Juan on the calices of Held (p. 12).

Karl Bauer (1932) has cited the work of Levi and his collaborators who have studied degeneration in peripheral nerve processes separated from their cell body, in tissue culture. Twelve hours following section, the distal segment resembled a 'pearl-string', whose swellings were in the process of fragmentary degeneration.

Hoff's work on bouton degeneration was first published in 1932. The stages of degeneration of the synapses in the spinal cord were recorded following posterior root section, longitudinal, and transverse sections of the cord. After twenty-four hours' degeneration the boutons were slightly

swollen and darkly granular. A thin filament with a moniliform swelling was reported in the centre of the bouton. Within forty-eight hours the boutons had become larger and almost opaque. The granular 'spatula-like' masses found at seventy-two hours have been taken by Hoff as criteria of degeneration, and his subsequent work has been with them .
At the end of four days he could be sure of complete degeneration, and at the end of six days, complete disappearance. In no cases could degenerated intracellular neurofibrils be seen. In a study of the boutons in the monkey's spinal cord, the same procedure gave similar results, the only change in technique being the injection of larger quantities of 10% chloral hydrate fixative to compensate for a larger animal. (On a recent visit to this laboratory, Dr. Laidlaw reported that some workers are now using 25% chloral hydrate for injection fixation.)

Hoff's work on monkeys has been confirmed by Foerster, Gagel and Sheehan (1933). Normal cells of 2μ by 4μ attained a maximal size of 4μ by 7μ in the stage of hypertrophic degeneration (figs. 9 a & b) .

Using a pyridine-chloral hydrate fixative, Sereni and Young (1932) observed the same phenomena of degeneration in the synapses in cephalopods (fig. 8) .
Normal boutons seemed to be of two sizes, 4.5μ by 2μ , and 6.5μ by 5μ . Most of the small boutons lying on the stellate ganglion were found by degeneration experiments to belong to the 'mantle connective'. Section of the 'stellar nerves' produced degenerative changes in their extremities in fifteen hours.

The process seemed to reach its height in twenty-four hours, and by the end of the seventh day the debris was completely cleared away. Regeneration took place at 7μ to 18μ per hour. Cutting the 'mantle connective' produced bouton changes in thirteen hours. Not all the boutons on the dendrites of the stellate ganglion degenerated, indicating that more than one system focussed its projections on the ganglion. This afforded an opportunity for comparing the swollen synapses with the normals. Prompt regeneration could be seen, a few fibres even developing in the reverse (cellulopetal) direction. (cf. Speidel, 1933). Measurements of 'degeneration time' showed that it varied inversely as the temperature of the water in which the cephalopods were kept.

The latest publication of Lawrentjew (1934) considers the boutons to be constant structures in the synapses of the autonomic nervous system (fig. 14). As a general rule, degeneration is amply visible six to eight days following axonal section. The first statement is sustained by the excellent work of Kolossow, appearing in Cajal's 'Travaux', 1932-33. The Gross-Bielschowsky technique has been used to demonstrate synaptic degeneration in the cervical sympathetic ganglia, the semilunar and inferior mesenteric and heart ganglia, and the ganglia of the intestinal tract, the genito-urinary tract, and the suprarenal plexus (fig. 16). In every case, contact rather than continuity has been reported.

The question might well be asked, "How can degeneration of experimental origin be differentiated from post-mortem changes in the synapse?". Hoff (1934) has shown that aseptic

removal of a portion of the spinal cord, followed by incubation at 37°C for twenty-four hours in sterile saline solution, produces a granular destruction without swelling of the boutons. This condition can be positively distinguished from the hypertrophy and increased silver affinity seen in bouton degeneration following an operative lesion.

The phenomenon of so-called 'transneuronal degeneration' has recently reappeared, and in view of its relevance to bouton degeneration we may digress briefly to examine the last reports. Sherrington (1932) tells of finding chromatolysis in a number of the ventral horn cells of the fifth lumbar segment of a monkey whose spinal cord had been sectioned at the level of the eighth thoracic vertebra thirteen days previously. Having ruled out any possible myelitis he states: "In a number of the cells the perikaryon has lost its normal Nissl bodies to a large extent, a great deal of it has become hyaline, or even vacuolated in appearance; the nucleus has become displaced and eccentric, even so as to lie quite to one side". The axones of the affected cells did not exhibit Wallerian degeneration, but there was evidence of functional change.

Niessl von Mayendorf (1934) has written of supposed 'transneuronal degeneration' found in three cases of 'softening of the brain', aged ninety years, eighty years, and fifty-three years. With much fanciful speculation he propounds "une nouvelle conception du neuróne" which concludes: "Le neurone n'est donc pas une cellule avec ses prolongements, mais une chaine de cellules dont le centre trophique est l'écorce cérébrale".

Those who oppose the neurone theory seize upon cases of 'transneuronal degeneration' as evidence in favour of the continuity of the nervous system. They have been well answered by Cajal (1934, p.134): "En réalité tous les elements nerveux en connexion intime souffrent par suite de la lésion des corpuscules associés dynamiquement, et qu'au bout de mois ou d'années ils peuvent tomber en atrophie et en dégénération par désuétude, à condition de ne point posseder d'autres connexions capables d'entretenir l'activité fonctionelle".

Conclusions based on central chromatolysis must be confirmed when the whole phenomenon is better understood. Minea (1934) has recently discovered that no chromatolysis of importance can be demonstrated in a cell whose axone has been severed, provided that a local anaesthetic is applied to the cut end. It is unfortunate that such a comprehensive term as 'transneuronal degeneration' has been applied to a process so little understood.

One would be justified in concluding from the literature that histological and pathological data are unequivocally in support of the neurone theory.

(c) Physiology.

"Each vesicle, or each portion of grey matter that establishes a continuity between the central termini of fibres, is not merely a connecting link: it is also a reservoir of molecular motion which it gives out when disturbed." Herbert Spencer.

Until recently, the emphasis in the study of synapses has been on the functional rather than on the structural side. Nerve-muscle preparations offered more interesting opportunities for observation to the physiological than did the microscope and histological preparation. This may be attributed in part to the inaccessibility of the synapses of the central nervous system, a situation which, despite improved methods of experimental neurosurgery, still exists.

Since the time of Marshall Hall, it has been recognized that while a nerve fibre may conduct an impulse in either direction, a reflex arc can only transmit in one direction. We know that stimulation of a nerve fibre gives an instant response, which ceases immediately the stimulus is removed. The reflex arc, however, is characterized by inertia which manifests itself in the time lag following a stimulus and the carry-over after stimulation is stopped. Reflex arcs are many times more susceptible to fatigue and to the action of drugs than are peripheral nerve fibres. Years of observation in both frog and mammalian laboratories have given us information on the phenomena of summation, reinforcement, inhibition and general functional properties of reflex arcs, without actually defining in more than abstract terms the structural basis of conduction across synapses.

At a time when biochemical and histological methods of investigation were coming to be applied to the nervous system, Scott (1905) formulated the following conception of 'neurodynamics': "Just as in ferment-forming cells we have nucleus, prozymogen and zymogen, so in nerve cells we have nucleus, pro- or Nissl substance and neurosomes. The process of excitation, like that of secretion, involves, I believe, the discharge of neurosomes in the region of the synapse. Since discharge in other cells means the using up of formed material, it must be an exhaustible process, and the process of complete recovery at the synapse must depend on the integrity of the connection of the synapse with the nucleus and cell body which are the original seats of formation of the material involved in the activity". Apropos of Scott's statement concerning the neurosomes in synaptic action, one should recall Held's discoveries of the neurosomes in the axone, and especially in nerve endings in muscle. Bartelmez (1933) has described a special arrangement of these mitochondria at the zone of interneuronal transfer.

Physical chemists have made great strides in an attempted explanation of the propagation of nerve impulses. Nearly all agree that in the synapse we are dealing with surface phenomena associated with the limiting membrane of the terminal feet, with the intermediary substance, and with the surface of the dendrites and body of the second cell. That these layers contain the secret of polarization in the reflex arc is evident from the work of Sybil Cooper (1929): "When two

afferent paths converge to cause excitation of the same motor unit each path is separated by an irreversibly conducting junction from that unit and from the other path. It is obvious from our results that afferent paths of equivalent sign do not join a neuropile net with conduction in all directions, because the impulse from A would then traverse not only the network termination of B but would traverse B itself, and render a stimulus to B ineffective". In summarizing the biophysical properties of the synapse, Sherrington has referred to the "surface of separation" which may "restrain diffusion, back up osmotic pressure, restrict the movement of ions, accumulate electric changes, support a double electric layer, alter in shape and surface tension with changes in difference of potential, alter in difference of potential with changes in surface tension or shape, or intervene as a membrane between dilute solutions of electrolytes of different concentration or colloidal suspensions with different sign of charge".

The fundamental property of a nerve impulse is its "conducted tendency to excite" (Davis, 1926). It is taken to be an energy liberation by the fibre itself. Lillie has associated each impulse with a discharge of ions, and Adrian has aptly described it as "a little patch of surface leakage spreading along the fibre and being sealed up again as soon as it has formed". It must be evident that the nerve impulse itself is only the messenger in the energy transaction, being infinitesmal in size compared with the energy released; and yet it is the mediation and correlation of these delicately graded impulses or currents which must take place in the synapse if muscular coordination throughout the body is to be attained.

The recent histological studies of the boutons terminaux by Hoff, and by Lorente de Nó have raised the question: "What is their function?". Creed (1931) writes: "The function of these synaptic connections in grey matter has been little discussed; they appear frequently to be regarded as no more than relay stations in which impulses are transferred to a new set of nerve fibres. A moment's reflection shows that such a view is totally inadequate and provides no explanation of their existence". Liddell(1934) replies that the boutons are the "agents of liaison" in the transmission of excitatory and inhibitory impulses at interneuronal connections: "The mechanism of excitation is believed to be concerned with the generation of a point of depolarization on the neurone's surface underneath each bouton. The area of surface in apposition to a bouton is the 'synapse', whose activities form the characteristics of central nervous action".

The boutons are now considered to be the active agents in the 'motoneurone pool' of Sherrington's 'central excitatory state'./ fig. 18. The activity of boutons is analogous to the effect produced by throwing stones into a pool. A single stone produces but a transitory ripple, but if enough stones are thrown in, in sufficiently rapid succession over a period, a commotion will be produced which may cause the water to exceed the limits of the pool. In the same way, if a sufficient proportion of the three hundred to three hundred and fifty boutons ending on a ventral horn cell fire together or in rapid succession, the 'central excitatory state' will exceed the 'threshold', and the neurone will discharge. A further simile has been used,

that of breathing on a mirror to produce droplets of water. The moisture condensed from a single breath will evaporate if left long enough, but if one breathes often enough, each will augment the last, resulting in the production of large drops of water. Sub-liminal stimuli may be augmented if enough of the boutons on a cell discharge sufficiently frequently. Hence, the properties of 'temporal' and 'spatial' summation can be accounted for in this theory of bouton function. Concerning the temporal and spatial nature of integration, it is interesting to record Hughlings Jackson's theory (1870): "Co-ordination in Space -- the power of using several muscles together for one purpose -- is brought about by groupings of fibres. Co-ordination in Time -- the process by which one movement follows another -- is brought about by relations betwixt ganglion cells".

Liddell suggests that it is unfair to assume that when a neurone is not discharging it is entirely at rest. He believes that the cell is constantly on the 'qui vive', and that dispersed localities of depolarization on its surface are occurring and recurring, but still below the threshold value. It then requires only a limited further depolarization to discharge completely. However, if by section of the spinal cord, by fatigue, anaesthesia or limiting of the blood supply, we reduce the condition of 'qui vive' in a cell, we must increase the number of bouton discharges to a much higher level than before in order to produce a discharge of the neurone. This state is analogous to 'spinal shock' or 'diaschisis' which Liddell attributes to the "passive withdrawal of excitation". Strychnine

would increase the sense of 'qui vive' in the neurone. 'Inhibitory boutons' are hypothecated as agents in the 'central inhibitory state'.

This theory, put forward by the Oxford group (see Creed et al, 1932) with an unparalleled experience in animal observation, throws light on the phenomenon of nervous integration, but not new light. For it was Hughlings Jackson who said nervous coordination was "based on the anterior horn cell". This "Argus-eyes observer with a passion for detail and a brooding intelligence" came to the conclusion that "not only had the nervous system been evolved from the primitive nervous system of the lower vertebrates by the addition of new parts, but he perceived physiological and phylogenetic differences in the various levels of the nervous system so constructed" (Foster Kennedy, 1935).

In 1920 Henry Head wrote: "Integration of function within the nervous system is based on a struggle for expression between many potentially different physiological activities.... Such integration is the task of the central nervous system and it is carried out by means of a series of receptors which guard certain synaptic junctions on the centripetal paths. There are in reality end-organs, exposed to the influence of the complex mass of afferent impulses generated in the peripheral nervous system by the impact of an external stimulus. They are not influenced directly by the forces of the world around; they react to the diverse physiological conditions produced by the action of physical stimuli on the receptive mechanism at the periphery. Like resonators placed in a concert hall each group picks up

those qualities to which it is attuned, and refuses those with which it is not in harmony. This process is repeated throughout the central nervous system until the final products of integration come to ..... excite those conditions which underlie the more discriminative or more affective aspects of sensations".

An excellent presentation of the present theory of integration is to be found in "Reflex Activity of the Spinal Cord" (Creed et al, 1932). Here Sherrington and his collaborators have correlated observations of function and structure in a way which advances the explanation for the integration of impulses one step further. "One neurone is usually connected functionally with a considerable number of others next 'downstream' to it, while these latter are themselves each subject to the influence of several 'upstream' neurones. Here we find the anatomical basis (a) for the experimental finding that a reflex response is rarely if ever confined to a single effector unit, and (b) for the principle of convergence of many afferent arcs on a single 'final common path'."

While no exact anatomical study has been made concerning the various 'families' of afferent impulses which converge on the ventral horn cell, we may deduce from Hoff's work (1934 b) that there are several. Lorente de Nó has advanced the idea that what we have called 'families' of boutons are grouped on a particular part of the cell body. Although he accepts Sherrington's conception of a 'central excitatory state' he cannot agree that the 'refractory state' concerns the cell body and its dendrites, but rather the axone.

Sherrington's statement of "overlapping" (fig. has been accepted by Lorente de Nó, and the arrangement of boutons on the perikaryon may prove to be the basis for it. Lorente de Nó further believes that this "partially shifted overlapping" is seen in every part of the central nervous system. The arrangement of boutons in the synapse may yet prove to be the anatomical counterpart of the vicarization of function so familiar to the neurosurgeon.

The structural basis underlying the 'central inhibitory state' has not been clarified, and some writers believe that we shall not find the answer in the synapse itself. Barron and Matthews (1935) working on cats and frogs have discovered that, in passing along a single fibre in the posterior columns, a rhythmic impulse is changed to an intermittent one. Their explanation is that the collaterals which such a fibre possesses are intermittently active, and produce an electrotonic block for short recurring periods in the main conducting path. They conclude: "Our results show that inhibition of conduction may occur commonly in the central nervous system at points distant from a synapse, and without the intervention of a synapse in the path in which the impulses are blocked. Moreover, they give direct evidence that what passes up the fibres of the spinal cord is far from being a simple sensory discharge from peripheral receptors, and that even in the posterior columns the messages are integrated to some extent inasmuch as the impulses passing up any single fibre may be modified by changes not affecting the receptor from which they come".

Fulton in 1926 substituted the term 'central excitatory substance' for Sherrington's 'central excitatory state'. Dale and his group in London have recently given experimental proof of at least a part of the theory of chemical mediation of nerve impulses, which has been proposed since the time when the nerves of the body were thought to be tubes transporting excitatory fluids. It is reasonable to expect that the central nervous system should have chemical transmitters similar in principle to those of the ? nervous system.

The superior cervical ganglion offers the most accessible field in which to study humoral transmission in synaptic structures. Kibjakow (1933) has studied this ganglion in cats, and concludes: "Wir glauben dass die funktionellen Eigenschaften einer Synapse des Zentralnervensystems ihre erschopfendste Erklärung finden bei Voraussetzung humoraler Übertragung der Erregung von Neuron auf Neuron".

Feldberg and Vartiainen (1934) have carried on perfusion experiments on the superior cervical sympathetic ganglion in cats with great success. Using warm oxygenated Locke's solution with a very weak concentration of eserine, as a sensitizer, the ganglion has been perfused while the preganglionic fibres were stimulated. Acetylcholine has been recovered in all cases, and it has been calculated on this basis that, for this ganglion, a single impulse at a single synapse liberates 10^{-15} grams of acetylcholine, or roughly three million molecules. Working with Brown, Feldberg (1935) has been able to demonstrate the excitatory effect of KCl on

sympathetic ganglia, with the production of acetylcholine in the nerve terminations. The amount of acetylcholine liberated increases as the concentration of the KCl up to a point, after which a decrease and a reversible paralysis of the ganglion is evident. Foster Kennedy (1935a) has referred to the many allergic manifestations in the nervous system, and it may well be that these arise from changes in the chemical transmitters or the content of the blood supply of the sympathetic ganglia.

Summary.

