known they appear as a secondary system of coarse fibres generally distributed through its entire thickness. Very few regions of the cortex reach this highest stage, however, even in the fully grown adult. The period from youth (eighteen years) to maturity is in general characterized by the gradual appearance of the fine fibres in the II. and III. Meynert layers, and the formation of the secondary fibre system of coarse fibres; but in the fully grown cerebrum there are regions which do not reach even the stage at which the fibres of the II. and III. Meynert layers become medullated.

The central gyri exhibit the most highly developed fibre systems. The addition of new medullated fibres may continue, according to Kaes, up to the fiftieth year of life, though this limit must certainly be subject to wide individual variations.

Flechsig[29] has shown that the cortico-petal projection fibres first became medullated in the regions of the cortex, which mediate sensations, and thus by following the process in these fibres, the sensorimotor areas of the cortex can be mapped out. Their demarcation occurs within the first three months after birth. The intervening portions of the cortex form the association centres of Flechsig and develop later. In the great sensorimotor region about the central fissure Passow[30] has been able to show that the association fibres are at maturity best developed in the more ventral portion which contains the (discharging) pyramidal cells controlling the movements of the lower arm, hand, and face. In the child at one and one-quarter years this layer is equally developed through the length of the gyri, that is, there is no differentiation between the cell groups controlling the leg and the proximal portions of the arm and those which control the distal portions of the arm, and from this we infer that the cortical control of the finer muscles and finer movements increases for some time after that for the coarser movements has been completed.

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BRAIN, HISTOLOGY OF.—METHODS.—The nerve tissues of the brain may be studied fresh or in frozen section, by maceration and isolation, or after hardening by various methods.

Fresh Tissues.—Nervous tissues from animals can be obtained immediately after death. In human beings a certain number of hours practically always elapse before the material can be removed from the body. Certain post-mortem alterations occur early, particularly in the most labile constituents of the nerve-cell protoplasm. But for ordinary histological purposes tissue obtained from the central or peripheral nervous system within twelve hours after death, provided the body has been kept cool, is sufficiently fresh for minute microscopic examination. For many studies, too, tissue removed as late as twenty-four hours after death, provided the body has been kept cool, is quite satisfactory, though one should be sceptical in such tissue in drawing conclusions from the condition of certain of the microscopic pictures, for example, those of the Nissl bodies, and the so-called fibrillary structures.

In examining fresh tissues either teased particles or sections made by the double knife of Valentine may be employed. The teasing method is particularly applicable to the study of the peripheral nerves. These may be observed in an indifferent fluid like physiological salt solution or blood serum, or after the addition of some reagent (acetic acid, osmic acid, caustic potash). Sections of entirely fresh tissue made with the double knife of Valentine are very fragile, besides being extremely difficult to prepare. They may be examined in physiological salt solution, care being taken that the cover slip is not pressed upon. Ehrlich's method of staining fresh masses of tissue will be referred to later.

Maceration and Isolation.—For the isolation of histological elements, especially in the central nervous system, the use of some macerating fluid is desirable. A large number of these have been employed, but only the more important ones need be mentioned. Osmic-acid solutions (one-tenth of one per cent.) work well, any fatty structures, including of course the myeline sheaths, being stained of a dark color. Müller's fluid and dilute chromic-acid solutions (one-one-hundredth of one per cent. to four-one-hundredths of one per cent.) are useful macerating reagents provided the fluids are not allowed to act too long. Tissues macerated in these solutions should be teased and examined at the end of twenty-four hours. Ranvier's alcohol (thirty-three and one-third per cent.) yields very good results.

The tissues intended for maceration should be divided into small pieces before being placed in the macerating fluid. At the end of the period of maceration gentle shaking in a test tube often facilitates the isolation of elements. A pipette may be used for transferring minute particles suspended in the fluid to the microscopic slide for examination. Coarser particles may be teased upon the slide or squeezed between the cover slip and the slide.

The fluid used for maceration may be employed as a mounting medium for the microscopic observations, but as a rule it is better to render the tissues somewhat transparent. For this purpose dilute solutions of acetic acid (one per cent.), saturated aqueous solution of potassium acetate, diluted glycerin, or salty glycerin may be used.

Hardened Tissues.—The three fluids most frequently employed at present for the hardening of nerve tissues are: (1) Müller's fluid, (2) alcohol, and (3) formol.

Müller's Fluid. This hardening reagent consists of potassium bichromate, 2.5; sodium sulphate, 1; distilled water, 100. If desired the sodium sulphate may be omitted, simple solutions of bichromate of potash appearing to act as well as the fluid prepared according to the original formula. Müller's fluid should be changed frequently during the hardening process. Hardening in this fluid requires from several weeks to several months at the ordinary temperature, though if the tissues be kept in the fluid in a thermostat at the body temperature the process may be much shortened. In summer the fluid is particularly prone to become contaminated with growths of fungi. This may be prevented by the addition of a small quantity of camphor. After Müller's fluid hardening, the tissues are best prepared for embedding by dehydration in alcohol, as a rule without previous washing out in water. Tissues hardened in Müller's fluid stain well in carmine and with Weigert's myeline stain. They are not suitable, however, for the application of finer histological methods, such as that of Nissl.

