ITALIAN RETROSPECT.

By W. Ford Robertson, M.D.

(Unavoidably postponed in publication.)

The Pericellular and Endocellular Reticula of Nerve-cells.—Professor Camillo Golgi has recently made two important communications to the *Società Medico-Chirurgica di Pavia* (19th April and 15th July, 1898), containing an account of certain structural features of the nerve-cell which he has observed in preparations by his chrome-silver method, or slight modifications of it. The first consists of a delicate investment of the cell body. Morphologically this investment presents itself in various aspects. It may have a finely reticulated structure, or form a continuous homogeneous layer, or be made up of small, closely set squames. The typical structure is reticular. The investment forms a complete cuirass, which clothes the cell body and the protoplasmic processes even to the subdivisions of the second and third order. It may be seen upon all varieties of nerve-cells, but most clearly in the large cells of the cord and other regions. Regarding previous observations of an investment of this kind, it is to be noted that Golgi, in a paper written in 1893, stated that he believed that the nerve-cells possessed a special investment, probably of the nature of neurokeratin. He attributed to the possible existence of such an investment, some weight in the way of argument against the theories of dynamic polarisation and of transmission of nervous impulses by contiguity. In 1895 Lugaro described appearances in the cells of the dentate nucleus of the cerebellum, suggesting the existence of a special investment. Two years later Martinotti described a reticulum at the periphery of the nerve-cells of the spinal cord of the dog, which he thought were composed of some kind of neurokeratin. Golgi admits that the isolating action which he and others have attributed to this supposed neurokeratin layer is still only an hypothesis.

The second structural feature is represented by a delicate and beautiful reticulum lying within the cell body. Its external aspect is sharply limited, and a clear zone of protoplasm lies between it and the surface of the cell. Towards its inner aspect, on the other hand, the threads of the network dip down to different levels. Sometimes there is a perinuclear clear zone, in other instances the threads appear to terminate in contact with the nucleus. Some of the threads end in little swellings. In the cells of Purkinje the whole reticulum is pyriform, in correspondence with the shape of the cell body, the various threads joining together and terminating in a point, or in a short filament, at the base of the large protoplasmic prolongations. He has found this endocellular reticulum in nearly all categories of nerve-cells of mammals, but has studied it chiefly in the cells of Purkinje and in those of the spinal ganglia. In the latter cells it presents rather the appearance of a convoluted irregular filamentous apparatus, especially in young animals, but connecting threads can generally be observed. He believes that this

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

endocellular reticulum is something absolutely distinct from either the Nissl bodies or the primitive fibrils of the nerve-cell.

In connection with this subject it is necessary to state here that Lugaro (*Rivista di Patologia Nervosa e Mentale*, 1898, f. 6, p. 265), shortly after Golgi made the first of his two communications, expressed the opinion that the pericellular investment does not constitute an integral part of the cell. He thinks that the appearance in question is due to the coloration of the interstitial tissues, or rather perhaps of an interstitial liquid. He also maintains that even if such an investment did exist, it would not necessarily present an obstacle to transmission by contact; and points out that if the theory of transmission by continuity is accepted as true, there is no need for an isolating investment for the nerve-cell. Regarding the endocellular reticulum, he does not commit himself to any opinion. It is also to be mentioned that Donaggio (*Rivista Sperimentale di Freniatria*, 1896, p. 862, and 1898, p. 460), using a modification of Ehrlich's methylene blue method, has obtained a demonstration of the presence in the nerve-cells of the higher Vertebrates of an uninterrupted reticulum extending throughout the cell body, on the one side forming connections with the nucleus, and on the other sending out delicate filaments which lose themselves in the surrounding tissues. He considers that up to a certain point this reticulum corresponds to the two described by Golgi. He can see no difference of structure between the internal reticulum and the external.

