A CONTRIBUTION ON THE MINUTE ANATOMY OF THE SYMPATHETIC GANGLIA OF THE DIFFERENT CLASSES OF VERTEBRATES.

G. CARL HUBER.

INTRODUCTION.

EVEN a cursory review of our knowledge of the minute anatomy of any tissue or organ teaches us that the various steps in the perfection of our knowledge are in a large measure synchronous with advances made in microscopical technic. This is, perhaps, to no one more clearly shown than to him engaged in neurological work. The introduction of solutions of osmic acid and gold chloride, the Weigert's haematoxylin method, the methods of Golgi and Ehrlich, and the numerous other special methods, have, each in its turn, directed the attention of investigators to results hitherto unattained. This is especially true of the chrome silver and the methylene blue method; the results obtainable by these two methods, variously modified (embracing, as they do, a large portion of our more exact knowledge of the shape of neurons, and especially of their relation to each other and to the myriads of cells under their influence), have led many workers to devote much of their time to the investigation of various portions of the central and peripheral nervous system of vertebrates, and to some extent, also, of the still larger group of invertebrates. Of this number many, no doubt, encouraged by the earlier results of Kölliker and Ehrlich, have used these methods for the investigation of the sympathetic or ganglionic nervous system. And it is gratifying to reflect that the researches of Kölliker, Ehrlich, Aronson, Arnstein, Smirnow, Cajal, Van Gehuchten, V. Lenhossék, Sala, Retzius, d'Erchia, Dogiel, and others have very much broadened our knowledge concerning this system, many points having been observed so often that they are beginning to be accepted as demonstrated facts.

My reason for presenting the following research may, if such reason be required, be briefly stated as follows :

It seemed to me desirable to confirm some of the observations previously made, with what seemed to me somewhat improved histological methods, hoping at the same time to throw new light on some of the still disputed questions, and especially to broaden our knowledge of these structures by systematically investigating the sympathetic ganglia of types from the several classes of vertebrates, using in each case the same methods.

During the investigation, material was obtained from the following vertebrates :

FISHES.

Ambloplites rupestris (Raf.), rock bass.

Micropterus dolomieu (Raf.), small-mouth black bass.

Perca flavescens (Mitch.), perch.

AMPHIBIA.

Rana Catesbiana. *Rana* Hal.

REPTILIA.

Chrysemys picta. *Chelydra serpentina*.

Emys melegaris.

BIRDS.

Gallus domesticus — chicken.

MAMMALIA.

Dog, cat, rabbit, and guinea pig.

The subject-matter to be discussed in this research will be taken up as follows :

- 1) A brief discussion of the methods used.
- 2) A descriptive account of the results obtained in the preparations made from each of the above classes of vertebrates, in the order above named, with the literature bearing on the subject.
- 3) The general conclusions which may be drawn.

Method.

In the earlier portion of this work both the chrome silver and the methylene blue method were used ; the former was, however, soon discarded, owing to its greater uncertainty ;

largely, however, because in my hands more definite results were obtained with the methylene blue method. For the past two years the latter method alone has been used, and the results here presented are based exclusively on observations made with it.

Ehrlich's methylene blue method has so often been modified in one way or another, that, in order to discuss it at all, it seems quite imperative to take it up historically. Riese (1) has, however, collected all the literature bearing on this method, appearing before 1891; for the earlier modifications, therefore, the reader is referred to this summary. Since that time the most important modification of the method has been suggested by Bethe (2), who, in recommending the use of ammonium molybdate to convert the unstable methylene blue compound, as formed in fresh tissues, into one practically insoluble in water, alcohol, and the imbedding media, has given us a method by which methylene blue stained tissues may be imbedded in paraffin, sectioned and counterstained at will. Too much cannot be said for this important addition to the method suggested by Ehrlich.

The fixative suggested by Bethe has the following composition:

Ammonium molybdate	1 gm.
Aqua dest.	10 c.cm.
Hydrogen peroxide	1 c.cm.
Hydrochloric acid	1 gtt.

Into this solution, cooled to $+2^{\circ}\text{C.}$, the stained tissues are placed for four to five hours; are then washed in water, dehydrated in alcohol, and imbedded in paraffin. Meyer (4) suggests the omission of the hydrogen peroxide from the above formula, because, as he believes, it has a bleaching action — “etwas bleichend gewirkt hat.” Bethe (3) in a more recent communication has, however, drawn attention to the fact that this strong oxidizer is not, as such, present in the fixing fluid, because it at once unites with the *molybdänsauren ammonium*, and this has in the main lost the properties of the hydrogen peroxide. For a further discussion of this question the reader is referred to Bethe's (3) recent article. I may, however, add that before Meyer's paper came into my hands similar conclusions had

been reached by me; namely, that better results were obtained when the hydrogen peroxide was omitted from Bethe's formula. It may be stated that it has always been my plan to expose the ganglia, or other tissues to be stained, to the air, either in the animal or on the slide, until all the elements which it was thought would stain were clearly seen under the microscope; or, in other words, until the leukobase, the tetramethyldiamido-thiodiphenylamin, which is formed by the living tissues when methylene blue comes in contact with them, is again oxidized, as it is in the presence of air. Hydrogen peroxide, or, as used in the formula, *hypermolybdänsauren ammonium*, would in such cases be unnecessary, and does, to some extent at least, as Meyer suggests, have a bleaching effect. I wish further to call attention to other modifications which Bethe (3) himself has suggested. He has tried to obviate the necessity of placing the tissues into an "ice-cool mixture," as his earlier method requires, by changing the unstable methylene blue stain, as found in fresh tissues, into one insoluble in water. To this end he places the fresh tissue in a saturated aqueous solution of ammonium picrate (suggested by Smirnow and Dogiel), and, at the expiration of fifteen to twenty minutes, transfers them to a solution prepared after one of several formulas, one of which is the following:

Ammonium molybdate	1 grm.
Aqua dest.	20 c.cm.
Hydrochloric acid	1 gtt.

Tissues two to three millimeters in thickness remain in this second solution three-fourths to one hour.

My experience with this modification of Bethe's earlier method has not been extended; as far as it goes, I have been led to conclude that the prefixation in ammonium picrate is open to a number of objections.

In the first place, picrate of ammonium seems, even in the short time the tissues are exposed to it, to act more or less as a macerating fluid, which is obviously an objection where the relation of end fibrillae to cells is to be investigated. Secondly, tissues prefixed in the picrate solution do not stain so readily as when the molybdate alone is used.

The methylene blue method, as used by me in this research, may be briefly described as follows: A 1%, 2%, or 4% solution of methylene blue (Grübler's rectificiert nach Ehrlich), made in a normal salt solution, was injected into a vein, easily accessible. In frogs, the large lateral cutaneous vein; in Reptilia, the jugular; in birds, the humeral (Owen); in Mammalia, the jugular or femoral, usually the former, were used. As to the percentage of the solution used, no definite statement can be made; the above solutions were used with equally good results in some experiments, and equally unsatisfactory results in others. In a general way it may be said that the stronger solutions stained more readily the cell body and branches of the sympathetic neurons, while the weaker solutions brought to view more clearly the pericellular plexuses; these statements are, however, open to many exceptions.

The quantity injected varied with the size of the animal used; from 2 to 4 c.cm. for a frog, to 60 to 80 c.cm. for a dog. The solution was allowed to flow into the circulation until the animal became blue, or until the heart's action was stopped.

Forty-five minutes to one hour after the injection, the ganglia or tissues to be examined were exposed; the larger ganglia, with the afferent and efferent nerves, were freed from the surrounding tissues, but not removed until they assumed a blue color, when they were excised and placed on a slide, and, if the staining seemed satisfactory, were placed in the fixative. Smaller ganglia were at once removed to a slide moistened with normal salt, and examined from time to time, until the stain was developed, when they were also placed in the fixative. The fixative used by me had the following composition:

Ammonium molybdate	1 gm.
Aqua dest.	10 c.cm.
Hydrochloric acid	1 gtt.

The molybdate is ground in a mortar and the water added; the solution is then removed to a flask and heated until perfectly clear; the hydrochloric acid is then added. The solution so made is placed in small glass jars, and these are surrounded

with snow or ice. The solution is best made some time before the injection, so that it may be properly cooled before using. In the fixative the tissues remain three to five hours. They are then washed in distilled water for about one hour and dehydrated and hardened in absolute alcohol, placed in xylol and imbedded in paraffin. As a rule, the ganglia were cut into serial sections, fixed to slide with the albumen fixative, and mounted in Canada balsam; other series were double-stained in alum carmine before mounting.

My experience as to the durability of preparations made in this way has not been the most satisfactory, although, as a rule, they have kept without fading for several months. Some of the sections (ganglia of Amphibia) have been well preserved for over two years, and are only now beginning to show signs of fading; others (sections of sympathetic ganglia of the chicken) began to fade at the end of several weeks. My experience would go to show that sections double-stained in alum carmine keep better than such as are stained only in the methylene blue. Why some sections should seem to fade more readily than others I am unable to say; it would seem, however, that sections thoroughly dehydrated and thoroughly washed in oil of bergamot and xylol fade less quickly than those where these precautions are not taken. Sections exposed to light fade more readily than those kept in the dark. Bethe (2) in discussing this phase of the matter says: "Die Haltbarkeit der Präparate, die mit der einfachen Fixation hergestellt sind, ist keine unbedingte. Sehr dicke Präparate zeigen oft schon nach zwei bis drei Monaten zuerst ein Dunkelwerden des Canadabalsams, auf den Trübung des Protoplasmas und Diffuswerden der Färbung folgt." "Schnitte halten sich besser, vielleicht deswegen, weil hier der Ueberschuss von Ammoniummolybdät, von dem in Verbindung mit Resten von Alkohol die Zerstörung auszugehen scheint, besser ausgewaschen wird."

In attempting to stain the sympathetic ganglia of fishes by injecting methylene blue into the circulation (through the caudal vein), it was found that the loose adipose tissue in the gill region, in front of the vertebral column, by the removal of which a number of small ganglia were brought to view, stained

so deeply that after an injection a search for them was usually fruitless. In fishes, therefore, it was found advisable to remove the ganglia, unstained, to a slide or watch crystal, and, following Dogiel's suggestion, to stain them in a $\frac{1}{2}\%$ solution of methylene blue in normal salt. A portion of a ganglion thus treated was usually stained in forty-five minutes to one hour, the staining being controlled under the microscope. The tissues so stained were then fixed in ammonium molybdate, the further treatment being as above described.

In attempting to stain the tissues on the slide, I have tried a number of methods, which, in the hands of other investigators, have given good results. I may especially mention the methods recommended by Lawdowsky (5). He suggests the dilution of the methylene blue with one of the following solutions: Blood serum, egg albumen, a $\frac{1}{10}-\frac{1}{5}-\frac{1}{2}\%$ solution of ammonium chlorate, or ferrum ammonium chloratum. The successful staining obtained by Lawdowsky has not been realized by me; on the contrary, results obtained with methylene blue diluted in normal salt have been much more satisfactory.

In closing the discussion of the methylene blue method, I need hardly add that, even with the greatest care, negative results are only too frequent. Why, when this method is used over and over again in exactly the same manner, in some instances successful staining is obtained, in others only failure, I am unable to say; but such is the case. Then, of course, it must be remembered that the investigator is always at a loss to know whether the preparation before him tells the whole story, or only a portion of it; whether, in other words, the structure before him is completely or only partially brought to view by the methylene blue.

SYMPATHETIC GANGLIA OF FISHES.

The sympathetic system of teleosts, to which subclass my investigations were confined, consists of a cephalic, a trunkal, and a caudal or post-anal portion, made up of a series of ganglia, united by intervening nerves into two cords lying close to the vertebral column. The cephalic portion extends forward

under the place of exit of the vagus, glosso-pharyngeal, and facial nerves, and sends branches to the trigeminus; at the point of union of the sympathetic with the cranial nerves mentioned, small sympathetic ganglia are usually found. At the anterior end of the trunk the two chains approach each other and present a relatively large ganglion — the splanchnic ganglion. In the trunk the two chains are found immediately under the vertebral column above the kidney, in which they may in part be imbedded. The caudal portion is found within the haemal arch, accompanying the aorta. The trunkal and caudal portions receive rami communicantes from the anterior roots of the spinal nerves. At the point of junction of the rami with the sympathetic cords small ganglia are found, which may be of microscopic size, or large enough to be recognized with the naked eye. This brief description of the sympathetic system of fishes is taken from Stanius's *Handbuch der Zootomie*, Zweiter Theil, Die Wirbelthiere, Zweite Auflage, pp. 143–146. The ganglia especially studied by me were the splanchnic and several small ganglia found in connection with the cardiac and intestinal branches of the vagus and the cells of Auerbach's plexus.

Literature. — The literature bearing on this portion of the subject is very meager. In the literature at my disposal I have found no observations on the structure of the larger sympathetic ganglia of fishes, made with the Golgi or methylene blue method. Monti (6) has used the Golgi method in studying the intestinal canal of teleosts with reference to its nerve supply, and has described small ganglia, composed of multipolar cells, possessing a single, unbranched axis-cylinder process. These ganglia take part in the formation of a plexus found in the submucosa, and from it fibers are given off which form a plexus in the muscularis mucosa. This latter plexus is continuous with a periglandular plexus, in which nerve cells, also with a single axis-cylinder process and surrounded with a very complicated network of fine fibrillae, are found. Sakusseff (7), a pupil of Dogiel, has stained the nerve plexus in the intestine of fishes with methylene blue. I was, however, not able to obtain the original article. Dogiel, in his brief reference to

this work, draws attention only to the fine fibers which leave the plexuses, passing through the mucosa to reach the epithelium.

The ganglia studied by me varied in size from such as were just recognizable with the naked eye to others about $\frac{1}{2}$ mm. in their longest diameter. They were most often recognized as spindle-shaped swellings in the course of one of the vagus branches. They were removed to a slide and stained in $\frac{1}{20}\%$ methylene blue solution in normal salt; fixed in ammonium molybdate and teased or sectioned, some of the sections being further stained in alum carmine. The ganglia are surrounded by a fibrous capsule, which is continuous with the perineural sheath of the nerves connected with the ganglion.

