

**METHODS OF  
STAINING  
THE NERVOUS  
SYSTEM.**

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**POLLACK.**





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METHODS OF STAINING  
THE  
NERVOUS SYSTEM.



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NERVOUS SYSTEM

W. J. W.

BY  
DR. BERNHARD POLLACK

TRANSLATED FROM THE SECOND GERMAN EDITION

BY  
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## AUTHOR'S PREFACE TO THE SECOND EDITION.

I HAVE so much the more willingly complied with my publisher's request to prepare a second edition, as it gave me the desired opportunity to introduce a large number of changes—on the one hand, numerous additions, and on the other, a small number of omissions.

Although there appeared to be no special reason for a complete revision of the volume, I hope that I have at least supplied some portion of the information required to bring it up to date. I have been delighted to receive valuable suggestions from various quarters, and now, as formerly, I must thank my honoured teachers, Wilhelm Waldeyer and Carl Weigert, very heartily for them. My hearty thanks are also due to Professor Edinger of Frankfort, Professor Krause of Berlin, Professor Laskowski of Genf, Professor Ziehen of Jena, and lastly to my friend, Dr. E. Flatau.

The section upon the treatment of the retina has been newly added.

It only remains for me to emphasise once more what I said in preface to the first edition, that I have no other purpose in this work than to supply the neurologist with a book of reference in as convenient a form as possible.

In conclusion, my sincere thanks are due to the publisher for the manner in which he has carried out my wishes.

POLLACK.

FIRST ANATOMICAL INSTITUTE,  
BERLIN, 30th November, 1897.



## PREFACE TO THE FIRST EDITION.

THIS little book is intended only for those engaged upon the microscopic examination of the central nervous system. This is as much as to say that it is not intended for the tyro in histology, and that it is not meant to be, or to replace, a text-book of microscopy. On the contrary, a large number of directions, which are to be found in every work dealing with the technique of microscopy, have either been purposely omitted or referred to so briefly as to show that a certain amount of previous knowledge and experience in the domain of technical microscopy has been taken for granted.

The real purpose of this collection is to give to those interested in the subject a convenient reference book, after the fashion of Goodall's admirable work. The great activity of neurologists of late years has gradually given to their subject an importance so great, and a field so extended, that it is not only impossible to retain in the memory all the methods, frequently so complicated, but it would also require more than one lifetime to verify everything that has been recently published.

My endeavour, at all events, has been to give chiefly such methods as may be regarded as a more or less certain and useful possession of our province. I trust that the omissions, which the difficulty of choice made unavoidable, may be overlooked. It seemed to me useful to give some methods shortly, and others in greater detail. Thus Nissl's stain, Marchi's method, and Golgi's chromate of silver method have been thoroughly gone into. Weigert's stain for the neuroglia, too, which, apart from abstracts in the journals, appears as yet in



almost no text-book of histology except Weigert's own great work, had to have a fitting description allotted to it.

For distinctness' sake, I have almost throughout employed the form of formulae which was proved so useful in von Kahlde's "Technik." In addition, I endeavoured to add certain critical remarks upon various methods, in which I had the friendly help of my friend, Dr. E. Flatau.

I offer my very sincere thanks to my honoured teachers, Herren Gehh-R. Waldeyer, and Weigert, for the friendly interest which they have so helpfully taken in my little work, which I hereby dedicate to them.

POLLACK.

FIRST ANATOMICAL INSTITUTE,  
BERLIN, 20th February, 1897.

### TRANSLATOR'S PREFATORY NOTE.

THAT this little book ran through a first edition, and passed into a second within a year of its publication, is the best proof of its popularity in Germany, and my apology for introducing it to the British public. My object has been to render the sense of the original as faithfully as possible, and, to do so, I have in many cases had to depart to some extent from literal exactitude. I trust that the book may prove as useful to readers in this country as it has already proved abroad.

WILLIAM R. JACK.

GLASGOW, August, 1898.



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## I.—METHODS OF SECTION OF THE BRAIN.

THE macroscopic or microscopic examination of the cerebral nervous system of vertebrate animals becomes possible on the fulfilment of certain preliminary conditions. These are: first, the opening of the skull or spinal column; second, the removal of the organs from the protective coverings surrounding them (among which the dura mater must be included); and, third, the disposition of certain incisions in such a manner as to permit the most accurate investigation of all internal parts; and, further, the preparation of microscopic sections, stained or unstained.

Although the first and second of these proceedings are relatively simple, and familiar to every anatomist, a brief sketch of the process may not be out of place.

The scalp having been divided by an incision running from one ear to the other over the vertex, and reflected forwards and backwards to the margin of the orbit and the external occipital protuberance, the temporal muscles are horizontally divided, and the vault of the cranium is removed by means either of the saw or of the chisel, the latter mode being preferred in France. As Déjerine rightly points out, the use of the saw may easily bring about an injury to the surface of the brain, especially as the skull, in the course of its circumference, varies in thickness and density. He would therefore limit its employment to those cases where fracture or other injury of the skull has previously occurred. It appears to me, however, that lesions can be avoided as easily with a little practice, care, and accurate observation of the changing tone of the saw, as with the chisel preferred by Déjerine.



The vault of the cranium having been removed, and the dura mater sufficiently examined for external abnormalities and alterations, the dura is then divided by scissors, either with a circular incision, or, better, with the familiar crucial incision. One may now proceed to remove the brain from the cranial cavity. For this purpose the left hand is introduced under the frontal lobes, the organ is carefully raised, the cerebral nerves at the base and the vertebral arteries are divided with the scalpel, the cerebellum is freed, and finally the medulla oblongata (or spinalis) is divided. With the support of the right hand, the whole brain can now be lifted out. It cannot be too much insisted upon that any tugging or tearing must, in all these manipulations, be carefully avoided. Apart from the fact that the cerebral peduncles might thus be injured, such rough manipulation may also give rise to artificial products which, at a later stage of the examination, and especially if Marchi's method be employed, may be the source of misinterpretation.

The opening of the vertebral column, the removal of the spinal cord, and the division of the nerve roots, are simple, and require no further exposition.

The manipulations which the brain has to undergo, after it has been placed in a hardening or fixing fluid, will be mentioned later. The incisions to be made in its substance must first be considered.

While normal anatomy still follows the general lines of the old method of Galen,<sup>1</sup> R. Virchow about fifty years ago devised a new method for pathological anatomy, calculated to give a complete view of any alterations, while preserving so far as possible the relationship of the parts. The essential features of the *method of Virchow* are the following:—

After the hemispheres have been pulled asunder at the longitudinal fissure, a vertical incision is made into the corpus callosum, by the side of the raphe, for the purpose of opening the body of the lateral ventricle. The anterior and posterior horns are exposed by horizontal incisions into the anterior and

<sup>1</sup>Siemerling and Edinger : Zeitschrift f. Psych. 1894 p., 349 ff.

posterior cerebral lobes. The septum pellucidum is seized with the left hand behind the foramen of Monro. The knife is passed through this aperture, and the corpus callosum is divided by an incision running obliquely forwards and upwards. All the parts (corpus callosum, septum pellucidum, fornix) are pulled away from the velum choroides. Passing from before backwards, the handle of the scalpel is inserted under the velum, which is pulled away from the pineal gland and corpora quadrigemina. By a long vertical incision the corpora quadrigemina and cerebellum are divided as far as the aqueduct of Sylvius and the fourth ventricle. The hemispheres are laid open by incisions passing from within outwards, so that each successive incision is carried over the middle of the surface already cut, and each new half is again redivided. The optic thalami and corpora striata are divided by radial incisions arranged in the shape of a fan, and having the crus cerebri as their common starting-point.

The *French method* permits of an accurate localisation of focal lesions, and is the best adapted for further microscopic investigation. By this method, the hemispheres, which have been separated from each other and freed from pia mater, are divided by means of frontal sections into several parts. The first incision is made about 5 cm. before the fissure of Rolando, the second 1 cm. before the parieto-occipital fissure. The hemisphere is thus divided into three regions—the pre-frontal, the occipital, and the fronto-parietal. The last is opened out by four further incisions. The first of these passes through the “feet” of the temporal convolutions (*coupe pédiculo-frontale*), the second through the ascending frontal (*coupe frontale*), the third through the ascending parietal (*coupe pariétale*), and the fourth through the “feet” of the parietal convolutions (*coupe pédiculo-pariétale*).

This method of *Pitres* has been somewhat modified by *Nothnagel*, who, after separating the hemispheres, divides each of them by partial incisions, running from above downwards, and in the main parallel to the fissure of Rolando. The starting-points for these incisions are formed by the genu and



splenium corporis callosi, immediately before or behind which, as the case may be, the incision falls. Nothnagel, like Pitres, makes in all six incisions, more closely related, however, to the given fixed points, and thus making it possible to name the part of the centrum ovale between every two incisions simply after the superficial parts of the brain contained between them. He gives the following names to the regions of the centrum ovale, counting from behind:—

- (1) Pars occipitalis.
- (2) „ parietalis.
- (3) „ centralis post.
- (4) „ „ ant.
- (5) „ frontalis post.
- (6) „ „ media.
- (7) „ „ ant.

The *method of Meynert* (Vierteljahrschrift f. Psych. 1867, II.) was invented for the purpose of weighing the different parts of the brain. By this method the organ is divided into cortex, caudex cerebri, and cerebellum. The pia mater is not removed.

This method of dividing the brain has not been widely followed. Other methods (Nauwerk, Sectionstechnik, Jena 1891, Burkhardt, Microtomie des frischen Hirns, Centr.—Bl. f. d. med. Wissenschaften, 1881, p. 529, and Byrom Bramwell, on a ready method of preparing large sections of the brain, Brain, vol. x., 1887-88, p. 435) may be passed over, and mention made only of the *combination of the methods of Virchow and Meynert* recommended by *Weigert*. The ventricles are opened from above, and the great ganglia are cut round by means of a curved incision, which lays open the entire inferior horn. The hemispheres are divided from without, as far as the central convolutions in frontal, and from that point in horizontal sections. By this method the brain can always be put together again.

The objects which have to be kept in view in the selection of one or other of these methods are very various, yet, according to Siemerling, two points may be regarded as mainly deter-

mining the choice. In cerebral affections, especially in the so-called focal lesions, the points of importance are the seat and the extent of the lesion. Bearing this in mind, the incisions must be made so as to give the greatest facilities for its ultimate localisation. Moreover, since the recent progress in neurology has been attained only by the aid of the highest developments of microscopic methods, it is desirable to keep in mind the possibility of an extensive microscopic examination of the brain.

The postulate of Virchow, now more than fifty years old, that “the individual peculiarities of the case must determine the method of examination,” applies to no organ more forcibly than to the brain.

Accepting the view that the macroscopic section of the brain must be adapted to the microscopic examination to follow, we may deduce the general law, that no one form of section can be considered as the best adapted to every case, but that certain special features must be kept in mind.

Virchow's method, indeed, permits of an accurate view of all the parts, but the division into small portions, hanging together only loosely by their connection with the pia mater, makes an accurate microscopic examination almost impossible. Serial sections, in any case, are completely excluded. But the division of the cortex by incisions, the direction of which is constantly changing, also increases, not infrequently, the difficulty of determining the seat of a lesion. The approximate certainty obtained by this method, that the internal parts of a brain are free from gross lesion, is no longer sufficient for the class of cases under consideration.

Meynert's method is better adapted for normal anatomy, and for the weighing of the various parts of the brain, as also for cases where the cortex and the rest of the brain have to be separately examined.

The method of Pitres-Nothnagel is that chiefly employed for the majority of internal organic lesions.

The importance of care in the removal of the brain and cord and all later procedures cannot be sufficiently emphasised, for many cases, which are recorded as abnormalities, have been artificially produced.



Ira von Gieson has shown how far the influence of careless handling can go in producing false impressions; how easily, for example, artificial heterotopia may be produced. Even an apparent duplication of the cord might be attained in this way.

It will be seen from the foregoing that the French method of frontal sections should be employed for later microscopic examination of the brain. The sections should be made as few as possible, especially in the ganglia. It is only after hardening has taken place that further sections should be made.

A few facts with regard to the weight of the brain in man and some of the higher animals may here be given. Broca determined the weight of the internal envelopes of the brain (along with the serum in the subarachnoid space) as 55.8 gm. in man, and 48.7 gm. in woman. The brain itself weighs on an average 1360 gm. in man, 1230 gm. in woman (according to other authors 1416 and 1260 gm.), and in the new-born infant 447.5 gm. It follows that the relative weight of the brain (*i.e.*, the relation of the weight of the brain to that of the whole body) is for the adult about 1 to 40 or 60, and for the infant 1 to 8.3 (according to Mies 1 to 5.9). The brain of the infant is therefore relatively much larger than that of the adult. Thurnam's calculation of the adult proportion as 1 to 33 and 1 to 31.9 is declared by Obersteiner to be valid only for very emaciated individuals.

The heaviest brain is the property—not of man—but of the elephant, whose organ attains a weight of 4000 to 4600 grms. Then follows the whale, with a weight of about 3000 gm. (Beauregard), and man occupies the third position. In the horse the weight is only 680 gm.—in the gorilla, about 500.

Although, generally speaking, a certain relationship appears to exist in the healthy man between size of brain and intelligence, yet no generally valid rules can be laid down upon this point. Gambetta's brain, for example, weighed only 1100 gm.—Turgenieff's nearly double as much, 2012 gm.; and, even in ordinary labourers, brains have been met with weighing over 2000 gm. But it is certain that "the brain

must exceed a definite minimal weight (1000 or 900 gm.), in order that the psychical functions may be performed in a normal way" (Obersteiner).

The two hemispheres are almost always of equal weight (except in the insane). Ogle states that the right hemisphere, in left-handed people, weighs a few grams more than the left, and, in right-handed people, this is reversed. Meynert has shown, with regard to the insane, that the weight of the brain is least in general paralytics, who are followed, in this respect, by chronic alcoholics.

To the neurologist it is often of interest and importance to know the cubic content of the interior of the skull, and to be able at once to draw certain conclusions therefrom. In the majority of the methods of Welcker usually employed (with sand, small-shot, &c.), there are, as may easily be understood, many sources of fallacy. Water is probably the most useful medium. According to Zanke's proposal, both parts of the skull (the vault, which has been removed, and the basal portion) are filled with water from a graduated glass, and the amount of water poured in gives the number of cubic centimetres of the cranial content. Before reading off the amount of water to be poured in, the empty canal of the spinal column must also be filled with water, or, if the spinal column has been previously opened, the foramen magnum must be plugged with cork or moist cottonwool. In a macerated skull, Zanke replaces the dura mater by a pig's bladder of the largest size and smallest possible thickness, which has been made pliable in water.

The importance of the numerical difference between brain-weight (taken as unity) and cubic contents of the skull is shown by the fact that, in paralytics, numerical differences of from 10 to 580 have been found.

Broca estimated the average content of 115 skulls of the 12th century at 1426 ccm., that of 125 skulls of the 19th century (all of which were those of Parisians) at 1461.5. He concluded from these facts, as is well known, that there had been a corresponding increase in brain-weight.



## HARDENING OF THE BRAIN FOR PURPOSES OF PRESERVATION.

Whether we desire to preserve the normal brain in a hardened condition for purposes of demonstration, or to add rare specimens to a museum collection, modern technique in either case demands that two conditions be as completely as possible fulfilled. These are—the maintenance of the original size and form, and the maintenance of the architectural structure. In other words, the prepared brain must differ to the smallest possible extent from the fresh one. Until a comparatively short time ago, it was not possible—and, indeed, it was not attempted—to fulfil these postulates. The specimens preserved in spirit in pathological collections prove this sufficiently. The constant endeavour and demand of the neurologist, however, is towards improvements in macroscopic, as well as microscopic, technique. Besides the simple process of hardening in alcohol and bichromate, there have thus arisen a number of processes, the usefulness of which has led to their permanent retention.

If the natural configuration is to be preserved, it must be borne in mind from the outset that the weight of the brain is very apt to cause it to flatten, unless care is taken to cover the bottom of the vessel, in which the hardening fluid is contained, with a thick layer of soft cotton wool. Even with this precaution it is difficult completely to avoid a slight degree of flattening. The proposal of Retzius is therefore to be recommended. The brain, which must be newly removed, and still within its envelopes, is suspended in the fluid by a thread attached to the basilar artery. If, in the process of hardening, the pia mater be removed, the frontal portion of the brain sinks downwards, owing to its greater weight. In this case, as in preparing the organ for staining, two points are of equal importance. The material must be as fresh as possible, and large quantities of fluid, frequently renewed, must be employed. The process most in use is the

*Method of Giacomini.*

- (1) The fresh brain is placed in a 10 per cent. solution of

chloride of zinc, in which it is frequently turned round. The pia mater is removed after eight or ten hours.

- (2) As soon as the brain has sunk downwards, which occurs in a few days, it is transferred to spirit, which must be changed twice or thrice in twelve days. The bottom of the vessel must be covered with cotton wool (see above).

- (3) The hardened brain is transferred to glycerine, to which 1 per cent. carbolic acid may be added. After complete saturation it sinks. It is then placed upon a slanting glass plate, that the superfluous glycerine may flow off, and the brain be dried in air. In this condition it may lie for many years exposed to the air. The length of time for which it is preserved depends upon the permanence of the action of the chloride of zinc.

If the brain be not quite fresh, it is recommended to inject by the carotid arteries, and at a moderate pressure, about 600 grm. of the chloride of zinc solution. If, later on, it should show a tendency to shrink, good results will be obtained from its re-immersion in glycerine. Instead of chloride of zinc, a 5 per cent. solution of carbolic acid may be employed. The spirit and glycerine should then be slightly acidulated with acetic acid.

If it be preferred to prepare the brain without the use of spirit, the second step in the process may be omitted.

The great advantage of Giacomini's method is that the brain practically does not shrink at all. With a certain firmness, it is also to some extent soft, and this permits of an examination of the sulci. The method is therefore well adapted to the study of the organ. Its only drawback is the stickiness produced by the treatment with glycerine. According to Bischoff, the first to use chloride of zinc for this purpose, the pia mater may be easily and cleanly removed.

*Method of Stieda.*

- (1) The brain is placed for twenty-four hours in a saturated solution of chloride of zinc.

- (2) The pia mater is removed, and the brain is transferred to 96 per cent. alcohol, which must be changed every five days.



(3) After it is sufficiently hardened (in about two or three weeks), it is transferred to turpentine, where it remains for two or four weeks. The more complete the dehydration, the more rapidly the turpentine penetrates. In this fluid the brain becomes again somewhat softer, but "transparent," and of a brownish colour.

(4) The brain is transferred to oil-varnish for two weeks, and is then allowed to dry in the air for one week on blotting paper.

This method is merely a modification of Giacomini's. It causes a cheesy consistency of the brain, and also a considerable shrinking, amounting to a fourth, or more, of the original volume. Better results would seem to be obtained by prolonging the immersion in turpentine, and shortening that in oil varnish.

A very good method, to be recommended for its simplicity, is the

*Method of Laskowski.*<sup>1</sup>

The fresh brain is placed for fifteen to twenty days in the following fluid:—

Formalin, - - - - -	2,0
Glycerini pur., - - - - -	100,0

The meninges must be previously removed. After this time the brain may be preserved either in air or in the same fluid. Laskowski at first employed carbolic acid in place of formol, in either of the two following formulæ:—Carbolic acid, 5,0; glycerine, 100,0; or glycerine, 100,0; alcohol (95 per cent.), 20,0; carbolic acid, 5,0; crystallised boracic acid, 5,0.

All these methods have the advantage that the organs, hardened by means of them, may be preserved in air.

*Method of Lenhossék.*

The brain is hardened in alcohol, chloride of zinc, or Müller's fluid, and afterwards, in any case, transferred to alcohol, to

<sup>1</sup> Laskowski: L'embaumement. La conservation des sujets et les préparations anatomiques, 1896. Prof. Laskowski was good enough to inform me privately of the use of formol in place of carbolic acid.

permit of the easier penetration of which the sulci are kept open with cotton wool. After the evaporation of the alcohol from the surface, the convolutions and the sulci are painted with a rather thin solution of celloidin. As soon as this is dry, the brain is transferred to, and preserved in, alcohol. For purposes of demonstration, it may be exposed to air for as long as two hours.

Retzius points out,<sup>1</sup> that a mixture of chromic and acetic acids with a little hyperosmic acid (in fact, a modified Flemming's fluid), is very suitable for the foetal brain, which becomes hardened soon after its injection into the umbilical vein or the aorta. Alcohol and chloride of zinc are not adapted to the foetal brain. But for embryos in the first month, a three or four per cent. solution of bichromate of potash is still better.

Besides bichromate of potash, Retzius also recommends for the brain of adults the most modern preservative, formol, although it occasionally produces superficial friable patches. The following combination, however, seems to him the most appropriate:—

Bichromate of potash, - - - - -	3—4,0
Formol, - - - - -	1,0
Distilled water, - - - - -	100,0

If this mixture be employed, the brain does not become so dark as when bichromate alone is used. The process of hardening lasts only two or three weeks. Above all, the architectural structure of the walls of the ventricles is well preserved, which is not the case when formol alone is used.

Other methods, for the most part not to be recommended, have been advocated by Flesch (glycerine), Schwalbe (paraffin), Broca (nitric acid), and Rosenbach (carbolic acid). They may be discarded in favour of those mentioned above.

The hardening action of alcohol and chromic acid will be discussed in the sequel.

THE PREPARATION OF MODELS OF PATHOLOGICAL SPECIMENS  
(P. BERLINER'S Method).

Models are prepared in the following way:—A plaster cast

<sup>1</sup> Retzius, Das Menschenhirn, 1896. Einleitung.



of the part to be reproduced, in this case the brain, is first made, and constitutes the negative form. If this cast be filled with wax, which has been liquified by heating on a waterbath, the positive form, or model, is obtained. The model is then painted *ad naturam*, in oil colours, after the original preparation. The more or less moist and glistening appearance, on the actual reproduction of which the life-likeness of the model very much depends, may be obtained by the addition of various solutions to the colours employed, or by the subsequent application of various kinds of lacquer.

## II.—HARDENING AND FIXING FLUIDS FOR THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM.

The number of methods of hardening adapted to our purpose, in contrast to those employed in general histology, is relatively small. If we disregard a few special methods (those of Nissl and Golgi), we are practically limited to bichromate of potash (Müller's and Erlitzki's fluids) and to formol (Blum). On account of its many advantages, formol has recently become so widely employed, that it appears to be at present the hardening material *κατ' ἐξοχήν*, and may therefore first be mentioned.

It is, however, a rule of primary importance that the brain, spinal cord, and nerves must be immersed in the fixing or hardening fluid immediately after their removal from the body, without coming into contact with water. Failure to obtain good results from staining, especially with the more complicated methods, is often due to a neglect of this precaution.

The first to use hardening fluids, for the purpose of obtaining delicate sections of the spinal cord, was Keuffel (1810)<sup>1</sup>, who even then employed perchloride solutions and dilute nitric acid. In the thirties, L. Jacobsohn discovered the hardening property of chromic acid, while Hannover first turned it to histological use. Bichromate of potash, however, was first used by Heinrich Müller, although the formula for

<sup>1</sup>Weigert; *Technik*. Merkel-Bonnet, 1895, vol. V.

the classic Müller's fluid is not to be found in his printed works (Weigert, *loc. cit.*) This salt has displaced chromic acid, which does not easily penetrate and makes the organs very brittle.

*Formol*<sup>1</sup> is a 40 per cent. watery solution of *formaldehyde* (a gas the formula of which is HCOH). At first introduced for purposes of disinfection, it was used by the elder Blum for macroscopic, and by his son for microscopic purposes. It is a clear fluid, possessing a characteristic penetrating smell. Its reaction is neutral or feebly acid.

Almost the only strength of formol employed for the nervous system is a 10 per cent. solution. According to Gerota, 40 to 50 per cent. solutions cause destruction and necrosis of the cells of all other organs except the nervous system. Formol has not the dehydrating properties of alcohol. It rapidly fixes the various parts of an organ in their original form, and imparts to them an elastic hardness, somewhat like that of gutta percha. In a 10 per cent. solution the weight of the organ becomes somewhat increased,<sup>2</sup> the colour is less altered than by other fixing fluids, and the pieces of tissue are saturated more rapidly than in alcohol, perchloride, &c.

One of its greatest advantages, however, is this, that after hardening in formol any one of the more important methods of staining may be employed, that of Nissl or that of Golgi; and methods adapted to the medullary sheaths and neuroglia, like that of Marchi—an advantage which, in association with the more rapid hardening, has probably contributed more than any other to the general acceptance of this reagent.

Besides the aqueous solutions, varying from 2 to 10 per cent., a 2 to 4 per cent. solution in 85 per cent. alcohol may also be employed. A combination of formol with Müller's fluid is recommended by Orth. It may also be combined with a simple solution of bichromate of potash. Orth's mixture, as it is called, is known in Germany as Formol-Müller, or briefly F.M., and consists of—

<sup>1</sup>The formol of the Hoechster Farbwerke is identical with the formalin of Schering of Berlin.

<sup>2</sup>Cf. chap. III.



Bichromate of potash, - - - -	2,5
Sulphate of soda, - - - -	1,0
Formol, - - - -	10,0
Distilled water, - - - -	100,0

When this fluid has stood for four days, a crystalline precipitate falls, the appearance of which indicates that the combination has lost its special effectiveness. The fluid must therefore always be freshly prepared, and, should its hardening effect not be sufficient, it must be soon replaced by a fresh supply. By this method, says Orth, small pieces of tissue (0.5 cm.) are fixed after only three hours in the incubator. Their consistency, for the purpose of section cutting, is admirable, and the more they have been dehydrated, the better they take on stains. The carmine stains are the most appropriate.

Among the numerous combinations and variations which formol has undergone, that of A. Marina, quite recently made public, is deserving of mention. Its object is to permit of staining the same pieces of the central nervous system either by Weigert's or Nissl's method. With this combination the author has also successfully employed the methods of Held and von Gieson.

In Marina's process portions of the brain, or the whole of it, are immersed in the fluid, which consists of alcohol (90 per cent.), 100 ccm., formol 5 ccm., chromic acid 0.1 ccm. Next day the pieces are cut up, and afterwards replaced in a quantity of the same fluid, freshly prepared. The fluid is changed every day for three days or at most five. The pieces are then transferred direct to wood or cork, and preserved in 90 per cent. alcohol, or in a 1 per cent. alcoholic solution of chromic acid, the alcoholic strength of which is 90 per cent. In cutting sections, the knife is moistened with 90 per cent. alcohol. Sections to be stained by Nissl's method or with thionine are kept in 96 per cent. alcohol, those intended for the neuroglia stain in chromogen solution, others in a 3 per cent. solution of bichromate of potash, with or without two or three drops of ammonia. The alcoholic solution of chromic acid may be kept in stock, but the formol should only be added as it is required.

*Müller's Fluid*<sup>1</sup> consists of—

Bichromate of potash, - - - -	2,5
Sulphate of soda, - - - -	1,0
Distilled water, - - - -	100,0

Many have of late preferred to replace the original Müller's fluid by a solution of bichromate of potash alone, the best strength being four or five per cent. The development of fungi—which does not necessarily indicate that the preparation is spoiled—may be prevented, although not invariably, by the addition of a little camphor or carbolic acid.

*Erlitzki's Fluid* consists of—

Bichromate of potash, - - - -	2,5
Sulphate of copper, - - - -	0,5
Distilled water, - - - -	100,0

This fluid, therefore, which was rescued by Weigert from neglect, differs from Müller's only in the substitution for the sulphate of soda of half the quantity of sulphate of copper. Its advantages consist in the rapidity of the hardening process, which is completed, in the incubator, in about five days, and at the ordinary temperature of a room in about ten. This advantage, however, carries with it the drawback that the tissues become too much shrunken.

It is best to prepare the necessary quantity afresh for each new preparation, and to renew the fluid every second day. The hardened pieces are then placed in alcohol of increasing concentration (70 per cent., 80 per cent., 90 per cent.), remaining for twenty-four hours in each of these fluids. The deposits, which are often to be seen in the sections, can for the most part be at least partially removed by washing in warm water, or water slightly acidulated with hydrochloric acid. Or, after hardening, and before the pieces are placed in alcohol, they may be treated with a 0.5 per cent. solution of chromic acid.

*Alcohol*, as has already been said, should not be employed for the hardening or fixing of the central nervous system, except when Nissl's method for the staining of nerve cells, or v. Lenhossék's thionine stain, which shows their structure, is to be

<sup>1</sup> First mentioned by Heinrich Müller, Würzburg (1859).



used. It is of course invariably employed in the after treatment of pieces of tissue, in whatever fluid they may have been hardened. If entire pieces are to be stained in carmine, it is used only after they have been stained. Among its disadvantages are the greater shrinking which the tissues undergo, and the cost as compared with bichromate of potash.

