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Original Articles and Clinical Cases.

THE CHROME-SILVER METHOD. A STUDY OF
THE CONDITIONS UNDER WHICH THE
REACTION OCCURS AND A CRITICISM
OF ITS RESULTS.

*(Being the Presidential Address to the Neurological Society
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THE silver-method, discovered by Professor Golgi, of Pavia, has thrown more light upon the structure of the central nervous system than all other methods, microchemical, physical, and experimental, put together. In the address which Professor Schäfer delivered as President of this Society, in 1893, he gave a masterly account of the logical effects which these discoveries must have upon our views as to the constitution of grey matter and as to its mode of action as an apparatus for the distribution of nerve impulses.

The modifications in our hitherto accepted beliefs upon which Professor Schäfer insisted were chiefly of a negative character, but there was enough of reconstruction to show that no question is of such burning interest as this to anatomists, physiologists, and psychologists, or shall we say, in one word, to neurologists.

In 1865, Deiters pointed out the fundamental distinction between the axis-cylinder process and the protoplasmic pro-

cesses of a nerve-cell. In 1871, Gerlach gave expression to the usually accepted view as to the structure and mode of action of the nerve-machine. Gerlach's theory was extremely simple, and yet it made all the processes of nerve activity, from reflex action to thought itself, easily explicable. We could, in fancy, follow the afferent impulse as it traces its way along lines of least resistance through the network of nerve-strands to the appropriate efferent fibre: nay, we could see impulses which had sunk out of consciousness, taking a dynamic attitude within the cells in which they had lain dormant, and starting on a fresh voyage through the network, coming into relation with other impulses similarly liberated—combining, disparting, interweaving the tissue of a dream or a thought. The apparatus by which these processes are carried out was supposed to consist of afferent fibres ending in a network which receives also the protoplasmic processes of the nerve-cells, of which the axis-cylinders become nerves. Nothing could be simpler or more satisfactory. Without some such conception, the brain, as a mechanism, becomes unthinkable, for it bears no relation in character to the products of its own activity.

But this simple faith received a terrible shock when, in 1875, Golgi introduced his method for colouring the brain with silver. This method revealed the existence of branching systems hitherto unsuspected—the collateral ramifications of axis-cylinder processes and of posterior root fibres—and, at once, our views as to the way in which grey matter does its work fell into a state of chaos.

Golgi himself could not get free from the prejudices in which he had been brought up, but he deduced from his facts the only conclusions which were logical from his point of view. He had discovered a second *plexus* formed by the collaterals. He did not want two; and, moreover, he traced the protoplasmic processes of the cells into connection with blood-vessels and ependymal cells. Hence he was justified in throwing Gerlach's plexus overboard, and accepting, as a reflex apparatus, in its stead, the plexus of collaterals. Protoplasmic processes he relegated to an ancillary position, for he regarded them as nutritional; the roots

by which cells suck up the nourishment they require both for themselves and for the long fibres to which they give rise.

Fancy the grand branching system of a Purkinje-cell reduced to the level of a vegetable root! The position seemed intolerable, and besides we have no analogy for supposing that any animal cell needs such a system of roots, however extensive may be the processes of the cell body which look to it for their nourishment, or however important their functions. Indeed, it might be pointed out that the nerve-cells which are responsible for the nutrition of the longest fibres of all, the cells of the root-ganglia, are described by Golgi himself as destitute of this apparatus for collecting nutriment. Again looking at the question from another point of view, the processes of the granule cells of the cerebellum seem to lose their meaning if the protoplasmic processes of the Purkinje-cells are incapable of collecting impulses; so, too, in the olfactory bulb the glomerular protoplasmic processes of the mitral cells seemed clearly to be the chief centripetal conductors, and not to play a subsidiary part as Golgi suggested. Then the axis-cylinder processes of the large cells of the anterior horn of the spinal cord do not carry collaterals; in this case, therefore, impulses carried to the cord by posterior roots or by descending pyramidal fibres can only be transferred to the efferent root fibres *viâ* the protoplasmic processes of their cells of origin.¹ These and a variety of other reasons justified histologists in rejecting the nourishment-collecting theory of the function of protoplasmic processes.

The changes in our views as to the mode of working of grey matter are sufficiently startling, however, even though we do not classify cell processes as functional and vegetative. With regard to many of the features revealed by the chrome-silver method we have no physiological theories at all, and some seem to be incapable of explanation. From

¹ Kölliker ("Zur feineren Anatomie des centralen Nervensystems," II., *Zeitsch. f. w. Zool.*, li., p. 27, 1891) says that he cannot find collaterals on the axis-cylinder processes of motor cells. I also have searched for them in vain. Golgi figures them. Ramón y Cajal says that he observed them both in the rat and in the dove. We may therefore conclude that they are present exceptionally.

every point of view, therefore, it is essential that we should come to understand the conditions under which this method works, and learn how far we are to trust its results.

The suspicion with which we approach it would be unwarrantable in the case of almost any other method; but in many ways this method seems to court suspicion. To begin with, it is so coarse, from the point of view of modern histological technique, that we can say without any want of gratitude to the histologist who gave us this marvellous process, that it can have been discovered only by accident. A block of tissue soaked in bichromate of potassium, or in a mixture of bichromate of potassium and osmic acid, is dropped into a solution of silver-nitrate. A copious precipitate of bichromate of silver of course occurs. We should have expected that this precipitate would be uniformly distributed throughout the mass, whereas we find that the bichromate of potassium leaves the mass more quickly than the nitrate of silver enters it, and that after all the chrome-salt, which is in solution at the time, is precipitated, the nitrate of silver continues to penetrate and is reduced by certain cells and fibres in the interior of the block. The addition of a very little formic acid (about 1 to 300 of the bichromate and osmic acid mixture) delays the precipitation of chromate of silver, inducing it to crystallise out instead, and so favours the entrance of the silver-nitrate; as a result the interior of the block is freer from granular deposit than when nitrate of silver alone is used.

The cells and fibres which reduce the silver-salt are marked out with marvellous delicacy and present figures of great beauty, but the success of the method depends upon its failure—its failure as a stain. If the colour were to be taken by all the elements, or even by all the elements of one kind, the result would be an impenetrable black mass. It is because it picks out one cell in fifty, or one in a hundred that a picture is obtained which conveys information as to the structure of the tissue.

The results obtained by this method are so conspicuous and diagrammatic that no special skill in microscopy is needed for their recognition. So diagrammatic are the figures that

those who first used the method thought it quite unnecessary to copy them. More effective pictures could be made by merely imitating their general style. For a long time histologists were in doubt as to how far the artist followed nature, and how far he might be said to keep step with, or even to precede, her. These jet-black figures, on a tawny ground, are particularly suitable for photography; and this, as it seems to me, is by far the best way of reproducing them. They should only be drawn by hand when, as is not infrequently the case, the sections are too thick to allow of their photographic reproduction.

I have no intention of recapitulating the results obtained by Golgi and his pupils, by Ramón y Cajal, Lenhossék, Kölliker, Retzius, Van Gehuchten and others who have used the method with conspicuous success. Exhaustive, analytical, and bibliographical notices are accessible to everyone.¹ My endeavour has been to ascertain the CONDITIONS UNDER WHICH THE METHOD WORKS; THE NATURE OF THE REACTION; and, incidentally, THE VALUE OF ITS RESULTS. It has made startling revelations. How far may they be trusted?

What is the nature of the reaction? Why does it select certain cells or fibres and neglect the rest? Are there any cases in which the results obtained by Golgi's method are incompatible with those obtained in other ways, and if the two results cannot be reconciled, which is to go to the wall? These are the problems which I set myself to work out, and I may as well say at once that I am as far from knowing the answers as when I started. I can only hope that the publication of my work may save some other investigators trouble.

¹ Cf. v. Lanhossék, "Der feinere Bau des Nervensystems im Lichte neuester Forschungen," Berlin, 1893. Kölliker, *loc. cit.*; also "Handbuch der Gewebelehre des Menschen," vol. ii.; "Elemente des Nervensystems," 1893. Retzius, "Biologische Untersuchungen," *Neue Folge*, iii., iv., v., vi. Cajal, "Les nouvelles idées sur la Structure du Système Nerveux," trans. by Azoulay, 2nd edition, Paris, 1895. Van Gehuchten, "Le Système Nerveux de l'homme," Lierre, 1893. Aldren Turner, "On recent applications of Golgi's Method to the Study of the Nervous System," *BRAIN*, 1893, vol. xvi., p. 259. Schäfer, Quain's "Anatomy," vol. iii., part 1, 1893. Obersteiner, "Anleitung beim Studium des Baues der Nervösen Centralorgane," 3rd edition, Leipzig, 1896.

Method.—The conditions necessary for success, as laid down by those who have chiefly used the method, may be summed up as follows :—

- (1) The subject should be a young animal or an embryo.
- (2) The tissue must be as fresh as possible.
- (3) It must be cut into thin blocks, not more than 2 mm. thick.
- (4) During hardening, the bottles must be kept in the dark¹ and warm.

(5) If embedded in celloidin, the embedding process must not occupy more than an hour (for fear of destruction of the stain by absolute alcohol), and the celloidin should be set with 70 per cent. alcohol, and cut under alcohol.

Probably all these precautions are desirable under certain conditions, but that none of them are essential is shown by the photograph (fig. 1) from one of the most successful preparations I have ever made. It was taken from the brain of a man who had been dead for more than twelve hours; it was hardened in the cold of winter, kept in the light, embedded in celloidin in the ordinary way (the process extending over two or three days), and cut frozen on a Swift's microtone.

We are still so far from understanding the nature of the reaction, that all variations of procedure are possible sources of instruction. My object being not to get good specimens, but to determine the conditions under which the reaction occurs, I endeavoured to ascertain the limits within which the process may be varied without abolishing the reaction, and have noted the effects of the several modifications.

First, as to *material*.

