Anatomy. — *"Is the post-embryonic growth of the nervous system due only to an increase in size or also to an increase in number of the neurones?"* (Second part). By ERIK AGDUHR. (Communicated by Prof. J. BOEKE).

(Communicated in the meeting of February 22, 1919.)

Mitoses.

In connection with these matters I have found specially interesting phenomena in the thoracic region of a puppy seventeen days old. The spinal ganglia were fixed according to FLEMMING's method, cut up in paraffin sections from 3 μ to 5 μ thick and stained with the iron alum hematoxilin of HEIDENHAIN. In these continuous series of sections I found a large number of mitoses — an approximate calculation showed that in a single one of these ganglia there were over two hundred mitoses. Figures 5, 6, 7, and 8 show how these mitoses appear in the preparation. One would be inclined at first sight to refer these mitoses, especially the ones reproduced in figures 5 and 6, to the large ganglion cells — the light field round the chromatin showing, of course, a rather diffuse transition to the rest of the protoplasm. Owing to the continuous series I was able, however, to follow the cells from one section to the other, and then I found that the real nuclei of these ganglion cells were not found in a stage of division, and that these mitoses must belong either to other small cells situated between the ganglion cell and its capsule or probably to cells that form the capsule itself. In fig. 7, on the other hand, merely from the sharp outline which the light field makes against the surrounding protoplasm it is clear that there can be scarcely any question of the existence of a mitosis in the ganglion cell — this was also confirmed by the investigation of the same ganglion cell in the preceding and following sections. In fig. 8 we have again an example of a cell which is going to divide mitotically, and which is situated outside the capsules of the surrounding ganglion cells. With regard to size it resembles most closely the cells in mitotical division in figures 6, 7, and 8, but on closer examination, for instance, if they are traced from section to section, one finds that it is surrounded by capsule cells. We thus seem to be quite justified in describing

this figure as a spinal ganglion cell at such an early stage of development that it had not lost its power of increasing in number through mitotical division. I found another mitosis of this kind in the series just mentioned. Among the other group of mitoses, namely those in cells that are situated inside or in the capsule of an older ganglion cell, my preparation shows at least a few forms in which one can clearly follow the capsule peripherally of the cell that is engaged in mitotic division and where the latter must therefore be situated beneath the capsule. There are thus good reasons to support the assumption that, even among this group of mitoses, some are to be referred to very young undifferentiated cells, which on good grounds — for instance on account of their position — can be assumed to develop into nerve cells. By far the larger number of mitoses are, however, undoubtedly to be referred to ordinary capsular cells. But is the difference between the capsular cells and the nerve-cells really so great? Are not the former perhaps to be regarded as matrix cells for the latter? I must leave these problems to a subsequent and more detailed account of this question and confine myself to saying that there are points in the preparation that support such an assumption [1]). These facts are all the more worthy of attention because, among the investigators who formerly looked for mitoses in spinal ganglia, FLEMMING [2]), DAAL and LENHOSSEK have been unable to show any in young animals. MÜLLER [3]), on the other hand, found them in new-born animals, but in no later age. The very large number of mitoses in the spinal ganglia shown in the present and other investigations of young animals clearly support the considerable post-embryonic increase in the number of capsular cells in this region, an increase that could scarcely be explained if the ganglia did not increase in number too. In my opinion the exceedingly great number of mitoses that are found in the spinal nerve-cells, according to what has been shown above, cannot possibly be explained by an increase in size merely of those spinal nerve-cells which were already present at birth. This is the less probable because the spinal ganglion-cells must decrease in number with the years, if new ones do not grow out and replace all those that degenerate and die away during post-embryonic life. And this degeneration of the nerve-cells is admitted and shown by all the chief investigators of this problem.

[1]) See addendum!

[2]) FLEMMING, DAAL and LENHOSSEK. Quoted from MÜLLER E

[3]) MÜLLER ERIK, Untersuchungen über den Bau der Spinalganglien Nord. med. Ark. Stockholm. Bd. 23. 1891.

That such degeneration is rather common is also proved by the fact that no slight number of cells in a spinal ganglion of even a young animal show signs of degeneration. The new growth in this region has thus the task not only of replacing the ganglion-cells that have been destroyed by degeneration, but also of increasing their number. A fairly considerable increase of this kind takes places, as is shown above, during the animal's period of growth. To judge from my preparations, nature seems in this generation to make use of both mitotic and amitotic division. In no case have I been able to refer the cells that show the latter type of division to such small forms as those in which mitoses occur; the former cells seem to belong to remaining ganglion cells that are somewhat older and sometimes, at least, with a certain degree of development, for I have been unable to find fully developed processes among them.

Amitoses.

Besides the figures of mitoses one also sees in the preparations in question figures of cells which produce a strong impression of being engaged in direct division. As shown below one sees cells that seem to be in different stages of this division. The cells of this type, however, always belong to the young ones, to those cells (in the silver-impregnated preparations) that have taken a very slight amount of silver or even none at all during the impregnation.

The different stages of a direct division which are found in my preparations appear as follows: One sees cells, in which the nucleolus is being divided or has just divided (fig. 1*d* and fig. 2*b*) and where the two nucleoli are still in each other's immediate neighbourhood. The two nucleoli then move away from each other and the nucleus begins to show signs of incision in the middle (see fig. 3*b* and fig 2*b*). After this there follows a complete division of the nucleus, which is also frequently accompanied by a division of the protoplasmic body, fig. 3*a* and fig. 2*a*. Fig. 3*a* must be interpreted as a young apolar ganglion cell in which, after the nucleus had first divided into two, the protoplasmic body began to divide in the middle, after which the two nuclei again began a new division. The preparations in which these observations were made were particularly well fixed and impregnated, so that it is fairly certain that there was no possibility of artificial products. Another thing that further supports the idea of natural formations is the fact that these figures above-mentioned do not occur in such very great numbers. It is true that there are many nuclei of ganglion cells (among the smaller ones) which have two

or more nucleoli, but there are fewer that show signs of division.