After this review of the literature on interneuronal connections, embracing their embryology, histology and physiology, we may conclude with Lewellys F. Barker: "As his neurones are, so the man is", and we might even say: "As his boutons are, so the man is".

Thirty years ago Lache wrote: "L'étude des neurofibrilles ouvre de nouvelles prospectives à l'horizon lumineux de la neurologie". The same could be said today. A discussion of the literature will be found in the general discussion which follows the section on experimental results.

Strangely enough, the work still to be done was envisaged half a century ago, even before the literature here reviewed was written or contemplated. "To define all these various organs with accuracy, to determine their intimate structure as well as their individual energy, and to trace the physiological and pathological alterations which they undergo during the natural processes of development, maturity, and decay, and in diseases to which they are subject, is the greatest problem for the anatomy and physiology of the 20th.

century; and when it is solved, a complete revolution in psychology must result." Julius Althaus, 1880.

EXPERIMENTAL PROCEDURE.

The experimental work was concerned with both normal and degenerating boutons terminaux.

(1) Staining normal boutons terminaux.

The first attempt to stain these synaptic end-feet was made in the spinal cord of the cat. The procedure as outlined by Hoff was successfully used here and was later applied to the cerebellar boutons with rather good results. The following was found to be the most consistent and satisfactory method, equally applicable to either spinal cord or cerebellum. Normal cats of 2.0 to 2.5 kgms. were used in every instance. Each animal was placed under ether anaesthesia and a longitudinal incision was made in the abdominal wall. A small canula was inserted into the abdominal aorta just above its bifurcation. All branches of the aorta supplying abdominal viscera were clamped. Special care was taken to 'shell out' the kidneys from their serous investment, to insure complete obstruction of the renal vessels by forceps. After the brachial arteries had been clamped two hundred and fifty cubic centimetres of Ringer's solution was slowly injected through the canula which was securely placed in the aorta. When the injection was well under way the inferior vena cava was incised allowing clear fluid to escape from it. Three hundred cubic centimetres of 10% chloral hydrate followed the Ringer's solution immediately and gradual rigor could be seen as the injection progressed.

With the injection completed the central nervous system was quickly removed and placed in a large excess of 10% chloral hydrate for twenty-four hours. A more complete fixation occurred when the cord had been cut into small blocks not more than the length of one spinal cord segment. The staining technique used was Cajal's 6A reduced silver method as in Bolles Lee, etc. The blocks were kept in 95% alcohol with seven drops of ammonia per 100 cc. for twenty-four hours. They were then blotted dry and individually wrapped in a small piece of filter paper and immersed in a 2% silver nitrate solution for seven days at 37°C. Good results were obtained by adding a small quantity of 95% alcohol to the silver bath and renewing the silver solution after four days. Fifty cc. of the 2% silver nitrate was found to be sufficient for three small blocks of spinal cord tissue. Following the silver the pieces were unwrapped and thoroughly washed in distilled water. An aqueous solution of 2% hydroquinone or 2% pyrogallic acid was used to reduce the specimens over a period of twenty-four to forty-eight hours. The blocks were washed, dehydrated and imbedded in paraffin. Serial sections at 15μ were found to be the best for microscopic analysis.

The failure of Cajal's method No. 6A to stain cerebral boutons terminaux in this laboratory necessitated other procedures.

1. CAJAL'S MODIFICATION FOR FROZEN SECTIONS (McClung's Handbook of Microscopical Technique, page 333, 1929) was first resorted to as a very selective stain for nerve fibres. The following use of the technique proved to be very successful

in cerebellar sections and was the first one to stain the boutons terminaux in the cerebrum, although only scantily.
The material is fixed in 20% formalin for three days or more.
Frozen sections are cut at 30 to 40μ.
Wash for several minutes in distilled water.
Place sections from four to six hours in the following: Silver nitrate 2%, 12 cc.; pyridine, pure (e.g. Kahlbaum) 7 to 10 drops; alcohol 97%, 5 to 6 cc. The sections became light brown in colour. This took place at room temperature and was still more rapid in the oven. Sections thus stained for twenty-four hours or more gave excellent results.
Place in absolute alcohol for three minutes.
Reduce in the following for three minutes: hydroquinone 0.30 gms.; distilled water 70 cc.; formalin 20 cc.; acetone 15 cc.
Wash in a large quantity of distilled water.
The sections were toned to advantage in a solution of gold chloride, one part to five hundred of water. After fifteen minutes in the gold they were fixed for one-half minute in a 5% solution of sodium hyposulphite. They were then washed, mounted on slides and dried carefully with filter paper. Dehydration in the alcohol and zylol or toluol followed.
Mounting was done in balsam.

2. After examining a slide stained by Rio-Hortega with 'double impregnation technique', it was decided to apply this procedure to both cerebellum and cerebrum. The following conditions, when carefully fulfilled, gave the most consistent and clear-cut pictures of the boutons in the brain.
The tissue is thoroughly fixed in formaldehyde.

Frozen sections are cut at 30μ and washed well.
Two per cent silver nitrate from twenty to twenty-four hours is necessary.
The sections are then washed in distilled water. They are placed in small thin pyrex dishes containing about 15 cc. of silver carbonate made according to the following directions: 5 cc. of 10% reagent primary standard silver nitrate is added to 15 cc. of 5% sodium carbonate and the mixture made up to 75 cc. Just enough ammonia is added to dissolve the precipitate. To each 15 cc. of this liquid used there is added five drops of pyridine. A single section is then placed in a small pyrex dish and heated to 45°C. at which time it usually comes to have a tobacco colour. After a thorough washing in distilled water, one of the following processes is used: (a) Wash and tone in gold; or (b) Reduce in 10% formalin, wash and tone in gold; or (c) Place in 50% alcohol one-half minute, then 95% alcohol one minute with slight heat. Reduce in 1% formalin. Wash and tone in gold.

(2) Staining degenerated spinal cord boutons.

A series of five cats was used in this investigation. Females of approximately two kilograms each were selected in order that the urine might be more easily expressed during the post-operative period. Two of the subjects had ether anaesthetic, while the other three were given intraperitoneally 0.5 cc. of "Dial" per kilogram of body weight. Using aseptic technique laminectomy was performed at the level of the first lumbar vertebra, the dura being exposed and the bleeding in the bone being stopped with bone wax. A similar exposure was

was made between the eighth and tenth thoracic vertebrae. In the lower exposure the dura was excised lateral to the dorsal spinal artery and reflected for 1 to 2 cms. An aneurysm needle carrying silk thread was passed around the cord intradurally. With slight tension on the thread, the cord was completely sectioned with a pair of fine scissors. The same procedure was carried out on the cord at the thoracic level already prepared, Bleeding was thus minimized and a comparatively dry field was the result.

The post-operative course of the animals was uneventful. All were given milk the first day, the cats which had been anaesthetized with ether being more alert. In all cases there was paralysis of the hind quarters and retention of urine.

The sacrifice of the cats was essentially the same as that described in the previous section. The tissue blocks were stained with Cajal's 6A technique, as previously described, and serial sections at 15μ were mounted on large slides for examination.

the method of sacrifice

EXPERIMENTAL RESULTS.

Normal boutons terminaux were seen to best advantage on the ventral horn cells of the spinal cord, where they appeared as smooth, ring-like, fibrillar structures, closely applied to the surface of the cell body or dendrites. In each case a bouton was attached to its specific neurofibril. Even in the most completely stained specimens, no fibrillar passage from the bouton to the intracellular fibrils of the ventral horn cell's body could be seen. The boutons terminaux were usually regularly spaced on a cell, whereas the boutons de passage had little or no orderly arrangement. Fig. 20 shows a typical arrangement of terminals on a large ventral horn cell, while Fig. 19 demonstrates their loop-like structure, a direct continuation of the fibril from the axis cylinder. Normal boutons are well contrasted with pathological in Figs. 24, 25 and 29. The presence of these two types on one cell indicates the variety in location of their cells of origin.

A microscopic examination of the boutons on ventral and dorsal horn cells in the serial sections revealed a sequence of degenerative modifications analogous to that described by Cajal (1928) for the peripheral stumps of sectioned nerve processes. Boutons which had been separated from their cell body for twenty-four hours showed a uniform swelling (Fig. 29). Their diameter increased from 2 to 2.4 on the average, and the loop-like appearance was gradually lost. This stage is an early form of what Cajal has aptly described as "premonitory of fragmentation".

After forty-eight hours, the degenerating bouton had lost its central hollow, and its circular configuration. It was characterized by irregular varicosities, which increased its breadth from 2.4 to 2.8 . In common with its adjacent neurofibrillar tissue the degenerating bouton showed an increased affinity for the silver salts.

The seventy-two hours stage proved to be one of the most interesting, both in shape and size. The transformation from a disc-like to a 'spatula-like' shape resulted in a noticeable increase in length, and also in area. This transition was usually very sharp and afforded unmistakable evidence and a good criterion of degeneration. The bouton at this stage still preserved the homogeneity of structure and stain already seen in the earlier stages.

By ninety-six hours, the bouton had become granular and increased in length and width. The relatively smooth contour was disrupted by large granules, ready to break away. This was the last stage in which it could be said that the bouton was an intact structure.

The changes seen after one hundred and twenty hours varied between rather wide limits, depending on the location of the cell studied relative to the cord section. The characteristic of this stage was the fragmentation of the formerly solid black mass. In some only the periphery had begun to break up, while in others clefts could be seen in the central part. More advanced still were those phases in which the body was completely disrupted. There was a considerable tendency

towards the circular shape again, though the increased diameter could be partly attributed to the interstices between the segments.

Finally, there occurred the familiar granular disintegration and total fragmentation of these structures. In a few cases the semblance of a bouton shape could be seen, but usually the granules were more widely dispersed and not easily recognizable.

As can be seen from Fig. 29, an average degenerating bouton passes through the processes of hypertrophy, elongation, fragmentation and granulation and from the circular to the ellipsoid and back to the circular shape.

DISCUSSION.

The fact that normal boutons terminaux have been stained in the spinal cord, cerebrum and cerebellum suggests that boutons may be the most typical endings of axis cylinders upon nerve cell bodies and processes. Their existence in the autonomic nervous system has already been cited, though there is no certainty that other less specific forms of terminals do not occur as well. In some preparations normal boutons of varying sizes have been seen in the same section. In every case, however, there has been no ground for confusing them with enlarged degenerating structures. In many sections studied, it would seem that the degenerating boutons and neurofibrils exhibited an increased affinity for silver.

In no case could it be said that a cell body on which degenerating bouton terminals were clustered was modified in any particular; nor could neurofibrillar continuity across synaptic surfaces be seen. In some instances, there were from twenty to thirty boutons visible at one level on one horn cell, which compares favourably with Hoff's estimate of three hundred to three hundred and fifty per ventral horn cell's total surface. The central filament with a small 'moniliform' swelling, described by Hoff, could not be seen in the centre of any bouton, normal or pathological. Concerning the experiments on degenerated boutons in the spinal cord, it should be clearly stated that the bouton method, as a method, is subject to the vagaries of all types of silver staining. It is reasonable to expect that the boutons terminaux undergo the changes described, whenever their axone is interrupted; but it would be unfair to assume that, because abnormal boutons were not stained by the silver method, no degenerative changes had taken place. In most cases it is impossible to stain more than a small proportion of the degenerated boutons around a cell, but those which do appear are usually well defined.

The interpretation of the present work is based on the assumptions (a) that boutons terminaux occurring in the dorsal, ventral and lateral horns are normally of the same size; and (b) that the terminal arborizations of an axis cylinder degenerate in one and the same manner, regardless of the finer architecture of their axone or the location of their cell of origin, whether it be a pyramidal or an intercalated cell.

While a discussion of the many hypotheses extracted from the literature would be impossible, it may be useful to consider the more enigmatic elements in the whole question of interneuronal connections. This is here attempted in a short treatment of the neurofibril theory together with a number of questions and suggested experiments.

An increasing amount of evidence forces one to consider the possibility that the individual neurofibril may be the functional unit of the nervous system. Barron and Matthews (1935) have described the strongly determinative influence of collaterals on their central nerve fibre, which suggests that in the final analysis the neurone acts only as an expression of its constituent neurofibrils. Such an hypothesis would predicate levels of integration from the single neurofibril to groups of neurofibrils, to a neurone, to combinations of neurones, to reflexes, to chains of reflexes and the sorting out of reflexes to the final common pathways of motor effector units of conduct.

No author has reported two or more neurofibrils ending on a single bouton, a fact that adds weight to the theory of the specificity of a single bouton for one neurofibril.

The question of the autonomous nature of each intracellular neurofibril is illuminated by Cowdry's statement (1932) that two neighbouring dendrites may not have the same physiological properties, a phenomenon which he calls 'selective irritability'.

The suggestion by Parker and others that neurofibrils are nutritive in function overlooks the fact that the axone is an example of closely packed neurofibrils, and at the same time is the centre of catabolic rather than anabolic processes. Carbon dioxide is given off, increasing with excitation and activity. The metabolism of nerve fibres is but one seventy-fifth that of grey matter (Gerard, 1931).

Kappers (1932) believes neurofibrils are the products of conduction. He has described their development around the diplosomes of the axone growth cone. The following statement (Kappers, 1934) throws some light on the problem of the independent functioning of a neurofibril within a neurone: "I refer here to the case that an axone frequently divides, its branches being connected with various muscles. In such a case a reflex, caused by the stimulation of the skin and reaching the motor cell from which all the branches originate, may cause a contraction of one muscle only. This point has been brought out by the experiments of Weiss". Concerning the presence of neurofibrils, Kappers has said (1932): "Although the neurofibrils are visible in living axones (De Renyi, 1929), the micellae definition - protein molecular aggregates that constitute them have hitherto escaped observation. Still their existence, be it in an ultravisible form, is made more probable since Heringa has demonstrated the micellae of connective tissue fibrils".

Gerard (1931) described experiments in which the principle of using heat production as an index of molecular volume involved in a metabolic reaction was applied to the

nervous system. In the case of conducted nerve impulses it has been found that the heat produced is indicative of a very small molecular volume having been affected, one twenty-thousandth of the area relation of the axolemma, and more in keeping with the surface measurements of a few neurofibrils, which may have been concerned in the transmission.

Gerard cites Erlanger, Bishop and Gasser (Am. J. Physiol., 78:574, 1926) in connection with the discovery that an impulse travelling up a sensory nerve is delayed 0.00014 second in passing the spinal ganglion. The crude sensory nerve fibre to all appearances has a T-shaped connection with the dorsal root ganglion. Therefore an impulse entering the ganglion would have to wait until the refractory period in the stem of the T was over before retracing the other 6 mm. of the 'detour' to return to the main stream, and thence into the cord. If the impulse had been transported by an individual neurofibril no conflict nor refractory period would have ensued. The impulse would then have travelled up the neurofibril, through the stem of the T, around the nucleus, back out the stem and on into the spinal cord. This hypothesis obviates the necessity of considering any possible refractory period, a most unlikely occurrence in such a small total delay as 0.00014 second. Bethe in 1898 and Steinbach in 1899 cut off the dorsal root ganglion in various lower forms and claimed to have found that conduction still occurred from receptor to spinal cord.

If it could be shown that the size of an axis cylinder is directly proportional to the number of contained neurofibrils,

a great deal of evidence could be brought in favour of the neurofibril as the unit of conduction, for axis cylinder diameter is in a general way related to conduction, notably in the large motor trunks.

It is an interesting phenomenon that axis cylinders should be constricted at the nodes of Ranvier, while the intracellular neurofibrils remain unchanged. Bethe (1903) claimed that the peripheral substance of a nerve fibre could be compressed down to one six-hundredth of its normal cross-section and yet conduction was possible as long as the neurofibrils were intact and undamaged.

The possibility of two types of transmission in the nervous system is strengthened by the finding that a nerve fibre begins activity within 0.0005 second after a stimulus is applied and ceases to function immediately the stimulus is removed. On the other hand, reflex firing begins slowly and continues for several seconds after stimulation stops.

Sixty years ago Max Schultze (cited by Parker, 1929) speculated on the specific function of the neurofibrils in the large ganglion cells of the Torpedo: "We may regard such a ganglion cell from which a peripherally directed nerve fibre proceeds, as representing the source and origin of this axis cylinder, but only in the sense that the fibrils which compose the axis cylinder are collected into a group from the arborescent processes of the cell; and thus the fibrils which are seen traversing the substance of the ganglion cell do not originate in the cell, but only undergo a kind of arrangement in it, and then pass to the axis-cylinder process, or extend

into the other branched processes". "On physiological ground also I maintain the possibility of isolated conduction in these constituent fibrils, even when no trace of interfibrillar substance is present."

It might be an appropriate place to put forward some questions which require answers, if future work in the field of interneuronal connections and the theory of neurofibrillar function is to be relevant and thereby valuable.

Why are there no boutons on the cone of origin of axis cylinders or on the axone itself? (See Cajal & Van Gehuchten)

Is this absence of boutons due to dynamic polarization of the cell, or to a lack of chromidial substance?

What purpose is served by a preponderance of potassium in the axis cylinder compared with the dendrites, and why is there no oxydase in axones?

What protective or insulating sheath has an axone as it leaves a nerve cell body, and how far along the axone does it extend?

Do dendrites end by means of boutons?