(a) *Age of Subject.*—For general purposes young animals are desirable. The method succeeds well with embryos after about half-time (in the case of the calf, horse, and other large animals); new-born animals give excellent preparations of cortex cerebri, spinal cord, &c.; nevertheless, the most comprehensive results are obtained with animals of some days old, *e.g.*, kittens of three to twenty days, puppies

¹ In his later publications, Ramón y Cajal says that it does not matter whether the preparations are kept in the dark or in the light.

of a fortnight, rats of eight to twelve days, hedgehogs of nine days, &c.

For the cells of the cerebellum and cortex cerebri, I prefer a full-grown animal.

For the collaterals of posterior root fibres, it seems to be necessary to use a young animal: but this rule does not hold good for the axis-cylinder collaterals of pyramids and Purkinje cells. I have obtained beautiful preparations of axis-cylinder collaterals from the brains of full-grown hedgehogs.

Ramón y Cajal and Van Gehuchten recommend the use of embryos or very young animals on the ground that it is the non-medullated, or not yet medullated, fibres that give the reaction; but neither of the histologists just quoted states explicitly, so far as I am aware, that medullated fibres will not take the stain. This is a matter to which I have given some attention, in the hope that it might throw light upon the nature of the reaction, and I have come to the conclusion that although embryonic fibres and non-medullated fibres give it best, it is also possible to obtain the reaction through the medullary sheath. When Weigert and Golgi preparations are compared, it is found that the stainings do not tally. In the cortex cerebri or cerebelli, for instance, the Weigert stain follows the fibre close up to its cell: while in the Golgi preparations, the axis-cylinder process is stained black for a long distance. Again, in the white matter of the brain, *e.g.*, the internal capsule, it often happens that the silver colours great masses of fibres where we have no reasons for supposing that non-medullated fibres preponderate.

(b) *Freshness*.—It is especially desirable for this method of preparation that the tissue should be fresh, since the mixture of osmic acid and bichromate of potassium penetrates very slowly. If a block of two millimetres in thickness is taken out of the hardening agent after twenty-four hours and cut across, the centre will usually be found to be perfectly uncoloured, and it is easily to be understood that many *post-mortem* changes may have occurred in the interval, especially if the bottle has been kept warm.

I prefer to open the chest of the animal before the heart has ceased to beat, and then, having thoroughly washed out the blood-vessels with salt solution under suitable pressure through a canula inserted in the aorta, to inject a considerable quantity of bichromate of potassium into the circulation.

In my first attempts to inject warm bichromate of potassium into the living vessels, I found that the small arteries closed so vigorously against the irritant fluid that it was impossible to drive it through. By the addition of 1 per cent. of ordinary fermentation (ethylidine) lactic acid to the salt-solution I succeeded in producing such a paralytic dilatation of the small vessels, as judged by the free outflow from the cut jugular vein, that when the bichromate solution was used it flowed through with the greatest facility. Tooth has found that the injection of morphia after the salt-solution is equally effective in producing paralysis of the arterioles.¹ Sometimes I use the bichromate and osmic mixture for the injection, but it does not appear to possess any advantage over the bichromate alone; while in the quantity I use (250 cc. of the mixed osmic and bichromate) the cost is considerable and the smell extremely disagreeable.

This question of the *penetration of the hardening fluid* is, I believe, a factor of great importance in determining the subsequent reaction.

The excellent results which are to be obtained with the hedgehog's brain seem to be due, at any rate in part, to its unusual firmness and permeability; the difficulty in staining the human brain, to the closeness of its texture.

The success of the method when applied to young animals appears to me to depend partly upon the ease with which the hardening fluid, and, subsequently, the silver-nitrate penetrate.

Of much more consequence, as guiding us in forming an estimate as to the amount of reliance which we are to place upon the appearances presented by Golgi-preparations, is the fact that, in any part of the brain, the staining is more abundant and conspicuous when the tissue presents an open tex-

¹ Tooth, *BRAIN*, vol. xv., 1892, p. 400.

FIG. 1.

A cell of Purkinje from the cerebellum of a man aged 45, together with the branching system of a second cell.

FIG. 2.

Adult hedgehog; cortex cerebri $\times 400$. The tissue was embedded in celloidin, thickened very gradually, the process extending over four days. A thin, flat section could be obtained in consequence. The thorns on the protoplasmic processes are well seen.

FIG. 3.

Skate (*Raia batis*); granular layer of the cerebellum, stained with the alum-carmin-Weigert method. The figure shows the nuclei of "granules" surrounding clumps of molecular substance. In the centre of the molecular clumps are seen the swollen axis-cylinders of the rosettes of Cajal's mossy fibres.

FIG. 4.

Adult hedgehog ; cerebellum $\times 408$. A single large cell of the molecular layer, with thorny protoplasmic processes and an axis-cylinder process from which a branch passes inwards to form a basket (only partially coloured) around a cell of Purkinje.

ture than it is where the tissue is dense. Many illustrations of this law may be cited; I will content myself with two which appear to me to be of the greatest importance: (*a*) In the cerebellum the thin sheet of tissue in which the bodies of the Purkinje-cells lie is extremely open, especially after the shrinking of the cells in the hardening agent. As a result we obtain in this layer an abundant staining of the fibrils which pass through it. Various appearances, often completely limited to this thin stratum, are seen in consequence. Sometimes the sheet is pierced by multitudes of vertical fibrils, the centrifugal fibrils of the "granules." At other times, either the branching processes of the tangential cells of the molecular layer or the branching processes of penetrating nerve-fibres are abundantly stained as they lie in this sheet.

The fascia dentata affords another illustration of the limitation of the stain to a layer. It usually happens, in a transverse section of the cerebrum, that we find that the cells of the granular layer of the fascia dentata, where it lies near the surface, are beautifully stained; the underlying cells of the nucleus fasciæ dentatæ may or may not be stained; the small pyramids of the granular layer where it lies at a distance from the surface are hardly ever stained, but we see in their place a quantity of penetrating fibres (fig. 7). The difference in staining of the fascia dentata in its two parts can only depend upon the relative freedom of access afforded to the reagents. Usually the fibres in the deep-lying fascia dentata are absolutely limited to this layer which is marked out by the total absence of stain on either side of it. It seems to me difficult to account for this limitation of the reaction on any hypothesis other than the one I have put forward, viz., that the reaction occurs most readily where the tissue is loosest in texture.

(2) The occurrence of the reaction does not appear to depend upon the tissue being fresh, for it can certainly be obtained a considerable time after death—at least two days in cool weather. If it were not for the slowness with which the hardening fluid penetrates, good results might, I believe, be relied upon with any tissue in which putrefaction had not already set up.

On the other hand, my experiments in injecting tissues with bichromate during life seem to show that absolute freshness has a good deal to do with the character of the results obtained. When the injection has been successful—and it is easy to tell from the appearance of the unstained nuclei whether this is the case—not only are deeply lying structures, such as the corpus striatum, stained before the pyramids, but all the cells show the most remarkable clearness of outline. The processes of the cells are sharp and clear cut, and there is in such preparations as I have made a total absence of “thorns” or “mossiness.” To this I shall refer later on.

(3) *Size of the Block.*—As observed by everyone, the amount of osmic and bichromate mixture used to harden the brain should be large relatively to the amount of tissue, but the brains of different animals vary immensely in their capacity for absorbing the reagent. Of all the animals I have used none allows the reagent to penetrate so easily as the hedgehog. A complete transverse section of the brain of the young hedgehog is easily stained throughout its whole extent, giving a perfect result, even though the slab of tissue be 3 or 4 mm. thick. I attribute this penetrability to the peculiar texture of the brain which, when fresh, is white, and so firm as to be convenient to handle.

If the blood-vessels are injected with warm bichromate of potassium, the amount of tissue hardened in one block may be almost indefinitely increased, since the nitrate of silver finds its way through any thickness of tissue, giving apparently a better result the farther it has to go.

(4) *Duration of Hardening.*—In one paper (*Rivista trimestriale de histologia*, March, 1889, p. 104) Ramón y Cajal gives the following general rule:—

For neurogleia, harden 12-20 hours.

„ nerve-cells „ 20-24 „

„ nerve-fibres „ 24-36 „

In another place (*La Cellule*, vii., 1891, p. 130) he amplifies his directions by pointing out that the time needed depends not only upon the structure which it is

desired to show, but also upon the kind of animal and its age. For example: "In the embryos of mice, rats and rabbits the cells impregnate in a very uncertain fashion; on the other hand, the epithelial elements, vessels and fibres colour constantly, provided the duration of the hardening is not prolonged beyond two to three days. The duration of the hardening of the blocks in the osmio-bichromate mixture ought to be prolonged to two, three to five days when dealing with new-born mammals of a certain size (rabbit, guinea-pig, cat), et, à plus forte raison, s'ils sont âgés de 8 à 15 jours."

"To succeed in colouring the cells of the molecular layer in the rabbit (eight days old) the hardening must be prolonged for nearly five days; while the collaterals of the white substance demand six or seven."

Lenhossék ("Der feinere Bau des Nervensystems," Berlin, 1893, p. 6) gives as the most favourable time for the human spinal cord—

Neurogleia, 2-3 days.

Nerve-cells, 3-5 days.

Nerve-fibres (collaterals), 5-7 days.