Alcohol. This reagent should be employed when the original Nissl procedure is to be applied, small pieces of the tissue being left in ninety-five-per-cent. alcohol for about three days (see Nissl's method). Alcohol hardening is also desirable if Weigert's fibrin stain or Gram's method of staining bacteria is to be utilized. The periphery of bits of tissue hardened in strong alcohol is likely to undergo marked contraction and distortion. The blood at the periphery of such tissue is also much altered. Artefacts in various parts of the tissue may result from the action of the alcohol.

Formol. At present solutions of this substance are much employed in neurological technique since tissues fixed in them can be stained by several different methods. Formol, or formalin, is a forty-per-cent. aqueous solution of formaldehyde. As a rule, ten-per-cent. solutions of formol are used for hardening pieces of the central nervous system. This corresponds to a four-per-cent. aqueous solution of the gaseous formaldehyde. The whole brain may be hardened more or less satisfactorily in such a solution, but it is better, as a rule, to more or less subdivide the organ. Formol does not contract the tissues hardened in it, but fixes them rapidly in their original form, thus differing markedly from many other hardening fluids. The fluid has a characteristic odor and gives off irritating fumes. Injury to the eyes must be carefully avoided when using it, and it is also well to keep the hands as much out of the fluid as possible, as sometimes a troublesome form of dermatitis is set up by it. After hardening in formalin for a few days the tissue is ready for further manipulation. If Weigert's myeline stain is to be employed the tissue may be placed in Müller's fluid or in his chromic acid mordant. The latter also suffices for the application of Weigert's neuroglia stain. Formalin tissues can also be used for studies by Marchi's method; the ordinary Marchi procedure is gone through with after the formalin hardening. Tissues prepared in formalin do not warp so much as those simply hardened in Müller's fluid. Very good Nissl preparations can also be made from formalin tissues provided they have been gotten fresh and cut into small pieces. The results are not equal, however, to those obtained after fixation in alcohol or sublimate. Marini's modification of the formalin hardening is especially recommended when it is desired to examine tissue by several different methods. This investigator recommends a fluid consisting of five per cent. formol with one-tenth of one per cent. chromic acid in ninety per cent. alcohol. Large pieces of the tissue put into this fluid for twenty-four hours are then removed and divided into small pieces. The latter are placed in more of the fluid freshly prepared, which is changed every day for from three to five days. The blocks of tissue are then fastened upon cork (without any other preparation), and kept in ninety-per-cent. alcohol or in a one-per-cent. solution of chromic acid in ninety-per-cent. alcohol. Sections for staining with methylene blue or thionin are placed in ninety-per-cent. alcohol; those for Weigert's myeline stain in Müller's fluid or in a three-per-cent. solution of potassium bichromate, while sections to which Weigert's method for studying neuroglia is to be applied are placed in a solution of chromogen.

When the material to be studied is not limited in amount it is preferable to use at least three hardening fluids: (1) alcohol for Nissl's method; (2) formol for Weigert's myeline stain, for Marchi's and for Golgi's methods; and (3) Weigert's formol-chrome-alum mordant for the neuroglia stain.

Sublimate solutions, Orth's fluid, Erlitzki's fluid, Flemming's mixture, Van Gehuchten's fluid are all recommended for special purposes. The formulæ for these fluids are to be found in the text books of histological technique. (See also article: *Histological Technique.*)

Embedding.—Three methods are ordinarily employed for embedding neurological tissues: (1) embedding in pith or gum; (2) celloidin embedding; and (3) paraffin embedding.

Pith or gum embedding may be dispensed with. Nissl, for his method, insists upon the avoidance of embedding entirely. He simply fastens the mass of tissue to the block by a solution of gum arabic. He fears that celloidin or paraffin embedding will interfere with the structure of the protoplasm of the nerve cells. He is here in error. Careful embedding in celloidin or paraffin, especially the latter, preserves beautifully the structure of the protoplasm.

Celloidin embedding is carried out as follows: After dehydration in absolute alcohol the tissues are left for twenty-four hours in equal parts of absolute alcohol and ether. They are then transferred to a very thin solution of celloidin dissolved in alcohol and ether. Here they remain until the thin fluid has penetrated the tissue. Small blocks are sufficiently penetrated if they remain for from two to five days. Large pieces, however, as, for example, a whole hemisphere of a baby's brain, must remain in a thin celloidin for a much longer time. The tissues are next transferred to a solution of celloidin which has the consistency of thick syrup. They need not remain in this fluid long; twenty-four to forty-eight hours suffice. There is a widespread idea that the longer tissues stay in thick celloidin the better are the sections obtained. The reverse rather is true, especially when we have to deal with large pieces. The penetration by the thin celloidin should, however, be complete. The thick celloidin may be allowed to stiffen very gradually through evaporation of the alcohol and ether, and a block of it containing the tissue be fastened by means of celloidin on a piece of cork or wood. Or it may be placed directly, without previous stiffening of the celloidin, on a block, allowed to stand in the air for a few minutes until the surface of the celloidin has hardened, after which it may be immersed in eighty-per-cent. alcohol. The blocks may be kept in eighty-per-cent. alcohol until the sectioning is carried out. Instead of celloidin, photoxylin may be employed if desired; it is more transparent than celloidin.