Alcohol is generally employed as absolute alcohol (99.8 per cent.), or as 96 per cent. alcohol, as it is usually sold. It is easy to obtain absolute from 96 per cent. alcohol by dehydration by means of copper sulphate which has been burnt white, and equally easy, by admixture with water, to obtain alcohol of less strength. Although it is of comparatively little importance to use instead, for example, of 60 per cent. alcohol, a fluid of slightly lower or higher percentage, Stöhr's formula for the alteration of the percentage may be given here. If  $p$ . represent the desired percentage, then

$$100 : 96 = x : p,$$

and if 90 per cent. alcohol be required—

$$100 : 96 = x : 90$$

$$96 \times \quad = 90 \cdot 100$$

$$x = \frac{9000}{96} = 93.7, \text{ or in round numbers, } 94.$$

In order to obtain 100 ccm. of 90 per cent. alcohol, therefore, 6 ccm. of water must be added to 94 ccm. of 96 per cent. alcohol.

In his "Technik" Mercier has calculated out a complete table for the difference percentages of alcohol, which, however, need not be given here.

Hardening in *corrosive sublimate* is another process not often employed. The following is the commonest solution:—

Corrosive sublimate, - - - 7.5.

Normal salt solution, - - - 100.0.

When this fluid has been boiled, the pieces of tissue are immersed in it, in the dark, for twenty-four hours, then thoroughly washed, and further hardened in alcohol of increasing concentration. The deposits, which come down in quantity, are

only partially removable by washing. To remove them, Bolles-Lee recommended the employment of Lugol's solution (iodine, 4.0, iodide of potash, 6.0, water, 100.0). But the results of staining are not always satisfactory; haematoxylin, carmine, &c., give too diffuse a stain. Overstaining with one of the aniline colours and subsequent differentiation in alcohol have been specially recommended for the demonstration of the cells (Goodall employed chiefly toluidine-blue).

*Zenker's solution*—

Corrosive sublimate,	- - -	5.0
Bichromate of potash,	- - -	2.5
Sulphate of soda,	- - -	1.0
Distilled water,	- - -	100.0
Glacial acetic acid,	- - -	5.0

This fluid consists, as will be seen, essentially of Müller's fluid and corrosive sublimate. The glacial acetic acid is added only shortly before use.

After fixation of the tissues, which occupies about fourteen days, they are washed in water, and hardened in iodised alcohol (alcohol 70 per cent., to which enough tincture of iodine is added to produce a port-wine colour), which must be frequently changed. By this process the sublimate is extracted, and the iodine must afterwards be similarly extracted from the tissues by placing them in 80 per cent. alcohol. The action of the acetic acid is to prevent the shrinkage and brittleness which follow the use of sublimate, while the iodised alcohol obviates the disadvantage that sublimate forms insoluble compounds with the albuminates, thus producing crystallisation in the interior of the tissues.

*Flemming's chromo-acetico-osmic acid*—

Solution of osmic acid, 2 per cent.,	-	4.0
Aqueous solution of chromic acid,		
1 per cent.,	- - -	15.0
Glacial acetic acid,	- - -	1.0

The pieces of tissue remain in this fluid from one to three days. They are then washed for several hours in running water, and subsequently hardened in alcohol of increasing



concentration. Flemming's solution is employed mainly to demonstrate the process of nuclear division.

*Friedmann's modification* of it deserves commendation—

Solution of osmic acid, 1 per cent.,	-	-	0,5
Solution of chromic acid, 1 per cent.,	-	-	7,0
Glacial acetic acid,	-	-	0,3

When this method is employed, the ground of the sections is somewhat less dark. The pieces are left in the solution up to twenty-four hours, and are then washed, and hardened in alcohol of increasing concentration. It is stated that sections stain better if the pieces have remained some time in alcohol.

*Fol's modification* of Flemming's solution—

Hyperosmic acid, 1 per cent.,	-	-	2,0
Chromic acid, 1 per cent.,	-	-	25,0
Acetic acid, 2 per cent.,	-	-	8,0
Water,	-	-	68,0

This combination has been recommended for cases in which it is desired to investigate, in its finest detail, the structure of portions of tissue obtained from the living or surviving nervous system. Should any cloudiness appear, the solution must be renewed. The preparation is usually hardened in twenty-four hours. After thorough washing, it is transferred to 80 per cent. alcohol.

*Osmic acid* (introduced by Max Schultze) is employed in 1 per cent. solution. The pieces of tissue to be hardened are immersed in it, and kept in the dark. Particularly small pieces should be chosen, as the acid has little penetrative power. They are allowed to remain in it from one to five days, then washed and transferred to alcohol.

Except in Exner's method for staining the medullary sheaths, osmic acid is little used for hardening purposes. It is of value chiefly in connection with the methods of Marchi and of Golgi and Cajal, but even in the latter it has no longer the importance at first ascribed to it. Its action upon the medullary sheath depends on a reduction of the hyperosmic acid to metallic osmium. It should be noted that fat globules take on an intensely black stain in osmic acid.

Ranvier was the first to use the vapour of the acid instead of the acid itself. This proceeding is to be recommended for tissues such, for example, as the retina.

*Rabl's fluid*—

Chromic acid solution, 0.3 per cent.,	-	200,0
Concentrated formic acid,	-	4.5 drops

The pieces are left in this from twelve to twenty-four hours, washed, and subsequently hardened in alcohol of increasing concentration.

*Merkel's fluid*—

Chromic acid solution,	-	-	} aa 1 in 400
Solution of chloride of platinum,	-	-	

The pieces are left from four to six days, and then transferred direct to alcohol of increasing concentration, in which they are hardened.

*Benda's fluid*—

(a) Nitric acid,	-	-	10,0
Distilled water,	-	-	90,0
(b) Solution of bichromate of potash,	-	-	1 part
Distilled water,	-	-	3 parts

The pieces are left for twenty-four hours in solution (a), and transferred, without washing, direct into solution (b). This is gradually made more concentrated, until it contains equal proportions of its two constituents. After about fourteen days the pieces are washed, and then hardened in alcohol of increasing concentration. This method is not adapted for embryonic tissues.

*Nitric acid.*—The pieces are left in a 10 per cent. solution from one to three hours, washed, and transferred to alcohol.

It is essential that all material used for the purpose of making stained preparations should be as fresh as possible. As a matter of practice, in the central nervous system of man there are always certain limitations in this regard. If we neglect experimental research in animals—from the data of which, very often, only partial influences can be drawn with



regard to man—we have to deal chiefly with nervous substance which, in the course of a number of hours after death, has already undergone certain changes. If we have to do only with old and chronic degenerative processes, or with processes no longer active—as in tabes and syringomelia, for example—this difference of hours is almost negligible. But a defect in the freshness of the material may become of the greatest import, if we desire, as with Nissl's method, to study the finer processes occurring in the cells.

It must, in fact, always be kept in mind that it is often impossible to say what the pathological process really is in this or that disease. It must always be kept in mind that—except in processes no longer active, which do not undergo alteration—it can really only be said that, so many hours after death, such and such changes were found as the result of examining stained sections. It is not always allowable to draw the conclusion that the condition was exactly similar in the living organ.

*Nissl's* method, which demands the freshest possible material, has also proved the first to open out the prospect that we may, in the future, understand the finer pathological processes.

In every case, therefore, with the exception demanded by Marchi's method in experimental investigations, the central nervous system, or its parts, must be placed as early and in as fresh a state as possible in the fixing or hardening fluid. *Any washing with water must be rigorously avoided.*

The fluids must always have several times the volume of the organ to be immersed in them, and, especially at first, must be frequently changed. A layer of cotton wool should occupy the bottom of the vessel. The constant endeavour of modern technique is towards refinement of the methods employed. Having regard to staining at a later stage, it is therefore necessary that the pieces of tissue shall be cut as small as possible, to further the more rapid, complete, and uniform penetration of the fluids. In the case of larger pieces, the connection of which it is desirable to preserve, incisions into the organ may

at least be made, and their number may be gradually increased during the first day of hardening. That their effect should not be entirely illusory, the separation of the parts may be maintained by the insertion of small pieces of cotton wool.

As in the case of the later process of staining, a considerable acceleration of the fixing and hardening action of the fluids may be obtained by means of warmth. The temperature, however, must not exceed 30° C. At the temperature of an ordinary room, the requisite degree of hardness is attained, in the case of a cerebral hemisphere, in about three months; in the case of the spinal cord, in about six weeks, if Müller's fluid or a 2 to 5 per cent. solution of bichromate of potash be employed. At 30° C. the result is attained in about a fourth of this time. It may be mentioned here that, in Weigert's method for staining the medullary sheaths, he has succeeded in reducing the hardening period to four or five days (see section on staining of the medullary sheaths).

After hardening in bichromate of potash, or formol and bichromate, has been completed, the pieces may, without washing, be transferred to alcohol. To prevent the formation of precipitates of bichromate, light, according to H. Virchow, must be excluded. Only those pieces of tissue, which it is intended to stain exclusively by means of carmine, may be left in the water after hardening, for carmine does not give good results with alcoholic preparations. The method of staining in bulk by means of an appropriate preparation of carmine, especially soda-carmine, may here be noted.

Mention must also be made, in connection with most of the varieties of staining, of a process which, simple as it is, deserves the widest observation and use. It is the process of the "double method," which Ramon y Cajal was the first to recommend in connection with Golgi's mode of staining, and which may be employed with great advantage in the other classic methods, such as Weigert's for the medullary sheaths and neuroglia, and Nissl's for nerve cells. The process of staining and differentiation should be twice or thrice repeated, until good results are obtained. The slight trouble is amply rewarded.



## EXAMINATION OF FRESH UNSTAINED SPECIMENS.

At the present date the nervous system is examined only occasionally before its tissues have been hardened and stained. The modern neuropathologist seeks to elucidate conditions which are imperfectly demonstrated, or not demonstrated at all, by the simple methods of teasing, maceration, and separation. But for the few cases in which staining may not be desired, or in which more primitive methods of examination may be preferred, the method of teasing has in the first place to be considered. With the help of scissors and forceps, a small piece of tissue is removed, and teased by means of needles on the slide. This should be done in normal salt solution. Should it prove too difficult to tease out the smallest constituents of the tissue, fluids adapted to its maceration or separation may be employed. They accomplish the required result in twenty-four hours. Only small quantities of fluid should be used.

Among those much in use the following may be mentioned:—

1. 33 per cent. alcohol (Rauvier).
2. Weak solutions of chromic acid (0.01 to 0.03 per cent.)
3. Müller's fluid.
4. 0.1 per cent. osmic acid.

The following re-agents are useful both in increasing the transparency of the preparations and in the clearer demonstration of some of their constituent parts:—

1. Glycerine, which should be diluted with an equal quantity of water, and may be used for hardened as well as for unhardened specimens.
2. Acetate of potash, in saturated aqueous solution (50 per cent.).
3. Acetic acid, in 1 or 2 per cent. solution.

Even fresh sections may be stained, if a drop of the staining fluid (methylene blue, methyl green, &c.) be allowed to flow from the margin towards the centre of the section. Haematoxylin, however, is not adapted to this purpose.

*Labelling the Tissues.*

Whatever number of jars one may possess, it is absolutely

necessary to label each piece of tissue before its immersion in the first solution employed. This may be done very simply, by marking the upper or under side, as one prefers, on the right and left, with a fine inserted bristle or with ink. If, at the same time, several pieces of tissue be dealt with, there may be drawn through the corner of each a thread, which carries a small piece of pasteboard with the designation of the tissue.

Particularly in the case of the spinal cord, the segment dealt with should at once be accurately labelled.<sup>1</sup> Pieces of the cortex may be marked by the number which they bear in Exner's plates.

The pieces, with their designation, are placed in the hardening fluid, in alcohol, in celloidin, and so on. When they are afterwards fixed on cork the label may be fixed along with them. To mark out the blocks of wood or cork, on which they are fixed, the so-called negro pencil<sup>2</sup> (No. 1 or 2), may be strongly recommended. It produces quite black and distinct characters, which resist the action of alcohol, but may be easily removed by sharp friction. The blocks may thus be used for new preparations, and fresh descriptions may be written upon them to any extent.

On the slide, sections are still marked, chiefly by means of labels, or by a coloured pencil, and occasionally by a diamond. None of these methods, however, is quite satisfactory. It would be better, if it is preferred to write in black, to use Chinese ink, or Krems white, where white is preferred. Schiefferdecker recommends Soennecken's drawing-pen, No. 144, and Schœbel a water-glass ink, *i.e.*, a combination of soluble glass and liquid China ink.

## EMBEDDING.

*The Celloidin Method.*<sup>3</sup>

Two solutions of celloidin must be kept in stock, the one thin, the other thickened to the consistence of syrup. The

<sup>1</sup> Even at the autopsy, it is advisable to indicate, by means of a thread, the position of the last dorsal root.

<sup>2</sup> A German pencil.

<sup>3</sup> First employed by Schiefferdecker.



celloidin is dissolved in equal parts of ether and absolute alcohol.

The piece to be embedded, after it has been dehydrated in alcohol (60-90 per cent.), is transferred for a day to absolute alcohol,<sup>1</sup> and then for a day to alcohol and ether. It is then placed for several days in the thinner, and afterwards for several days in the thicker solution. The longer the process lasts the more successful is the embedding. The celloidin, with the preparation, may be allowed to become solid in the vessel. A cube containing the preparation, and of any size that may be preferred, is then cut out, and fastened upon cork or wood by means of celloidin. Or the saturated preparation may be removed from the celloidin while it is still fluid, and fastened upon cork, the solution being freely poured over it. It is best to allow the piece to dry under a bell glass, *as slowly as possible*. Preparations made in this way become much firmer. The process of drying may even be extended over several days, when a consistence approaching that of paraffin is attained. The pieces are then kept in 80 per cent. alcohol, until they are cut. This hardens the celloidin. Instead of alcohol, pure chloroform may be used for this purpose, in which case the celloidin remains more transparent.

Absolute alcohol and oil of cloves dissolve out the celloidin from the sections. If this result is to be avoided, 96 per cent. alcohol, carbol-xylol, or some other oil, must be employed. On the other hand, certain aniline stains require the removal of the celloidin from the sections. In this case absolute alcohol and ether-alcohol, or oil of cloves, must be employed. The sections are stained only after they have again passed through absolute alcohol and water.

The entire process, then, is carried out as follows:—

1. Hardening in absolute alcohol.
2. Immersion in thin celloidin, then thick celloidin (one to five days in each for small pieces, several weeks or months for a cerebral hemisphere).

<sup>1</sup> Larger pieces require several days.

3. Placing the pieces on cork, drying, and preserving them in 80 per cent. alcohol.

4. Cutting sections, staining, washing, dehydration in 96 per cent. alcohol, and, if necessary, afterwards in absolute alcohol.

5. Treatment with oil or carbol-xylol, and mounting in balsam. Photoxylol has recently come much into use in place of celloidin, to which it is chemically nearly related. It possesses the advantage of greater transparency. It is very rapidly soluble in ether-alcohol, and is employed exactly in the same way as celloidin.

#### *The Paraffin Method.*

After complete dehydration in absolute alcohol (eight to twenty-four hours), the piece to be embedded, which should be as small as possible, is placed for a day in a bottle containing xylol. Turpentine, chloroform, or cedar oil may also be used. It is best to drop little pieces of paraffin direct into the bottle, until the solution is concentrated. The piece of tissue is then transferred to melted paraffin at about 50° C., in which it is kept on the thermostat for from two to twenty-four hours. It is advisable to change the paraffin once or twice. It is important, in order to obtain very thin sections, that the melting point of the paraffin should lie within certain limits. Two kinds, each with a different melting point (the higher from 55° to 60° C., the lower from 45° to 48°), should be kept in stock. These are mixed with one another in different proportions, with the object of employing in summer paraffin of a harder, in winter paraffin of a softer, consistence. The melting points of the two mixtures should be from 52° to 54° C., and from 48° to 50° C.

After thorough penetration, the fluid, along with the piece of tissue, is to be poured into a low-edged vessel. When the preparation has been located in any position that may be desired, the mass must be quickly solidified by pouring cold water over it. The opaque block, which may be of any size required, is cut out with a warm knife. It is then fixed in the clamp of the microtome, and cut by short strokes of the



knife, which must be placed obliquely, and not moistened. The sections can often be prevented from rolling up, by pressing them against the knife, during the act of cutting, with a brush or spatula. They are next placed in xylol, to dissolve out the paraffin, then in carbol-xylol, and then are mounted in dammar. If, however, they have yet to be stained, they are transferred from the xylol to absolute alcohol, and then to the staining fluid. The staining may also be previously done in bulk.

The paraffin method is little used for the central nervous system, except in certain processes, such as that of Held, and to obtain very thin sections. The prolonged heating at 50° C., the removal of the paraffin from the section, and the defective fixation of the more delicate tissue elements, are great disadvantages, especially as previous staining in bulk is for the most part inapplicable, and the after-staining of the brittle sections, even on the slide, is often difficult. Large sections, too, cannot be prepared by this method.

Frankl has recently constructed an apparatus designed to facilitate the process of paraffin embedding. It consists of a square plate of clear glass, 15 cm. in length, on which are laid four similar pentahedral blocks of glass, 1 cm. high, the upper and under surfaces of which are ground. Their clear, lateral surfaces are respectively 35, 30, 22, 19, and 14 mm. in length. By placing these blocks together five squares may be formed, varying in the length of their sides, and always leaving a cavity between. The melted paraffin is quickly poured into this cavity, the object placed in the desired position, and covered, if necessary, with a further supply of paraffin. In this way, fissuring of the block of paraffin is said to be entirely avoided.

The combined *celloidin and paraffin method* need be only briefly referred to. The process is as follows:—

1. The object is placed for some hours in ether-alcohol.
2. It is left in a moderately thick solution of celloidin till this has thoroughly penetrated (twenty-four hours).
3. It is soaked in oil of origanum, and then transferred to a mixture of oil of origanum and paraffin, which must not be heated to more than 40° C.
4. It is transferred to melted paraffin. The further steps are the same as in paraffin embedding.

Difficulties are now and then encountered in fixing paraffin sections on the slide, hence the multiplicity of methods which have been suggested. The most useful are those of Gulland, who employs water; Rabl, who employs a mixture of oil of cloves and collodion; Strasser (a mixture of collodion and castor oil), and Heidenhain. The essential point of the last is that, to avoid shrinkage, the water on the slide is allowed slowly to evaporate, at a temperature of not more than 35° C.

A process has been recently recommended by Albrecht and Stork which is said to obviate various inconveniences. It proves, also, to be a transformation of paraffin sections into celloidin sections. The process is as follows:—

1. A drop of water is spread over a slide which has been breathed upon. The section is then laid on the slide.
2. The section is pressed down with thin filter paper folded several times, on which about five drops of absolute alcohol have previously been allowed to fall.
3. The paraffin is dissolved out by xylol, and this is displaced by absolute alcohol.
4. A few drops of a quite thin solution of celloidin are poured over the section, the slide being held obliquely.
5. After treatment with 95 per cent. alcohol, the preparation is transferred to water. It may hereafter be dealt with in any manner that may be desired.

If those constituents of the tissue, which are soluble in alcohol and ether, are to be preserved, embedding in celloidin or paraffin is undesirable. If it is necessary, in such circumstances, to obtain thin sections, the process proposed by Döllken—embedding in a soda soap—may be employed. A 20 to 30 per cent. solution of caustic soda is heated to boiling point, and, while still boiling, as much castor oil is added as will leave a small excess of caustic soda. The solution must be allowed to boil for a little longer, and afterwards to cool and solidify. The excess of caustic soda solution must be pressed out of the resulting soap mass. For the purpose of embedding, Döllken uses a three to five per cent. solution of the soap, at a temperature of 35 to 40° C. Into this the pieces of tissue are to be transferred from formol or from Müller's fluid, with or without washing. They must be left in it, at the same temperature, from thirty-six to seventy-two hours. They must then evaporate until they become solid. The blocks, which



must be cut of a considerable size, are fastened upon wood by means of soluble glass. After drying, they must be cut with a dry knife. When transferred to water, the sections unfold themselves, and become flat. They must be thoroughly washed in water before they are stained.

Hardening and fixing are even more rapidly accomplished by acetone than by formol. Serial sections, 5 to 10  $\mu$  thick, may, in this way, be easily obtained.

#### *The Freezing Method.*

The piece of tissue must be as thin as possible (less than 5 mm.), to permit of the uniform action of the cold. It is placed in the freezing microtome, upon a metal plate, upon the lower surface of which an ether spray is directed. That the tissue may freeze hard enough for purposes of cutting, it must previously have been thoroughly soaked in water, and this purpose must be aided by the pressure of a spatula or the handle of a scalpel. If it has been previously hardened in spirit, or embedded in celloidin, it should remain in water for a whole night. If bichromate of potash had been used, a short soaking would be sufficient; and it could scarcely be short enough, if Weigert's stain for the medullary sheaths were afterwards to be employed.

Hamilton has recommended a special process for the embedding of tissues in celloidin, and their subsequent freezing—

1. The hardened piece of tissue is placed for three or four days in alcohol and ether-alcohol.
2. It is embedded in celloidin (four days).
3. The block of celloidin, containing the piece of brain, is transferred for one day to water, then for several to fluid A or B. Afterwards it is frozen and cut.

He recommends the two following as freezing fluids. Their employment involves no injury to the tissues:—

1. A syrup, composed of 28½ gm. pure sugar and 30 gm. water, which is to be saturated with boracic acid while it boils, and filtered after cooling.

2. A mucilage, composed of 456 gm. gum arabic, and 2400 gm. water, saturated with boracic acid and filtered as above.

Fluid A. Syrup, 4 parts; mucilage, 5 parts; water, 9 parts. This is to be boiled, saturated with boracic acid, and filtered after cooling.

Fluid B. 2 parts of fluid A; 1 part of syrup.

Fluid C. 4 parts of syrup; 5 of mucilage.

In the freezing process, A produces the greatest hardening, C the least. The pieces must remain in the fluid for at least a week.

No great practical advantage can be attributed to this method, as it is prolonged in operation, and as, in general terms, the freezing method may, for our purposes, be dispensed with.

#### MICROTOMES.

The instruments which are necessary for the purpose of section cutting are the microtome and the knife. It may be taken for granted that the principles, the structure, the technique, and the action of microtomes are known to readers of this book. I limit myself, therefore, to the statement that for large sections of the brain, the microtome made by Becker of Göttingen, and Gudden's microtome, made by Katsch of Munich, are the best adapted. For small sections, and for those of moderate size, the microtomes of Schanze of Leipsic, and of Miede of Hildesheim, are the best. For paraffin preparations, that of Jung, of Heidelberg, may be recommended.

The double knife, advocated by Valentin for the preparation of fresh sections, possesses only a historical interest. In the special province of neurology, it has become superfluous, owing to the introduction of the freezing microtome, and the very different acquirements of modern histologists.

The knife employed in section cutting cannot be too carefully made or guarded. If celloidin preparations are used, one must take care to cut with a gentle and continuous stroke, without pressure and without intermission.

#### WEIGERT'S METHOD FOR SERIAL SECTIONS.

In many cases, especially where ascending and descending degenerations of the cord have to be accurately traced, it is necessary to prepare an uninterrupted series of sections. That good results may be obtained, the embedding must be perfect and the knife in perfect order.



In spite of its simplicity, Weigert's method of preparing these sections is very well adapted to the purpose. Collodion is poured over one or more glass plates. The sections are removed from the knife on a strip of toilet paper, somewhat broader than the sections, in such a way that each successive section lies to the right of its predecessor. The strips are numbered and placed upon a plate, the surface of which is covered with several layers of blotting paper moistened with 80 per cent. alcohol. The sections should lie facing upwards. One or two such strips of sections are then laid upon the glass plate or the slide, the strip of curl paper, with the sections facing downwards, being placed upon the layer of collodion. The paper may now be easily removed, leaving the sections adhering to the collodion. A second layer of collodion is then quickly poured over them. One must not omit to number them by means of methyl-blue. The glass plate may now either be kept in 80 per cent. alcohol or transferred to the staining fluid, in which, especially if in the incubator, the mass of collodion soon separates *in toto* from the glass. The further treatment of the sections is carried out in the usual way.

Darkschewitsch has recommended, as the simplest method of preserving serial sections, that each section be separately removed from the knife on a numbered piece of blotting paper or toilet paper of appropriate size. The paper must previously have been well soaked in alcohol. The separate pieces, with the sections adhering to them, are then laid one upon the other in the order of numeration, and may be kept in alcohol as long as is desired in a glass jar of appropriate dimensions. The further steps of staining, and so on, are carried out without removing the sections from the paper.

Lissauer has recommended the following process for the preparation of large sections of the brain:—

1. Embedding in soft paraffin.
2. Cutting. The cut surface, *before* each section is made, must be covered with a thin layer of celloidin, or—and this is better—a strip of tissue paper, covered with an extremely thin

layer of a thickish solution of dextrin, must be stuck flat on the surface.

3. The section, which adheres to the paper, is fastened still more firmly by dipping it into celloidin, and goes through the further processes along with the paper. Once it has been fixed upon the slide, the paper may easily be dissolved off.

The preparation of complete sections through the whole human brain is generally very difficult, but satisfactory results may be obtained with the microtomes of Gudden and C. Reichert. In both cases the section is made under water. The process, as described by Pal, may here be given, especially as such sections are employed for purposes of demonstration.

The brain is injected with Müller's fluid, to which a quarter of its volume of a 5 per cent. solution of lysol has been added, and is then kept in the dark in the same fluid (bichromate-lysol) at the temperature of an ordinary room.

Each thinly cut piece is dried with blotting paper, and transferred (without dehydration), after it has remained a short time in absolute alcohol, to photoxylin. It is then stuck on to a roughened metal plate, which is fastened on a small piece of wood fitting the clamp of the microtome. Wooden plates are too apt to warp during the number of days required for the completion of the series. According to Pal, a section of the entire brain, which should not be thinner than 50  $\mu$ , may be cut in ten to fifteen seconds. The section is allowed to fall into water, and is taken up on toilet paper. To permit of good staining, it is placed upon a plate, the surface of which is covered with a mixture of dextrin and sugar candy. It adheres completely, when the paper is removed, to the layer of sugar on the plate. The preparation is dried, and a thin layer of photoxylin is poured uniformly over it. When this is dry, a roller is passed over it, and the plate is then put in water, in which the layer of dextrin dissolves. The preparation, with the adherent layer of photoxylin, falls off. As one of its surfaces is free, the section may easily be stained and differentiated. Finally, dehydration and cleaning are performed upon the slide, and the preparation is covered with a thin glass.



Generally speaking, the so-called "collodionage des surfaces," recommended by Duval, should be employed for all preparations of which it is desired to obtain large and thin sections, especially if the pieces crumble readily, or are apt to split up. Each freshly cut surface must be dried by blowing upon it, and covered with a very thin layer of collodion, to which the thin section afterwards adheres.

Finally, it may be mentioned here, that, especially in the case of large sections of the brain, one may often find it useful to replace the expensive cover glasses by plates of mica. The only objection to mica, that it is easily damaged, is not, in these cases, of great importance, for the finer details of cells or fibres are not usually looked to in the examination or demonstration of large sections. And, if one divides the mica with a fine scalpel, under flowing water, one can at any time procure, easily and cheaply, very thin plates, as large as may be desired.

#### SERIAL LONGITUDINAL SECTIONS THROUGH THE WHOLE SPINAL CORD (E. Flatau).

##### *Marchi's Method being Employed.*

In view of the great importance of experimental investigation of the secondary degenerations which follow upon vertical or cross sections of the cord, or of portions of the cord, it is important accurately to trace the degeneration, both of individual fibres and of entire tracts. Marchi's method has, of recent times, rightly been that chiefly, almost exclusively, employed for such investigations. It allows us to distinguish not only the localised but also the more diffuse degeneration of the medullary fibres. If, in such investigations, transverse sections were alone employed, it would be easy to fall into error, and to consider a diffuse degeneration to be present where it did not really exist.

Thus, if Marchi's method be employed, there are always to be found in transverse sections of the normal central nervous system, whether of man or animals, somewhat numerous,

scattered, black points, which are, for the most part, small and rounded, but may also present larger, irregular forms.

In the present position of our knowledge no reliable explanation of this can be offered. It is at all events of import for the experimental investigation of the spinal cord after operation that, in using Marchi's method for longitudinal sections of the cord, the characteristic degenerated fibres, and the chain-like arrangement of the myeline droplets, are, broadly speaking, absent. It is therefore desirable, in experimental work, that the investigation should not be limited to the preparation of transverse sections, but that serial longitudinal sections should also be made.

If, however, the spinal cord be divided into separate segments, and these be cut into a series of longitudinal sections, it is difficult to establish a continuous connection between the degenerated fibres, and especially between the single scattered fibres of different segments. For this reason, in investigating secondary degenerations in dogs, E. Flatau prepared uninterrupted longitudinal serial sections, extending from the medulla to the cauda equina. He used the following method:—

The spinal cord of the animal operated upon was removed entire two or three weeks after the operation. A weight (such as a piece of glass rod) was suspended from the cauda equina, to prevent the otherwise unavoidable curvature of the cord. Two threads, placed opposite to each other, were drawn through the dura mater of the upper part of the cord (the lower end of the medulla, or the upper end of the cervical cord). The cord was then suspended in Müller's fluid, in a glass cylinder about 40 cm. high, and 3 to 4 cm. in breadth. Sometimes it was suspended for a day in a 10 per cent. solution of formol before being placed in Müller's fluid. The threads were laid over the edge of the cylinder, and kept in position by a weighted glass cover, so that the cord hung free in the fluid, and in the middle of the cylinder.

