# STUDIES ON CEREBRO-SPINAL FLUID. NO. IV.*

## THE DUAL SOURCE OF CEREBRO-SPINAL FLUID.

LEWIS H. WEED.

(*From the Laboratory of Surgical Research, Harvard Medical School.*)

Introductory. — The results of observations upon the pathways of escape of cerebro-spinal fluid recorded in the preceding reports in this study have led to the conclusion that this fluid is returned to the major circulation through a mechanism of fluid passage into the great sinuses from arachnoidal villi. In addition to this chief absorption into the venous system there is also evidence of an accessory drainage into lymphatic channels. Furthermore, an experimental basis for the retrograde passage of fluid from the venous system into the subarachnoid spaces has been suggested. No support has been given by these observations to any theory of escape of cerebro-spinal fluid into either the cerebral veins or capillaries.

In the course of these studies upon the phases of the problems connected directly or indirectly with the cerebro-spinal fluid, interest turned many times to the possible sources from which this circumambient fluid might arise. Many of the experiments planned primarily to test Mott's[36] theories of the absorption of cerebro-spinal fluid by the cerebral capillaries not only served their original purpose, but also afforded interesting information regarding the origin of the fluid. In addition a new series of experiments were undertaken to ascertain the processes of elaboration of the fluid; the results of these two series of observations will be detailed here.

Since the discovery of the cerebro-spinal fluid there has existed a greater or lesser degree of uncertainty regarding its actual origin within the central nervous system. Haller[17] and Magendie,[31] to whom the greatest credit for its further

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description must be accorded, believed it to be the product of the cells of the leptomeninges. Faivre,[14] 1854, and Luschka,[29] 1855, were the first to suggest the choroid plexuses of the cerebral ventricles as the elaborators of the fluid, but aside from the glandular morphology of the plexuses no anatomical proof nor physiological evidence of such a function was presented. Another possibility as to the source of cerebro-spinal fluid was brought forward by the development of the conceptions of the perivascular canalicular system in the nervous tissue itself. For the first comprehensive description of these perivascular lymph spaces and their connections with the cerebro-spinal spaces we are indebted to His.[22] The double source of cerebro-spinal fluid from the choroid plexus and from the nervous tissue itself by way of the perivascular system has been accepted by the more recent authoritative writers (Mestrezat,[33] Plaut, Rehm, and Schottmüller[38]).

But analysis of the evidence which leads to the acceptance of this dual source shows that it is not supported by any incontrovertible observations; for the greater part this conception is based upon theories which have been suggested to account for isolated phenomena. The case is a strong one undoubtedly when one weighs the indirect evidence and gives credence to the intelligent hypotheses, but there is very little direct anatomical or physiological support to this assumption. The findings in this series of observations are presented in the hope that they will afford more direct and convincing evidence for the growing belief that cerebro-spinal fluid is a product of the choroid plexuses and of the nervous tissue.

The choroid plexuses as elaborators of cerebro-spinal fluid. — The adverse comments which have already been made regarding the present evidence of the sources of cerebro-spinal fluid surely may be applied to the observations reported in regard to the choroid plexuses as the source of the fluid. Clinically there is a fairly firm basis for such a belief; the hydrocephalus subsequent upon obstruction

within the intraventricular channels strongly argues for such a view.

The most convincing yet indirect substantiation of this function of the choroid plexuses is afforded by the work of Cappelletti,[6] of Pettit and Girard,[37] of Findlay,[15] of Meek,[32] and of Mott,[36] in which histological changes in the cells of these plexuses under varying functional conditions are reported. The first of these observers was able to increase the flow of cerebro-spinal fluid by the administration of certain alkaloids (pilocarpine, muscarin) and of ether. With this augmented rate of formation histological changes within the choroidal cells indicative of increased function are discernible. Dixon and Halliburton[13] have recently observed an increase in the formation of cerebro-spinal fluid after injection of dried extracts of brain and of the choroid plexus.

Apart from this histological change observable in the choroid plexus after the administration of the muscarin series there is not even any indirect evidence of value that this plexus is responsible for the formation of cerebro-spinal fluid. For the reported methods of obtaining the fluid and of recording its rate of flow all deal with the fluid obtained from the subarachnoid spaces. Hence the collected fluid may come not only from the choroid plexuses but also from the perivascular lymph spaces. That these spaces under physiological experimentation may yield considerable fluid was convincingly demonstrated by Spina.[49] The different pharmacological substances affecting the flow of the cerebro-spinal fluid may all do so by altering the cerebral capillary mechanism to such a degree that an increased flow of the fluid from the cannula is recorded.

The usual methods of studying the formation of cerebro-spinal fluid are those of introducing cannulæ into the subarachnoid spaces at some point or other. Cavazzani[9] studied the flow of fluid from an artificial cerebro-spinal fistula which he made in animals and his technic has been employed by other observers. Often the lumbar subarachnoid space is tapped and the fluid allowed to drop from a needle or from

a cannula. Even more generally used is the method of introducing a cannula or needle into the cerebellar cistern through the exposed occipito-atlantoid ligament. Dixon and Halliburton introduce the needle with attached glass cannula directly into the cistern after merely incising the skin in the appropriate situation.

It was felt that no advance could be made in this question of the formation of this fluid unless the perivascular canals could be excluded from the participation in the resultant changes in the rates of formation. The introduction of a hollow tube beneath the cerebellum, through the aqueduct of Sylvius, into the third ventricle was suggested by the method of causing a mechanical internal hydrocephalus devised by Dandy and Blackfan.[12] This introduction of a tube directly into the third ventricle has been found wholly possible and is such a simple procedure that it is reported as a physiological method of approaching the questions of the function of the choroid plexuses.

The customary method of making the observations on the rate of flow of cerebro-spinal fluid concerns initially an exposure of the occipito-atlantoid ligament. Dogs and large cats have been used, but the greater size of dogs makes them far preferable for the work. The ligament is best exposed by a midline skin incision, starting from the binaural line and running caudally, with subsequent division of the posterior spinal muscles in the midline. The muscles are then reflected laterally by dissection from the occipital bone on either side. In the earlier observations the bone was removed over the cerebellum, but as one acquires skill in the procedure this step may be omitted and the tube introduced directly through a slit in the exposed occipito-atlantoid ligament. The cerebellar cisterns are opened with care and the cerebellum gently elevated caudally away from the floor of the fourth ventricle. A tube of suitable dimensions is then gently inserted along the medullary floor and through the aqueduct of Sylvius. The walls of this aqueduct offer characteristic resistance to the passage of the tube; this gradually yields to continued gentle pressure. Finally, the

end of the tube comes to lie in the third ventricle; no fluid passes around it to escape into the fourth ventricle because of the apposition of the walls of the aqueduct to the tube. The cerebro-spinal fluid confined in the third and lateral ventricles can find exit only by way of the introduced tube, ensuring, therefore, no contamination of this fluid by the products of the perivascular system. Any fluid obtained must represent the secretion of the choroid plexuses alone (leaving the ependymal cells out of consideration for the moment).

