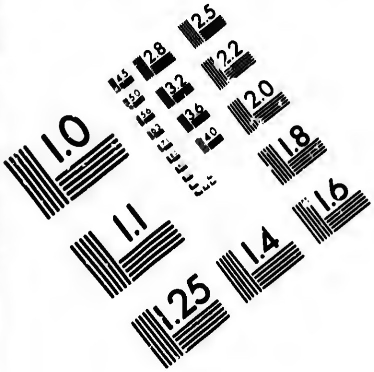
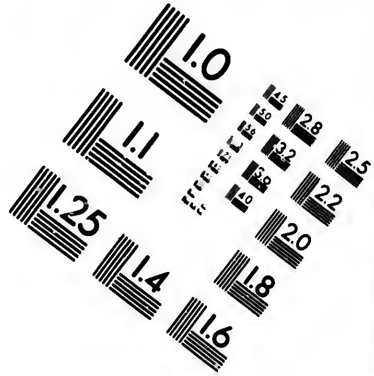

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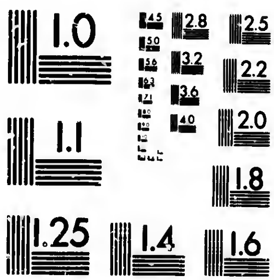

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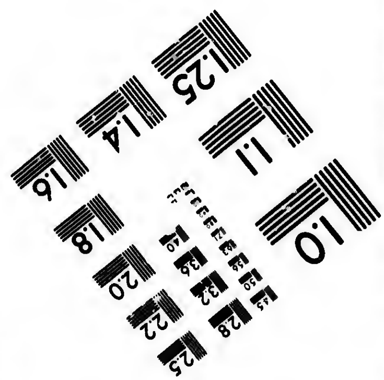

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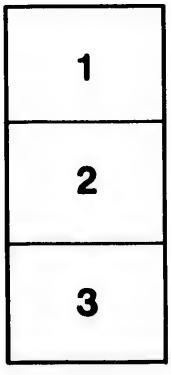

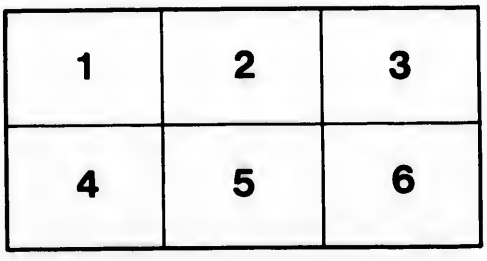

ON THE STRUCTURE, MICRO-CHEMISTRY AND DEVELOPMENT OF NERVE CELLS, WITH SPECIAL REFERENCE TO THEIR NUCLEIN COMPOUNDS

By F. H. Scott, B.A.

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UNIVERSITY OF TORONTO, June 3, 1899.

To the Registrar
University of Toronto

SIR,

I beg to report that Mr. F. H. Scott, B.A., has passed satisfactorily the examination in Physiology, his major subject for the degree of Doctor of Philosophy.

I beg to report also that Mr. Scott's thesis "On the Structure, Micro-Chemistry and Development of Nerve Cells with Special Reference to their Nuclein Compounds" is of distinction as a contribution to Physiology, and I recommend that it be accepted for the degree of Doctor of Philosophy.

Yours truly,

A. B. MACALLUM,

Professor of Physiology.

I HEREBY certify that the thesis above mentioned has been accepted by the Senate of the University of Toronto for the degree of Doctor of Philosophy in accordance with the terms of the Statute in that behalf.

JAMES BREBNER,

Registrar.

UNIVERSITY OF TORONTO, June 5, 1899.

ON THE STRUCTURE, MICRO-CHEMISTRY AND DEVELOPMENT OF NERVE CELLS, WITH SPECIAL REFERENCE TO THEIR NUCLEIN COMPOUNDS*

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The finer structure of the nerve cell has attracted a great deal of attention in the last few years, chiefly because the cell body contains masses that have a peculiar affinity for certain nuclear stains. These masses were first observed in 1882 by Flemming,[1] who was not certain whether they were nodular thickenings of the ordinary protoplasmic fibrillæ or independent structures. His preparations, however, were, for the most part, from material that had been fixed in chromic or osmic acid, and stained in hæmatoxylin or carmine, and for this reason the bodies in question did not exhibit any distinctive staining properties. It was reserved for Nissl[2] who examined the cells of the cerebral cortex of mammals after fixation in alcohol and staining in basic aniline dyes, to show that these bodies stain differently from the remainder of the cell protoplasm, and in fact resemble in this respect the large nucleolus. For this reason these structures are commonly called Nissl granules or "Schollen." Some observers have employed other names, such as tigroid bodies, chromophile corpuscles, basophile or basic substance, cytoplasmic chromatin, etc.

The variable form exhibited by nerve cells from different sources with respect to these granules makes the selection of a suitable name based on morphological data difficult, but for the purposes of this memoir as

*A short account of some of the facts recorded here was given for me by Prof. Macallum before the Fourth International Physiological Congress, Cambridge, 1898, and the British Medical Association, Edinburgh, 1898. See Journal of Physiology, XXIII, supp. p. 33, and British Medical Journal, September 17th, 1898.

1 Flemming, W., "Vom Bau der Spinalganglienzellen," Festgabe für J. Henle, p. 12, 1882.

Also: "Zellsubstanz, Kern and Zelltheilung," p. 41, 1882.

2 Nissl, Fr., "Ueber die Untersuchungsmethoden der Grosshirnde," Tagebl. der Versammlung deutschr Naturforscher, Strasburg, p. 506, 1885.

far as it pertains to the nerve cells of adults, the name of Nissl granules will suffice. It will be shown later that this name, in some cases at least, implies an incorrect inference as to the mode of occurrence of the chromophilous substance in the cell.

The chemical properties of the Nissl granules have been studied by Held,[3] Eve,[4] Mackenzie,[5] Bühler[6] and others. Held found the granules were soluble in dilute alkalies, did not digest in pepsin and hydrochloric acid, were not acted on by acids and gave no reaction with Millon's reagent, Adamkiewicz's or the xanthoproteic tests. Held, however, obtained a positive reaction for phosphorus by the employment of Lilienfeld and Monti's test for that element. He concluded from these reactions that the Nissl granules were of a nucleo-albuminous nature. Eve, however, was doubtful whether the granules were really dissolved in the alkali or were merely altered in their staining powers ; and found that after treatment with acids or salt solutions, the granules stain more diffusely. Bühler found the granules were soluble in salt solutions as well as in alkalies. It has recently been observed that nuclear chromatin gives with the Millon reagent a definite reaction, and Macallum[7] has shown that the reaction of Lilienfeld and Monti does not differentiate the phospho-molybdate, formed by the combination of the molybdate employed and the phosphorus of the cell, from the ammonium molybdate which has simply been absorbed and retained.

The only undisputed evidence, therefore, adduced by Held in favour of the nucleoproteid nature of these granules is their resistance to digestion. His conclusion is, however, further supported by the observation of Mackenzie who obtained, after treatment with acid alcohol, a reaction for iron in the granules.

In the present research the micro-chemistry of the nerve cell has been reinvestigated by the more recent methods and the results indicate that Held's conclusion is correct, although, as we have seen, based on insufficient grounds.

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3 Held, Hans, "Beitrage zur Structur der Nervenzellen und ihrer Fortsätze."

Erste Abhandlung, Archiv. f Anat. u. Phys., Anat. Abth., p. 396, 1895.

Zweite Abhandlung, ibid, p. 204, 1897.

4 Eve, F. C., "Sympathetic Nerve Cells and their Basophil Constituent in Prolonged Activity and Repose," Journal of Physiology, XX, p. 334, 1896.

5 Mackenzie, J. J., "Investigations in the Micro-chemistry of Nerve Cells," Report British Assn., Toronto Meeting, p. 822, 1897.

6 Bühler, Anton, "Untersuchungen über den Bau der Nervenzellen," Verhandlungen der Phys. Med. Gesell. zu Würzburg, XXXI, p. 285, Verlag von Stahel, 1898.

7 Macallum, A. B., "On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues," Proceedings of Royal Society of London, Vol. LXIII, p. 467, 1898.

The mode of occurrence of these granules in embryonic and foetal cells has evoked considerable interest. Vas[8] and Eve found the chromophilous substance uniformly distributed in the nerve cells of fœtal rabbits, and Szczawinska[9] observed the same for embryonic cells of selachians. Bühler noticed that the granules were entirely absent from the nerve cells at an early stage. Timofeew[10] observed that in the interval between the fourth and sixth day of incubation in the chick, the chromophilous substance increased markedly in amount and was uniformly distributed.

None of the above observers seem to have suspected any other than a cytoplasmic origin for this substance and none of them have followed out in detail the appearance of this substance in the cell. The nucleoproteid nature of these bodies suggested the nucleus as a possible source of the substance forming them, and this inference has been confirmed by a series of observations made on mammalian and avian embryos. Further evidence in support of the nuclear origin of these bodies is found by the examination of the structure of the nerve cells of animals in which no Nissl granules occur. These observations will form Parts 2 and 3 of the present memoir, while Part 4 will be devoted to the discussion of certain general considerations with respect to the structure of the nerve cell that have recently been the subject of much investigation.

The question of a good fixing agent for nerve cells has been discussed by many writers but more particularly by Flemming,[11] v. Lenhossek[12] and Held.[13] Flemming, v. Lenhossek and with them many others find that saturated aqueous sublimate is the most satisfactory fixing fluid for nerve cells. Held, believing in the foam-like structure of protoplasm, does not consider it as good as other fluids. Besides sublimate, Carnoy's fluid, Flemming's fluid and picrosulphuric acid are generally found to give good results. With all these fluids fair results were produced, but the sharpest granules and the clearest intergranular substance were obtained by using the modification of Foa's fluid as recommended by Bensley,[14] viz., equal

8 Vas, Friedrich, "Studien über den Bau des Chromatin in der Sympathischen Ganglienzelle," Arch. f. Mik. Anat., XL, p. 375, 1892.