Celloidin embedding is to be preferred when large pieces of tissues are handled, when the tissue is friable, or when the staining method to be employed is one involving considerable manipulation. It is always to be used when Weigert's myeline stain is to be applied.

For embedding in paraffin small pieces should be taken, thoroughly dehydrated in absolute alcohol (twenty-four hours), cleared in xylol or chloroform, and then passed through equal parts of the clearing reagent and soft paraffin, thence into melted paraffin (melting point of 52° C.) for two to eight hours, the paraffin being changed twice during this period. After complete penetration by the paraffin, best in the thermostat kept at the melting point of the substance, the piece of tissue is placed in a shallow dish and covered with paraffin. This is rapidly cooled with cold water until the paraffin stiffens, after which the paraffin block, properly trimmed, can be fastened on the platform of a microtome, when it is ready for cutting.

In warm weather it may be necessary to use a paraffin of higher melting point (60° to 65° C.); in cool weather, a paraffin of low melting point will be found useful (45° to 50° C.). If two kinds of paraffin, very soft and very hard, be kept in stock, it is easy, by mixing them in variable proportions, to make a paraffin of any desired melting point.

Paraffin is to be preferred when very thin sections are desired, or when staining *in toto*, before embedding, is possible. It is especially useful, therefore, in the study of the embryology of the nervous system, and also in the investigation of the finest histological details of the neural elements. It is one of the best methods which can be used when Nissl's method is to be applied.

A combination of the celloidin and paraffin methods may sometimes be used with advantage. The tissue after

dehydration is passed through equal parts of absolute alcohol and ether and then soaked for twenty-four hours in a solution of celloidin. The object is then cleared in oil of origanum, after which it is to remain in a mixture of oil of origanum and fluid paraffin for one or two hours at a temperature of 40° C. It is then placed in melted paraffin of a melting point suitable to the temperature of the time of year and embedded in paraffin in the ordinary way. Now that methods of fastening paraffin sections to the slide have been very much improved, this combination of celloidin and paraffin is very little in vogue.

Sectioning.—In making sections of nervous tissue we have to consider: (1) frozen sections; (2) sections after celloidin embedding, and (3) sectioning after paraffin embedding.

Frozen sections may be made of fresh tissues or of tissues which have been hardened in Müller's fluid or formalin. Frozen sections should be very thin, since to get the most out of them they have to be studied with high powers of the microscope, and unless the sections are thin this is of course impossible. An ether-freezing microtome may be employed, or, better still, a carbon-dioxide freezing microtome. If fresh tissues are used they should be transferred from the knife to physiological salt solution and mounted on a slide in the same fluid; if they have been fixed in formalin or Müller's fluid before freezing they may be examined either in Müller's fluid, Farrant's medium, or salty glycerin.

Celloidin Sectioning.—The sections may be made with any good microtome. The microtomes made by Bausch & Lomb, by Schanze, and by Jung are good. The knife must be kept very sharp, and it is important to have it constantly flooded with eighty-per-cent. alcohol. The whole edge of the knife should be made to cut from the heel to the tip. If very large sections are to be cut, it is best to use a microtome which is so arranged that the knife and tissue mass are entirely immersed in alcohol. Such microtomes are now available at a moderate cost. If sections of a whole hemisphere are to be made, it is important that the knife should be clamped at both ends; otherwise the vibration of the blade will lead to streakiness or even more marked irregularity in the thickness of the sections.