In the earliest observations a glass cannula of suitable bore and curvature was employed as the "ventricular catheter," but it proved partially unsuccessful. A soft rubber catheter of similar caliber likewise was unsuited for the purpose. It has been found that silk catheters (paraffined) are wholly satisfactory, especially when they are provided with a bougie tip. The caliber of the catheter naturally varies with the size of the animal used; for an ordinary dog of eight to ten kilograms body weight, a silk catheter (No. 8 French) is best adapted. Ordinarily, the catheter may be introduced without its bending, but at times it is necessary to insert a wire obturator to insure greater stability. This obturator may be removed as soon as the ventricle is entered.

The operative preparations in the first of these observations proved so extensive that a condition of low arterial blood pressure had resulted by the time that the ventricular catheterization was done. In subsequent experiments, with improvement in the operative technic, with practically no loss of blood, and with no evidence of medullary injury, the animals have shown blood pressures equal to those in the ordinary etherized animal.

Realizing the great possibility of error in any physiological deductions made from the rates of formation of the fluid in the cerebral ventricles under abnormal conditions of secretion, an attempt has been made throughout to standardize the resistance to flow in the catheters employed. This calibration has proved quite simple and the catheters are now all graded in terms of millimeters of water (the height of a column of water just sufficient to cause the fluid to

drop from the end of the catheter). In this way with known resistance in the cannula it is possible to maintain in the cerebral ventricles a normal cerebro-spinal fluid pressure and at the same time to secure the flow and to record its rate of formation. This maintenance of normal intraventricular tension is certainly of extreme importance in any study of the formation of cerebro-spinal fluid; its necessity has not been heeded by any of the previous workers in this field.

With this method, which permitted the securing of the fluid from the choroid plexuses alone, without admixture by the products of the perivascular system, it has been possible to obtain interesting data regarding the elaboration of the cerebro-spinal fluid. If a ventricular catheter be inserted of such resistance as to maintain normal intraventricular tension, fluid will flow drop by drop from the end of the tube. An initial, short enduring flow of several drops customarily follows the introduction; this is followed very soon by the establishment of a slow uniform elimination of the fluid, corresponding exactly to the rate of elaboration under the experimental conditions. (Any fluid coming from the intraventricular structures must necessarily be eliminated by the catheter, as it completely fills the aqueduct. This method of study consequently differs greatly from those previously employed, for in these the normal channels of escape from the subarachnoid space are intact and absorption must continue throughout the observation.) The fluid continues, without further interference, to flow for three or four hours, the rate of escape from the catheter being gradually slowed. The final cessation of flow is apparently due to an exhaustion of the choroid plexuses under the experimental conditions. At times the flow may not be uniform throughout the period of observation; definite periodical variations in rate may exceptionally be recorded.

The action of drugs and other substances upon the rates of excretion of this product of the choroid plexuses have been the subject of study. These results, together with the histological changes in the cells of the choroid plexuses

under the varying experimental conditions, will be presented in another communication. We are concerned here chiefly with the method of study and the fact that when the perivascular system is excluded it is possible to secure fluid from the chambers holding the lateral choroid plexuses — more direct proof apparently than has yet been offered in support of this function of elaboration.

The fluid obtained by the method of ventricular catheterization is, as far as we are able to ascertain, identical microscopically and chemically with normal ventricular fluid. The small quantities obtained, however, render it difficult to make an accurate chemical analysis.

There is also another possibility in such a method of a second ventricular source of the fluid. With a realization that the cells of the choroid plexus are merely highly differentiated ependymal elements it is not a great assumption to suppose that the ventricular ependyma may play some part in the elaboration of cerebro-spinal fluid. Our only test for such a function normally is histological; changes in the cell-activities may present microscopic alterations. These investigations have failed to show any such histological evidence of function; it is safe to conclude that any fluid elaborated in these ependymal cells must be very small in quantity as compared to the amount from the choroid plexuses.

The employment of this method of ventricular catheterization affords apparently evidence, of a more direct nature than any heretofore reported, that the choroid plexuses of the cerebral ventricles are the elaborators of the cerebro-spinal fluid. There is no histological evidence that the cells of the lining ventricular ependyma play any part in the formation of this fluid.

The drainage of the nervous tissue itself. — According to Mott[36] and to Mestrezat[33] the fluid spaces around the cerebral veins and arteries in their cortical course were first described by Robin, 1858. His[22] was able to demonstrate them in a striking manner and to connect them with the cerebro-spinal spaces. By puncture-injection into the

substance of the spinal cord or of the brain, His distended a complete perivascular network, more complex in the gray matter than in the white. The injection mass passed peripherally and finally came to lie beneath the pia where it spread out in a large sub-pial plexus. The presence of these sheath spaces is now generally acknowledged by all authorities. They are usually described as tubes lined by mesothelium, surrounding the arteries and veins as far as the finer subdivisions into capillaries. (Whether these sheath spaces are lined by mesothelium or by simple glial cells is still in doubt. His,[22] by intramedullary injections with silver nitrate solutions, demonstrated a mesothelial lining of the spaces. If we hold that silver reductions outlining definite polygonal cells are conclusive demonstrations of mesothelium, the findings all argue for this belief. But the reaction of these cells and the striking pictures about them, seen in many pathological lesions, incline one to the belief that they are enclosed merely by glia.) The existence of these spaces, long considered as artefacts, has been well demonstrated by Poirier and Charpy,[39] and later, in an excellent way, by Mott,[36] in cases of experimental cerebral anemia. That these spaces connect with the subarachnoid space has also been repeatedly shown. His' observations of a sub-pial connection are at variance with this subarachnoid connection, for he found a subpial plexus as the terminal point. It may be that His' injection mass passed along the outer wall of the sheath and consequently spread beneath the pia. Jacob,[23] Lewandowsky,[28] and Bruno [5] have furnished physiological and toxicological proof of the existence of these perivascular spaces. Milian,[35] in 1904, in a case of subarachnoid hemorrhage, found these spaces filled with blood; on the outer side of the clotted blood was a definite membrane, surrounded in turn by a clear space, which separated each vessel from the brain substance.

Pathology offers striking proofs of these perivascular spaces, for in many of the inflammatory conditions affecting the nervous tissue, the first exudation of corpuscular elements occurs around the vessels. This is especially well

brought out in the earlier lesions in clinical and experimental poliomyelitis. The usual course deals with the limitation of the inflammatory process to these spaces for a short time with later spread to the surrounding tissue.

As a more or less subsidiary finding in our earlier work upon the escape of cerebro-spinal fluid we have observed that these perivascular spaces are injected when the ferrocyanide solution is introduced, under certain pressure conditions, into the spinal subarachnoid cavity. This finding is quite constant when this method of injection is used, but when carbon granules in suspension are introduced the results are by no means constant.

The success of the method of injection depends in large measure upon the conditions of tension in the cranial cavity, as apparently it is quite easy to obliterate these perivascular spaces by increasing the pressure in the surrounding tissue. Hence, if pressure be applied experimentally in excessive degree to the cerebral tissue itself, a very different picture regarding the perivascular spaces may result. In typical observations in which the ferrocyanide solution was injected under very low pressures for several hours, practically no granules are found in the perivascular spaces. When a similar injection be made under moderate pressures (50 mm. Hg.) the precipitated material is found in the larger spaces down to the capillary bed. This extent of the injection we have not been able to attain with suspension of carbon granules when introduced into the spinal subarachnoid space. The spaces usually show strands running between the outer surface of the vessel and the inner surface of the sheath (Fig. 8); these may well account for the fact that the granular suspensions do not yield as extensive injections as do the solutions.