9 Szczawinska, W., "Recherches sur le systeme nerveux des Selaciens," Arch. de Biologie, XV, p. 463, 1897.

10 Timofeew, D., "Beobachtungen über den Bau der Nervenzellen des Spinalganglien und des Sympatheticus beim Vogel," Inter. Monat. f. Anat. u. Physiol, XV, p. 259, 1898.

11 Flemming, W., "Ueber den Bau der Spinalganglienzellen bei Saugethieren, und Bemerkungen über den der centralen Zellen." Arch. f. Mik. Anat., XLVI, p. 379, 1895.

12 v. Lenhossek, M., "Ueber den Bau der Spinalganglienzellen des Menschen," Arch. f. Psychiatrie, XXIX, p. 345, 1897.

13 Held, H., l. c. and Arch. f. Anat. u. Phys., Supp., p. 273, 1897.

14 Bensley, R. R., "Mammalian Gastric Glands," Proceedings of Canadian Institute, Vol. I, Part 1, p. 11, 1897.

parts of sublimate saturated in ninety-five per cent. alcohol and of a two per cent. solution of potassium bichromate in water. Small pieces were left in the freshly prepared mixture for two to four hours, washed in fifty per cent. alcohol, and then passed through the grades of alcohol. Material intended for chemical investigation was fixed in alcohol. The cells obtained from alcohol fixation are not materially different from those obtained with other fluids. The cone of origin and the process of spinal ganglion cells have nearly the same appearance in well preserved alcohol tissue that they have in sublimate material. Flemming's failure to get good results with alcohol may have been due to the circumstance that he did not leave his tissue in the alcohol for a sufficient time. Three days in alcohol as in Flemming's[15] method is not enough to insure complete coagulation of the proteids of the cell.

After fixing and hardening the material was imbedded in paraffin, using oil of bergamot for infiltration. Sections were attached to the slide by the distilled water method and stained.

I.—The Structure and Micro-Chemistry of the Nerve Cells of Mammals.

It is generally believed that three substances enter into the formation of the body of nerve cells: (1) the Nissl granules, (2) a spongioplasm that is generally believed to be fibrillar but which may be reticular, and (3) a hyaloplasmic ground substance in which the two former are embedded. As this structure is found in the nerve cells of mammals and the nerve cells of this class have been most frequently studied, they will form the subject of this section.

Material was used from the following animals:—man, ox, pig, sheep, dog, cat, rabbit, guinea pig and mouse. In most cases pieces from the cortex, cerebellum, cord, spinal and sympathetic ganglia were obtained and fixed in various fluids, but by preference in alcohol and the bichloride-bichromate mixture. The shape and distribution in the cell of the Nissl granules are best demonstrated by staining sections fixed to the slide for a few minutes in an aqueous solution of toluidin blue or methylene blue, but preferably in toluidin blue, which v. Lenhossek regards as a specific stain. After staining, the sections are differentiated in a mixture of aniline and alcohol, cleared in oil of bergamot and mounted in balsam. The results obtained with this method are similar in every respect to those obtained with the more laborious process of Nissl.

[15] L. c., p. 385.

Nissl[16] describes the bodies stained by his method, as having the form of larger or smaller, round, oval, spherical, often angular or irregular masses which have thread-like processes. These thread-like processes often unite the different masses into a true reticulum. Bühler[17] and Cox[18] for the spinal ganglion cells[19] and Flemming[20] for the cells of the cord of *Gadus* have noticed this reticulum of chromophilous substance. The reticular nature of this substance is frequently seen in the spinal or sympathetic ganglion cells, or in the cells of Purkinje in the cerebellum, and is occasionally seen in the cells of the cord and cortex. In sections stained with toluidin blue alone, the nucleus is seen as a clear space in the cell containing a large, round, deeply-stained nucleolus. There is usually nothing else stained in the nucleus, but occasionally there may be a faint bluish tint along certain lines.

If instead of employing toluidin blue alone, we use a cytoplasmic stain with it, we get the intergranular substance stained as well as the granules. The combination of eosin and toluidin blue, as employed by Mann, was the one used most frequently, although erythrosin and methylene blue, as employed by Held, give good results. Using these dyes we find the Nissl granules are stained blue, while the intergranular substance appears red. (Figs. 1 and 2). The nucleolus is also blue, but the blue is not the same as that of the Nissl granules, nor is the blue uniform throughout, for in many cases one can see a distinct red centre having a blue-stained layer on the outside. (Fig. 21). Probably the greatest change the addition of eosin to the stain has made in the appearance of the cell is in the nucleus. Here, instead of finding an unstained substance, one sees stretching from the nucleolus to the nuclear membrane a network of eosin-stained material. This substance is generally abundant near the nucleolus and adjacent to the nuclear membrane, while extending across the intervening space is a loose network of the same material. Sometimes, however, this material is found scattered throughout the nucleus in a finely granular form. This eosinophilous substance is generally more abundant in the nuclei of spinal and sympathetic ganglion cells than in the nuclei of cells of the central nervous

16 Nissl, F., "Mittheiiungen zur Anatomie der Nervenzelle," Allgemeine Zeitschrift für Psychiatrie, L, p. 372, 1894.

17 Bühler, l.c., p. 98.

18 Cox, W. H., "Die Selbstandigkeit der Fibrillen im Neuron," Internat. Monat. f. Anat. und. Phys., XV, p. 209, 1898.

19 Lugaro (Lo sperimentale, 1895), also observed the reticular nature of this substance. Quoted from Robertson, Brain, 1899, p. 212.

20 Flemming, W., "Ueber die Structur centraler Nervenzellen," Anat. Hefte Heft, XIX, p. 563, 1896. (Original inaccessible. Quoted from Bühler).

system. It has been suggested by v. Lenhossek[21] that this substance is the same as the lanthanin of Heidenhain[22] or the œdematin of Reinke.[23] This substance is undoubtedly a nuclein compound and is oxyphile, yet it will be seen later that it has very peculiar properties which distinguish it from all chromatins or other substances heretofore described, and I shall therefore call it for the present the oxyphile substance of the nucleus.

Staining sections in gentian violet or safranin and differentiating, gives figures almost similar to those obtained with toluidin blue alone, but if one fixes in Flemming's fluid and stains with his orange method, one finds the granules are a deep violet on a reddish ground, the nucleolus is red with an outer colouring of violet, while the oxyphile substance is also a deep violet. This method has given me some of my most instructive preparations, especially of spinal ganglion cells, the unattached sections of which may be left in the stains.

The iron-alum stain of Heidenhain has been extensively used by Flemming, v. Lenhossek and others to show the structure of the cell. As this stain colours the cytoplasm as well as the chromatin, it ought, in my opinion, to be used on nerve cells with care, for the granules are often fibrillar in character and with the iron-alum hæmatoxylin stain alone it is often impossible to distinguish the fine fibrillar processes of the granules from the intergranular substance. An after stain of rubin removes a great deal of the difficulty, as then the fine processes of the granules are stained like the granules themselves.

The granules in different classes of cells exhibit a variable affinity for the methyl green in the Ehrlich-Biondi combination, but such affinities are not constant. In this stain the nucleolus is generally greenish, but the green is unlike that in the nuclei of the neuroglia cells, a circumstance that v. Lenhossek has also noticed. There is usually no other green-staining substance except the nucleolus in the nucleus, but Levi,[24] Heimann[25] and Bühler have found such a substance. This of all staining mixtures is hard to manipulate, and one cannot lay any great stress on differences obtained with it.

21 V. Lenhossek, M., Arch. f. Psychiatrie, XXIX., p. 375.

22 Heidenhain, M., "Kern und Protoplasma," Festsch. f. Koelliker, p. 128, 1892, and Arch. f. Mik. Anat. XLIII.

23 Reinke, Friedrich, "Zellstudien," Arch. f. Mik. Anat., XLIII, p. 402, 1894.

24 Levi, G., "Su alcune particolarità di struttura del nucleo delle cellule nervose," Rivista di path. nervosa e mentale, 1896. (Quoted from v. Lenhossek, Arch. f. Psych., XXIX, p. 376).

25 Heimann, E., "Beitrage zur Kenntniss der feineren Struktur der Spinalganglien," Virchow's Archiv, CLII, p. 298, 1898.

26 Bühler, c., p. 46.

It has been shown by Macallum[27] that iron is a constant constituent of all chromatin. Mackenzie,[28] using the ferrocyanide method and the hæmatoxylin method of Macallum,[29] found the Nissl granules to contain iron. Using the hæmatoxylin method, which consists in keeping sections in acid alcohol (sulphuric acid 4, alcohol 100, by volume) for a few hours at 37° C., washing the acid out in alcohol and transferring them to an aqueous solution of hæmatoxylin, one finds the Nissl granules are stained bluish black, which is an indication that they contain iron. Besides the Nissl granules the nucleolus and the oxyphile nuclear substance have the same colour, showing that they also contain iron (Fig. 4). After the sections have been treated with the acid alcohol they may be transferred to acid ferrocyanide solution, when a Prussian blue reaction will be found in the three parts mentioned. The same result is obtained if teased-out cells are baked at 60° C. for several days in a mixture of ammonium sulphide and glycerine according to the method of Macallum, when the Nissl granules, nucleolus and oxyphile nuclear substance turn green, owing to the formation of ferrous sulphide. With any of these methods the Nissl granules are seen in the cell as masses or a reticulum. The appearances obtained by these methods are similar to those obtained by staining with toluidin blue alone, except that the oxyphile nuclear substance is also affected.