After twenty-four hours, the dura mater was cut through along the anterior and posterior surface of the cord, which was again immersed in the fluid. It remained there about two



or three weeks and was then taken out. The threads were fastened to a fixed support, and the cord was allowed to swing free in the air. It was split with Gräfe's fine cataract knife, longitudinally and in the middle line (anterior longitudinal sulcus and posterior longitudinal septum). It may be mentioned that this manipulation should be performed with the help of an assistant, the one operator devoting his attention to the anterior sulcus, the other to the posterior septum.

The object of splitting the cord in this manner is to facilitate the penetration of Marchi's fluid. The lowest part of the conus medullaris, in the cauda equina, is not split, in order that the connection of its parts may be there preserved, and that both halves may afterwards be easily rejoined.

The cord is now again suspended in the cylinder, filled this time with Marchi's fluid. It ought to be kept in a warm place, but must not be overheated. As in the treatment of the brain, it is advisable gradually to increase the concentration of the fluid in regard to osmic acid. The fluid is changed at first frequently, then less often. The smell of osmic acid must always be distinct.

According to the size of the animal operated on, the cord must remain in the fluid for a period of from three to five weeks. The further manipulations are to be carried out in the same cylinder, from which the preparation must not be removed. These manipulations are as follows:—washing for twenty-four hours in running water, immersion in alcohol, and embedding in celloidin.

When the cord is completely saturated with celloidin, it is removed, and fastened on a block of wood which has been specially prepared for the purpose. The large microtome of Becker should be used. The lower portion of the block, which is to be held by the clamp, and the plate which is to carry the cord, must be cut out of one piece of wood. The size of the former must correspond to the aperture between the teeth of the clamp, that of the latter to the length of the cord (about 35 to 40 cm. in length, and 5 cm. in breadth).

The part to be held by the clamp, which should be quadrilateral, is placed at an angle of about 45 degrees to the long axis of the stage, which is therefore not parallel to the direction of the knife, but lies at the angle mentioned. To support the preparation, a layer of solidified celloidin, of the proper length and breadth, and of a height of about 6 to 8 mm., is fixed upon the plate with collodion. The cord is then fastened upon this on its removal from the thick celloidin solution. After the preparation has completely hardened, the whole block is placed in a long glass vessel containing 80 per cent. alcohol.

If the knife be properly placed, the preparation of serial sections presents no special difficulty. The cut surface must be covered with a thin layer of collodion, as detailed above. The sections, which should be from 60 to 80  $\mu$  thick, are removed direct with the finger from the knife, and, for their further handling, transferred to alcohol and carbol-xylol. They are then placed upon slides of an appropriate length.

This method is of good service where multiple foci or pathological processes extending through the length of the cord, have to be traced and accurately localised. Hæmatomyelia, syringomyelia, multiple sclerosis, and so forth, may be mentioned as examples.

For the microscopic examination of such longitudinal sections, or other large preparations (complete sections of the brain, &c.), the apparatus recently advocated by Nebelthau is particularly adapted. Its essential principle is the free mobility of the plate carrying the preparation, and of the tube of the microscope.

#### THE OILS (CLEARING RE-AGENTS) AND RESINS.

The treatment which all stained sections must undergo after dehydration may be referred to in a few words. Dehydration itself is carried on in absolute alcohol, or, if it is desirable not to dissolve out the celloidin, in 96 per cent. alcohol. For this latter purpose Nikiforoff recommends a mixture of equal parts of chloroform and absolute alcohol.



Oil of cloves, in other ways so useful, has the property of dissolving celloidin, which is often troublesome. It also extracts the majority of the aniline dyes, so that the staining gradually becomes bad. It is, therefore, generally little suited for our purpose.

The other oils, as those of organum, bergamot, cajuput, and also turpentine, have no action on celloidin or aniline dyes, while oil of cedar acts upon the latter only. The best clearing re-agent, however, has proved to be xylol, which has, therefore, an established reputation. Complete dehydration in absolute alcohol must previously take place, and the section must not remain too long in the xylol, lest it shrink. If it is of importance to prevent solution of the celloidin, carbol-xylol is employed, according to Weigert's recommendation, in the proportion of one part of carbolic acid to three of xylol, after dehydration in 96 per cent. alcohol. Haematoxylin and carmine preparations are, by this method, completely cleared up in a few seconds. Or the section may be dried with blotting paper, after which xylol is dropped upon it. It is again dried, and xylol is dropped upon it for the second time. The sections become as clear as if absolute alcohol had been employed.

The carbol-xylol may, after filtration, be re-employed, if some burnt sulphate of copper be placed in the bottle. The latter must, so soon as it has become blue, be replaced by a fresh supply, just as in the process of preparing absolute alcohol. The following is a suitable mixture:—

Pure xylol, - - - - -	45,0
Carbolic acid, - - - - -	15,0
Burnt sulphate of copper, - - - - -	40,0

For staining with aniline dyes, aniline oil-xylol, in the same proportion of one to three, is used instead of carbol-xylol.

The further treatment of the sections, after they have been stained, is always the same. They must be completely dried by means of several layers of filter paper, mounted in dammar or Canada balsam, and covered.

To produce permanent preparations, such as the neurologist

almost exclusively works with, the resins must also be employed. Of these, for the most part, only the three following are in use—Canada balsam, dammar, and colophonium. Preservation of the sections in glycerine, as is sometimes done when Golgi's method is used, is always troublesome, and it requires many months for Venetian turpentine to become as hard as Canada balsam.

The Canada balsam must be dissolved in chloroform or xylol until a fluid of the desired consistence is obtained. Delicate stains often become paler if solutions of turpentine are employed.

Dammar is, by some, preferred to Canada balsam, as it is stated that the finer details of the preparation are more distinctly seen. It also hardens more rapidly. It is to be dissolved in equal parts of benzine and turpentine. Colophonium must be dissolved in benzine or chloroform. It is chiefly used in Nissl's method, and has the advantage that it does not become yellow in course of time.

### III.—CHANGES IN THE WEIGHT OF THE BRAIN AFTER PRESERVATION IN THE DIFFERENT MEDIA, ESPECIALLY FORMOL (After E. FLATAU).

In the macroscopic examination of the brain, and the determination of its weight, the question often arises, in how far the weight has altered during the shorter or longer time that it has remained in the different preserving fluids.

The brains of rare animals are often sent from distant countries to anatomical institutes and museums, and for various reasons their initial weight may not have been determined on the spot. Further, a brain may have been weighed *en masse*, and then preserved. If it be then divided into its several parts, the same question arises, should it be desired to determine the special weight of each. For these reasons, E. Flatau has made a study of the effect of the usual preserving fluids upon the weight of the brain.



In Donaldson's very careful work, the effect of the salts of chromic acid, and of alcohol, acting over various periods of time, has been determined with great exactitude. It is therefore only necessary here to state the effect of solutions of formol, of different percentages, and to compare it with that of alcohol and the salts of chromic acid, as given by Donaldson.

It has been shown that, in a 10 per cent. solution of formol, the weight of the human brain increases in the first month from 2 to 3 per cent., and after from five to fifteen months, has increased by only 1 per cent. of the original weight.

In a 5 per cent. solution, the weight increases in the first four days by 9 per cent.; after one month by 10 per cent.; after about five months by 7 per cent.; after about fifteen months by 6 per cent.

In a 1 per cent. solution, the weight increases in the first two days by 14 per cent.; after a month by 23 per cent.; after fifteen months, by 19 per cent.

The weight of a separate hemisphere increases in a 10 per cent. solution by 7 per cent. in the first three days; after a month by 4 per cent.; after about nineteen months by 2 per cent.; and in a 1 per cent. solution by 14 per cent. in the first three days; after a month by 20 per cent.; and after about nineteen months by 17 per cent.

In a 10 per cent. solution, the spinal cord increases in weight by 10 per cent. in the first three days; after fifty days by 14 per cent.; after nineteen months by 14 per cent.; and in a 1 per cent. solution by 11 per cent. in the first three days; after fifty days by 13 per cent.; after five months by 23 per cent.; and after nineteen months by 17 per cent.

It is evident from the foregoing, in the first place, that an inverse proportion exists between the percentage of the solution and the increase in weight. The less concentrated is the solution employed, the greater is the increase in weight.

Secondly, it is evident that the increase in weight may be represented by a curve, the beginning and the end of which (in our cases covering the space of a year and a half) are of

pretty nearly the same height, while its apex, at least in the case of the weaker solutions, is very considerably higher.

Finally, the increase in weight of the spinal cord appears to be much greater than that of the brain.

If we combine the data of Donaldson for alcohol and the salts of chromic acid with those obtained in the case of formol solutions, we may draw up the following table, showing at a glance the increase in weight at different periods of time in brains which have been removed twenty-four hours after death:—

CHANGES IN BRAIN-WEIGHT  
In the Different Preservative Fluids.

Number of days in fluid.	Loss in percentage in 96 per cent. alcohol.	Gain in percentage in bichromate solution (2½ per cent.)	Gain in percentage in formol solutions.		
			10 per cent.	5 per cent.	1 per cent.
1	- 7	-	-	+ 6	-
3	- 18	+ 21	+ 2	+ 9	+ 14
30	- 30	+ 32	+ 3	+ 10	+ 23
90	- 31	+ 32	+ 1.5	+ 9	+ 23
150	-	-	+ 1	+ 7	+ 22
450	-	-	+ 1	+ 6	+ 19
560	- 34	+ 31	-	-	-

#### IV.—THE DRAWING APPARATUS OF L. EDINGER.

This apparatus is specially designed for drawing objects under a low magnifying power. In contrast to the majority of the forms of apparatus employed, which are based upon the principle of the camera lucida, its principle is that of projection of the enlargement produced by the objective. It throws the image of an object, enlarged from two to thirty times, direct upon a sheet of drawing paper, upon which the outlines may be traced with the pencil. The enlargement is made by means of pocket-lenses. As Edinger describes it, light is thrown from a lens upon a mirror placed at an angle of 45°, whence it falls downwards upon the stand that carries the section. Below the



stand there is a lens-holder which may be moved upon a cogged wheel, and which carries several lenses. The image thrown by the lens upon the horizontal sheet of paper is sharp and clear, and perfectly reproduces the colouring.

It is thus possible to draw objects, the image of which is only very slightly enlarged. But the apparatus has also this advantage, that even after a protracted sitting the operator is not wearied. The simultaneous observation of the preparation and of the point of the pencil, required by other forms of apparatus, is done away with by objective projection.

#### THE PHOTOGRAPHY OF MACROSCOPIC PREPARATIONS.

Reference may here be made to the service which photography has hitherto rendered in the study of nervous anatomy, and to the future benefits promised by the further development of its technique. Here, too, the distinction between the photography of macroscopic and of microscopic objects must be kept in view.

To obtain a true reproduction of a fresh macroscopic object, it must be photographed in a position as nearly as possible corresponding to the natural position in the body. With this object in view, Flatau has constructed the arrangement detailed below, which permits, especially in the case of the brain, of a "vertical picture." It consists of three parts—

1. A large rounded wooden stand with a hole for the objective.
2. Two ferrule screws.
3. A metal tube, into and out of which a metal rod may be pushed or withdrawn, and fixed at any desired length by a screw.

The fresh brain is washed with water, and fixed with cement upon a black plate, in as natural a position as possible. The operator stands upon a high chair or on a table, and focusses the preparation, which should be upon a pivot-chair.

Such a vertical photograph may have a special value in the case, for example, of tumours of the brain. In no other organ is it so important to preserve the accurate relationship of the

parts as in the brain and spinal cord, where the most various lesions may be localised in a comparatively small space.

For the purposes of microphotography, the apparatus of EDINGER (or some other of the numerous and differently constructed forms of apparatus) is employed. It is superfluous here to describe this apparatus, which is sufficiently familiar to every one interested in the subject.

#### V.—METHODS OF STAINING.

The times have passed in which it was possible for the anatomist or pathologist to make important discoveries in unstained preparations, cut with a razor guided by his own hand. Since their departure further progress has been anticipated, and attained, only by the continual improvement and refinement of the methods of investigation. Thus the microtome has been gradually substituted for the hand, and its large plano-concave knife for the razor. When, by these means, sections had reached an almost ideal thinness, uniformity, and transparency, their various elements were differentiated, and made more easily recognisable by staining fluids.

Like the microscope, the microtome and its knife have reached a degree of perfection which it seems unnecessary, and scarcely possible, to exceed. The discovery of new methods of staining, or the improvement of those already devised, on the other hand, continually progresses, and especially in the microscopy of the nervous system its progress has been attended with the happiest results. The achievements of chemistry, and of the art of dying, from the point of view of commerce, admittedly aided in this result. For these purposes, as in the province of neurology, the manufacture of permanent dyes has a special importance.

It is known to everyone that the extraordinary progress of chemistry has proved of the greatest importance to the neurologist. Yet, in very many cases, it is impossible at the present date to say why this or that element of a tissue should behave



differently from another with regard to a staining fluid. The majority of stains have been discovered empirically, although they have often been perfected only by many researches and experiments with a definite end in view.

To those who desire a thorough acquaintance with the general principles of staining, and with the conditions essential to success, the admirable "Handbuch der Färberei," by Löwenthal, may be recommended. For special points, the many works of Carl Weigert are, in this respect, of inestimable value.

To produce the required reactions, various chemical re-agents, mordants, and colouring matters are employed. This division, however, cannot be rigidly insisted upon, as bichromate of potash, for example, is, on the one hand, a genuine colouring matter, producing chrome-yellow, and on the other is used by the neurologist as a mordant, not to mention its action as a hardening agent. Generally speaking, the chemical re-agents are employed only in the preparatory process, and to aid the action of the other substances.

The mordants are intended to form definite coloured compounds with the colouring matters, and to play their part in the final staining, as they become, in effect, a constituent portion of the stain. They thus form the so-called "Farblacke" (colour lake). Though they sometimes serve only to fix the stain, they often become essential parts of the ultimate result, as without a mordant the colouring matter gives no proper stain. Often enough with different mordants the same colouring matter may give different tints, which may not be equally true.

Regarding the modern detail of the process of staining, many pathologists have urged, even more forcibly than against the methods formerly employed, that in this way it is really only artificial products that are obtained. In a certain sense, the objection is undoubtedly correct. Should we be able in the future to examine all the permanent or temporary alterations in the elements of the central nervous system as they really are, and not as we artificially make them appear by the help of colouring matters, we shall have attained the fulfilment

of our ideal. Every neurologist, however, is aware that in a certain sense he has to deal with artificial products under the microscope. But he is also aware that the continual progress in the knowledge of the processes, whether of health or of disease, is due in great part to the work of men such as Weigert, Golgi, Ehrlich, and others, who have pointed out new paths of research.

It is not my intention to treat of these questions here at greater length, nor is this the place to do so. But it is perhaps to the purpose to mention in this place the postulates which Carl Weigert has laid down as essential to a successful staining.

It is easy to indicate the object aimed at in a good staining. It is simply this, that certain constituents of the tissues and cells should select the colouring matter from a solution, and form with it a compound as permanent and as deeply stained as possible. In other words, by means of a certain colour, the elementary constituents of the tissue must be made more distinct. But it is often not enough to present a single colour to the eye in the microscopic image; we employ double and even triple stains, according to the object we have in view.

Whatever our object be, however, we must be guided by the postulates of Weigert,<sup>1</sup> which, though originally laid down for a neuroglia stain, apply, *mutatis mutandis*, to any variety of stain.

1. The first requirement is that the stain must be *selective*, *i.e.*, that it shall not stain at the same time anything which might cause confusion, or prevent the distinctness and prominence of the element it is desired to examine. For a neuroglia stain, for example, Weigert states that no method should be employed in which the possibility of staining the axis-cylinders and nerve-cells cannot be definitely excluded.

2. The second important requirement is that the results of the method shall be reliable, *i.e.*, every section which has been properly prepared must show, in every part, every existing

<sup>1</sup> C. Weigert: Beiträge zur Kenntniss der normalen menschlichen Neuroglia, 1895. Frankfurt a. M.



portion of the tissue to be demonstrated. It is essential, in every case, that the success of the method must not be made to hang upon the passage of a very brief period of time in any of the processes to be gone through. A stain, the success of which is decided by a second, more or less, is worthless.

3. It is very desirable that the other elements of the tissue should be brought into view, as far as is necessary, for purposes of localisation.

If, for example, neuroglia fibres have to be demonstrated, the nuclei may, without inconvenience, be stained of the same colour, as no one would confuse a nucleus and a fibre. But in other cases a contrast-stain must always be employed.

4. The elements to be demonstrated must take on the stain as deeply as possible.

5. The preliminary steps and the preparation of the section should occupy as little time as possible, although it is much more important that the process should be certain than that it should be rapid.

In staining for the medullary sheaths, for example, the tissues may now be made ready for the stain in four days, without the aid of heat, while formerly weeks and months were necessary. (See "Staining for Medullary Sheaths.")

6. The tissues must not be injured by the manipulations which they undergo. Brittleness, shrinkage, and so forth, must be avoided.

7. It is desirable that the preparations should be permanent.

These postulates admittedly cannot all be equally fulfilled in every case, but no method should be regarded as perfect, unless, like the classic method for medullary sheaths, it meets all these requirements. At all events the pathologist demands at least a *selective* demonstration of the different tissue-elements. The more isolated is the staining, the easier is it to recognise pathological alterations.

From this point of view staining methods may be divided into those appropriate to the different elements of the nervous system, although it must be admitted that an absolutely selective stain, with which, as in Weigert's neuroglia method,

only the elements to be demonstrated appear in the preparation, is to be found in a very small minority of instances.

Staining methods are, however, divided into the following groups:—

1. Stains for nerve cells.
2. For the medullary sheaths.
3. For the axis-cylinders.
4. For the neuroglia.

The methods of Golgi and Ehrlich occupy a position by themselves.

#### A. STAINING THE NERVE CELLS.

The method of staining by means of carmine, introduced by Gerlach in 1854, remains to some extent in use at the present day. But of recent years the different kinds of carmine have lost so much of their importance, that some neuro-pathologists have almost entirely abandoned their use. For this there are various reasons. What sections stained with carmine show, may either be seen by the practised eye, even in unstained preparations, or may be shown by other methods. Further, the preparations of carmine seem of late years to have deteriorated in quality. Moreover, the use of alcohol before staining prevents a good demonstration of the tissue-elements. For this last reason, some employ this stain only in bulk, for pieces of tissue previously hardened in Müller's fluid. Finally, by the use of the methods of Nissl and Held, so perfect a demonstration of the inner structure of the nerve cell may be obtained as to make other methods unnecessary for its investigation. It is, however, necessary to mention the carmine stains in this place, as they are not altogether without importance in the survey especially of such nerve cells as have undergone pathological changes.

The best results are to be got from carmine<sup>1</sup> staining, if treatment with alcohol is omitted, *i.e.*, when the piece of tissue,

<sup>1</sup> Carmine is prepared by treating cochineal with alum. The powder is almost insoluble in water and alcohol, but readily soluble in ammonia. Cochineal is the dried female insect of *Coccus cacti*, a shield-louse living upon certain kinds of cactus. Its colouring ingredient is carminic acid.



after hardening, is placed entire in the staining fluid. Further detail is given below.

#### STAINING THE NERVE CELLS BY CARMINE AND HÆMATOXYLIN.

For staining in bulk, Beale's carmine, soda-carmine, in 1 per cent. solution, alum-carmine, and borax-carmine must be considered.

##### 1. *Ammonia-carmine* (Beale):—

Carmine, - - - - -	0,6
Liq. Ammon. fort., - - - - -	3,75

The solution is boiled for a few minutes, and the following is then added:—

Glycerine, - - - - -	60,0
Distilled water, - - - - -	60,0
Alcohol, - - - - -	15,0

The entire pieces remain from two to eight days in the solution, and are then washed out. They are afterwards hardened in alcohol, embedded, and cut.

Apart from the saving of time and trouble, the advantage of staining in bulk is that better results are obtained owing to the omission of alcohol. Small pieces, which should not be more than 1 cm. in length and 0.3 cm. in breadth, may be stained in from two to four days. They are then washed out and placed for twenty-four hours in 70 per cent. alcohol, to which 1 ccm. of hydrochloric acid is added. The acid is then removed by water, after which the pieces are hardened in alcohol and cut.

A drawback to *soda-carmine* is its high price (about three shillings per gramme).

2. *Alum-carmine*, as recommended by Grenacher, is less used, whether for staining in bulk or for separate sections. It is prepared by boiling 2 to 5 gm. of carmine with 100 ccm. of a 5 per cent. solution of alum for a quarter of an hour to an hour. The pieces remain in the stain from ten minutes to several hours, and are then washed out in distilled water and

at once embedded. There is no risk of overstaining, but, on the other hand, the solution is not adapted for objects that are difficult to stain, as the bluish-red colour of the nuclei is not sufficiently intense. The protoplasm stains bright red, while the ground substance hardly takes on the stain at all.

According to Upson, sections may be completely stained in five minutes if one to three drops of phospho-molybdic acid be added to every 5 ccm. of the alum-carmine solution.

Haug recommends the following combination:—

Carmine, - - - - -	1,0
Borax, - - - - -	1,0
Alum-carmine, - - - - -	2,0

These are rubbed together and boiled for half an hour with 100 ccm. of liquor aluminis acetici (P.G.) The solution is then filtered. It may be used in a few weeks, and keeps for a long time.

##### 3. A. *Borax-carmine* (aqueous solution):—

Carmine, - - - - -	0,5
Borax, - - - - -	2,0
Distilled water, - - - - -	100,0

The ingredients are mixed and heated to boiling point. 5 ccm. of dilute acetic acid are then added, while the fluid is stirred, until its colour resembles that of ammonia-carmine. It should be filtered in twenty-four hours.

##### B. *Borax-carmine* (Grenacher) (alcoholic solution):—

Carmine, - - - - -	2—3,0
Borax, - - - - -	4,0
Distilled water, - - - - -	93,0

After this has stood for two days, an equal quantity of 70 per cent. alcohol is added, and the fluid is filtered thirty-six hours afterwards.

The sections remain in one of these solutions from a quarter of an hour to ten hours. There is no risk of overstaining. They are differentiated, as above, in alcohol acidulated with hydrochloric acid. They are then washed in water, transferred to alcohol, and mounted in balsam.



C. *Neutral borax-carmin* (Nikiforoff):—

Carmin	-	-	-	-	3,0
Borax	-	-	-	-	5,0
Distilled water	-	-	-	-	100,0

The fluid is boiled, and ammonia is added. After it has evaporated to half its volume, some dilute acetic acid is added. This carmin is specially to be recommended for staining in bulk.

The process of staining in sections is simple, and in every case the same. It is as follows:—

1. Hardening in Müller's fluid.
2. Washing in water; subsequent hardening in alcohol; transference to ether-alcohol; and embedding in celloidin.
3. Cutting; staining in one of the carmin solutions (from a few minutes to twenty-four hours).
4. Washing in water, or, in the case of the compound carmin solutions, in alcohol acidulated with hydrochloric acid.
5. Transference to alcohol, then to one of the oils or to carbol-xylol, and mounting in Canada balsam.

The nerve cells and their nuclei take on the stain best, and become of a brilliant red colour. Then come the axis-cylinders and the neuroglia. The ground substance appears pink, and the medullary sheaths remain almost unstained.

Cells that have undergone pathological change are for the most part indicated by an intensely red colour, which, in transverse sections of the cord, for example, may often be distinctly perceptible to the naked eye.

The following kinds of carmin must be considered, as suitable for our purpose:—

4. *Ammonia-carmin* chanced to be the first form of carmin to be employed as a stain. It is prepared as follows:—The desired quantity of Naccarat's best French carmin is stirred up with a little ammonia, which is then diluted with distilled water until a dark red fluid is obtained. After filtration, the excess of ammonia is allowed to evaporate. The older the solution the better is its effect. The best results are obtained in staining, if a solution, diluted till it is of a rose colour, be

employed. Its action should be prolonged, and is aided by heat. Used in this way it possesses the practical advantage, which must not be overlooked, that the sections can be distinctly seen in the fluid. The waste of time spent in searching for them in a dark and opaque fluid like the other forms of carmin is thus avoided. The longer was the duration of the processes of fixing and hardening in bichromate, the longer does the staining take. When it is complete (in from twelve to twenty-four hours), the sections are washed, first in water, then in water containing about 1 per cent. of acetic acid (instead of which hydrochloric acid may be used). The acid is removed by prolonged washing out in distilled water (from one to twenty-four hours). The usual after-treatment follows, and the sections are mounted in balsam.

5. *Dry ammonia-carmin* (Hoyer).

An aqueous solution is prepared as follows:—

Carmin	-	-	-	-	1 gm.
Liq. Ammon. fort.	-	-	-	-	2 ccm.
Distilled water	-	-	-	-	8 ccm.

The excess of ammonia is removed by heat. After the solution has cooled it is mixed with about five times its volume of absolute alcohol. It is then filtered and allowed to evaporate to dryness. A powder is thus obtained which may be kept for months. A  $\frac{1}{2}$  per cent. aqueous solution of the powder is used as a staining fluid, and often gives good results even when it is freshly prepared. The intensity of the carmin stain is increased if a 1 per cent. solution of alum be previously used as a mordant. The sections should remain in this for a few minutes, and should be washed for a short time in water. But this process seems occasionally to make the differentiation less distinct.

6. *Uranium-carmin* (Schmaus)—

Carminate of soda, 1 gm., and oxide of uranium,  $\frac{1}{2}$  gm., are rubbed together and afterwards boiled for half an hour in 100 ccm. of distilled water. The solution is allowed to cool, and is afterwards filtered. The sections should be left from fifteen to twenty minutes in the stain, but even if they remain



for twenty-four hours they are not overstained. The celloidin does not take on the stain.

7. *Picro-carmin* (Ranvier)—

Carmine, - - - - -	1,0
Liq. Ammon. fort., - - - - -	3,0
Distilled water, - - - - -	10,0

After the ingredients have been dissolved by the aid of heat, 200 grm. of a saturated watery solution of picric acid are added, and the resulting fluid is evaporated to a third of its volume, and filtered. The sections are left in the stain for an hour and then transferred to glycerine containing 1 per cent. of hydrochloric acid. To this a little picric acid should be added. They are then washed for five minutes in water containing picric acid, dehydrated in alcohol similarly treated, and mounted.

In this way a kind of double-staining is attained. The nuclei are of a brownish-red, and the protoplasm is yellow.

8. *Soda-picro-carmin* (Loewenthal)—

Caustic soda, 1 grm., is dissolved in 100 ccm. of distilled water, and carmine, 0.4 grm., is added. The fluid is boiled for ten to fifteen minutes, and diluted with 100 ccm. of distilled water. A 1 per cent. solution of picric acid is then slowly added, until the point arrives at which the resulting precipitate ceases to be completely dissolved. The fluid is allowed to stand for three hours, and then filtered several times through the same filter paper. If it is kept for a considerable time it often becomes turbid.

If it is intended to procure permanent preparations there must be added to the water or to the alcohol, as in the fluids above mentioned, a little picric acid.

In good preparations the nerve cells are pink and their nuclei dark red. The nuclei of the neuroglia are bright red, the axis-cylinders somewhat darker, and the myelin yellow.

9. *Lithia-carmin* (Orth):—

Carmine, - - - - -	- 2,5—5,0
Saturated watery solution of lithium carbonate, - - - - -	100,0

The staining is performed just as with borax-carmin. The nuclei become deep red. Overstaining is easily corrected by differentiation.

10. *Picro-lithia-carmin* (Orth)—

The above solution of lithia-carmin,	1 part.
Saturated solution of picric acid, -	2 parts.

The staining lasts from six to twelve hours, and gives a result similar to that of lithia-carmin, except that the myelin is stained yellow.

Haug recommends, for preparations specially difficult to stain, the following solution, which may be kept for a very long time, and used as soon as it is prepared:—

Carmine, - - - - -	1,0
Chlorate of ammonia, - - - - -	2,0

These ingredients are rubbed together, and boiled in 100 ccm. of distilled water. After cooling 15 to 20 drops of Liq. Ammon. fort. and 0.3 to 0.5 grm. of lithium carbonate are added. The solution is then filtered. Sections are stained in a few minutes. The usual after-treatment follows.

11. *Alum-cochineal* (Csokor)—

One gramme of alum and one of cochineal are rubbed together, and dissolved in 100 ccm. of distilled water, which is evaporated to half its volume. After cooling and filtration, a little carbolic acid is added, to prevent the development of mould. In this case also overstaining is impossible.

The nuclei become violet, the body of the cell and the axis-cylinders of a reddish tint.