I shall discuss at greater length below some of the literature concerning direct post-embryonic division of nerve-cells. I will only mention here that RODHE [1]) describes four different types of amitotic division of the ganglion cells in full-grown evertebrates. PALADINO states that direct division is a very common way for young ganglion-cells in the higher vertebrates to increase.

In fig. 4*a* I reproduce a group of nerve-cells from a silver-impregnated spinal ganglion in a sixty days old puppy. In it the cells are packed close together into a formation shaped like a string of beads, lying within the same capsule. Between the cells at a few places one can also clearly see bridges of protoplasm, which connect cells that are close to each other. The series of sections of the spinal ganglia from this animal show numerous examples of similar groups (MÜLLER E.) of cells situated within the same capsule. I have obtained the impression, however, that they do not occur in equally great numbers in all the spinal ganglia of the same individual; similar groups of cells have been observed in puppies of six and seventeen days — but they were not so numerous as in the sixty days old animal [2]). In the 3,5 years old dog, among five spinal ganglia that were investigated, I did not come across more than a few of these groups of cells and in the five years old dog among a still greater amount of material, I did not succeed in finding such a group in more than a single place. It is thus an obvious assumption to regard these groups of cells as formations belonging to the post-embryonic growth of the spinal ganglia — forms produced by the spinal ganglion cells during the post-embryonic increase in their number.

In spite of the considerable number of works that have been published on spinal ganglia in the course of years, the information about these groups of cells to be found in this literature is exceedingly small. Before 1880, however, they had been observed by a number of investigators and were described most thoroughly by P. MAYER [3]). After that the subject seems to have been almost forgotten, until in 1889 and 1891 MÜLLER ERIK [4]) gave more thorough and valuable descriptions of similar groups of spinal ganglion cells within the same capsule. Since MÜLLER's description of these groups of nerve-cells they seem to have been neglected again in recent

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[1]) RODHE, Ganglienzellkern and Neuroglia. Ein Kapital über Vermehrung and Wachsthum der Ganglienzelle. Arch. f. mikr. Anat. Bd. 47.

[2]) The sixty days old dog was rachitic.

[3]) MAYER, S., Arch. f. Psychiatrie, Bd. 6, 1876.

[4]) MÜLLER, E., L c.

literature — I have not found a single mention of them in a whole series of recent publications on this subject that I have looked through. MÜLLER gives the name of *"Cellkolonien"* to these groups of nerve-cells and distinguishes between regular and irregular colonies. "Die ersteren" — the regular ones — "sind nach aussen durch eine cirkelrunde Kapsel vom selbigen Aussehen wie diejenige, welche die grossen Zellen umgiebt, begrenzt; innerhalb dieser Kapsel finden zich zwei, drei oder vier Zellen sehr regelmässig wie Sectoren um einen Mittelpunkt geordnet". MÜLLER also found bridges of protoplasm connecting the different cells of the colony with each other. I have not found in my preparations any colonies of cells which showed this regular arrangement of their cells, resembling a sector of a circle, although there are several figures of colonies in which the cells are very nearly equal in size; but in these cases they are situated side by side, although they do not always form such long rows as the one shown in fig. 4. Most of the colonies observed by me are quite clearly built up of cells that are different in size, and it seems as if one might place them all in the group that MÜLLER describes as *irregular*. With regard to the significance of these colonies MÜLLER writes: "Vielleicht steht das Vorkommen dieser Bildungen mit Regenerations-phänomenen in den Spinalganglien in Verbindung", but he points out that, as he had no opportunity of studying the processes of these cells, his statement on this point can only be a supposition. He continues: "So viel geht jedoch aus dem unbedeutenden Vorkommniss bei älteren Thieren von diesen Bildungen — Kolonien und Halbmonden — welche bei jungen Thieren zahlreich auftreten, hervor, dass sie Entwicklungsstadien von Ganglienzellen repräsentieren und ferner, dass die Entwickelung der Spinalganglien eine langsame ist, welche erst in späteren Zeiträumen von dem Leben des Thieres abgeschlossen wird."

In tearing preparations of older animals the same investigator found that the crescent-shaped cells that are situated within the same capsule as other ganglion cells, have no processes. These observations of mine, however, are not made from tearing preparations, in which one has of course always to reckon with the possibility of the removal of processes that have really been present, but are made from continuous series of intensely impregnated BIELSCHOWSKY-preparations, in which one can very easily look for these colonies section by section. In the series of sections from which fig. 4 is taken there is no trace of any processes. The spinal ganglion in question is intensely impregnated according to the method mentioned above. The impregnation is very successful; not only the axons,

but the neuro-fibrils appear exceedingly distinctly. One may thus postulate that if processes of the cells in this colony had really existed, they would also have clearly appeared in the sections. That these cells are likewise at an early stage in their development is indicated, in addition, by the fact that there are evident bridges of protoplasm between some of them. In this series of sections there are, however, colonies of cells which, as far as one can judge, are at later stages in their development — in these the different cells have processes, there are no bridges of protoplasm between them, and the future capsules of the separate cells exhibit the first traces of their development. In the cells of some of the colonies found in the 3,5 year old dog I have been able to show processes — there were also signs showing that these colonies were at a later stage of development than the one shown in fig. 4. In the five year old dog, as has been mentioned above, I found only a single colony of cells and no apolar cells. The results of counting the ganglion cells and their axons indicate, however, that there really are apolar cells here as well [1]). The purely morphological observations in the 3.5 and 5 year old dogs do not, of course, quite exclude the possibility of there being colonies of cells here as well at a very early stage of development, but with regard to this they indicate that in older animals these formations are relatively very rare. It is to be noted that such eminent investigators as KEY and RETZIUS [2]), SCHWALBE [3]) and of recent years RANSON [4]), are decidedly against the opinion that apolar cells are to be found in the spinal ganglia on the other hand. KÖLLIKER [5]), MÜLLER [6]) and others hold the opinion that such cells really exist. It would lead me too far from my real subject were I to discuss in detail the literature concerning apolar cells in the spinal ganglia. I must content myself with the references already given, and in connection with this point I wish to state that there are also investigators who have observed processes from cells in colonies similar to those described above; such are ARNDT [7]) and STIENON [8]) etc.