Using the test for phosphorus as described by Macallum,[30] the Nissl granules, nucleolus and oxyphile nuclear substance give a marked reaction for phosphorus, while the intergranular spongioplasm gives a faint reaction. (Fig. 3). For the purposes of this test, material that has been fixed in alcohol is extracted in a Soxhlet apparatus and imbedded in paraffin. Sections fixed to the slide are washed several times in distilled water to insure the absence of all alcohol, and then transferred to a solution of ammonium molybdate in nitric acid. After sections have been in this solution for some time they are brought into a solution of phenylhydrazin hydrochloride which reduces the phospho-molybdate to a greenish oxide of molybdenum but does not reduce the molybdate itself. Sections treated for a few minutes in the molybdate solution show little or no phosphorus. It is necessary to leave the sections in the molybdate solution for several hours in order to bring out clearly a reaction in the cell. After treating with phenylhydrazin hydrochloride the

27 Macallum, A. B., "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells," Quarterly Journal of Microscopical Science, Vol. XXXVIII, p. 175, 1895.

28 Mackenzie, l. c.

29 Macallum, A. B., "A New Method of Distinguishing between Organic and Inorganic Compounds of Iron," Journal of Physiology, XXII, p. 92, 1897.

30 Macallum, l. c.

sections are washed in water, dehydrated, cleared in oil of cloves and mounted in balsam. The preparations show that in the parts in which organic iron is present phosphorus occurs, and that a fainter reaction for phosphorus obtains in the spongioplasm.

Held[31] found the Nissl granules are not digested in pepsin and hydrochloric acid solutions. This is correct, but the oxyphile nuclear substance also digests and the nucleolus under certain circumstances disappears. This is an important fact and is the chief objection to calling the oxyphile nuclear substance oxychromatin, for chromatin is always considered to be indigestible. Held's figures seem to show that he obtained the same result on digestion. No mention is made of this in his text, but in the description of the cells given under his plate he adds, "Nucleolus und ein Theil der Kernmasse noch nicht verdaut," thus indicating that he considered it an ordinary circumstance for nuclear parts to digest.

The oxyphile nuclear substance digests very readily indeed, but it is doubtful if the disappearance of the nucleolus is really due to the digestion of its substance. I shall show later that the nucleolus has an oxyphile centre and it is probable that this centre would digest, thus liberating the whole nucleolus, if it were attached to the slide only by its centre. If on the other hand, the nucleolus is attached by its periphery it will not be removed. Sometimes the nucleolus, after digestion, appears as a shell The nucleolus is also very loosely attached to the nuclear network, a feature to which v. Lenhossek[32] has called attention and which will afterwards be discussed. After digestion the deepest iron-alum stain of Heidenhain or any other stain, will not show a reticulum in the nucleus, consequently there would be nothing to hold the nucleolus in its place in material digested in bulk. Considering everything, it is probable that the peripheral or basophile portion of the nucleolus is never digested. It is not the weak acid that affects the oxyphile nuclear substance, for one may leave loose sections in weak (0.2 per cent.) hydrochloric acid for days at 37° C. and yet the nucleus will contain oxyphile substance.

In the digestion experiments fresh material was sometimes first submitted to digestion and then hardened and imbedded, but generally the tissue had been fixed in alcohol beforehand. The material employed was in the form of sections attached to the cover glass or in thin pieces which were afterwards dehydrated and then imbedded.

31 Held, Arch. f. Anat. u. Phys., Anat. Abth., 1895, p. 396.

32 v. Lenhossek, M., Arch. f. Psych., XXIX, p. 373.

Held[33] found the granules are insoluble in weak or in concentrated mineral acids. Eve[34] observed that acids had a slight dissolving action on the granules.

Acids have a marked action on the granules and nuclear contents. One way to detect the presence of iron in the cell is to treat the sections with acid alcohol, when the iron can be detected by ordinary reagents (vide ante). If the sections are left in the acid for longer time all the iron will be extracted from the cell and then on staining them with eosin and toluidin blue, no blue-stained substance will be seen, the result being the same as if unaltered cells were stained with eosin alone. There are no vacuoles in the cell and the granules may still be detected with Heidenhain's hæmatoxylin stain. After all iron has been extracted from the cell the granules in the cytoplasm, the nucleolus and the oxyphile nuclear substance still contain phosphorus.

The action of alkalies on the cell has been studied by Held.[35] Eve,[36] Bühler[37] and Ruzicka.[38] Held and Bühler find the granules are first altered in their staining powers and then are soluble in alkalies, forming vacuoles in the cell where the granules were originally. Eve is doubtful whether the granules are soluble in the alkalies or are merely altered in their staining powers. Ruzicka finds the granules are insoluble in alkalies and are not altered in their staining properties.

After treatment with alkalies the granules, I find, do not stain blue with toluidin blue or do not give an iron reaction, but most cells do not contain vacuoles. Since alkalies detach sections from the slide, either loose sections of spinal ganglia were used, or thin pieces of material which had been fixed in alcohol were left in the alkali for the desired time and then washed, dehydrated and imbedded. As alkalies the following solutions were employed: sodium hydrate 0.25 per cent., 0.5 per cent. and 1 per cent.; potassium hydrate, 0.2 per cent. and 0.5 per cent. and lithium carbonate in saturated aqueous solution. Held[39] figures a vacuolated cell obtained by leaving a thin piece of cord in saturated lithium carbonate for four days. Material was left in lithium carbonate solution for as long as ten days and yet no vacuoles were

33 Held, Arch. f. Anat. u. Phys., Anat. Abth., 1895, p. 396.

34 L. c.

35 Held., H. Arch. f. Anat. u. Phys., Anat. Abth., 1895, p. 396, and 1897, p. 204.

36 Eve., l. c.

37 Bühler., l. c.

38 Ruzicka, Vladislaw, "Untersuchungen, über die feinere Structur der Nervenzellen und ihrer Fortsätze," Arch. f. Mik. Anat., LIII, P. 485, 1898.

39 Held, l.c., 1895, Fig. 10.

observed in many cells. Material has been left in the weak potash or soda solutions for seven days with the same result. After treatment with the alkali, staining with eosin and toluidin blue will produce the same result in the nerve cell as staining unaltered sections with eosin alone, except that the nucleolus will probably be quite blue. The nuclei of the neuroglia cells are still stained normally, as are also the nuclei of the cells of the walls of the blood vessels and of white blood corpuscles present in them, thus showing the stains are effective and would still bring out the granules if they were unaltered. Held[40] has also observed that after treatment with alkalies the nuclei of the neuroglia and connective tissue cells are unaltered.

If one treats cells altered in this way to determine the distribution of iron in the cell, one finds the nucleolus and oxyphile nuclear substance may still be quite rich in iron but the remainder of the cell is devoid of it. The neuroglia cells also contain iron, this showing that the reaction would still detect any iron if it were present in the cytoplasm of the nerve cells. After prolonged treatment with the alkali, the distribution of phosphorus is quite normal, as the granules in the cell, the nucleolus and oxyphile nuclear substance still give the phosphorus reaction.

One can obtain similar results if tissue is hardened in an alcoholic solution, containing a small percentage of alkali, such as Held employed, when the iron-holding substances of the nerve cell are extracted from the cytoplasm but the nuclei of the neuroglia cells are only slightly affected. A ten per cent. solution of lysol, which Reinke found to be a solvent of chromatin, was also used with the same result; it altered the staining properties of the nerve cell but did not affect those of the neuroglia cells.

Ruzicka used material that had been fixed in sublimate for his experiments. The mercurial compound of these granules is much less easily altered than the granules coagulated in alcohol; but if treatment with the alkali be prolonged the same result is obtained.

When the tissue is placed in the alkali it swells but shrinks again on placing it in alcohol. This swelling and shrinking causes clefts in the protoplasm of some cells. Although these clefts do not correspond in position or form with the granules in the cell, it seems probable they are the vacuoles noticed by Held and Bühler and considered by them as the spaces left by the dissolved granules. It seems highly improbable that

40 Held, Arch. f. Anat. u. Phys., Anat. Abth., 1897, p. 207.

alkalies should remove the granules, leaving vacuoles, but not the nucleolus or the oxyphile nuclear substance which are related substances.

The slight degree of alkalinity necessary to alter the granules suggested that the blood, which is really more alkaline than some of the solutions used, might act in a similar way. This was tried and found to be the case. After loose sections of a spinal ganglion that had been fixed in alcohol had been in fresh defibrinated ox-blood for twenty hours, the granules were altered in the same way as if they had been in potash or soda solutions for the same time. We thus find that the granules, as they occur in the cells after fixation, are altered by the animal's own blood.

Eve observed that salt solutions had little action on the granules, but Bühler found the granules were soluble in physiological salt solution in twenty-four hours, leaving vacuoles in the cell. My results coincide with those of Eve, for when fresh spinal cord and ganglia were left in salt solutions for as long as three days at room-temperature the substance of the Nissl granules was still present. The cells contained vacuoles, forcing the granules into distorted shapes, but the substance stained normally with toluidin blue, and contained iron. In one case, after material had been in the salt solution for three days, the granules were so altered that they would not stain with toluidin blue. On examination the salt solution used was found to be distinctly alkaline, but in all cases where neutral salt solution was used the substance of the granules was not removed.

Leaving fresh material in distilled water for five days at the temperature of the room does not alter the staining powers of this substance, although the cell may contain vacuoles. Hardening material, however, by putting it into boiling water, has an action on nerve cells somewhat similar to the action of dilute alkalies. If the boiling has been continued long enough the granules will not stain with basic dyes and the iron cannot be detected in them with the acid alcohol method. The distribution of phosphorus is, however, normal throughout the cell.