When I commenced this series of tests it was my intention to compare Golgi-preparations of all parts of the brain with sections stained according to my modification of Weigert's hæmatoxylin method.¹ I wished to ascertain whether the silver colours tissue only, or whether, as was suggested by Rossbach and Sehrwald, in 1888, it colours instead of, or in addition to, the neurogleia and nerve-tissue, lymph, or lymph-deposit, or lymph-coagulum which surrounds the nervous structures. I was soon convinced that

¹ My modification of Weigert's method depends upon a previous staining of the tissue *en bloc* in carmine-alum. The carmine-alum is made by boiling *an excess* of both carmine and alum in a porcelain dish for three or four hours, water being added from time to time. The fluid is allowed to stand for some hours, and then filtered. Blocks of tissue are soaked in carmine for twenty-four hours; sections are then made and stained according to Weigert's hæmatoxylin method; decolouration may be effected with either ferridcyanide of potassium or permanganate of potash, the solution being in either case of about half the strength usually recommended. All nerve-cells and their processes are stained black; medullated fibres are stained blue, and non-medullated fibres black (*cf.* the photograph, fig. 13).

while the black reduction-substance may lie entirely outside the nerve-elements, forming a case for them, or may both surround and penetrate the nerve-elements, it is, in the most successful preparations, limited to the nerve-elements themselves. I therefore abandoned the comparison with Weigert preparations. For the sake of the comparison, however, all my earlier preparations were made under rigidly uniform conditions, the tissue remaining in the hardening fluid for exactly five days. Among these preparations I find admirable examples of cells of all kinds, of granules, fibres, collaterals, &c.; and hence this test, as well as my recent experiments in which I have varied the time of hardening within the widest limits (one day to two months), fail to establish anything like a definite relation between length of hardening and elements stained. Preparations of the cortex of a twelve-day rat, which had remained in the bichromate and osmic mixture for two months, exhibit pyramidal cells with their axis-cylinders and collaterals with exceeding beauty and completeness.

Ramón y Cajal says that some elements may be lost because the hardening in bichromate of potassium has been too prolonged. Of this I have never seen proof.

That pieces of tissue which after three, four, or five days' hardening give very little reaction may give a good result at the end of ten days or a fortnight I am fully convinced, but I cannot lay down any law as to the form which the improvement will take.

The variability of the result leads us to hope that when we understand the conditions under which the reaction occurs we shall have in our hands a method with which to produce at pleasure a wide range of effects, and it is very greatly to be hoped that someone will undertake an exact series of observations upon its time- and temperature-relations.

Prolonged immersion in silver-nitrate does not appear to affect the reaction.

(5) *Temperature*.—I have hardened the tissues at every temperature from the cold of winter to 45° C. Warmth hastens the reaction, but it does not, so far as I can determine, affect its quality.

(6) *Light*.—As observed elsewhere the reaction occurs in bright sunlight as well as in photographic darkness.

(7) *Celloidin*.—The rules for embedding, as laid down by various observers,¹ present considerable discrepancy although all agree that the sojourn in the alcoholic solution of celloidin should be as short as possible. I have prolonged it up to four days without any perceptible deterioration, and with the obvious advantage that the celloidin when set and cut does not pucker but lies perfectly flat on the slide. The accompanying photograph (fig. 2) is from a section of a block placed for some hours in a mixture of alcohol and ether, to which celloidin was added in gradually increasing quantities until at the end of four days it was ready to set. It is a matter of great importance, if celloidin is to be cut frozen, that its constitution should be uniform. I am in the habit of gradually adding dry celloidin to the alcohol and ether in which the block is soaked. Not only does this ensure penetration, but the sections when cut lie perfectly flat. It is just as easy to make a section of the whole brain, either transverse or longitudinal, as to cut a minute piece.

Variations in the Hardening Agent.—There is little latitude for variation in this direction. A chromate of some kind must be used, and the potassic bichromate seems to answer better than other salts. Ammonic bichromate gives the reaction, but whilst the cells come out very clearly, and the fibres are clearer than with potassic bichromate, the finer structures are not seen.

Salts of chromium, other than the chromates, do not give the reaction. Chrome-alum, for example, which for some purposes is an excellent hardening agent, is, for this method, perfectly useless.

Osmic acid expedites the hardening, but does not take part in the reaction ; indeed, it may be replaced by formaline

¹Ramón y Cajal: "The pieces can be quickly embedded in celloidin or paraffin." Kölliker: "One hour in absolute alcohol and one hour in celloidin." V. Gehuchten: "Fifteen to twenty minutes in 96 per cent. alcohol, a quarter of an hour in absolute alcohol, a quarter of an hour in thin celloidin. The block is fixed on cork with celloidin, and immersed for a short time in 70 per cent. alcohol." Lenhossék recommends five minutes in a moderately thick colloidal solution; placing the object in elder-pith; setting celloidin with 80 per cent. alcohol, and cutting under alcohol.

(4-6 per cent.), as Durig¹ has shown. There is, therefore, no need for such a cumbersome name as bichrom-osmio-silver method (Retzius): the "chrome-silver method" best expresses the reaction.

Variations in the Silver-salt.—The only experiments I have tried have been in the direction of a salt more easily reducible than the nitrate.

(a) Silver-acetate gives the reaction, but does not pick out the finest markings, and is usually accompanied by a great quantity of deposit.

(b) Silver-nitrite, saturated solution, *i.e.*, about $\frac{1}{2}$ per cent. (with the addition of formic acid about 1 to 1,000 parts of the nitrite solution), gives an excellent result. The reaction seems to differ from the reaction obtained with the nitrate in certain important respects, and I am hoping that this will prove a useful modification of the process. So far as my observations go at present, they lead me to think that the nitrite gives a better reaction with the extremely minute fibrils than the nitrate; it gives a good preparation of collaterals; it shows granules lying in an abundant feltwork of fibrils; the branches of protoplasmic processes are more numerous, and their "thorns" more distinct than when nitrate is used.

The Nature of the Reaction.—The term "stain" has been generally abandoned since it is clear that the reaction which occurs when a tissue soaked in bichromate of potassium is plunged into a solution of nitrate of silver is not comparable to a process of staining by an organic stain. Ramón y Cajal speaks of the black colouration as a "deposit of chromate of silver."² Van Gehuchten refers to it as a "reduction." In fortunate sections the chief colouration is black, without a tint of either green or red. There is almost always in the same sections a red-brown colouration of blood-vessels and other structures similar to the effect produced upon fresh tissue (especially intercellular "cementing substance") by the action of silver-nitrate alone. The extreme blackness

¹ Durig "Das Formalin als Fixirungsmittel anstatt der Osmiumsäure bei der Methode Ramón y Cajal's," *Anat. Anz.*, x., No. 20.

² Ramón y Cajal, *La Cellule*, vii., p. 131 and elsewhere.

of the black deposit makes it quite clear that it is not a chromate of silver. It must be a reduced salt (sub-salt) of silver. That it is not a metallic oxide or a salt reduced so far as in photography is shown by the solubility of the deposit in hyposulphite of soda or dilute ammonia.

One of the most singular peculiarities of the deposit is its rapid disappearance when the section is covered with a coverslip. All the "staining" may disappear, if the section is mounted in ordinary Canada balsam, in four or five hours, although masses of black deposit remain permanently. Since most of the gum-resins have a weak oxidising action, the disappearance of the colour might be attributed to the oxidation of the reduced silver-salt. If, however, the process is watched under the microscope, appearances are noticed which indicate that the change is due, as Sanassa suggested, to physical causes—at any rate, in part. The colour first leaves the centre of cells or fibres. Dark brown granules then appear in the matrix in the vicinity of the structure which is losing its stain, and it would seem as if the deposit is withdrawn from the tissue by a mechanical force; perhaps its displacement is due to currents set up by the drying of the balsam at the edge of the coverslip. I find that when a slowly-setting turpentine balsam is used without a coverslip, the colour stays in the section, even though the balsam about it may remain liquid for days. On the other hand a large part of the stain is permanently retained in a covered section if the balsam has been evaporated until it sets in a brittle mass, and then re-dissolved in xylol or chloroform. Preparations made with nitrite are also comparatively durable, and the fact that hyposulphite of soda does not destroy all the colouration shows that the reduction is more complete in some parts (particularly the cells and their protoplasmic processes) than in others, and holds out a prospect that a modification will soon be discovered which will ensure the permanence of the preparations when covered with a cover glass. Various methods have been suggested for fixing the silver-deposit—methods which naturally occur to every photographer. It may be toned in an alcoholic solution of chloride of gold

(Obregia¹), or converted into a bromide which is subsequently reduced by light (Greppin²), or the reduction may be completed by sodic sulphide, eikonogen, or any other developer, and it may be mounted beneath a coverslip with or without subsequent toning with gold or with platinum. It is very easy in most cases to obtain a preparation which is permanent, although covered, but there is always, so far as my experience goes, a loss of delicacy. If reduction is completed by a developer, the ground-substance, which still retains traces of silver-nitrate, either free or in chemical union with proteids, is darkened. If development is not completed before toning, some of the finer markings are lost.

I find that good covered preparations may be obtained by staining in fuchsin, with subsequent treatment with picric acid in spirit, and washing. Such preparations are usually too dark, but may be of use in showing the position of the black structures in relation to other cells and fibres.

What is the nature of the chemical substance which, when united with a chrome-salt, reduces the silver? It might be *doubted whether a chrome-salt is necessary*, but such experiments as I have tried seem to show that it is. All my attempts to reverse the process, using the silver first and the bichromate of potassium afterwards, have failed, even when nitrate of silver or lactate of mercury was passed through the cerebral blood vessels. Again, if acetate of copper is used after the chromate it prevents the reaction unless the block is returned to the chromate for a time, in which case it takes the stain as usual.

The fact that the preparation for the silver-stain is effected by a chrome-salt suggests that the reduction or non-reduction of the silver-salt depends upon the extent to which the chromate is fixed by light. Chrome-salts, as is well known, are profoundly changed by the action of light. If a gelatine plate be immersed in a chromate and then exposed

¹ Obregia, "Fixierungsmethode der Golgischen Präparate," *Virchow's Arch.*, cxii., 1890, p. 387.

² Greppin, "Weiterer Beitrag zur Kenntniss der Golgischen Untersuchungsmethode der Centralen Nervensystems," *Arch. f. Anat. und Physiol.*, Suppl. Heft., 1889, p. 55.

FIG. 5.

Adult cat; cerebellum. Large cells of the molecular layer are seen forming baskets about the cells of Purkinje. The cells of Purkinje are not coloured, but portions of their branching systems are shown at the top of the molecular layer.

FIG. 6.

