

OBSERVATIONS ON THE INNERVATION OF THE INTRACRANIAL VESSELS.

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With Plate I.

The research, the results of which are here given, was begun some two years ago, at which time the only observations, familiar to me, dealing with the innervation of the vessels of the pia-mater and brain, were those of Lovell Gulland (1) and Obersteiner (2). Gulland (1), at the suggestion of Dr. Batty Tuke, examined the brain vessels, with a view of ascertaining "whether it was possible to demonstrate nerve-fibrils in the walls of the intracranial bloodvessels." "The methods employed were the Golgi method with various modifications and the Ehrlich methylene blue method. The brains examined were those of cats and rabbits, adult, young and embryonic and a number of human brains obtained as fresh as possible from the post-mortem room." His results are summarized by himself as follows: "The net result of all these observations was that neither by the silver, the mercury, nor the methylene blue method could I succeed in demonstrating any nerve-fibers in the walls of the pial vessels nor of the intracerebral vessels. In Golgi preparations, the bloodvessels, especially the smaller ones, are often impregnated as a whole; sometimes a partial impregnation of this sort gives an appearance like a nerve plexus. Again in the larger vessels especially, the longitudinal network of elastic fibers is often impregnated, but it is easy to distinguish this from the nerve plexus of ordinary arteries. The processes of the neuroglia cells attached to an artery and running along it, as they often do, for a little way, might also give rise to error."

Obersteiner (2) describes a preparation of the pia-mater, taken from the convexity of the human brain, stained in gold chloride, which has been in his collection for many years, and which contained a small vessel (figured by him), on which a number of relatively large nerve-fibrils were found. He states that in this preparation, only the coarser nerve-branches seemed stained, the finer branches, with their terminations remaining unstained. "Es ist also" (he adds) "damit der directe anatomische Beweis geliefert, dass die feineren intracraniellen Gefässe, wenigstens innerhalb der Pia mater, ihre eigenen Nerven besitzen. Daraus darf doch ohneweiters der Schluss gezogen werden, dass diesen Gefässen auch die Fähigkeit zukommt, sich activ, selbstständig zu contrahiren, respective zu dilatiren."

These contradictory statements, taken in connection with the fact that physiologists very generally deny the existence of vaso-motor fibers in the intracranial vessels—if one may be allowed to judge from their writings—led me to re-investigate the question under consideration. Sometime after the completion of my own observations, I was gratified to see a short abstract, in which Gulland (3) states that on renewed investigation he had been able to find nerves on the intracranial vessels. He there states that: "In material from human brains and those of dogs prepared by the Cox's method, given him by Dr. W. Ford Robertson, he found nerves on a number of the vessels, and saw that they presented the usual appearance of perivascular nerve plexuses." At the Edinburgh meeting of the British Medical Association, microscopical specimens were shown in proof of this fact. This abstract further states, that Dr. H. Morrison, of London, had also been able to demonstrate these nerves by Sihler's hæmatoxylin method. In consideration of the fact that my own work was practically completed before I became cognizant of the observations just given, and further, since the methods adopted by me seem to differ in every respect from those followed by the English observers, I see no inconsistency in presenting my own results at this time. The answer to the question of the existence or non-existence of vaso-motor nerves on the intracranial vessels is so necessary to a

correct understanding of the circulation of the brain, both in its physiological and pathological aspect, that corroborative results obtained in the investigation of this fundamental anatomic fact, can but extend and substantiate our knowledge of brain physiology.

In my own experiments I have used dogs, rabbits and cats. For staining the nerves I have used the methylen blue method of Ehrlich. After anaesthetizing the animal, the carotid was exposed in the neck, on one side, and a canula inserted cerebralward. I then injected of a 1% methylen blue solution, made up in normal salt solution, a quantity sufficient to tinge lightly blue the ear and eye of the side on which the injection was made. It was found expedient to remove the thoracic and abdominal viscera, and elevate the head of the injected animal for a few moments after the injection, to allow as much of the blood, as would do so, to drain from the head. Unless this is done there is always oozing and bleeding during the removing of the brain. Some thirty to forty-five minutes after the injection, the brain and cervical cord were exposed, and to insure against lacerating the pia-mater, the dura was, during this step, injured as little as possible. After removing the dura, the cord was cut about one inch below the junction of the vertebral arteries and the brain removed. The cerebellum was then cut off and an incision made into each cerebral hemisphere, and these reflected outward. The brain was then placed on a glass plate, base uppermost and exposed to the air for a period varying from five to thirty minutes or until it was found that the nerve fibers sought were stained. After some experimentation it was found that preparations of the pia-mater could be most easily made by dissecting off with curved scissors, pieces of the brain cortex, and after placing these on a large slide, pial surface uppermost, and covering them with a large cover glass, exerting pressure sufficient to press the brain tissue out from under the pia-mater.

Even a delicate pia-mater—from the rabbit for instance—may be treated in this way without laceration. This simple method has the advantage of giving preparations which may at

once be examined microscopically. Relatively large pieces of pia-mater may in this way be obtained, practically free from brain tissue. I have for instance a number of times been able to obtain, in one piece, the pia-mater from the base of the brain of a cat, with that covering the lower surface of the medulla and upper cervical cord, which, although slightly lacerated, would contain a complete circle of Willis with its main branches, the basilar and a portion of the vertebrals. When it was found that the perivascular nerves were well stained, the pieces of pia-mater were fixed either in an ammonium picrate solution, (Dogiel) or in the ammonium molybdate solution (Bethe). Some of the ammonium molybdate fixed tissue was hardened in alcohol, sectioned and counterstained in alum-carmine. I have given the method used by me somewhat in detail, since Gulland (1) mentions having used the methylen blue method in his research, without success however. If I read his account correctly, the methylen blue method used by Gulland, was the modification of Ehrlich's method suggested by Dogiel, according to which a dilute solution of methylen blue, made with normal salt solution, (1-1000 used by Gulland) is applied to the tissues. This method was used by me, with negative results.