Serial sectioning in celloidin is quite possible if care be taken. The most convenient method is that employed by Obregia. The glass slides upon which the sections are to be employed are coated twenty-four or forty-eight hours before they are to be used, with the following solution: 30 c.c. of a thick syrup made by dissolving powdered candy sugar in boiling distilled water is mixed with 10 c.c. of a thick solution of pure dextrin in distilled water and 20 c.c. of absolute alcohol. The glass slides after partial drying are quite sticky. On a large white plate two or three thicknesses of good toilet paper are thoroughly moistened with eighty-per-cent. alcohol; on the toilet paper an area is marked off in lead pencil which corresponds to the size of the large cover glass which is to cover the preparations on the slide. Each celloidin section, as it is cut, is taken up from the knife on a strip of moist toilet paper and transferred by means of this to the plate. Some regular arrangement of sections in the area marked off on the plate is to be maintained. When the area is full of sections the sticky side of the prepared glass slide is pressed firmly down upon the series of sections. On lifting the glass slide all of the sections adhere to the glass slide. With a sharp knife the celloidin protruding beyond the edge of the glass slide is trimmed off. After a moment's drying to permit any excess of alcohol to disappear, a thin solution of photoxylin is poured over the sections on the glass slide, care being taken to have the photoxylin solution evenly distributed. For this latter purpose it may be desirable to use a glass plate carefully levelled, upon which the glass slide may be laid, while the photoxylin is stiffening. As soon as the photoxylin has stiffened on the surface the glass slide with the sections now covered by a thin sheet of photoxylin can be thrown into a large dish of water. The water dissolves the sugar-dextrin and the photoxylin sheet floats off into the water. Each sheet of this sort may then be treated as a single section. Just before immersion in the water it is well to number the photoxylin sheet with some quickly drying oil paint. This method of serial sectioning of tissues embedded in celloidin is the best one I know of when the Weigert-Pal staining is to be employed. Weigert's celloidin method is also a very useful method, and Obregia's procedure is really only a modification of this.

Serial sectioning of tissues embedded in paraffin is a very simple matter, especially if the Minot or Blake-Minot microtome be used. It is possible with these microtomes to make serial sections of small pieces of tissue as thin as 1 μ or less. In order that the series may be satisfactory the temperature of the room and of the knife must be very accurately adjusted, so that they shall be of the proper temperature to suit the paraffin in which the tissue is embedded. In very cold weather and in very hot weather this is not always easy. When the conditions are perfect, however, each section lies flat and smooth, and the sections one after another hold together at their edges in the form of a delicate ribbon. The problem of transferring the ribbon to the slide and of fastening the sections upon the slide so that when the paraffin is removed no particle of tissue may be lost or displaced, has been variously solved. Three principal methods are employed: (1) Schallibaum's collodion; (2) Mayer's albumen, and (3) the water or alcohol method.

In 1883 Schallibaum recommended mixing one part of collodion with three or four parts of oil of cloves or oil of lavender. The mixture, which makes a clear solution, is spread thinly over the surface of a glass slide by means of a camel's-hair brush. Sections are placed upon the surface and gently warmed over a water bath for from five to ten minutes until the oil has evaporated. The sections adhere firmly and can be passed through almost any fluid without being removed. Absolute alcohol, or equal parts of alcohol and ether, will dissolve out the collodion, and hence in these fluids sections may be lost if they are left in them for a long time. The method is of especial advantage when tissues have been stained in bulk, but is not quite safe when the staining is to be done upon a slide.

Mayer's albumen is made as follows: 50 c.c. of glycerin is mixed with the same quantity of fresh egg albumen. To the mixture 1 gm. of sodium salicylate is added. The whole is well shaken and filtered into a clean bottle. Instead of salicylate of soda some other preservative, like carbolic acid or thymol, may be employed. The mixture is spread in a thin layer with a camel's-hair brush on the surface of the slide, each section being well pressed down upon the albumen with the brush. The sections are then warmed for a few minutes on a water bath, kept just above the melting point of the paraffin. As soon as the paraffin has melted the slide is put into xylol, which dissolves out all the paraffin, and then placed in absolute alcohol, after which it is passed through graded alcohols to water, and is then ready for staining. Care should be taken not to have too thick a layer of albumen, otherwise as it coagulates a slight opacity occurs which may interfere with the study of the finest structures in the cytoplasm or nucleus.

Most of the disadvantages of the above two methods are avoidable if the water or alcohol method be employed. In this method water or alcohol is placed upon a perfectly clean glass slide, the sections are arranged on it with the aid of a brush which has also been moistened with alcohol, the slide then warmed up to about 40° C. (never up to the melting point of the paraffin); the alcohol or water is allowed to evaporate slowly, best in the thermostat kept at about 37° C. for twenty-four hours, after which the slides may be raised in temperature to the melting point of the paraffin, transferred to xylol to remove the paraffin, thence to alcohol to remove the xylol, and afterward passed through graded alcohols to water, when the staining may be undertaken. The warm alcohol or water spreads the sections evenly, removing any creases which may have been present in the section

when it came from the knife. Care must be taken not to have too thick a layer of water or alcohol, or it will be found difficult to keep the sections in their proper order and position on the slide. The water or alcohol will not spread uniformly over the slide unless every particle of grease has been removed from the slide. Sections after hardening in most of the fluids employed in histological technique generally adhere very firmly to the slide by this method. An exception to this statement, however, has to be made with regard to tissues which have been fixed in solutions which contain chrome salts. Those tissues which have been hardened in Müller's fluid or in Flemming's solution do not adhere well, and it is useless to try to employ the method in such cases. One great advantage of the alcohol or water procedure is the absence of any precipitate or opacity due to foreign substances.

When the block of tissue to be cut in paraffin is large it is best not to try to make ribbons of the sections, but to remove each single section from the knife and put it in its proper place on the slide.

