A CONTRIBUTION TO THE BIOLOGY OF PERIPHERAL NERVES IN TRANSPLANTATION.[1]

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PLATES 44 TO 46.

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The large amount of experimental and clinical work in transplantation that has been done during the last few years has elucidated many essential points, and the transplantation of bone, skin, connective tissue, blood vessels, and glandular organs has assumed a practical value. This is not the case with peripheral nerves, possibly because their transplantation in human beings has been mainly heteroplastic.

The processes leading to the degeneration of a divided peripheral nerve are well known, and the work of Nageotte has recently added minute and important histological details to our knowledge of the cells of Schwann and the part played by them in the degeneration and regeneration of a peripheral nerve. I shall not comment upon attempts at transplantation of nerves in human beings. Of the experimental work in this field I shall mention only the results of those investigators who have made histological examinations of the transplanted pieces, especially for the Wallerian degeneration, and who have attempted, in the case of a negative result, to determine the variations in these processes; that is, in the Wallerian degeneration, produced by transplantation.

Huber[2] has examined histologically mainly heteroplastic transplanted segments of nerves, and concluded from his experiments that the degenerative process, which occurred in these pieces was very much like the true Wallerian degeneration, the various stages of which, however, succeeded each other more rapidly in the graft than in the peripheral part of a divided nerve.

[1] Aided by a grant from The Rockefeller Institute for Medical Research.

[2] Huber, G. C., *Jour. Morphol.*, 1895, xi, 629.

Ballance and Stewart[3] in their experiments on transplantation are reserved in their conclusions, expressing themselves as follows: The degeneration appears in the graft exactly as in the peripheral part of a divided nerve. The graft itself is a piece of dead tissue and is gradually absorbed and replaced like a clot by living tissue. The regeneration occurs later, but is not a result of the work of the cells of the graft itself. This conclusion seems to me to express two conflicting views, of which one, if true, necessarily excludes the other. If the degeneration of the graft appears exactly as in the peripheral segment of a divided nerve, then the segment is not dead. For the peripheral segment of a divided nerve is not dead. The axis cylinder dies and degenerates, but the cells of Schwann, on the other hand, after the division give evidence of their life by their proliferation.

Neither Ballance and Stewart nor Huber discriminated between the auto-, homo-, and heteroplastic transplantation. This was first done by Merzbacher.[4] In 1905 Merzbacher observed that the graft in auto- and homoplastic transplantations only degenerated in a typical way, whereas in heteroplastic transplantations there occurred in the fibers various regressive processes that resulted in the necrosis of the piece. In auto- and homotransplantations the grafts survive and therefore are capable of a true Wallerian degeneration, which can take place only in nerves in a condition of survival. In heterotransplantations the grafts die and cannot degenerate, but become necrotic; in these cases there is no, or only an insignificant formation of myelin ovoids. The conclusions of Merzbacher were confirmed by the experiments of Segale.[5]

Verga[6] in 1910 performed a series of homo- and heterotransplantations, bridging the central and peripheral part of a divided nerve by the graft. Verga found that the segments healed and always degenerated, and that from an anatomical standpoint there was no difference between the homo- and the heteroplastic graft.[7]

In 1911 Maccabruni[8] in Golgi's laboratory made a number of homo- and heteroplastic transplantations. His results are in accord with those of Huber and Verga, recording a typical Wallerian degeneration in homoplastic as well as in heteroplastic grafts. The center of both kinds of graft he found necrotic, possibly due to the lacking supply of nourishment. Thin grafts degenerated completely without necrosis. From the 8th to the 14th day he found proliferation of the cells inside the nerve fibers by means of karyokinetic division. The process has a slower course than in a degenerating nerve. Regarding the origin of the cellular elements inside the fibers, Maccabruni expresses some reservation. His preparations do not show whether these cells represent the syncytium of Schwann, or whether they are connective tissue cells.

[3] Ballance, C. A., and Stewart, P., *Rev. neurol.*, 1902, x, 860.

[4] Merzbacher, *Neurol. Centralbl.*, 1905, xxiv, 150.

[5] Segale, L., cited by Maccabruni, F., *Folia neuro-biol.*, 1911, v, 598.

[6] Verga, *Jahresber. f. Chir.*, 1910, xvi, 481.

[7] This publication is not available. In the summary of the article in the *Jahresber. f. Chir.* the cells of Schwann are not mentioned.