Diagrams of the three types of "basket" found in the cerebellum:—

- (a) The appearance most commonly observed.
- (b) The tassel of separate fibres figured by Cajal.
- (c) The tassel ending in a black mass beneath the cell of Purkinje and fibres streaming off from this mass into the granular layer.

FIG. 7.

Adult hedgehog; fascia dentata hippocampi. Showing on the superficial side of the fascia dentata the colouration of cells, while only fibres are stained in the part farthest removed from the surface. This indicates the effect which the texture of tissue has upon the chrome-silver reaction.

FIG. 8.

Rat, 4 days old; cortex cerebri. The apical process of one pyramid runs into the base of another more superficially placed.

FIG. 9.

Calf, six months (*Octus*), spinal cord. Showing the connection of anterior horn cells by stout processes.

FIG. 10.

Adult hedgehog; cortex cerebri. The vessels were injected with warm bichromate and osmic mixture. The protoplasmic processes of the pyramids are destitute of thorns.

FIG. 11.

Adult hedgehog; cortex cerebri. Showing the junction of Meynert's second and third layers. The protoplasmic processes of the pyramids are thorny.

to the light (as in one of the methods used in preparing plates for photo-printing), the chrome-gelatine compound upon which the light falls is rendered insoluble. It seemed possible that light penetrates the blocks of brain immersed in bichromate of potassium and reaches some cells while it is blocked out from others, but various experiments which I have made show that this is not the explanation. For instance, I divided a brain into two halves; put one half, in suitable pieces, into a bottle of bichromate and osmic which was enveloped in black paper and kept in the dark throughout all stages of the preparation, only bringing the pieces into the light when they were placed on the microtome. The other half of the brain was exposed throughout all stages of its preparation to the full light of a west window. The reaction succeeded equally well in the two cases, although the results were somewhat different. It seems unnecessary to record the differences in detail, since they may be attributed either to the caprice of the method, or to the fact that the bottle exposed to the light was warmed by the afternoon sun while the other bottle was kept at a uniform temperature.

The fact that in one part of a section the stain will mark out the nerve-cells and in another the neurogleia, precludes us from looking to any chemical body peculiar to nervous tissues as the attractive substance. The singular way in which in all sections one element, whether large nerve-cell, small nerve-cell, or nerve-fibre is picked out, while a dozen, a score, or perhaps hundreds of precisely similar elements are passed by, suggests that the condition of nutrition of the cell determines its reacting power.

Does the reaction depend upon the condition of nutrition of the elements?—To test this by exhausting one part with repeated stimulation while another part remained at rest was a natural suggestion; but the fact that the method so often selects the neurogleia cells led me to doubt whether any result would be obtained. It seemed better to try to affect the nutrition of the whole of the brain at once and profoundly. With this end in view, I determined to wash the blood out of the brain, and to compare this starved

brain with one removed from an animal before the cessation of the circulation.

(1) A stream of salt solution (.6 per cent.) at the temperature of the body, was passed into the aorta of a puppy for more than half-an-hour. The water left the jugular vein quite free from colour; but the brain took the silver reaction in the usual way.

(2) In the case of a second puppy, 1 per cent. of ordinary fermentation (ethylidene) lactic acid was added to the salt-solution. It was passed for more than half-an-hour, and appeared from the swelling of the body and the rapidity with which it left the pressure bottle to cause great dilatation of the blood-vessels. The brain took the reaction as usual.

These experiments, 1 and 2, were repeated in the hedgehog, and other animals.

(3) After using (in the case of an adult hedgehog) normal salt-solution for a short time, the pressure bottle was filled with a 10 per cent. solution of NaCl. It flowed freely through the vessels for more than half-an-hour. The body of the animal became greatly distended. All the connective tissues were found to be filled with transparent colourless jelly, resembling vitreous humour. The brain was semi-transparent, soft, and very difficult to handle, remarkably different from the ordinary hedgehog-brain, which is particularly firm and white. Nevertheless it took the reaction as usual so far as the selection and rejection of elements is concerned, although the appearance of the elements was changed in certain details.

The results of this last experiment seem to me very remarkable. The strong salt-solution must have dissolved and carried away a great quantity of albumins and globulins. All "cement-substance" or other lymph-deposit must have been dissolved. The softness of the brain showed that it had lost much of its substance, and yet it reacted to the bichromate and silver in the usual way. It seems quite to exclude any state of nutrition as a factor in determining reduction; at the same time it makes it very difficult for us, unless by a process of elimination, to put our finger upon the chemical substance which, when combined with a chrome-salt, reduces the nitrate of silver.

In face of this result, I did not think it worth while to carry out the experiments upon the effects of CO₂, morphia, chloroform, &c., which I had in contemplation.

INTERPRETATION OF RESULTS.

In summing up the contributions to our knowledge made by Golgi, Ramón y Cajal says¹ that the researches of this *savant* have placed *hors de discussion* the following facts:—

(1) The free termination of the protoplasmic expansions of nerve-cells, as well as of the processes of neuroglial, or spider cells.

(2) The existence in the axis-cylinder of ramified collaterals.

(3) The existence of two kinds of cellular elements, distinguished by the behaviour of their axis-cylinders; the cells in which this process loses its individuality by ramifying without reaching the white matter, and the cells of which the nervous process, despite its numerous collaterals, preserves its individuality, and continues into a tube of the white substance.

I should add to this catalogue of the great changes which the silver method has introduced into current nerve-histology:

(4) The existence in various parts of the central and peripheral nervous systems of a form of nerve-ending, first described by Ramón y Cajal the pericellular basket work; with the physiological inference that nerve impulses act upon (or from) nerve *cell-bodies*, through the medium of a brush of fibres.

(5) The conclusion that axis-cylinder processes, and their collaterals, as well as protoplasmic process have free terminations; that there is no such thing as anatomical continuity of nerve-elements, with the physiological consequence that transference of impulses occurs *in distans*.

(6) The great prominence given in making schemes of the several cortical fields other than the cerebellar cortex, to large nerve-cells, their axis-cylinder processes, and

¹ *La Cellule*, vii., 1891, p. 126.

collaterals; these elements, together with certain free-ending fibres, being held to constitute the reflex mechanism, to the exclusion of the "granules."

These changes, introduced by those who have worked with the chrome-silver method into our conception of the central nervous system, are of such importance that the grounds upon which they are based need the fullest investigation. It is our duty to assume a sceptical attitude of mind towards the new histology, in justice to the investigators who patiently laboured in this field before the introduction of the silver method. We fully realise that Golgi's predecessors did their work with tools which now seem primitive, but they worked without tiring, absorbed in their zeal for truth, and they deserve that the results of their labours should be upheld until swept from the field by an overwhelming weight of argument.

The absence of uncertainty, the simplicity, one might almost say the ingenuousness, of the silver-preparations carry all before them. It is difficult to resist the belief that they tell the truth, the whole truth, and nothing but the truth. For the purposes of this address, however, I have accepted the position of counsel for the defendants, and I beg to be allowed to lay before this court certain considerations which tend to invalidate the testimony of the plaintiff.

I will sum up the evidences of untruthfulness which tend to shake our faith in the chrome-silver method under three heads:

(1) The deposit is liable to spread beyond the limits of the elements which chiefly attract it. In some cases it stains, not the cell or the fibre, but the wall of the lymphatic space which surrounds it.

(2) The amount and character of the reaction depends upon the texture of the tissue in which it occurs. It is, therefore, liable to stop short at the edge of a favourable zone, giving an incomplete picture of the elements which it colours.

(3) *a.* It never takes all the elements of any one kind. *b.* It seldom shows heterologous elements in the same section with sufficient completeness to enable us to trace their connections. *c.* It does not stain the whole of the substance

which belongs to a cell or a fibre, but only part, and this apparently the neuromorphic not the conducting part.

We may now consider how far these three drawbacks to the method affect the credibility of the great conclusions which have been drawn from its results.

(1) *The Spread of Deposit*.—Of this we need say but little. Everyone who has worked with the chrome-silver method is on his guard against this danger. It is especially in the case of neuroglia that the deposit is apt to occur outside the cell. I am inclined to believe, to take an example from the neuroglia, that the extraordinary irregularities on the course of the central processes of Bergmann's cells of the cerebellum, as figured by Retzius,¹ are collections of deposit. It may also occur on nerve-elements; thus it serves to exaggerate the mossiness of Cajal's mossy fibres, although the existence of these "fibras musgosas" is now placed beyond dispute. In good preparations the branching fibres of this system are of exquisite delicacy and clearness of outline. In fig. 3 the thickenings at the points of division, which constitute the "rosettes," are shown by another staining method. In passing, it may be noted that it would be a great advantage if the term *rosette fibres* could be substituted for mossy fibres, since the thickened bunches which these nerves carry are unlike any kind of moss except, perhaps, certain lichens, and, besides, the term "mossy" is constantly and aptly applied to an entirely different appearance which distinguishes the protoplasmic processes of many large cells both nervous and epithelial.

The staining of the lymph-case in which cells and their processes lie is a phenomenon which has not received the attention it deserves. It is of very frequent occurrence in the cerebellum. With or without simultaneous staining of the cells of Purkinje, the wall of the space in which these cells and their chief processes lie is coloured, usually a full brown, but sometimes a very dark brown or even black. It is difficult to show this in a photograph, or even in a drawing, since the appreciation of the fact that one is

¹ Retzius, "Biologische Untersuchungen," *Neue Folge*, vi., 1894.

looking at a hollow case depends entirely upon variations of focus. I am, therefore, obliged to content myself with a verbal description. Owing, no doubt, to the fact that many fibrils pass through the case, its wall is always sculptured in an elaborate but variable pattern. It frequently extends, for a short distance, outwards along the course of the fibrils which pierce it. Of course it follows the outlines of the cell and its chief protoplasmic branches.