The existence of a perivascular plexus in the arteries carrying blood to the brain, seems beyond question. A number of anatomies consulted with reference to this point mention them. We read in Quain that the ascending carotid branches of the superior cervical ganglion, as they ascend into the skull with the internal carotid artery, divide into two parts, known respectively as the internal and external division. The terminal parts of these two divisions extend to the cerebral and ophthalmic arteries, around which they form secondary plexuses, those on the cerebral arteries ascending to the pia mater.

And again: "From the lowest cervical and first dorsal ganglion, slender grey branches ascend along the vertebral artery in its canal, forming a plexus (plexus vertebralis) around the vessels by their intercommunications, and supplying it with offsets. This plexus is connected with the cervical spinal nerves as they cross the vertebral artery, and its ultimate ramifications

are continued on the intracranial branches of the vertebral and basilar arteries."

Testus states that "in the course of the arteries of the cranial pia-mater numerous nerves, arising probably from the carotid plexus, may be found. Their branches have been found on the small branches of the pial vessels, even such as penetrate the convolutions. Nothing however is known about their mode of ending." He asks the question,— "What is the anatomical significance of the nerve plexus on the pial vessels?" And answers, "It is very rational to think, they terminate in the smooth muscle of the arteries, and that they become here, as in other parts of the body, regulators of the circulation." He further suggests the probability of there being nerves other than vaso-motor on the vessels of the pia-mater, namely sensory nerves, susceptible of being stimulated in the periphery and thus becoming, both in normal and pathological conditions, points of departure of reflexes. Kölliker refers to the nerves on the cranial vessels in the following words.— "An der Hirnbasis finden sich an den Arterien des Circulus Willisii viele ähnliche Geflechte" [Reference is here made to nerve plexuses on the vessels of the pia of the cord, first described by Purkinje] "welche mit Stämmchen von höchstens 67μ mit den verschiedenen Arterien, mit Ausnahme derer des Cerebellum, immer dem Verlaufe desselben folgend, durch die ganze Pia des Gehirnes sich ausbreiten, jedoch in ihren Enden nirgends erkennen lassen; doch verfolgte ich dieselben bis zu Arterien von 90μ und darunter in die Substanz des Gehirns hinein."

He further states that in the pia-mater of the brain, other than sympathetic fibers (Plexus caroticus internus, Plexus vertebralis), are to be found; quoting Bochdalek to the effect that he had traced numerous branches from the roots of four of the cranial nerves to the perivascular plexuses on the vessels of the base of the brain and the cerebellum. That there are nerve plexuses on the larger vessels of the base of the brain, has long been known. This is shown by the fact that Bourgery and Arnold described very delicate nerve fibrils passing from the carotid plexus to the anterior and middle cerebral and posterior communicating. In

successfully stained methylen blue preparations, perivascular nerves may be clearly seen on the vessels constituting the circle of Willis, and on the larger arterial branches that proceed from it, and may from here be traced on to the arteries of the pia-mater of the hemispheres. Even under low magnification, however, it becomes obvious that such perivascular nerves do not all present the same characteristics. On the one hand, and this more particularly on the larger vessels, well formed bundles of medullated nerve fibers are observed; on the other, plexuses of non-medullated nerve fibers. To anticipate somewhat, I may state, for considerations which will follow, that I have come to regard the medullated nerve fibers as sensory nerve fibers, the perivascular plexuses of non-medullated fibers as vaso-motor fibers. I shall therefore consider the nerves associated with the arteries of the cranial pia-mater under these two heads.

Sensory nerves. In all well stained methylen blue preparations of the pia-mater, whether of the dog, cat or rabbit, I have observed relatively large medullated nerve fibers accompanying the blood vessels, not only the larger vessels found on the base of the brain and medulla, but following the vessels over the convexity of the cerebrum to the great longitudinal fissure. These medullated nerve fibers reach the pial vessels, so far as I have been able to determine, from two main sources. A relatively large bundle of medullated nerves—sometimes several small ones—joins the middle cerebral just after it gives off the posterior communicating branch. This, as a rule, divides into two branches, one joining the middle cerebral, the other the posterior communicating artery. The other source of medullated nerve fibers is from one or several bundles of medullated nerve fibers, which proceeds upwards on the basilar artery until it divides into the posterior cerebrals; the nerve-bundles on the basilar undergo a similar division and follow the branches of this artery. The bundles of medullated nerves on the basilar artery are formed from such found on the vertebral arteries. Fig. 1 may serve to illustrate these statements. In the figure the circle of Willis with a portion of the basilar artery,