Does 'dynamic polarization' demand that the intracellular neurofibrils entering the cell <u>via</u> the dendrites must all leave the cell body <u>via</u> the axone?

What is the origin of intracellular neurofibrils which we see in the axis cylinder if they do not come from the dendrites?

How do boutons ending on dendrites contribute to the 'motoneuron pool'?

Do they augment the perhaps faint stimulus passing through the dendrite, or do they discharge minutely but over a considerable period of time, causing a constant current or a constant surface depolarization which prevents the passage of the stimulus down the dendrite?

The work of Bozler (as described by Parker, 1929) has suggested some experiments which might well be attempted in furthering our knowledge of neurofibrillar connections and functions. He found that by immersing the circular nerve band of the jellyfish Rhizostoma in hypertonic saline he could produce a herniation of a few individual neurofibrils through the fibre sheath. This could be repeated, with a view to cutting across the few neurofibrils thus isolated, and the synaptic degeneration resulting could be studied as an indicator of fibrillar connections. The ideal experimental animal would be one in which a simple, large synapse could be easily approached and studied, and its constituent fibril endings delineated.

A problem confronting any histologist studying boutons is whether or not cutting an axone close to its bouton termination gives a different picture of degeneration than cutting the axone at a considerable distance from its boutons. An answer to this question would be necessary before one could proceed to a classification of the families of boutons ending on anterior horn cells of the spinal cord. Such a classification might be carried out by removing different cortical areas on a series of cats, or confining the extirpations to a single cat, with definite and recognizable time intervals between.

The literature is still incomplete on such subjects as bouton changes in anemia, anaesthesia, trauma, heat, drugs, poisons, fatigue, and under continued electrical stimulation.

It might be practicable to use an ultra-centrifuge and a tissue containing a simple synapse from a large nerve cell, to force the nuclear elements of the cell to one end with such force that the intracellular neurofibrils would be disturbed, if not broken. Heavy intra vitam nuclear staining might aid in the force of the nuclear displacement.

Cowdry has suggested the value of the micro-incineration technique of Policard and Okkel (Anat. Rec., 44:349) as a means of determining chemical changes under different functional conditions in tissues in general, and its use in investigating chemical changes at the synapse might prove to be of value.

An account of the actual extent of the myelin sheath on nerve cell bodies and processes is very badly needed. Bartelmez (1933) has found some interesting facts about the protective covering of nerve fibres, chiefly its fading out as the site of synapse is approached. Only a technique which will stain protective coverings from the cell body to the last outposts, axonic and dendritic, will clear up the question of the convergence and divergence of neurofibrils, and nerve fibre branchings.

Recent work on the chemical phenomena associated with synaptic transmission (see Feldberg, J.Physiol., 1934) has suggested the possibility that inhibition is produced by

the excretion or injection into the synaptic region of an agent which immediately raises the threshold; and conversely that excitation may be attributed to a chemical promoter. The histology and chemistry of the synapse are inseparable.

DISCUSSION.

The fact that normal boutons terminaux have been stained in the spinal cord, cerebrum and cerebellum suggests that boutons may be the most typical endings of axis cylinders upon nerve cell bodies and processes. Their existence in the autonomic nervous system has already been cited, though there is no certainty that other less specific forms of terminals do not occur as well. In some preparations normal boutons of varying sizes have been seen in the same section. In every case, however, there has been no ground for confusing them with enlarged degenerating structures. In many sections studied, it would seem that the degenerating boutons and neurofibrils exhibited an increased affinity for silver.

In no case could it be said that a cell body on which degenerating bouton terminals were clustered was modified in any particular; nor could neurofibrillar continuity across synaptic surfaces be seen. In some instances, there were from 20 to 30 boutons visible at one level on one horn cell, which compares favourably with Hoff's estimate of three hundred to three hundred and fifty per ventral horn cell's total surface. The central filament with a small "moniliform" swelling, described by Hoff, could not be seen in the centre of any bouton, normal or pathological. Concerning the experiments on degenerated boutons in the spinal cord, it should be clearly stated that the bouton method, as a method, is subject to the vagaries of all types of silver

staining. It is reasonable to expect that the boutons terminaux undergo the changes described, whenever their axone is interrupted; but it would be unfair to assume that because abnormal boutons were not stained by the silver method no degenerative changes had taken place. In most cases it is impossible to stain more than a small proportion of the degenerated boutons around a cell, but those which do appear are usually well defined.

The interpretation of the present work is based on the assumptions (a) that boutons terminaux, occurring in the dorsal, ventral and lateral horns, are normally of the same size; and (b) that the terminal arborizations of an axis cylinder degenerate in one and the same manner, regardless of the finer architecture of their axone or the location of their cell of origin, whether it be a pyramidal or an intercalated cell.

While a discussion of the many hypotheses extracted from the literature would be impossible, it may be useful to consider the more enigmatic elements in the whole question of interneuronal connections. This is here attempted in a short treatment of the neurofibril theory together with a number of questions and suggested experiments.

An increasing amount of evidence forces one to consider the possibility that the individual neurofibril may be the functional unit of the nervous system. Barron and Matthews (1935) have described the strongly determinative influence of collaterals on their central nerve fibre, which

suggests that in the final analysis the neurone acts only as an expression of its constituent neurofibrils. Such an hypothesis would predicate levels of integration from the single neurofibril to groups of neurofibrils, to a neurone, to combinations of neurones, to reflexes, to chains of reflexes and the sorting out of reflexes to the final common pathways of motor effector units of conduct.

No author has reported two or more neurofibrils ending on a single bouton, a fact that adds weight to the theory of the specificity of a single bouton for one neurofibril.

The question of the autonomous nature of each intracellular neurofibril is illuminated by Cowdry's statement (1932) that two neighbouring dendrites may not have the same physiological properties, a phenomenon which he calls "selective irritability".

The suggestion by Parker and others that neurofibrils are nutritive in function overlooks the fact that the axone is an example of closely packed neurofibrils, and at the same time is the centre of catabolic rather than anabolic processes. Carbon dioxide is given off, increasing with excitation and activity. The metabolism of nerve fibres is but one seventy-fifth that of grey matter (Gerard, 1931).

Kappers (1932) believes neurofibrils are the products of conduction. He has described their development around the diplosomes of the axone growth cone. The following statement (Kappers, 1934) throws some light on the problem of the

independent functioning of a neurofibril within a neurone: "I refer here to the case that an axone frequently divides, its branches being connected with various muscles. In such a case a reflex, caused by the stimulation of the skin and reaching the motor cell from which all the branches originate, may cause a contraction of one muscle only. This point has been brought out by the experiments of Weiss". Concerning the presence of neurofibrils Kappers has said (1932): "Although the neurofibrils are visible in living axones (De Renyi, 1929) the micellae (definition = protein molecular aggregates) that constitute them have hitherto escaped observation. Still their existence, be it in an ultravisible form, is made more probable since Heringa has demonstrated the micellae of connective tissue fibrils".

Gerard (1931) described experiments in which the principle of using heat production as an index of molecular volume involved in a metabolic reaction was applied to the nervous system. In the case of conducted nerve impulses it has been found that the heat produced is indicative of a very small molecular volume having been affected, one twenty-thousandth of the area relation of the axolemma, and more in keeping with the surface measurements of a few neurofibrils, which may have been concerned in the transmission.

Gerard cites Erlanger, Bishop and Gasser (Am. J. Physiol., 78:574, 1926) in connection with the discovery that an impulse travelling up a sensory nerve is delayed 0.00014 second in passing the spinal ganglion. The crude sensory

nerve fibre to all appearances has a T-shaped connection with the dorsal root ganglion. Therefore an impulse entering the ganglion would have to wait until the refractory period in the stem of the T was over before retracing the other 6 mm. of the "detour" to return to the main stream, and thence into the cord. If the impulse had been transported by an individual neurofibril no conflict or refractory period would have ensued. The impulse would then have travelled up the neurofibril, through the stem of the T, around the nucleus, back out the stem and on into the spinal cord. This hypothesis obviates the necessity of considering any possible refractory period, a most unlikely occurrence in such a small total delay as 0.00014 second. Bethe in 1898 and Steinbach in 1899 cut off the dorsal root ganglion in various lower forms and claimed to have found that conduction still occurred from receptor to spinal cord.

If it could be shown that the size of an axis cylinder is directly proportional to the number of contained neurofibrils, a great deal of evidence could be brought in favour of the neurofibril as the unit of conduction, for axis cylinder diameter is in a general way related to conduction, notably in the large motor trunks.

It is an interesting phenomenon that axis cylinders should be constricted at the nodes of Ranvier, while the intracellular neurofibrils remain unchanged. Bethe (1903) claimed that the peripheral substance of a nerve fibre could be compressed down to one six-hundredth of its normal cross section and yet conduction was possible as long as the neurofibrils were intact and undamaged.

The possibility of two types of transmission in the nervous system is strengthened by the finding that a nerve fibre begins activity within 0.00005 second after a stimulus is applied and ceases to function immediately the stimulus is removed. On the other hand reflex firing begins slowly and continues for several seconds after stimulation stops.

Sixty years ago Max Schultze (cited by Parker, 1929) speculated on the specific function of the neurofibrils in the large ganglion cells of the Torpedo: "We may regard such a ganglion cell from which a peripherally directed nerve fibre proceeds as representing the source and origin of this axis cylinder, but only in the sense that the fibrils which compose the axis cylinder are collected into a group from the arborescent processes of the cell; and thus the fibrils which are seen traversing the substance of the ganglion cell do not originate in the cell, but only undergo a kind of arrangement in it, and then pass to the axis cylinder process, or extend into the other branched processes". "On physiological ground also I maintain the possibility of isolated conduction in these constituent fibrils, even when no trace of interfibrillar substance is present."

It might be an appropriate place to put forward some questions which require answers, if future work in the field of interneuronal connections and the theory of neurofibrillar function is to be relevant and thereby valuable.

Why are there no boutons on the cone of origin of axis cylinders or on the axone itself? (See Cajal and Van Gehuchten).

Is this absence of boutons due to dynamic polarization of the cell, or to a lack of chromidial substance?

What purpose is served by a preponderance of potassium in the axis cylinder compared with the dendrites, and why is there no oxydase in axones?

What protective or insulating sheath has an axone as it leaves a nerve cell body, and how far along the axone does it extend?

Do dendrites end by means of boutons?

Does "dynamic polarization" demand that the intracellular neurofibrils entering the cell via the dendrites must all leave the cell body via the axone?

What is the origin of intracellular neurofibrils which we see in the axis cylinder if they do not come from the dendrites?

How do boutons ending on dendrites contribute to the "motoneuron pool"?

Do they augment the perhaps faint stimulus passing through the dendrite, or do they discharge minutely but over a considerable period of time, causing a constant current or a constant surface depolarization which prevents the passage of the stimulus down the dendrite?

The work of Bozler (as described by Parker, 1929) has suggested some experiments which might well be attempted in furthering our knowledge of neurofibrillar connections and functions. He found that by immersing the circular nerve band of the jellyfish Rhizostoma in hypertonic saline he could

produce a herniation of a few individual neurofibrils through the fibre sheath. This could be repeated, with a view to cutting across the few neurofibrils thus isolated, and the synaptic degeneration resulting could be studied as an indicator of fibrillar connections. The ideal experimental animal would be one in which a simple, large synapse could be easily approached and studied, and its constituent fibril endings delineated.

A problem confronting any histologist studying boutons is whether or not cutting an axone close to its bouton termination gives a different picture of degeneration than cutting the axone at a considerable distance from its boutons. An answer to this question would be necessary before one could proceed to a classification of the families of boutons ending on anterior horn cells of the spinal cord. Such a classification might be carried out by removing different cortical areas on a series of cats, or confining the extirpations to a single cat, with definite and recognizable time intervals between.

The literature is still incomplete on such subjects as bouton changes in anaemia, anaesthesia, trauma, heat, drugs, poisons, fatigue, and under continued electrical stimulation.

It might be practicable to use an ultra-centrifuge and a tissue containing a simple synapse from a large nerve cell, to force the nuclear elements of the cell to one end with such force that the intracellular neurofibrils would be disturbed, if not broken. Heavy intravitam nuclear staining might aid in the force of the nuclear displacement.

Cowdry has suggested the value of the micro-incineration technique of Policard and Okkel (Anat. Rec., 44:349) as a means of determining chemical changes under different functional conditions in tissues in general, and its use in investigating chemical changes at the synapse might prove to be of value.

An account of the actual extent of the myelin sheath on nerve cell bodies and processes is very badly needed. Bartelmez (1933) has found some interesting facts about the protective covering of nerve fibres, chiefly its fading out as the site of synapse is approached. Only a technique which will stain protective coverings from the cell body to the last outposts, axonic and dendritic, will clear up the question of the convergence and divergence of neurofibrils, and nerve fibre branchings.

Recent work on the chemical phenomena associated with synaptic transmission (see Feldberg, J. Physiol., 1934) has suggested the possibility that inhibition is produced by the excretion or injection into the synaptic region of an agent which immediately raises the threshold; and conversely that excitation may be attributed to a chemical promoter. The histology and chemistry of the synapse are inseparable.

SUMMARY.

The degenerative changes noted in the boutons terminaux are in accord with the accepted observations on neurofibrillar degeneration in the peripheral segments of sectioned nerves.

The optimum period of degeneration of the boutons from the point of view of ease and certainty of recognition is from seventy-two to ninety-six hours in the spinal cord.

Criteria for the determination of the length of time which a bouton has been degenerating have been set forth, in order that the technique may be more widely applied, especially in cases where it is desired to differentiate between boutons of longer or shorter periods of degeneration.

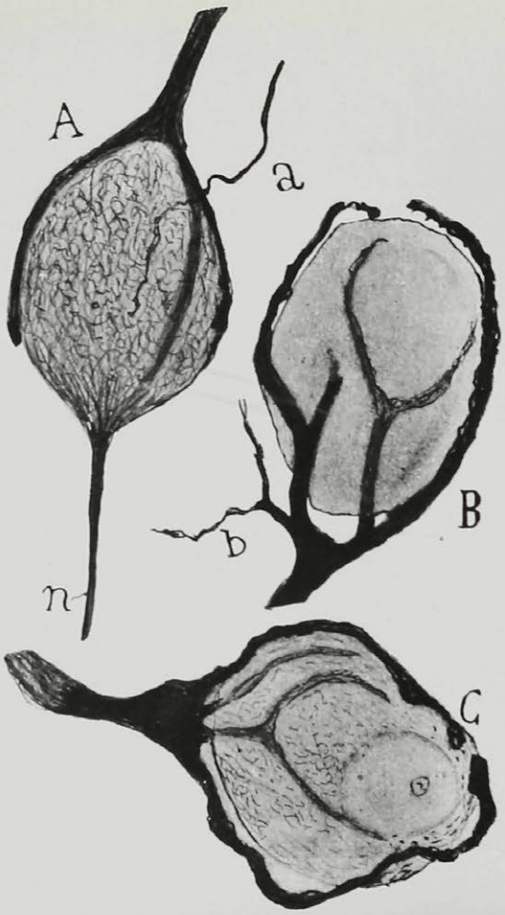


Fig. 15. — Calices de HELD du noyau du corps trapézoïde du chat: *A*, cellule dont le réticule se montre coloré, quoiqu'en un ton rouge; *B*, calices énergiquement imprégnés en un ton café foncé; *a*, fibre qui arrivait au calice depuis longue distance; *b*, branche émergente de la naissance du calice et se perdant dans le plexus interstitiel; *n*, axone bien coloré d'un neurone qui, sans le calice, pourrait simuler une connexion par continuité neurofibrillaire (méthode au nitrate-alcool-pyridine; réduction à l'hydroquinone).

Figure 1
(After Cajal - 1934)

Fig. 2. (After Held, (1897)).

(Fig. 8) Axis cylinder with neurosomes, cauda equina, dog. Palladium chlor. and glacial acetic. Paraffin at 1.5 u Fuchsin.

(Fig. 9) Dorsal horn cell of cord, dog. Chromic acid 1:2000, paraffin 1.5. Erythrosin-methylenblue.

(Fig. 11) From the trapezoid nucleus of growing dog, young. Picro-sulphuric acid, paraffin 5 u. Erythrosin-methylenblue. Endkorb.

(Fig. 12) Purkinje cell, human. Golgi sublimate; paraffin 10 u; weak erythrosin.

(Fig. 13) Glomerulus olfactorius, growing pup; Fuchsin, after Altmann.