After all that has been said about Cajal's *fibres grim-pantes* (kletterplexus, climber plexus), and especially since they have been carefully investigated by Retzius, and their authenticity is vouched for by so competent an histologist, I hesitate to suggest that they are an illusion due to the silver-staining of the lining of the lymph-case which surrounds the Purkinje-cell and its processes. If I saw the preparations from which Cajal and Retzius derive their descriptions, it is probable that I should recognise at once that such an explanation is out of the question. I content myself, therefore, with asserting that the only preparations of my own which show the kletterplexus may be explained in this way.

The apparent continuity of the plexus with a nerve is a great difficulty in the way of this explanation; but, on the other hand, Cajal's description of the nerves as "*fibres épaisses à myéline, peu ou pas ramifiées à leur passage à travers les grains,*" would apply equally well to the neural processes of the Purkinje-cells.

(3) Of much more consequence than this basket-work surrounding protoplasmic processes are the baskets supposed to be formed by axis-cylinder processes and their collaterals about the bodies of large nerve cells. They have been described in the cerebellum, the retina, sympathetic ganglia, and elsewhere. The ending of a nerve in a brush of fibrils which form a basket to invest the body of a nerve cell is a new conception in histology which we owe to the Golgi method. Have we any alternative but to accept the picture which the silver draws as a correct and complete account of the structure? I hope to show that we are *not compelled to believe that the baskets which surround Purkinje-cells are the end-brushes of nerves.*

The cells which form these baskets are found in the deeper parts of the molecular layer of the cerebellum. They were first described by Golgi as cells of his second type. No cells are easier to stain (figs. 4 and 5). In many preparations their processes form so dense a felt-work that the molecular layer is impenetrably black, although no other structures are coloured. Their full extension can only be seen in antero-posterior sections, irregularly stained.

The oval cell-body, as seen in section, gives origin to five or six processes, some of which run towards the surface, while others turn towards the granular layer. One of the processes extends horizontally for a great distance (1 to 2 mm. in the cat) giving off at intervals branches which descend towards the Purkinje cells. This long process is rightly called the axis-cylinder process of the cell; but it will be noticed (a) that although it is thin, round, and well-defined at its origin, it has a curious tendency to thicken as it goes on; (b) that it never acquires a medullary sheath (or if it has such a sheath this does not interfere with its colouration); and (c) it is possible that some of the other processes also take part in the formation of baskets.

As its collaterals approach the cells of Purkinje, each of them divides into a brush (the "basket"), the filaments of which end freely, and eventually the axis-cylinder process itself terminates in the same way. Ramón y Cajal, v. Gehuchten and others are perfectly explicit as to the free ending; but it is with regard to this free ending that I venture to mistrust the silver method.

If a number of specimens of baskets are examined, it will be found that much the commonest appearance is the ending of the branches which invest the cell of Purkinje in an irregular patch or blob beneath the cell (fig. 6a). This appearance is set aside as incomplete. On the other hand, instances will be found, every here and there, in which, beneath the cell of Purkinje, the basket-work, after forming a dense tuft, streams off amongst the granules as separate fibres (fig. 6c). We have, therefore, three forms of basket to select from—(1) the loose basket-work ending in a

blob beneath the Purkinje-cell; (2) the tassel of free-ending fibrils described by Ramón y Cajal (fig. 6b); and (3) the bunching together of the fibrils of the tassel and their continuation into the granular layer.

In deciding between these divers forms of basket, we need to give great weight to the influence which the open nature of the tissue forming the sheet of Purkinje-cells has upon the reduction of the silver-salt. It constantly happens that colouration is limited to this sheet—the fibres which traverse it being stained, while the molecular layer, on the one side, and the granular layer, on the other, are uncoloured. The weight which must be given to the mere physical texture of the sheet is well illustrated by the accompanying photograph (fig. 7), taken from a different part of the brain, in which the conditions are somewhat similar. Like the cerebellum on a small scale, the fascia dentata of the hippocampus consists of a layer of granules bearing a sheet of cells, each of which has an axis-cylinder process passing back through the granules and an elaborate system of branching processes which extend towards the free surface. The resemblance to the granular layer, the sheet of cells of Purkinje, and the molecular layer of the cerebellum, is very singular. This triple sheet of tissue is folded over the cortex margin or nucleus fasciæ dentatæ, and I have found in numbers of preparations of this region which I have coloured with the chrome-silver method, that whereas in the part of the fascia dentata which lies on the surface, the cells and their processes are beautifully defined, and only their axis-cylinder processes are to be seen in the granular layer, on the other side of the nucleus fasciæ dentatæ, where the tissue lies at a distance from the surface, no cells are stained, but numbers of fine fibrils are seen streaming through the granular layer. Now, it cannot be doubted that these fibrils extend beyond the limits of the granule layer, although it is only in this layer that they stain—owing, as I think, to the looseness of the tissue.

In the cerebellum, it is clear that the cells of Purkinje leave but little space for the passage through the sheet in which they lie, of the vast number of fibrils by which the

FIG. 12.

Skate (*Raja batis*) ; cerebellum. Sketch of the molecular layer, showing the union of horizontal fibres with the undulating protoplasmic processes of Purkinje-cells. In other parts of the preparation the processes were thorny. Here, horizontal fibrils of varying length take the place of thorns.

FIG. 13.

Lamb; cerebellum $\times 77$. The section was stained with the alum-carmin-Weigert method by myself, but I am indebted to Dr. Dallinger for permission to reproduce the photograph which appears on the frontispiece of his edition of Carpenter's "Microscope."

FIG. 14.

Rat, 4 days old; cerebellum. A group of granules with centripetal axis-cylinders. Two Golgi-cells are seen above the granules. The position of the cells of Purkinje is indicated by dotted lines.

FIG. 15.

Rat, 4 days old; cerebellum $\times 398$. Photograph of a group of granules, with centripetal axis-cylinders and a Golgi-cell.

FIG. 16.

Kitten, 3 days old; olfactory bulb $\times 248$. Showing mitral cells and granules.

molecular and granular layers are united. These fibrils seem to me to arrange themselves in two sets: (1) the tassels of the "molecular" cells which place themselves around the cells of Purkinje, and (2) the axis-cylinder processes of the "granules" which occupy the intervening spaces. Owing to the peculiar texture of the sheet, and especially to the facilities for the passage of the silver nitrate which its radial striation affords, it comes about that the staining is much more complete here than elsewhere; BUT IT DOES NOT FOLLOW THAT BECAUSE THE FIBRILS OF THE MOLECULAR CELL TASSELS CANNOT BE FOLLOWED INTO THE GRANULAR LAYER THEY END HERE.

The object of my work is to determine the conditions and limitations of the chrome-silver method. I have no intention of giving in this paper a detailed description of any particular nervous organ; nor do I contemplate making a list of all the supposed revelations of the method which seem to me to require confirmation. These three illustrations from the cerebellum are selected merely on the ground that they are representative of the three main directions in which the *method is likely to mislead*.

(b) *Isolation of Nerve-Elements*.—The great conclusion deduced from observations made with the chrome-silver method is the discontinuity of the end-brushes of fibres and the ramified processes of nerve-cells. This anatomical conclusion is of profound interest to all who endeavour to explain the action of the nervous mechanism, whether physiologists or psychologists, since like the theory of which the anatomical basis has just been discussed—the theory that nerve-fibres end freely in contiguity with the bodies of nerve-cells—it is completely subversive of all existing notions of nerve-conduction.

Gerlach¹ described the posterior roots of spinal nerves as ending by ramification in the grey matter; the anterior roots as starting in cells; between the two a plexus formed by the union of the terminal twigs of the afferent fibres, the protoplasmic processes of the motor cells, and thirdly, the

¹ Stricker's "Manual of Human and Comparative Histology," translated by Henry Power, London, 1872, vol. ii., pp. 327 *et seq.*

protoplasmic processes of smaller cells which have no axis-cylinder processes. The instance—the cells of Clarke's column—from which he endeavoured to prove that certain cells have no axis-cylinder processes, was unfortunate, since it has been shown, by the same methods which Gerlach himself used, that he was mistaken. However, the existence or non-existence of cells without axis-cylinder processes is not essential, and, for purposes of comparison, it is better to suppose that they do not exist.

The Gerlachian theory, then, stands thus: the grey matter is a network; long fibres of every degree, whether destined for other parts of the homologous network or for a heterologous network (say from the grey matter of the spinal cord to the cortex cerebri) or for a motor organ, start from cells; fibres from distant cells end by ramifications; there is no functional difference between protoplasmic and axis-cylinder processes. It is very important that this last point should be understood. A good deal has been made lately of the difference in direction of conduction. Ramón y Cajal has formulated a theory, “de la polarisation dynamique des éléments nerveux;” v. Gehuchten had already given expression to the same idea in coining the two terms “cellulipetal” and “cellulifugal”; but this difference in the direction of conduction, if it be absolute, which is open to question, is a trivial and accidental distinction. The function of a nerve fibril is to conduct. We should not expect to find a difference in structure or in behaviour to reagents between fibrils which conduct towards a cell and fibres which conduct away from a cell. The two processes of a cell of a root-ganglion are of equal standing; not one “protoplasmic” and the other “neural.”

The essential difference between Gerlach's teaching and the modern view (the view of Retzius, Ramón y Cajal, Kölliker, v. Gehuchten, and many others) turns upon this:—*Is the grey matter a network of continuous strands or is it a felt-work of interlacing but discontinuous fibrils?*

The importance of this distinction lies in its physiological application. If grey matter is a network, impulses are conducted through continuous circuits. If it is not a

network, their passage is an *actio in distans* (cf. Kölliker, *loc. cit.*, p. 35). The substitution of the term "contact" for "contiguity" does not help matters physiologically even if it were justifiable; whereas, anatomically, it is a gratuitous assumption. It is impossible for a microscopist to distinguish, in a preparation stained black, between *contact* and *continuity* of substance. Besides he has no right to conclude that the position of structures in a hardened and shrunken tissue is the position which they occupy during life. On the contrary, he knows that the tissue has lost lymph to the amount of at least one-third of its bulk.

The extreme difficulty of this conception justifies us in pushing our resistance to the verge of rebellion. I wish, therefore, to lay before you certain considerations which seem to justify us in calling for yet more evidence before we make up our minds.

