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HOW CAN WE PREPARE NEUROLOGICAL
MATERIAL TO THE BEST ADVANTAGE?

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NEUROLOGICAL studies have quite a number of peculiar difficulties.

A fair knowledge of the topographical anatomy, of physiology and pathology of the nervous systems offers about all the practitioner and clinician needs for diagnostical purposes and for the comprehension of his cases. Much of the contents of the standard works and monographs on the subjects mentioned forms so far merely crude material, waiting for the combining hand that furnishes the links for the whole chain. To study these details is too lengthy and inadequate a task for the practitioner; specialists have to step in, and it is with much justification that chairs for pure neurology have been founded in Clarke University and in the Chicago University for thorough scientific investigations, and that a few asylums in this country have their special pathologists giving all their time to these studies.

I have no doubt that many a physician has, or has had cases of great interest for neurological investigation. He would be anxious to give them a thorough study.

He makes, perhaps, an attempt, but not having the facilities and not being quite familiar with the methods and the literature, he fails to obtain a satisfactory result, and when the next case comes, he is not tempted to go through the same trouble again, as long as the prospects for the ultimate results are not better. He is not to blame for this. He needs help and somebody has to do this tedious work for him. I don't think that I overrate the interest in science of the American physician in believing that he will never mind going through the trouble of carefully preserving material, if two conditions are fulfilled: 1. If he knows an easy and safe method of preserving material, and, 2. If he can be satisfied that it will find a careful and well prepared investigator.

In the present note, I wish to give a short but exact description of the best methods of preserving neurological material and a few hints about the ways of sending specimens to a neurological laboratory. Many a physician will find a help in this; on the other hand, the success of neurological laboratories depends so largely on contributions of practitioners and clinicians that an appeal for co-operation is justified in every way.

I. PATHOLOGICAL MATERIAL.

As a rule, an autopsy should be made as soon as possible, at least within twelve hours, after death. In twenty-four hours, especially in summer, the brain may become useless.

For taking out the brain we adhere to the method which is universally used. The scalp is divided in a line which runs from one ear to the other over the vertex. The flaps are reflected together with the pericranium. The cranial cavity is opened by a circular incision with the saw, passing about 3 cm. (1") above the margin of the orbit and above the external occipital protuberance. Care is taken that the saw does not cut the *dura mater*. The inner table of the skull is better broken with the chisel.

The calvarium being loose we examine the skull for symmetry, note the condition of the sutures, the color and thickness of the external table, of the diploe and of the inner plate, the depth of the sulci of blood vessels, and the amount of adhesions of dura mater. I merely mention the normally strong adhesion between dura and skull in the child, which makes it necessary to remove dura and skull together with the brain.

We go over to the description of the dura: tension, color, degree of moisture; contents of longitudinal sinus; we then make a semi-circular incision of one side of dura (preference given to scissors) in order to see the inside of the dura before any blood may have soiled it; the vascularity, the moisture, and possible hemorrhages are noted; the other side of the dura is opened in the same way (following the line of section of the skull) and the inside is described.

The arachnoidea is controlled with regard to transparency, degree of moisture, vascularity, amount of adhesions to dura; for the latter purpose we cut the ant. attachment of the falx cerebri and draw the dura carefully backwards.

Now, we may proceed to the removal of the brain. We lift the frontal lobes, taking care not to tear the olfactory tracts and bulbs; and with a very sharp knife we divide the optic nerves of the pons and oblongata and finally sever the vertebral arteries and the spinal cord, taking care to make the section as little oblique as possible. No nerve should be *torn*, but all should be *cut* before the tension becomes dangerous.

The pia and the blood vessels of the basis are next examined, and for this purpose we open the fissure of Sylvius in its whole extent. After a careful review of all the superficial parts, nerves, peduncles, etc., for symmetry, etc., the real dissection may begin.

The following rule must be the guide for every dissection: A method can only be good if it does not destroy the mutual relations of the parts and moreover leaves them always in a condition which will make a

subsequent microscopical examination not merely possible, but most profitable.

Where we know with certainty what we are going to find ; for instance, where we see the pyramidal tract of one side degenerated, we know that we have to try to keep the whole stem intact in order to have an opportunity for a comparison of the two sides from the medulla up to the internal capsules. But in most cases we have no knowledge of what we might find, and in such cases, routine must dictate its best plan.