removed from the base of a cat's brain, was reflected by camera lucida. From the same preparation the nerve bundles are sketched in semi-diagrammatically, only a sufficient number of nerve fibers being sketched to give the general course of the larger bundles of medullated nerves. In the preparation a relatively large bundle of medullated nerves divides into two branches, the one, *a*, accompanying the posterior communicating branch. This bundle and its branches could be followed along this artery on to the posterior cerebral of the side on which the nerves are sketched and on to the posterior cerebral of the opposite side. In this bundle, I was able to count, at its origin, some twenty relatively large medullated fibers. The other branch, *b*, containing ten large medullated fibers, accompanies the middle cerebral. The nerve bundle, *c*, I regarded as a continuation of *b*, although the connection is not apparent, as the nerves on the intervening portion of the vessels were practically unstained. The bundle of medullated nerves on the basilar artery, in which I was able to count 15 large nerve fibers, divides into branches which follow the right and left posterior cerebral. The branch on the left side was traced to the middle of the posterior communicating artery. There are then, and this may be emphasized, no distinct areas of distribution for the medullated nerve fibers, accompanying the pial vessels and coming from the two sources above mentioned. The bundles of medullated nerve fibers on the vessels forming the anterior part of the circle of Willis, are distributed to half of the circle of Willis and from this to the arteries arising from it, and to some extent to the opposite side of the circle of Willis; the medullated nerves entering with the basilar artery are distributed, in part, at least, to the anterior portion of the circle of Willis.

The medullated nerve fibers here under discussion undergo repeated division, such division taking place at the nodes of Ranvier; the resulting branches now and then seem to run parallel for a distance, again diverging at acute or obtuse angles. Such branching is repeatedly observed at places where the artery divides, one branch of a dividing medullated nerve accompany-

ing respectively, each of the arterial branches. In the preparation from which Figure 1 was drawn, at the place of division of the nerve bundle into branches *a* and *b*, a number of dividing medullated nerves may be observed in which one branch passes to the middle cerebral, the other to the posterior communicating. Similar observations I have repeatedly made. Attention is drawn to this fact to show over how large an area a single medullated fiber (sensory fiber) may be distributed, and to show how futile it would be to attempt to localize pain in the pia-mater; for it must be understood that such diverging branches may now and then be traced in the pia-mater of the cat, for distances approaching an inch, without reaching their termination.

In preparations of the pia-mater, coming from the base of the brain and embracing the circle of Willis and the anterior and middle cerebrals and their main branches, the medullated nerve fibers, above described as found in connection with the arteries of the circle of Willis, may be traced in connection with the branches of the anterior and middle cerebral to the borders of the preparation. In preparation of the pia-mater from the lateral aspect of the hemispheres they are again found with the vessels and in suitable preparations may be traced to the longitudinal fissures. On the lateral surfaces of the cerebral hemispheres the medullated fibers accompanying the pial vessels appear as single fibers or small bundles consisting of two, three, four or five medullated fibers. The bundles of medullated nerve fibers, as also single fibers, accompanying the pial vessels undergo, as before said, repeated division, not only at points of division of the arterial branches but at other points along the course of the vessels. The branches of bundles of medullated fibers or single fibers wind around the vessels in long sweeps, and here and there exchange fibers, and in this way form plexuses of large irregular meshes, which surrounds the vessels.

I have in a number of experiments been able to obtain methylen blue preparations which show most clearly the mode of termination of the medullated nerve fibers under discussion. The most successful preparations come from the cat, through a

sufficient number of observations have been made on the dog and rabbit to enable me to say that the endings described for the cat hold good for these animals also.

The most successful preparation obtained, showing the mode of termination of the medullated fibers accompanying the pial vessels is reproduced in Fig. 2, which gives the ending of a single medullated nerve fiber as seen under the 1-12 in. oil immersion. The preparation was unusually fortunate, as owing to the caprice of the methylen blue method, in the region of the vessel—posterior cerebral—from which the drawing was made, beside the branches of this one nerve, only a few coarse medullated branches with some few non-medullated fibers were stained. As may be seen from this figure, the medullated fiber, after reaching the vessel, gives off a number of relatively large medullated branches which leave the parent fiber at nodes of Ranvier; these primary branches divide further into medullated fibers of a second order, and of somewhat smaller size, some of which divide still further into still smaller medullated branches. The medullated branches terminate at a longer or shorter distance from the parent fiber in fine, varicose, non-medullated, terminal fibrils, which may often be traced for relatively long distances before they end, and this usually in a small granule or nodule. Similar non-medullated fibrils proceed from the medullated fibers, at nodes of Ranvier. These terminal, varicose fibrils have, as the figure may serve to show, a course which may be parallel or oblique to the long axis of the vessel, and therefore not parallel to the long axis of the non-striated muscle cells of the vessel wall, a fact to which I draw attention at this time, for reasons which will appear later. Complete, or even nearly complete endings such as shown here I have not often met. In well stained preparations, varicose fibrils such as above described and having a similar course, are met with in connection with the pial vessels in all parts of the pia-mater of the cerebral hemisphere, even on vessels with a musculature consisting at most of two or three layers of non-striated muscle cells. It is often somewhat difficult, however, and again impossible, to trace them for distances long enough to ascertain their

origin. So that relatively infrequently can they be traced to medullated fibers, from which I believe they proceed. I believe that these varicose fibers may be differentiated from the vascular nerves which terminate in the non-striated muscle tissue and which will be considered under the head of vaso-motor nerves. As to the relation of the medullated nerves and their endings to the vessels and their structural elements, my observations are not so conclusive as to be entirely beyond question, especially as concerns their ending. In methylen blue stained preparations of the pia, mounted in ammonium picrate-glycerine, especially if such preparations have been mounted some days, in which case even relatively thick pieces of tissue become quite transparent, it may be seen that the larger and smaller bundles of medullated nerve fibers and the majority of the single medullated fibers are situated in the connective tissue adventitia of the vessels or in the neighboring fibrous tissue. This is especially well shown, when, in the field of observation, the nerves happen to lie by the side of the artery. As is well known, non-striated muscle cells are readily stained by methylen blue when applied to the fresh tissue. Such cells retain their color after fixation. This fact makes it easy to determine the relation of the medullated nerve fibers to the muscular coat of the vessel.