Held failed to obtain a Millon reaction in the granules. A Millon reaction may, however, be obtained throughout the cell body, the nucleolus and oxyphile nuclear substance, if sections of material fixed in alcohol are left in freshly prepared Millon reagent for several hours at room temperature.

Besides the granules, the nerve cells frequently contain a yellowish

pigment in their body. The pigment has been found to be especially common in man and monkeys. (Warrington).[41] The pigment present in the cells of a thoracic sympathetic ganglion of an ox, after it had been hardened in alcohol, gave the following reactions. It was still present after a one per cent. solution of potash had acted on loose sections for three days at room-temperature. It was not removed from the free sections by the action for a week of one per cent. hydrochloric acid solution, nor did it give, after the use of acid alcohol, any reaction for iron, which confirms what Warrington found for the pigment present in the nerve cells of man. It did, however, give a positive reaction for phosphorus, using Macallum's test.

Before leaving this section I would like to discuss the structure of the nucleolus. There is always one, and there may be several, nucleoli present in the nucleus of the nerve cells of mammals and in most other classes of animals; but there is rarely a nucleolus in the nerve cells of the Urodela and if present it cannot be distinguished with certainty from the remainder of the nuclear chromatin.

The nucleolus is considered by most observers to consist of a single substance which may be vacuolated. Several observers, however, have described the nucleolus as consisting of fine grains embedded in a ground mass. This view is supported by v. Lenhossek,[42] Held,[43] Ruzicka,[44] Obersteiner,[45] but more particularly by Timofeew[46] who says the nucleolus consists of basophile grains embedded in an oxyphile ground substance.

The nucleolus consists of two substances, but the relation of these two is different from that usually described. I find the nucleolus is a vesicle with an oxy-centre and a basophile covering.[47] This relation is often seen in sections stained with eosin and toluidin blue, or in material fixed in Flemming's fluid and stained with his orange method. A somewhat similar structure has lately been described by Heimann[48], who noticed the periphery of the nucleolus had a great affinity for stains.

This structure is best seen in the nerve cells of rodents but occurs in

41 Warrington, W. B. "On the Structural Alterations observed in Nerve Cells," Journal of Physiology, XXIII, 1898.

42. v. Lenhossek, l. c.

43. Held, Archiv f. Anat. u. Physiol., p. 207, 1897.

44. Ruzicka, Zeit. f. Wiss. Mikroskopie, p. 452, 1897.

45. Obersteiner, Zeit. f. Wiss. Mikroskopie, p. 60, 1898.

46. Timoteew, l.c.

47 Mackenzie also observed this relation in the nucleolus. Ora Communication, British Association Toronto Meeting, 1897.

48 Heimann, l. c.

all animals and in well-stained sections is easily observed. Vacuoles are also quite frequent in the nucleolus, a fact which has attracted the notice of several observers. That this is quite correct is shown by the action of alkalies or of digestive fluids on the nucleolus. The action of digestive fluids in sometimes leaving a shell of undigested material has been referred to, but the effect of alkalies is more convincing. Held found that after prolonged treatment in the alkali the nucleolus no longer stained with methylene blue, and he thought that this showed that the nucleolus was formed of fine grains embedded in a ground mass. Alkalies have an altering action on the nucleolus similar to that on the Nissl granules but the action must be prolonged. If tissue which has been fixed in sublimate is used the action is very slow and one can often find the outer covering of the nucleolus broken, between the portions of which the oxyphile centre may be seen. This structure can be seen in sections stained with eosin and toluidin blue, or in iron-alum hæmatoxylin, but the clearest way of demonstrating it is the gold method of Apathy.[49] Figs. 9 and 10 are the nuclei of cells that have been treated with potash and then stained with this method. The oxyphile centre can be seen between the pieces of the basophile covering which has undergone fragmentation.

The above considerations render it clear that there are at least three distinct nuclein compounds in nerve cells, the Nissl granules, the basophile covering of the nucleolus and the oxyphile nuclear substance. Each of these bodies contains iron and phosphorus, the usual constituents of many nucleo-proteids. Van Gehuchten[50] and Cajal[51] believe the nuclein is condensed into the nucleolus, while v. Lenhossek maintains that the nerve cell does not contain true nuclein or chromatin. There seem to be many different nuclein compounds in different cells, but we shall see that for the nerve cells these different nuclein compounds are genetically related, and that intermediate substances are found in the nerve cells of different animals.

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49 Apathy, Stefan, "Das leitende Element des Nervensystems, etc," Mitth. aus der Zool. Station zu Neapel, XII, p. 495, 1897.

50 Gehuchten, A. van, "L'anatomie fine de la cellule nerveuse,' La Cellule, XIII, p. 313, 1897.

51 Cajal, S. R., Revisto Trimensal Micrografica, 1896. (Original inaccessible, quoted from van Gehuchten).

II.—The Development of Nerve Cells with Special Reference to the Development of the Chromatic Substance of the Cell Body.

Several attempts have been made to determine the origin of the Nissl granules, but all have failed to detect it. Vas made some interesting observations on the chromatin of fœtal sympathetic ganglion cells but did not attempt to ascertain the origin of the chromatic substance of the cell body. Eve found the chromatic substance completely filled the cell body at an early date. The cells of the vagus ganglion were the first to show an appearance like the adult cell with regard to the distribution of this substance. Szczawinska, working with selachian embryos, did not trace it further than the stage in which the cells were uniformly stained. Bühler[52] states that fœtal cells are devoid of granules, but does not ascertain the origin of the granular substance. He did, however, notice that the nuclei of young nerve cells are basophile and gradually become oxyphile as development proceeds. Timofeew observed that in chick embryos the basophile substance increased markedly in amount in the cells of the spinal ganglia, between the fourth and sixth day of incubation. He says nothing of its origin and evidently considers it cytoplasmic.

The chromatic substance which forms the Nissl granules is undoubtedly derived from the nuclear chromatin. A series of pig embryos from 7mm. onward to birth was the chief material used for these observations, but calf, sheep, rabbit and chicken embryos were used to confirm the results.

The embryos were fixed in the bichloride-bichromate mixture or in picro-corrosive fluid. Material intended for chemical methods was fixed in alcohol.

The development of the chromatic substance which forms the Nissl granules is closely connected with the morphological development of the cell. His[53] showed that the neuroblasts are derived from cells, lying, in mammals, next the medullary canal, which he calls germinating cells. These cells have a protoplasm which may be divided into an outer clear

52 L. c., p. 46.

53 His, W., "Die Neuroblasten und deren Entstehung im embryonalen Mark," Arch. f. Anat. u Phys., Anat. Abth., 1889, p. 249.

Also, "Histogenese und Zusammenhang der Nervenelemente," Arch. f. Anat. u. Phys., Supp. 1890 p. 95.

and an inner granular layer. The next distinct stage which His distinguishes in the development of the nerve cell is the neuroblast phase. Here an oval nucleus bears a conical cell body, and this in turn is continued into a long process. The nucleus is moderately rich in chromatin of which there are several masses united by a filament. There are no protoplasmic processes and the protoplasm around the nucleus is very scanty. The neuroblasts arise in the inner layer from the germinating cells and pass out secondarily into the mantle layer of the wall. In the transformation of the germinating cells into neuroblasts His distinguishes five stages:

(1). Germinating cells of round form with a broad protoplasmic body.

(2). Germinating cells of round form with initial point and broad protoplasmic mantle.

(3). Intermediate cells of pear shape with little protoplasm around the unclosed nucleus. The cytoplasm is continued into a long process and the cells may still lie close to the internal membrane.

(4). Intermediate cells of pear shape with closed nucleus, deeply staining, outer cone, but little protoplasm around the remainder of the nucleus.

(5). Finished neuroblast.

My observations confirm those of His on the origin of the neuroblasts and in addition show the fate of their chromatin, a point not touched upon by His.

Germinating cells occur in the pig from the earliest stage procured by me (7mm.) to that of 18mm. length. If a section of a cord of, *e. g.*, a 10mm. pig, is stained with eosin and toluidin blue, one finds that all the blue-staining substance in the germinating cells is confined to the chromatin of their nuclei. The reactions for iron demonstrate that the cytoplasm is devoid of substance containing this element. At this period all iron-holding material is confined to the chromatin of the nucleus.

These cells are of round or oval shape, (Fig. 12), and are in some stage of mitosis. The cytoplasm is free from iron-holding material, or from material staining with toluidin blue. The cells are sometimes in the loose-skein phase, sometimes in the dyaster stage but most frequently in the equatorial-plate phase. The cells are generally in the equatorial plate phase when the process begins to be formed. As the cone increases the chromatin becomes excentric (Figs. 13 and 14). There is

still no nuclear membrane and still no iron-holding nuclein compounds in the cell body or process. The cell bodies appear, quite frequently, reticulated.

The equatorial-plate stage is soon passed, and the chromatin begins to distribute itself in the nucleus. Various steps in the distribution of the chromatin may be followed until a stage, such as is represented in Fig. 15, is reached. By this time a nuclear membrane has been formed. Usually several masses of chromatin are found touching the nuclear membrane, while others are found towards the centre of the nucleus, but all seemingly connected by filaments. There is, as yet, no oxyphile substance in the nucleus. These cells are usually found under the membrane of the medullary canal but in very young embryos they occur in the mantle layer. The nucleus is entirely excentric and the cell body runs out into a long process. The cell body and process are still entirely free from iron-holding nuclein compounds.