*Hæmatoxylin* was introduced by Waldeyer in 1865. It is used at the present time to demonstrate the finer details of the structure of the nucleus and of the body of the cell. It is to be specially recommended in the study of the ground substance of the nerve cells, and of the fibrillæ which traverse it. For this purpose it may be employed, as Flemming and others recommend, after fixing in corrosive sublimate, in the form of Heidenhain's iron-hæmatoxylin, or of Delafield's hæmatoxylin, used in gradually increasing strength.



Hæmatoxylin is the colouring matter of logwood. In 1810 Chevreul found in it a yellowish-white crystalline body, which rapidly darkened when exposed to air, especially in the presence of ammonia. It is transformed by oxidation into the proper colouring matter of prepared logwood, the so-called hæmatein of Erdmann.

Logwood itself is perhaps the most important of all colouring matters. Its colouring ingredient, though red, gives, in combination with mordants, blue, violet, or black stains. Logwood (or campeachy wood) is the central wood of the stem of hæmatoxylon campechianum (a leguminous pine), stripped of the bark and sap-wood.

With the exception of Ehrlich's solution, which remains unchanged and active for many years, hæmatoxylin solutions undergo decomposition after a certain time. Generally speaking, they are not at their best until a few weeks after they have been prepared. *In contrast to carmine, hæmatoxylin in solution very readily produces overstaining.*

#### 1. Alum-hæmatoxylin (Boehmer)—

A 1 per cent. solution of hæmatoxylin in 10 ccm. of absolute alcohol, and a 1 per cent. solution of alum, should be kept in stock. A few days before the stain is to be used, enough of the hæmatoxylin is added to the alum solution to produce a violet tint. This fluid is exposed to the light for a few days, which causes it to darken still further. It may then be used. After some weeks the solution may become too deeply stained, but this may be avoided by further dilution with the alum solution. The staining is carried out as follows:—

(1) The sections remain from one to three minutes in the stain.

(2) They are thoroughly washed out, and left in distilled water for twenty-four hours.

(3) They are then transferred to alcohol, and afterwards to oil of origanum, and to Canada balsam.

The nuclei are of a bluish-violet tint, and the protoplasm light blue.

Sections of pieces that have been hardened in bichromate must be washed out before they are placed in the stain. It is also advisable not to transfer the sections direct from alcohol to the stain, but to leave them first for a short time in water or in a 1 per cent. alum solution.

It should be noted that, after removal from the stain, the sections darken still further in water. They should not, therefore, be left too long in the hæmatoxylin. But if this has happened, and overstaining has been the result, it may be rectified by the use of a 1 per cent. alum solution, after which the sections are washed out in distilled water.

Treatment with acid is for the most part superfluous, although some employ one drop of hydrochloric acid in 50 ccm. of distilled water. If this be done, the acid must be afterwards neutralised, *e.g.*, with ammonia. As clove oil appears to reduce the permanence of the hæmatoxylin stain, origanum oil should be used in its place.

#### 2. Hæmatoxylin (Delafield)—

Two grammes of hæmatoxylin, dissolved in 10 ccm. of absolute alcohol, are mixed with 200 ccm. of a saturated solution of ammonia-alum, exposed to light in an open bottle, and filtered four days afterwards. 100 ccm. of alcohol, and the same quantity of glycerine, are then added. If the colour has become dark the fluid is again filtered, and the bottle is closed by a stopper. The solution may be used in a few months, a small quantity of it being added to a considerable amount of water. The same precautions must be taken as with alum-hæmatoxylin.

#### 3. Acid hæmatoxylin (Ehrlich)—

Hæmatoxylin,	-	-	-	-	-	6,0
Distilled water,	-	-	-	-	} aa	300,0
Absolute alcohol,	-	-	-	-		
Glycerine,	-	-	-	-		
Glacial acetic acid,	-	-	-	-	-	20,0

Alum in excess.

The solution is filtered and exposed to light for two or three weeks, becoming darker during this period.

This hæmatoxylin of Ehrlich is distinguished from the others by the greater rapidity of the staining process, and the almost complete permanence of the result. After they are stained the sections are washed in water, in which they darken. They are then mounted. All the nuclei are stained



a deep blue. The bodies of the cells are light blue, or may not be stained at all.

For objects which are difficult to stain Haug recommends the following solution, the action of which is very rapid:—

Hæmatoxylin, - - - - -	1,0
Absolute alcohol, - - - - -	10,0
Liquor aluminis acet. (P.G.), <sup>1</sup> - - -	200,0

This stain, which is at first blue, becomes in a few weeks dark brown. The addition of carbonate of lithia promotes the success of the stain. The sections may afterwards be differentiated in alcohol to which hydrochloric acid has been added. They are then washed out and mounted.

In conjunction with the above nuclear stains the aniline colours and their combinations may here be shortly described, as they are also to be regarded as nuclear stains.

Goodall has made extensive experiments with these reagents, both on pieces of tissue hardened in bichromate and on those hardened in sublimate. The pieces must be afterwards treated with alcohol. From his results it follows that, as a general rule, the process of staining is more rapid after hardening in sublimate than after hardening in bichromate. The following *aniline stains*, as mentioned by him, may here find a place:—

1. *Aniline blue-black* (the English preparation)—

Aniline blue-black, - - - - -	0,25
Distilled water, - - - - -	100,00

The sections are stained for from half an hour to an hour. Overstaining must be avoided, as it is impossible to differentiate sufficiently or completely to extract the excess of stain. The sections must be washed out and transferred to alcohol, then to creosote or one of the oils, but not to oil of cloves, which in time alters the colour. They are then mounted in Canada balsam. For objects which are difficult to stain, Goodall recommends a  $\frac{1}{8}$  to  $\frac{1}{3}$  per cent. solution in absolute alcohol, which acts more rapidly and with greater intensity, while staining the celloidin less deeply. It brings out the nerve cells with their nuclei

<sup>1</sup>Sulphate of aluminium, 300; acetic acid, B.P. (by weight), 327; precipitated carbonate of calcium, 130; water, 1000,0.—*Tr.*

and processes, the axis-cylinders, and the nuclei of the neuroglia and blood vessels. Their tint varies from a bluish-grey to a deep blue. The axis-cylinders and the nuclei of the nerve cells are the most deeply stained.

Sublimate preparations do not give such good results as those hardened in bichromate.

*Bevan Lewis's* modification for the cerebellar cortex:—

The sections are to be stained in the watery solution, washed out, and transferred to a 2 per cent. solution of chloral hydrate, in which they remain from twenty to thirty minutes. Afterwards they are differentiated in a mixture of equal parts of oil of cloves and 2 per cent. solution of chloral hydrate, to which absolute alcohol has been added until a clear solution is obtained. The process should be constantly watched under a low power of the microscope. The sections are afterwards transferred to absolute alcohol, oil of cloves, and Canada balsam.

*Indulin* is used in the same way as aniline blue-black.

2. *Aniline blue* is employed in a very dilute watery solution. The sections are stained for five to ten minutes. The other steps in the process are the same as those given above.

In contrast to the other aniline stains these two are unaffected by alcohol. Hardening by sublimate cannot be recommended if these stains are to be used.

3. *Toluidin blue*—

Toluidin, - - - - -	0,25
Distilled water, - - - - -	100,00

The addition of a little alcohol promotes the solution of the powder. The staining lasts from twenty-four to forty-eight hours, or, if the tissues have been hardened in sublimate, for a shorter time. The sections must then be washed out, and transferred first to 96 per cent. alcohol and then to absolute alcohol. When they give off no further cloudiness they are transferred to xylol and then to Canada balsam.

The section is of a pale blue or purple tint. The nuclei of the nerve cells, the neuroglia, and the blood vessels, are dark blue or purple, and the protoplasm of the nerve cells is somewhat paler. If the tissue has been hardened in bichromate it



a deep blue. The bodies of the cells are light blue, or may not be stained at all.

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Absolute alcohol, - - - - -	10,0
Liquor aluminis acet. (P.G.), <sup>1</sup> - - - - -	200,0

This stain, which is at first blue, becomes in a few weeks dark brown. The addition of carbonate of lithia promotes the success of the stain. The sections may afterwards be differentiated in alcohol to which hydrochloric acid has been added. They are then washed out and mounted.

In conjunction with the above nuclear stains the aniline colours and their combinations may here be shortly described, as they are also to be regarded as nuclear stains.

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The section is of a pale blue or purple tint. The nuclei of the nerve cells, the neuroglia, and the blood vessels, are dark blue or purple, and the protoplasm of the nerve cells is somewhat paler. If the tissue has been hardened in bichromate it



is said that the result is not so good, as the stain becomes too diffuse.

#### 4. *Victoria blue*—

The hardening should take place in sublimate, and the sections should be stained in a dark blue solution for forty-eight hours. They are afterwards treated as above mentioned. The result is the same as in the case of toluidin blue, except that the body of the nerve cell is less decolourised. Hardening in bichromate does not give such good results.

#### 5. *Safranin*—

A deep red watery solution should be used, or a solution in equal parts of water and alcohol. The sections are left in the stain from twelve to twenty-four hours, but their treatment should be otherwise as above detailed. For differentiation, as in the case of borax-carmin, alcohol, to which hydrochloric acid has been added, is sometimes recommended. It should be followed by absolute alcohol. This process is chiefly employed after the tissues have been fixed in chromo-acetico-osmic acid. Hardening in sublimate is not advisable.

#### 6. *Dahlia*—

A deep red watery solution should be used, and the sections may be stained for as long as forty-eight hours. Overstaining may be rectified as in the case of toluidin blue. Equally good results are obtained whether the tissues have been hardened in sublimate or bichromate.

#### 7. *Gentian violet*—

A deep violet watery solution is used, and the sections are stained for as long as forty-eight hours. Otherwise they are treated as above, or alcohol acidulated with hydrochloric acid may be used. The same results follow hardening whether in sublimate or bichromate.

#### 8. *Methyl violet*—

A deep violet solution in equal parts of water and alcohol is used. Staining lasts for two or three days. The after-treatment is as above. Sublimate gives better results than bichromate.

#### 9. *Methylene blue*—

A deep blue watery solution is used, and the staining process lasts forty-eight hours. The after-treatment is as above. This method is specially adapted for staining the nuclei of the neuroglia. The same results follow hardening whether in sublimate or bichromate.

#### 10. *Congo red*—

A deep red solution in equal parts of alcohol and water is employed. The staining process lasts from twelve to eighteen hours. The reddish-brown sections are washed out in water and alcohol, then differentiated for a few hours in alcohol acidulated with hydrochloric acid (as in the borax-carmin process). They are then washed in water, transferred for a short time to alcohol and then to xylol, and are finally mounted in Canada balsam. The axis-cylinders appear dark brown, the nerve cells and neuroglia cells dark brown or purple, while the ground substance is paler. The axis-cylinders are particularly distinct.

Nissl recommends the following method:—

The pieces should be hardened in bichromate of potash and transferred to 95 per cent. alcohol. The sections are stained for 72 hours in a solution of Congo red (5,0 to 400,0). They are then transferred to 95 per cent. alcohol, in which they remain from five to ten minutes, and are then left for six hours in nitric acid-alcohol (3,0 to 100,0). From this they are retransferred to alcohol, then to oil, and finally mounted in Canada balsam. Hardening by sublimate is not to be recommended.

#### 11. *Methyl green*—

This substance is not so often employed, as the stain is rapidly extracted both in water and alcohol. Erlitzky, however, frequently used it for pieces hardened by his fluid. He employed a 1.5 or a 2 per cent. alcoholic or aqueous solution. The staining process lasts from twelve to twenty-four hours.

It is of value as a nuclear stain. To a strong watery solution 1 per cent. of acetic acid should be added, and



the sections should be washed out in slightly acidulated water.

Of the very numerous combinations of aniline stains, the following, which are preferred by Goodall, may be mentioned as of importance:—

1. *The Ehrlich-Biondi mixture* (methyl-green, acid-fuchsin, and orange). The pieces are hardened either in sublimate or bichromate. The staining process lasts from six to twenty-four hours. The sections are then washed out, transferred to alcohol and xylol, and mounted in Canada balsam.

The section has a general reddish tint. The nerve cells are of a pale violet colour, and their nuclei deep violet. The nuclei of the neuroglia are bluish-green, and the vessels dark red.

2. *Gentian violet and eosin*—

After hardening in bichromate the sections are stained in an alcoholic solution of gentian violet, diluted to half strength with water, and placed in alcohol or acidulated alcohol until they are completely differentiated. They are then stained for one or two minutes in a fairly strong watery solution of eosin, after which they are quickly dehydrated in alcohol, transferred to xylol, and mounted in Canada balsam.

A number of combinations, which cannot be overlooked, are employed for the special purpose of counter staining. Many of them, however, are of no great importance for the attainment of the real object of investigation, namely, the promotion of our knowledge of the histology and the pathological changes of the nervous system. The technical details of their employment are for the most part simple, and are based throughout upon the usual preliminary hardening in bichromate and after-hardening in alcohol.

1. *Hæmatoxylin and picric acid*—

The sections are stained for a quarter of an hour in hæmatoxylin (*e.g.*, Ehrlich's), washed out, and placed for a few minutes, until the blue colour has given place to a yellowish tint, in alcohol made yellow by the addition of crystals of

picric acid. They are then transferred to xylol, and mounted in Canada balsam.

The nerve cells appear yellowish, and their nuclei of a pale violet tint. The nuclei of the neuroglia and blood vessels are deep violet, and the ground substance yellow.

2. *Carmine and picric acid*—

The sections are stained in carmine, and treated as above. When the red section has become reddish yellow it is transferred to xylol and mounted in Canada balsam.

The nerve cells appear red, and their nuclei deep red. The ground substance is paler. The nuclei of the neuroglia are not distinct.

3. *Hæmatoxylin and eosin*—

The sections are stained for a quarter of an hour in Ehrlich's hæmatoxylin and then washed out. The counter stain is a weak watery solution of eosin, in which they are left for a few minutes until the blue colour has given place to a reddish violet. They are then transferred to xylol and mounted in Canada balsam.

The nerve cells are red and their nuclei pale violet. The nuclei of the neuroglia and blood vessels are deep violet, and the ground substance pale red.

This combination gives the best microscopic pictures, and is therefore among those most in use. Goodall, however, also recommends the following combination as very good:—

4. *Hæmatoxylin and benzo-purpurin B*—

The sections are stained for ten minutes in Ehrlich's hæmatoxylin and then washed out. The counter stain is a weak watery solution of benzo-purpurin B, in which the sections remain for a few minutes. They are washed out, transferred to alcohol and to xylol, and mounted in balsam. The nerve cells are reddish and their nuclei pale violet. The nuclei of the neuroglia and blood vessels are deep violet and the ground substance reddish violet.

5. *Hæmatoxylin and aniline blue-black*—

The sections are stained for a few minutes in hæmatoxylin, then for a few seconds in 0.5 per cent. watery solution of



aniline blue-black. They are washed out and treated as above.

Apart from the rapidity of the staining process in this combination the contrast is extremely sharp, as aniline blue-black stains principally the nerve cells, and the hæmatoxylin those of the neuroglia. These stains seem to be particularly well suited to the cerebellar cortex (Purkinje's cells).

6. *Hæmatoxylin and safranin*—

The sections are stained for a few minutes in hæmatoxylin, washed out, and transferred to the counter stain, a solution composed as follows:—Safranin, 1,0; absolute alcohol, 100,0; distilled water, 200,0. They are again washed out, and treated as above.

The nerve cells and axis-cylinders are pale red, all the nuclei are violet, and the ground substance is reddish.

7. *Aniline blue-black and picro-carmin*—

The sections are stained from a quarter of an hour to half an hour in picro-carmin, then transferred direct to a  $\frac{1}{4}$  per cent. watery solution of aniline blue-black. In about ten minutes they appear of a deep violet tint. The further treatment is as above.

The nuclei of the blood vessels are particularly sharply defined in the midst of the other structures, which are stained violet.

8. *Picro-carmin and aniline green*—

The sections are stained in picro-carmin, in the incubator, from a quarter of an hour to half an hour. They are then washed out in acidulated water and rapidly passed through distilled water. The counter stain is a watery solution of aniline green (1 to 1000), in which they remain for twenty-four hours. They are then washed in water. The further treatment is as above.

This method is specially recommended for the examination of the structure of the cerebellum under the low power. The cells of Purkinje and the blood vessels appear red, the axis-cylinders dark green, and the myelin pale green.

9. *Carmin and aniline blue* (Duval)—

The sections are stained in carmin, washed out, and counterstained for five minutes in the following solution:—

Saturated alcoholic solution of			
aniline blue,	-	-	10 drops.
Absolute alcohol,	-	-	10,0

They are cleared in turpentine, without any further treatment with alcohol, and mounted in balsam.

The sections appear dark violet. The nerve cells and axis-cylinders are reddish violet, the nuclei of the neuroglia blue, and the blood vessels bluish violet.

10. *Borax-carmin and picro-carmin*—

One may stain at pleasure first in one or the other re-agent, or a mixture of both may be employed, a few drops of picro-carmin being added to a tumbler of borax-carmin. The sections are washed out, dehydrated in picric-acid alcohol, transferred to xylol, and mounted in balsam. The results are much the same as those of carmin and picric, but the nuclei are more sharply defined.

11. *Borax-carmin and indigo-carmin*—

The sections are stained for some hours in Grenacher's borax-carmin, and differentiated in acidulated alcohol. They are then washed out, and placed for from ten to twenty hours in the counterstain, a dark blue alcoholic solution of indigo-carmin. They are again washed out, transferred to alcohol and to xylol, and mounted in balsam.

The nerve cells appear blue, and their nuclei red. The nuclei of the neuroglia and blood vessels are red or violet, the ground substance bluish green, and the medulla green.

#### NISSL'S METHOD FOR STAINING NERVE CELLS

1. The fresh material is hardened in 96 per cent. alcohol.
2. Without embedding, the pieces are fixed upon cork by means of gum arabic or fish-lime, and are then cut.
3. The sections are stained in a watch-glass with a solution of methylene blue, which is held over the flame of a spirit



lamp till bubbles are given off (at 65° to 70° C.). The staining solution consists of—

Methylene blue B. (patent),	-	-	3,75
Grated Venice soap,	-	-	1,75
Distilled water,	-	-	1000,0

4. The sections are differentiated in a mixture of aniline oil, 10 parts, and 96 per cent. alcohol, 90 parts. The process is continued until the large clouds of stain cease to be given off. The aniline oil and the differentiating fluid must be protected from the light by keeping them in dark bottles.

5. The sections are very thoroughly dried upon the slide, and rendered transparent by oil of cajuput. They are again dried with blotting paper.

6. Benzine is poured over them, and they are mounted in colophonium dissolved in benzine. The benzine gases are driven off by heating over the lamp.

This method of Nissl's gives a demonstration of the finer structure of the nerve cells, such as has never before been attained by any other method. In particular, commencing degenerative processes in what he calls "the portions of tissue capable of taking on the stain" have been for the first time revealed to us by the use of this method.

In the examination of the nerve cells, especially by Nissl's method, a number of directions, given by Goldscheider and Flatau,<sup>1</sup> must be followed out.

1. The entire spinal cord (or portions of it not more than 2 cm. long) is placed in alcohol for five or ten minutes. Cotton wool should previously be laid on the bottom of the vessel.

2. After five or ten minutes the cord is divided into thin sections, measuring 2 or 3 mm. These are dried with blotting paper, and one surface (the proximal or distal, as may be preferred) is marked in the white substance with a point of ink. Hardening for fifteen to twenty hours is sufficient for these thin sections.

<sup>1</sup> Goldscheider and Flatau. Normale und Pathologische Anatomie der Nervenzellen. 1898.

3. The pia mater is now removed with a fine pair of forceps, beginning at the longitudinal sulcus. The little pieces are dried on blotting paper, and fastened on cork by a very thin layer of fish-lime. They should be gently pressed down with the finger. Ninety-six per cent. alcohol is now dropped upon them.

4. The corks, carrying the pieces, are placed in 96 per cent. alcohol. The pieces may be cut after half an hour's immersion.

5. The sections, which should be from 10 to 20  $\mu$  in thickness, are transferred to methylene blue. *It is not necessary that bubbles should be given off.* It is sufficient that the fluid should be gently heated until steam arises. *The sections should remain upon the surface.*

6. They are placed for half a minute to a minute in aniline oil and alcohol, returned to methylene blue (the double method of staining), and then again to aniline oil and alcohol. The *white* substance must appear transparent. It is advisable to immerse the sections in alcohol for some hours before staining.

7. The sections are passed through several watch-glasses containing aniline oil and alcohol, and are then, to render them more durable, very firmly dried upon the slide with several layers of fine blotting paper. They have now the glittering appearance of mother-of-pearl.

8. Cajuput oil is applied for a short time. They are quickly dried, benzine is poured over them, and they are mounted in colophonium dissolved in benzine.

If, at a later date, crystals should form in the preparations, they may be got rid of by heat. Instead of direct fixing in alcohol, the pieces may first be fixed for one or two days in 10 or 20 per cent. formol.

Nissl's method of staining, like thionin and toluidin blue, serves to demonstrate the formed elements of the tissue capable of taking on the stain (Nissl's cell-corpuscles).

In the methods of Flemming and Held the interstitial substance and ground substance are also stained.

Flemming employed in fixing chromic acid, chromo-acetic



acid, and especially a concentrated solution of corrosive sublimate. Among stains, besides safranin and gentian violet, he used most frequently Heidenhain's iron hæmatoxylin, and for sublimate preparations Delafield's progressive hæmatoxylin stain.

We do not yet possess a specific method for the demonstration of the fibrillæ of the nerve cells. So far, they have perhaps been best demonstrated by Heidenhain's sublimate and iron hæmatoxylin process, in which the iron solution is extracted with acetic acid until the preparation appears of a pale bluish grey. Flemming states that the fibrillæ can also be brought into view if, *e.g.*, the spinal ganglia be hardened for three days in 90 per cent. alcohol, the strength of which is gradually increased, and embedded in paraffin or celloidin. The sections should be stained for a few hours in a weak solution of Delafield's hæmatoxylin. The fibrillæ are distinct, but not so dark as in sublimate preparations.

Becker's method of demonstrating the fibrillæ by means of copper hæmatoxylin is not yet published.

Von Lenhossék obtained good results in demonstrating the structure of the nucleus by means of iron hæmatoxylin. The nucleus and the nucleoli may be stained by Lenhossék's toluidin blue and eosin, or by Held's erythrosin methylene blue.<sup>1</sup>

<sup>1</sup>In order so far as possible to exclude artificial products in interpreting the significance of the appearances observed, Nissl has employed the expedient of replacing the actual nerve cells occurring in the tissue by an equivalent of these cells. By this is to be understood the microscopic image of the nerve cells existing in the tissue of an animal killed in a certain way. We are aware, by experience, that this is reproduced with a constant regularity if the tissue is subjected to a certain treatment and certain premisses be observed. The few forms which deviate from the equivalent image, which are to be referred to some still unknown effect of the methods employed, are not considered in this connection, but are to a certain extent excluded. In consequence of this attitude, the question of the existence of artificial products becomes objectless. For, if all the images of nerve cells are equivalent images, it is clear that every deviation from the equivalent can find its origin only in the cell itself.

It cannot be denied that, by means of this expedient of Nissl's, we are able to eliminate or to negate certain objections and certain sources of error.

Ramón y Cajal recommended hardening, first in sublimate and afterwards in alcohol.

#### NISSL'S SECOND METHOD OF STAINING THE NERVE CELLS.

1. The tissues are hardened in 96 per cent. alcohol.
2. They are cut, and the sections stained over the flame in a concentrated watery solution of fuchsin or magenta until steam arises.
3. They are washed out for one or two minutes in absolute alcohol.
4. They are transferred to oil of cloves and mounted in Canada balsam. Pieces of tissue intended for Nissl's stain may also be treated with Weigert and Pal's stain for the medullary sheaths, if they have undergone a previous hardening in formol. According to H. Gudden the sections must, in this case, be laid for ten hours in a 0.55 per cent. solution of chromic acid at the ordinary temperature of a room. They are then washed in water and soaked for a short time in 80 per cent. alcohol. They behave, thereafter, like preparations hardened in Müller's fluid, indeed, if a few drops of diluted nitric acid be added to the hæmatoxylin, they stain much better than such preparations (*cf.* Marina, p. 19).

#### *Sadorsky's modification of Nissl's method (formol process).*

1. The pieces are hardened in 10 per cent. formol for three or four days.
2. They are placed in 96 per cent. alcohol for two days, and in absolute alcohol for three. They are then embedded in celloidin.
3. The sections are stained in a 1 per cent. solution of methylene blue, or in a saturated solution of fuchsin in 5 per cent. carbolic acid.
4. They are differentiated with a 1 per cent. solution of glacial acetic acid until the grey matter can be distinguished from the white.
5. They are transferred to absolute alcohol and to xylol, and then mounted in balsam.

#### *Staining with thionin*<sup>1</sup>—

In the case of preparations hardened in alcohol, as in Nissl's

<sup>1</sup>Thionin, or Lauth's violet, is a basic derivative of tar. It is chemically closely allied to methylene blue, which is derived from it. Both pigments are indamines. Toluidin blue also belongs to the thionin group. Thionin was used for the first time by P. Ehrlich, with the object of staining the living nervous system. For this purpose methylene blue is also employed.



method, the thionin stain recommended by Weigert and Hoyer may be mentioned, as it is of great service in demonstrating Nissl's corpuscles. The process is as follows:—

1. The tissues are hardened in 96 per cent. alcohol, or in absolute alcohol; and previous to this process they may, if it be desired, be hardened for two days in a 50 per cent. solution of formol.

2. They are embedded in celloidin, or, if very thin sections be required, in paraffin.

3. The sections are stained for five minutes in a concentrated watery solution of thionin.

4. They are quickly rinsed, and then differentiated in a solution consisting of aniline oil, 1,0; absolute alcohol, 9,0.

5. They are cleared in cajuput oil, transferred to xylol, and mounted in xylol-balsam.

As in the case of Nissl's stain, the permanence of the thionin stain is often limited. It may disappear within a short time.

Held has supplied us with a double stain for the nerve cells and their processes. Its results are so good that it cannot be passed over. It demonstrates especially the otherwise unstained protoplasmic masses between the so-called Nissl's bodies.

*Held's modification of Nissl's method—*

1. The tissues are embedded in paraffin, and the sections, which should be from 1 to 10  $\mu$  in thickness, are fastened on the slide with diluted alcohol.

2. The sections are stained with the following solution of erythrosin:—

Pure erythrosin,	-	-	-	-	1,0
Distilled water,	-	-	-	-	150,0
Glacial acetic acid,	-	-	-	-	2 drops.

They are left in this stain for one or two minutes, the fluid being gently heated.

3. The sections are washed in water, and afterwards stained with the following counterstain:—

(a) Aqueous solution of acetone (1 to 20),	} Equal parts.
(b) Nissl's solution of methylene blue,	

This solution should be strongly heated, and the staining should be continued until the smell of acetone is no longer perceived.

4. The fluid is allowed to cool, and the sections are differentiated in a 0.1 per cent. solution of alum until they again become reddish. The time varies from a few seconds to a few minutes.

5. The sections are rinsed and transferred to absolute alcohol, then to xylol. They are mounted, as in Nissl's process, in colophonium dissolved in benzine.

Nissl's corpuscles are stained of a blue or a pale violet colour, while the interstitial substance is of a brilliant red. The nuclear membrane and the body of the nucleus are red; the nucleoli, blue; and the adjoining granules, violet.

For fixing, Held recommends that the tissues should be placed for twenty-four hours in picro-sulphuric acid. They are then washed in water, or at first in 20 per cent. alcohol, the strength of which is gradually increased by 10 per cent. at a time until absolute alcohol is employed. They are then several times treated in alcohol-xylol, and embedded in paraffin.

In place of alcohol, solutions of acetone may be used. These are followed by acetone-xylol, warm xylol, and xylol-paraffin. Embedding in paraffin then takes place.

STAINING THE NERVE CELLS WITH TOLUIDIN BLUE

(von Lenhossék, Hoyer).

1. The pieces are fixed for twenty-four hours in a concentrated sublimate solution (about 5 per cent.).

2. They are hardened in increasingly concentrated alcohol.

3. They are carefully embedded in paraffin with the aid of chloroform.

4 They are cut into sections 5  $\mu$  thick, which are fixed on the slide with distilled water (Gulland's method). The paraffin is extracted with xylol and iodised alcohol.

5. The sections are stained for several hours with a concentrated watery solution of toluidin blue.



6. They are differentiated in aniline-alcohol, and counterstained in an alcoholic solution of eosin, or in erythrosin.

7. They are quickly dehydrated in absolute alcohol, transferred to xylol, and mounted in xylol-balsam.

Nissl's corpuscles become, with this stain, of an intense dark blue colour. After differentiation in alcohol the ground substance is almost colourless, and takes on the counterstain.

Hoyer and von Lenhossék state that toluidin blue is an even more specific stain for Nissl's corpuscles than thionin or methylene blue.

It must specially be observed that the sections must never be allowed to dry while they are being transferred from one fluid to another. If they be, the integrity of the cells may easily be damaged. Consequently, in the whole process of after-treatment, blotting paper must not be used. Although this method gives very beautiful results, the preparations are not permanently durable. It is especially valuable for the cells of the spinal ganglia, but also for all central nerve cells, which are essentially of similar structure.