In sections, sympathetic nerve cells, medullated fibers, very small medullated fibers, and Remak's fibers may be seen.

Shape and Structure of the Ganglion Cells.—The shape of the nerve cells in the sympathetic ganglia of fishes varies. The cell body may be more or less regularly round or oval, and from it one or several processes may have their origin. The cells may therefore be unipolar or multipolar. In cells not too deeply stained in methylene blue the protoplasm appears granular, the granules staining more deeply than the remaining portion of the cell. The granules are very small, and evenly distributed through the protoplasm. The granules are probably the chromophile granules described for other nerve cells. In case the cell is deeply stained, it assumes a diffuse blue color, the granules showing only very indistinctly. In my preparations the nucleus was sometimes stained more deeply, again less deeply than the protoplasm, usually showing no distinct structure, although in preparations double-stained with alum carmine a nucleolus may now and then be made out. The cell body is invested in a nucleated capsule.

The neuraxis arises from a cone-shaped extension of the cell body. (See Pl. III, Fig. 1, *a-e*.) In sections it is sometimes difficult to make out with any degree of certainty which of the several processes is the neuraxis; for instance, *e*, of the above figure. In the neighborhood of the cell the neuraxis is non-medullated; whether at some distance from the cell it

becomes surrounded with a sheath of myelin, I have been unable to determine.

The dendrites vary in number; four to six are usually seen. They are quite short, and in my preparations do not undergo much branching; this of course may be due to imperfect staining. The dendrites terminate between the ganglion cells, are extra-capsular, and form a loose network, the arrangement of which depends in part on the number and relative position of the adjacent neurons.

Medullated Fibers of the Ganglion.—In sections a large number of medullated fibers are seen in the sympathetic ganglia of fishes. Many of these pass through the ganglia without giving off any branches. This is especially the case in the smaller ganglia, which are recognized as spindle-shaped enlargements in the course of a nerve trunk. Other medullated fibers, which give off one or several branches in the respective ganglion, are frequently met with. These branches, which are usually smaller than the parent fiber, end in a network on the cell bodies of the sympathetic cells. This network varies much in complexity; and in double-stained preparations it may easily be seen that this network is *intra-capsular*. It may be quite simple, as may be seen in Pl. III, Figs. 2 and 3, where only the network is shown. In Fig. 2, *a*, the portion of the neuraxis shown (a collateral branch of a medullated fiber), divides into four smaller branches, which anastomose to form a pericellular plexus; in Pl. III, Fig. 3, both the neuraxis and the terminal fibrillae are very small and beset with varicose enlargements. This pericellular plexus enclosed a long oval cell, lying between the nerve fibers of a ganglion; the cell was only faintly perceptible and is outlined in black; the processes were not made out. In Fig. 4 is shown a more complicated ending. The large axis cylinder, *a*, which was surrounded by a medullary sheath (not shown in the figure), breaks up within the capsule into a number of branches, some of which pass over the cell, others ending between the cell and the capsule in a number of branching, twisted, or coiled fibrillae. In Pl. III, Fig. 5, the same cell is again represented, after staining in alum carmine (the preparation from which Fig. 4 of Pl. III was sketched was broken

down, stained, and remounted). The latter figure may serve to show that the end fibrillae shown in Pl. III, Fig. 4, terminate between nuclei found within the capsule. In all instances (see also Pl. III, Figs. 6 and 7) where this more complicated ending was observed, the cell body of the sympathetic cell seemed imbedded in these nuclei; some of which, no doubt, belong to the cells of the capsule; others, if my observations are to be relied on, are within the capsule and seem to be grouped more particularly about that portion of the sympathetic cell from which the neuraxis arises. The number of the nuclei varies; in Pl. III, Fig. 6, sketched from a cell obtained by teasing a small ganglion, and which was completely isolated from the surrounding structures, I estimated that ten to twelve nuclei are within the capsule. The capsule enclosing this cell (*c*) was very clearly made out. The branches of the nerve fiber ending within the capsule are clearly shown in the figure, they alone being stained in methylene blue, the other structures taking the alum carmine. In Pl. III, Fig. 5, above referred to, and in Pl. III, Fig. 7, the number of these nuclei is far greater. In the latter figure, which was also sketched from a teased preparation, may be seen the neuraxis of a large medullated fiber, from which two non-medullated collateral branches (*a'* and *a''*) are given off, these terminating within the capsule of the sympathetic ganglion cells (*A* and *B*) in a system of varicose end branches, the majority of which end between the intracapsular nuclei.

It may be of interest to note that Arndt (8) has diagrammed a cell which resembles very closely the one shown in Pl. III, Fig. 6. The cell referred to is reproduced by him in Pl. XIV, Fig. 38. Arndt, in his account, describes it as coming from a sympathetic ganglion of *Perca*, macerated in a $\frac{1}{10}\%$ solution of acetic acid.

I have not been able to formulate any definite conclusions as to the nature of the nuclei above mentioned. I beg, however, to be allowed to give expression to a hypothesis which has often suggested itself in studying the preparations made from the sympathetic ganglia of fishes, but more particularly from similar preparations made from Reptilia; namely, that these

nuclei may belong to very much branched neuroglia cells, only the nuclei staining in the preparations at my disposal. This hypothesis is here only mentioned, and will be further discussed subsequently.

The medullated fibers ending in the pericellular plexuses may now and then be traced into some nerve root coming to the ganglion; their further course has not been ascertained.

A few very small medullated fibers are occasionally stained in sections of the sympathetic ganglia of fishes. My observations on these do not allow of any definite statement concerning them.

The non-medullated fibers seen in the ganglia are no doubt, to a large extent, the neuraxes of the sympathetic cells, constituting the ganglia, although in sections it is an exceedingly difficult task to trace such non-medullated fibers to a ganglion cell; I was able to do so only a few times. Small bundles of them may be traced into the nerves leaving the ganglia.

SYMPATHETIC GANGLIA OF AMPHIBIA.

The sympathetic system of the frog comprises a series of ganglia, lying on each side of the vertebral column, united by intervening nerves to form the ganglionated chains. The number of sympathetic ganglia usually corresponds to that of the spinal nerves, ten pairs of ganglia being found. Numerous smaller ganglia are found in the walls of the various organs — Bidder's and other ganglia in the heart; ganglia in the lungs, the pharynx, the intestinal canal, and the bladder. Sections were usually made of the larger ganglia — the first and second (this being the largest), and seventh, eighth, and ninth. The last three, on account of their size, the length of their rami, and their exposed position, are very easily found.

The methylene blue method was used to the exclusion of other methods. The stain was injected into the circulation of the living frog. The larger ganglia were fixed in ammonium molybdate, sectioned, and some of the sections double-stained in alum carmine. The smaller ganglia were fixed in ammonium picrate and cleared in glycerine.

Literature. — The ganglion cells of the frog's sympathetic were first described by Beale and Arnold. In Beale's (9) account the following statement may be found: "In the fully formed cell a fiber comes from the center of the cell (straight fiber), and one or more fibers (spiral fibers) proceed from its surface." "These are wound spirally around the first fiber." Arnold (10) described similar bodies in connection with the nerves in the frog's lung, believing them to be peculiar to this organ. In Arnold's (11, 12) later publications he states that these cells were found generally in the sympathetic system of the frog, and gives a much more detailed account of the straight and the spiral processes. The straight fiber he was able to trace through the protoplasm of the cell to the nucleolus, while the spiral fiber had its origin in a network of fine fibrillae connected with the branches of the nucleolus. (See Pl. I, Figs. 3, 4, and 6, Virchow's *Archive*, Vol. XXXII.) Courvoisier (13) corroborated many of the observations made by Arnold. His results may be summed up as follows: The straight fiber begins or ends in the nucleus, while the spiral fiber has its origin in a network which is connected to the nucleolus by "root fibers" — *Wurzelfäden*. This network gives origin also to "commissural fibers," which connect the network of adjacent cells. This network was also described by Kollman and Arnstein (14). On the other hand, Schwalbe (15) stated that the sympathetic ganglia of frogs contained two kinds of cells — bipolar cells, with straight and spiral fibers, the latter arising from the cell body and making only a few turns around the straight fiber, and unipolar cells, with only the straight process, with a prominent capsule, the thickenings or folds of which may simulate a spiral fiber. This latter form Schwalbe believed to be the more common.

Arndt (16), while not denying the nervous nature of the spiral fiber, suggests that the fibrillar structure of the capsule may in some instances be mistaken for a spiral fiber, inasmuch as the fibrillae of the capsule are now and then arranged transversely to the axis of the straight fiber as it leaves the cell.

Axel, Key, and Retzius (17) made further contributions by showing that the spiral fiber became invested with a medullary

sheath at some distance from the cell body; thus for the first time pointing out conclusively its nervous nature.

The true nature of the spiral fiber and the network, which had been described by some of the earlier investigators, and their relation to the protoplasm and the straight process of the cell, were, however, not understood until Ehrlich's methylene blue method became known. Ehrlich (18), in his first communication on the reaction of methylene blue on living nerve tissues, gives a very clear description of the spiral fiber and network. I take the following brief account from this article: "The spiral fiber, which alone was stained blue, divides into fine fibrillae, which unite to form a network, which network may enclose the entire cell or only a portion of the cell. From this network fine fibrillae are given off, which end on the cell in small terminal swellings. This mode of ending of the spiral fiber was observed in all the larger ganglia examined; also in the smaller ganglia found in the heart, bladder, lung, and pharynx." The fact that the spiral fiber was medullated led Ehrlich to believe that it was a cerebro-spinal fiber, and that its course was centralward, and to express the opinion that it conveyed impulses from the nerve centers to the sympathetic ganglia through the network about the cell. Ehrlich's observations were soon corroborated by Aronson (19), and by Arnstein and his pupil Smirnow. In the account given by Arnstein (20) the network formed by the end branches of the spiral fiber is described as a closed one; the terminal fibrillae mentioned by Ehrlich and Aronson were only occasionally seen, and then only when the network was imperfectly stained. Arnstein states that they found it extremely difficult to determine which direction—centralward or toward the periphery—the spiral fiber takes on leaving the cell; often it was impossible to decide. In the sympathetic cells found in the pharynx of the frog, the spiral fiber went to the periphery, often branching in its course. Retzius (21), in a short but very comprehensive account of his observations on methylene blue stained sympathetic ganglia of the frog, adds a number of details to observations previously made. He here points out that the terminal network of the spiral fiber is always within the capsule of the

ganglion cell. The network is described as a closed one with varicose fibrillae. At the nodal points of the network, nodular enlargements are often seen; these vary in shape and number. He further observed that the spiral fiber became myelated at some distance from the cell, and that it often divided into two branches, — "*tubes en T*," — the two arms being often traced in divergent directions. (See Fig. 3 of his article.) Retzius looks upon the spiral fiber as of cerebro-spinal origin, and reaches the following conclusions as to its function: "Nachdem die Spiralfasern sich getheilt haben, schicken sie den einen Arm an eine sympathische Ganglienzelle, um in dieser Weise eine Verbindung mit ihr einzugehen."

Lawdowsky (22) (whose account I have not been able to obtain) and Feist (23) have also written on the frog's sympathetic. The latter's observations corroborate in the main those made before him; he finds, however, a different explanation for the network and spiral fiber. Feist, while admitting that the spiral and network often stain alike, does not regard this as sufficient reason for regarding them as identical in structure. The conditions, he says, may just as well be the following: "The ganglion cell is united to the capsule through a layer of protoplasm, arranged in the form of a network. The spiral fiber, as it approaches the cell, pierces the protoplasmic network at some nodal point and then enters the substance of the cell."

Feist (23) also finds, as his Figs. 20, 21, and especially 22, may show, that the straight process undergoes division at some distance from the cell. As a result of his investigations he reaches the following conclusion, which is so at variance with results previously obtained that it will be given in full: "Nach dieser Beobachtung rückt die bipolare Ganglienzelle des Froschsympathicus in das Schema der multipolaren Hirn- und Rückenmarksganglienzellen der Säuger ein, nur mit dem Unterschiede, dass bei jener die verästelten Fortsätze nahe bei einander von einer weit vom Centrum der Zelle entfernten Stelle, der Theilungsstelle der geraden Faser, abgehen, welche letztere hiernach nur eine lang ausgezogene Partie der Zellsubstanz darstellt." If I understand Feist correctly, the spiral

process would thus become the neuraxis of the cell, the straight process, with its branches, the dendrites; a conviction entirely at variance with the facts in the case, as will be subsequently shown.

Before concluding the review of the literature bearing on the structure of the sympathetic ganglia of Amphibia, mention must be made of Smirnow's (24) later publications on the subject. I wish, however, at this place to draw attention only to that portion of his writings in which he discusses the relation of the network to the spiral fiber and its further course after leaving the cell.

According to Smirnow, the spiral fibers extend toward the periphery, and are, according to the location of the nerve cell, distributed to various peripheral tissues. "They form anastomoses with other cells, in so far as they may join the processes of other cells; or may become continuous with the network enclosing other cells; or they may be distributed to heart muscle or form vasomotor fibers to the blood vessels. The distribution of the spiral fibers is such that, as a rule, a portion of it serves to form anastomoses with other cells, while the remaining portion goes to muscle tissue or to blood vessels."

Nearly all writers who, after Ehrlich, have used the methylene blue method in their investigation of the sympathetic ganglia of Amphibia, are agreed that the spiral fibers and the network enclosing the cell body are continuous, and that they stain more readily than do the other structures of the ganglion. There exists, however, a difference of opinion as to the nature of the spiral fibers; Ehrlich (18), Aronson (19), and Retzius (21) looking upon them as cerebro-spinal fibers, while Arnstein (20), Smirnow (24), and Feist (23) ascribe to them other properties.

The results obtained by the writer will be taken up as follows:

- 1) Sympathetic ganglion cell of Amphibia, its structure, the straight process and the capsule of the cell.

- 2) The spiral fibers and the pericellular network and the course of the spiral fiber after it leaves the straight process.