(1) The belief in the discontinuity of the elements is based chiefly, although not entirely,¹ upon results obtained with the chrome-silver method. Certainly the figures which this method gives appear discrete; but what is proved by a stain which picks out one cell-system and leaves a number of similar cells uncoloured? In no preparation of the cortex cerebri, for example, could I find fewer than seven pyramids unstained for every one stained; usually the proportion of unstained cells to stained cells is much greater. Supposing for a moment that all these pyramids are united together by their protoplasmic processes, should we recognise it in a preparation in which every stained cell is surrounded by seven cells which are unstained?

Gerlach's theory, however, did not imply that *homologous elements* are united together. It is more intelligible on the supposition that the large cells (the nutrient cells of long fibres) are not directly connected with one another. Now when we endeavour to obtain evidence as to the union of heterologous elements we find that the Golgi preparations are almost silent. Hardly ever are two distinct kinds of nerve element, say branching fibres and nerve-cells, or large

¹ Forel in 1887, Retzius (using Ehrlich's method), and His (as the result of embryological research) were led to doubt the existence of the network.

nerve-cells and granules, properly and completely stained in the same part of any preparation, or even in the same preparation.

(2) Even with the chrome-silver method a coarse union between similar cells is shown to be by no means uncommon. These connections appear to be freaks in development, and are of no interest except as showing that intercellular union is possible. In fig. 8 I show a case in which the apical process of one pyramid ran into the base of another; careful focussing left no doubt as to their actual continuity. Union between two cells of Purkinje by means of a thick arched process is not uncommon. Fig. 9 shows three cells of the spinal cord connected by stout protoplasmic processes. I have seen two granules in the olfactory bulb of an adult animal united by a stout bridge.

(3) *Thorns* (épines) on the protoplasmic processes of pyramids and cells of Purkinje were first described by Ramón y Cajal, but no attempt has been made to determine their significance. I am inclined to think that these thorns will eventually give the key to the problem.

Thorns are very minute lateral branches of the protoplasmic processes of certain cells; especially pyramids (figs. 2, 11), and cells of Purkinje. They often occur at very regular intervals. Sometimes they are opposite but usually they are roughly alternate. Their length varies from less than 1μ up to 5 or 6μ ; on the peripheral processes of the granules of the olfactory bulb they often measure 10 or 12μ (fig. 18). They usually terminate in minute knobs. Sometimes the knobs alone are visible. Occasionally a clear line can be recognised carrying a round dot in its centre or at one end.

Are the thorns artifacts? They certainly are most distinct in imperfectly hardened tissue. When I first succeeded in making a thoroughly successful injection of the brain with bichromate of potassium I was much struck with the fact that the processes of the pyramidal and other cells were perfectly clean cut and destitute of thorns (fig. 10), whereas tissue which had been purposely left for twenty-four hours before immersion in the osmio-bichromate mixture showed the thorns in a very marked manner (fig. 11). So, too, did

the brain injected with strong salt-solution. This led me to think that the thorns were artificial products. On the other hand, their great regularity is opposed to an artificial origin, and besides, they are not limited to preparations made with the chrome-silver method. If a section of the molecular layer of the cerebellum is deeply stained with hæmatoxyl in and then broken up with needles in glycerine, the thorns on the processes of the Purkinje-cells are beautifully distinct, although they are irregular in length and do not end in knobs.

It seemed to me that the thorns must indicate an actual union between certain unstained filaments and the protoplasmic processes. I sought, therefore, to ascertain the nature of these unknown filaments by studying the cortex of the cerebellum. As everyone knows, the molecular layer of the cerebellum exhibits a very regular striation parallel with the surface. This is seen to perfection in the cerebellum of a cartilaginous fish. In the skate, for example, the undulating processes of the cells of Purkinje, which have a radial arrangement, cross the tangential striæ at right angles, making with them a diaper pattern of the utmost regularity. Near the dorsal commissure, the cells of Purkinje are absent, and only the tangential striæ are present. In the cerebellum of the skate (fig. 12), coloured with the chrome-silver method, I am able to show in place of thorns on the Purkinje-cells, long striæ streaming away from the protoplasmic process on either side. Unfortunately, I have not succeeded in following the axis-cylinder processes of the granules of the fish's cerebellum from the granules to their divarication in the molecular layer, nor had Fusari¹ better fortune. Schaper² traced the fibre into the molecular layer, and observed its divarication, but could not follow it to its termination. We may therefore conclude that the arrangement of the axis-cylinder is the same as in birds and mammals, and I think that there is little doubt but that the tangential fibres which I observe in direct continuity with

¹ Fusari, "Untersuchungen über die feinere Anatomie des Gehirnes der Teleostier," *Int. Monatschr.*, 1887.

² Schaper, "Zur feineren Anatomie des Kleinhirns der Teleostier," *Anat. Anzeiger*, Nos. 21 and 22, 1893.

the protoplasmic processes of the Purkinje-cells, are the axis-cylinder processes of granules.

It is most probable that the tangential striæ seen in carmine or hæmatoxylin stained sections of the cerebellum are the tangential fibres to which the axis-cylinder processes of the "granules" give rise, by their division, in the form of a T or a Y. This T-division was first described by Ramón y Cajal, and has been observed in a large number of animals by Pedro Ramón. In many of my own preparations, the delicate axis-cylinder is to be seen commencing in the granule, passing between the cells of Purkinje, and divaricating in the molecular layer. It is important to notice that the branching system of Purkinje-cells, and of the "molecular" cells are arranged at right angles to the folia, the longitudinal fibrils in the direction of their long axes.

This brings us back to the old view as to the plan of structure of the cortex cerebelli, first defined by Boll and often reiterated by myself and many others. Afferent fibres branch among the granules; delicate processes from the granules stream into the molecular layer between the cells of Purkinje; dividing at right angles (as demonstrated by Cajal), they are collected by the protoplasmic processes of the Purkinje-cells, from the base of each of which a neural process passes into the medullary centre. This seems to be the basis of the reflex machine—the system of primary couples—to which the bracket-like cells of the molecular layer, the collaterals of the neural processes of the cells of Purkinje, the cells of Golgi, the fusiform cells, the scattered large cells of the granular layer, and other elements, must be fitted in as our knowledge of the connections of these elements becomes more complete.

If in the cortex cerebelli a *direct continuity of structure is established from the granules to the cells of Purkinje*, a similar continuity must be looked for in the cortex cerebri, and may be expected in the central grey tube.

The fact that the silver deposit extends so short a distance along the tangential fibrils as to give to the protoplasmic process merely a "thorny" appearance, seems to

indicate that GREY MATTER CONSISTS OF TWO SUBSTANCES WHICH BEHAVE DIFFERENTLY TO THE CHROME- OR SILVER-SALT. The ending of the thorn in a knob, as well as the frequent varicosities in the course of all other delicate fibres, especially at the points at which collaterals are given off from axis-cylinder processes, indicates that the substance which reduces the silver-salt is fluid or semi-fluid. The reduction of the silver by neuroglial cells, and the frequent appearance of the deposit outside nerve cells, show, I think, that it is *not the nerve strand properly so-called, i.e., the conducting fibril,*¹ *which reacts to silver, but the fluid or semi-fluid substance—the neuroplasm—in which it is embedded.* If this hypothesis be correct, it may be applied in many ways to explain the fact that a direct continuity of heterologous nerve elements has not been observed (except by myself in the case of the cerebellum) with Golgi's method.

The chief difficulty which I see in the way of this explanation is the fact that many ependymal cells are quite as thorny as the protoplasmic processes; but this difficulty has to be faced whatever view is held as to the meaning of the thorns. No doubt the ependymal cells are constructed upon the same plan as the web which they serve to support.

It will, no doubt, be urged in opposition to my theory, that it is not only Golgi's method which seems to demonstrate the discontinuity of nerve elements; the same thing is exhibited by Ehrlich's method, which has given such admirable results in the hands of Retzius; but I maintain that this fact is confirmatory of my hypothesis. Methyl blue injected *intra vitam* acts very quickly upon the nerves, while they are in a living state. It is much more likely that the stain is taken up from the circulation by the nutritional protoplasm of the nerves than that it affects the conducting fibrils.

The fact that NO THORNS ARE TO BE SEEN WHEN THE BRAIN IS INJECTED WITH BICHROMATE shows that the outward spread of the neuroplasm along the nerve-fibrils occurs *post-mortem*. We should, therefore, expect that Ehrlich's

¹ The question as to whether the fibrils are solid (Schultze) or hollow tubules (Nansen, Schäfer) does not affect the theory.

method would show the nerve-elements as still more discrete than they seem to be when prepared in the ordinary way by the chrome-silver method after death.

Lastly, I submit that even from the point of view of those who work chiefly with Golgi's method, it is a bold assumption that this method shows us the ultimate termination of nerve-fibrils. Speaking for myself I may say that the thought which a good Golgi-preparation of the spinal cord suggests is not, how complete the picture, but how much there must be that one does not see! The amazing delicacy of the last divisions of the collaterals impresses upon one's mind the fact that it is hopeless to try to follow them to their termination since the method is incapable of showing their ultimate subdivisions.

Still more forcibly is the inadequacy of the method brought out by a study of the cortex cerebri. Every here and there (especially when the tissue has remained a long time—say six weeks—in bichromate and osmic) occur masses of fibrils—looking like the mycelium of a fungus. The centre of the mass is an impenetrable tangle, and only at its margin can the separate fibrils be seen; it brings home to one the fact that admirable and diagrammatic as are the partial pictures, they give a very incomplete and inadequate representation of the infinitely complex tissue of the brain.

Granules.—Perhaps the greatest service which Golgi's method has rendered to histology is in giving us accurate information with regard to the nature and form of these minute nerve cells. All attempts to isolate the granules by teasing had failed; fragments only of their processes remained attached to the cell-body. Owing to the closeness with which they are massed together there was no hope of our being able to make out their form by studying them in sections. The chrome-silver method came to our rescue; picked out one granule in a hundred and depicted it with diagrammatic simplicity.