For the nerve cells M. Heidenhain has recommended hardening in sublimate, staining in iron hæmatoxylin, and counterstaining in eosin.

*Cox's method for the fibrillæ lying between Nissl's corpuscles—*

1. The tissues are hardened for two or three days in either of the following solutions:—

	I.	II.
Saturated solution of corrosive sublimate, - - - -	30	15
Solution of chloride of platinum, 5 per cent., - - - -	—	15
Solution of osmic acid, 1 per cent., - - - -	10	10
Glacial acetic acid, - - - -	5	5

2. They are embedded in paraffin.

3. The sections, which should be 5  $\mu$  thick, are fastened on the slide, and placed for eight hours in a 20-25 per cent. solution of tannin.

4. They are washed out, and stained with indoin blue or methylene blue. (a) Indoin blue.—The sections are placed for five to ten minutes in a 5 per cent. solution of tartar emetic. They are then washed out for ten minutes, and left from twelve to eighteen hours in the following mixture:—

Solution of alum, 5 per cent., - - -	10,0
Merk's indoin blue BB., 5 per cent. solution, - - - -	20,0

(b) Methylene blue.—The sections are placed for five to ten minutes in a 2.5 per cent. solution of oxide of iron and sulphate of ammonium. They are then washed out for ten minutes, and left from twelve to eighteen hours in the following mixture:—

Solution of phenol, 2 per cent., - - -	15
Alkaline solution of methylene blue, - - -	1-2

The latter ingredient is composed as follows:—

Methylene blue, - - - -	1
Carbonate of potash, - - - -	1
Distilled water, - - - -	100

This solution must be boiled for five minutes.

The staining solutions should be mixed only a short time before use.

After the excess of water has been removed by filter paper, the preparations are transferred to xylol-alcohol (xylol 3, alcohol 2), then to xylol, and finally mounted in balsam. If it should be necessary to decolourise, it may be done by Unna's alum-aniline.

*Von Lenhossék's method for the pigment in the cells of the spinal ganglia—*

1. Paraffin sections are prepared in the usual way.
2. They are fixed upon the slide, and left over night in a concentrated alcoholic solution of aniline blue.
3. They are washed out, and differentiated in absolute alcohol. Only the pigment retains the deep blue or almost black aniline stain.

4. To show the outlines of the cell, they are counterstained with eosin or erythrosin.

The cells of the human spinal ganglia are deeply pigmented,



the amount of pigment increasing with age. According to von Lenhossék, they are not stained by basic aniline dyes.

*Roncoroni's method for the nerve cells—*

1. Small pieces, about  $\frac{1}{2}$  cm. in size, are hardened in a mixture of equal parts of Müller's fluid and a 0.8 per cent. solution of platinum subchloride.

2. The fluid is changed five hours afterwards, and again at the end of the first, second, and third days. The pieces may now be bisected or divided into four.

3. After five or six days they are transferred to a simple 0.8 per cent. solution of platinum subchloride, in which they are left for one or two days.

4. They are washed in water for half an hour, transferred to alcohol, and embedded in celloidin.

5. The sections are stained from twenty to thirty hours in alum hæmatoxylin.

6. They are washed for twenty-four hours, and differentiated as in Pal's modified method.

7. They are washed out, dehydrated, and transferred to xylol. They are then mounted in Canada balsam.

As all the nerve cells are stained, the sections, on account of the number of these cells, must not be more than 5 to 10  $\mu$  in thickness. Otherwise the microscopic image will be confused.

The alum-hæmatoxylin solution is prepared as follows:—To 150 gm. of a hot saturated solution of alum, there are added, after the fluid has been cooled and filtered, 5 drops of a 1 per cent. solution of lithium carbonate, and 1 gm. of hæmatoxylin, dissolved in 10 gm. of absolute alcohol. The solution is ready for use in twenty days.

The sections are differentiated in a 0.1 per cent. solution of permanganate of potash, at a temperature of about 30° C. They are then transferred for two seconds to a solution of equal parts of oxalic acid (0.2 per cent.) and sulphide of potash (0.2 per cent.) in 100 parts of water, and afterwards for ten minutes to an hour to a 1 per cent. solution of lithium carbonate.

The protoplasmic processes of Purkinje's cells are stained of a coffee-brown colour, and Purkinje's cells themselves blue. The nuclei of the neuroglia, and the axis-cylinders, are intensely blue.

In order to stain the nerve cells in contrast to the neuroglia, Rehm's method, as recommended by Goodall, may be employed.

*Rehm's method—*

1. The tissues are hardened in 96 per cent. alcohol, and afterwards in absolute alcohol.

2. They are fastened on cork, with or without embedding in celloidin. They are then cut.

3. The sections are stained for at most half a minute in a warm solution of methylene blue (0.1 per cent.).

4. They are differentiated in 96 per cent. alcohol. The nerve cells are sharply defined by the stain, as the observer may even at this stage convince himself with the aid of the microscope.

5. The sections are transferred for a quarter of an hour to half an hour to a 0.1 per cent. solution of fuchsin in 96 per cent. alcohol.

6. They are washed for about a minute in alcohol, until the red clouds of stain cease to be given off.

7. They are transferred to oil of cloves, thoroughly dried with blotting paper, and mounted.

In a successful preparation the nerve cells are blue or bluish red, and the neuroglia deep red.

In normal cases the nuclei of the nerve cells are unstained, but the nucleoli are blue. In pathological cases red bodies are often to be seen in the nuclei of the nerve cells.

It may be pointed out that, in comparison with the time required for the action of the fuchsin, the staining with methylene blue is of extremely short duration. It must also be noted that, while oil of cloves extracts fuchsin, oil of origanum extracts methylene blue. Therefore, if a section is too blue, it should be treated for a short time with oil of origanum; if too red, with oil of cloves.

*Azonlay's method—*

1. The tissues are hardened in Müller's fluid. They are then embedded and cut.

2. The sections are washed out, and stained on the slide for two or three minutes with a  $\frac{1}{2}$  per cent. solution of vanadate of ammonium.

3. They are washed out with a few drops of distilled water.

4. A few drops of a 2 $\frac{1}{2}$  per cent. solution of tannin are poured over them, and left for two or three minutes.

5. They are washed out as in process (3).



The steps from (2) to (5) are repeated as often as may be necessary to produce a greenish-black appearance of the nerve cells and axis-cylinders. The usual further treatment with alcohol, &c., follows.

*Kronthal's method—*

A very good view of the nerve cells may be obtained, without hardening, if the process recommended by Kronthal be employed. A particle of grey matter from the cerebral cortex or the spinal cord is teased upon a cover-glass, and gently compressed by laying another cover-glass on the top. The two glasses are then slid apart, exactly as in staining for micro-organisms, and the dried grey matter is stained with one or two drops of a watery solution of methylene blue (0.5 per cent.). This is poured off in about a minute, and after the preparation is dry, it is mounted in balsam on the slide.

Besides the nerve cells, the axis-cylinders and the nuclei of the neuroglia are stained when this method is employed.

*Rosin's method of staining—*

Different combinations are employed, according as sections treated by alcohol alone, or those which have been embedded in celloidin, have to be stained. The Ehrlich-Biondi mixture, indeed, is adapted for either case. It is a re-agent composed of acid fuchsin, methyl orange, and methyl green, the two first ingredients being acid and the last a base. The mixture itself has a neutral re-action. The different elements of the tissues select from this fluid, as the case may be, the basic or one of the acid ingredients. Rosin therefore takes the view that they contain acidophil, basophil, or even neutrophil substances.

For sections without celloidin he employs the following:—

*Solution A.*

Ehrlich-Biondi mixture,	-	-	-	0,4
Distilled water,	-	-	-	100,0
0.5 per cent. solution of acid fuchsin,	-	-	-	7,0

The sections should be stained for five minutes.

For celloidin sections he employs—

*Solution B.*

Solution A,	-	-	-	-	4,0
0.5 per cent. solution of acid fuchsin,	-	-	-	-	1,0

1. The sections should be stained for one minute.

The after-treatment is the same in both cases, and is as follows:—

2. The sections are rapidly washed in distilled water in two watch-glasses (one or two minutes).

3. They are washed out *for ten seconds* in solution of acetic acid (one drop of the glacial acid to 100 ccm. of distilled water).

4. They are replaced for a minute in distilled water, to remove the acetic acid.

5. They are transferred to absolute alcohol, where they remain until the violet colour is no longer given off (two or three minutes).

6. They are transferred to xylol, and mounted in balsam.

The difficulty of obtaining good preparations is perhaps increased by the rapidity of the third step in this process. A few seconds more or less may make success doubtful. The method, however, seems to be really useful, especially for an organ so complicated as the retina.

The preparations remain good for years. The staining fluid, however, does not retain its properties for more than three months.

Celloidin sections, after being removed from alcohol, should be placed in water for a minute or two before they are transferred to the stain.

Rosin states that his method possesses the following advantages:—

1. Degenerations are sharply marked out.

2. Extravasations of blood are well seen; and new-formed blood vessels are to be recognised by the purple colour of the wall of the vessel.

3. An increase in the nuclei is distinctly shown by the bluish-green stain they take on.



4. In alcohol preparations, the structure of the nerve cells and of the nuclei is clearly seen.
5. The nerve cells and the cells of the neuroglia are easily distinguishable (though the fibres proceeding from them admittedly are not).
6. Exudations into the central canal are to be recognised by the red stain imparted to their albumen.

WEIGERT'S METHOD OF DEMONSTRATING KARYOKINESIS IN THE  
CENTRAL NERVOUS SYSTEM.

1. The tissues are hardened in 96 per cent. alcohol, as in Nissl's method.
2. The sections, which should be as thin as possible, are placed for half an hour in Rademacher's tincture of iron.
3. Their surface is rinsed in water.
4. They are stained for half an hour in the following solution:—

Hæmatoxylin,	-	-	-	-	-	1,0
Absolute alcohol,	-	-	-	-	-	10,0
Distilled water,	-	-	-	-	-	100,0

5. They are washed, and then differentiated for a short time in acidulated alcohol (hydrochloric acid 1,0, alcohol (70 per cent.) 100,0).
6. They are placed for ten minutes in water. They are then transferred to alcohol, and afterwards to oil. Finally they are mounted in balsam.

*Demonstration of the mitoses in the embryonic nervous system.*

*A. Merk's method—*

1. The pieces are fixed in 12 cmm. of the following fluid, in which they should be left for one or two days:—

Solution of chromic acid (2 per cent.),	-	-	-	-	7,5
Glacial acetic acid,	-	-	-	-	1,0
Water,	-	-	-	-	3,5

This is mixed shortly before use with  
Solution of osmic acid (1 per cent.), - 8,0

2. The sections are stained in a solution of safranin in 33 per cent. alcohol. They should be left from twelve to twenty-four hours.
3. They are decolourised in alcohol acidulated with hydrochloric acid.

*B. Altmann's method—*

1. The pieces are fixed in nitric acid (sp. gr. 1.02). In this they remain for three or four hours, and are afterwards transferred to alcohol, where they may be left as long as is desired.
2. They are overstained in hæmatoxylin.
3. They are decolourised in alcohol acidulated with hydrochloric acid. Ziehen states that Altmann's method may be used not only in this way, but even if the object (for example, the human embryo) has not been previously treated with nitric acid.

*GOLGI'S METHOD (the chromate of silver method of 1873).<sup>1</sup>*

The exceptional position which this method has attained requires that it should be separately discussed. This is the more necessary, as the "stain," so far as it can be regarded as a stain at all, brings into prominence the most different elements of the nervous system, whether they be, or be not, nervous elements. It is impossible, therefore, to classify along with others this remarkable method, which is on the one hand non-selective, and on the other selective in a manner which is not subject to a definite rule.

To commence with the technical procedure, it is as follows:—<sup>2</sup>

Pieces of the fresh nervous system, which should be as small as possible (2-8 mm.), are at once placed in the following solution:—

Solution of bichromate of potash	-	-	-	-	4 parts.
(3 per cent.),	-	-	-	-	1 part.
Solution of osmic acid (1 per cent.),	-	-	-	-	1 part.

<sup>1</sup>Cf. C. Weigert: Merkel Bonnet, *Ergebnisse der Anatomie und Entwicklungsgeschichte*. 1896. Vol. v. Article on "Technik."  
<sup>2</sup>Throughout the process, metal needles must be replaced by small rods of glass or horn.



In this they must be left, in the dark, from two to eight days. The length of time depends upon the element which is to be demonstrated. Von Lenhossék states that for the neuroglia two to three days are necessary, for the nerve cells three to five days, and for the nerve fibres five to seven days.

The solution must always be employed in a fresh state, and in large quantities (10 to 50 times the volume of the pieces). Fluid which has been once used, however, may still serve to rinse fresh pieces of tissue, or in the performance of the "double method."

The pieces are quickly rinsed in water, or in silver solution which has been already used. They are then transferred to the solution of silver nitrate (0.6 to 1 per cent.), to which it is superfluous to add acetic acid. This solution may be used even when it is a few weeks old, but it must be kept in the dark. Like the former, it is also to be used in large quantities. It is best to suspend the pieces in the fluid by means of a thread attached to the cork.

After from two to six days, they may either be cut at once, or previously undergo further hardening (from a quarter of an hour to half an hour) in 96 per cent. or in absolute alcohol, which must be free from chlorides. The operator should convince himself, by making small incisions, that the fluid has produced a sufficient re-action in the deeper parts. If it has not, the pieces should be returned for one to three days to the bichromate and osmic acid solution, and then replaced in the solution of silver nitrate.

The pieces may be cut either by hand, in which case they are clamped between bits of hardened liver, or they may be rapidly embedded in celloidin, a process which may last from five to thirty minutes. In the latter case they are transferred for a short time to 80 per cent. alcohol and then cut. The sections are placed for a moment in absolute alcohol, and then in oil of bergamot. Finally they are dried with blotting paper on the slide, and then mounted in xylol-dammar, which is allowed to dry in the incubator at a temperature of 40° C. No cover-glass is to be applied. If the sections have been

mounted upon the cover-glass this must be fixed to the slide by two slips of glass, so applied as to allow air to pass between the slide and the dammar which carries the preparation.

This is the so-called "rapid method of Golgi," and has been attributed to Ramón y Cajal. It first appears, however, in Golgi's work, published in 1885—"Sulla fina anatomia degli organi del sistema nervosa centrale."

This method, now in such general use, is the third of those published by Golgi. It is remarkable that both Weigert and Golgi, to whom we owe two of the most important in the whole range of methods of staining, those, namely, for the medullary sheaths and for the nerve cells, should have attained their object only with the third method published by them.

Briefly, Golgi's two first methods involve the following procedures:—

1. The "slow" method (1873)—

Small pieces, which must be as fresh as possible, were hardened in a solution of bichromate of potash, commencing with a 2 per cent. and increasing in strength up to a 5 per cent. solution. According to the temperature, from fifteen to forty-five days were required; in the incubator (which Weigert recommended), eight to ten days were sufficient. The pieces were then placed for a few days in a 0.75 per cent. solution of silver nitrate, or for two or three weeks in a solution of corrosive sublimate (0.25–0.5 per cent.) The latter had to be changed every day, and pieces, at first brownish red, were externally decolourised by its action.

2. The "intermediate" method (1880) is distinguished from that at present in use only in this, that the process of hardening by bichromate and osmic acid is carried out in two separate stages. The pieces are first left from four to five days only in a 2 per cent. solution of bichromate of potash, and are then transferred to the mixture of 2 per cent. bichromate solution (8 parts) and 1 per cent. solution of osmic acid (1 part). In this they remain from twenty-four to thirty hours. The treatment with nitrate of silver afterwards follows, as detailed above.



According as nitrate of silver or perchloride of mercury is employed, the methods are known as the silver method and the sublimate method. It will be seen, however, from what has gone before, that in both cases the technical procedure is almost identical.

Finally, Golgi's recent modification of his sublimate method has still to be mentioned.

*Golgi's modification of his sublimate method—*

1. The tissues are hardened and fixed as before (in Müller's fluid and sublimate).

2. They are cut, and the sections are washed out in distilled water.

3. They are left for two minutes or longer in a gold fixing bath, in which they become black.

4. They are washed out in distilled water for a very long time, then transferred to alcohol and oil of cloves, and mounted in balsam.

The gold fixing bath is that which photographers employ in preparing prints upon aristo paper.

As the result of these manipulations, the nerve cells and their processes, and the cells and fibres of the neuroglia, are stained black or blackish brown, and are presented as a connected whole with a distinctness unattainable by any other method. As von Lenhossék puts it, in successful preparations "fibres and cells are presented to the eye in their entirety. The former may be traced to the terminal dendron, and the latter show the entire extent of their protoplasmic processes and their nerve process, exactly as when the method of isolation has been successfully employed."

It is natural that a method which, like Golgi's, has opened out new lines of investigation, and has led, within comparatively few years, to the most amazing results, should already have undergone an immense number of modifications. This will the more easily be understood if we reflect that there exist in the method a large number of inconveniences which it is the ardent endeavour of the investigator to eliminate.

Only a few of the numbers of modifications need be mentioned here.

*Obregia's modification—*

1. The preliminary treatment is the same as in Golgi's method (silver or sublimate preparations).

2. The sections are placed in absolute or 96 per cent. alcohol, and afterwards for half an hour in the following solution, which must be freshly prepared:—

Solution of chloride of gold (1 per cent.),	8-10 drops.
Absolute alcohol,	10 ccm.

The solution should at first be exposed to diffuse daylight, but after the sections are placed in it, it must be kept in the dark.

3. The sections are rapidly rinsed in 50 per cent. alcohol and in water.

4. They are transferred for five or ten minutes to a 10 per cent. solution of sulphite of soda.

5. They are thoroughly washed out in water.

6. If it be desired, they may now be counterstained with carmine hæmatoxylin, or by Weigert's process.

7. They are transferred to alcohol, then to creosote or xylol, and mounted in balsam. A cover-glass is used.

The advantages of this modification are that it permits of counterstaining and of the employment of a cover-glass.

*Flehsig's modification—*

1. The tissues are hardened in a 2 per cent. bichromate solution.

2. They are impregnated with sublimate.

3. The sections are placed in 96 per cent. alcohol.

4. They are stained for from three to eight days, at a temperature of 35° C., in the following solution:—

Pure extract of Japanese red-wood,	1,0
Absolute alcohol,	10,0
Distilled water,	100,0
Saturated solution of Glauber's salt,	5,0
Saturated solution of tartaric acid,	5,0

5. The sections are separately transferred, each to 3 ccm. of



a 0.25 per cent. solution of permanganate of potash, in which they are left until it has lost its bluish tint.

6. They are decolourised in the following solution:—

Oxalic acid, - - - - -	1,0
Sulphite of potash, - - - - -	1,0
Distilled water, - - - - -	200,0

Both (5) and (6) are repeated until the section is no longer yellow.

7. The sections are transferred to the following mixture:—

Solution of chloride of gold and potassium (1 per cent.), - - - - -	5 drops.
Absolute alcohol, - - - - -	20,0

in which they are left until the precipitates of sublimate, which appear white by reflected light, have become deep black, and the bundles of nerve fibres, at first red, have taken on a bluish tint.

8. They are rapidly washed out in the following fluid:—

Solution of cyanide of potash (5 per cent.),	1 drop.
Distilled water, - - - - -	20,0

The section must float upon the surface.

9. They are transferred to absolute alcohol, then to oil of lavender, and are finally mounted in balsam.

All the nerve fibres appear carmine-red, and the nerve cells and their processes deep black.

*Ziehen's method—*

1. The fresh pieces of tissues are hardened, without treatment by bichromate, in the following solution:—

Solution of chloride of gold (1 per cent.),	} Equal parts.
Solution of corrosive sublimate (1 per cent.),	

They are kept in this fluid, which must be frequently changed, from three weeks to five months, when they become of a reddish-brown colour.

2. They are fixed upon cork without embedding, and cut under alcohol.

3. They are transferred to Lugol's solution, diluted with four volumes of water, or to tincture of iodine, diluted with four volumes of alcohol. In this they remain for a time

varying with the thickness of the section. The body of the cell ought to be translucent, and of a bluish-black colour.

4. They are transferred to absolute alcohol and oil of cloves, and mounted in balsam.

The cells and their processes, and the fibres, all appear bluish grey. They are stained in greater numbers than if Golgi's method be used. Ziehen states also that his method has this advantage over Golgi's, that, apart from the greater permanence of his preparations, the medullated fibres are stained.

According to *Cox*, hardening and staining with sublimate may be carried on simultaneously, if small pieces of the cortex be left from two to five months in the following fluid:—

Solution of bichromate of potash (5 per cent.),	20,0
Sublimate solution (5 per cent.), - - - - -	20,0
Solution of chromate of potash (5 per cent.), -	16,0
Distilled water, - - - - -	40,0

The bichromate solution must not be added until after the fluid has been diluted by the addition of the water. The pieces are afterwards treated with ammonia or other alkalies. Impregnation takes place only if the re-action of the hardening fluid is as feebly acid as possible. It depends upon the formation of an oxide of mercury, which yields a black mercurio-amide in combination with ammonia.

The pieces must be cut with the freezing microtome, as alcohol does them too much damage.

The sections are placed for one or two hours in a 5 per cent. solution of carbonate of soda, or in a solution of ammonia. They are washed out, rapidly dehydrated, and cleared with oil, which must be removed by filter paper. They are then covered with a thin layer of a rapidly-drying resin, such as the following:—

Resina sandaraca (P.G.), - - - - -	75 parts.
Camphor, - - - - -	15 "
Turpentine, - - - - -	30 "
Oil of lavender, - - - - -	22.5 "
Absolute alcohol, - - - - -	75 "
Castor oil, - - - - -	5—10 drops.



The great importance of Golgi's method is proved by the number of modifications which it has already undergone; but they also clearly prove the imperfections inherent in it. These imperfections are the following:—Owing to the intensely black staining of the cells and the branches proceeding from them, it is impossible to investigate the finer structural detail of the elements thus impregnated. Further, the capriciousness of the method, the precipitated deposits on the sections, and their defective durability, constitute additional imperfections. Lastly, the use of cover-glasses in mounting is attended with difficulty.

The first of these objections may be overcome by the use of the other methods of staining, by which we can now so freely examine the detail of the structure of the tissue elements.

Ramón y Cajal has shown that the stain is less capricious in the case of the brains of young animals or of embryos, in which it is much more certainly successful than in the adult brain. The addition of formic acid to the silver solution has proved to be superfluous. On the other hand, the employment of the "double (or triple) method" introduced by Cajal produces a remarkable improvement in the results.

Up to the present time no satisfactory means of dealing with the deposits has been found, and it has proved equally impossible to render the preparations permanent. They often readily decompose.

Tal used sulphide of soda: Greppin treated the sections for 30 or 40 seconds with a 10 per cent. solution of hydrobromic acid, in which they remained till they became white. He then washed them out and mounted them. The results were even better, if the process was carried out in direct sunlight.

Greppin also combined the method for staining the medullary sheaths with Golgi's method, placing the sections (before or after treatment with hydrobromic acid) for twenty-four hours in a  $\frac{1}{2}$  per cent. solution of chromic acid. The further treatment was as usual.

Held, and later Obregia, employed, as is done in photography, five drops of a 1 per cent. solution of chloride of gold and potassium in twenty grm. of absolute alcohol.

On account of the price, the employment of osmic acid is

objectionable. But its value has been over-estimated, as Weigert found some time ago that it was quite unnecessary for the success of the impregnation, which could be quite well attained if the tissues were treated simply with formol and the salts of chromic acid. Held, indeed, obtained good results without formol, by the use of these salts alone. In the use of this method, the perfection of the stain appears to depend particularly upon the absolute freshness of the material employed, although in isolated cases good results are reported where the material has not been quite fresh. Golgi has been successful, especially if the weather were cold, with tissues treated two days after death.

It may be seen from these two points—that in many cases excellent results may be obtained without the use of osmic acid, and that the material may in certain circumstances be used even when it is not quite fresh—how far we still are from an explanation of the secret of the chromate of silver method. The experiments of Flatau and those of Kopsch, among others, have proved the truth of the latter assertion. In the case of material which was from twenty-four to forty-eight hours old, Kopsch employed formol for the "rapid method" of impregnation. The following is the process:—

1. The pieces are hardened for twenty-four hours in a solution of formol and bichromate of potash (formol 10,0, solution of bichromate of potash (3.5 per cent.), 40,0). The ingredients should be mixed shortly before use.

2. They are transferred to a 3.5 per cent. solution of bichromate of potash for three to six days, and thereafter to the silver solution. The consistency of the tissue is well adapted for cutting, and the deposits are not very numerous.

The sublimate method, in Cox's modification given above, was strongly recommended by W. Krause. Along with the "rapid method" of Golgi (Golgi and Cajal), it is that at present most in use, for the reason that a larger number of the tissue elements are impregnated, and that, as Lenhossék states, the sections may afterwards be stained in other ways, with alum-carmin, for example.

The advantages of Golgi's method have been already mentioned. It is much to be wished that the method could be so developed, without detriment to these advantages, as to



be serviceable for pathological purposes. Although Flatau's experiments in this direction have resulted in very beautiful preparations, the length of time required (a year) makes his method unavailable.

A large number of theories has been formed to explain the real inner process of the stain—why the tissue-elements should be impregnated so capriciously, on the one hand so completely, on the other hand according to no definite rule. According to Weigert, however, we must be content to accept the view that the great irregularity in the impregnation of the elements of the central nervous system depends upon an irregularity of the penetration of the fluids required. As bichromate of potash is known to penetrate very regularly, the silver or the mercury employed must be held responsible for this irregularity. If we wish to understand the method, we can only accept the statement of Weigert, "that the elements adapted for impregnation by Golgi's method possess the faculty of producing in their substance a very fine and peculiar precipitate of chromate of silver, when treated by one or other of the methods discovered by Golgi."

#### EHRlich's INTRA VITAM METHYLENE BLUE METHOD.

The method of staining living nervous tissue by subcutaneous injection of methylene blue, initiated by Ehrlich in 1886, forms an important step in advance, the range of which cannot even yet be completely determined.

As in the case of many important ideas, the method of carrying it into operation could not at first be otherwise than inadequate, and just as was the case with the equally important discovery of Golgi, years passed before the method came at all into general use. Interest in the matter, however, was never entirely lost. From the first papers on this subject (those of Hans Aronsohn) until the present day, it has been more and more widely recognised that we have here to do with a discovery as original as the happy completion in Golgi's method of the results we have already detailed.

There is a number of names associated with this *intra vitam* method, and of the authors who have written upon the subject, each has contributed more or less to the elimination of its difficulties. There were two of these, in particular, which it seemed at first impossible to overcome, namely, the fixing of the tissue, and the preparation of sections. Now, however, both of them have been so far vanquished, thanks to the untiring labour of workers like Dogiel and Bethe. Many and various methods and modifications have been published, but, as in Golgi's method, it is not advisable here to give all, or even any large number, of them. Such a step would not add to the clearness of the description. The author, therefore, begs to be allowed to give too little, in place of confusing the issue by giving too much. He intends to describe the method as it has taken shape in the last publications of Bethe. It is to him that we owe the discovery that methylene blue forms with molybdic acid a compound insoluble in alcohol, and it is he, in consequence, who has satisfied the important postulate that sections must be preparable from tissues treated in this manner. We have, on the other hand, to thank Dogiel for the method of fixing by means of picrate of ammonia.

#### *Bethe's method of intra vitam staining with methylene blue—*

1. A subcutaneous injection of a saturated solution (saturated at 37° C.) of methylene blue BX. is employed. Of this, doses of 2 ccm. are given at intervals of from a quarter of an hour to half an hour. The animal dies after from three to six injections.

2. The pieces, which should be as small as possible, are fixed, to begin with, for ten to fifteen minutes in a concentrated watery solution of picrate of ammonia, in which they are left till a violet colouration appears.

3. They are not rinsed, but are definitely fixed in one of the solutions of ammonium molybdate given below, or in a solution of phosphomolybdate of soda. The process lasts from one to twelve hours.



4. The sections are washed out in water, dehydrated in alcohol, transferred to xylol, and mounted in xylol balsam; or the pieces may be embedded in paraffin.

5. If it be desired, they may be counterstained with alum-carmine, alum-cochineal, or the neutral aniline colours.

This is an outline of the external process.