Sympathetic Ganglion Cell of Amphibia. — It may be stated at the outstart that the writer regards the sympathetic neuron

of Amphibia as a unipolar cell, adopting in this respect Kölliker's (25) view. In methylene blue stained preparations the cell body of such neurons remains, as a rule, wholly unstained. In such sections, double-stained in alum carmine, its outline may, however, be clearly made out, as Pl. III, Figs. 9 and 10, may show.

In such sections it may be seen that the cell body is more or less regularly round or oval, becoming attenuated at one end, from which portion arises the straight process, *a*, of Pl. III, Figs. 9 and 10 respectively. The straight process often remains unstained; in some cells, however, it is stained faintly blue, and in such cases it gradually loses its blue color as it approaches the cell body, becoming continuous with the drawn-out portion of the cell. Smirnow (24) states that the straight process may in some cases take on a medullary sheath (see Fig. 16 of Smirnow's article). This I have not seen. In sections only relatively short, straight processes are seen; they are not, as far as my observations go, medullated near the cell body. In sections of the nerve roots coming from the sympathetic ganglia, very small medullated fibers may now and then be seen, no doubt medullated sympathetic fibers such as Smirnow describes, and as were seen many years ago by Kölliker and others in the sympathetic nerves of the frog. The great majority of the sympathetic fibers of the frog are, however, non-medullated. Schwalbe, Feist, and Smirnow state that the straight process may undergo branching at a variable distance from the cell body. The former (Schwalbe and Feist) regard the straight process as a dendrite, if I understand Feist correctly, as has been stated in the review of the literature. Smirnow (24) likens the branching seen by him (Fig. 11 of his article) to a "T"-shaped process, as seen in the spinal ganglia. He leaves it an open question whether all straight processes divide in this way.

In sections of larger ganglia such a division was never seen by me. The straight process is here, as a rule, so indistinctly stained that it could not be traced for any distance. In surface preparations of the bladder and pharynx, stained, *intra vitam*, in methylene blue, fixed in ammonium picrate, and cleared in

glycerine, I was, however, able in a number of instances to trace the straight process of a sympathetic cell for some distance from the cell body, and in one preparation of the pharyngeal mucous membrane and the oesophagus of a frog prepared as above stated, a straight process coming from a sympathetic cell, which divided into two branches, was clearly made out. These branches could be traced toward a small gland situated in the upper part of the oesophagus, where both underwent a second division; from here one of the resulting branches could be traced on to one of the alveoli of the gland, where it was lost to view. The appearance here presented seemed to indicate that the straight process extended toward the periphery, and in this case innervated the small gland above mentioned, although the ultimate endings on the gland cells could not be made out. In the few cases seen by me, where the straight process branched, this did not simulate a "T"-shaped branching as described by Smirnow; the branching was usually at an acute angle. The arrangement seen by him I should regard as accidental. The observations made by me led to the conclusion that the straight process is the neuraxis of the unipolar sympathetic cell of Amphibia, innervating gland and non-striped muscle tissue, as my observations on the bladder of the frog would show. This conclusion is, therefore, wholly in accord with the following statement made by Kölliker (25): "Die geraden Fasern sind nicht Dendriten oder Protoplasmafortsätzen zu vergleichen, sondern einfach Achsencylinderfortsätze der betreffenden Zellen, die zu Muskelfasern treten und hierbei Verästelungen zeigen."

The statement has been made that the sympathetic neurons of Amphibia were unipolar cells. As an exception to this, I may say that the sympathetic cells found in the wall of the intestinal canal (stomach, small and large intestine) of frogs are distinctly multipolar, as the two cells reproduced in Pl. III, Fig. 11, may show. These cells were sketched from a preparation of the large intestine of a frog, stained, *intra vitam*, in methylene blue, and fixed in ammonium picrate; in the figure the purplish color assumed by methylene blue stained tissue when fixed in the picrate is not reproduced. The cell body of

these neurons is very irregular; the neuraxis, *a*, which in the cells sketched could be traced for a long distance as an exceedingly fine fiber, was lost in the network of Auerbach's plexus. The dendrites, *b*, have a very irregular, jagged appearance, perhaps better shown in the figure than can be described. Such multipolar cells were seen only in the intestinal canal; in all other sympathetic ganglia of the frog known to me the cells are unipolar.

Pericellular Network and Spiral Fiber.—In well-stained methylene blue preparations of any sympathetic ganglion of a frog it may be seen that the cell body of each neuron constituting said ganglion is enclosed in a pericellular network or plexus. In such preparations, double-stained in alum carmine, it may readily be seen that this network is intra-capsular (see Pl. III, Figs. 9 and 10, *c*, capsule of cells), and is in contiguity with the enclosed cell body, but never continuous with it. The arrangement and structure of the fibrillae constituting the network are very variable, as may be seen from Pl. III, Figs. 8–10. The fibrillae may be very loosely or more densely woven; their arrangement seems largely accidental. The fibrillae may be smooth or varicose. This apparent difference in structure seems to depend somewhat on the length of time the tissues are exposed to the air before fixing them. It will be remembered that with this method the nerve tissues are usually almost colorless about one hour after the injection, the interval of time allowed to elapse before the ganglia are exposed; if, on exposing the ganglion to the air, they become blue in a few moments, as they often do, the fibrillae of the network have a much smoother appearance than when it is necessary to expose them ten, fifteen, or even twenty minutes before the stain has developed, in which case the fibrillae usually present a very varicose appearance. Now and then quite large nodular masses, stained deeply blue, may be seen at some nodal points in the pericellular network, *d*, of Pl. III, Figs. 8 and 9; Retzius (21) has also described such nodular enlargements. The question as to whether the network is an open one, as described by Ehrlich (18), Aronson (19), and Lawdowsky (22), or a closed one, — Arnstein (20), Smirnow (24), and Feist (23), — has, I believe, been most cor-

rectly answered by Retzius (21), who states that, as a rule, the fibrillae form a closed network, but that now and then free-ending fibrillae may be observed. In Pl. III, Fig. 8, which represents a portion of a section of the sympathetic ganglion on the second spinal nerve of a frog, in which the structures were very well stained in methylene blue, I have indicated by the letter *e* a number of instances where fibrillae seem to end free, usually in a small nodular enlargement. That the network is continuous with the spiral fiber seems evident to all investigators, with the exception of Feist (23), who have studied the sympathetic ganglia of Amphibia with the methylene blue stain. Most often the network and spiral are alone stained, and in sections of $20\ \mu$ to $40\ \mu$ thickness the spiral fiber may often be traced with the utmost clearness through the "granular mass" into the fibrillae of the network; the granular mass, *g*, of Pl. III, Figs. 8-10, forming a more densely woven portion of the network, as has been pointed out by Retzius (21).

My own observations on the course of the spiral fiber after it leaves the straight process lead me to conclude that its course is centralward; or, to express it in another form, the pericellular plexus and spiral fiber are parts of a neuron, the cell body of which is situated more centrally than the respective sympathetic ganglia, in which the structures are found. These conclusions are based on observations made on ganglia prepared in the following way: a small quantity ($\frac{1}{2}$ to 1 c.cm.) of a 1% solution of methylene blue was injected into the circulation of a small frog. At the expiration of about an hour the entire sympathetic system was exposed. The entire sympathetic chain on one side, with the rami communicantes and segments of the spinal nerves from which they had their origin, was then removed and placed on a slide moistened with normal salt. As soon as the stain seemed developed as much as it would, the preparation was fixed in ammonium picrate and cleared in glycerine. When properly fixed and cleared, the entire chain was placed on a slide, where all the surrounding tissues were removed with teasing needles, care being taken not to tear any of the rami from the ganglia. The entire chain, thus freed from all connective tissue, was then mounted in ammonium

picrate and glycerine. By injecting only a small quantity of the methylene blue stain, as above stated, now and then only a few nerve fibers were stained in some of the ganglia of the chain, the ganglia clearing in the glycerine to such an extent that such fibers could be followed for long distances, even under the oil immersion. In such preparations it was often possible to trace a medullated fiber from a white ramus into a ganglion, or from the interganglionic nerve trunk into the respective sympathetic ganglion, and through several branchings to its ending in a spiral and pericellular network.

Some of my best examples are reproduced in Fig. I. I may say that the fibers here sketched were drawn under a $\frac{1}{12}$ in. oil immersion lens, with the camera lucida, and drawings reduced 5 or $2\frac{1}{2}$ times, as stated in the description of the figure. In the above figure, x by the side of a fiber indicates a node of Ranvier, and y the change of a medullated to a non-medullated fiber. In fiber A is shown the neuraxis of a large medullated fiber, which entered the first sympathetic ganglion through a nerve uniting the ganglion with the vagus ganglion; the fiber was traced for a distance much greater than shown in the figure. Soon after entering the ganglion this nerve divided into two branches, both of which were medullated. The right branch was traced through three nodes of Ranvier, when it became non-medullated. After a short distance this non-medullated fiber divided into two branches, one of which was traced into a spiral fiber and pericellular basket; the other terminated abruptly. The left branch of fiber A was traced through four nodes of Ranvier, where a small collateral branch was given off (b in the figure), which could be followed for only a short distance. At the succeeding node the fiber became non-medullated and divided into three branches, c , d , and e . Branch c was traced to a pericellular network; branch d , reflected upon itself at d' , divided into two branches, one of which ended in a spiral and network; branch e was followed a long distance, when it also terminated in a spiral and pericellular network. Fibers B , C , and D are sketched from the eighth sympathetic ganglion. In each case the fiber sketched shows only a short segment of the neuraxis before branching; these

FIG. I. — Spiral fibers from sympathetic ganglia of frog. (See text for description.)

were traced in every instance into a white ramus of the ganglion.

Fiber *E* may be explained somewhat more fully, as it explains, I believe, some observations made by Retzius (21) and Smirnow (24). This fiber entered the eighth sympathetic ganglion through a white ramus, and could be traced through a number of nodes of Ranvier, only two of which are shown. At the second node shown in the figure there was given off a non-medullated collateral branch, which terminated in a spiral and pericellular plexus; two nodes further on the fiber became non-medullated, ultimately branching into two branches, each of which terminated in a spiral and plexus. In a teased preparation, showing only the upper portion of the fiber, it may easily be seen how one might be led to say that the spiral fiber branched "*en T*," as Retzius (21) has done, and diagrammed in Fig. 3 of his article. Arnstein (20) and Smirnow (24) have also described a branching of the spiral fibers; they, it will be remembered, assume that the spiral fiber has a peripheral course. Such observations were made, I believe, on only partially stained fibers. Whenever I was able to trace a spiral fiber to a medullated fiber which had a peripheral course, such medullated fiber always terminated in a spiral in some more distal portion of the ganglion.

The anastomosis between pericellular baskets of adjacent ganglion cells, described by Smirnow (24) and long ago mentioned by Courvoisier (13) and others, I have never seen in sections. In methylene blue preparations of whole ganglia, cleared in glycerine, I have now and then seen what might be regarded as an anastomosis between two baskets. It would not, it seems to me, deserve the importance given it by Smirnow (24).

The observations above mentioned seem to me to present very strong evidence in favor of the hypothesis long ago expressed by Ehrlich (18) and Retzius (21), namely, that the spiral fiber was the ending of a cerebro-spinal fiber. They seem to me to show also that the course of the spiral fiber, after it leaves the straight process, is centralward. I have attempted by the degeneration method to determine this more clearly by

experiments of the following nature: the spinal cord of frogs was destroyed by inserting a hot needle through the peripheral end of the spinal canal; anaesthetized frogs stand this very well. In the frogs so operated upon, the posterior extremities seemed entirely paralyzed. At the end of five, ten, and twenty-five days a certain number of frogs were injected with methylene blue, with a view of staining the sympathetic ganglia. It was found, however, that even in frogs in which a portion of the cord had been destroyed twenty-five days previous to the injection, the nerves were not as yet degenerated; even the motor endings, in the sartorius, for instance, could be stained very easily. The sacral sympathetic ganglia showed, in all but one instance, the spiral fibers. This experiment (of ten days' duration) seemed at first conclusive, as only a few spiral fibers and baskets were stained; the unipolar ganglion cells stained much more readily than usual. Later experiments, where a longer time elapsed between the primary operation and the injection, did not, however, corroborate the above result. It may be stated that not much reliance can be placed on these experiments, as they were made on "winter frogs" in the early spring, at a time when it is well known that their metabolic changes are at their lowest. This, I think, may account for the fact that no degeneration of nerves was observed, dissection showing that the posterior portion of the spinal cord was completely destroyed. It is my aim to repeat such experiments on more suitable frogs.

The following brief summary of the results obtained in the study of the sympathetic ganglia of Amphibia may here be in place:

- 1) The sympathetic neurons of the frog are unipolar cells, the neuraxes of which (straight processes) innervate involuntary muscle and gland tissue (Kölliker).

- 2) Entering the sympathetic ganglia through the white rami, or through the interganglionic nerves, are seen medullated fibers, probably of cerebro-spinal origin, which fibers divide in the ganglion into two, possibly three, fibers, also medullated; at various places in the course of these fibers non-medullated, collateral branches are given off, which, with or without further

branching, end in spiral fibers and pericellular plexuses. After a longer or shorter course in the ganglion, the medullated branches lose their medullary sheath, and, as non-medullated fibers, also end in spiral and pericellular plexuses.

3) The pericellular plexuses surround the cell bodies of the sympathetic neurons, and are always intra-capsular. They represent the ending of cerebro-spinal fibers in the ganglion.

SYMPATHETIC GANGLIA OF REPTILIA.

In the tortoise the sympathetic chain begins in the superior cervical ganglion. The nerve connecting this ganglion with the succeeding one runs in close relation to the pneumogastric to about the middle of the neck, where it leaves the nerve, and, passing outwards and backwards, reaches the middle cervical ganglion; in *Chelydra serpentina*, a ganglion nearly a centimeter in length. From this ganglion several branches are given off, the largest of which passes toward the axilla and ends in the inferior cervical ganglion. Crossing the brachial plexus, the nerve which forms the continuation of the cord ends in the stellate ganglion. The relative size of these three ganglia differs in the several species studied; also, to some extent, in the same species. They are all, however, relatively large, and for this reason were used almost exclusively. They may be readily exposed by removing the plastron and pushing aside the tissues of the neck. From the stellate ganglion two filaments run back, one on each side of the subclavian artery, to a small ganglion which lies on the first dorsal nerve. In the succeeding dorsal segments are found small sympathetic ganglia, resting on the spinal ganglia. The sympathetic cord then runs back and becomes connected with sacral nerves. The two chains may then be traced as a single nerve into the tail, a number of small ganglia being found here. The account of the sympathetic system of the tortoise has been taken, with almost no alteration, from Martin and Moal's *Handbook of Vertebrate Dissection*, Part I: How to Dissect a Chelonian. Their description has reference more especially to the red-bellied slider terrapin (*Pseudemys rugosa*); their description

has been followed, as a number of dissections of the species worked on by me seem to agree with it in all essentials.