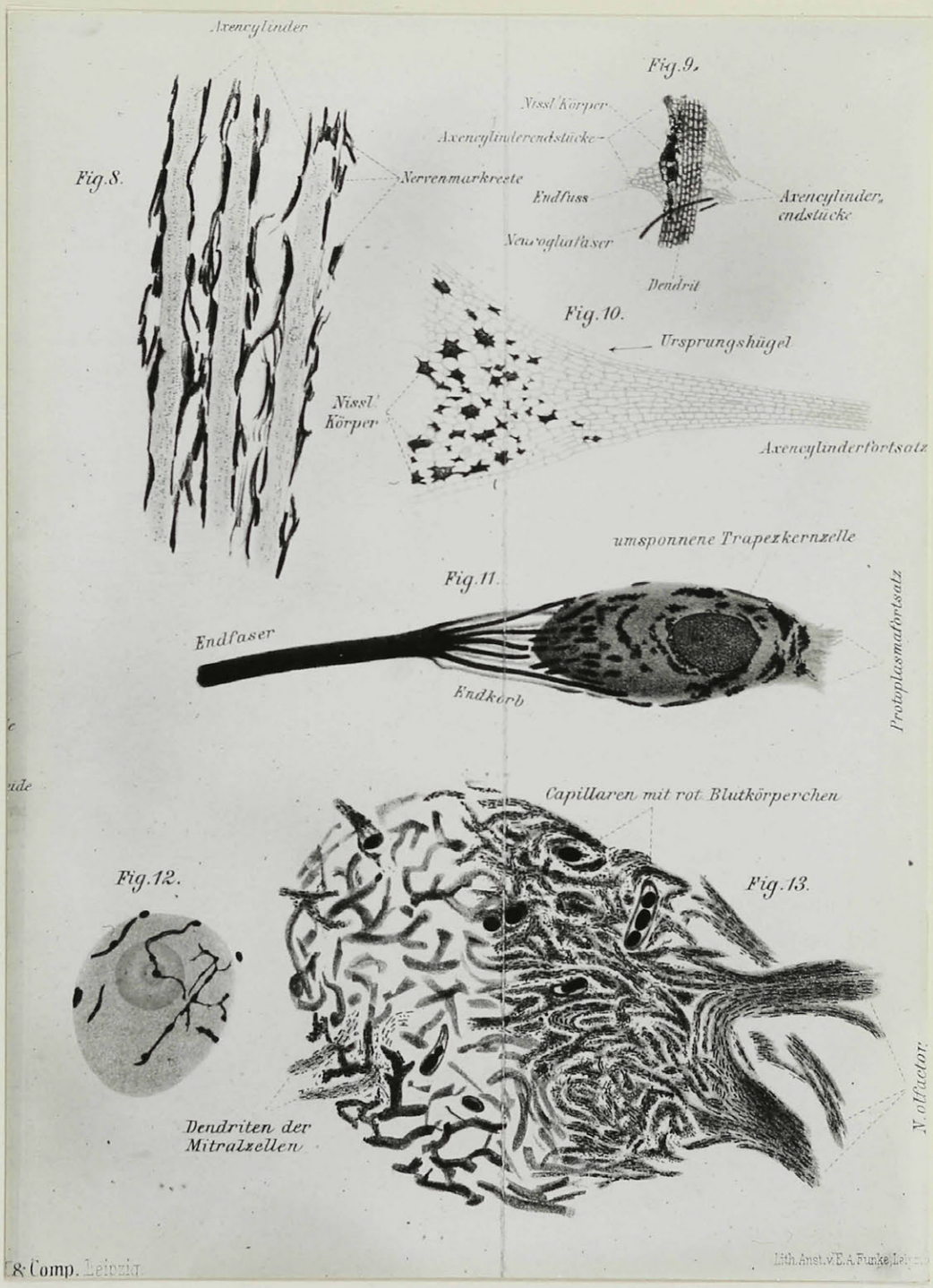


Figure 2
(After Held - 1897)

Fig. 3. (After Held, (1897)).

(Fig. 1-3, 5, 6) Spinal cells, dog. Chrome-osmium of Altmann, picro-alc. differentiation.

(Fig. 4) Purkinje cell, dog. Alc-chloroform; erythro-methylenblue.

(Fig. 7) Purkinje cell, dog. Chromosmium and acid Fuchsin. Axis cylinder of end bodies.

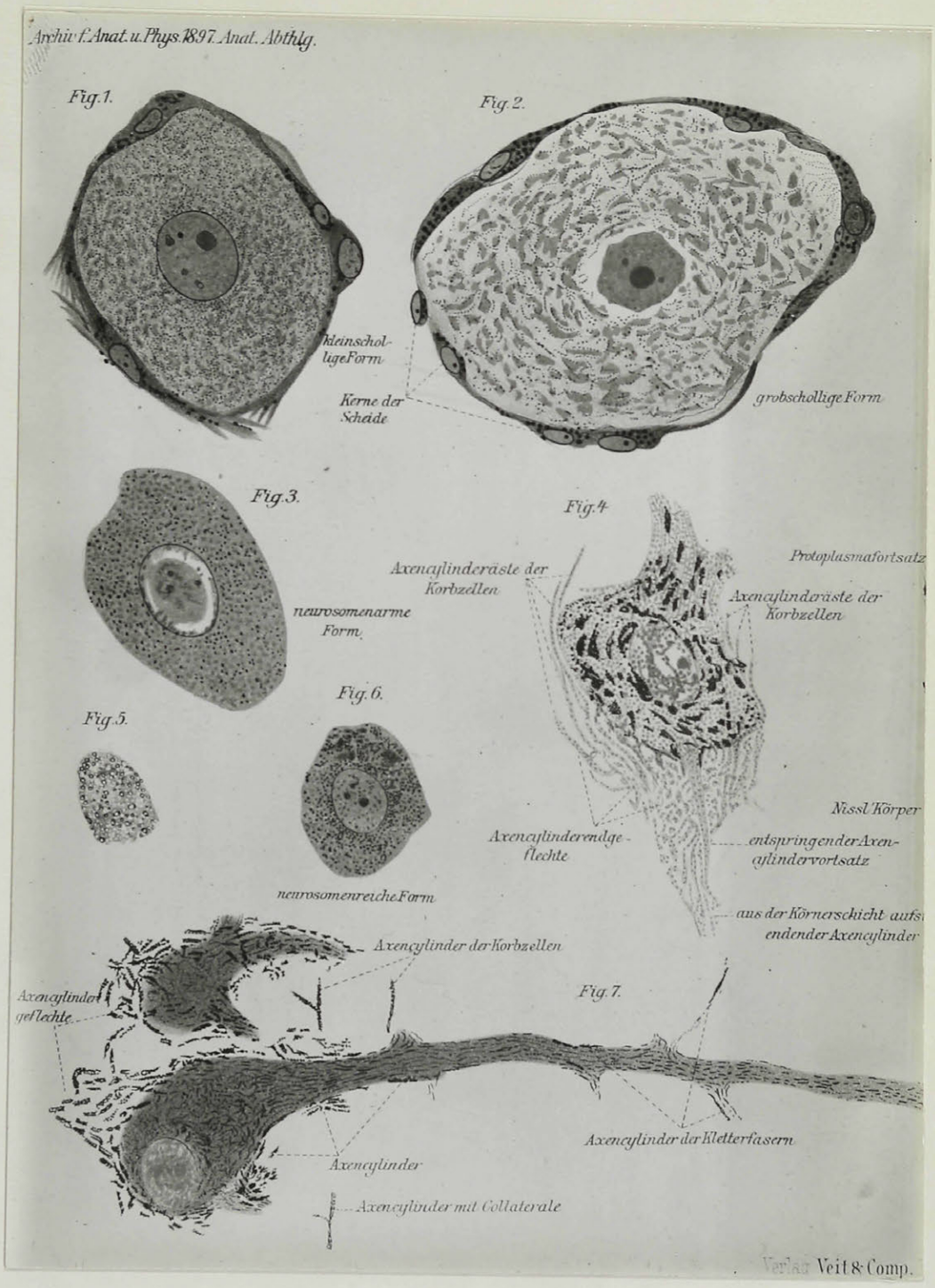


Figure 3
(After Held - 1897)

Figure 4
(After Karl Bauer - 1932)

Fig. 5. (After Max Wolff (1904-05)).

XVI. Dendrittenquerschnitt aus der unteren Olive der Katze mit deutlich Sich abhebender Hyaloplasmazone. Das endoplasmatiche Wabenwerk ist nicht mitgezeichnet.

XVII. Eine tiefer liegende optische Ebene desselben (XVI) Dendritenquerschnitts. Die Hyaloplasmaschicht ist sehr Schrag, fast parallel getroffen. Die beiden Endfusseaxone zweigen sich von einem links in die Tiefe steigenden Nervenbundel ab.

XVIII. Endfuss einer benachbarten Stelle senkrecht zur Zelloberflache geschnitten.

XIX. Mikrophotogramm aus derselben Gegend. Unretouchiert. Leitz, 1/12 Öl-immersion, okular 5.

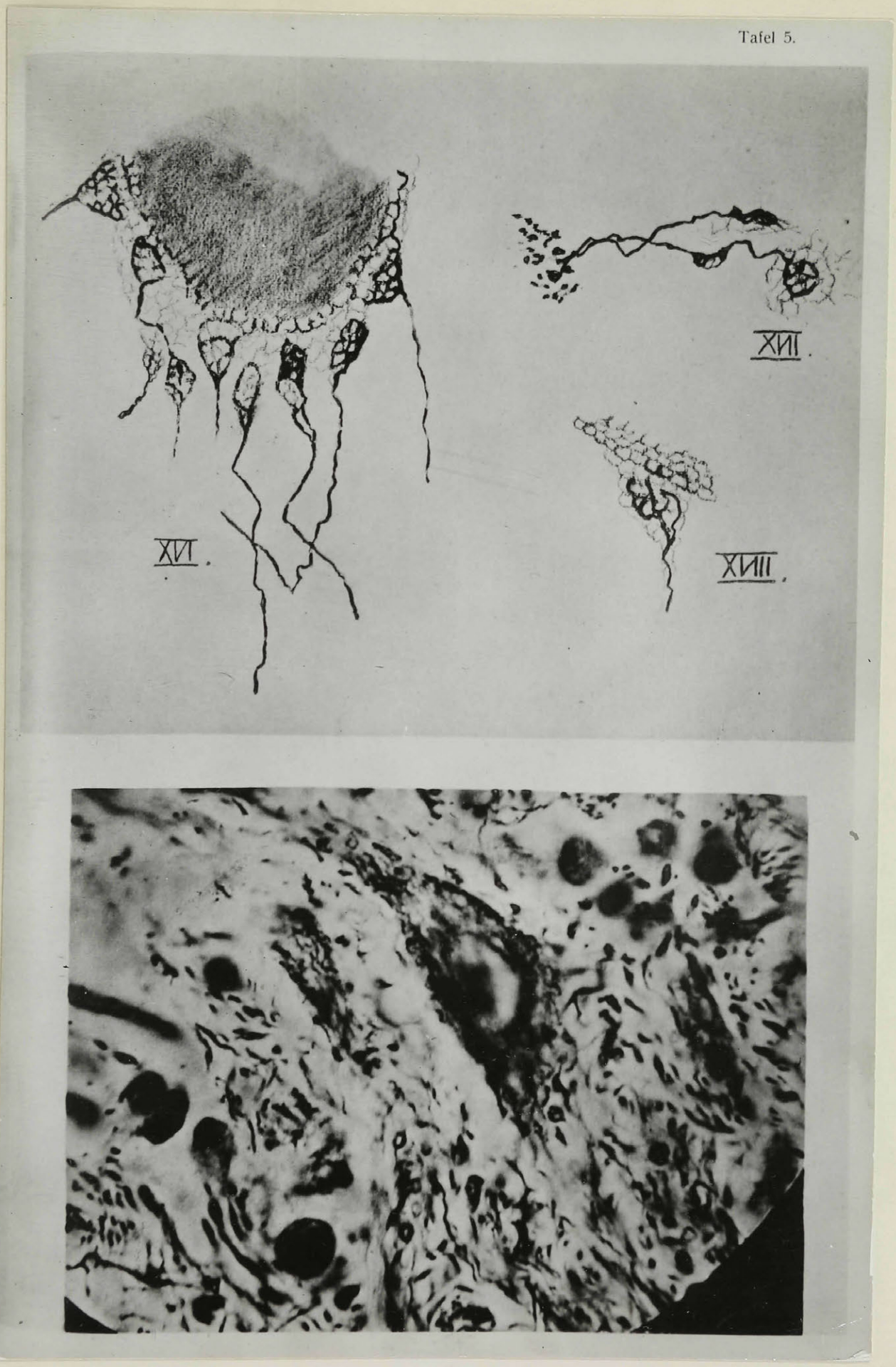


Figure 5
(After Max Wolff - 1904)

Fig. 6. (After Tiegs (1929)).

(Fig. 1) Mauthner cell of young trout (Bielschowsky preparations, obj.3mm. oc. x 15). The intracellular fibrils are impregnated only in places. Those of the large axon (a) are well shown. The cell is surrounded by a dense mass of nerve fibres, but only those at the lower part of the cell are true nerve terminals. They taper out into delicate threads and at least two of them pass into the protoplasma of the cell. In the region where they do not pass in, the neurofibril staining has failed.

(Fig. 2) Cell of tangential nucleus of young trout (Bielschowsky, obj.3mm. oc. x 15). From the swollen terminal several fibres pass into the substance of the cell; the figure cannot make it clear that the fibrils are not on the surface of the cell.

(Fig. 3) The same from nearly hatched trout (Cajal preparation, obj.3 mm. oc. x 15). The fibrils pass from the terminal through the substance of the cell into the axon (a).

(Fig. 4) Cell from trapezoid nucleus of adult cat (Bielschowsky, obj.3 mm. oc. x 15). With the terminal calyx of Held numerous fibrils are connected and pass upwards into the cell axon (a).

(Fig. 5) The same, seen in a transverse section. From the coarse pericellular terminal, firm fibrils are seen passing into the substance of the cell. (Bielschowsky, obj.3 mm. oc. x 15).

(Fig. 6) Termination of basket fibres on body of a Purkinje cell of rabbit. (Bielschowsky, obj.3 mm. oc. x 15).

(Fig. 7) A terminal "end-foot" of Held on surface of spinal cord cell, showing passage of neurofibrils into the substance of the cell. (Cat: Bielschowsky, obj.3 mm. oc. x 15).

(Figs. 8-9-10.) Connection between collaterals of Mauthner's fibre (m) and dendrites of spinal cord cells (amblystoma larva). In fig. 8 discontinuity is seen; in fig. 9 the more complete staining shows a complex fibril system passing across the junctional tissue connecting the collateral with the fibrils of the dendrite; in fig. 10 an exceedingly simple connection is seen. (Bielschowsky, obj.3 mm. oc. x 15).

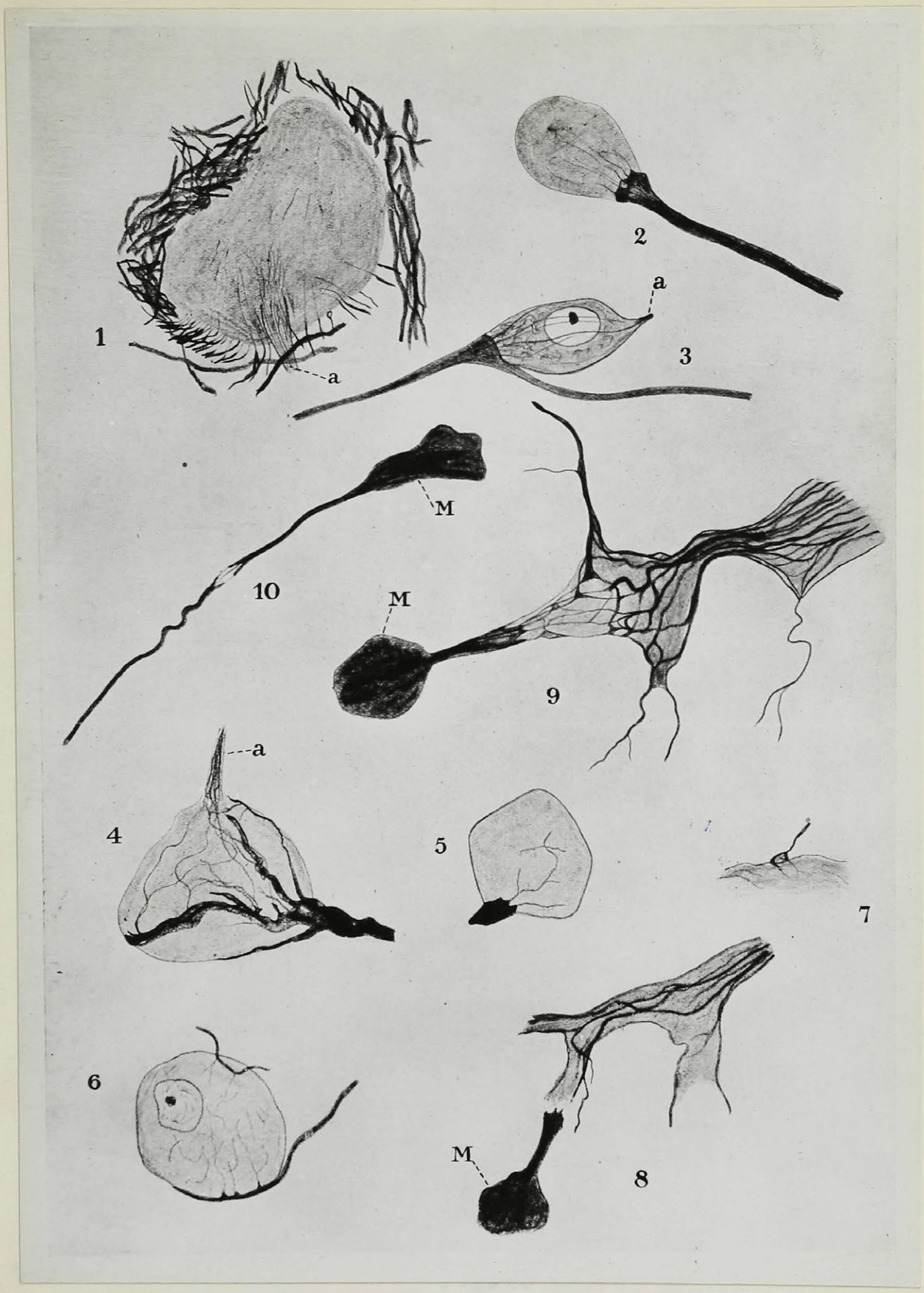


Figure 6
(After Tiegs - 1929)

FIG. 5. (*a*) Section of cell in dorsal region of grey matter in spinal cord of adult cat, showing 'boutons terminaux' in contact with the cell surface.