These perivascular sheaths have generally been designated "perivascular lymph spaces," or "perivascular lymphatics," terms which are obviously unsatisfactory as they indicate a lymph content in these vessels or sheaths. Until recently it has been believed on theoretical grounds that they carry into the cerebro-spinal space the fluid waste products of

nervous activity, but Mott's later theory of absorption is against such a view. The observations upon the theories of the flow in these perivascular spaces toward the subarachnoid space were first made by Spina.[49] This worker noted a punctate exudation of a clear limpid fluid from the exposed arachnoid membrane, immediately following a marked rise in the peripheral blood pressure. The methods which he employed for causing this rise in blood pressure (usually from 150 to 200 mm. Hg.) were the injection of extract of the suprarenal glands and high ligature of the aorta. An intense cerebral congestion and rise in intracranial tension followed these injections and an exudation of the fluid from the perivascular system quickly ensued. Lewandowsky,[28] accepting Spina's evidence as conclusive, hypothecated that cerebro-spinal fluid was really the lymph of the cerebro-spinal axis and the product of the nervous tissue itself, as poured into the subarachnoid space by the perivascular channels.

In our opinion these sheaths do carry away the waste products of the nerve cell metabolism, contributing, in part, to the formation of cerebro-spinal fluid. The fluid, then, obtained by lumbar puncture, represents not only the secretion of the choroid plexuses, but also the fluid waste products of nerve cell activity, poured into the subarachnoid spaces by way of the perivascular channels. But can these channels be designated as lymphatic? If this were the case we should expect a content in lymph, — cellular and containing many coagulable elements. The protein of such a tissue juice of lymph should suffice to keep patent the perivascular spaces after fixation for microscopic work. Ordinarily, however, these spaces appear as potential spaces and are not distended, indicating clearly that the coagulable elements are very slight. This is in keeping with our present knowledge of the chemistry of cerebro-spinal fluid. Of course, it may be argued that the fluid waste of nerve cell activity represents the lymph of nervous tissue, just as much as thoracic duct lymph serves as the fluid carrier of waste products of other body tissue. To differentiate these two

widely different kinds of lymph seems rather forcing the continued use of the term "lymph" when applied to this fluid content of the perivascular "lymph" spaces.

After a typical spinal subarachnoid injection of the ferrocyanide solution under moderate pressure (50 mm. Hg.) study of the cerebral cortex shows Prussian blue granules heaped up in the perivascular spaces about the veins and arteries (Fig. 9). The injection mass about these cerebral vessels may be traced from the granular collections in the subarachnoid spaces over the convolutions and is directly connected with it. Following the vessels peripherally (*i.e.*, toward the capillary bed) the granules can in some cases be identified in a continuous collection as far as the capillaries. Study of the capillaries reveals granules collected just outside of the endothelial wall in a pericapillary space. Mott has described these spaces as showing very clearly in the brains of animals in whom experimental cerebral anemia has been produced. The amount of the injected and precipitated salt about the capillaries is usually small, but it is very evenly distributed along the course of the vessel. In a certain number of our preparations, made in the routine way (under pressures of 50 mm. Hg.), it was found that similar, fine, diffuse collections of precipitated granules occurred around the nerve cells. (A very interesting finding of possible importance in the ultimate solution of cell-chemistry is that of the differential staining of large ganglion cells of the cortex under certain experimental conditions. In the routine low-pressure preparation, in which there is no injection of the perivascular system, none of the neurones are affected. But in those ferrocyanide injections of these intracortical channels in rare instances certain ganglion cells may show, in addition to the perineuronal injection, finely divided granules of Prussian blue in the cytoplasm. In other cases only the nucleus may have the precipitate within it or there may be only a perinuclear ring of the granules. The cause of this selective absorption of the ferrocyanide solution, rare as it is, appears to lie in the cell-chemistry. It has been repeatedly recorded that such diffuse staining

occurs after a ferrocyanide injection in dead tissues and the preparations made intra vitam which show this cytoplasmic or nuclear infiltration are those in which the fixative reached the tissue tardily. Neurogliar cells never show the phenomenon. Usually only an occasional neurone is found to be affected, surrounded by other cells which are enclosed in a perineuronal injection. The explanation of the phenomenon to which we incline is that these affected cells die before the fixative reaches them and that in consequence they absorb the ferrocyanide as any dead tissue. Possibly actual necrobiosis is unnecessary; the one requisite may be an altered cell metabolism. In these cases of perverted cell-chemistry the ferrocyanide may act as a vital stain but much more likely as an agonal perfusion mass.) For the most part the granules adhered to the outer surface of the body of the cell, appearing as a diffuse stain under low magnification but showing the uniform collections of granules on the cell periphery under oil immersion (Fig. 10). Along the course of the axones as they leave the nerve cell body, the granules are continued in the form of a uniform collection about the fiber. There is good evidence, moreover, that the dendrites also possess granular deposits about them, but this finding is rather rare as contrasted to the more frequent pericapillary injection.

The preparations in which these perivascular and perineuronal collections of granules are found show on macroscopic examination a dense collection of the Prussian blue at some distance from the surface of the convolution. Between this zone of heavy precipitate and the surface there is an area of lighter staining with here and there denser perforating strands. On microscopic examination there appears, on low magnification, an apparent diffuse staining in the peripheral zone, with denser collections in the zone of the pyramidal cells. This supposed staining with the precipitate is found under higher power to be due to the occurrence of the granules around nerve elements, — cell-bodies, axones, and dendrites. The perforating blue strands are readily identified as the larger collections of Prussian blue

which lie in the perivascular spaces. There is no evidence that this stained appearance of the cortical zones is due to the absorption of the injection fluid as a dyestuff, for even without the microscopic evidence of a pericellular distribution, the fact that beneath the surface zone there occurs a much denser line of precipitate speaks against such a conception. For if homogeneous tissue like that of the nervous system were to absorb dyestuffs the absorption would be practically uniform, dependent upon the chemistry of individual cells. Differentiation might occur between the absorption by nerve and neuroglia cell, but such difference would hardly affect the macroscopic evidence. This phase of the subject has been discussed in foregoing paragraphs.

We found, then, in our medium pressure preparations evidence of a pericellular or, rather, a perineuronal injection from the subarachnoid spaces. Besides, there was also equally good evidence of a pericapillary distribution, with the granules passing from the pericapillary to the perineuronal spaces, or, at least, with connections between them. This is wholly in accord with the evidence of such a communication between perineuronal and pericapillary space, as worked out by Mott, in those animals in which all the potential spaces were enlarged by producing cerebral anemia. Apparently, therefore, by this ferrocyanide method, we have been able to inject the perivascular spaces to the capillary bed and also to inject the perineuronal spaces.