The sympathetic ganglia in the pharynx, oesophagus, in the heart (see Gaskall, *Journal of Physiology*, Vol. V), in the wall of the stomach and small intestine, were also studied. The *intra vitam* methylene blue method was alone used. The ganglia were fixed in ammonium molybdate, sectioned, and some of the sections mounted at once in balsam; others were double-stained in alum carmine before mounting in balsam.

In the literature at my disposal I have not seen any reference to observations made on the sympathetic ganglia of Reptilia with either the methylene blue or the chrome silver method. The older literature was not searched carefully enough to say whether these structures have been examined at all; this did not seem necessary, as such references would have no direct bearing on the observations here recorded.

Sympathetic Neurons of Reptilia: Tortoise.—The sympathetic neurons of the Reptilia studied vary greatly in shape and size. Their shape may be multipolar, bipolar, or unipolar. Multipolar cells are found in all ganglia, and in well-stained cells the dendrites and neuraxes can usually be differentiated. Only one neuraxis is found; this may arise from the cell body, as is the usual way, or from a dendrite at some distance from the cell body.

Bipolar cells are not numerous, and are usually found near one of the poles of a ganglion, between the efferent or afferent nerve fibers. Such cells are now and then seen in peripheral nerves some distance from a ganglion; they are now and then seen in the nerves found in the oesophageal mucosa. Cells which I have classed as unipolar belong to the largest cells seen; their peculiarity calls for a somewhat more extended description. These cells belong to the largest cells found; they are much more numerous in the larger ganglia of the chain, although they have often been recognized in many of the smaller ganglia. The cell body of such cells (see Fig. II, and Pl. IV, Fig. 12) is usually more or less regularly round or oval, and very often presents a depression at one side of the cell body, from which arises one large process. This process

varies greatly in shape and in the course it assumes after leaving the cell body. It may be quite straight (Fig. II, *A*, and Pl. IV, Fig. 12, *A* and *B*), and break up at a variable distance from the cell into secondary branches; or it may assume a tortuous course (Fig. II, *B*, *C*, and *E*), and may (see figure) be partly wound around the cell or twisted upon itself. This

FIG. II.—Sympathetic neurons of Reptilia. (See text for description.)

process may again be quite short (Fig. II, *D*), and break up at once into a number of large secondary branches. In such cells the neuraxis may arise directly from this large process, *a*, of cell *B*, Pl. IV, Fig. 12, and *a*, of cells *B*, *C*, and *E*, of Fig. II, in which case the large process becomes gradually attenuated and stains more deeply; or it may arise from one of the dendritic branches at some distance from the cell body,

as in cell *A*, Fig. 12 (in which case the neuraxis was recognized by a nucleus of the sheath of Schwann, *b*, of figure), or again, cell *D* of Fig. II, where the neuraxis was recognized by its greater affinity to the methylene blue. It should be stated that the figures here reproduced were sketched from sections of sympathetic ganglia of *Chrysemys picta* and *Chelydra serpentina*, which accounts for the abrupt ending of some of the processes. The facts here given have been abundantly verified on fresh tissues.

In methylene blue stained preparations of sympathetic ganglia of Reptilia, examined fresh or in sections of such ganglia fixed in ammonium molybdate, the chromophile granules of the protoplasm are clearly seen. These granules vary in shape and size, and are distributed more or less evenly through the cell protoplasm, leaving free, however, a narrow zone immediately surrounding the nucleus, as may be seen in Pl. IV, Fig. 12.

The cell body of the sympathetic neuron of Reptilia is surrounded by a nucleated capsule, which seems continuous with the neurolemma of the neuraxis of said cell; although my observations on this point are not conclusive.

Within the capsule of many of the sympathetic cells (and this is especially true of the large unipolar cells above described) are found a varying number of nuclei, the nature of which has not been clearly determined. They are always recognized in sections of ganglia stained in methylene blue and double-stained in alum carmine; they may, however, also be seen in sections of ganglia hardened and stained in the more usual methods. In discussing similar nuclei found within the capsule surrounding the sympathetic ganglion cells of fishes, it was mentioned that these nuclei were looked upon as nuclei of neuroglia cells. Cajal has described cells which he regards as neuroglia cells in the superior cervical ganglion, and Dogiel (40) has drawn attention to spindle-shaped or star-shaped cells with long branching processes found in the sympathetic ganglia of Mammalia. Such cells, stained in methylene blue, are shown by Dogiel in Pl. XIV, Fig. 15. They are described as forming a network outside of the capsule of the ganglion cells. By reason of the fact that neuroglia cells are not stained in methylene blue,

Dogiel is inclined to think that the cells described by him do not belong to neuroglia tissue. He likens them to the branched connective tissue elements, which are found on the membrana propria of gland alveoli, which, as he states, are also occasionally stained in methylene blue. The cells in question have never been stained by me in methylene blue. This may be due to the fact that in all my work the methylene blue solution was injected into the living animal, in which case, as is well known, many elements do not stain as readily as when the methylene blue solution is added directly to the freshly removed tissues. My only reason for suggesting that the nuclei found within the capsule of many of the sympathetic cells of Reptilia are nuclei of neuroglia cells is based on some observations made on ganglia hardened in Müller's fluid, imbedded in paraffin and sectioned, the sections being then stained in haematoxylin and double-stained in the acid fuchsin—picric acid solution suggested by Van Gieson. In such sections are seen a large number of fine fibrillae, which seem to be more or less intimately connected with the nuclei above mentioned. These fibrillae are not, I believe, nerve fibrillae, belonging to the pericellular plexus presently to be described; their smooth and regular contour and their apparent connection with the nuclei would exclude such a supposition. Time has not been at my disposal to consider these nuclei more fully. I mention the above hypothesis in the hope that it may stimulate further inquiry into their structure.

In the sympathetic ganglia of Reptilia, stained in methylene blue, medullated nerve fibers, which enter the ganglia through their rami, are abundantly found. Many of these medullated fibers have been traced into intra-capsular, pericellular plexuses. Such pericellular plexuses may be described under two heads:

a) Simple pericellular plexuses, without spiral fibers, surrounding, as a rule, the cell body of multipolar or bipolar ganglion cells.

b) Complex pericellular plexuses, with spiral fibers, more often found surrounding the cell body of the large unipolar cells above mentioned.

The pericellular plexuses of the simpler type resemble very closely those to be described for birds and mammals. They are found in all sympathetic ganglia, but are relatively more numerous in the smaller ganglia. When the shape of the enclosed cell can be ascertained, it will, as a rule, be found to be multipolar. But as the sympathetic cells are generally only imperfectly stained, when the pericellular plexuses are well stained, the enclosed cells are usually not clearly made out. The above statement may, therefore, be open to exceptions. In Pl. IV, Fig. 13, three of the simpler pericellular plexuses are sketched. In each case the non-medullated axis-cylinder (a collateral branch or end branch of a medullated fiber, as one may occasionally trace such a branch to a medullated fiber) breaks up, just before or after it pierces the capsule (not shown in the figures), into several secondary branches, which, after further division, are woven into a network varying greatly in complexity and arrangement. The fibrillae of the network are often very varicose, showing quite large nodular enlargements. This network or plexus is generally seen as a closed one; only here and there some of the fibrillae seem to end free. (See *b* of Fig. 13.) These plexuses are always intra-capsular, and surround the cell body of a sympathetic cell; the capsule, though often not stained, may be recognized if much of the light is cut off.

The more complex pericellular plexuses vary greatly in structure; they are usually found, as above stated, surrounding the large unipolar cells. Their structure may best be made clear by reference to a number of figures showing some of the different types found. In Pl. IV, Fig. 16, the relation of such an end plexus to the enclosed ganglion cell and capsule is well shown. The axis-cylinder of the nerve fiber (*a*) ending in the plexus breaks up into several branches, which are wound around the single process of the sympathetic cell enclosed in its end plexus. From these spirally arranged branches secondary branches are given off, which, on approaching the cell body of the sympathetic cell, are woven into a loose basket-like network. Very often the axis-cylinder ending in such pericellular plexuses is spirally wound around the process of the sympa-

thetic cell before breaking up into secondary branches. The number of such spirals and their arrangement are subject to much variation. In Fig. 14 an axis-cylinder with four spiral turns is shown. In Fig. 15 is shown a long spiral, which surrounds a long and straight process of a unipolar sympathetic ganglion cell. In Pl. IV, Fig. 17, we may see a portion of four pericellular plexuses; special attention is, however, drawn to the one in connection with the axis-cylinder showing the spiral turns. The spiral fiber, *a*, here shown is a collateral branch of a medullated fiber, *a'*; the collateral branch, after making many turns around the process of the enclosed sympathetic cell (the process and cell body of the sympathetic cell are not shown in the figure; they can, however, be quite clearly made out in the section), breaks up into a number of fine varicose fibrillae, which form the pericellular plexus. Attention needs further to be drawn to the network of fine fibrillae seen within the spiral fiber. Many of these fine fibers branch from the spiral fiber. Their arrangement is, however, so complex that they can be followed for only a short distance. Such intra-spiral fibers were seen only a very few times, when the preparations seemed very well stained. They form a network which is in very close proximity to the straight process of the enclosed sympathetic cell, and suggest the possibility that the process may be stimulated directly through the intra-spiral fibrillae. This figure shows further some very small varicose fibers (*c*), which enter the spiral from without. Where these fibers come from, and the nature of their ending, I have not been able to ascertain. I should like to suggest that such fibers may be sympathetic fibers ending on the sympathetic cell or on one of its large processes. I possess, however, very few observations that would tend to strengthen such a view. My reason for suggesting the hypothesis is based on the fact that, in birds and Mammalia, end branches of sympathetic fibers would in some cases seem to end on dendritic branches of other sympathetic cells, as will be stated in the following pages. In Pl. IV, Figs. 14-16, only the spiral fiber and the pericellular plexuses are shown; their arrangement and structure are more easily seen when the other parts of the ganglion are

not sketched. In Pl. IV, Fig. 18, I have reproduced a sympathetic cell with pericellular plexus, taken from one of my most successful preparations, where the structures other than the spiral fiber and pericellular plexus may be seen. This sketch is made from a section of an inferior cervical ganglion of *Chelydra serpentina*, stained in methylene blue and double-stained in alum carmine. In this sketch the cell body of the sympathetic cell came out more deeply colored than in the section; in all other respects the coloring and arrangement of nerve fibers and nuclei were followed as accurately as possible. In this figure may be seen *B*, the cell body, *C* and *D*, the nucleus and nucleolus, and *A*, the process of a large uni-polar sympathetic cell. The capsule, *c*, was clearly made out and could be followed for some distance along the process, when it became unobservable. Three medullated fibers, *a*, *a'*, and *a''*, take part in the formation of the spiral and pericellular plexus. Their interlacement is, however, such that they could be followed for only a short distance. At their distal end these spiral fibers break up into secondary branches, which become very varicose and, after further branching, form a basket-like network which surrounds the cell body of the sympathetic cell, the arrangement of which is better shown in the figure than I can describe it. The figure shows most clearly that this network is intra-capsular. The figure may further serve to give an idea of the number and the arrangement of the intra-capsular nuclei. These, as has been previously observed, are regarded as nuclei of neuroglia cells. Fig. 19 has been added to give the general appearance of a methylene blue preparation of a sympathetic ganglion of a tortoise, double-stained in alum carmine. In this section the processes of the cells were only partly stained, and the fact that this sketch is from a section will explain the abrupt ending of some of the branches. The figure shows three of the more complex pericellular plexuses, *A*, with spiral fibers, and one simpler pericellular plexus, *B*; shows also the arrangement of the medullated and the non-medullated (varicose fibers in the figure) fibers and of the dendrites as seen in sections.

My reasons for assuming that the intra-capsular, pericellular

plexuses above described are the endings in the sympathetic ganglia of Reptilia of cerebro-spinal fibers may briefly be stated as follows :

a) The medullated fibers in the sympathetic ganglia may, from their size and structure, be regarded as fibers of cerebro-spinal origin.

b) In serial sections of well-stained, sympathetic ganglia, single medullated fibers, or small bundles of such, may often be traced from the white rami into the ganglia, where they undergo division, or give off collateral branches, and may now and then, as shown in Pl. IV, Fig. 17, be traced into a spiral fiber and pericellular plexus.

c) The small dorsal sympathetic ganglia of the Reptilia investigated are in close apposition to the spinal ganglia. By removing the bodies of the vertebrae, the spinal cord, with the roots of the spinal nerves, the spinal and sympathetic ganglia may easily be exposed. After exposing the structures as above indicated, I have, in a large number of experiments, divided the roots of the spinal nerves close to the spinal cord, and removed these with the spinal and sympathetic ganglia, with short segments of the nerves uniting the sympathetic ganglion to the sympathetic chain, and with a short portion of the spinal nerve. The tissues so removed were then fixed in ammonium molybdate, imbedded in paraffin, and cut in serial sections. As a rule, in the sections so obtained only a few of the nerve fibers and nerve cells were well stained in methylene blue. In some series, however, the staining was such that small bundles of medullated fibers could be traced from the anterior root into the sympathetic ganglion. The writer (26) has already drawn attention to the fact that in the dorsal spinal ganglia of Reptilia, stained in methylene blue, a small group of multipolar cells was found within the connective tissue capsule of the spinal ganglion. This group of multipolar cells is found on the ventral side of the ganglion, immediately under the capsule of the ganglion, and separated from the sensory cells by a connective tissue septum. Into this group of cells, which, by reason of their shape and structure, are looked upon as sympathetic cells, I have several times been able to trace medullated fibers coming

from the anterior root; and now and then such a fiber could be traced into a pericellular plexus of the simpler variety.