Where our microtomes are sufficient to make large sections of the stem, even in the region of the corpora striata, it is no doubt best to dissect the brain after Meynert's method, because it leaves all those parts together in which we can profit through a comparison of the two sides of a section. In the hemispheres, this becomes too difficult ; therefore, with the exception of a series of cases, of which I shall speak in connection with Siemerling's method, we prefer to give up this comparison and to examine each hemisphere separately.

Before beginning the description of Meynert's method we may remember the following rules :

1. Two good scalpels and two or three pairs of forceps, a thin bladed brain knife and a pair of scissors are the instruments necessary.

2. The brain substance is so soft and easily bruised, that it is to be handled with the utmost care and not more than absolutely necessary.

3. A clean cut with a sharp instrument does not do as much harm for the subsequent examination as undue pressure with the fingers and bruises and lacerations with blunt instruments.

4. We may subdivide the sections into such as will merely help us to obtain pieces of the brain that can be easily hardened and microtomitized without further subdivision, and into such as help us to discover pathological lesions.

5. The sections of routine should pass through parts which are of little interest and strictly avoid parts of

great importance. They do, therefore, not follow strictly the anatomical limits of the portions of the brain, but are chosen empirically. They should expose the important structures, but not injure them.

6. If this routine method leads us on the way to some pathological lesion, we had better leave the part intact until it is ready for preparation with the microtome. The naked eye examination has no other purpose but to decide whether subsequent examination with the microscope is necessary and profitable. As soon as this question is decided in the affirmative we should not cut any further. Hence, if we find after separating the hemispheres from the stem that there is such a lesion, we check our desire of cutting into it, and give a simple description of what we can see superficially, leaving the rest to the microtome. If we don't see any reason for such a thorough subsequent examination, we make further incisions, which we might call search sections; they must be methodical, *i. e.*, not interfere materially with the subsequent microscopical study, if such would become necessary in the end.

7. The reason for limiting the number of sections to a minimum is given by the fact that even the cleanest section destroys a certain number of histological elements; especially the white substance suffers somewhat by the protrusion of myeline, though not so much in the brain as in the spinal cord, which, on account of its narrow and unyielding coat of pia, is more apt to suffer.

8. Every sign of disease of a special part should, within reasonable limits, be a contra-indication, not an indication, for incision, as it may spoil the chances for making serial sections and a reconstruction of the whole. In the following description of Meynert's method of dissecting the brain, I shall try to revive the knowledge of those anatomical data which seem to be absolutely indispensable for the understanding of Meynert's plan. His idea is to separate the mantle of the fore-brain from the brain stem. He does, however, not follow pedantically

the anatomical limits, but he leaves the Island of Reil in continuity with the basal ganglia.

We may proceed in the following way: We open first the fissure of Sylvius in its full extent and we uncover carefully the Island of Reil and its limiting fissures. The limits of the Island of Reil form the landmarks of our dissection. It consists of a distinct triangle of short gyri, which mark, so to say, the lateral surface of the corpus striatum. The longitudinal arm of the triangle is parallel to the lateral border of the lateral ventricle; the anterior arm is nearly parallel to the gray matter of the corpus striatum, which projects somewhat into the frontal lobe, and the posterior arm is parallel to the inferior horn of the lateral ventricle.

For reasons to be mentioned below, we begin to separate first the frontal lobe from the corpus striatum. For this purpose we separate the two frontal lobes in order to uncover another guide, the knee of the corpus callosum. About one cm. behind it, we shall reach the ant. cornu of the lat. ventricle. We seize the frontal lobe, one finger in the longitudinal fissure, the other in the ant. limiting sulcus of the Island of Reil. We cut from the anterior border of the anterior perforated space, just behind the point of fixation of the olfactory tract, in a nearly horizontal direction forward until about one cm. behind the knee of the callosum we turn vertically upon the ventricle. This curve is made in order to avoid cutting into the caudate nucleus, whose head projects so far into the frontal lobe. And it will be seen that the anterior limiting sulcus of the Island indicates just the right curve for the section. If the knife goes too far forward (beyond the callosum) we risk not meeting the anterior cornu of the ventricle.

When cutting into the ventricle in the way described, we obtain information on several points:

1. The resistance which the knife meets informs us as to the toughness and tension of the ependyma, the real wall of the ventricle.

2. The amount of the fluid that escapes informs us

on the tension and the amount of cerebro-spinal fluid accumulated in the ventricle.