With such a method of fluid injection and subsequent precipitation the possibility of obtaining further information with regard to Mott's conception of absorption of cerebrospinal fluid by the cerebral capillaries seemed not unlikely. However, with our evidence that the major mechanism of drainage lies in the arachnoidal villus it was difficult to conceive of this other possibility. For in these preparations (with injection pressures of 50 mm. Hg.) there was no evidence of granules passing into the cerebral capillaries even when the pericapillary spaces were filled. Hence, it seemed most unlikely to us that this could be a normal process, especially when one realizes that the pressure in

the cerebral capillaries is considerably higher than the cerebro-spinal tension. Far more likely is it that fluid leaves the cerebral capillaries, circulates in the pericapillary and perineuronal spaces, yielding nourishment and receiving waste products, finally leaving the tissue by the pericapillary and perivascular space to reach the subarachnoid cavities over the surface. Thence, absorption into the venous sinuses would take place. In our preparations, made in this manner, the increased pressure of injection was apparently sufficient in these cases to replace the fluid in the perivascular and perineuronal spaces, without causing it to flow into the capillaries. This conception receives much support from the realization that in the ferrocyanide injections made for several hours (under pressures of 150 to 180 millimeters of water) there is no evidence of any precipitate in any of the terminals of this complex perivascular system. Hence, it seems most likely that the flow in these spaces must be toward the subarachnoid space as first evidenced by Spina[49] and by Lewandowsky.[28]

In the course of experiments with the ferrocyanide method to demonstrate the perivascular and perineuronal spaces, use was made of the dilatation of these potential sheaths by the production of cerebral anemia. This dilatation of the perivascular canals in anemic brains is of considerable physiological interest. As has been repeatedly pointed out, the physics of closed cavities, filled with fluid, must be considered in dealing with the cerebro-spinal axis. With the reduction, in anemia and in exsanguination, of the vascular pressure to practically zero, the intramedullary pressure throughout the axis becomes reduced to a corresponding level. In other parts of the body the organ, deprived of its blood, would become smaller. This is obviously impossible within a closed inelastic system such as the cerebro-spinal, and the brain attempts to compensate for this tendency by aspirating fluid from any available part. Ordinarily, in death, the cerebro-spinal fluid is aspirated by the brain to a greater or less degree.

Instead of ligating two carotids and one vertebral artery, as in Mott's work, we attempted, in early experiments, to

secure a greater degree of anemia by exsanguinating an animal by opening both carotids and both jugulars. An injection of a two per cent ferrocyanide and iron mixture was then made into the spinal subarachnoid space under high pressure (100 mm. Hg.). In this way the cerebro-spinal fluid was replaced by the ferrocyanide solution and the pressure conditions maintained at levels as different as possible. For with the carotids and jugulars open the cerebral arterial and venous tensions must have approached zero, while the subarachnoid pressure was maintained at one hundred millimeters of mercury. The results of these observations were not wholly satisfactory as frequently the brain appeared collapsed without any evidence of a perivascular injection.

In later experiments it was found that intense dilatations of these spaces occurred if the cerebro-spinal fluid was previously replaced by the ferrocyanide solution (under pressures of 200 to 250 mm. $H_2O$) and the cerebral anemia subsequently caused. It appeared likely that the high subarachnoid pressure in the earlier experiments compressed the nervous tissue and to some extent obliterated the customary dilatation, whereas in the mere replacement of the fluid, the anemic, "thirsty" brain aspirates sufficient fluid from the subarachnoid spaces. A more complete injection undoubtedly results from this second procedure than from the first. In addition, far more reliable results are obtained in this latter way than in the former, where the pressure relations are so abnormal.

These experiments afforded uniformly satisfactory results when the cerebral tissue was examined microscopically. Intense dilatation of all perivascular spaces, including those about the capillaries, and injection of the perineuronal spaces were found in all cases. Most characteristic of all the features, however, was the fact that the cerebral capillaries and veins showed in their lumina collections of ferrocyanide granules. Were these the result of retrograde injection from the sinuses? Against this possibility is the fact that the carotids and jugulars were both opened, making the point of lowest pressure in the neck and not in the cranial cavity, so that any

ferrocyanide solution which passed into the sinuses must necessarily pass down the neck and not toward the capillary bed. Still further evidence of this was furnished by the frequent discovery of capillaries which showed in their wall (Fig. 6) masses of precipitated Prussian blue passing into the lumen. This finding should be expected under the pressure conditions in these experiments. With the vascular tension approximating zero and with the cerebro-spinal pressure maintained at two hundred to two hundred and fifty millimeters $H_2O$ by the injection fluid, it seems likely that one should find a passage of the injection fluid into the vascular system at the point of lowest resistance. This point is apparently in the cerebral capillaries; the microscopic evidence is wholly in accord with this view. No evidence indicative of a passage of the injection fluid into the cerebral veins was anywhere found, even though the granules were apparent within the capillary. The results then indicate a passage of the injection fluid into the vascular system through the walls of the cerebral capillaries with subsequent transit within the veins and arteries toward the point of zero pressure in the neck.

This passage of the injection fluid into the cerebral capillaries has been obtained in a few cases in which spinal subarachnoid injections under very high pressures (100 to 150 mm. Hg.) have been made with the ferrocyanide solution. Some of these cases show an extreme perivascular injection; others exhibit no evidence of this. The difference in results, as has already been pointed out, is probably to be accounted for by the fact that in some cases the cerebral tissue is collapsed by the high pressures; in others the injection gains the perivascular system and distends it. The condition of the nervous tissue itself at the inception of these agonal injections probably determines which picture will result. In some cases certain areas in the cerebral cortex show a perivascular injection, while in other areas these canals are collapsed.

Such evidence indicates that when the experimental cerebro-spinal tension is very high or when an anemia of the

nervous system is occasioned, fluid can pass directly into the cerebral capillaries from the subarachnoid space, the pathway being along the perivascular spaces. These two findings — absence of evidence of capillary absorption in the low-pressure preparations and proof of such a pathway only in the high tension observations and in those with "thirsty" brains — offer arguments against Mott's view of the normal process of drainage of cerebro-spinal fluid; for we were unable to secure this passage of injection fluid into the capillaries under experimental conditions which approximated the normal; this should have been possible were Mott's conception of the drainage of cerebro-spinal fluid correct. It seems most likely that actually the flow is from the nerve cell and cerebral capillary toward the subarachnoid space and not from the space toward the capillary.

The space about the nerve cells is probably chiefly potential in character, filled during life by a very thin layer of fluid. His,[22] in 1865, was the first to describe these "pericellular spaces." He was able by puncture-injection to demonstrate them about the neurones of the spinal cord and of the cerebral cortex; the spaces, moreover, connected with the perivascular network which he described. But since his time most observers have regarded them as artefacts until Mott presented his evidence for their actual functional existence. The fact that they can be injected by ferrocyanide solutions adds another argument against their being artefacts. Undoubtedly, the early contention that they were lined by mesothelial cells was incorrect, as all the evidence argues for these cells as neurogliar elements grouped about the nerve cell. Both the perineuronal and the pericapillary spaces are unlined except by the loose stroma of the nervous tissue; both apparently function actively.

Another finding of possible importance in the nervous tissue in which a ferrocyanide aspiration of the perivascular system has occurred is the widespread distribution of the granules in very minute traces through the stroma of the cerebral cortex. The granules in such an observation are obviously heaped up in the larger perivascular and pericapillary

channels and also in the perineuronal spaces; these are the essential channels as determined by the quantities of the injection fluid. No Prussian blue granules can be made out in any definite relation to the individual neuroglia cells, but the generalized distribution, while small in amount, as compared to the collections in the perivascular system, undoubtedly indicates that this system is connected also with the supporting neuroglial fibrillar structures of the nervous tissue. This relatively insignificant amount of the neuroglial injection might be expected when one considers the relative metabolism of nerve and glial cell.