Such observations, it seems to me, point conclusively to the cerebro-spinal origin of the medullated fibers ending in the ganglion in intra-capsular, pericellular plexuses.

The non-medullated fibers seen in sections of a ganglion wind their course through the ganglion between its constituent cells. Many of these are no doubt the neuraxes of the sympathetic neurons of the ganglion, although it is very seldom that in section such fibers can be traced to a ganglion cell. This may, however, now and then be done, as may be seen from Pl. IV, Fig. 12. Serial sections show further that some non-medullated fibers enter the ganglia through the interganglionic cords. Their mode of ending in the ganglia has not been definitely ascertained, unless possibly that shown in Pl. IV, Fig. 17 (fibers *c*), and described with that figure, may be their mode of ending.

A brief summary of the observations made on the sympathetic ganglia of Reptilia above described may here be given:

1) The sympathetic neurons of Reptilia may be multipolar, bipolar, or unipolar. The first two types need no further mention. The unipolar cells, very characteristic for the sympathetic ganglia of Reptilia, are relatively large, possessing one large process, varying in length and its relation to the cell body of the respective neuron. All these cells have one neuraxis, which near the cell is non-medullated; whether at some distance from the cell it becomes invested with a sheath of myelin I am unable to say. The neuraxis arises from the cell body or from some one of the dendrites. The number of dendrites varies. The cell body of all sympathetic cells of Reptilia is surrounded by a nucleated capsule.

2) Medullated fibers, no doubt of cerebro-spinal origin, end in the ganglia, either in simple pericellular plexuses surrounding the multipolar or bipolar cells, or in more complex pericellular plexuses with spiral fibers surrounding the cell body of the unipolar cells. These pericellular plexuses are intra-capsular. The medullated fibers branch in the ganglia, and may thus influence a number of sympathetic cells through their several

pericellular plexuses; each branch probably having such an ending.

3) The ending of the non-medullated fibers in the ganglion has not been determined definitely.

SYMPATHETIC GANGLIA OF BIRDS.

The observations here recorded were made on half-grown chickens. A 1% to 4% solution of methylene blue in normal salt was injected into the humeral vein (Owen), just above its division over the distal end of the humerus. The methylene blue was allowed to flow into the circulation as quickly as possible; as in birds, a small quantity of the solution causes a stoppage of the heart. At the expiration of thirty to forty minutes the sternum with the attached muscles was removed, and the heart and lungs displaced to one side. In this way the dorsal sympathetic ganglia are readily exposed. In the chicken the dorsal sympathetic ganglia rest on the spinal ganglia, so that a white ramus as such is wanting. The sympathetic ganglia may, therefore, be best removed with the spinal ganglia; this was usually done. The tissues so removed were fixed in ammonium molybdate, the further treatment being as previously indicated. It should be stated at the outstart that the results obtained on the sympathetic ganglia of birds has been far from what might be desired. And this for two reasons:

1) I have found it very difficult to stain these structures at all well in methylene blue. Very generally, all the structures of the ganglion stain a diffuse blue; in which case, sections show scarcely more than may be seen in sections of these ganglia stained with the ordinary stains.

2) The few sections that were well stained would fade in a few days or weeks, no matter what care was taken. In some few instances, however, results which may be compared with those obtained on other vertebrates have been at my disposal.

In the literature I have been able to consult I find no reference to observations made on the sympathetic ganglia of birds with the methylene blue method, although these ganglia have been stained by Cajal (27), Retzius (28), and Lenhossék (29).

Ramon y Cajal's studies on the sympathetic system were published in three papers, which followed each other in close succession. In his second communication, to which I wish here to refer more particularly, he discusses observations made on dove embryos of the fourteenth and sixteenth day, and chick embryos of the seventeenth and eighteenth day. He here states that the sympathetic cells are multipolar, and that all the processes have the character of nervous processes. He describes long and short processes; the latter end free within the ganglion, the former pass beyond the bounds of the ganglion. In his third communication Cajal retracts in part these statements, and states that while the cells are multipolar, the great majority possess one axis-cylinder branch, the others being dendrites. In a brief account of his observations made on the sympathetic ganglia of vertebrates, Retzius refers in a few lines to those made on chick embryos of the fifteenth to the eighteenth day, in which he merely confirms the conclusions ultimately reached by Cajal as above given.

Lenhossék has studied the sympathetic ganglia of embryo chicks of the tenth and fifteenth day; the best results were obtained with the older embryos studied.

In cross-sections of chick embryos of the above age, stained after the double Golgi method, Lenhossék made the following observations:—Both in the cervical and thoracic regions a ramus communicans is wanting; the sympathetic ganglion lies directly on the median side of the ventral spinal nerve. In the thoracic region the spinal nerve divides into a smaller dorsal branch and a relatively larger ramus ventralis, on which the sympathetic ganglion is found. These observations I can to some extent corroborate on the grown chicken, although, as above stated, I find the sympathetic ganglion, or a portion of it, resting on the spinal ganglion. Lenhossék describes multipolar cells, with one neuraxis and several dendrites, five to ten in number. The dendrites are described as quite thick and relatively short, some ending free without any branching.

The neuraxes are described as taking one of three paths:

- 1) Rarely into one of the peripheral branches of the ganglion going to the viscera.

2) In frontal sections of chick embryos of the fifteenth day it was seen that some of the neuraxes passed up or down in the ramus internodalis, and entered one of the contiguous ganglia.

3) Many of the neuraxes take a peripheral course in the ventral branch of the spinal nerve of the segment.

Both Cajal and Lenhossék were able to trace cerebro-spinal nerve fibers into the sympathetic ganglia of the chick. The latter describes these as coming from the ventral ramus of the spinal nerve, as branching in the ganglia, and as terminating in a "free ending." Cajal was able to trace such fibers to the anterior root, while Lenhossék is inclined to regard them as coming from the posterior root. Neither of these investigators describes pericellular plexuses in the sympathetic ganglia of birds.

The results obtained by me with the methylene blue method confirm in many particulars the observations made by Cajal and Lenhossék with the Golgi method. I also find the sympathetic neurons multipolar, with numerous dendrites and one neuraxis. The body of such neurons may be irregularly round or oval, or of a triangular shape. In ganglia stained in methylene blue, and in those so stained and fixed in ammonium molybdate and sectioned, chromophile granules may be seen in the protoplasm of the ganglion cell, if the staining is not too intense or diffuse, as is often the case. These granules are very fine and evenly distributed through the protoplasm, as (Pl. V, Fig. 21) a portion of a section of one of the ganglia of the dorsal sympathetic chain may show.

The dendrites, as Lenhossék has correctly stated, are short and thick and not prone to much branching. They form an interlacing network between the cell bodies of the ganglion cells. As the cell bodies of such cells are surrounded with a nucleated capsule (see *c* of Fig. 22), this dendritic plexus is extra-capsular.

Cajal mentions this arrangement of the dendrites, formed by the "short process," and describes it as pericellular nests — "nido pericellular." He further suggests that through such pericellular nests ganglion cells may be physiologically associated.

Van Gehuchten, Sala, and Dogiel have observed a similar arrangement of the dendrites of the sympathetic cells in the ganglia of Mammalia; they, however, regard the nest-like arrangement as accidental. A study of Pl. V, Fig. 21, may show that the latter interpretation seems the more plausible one, and especially if we take into consideration that such dendrites are extra-capsular. It is further to be remembered that if these dendrites have the power to conduct nervous impulses, as they no doubt may have, they would, reasoning from analogy, conduct toward the cell body—be “cellulipetal”; and I am at present not aware of any instance where a dendrite is stimulated by the cell body or dendrites of another neuron. We may thus assume that the arrangement of the dendritic branches of the sympathetic neurons in the sympathetic ganglia of birds is mainly accidental, depending in a great measure on the relative position of contiguous ganglion cells. Participating in this network between the ganglion cells as above described, are found non-medullated and medullated nerve fibers. The former are, no doubt, in part the neuraxes of the sympathetic cells of the ganglion. In serial sections, however, it may be seen that some of the non-medullated fibers enter the ganglia from without; this, both Cajal and Lenhossék have described. The latter pictures in his article (Fig. 13) a bundle of such fibers entering a ganglion. This figure, as the descriptive text shows, was sketched from a dorsal sympathetic ganglion of a 14-day chick. Lenhossék has this to say concerning these fibers: “Wir haben es hier offenbar mit den Fortsätzen von anderweitig, etwa in den visceralen Ganglien gelegenen sympathischen Zellen zu thun.” He goes on to say that the ending of these fibers is by a simple end brush and not by an end basket; and, to quote again: “Wobei Endäste manchmal auffallende Verdickungen zeigten, an den Stellen, wo sie sich an die Zellen anlegten.” An ending such as here described has not been seen by me. The non-medullated fibers, as far as I have been able to determine, are always extra-capsular, and do not therefore end on the cell body of the sympathetic cells. A few times, however, an ending such as shown in Pl. V, Fig. 20, has been seen by me. In this figure, which is a portion of a section,

of 20 μ thickness, of one of the dorsal sympathetic ganglia, is shown a sympathetic nerve cell with several dendrites. On one of the branches, *d*, is shown the ending of a non-medullated fiber, *u*; the non-medullated fiber being stained somewhat more deeply than the dendrite. In a careful search of many preparations, only a few such endings have been found; yet in relatively thin sections, studied under $\frac{1}{12}$ -inch oil immersion, they have now and then been quite clearly made out.

Concerning the medullated fibers found in the sympathetic ganglia of birds, the following observations have been made:

It will be remembered that the sympathetic ganglia of the bird lie on the ventral branch of the spinal nerve and partly on the ventral side of the spinal ganglia, and are removed with these structures. In such preparations, stained in methylene blue and examined before fixing, even under a low power, the multipolar-sympathetic cells may be made out with certainty, and axis-cylinders of medullated fibers may now and then be traced between such multipolar cells. Cajal, it will be remembered, describes such fibers as coming from the anterior roots, while Lenhossék regards them as coming from the posterior roots, as may be gathered from the following statement made by him: "In zwei Fällen, schien es mir, als handelte es sich gerade umgekehrt um Fasern die aus dem Spinalganglion kommen, also um sensible Fasern, doch kann ich dies nicht mit voller Bestimmtheit vertreten." In ganglia removed as above stated, and examined before fixing, and especially in those where only a few nerve fibers were stained, I have several times been able to trace axis-cylinders of medullated fibers ending in the sympathetic ganglia toward the anterior root; this may also be seen in serial sections of the sympathetic ganglia and the structures in connection with them. My observations, as far as they go, are therefore in accord with those given by Cajal.

There is no doubt that many of the medullated fibers ending in the sympathetic ganglia of birds, do so in pericellular plexuses, although Cajal, Retzius, and Lenhossék were unable to see them in Golgi preparations of these structures. The latter says in this connection: "Von einer faserkornartigen Anordnung im Inneren des sympathischen Ganglion ver-

mochte ich beim Hühnchen nichts wahrzunehmen; es handelte sich immer um einfache Aufzweigungen." The pericellular plexuses seen by me are the endings of collateral branches, or ultimate endings of medullated fibers. A number of such endings are reproduced in Figs. 20-22. In Fig. 20, from the medullated fiber *A*, is given off at the node of Ranvier, *x*, a non-medullated collateral branch, *a*, which may be traced into two pericellular plexuses. Fig. 22 was sketched from a section of a methylene blue stained, dorsal sympathetic ganglion of a chicken, fixed in ammonium molybdate, the sections being further stained in alum carmine. Here the relation of the pericellular plexus to the cell body of the enclosed cell and its capsule is clearly shown.

The pericellular plexuses observed in birds are of a relatively simple structure. The end branch, or collateral branch destined to form such an ending, divides, just before or after it reaches the capsule of a sympathetic cell, into a number of fine varicose fibers which are woven into a very loose plexus and often end free on the cell.

My observations on the sympathetic ganglia of birds may be briefly summarized as follows:

- 1) The sympathetic neurons are multipolar, with one neuraxis and several dendrites. The cell body of such a neuron is enclosed in a nucleated capsule.
- 2) The non-medullated fibers entering the ganglia from without, end, after branching, on the dendritic branches of the sympathetic neurons.
- 3) The medullated fibers of cerebro-spinal origin, which enter the ganglia, most probably from the anterior roots, end, after branching in the ganglia, in pericellular plexuses of a relatively simple structure. These plexuses surround the cell bodies of the sympathetic neurons and are intra-capsular.

SYMPATHETIC GANGLIA OF MAMMALIA.

The sympathetic ganglia of Mammalia have been investigated with the Golgi method by Kölliker (30-33), Ramon y Cajal (34-36), Retzius (28), Van Gehuchten (37), Sala (38), d' Erchia

(39), and Lenhossék (29); and with the methylene blue method by Aronson (19) and Dogiel (40, 41). Each writer has in turn reviewed the work of those who have preceded him, to such an extent that a special review of the literature seems here uncalled for. The observations made by others will thus be considered in giving my own results.

The observations here to be recorded cover a period of now nearly three years; in the earlier portion of this work the Golgi method was to some extent used, but in the last two years the *intra-vitam* methylene method alone was used, and the results to be recorded pertain exclusively to observations made with it.

The animals investigated varied in age from such as were two or three weeks old to such as were full grown. The methylene blue solution was injected through the jugular or femoral vein, usually the former; the quantity varying with the size of the animal.

The ganglia studied were the superior and inferior cervical, the stellate ganglion, the smaller ganglia of the chain, and many of the peripheral ganglia. These were exposed forty-five minutes to an hour after the injection; were fixed in ammonium molybdate and studied in sections, either stained only in the methylene blue or double-stained in this dye and alum carmine.