This relation holds good for the smaller as well as the larger pial vessels. However, to make certain of this fact, especial attention was paid to it, in a number of series of sections of pia containing vessels. The tissue was stained in methylen blue, controlled under the microscope before fixing, fixed in ammonium molybdate, hardened in alcohol, embedded in paraffine and cut serially. The sections were then fixed to slides and counter-stained in alum carmine. In cross or oblique sections of vessels, in pial tissue thus prepared, the medullated nerve fibers were found in the adventitia. It was however much more difficult to reach a definite conclusion as to the disposition of the terminal branches of these medullated fibers, and their relation to the structure of the vessel wall. By studying surface preparations of the pia, the conviction is gained that the

majority of the slender, varicose fibrils, terminal branches of the medullated nerves, end in the adventitia of the vessel or in the surrounding connective tissue. When such varicose fibrils are seen by the side of the vessel, their termination in the connective tissue seems often easy to make out. When on the surface of the vessel presented to the observer, careful focusing will usually enable one to determine that, for the greater portion of their course, at least, they are not in the muscular layer of the vessel, although they are often in focus at the same time as is the superficial part of the muscular coat. This statement has reference especially to the larger vessels. In sections, prepared as above mentioned, I have now and again found relatively long segments of varicose nerve fibres in the adventitia, having a course parallel to the long axis of the vessels. Sections are however not so serviceable as surface preparations in the elucidation of this point, as it is impossible to obtain in a section segments of nerve long enough to form an idea concerning their course and relation to other nerve fibers.

So far as I may judge from my own observations, I believe I am warranted in making the statement that many at least of the non-medullated, terminal branches of the medullated fibers accompanying the vessels of the pia, end in the adventitia of the vessels or the fibrous tissue surrounding them; whether all do or whether some of them terminate in the muscular coat or even under the endothelium, I must leave as an open question.

In a number of preparations, medullated nerve fibers and their terminal branches were seen in the pia-mater some distance removed from vessels. This I have seen more frequently in the pia of the base of the brain and especially that portion enclosed in the circle of Willis. In the pia covering the lateral surfaces of the cerebral hemispheres such medullated nerve fibers have not been found frequently, but often enough to warrant the statement that here also the medullated nerves terminate in the pia in regions free from vessels. No medullated nerve fibers were found accompanying the veins of the pia. These veins, as is well known, have no muscular coat, and are therefore easily distinguished from the arteries in which the

muscle cells are readily stained. As previously stated, the medullated nerve fibers found in the pia-mater have been interpreted as sensory in function. My reasons for this assumption are based on their histological appearances, the nature of their endings and their distribution, and may be summarized as follows:

1. These nerves are distinctly medullated, a fact which is readily made out in methylen blue preparations, although as a rule only the neuraxes are stained. In methylen blue preparations the neuraxes of the medullated fibers stain more deeply at the nodes of Ranvier, and at the nodes present an appearance which suggests the Fromman's crosses obtained when treating medullated nerves with a silver nitrate solution. In more deeply stained methylen blue preparations the myelin of medullated nerves stains faintly blue and may then be readily recognized.

2. The medullated nerves in the pia appear, as has been stated, in relatively large bundles, especially in connection with larger vessels at the base of the brain; these bundles branch and anastomose forming plexuses, with wide meshes, which surround the vessels.

3. The neuraxes of these medullated fibers are much larger than the neuraxes of sympathetic neurons or white rami fibers known to me. Accurate measurements have not been made, yet I feel justified in making this comparison, basing my judgment on a somewhat extended experience with the size and appearance of sympathetic and white rami nerves as seen when stained with methylen blue. The possibility of their being cerebro-spinal motor fibers may, I believe, be dismissed without further comment.

4. These medullated fibers branch and re-branch and present the short internodal segments, usually observed when sensory nerve fibers approach their termination.

5. The non-medullated, varicose, terminal branches of the medullated fibers, the majority of them, at least, end in fibrous tissue—adventitia of vessels or in pia. These terminal fibrils are in the main parallel to the vessels or cross them

obliquely, and are thus unlike the terminal branches of the vaso-motor fibers which end in the muscular coat. These latter course along in the intercellular cement between the muscle cells and are parallel to them, that is, at right angles to the long axis of the vessels. This difference—the mode of distribution of the medullated nerve fibers and the vaso-motor is clearly seen in methylen blue preparations of the pia-mater, in which both sets of endings are stained on the same vessel.

6. Where the entire ending of the medullated fibers is made out, as in the preparation from which Fig. 2 was drawn, the ending resembles the peripheral termination of sensory nerves with free endings in other parts of the body, and is identical with the ending of sensory nerves found in the dura mater, to be described later. I have therefore looked upon the medullated nerve fibers found in the pia as sensory, since their arrangement, size and mode of ending make it improbable that they are the neuraxes of sympathetic neurons or of white rami neurons. The term *sensory nerve fibers* is here used in the sense commonly ascribed to it. Reference is had, to the dendrites of neurons in the cerebral or spinal ganglia with T-shaped processes.