This whole accessory fluid system of the cerebro-spinal axis — an intramedullary canalicular system — undoubtedly possesses an active function in maintaining the metabolic exchange and elimination of the nerve cells. Throughout the body in other tissues there is a chief and accessory circulation. For in these other tissues of the body there is in addition to the blood capillary a lymph capillary with the tissue juice or plasma playing the intermediate part in exchange. Nervous tissue lacks entirely the lymphatic system; it would appear that its place is taken by the perineuronal, pericapillary, and perivascular system with its contained fluid, and that this fluid is poured into the subarachnoid space, where it mixes with the fluid from the choroid plexuses.

## CONCLUSIONS.

(1.) Cerebro-spinal fluid appears to be derived from two sources: (*a*) The choroid plexuses in the cerebral ventricles; (*b*) the perivascular systems of the nervous tissues.

(2.) No evidence is afforded by these observations of any absorption of cerebro-spinal fluid into the cerebral capillaries.

(3.) Under certain pressure conditions an extensive injection of the perivascular system from the subarachnoid spaces can be secured by the ferrocyanide method.

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## KEY PLATE TO FIGURE REFERENCES.

| | |
|---|---|
| ac | arachnoidal cells. |
| am | arachnoid membrane. |
| av | arachnoid villus. |
| ax | axone of nerve cell. |
| bn | brain substance. |
| ca | cerebral artery. |
| ci | internal carotid artery. |
| cs | cavernous sinus. |
| cv | cerebral vein. |
| dc | diploetic communicating vein. |
| dm | dura mater cerebralis. |
| dt | diverticulum of superior longitudinal sinus. |
| dv | dural vein. |
| ec | endothelial cell. |
| fx | falx cerebri. |
| gc | germinal center of lymph node. |
| gm | granular material (precipitated Prussian blue). |
| ls | lymph sinus. |
| lv | lymph vessel. |
| mc | mesodermal cells. |
| nc | nerve cell. |
| ne | nasal epithelium. |
| og | olfactory gland. |
| on | olfactory nerve. |
| pc | pericapillary space. |
| pm | pia mater cerebralis. |
| pn | perineuronal space. |
| pv | perivascular lymph space. |
| rc | red blood corpuscles. |
| sa | subarachnoid space. |
| sd | subdural space. |
| ss | superior longitudinal sinus (sagittal). |

## DESCRIPTION OF PLATES.

### PLATE I.

FIG. 1. — x 30. An arachnoid villus (av) is shown approaching the lateral dural wall of the superior longitudinal sinus (ss). The specimen is taken from a cat which had been injected with a two per cent solution of potassium ferrocyanide and iron ammonium citrate into the lumbar subarachnoid space under a pressure of 180 mm. $H_2O$ for four hours. The Prussian blue granules after precipitation in situ are reproduced in black in the subarachnoid space. Most of these granules are still far from the endothelial wall of the sinus, but a few appear in isolated arachnoidal clumps near the sinus lumen. The clear zone of arachnoid cells over the granules in the subarachnoid space and the absence of the granules in the subdural space (sd) is well shown.

FIG. 2. — x 30. The drawing is made of a section taken from the same animal as Figure 1. The same villus is shown in its posterior portion, but it now possesses but little connection with the lateral wall of the superior longitudinal sinus (ss). The arachnoidal tissue has, however, approached the endothelial lining of the sinus and the ferrocyanide injection mass appears heaped up along the sinus wall. Under higher powers the transit of the precipitated fluid through the cells can be seen. A very large opening of the sinus communicating with the diploetic channels (dc) is given with one of its walls showing the granular accumulations (gm) of Prussian blue reproduced in black.

## PLATE II.

FIG. 3. — x 40. An arachnoid tuft occupies the whole lateral wall of the superior longitudinal sinus while a diverticulum of the sinus has arachnoid cells in close approximation to its endothelium. The specimen was prepared by injecting a one per cent solution of potassium ferrocyanide and iron ammonium citrate under a pressure of 150 mm. $H_2O$ for four hours. The strand-like character of the canine villus is well given. Throughout, the precipitated Prussian blue granules are everywhere shown in black. The absence of the granular material in the subdural space (sd), in the cerebral veins (cv), in the dense strands of dura (dm), in the substance of the brain (bn), and in the enclosing arachnoid mesothelium (am) is well illustrated. The universal occurrence of the granules in the subarachnoid space and in the arachnoidal villus is to be noted. A few granules are shown in the lumen of the sinus.

FIG. 4. — x 300. A drawing under higher power of the field outlined in Figure 3. The precipitated Prussian blue (black granules in the reproduction) is shown throughout the large cells comprising the arachnoid villus. The absence of the precipitate from the true dural tissue which surrounds the arachnoidal elements is quite striking. The mesodermal cell layer (mc) (vascular endothelium and arachnoidal mesothelium) is shown in the area of fluid transit. The passage of the fluid through this cell-membrane is reproduced in the resultant precipitate seen in the cells and between the cells. The granules also appear lying with the red blood cells in the lumen of the diverticulum of the sinus (dt).

## PLATE III.

FIG. 5. — x 40. Drawing of the lateral wall of the superior longitudinal sinus of an infant (9 months of age). The superior longitudinal sinus (ss) on the one side, with the subdural space (sd) on the other, gives the relations of the section. The dural wall is pierced by many diverticula of the sinus. On the subdural side of the wall arachnoidal cells (ac) are shown invading the dural connective tissue and developing into a typical arachnoid villus (av). The myxomatous character of the villus itself is well given.

FIG. 6. — x 900. The specimen prepared by maintaining the subarachnoid pressure in an exsanguinated animal shows an intense injection of the whole perivascular system. This drawing is of a cerebral capillary

and portrays the distension of the pericapillary space (pc), which is filled in part with the precipitated Prussian blue (reproduced in black). These granules in the pericapillary space adhere in their precipitation from the solution to either wall of the space in accordance with physical laws. The endothelial wall of the capillary is shown cut across in one area with red blood corpuscles (rc) appearing in the drawing; the injection mass is found in the capillary lumen outlining the corpuscles and also traversing the capillary wall. For the most part the drawing shows only the external surface of the capillary endothelium (ec). The injection of the perineuronal spaces which appears in this specimen is not shown because of the possible confusion in the resultant picture.

PLATE IV.

FIG. 7. — x 275. The section, cut in the sagittal plane, shows the dural walls of the cavernous sinus with the included arachnoid tissue and the internal carotid artery. The comparatively non-cellular dural tissue (dm) is contrasted with the cellular arachnoidal tufts (av) which are filled with the Prussian blue precipitate (gm). The specimen is from a dog in which an injection under low pressures had been made for several hours into the spinal subarachnoid space. In some areas the granules are heaped up in the villi at the lumen of the sinus; in other sections the granules are found lying free in the lumen. The lumen of the sinus is divided into many compartments by both dural trabeculæ and arachnoid villi (av).