Since our knowledge of the granules commences with the introduction of Golgi's method, it is superfluous to quote the views of observers who had not this means of observation at their disposal. Granules were called myelocytes, connec-

FIG. 17

Adult cat; olfactory bulb. At the bottom of the photograph are seen the glomeruli. The mitral cells are very imperfectly coloured. Above the mitrals is seen a single cell of the type for which I suggest the name "bracket-cells." Its protoplasmic processes extend horizontally for more than 3 mm. Its axis-cylinder was centripetal. Numerous granules are seen above this.

FIG. 18.

Kitten, 3 days old; olfactory bulb. Showing mitral cells and the extremely thorny protoplasmic processes of the granules.

FIG. 19

Olfactory bulb ; granules with axis-cylinders selected from various preparations :

1-4. Hedgehog ($9\ \mu$ to $11\ \mu$).

5. Kitten, 8 days old ($10\ \mu$).

6, 7. Rat, 12 days old ($12\ \mu$ and $15\ \mu$).

By an oversight 1, 2, 3 and 4 are drawn with their central ends upwards ; 5, 6 and 7 with their central ends downwards.

FIG. 20

Photograph of the granule 19, 7 (reversed).

tive tissue corpuscles, lymph-corpuscles, "doubtfully nervous" elements, and so forth, by men who would be the first to acknowledge that their nervous nature is now placed beyond any possibility of doubt. It is a little strange to find the hypothesis put forward in a recent memoir that they are nerve-cells which have remained behind in an embryonic condition.¹

There are four situations in which granules are found in dense masses: (1) the olfactory bulb; (2) the retina; (3) ? the fascia dentata; (4) the cerebellum. They are very numerous in the occipital region of the cortex cerebri. That all these small nerve cells bear a similar relation to afferent fibres is not improbable; but whether on this account they should all be described as homologous is a question which cannot be answered until we have further information. In the cerebellum the granules are arranged about clumps of molecular substance (figs. 3 and 13). So far as we can see, they are all of one kind, but Denissenko² thought that he could distinguish a second type of nucleus by the use of eosin. I have never been able to find these eosinophile nuclei of Denissenko, although I see in the centre of each clump of molecular substance the structures which he mistook, as I think, for nuclei. These structures are usually smaller than the nuclei, homogeneous and pale; sometimes single, and round, they often appear as rods, and very frequently they are divided into several parts (fig. 3).

When I first observed these structures in sections stained with eosin, or better still with the alum-carmin-Weigert

¹ Chatin, "Études d'histologie Zoologique sur la forme dite Myélocyte," Paris, 1890.

I am not quite sure that I have given a fair representation of Chatin's views. The following quotations show that Chatin looks upon the granules as identical with the large nerve cells when in an embryonic stage; but he does not state explicitly that either the granules are capable of growing into larger cells, or that they are larger cells which have remained in an embryonic, and therefore functionless, condition. "Ce qui mérite d'être noté, c'est, l'identité morphologique qui se manifeste constamment entre tous ces jeunes éléments nerveux, quelle que soit l'espèce animale sur laquelle on en suit l'évolution." "Il ne suffit pas d'avoir montré l'exacte signification du myélocyte, il faut encore établir qu'il ne peut aucunement représenter un type distinct."

² "Zur Frage über den Bau der Kleinhirnrinde bei verschiedenen Klassen von Wirbelthieren," *Archiv. für Mikr. Anat.*, Bd. xiv., 1877, p. 203.

method, I concluded that they were not nuclei, but did not like to hazard the suggestion that they were, as I thought them to be, swollen axis-cylinder processes at their points of division. The use of the chrome-silver method shows, however, that this is really the case. They are the dilated fibres of the rosettes which occur on the course of Ramón y Cajal's mossy fibres (*fibras musgosas*).

The granules arranged around each molecular clump give protoplasmic processes to it (as well as to three, four, five, or even six other clumps). Their protoplasmic processes end in curious bent claws which are in relation with the processes of the rosette. Whether this connection is one of contact only or of direct continuity remains to be proved.

If we neglect for a moment the nuclei which have been supposed to occur within the molecular clumps, and eliminate, of course, the nuclei of the superficial "Golgi cells" and the scattered large cells, we have to deal with a vast number of apparently similar nuclei. The question arises: Are all these of one kind? The way in which the chrome-silver method picks one granule out of several hundred, is more remarkable even than its capricious selection of other kinds of cells; I have never seen the granules of the cerebellum stained in masses, as often occurs in the olfactory bulb. They are always disposed at fairly regular intervals. All observers who have worked hitherto with the chrome-silver method have concluded that only the type of granule with centrifugal axis-cylinder is present. I shall show in a moment that granules of quite different form with centripetal axis-cylinders are also to be found.

In addition to its short protoplasmic processes already described, each granule (of type 1) has a single axis-cylinder process of exquisite delicacy which makes its way into the molecular layer and ends, as Ramón y Cajal discovered, by dividing at right angles to the planes of the Purkinje-systems.

The enormous number of radial fibrils which in some sections are seen making their way to the molecular layer between the cells of Purkinje as well as the equally numerous tangential fibres found in the molecular layer in other

sections, evidently require a vast number of granules to give them origin.

GRANULES WITH CENTRIPETAL AXIS-CYLINDERS.

It is late in the day to describe a new kind of cell in the cerebellum, but it is a curious proof of the caprice of the chrome-silver method that these granules should not have shown themselves to other observers. In one set of preparations from a four-day rat, they occur in large clusters about the apices of the convolutions. I have not yet had time to look for them in other subjects.

These new granules are quite unlike any hitherto described, nor can they be confused with the fusiform cells described by Golgi. Golgi's cells are much larger ($20\ \mu$), with long protoplasmic processes from each end of the spindle, and a fibre attached to the side of the cell. The new granules have a transverse diameter of $9-12\ \mu$. From the apex, a branching protoplasmic process runs towards the periphery (figs. 14, 15), while an axis-cylinder process is attached to the base. The axis-cylinder frequently gives off a recurrent branch near the granule, and passes on towards the white matter where or near which it divides.

What are the *central connections of the granules*?

From the medullary substance of the *arbor vitæ* a single medullated nerve fibre traverses the granular layer to reach each Purkinje cell; but there can be no doubt that many fibres are left over after all the Purkinje cells are provided for. Amongst these are the mossy or rosette fibres, which form an extremely complicated and delicate branching system among the granules. In animals in which the medullary fibres separate the granular layer from the cells of Purkinje, some fibres turn towards the molecular layer, whilst others turn towards the granules. This is seen especially well in cartilaginous fishes and in some reptiles, for example, the crocodile. The fibres which turn towards the granules divide frequently, and it is on this account, probably, that there is a marked difference in size between the fibres near the cells of Purkinje and those near the granules. The former are much the larger.

Ramón y Cajal has assigned to the granules with centrifugal fibres their proper position as elements on the ascending limbs of the cerebellar reflex arcs.

The *granules of the olfactory bulb* have not been treated with so much respect as those of the cerebellum. Many considerations show us that the granules of the bulb cannot be regarded as strictly homologous with the granules of the cortex cerebelli or cortex cerebri. The organ in which they are found is widely dissimilar from the cortex, homologous with a peripheral nervous organ—the retina—and not with a central or secondary nerve field.

Nevertheless, the granules of the olfactory bulb (figs. 16, 17, 18, 19) have sufficient in common with the granules of the cerebellum to entitle them to the full status and privileges of nerve cells. To simplify the diagram of the olfactory bulb by leaving out the granules, as v. Gehuchten has done,¹ is to complicate matters for future generations of histologists:—
“Les cellules mitrales sont donc les véritables cellules olfactives du bulbe. Loin d'être, comme Golgi le croit, une voie de transmission accessoire, elles forment l'unique route par laquelle l'ébranlement nerveux d'une fibrille olfactive peut être sûrement conduit jusqu'au cerveau.”²
This seems a little hard upon the granules, not to mention various other kinds of nerve-cell, which together play a much larger part in the formation of the olfactory bulb than do the mitral cells!

The granules of the olfactory bulb usually appear as minute round, fusiform or triangular cells with one very long peripheral process, which passes through the layer of mitral cells, and often approaches very near to the glomeruli, and three or four much more delicate central or basal processes which are often coloured only for a short distance. Occasionally the central processes are long and branch frequently. Sometimes the round cell-body lies on the side of a process which then extends for a long distance centrally as well as peripherally. It is rather remarkable that the peripheral

¹ Cf. Van Gehuchten, “Système Nerveux de l'homme,” Lierre, 1893, figs. 390-393.

² V. Gehuchten and Martin, *La Cellule*, vii., 1891, p. 232.

process is stained much more easily than the central processes.

Both peripheral and basal processes are thorny (fig. 18); the thorns on the sub-divisions of the peripheral process, after it has passed the layer of mitral cells and entered the stratum gelatinosum of Lockhart Clarke, are remarkably long (6-12 μ).

Golgi is doubtful as to the nervous nature of these cells, although he thought he saw in certain rare cases an axis-cylinder process which gave off collaterals and joined bundles of nerve fibres; as did also its collaterals.¹

Ramón y Cajal failed to find any axis-cylinder processes, but he nevertheless considers that the form of the cells and the termination of their apical processes in close relation with the protoplasmic processes of the mitral cells justify us in considering the granules as nervous. He homologises them with the "spongioblasts" of the retina as amacrine cells—*i.e.*, cells destitute of axis-cylinder processes.

V. Gehuchten and Martin, on the other hand, consider that since the granules have no axis-cylinder processes they are not nervous: "Du moment que, à l'exemple de Ramón y Cajal, on est disposé à ne tenir aucun compte de la présence ou de l'absence d'un prolongement nerveux pour déterminer le nature spéciale d'un élément des centres, on perd par le fait même tout motif pour ne pas considérer comme étant de nature nerveuse non seulement les cellules épendymaires . . . mais aussi un grand nombre de cellules de neuroglie."