Vaso-motor nerves. On the vessels of the pia-mater, from the larger vessels constituting the circle of Willis, to vessels with a muscular coat of not more than two layers of involuntary muscle cells, I have found perivascular nerves arranged in the form of a plexus, which in every respect resemble the perivascular nerves—vaso-motor nerves—found in the wall of vessels in other parts of the body. They are non-medullated and arranged in the form of plexuses which surround the vessels. On the internal carotid artery, as soon as it leaves the carotid canal, and on the vertebral after appearing in the spinal canal, such perivascular plexuses are observed, and these may in suitable preparations be traced on to the anterior and middle cerebral and posterior communicating on the one hand, and basilar and posterior cerebral on the other hand, and from the vessels of the circle of Willis to the branches that arise therefrom. On the

larger pial vessels, these non-medullated, varicose nerves form a primary plexus in the adventitia, the strands of which consist of small bundles of non-medullated fibers, and of single fibers. The small bundles of non-medullated nerves undergo repeated division; these, with the single fibers which also branch frequently, form an interlacing network—a nerve plexus. Within this plexus is formed a second, though not so well defined plexus, which would seem to lie just external to the muscular coat. From this, the terminal fibrils which end in the muscular tissues of the vessels, are given off. In Fig. 3 is sketched a short segment of one of the larger branches of the middle cerebral in the pia of a cat. In the figure the nerve plexus on that portion of the wall of the vessel turned toward the observer is reproduced. In the preparation from which this sketch was made, the sensory fibers were not well stained, their endings not at all, in the region of the vessel shown in the figure. It may serve to show the richness of the nerve supply of the pial vessels. The plexus in the adventitia being particularly well shown, the terminal fibrils could not be clearly made out with the magnification used, and are therefore not shown in the figure. In the smaller pial vessels with a muscular coat consisting of two to four layers of involuntary cells, only one plexus of non-medullated nerves is made out. In Fig. 4 is reproduced such a plexus. The sketch was made from a methylen blue preparation of the pia of a dog as seen under the 1-12 in. oil immersion. In one portion of this small artery, the non-striated muscle cells were well stained—not shown in figure—and in optical section it was made out that in this portion of the vessel there were present two layers of involuntary muscle cells in the media. I have found it much more difficult to stain the perivascular nerves on the smaller vessels of the pia; yet they have been found often enough in the dog, cat and rabbit in the pia-mater removed from various regions of the cerebral hemispheres so that I conclude that they are generally present, and that the difficulty experienced in staining them is due largely to the uncertainty of the method used.

That the ultimate branches of the fibers forming the perivas-

cular plexuses here discussed terminate in the muscular coat of the pial vessels, one may often observe in well-stained methylen blue preparations mounted in ammonium picrate-glycerine. In such preparations, by careful focusing, very delicate varicose threads are now and then found in the muscular coat, running parallel to the long axis of the involuntary muscle cells. To make certain, however, the serial sections, to which reference has previously been made, were studied with special reference to this point. In such sections—double-stained in methylen blue and alum carmine—very delicate varicose fibrils, stained blue, are found between the non-striated muscle cells of the vessel wall, which are stained red. And here and there in fortunate sections, these were seen to give off short lateral twigs terminating in fine granules on the muscle cells; in other words, the ultimate ending of the nerves in involuntary muscle tissue. To one familiar with the appearance of methylen blue preparations, fixed, hardened, sectioned and counterstained as above described, blue granules or even short blue fibrils do not of necessity indicate the presence of a nerve ending; for, unfortunately, tissues other than nervous stain blue and blue granules which appear to be nothing but precipitate are often seen. This I have tried to bear in mind, and only such granules or small end-discs, were accepted as the ultimate ending of perivascular nerves in the muscular coat of the pial vessels, when I was able to trace a connection between them and a blue fibril which from its varicosity, color reaction and other appearances I was led to regard as a nerve fibril. The arrangement and mode of ending of the perivascular nerves in the pia-mater being in every respect like that of vaso-motor nerves in other parts of the body, I feel warranted in placing them in the same category.

Assuming that the non-medullated nerve fibers of the perivascular plexus found on the carotid artery were the neuraxes of sympathetic neurons, the cell bodies of which were situated in the superior cervical ganglion, and that those found on the vertebral arteries were the neuraxes of neurons, the cell bodies of which were to be found in the inferior cervical and stellate ganglia, I extirpated these ganglia in pairs, with the hope that the

resulting degeneration would enable me, by exclusion, to ascertain the area of distribution of the neuraxes of the sympathetic neurons removed in the respective ganglia extirpated. Tuckett (8) has shown,—“that where non-medullated nerves degenerate, the core of the fibers of Remak, staining with methylen blue, disappears, while the sheath and nuclei are unaffected by cutting them off from their trophic nerve-cells. Degeneration is shown, by the histological changes and physiological phenomena, to begin about the twenty-fourth, while loss of irritability and conductivity is complete by the fortieth hour.” The experiments on *extirpation* of the ganglia were all made on cats. The operated animals were kept for a time varying from 3 to 49 days (see table) after *extirpation* of the ganglia. The results obtained in this portion of my investigation are so meager, that it does not appear to me advisable to give the protocol of these experiments in detail; I have therefore grouped the salient points in the table opposite.