Lugaro's Theory of Cerebral Activity and Sleep.—Lugaro ("Sulle Modificazioni Morfologiche Funzionali dei Dendriti delle Cellule nervose,"—*Rivista di Patologia Nervosa e Mentale*, 1898, f. 8) has lately advanced a new theory in explanation of the different phases of cerebral activity and sleep, which, if it can be accepted, must rank as one of the most important contributions that has yet been made to our knowledge of cerebral physiology. This theory, which is based upon experimental observations of a most striking character, relates to morphological alterations in the processes of the nerve-cells. The question of the occurrence of such alterations is one that has attracted a considerable amount of attention since Wiedersheim, in 1890, asserted that he had been able to observe movements in the cells of the superior œsophageal ganglion of *Leptodora hyalina*. Rabl-Rückhard, Lépine, Duval, Van Gieson, and others have contended that nerve-cells are capable of amœboid movements, and that hysterical paralysis, sleep, certain phenomena of insanity, &c., are to be attributed to contractions of the protoplasmic processes. Others have endeavoured to obtain experimental evidence of such contractions from the action of narcotics and excessive stimulation of the nerve-cells. Some of these investigators, including Ramon y Cajal and Azoulay, have obtained entirely negative results. Others, including Demoor, Stefanowska, Querton, and Manouelian, have observed varicosity of the protoplasmic processes and loss of the gemmulæ under such conditions. Lugaro, whose own first experimental observations upon the subject yielded no positive results, contends in the paper under notice that these experiments cannot be regarded as decisive. The technic employed gives no guarantee that the nerve-cells are fixed in any particular functional state. Further, it has been conclusively demonstrated that varicosities

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

of the processes are frequently produced by the methods of fixation adopted by all of these investigators. Lugaro has brought to his aid a new method of experimentation, the evidence furnished by which seems unassailable. He injected with Cox's fluid the carotid artery of thirteen living dogs, some of which were in a normal condition, others narcotised by chloral, ether, chloroform, or morphia. The injection caused instantaneous death, and effected an immediate fixation of the cerebral tissues in virtue of the sublimate contained in the fluid. Pieces of the brain were afterwards placed in the same fluid for the two or three months necessary to obtain a black reaction in the nerve-cells.

Lugaro summarises his results as follows:—(1) General absence of grave alterations affecting the cellular bodies, the protoplasmic trunks, and the larger branches. (2) Presence of naked and varicose prolongations in very small numbers, and diffusely localised in two of the dogs killed in a state of consciousness, and in three under morphia. (3) Paucity and even complete absence of gemmulæ on many protoplasmic branches in animals killed in a state of consciousness. (4) Presence of slight varicosities, covered with gemmulæ, in these same animals, but especially in those under morphia. (5) Almost complete absence of varicosity, and luxurious investment with gemmulæ in the dendrites of the dogs profoundly narcotised or killed by chloroform, ether, or chloral. He points specially to the general absence of grave alterations of the cell bodies and the large protoplasmic trunks as confirming his oft-expressed opinion that such alterations in Golgi preparations are to be attributed in most instances to defects of fixation. He discusses at great length the interpretation of his experimental results, but it is impossible here to do more than give a short outline of his conclusions.

Retraction of the gemmulæ and the formation of varicosities are two independent processes. The former is related to the physiological action of stimuli, and takes place rapidly, its duration corresponding to the duration of the stimulus. The latter is consecutive to a prolonged state of excitement, occurring therefore in dogs under morphia, which acts upon these animals as a violent stimulant rather than as a narcotic. Since retraction of the gemmulæ is an expression of activity, and the presence of varicosities in the fine branches an expression of fatigue, it is natural that both should be wanting in profound narcosis. It is probable that in physiological conditions the mobility of the neurons is limited to these short and rapid movements of the terminal appendages, and that more ample modifications, such as the formation of varicosities, do not occur as part of the normal mechanism of psychical activity. When a nerve-cell is in a state of excitement, all of its connections do not come into play: if this were so there would not be a serial and logical association,—that is to say, one regulated by the laws corresponding to those that regulate objective facts which through experience have impressed themselves on the cerebral organisation; but a chaotic association, owing to the rise in the mind of elementary representations increasingly numerous, and growing with formidable geometrical progression at every transmission from element to element. It is necessary, therefore, that in functional activity the great majority of possible connections should be suppressed, and that these should be restored