Some of the results here to be given were known to me before Dogiel's (40) article (from which I shall quote freely and to some extent follow) reached me. It seems, nevertheless, advisable to give them, for, notwithstanding the fact that we have both used the methylene blue method in our investigations, Dogiel's observations were made on tissues stained on the slide and fixed in ammonium picrate; and, furthermore, his observations pertain more particularly to the smaller peripheral ganglia (wall of the gall bladder and so forth), where it is possible to study the ganglia as a whole. He was in this way able to obtain preparations of sympathetic neurons, which for completeness of staining, judging from his pictures, seem not to have been equaled. My own observations, as above stated, were made on sections, usually double-stained in alum carmine; and while

in such sections the sympathetic cells are not so clearly shown, by reason of the fact, that many of the processes are cut from the cells, yet the relation of the dendrites and pericellular plexuses to the cell body and capsule of the sympathetic cells of the ganglion is more clearly shown than in ganglia studied as a whole.

SYMPATHETIC NEURONS OF MAMMALIA.

All writers who have reported observations made with the Golgi or methylene blue method are agreed that the great majority of the sympathetic neurons are multipolar. Dogiel (40) states that near the poles of the sympathetic ganglia bipolar and unipolar cells are to be found. Their number is relatively small. My own observations lead to the conclusion that such cells are usually found between the afferent and efferent nerve fibers of the ganglion. In the protoplasm of all these cells chromophile granules are seen ; in this respect my observations are in accord with those made by Dogiel (40).

The nucleus is usually only imperfectly stained in methylene blue, more often of a diffuse blue, which may be of a darker or a lighter hue than the cell body. Nucleoli are only rarely seen with this stain, although they are readily found in preparations stained in alum carmine.

In some Mammalia, namely, the rabbit, hare, and guinea pig, sympathetic cells with two or even three nuclei are found, as has been shown by Remak (44), Guye (42), Schwalbe (15), and more recently Apolant (43). Schwalbe and Apolant explain this curious phenomenon by stating that it is the result of an incomplete cell division, the nucleus dividing but not the protoplasm, and the latter has shown that the multiplication of the nuclei takes place by an amitotic cell division, which may be recognized in embryo rabbits as early as the third week. Apolant reaches the following conclusions concerning this point: "Ich glaube daher, dass die Bildung der beiden Kerne in einem innigen Zusammenhange mit den Wachstumsverhältnissen der Zelle steht, der Art, dass die anfängliche, überwiegende Ausbildung des Kernes zu einer Theilung desselben führt, welche

ihrerseits die Veranlassung zu einem stärkeren Wachsthum der Zelle abgiebt. Ich vindicire also dem Process keine functionelle, sondern lediglich eine biologische Bedeutung für die Zelle." My own observations on sympathetic ganglion cells with more than one nucleus were made on ganglia of the guinea pig stained in methylene blue, a method which, in the hands of Apolant, gave only unsatisfactory results; they may, therefore,

FIG. III. — Sympathetic neurons of guinea pig. (For description see text.)

receive this brief mention. The majority of my preparations were from the solar ganglion. The larger number of the sympathetic neurons in this ganglion are multipolar, with two and sometimes three nuclei. Such cells are reproduced in *A* and *B* of Fig. III; as may be seen from this figure, these cells differ from sympathetic cells found in other Mammalia only in having more than one nucleus. The number of dendrites varies; only one neuraxis is made out (*a*, in figure), if the cells are well stained.

In *D* of Fig. III is shown a multipolar cell with two nuclei, where the portions of the cell body containing the nuclei are united by a band of protoplasm, giving the appearance of a cell in the later stages of cell division. This condition was seen only a few times, and is very much like that shown by Apolant in Figs. 7 and 8 of his article. Not all the multipolar cells have two nuclei, as may be seen in cell *C* of the above figure. Mononuclear multipolar cells are, however, rarely seen. Bipolar cells are proportionally not more numerous in the rodents above mentioned than in other Mammalia. They are found near the poles of the ganglia, between the afferent and efferent nerves, and may be mononuclear or possess two nuclei. In order to close the discussion of these cells I may be allowed to anticipate somewhat, and state that I have often found the multipolar cells, with two or more nuclei, surrounded by a pericellular plexus; one such is shown in *E* of Fig. III.

I must, therefore, agree with Apolant when he states that the presence of two or more nuclei in the sympathetic cells of the rodents under discussion is not to be looked upon as expressing a degenerative process. In all other respects the structure of these cells, — the presence of chromophile granules, — as also the structure of the ganglia taken as a whole, is identical with the structure of the sympathetic cells and ganglia of other mammals, in which the sympathetic neurons have only one nucleus.

The Dendrites. — In the sympathetic cell near the center of the ganglion, the dendrites, the number of which varies, may arise from any part of the cell body. In the peripheral cells, as Dogiel (40) has correctly stated, the dendrites are usually given off from that portion of the cell body pointing toward the center of the ganglion. The dendrites branch and rebranch and form between the ganglion cells an intercellular plexus. This plexus is well shown in Fig. 26 — a portion of a section of the solar ganglion of a cat. It may here be seen that the dendrites intertwine in such a way as to leave open spaces, in which the cell body of one, two, or perhaps three sympathetic cells are found. This basket-like arrangement is what Ramon y Cajal has described as pericellular nests. The dendrites do not,

however, come in contact with the cell bodies of the sympathetic cells, but are separated from them by their capsule.

Dogiel (40) has further described a "general peripheral plexus," situated under the fibrous capsule of the ganglion. In the formation of this kind of plexus, dendrites from nearly all the cells of the smaller ganglia and many of the cells of the larger ganglia take part, the dendrites from the cells situated more centrally in the ganglia winding their way out until the plexus is reached.

Neuraxis. — It is now very generally conceded that the sympathetic neurons of Mammalia possess one neuraxis — axis-cylinder. This may arise from the cell body or from a dendrite at a variable distance from the cell body. In making the latter statement I have been guided largely by observations made by others, as in sections where, as a rule, only relatively short segments of the axis-cylinder are met with, it is often exceedingly difficult to classify the processes of any particular sympathetic cell. In most preparations the axis-cylinder branches are not stained in any way characteristically. Sometimes, however, as may be seen in Pl. V, Fig. 26, they stain a deeper and more purple shade of blue, which color differentiation may aid in making out which one of the several processes of a sympathetic cell is the axis-cylinder. In such sections I have been able to confirm the statements made above. Near the cell body the neuraxes of the sympathetic cells of Mammalia have a very regular contour and maintain about the same size; and Dogiel (40) has described a very delicate longitudinal striation, caused by a deeper staining of the ultimate fibrillae of the axis-cylinder.

Kölliker (32), who has for many years paid especial attention to the structure of the neuraxes of sympathetic cells, has quite recently summarized his observations as follows:

- 1) The sympathetic nerve fibers (axis-cylinder processes of sympathetic cells) are in many cases surrounded by a very delicate sheath of myelin.

- 2) In some of these fibers the sheath of myelin accompanies the neuraxis to its periphery — nerve fibers from the ciliary ganglion and the pilo-motor nerves of the cat.

3) In other instances the medullary sheath is sooner or later lost, the neuraxes continuing as Remak's fibers — fibers going to the intestine, liver, and spleen.

4) In many cases the neuraxes of sympathetic neurons are non-medullated throughout. This, it would seem to me, is the structure of the neuraxes of sympathetic cells in the peripheral ganglia — those of the heart, salivary glands, intestine, bladder, etc.

Lenhossék (29) and Dogiel (40) have described the giving off of collateral branches from the axis-cylinders of the sympathetic cells of mammalia; such branches have been traced into the intercellular plexus; their mode of ending has not, however, been determined.

Before closing the discussion of the sympathetic neurons in Mammalia, it is necessary to mention some cells found in the sympathetic ganglia, which Dogiel (41) has recently described as sensory sympathetic cells. These cells are said to have the following structural peculiarities: The cells are multipolar, with one to sixteen dendrites and one neuraxis; they are characterized by the structure of the dendrites, which are longer and more slender than the dendrites of the other sympathetic cells. These dendrites ramify in the ganglion and may often be traced into one of the nerve trunks connected with the ganglion, in which they may often be followed for long distances. In the ganglia of Auerbach's plexus such dendritic processes could be followed from a ganglion into one of its nerve roots, and then some into the mucosa, others into the submucosa, and so on. The neuraxes of such cells are described as coming from the cell body or from some dendrite; in the smaller ganglia such cells have non-medullated axis-cylinders; in the larger ganglia of the chain this process becomes surrounded with a medullary sheath some distance from the cell body of the sympathetic cell from which it arises. In the plexuses of the intestine Dogiel was able to trace the neuraxes of such sensory sympathetic cells through several ganglia. In the ganglia through which they pass, or in which they end, collateral branches are given off which terminate in the intercellular plexus. Dogiel suggests that such cells may form the anatomical basis for certain phe-

nomena, such as peripheral reflexes, etc., which have been observed in the sympathetic system. My own observations, based almost entirely on sections, do not give me sufficient evidence on which to judge these interesting and, if corroborated, most important observations which Dogiel has given us. Cells with long, slender processes, such as he has described, have now and then been seen by me, but I have not been able to trace these processes for any distance. I may, therefore, dispense with any further discussion of these so-called sensory sympathetic cells.

Non-Medullated and Medullated Nerve Fibers in the Sympathetic Ganglia. — In sections of sympathetic ganglia stained in methylene blue, smaller and larger medullated fibers and non-medullated fibers may be seen between the ganglion cells. These have been observed by Kölliker, Ramon y Cajal, Lenhossék, Sala, Van Gehuchten, and others, in Golgi preparations of the sympathetic ganglia of Mammalia, and by Aronson and Dogiel in methylene blue stained preparations of these structures. In serial sections of the chain ganglia removed with the white rami, stained in methylene blue, bundles of medullated fibers may readily be traced from the white rami into the ganglia, although it is not always easy to trace individual fibers for any long distance. In the ganglia such medullated fibers are seen branching into two or three branches, which may or may not be medullated, and in a number of instances such branches were traced to a sympathetic cell, where, after further branching, they terminated in a pericellular plexus which surrounded the cell body of the sympathetic cell. In well-stained preparations such pericellular plexuses are easily found, although in sections it is not always easy to connect such plexuses with any particular nerve fiber. These pericellular plexuses vary much in complexity and in the arrangement of fibrils which form them. They may be very loosely woven, or, again, made up of a large number of fibrils. The fibrils may be quite smooth, or show numerous and large varicose enlargements. The ones shown in Pl. V, Fig. 23, a small portion of a section of the stellate ganglion of a dog, stained in methylene blue and alum carmine, may be looked upon as presenting the general appear-

ance of the pericellular plexuses in the sympathetic ganglia as seen by me. In double-stained sections there can be no doubt that the pericellular plexuses are in contact with the cell body of the sympathetic cell—are intra-capsular. This agrees with the following statement found in Dogiel's (40) account: "Das-selbe" (speaking of the pericellular plexuses) "liegt unmittelbar der Oberfläche der Ganglienzellen an und befindet sich, wie mir scheint, zum Unterschiede von dem intercellularen Geflecht nicht über, sondern unter der Zellenhülle." The fibers terminating in the pericellular plexuses are usually non-medullated for some distance from the cells around which they end (*a*, Pl. V, Fig. 23), although in a few instances they are medullated to the point where they pierce the capsule, *b*, of the same figure. In some few instances much more complicated pericellular plexuses were seen, in which the fiber or fibers terminating in such plexuses were spirally wound around some process, probably the axis-cylinder branch of the cell enclosed by the pericellular plexus. One such ending is shown in Pl. V, Fig. 24, taken from the stellate ganglion of a dog. In the few instances seen by me the network resulting from the division and redivision of such spiral fibers is much more complicated than the pericellular plexuses usually observed. The fibrils are very varicose, often presenting quite large nodular enlargements. Aronson (19) has described spiral fibers in the sympathetic ganglia of the rabbit. His description leads one to infer that they are quite common. A comparison of the figures given by Aronson (especially Fig. 1) with my Pl. V, Fig. 24, may suffice to show that very dissimilar structures are spoken of in the two accounts. Spiral fibers, such as he describes, formed by one, two, or three varicose fibers, twisted once, twice, or three times around the neuraxis of the sympathetic cell and terminating in an ordinary pericellular plexus, have now and then been seen by me. In this account, however, the term "spiral fiber" is confined to such as are diagrammed in Pl. V, Fig. 24, ending in a more complex pericellular plexus. These were only rarely seen by me. They resemble, to some extent, the more complicated pericellular plexuses, with spiral fibers, described for Reptilia.

Pericellular plexuses, enclosing the cell body of the sympathetic neurons of Mammalia, have been described for nearly all sympathetic ganglia in all parts of the sympathetic system; in the cranial sympathetic ganglia as follows: Ciliary ganglia by Kölliker (32) and Michel; sphenopalatine by Lenhossék (29); and submaxillary and sublingual by myself (45); in the large ganglia of the chain by a number of investigators, beginning with Aronson (19); in the respiratory passages by Arnstein (46); in the heart of the rabbit by Aronson (19); intestinal canal and other peripheral ganglia by Dogiel; in the suprarenal by Dogiel (47); in the epididymis by Timofeew (48); and have further been seen by me in the bladder and oesophagus of the cat. In all the Mammalia studied, and in all the ganglia, they have essentially the same structure, and so far as my observations go, in sections of the ganglia of the sympathetic chain and various peripheral ganglia of the dog, cat, rabbit, and guinea pig, the pericellular plexuses are always intra-capsular. Sometimes only one fiber may end in a pericellular plexus; sometimes two, three, or even more fibers may take part in the formation of one plexus; these fibers are always, so far as may be gathered from the investigations on the sympathetic ganglia of Mammalia, collateral or end branches of medullated fibers.

So far as concerns the small medullated fibers, which may be traced from this or that nerve root of a ganglion into said ganglion, my own observations confirm wholly the account given by Dogiel (40). Such fibers may readily be seen in sections of methylene blue stained ganglia, where they are found branching and rebranching, and forming, with the dendritic processes of the ganglion cells, what Dogiel has described as the intercellular plexus; this plexus is always extra-capsular and may be seen in Pl. V, Fig. 26—a portion of a section about $20\ \mu$ in thickness. In sections one-third or one-half that thickness I have now and then observed what I have looked upon as the ending of the fibers under discussion. In Pl. V, Fig. 25, is reproduced a cell from a section about $10\ \mu$ in thickness of the solar ganglion of a cat stained in methylene blue. The cell body of this cell was deeply stained, its neuraxis, *a*, and dendrite, *b*, not so deeply; these could, however, be clearly

made out. A very small non-medullated fiber, *c*, could be traced with the utmost clearness to its ending on one of the protoplasmic branches of the cell in question; this non-medullated fiber terminated in two very small nodular enlargements, which were in contact with this dendrite, as shown in *c'* of this figure. In a number of instances such endings were made out, so that I feel justified in concluding that at least some of the non-medullated fibers in the ganglia, possibly also the terminal branches of the *small* medullated fibers, terminate in the ganglia after this manner.

