REGENERATION OF AXIS CYLINDERS IN VITRO.

Second Communication.*

By RAGNVALD INGEBRIGTSEN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 27 to 31.

In a previous article[1] I described certain observations on cultures made from pieces of the central nervous system of young mammals, cultivated in coagulated plasma. Most of the observations were made from living (unstained) material. Further study of the cultures, carried on with different methods of fixation and staining, has confirmed the observations made on unstained preparations; namely, that nerve fibers grow out from pieces of the cerebellum and spinal ganglia of young mammals, when cultivated in plasma. Certain other phenomena of growth have also been revealed. This paper, however, will be limited to a description of the outgrowth of nerve fibers and their appearance in stained specimens.

As young dogs were not available, young cats and guinea pigs were employed in most of the experiments. The cultures were chiefly made from pieces of cortex of the cerebellum and spinal ganglia.

Held's pyridin method was found to be the only practicable one for fixing the cultures. It causes no precipitation in the plasma, and the subsequent staining by Cajal's silver nitrate method, when successful, has given good results. In good preparations the fragment of tissue stains dark brown or black and the surrounding plasma a light reddish brown. The black or dark brown nerve fibers which have grown out are easily discernible against this background.

Cerebellum.—The network of dark brown or black nerve fibers

* Received for publication, June 17, 1913.

[1] Ingebrigtsen, R., *Jour. Exper. Med.*, 1913, xvii, 182.

contained in the tissue can be observed in the thinner part of the transplanted fragment, especially along the border lines adjoining the plasma. In some preparations the new fibers in the plasma can be determined as a direct outgrowth or continuation of fibers contained in the tissue itself (figure 1). In other preparations this direct connection is not visible, either because the original fragment fails to reveal individual fibers or because it is surrounded by other newly grown tissues (connective tissue, glia), which cover the nerve fiber at the point where it emerges from the brain and enters the plasma (figure 2).

From this point the nerve fiber often proceeds by means of a terminal bulb, which in the living culture possesses ameboid movements. In stained preparations the terminal bulb usually takes on a somewhat lighter shade of brown than the fiber itself and shows an even, slightly granular structure. It is ovoid or globular in shape with a diameter of two to three microns. No capsule has ever been seen surrounding it. This end bulb is not, however, invariably present; sometimes the fiber proceeds with a point, and in one preparation the swelling at the end of the fiber is probably produced by a minute loop formed by its point (figure 3).

Throughout their course the fibers are either evenly cylindrical (figure 2) or provided with varicosities, which have much the same appearance as the terminal bulb (figures 1 and 4).

Spinal Ganglia.—While the cultures of cerebellum in this series of experiments with stained specimens have shown only a scant number containing newly grown nerve fibers, the cultures of spinal ganglia give better results both as regards the number of successful cultures and the number of nerve fibers growing out in each culture.

The technique employed for the preparation was the same in both cases: the pieces of cerebellum, as well as of the spinal ganglia, were taken from the living etherized animal, put directly into Ringer solution, where they were cut, and from there transferred to the plasma.

The success of the operation depends upon the rapidity with which it is carried on, at least in the case of the cultures of the cerebellum, twelve to fifteen seconds being the longest lapse of time permissible between the removal of the tissue from the animal and

its inoculation into the plasma. In the case of the spinal ganglia the interruption of the circulation and the transplantation to the new medium is more easily withstood without injury.

Most of the cultures of spinal ganglia, as well as those of cerebellum, were fixed and stained after two or three days' incubation, in order to be sure that degenerative changes of the newly grown fibers had not occurred; as a rule, these changes begin to appear on the fifth and following days at an incubation of 39° to 40° C. On the other hand, the growth of the nerve fibers is most rapid during the first two days, and on the second day, in particular, they attain, if not their full size, at least almost their entire length.

In the cultures of spinal ganglia, as well as in those of cerebellum, the nerve fibers in the plasma in several preparations have grown out as a direct continuation of the fibers in the original piece, and the outgrowth of new fibers is especially abundant in places where parallel and transversely cut nerve fibers radiate out against the plasma. The nerve fibers are also seen to grow out from masses of ganglia cells, situated at the edges of the fragment (figure 5).