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

whenever the active state of the element ceases, in order to render possible the reception of new stimuli from any other province. Every nervous element, stimulated in any part of it, must, while it is engaged in the elaboration of the stimulus received, retract all its drawbridges, in order to impede the access of other stimuli which might deviate or suppress the first. The spaces interposed may still be forded by stronger stimuli, which, as it were, demand the attention of the element. Thus are to be explained the occurrence of a logical association of ideas, the fixing of the attention on a particular set of ideas, the incapacity of ordinary stimuli to enter into the field of consciousness when the attention is directed to another object; and, on the other hand, how stimuli of abnormal intensity are able nevertheless to force their way in, to break the pre-existing current of ideas, and to initiate another current of psychical associations. In normal conditions of psychical activity, expansion and retraction of the gemmulæ must be diffused and active to a degree hardly conceivable. Every elementary state of consciousness is accompanied by modifications of the spines in a very large number of elements. On the other hand, these modifications do not last longer than the said fact of consciousness, and the gemmulæ quickly reassume their state of expansion ready to receive new impressions. Sleep is not associated with contraction of the protoplasmic processes or the gemmulæ. The interneuronic suspension of contact is quite a secondary matter in this relation. The characteristic condition is a general expansion of the gemmulæ, with exhaustion of their contractility at the beginning, and restoration of their capacity to contract at the end of sleep. This expansion of the gemmulæ implies multiplication of contacts and wide diffusion and consequent weakening of stimuli, which therefore subside without provoking reaction. It may be of advantage to give here a literal translation of some of Lugaro's statements upon this point. "If the cerebral organ is fatigued, if in it there abound those regressive products which are an obstacle to functional activity, under the action of stimuli there takes place more torpidly and more incompletely that retraction of the spines which isolates the systems of neurons functionating, and which secures the momentary autonomy of the functional association and of the corresponding psychical process. It follows that the logical succession of ideas and the activity of the attention are rendered more and more difficult; association becomes more and more delayed and incoherent. . . . . What can be done by the autotoxic action of regressive products can be done also by inactivity of the nervous elements from want of stimuli. If the representative psychical activity is weak, and there is a scanty inflow of external stimuli, the central elements assume their attitude of repose—that is of greatest expansion,—contacts are multiplied, the nervous processes become more and more dispersed and incoherent, the associations become enormously diffused, the stimuli subside without provoking reaction, and there results the unconsciousness of sleep."

It is evident that these new conceptions of the physical basis of cerebral activity and sleep have far more than a mere physiological interest. They have a most important bearing upon many obscure

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

problems in the domain of mental disease, and therefore deserve the very careful consideration of every alienist.

Giuseppe Levi's Recent Work upon Nerve-cells.—During the course of the last three years Dr. Giuseppe Levi, of Florence, has published in the *Rivista di Patalogia Nervosa e Mentale* a very important series of papers upon the histology of the nerve-cells. It is surprising how little attention has been paid to this observer's work outside of Italy. In this country it seems still to be almost unknown, while in a recent German text-book, purporting to give an account of the normal histology of the nerve-cells in the light of the most recent researches, Levi's name is mentioned only once, and that in connection with a statement of his views on a particular point which is the direct opposite of that actually made. Yet it is certain that during the last six years, in which the normal structure of the nerve-cell has been investigated by so many workers, there is no one who has made a larger or more valuable contribution to our knowledge of the subject than this Italian neurologist. His studies, which, like most of those of Lugaro, have been carried out in Professor Tanzi's laboratory, include that of the nucleus of the nerve-cell. Our knowledge of this portion of the neuron was for long very vague and unsatisfactory, but Levi has cleared away many erroneous conceptions regarding it, and has given us a most minute and lucid description of its intimate structure in different forms of nerve-cells. It may justly be said that he has done for the nucleus of the nerve-cell what Nissl did for the chromophile constituents of the protoplasm.

It is impossible to give here more than a brief summary of each of the eight papers which Levi has published. Such a summary can, unfortunately, give only a very imperfect idea of the painstaking character of his work, and of the vast range of his original observations.