[8] Maccabruni, *loc. cit.*

Besides the work just mentioned, experimental transplantation of nerves has been performed by Gluck,[9] Kilvington,[10] and Duroux.[11] The results of these investigators are encouraging as far as the function is concerned. Neither Kilvington nor Duroux, however, made histological examinations of the transplanted pieces, and the interpretation and the conclusions drawn from his material by Gluck concerning the processes of regeneration are not convincing. The lack of proof, in the work of Gluck, that the graft is different from dead material has caused Kölliker to remark that the nerve bridge must be supposed to prevent the regeneration of the peripheral part instead of facilitating it, and Kölliker advocates the bridging of the defect by strands of catgut or tubes as superior to the application of a graft of nervous tissue.

In the problem of transplantation of nerves the question of the fate and survival and multiplication of the cells of Schwann is of importance. The solution of this point, which is the only reliable sign of the survival of the transplanted piece, gives the key to the problem and will influence the procedure of surgeons in cases of nerve defects. If the grafts die and become necrotic they are no more suitable for bridges than strands of catgut. If it is true, on the other hand, that the grafts do survive, the statement of Kölliker lacks support, and in bridging nerve defects grafts of peripheral nerves must be preferred to dead material.

I have experimented on the sciatic nerve of rabbits, from which pieces 2 to 3 cm. long are taken out and then either reimplanted into the same animal, united to the cut ends of the nerve by means of a single silk suture (autoplastic), or implanted into the sciatic nerve of another rabbit (homoplastic), or into guinea pigs (heteroplastic). I have made three series of experiments. In each series I have operated on several animals and the transplanted pieces were removed for histological examination at different intervals (4, 8, 12, 16, or 20 days) after the transplantation.

Then the pieces were treated in the way indicated by Nageotte, which has given excellent results in his study of the Wallerian degeneration. The grafts were hardened in Dominici's solution, next they were dissociated by means of needles as far as possible, and stained by hematoxylin before passing through alcohol and mounted in cedar oil. From some cases sections were prepared and treated according to the method of Marchi.

[9] Gluck, *Jahresber. f. Chir.*, 1895, i, 282.
[10] Kilvington, B., *Brit. Med. Jour.*, 1908, i, 1414.
[11] Duroux, E., *Lyon Chir.*, 1912, viii, 562.

I wish to emphasize the necessity of making dissociation preparations, if one wishes to make indisputable observations on the cells of Schwann. In such preparations only we are sure that a certain cell belongs to a certain fiber, and in this case only can we count the accurate number of cells in each individual fiber. I shall give a summary of my results from each of the three series, beginning with the autoplastic transplantations.

Autoplastic Transplantations.

In this series the examination of the graft four and six days after transplantation (Fig. 4) reveals a process which is not very different from the ordinary Wallerian degeneration (Figs. 1, 2, and 3). The nuclei of the cells of Schwann have fallen in towards the center of the fibers between two myelin ovoids. The nuclei are richly provided with chromatin and are embedded in protoplasm. In a few fibers from the sixth day two or three nuclei are observed close to each other, indicating that multiplication of these cells has already begun. Between and in the myelin ovoids there are immigrated mononuclear cells of a type quite different from the cells of Schwann. The nuclei of these cells are smaller and richer in chromatin than the nuclei of the cells of Schwann. They present phagocytic properties filling their cell bodies with fragments of myelin, and Nageotte, who found them in the Wallerian degeneration, called them "*corps granuleux.*" Probably they are lymphocytes.

The only difference between the autoplastic graft from the fourth and sixth days and the peripheral part of a divided nerve is that in the latter the formation of myelin ovoids is more advanced than in the graft. In the examination of the grafts in later stages, we find this feature again and again. After eight days we find a degenerative process resembling a somewhat delayed Wallerian degeneration. A graft from the eighth day is in as degenerative a stage as a peripheral nerve from the fifth to sixth days. But in some fibers we observe no degeneration at all; there is no formation of myelin ovoids and they look perfectly normal. These fibers belong to the central parts of the graft, and judging from the appearance

of Marchi preparations, these fibers later become necrotic. This is true about the central fibers of homoplastic grafts as well as autoplastic. On the twelfth day we find as pronounced a Wallerian degeneration as on the eighth or ninth day. A large number of myelin ovoids has been formed, and there are many "*corps granuleux*" and numerous nuclei of the cells of Schwann.