As the cells pass outward into the mantle layer and become older, the substance having affinity for toluidin blue in their nuclei disappears and a substance with more affinity for eosin takes its place. Synchronous with this change, a substance with great affinity for toluidin blue appears in the cell around the nucleus (Figs. 16 and 17). With the appearance of this substance in the cell body iron may be detected there for the first time. In this stage, which would correspond to stage 4 of the series described by His, there are several granular masses in the nucleus with marked affinity for toluidin blue, but the most of the nuclear chromatin stains intermediate between the red and the blue.

As development proceeds (Figs. 18 and 19) the basophile substance in the nucleus continues to decrease, while the basophile substance in the cell body increases, and as it does so the affinity of the nuclear chromatin for eosin also increases correspondingly. One part of the chromatin does not alter but remains basophile and constitutes ultimately the peripheral portion of the nucleolus. Figs. 18 and 20 represent cells from the same embryo. The one indicates the distribution of the oxyphile and the basophile parts, while the other shows that both contain iron.

In Figure 21 is represented a cell from the medulla of a 32mm. pig embryo. The basophile substance forms a homogeneous mass filling the cell body. The cell may be said to have undergone at this time complete development of its chromatic substance, for now the nuclear oxyphile substance is completely digestible, and it stains like the substance found in the nuclei of adult mammalian nerve cells.

These facts indicate that the three nuclein compounds of the adult nerve cell, the Nissl granules, the nucleolus and the oxyphile nuclear substance, are derived from the chromatin of the nucleus of the germinating cell. This chromatin divides into two parts, each containing iron and phosphorus, but the one is oxyphile and remains in the nucleus, while the other is basophile and diffuses into the cell body and becomes the Nissl granules. The nucleolus seems to correspond in character to the chromatin of such a stage as is represented in Fig. 15, where little change has occurred from the equatorial-plate phase.

The cell body is filled with diffused chromatin before the protoplasmic processes are formed, but as the cell grows and the protoplasmic processes arise, the diffused chromatin is formed into masses and these in turn into smaller pieces until the size observed in the adult is reached. The breaking up of the diffused chromatin into masses is probably due to growth, and not to functional activity as one might think from the results obtained by Vas and Eve, for the ganglion cells of the retina of a fœtal calf of 60 cm. were distinctly granular. If the process of fragmentation proceeds far enough, the masses will be isolated in the cell, but if not they will constitute a reticulum. No evidence was observed of a connection persisting between this diffused chromatin and the nucleus.

One criticism of the observations of His is necessary. His stage 4 in the development of the neuroblast should succeed his stage 5, for the description of the latter stage is of a cell in which the basophile substance has not yet diffused from the nucleus, while the description of the former stage is of a cell in which this diffusion has taken place. That such a mistake might arise is seen from the fact that the basophile substance is not distinct in the cell body for a long time after neuroblasts are formed and have migrated into the mantle layer. Thus germinating cells are found in all the stages from the earliest procured (7 mm.) to that in which the embryos are about 18mm. long, while neuroblasts of the type that His describes as developed are abundant in the mantle layer at 7mm. and continue to be so until the formation of neuroblasts ceases. The chromatic substance, however, is not abundant in the cell body till the embryo is about 15mm. long. Consequently the process of transformation and diffusion of the chromatin is going on while the embryo grows from at least 7mm. to 15mm. In an embryo of 15mm. there are still many neuroblasts in the mantle layer that have not a distinct colourable cone but only a thickened mass of basophile substance around the nuclear membrane.

A single section of an embryo pig from 15 to 18mm., since it contains

germinating cells and neuroblasts with diffused chromatin, shows all stages in the process of transformation and diffusion of the chromatin. This circumstance enables one to be sure that the gradual loss of affinity of the nucleus for basic dyes is not due to overstaining in eosin, for after the chromatin begins to change, an overstaining with eosin will make it red, but a shorter time in the eosin will colour it purplish. If one examines a section in which all stages of the diffusion of the chromatin are seen, one can easily see the great affinity the nuclei of the nerve cells next the medullary canal have for toluidin blue. The nuclei of cells lying farther from the canal have less and less affinity for basic dyes, but one can observe that with this loss of affinity on the part of the nucleus for these dyes, a substance with marked affinity for them appears in the cytoplasm. This substance is first seen as a thickened nuclear membrane, but as more of it diffuses from the nucleus it takes the form of a distinct cone in the cytoplasm forming a cap on the nucleus.

These observations have been confirmed on the cells of the cortex, cerebellum and spinal ganglia of mammals and also on the cells of the chicken. The chick embryos are not as suitable as mammalian embryos for following this process, on account of the general distribution of yolk nucleo-proteids, but the stages can be distinctly observed.

The chromatic material appears in the cytoplasm of the cells of the medulla before it appears in those lower down in the cord, but my observations on the rate of the development of the material in the different centres are incomplete.

The action of dilute alkalies on the cell varies with the degree of its development. At a stage such as is represented in Figure 21 the action of alkalies is similar to their action on adult cells. After treatment with alkalies the cell body would not stain with toluidin blue or did not contain iron, but the body of the cell still remained. If the view of Held and Bühler regarding the nature of the Nissl granules is correct, then the whole cytoplasm should have disappeared.

Alkalies are very slow in altering the staining powers of the chromatin in mitosis. The nuclei of germinating cells and of the nerve cells in which the kinetic chromatin was only slightly altered, contained a large quantity of material that stained with toluidin blue and proved to be iron-holding, after small pieces of tissue, that had been fixed in alcohol, had been treated with potash (0.2%) for six days. In this same solution the Nissl granules had been altered so that they would not give their

ordinary reactions in six hours, but the nucleolus of the nerve cell and the nuclei of the neuroglia cells manifested their ordinary reactions, although more diffusely, at the end of the six days.

On digestion of material which had been fixed in alcohol there was no appreciable effect on the nuclein compounds until a stage of which Figs. 19 and 21 are representations. In the stage illustrated in Figure 21, which is from the cord of an embryo of 32mm., all the oxyphile nuclear substance digests as in the adult, but in stages before this one, there is always some substance which does not disappear on digestion. After digestion the periphery of the nucleolus always remains. In this respect as well as in all its other reactions it resembles the chromatin found in primitive nerve cells.

In order to facilitate reference to the different stages through which the chromatic substance passes in getting into the cytoplasm of the nerve cell I shall adopt the example of His and divide the process (arbitrarily) into different stages.

Stage 1. Germinating cells (Figs. 12, 13, and 14), stages 1, 2, and 3 of His. The chromatin is confined to the nucleus and is in mitosis. Weak alkalies alter this substance very slowly. Digestion dissolves the cell body but does not alter the staining power of the chromatin.

Stage 2. Neuroblast stage (Fig. 15), stage 5 of His. The chromatin is confined to the nucleus but is broken into masses. A nuclear membrane has been formed and the greater portion of the chromatin is distributed around the membrane. Alkalies and digestive fluids have little or no power to alter the reactions of this substance.

Stage 3. (Figs. 16, 17, 18, 19 and 20), stage 4 of His. Some of the kinetic chromatin is transformed into two other kinds, an oxyphile and a basophile. As the chromatin alters, the basophile part diffuses into the cytoplasm but the oxyphile substance remains in the nucleus. Most of the chromatin alters at the same rate but there may still be masses in the nucleus with marked affinity for basic stains. Alkalies have an altering action on the diffused-out chromatin in extracting the iron from its substance, thus changing its staining reactions. Digestive fluids affect the nucleus but still leave the basophile parts behind.

Stage 4. (Fig. 21). The transformation of the kinetic chromatin into the oxyphile and basophile kinds is now completed and the diffused basophile part fills the whole cell body. Alkalies alter the chromatin, especially the diffused part. Digestion dissolves the oxyphile substance

completely but leaves the diffused substance and the periphery of the nucleolus unaffected.

Stage 5. Adult cell. Differs from stage 4 only in the distribution of the chromatin which has diffused into the cytoplasm.

III.—On the Structure of the Nerve Cells in Other Classes of Animals.

When such a remarkable change as that described in the development of the Nissl granules occurs in mammals and birds, one would expect to find some trace of it in the nerve cells of other animals. With this in view the nerve cells of adult animals of the different classes were examined. Before giving the results it would be advisable to make as complete a list as possible of those forms in whose nerve cells a substance analogous to that of the Nissl granules has been found. The following authors have dealt with the structure of the nerve cells of different mammals:—Flemming,[54] Nissl, Vas Mann, Dogiel,[56] Held, Eve, v. Lenhossek,[57] Lugaro,[58] Cajal, van Gehuchten, Cox, Bühler, Heimann, Rohde,[60] Ruzicka and many others. In all cases recorded for mammals the nerve cell contained the granules. The only exception to this statement is that of Reinke who, in the discussion of v. Lenhossek's paper before the Anatomische Gesellschaft, stated that he had found large nerve cells in the ganglion of a cat free from granules. In all ganglion cells of the cat examined . .sl granules were present, and it is probable the cells found by Reinke were in a diseased or otherwise altered ganglion.

The nerve cells of the animals below mammals have been studied with methods that would show the Nissl granules as follows:—

From Aves: in the pigeon,[61, 62, 63] raven,[62] and fowl.[63]

54 Flemming, l.c., and Archiv f. Psychiatrie, XXIX, p. 969, 1897.

55 Mann, Gustav., Journal of Anatomy and Physiology, XXIX, p. 100, 1894.

56 Dogiel, A. S., Archiv f. Mik. Anatomie, XLI, p. 62, 1893.

57 v. Lenhossek, M., l.c., also Verhand. der Anat. Gesellschaft, 1896, p. 9.