In his first paper (*loc. cit.*, 1896, f. 4) Levi deals with certain structural features of the nucleus of the nerve-cell. He regards Rosin's opinion that the nucleus and nucleolus of the nerve-cell are neutrophile as quite untenable, being out of harmony with modern views of the structure of nuclei and nucleoli in general. He thinks that this observer has been misled by his use of Biondi's stain in a concentrated solution, which fails to give an elective coloration with basophile and acidophile substances. He has himself used Biondi's stain in a very dilute solution, and has obtained results entirely different from those of Rosin. He finds that all the elements of somatochrome nerve-cells, including the Nissl bodies, the nuclear membrane, the nuclear reticulum and granules, and the nucleolus (or nucleoli, since it is not rare to find a double nucleolus), are acidophile in reaction, with the exception of from two to four small particles adherent to the nucleolus which are basophile. These basophile particles, which he regards as composed of nuclein (chromatin), may be in the form of semilunar bands (often surrounding the nucleolus like a ring), or of spherical or irregular masses projecting from the nucleolus. He has never seen them detached from the nucleolus, and regards them as morphologically part of it. The irregular acidophile particles in the nucleus, often little inferior in volume to the nucleolus, and described by some as accessory nucleoli, are never surrounded by basophile particles. All the chromatin of the nucleus is represented by the little particles attached to the nucleolus. Nissl and

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

Dehler had previously described a deeply coloured zone at the periphery of the nucleolus in preparations stained with basic dyes, but its nature was not correctly interpreted, as elective staining methods were not employed. Levi has further observed that while in the resting condition of the nerve-cells of the spinal ganglia of the guinea-pig the nuclein is in the form of rounded particles projecting from the surface of the nucleolus; after stimulation of the sciatic nerve these particles in the related spinal ganglia have assumed the form of semilunar bands. This change of form he believes to be due to the enlargement of the nucleolus which Mann and Lugaro have shown to occur during functional activity (causing elongation of the nuclein particles). The nuclei of the *Kernzellen* and granules have distinctive features sufficient to constitute each of them a separate group of cells.

The second paper (*loc. cit.*, 1896, f. 5) contains an important contribution to our knowledge of the functional changes occurring in nerve-cells. Levi has endeavoured to further elucidate this subject by investigating the intra-cellular products of metabolism, as has already been done with much success in the case of certain other tissues. He found fixation in Herrmann's solution and staining by Galeotti's acid fuchsine and methyl green method best adapted to his purpose. His experimental observations were made upon the seventh lumbar ganglion of rabbits. In the protoplasm of the nerve-cells of the normal ganglion he found a certain number of minute granules stained deeply by fuchsin and lying between the primitive fibrils, which, on the other hand, along with the Nissl bodies, were stained by the methyl green. In the resting condition of these cells (attained by section of the sciatic nerve, with examination of the cells after two days) the fuchsinophile granules were diminished in numbers. On the other hand, after electrical stimulation of the sciatic nerve they were greatly increased in number and in size, many of them being elongated. The maximum was reached after stimulation for about two hours. Levi regards these fuchsinophile granules as a true product of the metabolism of the cell. This paper is illustrated by a very beautiful coloured plate.

The third paper (*loc. cit.*, 1896, f. 10) is a short note upon the proliferative capacity of the nerve-cell, a question upon which there has been the greatest difference of opinion among those who have written concerning it. Levi refers to the difficulty that there is in being certain that any cells observed to be undergoing proliferation are really nerve-cells. This difficulty he has himself successfully overcome by special methods. His experiments were conducted upon the cerebral cortex of guinea-pigs, and their results, so far as they are recorded in this paper, prove that at least karyokinesis can take place in nerve-cells. The strictly limited character of this proliferative process is shown in a later paper.