In summarizing the results given in this table, attention may be drawn to the fact that in experiments where nerves in the pial vessels were stained, after the *extirpation* of sympathetic ganglia in the neck or thorax, the medullated nerve fibers accompanying the basilar or internal carotid and its branches were stained. The *extirpation* of the ganglia, did in no way alter their reaction to the methylen blue. This I take it may be put forth as an additional argument, to those already given, that these medullary nerve fibers are sensory. As to the distribution of the vaso-motor fibers, the results are not conclusive. A study of the table will show, that after *extirpation* of the superior cervical ganglia the vaso-motor fibers on the anterior half of the circle of Willis and the branches of the middle cerebral seem less numerous or entirely wanting; the same is true of the basilar artery and its branches after *extirpating* the inferior cervicals or stellates or both. Yet after making comparisons between the preparations obtained in these experiments and those obtained from normal animals, I am free to admit that the above conclusions do not seem justified. It should be stated that in this research some sixty animals were used, and of this number

TABLE I. GIVING SYNOPSIS OF EXPERIMENTS ON EXTIRPATION OF SYMPATHETIC GANGLIA.

No.	Ganglia removed.	Length of time intervening between extirp. of ganglia and meth. blue injection.	Examination of dist vessels resulted as follows:				Remarks.
			Vertebral and basilar.	Circle of Willis.	Middle cerebral and branches.		
1	L. and R. Sup. cerv.	Cat dead next morn.	_____	_____	_____		
2	" " "	Cat dead next morn.	_____	_____	_____		
3	" " "	25 days.	Sensory fibers well stained. A few vaso-motor fibers	Sensory fibers well stained. Some few vaso-motor fibers on internal carotid branches of the circle of Willis, especially post-com.	Sensory fibers well stained. No vaso-motor on middle cerebral on convexity of hemispheres	{Methylen blue staining unsuccessful. Vessels stained deeply and diffusely blue	
4	" " "	Cat dead second day	_____	_____	_____	{Methylen blue staining unsuccessful. Vessels stained deeply blue	
5	" " "	3 days	_____	_____	_____	{Methylen blue staining unsuccessful. Vessels stained deeply blue	
6	" " "	49 days	_____	_____	_____	No nerves stained	
7	" " "	9 days	_____	_____	_____	No nerves stained	
8	L. and R. superior and middle cervical	Dead third day	_____	_____	_____	No nerves stained	
9	L. and R. stellate	5 days	_____	_____	_____	No nerves stained	
10	L. and R. stellate	8 days	_____	_____	_____	Basilar and circle of Willis stained deeply and diffusely blue	
11	" " "	4 days	_____	_____	_____	Basilar and circle of Willis stained deeply and diffusely blue	
12	" " "	7 days	_____	Sensory and vaso-motor fibers on anterior half of circle of Willis.	Sensory and vaso-motor fibers	Basilar and circle of Willis stained deeply and diffusely blue	
13	L. and R. sup. cerv.	9 days	_____	_____	_____	Circle of Willis stained deeply blue	
14	" " "	3 days	Sensory fibers.	No nerves stained	Right side sensory fibers well stained. Left side only a few	Nerves not well stained	
15	L. and R. stellate	9 days	Vasomotor fibers?	No nerves stained	_____	Nerves not well stained	

only in a small percent. did I obtain results which might be looked upon as satisfactory, and by this is meant that only in a few experiments were the nerves on the larger as well as the smaller vessels, and in different regions of the pia-mater well stained. In a much larger number of experiments they were well stained in certain regions, sometimes on one side and not on the other, again on one portion of the circle of Willis and not on the remaining portion, sometimes medullated, sensory fibers and not vaso-motor or vice versa, etc.; although after some preliminary experimentation, the same method—strength of solution, quantity, time of exposure, etc.—was used in all the experiments.

It seemed therefore ill-advised to continue this line of experimentation. Before leaving, however, these experiments, attention may be drawn to the fact that after the *extirpation* of the ganglia as above described, in nearly every experiment certain vessels of the pia stained a peculiar, greenish blue color, quite unlike the deep blue color seen after the injection of methylen blue into normal vessels. It is to be born in mind that the muscle cells of the vessels show a selective action toward the methylen blue. I am not prepared to say that this change in the reaction of the muscle cells toward the methylen blue, after the *extirpation* of the sympathetic ganglia of the neck, indicates a chemical change in the protoplasm of these muscle cells, perhaps as a result of trophic influences interrupted at the removal of the cell bodies of sympathetic neurons, sending neuraxes to these vessels. I offer this as a possible explanation of a phenomenon observed in many of the experiments here under discussion.

In the preceding pages I have drawn attention to the nerves in the pia-mater of the cerebral hemispheres, and more particularly to the nerves,—sensory and vaso-motor,—found in conjunction with the middle cerebral and its branches. I can not state positively that I have been able to trace any such nerves into the substance of the brain. In preparations of the pia, prepared after the method described (namely, removing the pia-mater with a portion of the brain cortex to a slide and

pressing the brain tissue out from under the pia) it is often impossible to differentiate between the small arterial branches in the pia-mater and those which penetrate the brain substance—intra-cerebral branches. As previously stated, small vessels with two to three layers of involuntary muscle cells in the media possess vaso-motor nerves. Whether some of these arterioles were intra-cerebral, I can not say; it seems however very probable, as such vessels correspond in size and structure of coats to many of the intra-cerebral arterioles found in the cortex. Kölliker (6) states that he was able to trace perivascular nerves into the substance of the brain.

