REGENERATION IN THE BRAIN OF AMBLYSTOMA

I. THE REGENERATION OF THE FOREBRAIN

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FOUR FIGURES

Up to the present time the data dealing with regeneration in the central nervous system have been exceedingly conflicting. The early workers reported the regeneration of various definitive parts after they were extirpated. As far back as 1890 Danielewsky found that the removal of the cerebral hemispheres of the frog resulted in the formation of a 'cerebral mass' which he believed contained embryonic nerve cells, though this mass was in no way a new hemisphere. More recently Bell, in 1907, removed the 'lateral half' of the brain of the frog and found that the brain nearly always is reformed though never does it reach the normal size.

On the other hand, Schaper ('98) found that the removal of portions or of the entire brain of *Rana esculenta* was never followed by regeneration. Rubin in 1903 working with *Rana fusca* larvae found that no regeneration occurred after the removal of the brain or of parts of it.

The results of experiments published elsewhere (Burr '16) show conclusively that the nasal placode of *Amblystoma* and also of the frog does not regenerate when it is completely removed. In addition, the large amount of work by Lewis and others has shown that the eye will not regenerate when all of the anlage is removed. It was deemed probable, therefore, that complete extirpation of the cerebral hemisphere would not be followed by even a partial regeneration of the part removed. The discrepancy in the results previously reported might be due to incomplete operations. The regeneration of parts of

the brain would then be due to the fact that the entire substance of the hemisphere was not removed, enough being left to carry the regeneration through to a considerable degree of completeness. A condition similar to this has been shown by Harrison to exist in the extirpation of limb buds in *Amblystoma* (Harrison '15).

So far as it has been possible to ascertain, no attempt has previously been made to control the stimulus afforded by the functional activity of the end organ normally connected with the part of the central nervous system removed. Extirpation of a portion of the brain has invariably carried with it the removal of the end organ. It is a little difficult then to see why a part should regenerate when its activity has ceased owing to the removal of the end organ.

In order to obtain some answer to the above question the following experiments were performed on *Amblystoma* larvae. Two series of operations were undertaken on embryos possessing neither peripheral nerves nor a circulatory system. In the first of these the right cerebral hemisphere and the right nasal placode were extirpated, the cut that severed the hemisphere from its connections passing directly in front of the optic stalk. In the second series the right telencephalon was removed but the right nasal placode was left in position. This was accomplished by turning back the flap of skin containing the placode and removing the underlying forebrain. The flap was then returned to its former position and held in place until the wound had healed. Great care was taken to remove all of the cells of the cerebral hemisphere in all the operations.

The above experiments subject the brain tissue left by the extirpation of the hemisphere to two conditions. In the first series of operations the nervous tissue is left to regenerate without the possibility of any stimulus from the end organ that is normally connected with it. In the second series the end organ, the nasal placode, is left in its normal position and may therefore act as a stimulus to the nervous tissue (Burr '16).

In the first series of experiments in which the right hemisphere and the right nasal placode were removed the wound usually

healed within the first twenty-four hours. Five days after the operation a new wall had formed connecting the wall of the right diencephalon with the wall of the left telencephalon. At first this wall consists of a narrow band of new cells bridging the interventricular foramen. As growth proceeds the narrow

Fig. 1 Transverse section of embryo five days after operation, by which the cerebral hemisphere was removed, leaving the nasal placode in place, showing curtain of cells across the interventricular foramen. $\times 50$.

band of cells is drawn out into a thin plate, never more than two or three cells thick, stretching across the foramen (figs. 1 and 3).

A careful inspection of a series of operated larvae makes it quite clear that the new tissue thus formed is derived from the primary ependymal cells that line the neural tube. Figure 2 drawn from a section of an operated larva of the second series shows the origin of this new tissue from the margin of the dien-

cephalon. It is composed entirely of the typical columnar cells that make up the ependyma. As growth proceeds the cells of the new membrane lose their columnar shape and become metamorphosed into flattened quadrilateral cells. In the three months old larva, a section of whose brain is shown in figure 3, it is evident that this metamorphosis is accompanied by a thinning of the tissue so that at this time the membrane is

Fig. 2 Portion of the regenerated curtain of an embryo seven days after the same operation as in figure 1, showing regeneration from the margin of the diencephalon. $\times 50$.

composed of only a single layer of cells whose somewhat elongated nuclei are parallel to the surface.

It is evident from the above that the regenerated portion is not made up of nerve cells but rather of the primitive germinal epithelium which lines the neural tube, the only regeneration that takes place being that necessary to close the wound made by the removal of the hemisphere. We have then, forming as a result of the operation, a curtain of primary ependyma across the interventricular foramen. This is not nervous and hence constitutes no true regeneration of the telencephalon.