The next two papers (*loc. cit.*, 1897, f. 5 and 6), which are illustrated by two very fine coloured plates, deal with the comparative histology of the nerve-cells of the Vertebrates, and give a record of researches which must have been of the most laborious nature. The author has studied the cytological characters of the nerve-cells in all classes of Vertebrates excepting birds, using chiefly sublimate fixation, and staining with the Ehrlich-Biondi reagent, thionine and Delafield's hæmatoxylin. He

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

first gives a minute description of the structural features of the nerve-cells in a representative of each class, and then occupies himself with a lengthy synthesis of his results, together with a critical comparison of them with the conclusions of other workers. He considers that nerve-cells have still been too little studied to allow of a satisfactory classification of them being made. He retains merely for convenience the classification of Nissl into *Somatozellen* (somatochrome cells), *Kernzellen*, and *granula*, although the criteria of his distinctions are different. Taking first the somatochrome cells, he distinguishes in their protoplasm two constituents which, on the ground of their diversity of reaction with the method of Nissl, may be termed respectively the chromophile part and the achromatic (or fundamental) part. The chromophile substance has only very weak basophile properties. With Biondi's staining method it retains the acid colour and not the basic. It is essentially composed of minute granules. These are generally aggregrated into masses which have different forms in different animals. He thinks that we are not justified in attributing any great importance to the arrangement of this chromophile substance, much less in making of it a criterion for the distinction of nerve-cells, as Nissl has done. He has observed that the chromophile particles are more minute the more voluminous is the cell, independently of the position the animal occupies in the zoological scale. Dealing next with the structure of the fundamental part of the protoplasm, he refers to the views advanced by the various authorities who have written about the subject. The great majority of these writers agree in maintaining that this portion of the cell body has a fibrillar structure, but as regards the arrangement of the fibrils there is much difference of opinion. Levi has been able to throw important light upon this question by observation of the fact that there are certain animals in which some of the nerve-cells (such as those of the spinal ganglia of *Bufo* and *Zamenis*) are specially well adapted for the study of these fibrils. He believes that what appear on first view to be single uninterrupted fibrils are really composed of a bundle of discontinuous fibrillæ. The bundles of fibrils contained in the dendrites are continued into the superficial portion of the cell body, sometimes passing from one dendrite to another, sometimes turning towards the deeper portion of the cell, where the fibrils cross in all directions, forming a true network with large meshes. He thinks that in the dendrites also the fibrils form a reticulum, the meshes of which, however, are very long. He agrees with the opinion of Lugaro that the fibrils of the axis-cylinder process originate not from the parallel fibrils of the peripheral portion of the cell, but from the reticulum in the deeper part of the cytoplasm. He maintains that the "centrosome and sphere," described by Lenhossék and others, in the protoplasm of the nerve-cells of certain of the lower Vertebrates, are nothing more than peculiar vertices of fibrils which he has himself observed in certain of these animals. With regard to the relations between the fibrils and the chromophile substance, he thinks it probable that in the majority of instances the former constitute a woof which passes across the particles of the latter in every direction. He confirms the description of the nucleus of somatochrome cells given in his first paper, especially as regards the centralisation of the chromatin.

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

He finds the same structural features in the nuclei of this class of nerve-cells in all the animals he has studied. Passing next to the *Kernzellen*, Levi states that a few chromophile granules may be distinguished in their scanty cytoplasm, but he has been unable to see fibrils. With regard to the nucleus, the membrane presents the same characters as in the somatochrome cells, but centralisation of the chromatin is very partial and not constant. In the granula of the cerebellum, olfactory bulb, and retina the cytoplasm entirely eludes observation. It is doubtful if the nuclear membrane is always acidophile. Within the nucleus acidophile substance is always present, but it is very scanty. Chromatin is, on the other hand, abundant. The nuclei of neuroglia-cells present a nuclear membrane composed of chromatin, and a dense reticulum composed of the same substance. There is, however, no precise differential criterion by which it is possible to distinguish them from the granules. Levi believes that to complexity of structure in a nerve-cell corresponds the elevation of its function. At the same time he considers that differences of functional elevation of the nerve-centres depend not upon differences in the complexity of structure of the individual elements, but upon the multiplicity of the connections that these contract. He enumerates the morphological characters of the nerve-cell which are associated with its higher differentiation, referring specially to what he terms the "centralisation of the constituents of the nucleus," a condition which, as already indicated, occurs to the greatest extent in the somatochrome cells. In these cells the reproductive capacity is lost, being apparently entirely sacrificed in favour of the performance of their special function.