Flatau, of Berlin, has recently described a method by means of which serial longitudinal sections through the whole spinal cord can be prepared. It is especially applicable when it is desired to use the method of Marchi for the study of recent secondary degenerations. The spinal cord of the animal which has been submitted to experiment is removed whole about fifteen or twenty days after the operation. A weight is attached to the cauda equina in order to avoid twistings or distortions of the cord. Two threads are drawn through the dura mater of the upper part of the cord, and the whole spinal cord suspended in a glass jar 3 or 4 cm. broad and about 40 cm. long filled with Müller's fluid. The threads are drawn out of the mouth of the glass jar so that they are held in place by the cork when this is put in. Twenty-four hours later the dura mater is cut open along the ventral and dorsal surface and the Müller's fluid is renewed. The cord remains in this fluid for from two to three weeks, after which it is taken out and hung by means of the threads on a stand where it swings free in the air. It is then split lengthwise through the ventral longitudinal sulcus and the dorsal longitudinal septum by means of a Graefe's cataract knife, in order that Marchi's fluid may better penetrate the tissue. The splitting is not carried as far as the lowest part of the conus medullaris. The spinal cord thus retains its connection in its lower part, and the two halves can later be very easily applied to each other. The spinal cord is next hung in a tall glass jar filled with Marchi's fluid and kept in a warm place. Flatau recommends gradual increase in the strength of the osmic acid of the solution with frequent changing of the fluid. He leaves the tissue in the Marchi's fluid for from three to five weeks, after which the cord is washed for twenty-four hours in running water, dehydrated in alcohol, and embedded in celloidin. The tissue is then placed upon a specially prepared block of wood and fastened in Becker's microtome. The sections are cut of a thickness varying from 60 to 80 μ, are dehydrated in alcohol, cleared in carbol xylol, and mounted in balsam on long glass slides.

Staining Methods.

These may be divided into: (1) those used for staining masses of tissue in bulk, and (2) those used for the staining of sections.

Staining in Bulk.—This method is particularly applicable to the staining of the whole nervous system, for example, of embryos or of small animals where serial sections are to be made. For ordinary purposes, perhaps no better stain is available than the alum cochineal of Czokor or Partsch. Powdered cochineal is boiled for a long time in a five-per-cent. solution of alum. After filtration some preservative like salicylic acid is added to prevent the growth of moulds or bacteria. Herrick recommends sulphate of aluminum instead of alum.

Alum cochineal stains very well after nearly all kinds of fixation and hardening. According to the size of the mass of tissue to be stained it is left in the alum cochineal for from one to four days; then washed out thoroughly in water and passed very gradually through graded alcohols, embedded in paraffin, after which serial sections may be made. Grenacher's alum carmine may also be used if desired. Beale's ammonia carmine and Grenacher's borax carmine are also much employed for staining in bulk. If desired tissues may also be stained in bulk by one of the various hæmatoxylins which have been introduced. The formula of Boehmer and that of Delafield are especially to be recommended (*vide infra*).

Golgi's method and Ehrlich's method may be considered as methods for staining in bulk. They will be described further on.

Staining of Sections.—For the staining of sections very different methods have been employed, some being suitable for the demonstration of one element, others for the demonstration of other elements in the tissue. Every attempt has been made by neurologists to devise elective staining methods suitable for the demonstration of the various constituents in the nervous organs. Sections may be stained upon the slide after fixation thereon, or they may be first stained and afterward transferred to the slide.

It will be convenient to divide the staining methods as follows: A. Methods which give information concerning the external form of the nerve units or neurones. B. Methods which demonstrate the internal morphology of the neurones. C. Methods which demonstrate especially the myeline sheaths. D. Methods especially devised for the demonstration of axones or axis-cylinder processes. E. Methods especially applicable to neuroglia. F. Methods for demonstrating certain fibrillary and reticular structures.

A. Methods Which Give Information Concerning the External Form of the Nerve Units or Neurones.—The method above all others which has been of service in recent years in demonstrating the external morphology of the nerve cells and their processes is that introduced by Golgi and the various modifications which his method has undergone in the hands of other investigators.

The slow method is carried out as follows: The fresh, small pieces not over 1 to 1.5 cm. large are immersed in a large quantity of a two-per-cent. solution of bichromate of potash, where they remain for a month in summer or two months or more in winter, the strength of the solution being gradually increased to five per cent. The pieces are then placed in a 0.75 solution of silver nitrate, where they remain for from twenty-four to forty-eight hours, after which sections are cut.

The second method of Golgi is sometimes known as the mixed method; small pieces of tissue remain for three or four days in bichromate of potash solution, after which they are transferred to a mixture of two parts of a one-per-cent. acid solution and eight parts of a two-per-cent. bichromate of potash solution. Here they remain for from three to eight days, after which they are passed through a silver bath as by the other method.