[1]) These and other explanatory details will be given more fully in a forthcoming and more complete work.

[2]) KEY and RETZIUS. Studien in d. Anat. d. Nervensyst. u. Bindegewebe, Bd. 2, 1876.

[3]) SCHWALBE, Arch. f. Mikr. Anat. Bd. 4; 1868.

[4]) RANSON, L. c.

[5]) KÖLLIKER, Handbuch der Gewebelehre, 5 Aufl., 1867, quoted from MÜLLER E.

[6]) MÜLLER, E., l. c.

[7]) ARNDT, Archiv f. Mikr. Anat., Bd. 10, 1873.

[8]) STIENON, Annales de l'université libre de Bruxelles, 1880, quoted from MÜLLER E.

If now we summarize the observations that have been made and given above on these colonies of cells and the processes of the cells that belong to them, it seems to be clearly shown *that some at least of the apolar cells in these colonies grow out to new neurones during the postembryonic growth of the animal.* On the other hand it does not seem to me so easy to decide how these colonies of cells arise. The way is perhaps that small cells from the capsule cells which have been developed mitotically, or are at least situated within the capsule, grow out into new ganglion cells, which are added to other ganglion cells already existing within the same capsule. Might not a relatively large ganglion cell, which in some respects is at an earlier stage of development — for instance, apolar — increase in number and become one of these colonies of cells by means of amitotic divisions. I have not been able to decide with certainty whether one or the other or both of these methods of formations occur, though, as a matter of fact, there are signs in my preparations to support the idea that both these methods of formation may occur.

If, as seems to be shown above, a new formation of neurones in the spinal ganglion really occurs post-embryonally, one would and might, of course, also expect to find, during post-embryonic life, figures of growing axons in the peripheral nerves. I have examples of such claviform figures, which are quite evident in silver-impregnated preparations of, for instance, the dorsal and ventral roots of young dogs. More details of this will, however, be given below.

I consider that I have now shown that the cells in the spinal ganglia sufficiently explain the origin of the actually existing and fairly considerable post-embryonic numeric growth of axons in the dorsal roots of the spinal nerves. I shall now pass on to examine to some extent in connection with those matters the

Medulla spinalis.

There is but exceedingly scanty information about post-embryonic divisions of the ganglion cells of the central nervous system to be found in literature, and the existing accounts are not generally admitted to be correct. These accounts, however, take two directions. Some investigators maintain that the cells in this region divide by means of mitoses, others say that the usual method of increase in this case is that of amitotic cell division.

Mitoses.

ALLEN[1]) states that in the spinal cord of an "albino rat" twelve

[1]) ALLEN, EZRA. The cessation of mitosis in the central nervous system of the *Albino rat;* J. Comp. Neurol. Vol. 22, pp. 547—568, 1912.

days old he found (counting in mm³) 46 mitoses in the cervical, 75 in the thoracic and 14 in the lumbar region, but that in an animal twenty days old he could not show a single one.

HAMILTON [1]) found in thirteen succeeding sections, 6,75 μ thick, from the medulla spinalis of a four days old rat mitoses in the ependyma and 64 situated extraventricularly.

ADDISON W. H. F. [2]) found in an "albino rat" nearly 22 days old mitoses "in the other granule layer" of the cerebellum.

SCLAVUNOS G. [3]) has observed mitoses in the central nerve system of new-born dogs.

SUGITA NAOKI [4]), who has studied the post-embryonic growth of the cortex of the brain in the "albino rat", found that the value for the number of cells in this region in the ten days old animal was 1,9 × the value at birth, and that the number of cells increases further during the next ten days and is complete at twenty days. After this time the number of cells is practically constant and the number of cells in the fully-grown state is approximately twice as great as at birth. These calculations are based on the determination of the number of cells in only two layers at only one place and therefore their general value may be questioned. S. has, however, previously shown by measurements made at different places on the cortex of the brain that it undergoes the same relative increase in thickness between birth and maturity. S. considers that the values obtained may therefore with great probability be generalized for the whole cortex. With regard to the way in which such a post-embryonic increase in the number of cells in the cortex takes place one can, of course, herein supported by ALLEN, who in 25 days old specimens of the "Albino rat" found as many as 27 mitoses per mm³ of tissue in the cerebrum, consider that it is due to mitotic division.

The values given for the number of mitoses and for the increase in number of the cells in the central nervous system do not refer to any definite number of cells, but apply to all the cells taken together, and thus do not exclude an increase in the number of

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[1]) HAMILTON, ALICE, The division of differential cells in the central nervous system of the white rat. J. Comp. Neur., Vol. II, pp. 297—320, 1901.

[2]) ADDISON, W. H. F., The development of the *Purkinje* cells and of the cortical layers in the cerebellum of the *Albino rat*. J. Comp. Neurol. Vol. 21, pp. 459—487.

[3]) SCLAVUNOS, G., Ueber Keimzellen in der weissen Substanz des Rückenmarks von älteren Embryonen und Neugeborenen Anat. Anz., Bd. 16, 1899.

[4]) SUGITA NAOKI, Comparative Studies on the growth of the Cerebral cortex III, IV and VI, Journ. Comp. Neur. Vol. 29, 1918.

both glia and ganglion cells. The mitoses found in the central nervous system of young animals do not seem to refer to so-called neuroblasts (His) [1]), but the preparation indicates that Koelliker [2]) is right when, partly by reasoning and partly by direct observations, he comes to the conclusion that those "Keimcellen" that are in mitosis are undifferentiated epithelium-cells, which give rise to both glia and ganglion cells. Schaper [3]) arrives at the same result by his investigations of the course of differentiation in the central nervous system of the trout. We thus seem to be justified in postulating as a fact that as long as mitoses can be shown in the central nervous system a new formation of ganglion cells is also taking place.