58 Lugaro, Rivista di pathologia nerv e. ment., 1896, p. 12.

59 Cox, W. H., l.c. and Anat. Hefte, X, Heft 31, p. 73, 1898.

60 Rohde, E., "Die Ganglienzelle," Zeit. f. Wiss. Zool., LXIV, p. 697, 1898.

61 Held, l.c.

62 Bühler, l.c.

63 Timofeew, l.c.

From Reptilia: in the ganglia of *Testudo*, *Emys*, *Uromastix*, and *Agama*.[64]

From Anura: in *Rana*,[65, 66, 68] and *Bufo*.[67]

From Pisces: in the electric lobe of *Torpedo*,[69] in *Gadus*,[70] in *Leuciscus*,[71] in *Cyprinus*,[72] in *Alopias*.[73]

From Invertebrates: in the crayfish,[74] earthworm,[74, 75] molluscs[74, 76] and insects.[74]

With the exception of Szczawinska, who states that granules are not present in a few cells of some rays, all these observers find a substance analogous to that of the Nissl granules present in the nerve cells of the different animals examined. I think the reticulum, in the cells without granules which Szczawinska figures, is made up of this chromatic material, for one frequently finds the chromatic substance in such a reticulum in some of the nerve cells of the earthworm, *Limax* and *Limnæa*, while neighbouring cells have the chromatic substance in a granular form. In any case the observations on the occurrence of this substance in the nerve cells of the pigeon, frog, earthworm, crayfish and various molluscs were confirmed.

The nerve cells of other forms which have not been studied, so far as I know, by others, were examined, and this chromatic material was found in the following forms:

From Reptilia: cells of cord and cortex of *Chrysemys picta*.

From Ganoidei: cells of cord of *Amia calva*.

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64 Pugnat, Charles Amedee, "Recherches sur la structure des cellules des ganglions spinaux de quelques reptiles," Anat. Anz., XIV, p. 89, 1898.

65 v. Lenhossek, M., "Centrom und Sphäre in den Spinalganglienzellen des Frosches," Arch. f. Mik. Anat., XLVI, p. 345, 1895.

66 Dehler, Adolf., "Beitrag. zur Kenntniss von feineren Bau der sympathetischen Ganglienzellen des Frosches," Arch. f. Mik. Anat., XLVI, p. 724, 1895.

67 Bühler, l.c.

68 Rohde, l.c.

69 v. Lenhossek, M., "Der feinere Bau, etc.," p. 159, 1895.

70 Flemming, l.c.

71 Bühler, l.c.

72 Szczawinska, l.c.

73 Paladino, G., "Sur la constitution morphologique du protoplasma des cellules nerveuses," Arch. It. de Biol., XXIX, p. 60, 1898.

74 Pflüke, Max, "Zur Kenntniss des feineren Baues der Nervenzellen bei Wirbellosen," Zeit. f. Wiss. Zool., LX, p. 500, 1895.

75 Eve, l.c.

76 McClure, Charles F. W., "The Finer Structure of the Nerve Cells of Invertebrates," Zeologische Jahrbücher, Abth. für Anatomie, XI, p. 13, 1897.

From Teleostei: cells of cord of *Amiurus catus*.

In all cases this substance in the cell body, although distributed differently, stained with toluidin blue and gave the reactions for iron and phosphorus. In all cases tried the substance was found to be insoluble in pepsin and hydrochloric acid but to be easily altered by dilute alkalies.

The widespread occurrence of this substance in such diverse forms has been taken by some (Rohde,[77] Marinesco[78]) to indicate that this material is an essential constituent of all nerve cells. This, however, is not the case, for in 1895 Bühler[79] described the cells of the forebrain of *Lacerta agilis* as frequently devoid of Nissl granules, and I find that the vast bulk of the nerve cells of the Urodela are absolutely devoid of them. It will therefore be necessary to enter into a detailed account of the nerve cells of these forms.

Several specimens of *Necturus*, *Amblystoma*, *Plethodon* and *Diemyctylus* were obtained and the cord, brain and ganglia fixed in different fluids. A series of a *Salamandra* larva was also examined[80] and series of larval *Amblystomata* of various ages were made. The nerve cells of all these different forms were found to correspond in their structure and characters.

In the nerve cells of these animals the cytoplasm, instead of holding granules which contain iron and phosphorus and which stain with basic dyes, is often free from iron, phosphorus or substance staining with toluidin blue, and on the other hand, their nuclei, instead of containing very little basophile substance, abound in granules of such basophile material. This is true of ganglion, retinal and central nerve cells.

If one fixes in Flemming's fluid and stains with his orange method there is no gentian-stained substance in the cell body while the nucleus is filled with granules and threads which stain deeply with the gentian. If instead of the orange method one uses safranin and light green, according to Benda's process, one finds all the substance staining with safranin confined to the nucleus.

In material that has been fixed in alcohol or in sublimate, and stained

77 Rohde, l.c.

78 Marinesco, G, "Recherches sur la biologie de la cellule nerveuse," Arch. f. Anat. und Phys., Phys. Abth., 1899, p. 89.

79 Bühler, Anton, "Protoplasma-Structur in Vorderhirnzellen der Eidechse,' Verh. d. phys. med Ges., Würzburg, Stahel, 1895.

80 For this privilege I am indebted to Dr. J. Stafford.

with eosin and toluidin blue, there is in the bodies of most nerve cells no blue-stained substance, while the nucleus is full of blue-stained granules and threads (Fig. 5). On staining sections in the Ehrlich-Biondi mixture, one finds the cell body is red, but all the nuclear chromatin is greenish, and there is no difference in the staining reactions of the nuclei of nerve and neuroglia cells such as is found between these cells in mammals.

The reactions for iron (Fig. 7) and for phosphorus (Fig. 6) show there is no iron and little phosphorus in the bodies of most nerve cells.

In a few cases a little basophile substance was observed in the cell body. In these the cytoplasm also contained a slight amount of iron and phosphorus-holding substance, but the much greater part of this substance, or of the substance staining with basic dyes, is in the nucleus A sufficient number of specimens to determine the cause of the presence or absence of this slight amount of basophile substance in the cytoplasm have not been examined, but when it is present, it is most frequently diffuse and not in granular form, although the latter, in rare cases, has been seen.

On digestion little material is dissolved from the nucleus, but the oxyphile substance, which was present in traces previously, has now disappeared (Fig 9). Those cells which contain a little basophile substance in the cytoplasm retain it after digestion.

The action of alkalies on the nerve cells of these animals is similar to their action on the neuroglia cells of the adult, or on the nerve cells of embryo mammals. Thus, after six days in a solution of potassium hydrate (0.2%) the nuclei still held a large quantity of material which contained iron and phosphorus, and which stained with toluidin blue. This same solution had removed all the basophile material from the cytoplasm of the nerve cells of adult mammalia in a few hours, but the nucleolus of the nerve cell and the neuroglia cells stained with basic dyes after six days, and the same was true of the embryonic nerve cells of mammals. The nuclei of the neuroglia cells of these Urodela, as in mammals, resist the action of alkalies. There is, therefore, in the former, no difference with respect to the action of alkalies between the nuclei of nerve and neuroglia cells. The slight amount of the basophile material present in some cells is easily and quickly altered by the alkali.

For some reason, the transformation and diffusion of the chromatin has not proceeded, in the cells of the Urodela, past a certain stage, cor-

responding to stage 2, given above for mammalian development. Compare Fig. 15, which is the nerve cell of an 11mm. pig, with Fig. 5, which is the motor nerve cell of an adult Necturus, fixed in the same fluid and stained with the same dyes. Besides the staining reactions the effect of alkalies or of digestive fluids is practically the same in both cases.

Levi[81] has examined the nerve cells of different types of Vertebrata (*Vespertilio, Cavia, Canis, Bos, Testudo, Zamenis, Rana, Triton, Proteus, Spelerpes, Tinca, Raja, Scyllium, and Petromyzon*), and has noticed the peculiar nature of the nucleus in the cells of the Urodela. He offered no explanation of the peculiarities of these cells, nor did he draw any conclusion about the nature of the substance in the cytoplasm of other forms.

A comparison of nerve cells of larval *Amblystomata* with those of the adult form shows them to be exactly similar. There is no transformation, except to a slight degree, of the chromatin into an oxyphile and a basophile part. I have noticed that those cells of the adult that had a little basophile substance in the cell body also had some oxyphile substance in the nucleus.

There are other forms that have not yet reached the adult or mammalian degree of differentiation. Thus in *Limax* and *Limnæa* (and from the descriptions of Pflüke and McClure, in all Gasteropods) the cells have stopped developing at a stage between 3 and 4 of the mammalian development. There is a quantity of iron and phosphorus-holding substance in the body of the nerve cell, but the nuclear chromatin is peculiar. It is not affected by digestive fluids, it stains green with the Ehrlich-Biondi stain, it stains with safranin, and is generally purplish with eosin and toluidin blue, although, by long action of eosin and short action of toluidin blue, it may be quite red.

Other forms (earthworm and crayfish) were also noticed to vary slightly from the mammalian type. I believe that if the nerve cells of all adult animals were examined, one would find a complete series in the diffusion of the chromatic substance to the cytoplasm.

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81 Levi, G., "Su alcune particolarita di struttura del nucleo delle cellule nervose," Rivista di patologia nervosa e mentale, Vol. I, p. 141, 1896.

Also: "Ricerche citologiche comparate sulla cellula nervosa dei Vertebrati," Ibid., Vol. II, pp. 103 and 244, 1897.

(Both papers inaccessible, quoted from the Zoologischer Jahresbericht for 1896 and 1897.)

IV.—Some General Considerations on the Structure of the Nerve Cells.

It may seem strange to revert to this subject, but owing to the fact that the Nissl granules were thought to be cytoplasmic structures, several views concerning the structure of nerve cells have been advanced that would not have been if the true nature of the granules had been known.