After the sixteenth and twentieth days (Figs. 5 and 6) the nuclei of the cells of Schwann are arranged in long rows inside the sheaths of Schwann with continuous protoplasmic bridges about and between them; the only difference from the Wallerian degeneration of the same stage is that in the latter the absorption of myelin fragments is a good deal more advanced than in the graft.

Homoplastic Transplantations.

I shall next describe the results of my homoplastic transplantations.

In almost every respect the preparations from the fourth and fifth days (Figs. 7 and 8) resemble the picture of a nerve on the fourth and fifth days of Wallerian degeneration. The nuclei of the cells of Schwann have fallen in towards the center of the fibers, and are richly provided with chromatin. A formation of myelin ovoids has started and only a few immigrated cells are seen. In one of the preparations from the fourth day we find a cell of particular interest. In one of the fibers (Fig. 8) we observe three oval nuclei of Schwann close to each other, and also a large darkly stained cell including a nucleus in mitotic division. This cell gives proof that the cells of Schwann multiply in a homoplastic transplanted graft. The increased number and the long rows of nuclei of Schwann in individual fibers already give strong evidence for the probability of such a conclusion, but the mitotic figure presents a picture from a stage of the process itself. We do not hesitate in the identification of this cell. The well outlined protoplasmic body of the cell, which is never observed in the immigrated cells during their mitotic division, determines that the cell is really a cell of Schwann.

I have never observed in the grafts in autoplastic transplantation such a mitotic division of the cells of Schwann. But mitotic divi-

sions are not easily found and are not frequently seen in the cells of Schwann during their proliferation in the ordinary Wallerian degeneration. Accordingly there is no reason to doubt that mitotic divisions may also be observed in autoplastic transplantations, since we know that grafts in such conditions are best fit for survival.

During the first ten to eleven days the degenerative process in the homoplastic transplanted grafts appears mainly as in autoplastic transplantations; that is, like a Wallerian degeneration, only a little more slowly. Myelin ovoids are formed, the cells of Schwann multiply, and immigrated phagocytic cells loaded with fatty granules are seen in the fibers (Fig. 9). From the eleventh to twelfth days, these cells are present in a number considerably exceeding those in the autoplastic grafts. They are steadily increasing in number, and from the sixteenth to eighteenth days (Fig. 10) they form a marked feature in the whole picture.

It is possible that the presence of these numerous immigrated cells in homoplastic grafts—cells provided with phagocytic properties and probably of lymphocytic origin—are playing some part in the mechanism of immunity against homoplastic transplantation of tissue in general. For in transplantation of organs and tissue there is a marked difference between the final result of homoplastic and autoplastic transplantation (the kidneys, for instance). But I do not wish to enter upon further discussion of the importance of the phagocytic cells. I wish only to add that in homoplastic nerve grafts the nuclei of Schwann from the eighteenth to twentieth days are pale and faintly stained and are evidently in a necrobiotic condition, which is possibly dependent upon the presence of the lymphocytes.

Heteroplastic Transplantations.

In heteroplastic transplanted nerves an abundant formation of myelin ovoids occurs during the first four to five days. But later these grafts do not resemble either the autoplastic or homoplastic transplanted pieces or nerves in Wallerian degeneration. There is no proliferation of the cells of Schwann. These cells, on the contrary, are faintly stained or have completely disappeared. From the eighth and tenth days the contents of the fibers consist mostly of

irregularly broken up pieces and fragments of myelin and protoplasm and the whole fiber looks necrotic (Fig. 11).

In later stages we find between and in the fibers numerous immigrated cells, and from the sixteenth to eighteenth days the graft on gross examination is yellowish, soft, and necrotic, or it is encapsulated by young connective tissue.

SUMMARY.

In autoplastic transplanted nerves a degenerative process occurs which resembles the ordinary Wallerian degeneration, but appears a little more slowly than the latter. The cells of Schwann are in a condition of survival and are capable of multiplication after the transplantation.

In homoplastic transplanted nerves I have found a degenerative process resembling a Wallerian degeneration, somewhat delayed. The cells of Schwann multiply, and for some time at least are in a condition of survival. After twelve to fourteen days an abundant and increasing immigration of lymphocytes is observed, and from the eighteenth day the cells of Schwann develop a necrobiotic appearance.