The frontal lobe of the other side is detached next. After this we proceed to the detachment of the temporal lobes from the brain stem. First of all we consider once more the lateral landmark, the posterior limiting sulcus of the Island. Next, we sever the union between the uncus of the temporal lobe and the optic tract, and the knife opens the inferior cornu of the lateral ventricle. The bridge between the posterior limiting sulcus of the Island and the inferior cornu being very narrow we cut it down in its whole extent. After this we lift the cerebellum with the medulla and pons, divide the pia which united the posterior border of the hemispheres and the splenium of the callosum with the cerebellum, and we thus uncover the corpora quadrigemina and the epiphysis. Cutting further, between epiphysis and callosum, we open the velum interpositum (the roof of the third ventricle) and thus enter the third ventricle.

We find the corpus callosum, the lyra and the fimbria of the fornix uncovered, and between the margin of the fimbria and the optic thalamus we see a bluish membrane extend, the pia with the choroid plexus. The latter ventricle is not open before this membrane is cut or torn.

We elevate the brain stem further, and, in a way, luxate the optic thalamus from the excavation of the lat. ventricle, so as to get access to the roof of the lateral ventricle. By severing the tissue which lies between the lateral margin of the ventricle and the longitudinal limiting sulcus of the Island of Reil, we succeed in peeling out the whole corpus striatum without injuring any of its ganglia. The only thing that remains to be done is to cut through the fornix and septum lucidum.

We have thus obtained :

1. The two hemispheres united in the middle line by the corpus callosum.

2. The brain stem including the Island of Reil.

For the examination of the hemispheres you may follow this plan:

Compare the breadth of the lateral ventricles. If one of the ventricles is distended, you find the distance from the middle line to the lateral border of the ventricle larger than on the other side (give measures in mm.)

Examine the plane of section for degeneration and compare the fornix of both sides.

Cut the corpus callosum in the middle line and ascertain whether the section shows any degenerated strands. Test the different lobes for adhesion of pia. For the inspection of cortex and centrum semiovale you divide the hemisphere. 1. Before the præcentral sulcus. 2. Behind the post-central sulcus and a superficial transverse section into the occipito-temporal lobe. In this way we obtain the motor area and the central gyri in one piece, the frontal lobe in another, and the occipito-temporal lobe in a third. Whereas additional search sections are made in a transverse direction in the frontal and occipito-temporal lobes, it is better to make them in the central lobe perpendicularly on the fissure of Rolando, in order to have a cross-section of both central gyri. The search sections must be made so that the parts are not completely severed, but may always be recognized in their mutual topographical relations.

In this part of the dissection I do not adhere to Meynert's plan, but follow more the directions of Weigert. Meynert separated the hemispheres in the fissure of Rolando and thus destroyed more or less the important relations of the motor areas.

Let us go over to the brain stem. An accurate inspection especially with regard to the symmetry of the parts should be the first thing. If there is anything abnormal in the brain stem as atrophy of nerve centres, cysts and secondary degeneration, we shall discover them in this way without injuring the stem at all. The only incision which should be generally made for facilitating the inspection, is a longitudinal section through

the worm of the cerebellum, by which the fourth ventricle is freely exposed. We inspect the convolutions of the Island of Reil, the corpora striata, internal capsules, optic thalami, the corpora quadrigemina, the fourth nerve, the fourth ventricle and the hemispheres of the cerebellum. On the basal aspect we review the optic tracts, the tuber cinereum and corpora albicantia s. mammillaria, the crura and the 3d pair, the pons with the 5th pair, the 6th; the olivary bodies and 7th, 8th, 9th, 10th, 11th, and 12th nerves. Search-sections, if necessary, are made in planes which are exactly perpendicular on the axis of the brain stem. As an anatomical guide in the dissection and topography of the brain stem, I should recommend the splendid atlas of Bruce; for the anatomy of the fore-brain, I have to refer the reader to one of the text-books, the first part of the third volume of Quain's Anatomy (edited by Schaefer), or Edinger or Obersteiner.

It is advisable to measure the crebro-spinal fluid, which escapes during the dissection of dura and brain, and to weigh the parts of the brain: the hemispheres, the stem and cerebellum. The brain is then put at once into a large quantity of Müller's fluid (two or three gallons) on several layers of filtered paper. The fluid should be changed the 2d, 4th and 7th days, and afterwards once a week. The first few days the brain substance becomes rather soft and should not be manipulated at all; it is, therefore, advisable to change the Müller's fluid with a syphon arrangement. In the third or fourth week the brain contains a consistence which is very good for dissection. The tissue elements are sufficiently fixed; protrusion of myeline is scarcely observed, and a section made at this time will remain smooth. It seems to me that this is the best time for transportation. For this purpose a tin pail is made, slightly larger than absolutely necessary for the bulk of the brain, which is enveloped in absorbent cotton. The pail is filled up with Müller's fluid, the lid is soldered and the pail packed in a box of wood.