I have repeatedly attempted to ascertain whether perivascular nerves were to be found on the vessels of the choroid plexus in the lateral ventricles. My results have thus far, however, been negative. This, I believe, is in part at least due to the fact that in methylen blue preparations—both when the methylen blue is injected into the circulation and when applied in very dilute solution (1 to 1000 in normal salt solution) to the fresh choroid plexus—the epithelial cells covering the vessels of the choroid plexus stained so deeply that the underlying structures could not be made out with any degree of clearness.

As to the nerves in the pia-mater of the cerebellum, my results have not been so conclusive as might be desired. Sensory nerves have now and then been traced by the side of the arteries of the pia-mater of the cerebellum; these end as do similar nerves in the pia-mater of the cerebral hemispheres. Vaso-motor nerves have been met with in only a few cases, and then only in small numbers and not so clearly stained as in other parts of the pia.

Nerves of the Dura-mater. The presence of nerves in the dura-mater seems beyond question. Their origin, course and to some extent distribution have been known to anatomists for many years. It is not my purpose at this time to concern myself with these grosser anatomic facts, as my observations have not been of a nature to give me data for this purpose. In a number of experiments on dogs, cats and rabbits, I have

obtained well-stained preparations of the dura-mater after the injection of a methylen blue solution with a view of staining the nerves of the pia-mater.

In a number of such preparations the terminations of the dural nerves were well stained and it is to this that I propose to confine my remarks. Alexander (9) made observations with the gold chloride method on the nerves in the dura of the dog, Guinea-pig, rabbit, rat, mouse, dove and frog, and found two kinds of nerves,—vessel-nerves and dural-nerves. He then states that the arteries of the dura, to their microscopic branches are accompanied by two small nerve bundles which run parallel to the vessel. These bundles become smaller toward the periphery until ultimately only single, medullated nerves are found by the side of the vessels. From these medullated fibers, non-medullated branches are given off which go toward the vessels and terminate thereon; their mode of ending he could not ascertain. The dural nerves, he states, have their origin directly from the larger nerve bundles or from those following the vessels. These bundles of medullated nerves, after division, end in non-medullated fibers which form plexuses, often with very narrow meshes. These plexuses seemed in no way connected with the vessels. Whether this was a true plexus or only a network could not be ascertained. Alexander, however, if I read him correctly, inclines to the former view.

My own observations led me to the conclusion that in the dura mater of the dog, cat and rabbit two kinds of nerves were to be found :

- a.* Sympathetic nerves forming peri-vascular plexuses.
- b.* Medullated, sensory nerves terminating in the dura.

The sympathetic nerves form peri-vascular plexuses, which in every respect resemble those already described for the pia-mater and those found about arteries in other parts of the body. They have been found most clearly stained on the middle meningeal artery; this seemed to me, however, accidental, as the portion of the dura containing this vessel and its branches is more easily removed and seemed better stained than other parts of the dura.

The peri-vascular plexus on the middle meningeal has been observed on this vessel as soon as it reaches the dura, and I have been able to trace it along its course until the arterial branches were reached having a musculature consisting of two layers of involuntary cells. My reason for believing the non-medullated nerves, which form this peri-vascular plexus, to be neuraxes of sympathetic neurons, is the fact that in some of the best stained preparations, fine nerve fibrils, coming off from the perivascular plexus, could be traced into the muscular coat of the vessels, where such fine nerve fibrils assumed a course parallel to the long axis of the involuntary muscle cells of the vessel wall, between which they seemed to terminate. For such a perivascular plexus see Fig. 5.

The medullated nerves of the dura which, with other observers, I regard as sensory, comprise the two kinds of nerves described by Alexander. Bundles of medullated nerves accompanying the arteries are readily stained in methylen blue; two such bundles are shown in Fig. 5. In portions of the dura free from larger vessels, are found bundles of medullated nerves (see Fig. 6) having a course quite independent of the vessels; these I take to be the dural nerves described by Alexander. In their mode of ending I find no difference between the medullated nerves of the dura accompanying the vessels and those having a course independent of them. In each case the medullated nerves, after branching here and there at the nodes of Ranvier, lose their myelin and continue as non-medullated fibers. These non-medullated fibers after further branching, terminate in long varicose fibrils, which may now and then be traced through several fields of the microscope when the preparation is viewed under a magnification of about 400 diameters.

These non-medullated terminal branches interlace in every possible manner, as may be seen in Fig. 6, giving an appearance which is commonly termed a nerve plexus; the identity of the nerve fibrils is, however, not lost in this "plexus" as one meets with no anastomoses of the nerve fibrils. The medullated fibers accompanying the arteries of the dura often terminate over them or in such a way as to surround the vessels; their term-

inal branches do not however form perivascular plexuses; the plexus formed by the sympathetic fibers seems quite independent of them. The one set of fibers—vaso-motor or sensory—is often stained to the exclusion of the other, the sensory fibers staining more readily than the vaso-motor nerves.

I have not been able to trace in its entirety the end-brush of a single medullated nerve fiber terminating in the dura. In a well stained preparation, the varicose terminal branches of the sensory fibers are so interwoven that the ones coming from a single medullated nerve fiber can not be separated from the others. There would seem to be no doubt, however, that the termination of the sensory fibers of the dura is an end-brush spread over a relatively large area, the component parts of this end-brush being very slender, varicose fibrils which terminate in the connective tissue of the dura.