Figure 7
(After Hoff)
(in Creed et al - 1932)

Fig. 8. (After Sereni and Young, (1933)).

(Fig. 24) Degenerating terminal boutons from neuropil of stellate ganglion, 15 hours after section of mantle connective. Cajal's stain. Octopus vulgaris. Zeiss, apo. 90.1,3.

question. The present experiments show clearly that in the molluscs studied degeneration does not extend across synapses.

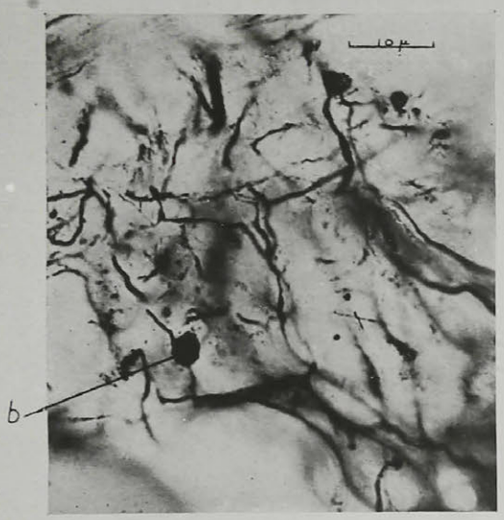


Fig. 24. — Degenerating terminal boutons from neuropil of stellate ganglion, 15 hours after section of mantle connective. Cajal's stain. *Octopus vulgaris*. Zeiss apo. 90 1,3.

c. *Double Section of the Mantle Connective.* — The results of the experiments in which the mantle connective and stellar nerves were cut indicate that the spheres which are seen in the peripheral stumps are formed at the cut surface whenever a fibre is isolated from its cell body. In order to confirm this hypothesis the mantle connective was cut in two places about 1-2 cm. apart, so that it was possible to be certain that the fibres in the intermediate portion were isolated from all connection with cell bodies. As was expected necrotic processes leading to the formation of degeneration spheres were seen in all the fibres at both ends of the isolated piece of nerve (Figs 13 and 26) as well, of course as in the peripheral stump which remained attached to the ganglion.

III. — Conclusions from Histological Changes

The histological examination of the nerves and ganglia after section of the mantle connective and stellar nerves has shown that the nervous system of Cephalopods is built of separate units, so that after section of an axon the isolated portion degenerates up to its ending on another neuron or muscle cell, the degeneration not extending into the next cell of the chain.

It is also possible to make several deductions as to the

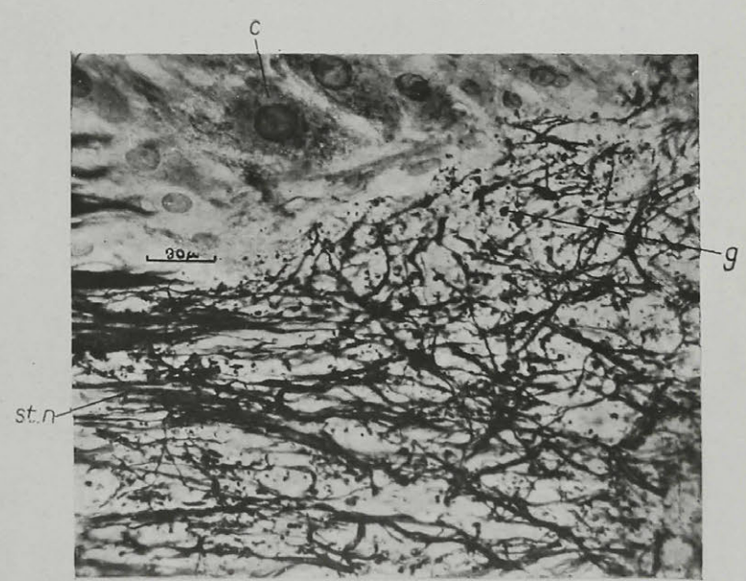


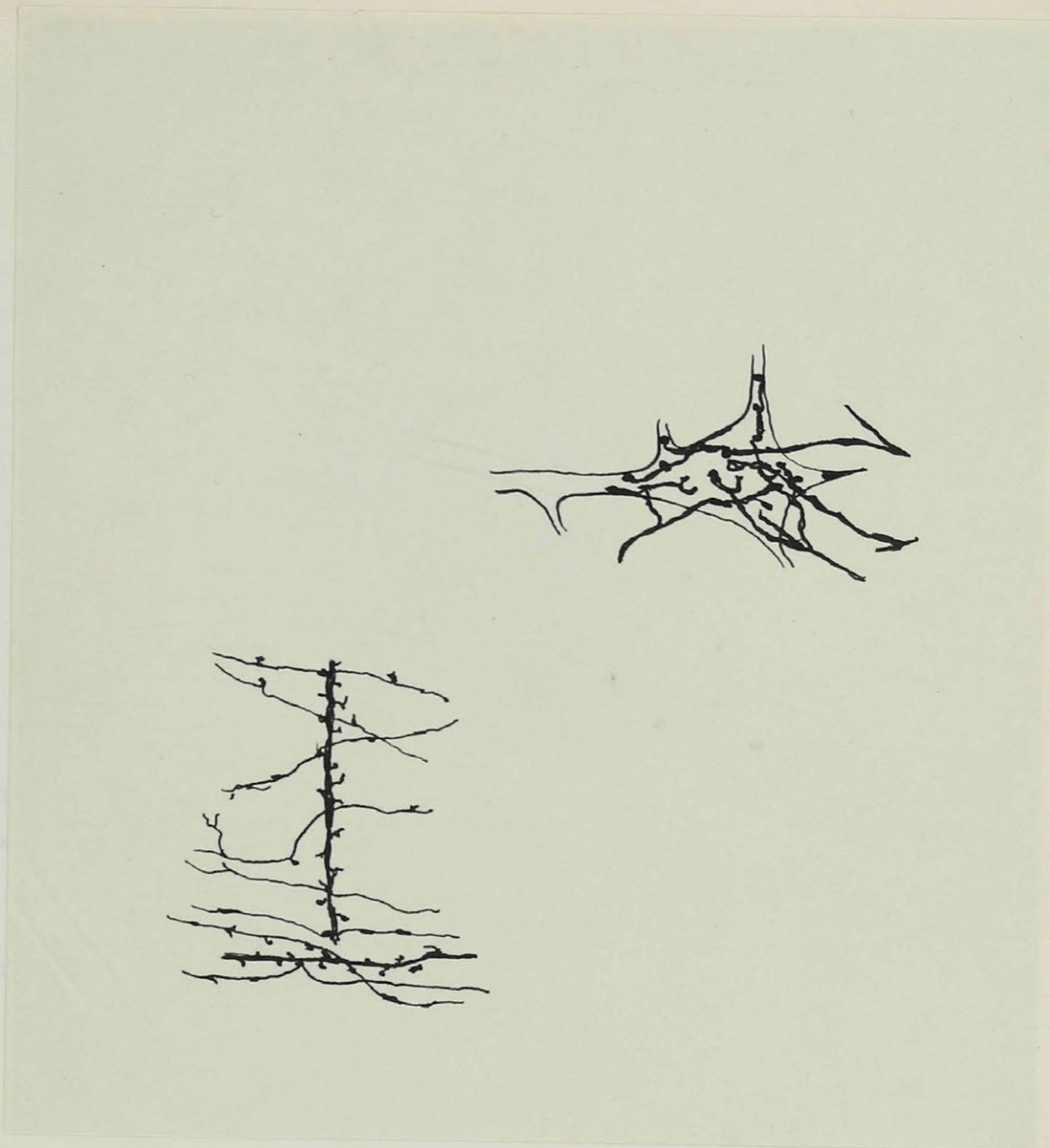
Fig. 28 — Granules in neuropil of stellate ganglion, 22 hours after section of mantle connettive. *Octopus vulgaris*. Cajal's stain. Photo. Watson. 1/6 in. *c.*, cell layer of ganglion: *g.*, degeneration granule; *st, n.*, stellar nerve.

Figures 8 a & b
(after Sereni & Young - 1933)

Abb. 4a. Pathologisch veränderte, aufgetriebene, intensiv imprägnierte Endösen an Zellen der Clarkeschen Säule. (Cajal-Modifikation 6 A. Rhesus.) Vergrößerung 900fach.

Abb. 4b. Pathologisch veränderte, aufgetriebene, intensiv imprägnierte Endösen an Zellen der Ckarkeschen Säule. (Cajal-Modifikation 6 A. Rhesus.) Vergrößerung 1000fach.

Figures 9 a & b
(after Foerster, Gagel and
Sheehan - 1933)



Figures 10 a & b
(after Lorente de Nó - 1933)

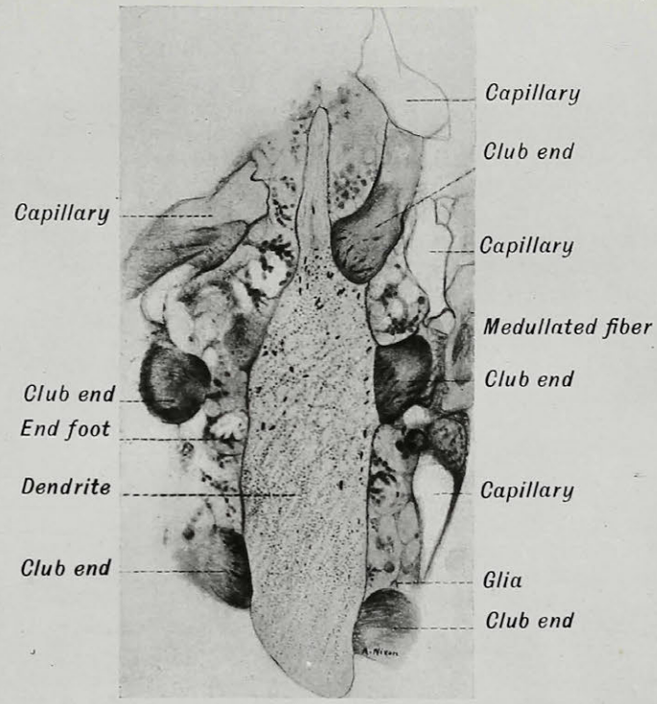


Fig. 173.—Five large, clublike terminations of eighth nerve fibers on the dendrite of a large nerve cell. The fixing fluid was introduced into the living brain through the blood vessels. Preparation stained to show mitochondria which appear as short dark rods. 1285 ×. After Bartelmez and Hoerr.

As already mentioned, degeneration or chromatolysis resulting from injury to a neuron does not ordinarily cross the synaptic barrier and affect other neurons which are connected with it in the same chain of conductors. Nervous impulses may traverse the synapse, but the trophic influences which maintain the life of the cell do not.

Figure 11
(after Bartelmez & Hoerr - 1933)

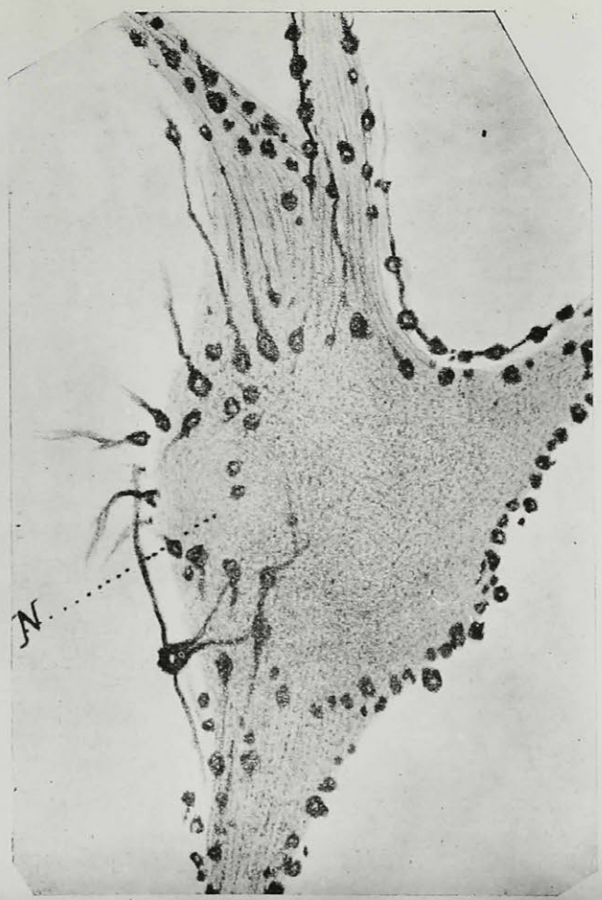


Fig. 1. — Myélite aiguë. Cellule de la corne antérieure. N. noyau représenté par une tache moins colorée. Le réseau du cytoplasma, disparu est remplacé par de fines granulations denses et bien colorées. A la surface de la cellule et des prolongements protoplasmiques, il existe un grand nombre de boutons terminaux bien colorés, de volume normal ou à peu près.

Figure 12
(after Marinesco - 1904)

Fig. 13. (After Achucarro (1909)).

(Fig. 1 and 2) Anterior horn cells of dog dead of rabies. Normal intracellular neurofibrils but pathological swelling of endfusse.

(Fig. 6) Large pyramidal cell of cortex of 28-year-old man dead of rabies, showing fatty degeneration with displacement of neurofibrils.

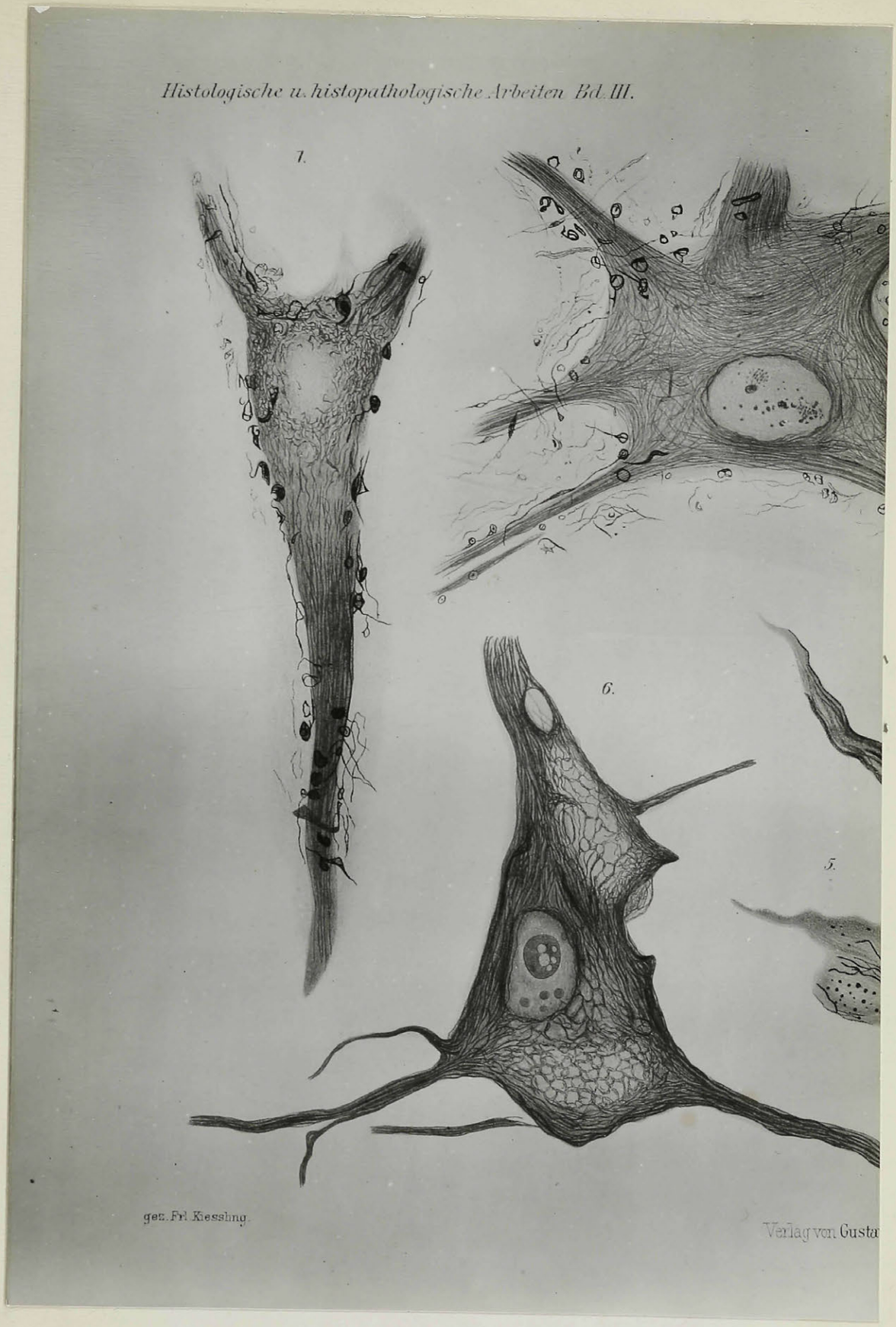


Figure 13
(after Achucarro - 1909)