The rapid method of Golgi, which is the one so much used by Ramón y Cajal, is carried out as follows: Very small pieces of tissue are placed directly in a mixture of one part of one-per-cent. osmic acid solution and four parts of a three-and-one-half-per-cent. solution of bichromate of potash. In this fluid they remain for a few days, after which they are passed through the 0.75-per-cent. solution of silver nitrate. The impregnation of the nervous elements varies according to the length of time the tissues are immersed in the first solution. Thus in the human spinal cord, neuroglia is best stained by an immersion of from two to three days; the cell bodies of neurones by an immersion of from three to five days; axones and collaterals by an immersion of from five to seven days.

The so-called double method gives very good results with some tissues; the bits of tissue after having been passed through the procedure above described are sub-

jected at once to a repetition of the process, after which the sections are cut.

Cox's modification of Golgi's method is very valuable when it is desirable to impregnate a large number of elements in the tissue. He proceeds as follows: Bits of fresh tissue are immersed for from two to three months in the following fluid: bichromate of potash (five-per-cent solution), 20 parts; bichloride of mercury (five-per-cent. solution), 20 parts; distilled water, 30–40 parts; chromate of potash with strong alkaline reaction (five per-cent. solution), 16 parts. The bits of tissue are then washed for half an hour in ninety-per-cent. alcohol to remove the excess of sublimate, after which sections are cut and mounted by Golgi's method. The sections can be counterstained, if desired, with carmine or hæmatoxylin.

Ehrlich's Vital Staining with Methylene Blue.—This method, introduced by Ehrlich in 1886, has been of very great importance, inasmuch as it is an elective stain for nerve elements in fresh tissues. Ehrlich injected a solution of methylene blue dissolved in salt solution into the vessels of animals, and found that the axis cylinders of many of the nerve fibres as well as numerous nerve endings stained after a time when the animal was killed and the tissues exposed to the air. While the nerve elements stain of a deep blue color, the other structures in the tissue remain but little or not at all affected. The staining was transient. S. Meyer recommends subcutaneous injection of the methylene blue, using 2 c.c. of a saturated solution beneath the skin every fifteen minutes until the animal dies. A method of fixation was introduced by Dogiel. This investigator laid the pieces of tissue after staining in an aqueous solution of ammonium picrate. A much better fixing method is that of Bethe, who proceeds as follows: the tissue at the acme of staining is immersed in the following mixture: ammonium molybdate, 1 gm.; distilled water, 10 c.c.; hydrogen peroxide, 1 c.c.; pure hydrochloric acid, 1 drop. The fluid must be well cooled before the stained tissues are immersed in it, after which they remain immersed for from two to five hours in the ice box. They are then removed from the refrigerator, left at the room temperature for a few hours, washed for thirty minutes in distilled water, dehydrated quickly in cool alcohol, cleared in xylol, embedded in paraffin, and finally, after sectioning, mounted in balsam. Alum cochineal may be used as a contrast stain. Neuroglia does not stain by Ehrlich's method. A slight modification has been introduced by Ramón y Cajal in order to demonstrate the gemmules or lateral buds on the dendrites of the primary cells in the brain. He uses a saturated solution of Grübler's methylene blue by covering thin pieces of the fresh brain with the fluid by means of a small brush. The fluid is allowed to act for three-quarters of an hour, after which the tissues are washed in salt solution and fixed in the following modification of Bethe's fluid: ammonium molybdate, 1 gm.; distilled water, 10 c.c.; pure hydrochloric acid, 1 drop. After fixation for from two to three hours the excess of ammonium molybdate is removed by washing in water. The tissues are then hardened for from three to four hours in a forty-per-cent. solution of formol to every one hundred parts of which five parts of a one-per-cent. solution of platinum chloride have been added. The formol is washed out by passing it through an alcoholic platinum chloride solution, after which the tissues are embedded in paraffin; thick sections are made. They are passed through absolute alcohol to which platinum chloride has been added, cleared in xylol, and mounted in balsam.

Other methods which stain the nerve-cell protoplasm deeply, leaving the fibres almost unstained, are of value for demonstrating the external form of the nerve cells. These all, however, illustrate also the internal morphology of the nerve cells, and the methods will accordingly be described under the next heading.

B. Methods Which Demonstrate the Internal Morphology of the Neurones.—The methods to be considered here are those which bring out especially well the nuclear and cytoplasmic structures. Generally speaking, it may be said that basic dyes stain the chromatic part of the nucleus, and certain substances (*e.g.*, the Nissl bodies or tigroid masses) in the cytoplasm, while acid dyes stain the other cytoplasmic structures and some of the so-called achromatic substances in the nucleus. It is not unusual in staining methods to employ combinations of the two procedures. Thus, in the much-used hæmatoxylin and eosin stainings, the hæmatoxylin differentiates the chromatic part of the nucleus, while the eosin stains more particularly the protoplasm. Again, when methylene blue and erythrosin are employed, the methylene blue stains the nucleoli, the chromatin of the nuclei, the tigroid bodies, and the neurosomes of the protoplasm, while the erythrosin stains the meshwork-like structure which is visible in the cell protoplasm as well as certain constituents of the nucleus. Sometimes structures are present in the cells or nuclei which take up both kinds of dye and assume a color intermediate between them. The most common stains perhaps are the carmine and cochineal stains, hæmatoxylin and its derivatives, and various aniline dyes.