In giving my own conclusions as to the nature of the fibers ending in the sympathetic ganglia of Mammalia, I can do no better than to quote the conclusion reached by Dogiel (40) concerning this point, which is as follows: "Die feinen Fasern, welche in den Ganglien mit intercellularem Geflechte endigen, zu den sympathischen, augenscheinlich vorzugsweise markhaltigen Fasern gehören, die dicken Fasern aber, deren Endverzweigungen in den Ganglien pericellulare Geflechte bilden, zu den markhaltigen Fasern zu rechnen sind, welche aus dem Cerebrospinalsystem entspringen." These pericellular plexuses, as already stated, are always intra-capsular.

GENERAL CONCLUSIONS.

In these general conclusions my aim is to be as brief as possible. Two reasons may here be given in justification of this: (1) the results obtained in each of the vertebrate classes studied have to some extent been summarized in the foregoing pages; (2) the writer has, in a series of "Special Lectures on the Sympathetic Nervous System," given before the medical students of Michigan University, and published in the *Journal of Comparative Neurology*, Vol. VII, No. 2, September, 1897, dwelt more fully on many of the questions which will here be touched upon.

From a study of the sympathetic ganglia of vertebrates the following facts concerning the shape and structure of the sympathetic neurons, and the nerves ending in the ganglia, may be deduced:

1) In all vertebrates, excepting the Amphibia, the great majority of the sympathetic neurons are multipolar, possessing a varying number of dendrites; but each cell has only one neuraxis. In Amphibia the sympathetic neurons, with the exception of those found in the coats of the intestinal canal and stomach, which are multipolar, are unipolar cells, as Kölliker has previously stated. In vertebrates other than Amphibia, some few unipolar and bipolar sympathetic neurons are to be found.

2) In all the vertebrates examined chromophile granules were found. One relatively large nucleus is the very general rule; the sympathetic cells of rabbits, hares, and guinea pigs forming an exception; many sympathetic cells with two or, occasionally, even three nuclei being here found.

3) The neuraxes of sympathetic cells may be medullated throughout, medullated for only a portion of the process, or non-medullated throughout; the medullary sheath, if present, forms a relatively thin layer, thinner than in the small medullated cerebro-spinal fibers. (The above statements are taken from Kölliker's writings.)

The neuraxes of sympathetic neurons have one or the other of the following distributions:

- a) To involuntary smooth muscle or heart muscle.
- b) To glandular tissues.
- c) To other sympathetic ganglia (?).
- d) To the spinal ganglia.

In the special lectures above alluded to, the writer has discussed, somewhat at length, each of the above possible modes of termination of the neuraxes of sympathetic neurons, considering also the literature bearing on this subject. It may here suffice to add that Arnstein (46) has recently traced, and pictured the neuraxis of a sympathetic neuron ending in smooth muscle tissue; the writer has traced small branches of non-medullated fibers from some of the small ganglia found in the cat's auricle to their ending on heart muscle, and also the neuraxes of sympathetic neurons of the sublingual ganglion (Langley) to the epilamellar plexus surrounding alveoli of the gland of the same name; from Dogiel's (40) work, some of

which I have corroborated, the conclusion may be drawn that neuraxes of sympathetic neurons (especially those surrounded by a thin layer of myelin) end in the intercellular plexuses of sympathetic ganglia, probably on the dendrites of sympathetic cells; and, finally, Ramon y Cajal's (36) and Dogiel's (49) observations on the ending of sympathetic nerve fibers in the spinal ganglia of Mammalia support the view that the neuraxes of sympathetic neurons end in the spinal ganglia.

4) The cell bodies of sympathetic neurons are surrounded by a nucleated capsule, which apparently has the same structure in all vertebrates.

5) In the sympathetic ganglia of all vertebrates studied by others and myself (speaking here of results obtained with either the Golgi or the methylene blue method), medullated fibers ending in pericellular intra-capsular plexuses have been found. These are, in all instances described, of essentially the same structure. I am well aware, as the accompanying plates may show, that the relation of the pericellular plexuses to the cell bodies of the enclosed sympathetic neurons varies somewhat in the different vertebrates, as do also the course, structure, and relation to other processes of the nerve fibers ending in such pericellular plexuses; yet these differences are not essential and important. The question now arises, What is the origin of the medullated fibers thus ending in the pericellular plexuses? "Bei der Ermittlung der schwierigen, hier zur Sprache kommenden Verhältnisse haben sich" (as Kölliker aptly states) "die Physiologie und die feine Anatomie brüderlich die Hand gereicht." The anatomical side of the question has been repeatedly touched in the preceding pages, where it may have been seen that abundant observations have been made, both with the Golgi and methylene blue method, to show that such medullated fibers enter the ganglion either through the white rami (chain ganglia) or through some nerve root of the ganglion. Once in the ganglion, the medullated fibers have been shown to branch and even rebranch before ending in the pericellular plexus.

Physiologists have long known that all sympathetic effects, which may be produced by stimulating a sympathetic nerve in any region, may also be produced by stimulating some spinal

nerve within the vertebral canal. This, as is now well known, and first clearly shown by Gaskell (50), is explained by the fact that certain small medullated fibers, which leave the cord through the anterior roots of certain spinal nerves—first dorsal to the third or fourth lumbar inclusive—reach the sympathetic ganglia through the white rami communicantes. These fibers end, not only in the ganglia of the chain, but also may be traced into the pre-vertebral and even the peripheral ganglia, as the following statement taken from Gaskell's account may show:

"The white rami communicantes are formed by an outflow of medullated fibers from both the anterior and posterior roots of the spinal nerves between the second thoracic and second lumbar inclusive, which medullated fibers pass not alone into their metameric sympathetic lateral ganglia, but also form three main streams, upwards into the cervical ganglia, downwards into the lumbar and sacral ganglia, and outwards into the collateral (pre-vertebral) ganglia. The white rami communicantes alone constitute the rami viscerales of the morphologist. The outflow of visceral nerves from the central nervous system into the so-called sympathetic system takes place by their means alone."

That these small medullated fibers are the fibers which, in all vertebrates studied, end in the sympathetic ganglia in pericellular plexuses, may now be assumed with much certainty; and this for two main reasons:

a) Medullated fibers have been traced from the white rami into the sympathetic ganglia, or from some nerve root into the peripheral ganglia, and have been seen to end in pericellular plexuses, as repeatedly stated.

b) The researches of Langley (51), Anderson, and Dickinson have taught us that in nicotin we have a drug which shows most clearly that the action of such fibers is interrupted in the sympathetic ganglia; that the sympathetic effects which may be obtained on stimulating a cerebro-spinal nerve, containing the small medullated fibers first described by Gaskell, or a white ramus made up of such fibers, cannot be obtained if a solution of nicotin of proper strength be injected into the circulation of the animal or be applied to the sympathetic ganglia with which such fibers are connected.

Such observations led Langley to conclude that the nerve fibers, issuing from the cord and reaching the sympathetic ganglia through the white rami, had each a sympathetic nerve cell in its course, which sympathetic cell was paralyzed by nicotin. Langley has suggested the term pre-ganglionic fibers or pre-cellular fibers to designate the efferent medullated fibers before they reach the nerve cells, and post-ganglionic or post-cellular fibers after they have traversed the nerve cells. Taking these statements into consideration, it would seem reasonable, to say the least, that the pre-ganglionic fibers are the medullated fibers which end in the sympathetic ganglia in pericellular plexuses, and the post-ganglionic fibers, the neuraxes of the sympathetic neurons of said ganglia.

It is not, however, to be supposed that such pre-ganglionic fibers end only in the chain ganglia in pericellular plexuses. Small medullated fibers may, as we have quoted from Gaskell, be traced into the peripheral ganglia, and the mere fact that in such ganglia pericellular plexuses, similar in structure to those found in the chain ganglia, have been described as the endings of medullated fibers, would warrant the conclusion that many of the pre-ganglionic fibers reach also the peripheral ganglia. Kölliker (33) has stated the fact so tersely that I may be allowed to quote again from him: "Hierbei ist der Verlauf derselben" (meaning here the pre-ganglionic fibers) "ein längerer oder kürzerer. Die einen enden an den nächstgelegenen Ganglienzellen, andere durchlaufen mehrere Ganglien, bevor sie zu ihren Endigungen gelangen und können hierbei durch Collateralen auf eine Mehrheit von Zellen einwirken. Noch andere endlich finden erst an den am meist peripherisch gelegenen Ganglien ihr Ende, wobei es unentschieden bleibt, ob sie in ihrem Verlaufe auf zwischengelegene Zellen einwirken."

These observations warrant, it seems to the writer, the following statements: the sympathetic neurons form the peripheral links in a nerve chain, of which the second link is formed by a neuron, the cell body of which is situated in the cerebro-spinal axis, the neuraxes of which form the pre-ganglionic fibers ending in pericellular plexuses, which unite the two links physiologically.

It is probable, then, that in all vertebrates this statement holds good: an impulse, leaving the cerebro-spinal system, and having sympathetic effects, is transferred from a pre-ganglionic fiber to one or several sympathetic cells which convey it along their neuraxes to the periphery.

The writer would here again offer the suggestion first made in the lectures above referred to, that nicotin does not primarily paralyze the sympathetic cells of the sympathetic ganglia, but the pericellular plexuses, the endings of the pre-ganglionic fibers in the sympathetic ganglia. This theory, for it is but a theory, is based on the analogy which exists between the physiological action of nicotin and certain other drugs, notably curare. The latter drug, as is well known, paralyzes the motor endings in striped muscle, and has an action very similar to nicotin on the sympathetic ganglia; on the other hand, nicotin paralyzes also the motor endings in striated muscle, not quite so readily as curare, but in a similar manner; its action on the sympathetic ganglia has already been explained. It would seem reasonable, therefore, to suppose that, in both cases above alluded to, curare and nicotin paralyze the ending of the cerebro-spinal fiber; in the one case, the motor ending in striped muscle; in the other case, the pericellular plexuses in the sympathetic ganglia.¹

The question as to whether the neuraxes of sympathetic cells may end in other sympathetic ganglia, and may in this way influence other sympathetic cells, seems as yet open to discussion. Kölliker and Langley are of the opinion that the neuraxes of sympathetic neurons end always in the periphery, in involuntary muscle, gland tissue, etc.; while Dogiel, with whom my own observations on this point lead me to concur, believes that the fine fibers, which end in, and help to form the intercellular plexus of the sympathetic ganglia, are the neuraxes of sympathetic neurons, more especially the myelinate ones. They end, I believe, on the dendrites of the sympathetic neurons of the ganglion.

HISTOLOGICAL LABORATORY, UNIVERSITY OF MICHIGAN,
July, 1897.

¹ In his later writings, Professor Langley gives this explanation of the action of nicotin on the sympathetic ganglia.

LITERATURE CONSULTED.

NOTE.—In a number of instances the reference number is repeated, as the same thought is expressed in two or more articles by the writers mentioned.

METHOD.

1. '91 RIESE. Zusammenfassendes Referat über die vital Methylenblaufärbung des Nervengewebes. *Centralblatt f. allgemeine Path. u. path. Anatomie.* Vol. ii, Nos. 20, 21.
2. '95 BETHE. Studien über das Centralnervensystem von Carcinus Maenas nebst Angaben über ein neues Verfahren der Methylenblaufixation. *Archiv f. mikr. Anat.* Vol. xlv.
3. '96 BETHE. Eine neue Methode der Methylenblaufixation. *Anat. Anzeiger.* Vol. xii, No. 18.
4. '95 MEYER. Die subcutane Methylenblauinjection, ein Mittel zur Darstellung der Elemente des Centralnervensystems von Säugethieren. *Archiv f. mikr. Anat.* Vol. xlv.
4. '96 MEYER. Ueber eine Verbindungsweise der Neuronen. Nebst Mittheilungen über die Technik und die Erfolge der subcutanen Methylenblauinjection. *Archiv f. mikr. Anat.* Vol. xlvii.
5. '95 LAWDOWSKY. Zur Methodik der Methylenblaufärbung und über einige neue Erscheinungen des Chemotropismus. *Zeit. f. wiss. Mikr.* Vol. xii.

FISHES.

6. '95 MONTI. Contributo alla conoscenza dei nervi del tubo digerente dei pesci. *Rendiconti del r. Istituto lombardo di sc. e lettere.* Serie ii, Vol. xxviii.
7. '95 SAKUSSEFF. Comptes rendus des séances de la société impériale des natur. de Saint-Petersburg. December, 1895. Taken from Dogiel, Zwei Arten sympathischer Nervenzellen. *Anat. Anzeiger.* Vol. xi, No. 22. 1896.
8. '74 ARNDT. Untersuchungen über die Ganglienzellen des Nervus sympathicus. *Archiv f. mikr. Anat.* Vol. x.

AMPHIBIA.

9. '63 BEALE. On the Structure of the So-called Apolar, Unipolar, and Bipolar Nerve-Cells of the Frog. *Quart. Journ. of Micr. Sci.* New Series. Vol. iii.
10. '63 ARNOLD. Zur Histologie der Lunge. *Virchows Archiv.* Vol. xxviii.
11. '65 ARNOLD. Ueber die feineren histologischen Verhältnisse der Ganglienzellen in dem Sympathicus des Frosches. *Virchows Archiv.* Vol. xxxii.