In Prenant [4]) we read as follows: *a.* "Les cellules nerveuses, en se différenciant, ont perdu le pouvoir de se reproduire, *b.* Les rares multiplications qu'il a été possible d'observer dans les cas de cicatrisation de portions du névraxe, appartiennent à la neuroglie (Valenza, Marinesco, Monti); *c.* Enfin il n'est pas exclus que les quelques mitoses observées doivent également être assignées à la neuroglie". Among the investigators who do not seem to be able to admit the possibility of an increase of the neurones during post-embryonic life I want to mention also Bizzozero [5]) and Marinesco [6]). In deciding such matter these authors seem more or less to have proceeded from the idée préconçu that the neurons have a very long life and are nearly perpetual. They consider that this is an absolutely necessary qualification if the individual is to perserve its psychical inheritance, to form associations of ideas, and for memory in general. A close study of suitable preparations of, for instance, the spinal cord from animals of different ages will soon convince us that this does not quite agree with the real facts. For in these preparations one finds not infrequently figures of ganglion cells which are degenerating as well as those which indicate generation. Nor is the literature on the subject without scattered statements about observations of such degeneration in the central nervous

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[1]) His, Die Neuroblasten und deren Entstehung im embryonalen Mark. Arch f. Anal. u. Entwickelungsgesch. 1889.

[2]) v. Koelliker, Gewebelehre, Bd. 2, 1893.

[3]) Schaper, Archiv. für Entw. mech. der Organ. Bd. 5.

[4]) Prenant, Histologie et Anatomie microscopique, t. II, p. 353, 1911..

[5]) Bizzozero, G, Accrescimento e rigenerazione nell'organismo (Conférénce du Prof. G. Bizzozero au Congrés international tenu à Rome en 1894). Voir, en outre, dans le 2e volume des oeuvres scientifiques du même auteur publié a Milano en 1905, et dans les Arch. ital de Biol. t. XXI, p. 93, quoted from Paladino.

[6]) Marinesco, G., La cellule nerveuse, Vol. 1, p 400, Paris 1909.

system. Among the investigators who have made such observations we mention RETZIUS, v. GEHUCHTEN, RAMON Y CAJAL, DEJERINE.

The presence of degenerating nerve elements in individuals that are growing also renders the possibility of a regeneration of such very probable. If there is no regeneration, the nerve elements would, of course, decrease during growth—a phenomenon that is not indicated by any recorded observations. The probability of generation becomes certainty, however, when one investigates suitable preparations from the central nervous system, for instance from the spinal cord of animals at various post-embryonic ages. Such preparations show numerous figures of new growth, which seem to me sufficient to explain not only how degenerated ganglion cells are replaced, but also how the increase in nerve fibres in the central roots arises, which I proved above to exist during the period of growth.

I have made suitable preparations for these investigations from the spinal cord of toads, mice, rats and dogs of different post-embryonic ages. The material was fixed either in FLEMMING's or ZENKER's fixing liquids and the paraffin sections cut from it were impregnated either with HEIDENHAIN's iron-alum hœmatoxylin or with ERLICH's acid hœmatoxylin. I have in addition, quite excellent BIELSCHOWSKY-preparations from this material.

In the hœmatoxylin-impregnated preparations from toads 2 cm. long (from neck to sacrum) and ten days old mice I found some — but very few — mitoses. On the other hand I have not found any certain examples of such mitoses in the older individuals of this species nor in six and seventeen days old dogs or in full-grown ones. In a young mouse 23 days old (*Mus musc. var. albus*) I found three appearances, which are reproduced in figs. 9 and 11. The figures are carefully drawn from preparations — which are from the material that was fixed by FLEMMING's method — and, at the first glance, certainly produce the impression of being mitoses, and it is possible, of course, that this is the case. A number of facts seem to me, however, to render this doubtful; these are first, that I have not found any more mitoses in this animal and, secondly, that in other mice of equal age, in which the material was fixed according to ZENKER's method — this method gave better and finer results — and impregnated in the same way, I have not found any trace of mitoses. In any case I have not found any appearance of a mitosis in preparations of the spinal cord of white mice more than 24 days old. My observations of mitoses in the spinal cord of growing individuals thus agree on the whole with those previously made by other in-

vestigators. As far as the animals investigated by me are concerned, an increase in the number of neurones by means of mitotic division of nerve cells seems thus to be concluded during the first month of post-embryonic life. DONALDSON's [1]) statement: "Moreover, in the case of the albino subjected to modifying conditions after 30 days of age, *the number of neurones is already complete at this age, so that the changes induced are again merely of size*[2]), unless some neurones should have been destroyed," is an assertion that I cannot agree with, as far as my material is concerned, and I am inclined to think that it does not describe the conditions in any animal. If one gives a strict definition of a neurone as being a nerve-cell with its processes, one of which is an axon and the others dendrites, and one adds to this the generally accepted condition, which by means of the evidence put forward about it, has almost become a certainty, namely that one cell in the ventral horn does not send more than one axon out into the ventral root and that the axons do not show any T-division on their way through this root, the considerable post-embryonic increase in the number of axons in this region, which has been shown above to be an actual fact, is a proof of the real existence of an increase in the number of neurones during a considerably longer period of development than the one given by DONALDSON.

The Wistar school (DONALDSON and others) have, as has been stated above, with their splendid statistical and experimental investigations found, by means of the methods they have used (staining of medullary sheaths), that post-embryonic growth in the nerve roots is principally merely an advancing myelinisation. The most important of all the changes that take place during this process, namely the post-embryonic growth in the number of axons, has quite escaped their notice. There was therefore no need to look for an increase in the number of neurones going on for a longer time postembryonally than the time during which the mitosis in the central nervous system showed clearly that an increase of this kind really existed. But is mitosis the only way in which an increase or a new formation of the cells in the central nervous system can take place?