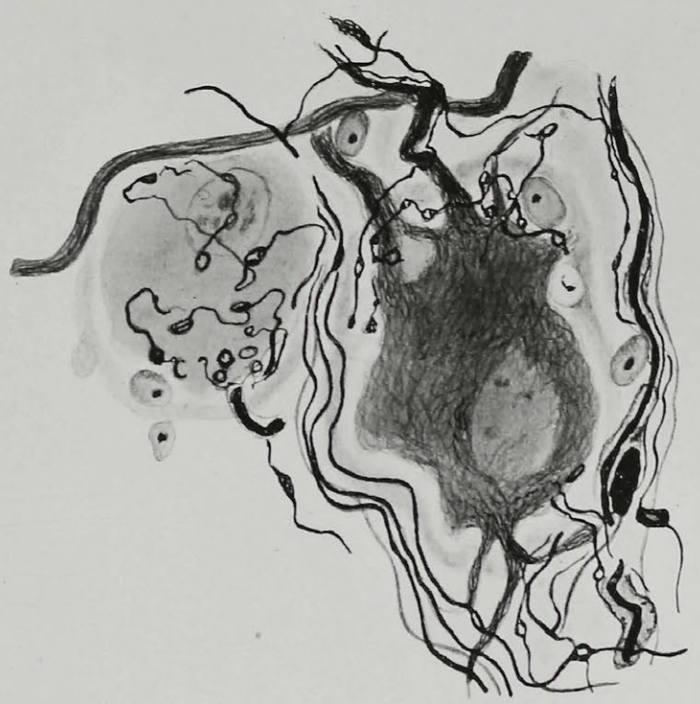


Abb. 13. Nervenzellen aus einem kleinen Nervenknötchen des rechten Vorhofes. Epikard (Katze). 4 Tage nach Durchschneidung des rechten N. vagus. Die perizellulären Apparate — »Endkörbe« — auf den Nervenzellen sind gut sichtbar. Bielschowsky-Gros. Hämatoxylin. Vergr. 1050fach.

Figure 14
(after Lawrentjew - 1929)

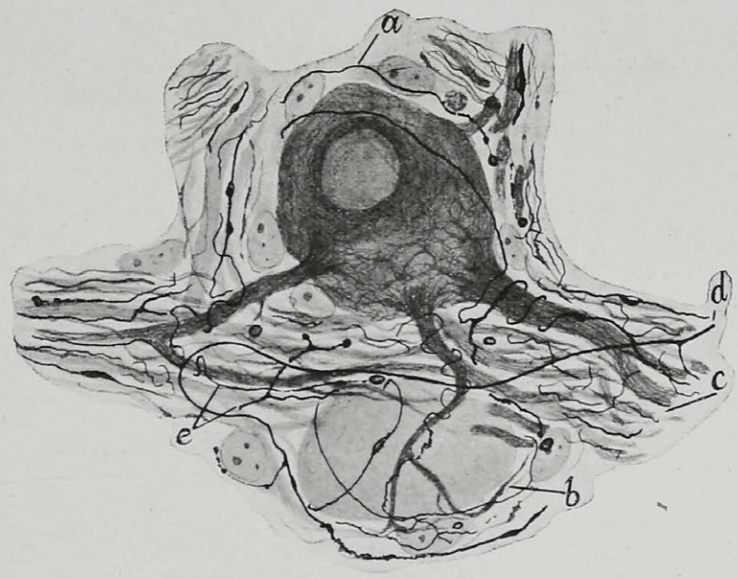


Fig. 5. — Portion de ganglion cervical supérieur du chat sacrifié 16 heures après la section du trone cervical. — *a, d, e,* branches terminales des fibres préganglionnaires en état granuleux et avec hypertrophie et ségrégation de quelques branches terminales; *c,* tractus dendritique avec anneaux isolés et rameaux granuleux; *b,* nid dendritique péricellulaire avec une fibrille préganglionnaire granuleuse. (Voir d'autres explications dans le texte). Méth. de CAJAL, pyridine; obj. apoc. 1,30; 2 mm. × o. c. 20 (— 1/5).

Figure 15
(after De Castro - 1930)

Fig. 13. — Le bonnet. Dégénération des péricellulaires sur les cellules du ganglion du plexus d'AUERBACH, 71 heures après la section du nerf pneumogastrique blessé. (Zeiss. Imm. Ap. 3 mm. K. Oc. 8.)

Fig. 14. — Le bonnet. Dégénération des appareils péricellulaires du plexus d'AUERBACH, 71 heures après la section du nerf pneumogastrique blessé. (Zeiss. Ap. 3 mm. K. Oc. 8.)

Figure 16
(after Kolossow - 1933)

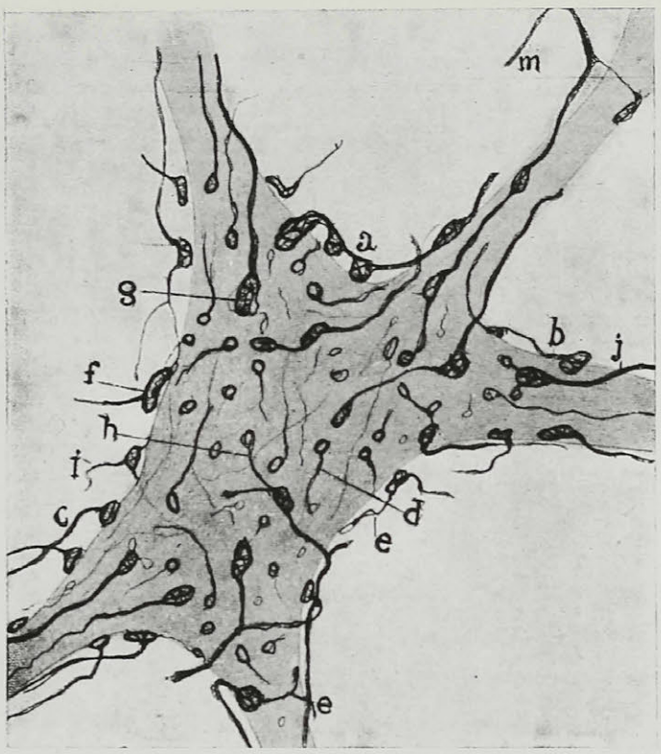


Fig. 28. — Détails du nid péricellulaire d'un neurone moteur appartenant à la moelle du chien adulte (quatrième formule): *a*, *b*, gros bulbes terminaux; *d*, anneaux terminaux délicats; *e*, fibrilles fines et pâles, émanées d'un bulbe terminal; *f*, *g*, *h*, autres bulbes émettant des branches secondaires fines.

Figure 17
(after Cajal - 1934)

purely excitatory for the muscle in question, and that a break-shock series stimulating it be reduced in strength with consequent decrease in number of its stimulated fibres the decrease being among the smaller of the fibres previously excited. The field

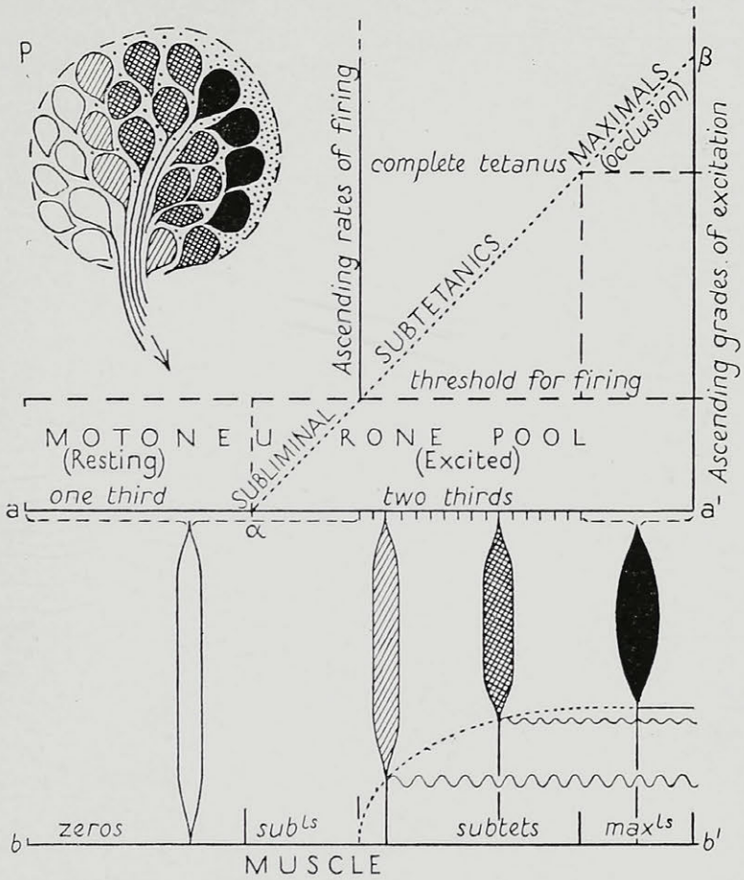


FIG. 61. Scheme of distribution of excitement in a reflexly active motoneurone pool of a muscle. Grades of excitement plotted against numbers of motoneurones [abscissa *a a'*] and of motor-units [abscissa *b b'*]. $\alpha\beta$ denotes excitements in active fraction of pool.

and density of the exciting central terminals are thus reduced. If we imagine this to reduce existing excitements by a given, say just subliminal amount, we may sample its effect on the various motor units along the scale of excitation of the chart. Subjected to this reduction the old subliminal fringe drops out altogether. The total excited field thus shrinks, i.e. the point α in the

Figure 18
(after Creed et al - 1932)

Figs. 19 and 20.

Normal boutons terminaux on ventral horn cells of the spinal cord in the cat.

Fig. 19. A thin fibril ending in a loop-like bouton.

Fig. 20. Note the arrangement of the boutons on the cell body and dendrites.

Magnification: 2900 diameters.

Figure 19

Figure 20

Fig. 21.

Terminal boutons 24 hours after section of their axis cylinders. Their ring-like appearance is preserved, though they are considerably swollen. The normal bouton on the centre of this anterior horn cell is easily differentiated.
Magnification: 2900 diameters.

Figure 21

Fig. 22.

Degenerated terminals 48 hours after section of the spinal cord. Large anterior horn cell with opaque boutons in contact with the periphery.
Magnification: 2900 diameters.

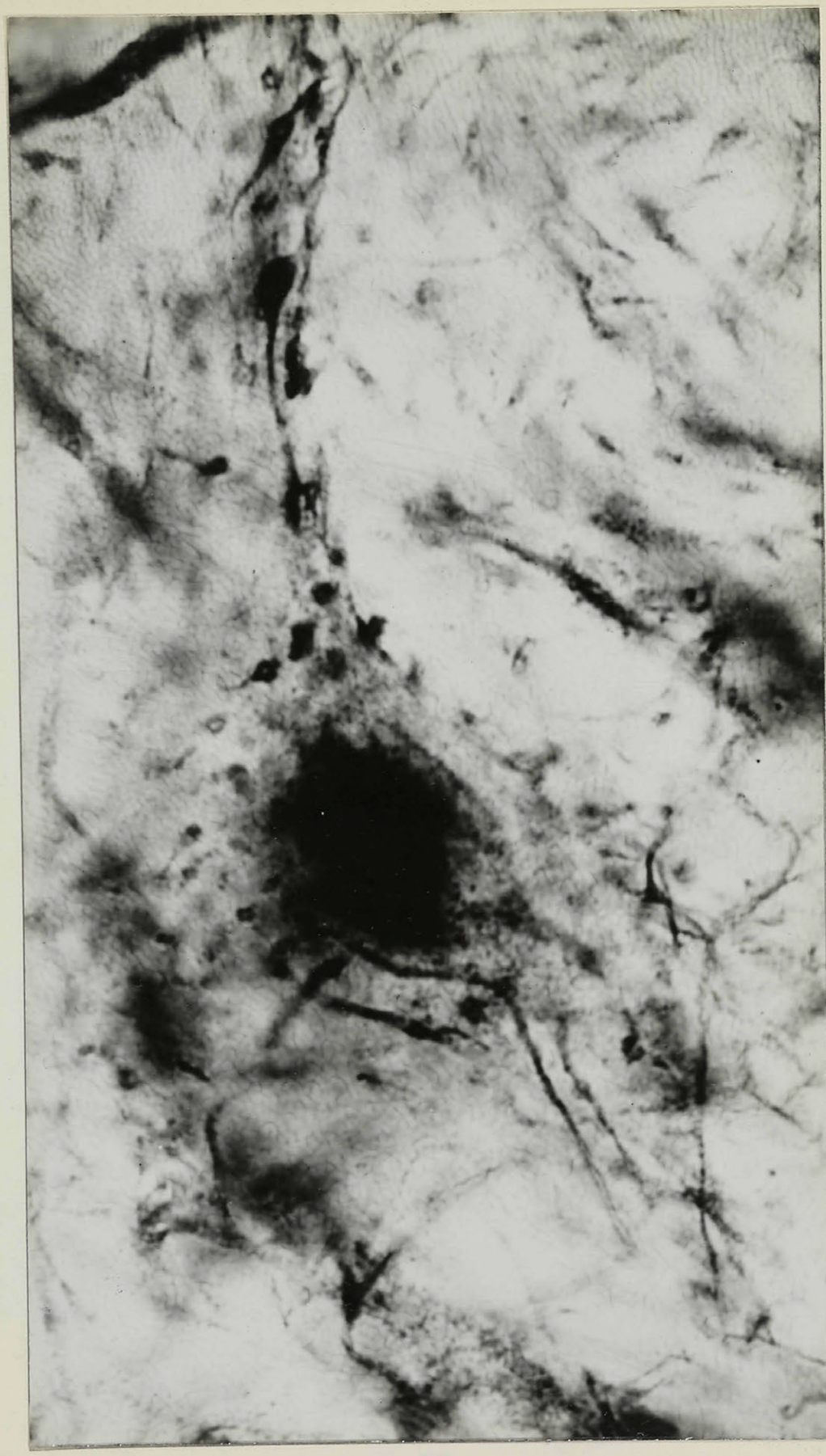


Figure 22

Fig. 23.

Elongated boutons which have degenerated over a period of 72 hours. These "spatula-like" structures stand out against the cell background. The former circular configuration has disappeared.
Magnification: 2900 diameters.

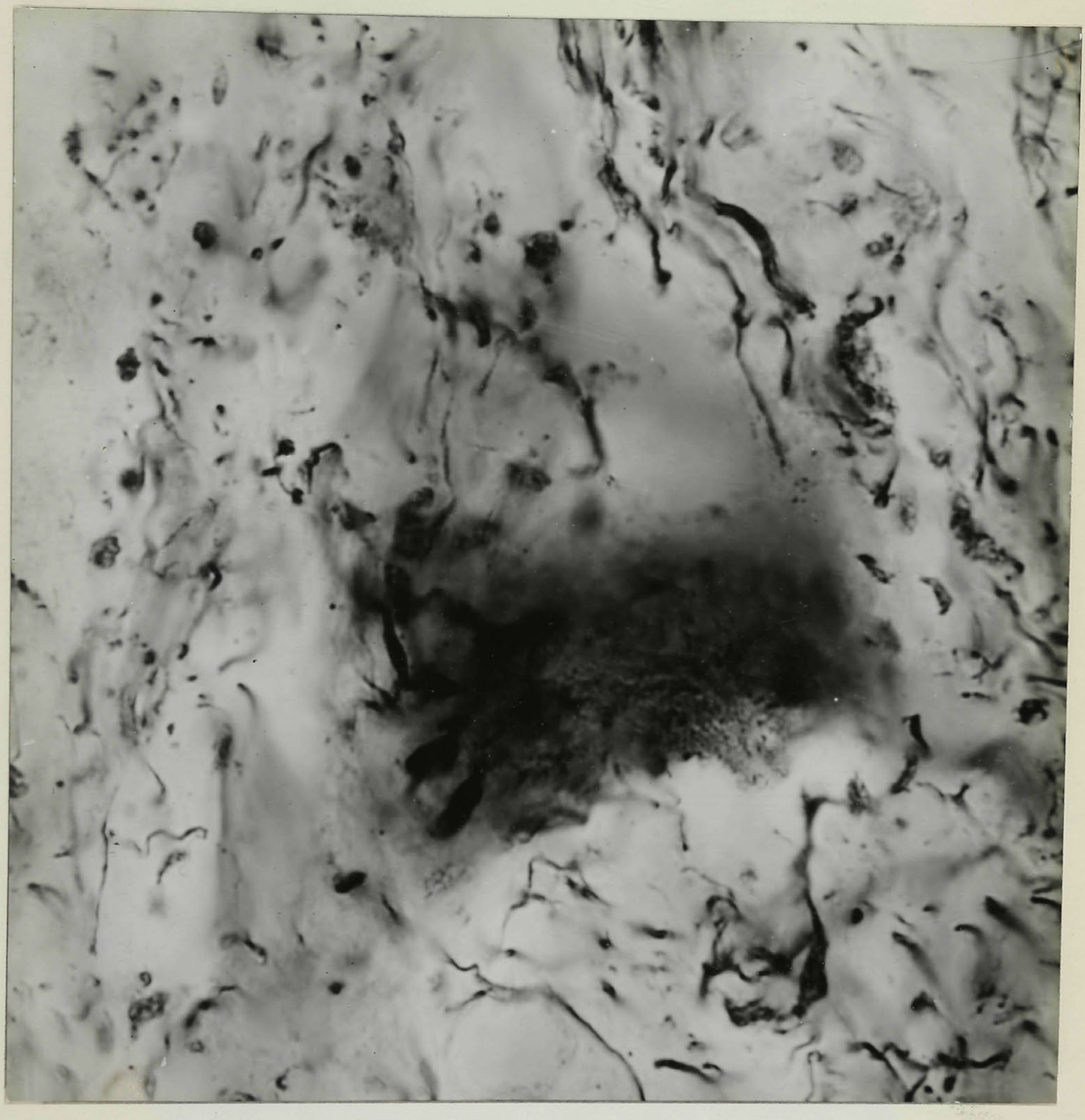


Figure 23

Fig. 24.