In heteroplastic transplanted nerves numerous myelin ovoids are formed during the first four to five days, but there is no proliferation of the cells of Schwann, and no Wallerian degeneration is seen. The graft becomes necrotic within about two weeks.

The formation of ovoids that occurs during the first four to five days after the performance of the heteroplastic transplantation does not reveal the condition of the life of the graft. This formation of myelin ovoids is found in the nerve fibers when they have been kept in an incubator for twenty-four hours in Ringer solution (Nageotte[12]) or in homologous or heterologous serum, but it is not found in the fibers after their incubation in isotonic salt solution (Nageotte), the presence of calcium being necessary for the occurrence of ovoid formation.

[12] Nageotte, J., *Compt. rend. Soc. de biol.*, 1910, lxix, 556.

CONCLUSIONS.

Heteroplastic transplanted nerves become necrotic. They are unsuitable for bridges in cases of nerve defects, and my results explain the failure of the attempts at heteroplastic transplantation of nerves in human beings.

If we wish to bridge a nerve defect by implantation we must use autoplastic or homoplastic grafts. The occurrence of a Wallerian degeneration in these grafts during the first two to three weeks after the transplantation should make bridging a promising operation; for in this period the grafts resemble the peripheral part of a divided nerve and must be assumed to be capable of regeneration, and thus are very different from dead material.

I have studied the process of regeneration, and shall communicate in a future article my results of bridging defects, which are encouraging as far as the function is concerned.

My results with homoplastic transplantation of nerves have a bearing on the homoplastic transplantation of limbs, which has been successfully performed in dogs by Carrel. None of his dogs lived long enough to show any function of the transplanted leg. The practical value of this operation is dependent, of course, upon the return of function, and especially on the regeneration of the nerves in the transplanted leg. The results with homoplastic transplantation of nerves seem to indicate the possibility of a regeneration of the nerves in a homoplastic transplanted leg.

EXPLANATION OF PLATES.

PLATE 44.

Figs. 1, 2, and 3 are nerve fibers from the peripheral part of divided nerves. Wallerian degeneration in different stages.

FIG. 1. Wallerian degeneration, 4th day.

FIG. 2. Wallerian degeneration, 7th day.

FIG. 3. Wallerian degeneration, 14th day. Multiplication of the nuclei of Schwann, numerous immigrated cells (*"corps granuleux"*).

FIG. 4. Nerve fibers from a graft, 6 days after transplantation (autoplastic). Multiplication of the nuclei of Schwann.

FIG. 5. Nerve fiber from a graft, 16 days after transplantation (autoplastic). Long rows of nuclei of Schwann. Some immigrated cells (*"corps granuleux"*).

PLATE 45.

FIG. 6. Nerve fiber from a graft, 18 days after transplantation (autoplastic). Multiplication of the nuclei of Schwann. Reduction of the myelin ovoids. Numerous immigrated cells.

FIG. 7. Nerve fibers from a graft, 4 days after transplantation (homoplastic). The nuclei of Schwann have fallen in towards the center of the fibers, embedded in protoplasm.