Scattered statements in the literature exist to the effect that a new formation of nerve-cells may also take place by means of

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[1]) DONALDSON, H. H., HATAI, S. and KING, H. D. Post-natal growth of the Brain under several experimental conditions. Studies on the albino rat. Journ. Nerv. and Mental Disease. Vol. 42, 1915.

[2]) The italics are mine.

Amitotic division.

Most investigators believe, with FLEMMING, that mitotic cell division is the only way in which a new growth in a healthy body can take place. And it is generally admitted that amitotic cell division occurs only in pathological tissues and, apart from this, only in cells that have a very short life. As has been pointed out above, the nerve cells are generally admitted to have a life equal in length to that of the individual; it is therefore obvious that any idea of an increase in these by amitotic division must be out of the question. And I must myself confess that the idea of the permanence and high position of the neurones among the cells in general has become so deeply rooted through studying handbooks of medicine as well as the majority of special treatises on this subject that it is really difficult to get accustomed to the idea that there may be another possibility for the increase in the nerve-cells than mitotic division. If, however, one comes quite freely, as I did, to the problem of explaining the actually existing increase in the nerve-fibres during the whole post-embryonic development, and finds that this explanation has to be sought in an increase in the number of the neurones and not in a cleavage of the axons — and this at the same time as one finds signs of how a large number of the nerve cells are degenerating and dying away, then of course the new formation of ganglion cells, even after mitoses no longer occur in these regions, must be considerable. There are also in the central nervous system, as will be described in more detail below, appearances that seem to indicate that amitotic division of young cells really takes place there. Observations pointing in this direction have already been made and described in literature, although this information seems to have attracted but little attention.

ROHDE [1]) described in 1896 how ganglion cells in invertebrates increase by amitotic division. R. distinguishes four different types of such a division in these animals. As invertebrates have not been the object of my investigations in this matter, I cannot criticize R's statements, although some of them seem somewhat strange.

PALADINO [2]) (1914) describes amitotic division of cells in the central nervous system of vertebrates. P. states that the neurones degenerate and perish, and in connection with this there is a new development of nerve elements. There are good reasons for believing that this

[1]) ROHDE, l. c.

[2]) PALADINO, l. c.

development takes place by means of the activity of the ependyma and to a subordinate and limited extent by means of direct division. Where these elements exist they sink down and gradually disappear, sending off a first process, which grows and is lengthened, while others are also developed, so that gradually a multipolar cell arises. "Avant d'arriver à cette différenciation, ces éléments se divisent ça et là par scission directe, qui, ou bien se complète — et alors les nouveaux éléments restent en connexion avec un des prolongements — ou bien ne s'achève pas, et on a alors des formations gemellaires de divers degré. Ces faits peuvent s'observer le long de la moelle épinière d'individus d'âge différent et dans des préparations obtenues avec des séries de sections frontales et avec les divers colorations". PALADINO accompanies his statement with a figure to show how the epithelium-cells (ependyma) are further differentiated and move down into the surrounding tissue. On the other hand it is to be regretted that P. did not add a figure showing a cell engaged in direct division and that he did not give a more detailed description of the amitoses in the central nervous system observed by him.

The more thoroughly I study my preparations from the central nervous system of animals of various post-embryonic ages, the more convinced am I that PALADINO is right in his statements as given above. In these preparations of mine I have found, in a number of places, appearances that indicate, just as clearly as P.'s figure, a movement of cells from the ependyma into the surrounding tissue. These appearances are not, however, found continuously along the whole central canal, but occur scattered here and there — this too agrees with P.'s statements. On the other hand, with regard to figures of direct cell division, I have observed a great many which, in my opinion, are to be interpreted in this way. And as a matter of fact I have obtained series of such appearances which show the different stages of a direct cell division. Notches, indentations and irregularities in shape occur very often in the nuclei of the nerve cells. If, however, such appearances be examined more closely, we shall find in most cases that they cannot be counted as figures of amitotic divisions. Thus figures which may with a great degree of probability be considered as stages of amitotic cell divisions do not occur in such abundance in my preparations of the spinal cord from the above-mentioned animals. Fig. 13 shows a type of these notches, which are very common in the nuclei of ganglion-cells, but which, as far as one can see, have nothing at all to do with amitotic divisions of the cells. Figs. 14 and 15 are cell-plasmodia or syncytia, of which one often sees examples, especially close to the ependyma. The

syncytium in fig. 14 was situated immediately beneath the ependyma, and that in fig. 15 in the dorsal horn of the spinal cord in a young white mouse ten days old. Figures 1*b*, 2*a*, 3*a*, 9*b*, 10, 14, 15, 16, 17, 18, 19, 20, and 21 are pictures of different stages of young nerve cells engaged in amitotic division. These figures are all drawn from appearances in the spinal cord of a white mouse, the two first from an animal 24 days old and the others from 10 days old animals. In the material from toads and dogs that was investigated, similar appearances to those in the white mouse have been found to about the same extent. Fig. 9*b* shows one stage of direct cell division which in my opinion is very rare; I myself have only found this single case. Fig. 20 shows the most advanced incision usually seen. Transitional stages between this and complete division of the nuclei occur exceedingly seldom. I obtained a particularly welcome opportunity through Professor BOEKE's great kindness during my visit to Holland last summer — of observing in eelembryos that it really is a fact that the appearance of amitoses is very rare in cases where the daughter-nuclei show only very narrow communicating bridges between each other. It is, as we know, generally recognized that the nuclei in the myogene tissue increase by direct division during a later stage of its differentiation into muscular fibres. Eel-embryos are particularly suitable for the study of this development (GODLEWSKI E. [1]). BOEKE's very fine preparations of these embryos showed in this region numerous nuclei engaged in amitotic division. It is worthy of note that here too, among this mass of nuclei in amitotic division, no stage could be discovered in which the nucleus showed a far advanced incision — and consequently a very small communicating bridge between the two daughter-nuclei. — Accordingly, after studying this material, I was inclined to assume that the last part of the process of division took place rapidly, without any narrow drawn-out communicating bridge between the daughter. nuclei being formed. With this in view, it is not strange that I looked upon the appearances that form the basis of fig. 21 with a certain amount of surprise and doubt. Does this figure really show stages of amitotic cell division or are they only artifical products? The preparations were well fixed and as a matter of fact do not support the idea of there being artificial products. The nucleoli show a particularly great generative tendency. If we

[1]) GODLEWSKI, E Ueber Kernvermehrung in den quergestreiften Muskelfasern der Wirbeltiere, Bull. intern. de l'Academie des Scien. de Cracovie, 1900.