Hardesty ('04) has shown that the dividing elements of the primary ependyma, the germinal cells, give rise to a nuclear layer just outside of the ependyma from which later develop the neuroblasts and the spongioblasts. It is conceivable that the differentiation of the nuclear layer into nerve cells results from the stimulus derived from the ingrowth of nerve fibers

Fig. 3 Transverse section of embryo without the right hemisphere or nasal placode three months after the operation, showing the thin curtain across the interventricular foramen. $\times 50$.

from other definitive parts of the nervous system. Evidence of this is seen in the fact that the fiber tracts of the telencephalon do not develop until the olfactory nerve fibers have grown into the peripheral part of the hemisphere from the nasal placode. The possibility of such a stimulus has been used by Kappers ('14) in his neuro-biotaxis theory of the phylogenetic migration of nuclear centers in the central nervous system.

On the other hand Harrison ('10) has shown that embryonic nerve cells taken from the medullary cord of frog larvae will produce protoplasmic nerve processes when cultivated in vitro, but it is manifestly impossible to determine by such methods the extent to which development will proceed without the intervention of functional activity. As has been shown elsewhere (Burr '16), development and differentiation of the central nervous system will progress to a certain point without the presence of a functioning end organ. Beyond this point functional activity is essential for continued growth. In the experiments just referred to, the telencephalon was quite completely organized without the ingrowth of the olfactory fibers, but this nerve is not the only one whose fibers penetrate the hemisphere, for Herrick ('10) has shown that in the posterior third of the telencephalon of *Amblystoma* there are at least two centers that are directly connected with the hypothalamus and the pars dorsalis thalami by ascending projection fibers. Hence it seems probable that the apparent self-differentiation of the telencephalon may be due in some degree to the presence of the forward growing nerve fibers which establish this connection, the functional stimulus imparted by them being sufficient to carry differentiation some distance. Experimental evidence that is in favor of this view is seen in the fact that in the posterior margin of the curtain which develops across the foramen of Monro on the removal of the telencephalon and nasal placode, there appears in the older larvae a rounded mass of nerve cells and fibers. This mass is in direct communication with the nucleus habenulae and the hypothalamus, a tract of fibers reaching it from each of these regions. Evidently the neuroblasts at the posterior margin of the interventricular foramen have been stimulated to further development by the ingrowth of axones from lower centers. This mass is, then, in all probability the rudiment of the primitive pallium.

The second series of experiments involved the removal of the right hemisphere without the removal of the nasal placode. The results here are definite and conclusive. A curtain of ependymal cells is formed across the foramen of Monro as in the

previous experiment. The healing of the wound brings the nasal placode back to approximately its normal position though a slight shifting anteriorly has been noted. Five days after the operation the curtain is composed of a single layer of cells (fig. 1). Two days later this single layer of cells has become converted into a thickened mass of columnar cells that are evidently of ependymal origin (fig. 2). By the time the twelfth day is reached the olfactory nerve has established a connection with the curtain, which has in the mean time increased considerably in size. The establishment of the nervous connection between the nasal placode and the telencephalon takes place in the normal larva about the tenth day, only a slight delay in this union occurring as a result of the operation. In the unoperated larvae the nasal placode lies in close apposition to the thick wall of the telencephalon, the olfactory fibers growing into the hemisphere at the point of contact. In the operated forms the nasal anlage lies close to the newly formed curtain, which as indicated above shows a slight thickening not seen in the operated larvae possessing no nasal placode. This thickening anticipates the ingrowth of the olfactory nerve by several days. It is not possible at this time to give any explanation of this thickening, though it is conceivable that the presence of the olfactory anlage stimulates the ependymal cells to grow and divide through some mechanical or chemical factor.

Two days after the ingrowth of the olfactory nerve the new telencephalon, as it must now be called, has reached quite an advanced stage of development. During these two days the thickened curtain has been differentiated into a small but nevertheless typical telencephalon. Neuroblasts are present and a ventricle has appeared. Outwardly the form has changed. A forward growth has converted the thick flat plate of cells into an ovoid mass of neuroblasts and spongioblasts. The ingrowth of the olfactory nerve on the lateral aspect of the mass is accompanied by the appearance of the large lateral forebrain tract (fig. 4). Almost simultaneously there appears from the dorsal aspect of the olfactory bulb the tractus olfactorius dorso-lateralis and from the ventral aspect the tractus olfactorius

medialis. At this time the new telencephalon is similar in organization to its fellow but it is considerably smaller in size. From this time on growth and differentiation continue in both hemispheres in an entirely normal fashion, though apparently there is to some extent a compensating increase in the rate of growth of the new telencephalon for in the oldest larva of the

Fig. 4 Transverse section of an embryo one and one-half months after the operation showing the regenerating telencephalon as a result of the presence of the nasal placode. $\times 44$.

series, a larva some three and a half months old, the new hemisphere had completely regenerated and could not be in any way differentiated from its fellow.

SUMMARY

It is evident from the above experiments that the forebrain of *Amblystoma* will not regenerate when it and its functional end organ are completely extirpated. The healing of the wound

results in the formation of a curtain across the interventricular foramen derived from the ependyma lining the neural tube. If, on the other hand, the forebrain is removed without removing the end organ, the nasal placode, the presence of the latter acts as a stimulus to the regeneration of a new telencephalon through the ingrowth of the olfactory nerve. The pallial region of the telencephalon is regenerated in all cases owing to the stimulus afforded by the forward growth of axones from lower centers in the brain and cord.

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