Anterior horn cell of a cat sacrificed 96 hours after section of the spinal cord. The photograph shows a normal bouton adjacent to the greatly enlarged degenerated ending. Magnification: 2900 diameters.

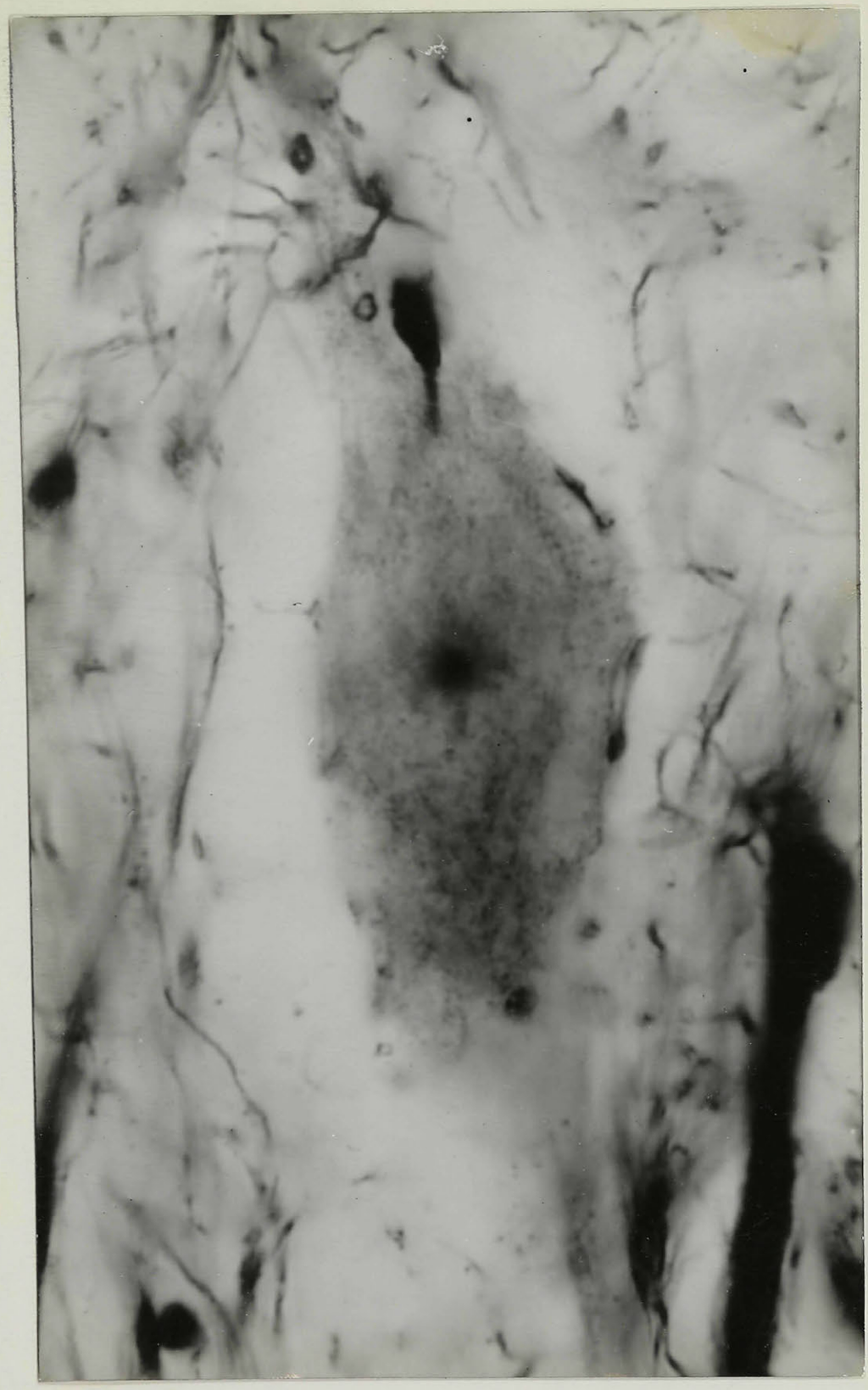


Figure 24

Figs. 25 to 28.

Final stages in the degeneration of boutons terminaux 120 hours after section of the spinal cord.

Fig. 25. Anterior horn cell, at the lower edge of which a greatly enlarged and granular bouton can be seen. The pericellular endings reach their greatest length at this stage early in the 5th day of degeneration.

Fig. 26. Fragmentation of a bouton on the anterior horn cell at the left margin of the photograph, still more advanced in the cell at the right margin.

Figs. 27 and 28. Granulation and final disappearance of boutons from the anterior horn cells.

Figure 25

Figure 26

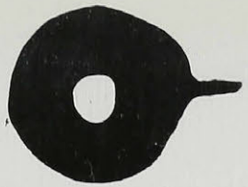
Figure 27

Figure 28

Fig. 29.

Diagrammatic drawing to show the stages of bouton degeneration. The transition from the circular shape to the ellipsoid and back to the circular can be seen. Measurements of the cells from which these sketches were made are given in each case, together with the approximate area. Degenerative changes in the bouton are most marked between the periods of 72 and 96 hours.
Magnification: 10,000 diameters.

NORMAL
2u x 2u
4·00 SQ.u



24 HOURS
2·4u x 2·4u
5·76 SQ.u

48 HOURS
2.8u x 2·8u
7·84 SQ.u

72 HOURS
4.1u x 2·04u
8·20 SQ.u

96 HOURS
4.5u x 2·4u
10·8 SQ.u

120 HOURS (a)
4·8u x 2·8u
11.5 SQ.u

120 HOURS (b)
4.5u x 2·8u
12.6 SQ.u

120 HOURS (c)
4.1u x 4.1u
16·8 SQ.u

120 HOURS (d)
GRANULATION

x 10,000

Figure 29

BIBLIOGRAPHY

(1)

Achucarro, N.	1909	Zur kenntnis der path. histologie des zentralnervensystems. Nissl's Histologische u. Pathol. Arbeiten. 3:142. Jena.
Althaus, J.	1880	The functions of the brain. A popular essay. Longmans, London.
Antoni, N.	1908	Die frage von einen neurofibrillaren kontinuität im zentralnervensystem der wirbeltiere. Folia neuro-biologica, 2.
v. Apáthy, S.	1891	Proc. Internat. Congress Zool. Cambridge.
Ariens Kappers, C.U.	1932	Principles of development of the nervous system. In Cytology and Cellular Pathology of the Nervous System (Penfield). Vol.1, 1932.
Ariens Kappers, C.U.	1933	La correlation entre le procès de conduction et la croissance du nerf. Psychiat. en Neurol. bl. 37:579.
Ariens Kappers, C.U.	1934(a)	Differences in effect of various impulses on structure of central nervous system. Irish J. M. Sc. 1934:495.
Ariens Kappers, C.U.	1934(b)	Special volume in honour of -- Psychiat. en Neurol. bl. 38:265.
Ashby, W.R.	1934	On the nature of inhibition. A review. J. Ment. Sc. 80:198.
Auerbach, L.	1897	Farbung fur axencylinder und ihre endbaumchen. Neurol. Zentralbl. 4:60.

BIBLIOGRAPHY

(2)

Auerbach, L.	1899	Das terminale nervennetz in seinen beziehungen zu dem ganglienzellen der centralorgane. Monatschr. f. Psychiat. u. Neurol. 6:191.
Auerbach, L.	1927	Das histologische substrat des polarisierten nerven. Zeitschr. f. Zellforsch. u. Mikr. Anat. 5:386.
Azoulay, L.	1905	Les neurofibrilles d'apres la methode de Cajal. Presse Medicale, No. 2:9, No. 80:635.
Bacq, Z.M.	1932	Le transport humoral des excitations nerveuses. Essai de physiologie comparee. Ann. de Physiol. 8:917.
Ballantyne, F.M.	1925	Continuity of the vertebrate nervous system. Trans. Roy. Soc. (Edinb.) 53:663.
Barker, L.F.	1909	The nervous system. New York. 1909. 1122 pp.
Barron, D.H. and B.H.C. Matthews	1935	Conduction in the spinal cord. J. Physiol. 84: 9P.
Bartelmez, G.W.	1915	Mauthner's cells and the nucleus motoris tegmenti. J. Comp. Neurol.
Bartelmez, G.W.	1920	The morphology of the synapse in vertebrates. Arch. Neurol. & Psychiat. 4:122.
Bartelmez, G.W. and N.L. Hoerr	1933	The vestibular club endings in ameiurus. Further evidence on the morphology of the synapse. J. Comp. Neurol. 57:405.

BIBLIOGRAPHY

(3)

Bauer, Karl	1932	Beobachtungen uber das wachstum von nervengewebe 'in vitro'. Ztsch. f. Mikr. Anat. Forsch. 28:47.
Beccari, N.	1907	Richerche sulle cellule e fibre del Mauthner e sulle loco connesione in pesii ed anfibri. Arch. Ital. Anat. e Embr. 6:
Beccari, N.	1918	Arch. Ital. di Anat. e di Embr. 17:239.
Bielschowski, M.	1905(a)	Referat. Neurol. Centralbl. 24:158. Note: An excellent analysis of the recent work on the histology of the synapse by Van Gehuchten (1904), Held (1904) and Wolff (1905).
Bielschowski, M.	1905(b)	Die histologische seite der neuron-lehre. J. F. Psychol. u. Neurol. 5:128.
Bielschowski, M. and M. Wolff	1905(c)	Zur histologie der kleinhirnrinde. J. Psychol. u. Neurol. 4:
Bielschowski, M.	1931	Nerve silberimpragnationsversuche zur darstellung der neuroglia und deren ergebnisse. Ztschr. f. d. ges. Neurol. u. Psychiat. 135:253.
Bishop, G.H. and Heinbecker, P.	1930	Differentiation of axone types in visceral nerves by means of the potential record. Am. J. Physiol. 94:170.
Bjork, A. and N. Antoni	1906	Beobachtungen im trapezkern des kaninchens. Anat. Anz. 29:300.
Boeke, J.	1929	The nature of the interneuronal connections (synapses). Proc. Konink. Akad. v. Wetenschap. Amsterdam. 32:pt.2, No.6.

BIBLIOGRAPHY

(4)

Boemke, F.	1934	Artificial production of histological changes in the brain and spinal cord by electric current. Virchow's Archiv. f. Path. Anat. 293:180.
Bok, S.T.	1915	Die entwicklung der hirnnerven und ihrer zentralen bahnen. Die stimulogene fibrillation. Folia Neurobiol. 9. (cited by Kappers)
Bozler, F.	1927	Untersuchungen uber das nervensystem der Coelenteraten. Ztschr. f. Zellforsch. u. Mikr. Anat. I Teil, 5:244.
Bremer, F.	1930(a)	Nouvelles recherches sur le systeme nerveux mammalian. Compt. Rend. Soc. de Biol. 104:510.
Bremer, F. and G. Homes	1930(b)	Interpretation theoretique de la sommation d'influx nerveux. Compt. Rend. Soc. de Biol. 104:806.
Cajal, Ramon y	1934	Les preuves objectives de l'unité anatomique des cellules nerveuses. Travaux du Lab. Rech. Biol. Univ. Madrid. 29:1.
Cameron, J.	1906	The histiogenesis of nerve fibres. J. Anat. & Physiol. 40:pt.2.
Cannon, W.B.	1934	The story of the development of our ideas of chemical mediation of nerve impulse. Am. J. M. Sc. 188:145.
Coghill, G.E.	1924	Correlated anatomical and physiol logical studies of the growth of the nervous system in amphibia. J. Comp. Neurol. 37:37.

BIBLIOGRAPHY

(5)

Cole, E.C.	1930	Anastomosing neurones in the myenteric plexus of the human sigmoid flexure. J. Comp. Neurol. 50:209.
Collins, J.	1906	Neurones and neurofibrils. Med. Record. 69:958.
Cooper, Sybil	1929	The interaction between two trains of impulses converging on the same motoneurone. Proc. Roy. Soc. Lond. B.105.
Cowdry, E.V.	1932	The neurone. General character. In Cytology and Cellular Pathology of the Nervous System (Penfield). Vol.1, 1932.
Cox,	1897	Die selbstandigkeit der fibrillen im neuron. Internat. Monatschr. f. Anat. u. Physiol. 15:
Creed, et al.	1932	Reflex activity of the spinal cord. Creed, Denny-Brown, Eccles, Liddell, and Sherrington. Oxford, 1932. 183 pp.
Dale, H.H.	1934	Chemical transmission of the effects of nerve impulses. Brit. M. J. 1:835.
Davenport, H.A.	1929	Silver impregnation of nerve fibres in celloidin sections. Anat. Rec. 44:79.
Davenport, H.A.	1930	Staining nerve fibres in mounted sections with alcoholic silver nitrate solution. Arch. Neurol. & Psychiat. 24:690.
Davis, H.	1926	The conduction of the nerve impulse. Physiol. Rev. 6:547.

BIBLIOGRAPHY

(6)

De Renyi, G.S. 1932 Architecture of the nerve cell as revealed by microdissection. Special Cytology (Cowdry). 3:1369.

Dogiel, 1893 Zu frage uber den bau der nervenzellen, achsenzylinder fortsatzes. U.S.W. Arch. f. Mikr. Anat. 41:

Doi, M. 1932 Experimental untersuchen uber die nervenzellensynapse mit besonderer berucksichtigung der neurosomen Held's. Mitt. uber Allg. Path. u. Path. Anat. vii, 1.

Donaggio, A. 1905 Reseau fibrillaire. Rev. Neurol. & Psychiat. 1905:81.

Eccles, J.C. 1934 Synaptic transmission through a sympathetic ganglion. J. Physiol. 81:8 P.

Feldberg, W. and J.H. Gaddum 1934 The chemical transmitter at synapses in a sympathetic ganglion. J. Physiol. 81:305.

Feldberg, W. and A. Vartiainen 1934 Further observations on the physiology and pharmacology of a sympathetic ganglion. J. Physiol. 83:103.

Feldberg, W. and G.L. Brown 1935 Effect of potassium chloride on a sympathetic ganglion. J. Physiol. 84:12 P.

Foerster, O., O. Gagel and D. Sheehan 1933 Veranderunden an den endosen im ruckenmark des affen nach hinterwurzeldurchschneidung. Ztschr. f. Anat. u. Entwicklngsgesch. 101:553.

Foerster, O. and O. Gagel 1934 Die tigrolytische reaktion der ganglienzelle. Ztschr. f. Mikr. Anat. Forsch. 36:567.

BIBLIOGRAPHY

(7)

Forbes, A., O.C. Smith et al.	1933	The cerebral inhibitory mechanism investigated by means of antidromic impulses. Am. J. Physiol. 103:131.
Fragnito, O.	1905(a)	Su le vie di conduzione nervosa extra-cellulari. Riv. Sper. di Freniat. 31:
Fragnito, O.	1905(b)	Sur le genese des fibres nerveuses centrales et sur leur rapport avec les cellules ganglionaires. Annali di Nevroglia. 23:Fasc.1-2.1.
Fromont, M.	1893	Demonstration anatomique de la recurrence nerveuse. Compt. Rend. Soc. de Biol. 9:5:220.
Fulton, J.F.	1926	Muscular contraction and the reflex control of movement. Baltimore. Williams & Wilkins Co. 349 pp. cited by Cannon (1934).
Fulton, J.F.	1931	The functional activity of single units in the central nervous system. Science. 73:685.
Fulton, J.F.	1934	The principles of bibliographical citation. Bull. Med. Libr. Assn. 22:183.
Gerard, R.W.	1930	Nerve conduction in relation to nerve structure. Quart. Rev. Biol. 6:
Golgi, C.	1906	La doctrine du neurone. Theorie et faits. Les Prix Nobels.
Grigorieff, L.M.	1929-30	Wachstum und differenzierung der nervengewebes und seine beziehung zu anderen geweben unter bedingungen der kultur 'in vitro'. Anat. Anz. 68:129. (cited by Kappers)

BIBLIOGRAPHY

(8)

Gurewitch, M.J. 1908 Zur morphologie des fibrillaren apparates der nervenzellen in normale und pathologischen zustande. Folia Neuro-Biol. 2:197.