[2]) GODLEWSKI, E. Die Entwicklung des Skelet- und Herzmuskelgewebes der Säugethiere. Arch. f. micr. Anat. B. 60, 1902.

add that this picture is the only one among my extensive material in which I found such far advanced incisions in the nuclei, these facts certainly support the idea that there really are natural formations. All these cells that show signs of amitotic division are very young. Some of them have no signs at all of processes (fig. 19 and 20), while others show indications of the beginning of a development of these (figs. 9*b*, 16, 17, 18, and 21). I can agree with PALADINO's statement quoted above that it is only before the differentiation of the processes that amitotic division takes place. On the strength of the appearances in this material I am of the opinion that the amitotic division proceeds in the way:

a) The nucleolus [1]) increases in length and begins to show incisions in the middle; this incision becomes deeper and deeper (figs 16 and 17) and finally we have a division into two nucleoli, each of which moves to an end of the nucleus of the cell, which has begun to become drawn out into a more or less oval formation. The nucleoli often exhibit a continued power of generation even after they have moved out to the future daughter-nuclei; it is this that causes us often to see in such daughter-nuclei either one nucleolus engaged in direct division or else several nuclei, a number of which may be seen moving out of the nucleus. I have not been able to decide with certainty whether the filaments (nuclear fibres) of the nucleus thereby have any specific function. It is a fact, however, that there are sometimes appearances which point to this being really the case (fig. 16 and 17). *b*) The drawn-out, elliptical nucleus begins to show signs of incision in the middle (fig. 16, 17, and 18). This incision usually takes place in the middle, so that the two daughter-nuclei are equally large. There are, however, figures showing the existence of a slight dissymmetry (fig. 18). The incision grows deeper, but is not as a rule, however, deeper than is shown in fig. 20, the connection between the nuclei being retained. Incision of the nuclei as far advanced as that shown in figs. 9*b* and 21 is exceedingly infrequent and these figures are the only ones I found of this type. There are also figures that indicate that the fibres of the nucleus may have something to do with the division of the nucleus. *c*) If the cell in which the nucleus divides amitotically is at a very early stage of development, a cleavage of the protoplasm does not, in most cases, ensue, but a cell plasmodium arises. These cells are

[1]) It should, however, be noted that such phenomena of new growth often appear in the nucleoli without the nucleus otherwise showing any signs of an amitotic division.

then situated most frequently in the neigbourhood of the central canal (fig. 15). If the cell is at a somewhat later stage of development, an incision of the nucleus is usually accompanied by a division of the surrounding protoplasm, which even at the same stage shows protoplasmic processes engaged in development (fig. 10). Cells of this last type are situated farther away from the central canal.

It is noteworthy that the structure of the nucleus in the cell engaged in amitotic division seems to be relatively intact in comparison with the corresponding condition in mitotic cell division.

With regard to the degree of the neurone formation I think that, on the ground of the reasons given above, I may go a step further than PALADINO when he writes: "En conclusion, le tissu nerveux ne fait pas exception à la loi, d'après laquelle tout tissu vit dans l'ensemble et se renouvelle isolément, pour remplacer les éléments qui se détériorent et se détruisent; en d'autres termes, le tissu nerveux, lui aussi, est un siège de *regénération* pour ainsi dire *restauratrice.*" It seems to follow from what has been shown above that we are not dealing with merely a restoration of, but also with an increase in the number of neurones.

In order to complete this survey I shall add the results of my investigations of the