For fixing (procedure No. 3) Bethe recommends one of the following solutions, or others varied upon similar lines:—

I. Ammonium molybdate, - - -	1,0
Distilled water, - - -	20,0
Hydrochloric acid (P.G.), <sup>1</sup> - - -	1 drop
II. Ammonium molybdate, - - -	1,0
Distilled water, - - -	10,0
Solution of chromic acid (2 per cent.),	10,0
Hydrochloric acid, - - -	1 drop
III. Ammonium molybdate, - - -	1,0
Distilled water, - - -	10,0
Solution of osmic acid ( $\frac{1}{2}$ per cent.),	10,0
Hydrochloric acid, - - -	1 drop
IV. Phosphomolybdate of soda, - - -	1,0
Distilled water, - - -	20,0
Hydrochloric acid, - - -	1 drop
V. Phosphomolybdate of soda, - - -	1,0
Distilled water, - - -	10,0
Solution of chromic acid (2 per cent.),	10,0
Hydrochloric acid, - - -	1 drop
VI. Phosphomolybdate of soda, - - -	1,0
Distilled water, - - -	10,0
Solution of osmic acid ( $\frac{1}{2}$ per cent.),	10,0
Hydrochloric acid, - - -	1 drop

Bethe's object in this method of fixing was, first, to do away with the process of freezing which had previously been necessary, and secondly, to obtain certain results in the case even of objects rebellious to treatment. It suggested itself to him, and it was found to be desirable, "to make at first a readily soluble compound of the methylene blue,

<sup>1</sup>Sp. gr., 1.124. *Tr.*

which always gives an even result, and afterwards to convert this into a compound soluble with difficulty." The picrate of ammonia serves this purpose, forming with methylene blue a compound almost insoluble in water, but readily soluble in alcohol. If treated with ammonium molybdate, this compound is converted, even without the aid of heat, into a molybdate, and the process is more rapid if the solution of ammonium molybdate be strongly acid. The same is true of the conversion of the picrate of methylene blue into a phosphomolybdate.

Bethe prefers, as a rule, the first three methods of fixing, the others not being so resistant to the action of alcohol. The third and sixth formulæ are adapted only for sections, and for thin preparations of an entire organ. They are the best fixatives. The time required for the process of fixing depends upon the size of the object; as a rule from three quarters of an hour to an hour is sufficient. In the case of the third and sixth formulæ it is advisable to give from four to twelve hours in order that the osmic acid may produce a sufficient darkening.

The ammonium molybdate or phosphomolybdate of soda are dissolved in water, with the aid of heat, applied until no turbidity remains. When hydrochloric acid is added to the former solution white clouds of free molybdic acid are formed, and redissolve upon shaking, forming the acid salt. When hydrochloric acid is added to the solution of phosphomolybdate of soda a yellow discolouration appears, due to the formation of free phosphomolybdic acid. On shaking, the colour disappears, and the acid salt is formed.

This *intra vitam* method gives very good results in the adult animal. In contrast to Golgi's method, the neuroglia is not stained, and only nervous tissue is to be seen. The success of the staining depends upon the quantity of colouring matter, or, in other words, on the time the animal remains alive. It is therefore advisable, according to S. Meyer, that the solutions should be as strong as possible (5 to 6 per cent.).



Ramón y Cajal has somewhat modified the method of Ehrlich and Dogiel, in order to demonstrate the collaterals, and he has succeeded in confirming the observations which he made with Golgi's method. His process is as follows:—

1. Thin pieces of the fresh brain of a rabbit are painted, by means of a camel's-hair brush, with a saturated solution of B. Grüber's methylene blue (or powdered methylene blue is dusted over them). In three quarters of an hour they are quickly washed in weak salt solution.

2. They are fixed in the following solution:—

Ammonium molybdate,	-	-	-	10,0
Distilled water,	-	-	-	100,0
Hydrochloric acid,	-	-	-	10 drops

The process lasts from two to three hours.

3. The excess of ammonium molybdate is removed by washing in water, and the pieces are hardened for from three to four hours in the following solution:—

Formol,	-	-	-	-	40,0
Distilled water,	-	-	-	-	60,0
Solution of chloride of platinum (1 per cent.),	-	-	-	-	5,0

4. They are quickly washed out to extract the formol, placed for a few minutes in an alcoholic solution of chloride of platinum ( $\frac{1}{3}$  per cent.), and embedded in paraffin.

5. The sections, which should be thick, are dehydrated in absolute alcohol, to which chloride of platinum ( $\frac{1}{3}$  per cent.) has been added. They are transferred to xylol, and mounted in balsam.

The chloride of platinum is said in the first place to act as a fixative, and, secondly, to increase the insolubility of the compound formed by the molybdate of ammonia with the methylene blue, so that it cannot be acted upon by water, formol, alcohol, &c.

The important question, why the nerves stain in methylene blue, has been answered by the experiments of Ehrlich himself. He finds that the chemical peculiarity of this substance, namely, the sulphur group which it contains, determines the staining of the nerves.

As in the re-action occurring in Golgi's method, we find in Ehrlich's that in the most successful preparations only a portion of the nerve-fibres is to be seen, and that they are apparently stained in a quite unselective manner. Even in his first publication, Ehrlich endeavoured to find the explanation, and he finally adopted the view that the re-action of the nervous system to methylene blue depends upon two conditions—saturation with oxygen and an alkaline re-action. It may be pointed out that, as a matter of fact, the cerebral cortex must contain substances of an alkaline re-action, for Liebreich and Langgendorf found that fresh pieces of the cortex turned litmus blue. But Lieberkühn and Edinger, using an infusion of alizarin, noticed that the brain became yellow after the introduction of a violet soda compound, and referred this to an acid re-action of the cortex. One must, therefore, admit the existence of substances, some of which have an acid, and some an alkaline re-action. If we further admit the existence of substances with a neutral re-action, we come with Ehrlich to the conception that in the nervous system there are, according to the degree of functional activity, various degrees of alkalinity, which, in conjunction with the changes in the amount of oxygen, determine what foreign bodies may be taken up in the special parts of the nervous system.

#### ADDENDUM.—THE TREATMENT OF THE RETINA.

It is perhaps of service to insert in this place a section dealing specially with the staining of the retina, an organ in many ways the most marvellous of the entire body.

In no part of the central nervous system, perhaps, have so many important researches and discoveries of a general anatomical and physiological nature been made; in no part, perhaps, have the two most important methods, those of Golgi and Ehrlich, within the last ten years proved themselves of such service, as in this, the only free-lying part of the central nervous system, no other part of which is so easily obtained in a fresh condition.

It will be understood that I do not make any claim to give in this place a complete description of the methods of dealing with the retina. It is not my intention to do so, nor would it be to the purpose. It would scarcely be possible to do so without going more deeply into the microscopic anatomy of



so complicated an organ. Almost every animal has its own peculiarities, and new researches are daily bringing us new knowledge and new results. Hardly in any other organ are the views of the workers so conflicting as in the case of the retina.

In order to obtain a first survey of the structure of the retina, Ranvier recommends that the research should commence with *Triton cristatus*, an animal in which the layer of visual cells and other peculiarities are distinctly seen. Both the eyes of the animal are removed, and one is placed in a little bottle closed by a cork, and containing about 1 ccm. of a 1 per cent. solution of osmic acid. The eye is left in the fluid for twenty-four hours, and then cut across the equator and macerated in water for two or three days. A piece of retina is then cut out, and teased upon the slide in a drop of water. The elements which have been thus isolated are stained with picro-carmin and mounted in glycerine. By the help of this method of isolation, the structure of the rods and cones, which float for the most part free and separate from the cells, may be distinctly recognised.

The other eye is exposed to the vapour of osmic acid, being fixed by means of a needle to the under surface of the cork, which is then replaced. The vapour of the acid readily penetrates the thin sclerotic, quickly reaches the retina, and fixes it, for the most part, within ten minutes. The eye is then transferred to one-third alcohol, and divided at the equator with a fine pair of scissors. After the lapse of a few hours, the posterior pole is placed for several hours in a 1 per cent. solution of picro-carmin, from which, for definitive fixing of the elements, it is transferred direct to the osmic acid solution. It is then washed out in water and further hardened in alcohol. Embedding follows, and also the preparation of sections, which must pass vertically through the optic nerve. The sections are received in alcohol, transferred to water, and mounted in glycerine.

Of the modern workers there are two in particular whose researches take an especially prominent position, Ramón y Cajal

and Dogiel. The former is to be noted for his very successful use of Golgi's chromate of silver method, and Dogiel for his special modification of Ehrlich's methylene blue stain.

Both methods, chromate of silver and methylene blue, are equally well adapted to the retina, as both permit of making either transverse or longitudinal sections. Among mammals, v. Lenhossék<sup>1</sup> recommends the albino rabbit as specially fitted for the study of the retina. In this animal the supporting cells of Müller are the most easily impregnated. In almost every case they are to be recognised in the form of thick parallel bars traversing the retina. After them the so-called bipolar cells and the "spongioblasts" of H. Müller are the next to be impregnated.

Ramón y Cajal,<sup>2</sup> to whom we owe an important work on the retina of vertebrates, gives therein the following technical hints (v. Lenhossék):—

The more delicate a retina is the less is it adapted for Golgi's method. The retina of large animals is therefore the most suitable. The eye is divided in the frontal plane, the vitreous humour is removed, and the retina is gently removed from the choroid and optic nerve by means of a forceps and a pair of scissors. In order to avoid the formation of superficial precipitates, Cajal now carefully rolls the retina into a small cylindrical or spherical mass (*procédé d'enroulement*), which he dips for a second into a thin solution of celloidin. He then leaves the little mass for a few seconds in the air, until the celloidin is half solid, and then places it in Golgi's fluid. In the case of the eyes of large animals only a portion of the retina is thus rolled up (Kallius expresses himself, however, as not completely satisfied with this process). The "double" method appears to v. Lenhossék to be especially useful for the retina. He carries it out as follows:—

1. The retina is laid for from twenty-four to forty-eight hours in Golgi's mixture.

<sup>1</sup> Von Lenhossék: *der feinere Bau des Nervensystems im Licht neuester Forschungen*, Berlin, 1895.

<sup>2</sup> Ramón y Cajal, *La rétine des vertébrés*. *La Cellule*, vol. ix., 1893.



2. It is impregnated for twenty-four hours in a 1 per cent. solution of silver nitrate.

3. It is again laid for from twenty-four to thirty-six hours in a weaker solution of osmic acid and bichromate (1 per cent. osmic acid, 1,0; 2.5 per cent. bichromate of potash, 10,0).

4. It is once more treated for twenty-four hours with the silver solution.

The process may sometimes be advantageously repeated a second time, thus making a "triple" out of the "double" method.

As the result of his researches, Kallius<sup>1</sup> considers it important for the success of the impregnation that the time during which the mixture of Golgi and Cajal is used should be carefully observed. He took retinæ in as fresh a state as possible, and allowed them to remain in the fluid for from twelve to seventy-two hours. In this way he often found that only cells of a special kind were stained. For example, after twelve hours it was frequently only the rods and cones and a few bipolar cells that were impregnated; after twelve hours other bipolar cells and the so-called spongioblasts. Then followed the ganglion cells of the optic nerve, later the nerve-fibres, and, when the ganglionic elements no longer stained well, the supporting cells were brought into view.

In opposition to van Gehuchten, he considered that it was not necessary to leave the retina for more than twenty-four hours in the solution of silver nitrate, but he thought it often advantageous to substitute bichromate of soda or ammonia for the bichromate of potash.

To obviate the precipitation of bichromate of silver, he covered the surface of the retina facing the vitreous humour with as thin as possible a layer of gelatine before placing it in the silver solution. He rejects Cajal's method of rolling up the retina, because it cannot afterwards be flattened out, and it is therefore impossible to prepare and examine a series of horizontal sections through its whole thickness.

<sup>1</sup> Kallius: Untersuchungen über die Netzhaut der Säugethiere. Merkel-Bonnet iii., 1894.

The retina is often somewhat curved or slightly rolled up. In order to obtain good transverse or horizontal sections it must then be quickly embedded between two blocks of celloidin, on each of which a flat surface has been cut. These surfaces are made sticky by dipping the blocks in ether-alcohol or collodion. Kallius then reduces the sections with hydrochinon in the manner described by him, and mounts them in balsam.

The process of Kallius depends upon a reduction of the silver, for which purpose the so-called "quintuple hydrochinon developer" of the photographers is employed. Its composition is as follows:—

Hydrochinon, - - - - -	5,0
Sulphite of soda, - - - - -	40,0
Carbonate of potash, - - - - -	7,5
Distilled water, - - - - -	250,0

Of this 20 gm. are added to 230 gm. of distilled water. Before use a small quantity of this fluid is mixed with a third to a half of its volume of alcohol, that no diffusion may take place out of the section saturated with alcohol. After several minutes the sections, which have become greyish black, are placed in 70 per cent. alcohol, then left for five minutes in a watery solution of sodium monosulphide (10 to 50 of distilled water), in which all the bichromate of silver is dissolved, but not the reduced metallic silver. Finally the sections are washed out in water for twenty-four hours, and counterstained as the operator may desire.

Kopsch<sup>1</sup> recommends that several pieces of retina should be laid in a vessel containing the bichromate solution, and that after two days one or two pieces should be daily transferred to the silver solution. On the second day the rods and cones, and Müller's fibres, are stained almost alone. The bipolar cells and spongioblasts take on the stain after from three to six days in bichromate.

Nissl's methylene blue stain, the thionin stain, and, above all, the Ehrlich-Dogiel method, are especially used for the nerve cells. Dogiel<sup>2</sup> himself is of opinion that in the retina the Ehrlich-Dogiel method completely replaces Nissl's. The retina must be laid upon the slide with the layer of nerve fibres facing the observer, so that it is always in connection with a

<sup>1</sup> See Kopsch's modification of Golgi's method.

<sup>2</sup> Dogiel. Die Structur der Nervenzellen der Retina. Arch. f. micr. Anat. 1895.



smaller or larger quantity of the vitreous humour, and the staining solution does not come into direct contact with it. According to Dogiel, the solution should be  $\frac{1}{10}$  to  $\frac{1}{18}$  per cent., and it operates sufficiently in from twenty to forty minutes. To determine the moment at which the preparation should be fixed, it must from time to time be examined, uncovered, with the low power, but the position of the retina upon the slide must undergo no further alteration. It must be fixed upon the slide by means of five or six drops of picrate of ammonia, and this process must occupy not more than three or four hours. Thereafter a few drops of a mixture of picrate of ammonia and glycerine are added to the picrate of ammonia, and the preparation is allowed to stand from eighteen to twenty hours. Finally it is mounted in the same solution.

For staining the retina with methylene blue, Apáthy<sup>1</sup> recommends medicinal methylene blue, free of chloride of zinc, of the strength of 1 to 1000 of normal salt solution. The retina is placed upon the slide with its inner side downwards, and covered with a thin layer of the vitreous humour. The methylene blue is then poured round it. The preparations must be prevented from drying. They are left for four or five hours, and the progress of the stain is from time to time observed under the microscope. For the purpose of fixing, Apáthy added a few drops of the strongest liquor ammoniæ to the picrate of ammonia, and he mounted the preparations in a solution of gum arabic and sugar, in which they keep better than in glycerine.

Prenant did not stain upon the slide, but injected the solution of methylene blue by means of a Pravaz syringe, direct into the posterior chamber, so that the jet of fluid fell upon the retina (if it be desired, some of the vitreous humour may previously be removed by the syringe). About two hours after the injection the eye was opened at its equator, and the retina was detached and spread out upon the slide until the blue stain made its appearance. It was fixed with Apáthy's modified picrate of ammonia, and mounted in glycerine.

<sup>1</sup> Apáthy, Methylenblau. Zeitschr. f. wissenschaftl. Micr., 1892, ix.

If we disregard the results of Golgi's and Ehrlich's methods as applied to the retina, the most conspicuously interesting question at the present day is the special staining of the rods and cones to show the action upon them of light and darkness. Very thorough researches on the subject are published in the works of Pergens, of the Institut Solvay, to which I therefore refer the ophthalmologist.

The majority of the important stains for the rest of the central nervous system are more or less adapted to, and employed for, the retina. These are Weigert's stain for the medullary sheaths, Nissl's and Held's stains for the nerve cells, the carmine and hæmatoxylin stains, the Ehrlich-Biondi mixture, and Rosin's method; Weigert's neuroglia stain does not yield any important results. Osmic acid or its vapour (Ranvier's method), picric acid, nitric acid, and all the modifications of bichromate of potash, are all in different ways serviceable in the examination of the retina.

Angelucci employs nitric acid in combination with Müller's fluid, fixing the retina for half an hour to an hour in a 3 per cent. solution of nitric acid, and washing it out for ten days in Müller's fluid, after which it is further hardened in alcohol.

Schaffer recommended, in the use of Weigert's stain for the medullary sheaths, that the celloidin sections, after cutting, should again be placed over night in a 1 per cent. solution of chromic acid, and, after a brief dehydration, stained for twenty hours in Kulschitzky's solution of acetic acid hæmatoxylin. They should then be differentiated for twelve hours in Weigert's solution of borax and ferricyanide of potash, after which they should be rinsed and mounted. The cones appear quite dark, almost black, while the rods and the external nuclear layer are light brown.

Flesch also used a modified Weigert's stain, and found that it gave excellent results in the layer of rods and cones, the external segments of which took on a very dark violet colour.

*It is to be noted in general that the outer and inner segments of the rods behave differently to the majority of the stains.*



In the case of carmine, for example, the inner segments alone are stained, and that very deeply, so that the outer segments are sharply demarcated. On the other hand, osmic acid stains the outer segments black, while the inner are unstained (at least in the majority of amphibia).

The process used by Birnbacher as a colour re-action for the retina after exposure to light, or after being kept in the dark, must still be mentioned.

1. The eye is fixed in 3.5 per cent. nitric acid (or, in the dark, from six to twenty-four hours in a concentrated solution of corrosive sublimate).

2. It is divided at the equator, and the posterior section is washed in running water for six hours.

3. It is hardened in alcohol of increasing concentration (50, 70, 80, 90, 96, and 99.8 per cent.).

4. The retina is removed from the hinder section, divided, embedded in celloidin, and cut in a direction perpendicular to the surface.

5. It is stained in a concentrated alcoholic solution of eosin-extra-yellow, and must be overstained for half an hour.

6. It is decolourised in 96 per cent. alcohol, which must be frequently changed. The sections appear of a bluish pink. They are passed through oil of cloves, and mounted in dammar.

Of the combinations of stains, Ehrlich-Biondi's (Biondi-Heidenhain's) gives specially good results. If the retina has been exposed to light the cones become green; if it has not, they are yellow.

#### B.—STAINING OF THE MEDULLARY SHEATHS.

Of all the methods of demonstrating the medullary sheaths, the hæmatoxylin stain of Carl Weigert (1884), which has become classic, is the chief.

Twice before the publication of this method Weigert attempted to demonstrate the sheaths of medullated nerves. On the first occasion (1882) he used acid fuchsin, on the

second ordinary fuchsin, followed by differentiation in hydrochloric acid. It was only on the third attempt that he employed colouring matters which formed a lake, *i.e.*, which formed typical compounds with metallic salts.

This method, which, like that of Golgi, proves the change it heralds by the extraordinary number of modifications it has undergone, involves the following steps:—

1. The pieces are hardened in Müller's or Erlitzky's fluid.

2. They are transferred direct to alcohol, and afterwards embedded in celloidin.

3. The pieces, or the individual sections, are treated with a saturated solution of acetate of copper, diluted with one volume of water. They must be left in this solution, in the thermostat, for twenty-four hours.

4. Section is performed under alcohol, and the sections are stained for a period varying from twenty minutes to twenty-four hours in the following solution:—

Hæmatoxylin,	-	-	-	-	-	1,0
Absolute alcohol,	-	-	-	-	-	10,0
Carbonate of lithia,	-	-	-	-	-	1,0
Distilled water,	-	-	-	-	to make	100,0

5. The sections are transferred to water and washed out. They are differentiated in a solution consisting of—

Borax,	-	-	-	-	-	2,0
Ferricyanide of potash,	-	-	-	-	-	2,5
Distilled water,	-	-	-	-	-	100,0

6. They are washed out and transferred to 96 per cent. or absolute alcohol, then to xylol or oil of origanum. Finally, they are mounted in Canada balsam.

The medullated nerve fibres appear of a blue-black tint on a brownish-yellow ground.

It is best to keep in stock the basis of the staining solution, consisting of hæmatoxylin and absolute alcohol, and to add, when the solution is required for use, the water and the carbonate of lithia (this last in the proportion of 1 to 100). From the action of the carbonate of lithia, the colour, previously reddish, becomes of a dark bluish-violet tint. The



differentiation becomes sharper, and may be better measured off the more the differentiating fluid is diluted. The grey matter takes on a light brown colour, the white a dark violet. If the sections should be thickish, and should differentiate badly, they are replaced in alcohol for twenty-four hours, and then returned to the solution of borax and ferricyanide of potash. To show the nerve cells and their nuclei, they may be counterstained with alum-carmin, picro-carmin, or lithia-carmin.

If the sections are to be stained in other ways as well, the copper process must not be applied to the entire piece of tissue, but only to those sections which it is intended to stain with hæmatoxylin.

Once used, the hæmatoxylin solution cannot be re-employed, but must be poured away.

The process was perfected by Weigert himself, in his work on the neuroglia (1895), by means of a step, the value of which cannot be over-estimated, as it accelerates the fixing and mordanting of the tissue, which occupies only from four to five days. The following solution is employed:—

Bichromate of potash (or of soda or ammonium),	-	-	-	-	5,0
Chrome-alum,	-	-	-	-	2,0

These ingredients are dissolved in 100,0 of boiling water, to which 10 grm. of a 10 per cent. formol solution are added. Or the hardening may previously be separately carried out in this 10 per cent. formol solution, when the mordanting follows after.

The solution of acetic acid, oxide of copper, and chrome-alum, devised for the neuroglia (*q.v.*), may also be recommended in the staining of the medullary sheaths. It gives rise to no precipitation in the pieces of tissue which have been treated with bichromate, and has, on the other hand, the advantage as compared with solution of Rochelle salt, that it is unnecessary afterwards to treat the tissues with a watery solution of the copper salt (Weigert).

*The following points of importance, whether for this original*

*method of Weigert or for its modifications, are always to be kept in mind:—*

1. The preparations must not be mounted immediately after differentiation is completed, but are to be left for two or three days in water, which must be frequently changed. Only in this way can fading of the colour be avoided. The sections become more deeply stained by leaving them in water.

2. The use of the "double" or "triple" method, which is to be generally recommended for most stains, is necessary in all cases where the sections are thickish.

3. In Pal's modification of the process, almost every separate section must be treated with a separate portion of freshly prepared differentiating fluid.

Berkley stated that very good results could be obtained if the tissues were hardened in Flemming's chromo-acetico-osmic acid. The pieces are left for thirty hours in the fluid, at a temperature of 25° C., and are transferred direct to absolute alcohol, where they remain for twenty-four hours, in which time the alcohol must be twice changed. After they have been embedded and cut, the sections are received in water, and are left over night in the acetate of copper solution. They are quickly washed, and stained in a solution of hæmatoxylin, prepared as follows, which has been allowed to cool:—50 ccm. of water are boiled for a short time with 2 ccm. of a saturated solution of carbonate of lithium. 2 ccm. of the usual alcoholic solution of hæmatoxylin (1 in 10) are then added.

The sections are left in the stain, at a temperature of 40° C., for twenty minutes. They are then washed out and differentiated. The further treatment is as above.

To obviate differentiation, Weigert himself, in 1891, devised a method, the results of which are not inferior to those of Pal's method, though the preparations obtained do not keep well.

*Weigert's Stain for the Medullary Sheaths, without Differentiation.*

1. The tissue is hardened in bichromate of potash solution.
2. It is embedded in celloidin.
3. The pieces are placed for twenty-four hours in a fluid, at a temperature of 35° C., composed of equal parts of a



solution of neutral acetate of copper, saturated in the cold and filtered, and of a 10 per cent. watery solution of Rochelle salt. The fluid is maintained at the required temperature in the incubator.

4. The pieces are placed for twenty-four hours in a simple watery solution of neutral acetate of copper, and kept in the incubator.

5. They are rinsed, and transferred for from half an hour to an hour to 80 per cent. alcohol. They are then cut.

6. The sections are stained in nine parts by volume of solution A, and one part by volume of solution B.

Solution A consists of 7 ccm. of a saturated solution of lithium-carbonate, and 93 ccm. of distilled water. Solution B consists of 1 gm. of hæmatoxylin and 10 gm. of absolute alcohol. The two solutions are mixed shortly before use.

7. The sections are washed out and transferred to 90 per cent. alcohol, then to aniline-xylol (2 of aniline to 1 of xylol). Afterwards they are transferred to xylol and mounted in balsam.

The most delicate medullated fibres are stained in from five to twenty-four hours. They appear black on a pale red ground. If the sections are too thick, they may be differentiated in borax and ferricyanide of potash.

*Pal's modification of Weigert's method—*

1. The tissues are hardened in Müller's fluid. The further steps are the same as in Weigert's method, omitting the treatment with acetate of copper.

2. The pieces are cut, and if the sections are not of a greenish colour, proving that they contain sufficient bichromate, they are again transferred for twenty-four hours to the bichromate of potash solution or to a very weak solution (0.3 to 0.5 per cent.) of chromic acid in 70 per cent. alcohol.

3. The sections are stained in Weigert's solution of hæmatoxylin for from twenty-four to forty-eight hours. If the process be carried out in the incubator it lasts for about an hour.

4. The sections are washed out in water, to which 4 per cent. of the carbonate of lithia solution has been added.

5. They are differentiated for twenty or thirty seconds in a freshly prepared solution of permanganate of potash (about  $\frac{1}{3}$  per cent.). When this process is completed the grey matter is of a yellow tint.

6. The sections are washed out, and further differentiated, as Lustgarten advised, in the following solution:—

Oxalic acid,	-	-	-	-	1,0
Sulphite of potash,	-	-	-	-	1,0
Distilled water,	-	-	-	-	200,0

The grey matter is decolourised in a few seconds, and the white becomes blue-black. If this result does not follow, the sections may again be placed in the permanganate of potash solution and the oxalic acid fluid.

7. They are washed out, and, if desired, they may once more be placed in a strong lithium solution for from five to thirty minutes. In this the stain becomes more intense. They are again washed out, transferred to alcohol and to xylol, and mounted in balsam.

The medullary sheaths appear black or blue-black, and all other nervous elements are decolourised. Counterstaining with alum-carmin, borax-carmin, or picro-carmin, may therefore be employed to more advantage than in the original method.

If Pal's modification is successful, it gives extremely good results, but even when great care is taken, it is always possible that the differentiation may be too strong, and that the most delicate fibres may be decolourised.

It has been recommended, in order to be able to use the hæmatoxylin solution several times, to stain the sections first in the hæmatoxylin alone, and afterwards to leave them in saturated solution of lithium-carbonate until they appear sufficiently black.

*Kulschitzky's modification—*

1. The tissues are hardened in Müller's or Erlitzky's fluid.
2. They are embedded and cut.



3. The sections are stained for from one to twenty-four hours in the following solution:—

Hæmatoxylin (dissolved in absolute alcohol),	- - - - -	1,0-2,0
Acetic acid (2 per cent.),	- - - - -	100,0

4. They are decolourised in the following:—

Saturated solution of carbonate of lithia,	- - - - -	100,0
Solution of ferricyanide of potash (1 per cent.),	- - - - -	10,0

5. They are carefully washed out, transferred to alcohol and to oil, and mounted in balsam.

The medullated nerve fibres appear deep blue or dark violet, all other tissues being colourless or pale yellow.

The stain originally employed was a solution composed as follows:—

Hæmatoxylin (dissolved in absolute alcohol),	- - - - -	1,0
Saturated solution of boric acid,	- - - - -	20,0
Distilled water,	- - - - -	80,0

This solution must be slightly acidulated with acetic acid immediately before use. It is at first yellow, and may be used after it has become red (in about three weeks).

*Wolter's method* (Kulschitzky-Wolters)—

1. The pieces are hardened in Müller's fluid, embedded, and cut.

2. The sections are stained for twenty-four hours (in the incubator) with Kulschitzky's hæmatoxylin solution.

3. The sections are then dipped in Müller's fluid, and differentiated as in Pal's method.

4. They are washed out, transferred to alcohol and to xylol, and mounted in balsam.

*This combination of two modifications is much to be commended on account of the beautiful results it gives. Even the most delicate fibres appear of a beautiful blue-black colour, while the nerve cells are brownish yellow.*

For this modification Kaes has recommended the substitution of Flemming's solution for the Müller's fluid. In this way the tangential fibres are said to be especially well shown.

*Vassale's modification of Weigert's method*—

1. The pieces are hardened, &c., as in the original method.

2. The sections are stained for three to five minutes in a 1 per cent. watery solution of hæmatoxylin.

3. They are rinsed, and transferred for three to five minutes to a saturated solution of neutral acetate of copper.

4. They are washed out, and differentiated in Weigert's solution of ferricyanide of potash.

5. They are washed out, transferred to alcohol and to carbol-xylol, and mounted in balsam.

The great simplicity of this method is of advantage. The chance of precipitation of the hæmatoxylin is also done away with, and the sections, owing to their shorter stay in the staining fluid, are less brittle. The stain should always be employed as freshly prepared as possible.

*Lissauer's modification*—

1. The preliminary treatment is as in Weigert's original method.

2. The sections are transferred to a 1 per cent. solution of chromic acid, which is warmed until bubbles appear.

3. They are washed out and stained in Weigert's hæmatoxylin solution, which must also be warmed until bubbles appear.

4. They are differentiated as in Pal's method.

This "rapid" modification is of especial importance in the treatment of brains which have lain too long in bichromate solutions and in alcohol.