FIG. 8. — x 290. The specimen, from which this drawing of the olfactory mucous membrane has been made, is from the same animal which furnished the lymph node for Figure 11. The nasal epithelium (ne) with its covering of mucus appears overlying large lymphatic vessels which are surrounded by masses of the Prussian blue (gm) in the intercellular stroma. The olfactory nerves (on) have a few granules in a perineural relationship, but the olfactory glands (og) have none about them. This accumulation of the precipitated ferrocyanide solution in the stroma should be noted.

PLATE V.

FIG. 9. — x 290. An injection of the "perivascular lymph spaces" about a cerebral artery has been reproduced. The specimen was injected by a replacement of the cerebro-spinal fluid over the hemispheres and in the basilar cisterns with a ferrocyanide solution with subsequent causation of cerebral anemia by exsanguination of the animal. The nervous tissue, being confined in a closed cranium, then aspirated the ferrocyanide solution as shown (cf. text). The sharp limitation of the ferrocyanide precipitate (here given in black) to the perivascular canals and the trabeculation of this channel should be noted.

FIG. 10. — x 900. Drawing under oil immersion of two nerve cells (nc) from the same specimen from which Figure 9 was obtained. In this anemic brain the perineuronal spaces (pn) are well dilated and show up the walls and upon the outside of the nerve cells deposits of the precipitated

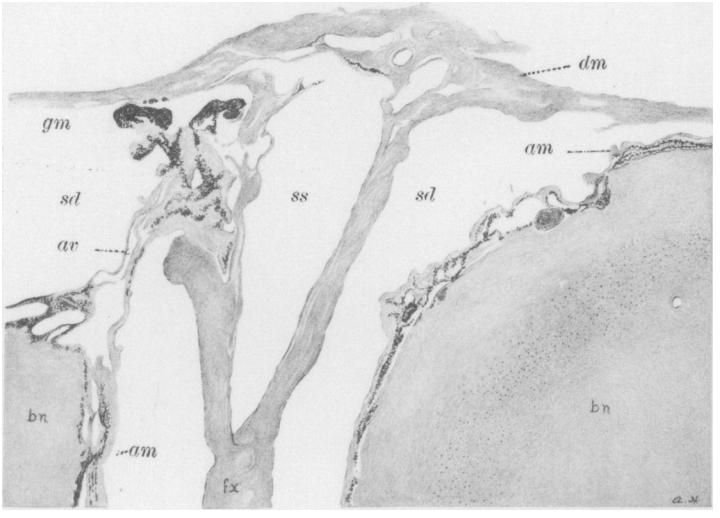
Prussian blue. None of the ferrocyanide occurs within the cell body, but it is a mere mechanical precipitation of the solution in the perineuronal space. The axone of one of these ganglion cells shows for a short distance the typical perineuronal injection.

FIG. 11. — x 300. The drawing is made of a cervical lymph node from a dog in which an injection of a ferrocyanide solution under a pressure of 160 mm. $H_2O$ had been made for three hours and thirty minutes. The lymph sinus (ls) between two germinal centers (gc) is reproduced with the characteristic Prussian blue granules (gm) adhering to the trabeculæ, which traverse the sinus. The absence of the precipitate in the lymphatic cells is noticeable.

## PLATE VI.

FIG. 12. — x 275. In the midst of dense dural connective tissue strands (dm) is a chain of arachnoidal cells (ac) containing in their cytoplasm and about them, collections of precipitated Prussian blue granules (reproduced in black). The freedom of the dural tissue from the injection mass is well shown as is the relation of the granular material to the vessels in the dura. The specimen was prepared by making a spinal subarachnoid injection of a solution of the ferrocyanide solution under low pressure.

FIG. 13. — x 60. A drawing of the motor area in the superior longitudinal sinus of a monkey. A portion of the great sinus (ss) is shown together with a part of a lateral diverticulum (dt) of this vessel. This diverticulum, a "lacuna lateralis" in the monkey, is invaded by a myxomatous arachnoid villus (av). Likewise, in the lateral wall of the great sinus there appears a similar arachnoid structure, surrounded by the dense strands of dural tissue (dm). A typical arachnoid villus (av) is seen leaving the arachnoid membrane on its course to the sinus lumen.

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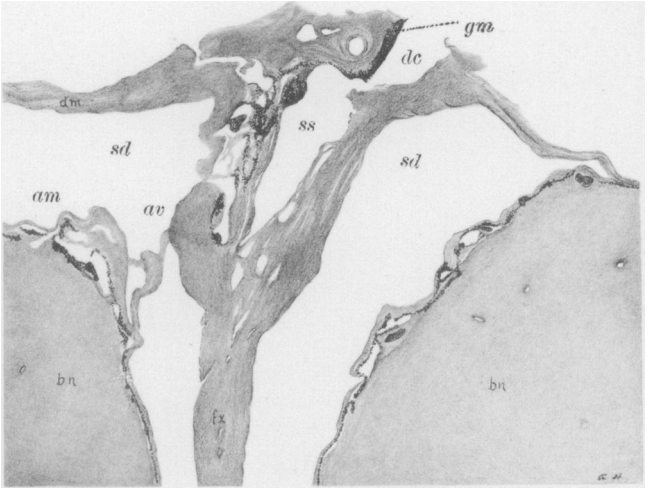


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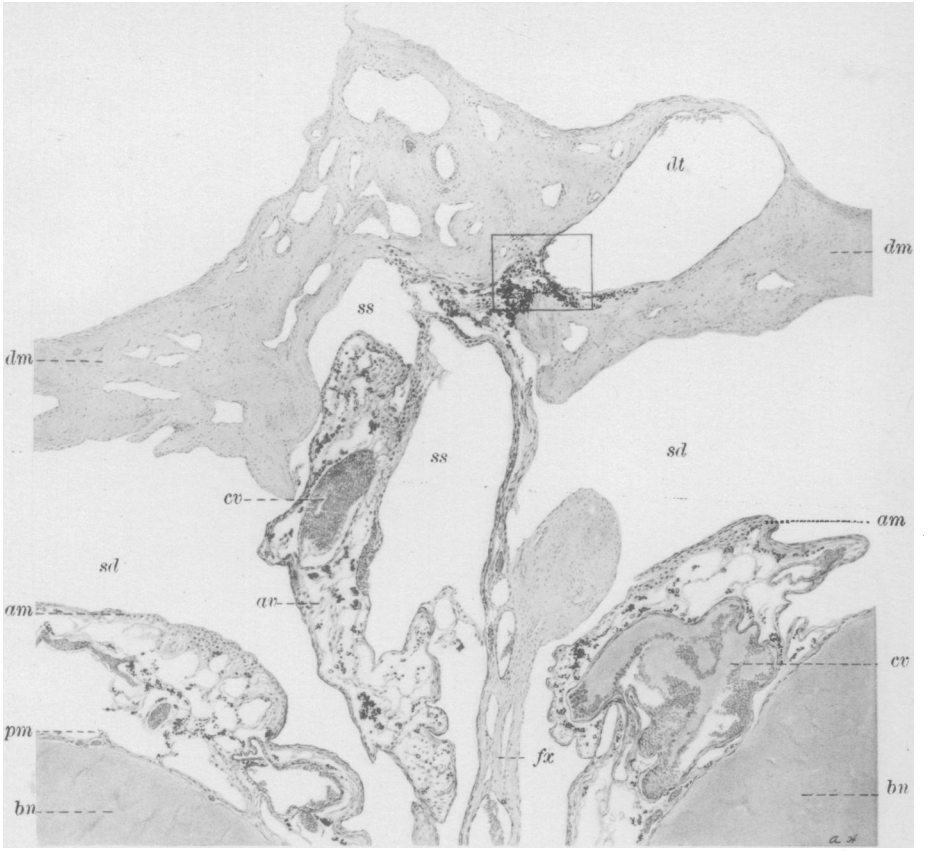