Appearances of growth

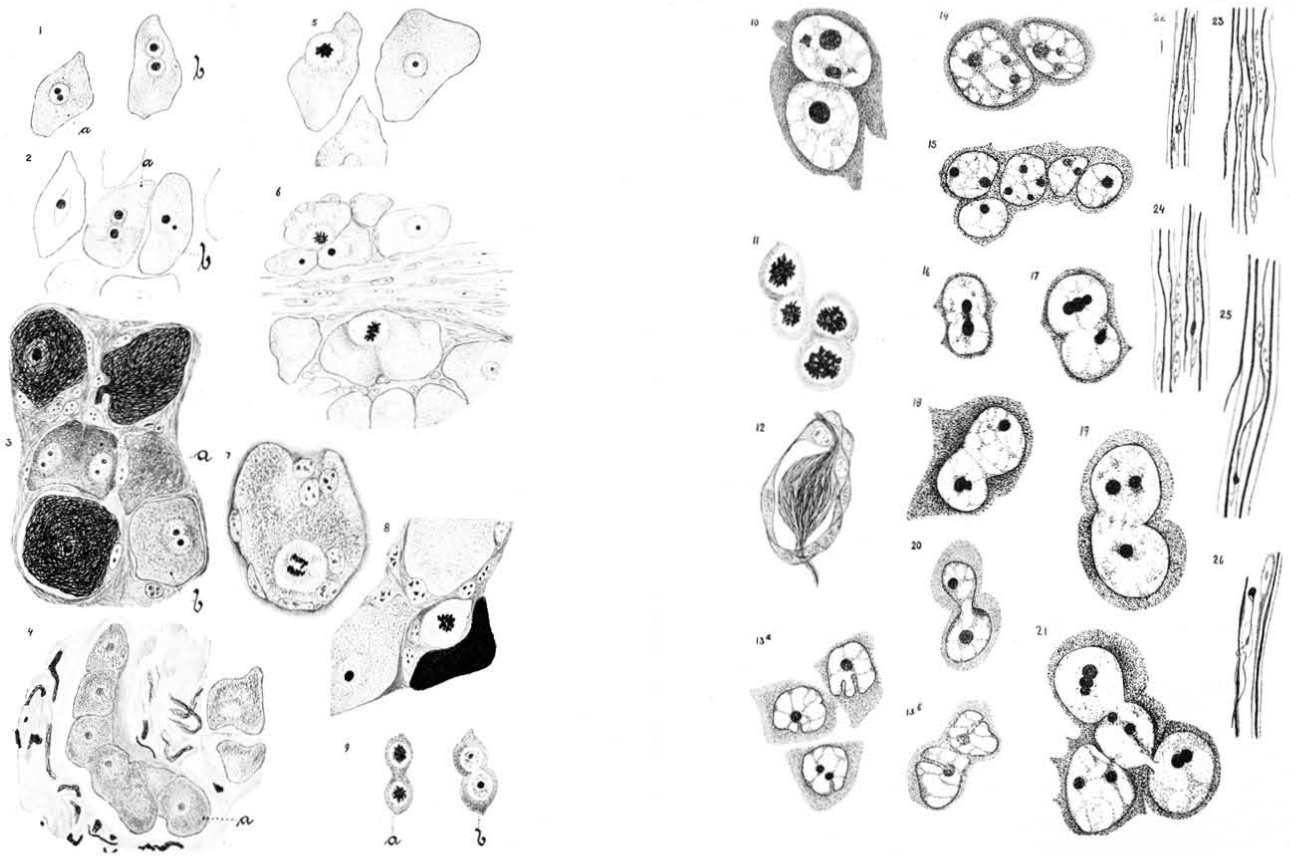
of the axons in the dorsal and ventral roots of the spinal nerves. As has already been shown above, the calculations of the number of the nerve fibres in cross-sections of the dorsal and ventral roots of the spinal nerves made at a, a_1 and b, b_1 text fig. 1 gave such values that one might expect that figures of growth might also really be shown in longitudinal sections of these roots. Silver-impregnated roots from some intact lumbal nerves of a 17 days old dog were set up in series of sections of suitable thickness, and then the preparations were searched for figures of growth. These investigations showed the occurrence of a large number of figures of nerve-fibres free from medullary sheaths, whose ends are situated between the two section surfaces of the preparations; the shape of these ends shows that they could scarcely be due to the nerve-fibres having been cut off when the sections were cut. Of the different shapes that the ends of these nerve-fibres take I will only mention one here, namely, that which shows a swelling at the point; this swelling has in most cases a claviform shape (figs. 22, 23, 24, 25, and 26). The nerve-fibres in these cases were very fine, and showed repeated convolutions during their course (fig. 26). A large number of such nerve-

fibres with a winding course were to be found in my preparations, although I could not find the free end of all of them. Of these figures of growth at least those that form the basis of figs. 22, 25, and 26 may be considered as being absolutely reliable. These figures resemble, of course, those usually found in preparations of nerves engaged in regeneration (in the regeneration of a peripheral end of a nerve, being produced experimentally), BOEKE, RAMON Y CAJAL, etc. We thus arrive at the interesting fact that in the roots of the segmental nerves of fully intact animals as old as those we are dealing with there really exist neurites engaged in growth, and also a new formation of neurones — a phenomenon that must be considered of fundamental importance for a comprehension of the post-embryonal growth of the whole individual.

Résumé and conclusions.

The investigations of the material in question have shown that the post-embryonic growth of the peripheral nerves is not due — as far as the axons are concerned — solely to an advancing myelinisation (DONALDSON, etc.) and an increase in the thickness of the separate axons, but is also due to an increase in the number of axons. This increase in the number of axons is, however, relatively larger during the earlier than during the later post-embryonic period of the animal's development. It is of special interest to note that the results of counting the axons show that the increase in the number of axons goes on for a considerable length of time during the post-embryonic life of the individual (see the table). This post-embryonic period during which an increase in the number of nerve fibres in the roots of the spinal nerves takes place is many times longer than that during which mitoses can be shown in the spinal ganglia and the spinal cord.

Investigations carried out with the object of explaining the method in which such a post-embryonal increase in the number of axons arises have shown that it can *not* be explained by means of *T*- or *Y*-division of the nerve fibres or by assuming that the same nerve cell sends off more than one axon, but that the explanation must be sought *in a real increase in the number of the neurones*. This increase in the neurones seems to a great extent to be due to the fact that from young cells lying in reserve processes are developed, among which the so-called axons grow out in, among other regions, the roots of the nerves and the peripheral nerves. Probably the young cell material in the spinal cord comes from undifferentiated



EXPLANATION OF FIGURES.

Figures 1, 2, 4, 5, 6, 9, 10, 11, 22, 23, 24 and 25 are drawn after magnifying with LEITZ immers. 1/12 and ocul. 4 and using REICHERT's drawing apparatus.

Figures 3, 7, 8, 12 and 26 are drawn after magnifying with ZEISS apochr. immers.; 2 mm. Apart. 1.3 and Comp. ocul. N°. 6, with the help of Abbe's drawing apparatus.

Figures 13, 14, 15, 16, 17, 18, 19, 20 and 21 are drawn after magnifying with ZEISS Apochr. imm., 2 mm. Apart. 1.3 and Comp. ocul. N°. 12, with the help of LEITZ's drawing apparatus.

Fig. 1. Repr. 2/3. Gangl. spin. (Fix. FLEMMING staining Jhtx and Eos.) of a 17 days' old dog. *a*. Ganglion cell with division of the nucleolus just started. *b*. Nucleus dividing amitotically. This small distinct collum-formation between the nuclei is very rare.