12. '67 ARNOLD. Beiträge zu der feineren Structur der Ganglienzellen. *Virchows Archiv*. Vol. xli.
13. '66 COURVOISIER. Beobachtungen über den sympathischen Gränzstrang. *Archiv f. mikr. Anat.* Vol. ii.
14. '66 KOLLMAN and ARNSTEIN. *Zeit. f. Biologie*. Vol. ii.
15. '68 SCHWALBE. Ueber den Bau der Spinalganglien nebst Bemerkungen über die sympathischen Ganglienzellen. *Archiv f. mikr. Anat.* Vol. iv.
16. '74 ARNDT. Untersuchungen über die Ganglienkörper des Nervus sympathicus. *Archiv f. mikr. Anat.* Vol. x.
17. '76 AXEL KEY and RETZIUS. Studien in der Anatomie des Nervensystems und Bindegewebes. Vol. ii.
18. '86 EHRLICH. Ueber die Methylenblaureaction der lebenden Nervensubstanz. *Deutsche med. Wochenschrift*. Vol. xii, No. 4.
19. '86 ARONSON. Beiträge zur Kenntniss der centralen und peripheren Nervenendigungen. *Inaug. Dis.* Berlin, Aug. 14.
20. '87 ARNSTEIN. Die Methylenblaufärbung als histologische Methode. *Anat. Anzeiger*. Vol. ii.
21. '89 RETZIUS. Zur Kenntniss der Ganglienzellen des Sympathicus. *Verhand. d. Biol. Vereins in Stockholm*. Vol. ii, Nos. 1, 2. November.
22. '89 LAWOWSKY. Weitere Beobachtung über Nervenendigungen, etc. *Beilage zum lxi. Bande der Denkschriften der Kaiserlichen Akad. d. Wissensch. zu St. Petersburg*. 1889. (Russian.) Taken from Smirnow.
23. '90 FEIST. Beiträge zur Kenntniss der vitalen Methylenblaufärbung des Nervengewebes. *Archiv f. Anat. u. Physiol.* Anat. Abtheil.
24. '90 SMIRNOW. Die Structur der Nervenzellen im Sympathicus der Amphibien. *Archiv f. mikr. Anat.* Vol. xxxv.
25. '96 KÖLLIKER. Handbuch der Gewebelehre des Menschen. Sechste umgearbeitete Auflage, zweiter Band, zweite Hälfte, p. 864.
26. '96 HUBER. The Spinal Ganglia of Amphibia. *Anat. Anzeiger*. Vol. xii.

BIRDS.

27. '91 RAMON y CAJAL. Algunos detalles sobre las células simpáticas. *Pequeñas contribuciones al conocimiento del sistema nervioso*. Vol. vi, pp. 56-58. Barcelona. 1891. Taken from *Ergebnisse der Anat. u. Entwicklungsgeschichte*. Vol. ii. 1892.
28. '92 RETZIUS. Ueber den Typus der sympathischen Ganglienzellen der höheren Wirbelthiere. *Biol. Unters.* Neue Folge. Vol. iii.
29. '94 v. LENHOSSÉK. Ueber das Ganglion Sphenopalatinum und den Bau der sympathischen Ganglien. *Beiträge zur Histologie des Nervensystems und der Sinnesorgane*. Wiesbaden.

MAMMALIA.

- '86 ARONSON. (19)
30. '89 KÖLLIKER. Histologische Mittheilungen. *Sitzungsber. der phys.-med. Ges. zu Würzburg.* 1889.
31. '92 KÖLLIKER. Sympathische Zellen aus dem Ganglion cervicale supremum und aus dem Ganglion solare des Kalbes. Demonstrated and described at the sixth meeting of the "Anatomische Gesellschaft" in Wien, June 7-9. *Verhandlungen.* 1892.
32. '94 KÖLLIKER. Ueber die feinere Anatomie und die physiologische Bedeutung des sympathischen Nervensystems. "Gesellschaft Deutscher Naturforscher und Aerzte." *Verhandlungen.* 1894. Allgemeiner Theil.
33. '96 KÖLLIKER. Handbuch der Gewebelehre des Menschen. Sechste umgearbeitete Auflage, zweiter Band, zweite Hälfte, pp. 850-864.
34. '91 RAMON y CAJAL. Estrutura y conexiones de los ganglios simpáticos. *Pequeñas contribuciones al conocimiento del sistema nervioso.* Barcelona. 1891.
35. '91 RAMON y CAJAL. Estructura del gran simpático de los mamíferos. *Gaceta sanitaria* del 10 Diciembre, 1891. Taken from *Ergebnisse der Anat. u. Entwicklungsgeschichte.* Vol. ii. 1892.
36. '93 RAMON y CAJAL. Neue Darstellungen vom histologischen Baue des Centralnervensystems. *Archiv f. Anat. u. Physiol.* Anat. Abtheil.
37. '92 VAN GEHUCHTEN. Les cellules nerveuses du sympathique chez quelques mammifères et chez l'homme. *La Cellule.* Vol. viii. 1892.
- '92 RETZIUS. (28)
38. '93 SÁLA. Sur la fine anat. des ganglions sympathiques. *Arch. Ital. de Biol.*
- '94 v. LENHOSSÉK. (29)
39. '94, '95 D'ERCHIA. Struttura del ganglio ciliare. *Monitore Zoologico Italiano.* Nos. 9, 10, 1894, and No. 7, 1895.
40. '95 DOGIEL. Zur Frage über den feineren Bau des sympathischen Nervensystems bei den Säugethieren. *Archiv f. mikr. Anat.* Vol. xvi.
41. '96 DOGIEL. Zwei Arten sympathischer Nervenzellen. *Anat. Anzeiger.* Vol. xi.
42. '66 GUYE. Die Ganglienzellen des Sympathicus beim Kaninchen. *Med. Centralblatt.*
43. '96 APOLANT. Ueber die sympathischen Ganglienzellen der Nager. *Archiv f. mikr. Anat.* Vol. xlvii.
44. REMAK. Quoted by Schwalbe. (15)

45. '96 HUBER. Observations on the Innervations of the Sublingual and Submaxillary Glands. *Journ. of Experimental Medicine.* Vol. i, No. 2.
46. '97 ARNSTEIN (ADAM PLOSKKO). Die Nervenendigungen und Ganglien der Respirationsorgane. *Anat. Anzeiger.* Vol. xiii.
47. '95 DOGIEL. Zur Frage über die Darmgeflechte der Säugetiere. *Anat. Anzeiger.* Vol. xi.
48. '94 TIMOFFEY. Zur Kenntnis der Nervenendigungen in den männlichen Geschlechtsorganen der Säuger. *Anat. Anzeiger.* Vol. ix.
49. '96 DOGIEL. Der Bau der Spinalganglien bei den Säugetieren. *Anat. Anzeiger.* Vol. xii.
50. GASKELL. On the Structure, Distribution, and Function of the Nerves which innervate the Visceral and Vascular Systems. *Journ. of Physiology.* Vol. vii.
51. '89 LANGLEY and LEE DICKINSON. On the Local Paralysis of Peripheral Ganglia, and on the Connection of Different Classes of Nerve Fibers with them. *Proceed. of the Royal Society of London.* Vol. xlvi, p. 423.
- '90 LANGLEY and LEE DICKINSON. On the Progressive Paralysis of Different Classes of Nerve Cells in the Superior Cervical Ganglion. *Proceed. of the Royal Society of London.* Vol. xlvii, p. 379.
- '90 LANGLEY. On the Physiology of Salivary Secretion. *Journ. of Physiology.* Vol. xi.
- '92 LANGLEY. On the Origin from the Spinal Cord of the Cervical and Upper Thoracic Sympathetic Fibers, with Some Observations on White and Gray Rami Communicantes. *Phil. Trans. of the Royal Society of London.* Vol. clxxxiii.
- '92 LANGLEY and ANDERSON. The Action of Nicotin on the Ciliary Ganglion, and on the Endings of the Third Cranial Nerve. *Journ. of Physiology.* Vol. xiii.
- '95 LANGLEY. A Short Account of the Sympathetic System. Physiological Congress, Berne. Further references to Langley's work may be found in *Journ. of Physiology.* Vols. xiv, xv, 1893; xvi, xvii, 1894; xviii, xix, 1895; xx, 1896.

The literature which has appeared since this article was sent to the editor will receive consideration in a future publication. — G. C. H.

EXPLANATION OF PLATE III.

All figures, with the exception of Nos. 1, 19, and 26, were sketched with the aid of camera lucida under a $\frac{1}{2}$ -inch oil immersion and No. 1 eyepiece, the image being reflected to the table, this giving a magnification of 890 diameters. Figs. 1, 19, and 26 were sketched under a No. 7 objective and No. 1 eyepiece, with about 400 diameters amplification.

FIGS. 1-7. *Sympathetic Ganglia of Fishes.*

FIG. 1. Sympathetic neurons from sections of sympathetic ganglia, stained in methylene blue, of small-mouth black bass (*Micropterus dolomieu* Raf.); *a*, *b*, and *c*, unipolar cells; *d* and *e*, multipolar cells.

FIGS. 2, 3. Pericellular plexuses found in sections of sympathetic ganglia of black bass. Methylene blue stain. *a*, neuraxis, ending in pericellular plexus. In Fig. 3 the enclosed ganglion cell is faintly indicated in black.

FIG. 4. From teased preparation of sympathetic ganglion of black bass, stained in methylene blue, fixed in ammonium molybdate and hardened in alcohol. *c*, sympathetic cell; *n*, nucleus; and *c*, its capsule; *a*, medullated fiber terminating within the capsule in pericellular plexus.

FIG. 5. The same cell, double-stained in alum carmine. Some of the end branches of pericellular plexus terminate between the intra-capsular nuclei.

FIG. 6. Completely isolated cell, obtained by teasing a sympathetic ganglion of black bass, stained in methylene blue and alum carmine. *a*, cell body of a sympathetic neuron; *b*, medullated fiber ending within capsule *c*.

FIG. 7. From the same ganglion from which Fig. 6 was taken. *A*, large medullated fiber giving off two collateral branches, *a'* and *a''*, ending within the capsules of cells *A* and *B*, respectively.

FIGS. 8-11. *Sympathetic Ganglia of Amphibia.*

FIG. 8. Small portion of a section of sympathetic ganglion of *Rana Catesbiana*, stained in methylene blue; only spiral fibers and pericellular plexuses stained. Shows the connection of spiral fiber, *a*, with pericellular plexus, the structure of which is well shown in this figure. In *d* we may see one of the large nodular swellings now and then seen; *e*, free ending of fibril of the pericellular plexus.

FIGS. 9, 10. Two ganglion cells from section of sympathetic ganglion of *Rana C.*, stained in methylene blue and alum carmine. *a*, neuraxis of unipolar cell; *b*, sheath nuclei of the neuraxis; *c*, capsule; *d*, nodular swellings in pericellular plexus; *e*, free endings of fibrils of plexus; *s. f.*, spiral fibers.

FIG. 11. Sympathetic neurons of Auerbach's plexus of large intestine of *Rana C.*, stained in methylene blue and fixed in ammonium picrate. *a*, neuraxis; *b*, dendrites.

EXPLANATION OF PLATE IV.

FIGS. 12-19. *Sympathetic Cells of Reptilia.*

FIG. 12. Two sympathetic cells from the inferior cervical ganglion of *Chrysemys picta*; methylene blue stain. *a*, neuraxis; *b*, sheath nucleus of neuraxis; *c*, clear zone of protoplasm surrounding nucleus; protoplasm shows chromophile granules.

FIG. 13. From section of dorsal ganglion of *Chelydra serpentina*, stained in methylene blue. Simple pericellular plexuses. *a*, neuraxis of fiber ending in pericellular plexus; *b*, free endings of fibrils.

FIGS. 14-17. More complicated pericellular plexuses, with spiral fiber. All sketched from sympathetic ganglia, *Chelydra serpentina*, stained in methylene blue.

FIG. 18. From section of sympathetic ganglion of *Chelydra serpentina*, stained in methylene blue and alum carmine. *A*, neuraxis; *B*, cell body; *C*, nucleus; and *D*, nucleolus of large unipolar sympathetic neuron. *a, a', a''*, three neuraxes of medullated nerve fibers, forming a spiral and ending in pericellular plexus; *c*, capsule.

FIG. 19. A portion of a section of an inferior cervical ganglion of *Chelydra serpentina*, stained in methylene blue and alum carmine; showing a number of more complicated pericellular plexuses with spiral fibers, *A*, and one simpler plexus without spiral fiber, *B*. Also the network of medullated and non-medullated fibers between ganglion cells.

EXPLANATION OF PLATE V.

FIGS. 20-22. *Sympathetic Ganglia of Birds.*

FIG. 20. From methylene blue stained section of one of the dorsal sympathetic ganglia of chicken. *A*, medullated fiber, with node of Ranvier at *x*, at which place is given off a non-medullated collateral branch, *a*, which terminates in two pericellular plexuses; *n*, a non-medullated, probably a sympathetic nerve, ending on *d*, the dendrite of the sympathetic cell.

FIG. 21. A portion of a section of a dorsal sympathetic ganglion of chicken, given to show the arrangement of the multipolar cells and the "intercellular plexus," formed by the dendrites, medullated and non-medullated fibers of the ganglion; *b*, pericellular plexus. The capsules of the sympathetic cells were not stained, and therefore not shown in the figure.

FIG. 22. Three sympathetic cells, with pericellular plexuses from dorsal ganglion of chicken. Methylene blue and alum carmine. *a*, neuraxis of nerve fiber ending in pericellular plexus; *c*, capsules of sympathetic cells.

FIGS. 23-26. *Sympathetic Ganglia of Mammalia.*

FIG. 23. A portion of the stellate ganglion of dog. Methylene blue and alum carmine. *a*, non-medullated fiber, collateral branch of pre-ganglionic fiber ending in pericellular plexus; *b*, fiber ending in pericellular plexus which is medullated to within a short distance of the capsule.

FIG. 24. Sympathetic cell from stellate ganglion of dog (methylene blue and alum carmine), showing a more complex pericellular plexus with spiral fibers.

FIG. 25. Sympathetic neuron from semilunar ganglion of the cat, methylene blue staining. *a*, neuraxis; *d*, dendrite; *c*, non-medullated, probably sympathetic fiber ending on dendrite at *c'*.

FIG. 26. A portion of a section of semilunar ganglion of cat, methylene blue staining, showing arrangement of sympathetic cells and "intercellular network" formed by the dendrites of the sympathetic cells and the medullated and non-medullated fibers of the ganglion.