I should have thought with Ramón y Cajal that the general appearance and disposition of the granules of the olfactory bulb sufficiently testify to their nervous nature; although the presence of an axis-cylinder may be needed to place it beyond a doubt. For the axis-cylinder I have sought very carefully in a large number of different prepara-

¹ Golgi ("Untersuchungen über den feineren Bau des Centralen und peripherischen Nervensystems"; German translation, Jena, 1894, p. 52) says: "Ob auch ein anderer, nervöser Fortsatz besteht, Kann ich nicht sagen. Allerdings habe ich in einigen Fällen beobachtet, dass einer der Fortsätze, welcher gewöhnlich aus dem Mittleren Theile der Basis der Pyramide entspringt, etwas homogener aussieht, als die anderen . . . ."

tions, with the result that I can almost always find it in the case of a certain number of the granules in any given section of the olfactory bulb (fig. 19).

The axis-cylinder is a short curved process attached to the side of the granule. As it leaves the granule it twists among the other granules which are densely packed together, and is soon lost to view. I have failed to determine its further course, although I notice that it inclines towards the periphery, but I would call attention to the fact that the tissue which separates the plates of granules is occupied by a dense feltwork of fibres; some derived from the basal protoplasmic processes of the granules, but the rest distinguished by the extreme delicacy which is characteristic of axis-cylinders and their collaterals. Occasionally the axis-cylinder process divides at right angles, very near the cell, one division running centrally, and the other peripherally.

Solitary granules are to be found at the margin of the special layer of the olfactory bulb which contains mitral cells and glomeruli. Their axis-cylinder processes may be followed for some distance, but I have not succeeded in ascertaining their destination.

Fig. 20 shows the axis-cylinder process of a cell of the olfactory bulb of a twelve-day old rat; but I am not certain whether this cell ought to be regarded as a granule. It lay at some little distance from the sheet of mitral cells, giving protoplasmic processes in each direction; those which approached the mitral cells being richly branched. Its axis-cylinder process *ran peripherally* and could be followed almost up to the mitral cell layer. The cell was about half as large again as the ordinary granules, and yet I think that the disposition of its protoplasmic processes and the course of its axis-cylinder process entitled it to be classed with them. It is worthy of note that the axis-cylinder processes of several other granules were distinctly visible in this preparation, and that they also curved peripherally.

If the cell I have photographed be not a granule it belongs to a kind of cell not described hitherto. It is too small for one of the multipolar cells, often of a fusiform shape, described by Golgi, who says, besides "Der nervose

Fortsatz entspringt Gewöhnlich von der nach dem Centrum gerichteten Seite der Zelle, bisweilen auch seitlich, um sich dann nach innen umzubiegen" (*loc. cit.*).

In passing it may be noticed that I do not find any description of the immensely large "bracket-cells," which are common features, at any rate, in both cat and hedgehog. They lie on the central side of the mitral cells, and their branching processes often extend horizontally for a distance of 2 to 3 mm. (fig. 15).

The possibility of the existence in any situation of amacrine nerve cells is too wide a question for discussion here; but it does not appear to me probable that they are to be found in the olfactory bulb. The observation of Pedro Ramón¹ that the lower the animal in the vertebrate scale the less developed are the basal processes of the granules of the bulb, seems to dispose of the idea that these cells are bipolar in the sense of having protoplasmic processes for both cellulipetal and cellulifugal conduction; it makes the existence of an axis-cylinder process for cellulifugal conduction the more necessary.

As everyone knows, the overlapping plates of granules constitute the most conspicuous feature of the olfactory bulb. It seems to me impossible to doubt that they take a large and important part in the constitution of the bulb as a nervous mechanism. A diagram of the structure of the bulb from which they are omitted is not only inadequate but misleading.

Apart, however, from the question as to whether, in the case of the olfactory bulb, we can or cannot prove that the granules are nervous, I wish to claim for them, on general grounds, a much more important rôle in the nervous mechanism than has been conceded to them by any observer who has up to the present made use of the chrome-silver method.

No one now doubts the nervous nature of the granules of the cerebellum. Yet other granules which, when prepared with carmine, picrocarmine, or any other stain very closely

¹ Ramón y Cajal, "Nouvelles Idées," p. 108.

resemble the granules of the cerebellum, are either set aside as non-nervous, or ignored altogether. It is instructive to compare a carmine-stained section of the cortex cerebri with any of the pictures which have been published of the same tissue prepared with the chrome-silver method. In the chrome-silver preparations the granules are conspicuous by their absence; whereas in a carmine preparation of the occipital cortex, or of the cortex about the calcarine fissure, their importance is beyond dispute. It is greatly to be desired that some one should work out the connections of the granules of the cortex cerebri with the aid of the chrome-silver method.

The Granules of the Fascia Dentata.—I have tried in vain to colour these granules with silver.

It may be doubted whether there are any granules in this layer. Sala¹ omits them altogether when recording the results of his observations with the chrome-silver method; but there seem to me to be strong reasons for thinking that the greater number of the nuclei of the "granular layer" of the fascia dentata belong to granules.

The cells of this layer, when coloured with silver, very closely resemble miniature Purkinje-cells. The body is rounded or oval; from its base an axis-cylinder process passes towards the nucleus fasciæ dentatæ. Its apex gives rise to a rich branching system (fig. 21). Sala figures these cells as placed at very regular intervals, as my preparations also show them. The coloured cells lie almost exclusively on the surface of the granular layer, as if they differed in nature from the "granules" which lie below them. In my description of the fascia dentata prepared with the carmine Weigert stain² I state that the average numerical relation of the small pyramids to the granules is as 1 to 8. This number coincides approximately with the relation between the unstained nuclei and the stained cells in my chrome-silver preparations.

A third reason for considering the cells of the fascia

¹ *Zeitschr. f. w. Zoologie*, vol. lii., 1, p. 18.

² Hill, "The Hippocampus," *Phil. Trans. of the Royal Society of London*, 184, B. 1893, p. 389.

FIG. 21.
Adult hedgehog; fascia dentata hippocampi $\times 310$.

FIG. 22.
Adult hedgehog: subiculum cornu Ammonis. Showing double pyramids.

FIG. 29.

Adult hedgehog; nucleus fascial dentata $\times 83$. Showing multi polar cells.

FIG. 24.

Adult hedgehog; spinal cord. Cells of the anterior horn. A single nucleus is also coloured.

FIG. 25.

Rat, 8 days old; cortex cerebri $\times 155$. The collaterals on the axis-cylinders of two pyramids are well seen.

FIG. 26.

Adult hedgehog; nucleus tenticularis. The blood-vessels were injected with warm bichromate of potassium. The reaction shows cells of Golgi's type II., and also very faintly the axis-cylinders of the fibres which constitute the bundles of the internal capsule.

ALL THE PHOTOGRAPHS ARE ABSOLUTELY UNTOUCHED BY HAND.

dentata as granules is also brought out in the paper just alluded to, although its value as a proof is less obvious. The development of the fascia dentata is exactly proportional to the development of the olfactory bulb. It is conceivable that the granules in the two situations belong to the same formation.

It may not be out of place in this description of the fascia dentata to call the attention of histologists to the illustration which the hippocampus affords of the extreme difficulty of basing homologies upon the form and general appearance of nerve-cells. As the hippocampus is approached, the pyramids of the cortex cerebri give place to the remarkable "double" pyramids of the subiculum cornu Ammonis (fig. 22); at the nucleus fascia dentata these are transformed into cells, which might easily be mistaken for the motor cells of the spinal cord (fig. 23); in the fascia dentata (fig. 20) we have a type of cell for which we most easily find an analogy in the cortex cerebelli.

Summary.—The method of colouration introduced by Professor Golgi, of Pavia, has yielded, to those who have had the foresight to apply it persistently, an undreamed of harvest. In particular Santiago, Ramón y Cajal is to be congratulated upon the wealth of new facts which it has enabled him to discover. These are, I believe, only a first instalment of the revelations in nerve-histology which the method is destined to make.

I have used it, not with the hope of making discoveries, but with a view to ascertaining the conditions of its action and the value of its results when compared with those which other methods afford. In other words, I have tried to discover its limitations—its weakness rather than its strength.

The subject is so large, and my results so fragmentary, that I hesitate to lay them before you, and I should not, assuredly, have embodied them in their present incomplete form in a memoir. When, however, you did me the great compliment of electing me as your President, I felt that you would expect me to bring before you such results of my personal work as are likely to be of greatest interest to neurologists in general.

The chrome-silver method has completely transformed our conception of the nature of the nervous mechanism. Whatever branch of neurology we may happen to pursue, we need to lay afresh the foundation upon which it rests. The new method of colouration has revealed to us the fact that grey matter is far more complex in its structure than histologists imagined. We are also asked to throw aside all preconceived ideas as to its mode of working, and to believe that its various elements are not in structural continuity. It is on this latter point that I wish you to suspend your judgment, and in favour of my *caveat* I lodge the following considerations:—

(1) The place of the granules in the mechanism has not yet been ascertained except in the case of the cerebellum. In all cortical fields they are in intimate relation with the bulk of the ascending or afferent fibres. How close their connections with these fibres may be, still remains to be determined. Their axis-cylinder processes are also in intimate relation with the protoplasmic processes of the cells which give origin to efferent fibres.

(2) The thorns on the protoplasmic processes of nerve cells are not artifacts, nor are they, on the other hand, processes which the chrome-silver method reveals in their entirety. It is not impossible that they contain the ends of the axis-cylinder processes of the granules surrounded by an extension, or *post-mortem* overflow of the neuroplasm of the larger nerve cell.

If a direct continuity of nerve substance between axis-cylinder processes of granules and the protoplasmic processes of nerve cells be proved to exist, it may be looked for also along all other lines of impulse reflection, and Gerlach's plexus—with all the modifications in form introduced by the chrome-silver method—may be a true expression of the structure of grey matter as a nervous mechanism after all.