The essential points in the preservation of entire or dissected brains are the following: Müller's fluid is the best hardening fluid, in fact, the only one that comes into question,

It consists of bichromate of potash, $2\frac{1}{2}$ parts; sulphate of soda, 1 part; distilled water, 100 parts by weight; best made about six days before use and then filtered. It should be prepared in large quantities and not be used too economically.

Brain substance should never come into contact with water.

When entire brains (for the study of convolutions, etc.) are preserved, the pia matter should be removed, because this facilitates the impregnation with Müller's fluid. Where a microscopical examination of the cortex is necessary, the pia should *not* be removed; but numerous incisions with a sharp knife should prepare free openings to the hardening fluid.

A few words on other methods will characterize my position to them. The method recommended by Blackburn is based on the general rule that all cavities should be opened and inspected before actual section of the part. This advice is good in some respects where the ventricles are of great interest for the pathology of a certain brain, for instance, hydrocephalus, but even there, the unavoidable mutilations, for instance, of the mid brain, is not worth the plus of insight into the cavities; the brain substance will always be the most important thing of the brain, and the ventricles are to be sacrificed, if necessary. This does not, however, occur with Meynert's method. With the exception of the aqueduct of Sylvius, which, from a pathological point of view, is of no importance compared with the surrounding tissue, all the ventricles are freely opened; moreover, we get a very good idea of the pressure within the lateral ventricle, if we open it first in the frontal lobe, not as Blackburn advises in his description of Meynert's method, from the transverse fissure, where we are very apt to open in the same time the third ventricle. Blackburn

separates the brain stem above the pons; the upper portion is divided in the middle line, and the mid-brain, the regio subthalamica and other important regions are lost for comparative study of the two sides. This is a very serious objection for those who wish to study more the course of degenerating tracts than the mere untographical histology of pieces of brain substance. Meynert's method gives us a good chance for both. A simpler method, which might be especially recommendable where the symmetry of the parts is of great importance, is the one proposed by Siemerling. After a careful inspection of the surface and of the vessels, the pia is examined for adhesions, then the brain is divided into frontal sections with a brain knife, beginning at the frontal pole. The sections should not be too thin, because it would be too difficult to make them equal on both sides. It is best to cut from the basis towards the convexity, as it is easier to ascertain the exact localization. S. chooses the following points: 1, directly behind the knee of the callosum; 2, before the chiasma; 3, immediately behind it; 4, through the corpora albacantia; the splenium of the corpus callosum is then divided in the middle line and the brain stem is detached; each occipital lobe is examined apart, and the brain stem may be preserved for microscopical examination.

This method may be recommended instead of Blackburn's, where the pathologist does not care for making the decidedly far more elegant and instructive dissection after Meynert, for instance, in general hospitals, in cases where no brain lesion can be expected. In cases of brain disease it may be justified by tumors, but even there the chance of easy comparison of the symmetry of the brain-mantle is paid too much for, as the basis, the subthalamie region, is too badly injured.

Virchow's method had better be altogether abandoned, as it slices the brain nearly regardless of anatomy and localization and destroys every chance of a microscopic examination.

Hamilton's method of injecting the brain with

Müller's fluid is certainly excellent ; but on the whole, it will require too much space and attention and give no insight into the fresh brain at all.

What brains are worth a thorough microscopic examination ? Fresh and old local lesions of any part of the brain make the whole brain interesting on account of the secondary degenerations. In old standing cases of hemiplegia some of the most important facts have been found the last year, for instance, the sensory tract that runs from the nuclei of Burdach and Goll, as far up as the motor area.

Another point worth study would be the degenerations in cases of aphasia, and in localized lesions of any part of the brain.

Difference in size of any two symmetrical parts of the brain may give a key to the topographical study of these connections.

Atrophy of one or more cranial nerves is always of the greatest interest, because there is considerable confusion in the literature with regard to the origin of the facial, and of other nerves ; moreover, every brain of a microcephalic individual, of idiots, of members of rare races (Indian, Asiatic tribes, negroes, etc.), has an interest for the study of the convolutions and should, partially deprived of the pia, be preserved as a whole.