As the preceding pages have shown, the writer has endeavored to answer the question of the existence or non-existence of vaso-motor nerves on the intracranial vessels, not by results obtained in physiological experimentation, but rather from observations made by the aid of our modern histological methods and the microscope. A knowledge of the existence of vaso-motor nerves on the intra-cranial vessels must of necessity weaken the position of physiologists, who, as a result of physiological experimentation, no matter how carefully planned and well executed such experiments may seem to be, deny the existence of such nerves. As the writer has conducted no physiological experiments in the investigation, for the reason that he has felt himself incapable of undertaking them, he deems it presumptuous to discuss the results and conclusions of physiologists, who, from their observations, have gained the conviction that the intra-cranial vessels were lacking in a vaso-motor nerve supply. For a consideration of this phase of the question under discussion I would refer the reader to the recent communication of Leonard Hill (10), in which admirable work, he has summarized his own numerous investigations in this field and those of his co-workers and has given us a most critical digest

of the literature bearing on this subject. Before concluding this paper it may, however, not be amiss to state briefly what may be regarded as the consensus of the opinions of physiologists on the question of the vaso-motor nerve supply of the intracranial vessels, and in doing this I quote from Hill, whose extended observation and knowledge of the literature enable him to make such a summary much better than I can.

“No evidence has been found of the existence of cerebral vaso-motor nerves: either by means of stimulation of the vaso-motor center, or central end of the spinal cord after division of the cord in the upper dorsal region: or by stimulating the stellate ganglion, and, that is to say, the whole sympathetic supply to the carotid and vertebral arteries.” “In every experimental condition the cerebral circulation passively follows the changes in the general arterial and venous pressures.” “There is no compensatory mechanism by which the intracranial pressure is kept constant.” And to quote still further, Hill states that in “turning to the evidence of past workers, I find that Nothnagel and Akermann obtained at times, on stimulating the cervical sympathetic, evidence of dilatation of the pial vessels. Recently Cavazzani found evidence of both cerebral constrictor and dilator fibers in this nerve; the methods of these workers are valueless in deciding such a question. Schultz, Riegel and Jolly, Cramer, v. Schultén, Gaertner and Wagner, Hürthle, Roy and Sherrington have obtained no positive evidence of any active cerebral effect from stimulation of this nerve.” In this connection it may be interesting to note that Hill states that “in a recent exhaustive research, Gulland has failed to demonstrate by every known histological means the existence of any vaso-motor nerves in the pial vessels.” This latter statement at least needs modification, since, as has previously been shown Gulland himself admits the presence of perivascular nerves on the pial vessels.

The statement above made may serve to emphasize the view previously expressed, that physiologists very generally deny the existence of vaso-motor nerves in the pial vessels. The fact that nerves, which in every respect—considered struc-

turally—resemble vaso-motor nerves found in other organs, have in a number of instances been found in the pial vessels, suggests at least the advisability of a renewed investigation of this problem at the hands of the physiologist, if indeed, considering the complexity of the problem, the question may be decided from this standpoint. I am reminded here of a similar doubt expressed by Obersteiner (2) in the article from which I have already quoted freely. After stating, and agreeing in this respect with Mosso, that the vessel-nerves of the brain are easily fatigued and fail to respond at a time when vaso-motor nerves still react in other organs, he adds: “Diese besondere Ermüdbarkeit könnte vielleicht auch zur Erklärung der negativen Versuchsergebnisse mancher Forscher herangezogen werden. Jedenfalls gestatten alle derartigen experimentellen Ergebnisse mancherlei Auslegung und sind nicht geeignet eine entschiedene Beantwortung der Frage nach dem Vorhandensein von Nerven an den Gehirngefäßen zu ermöglichen.”

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9. *Alexander*: Bemerkungen über die Nerven der dura Mater. *Archiv für Mikroskopische Anatomie*. Vol. XI, 1875. Page 231.
10. *Leonard Hill*: *The physiology and pathology of the cerebral circulation*. London, 1896. References to other papers mentioned may be found in this volume on pages 202-204.

DESCRIPTION OF FIGURES.

PLATE I.

Fig. 1. Circle of Willis and basilar artery of cat. Showing in a semi-diagrammatic way the distribution of the sensory nerve fibers. Only a few of the stained fibers sketched to give their course and distribution. 1. anterior cerebral; 2. middle cerebral; 3. posterior communicating; 4. posterior cerebral; 5. basilar. For letters see text.

Fig. 2. Portion of posterior cerebral artery of left side of cat showing the termination of a sensory nerve fiber. Nerve with ending in the adventitia of vessel. Methylene blue preparation fixed in ammonium picrate and mounted in ammonium picrate glycerine. Magnification 900 diameters. Reduced to one-fourth.

Fig. 3. One of the larger branches of middle cerebral of a cat. Methylene blue stain. Shows perivascular vaso-motor plexus. Magnification 450 diameters. Reduced to one-half.

Fig. 4. Small pial vessel from dog. Vessel has two layers of involuntary muscle cells. Shows perivascular vaso-motor plexus. Magnification 900 diameters. Reduced to one-half.

Fig. 5. Middle meningeal of cat. Methylene blue stain. Two bundles of medullated nerve-fibers—sensory fibers—accompanying it; *m.* vaso-motor plexus in vessel. Magnification 150 diameters. Vaso-motor plexus sketched in from higher power. Reduced to one-half.

Fig. 6. From surface preparation of dura of cat. Methylene blue preparation. *M.* bundles of sensory, medullated fibers. Varicose fibers in sketch terminal branches of sensory dural nerves. Magnification 450 diameters. Reduced to one-half.