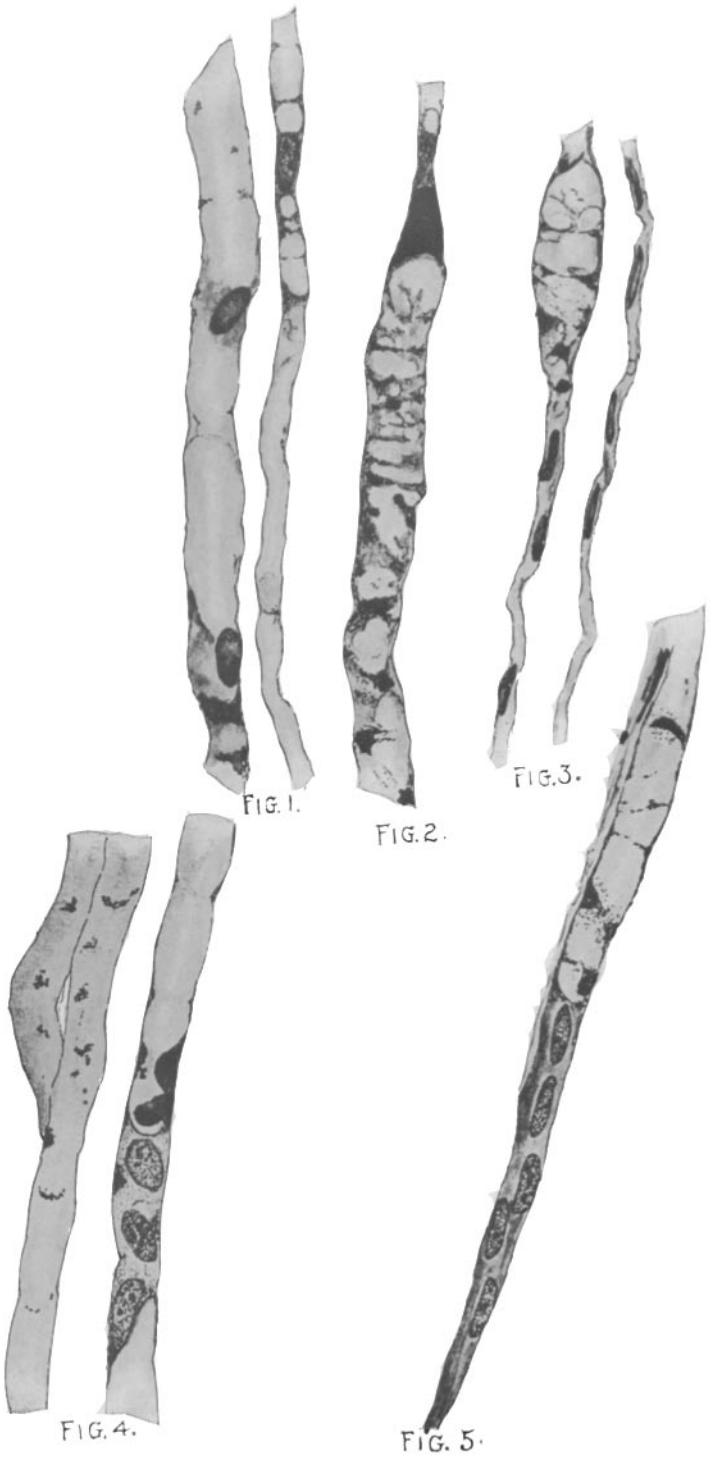
FIG. 8. Nerve fibers from a graft, 4 days after transplantation (homoplastic). In one of the fibers a mitotic figure is seen in a cell of Schwann.

FIG. 9. Nerve fibers from a graft, 10 days after transplantation (homoplastic). Multiplication of the nuclei of Schwann. Immigrated small darkly stained cells (*"corps granuleux"*).

PLATE 46.

FIG. 10. Nerve fiber from a graft, 18 days after transplantation (homoplastic). Multiplication of the nuclei of Schwann. These nuclei are pale and faintly stained. A large number of immigrated cells is seen.

FIG. 11. Nerve fibers from a graft, 10 days after transplantation (heteroplastic). No cell is seen. The fibers appear necrotic.



(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)

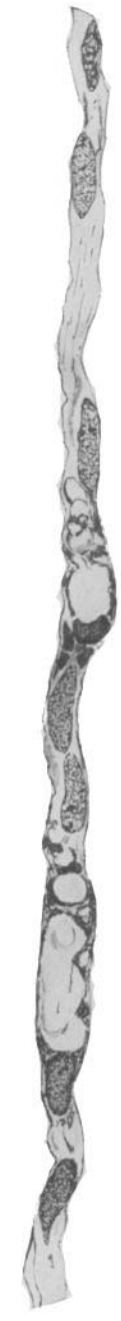


FIG. 6.

FIG. 7.

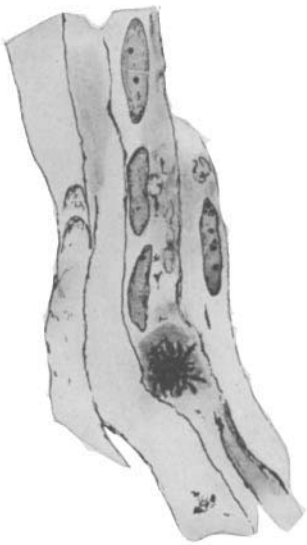


FIG. 8.

FIG. 9.

(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)

FIG. 10.

FIG. 11.

(Ingebrigtsen: Biology of Peripheral Nerves in Transplantation.)