*Schäfer's modification* seems to be highly commendable—

1. The pieces are hardened, embedded, and cut, as in Weigert's method.

2. The sections are transferred from water to Marchi's fluid, where they remain for some hours.

3. They are washed out, and stained in a solution of one grm. of hæmatoxylin in a little absolute alcohol, to which 100 ccm. of a 2 per cent. solution of acetic acid are added. The sections remain over night in the stain.

4. The sections, which have become black, are washed out. The further treatment is as in Pal's method.



*Kaiser's modification by means of Marchi's fluid—*

1. Small pieces are hardened for three days in Müller's fluid. They are then still further divided, until they are extremely small, and replaced for six days longer in Müller's fluid.

2. They are transferred to Marchi's fluid, in which they remain for eight days.

3. They are washed out, transferred to alcohol, embedded in celloidin, and cut.

4. The sections are placed for five minutes in the following solution:—

Liquor ferri sesquichlorati (P.G.),	-	10,0
Distilled water,	- - - -	10,0
Rectified spirit,	- - - -	30,0

5. They are transferred to Weigert's hæmatoxylin solution, and warmed for five minutes in a fresh portion of the stain.

6. They are washed out, and differentiated as in Pal's modification.

7. They are transferred for a short time to water to which a little ammonia has been added.

8. They are washed out, transferred to alcohol and to xylol, and mounted in balsam.

In this modification, therefore, the copper acetate is replaced as a mordant by a solution of chloride of iron.

The medullary sheaths appear of a blackish brown or deep black tint.

*The method of Adamkiewicz—*

1. The pieces are hardened in Müller's fluid, embedded, and cut.

2. The sections are transferred to water slightly acidulated with hydrochloric acid.

3. They are stained in a dark red watery solution of safranin no. 0.

4. They are washed in alcohol, and afterwards in absolute alcohol slightly acidulated with nitric acid.

5. They are treated with oil of cloves until no more of the red colouring matter is given off. They are then mounted in balsam.

Normal medullary sheaths are stained red, but those which are pathologically altered are not stained. The nuclei of the nerve cells and of the cells of the neuroglia and vessels appear bluish violet.

*Exner's method—*

1. Fresh pieces are placed in ten times their volume of a 1 per cent. osmic acid solution. They must be kept in the dark. The osmic acid is renewed on the second and fourth days.

2. After from six to ten days they are washed out. The pieces are fastened on cork and further hardened in alcohol. They are then cut.

3. The sections are transferred to glycerine, to which one drop of ammonia (liquor ammoniae fortissimus, 10,0; water, 50,0) is added on the slide.

4. The excess of fluid is removed with blotting paper. When the section is completely clear the cover-glass is put on.

The fibres appear greyish black. As the preparations are not permanent, and the ammonia causes the section to swell up and thus increase in thickness, the method need not receive much consideration.

*The method of Pal and Exner* is of service in obtaining permanent preparations—

1. The pieces are hardened as in Exner's method.

2. They are washed out for two minutes in absolute alcohol, and embedded.

3. The sections are placed in a mixture of glycerine and water (three to one). The glycerine is washed away by water.

4. The sections are differentiated, as in Pal's method, and then washed out.

5. They are counterstained with picro-carmin, transferred to alcohol and to xylol, and mounted in balsam.

Hans Aronson has employed gallein for the medullated fibres, in the following combination:—

Gallein paste,	- - - -	3-4 ccm.
Absolute alcohol,	- - - -	20,0
Distilled water,	- - - -	100,0
Concentrated solution of carbonate of soda,	- - - -	3 drops.



The preliminary treatment and the differentiation are the same as in Pal's modification of Weigert's method. After differentiation the sections are placed in soda until they become red.

*Heller's osmic acid method* of treating the medullary sheaths must here be added. The technical steps to be carried out are given further below. He points out that, apart altogether from the ease with which this stain may be combined with nuclear stains, it may be of advantage to examine the changes in the medullary sheaths both by osmic acid and by hæmatoxylin, in series of sections following one upon the other. The results of the two stains may thus be compared and the one checked by the other.

Heller's method has, besides, been modified, and its duration has been shortened by Robertson (A modification of Heller's method of staining medullated nerve fibres. *Brit. Med. Journ.*, 1897, p. 651) in the following manner:—

1. The pieces are hardened in a modified Weigert's solution of chrome-alum and copper, as follows:—

Chrome-alum,	-	-	-	-	2,5
Acetate of copper,	-	-	-	-	5,0
Acetic acid,	-	-	-	-	5,0
Formol,	-	-	-	-	2-10,0
Distilled water,	-	-	-	-	100,0

2. They are washed out in water, and embedded in celloidin.

3. The sections are placed in a 1 per cent. osmic acid solution, then in a 5 per cent. solution of pyrogallol. They remain in each fluid for half an hour.

4. They are transferred to a 0.25 per cent. solution of permanganate of potash, in which they remain from one to four minutes, and are then placed for five minutes in a 1 per cent. solution of oxalic acid. *They must be washed out after each process.*

5. They are transferred to alcohol, and then mounted. Robertson extols this stain especially for the delicate medullated fibres of the brain. Counterstaining with hæmatoxylin shows the nerve cells very well.

#### *Marchi's method*—

1. Small pieces are fixed in Müller's fluid for at least eight days.

2. They are transferred to a freshly prepared mixture of Müller's fluid and a 1 per cent. solution of osmic acid in equal parts. In this they are left for from six to twelve days.

3. They are washed out in running water for twenty-four hours.

4. They are hardened in alcohol, embedded in celloidin, cut, and mounted.

Degenerated medullary sheaths appear black, and everything else pale yellow, sometimes with a greenish lustre.

This method, a combination of the processes of hardening and staining, is of the greatest value and importance, and demands the observance of a number of special measures.

The animal must be left for twenty-four hours after death, before the brain and spinal cord are removed. The removal, to avoid any tearing, must be very cautiously performed, for a tear may very easily give rise to a false interpretation of the results of staining.

The fluid containing the pieces of tissue, which must be very thin (2 to 3 mm.), is placed in the incubator at a temperature of from 25° to 30° C. Care must be taken to renew the fluid as soon as the smell of osmic acid disappears. The pieces must rest, so far as possible; obliquely, and not upon their flat surface. The equal penetration of the fluid, which, at the temperature of a room, requires a longer time, must be aided by frequent agitation.

The sections themselves may be thick (from 50 to 80  $\mu$ ). Degenerations, not more than some months old, are to be recognised by the black clumps of myelin and detritus. Fatty degenerations of the nerve cells and blood vessels are also distinctly seen. In experimental work it is best to keep the animals alive only for two or three weeks. In human pathology one may count upon good results even if the process has lasted for several months.

The sections may be counterstained by Weigert's method, by carmine, or by van Gieson's method.

The degenerated portions of the degenerate medullated nerves are arranged in the manner of a chain. For the examination of the brain (or of the caudex cerebri), the pieces, which must not be over 2 mm. in thickness, should be left from four to six weeks in Marchi's fluid, while eight to twelve



days are sufficient for the spinal cord and peripheral nerves. In the former case the fluid should at first be little concentrated (1 to 3), and the concentration should be gradually increased until a proportion of 1 to 2, or 1 to 1, is attained. The fluid must be frequently changed. In about four weeks the operator should make incisions into the brain substance to ascertain whether the fluid has sufficiently penetrated the central parts.

In order to increase the blackening of the pieces of spinal cord, Azoulay has made use of the property of osmic acid to become black in the presence of tannin. After previous treatment with Müller's fluid and alcohol, the sections, which must be very thin, are blackened for a short time in a solution of osmic acid (1 in 500), and then warmed for about five minutes in a 5 per cent. solution of tannin. They are then washed out, and, if it be desired, counterstained with carmine. They are mounted in the usual way. Only the myelin is stained black.

According to Edinger, the method gives good results in the case of the medium-sized and coarser fibres, but is not so successful for the delicate fibres as the hæmatoxylin method. It is admirable for preparations treated by Marchi's method, as by its use the lacunæ, where the products of degeneration have already been absorbed, can easily be recognised. But it may also be used for preparations treated by Golgi's method. Obersteiner recommends it, especially for nerve roots and peripheral nerves.

One further process recently given by Allerhand is of importance.

*Allerhand's method—*

1. The pieces are hardened in Müller's fluid or in alcohol, embedded, and cut.

2. The sections are placed for fifteen or twenty minutes in a 50 per cent. solution of the liquor ferri sesquichlorati (P.G.). The reduction of the metallic salt is more easily attained if the fluid be slightly warmed.

3. The sections are rinsed for a short time and transferred

to a 20 per cent. solution of tannin. In this they must be left in the incubator for from one to two hours. The solution must have been exposed to light for about three weeks, and must have just begun to decompose. Fungi begin to appear when the process of decomposition is sufficiently complete. Thereafter the solution must be filtered.

The differentiation begins almost at once, and in this process the iron salt is reduced by the tannic acid. The sections become of a dark blackish-blue colour. The "double" method may be used, the sections being replaced in the iron solution.

4. The sections are differentiated as in Pal's process, but, in order to save time, with solutions of double the strength.

5. They are washed out and transferred for a few minutes to a 0.5 per cent. solution of acetic acid. They are then transferred to alcohol and to xylol, and mounted in balsam.

The method therefore depends essentially upon double staining with iron chloride and tannin, and differentiation by Pal's method. The medullated fibres, and also the tangential fibres, appear of an intense blue-black colour on a colourless ground. The nerve cells, with their processes, are also distinctly seen, the nucleoli being black. They are even more distinct in alcohol preparations, while, if bichromate has been used, the fibres are more prominent.

In the case of hardening in alcohol, the same pieces are to be used for staining the nerve fibres as for Nissl's method. It may be doubtful what constituent of the nerve fibres is stained in this case, as the medullary sheath is in large part destroyed by extraction of the fatty myelin, and therefore cannot appear stained blue *in toto*. Allerhand considers this remnant of the medullary sheath to be related to the stroma of neurokeratin described by Kühne and Ewald, and regards the two concentric rings seen in alcohol preparations as transverse sections of the external and internal horny sheath of the medullated fibre. But the axis-cylinder also appears in alcohol preparations, although not always, as a shrunken serrated structure, stained intensely blue.



In this "method of impregnation," solutions which have been once used may be several times re-employed. Tannin, especially, seems to gain in staining power (*i.e.*, reducing power) by repeated use.

### C.—STAINING THE AXIS-CYLINDERS.

Numerous as are the methods of staining the axis-cylinders which we possess, the fact must be emphasised that not one of them is even approximately satisfactory. The discovery of a method permitting us to trace the axis-cylinder proper in its degeneration and also in its structure, as Weigert's stain and Marchi's method do for the medullary sheaths, would be a very great service, especially for the purposes of pathology.

#### *Upson's stain* (method A)—

1. The pieces are hardened in the dark in bichromate solution (at first 1 per cent., then 2 per cent., to 2.5 per cent.) Over-hardening is injurious.

2. They are washed in water and placed for two or three days in 50 per cent. alcohol, which must be frequently changed. They are then transferred to 96 per cent. alcohol, in which they remain till a green colour appears (from two to four weeks). The alcohol must be frequently changed.

3. The pieces may or may not be embedded. Sections are cut, and it is best to stain them at once, without further treatment with alcohol, in the following solution:—

Chloride of gold, - - - - -	1,0
Distilled water, - - - - -	100,0
Hydrochloric acid, - - - - -	2,0

They become yellow after one or two hours.

4. The sections are rinsed and transferred for a minute to a 10 per cent. solution of caustic potash, to which a trace of ferricyanide of potash may be added shortly before use.

5. They are washed out and transferred for half a minute to a 10 per cent. solution of caustic potash. They are again washed.

6. They are reduced in the following solution, which must be freshly prepared:—

Sulphurous acid, - - - - -	5,0
Tincture of iodine (3 per cent.), - - - - -	10-15 drops

These ingredients are mixed, and 1 drop of liquor ferri chloridi is added. The section becomes pink.

7. The sections are washed, transferred to alcohol and to oil of cloves, and mounted in balsam.

Glass must be substituted for metal instruments, and the sections must be kept in the dark.

#### Method B—

1. The preliminary treatment is as in method A.

2. The sections are placed for two hours in the following solution:—

Hydrochloric acid, - - - - -	2 drops
Solution of chloride of gold (1 per cent.), - - - - -	5,0
Saturated solution of vanadate of ammonia, - - - - -	10 drops

3. They are rinsed for a short time, and transferred for one minute to the following solution, which must be freshly prepared:—

10 per cent. caustic potash, - - - - -	5,0
Vanadate of ammonia, - - - - -	a trace
Solution of permanganate of potash (10 per cent.), - - - - -	10 drops

4. They are rinsed, and then reduced in a freshly-prepared solution, composed as follows:—

Zinc solution (a), - - - - -	15 drops
Distilled water, - - - - -	3,0
Iron solution (b), - - - - -	5 drops
Sulphurous acid, - - - - -	3,0

When the acid is added, a copious precipitate is formed. At that moment the section is to be placed in the solution, which is then at its strongest. The section becomes purple.

5. It is then washed, transferred to alcohol and to oil, and mounted in balsam.



The zinc solution (a) is prepared by adding to a certain quantity of a 3 per cent. solution of tincture of iodine enough chloride of zinc to produce a whitish-yellow colour. Solution (b) is a saturated watery solution of phosphate of iron.

The nerve cells are stained along with the axis-cylinders. Upson remarks that the success of the stain is determined by special foreign bodies in the gold solution. A pure solution of chloride of gold, carefully neutralised, stains little or not at all. The more or less good result of the stain depends upon the presence of hydrochloric acid or other impurity in the solution. The best results, however, are obtained by the addition of acids or metallic salts, or both, to the gold solution.

*Gerlach's method—*

1. The pieces are hardened in a solution of bichromate of ammonia, at first weak, and afterwards concentrated up to 2 per cent.<sup>1</sup>

2. The pieces are reduced to as small a size as possible, and once more hardened for a short time.

3. They are cut by hand, being held under water between clamps of liver. Alcohol should not be used.

4. The section is transferred to a solution of chloride of gold and potassium (one in ten thousand), slightly acidulated with hydrochloric acid. In this it remains for twelve hours.

5. It is washed in a solution of hydrochloric acid (one in fifteen hundred).

6. It is placed for ten minutes in the following solution:—

Hydrochloric acid,	-	-	-	-	1,0
Alcohol (60 per cent.),	-	-	-	-	1000,0

7. It is transferred to alcohol and oil of cloves, and mounted in balsam.

The method is uncertain. The results are, perhaps, better in the embryonic brain. According to Goodall, it appears that Boll obtained good results in the fresh brain of the mouse, and Gerlach in the human cortex.

<sup>1</sup> Boll advises that the solution should at first be even weaker than 1 per cent., and that, after a few hours, a 1 per cent. solution should be used. After a few days this should be replaced by a 2 per cent. solution, but the whole process should last only eight days.

*Freud's method—*

1. The pieces are hardened in Müller's or Erlitzky's fluid. If desired, they may be further hardened in alcohol.

2. They are washed out, embedded, and cut. The sections are stained for from three to five hours in a 1 per cent. solution of chloride of gold.

3. They are washed out and placed for three minutes in the following solution:—

Solution of caustic soda,	-	-	-	-	1,0
Distilled water,	-	-	-	-	5,0

4. They are washed and transferred to a 10 per cent. solution of iodide of potash, in which they are left for from five to fifteen minutes, assuming a reddish-violet colour.

5. They are washed, transferred to alcohol—the concentration of which is gradually increased—then to xylol, and finally they are mounted in balsam.

The results are variable. In good preparations the axis-cylinders are seen to be stained of a dark reddish blue, or even black. The nerve cells are sometimes unstained and sometimes prominent, being of a distinct reddish tint. Freud states that the latter result occurs only in the adult. The durability of the preparation extends at least over many months.

Metal instruments must be avoided, and replaced by glass. The stain becomes more selective if the gold chloride solution be mixed with an equal volume of 96 per cent. alcohol.<sup>1</sup>

The stain appears to succeed best with fresh material which has not yet been hardened (Kahlden).

*Van Gieson's method—*

1. The pieces are hardened in Müller's fluid.

2. They are embedded and cut.

3. The sections are stained for from three to five minutes in Delafield's hæmatoxylin or another alum-hæmatoxylin.

4. They are thoroughly washed out.

5. They are stained in a mixture of saturated solution of

<sup>1</sup> Cohnheim was the first to use the gold method, employing it for the nerves of the cornea. The sections were placed in a 0.5 per cent. solution of gold chloride, and then in water, to which a few drops of acetic, hydrochloric, or formic acid had been added.



picric acid with saturated solution of acid fuchsin. The mixture must be of a dark red colour.

6. They are washed out for a short time, transferred to alcohol and to oil of origanum, and mounted in balsam.

The axis-cylinders appear deep red, the medullary sheaths yellow, the neuroglia reddish, and the nuclei bluish violet. Sclerosed parts are of an intense red.

This method was originally given by van Gieson for amyloid disease, the amyloid substance becoming light red. It was Paul Ernst who employed it specially for the central nervous system.

*Stroebe's method—*

1. The pieces are hardened in Müller's fluid.
2. They are transferred to alcohol, and embedded in celloidin.
3. The sections are stained for from fifteen minutes to an hour in a saturated watery solution of aniline blue, in which they become blue-black.
4. They are rinsed and differentiated in absolute alcohol (to which 20 to 30 drops of a 1 per cent. caustic potash-alcohol<sup>1</sup> are added) until they are transparent and of a pale brownish red. The process lasts from one to several minutes.
5. They are washed out, when they become pale blue.
6. They are placed for from fifteen to thirty minutes in a dilute solution of safranin as a counterstain.
7. They are transferred to absolute alcohol and to xylol, and mounted in balsam.

In this process the axis-cylinders and the fibres of the neuroglia are always stained blue, while the medullary sheaths, the protoplasm, the ground substance, and the nuclei take on the yellowish-red counterstain. Sometimes the nuclei may also show the blue stain. Absolute certainty is not attributable to the method.

*Benda's iron-hæmatoxylin method—*

1. The pieces are hardened in Müller's fluid, or another fixing fluid.

<sup>1</sup> Caustic potash-alcohol is prepared by adding 1 grm. of caustic potash to 100 ccm. of alcohol. The fluid is allowed to stand for twenty-four hours, and then filtered.

2. They are embedded and cut.

3. They are mordanted for twenty-four hours in a solution of equal parts of liquor ferri sulf. oxydati (P.G.) and distilled water, or in one part of the iron solution to two of water.

4. They are carefully washed out, first in distilled water, and then in ordinary water.

5. They are stained in a 1 per cent. watery solution of hæmatoxylin until they become black.

6. They are differentiated in 30 per cent. acetic acid. The further treatment is as usual.

*Schmaus's methods—*

1. Uranium-carmin is employed, without specially staining the celloidin.

One grm. of soda-carmin is rubbed up with 0.5 grm. of nitrate of uranium, and boiled for half an hour with 100 ccm. of water. The solution is allowed to cool, and then filtered. Sections of the spinal cord are stained in from fifteen to twenty minutes.

The pieces are hardened in Müller's fluid, and must not be dehydrated.

2. Grübler's black-blue is also to be recommended for the axis-cylinders. It is used in the form of a 0.25 per cent. solution in 50 per cent. alcohol, to which a little picric acid is added. Sections are to be stained for an hour, washed out in water, and dehydrated in alcohol. This process permits of treatment by acetate of copper if desired.

*Paladino's method—*

1. The pieces, which should be as thin as possible, are hardened in Müller's fluid.

2. They are washed out, and dehydrated in 96 per cent. or in absolute alcohol.

3. They are successively boiled for an hour in each of the three following fluids:—absolute alcohol and benzol, benzol, and finally absolute alcohol.

4. They are placed for from four to eight days in a solution of chloride of palladium (one in a thousand) to which a few drops of hydrochloric acid are added.



5. They are transferred for from one to four days to a 4 per cent. solution of iodide of potash, which must be used in the smallest possible quantity, as otherwise the iodide of palladium is re-dissolved.

6. They are placed in 80 per cent. and in 96 per cent. alcohol. They are then embedded. Paraffin must not be used, or the stain will not be successful.

7. Sections are cut, transferred to alcohol and to oil, and mounted in balsam.

The section now appears yellowish brown or dark brown. The axis-cylinders are stained, but so too are the neuroglia and medullary sheaths.

By this method Paladino believes that he has demonstrated certain relationships between the neuroglia and the medullary sheaths, regarding the latter as a continuation of the neuroglia. He states that they also contain neuroglia cells.

*The aniline blue-black method—*

1. The pieces are hardened in Müller's fluid, and afterwards in alcohol, or in sublimate, and afterwards in alcohol. They are then embedded and cut.

2. The sections are stained for an hour in a 0.25 per cent. watery solution of aniline blue-black, to which a little alcohol may be added. The English preparation must be used.

3. They are washed out, transferred to alcohol and to creosote or origanum oil, and mounted in balsam.

The axis-cylinders and the nuclei of the nerve cells are stained deep blue. The nerve cells and the nuclei of the neuroglia are bluish grey.

Hardening in bichromate gives better results than hardening in sublimate, which, however, generally permits of more rapid staining.

A similar stain may be obtained with indulin and aniline blue.

*The nigrosin method—*

1. The pieces are hardened in Müller's fluid, embedded, and cut.

2. The sections are stained for ten minutes in a concentrated watery solution of nigrosin.

3. They are washed out in equal parts of water and alcohol, transferred to alcohol and to origanum oil, and mounted in balsam.

Besides its simplicity, the method has the advantages of giving a very good general view, and possessing a constant result.

*Sahl's method—*

1. The pieces are hardened for as long as possible in Müller's fluid. They are washed out for a few minutes, embedded, and cut.

2. They are stained for several hours in the following solution:—

Saturated watery solution of methylene	
blue, - - - - -	24 ccm.
Solution of borax (5 per cent.), - - -	16 ccm.
Distilled water, - - - - -	40 ccm.

3. They are washed out in water, then in alcohol, until the grey matter can be clearly distinguished from the white. They are then transferred to oil of cedar, and mounted in balsam.

The axis-cylinders and the nuclei of the neuroglia are dark blue, the nerve cells greenish blue, and the ground substance pale blue.

As in the majority of aniline stains, too great a decolourisation may readily be produced by the action of the alcohol. The section must therefore be placed in the oil of cedar as soon as possible. Any bacteria that may be present are well shown by this method.

*D.—STAINING THE NEUROGLIA.*

*Weigert's neuroglia stain<sup>1</sup>—*

1. The pieces are fixed and mordanted for eight days in a solution of acetate of soda and chrome-alum, to which is added 10 per cent. formol.

<sup>1</sup> Weigert: Beiträge zur Kenntniss der normalen menschlichen Neuroglia. Frankfurt a.M., b. Diesterweg, 1895.



2. The pieces are prepared for section by the celloidin method. This process should occupy three days.

3. Sections are made.

4. They are reduced by permanganate of potash, and by chromogen solution, with the help of a mixture of formic and sulphurous acids.

5. The capacity of the neuroglia for taking on the stain, and of the nervous elements for taking on the counterstain, is increased by the aid of a simple watery solution of chromogen (5 per cent.).<sup>1</sup>

6. Weigert's method for fibrin is employed in a modified form.

The total duration of the process is twelve days. The steps from (3) to (6) occupy one day altogether.

This method, finally published by Carl Weigert in November, 1895, after the labour of seven years, marks so great a step in the province of neurology that it must be set forth in detail.

The principal measures to be observed in the staining of the neuroglia may be divided into three or four parts—

1a. The pieces removed from the central nervous system must be fixed.

1b. They must be mordanted with highly oxidised metallic compounds. Both processes may, if desired, be combined in one. 2. The metallic compound must be reduced. 3. The staining process follows.

1. *Fixing and mordanting*—

(a). These two procedures may be carried out separately or in combination.

The former alternative may be adopted, if it be also desired to treat the preparations by other methods, such as those of Marchi, Golgi, and Nissl, or the method for the medullary sheaths. In such a case the pieces are fixed with a 10 per cent. solution of formol, as weaker solutions do not fix them sufficiently. The material must be quite fresh, and the pieces must be placed in the fixing fluid only after they have been divided into portions of the smallest possible size, and not

<sup>1</sup> See footnote on p. 120.

more than half a centimetre thick. In pieces larger than this the stain is no longer certain.

For the purpose of hardening, shallow vessels should be used, on the bottom of which blotting paper must be placed, to prevent distortion of the thin pieces. The formol solution must be renewed only on the second day. As a rule, four days suffice for the hardening, but the pieces may remain in formol for years without losing their capacity for taking on the stain.

If, however, it is not intended to use any other stain than that for the neuroglia, it is better to omit the simple formol solution and to place the fresh pieces at once in the solution of copper and chrome-alum described below. To this solution a 10 per cent. solution of formol must then be added. The fluid must be changed on the second day.

(b). Mordanting may be performed either upon fresh pieces, or on those hardened in formol. Even pieces mordanted with chrome salts permit of staining the neuroglia, if a saturated (about 5 per cent.) solution of bichromate of potash be employed in place of the usual Müller's fluid. In this case, however, one is never quite certain that the axis-cylinders will not also be stained.

Weigert, then, has given the following solution as a typical mordant for the neuroglia:—

Acetate of copper,	-	-	-	5 per cent.
Ordinary acetic acid,	-	-	-	5 „
Solution of chrome-alum,	-	-	-	2.5 „

The chrome-alum is first boiled in water (if dissolved in the cold a precipitate is formed), and while it is absolutely boiling the flame is put out, and first the acetic acid and then the acetate of copper, which must be finely powdered, are added. The mixture is briskly stirred with a glass rod, and allowed to cool.

This solution may also be used for staining the medullary sheaths, as it produces no precipitate in pieces treated by bichromate, and, on the other hand, it has the advantage over Rochelle salt that it is unnecessary to carry the treatment by copper further, by the use of a simple watery solution of the copper salt.



In this solution, to which 10 per cent. of formol must be added, the pieces are left for eight days at the temperature of a room. If they remain longer, it does no harm. They are then rinsed, dehydrated, and embedded in celloidin.

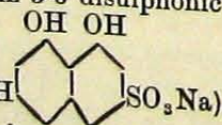
2. *Reduction.*—This process is different for preparations treated by chrome salts and for those treated by acetate of copper. In the case of the former the neuroglia, up to this point, is not sufficiently stained. Lustgarten's process (permanganate of potash and sulphurous acid) may be recommended as an energetic method of reduction for sections treated by copper. It has been somewhat modified by Weigert, who uses chromogen<sup>1</sup> to obtain the counterstain.

As the watery solution of chromogen is not sufficiently energetic in its action, a watery solution is made, containing 5 per cent. of chromogen and 5 per cent. of formic acid. This is filtered, and before it is used, 10 ccm. of a 10 per cent. solution of sulphite of soda (as employed in photography) are added to 90 ccm. of this fluid.

The sections are first placed for about ten minutes in a solution of permanganate of potash (about  $\frac{1}{3}$  per cent.), which is afterwards carefully poured off. They are then washed out in water, which is also poured off, and then the reducing fluid is poured over them. The sections, which were previously brown, are soon decolourised, but it is best to leave them from two to four hours in the solution.

If the sections be now stained the neuroglia fibres are blue, and the connective tissue is colourless. But if it is not important that it should be colourless, the procedure which follows is to be recommended. After its employment, the collagenic tissue no doubt becomes blue, with a faint tinge of violet, but, on the other hand, the neuroglia fibres take on a much darker stain, and the most delicate of them are distinct and prominent. The nerve cells, the cells of the ependyma, and the thicker axis-cylinders take on a yellow tint. On account of these numerous

<sup>1</sup> Chromogen is a naphthalin compound, viz., the acid sodium salt of 1-8 dioxynaphthalin 3-6 disulphonic acid (as is shown by the follow-

ing formula:—. The solution has an acid reaction, and is a reducing agent.

advantages, therefore, counterstaining should always be carried out according to the following method:—

*Counterstaining.*—After the sections have twice been rinsed in water, they are left over night (the longer the better) in a simple saturated watery solution of chromogen (5 per cent.), which must previously be carefully filtered. They must thereafter be twice rinsed, and then stained at once, as they easily lose their capacity of taking on the stain, whether in water or alcohol. They may, however, be kept for days in oxalic-acid alcohol (80 per cent. alcohol, 90 ccm., and 5 per cent. oxalic acid, 10 ccm.). After its use the stain is perhaps even more permanent. Weigert has of late been using picric-acid alcohol, made up as follows:—

Saturated solution of picric acid,	-	10 ccm.
Soda-carmin (1 per cent. solution),	-	2 „
Alcohol (96 per cent.),	-	90 „

3. *The stain proper* (modified fibrin method).—Instead of the watery solution of methyl violet, a solution in 70 to 80 per cent. alcohol is employed. It must be saturated while hot, and decanted after cooling, leaving behind the precipitate in the bottom of the vessel. No aniline oil is added, but 5 per cent. of a 5 per cent. solution of oxalic acid may be added to increase the permanency of the preparations. The solution of iodine and iodide of potash employed is the saturated solution of iodine in 5 per cent. solution of iodide of potash, as originally published. Aniline-xylol must be used in equal parts (not in the proportion of two to one, as in other cases).