Carmine and Cochineal Stains.—These have already been referred to above. Alum carmine, alum cochineal, and borax carmine are important stains, and are all applicable to tissues which have been hardened in formalin or in Müller's fluid. A large part of the older work on the nervous system was carried out with the aid of such stains. Various picrocarmines have been used, but in the writer's experience they possess no distinct advantages and can well be omitted from ordinary technique. Carmine is of no value for demonstrating the finer structures in the nerve-cell protoplasm or in the nerve-cell nucleus. It is, however, generally useful when simply the gross relations of the white and gray matter need to be studied.

The Hæmatoxylin Stains.—Hæmatoxylin is an exquisite nuclear stain and differentiates the structures in the nuclei far better than does any of the carmine stains. Tissues left long in hæmatoxylin solutions are usually overstained, and it is accordingly desirable to differentiate, after staining, with some decolorizing agent like acid alcohol. The two best solutions of hæmatoxylin for ordinary work are those of Boehmer and Delafield.

Boehmer's hæmatoxylin is made as follows: two solutions are kept ready: (*a*) a ten-per-cent. solution of hæmatoxylin in absolute alcohol; (*b*) a one-per-cent. solution of alum. A few days before it is desired to use the stain one adds enough of the first solution to the second to make the color a bright violet. The fluid is then exposed to the light for a few days, by means of which it is turned much darker. Sections can be stained in the fluid for from two to five minutes, after which they are quickly differentiated in one-per-cent. solution of hydrochloric acid in seventy-per-cent. alcohol. They are then thoroughly washed out in water and allowed to remain in the water until they turn blue (fifteen minutes); they can then be dehydrated (after previous counterstaining, if desired), cleared in creosote or oil of origanum, and mounted in balsam. The nuclei are stained of a deep blue violet color, while the protoplasm retains only a light blue tinge.

Delafield's hæmatoxylin is made as follows: 2 gm. of hæmatoxylin are dissolved in 10 c.c. of absolute alcohol. This solution is mixed with 200 c.c. of an ammonia-alum solution. The mixture is exposed to the light in an uncorked vessel for four or five days, after which it is filtered; 100 c.c. of alcohol and the same quantity of glycerin are then added. The fluid is again filtered and the bottle corked. It is then permitted to stand for a long time, at least six weeks, generally several months, in order to ripen. It is then ready for use. It is employed in much the same way as Boehmer's hæmatoxylin.

Ehrlich's acid hæmatoxylin has been much recommended, but possesses no especial advantages over the two formulæ given above. It is stated, however, that it stains more quickly and that the staining is more permanent.

The iron hæmatoxylin stains are very valuable as nuclear dyes and for the demonstration of particular elements of the cytoplasm, especially the centrosomes. The method of Heidenhain or that of Benda may be employed. According to Heidenhain, sections hardened in sublimate are immersed for from half an hour to three hours in a one-and-one-half to four per-cent. solution of ferric alum (clear violet crystals). After washing with water the sections are stained for thirty minutes in an aqueous 0.5-per-cent. solution of hæmatoxylin. They are then quickly washed in water, immersed again in a solution of ferric alum, which removes a part of the stain, the differentiation being followed under the low power of the microscope. After the differentiation has been completed the preparations are washed for fifteen minutes in running water, dehydrated, cleared, and mounted in balsam. The method is applicable to sublimate or alcohol material, but gives less good results with tissues hardened in chrome salts. It is best in using it to make sure that the sections are thin. They should not be thicker than 5 μ. A contrast stain may be used if desired.

Benda's method also gives very good results and is applicable to tissues fixed in all of the ordinary reagents. Sections are immersed for twenty-four hours in the liquor sulfurici oxydati of the German Pharmacopœia, diluted with one or two volumes of water. After thorough washing, first with distilled water and afterward with water from the tap, the sections are stained in a one-per-cent. aqueous solution of hæmatoxylin until they turn of a deep black color. After rinsing in water they are then differentiated in a thirty-per-cent. aqueous solution of acetic acid, the decolorizing process being carefully controlled by means of the low power of the microscope. The method is very valuable for the study of the central nervous system and the peripheral nerves. Acid fuchsin or eosin may be used with advantage as a contrast stain. Mayer's hæmalum may be used instead of the ordinary hæmatoxylin solutions. The particular application of hæmatëin, by Apáthy, for the demonstration of special elements will be referred to further on.

Weigert's hæmatoxylin method for demonstrating karyokinetic figures is as follows: tissues which have been hardened in ninety-six-per-cent. alcohol are cut into very thin sections and placed in tinctura ferri Rademacheri for thirty minutes. They are then washed quickly in water and stained for half an hour in a fluid of the following formula: hæmatoxylin, 1; absolute alcohol, 10; distilled water, 100. The sections are washed in water, differentiated in feeble acid alcohol, after which they are allowed to remain in ordinary tap water for ten minutes. They are dehydrated in absolute alcohol, cleared in xylol or some essential oil, and mounted in balsam.