The first question is, whether the Nissl granules are formed elements of the cell body, or are precipitated while the cell is dying or when it is affected by the fixing agent. Held[82] accepts the latter explanation, as he claims to have seen fresh cells in which there were no granules but a homogeneous cell body. On standing for a few minutes the cells become granular, thus showing the granules were precipitated while the cells were dying. On adding water to the cells they become vacuolated, but the vacuoles would collapse on adding a fixing agent, thus leaving the granules around a vacuole. Held also believes that the granules are soluble in alkalies, and that the normal reaction of the nervous system is alkaline, but it becomes acid shortly after death, and that this is the reason the cells contain vacuoles in tissue hardened in alkaline alcohol.

v. Lenhossek and Flemming say the granules are visible in the fresh condition shortly after death. Each animal has a typical form of granule in the spinal ganglion cell, whatever fixing fluid has been used, which could not be if the granules were precipitated either in dying or with the acid reagent.

Bühler maintains that the granules are not seen in a fresh state, or even in a fixed condition, but this is no argument for their non-existence in the living cell, for the nucleus is often invisible in a fresh state.

Ruzicka believes the granules are only due to differentiation in staining, because if you overstain you do not see them, and if you extract too much they are invisible.

I agree with Held and Bühler that these granules are invisible in a fresh condition, and with Bühler also that the granules are hardly visible as such in the fixed cell. If one examines an unstained section of a spinal

82 L. c., 1895.

ganglion, one cannot be sure that there are distinct granules in the cell, but the chromatic substance is responsible for its optical appearance, for the periphery of the cell is homogeneous, and does not resemble the central parts where we usually find the granules. The cone of origin, and the layer around the nucleus, described by v. Lenhossek as free of granules, are also homogeneous.

For observing fresh nerve cells I used the retina, because one can examine nerve cells in this organ more easily and more quickly than in any other place, and because the retina is transparent, and does not need to be crushed or removed from its normal medium for examination. The eye was generally excised immediately after death, but it was often half an hour before it was opened and the retina placed in some vitreous humour. It was laid on the slide with its nerve-fibre layer uppermost, and a cover slip placed upon it. Observing such a preparation, one can frequently detect absolutely no structure in the retina, other than the blood corpuscles in the vessels, even with the best lenses. After a few minutes, the rods and cones come into view, and then, after a considerable time (sometimes an hour) the outlines of the ganglion cells appear, but for a longer time the cells themselves are homogeneous. Eventually the nuclei of these cells become visible, and still later the cytoplasm becomes turbid.

One might quite as properly contend that the retina did not exist in life except as a homogeneous substance, and that the cells were precipitated in dying or by the fixing reagent, as that because the Nissl granules are not seen in a fresh condition, they are not formed elements of the cell. We have seen that the granules are not soluble in alkalies, so that argument of Held's on the present point is valueless.

Since it might be argued that it was one of the properties of the retina to be transparent the cells of the cord and cortex of young animals also were examined. These were killed by decapitation, the skull or vertebræ opened and a small piece of tissue taken and put in a drop of methylene blue. A cover was placed on the preparation and gently pressed till the latter was transparent enough for observation. The cells were found to have a granular appearance, resembling what would be found if the tissue had been fixed, embedded and stained with toluidin blue, and this within two minutes of death.

Held lays stress on the fact that different fixing agents produce a different form of granule. It is well known that different fixing fluids

83 Turner using methylene blue on fresh brain has observed the normal appearance of the cells shortly after death, ; Brain, part I, page 100, 1899, also Journal of Mental Science, 1898.

produce slight differences in distribution of all chromatin, and different fixing fluids also form a slightly different intergranular substance which would cause the granules to have a different appearance.

Putting all things together we may conclude that during life the granules have the same refractive index as the remainder of the cell, but that they are formed elements in the cytoplasm as much as ordinary chromatin is a formed part of the nucleus. It is probable that all chromatin is more or less plastic, for different fixing fluids produce a slightly different disposition of chromatin in the nuclei of all cells. It seems to me to be impossible to answer Flemming's objection that the cone of origin of the process of spinal-ganglion cells is always free of granules, if the latter are precipitated elements in the cell.

Many authors, including De Quervain,[84] Held, Flemming, v. Lenhossek and others, consider the Nissl granules are made of fine particles embedded in another substance. It is true that the Nissl granules, in the different cells, but more particularly in the spinal-ganglion cells, do not appear homogeneous. Is this due to one kind of substance embedded in another different substance, or is it due to irregularities in contour of the same substance? I think the latter is the correct explanation. In sections 1μ thick and stained with eosin and toluidin blue, iron-alum hæmatoxylin or other dyes, or treated to liberate the "masked" iron, the same result was always obtained; the granules appeared homogeneous but of different densities. The edges of the granules are never straight, a circumstance that many have noticed, and thus a section of the cell must contain different thicknesses of the material. The granules often contain vacuoles, which would also tend to give them a heterogeneous appearance. The vacuolated appearance is also due to inequalities of the surface of the granules, for one can see in almost every preparation how a section at right angles to the plane being examined would appear to leave cavities in the chromatic material.

I do not intend to discuss in this paper the arrangement of the Nissl granules in the cell, and shall refer only to the presence or absence of these granules in the axis cylinder and cone of origin of this process. The history of development would tend to show that the Nissl granules would not be found in the axis cylinder process, and this is what all observers who have worked with material that had been fixed and then

84 De Quervain, Fritz, "Ueber die Veränderung des Centralnervensystems bei experimenteller Kachexia thyreopriva der Thiere," Virchow's Archiv. CXXXIII, p. 527, 1893.

stained, have observed. Dogiel,[85] however, finds the cone of origin and axis cylinder itself are finely granular, and considers the Nissl granules are formed by the running together of these fine grains. Dogiel's method consists in staining the fresh material in methylene blue, fixing in ammonium picrate, and transferring to a mixture of ammonium picrate in glycerine, where the tissue remains for some time, in order to get sufficiently transparent for examination.

Using this method on the retina, and spinal and sympathetic ganglia, one obtains figures of cell-structure exactly resembling the figures of Dogiel. In this way spinal ganglion cells were obtained with the cone of origin, and the process filled with bluish-black grains resembling some of Dogiel's figures. This structure must be considered entirely artificial, for these grains occur more or less uniformly throughout the whole preparation, and the examination of tissue before and after fixation shows that they are formed by the precipitation of uncombined colouring matter. Examining the cells, stained in methylene blue, but not fixed in the picrate, one sees they are either granular, *i.e.*, the Nissl granules only are stained or they are uniformly stained, *i.e.*, the intergranular substance is stained as well as the granules, but if one puts the same cells through the fixing process, one finds fine dots of precipitated colouring matter all over the cells. This can be most easily followed in the retina, as little or no teasing of the preparation is necessary, and errors from that source are avoided. If one stains a retina with methylene blue, and examines it after washing as much of the colour as possible out of the preparation, one will find the nerve fibres are uniformly stained ; but, if one puts the same retina through the fixing process, and then examines again, one sees the nerve fibres are filled with spindles and round masses resembling what Dogiel figures. The same change may be followed in sympathetic and spinal ganglia, in which uniformly stained cells become covered with precipitated colouring matter in the process of fixation. The Nissl granules have, in the fixed preparations, a different tint from this precipitated colouring matter, and could not be formed by the running together of these masses, even if the latter were elements of the cell. From my observations on preparations stained by Dogiel's process, I have concluded that his method is one of the best to show the morphological connections between the

85 Dogiel, A. S., l. c. and "Die Structur der Nervenzellen der Retina," Archiv. f. Mik. Anat., XLVI, p. 394, 1895.

Also : "Zur Frage uber den feineren Bau des Sympathischen Nervensystems bei den Säugethieren," Arch. f. Mik. Anat., XLVI, p. 305, 1895.

Also : "Zur Frage uber den feineren Bau der Spinalganglien und deren Zellen bei Säugethieren," Inter. Monat. f. Anat. u. Phys., XIV, p. 73, 1897.

cells, but that it gives entirely artificial appearances in the cytoplasm of the cells.

The true structure of the cytoplasm of nerve cells has been the object of much investigation by Flemming, v. Lenhossek, Dogiel, Held, Lugaro, Cajal, Marinesco, van Gehuchten, Cox, and many others, in fact, nearly all the works mentioned contain references to it, and there are good reviews of the literature in van Gehuchten, and in Goldscheider[86] and Flatau. The question is whether there are independent fibrillæ, or fibrillæ forming a reticulum in the cell, or whether the cytoplasm has a foam-like structure.

In this paper I do not intend to discuss the structure of the cytoplasm but shall point out, that since the substance of the Nissl granules does not diffuse into the cell body before the structure of the cytoplasm is determined, (in other words, these are superadded to the cytoplasm), they cannot be a part of the fibrillæ or reticulum. Thus the Nissl granules are not thickenings of the protoplasmic fibrillæ, or are not the nodal points of the cytoplasmic reticulum, but are independent of the cytoplasmic structure; and although the fibrillæ, if they exist, might even run through the granules, they would never lose their independence. Several of the above-mentioned authors have reached the same conclusion, but could give no definite proof of its truth.