The nerve fibers growing out from the spinal ganglia generally reach a greater length than those from the cerebellum, many of them attaining 500 to 600 microns after forty-eight hours. The different fibers vary somewhat in thickness, and the heaviest ones branch several times. They are mostly cylindrical and absolutely even throughout their course, but they bend and curve in various directions, and sometimes spirally. Most of them end in a point. In one preparation most of them end in a bulb, and in this case the fibers are at intervals provided with varicosities, spindle-shaped, ovoid, or globular in form. These have a slightly granular structure and the centers stain somewhat darker than the peripheral parts.

In none of the stained cultures, whether taken from the cerebellum or the spinal ganglia, have I observed a true anastomosis. In the previous paper I described the existence of anastomoses between some of the fibers in the living cultures. This phenomenon has not been confirmed in the stained specimen, and its interpretation will therefore require further study. It may be merely a coalescence between two fibers situated closely together.

The nerve fibers from the cerebellum, as well as those from the

FIG. 1.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

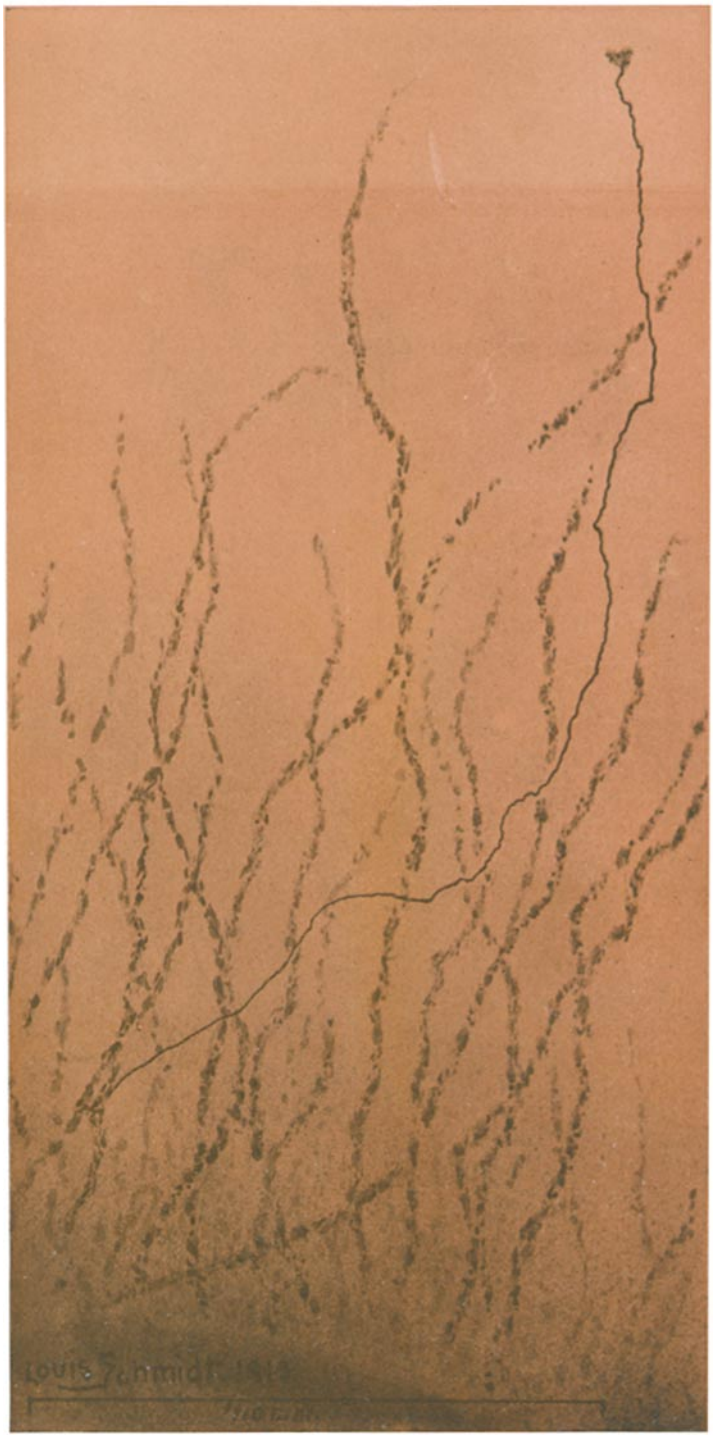

FIG. 2.

Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

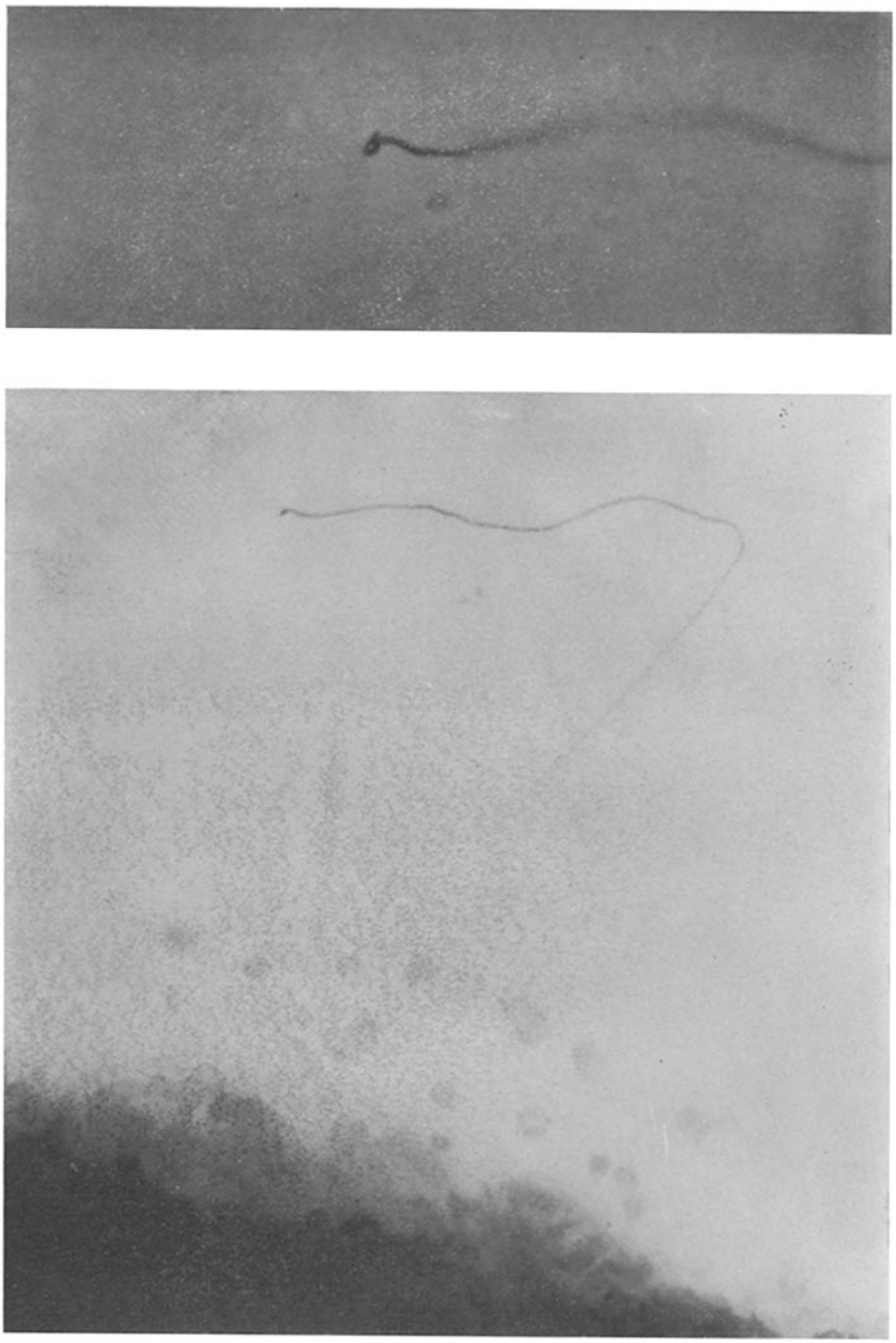

FIG. 3.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

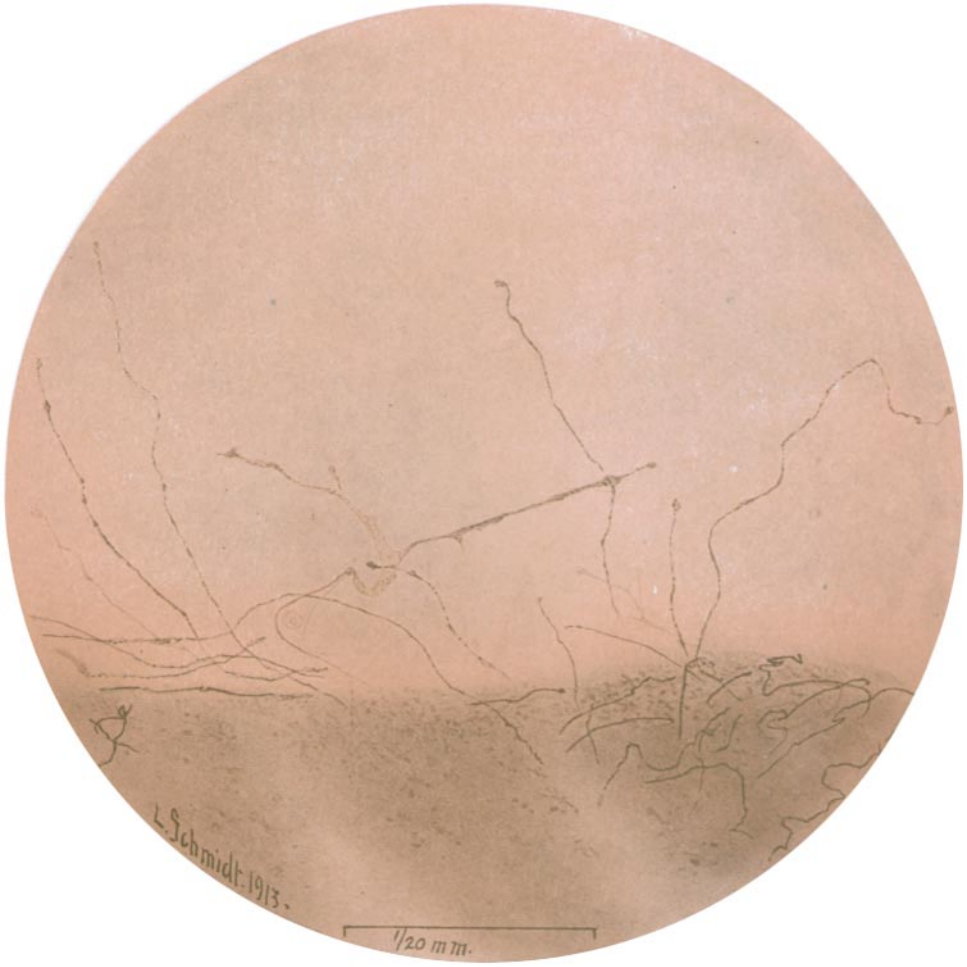

FIG. 4.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

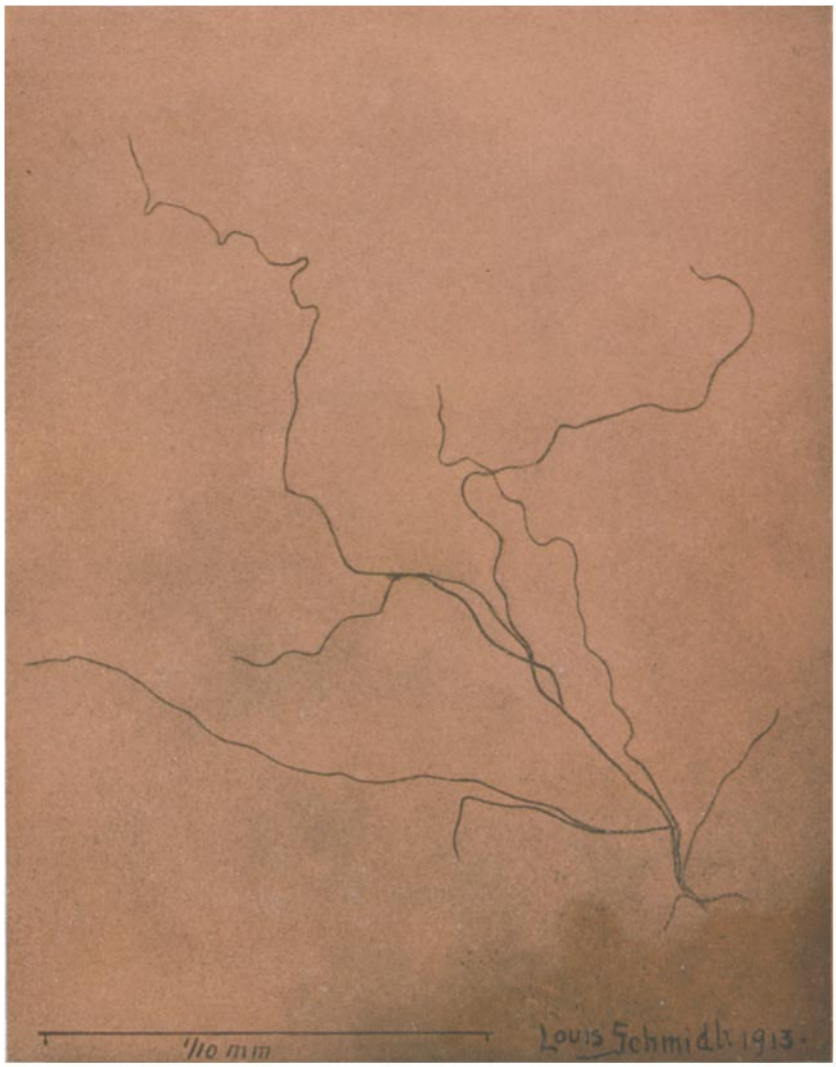

FIG. 5.

Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

spinal ganglia, emanate freely into the plasma without the support or attachment of any other structure. They are not accompanied by other tissue; sometimes they are surrounded by connective tissue cells or glia cells, but their growth begins earlier and does not depend upon the presence of these structures. In fact, cultures with rich growths of connective and glia tissues rarely contain newly grown nerve fibers.

SUMMARY.

It has been shown for the first time that nerve fibers grow out from pieces of cerebellum of young cats and guinea pigs, when cultivated in coagulated plasma.

The same phenomenon has been observed in cultures of spinal ganglia.

The nerve fibers do not anastomose and they extend into the plasma unaccompanied by structures of any kind.

EXPLANATION OF PLATES.

Figures 1, 2, 4, and 5 are camera lucida drawings focused on different planes of the plasmatic medium, in order to bring out the nerve fibers throughout their entire length.

PLATE 27.

FIG. 1. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 28.

FIG. 2. Two day old culture of cerebellum of a guinea pig eight days old. A single nerve fiber is seen surrounded by other structures, probably neuroglia.

PLATE 29.

FIG. 3. Photographs of a two day old culture of cerebellum of a cat six days old. The photographs are taken at two different magnifications, 1,300 and 1,900 diameters respectively.

PLATE 30.

FIG. 4. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 31.

FIG. 5. Two day old culture of spinal ganglia of a guinea pig six days old.