The Aniline Stains.—Of the aniline dyes the most useful in histological work are methylene blue, gentian violet, thionin, and toluidin blue. Aniline blue black, and saffranin have also been employed. The triple stain of Biondi-Ehrlich, which consists of a mixture of methylene green, acid fuchsin, and orange G, is of advantage for the differentiation of some elements, but is especially difficult to apply successfully to the staining of tissues. Saffranin is particularly valuable as a nuclear stain, especially in the study of karyokinesis. Methylene blue is most employed in the study of the nervous system for the staining of the nerve cells according to the method of Nissl or some modification of this method. Thionin may, if desired, be used in its place. Toluidin blue also appears to yield equally good results.

Nissl's Method of Staining with Methylene Blue.—Nerve tissues as fresh as possible are hardened in ninety-six-per-cent. alcohol for three days. After the hardening has been completed small blocks of tissue are fastened to cork by Weigert's method with gum arabic without embedding. The sections are cut in the ordinary microtome, placed in ninety-six-per-cent. alcohol, and stained in a watch glass of the dye. The dye has the following constitution: methylene blue B. pat., 3.75; Venetian soap, 1.75; distilled water, 100. A watch glass full of this dye containing the sections is heated over a spirit flame until small bubbles arise, making a crackling noise. The temperature corresponds to about 65° or 70° C. The sections are then transferred to a differentiating fluid which has the following composition: colorless aniline oil, 10 parts; ninety-six-per-cent. alcohol, 90 parts. The aniline oil must not only be colorless at first but must be kept carefully protected from the light. The process of differentiation of the sections is considered complete when no more coarse clouds of color go off into the fluid.

The differentiated sections are placed upon glass slides, carefully dried with filter paper, cleared in oil of cajeput, and again dried with filter paper.

The slide is then flooded with benzin, after which benzin colophonium is placed over the sections, and the whole slide is heated until all the benzin gas has been driven off. Should the material catch fire no harm will be done provided the flame is blown out immediately.

To make the benzin colophonium, one adds benzin to pure white rosin and allows the mass to stand for twenty-four hours. A fluid, transparent substance should be obtained which is ready for use. If it is too thin it may be allowed to evaporate until of the correct consistency; if too thick, more benzin may be added.

With this method the tigroid masses in the cytoplasm are exquisitely demonstrated. The method is especially valuable for the pathology as well as for the anatomy of the nerve cell.

Held's Modification of Nissl's Method.—This investigator, after hardening in alcohol, sublimate, Van Gehuchten's fluid, or other fluids, embeds small blocks of tissue in paraffin and cuts sections of a thickness varying from 1 to 10 μ. For the finest histological details sections of 1 μ or less are sometimes valuable, but for general pathological studies thicker sections are of much greater service. A thickness of from 15 to 30 μ is by no means too great for most purposes.

The paraffin sections are fastened upon the slide by the alcohol method (*vide supra*).

The sections, after they have been fastened to the slide, are first stained in the following solution: erythrosin, 1; distilled water, 150; acetic acid, 2 drops. This stain is permitted to act for a minute or two, and may be slightly warmed. Sections are then washed in water, after which they are stained in methylene blue. Held's staining fluid consists of equal parts of the methylene-blue solutions used by Nissl (*vide supra*) and a five-per-cent. aqueous solution of acetone. The sections are heated in this staining fluid and the heating is continued until all smell of acetone disappears. The sections remain in the fluid while it cools, after which they are differentiated in a one-tenth of one-per-cent. solution of ordinary alum. The process of differentiation requires from a few seconds to several minutes, according to the nature and thickness of the section. When the differentiation is complete sections are quickly washed in water, dehydrated as quickly as possible in absolute alcohol, passed through xylol for clearing, and then mounted in benzin colophonium according to the method advised by Nissl.

The Nissl bodies are stained of a deep blue color, sometimes showing a slight violet tint. The ground substance of the protoplasm stains red, as do the nuclear membrane and certain other structures in the nucleus. The nucleolus itself stains blue, the neurosomes stain violet, as do also the accessory nucleoli.

Mann's Method of Staining with Eosin and Toluidin Blue.—The sections are fixed in sublimate solution, sectioned in paraffin, fastened upon the slide, treated with iodine to remove the sublimate, and then washed for two minutes in water. The sections which are still somewhat yellow are then placed for from five to ten minutes in a one-per-cent. aqueous solution of eosin. They are then washed in ordinary water, after which they are stained in one-half-per-cent. aqueous solution of toluidin blue for from twenty to thirty minutes. The sections are then quickly washed off in distilled water to remove the excess of toluidin blue, quickly dehydrated in absolute alcohol, during which blue clouds of color go off.