The sections are stained upon the slide. To prevent folding they must be placed in water in a large vessel, from which they are to be removed by the slide, which must be previously rubbed with alcohol. The section is dried, and the methyl violet solution is dropped upon it. It is stained almost at once. It is then dried, and the solution of iodine and iodide of potash is dropped upon it, and immediately poured off.

The section must now be very thoroughly washed out with aniline-xylol, and before it is mounted in balsam it must be once more very carefully washed out with pure xylol, as other-



wise the preparation is not permanent. In this respect the neuroglia is much more sensitive than fibrin. The preparations also keep better, curiously enough, if they are exposed for some days to daylight. In this they contrast with preparations made by the method of Golgi and Cajal. Weigert has recently recommended amber varnish in place of the Canada balsam. It must, however, be warmed over the flame upon the slide.

This admirable method, at least in the form just detailed, does not yet yield results of absolute mathematical certainty; but, from private communications, it appears that Weigert, by using another mordant, has at last discovered the desirable completing steps, and has made the method applicable to all animals (which was at first impossible), while up till now it was almost exclusively the human central nervous system that re-acted.

The enormous advantage and the great importance of this selective stain for the neuroglia lie in this, that it was sought for and discovered as a method for pathological purposes. At the same time Weigert has, with the help of his method, quite recently solved the very vexed question of the structure and nature of the neuroglia, showing that its fibres are differentiated from the protoplasm, and that the neuroglia is not a nervous substance.

*Beneke's stain—*

1. The tissues are hardened in alcohol, and embedded in paraffin.

2. They are cut, and the sections are freed from paraffin upon the slide in the usual way.

3. They are stained from ten to twenty minutes in a watery solution of aniline gentian-violet, made up as follows:—

Aniline, - - - - -	10,0
Distilled water, - - - - -	100,0

The solution is filtered, and 5 to 10 drops of a concentrated alcoholic solution of gentian-violet are added.

4. The sections are washed, and placed for a minute in Lugol's solution—

Iodine, - - - - -	4,0
Iodide of potash, - - - - -	6,0
Distilled water, - - - - -	100,0

5. They are thoroughly dried with several layers of blotting paper.

6. They are placed in aniline-xylol (two to three). The violet colour disappears.

7. They are placed in xylol as soon as they are dehydrated and cleared. They are then mounted in balsam.

The fibres and cells of the neuroglia are bluish violet or reddish. Except the nuclei of the nerve cells, the nervous elements proper remain unstained.

Like Weigert's selective neuroglia stain, this stain is ultimately based upon Weigert's fibrin method, save that in the latter the proportion of aniline to xylol is as two to one, the aniline forming the decolourising element.

The stain seems to give permanent preparations, but in view of Weigert's new method it may be dispensed with.

*Kulschitzky's stain—*

1. The pieces are hardened in the dark in Kulschitzky's solution.<sup>1</sup>

2. They are transferred to 96 per cent. alcohol, without being previously washed out. They are then embedded in paraffin and cut.

3. They are stained for a few seconds in the following fluid:—

Patent säurerubin, - - - - -	0,25
Saturated solution of picric acid, 2 per cent. solution of glacial acetic acid, aa	100,0

4. They are transferred to 96 per cent. alcohol, absolute alcohol, and xylol, and mounted in balsam. The sections must be very thin.

<sup>1</sup> Kulschitzky's solution is a saturated solution of bichromate of potash and copper sulphate in 50 per cent. alcohol. Before use 6 drops of glacial acetic acid are added to 100 cm. of the solution. It must be kept in the dark.



The neuroglia appears reddish violet. If the stain be used for a short time only, the nervous elements are scarcely visible; otherwise they are stained yellowish red.

Kulschitzky employs of late the following staining fluid:— 96 per cent. alcohol, 100 ccm.; patent saürerubin solution (as above), 3 to 5 ccm. In this case the process of staining lasts half an hour or more.

#### STAINING THE PERIPHERAL NERVOUS SYSTEM.

In the staining of the peripheral nervous system we have to deal, *mutatis mutandis*, with the same methods and the same modes of treatment as those employed for the central nervous system.

The method of teasing may in this case be used to a greater extent than in the central nervous system. The sole precaution to be observed is, that only short sections of the nerves should be dealt with, as longer sections are difficult to separate.

Besides Golgi's chromate of silver method, and Ehrlich's process for the subcutaneous injection of methylene blue, we may here mention, as the principal staining methods employed, those of Weigert and Marchi, with Azoulay's modification (see p. 108), and further, the carmine, hæmatoxylin, and nigrosin stains.

The method of Chr. Sihler may be employed to demonstrate the nerve endings in muscular fibres and blood vessels. The process consists of three parts—

1. Maceration.
2. Staining the loosened muscle bundles with hæmatoxylin.
3. After-treatment with acetic acid to get rid of over-staining.

The macerating fluid is as follows:—

Ordinary acetic acid,	- - -	1 part.
Glycerine,	- - -	1 „
Watery solution of chloral hydrate (1 per cent.),	- - -	6 parts.

The staining fluid is as follows:—

Ehrlich's hæmatoxylin,	- - -	1 part.
Glycerine,	- - -	1 „
Watery solution of chloral hydrate (1 per cent.),	- - -	6 parts.

The pieces are left for eighteen hours in the macerating fluid, and are then placed for from one to two hours in glycerine. The muscle bundles are split up, and placed for from three to ten days in the staining fluid. They are then transferred to glycerine (which must be frequently changed), teased out, and treated with acetic acid. The longer they remain in glycerine, the greater is the success of the stain.

When the muscle bundles are taken out of the staining fluid, everything is uniformly blue, and only the nuclei are dark blue. Through the action of the acetic acid the mass of the muscular tissue gives up the stain, while the skeletal structure of the fibres, and the nerve fibres, retain it.

The muscle fibres, accordingly, are faintly blue, with transverse and longitudinal striæ of a darker blue. The non-medullated nerves are similarly stained, while the medullated nerves appear dark, and all the nuclei blackish blue.

The method appears to be simple and certain in its results. It demonstrates almost everything to be seen by the use of the more difficult gold methods.

*Ranvier's gold stain*—

Fresh pieces of tissue are teased, and placed for five or ten minutes in lemon juice, which must be freshly expressed, and filtered. When they have become transparent, they are washed out and transferred to a 1 per cent. watery solution of chloride of gold, in which they remain for from ten minutes to an hour. They are once more dehydrated, and transferred to a dilute solution of acetic acid (2 drops to 50 ccm. of distilled water). Under the influence of light, the gold is reduced in this solution in from twenty-four to forty-eight hours.

The pieces, which are stained a reddish violet, are hardened in alcohol. Sections may then be made, or they may be further teased out.



A further method of staining with gold has been given quite recently by von Frey. He began at first with the method which Muschenkoff<sup>1</sup> had recommended for the demonstration of the nerve endings in striated muscle. In this method a 2 per cent. solution of bichromate of ammonia was used before the employment of the acid and of the gold. Von Frey, however, found that this process was not adapted to the human skin.

*Von Frey's gold stain* for the demonstration of medullated nerves and their nerve endings.

Small pieces are hardened for a considerable time in a 2 per cent. watery solution of bichromate of ammonia, washed out for about ten minutes in running water, and transferred to a gold bath containing 1 per cent. of chloride of gold and 1 per cent. of hydrochloric acid. After an hour the pieces are superficially rinsed and then placed in a  $\frac{1}{50}$  per cent. solution of chromic acid, which is kept in the dark. Reduction goes on slowly. Twenty-four hours afterwards there follows treatment with the hyposulphite of soda used by photographers, to remove the gold not yet reduced.

This method of fixing is best carried out upon sections, which must not, however, be embedded, but must be cut by the freezing microtome.

Washing out, and the employment of the incubator, must be avoided. Frey previously hardens the pieces in the refrigerator for at least two weeks. The course and the branchings of the medullated nerves are most distinctly shown in thick sections.

It must be emphasised that we have not here to do with a structural stain, as is the case with the other gold stains, but with a stain by precipitation, certain spaces, like that of the medullary sheath, becoming filled with the granules of gold.

If the excess of gold be not removed by fixing out, there appears as a result of the action of light, and especially soon if the tissues are mounted in balsam, a brownish-red or violet colouration of the other constituents of the skin as well, and this is a structural stain.

<sup>1</sup> Muschenkoff, Zeitschr. für wissensch. Microscopie, V. p. 52.

For the demonstration of the medullated nerves of the skin in hardened preparations there may also be recommended

*Heller's method—*

1. The pieces are hardened in Müller's fluid for from one day to several months.

2. They are cut with the freezing microtome. The sections should not be too thin.

3. They are washed out, and transferred to 1 per cent. osmic acid, in which they must be kept at a temperature of 37° C. for one or two days.

4. They are reduced in the following solution:—

Sulphite of soda,	-	-	-	-	125,0
Carbonate of soda,	-	-	-	-	70,0
Distilled water,	-	-	-	-	500,0
Pyrogallic acid,	-	-	-	-	15,0

In this the sections become dark, or quite black.

5. They are differentiated with permanganate of potash. The solution should be of a pale violet tint.

6. They are transferred to a 1 or 2 per cent. solution of oxalic acid.

7. They are preserved in glycerine. If Canada balsam is to be employed, they must previously be slowly dehydrated and cleared in oil of cloves.

The nerves appear deep black, and the other tissues greenish yellow. The fat is also stained black.

With this method it is not important that the material should be absolutely fresh. It is not possible to embed the pieces in celloidin, as ether and alcohol dissolve the myelin, and thus, in contrast to what occurs in the central nervous system, render the osmic acid re-action nugatory. On the other hand, the method gives good results in the central nervous system with sections that have already been prepared for Weigert's method. In this treatment of the central nervous system with osmic acid, however, thorough dehydration between the various manipulations is necessary, as otherwise injurious processes of reduction and oxidation may go on even after the preparations are completed.



## VI.—GENERAL PRACTICAL REMARKS ON THE TREATMENT OF THE NORMAL AND PATHOLOGICAL CENTRAL AND PERIPHERAL NERVOUS SYSTEM.

The treatment of the nervous system, especially the central nervous system, differs so materially from that of other organs that it is necessary to give a few suggestions for its examination.

If the material is to be used only for *macroscopic* purposes, for example, in examining the central nervous system from the point of view of comparative anatomy, and particularly in the case of the brain, it is best to place it in 5 to 10 per cent. formol.

In the case of the larger brains (man, the ape, &c.), it is useful to remove the *pia mater* in the first few days after they have been placed in formol.

According to the experience gained in our laboratory, the brains may remain for years in the formol solution without undergoing material shrinkage. The form of the brain remains unchanged, and there is no marked flattening. The weight of the brain, if the organ be fresh to commence with, is very little altered after remaining in 10 per cent. formol solution for almost two years. It increases by about 1 or 2 per cent., as detailed above.

In the macroscopic examination of smaller brains (cat, rabbit, &c.), the removal of the *pia mater* is attended with certain difficulties. If, then, it is desired to study the convolutions or to photograph the organ itself, it is of advantage to place the fresh brain for twenty-four hours in a saturated watery solution of chloride of zinc, in which it at first floats, and then sinks gradually to the bottom, which must be covered with cotton wool. In twenty-four hours the *pia* is easily removable, the organ having shrunk. The brain, after removal of the *pia*, may then be kept in formol solution.

Although various authors state that the colour of the grey

matter is not altered by formol, this is only true for short periods of time (not more than a few months). If a longer time has elapsed, the finer differences of colour between the white and the grey matter disappear, although a coarser difference may still be perceptible when two years have passed. The brain may also be preserved, as is well known, in alcohol, chloride of zinc, saturated salt solution, and other fixing fluids, but none of these possesses the advantages of formol.

Another method is also adapted to the macroscopic demonstration of sections of the brain and spinal cord. It is that of placing the organs for two or three months in a salt of chromic acid, and preserving them afterwards in 80 to 90 per cent. alcohol. The coarser markings of the grey and white matter are more distinctly shown than by formol.

The following suggestions may be of service in the treatment of tissues for the purposes of microscopy:—

The special methods of examination must be determined by the pathological difference between individual cases.

It may be said that, upon the whole, Weigert's method for staining the medullary sheaths and the neuroglia, and the carmine stains, are of chief importance in the case of chronic processes which have lasted for many years. If, on the other hand, the disease be more acute, the methods of Marchi and Nissl must never be neglected.

*Except in quite chronic diseases, no examination should be considered as complete in which these two important methods (Marchi's and Nissl's) have not been employed.*

With regard to the degeneration of the medullary sheaths, modern investigations have shown that, by the use of Marchi's method, certain evidence of degeneration can be found where Weigert's method fails to show it. Upon this point pathologists should call to mind the observation of Mendel, that a method, which is brilliantly successful when its results are positive, must not be considered as decisive if it gives negative results.

Weigert's method demonstrates very clearly the more localised and older degenerations, but not so certainly the recent destruction of myelin or the more diffuse and disseminated



degenerations, the demonstration of which is the province of Marchi's method.

It must, however, be emphasised in this place, that the microscopic images given by Marchi's stain must be interpreted with great and critical care. Scattered clumps, lying loosely, and for the most part small and roundish, may be found even in the normal brain or cord. It is for this reason important to make longitudinal as well as transverse sections, for the *chainlike* arrangement of the degenerated clumps is very characteristic of a genuine degeneration.

Marchi's stain should not, however, be neglected even in chronic diseases, as in such cases pathological changes of a more recent date may accompany those that are older.

In the same way Nissl's method may be used in older pathological processes, although its principal use is for the more recent. This method and its modifications are, however, quite indispensable in all the processes of intoxication and infection in which the changes in the nerve cells are the chief point to be determined, for this method alone can at present give us a reasonably certain insight into the more delicate structural alterations of the body of the cell and of its protoplasmic processes. The carmine stains, which are employed for tissues hardened in salts of chromic acid, can never give results that are free from objection, because the changes in the nerve cells (few even when other methods are used) may be, for the most part, considered as artificial products of the hardening process, and are not completely unobjectionable. *The use of carmine to determine the more delicate structural alterations of the nerve cells must therefore be condemned.*

Some examples may serve to illustrate the above remarks.

If the case is one in which a local injury to the nervous substance happened a long time, it may be years, ago (as in traumatic destruction of the cerebral cortex, or an old hæmorrhage into the brain), and if it be desired to demonstrate the resulting secondary degeneration, the method of Weigert, or the carmine stain, should be employed. In such a case, little if any result is to be expected from Marchi's method.

If, on the contrary, we have to deal with an acute illness, in which the fatal termination is rapid (traumatic lesion of the brain or cord, acute myelitis, acute poliomyelitis, &c.), besides the usual methods, Marchi's and Nissl's stains would be of chief importance.

Similarly, in chronic affections of the central nervous system, in which new steps in the progress of the disease may always occur (multiple sclerosis, syringomyelia, bulbar paralysis, amyotrophic lateral sclerosis, &c.), Marchi's stain must be employed besides Weigert's, and Nissl's method, in turn, besides the carmine stains. Nissl's method should therefore be employed chiefly if the case be one of lesion of the nerve cells, produced by general causes (infection, intoxications), or when it is a question of determining the disease of the nerve cells as a part of the neuron (examination of the nervous nuclei after amputations, neuritis, &c.).

In disturbance of the mental functions, and especially in chronic forms of mental disease (*e.g.*, general paralysis), besides Weigert's and Marchi's methods for the examination of the medullated fibres, Nissl's should be used for the determination of changes in the nerve cells, and Weigert's neuroglia method to show the existence of large defects of the nerve substance and the complementary proliferation of the neuroglia.

It is apparent, even from these brief examples, that every case must be considered by itself with regard to the methods to be principally employed, and that any general scheme must be sedulously avoided. Otherwise important changes may easily be overlooked.

With regard to the microscopic examination of the normal central nervous system, there is very little need for unstained or teased-out preparations since modern technique has become so much refined. Their use, however, is still justifiable for the peripheral nervous system.

The methods used in our laboratory for the demonstration of the separate normal constituents of the nervous system are chiefly the following:—



A.—*For the central nervous system.*

1. For the nerve cells.  
Nissl's method, Held's method.  
The carmine method, especially staining in bulk with soda carmine.  
The nigrosin method.  
Golgi's method.  
Ehrlich's methylene blue method (if the material be fresh).
2. For the medullary sheaths.  
Weigert's method, especially in the modification of Kulschitzky-Wolters.  
The carmine method.
3. For the axis-cylinders.  
The carmine method.  
The nigrosin method.  
Van Gieson's method.  
Ehrlich's methylene blue method (if the material be fresh).
4. For the neuroglia.  
Weigert's method.
5. For the nuclei.  
Alum-hæmatoxylin.

B.—*For the normal peripheral nerves.*

1. For hardened nerves, the above methods for the medullary sheaths and axis-cylinders.
2. For fresh nerves, treatment with 1 per cent. osmic acid and teasing out in glycerine to show the medullary sheaths, or solution of nitrate of silver and teasing out in glycerine as before.

## VII.—BIBLIOGRAPHY.

- Boehm und Oppel. Taschenbuch der microscopischen Technik, 1896.
- Fol. Lehrbuch der vergleichenden microscopischen Anatomie, 1896.
- Friedländer-Eberth. Microscopische Technik, 1895.
- Goodall. The microscopical examination of the human brain. London, 1894.
- Israel. Practicum der pathologischen Histologie, 1893.
- von Kahliden. Technik der histologischen Untersuchung pathologisch-anatomischer Präparate, 1895.
- Loewenthal. Handbuch der Färberei der Spinnfasern, 1895.
- Mercier. Les coupes du système nerveux central. Paris, 1894.
- Ranvier. Technisches Lehrbuch der Histologie, 1888.
- Rawitz. Leitfaden für histologische Untersuchungen, 1895.
- Stöhr. Lehrbuch der Histologie und der microscopischen Anatomie des Menschen mit Einschluss der microscopischen Technik, 1897.
- 
- Albrecht and Störck. Beitrag zur Paraffin-Methode. Zeitschrift für wissenschaftliche Microscopie und microscopische Technik. XIII.
- Allerhand. Eine neue Methode zur Färbung des Central-Nervensystems. Neurolog. Centralbl., 1897, p. 727.
- Apáthy. Methylenblau. Zeitschrift für wissenschaftliche Microscopie und microscopische Technik, 1892.
- Aronson. Ueber die Anwendung des Galleins für die Färbung des Centralnervensystems. Centralbl. f. med. Wissensch., 1890.
- Azoulay. Markscheiden- und Nervenzellenfärbung. Neurol. Centralbl., 1895. No. 7.
- Berkeley. Die Osmium-, Kupfer-, Hæmatoxylinfärbung. Neurol. Centralbl., 1892.



- Berliner. Plastische Reproduktion pathologisch-anatomischer Präparate. *D. med. Wochenschr.*, 1892. No. 48.
- Bethe. Eine neue Methode der Methylenblaufixation. *Anat. Anzeiger*, 1896.
- Cajal, S. Ramón y. Modification der Ehrlich-Dogiel'schen Methylenblaufärbung. *Rivist. trimestr. microscop.* Vol. I., Fasc. 2 y 3. *Neurol. Centralbl.*, 1896, p. 1028.
- Die Retina der Wirbelthiere, 1893. (Translated into German by R. Greeff.)
- Cox. Imprägnation des Central-Nervensystems mit Quecksilber-Salzen. *Arch. f. microscop. Anat.* XXXVII. 1891.
- Ueber den fibrillären Bau der Spinalganglienzellen. Festschrift herausgegeben von dem Niederländischen Psychiatr. Verein, anlässlich des 25 jährigen Bestehens. 's Hertogenbusch, 1896, p. 227.
- Déjerine. Anatomie des centres nerveux. Paris, 1895.
- Döllken. Einbettung von Gewebstheilen ohne Alcohol-härtung. *Zeitschr. f. wissenschaft. Microscopie und microscop. Technik.* XIV.
- Dogiel. Structur der Nervenzellen der Retina. *Arch. f. microscop. Anat.*, 1895. Bd. 46.
- Donaldson. Preliminary observations on some changes caused in the nervous tissues by re-agents, commonly employed to harden them. *Journ. of morphology*, vol. IX. Boston, 1894.
- Edinger. Bau der nervösen Centralorgane. Leipzig. F. C. W. Vogel, 1894.
- Sammelreferate in Schmidt's Jahrbüchern.
- Zeichenapparat. *Zeitschr. f. wissenschaft. Microscopie und microscop. Technik.* VIII. 1891.
- Ehrlich. Ueber die Methylenblaureaction der lebenden Nervensubstanz. *D. med. Wochenschr.*, 1886.
- Flatau. Das Photographiren macroscopischer Präparate. *Internat. med.-photogr. Monatsschr.*, 1895.
- Serien-Längsschnitte durch das ganze Rückenmark. *Anat. Anzeiger*, 1897.
- Veränderungen des Hirngewichts in Formollösungen. *Anat. Anzeiger*, 1897.

- Flemming. Die Structur der Spinalganglienzellen bei Säugethieren. *Arch. f. Psychiatrie*, 1897, p. 969.
- *Ergebn. der Anatomie und Entwicklungsgeschichte*, 1896, p. 277.
- *Anatom. Hefte*, 1896, p. 568.
- *Arch. f. microsc. Anat.*, 1895. Bd. 46, p. 379.
- Franke. Einbettklötze für Paraffinobjecte. *Zeitschr. f. wissenschaft. Microscopie und microscop. Technik.* XIII.
- V. Frey. Eine Goldfärbung des Nervenmarks. *Arch. f. Anat. und Physiol.*, 1897. Supplementband (*Anatom. Abtheilung*).
- Gerota. Contribution à l'étude du formol dans la technique anatomique. *Journ. internat. d'Anatomie et de Physiol.* T. XII. F. 3, 1896 (Bibliography).
- Giacomini. Guido allo studio delle circonvoluzioni cerebrali dell'uomo. II. edizione. Torino, 1884.
- van Gieson. A study of the artefacts of the central nerv. syst. The topographic alterations of the grey and white matters of the spinal cord caused by autopsy bruises. New York, Appleton & Co., 1892.
- Goldscheider und Flatau. Normale und pathologische Anatomie der Nervenzellen. Berlin. Kornfeld, 1898.
- Golgi. Untersuchungen über den feineren Bau des centralen und peripherischen Nervensystems. Jena, 1894. (Gesammelte Abhandlungen.)
- Greppin. Beitrag zur Kenntniss der Golgi'schen Untersuchungsmethoden. *Arch. f. Anat. u. Physiol.*, 1889.
- Gudden. Anwendung electiver Färbemethoden am im Formol gehärteten Centralnervensystem. *Neurolog. Centralbl.*, 1897. No. 1.
- Held. Structur der Nervenzellen. *Arch. f. Anatom u. Physiol.*, 1897. III., IV.
- Heller. Osmirung des Rückenmarks. *Berl. klin. Wochenschr.*, 1895. No. 50.
- Hill. The chrome-silver method. A study of the conditions under which the re-action occurs and a criticism of its results. London, 1896.



- Kallius. Untersuchungen über die Netzhaut der Säugethiere. Merkel-Bonnet's Ergebnisse, 1894. III.
- Kantorowicz. Thioninfärbung. Centralbl. f. Allgem. Pathologie, 1894.
- v. Kölliker. Gewebelehre, II. Theil, 1896.
- Kopsch. Erfahrungen über die Verwendung des Formoldehyds bei der Chromsilber-Imprägnation. Anat. Anzeiger, 1895. XI.
- Krause. Bau der Retina. Schmidt's Jahrbücher. CCXLIX.
- Kulschitzky. Ueber eine neue Methode der Hämatoxylinfärbung. Anatom. Anzeiger, 1889. IV.
- Laskowski. L'embaumement; la conservation des sujets. Genève, 1886.
- v. Lenhossék. Der feinere Bau des Nervensystems im Lichte neuester Forschungen, 1895.
- Bau der Spinalganglienzelle des Menschen. Arch. f. Psych., 1897. Bd. 29.
- G. Mann. Ueber die Behandlung der Nervenzellen für experimentell-histolog. Untersuchungen. Zeitsch. f. wissenschaft. Microscopie u. microsc. Technik, 1895. Bd. XI.
- Marina. Fixationsmethode für Nissl's und Weigert's Färbung. Neurolog. Centralbl., 1897. No. 4.
- Merk. Darstellung der Mitosen im embryonalen Nervensystem. Denkschrift d. Acad. d. Wissensch. zu Wien, 1888.
- Meyer, Semi. Vitale Methylenblaufärbung. Arch. f. microsc. Anat., 1896. Bd. 47.
- Muschenkoff. Darstellung der Nervenendigungen im quergestreiften Muskel. Zeitschr. für wissenschaftl. Microscopie. V.
- Nebelthau. Microscop und Lupe zur Betrachtung grosser Schnitte. Zeitsch. f. Wissensch. Microscopie und microscop. Technik, 1897. XIII.
- Nissl. Veränderungen der Nervenzellen nach experimentell erzeugter Vergiftung. Neurolog. Centralbl., 1896.
- Nervenzellenanatomie. Neurolog. Centralbl., 1896.
- Nothnagel. Topische Diagnostik der Gehirnkrankheiten, 1879.

- Obersteiner. Anleitung beim Studium des Baues der nervösen Centralorgane im gesunden und kranken Zustande, 1895.
- Obregia. Schnittserien. Neurolog. Centralbl., 1890.
- Orth. Ueber die Verwendung des Formaldehyds. Berl. klin. Wochensch., 1896. No. 13.
- Pal. Ueber ein neues grosses Microtom für Gehirnschnitte. Zeitsch. f. wissenschaft. Microscopie und wissenschaft. Technik, 1893. X.
- Pergens. Action de la lumière sur la rétine. Trav. fait à l'Institut Solvay, 1896.
- Action de la lumière colorée. Trav. fait. à l'Institut Solvay, 1897.
- Pitres. Recherches sur les lésions du centre ovale des hémisphères cérébr. étudiées au point de vue des localisations cérébrales. Thèse de Paris, 1877.
- Pollack. Neuroglia und Neurogliafärbung. Arch. f. microsc. Anat., 1896.
- Sammelreferat in Monatsschr. f. Psychiatrie und Neurologie, 1897.
- Retzius. Das Menschenhirn, 1896.
- Robertson. A modification of Heller's Method of staining medullated nerve fibres. Brit. Med. Journal, 1897, p. 651.
- Roncoroni. Colorazione dei Prolungamenti protoplasmatici delle Cellule di Purkinje e dei cilindrassi preprio induramento dei pezzi in cloruro di platino e liquido del Muller. Arch. per le scienze medicale vol. XX. 1896. No. 9.
- Scarpattetti. Ueber die Anwendung electiver Färbemethoden am in Formol gehärteten Centralnervensystem. Neurolog. Centralbl., 1897. No. 5.
- Schaper. Zur Sublimatfixation. Anatom. Anzeiger. XIII. No. 17.
- Schiefferdecker. Signiren von Präparaten. Zeitschr. f. wissenschaft. Microsc. u. microsc. Technik. XIII.
- Schmaus. Technische Notizen zur Färbung der Axencylinder. Münch. med. Wochenschr, 1891. No. 8.
- Schoebel. Signiren von Präparaten. Zeitschr. f. wissenschaft. Microscopie u. microsc. Technik. XIII.



- Siemerling-Edinger. Die Technik der Gehirnsection. Zeitschr. f. Psychiatrie, 1894.
- Strasser. Schnittserien, ihre Nachbehandlung bei Paraffin-einbettung. Zeitschr. f. wissensch. microscopie, 1891.
- Stroebe. Axencylinderfärbung. Centralbl. f. allgemeine Pathol. und pathol. Anatomie, 1893. IV.
- Tandler. Zur Technik der Celloidinserien. Zeitschr. f. wissensch. Microscopie und microscop. Technik. XIV.
- Virchow. Sectionstechnik.
- Wallenberg. Directe Copie gefärbter Schnittpräparate des Centralnervensystems. Internat. photograph. Monatschrift für Medicin und Naturwissenschaften, 1896.
- Weigert. Markscheidenfärbung. D. med. Wochenschr., 1891. No. 42/43.
- Technik. Merkel-Bonnet. Ergebnisse der Anatomie und Entwicklungsgeschichte, 1893. III.
- Die Golgi'sche Methode. Merkel-Bonnet. Ergebnisse der Anatomie und Entwicklungsgeschichte (with a Bibliography), 1895. V.
- Beiträge zur Kenntniss der normalen menschlichen Neuroglia. Frankfurt a.M., 1895.
- Wolters. Drei neue Methoden für Mark- und Axencylinderfärbung. Zeitschr. f. wissensch. Microscopie, 1890.
- Zanke. Messung des Schädelinnenraumes. Neurolog. Centralbl., 1897. No. 11.
- Ziehen. Eine neue Färbungs-Methode des Centralnervensystems. Neurolog. Centralbl., 1891.

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