The *spinal cord* has been made the subject of such an interesting study by Dr. Ira van Gieson, that I need hardly give a long description of the post-mortem examination of this organ. The removal of the cord is the most distressing part of an autopsy ; if it is not done properly, numerous and very misleading mutilations of the cord may make a subsequent study impossible. Every method has a danger of causing bruises of the cord, before all those methods which allow the application of much force, such as the use of the chisel and of huge bone shears. Dr. van Gieson is right to insist on the use of a single-bladed (or double-bladed) curve-saw, and on dividing with it as much as possible of the vertebral arches. If the normal lordosis of the spine in the

lumbar and cervical region is corrected by sufficiently high logs of wood, it should not be too difficult to perform the operation with approximate accuracy. The incompletely sawed laminæ may be divided with not too bulky shears, such as we use for the ribs. In removing the spinal processes there is always a danger of using pressure on the dura which may cause diffuse bruises of the cord.

In removing the cord we have to avoid bending, twisting or stretching it too severely. For this purpose we avoid touching the cord altogether and seize the dura, keeping it straight under moderate tension. Much discretion should be used in making transverse sections of the cord. If it is very soft or where we discover at once on the surface some abnormality, sclerosis or gray degeneration, this should be an inducement to let the cord alone, to hang it up together with the dura, carefully, in a glass tube containing Müller's fluid. It is always much wiser to make transverse sections only after the tissue is sufficiently fixed, because with the fresh cord the myeline is pressed out, however sharp the knife may be. Where nothing abnormal is suggested by mere inspection, transverse incisions must be made with the utmost discretion. The spinal cord should never be put into the same jar with the other organs.

I should not warn particularly against longitudinal sections of the cord and bulb, if I had not seen precious material practically destroyed in this way by well meaning but badly informed physicians.

In order to mail a cord it is safest to keep it in an abundant quantity of Müller's fluid for about three weeks, then make a sufficient number of transverse sections, leaving the dura intact (for orientation), curl it up in a loose spiral, surround it with cotton, and mail it in Müller's fluid.

It is scarcely necessary to add that every piece should be carefully labelled. Cords of special interest, besides general paralysis, tabes, etc., are those of amputated (amputation of humerus or thigh), jongleurs, etc.

2. EMBRYOLOGY.

Partly in the range of pathology, in the subdivision of teratology, we find a great number of specimens that should come into the neurological department instead of being abandoned to the bacteria of putrefaction, those monsters which often furnish most suggestive malformations of the nervous system, anencephalus, spina bifida, etc. It is evident that these abnormalities require a well trained student if the material should be properly used.

But also the ordinary material, the nervous centres of children from two years back to the new born, the foetus of 9, 8, 6 months; well, every stage of the foetal development opens a wide field for investigation.

Whereas in the strictly pathological portion of neurology, lunatic asylums, poor houses and general hospitals, furnish the greatest number of cases, this part is largely depending on the benevolence and interest of the practitioner.

First, a few remarks on the youngest stages of embryo:

The scientist cannot help having a feeling of gratitude towards members of the profession who come to assist by furnishing material of this kind. These specimens cannot be obtained for money; but we depend altogether upon the kindness of the physician. Now, everybody knows the difficulty in obtaining those small forms, often expelled in blood clots, often hidden by the people and often too far destroyed by putrefaction. We need not wonder at the physician who was successful to obtain a fine specimen and then wishes to keep it, to withhold it from the microtome; still, I have no doubt there are many who wish to contribute their share to scientific investigation, if they just knew how to dispose of the material. It is remarkable what collections Prof. His could make in the small city of Basle, a place of about 40,000 inhabitants, where abortions are relatively much rarer than in this country. Following the great experience of His, I wish to give the following rules for

preserving embryological material. Embryos of the first two months: Whatever the condition of the material may be, it may be too old, torn, spoiled by blood, it still may be of value in some way and is worth being preserved (malformations are very frequent, especially in the expelled foetus). The specimen should not be washed; water spoils it; for fixation it is put into a 10% solution of nitric acid for about fifteen minutes; then it is preserved in 60% alcohol. This same method may be sufficient for embryos of three and four months, but it will be advisable to open the skull carefully in order to give access to the fixation fluid.

In specimens of the fourth month, we find the first development of medullated sheaths. This gives a strict indication for the use of Müller's fluid; the skull should be carefully opened, and also the spine in several places, carefully avoiding bruises of brain and cord, and the specimen is put into an *abundant* quantity of Müller's fluid. The latter should be changed on the 2d, 4th and 8th day. In three or four weeks the specimens can be mailed in a wide-mouthed bottle or tin box. The stages from the 5th month upward are exceedingly important, and also the brains and cords of children up to three years, because of the relative simplicity of the structure.