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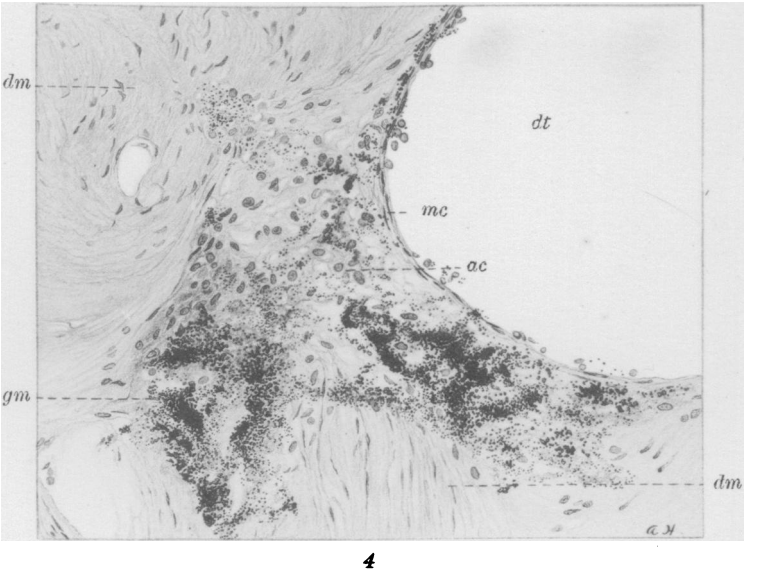


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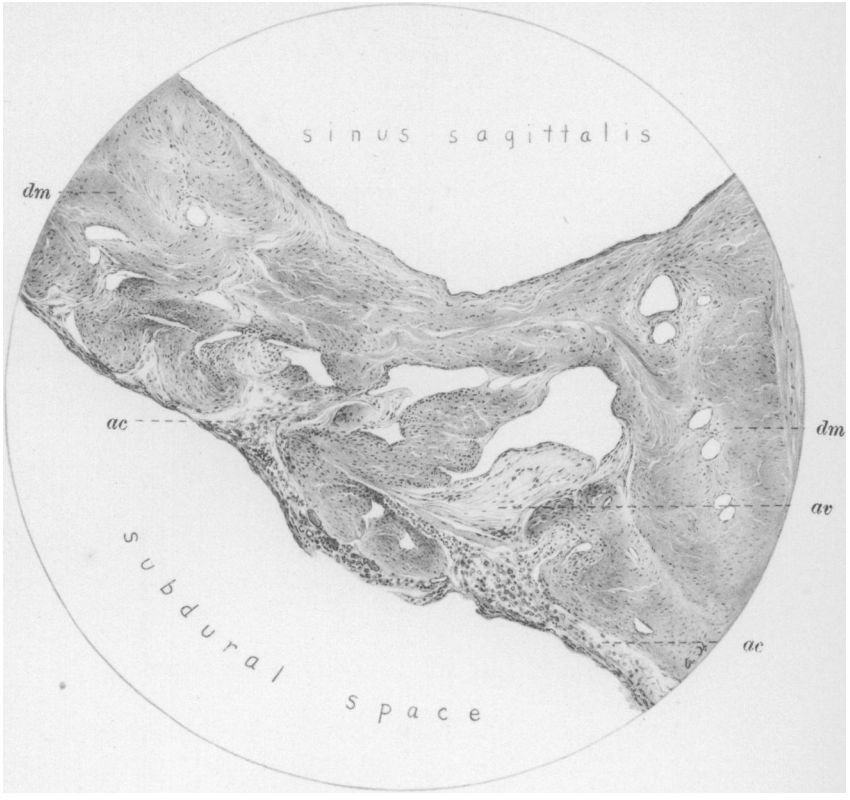
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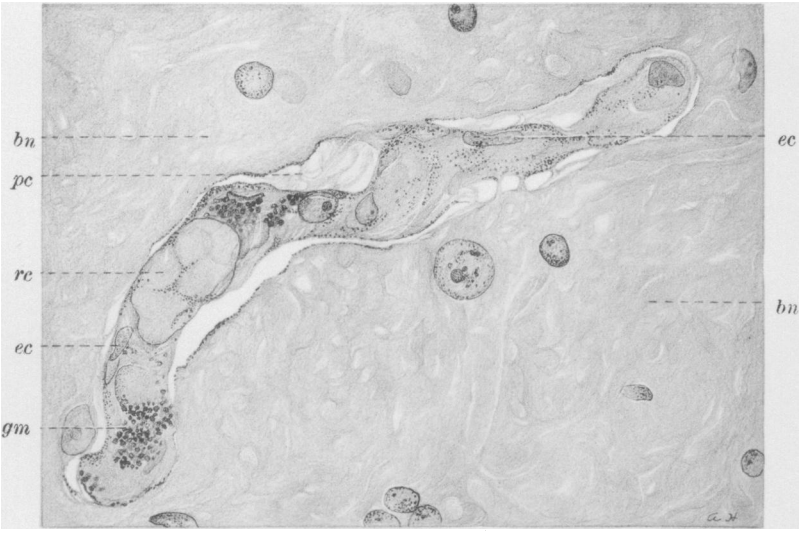
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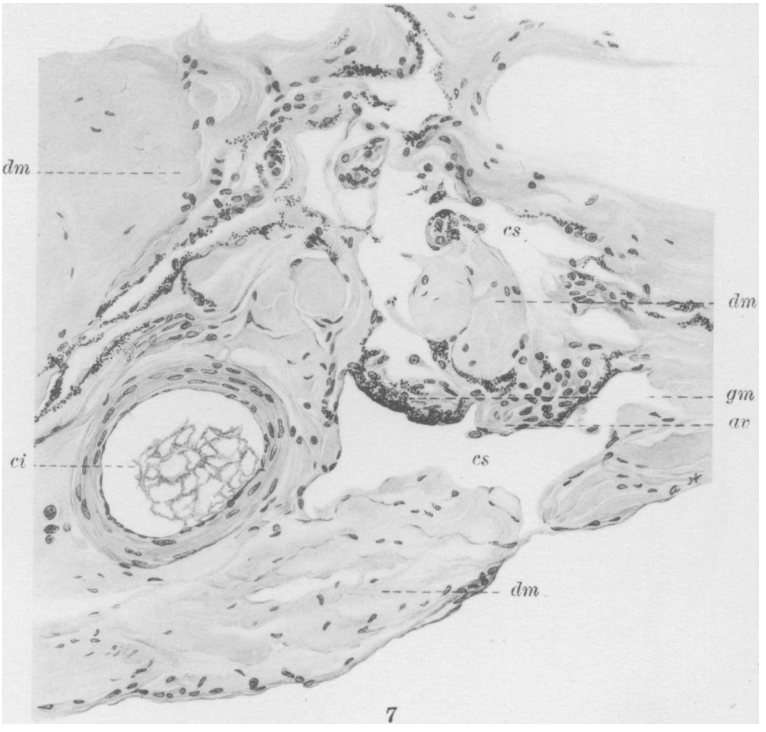