Hanstrom, B. 1928 Some points on the phylogeny of nerve cells and of the central nervous system of invertebrates. J. Comp. Neurol. 46:475.

Head, Henry 1920 Studies in neurology. Oxford University Press. 2 vol.

Held, H. 1897 Beitrage zur struktur der nervenzellen und ihrer fortsatze. Arch. f. Anat. u. Physiol. Anat. Abt. 259.

Held, H. 1902 Uber den bau der grauen und weisen substanz. Arch. f. Anat. u. Physiol. Anat. Abt. 189.

Held, H. 1904 Zur weitern kentniss der nervenendfusse und zur struktur der sehzellen. Abhandlungen der Mat-Phys. Kl. d. K. Geselschaft der Wissenschaften. 29:145.

Held, H. 1929 Die lehre den neuronen und vom neurocyncitium und ihre heutiger stand. Fortschr. der naturwiss. Forschung. N.F. 8.

Heringa, G.C. 1917 The intraprotoplasmic position of the neurofibrils in the axon and in the end organs. Verhandel. d. Kon. Akad. v. Wefensch. Amsterdam. 25: (cited by Kappers)

Herrick, C.J. 1931 Introduction to neurology. 5th ed. 417 pp. Philadelphia.

BIBLIOGRAPHY

Hoff, E.C.	1932(a)	Degeneration of the boutons terminaux in the spinal cord. J. Physiol. vol.74, 4 P.
Hoff, E.C.	1932(b)	Central nerve terminals in the mammalian spinal cord and their examination by experimental degeneration. Proc. Roy. Soc. B 3:175.
Hoff, E.C.	1932(c)	Unpublished observations.
Hoff, E.C.	1933	Interneuronal connections of the human spinal cord. Am. J. Physiol. 105:53.
Hoff, E.C.	1934(a)	Corticospinal fibres originating in the premotor area and their terminal distribution. Am. J. Physiol. 109:54.
Hoff, E.C.	1934(b)	Spinal terminations of the projection fibres from the motor cortex of primates. Brain, vol.57:454.
Hoff, E.C., H.E. Hoff, and D. Sheehan	1934(c)	Reflex interruptions of rhythmic discharge. J. Physiol. 83:185.
Hoff, E.C.	1935	Corticospinal fibres arising in the premotor area of the monkey; distribution of bouton terminations. Arch. Neurol. & Psychiat. 33:687.
Hoff, H.E. and J.C. Eccles	1932	Rhythmic discharge of motoneurones. Proc. Roy. Soc. Lond. B.110:483.

Hoff, H.E., E.C. Hoff, P.C. Bucy, and J. Pi-Suner. 1934 Production of silent period by synchronization of discharge of motor neurones. Am. J. Physiol. 109:123.

Holmgren, E. 1905 Über die sogenannten nervenendfusse, (Held). Jahrbuch. f. Psychiat. u. Neurol. 25: Fasc.1.1.

Ingvar, S. 1919-20 Reaction of cells to galvanic current in tissue cultures. Soc. Exp. Biol. & Med. 17-18:198.

Ingvar, S. 1923 Centrifugation of the nervous system. Arch. Neurol. & Psychiat. 10:267.

Jackson, Hughlings 1884 Evolution and dissolution of the nervous system. The Croonian Lectures. Brit. M. J. 1:591.

Jaderholm, G.A. 1905 Endozellulare netze oder durchlaufende fibrillen in den ganglienzellen. Arch. f. mikr. Anat. 67:82.

Joris, H. 1903 Nouvelles recherches sur les rapports anatomiques des neurones. Mem. Couron. Acad. Roy. de Med. Belgique. 18:1. (Excellent bibliography)

Kennedy, Foster 1935(a) Allergic manifestations in the nervous system. Trans. Am. Neurol. Assn. 1935.

Kennedy, Foster 1935(b) "Hughlings Jackson", New York Academy of Medicine, April 4, 1935.

Kibjakow, A.W. 1933 Uber humorale ubertragung der erregung von einem neuron auf das andere. Pflug. Arch. f. d. ges. Physiol. 232:432.

BIBLIOGRAPHY

(11)

Kolossow, N.G. 1933 Observations concernant l'innervation de la voie digestive chez les ruminants. Trav. du. Lab. Rech. Biol. Univ. Madrid. 28:345.

Kondratjew, N. 1933 Nature of correlations in vertebrates. Ergebn. d. Anat. u. Entwicklungsgesch. 30:341.

Kraus, W.M. and A. Weil 1926 Human adult and embryo anterior horn cells. Arch. Neurol. & Psychiat. 15:686.

Kubie, L.S. and D. Davidson 1928 The ammoniacal silver solutions used in neuropathology. Their staining properties, chemistry and methods of preparation. Arch. Neurol. & Psychiat. 19:888.

Lache, J.G. 1905 Sur les neurosomes de Hans Held. Compt. Rend. Soc. de Biol. 58:1004.

Lache, J.G. 1906(a) Sur les boutons terminaux de la cellule nerveuse. Compt. Rend. Soc. de Biol. 60:381.

Lache, J.G. 1906(b) Alterations cadaveriques des neurofibrilles. Rev. Neurol. 14:209.

Lawrentjew, B.J. 1925 Uber die erscheinungen der degeneration und regeneration im sympathischen nervensystem. Ztschr. f. Mikr. Anat. Forsch. 2:201.

Lawrentjew, B.J. 1934 Exp.-morphol. studien uber den feineren bau des autonomen nervensystems: weitere untersuchungen uber die degen. und regen. der synapsen. Ztschr. f. Mikr. Anat. Forsch. 16:383.

Leone, F. 1928 Richerche sull'apparato neuro-fibrillaire nell'intossicazione da tabacco è da tabacco ed alcohol. Rass. di studi Psichiatr. 17.2.136.

Liddell, E.G.T. 1934 Spinal shock and some features in isolation-alteration of the spinal cord in cats. Brain. 57:386.

Llombart, A. 1935 Farbung der nervendigungen mit silbercarbonat. Zentralbl. f. d. ges. Neurol. u. Psychiat. 75:631.

Lorente de No, R. 1934 Studies on the structure of the cerebral cortex. J. f. Psychol. u. Neurol. Bd.45 & 46.

Lugaro, E. 1904 Sur l'etat actuel de la theorie de neurone. Archiv. di Anat. e di Embryol. 3:412.

MacDonald, J.S. 1905 The structure and function of neurofibrils. Proc. Roy. Soc. B.76:322.

Magenot, G. 1926 Communications intercellulaires et synapses. Bull d'Histol. Applig. a la Physiol. 3:142.

Mahaim, A. 1905 Les terminations cylindraxiles pericellulaires de Held. Bull. Acad. Roy. de Med. Belgique. 1905. IV Series. 19:256.

Malone, E.F. 1928 The general relation of histological character to function in mammalian neurons. Special Cytology (Cowdry) vol.2:991.

Marinesco, G. 1904 Recherche sur la structure de la partie fibrillaire des cellules nerveuses a l'etat normal et pathologiques. Rev. Neurol. 12:405.

Marinesco, G. 1904-05 Several contributions on the pathology of neurofibrils were presented before La Societe de Biologie during 1904 & 1905. See contemporary Compt. Rend. Soc. de Biol. & Rev. Neurol.

Marui, K. 1918 On the finer structure of the synapse of the Mauthner cell with especial consideration of the Golgi net of Bethe, nervous terminal feet and the nervous pericellular terminal net of Held. J. Comp. Neurol. 30:127.

Marui, K. 1918-19 The effect of overactivity on the morphological structure of the synapse. J. Comp. Neurol. 30:253.

Matsumoto, T. 1920 Granules, vacuoles and mitochondria in sympathetic nerve fibres cultivated 'in vitro'. Bull. Johns Hopkins Hosp. 31:91.

Maximow, Alex. 1928 Development of argyrophile and collagenous fibres in tissue cultures. Proc. Soc. Exp. Biol. & Med. 25:439.

von Mayendorf, N. 1934 New conception of neurons. Rev. Neurol. 1:1024.

Meyer, S. 1896 Uber die verbindungsweise der neuronen, etc. Arch. f. Mikr. Anat. 47.

Michotte, A. 1904 Histologie fine de la cellule nerveuse. Le Nevraxe. 6:239.

Minea, I. 1934 Sur la signification de la "reaction a distance" des cellules nerveuses. Compt. Rend. Soc. de Biol. 117:1265.

Molhant, M. 1911-12 Le noyau ventral du vague. Le Nevraxe. 12-13:268.

Monckeberg, G. and A. Bethe 1899 Die degeneration der markhaltigen nervenfasern der wirbeltiere unter haupt sachlicher berucksichtigung des verhaltnisses der primitiv-fibrillen. Arch. f. Mikr. Anat. 54:135.

Monnier, A.M. 1933 Etude exp. du mechanism physico-chemique de la subordination nerveuse. Arch. Internat. de Physiol. 37:337.

Moruzzi, G. 1935 Ricerche sperimentali sulle degenerazioni transneuroniche. Zentralbl. f. d. ges. Neurol. u. Psychiat. 75:641.

Mott, F.W. 1912 The biophysics and biochemistry of the neurone. Brit. M. J. 2:780.

Nageotte, J. 1905 La structure fine du systeme nerveux. La Revue des Idees. 1905.

Nageotte, J. 1922 L'organisation de la matiere dans ses rapports avec la vie. Paris, 1922. 560 pp.

Nansen 1888 Die nervenelemente, ihre structur und verbindung in Z.N.S. Anat. Anz. 1888.

Nikolajew, W. 1893 Zur frage die innervation des froschherzens. Arch. f. Physiol. suppl. to 1893:67.

Paladino, G. 1913 Continuity in the vertebrate nervous system and the mutual and intimate connections between neuroglia and nerve cells and fibres. Review of Neurology & Psych. 11:

BIBLIOGRAPHY

Parhon, C. and M. Goldstein 1905 — Lésions secondaires des cellules motrices. Rev. Neurol. 4:205.

Parker, G.H. 1929 — The neurofibril hypothesis. Quart. Rev. Biol. 4:155.

Parker, G.H. 1935 — Neurohumors: Novel agents in the action of the nervous system. Science, 81:

Penfield, W.G. 1921 — The Golgi apparatus and its relationship to Holmgren's trophospongium in nerve-cells. Comparison during retispersion. Anat. Record. 22:57.

Peterfi, T. and S.C. Williams 1934 — Electrical excitation of cells in tissue cultures. Arch. f. Exper. Zellforsch. 16:230.

Prentiss, C.W. 1903 — Über die fibrillengitter in dem neuropil von Hirudo und Astacus. Arch. f. mikr. Anat. 62:592.

de Renyi, G. 1929 — Structures of cells in tissues as revealed by microdissection. J. Comp. Neurol., 47 & 48.

Retzius, G. 1908 — The principles of the minute structures of the nervous system as revealed by recent investigations. Croonian Lecture. Proc. Roy. Soc. v. 80:B.

Rodriguez-Perez, A. Pedro 1934 — Les terminaisons nerveuses dans les muscles des poissons. Quelques

dance du neurone. Trav. du Lab. (d'Histol.) Rech. Biol. Univ. de Madrid. 29:207-234.

BIBLIOGRAPHY

Rosenblueth, A. and D. McK. Rioch 1933 — Temporal and spatial summation in autonomic systems. Am. J. Physiol. 106:365.

Schaffer, C. 1905 — Recherches sur la structure dite fibrillaire de la cellule nerveuse. Rev. Neurol. 21:1021.

Scott, F.H. 1905 — On the metabolism and action of nerve cells. Brain. 28:506.

Sereni, E. and J.Z. Young 1932 — Nervous degeneration and regeneration in cephalopods. Pubbl. d. Staz. Zool. di Napoli. 12: Fasc.2.

Sherrington,C.S. and D.E. Denny-Brown 1928 — Subliminal fringe in spinal flexion. J. Physiol. 66:175.

Sherrington,C.S. 1931 — Quantitative management of contraction in lowest level co-ordination. Hughlings Jackson Lecture. Brain. 54:1.

Sherrington,C.S. 1932 — Chromatolysis of motor-horn cells. J. Physiol. 75:11 P.

Smirnow, A. 1890 — Die struktur der nervenzellen im sympathicus der Amphibien. Arch. f. mikr. Anat. 35:407.

Speidel, C.C. 1933 — Studies on living nerves. Am. J. Anat. 52:1.

Speidel, C.C. 1934 — Growth, irritation and repair of nerves. Arch. f. Exper. Zellforsch. 15:328.

Stefanowska, M. 1897-98 — Les appendices terminaux des dentrites cérébraux et leurs différents états physiologiques. Institut Solvay. Trav. de Lab. 3:1.

BIBLIOGRAPHY

Stohr, Ph. Jr. 1923 Ztschr. f. Anat. u. Entwicklngesch. 69:181.

Stough, H.B. 1930 Polarization of the giant nerve fibres of the earthworm. J. Comp. Neurol. 50:217.

Strongman, B.T. 1917 A preliminary exp. study on the relation between mitochondria and the discharge of nervous activity. Anat. Rec. 12:167.

Szepsenwol, J. 1934 Les relations entre la différenciation neurofibrillaire de la cellule nerveuse et son entrée, en activité. Compt. Rend. Soc. de Biol. 115:1021.

Tiegs, O.W. 1926(a) On the inadequacy of the conception of the neurone as the unit of conduction of the nervous system. Austral. J. Exp. Biol. & Med. Sc. 3:45.

Tiegs, O.W. 1926(b) The structure of the neurone junctions of the spinal cord. Austral. J. Exp. Biol. & Med. Sc. 3:69.

Tiegs, O.W. 1926(c) Further remarks on the structure of the spinal cord. Austral. J. Exp. Biol. & Med. Sc. 3:161.

Tiegs, O.W. 1927 (a) A further note on the structure of the neurone junctions of the spinal cord. Austral. J. Exp. Biol. & Med. Sc. 4:25.

Tiegs, O.W. 1927(b) The structure of the neurone junctions in sympathetic ganglia and in the ganglia of Auerbach's plexus. Austral. J. Exp. Biol. & Med. Sc. 4:79.

BIBLIOGRAPHY

Tiegs, O.W.	1927(c)	A critical review of the evidence on which is based the theory of discontinuous synapses in the spinal cord. Austral. J. Exp. Biol. & Med. Sc. 4:193.
Tiegs, O.W.	1929(a)	The microscopic structure of neurone junctions in the central nervous system. Proc. Roy. Acad. Amsterdam. 32: pt.1.
Tiegs, O.W.	1929(b)	On the neurofibrillar structure of the nerve cell. Austral. J. Biol. & Med. Sc. 6:111.
Tiegs, O.W.	1931	A study of the neurofibril structure of the nerve cell. J. Comp. Neurol. 52:189.
Tricomi-Allegra, G.	1904	I calici di Held nei centri agustici. Le Nevraxe, 6:157.
Turner, John	1905	Concerning the continuity of the nerve cells. J. of Mental Sc. 51:258.
Van Gehüchten, A.	1904(a)	Structure interne des cellules nerveuses. Le Nevraxe, 6:88.
Van Gehüchten, A.	1904(b)	Boutons terminaux et réseau pericellulaire. Le Nevraxe, 6:219.
Van Gehüchten, A.	1911	Anatomie et histologie du domaine du trijumeau. Le Nevraxe, 12:83.
Vizioli and Gozzano	1930	La resistenza delle neurofibrille nelle alterazione a tipo retrogrado delle cellule nervose. Boll. Soc. Ital. Biol. Sper. 5:1031.
Weiman, W.	1925	Studien an der Z.N.S. des mehschen mit mikrophotographie in ultraviolettenlicht. Ztschr. f. d. ges. Neurol. & Psychiat. 98:347.

BIBLIOGRAPHY

Weiss, P.	1926	The relations between central and peripheral coordination. J. Comp. Neurol., 40:241.
Windle, W.F. and S. Clark	1928	Observations on the histology of the synapse. J. Comp. Neurol. 46:153.
Windle, W.F.	1930	Normal behavioral reactions of kittens correlated with the post-natal development of the nerve-fibre density in the spinal grey matter. J. Comp. Neurol. 50:479.
Wolff, M.	1903	Über die kontinuität des peri-fibrillären neuroplasmas (Hyalo-plasma Leydig-Nansen) vorl. Mitteilung. Anat. Anz. 23:20.
Wolff, M.	1904	Das nervensystem der polypoiden Hydrozoa und Scyphozoa. Ztschr. f. allg. Physiol. 3: (cited by Kappers)
Wolff, M.	1905	Zur Kenntnis der Heldschen nerven-endfusse. J. f. Psychol. u. Neurol. 4:144.
Young, J.Z.	1933	On the cytology of the neurons of cephalopods. Quart. J. Micr. Sc. 75:1.