In his next paper (*loc. cit.*, 1898, f. 3) Levi returns to the subject of the reproductive capacity of nerve-cells. He gives an account of the observations already recorded, pointing out that the results obtained have been different according to the organ chosen for experimentation, and the animals upon which the researches were carried out. The object that he set before himself was different from that which other investigators have had in view, namely, to study the process of karyokinesis in nerve-cells, admitting that it occurs, and to observe what deviations from the normal it presents. He produced aseptic wounds in the cortex of guinea-pigs, and killed the animals in from one to twenty days after the operation. He fixed the tissues in sublimate and stained with the Biondi-Heidenhain reagent. He states his grounds for being satisfied that the cells in which he observed karyokinetic figures were nerve-cells, and not merely leucocytes or neuroglia cells. It is perhaps unnecessary to repeat here the minute description which Levi gives of the process of karyokinesis in nerve-cells, 'more especially as such a description would be somewhat difficult to follow without the beautiful coloured illustrations which accompany his paper. It is sufficient to state that he finds that the process presents in its early phases certain special features which depend upon the peculiar structure of the nucleus already referred to. Of more general interest are certain minor observations and deductions contained in the latter part of his paper. He maintains that karyokinesis cannot occur in highly differentiated nerve-cells, such as the large cells of the cord and brain. He has only been able to observe the process in the medium-sized and

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press

small pyramidal cells of the guinea-pig's cortex. Although he has never seen complete division of these cells, he is inclined, from the advanced stages of karyokinesis which he has observed, to think that it occurs. It is, however, very probable that this multiplication of nerve-cells is simply a reaction to the stimulus which has affected them, and that it does not lead to a stable regeneration. In favour of this view is the fact, noted by many observers, that the proliferation of the nerve-cells is most active from the second to the fifth day, afterwards diminishing gradually, and ceasing about the twentieth day.

Levi's next paper (*loc. cit.*, 1898, f. 7) deals once more with the structure of the nucleus of the nerve-cell, and is mainly controversial in character. He refers to the fact that his statement that the nuclear reticulum is always acidophile in reaction, has been confirmed by Lenhossék, Ramon y Cajal, and Van Gehuchten, while his description of the nucleolus has been endorsed by the first-named authority. He criticises a recent paper by Bühler in which views are expressed contrary to his own, and maintains, in opposition to the opinion of this German observer, that the basophile character of the nucleolar particles is absolute. He also enters into a controversy with Ramon y Cajal regarding the structure of the nucleolus, maintaining, against the opinion of this authority, that it consists of two essentially different substances, namely, a peripheral portion composed of nuclein, and a central portion with acidophile reaction.

Levi's most recent paper (*loc. cit.*, 1898, f. 10) deals with the morphological modifications occurring in the nerve-cells of cold-blooded animals during hibernation. Levi and others have observed that there are no appreciable structural modifications in the nerve-cells of warm-blooded animals associated with this state. Birhler has already described certain appearances in fresh sections of the spinal ganglia of the frog associated with hibernation, but he does not record any special features recognisable in preparations fixed and stained. Levi's observations were made chiefly upon the toad, the cells of the anterior horns of the cord and those of the spinal ganglia being selected for study. His main conclusions are as follows.

The chromophile substance diminishes greatly during hibernation; in the spinal ganglia it at the same time becomes strongly basophile in reaction; it gradually increases when hibernation ceases. During hibernation droplets of fat occur in the cytoplasm, and the interfibrillar fuchsinophile granules are diminished. The partial loss of the chromophile elements during hibernation allows the fibrils of the cytoplasm to come very clearly into view. Levi has taken advantage of this circumstance to make a further study of the arrangement of these fibrils, describing it in great detail, and comparing the results of his observations with those of Bühler. He concludes, after discussing the question from many points of view, that the morphological modifications observed can only be regarded as an expression of the difference in the quantity of functional energy that the nerve-cells are capable of displaying in the hibernating state, the great diminution of the chromophile substance corresponding to the torpor and diminished excitability of the animals in winter, its increase to the condition of vivacity which they exhibit in spring and summer.

https://doi.org/10.1192/bjp.45.189.403 Published online by Cambridge University Press