Fig. 2. Repr. 2/3. Gangl. spin. (Fix. and staining the same as the preceding) from the same section series as in fig. 1. *a*. Ganglion cell dividing amitotically. *b*. Ganglion cell in which the nucleus has two nucleoli.

Fig. 3. Repr. 2/3. Gangl. spin. (Silver-impregnated according to the BIELSCHOWSKY-method with my own modifications) of a six days' old dog. *a*. Ganglion cell dividing amitotically. *b*. Ganglion cell with the beginning of amitotic division of the nucleus.

Fig. 4. Repr. 2/3. Gangl. spin. (Impr. as in fig. 3) of a sixty days' old dog. *a*. Colony of apolar cells. Between the three cells in the middle there are bridges of protoplasm.

Fig. 5. Repr. 2/3. Gangl. spin. (Fix. and staining as in fig. 1) of a 17 days' old dog. The ganglion cell on the left imitates a mitosis. The mitosis in reality belongs to a capsular cell.

Fig. 6. Repr. 2/3. Gangl. spin. (Fix. and stain. as in fig. 1) of a 17 days' old dog. The mitoses in the capsular cells or in cells subcapsularly situated, which have a tendency to become ganglion cells (?).

Fig. 7. Repr. 1/2. Gangl. spin. (Fix. and stain. as in fig. 1) of a 17 days' old dog. Mitosis in the capsular cell.

Fig. 8. Repr. 2/3. Gangl. spin. (Fix. and stain. as in fig. 1) of a 17 days' old dog. Mitosis in a very young ganglion cell.

Fig. 9. Repr. 1/2. Spinal cord. (Fix. and stain. as in fig. 1) of a 24 days' old *Mus musculus var. albus*. *a*. Mitosis. *b*. Amitosis in a young nerve cell.

Fig. 10. Repr. 2/3. Spinal cord (Fix. and stain. as in fig. 1) of a 24 days' old *Mus musculus var. albus*. Syncytium or plasmodium of young nerve cells.

Fig. 11. Repr. 1/2. Spinal cord (Fix. and stain. as in fig. 1) of a 24 days' old *Mus musculus var. albus*. Mitosis in the nerve cells.

Fig. 12. Repr. 2/3. Spinal ganglion (Imprägn. as in fig. 3) of a 17 days' old dog. Bipolar ganglion cell.

Fig. 13. Repr. 2/3. Spinal cord (Fix. and stain. as in fig. 1) of a full-grown M. musc. v. alb. An example of fairly frequently occurring indentations on nuclei of nerve cells; in my opinion these indentations have very probably no connection with amitotic division.

Fig. 14. Repr. 2/3. Spinal cord (Fix. and stain. as in fig. 1) of a ten days' old *Mus musculus v. albus*. An example of a stage in amitotic (?) division in which the nuclei are quite separated but the protoplasmic body is not quite divided.

Fig. 15. Repr. 2/3. Spinal cord (Fix. and stain. as fig. 1) of a ten days' old *Mus musc. var. albus*. Cell plasmodium or syncytium, situated just ventrally of the canalis centralis.

Fig. 16. Repr. 2/3. Spinal cord (Fix. and stain. as in fig. 1) of a ten days' old Mus musculus v. albus. An example of a cell at an early stage of amitotic division.

Fig. 17. Repr. 2/3. Spinal cord (as in the preceding figure). An example of direct division of the nucleoli and a somewhat later stage than in the proceding figure of direct division of the cell in its entirety.

Fig. 18. Repr. 2/3. Same material as in the preceding figure. Young ganglion cells (neuroblast) engaged in direct division.

Fig. 19. Repr. 2/3. Same material as in the preceding figure. A cell engaged in direct division.

Fig. 20. Repr. 1/2. Spinal cord (Fix. and stain. as in fig. 1) of a 24 days' old *M. musc. v. alb*. A young nerve-cell in a far advanced stage of direct division.

Fig. 21. Repr. 2/3. Same material as in fig. 16. Young nerve cells from the base of the dorsal horn in a very far advanced direct division into four.

Figs. 22, 23 and 24 are probable and figs. 25 and 26 certain figures of growth in the roots of the seventh lumbar nerve in a 17 days' old dog. The material is silver-impregnated according to my modifications of the BIELSCHOWSKY-method.

cells in the ependyma and that in the spinal ganglia from undifferentiated cells among the capsular cells. These cells increase during their differentiation into ganglion cells, among others, partly by means of mitotic division and as far as I can see from my preparations also partly by means of amitotic division. This post-embryonic increase in the number of the cell-material is greater during the first month of post-embryonic life, but seems to continue afterwards as well. It is only during the first month of the post-embryonal life of the individual that one sees mitoses in these cells, but even during its continued life cell-division seems to occur; it then takes place amitotically. These new ganglion cells that have arisen by mitotic or amitotic division seem to develop into neurones, which not only replace older neurones that have been destroyed by degeneration (PALADINO), but also help to increase the absolute number of neurones.

Figures of growth for the axons have been shown morphologically in the dorsal and ventral roots of the lumbar nerves of a 17 days old dog [1]). These figures of growth have been, among various other shapes, claviform — thus under completely physiological conditions the same shape is found for the figures of growth of the axons as is usually found in experimentally produced regeneration of peripheral nerves.

Addendum.

It seems as if the post embryonal increase in the neurones can be effected by external influences. Thus, for instance, it has appeared that in growing animals (among others *Mus musculus var. albus*) the increase in the number of axons can be intensified by suitably adapted and gradually increased training. If, on the other hand, the training has been made too intense, quite a contrary result is obtained — the number of axons has been found to be relatively less in these animals than in the controlling animals. During my continued investigations of this problem I have succeeded in showing, in, among other animals, a 3,5 year old dog, numerous transitional stages from indifferent cells — as large as small capsular cells — to fully developed ganglion cells. These different transitional stages have been examined with regard to the position, size, offshoots and neuro-fibrillar structure of the cells. These questions will be dealt with more fully in a later and more complete account.

[1]) This is the oldest animal that I have investigated so far with regard to this.