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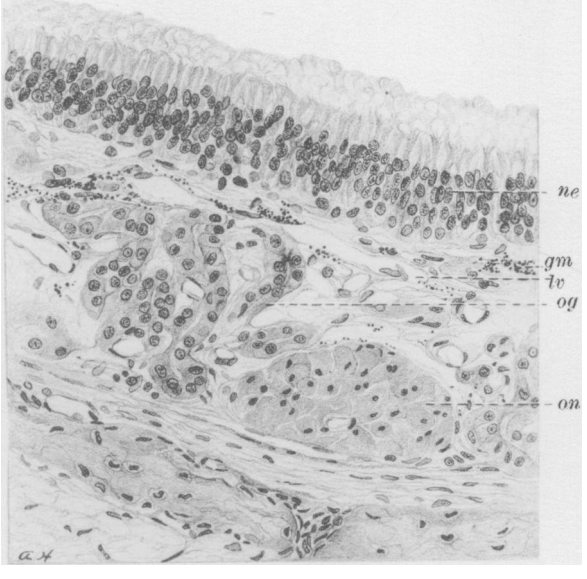


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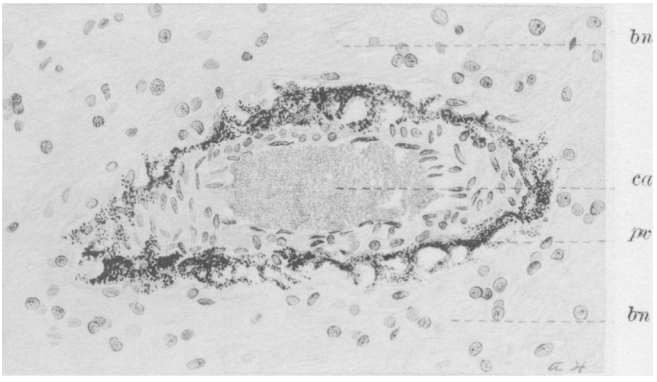


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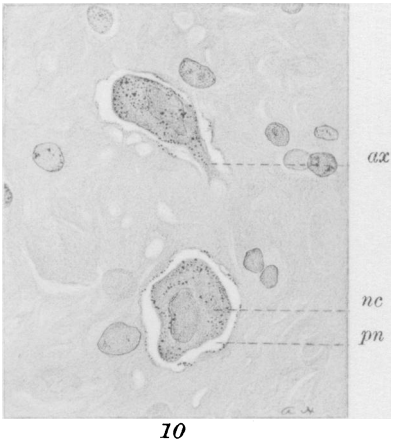


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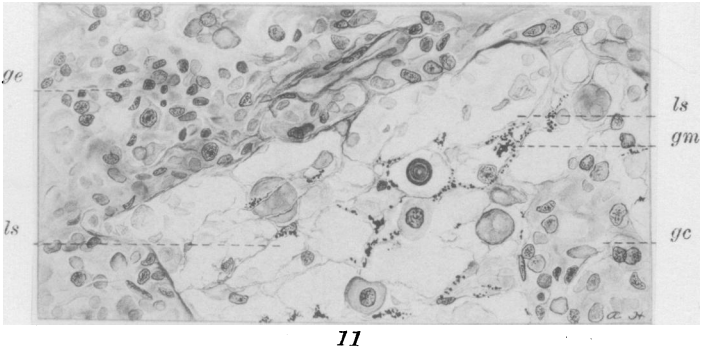


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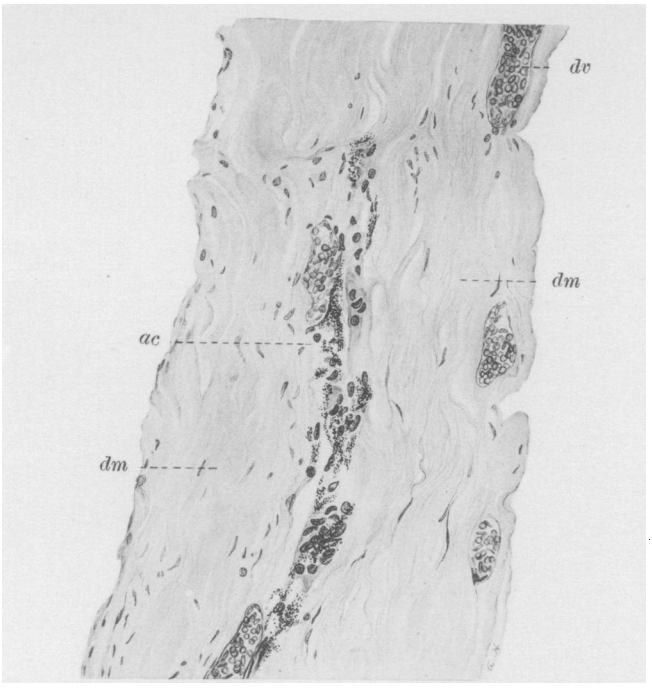


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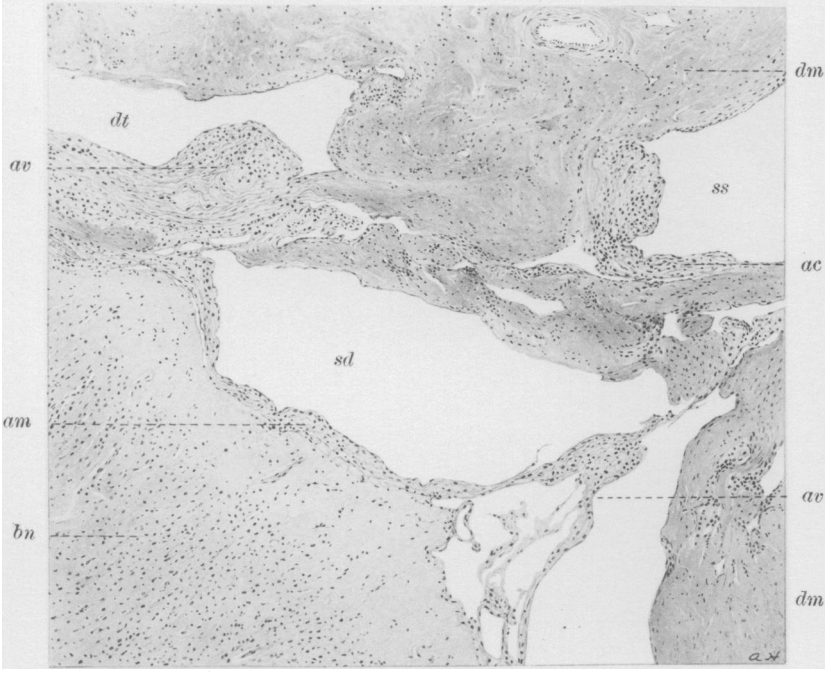


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