No definite conclusion has been reached as to whether the nucleus keeps sending new material from the nucleolus to the cytoplasm, during the life of the cell. If it does give out new material to the cytoplasm it certainly does not do so in the manner described by Rohde. The latter has described the migration of the accessory nucleoli into the cell body to become the Nissl granules, and the migration of the ordinary nucleoli to become the nuclei of neuroglia cells. He used iodine green and fuchsin as stains, and found the accessory nucleoli (which are only masses of oxyphile substance) resembled in their staining power the Nissl granules. Iodine green and fuchsin form a difficult combination to differentiate exactly, and the two appearances described by Rohde[87] can be obtained by a little longer or shorter differentiation; in any case, the resemblance of the staining properties of the oxyphile nuclear substance to the Nissl granules is much better seen by using Flemming's orange method (vide ante). Rohde says that by staining with iron-alum hæmatoxylin and long differentiation, the accessory nucleoli retain the stain longer than any other part, and thus the process of migration of

86 Goldscheider und Flatau, "Anatomie der Nervenzellen," Berlin, 1898.

87 L. c., p. 705.

these bodies can be easily followed. I find it is not the accessory nucleoli that retain the stain but the ordinary nucleoli themselves. In all places examined by Rohde (spinal and sympathetic ganglia) it is well known that several nucleoli are found, as can be easily seen by staining with toluidin blue. In the cells of the cord where one nucleolus is the rule, it is this nucleolus which retains the iron-alum hæmatoxylin stain, and not the neighbouring oxyphile substance.

If these nucleoli which retain the hæmatoxylin stain are outside the nuclear membrane they are artificially brought there. One can sometimes find, as v. Lenhossek has pointed out, the nucleolus pulled out of the spinal ganglion cell. He believes this occurs because the nucleolus is loosely attached to the linin thread, or because the nucleolus is very hard under certain conditions. I have never seen a nucleolus outside the nuclear membrane in the cells of the cord, and in ganglia that have been fixed in sublimate this appearance is far more common than it is in material that has been fixed in alcohol. The fact, (and I have carefully examined my preparations to see that it is a fact), that where more than one cell have their nucleoli displaced in the same section the direction of the displacement of the nucleoli is always the same, shows that these have been displaced in cutting. One can make the appearance of migrating nucleoli quite common, if one cuts sections, 1 or 2 μ thick, of ganglia fixed in sublimate, but all the apparent migration is in the same direction. If, however, thicker sections are cut, or if material that has been fixed in alcohol is used, the appearance may be said to be non-existent.

Holmgren[88] also believes in the migration of formed masses of the nuclear chromatin to the cytoplasm. In the cells of the spinal ganglia of *Lophius* he has described the migration of the chromatin out of the nucleus to form the Nissl granules, the migration of accessory nucleoli, and the passage of the Nissl granules back into the nucleus These changes are brought about through the agency of the micro-centre with its radiating threads, and are supposed to be different stages in the activity of the cells. Some of the cells observed so differed from the usual condition that they could only be considered as dying, and yet it is from cells in the same ganglia that these changes are described. Holmgren tries to justify his position by a study of the cells of *Acanthias*, *Gadus*, *Raja*, and *Rana*, in which similar conditions were observed. In the spinal ganglion cells of *Rana* I have never observed such conditions, except in cases which are manifestly artifacts made in cutting, as

88 Emil Holmgren, "Zur Kenntniss der Spinalganglienzellen von Lophius piscatorius Lin." Anat. Hefte, XXXVIII, p. 71, 1899.

described before, nor have I noticed any difference in the staining power of the nuclear membrane next the micro-centre, although I have observed many of the different conditions of the cell with respect to the distribution of the granules described by Holmgren. Thus, after the chromatin has once diffused from the nucleus, nothing occurs, in my opinion, to indicate the renewal of the granular substance from that organ. I do not deny that such renewal may take place, but if it does, it is in solution and not in formed masses. Further investigation, however, is necessary to decide this point.

Concerning the reason for the diffusion of the chromatin from the nucleus, it may be to aid physiological action, for it is a general rule, which no physiologist would now deny, that an iron-holding nucleo-proteid is necessary for the cell to carry on its normal function. These compounds are generally confined to the nucleus, but they occur in the cell body of all gland cells. It seems to me that it would aid physiological action in having these nuclein compounds in direct contact with the cytoplasm of the nerve cells, for in this case the cytoplasmic action would not be delayed by immediate participation of the nucleus. Thus cytoplasmic impulses may pass from one process of the cell into another without going through the nucleus, which could not happen if the chromatin had remained in the latter.

V.—Conclusions.

The Nissl granules are of a nucleo-proteid nature, since they contain "masked" iron and organic phosphorus, and are derived from the nuclear chromatin of the germinating cells. Pepsin and hydrochloric acid do not dissolve them, nor are they dissolved by alkalies or acids which, however, liberate the iron, and in consequence of this their staining reactions are altered. Digestion with pepsin and hydrochloric acid does not affect the occurrence of iron and phosphorus in the granules.

The nucleolus consists of an oxyphile centre with a basophile covering. The basophile covering seems to correspond to the original kinetic chromatin of the germinating cell. It contains iron and phosphorus, and alkalies extract the iron very much more slowly from it than they do from the Nissl granules.

The oxyphile nuclear substance is also a nuclein compound since it contains iron and phosphorus. It is readily dissolved in pepsin and

hydrochloric acid. It is altered but not dissolved by acids and alkalies, which liberate the iron from it. The alkali acts much more slowly in removing the iron from this substance than from the Nissl granules.

The three nuclein compounds of the adult nerve cell are derived from the mitotic chromatin of the primitive nerve cell. It follows from this that the Nissl granules are constituted of chromatin that has diffused from the nucleus into the cytoplasm.

A substance analogous to that of the Nissl granules is found in the nerve cells of most animals, but not in all, as it is rarely present in the nerve cells of the Urodela. Those animals, whose nerve cells are devoid of this material, have chromatin in the nuclei of such cells similar to that found in the nuclei of the cells of other tissues.

The Nissl granules are morphological elements of the cell, and consist of one substance. They have the same refractive index as the cytoplasm during life, and are not found in the axis cylinder process.

All the results obtained go to support the view that all iron-holding nuclein compounds are derived from pre-existing ones, and in mitosis all the iron-holding substance of the cell is confined to the nuclear chromatin.

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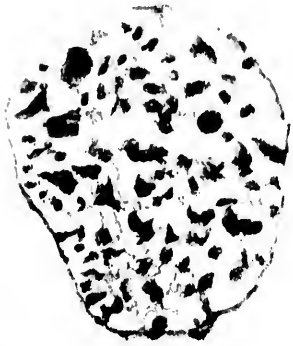

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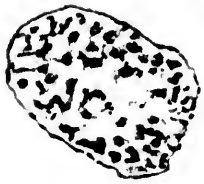

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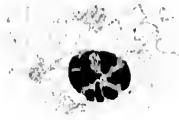

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EXPLANATION OF PLATE

NOTE.—All figures were drawn to the same scale with the aid of Abbe's camera lucida, as seen under the Leitz 1-12 homogeneous immersion lens, with compensation ocular 12 of Zeiss.

FIG. 1.—Motor nerve cell of cat. Alcohol, eosin, and toluidin blue.

FIG. 2.—Spinal ganglion cell of frog. Bichloride-bichromate, eosin, and toluidin blue. (To show the occurrence of granules in the Anura, as distinguished from the Urodela.)

FIG. 3.—Motor cell of cat. Alcohol, ammonium molybdate in nitric acid 7 hours, phenylhydrazin hydrochloride.

FIG. 4.—Motor cell of cat. Alcohol, acid alcohol 5 hours, hæmatoxylin.

FIG. 5.—Motor cell of *Necturus maculosus*, Raf. Bichloride-bichromate, eosin and toluidin blue. (Many cells have far more basophile substance in their nucleus than this one, which was selected to show the cytoplasm. It is the only cell in which anything like a fibrillar structure was observed.)

FIG. 6.—Motor cell of *Necturus*. Alcohol, ammonium molybdate in nitric acid 7 hours, phenylhydrazin hydrochloride.

FIG. 7.—Motor cell of *Necturus*. Bichloride, acid alcohol 6 hours, potassium ferrocyanide.

FIG. 8.—Motor cell of cat. Alcohol, section digested in pepsin and hydrochloric acid 20 hours, ammonium molybdate in nitric acid 12 hours, phenylhydrazin hydrochloride.

FIG. 9.—Motor cell of *Necturus*. Alcohol, eosin and toluidin blue.

FIGS. 10 and 11.—Nuclei of motor cells of dog. Bichloride-bichromate, piece ½mm. thick treated with sodic hydrate 0.2% for 6 hours, section 0.5% gold chloride 20 hours, formic acid very dilute in light, 10 hours.

FIG. 12.—Germinating cell, 7mm. pig. Bichloride-bichromate, eosin and toluidin blue.

FIG. 13.—Germinating cell, 11mm. pig. Bichloride-bichromate, acid alcohol 6 hours, potassium ferrocyanide, eosin.

FIG. 14.—Germinating cell, 11mm. pig. Bichloride-bichromate, acid alcohol 6 hours, hæmatoxylin, eosin.

FIG. 15.—Neuroblast from 11mm. pig. Bichloride-bichromate, eosin and toluidin blue.

FIG. 16.—Neuroblast from 14mm. pig. Bichloride-bichromate, eosin and toluidin blue.

FIG. 17.—Neuroblast from 15mm. pig. Bichloride-bichromate, eosin and toluidin blue.

FIG. 18.—Neuroblast from 15mm. pig. Bichloride-bichromate, eosin and toluidin blue.

FIG. 19.—Neuroblast from 15mm. pig. Bichloride-bichromate, long stain in eosin, short stain in toluidin blue. The oxyphile nuclear substance appears redder than it would be if the time of staining had not been altered. In this respect it resembles the nuclear substance of *Limax*. (See text.)

FIG. 20.—Neuroblast of 15mm. pig. Bichloride-bichromate, acid alcohol 5½ hours hæmatoxylin.

FIG. 21.—Neuroblast from 32mm. pig. Alcohol, eosin and toluidin blue.