with an intravenous injection, the time may be even shorter than this. The fatal dose may vary from eight to one hundred milligrams according to the purity of the poison or the mode of administration. While the fatal dose may be small the range between that necessary to induce the first and second stages and that necessary to kill may be wide. With one preparation seventy milligrams was required to kill, but five milligrams developed the first and second stages in pronounced forms.

Death is due to failure of respiration and the heart often continues to beat for some minutes after respiration has ceased. It seems most probable that death is due to the direct action of the poisons on the respiratory center. It is inferred from the readiness with which recovery may follow non-fatal doses that the poison cripples but does not destroy the cells of the respiratory center.

All attempts to produce antitoxins with these proteid poisons have, so far, failed. It is true that repeated treatments of animals with non-fatal doses of the poisons from the colon and typhoid bacilli enable animals to bear from two to four times the ordinarily fatal doses of living cultures of these bacteria, but this seems to be due to an increased resistance rather than to a true immunity. This condition is not specific and may be induced by the poisons obtained from peptone or egg white, as well as with that obtained by cleavage of the homologous bacterium.

Attempts have been made to ascertain the chemical constitution of the proteid poisons by splitting them up with mineral acids but at present these experiments have not yielded satisfactory knowledge and work along this line is being continued. The physiologic action of the proteid poisons leads to the suspicion that they contain a neurin group, but so far we have not been able to demonstrate the presence of such a body.

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Observations on the living developing nerve fiber.

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The immediate object of the following experiments was to obtain a method by which the end of a growing nerve could be

brought under direct observation while alive, in order that a correct conception might be had regarding what takes place as the fiber extends during embryonic development from the nerve center out to the periphery.

The method employed was to isolate pieces of embryonic tissue known to give rise to nerve fibers, as for example, the whole or fragments of the medullary tube, or ectoderm from the branchial region, and to observe their further development. The pieces were taken from frog embryos about 3 mm. long, at which stage, *i. e.*, shortly after the closure of the medullary folds, there is no visible differentiation of the nerve elements. After carefully dissecting it out the piece of tissue is removed by a fine pipette to a cover slip upon which is a drop of lymph freshly drawn from one of the lymph sacs of an adult frog. The lymph clots very quickly, holding the tissue in a fixed position. The cover slip is then inverted over a hollow slide and the rim sealed with paraffine. When reasonable aseptic precautions are taken, tissues will live under these conditions for a week and in some cases specimens have been kept alive for nearly four weeks. Such specimens may be readily observed from day to day under highly magnifying powers.

While the cell aggregates, which make up the different organs and organ complexes of the embryo, do not undergo normal transformation in form, owing no doubt in part to the abnormal conditions of mechanical tension to which they are subjected, nevertheless the individual tissue elements do differentiate characteristically. Groups of epidermis cells round themselves off into little spheres or stretch out into long bands, their cilia remain active for a week or more and a typical cuticular border develops. Masses of cells taken from the myotomes differentiate into muscle fibers showing fibrillæ with typical striations. When portions of myotomes are left attached to a piece of the medullary cord the muscle fibers which develop will, after two or three days, exhibit frequent contractions. In pieces of nervous tissue numerous fibers are formed, though owing to the fact that they are developed largely within the mass of transplanted tissue itself, their mode of development cannot always be followed. However, in a large number of cases fibers were observed which left the mass of nerve tissue and ex-

tended out into the surrounding lymph clot. It is these structures which concern us at the present time.

In the majority of cases the fibers were not observed until they had almost completed their development, having been found usually two, occasionally three and once or twice four days after isolation of the tissue. They consist of an almost hyaline protoplasm, entirely devoid of the yolk granules, with which the cell-bodies are gorged. Within this protoplasm there is no definiteness of structure; though a faint fibrillation may sometimes be observed and faintly defined granules are discernible. The fibers are about 1.5–3 μ thick and their contours show here and there irregular varicosities. The most remarkable feature of the fiber is its enlarged end, from which extend numerous fine simple or branched filaments. The end swelling bears a resemblance to certain rhizopods and close observation reveals a continual change in form, especially as regards the origin and branching of the filaments. In fact the changes are so rapid that it is difficult to draw the details accurately. It is clear we have before us a mass of protoplasm undergoing amœboid movements. If we examine sections of young normal embryos shortly after the first nerves have developed, we find exactly similar structures at the end of the developing nerve fibers. This is especially so in the case of the fibers which are connected with the giant cells described by Rohon and Beard.

Still more instructive are the cases in which the fiber is brought under observation before it has completed its growth. Then it is found that the end is very active and that its movement results in the drawing out and lengthening of the fiber to which it is attached. One fiber was observed to lengthen almost 20 μ in 25 minutes, another over 25 μ in 50 minutes. The longest fibers observed were 0.2 mm. in length.

When the placodal thickenings of the branchial region are isolated, similar fibres are formed and in several of these cases they have been seen to arise from individual cells. On the other hand, other tissues of the embryo such as myotomes, yolk endoderm, notochord and indifferent ectoderm from the abdominal region do not give rise to structures of this kind. There can therefore be no doubt that we are dealing with a specific characteristic of nervous tissue.

It has not yet been found possible to make permanent specimens which show the isolated nerve fibers completely intact. The structures are so delicate that the mere immersion in the preserving fluid is sufficient to cause violent tearing and this very frequently results in the tearing away of the tissue in its entirety from the clot. Nevertheless, sections have been cut of some of the specimens and nerves have been traced from the walls of the medullary tube, but they were in all cases broken off short.

In view of this difficulty an effort, which resulted successfully, was made to obtain permanent specimens in a somewhat different way. A piece of medullary cord about four or five segments long was excised from an embryo and this was replaced by a cylindrical clot of proper length and caliber, which was obtained by allowing blood or lymph of an adult frog to clot in a capillary tube. No difficulty was experienced in healing the clot into the embryo in proper position. After two, three or four days the specimens were preserved and examined in serial sections. It was found that the funicular fibers from the brain and anterior part of the cord, consisting of naked axones without sheath cells, had grown for a considerable distance into the clot.

These observations show beyond question that the nerve fiber develops by the outflowing of protoplasm from the central cells. This protoplasm retains its amœboid activity at its distal end, the result being that it is drawn out into a long thread which becomes the axis cylinder. No other cells or living structures take part in this process. The development of the nerve fiber is thus brought about by means of one of the very primitive properties of living protoplasm, amboeoid movement, which, though probably common to some extent to all the cells of the embryo, is especially accentuated in the nerve cells at this period of development.

The possibility becomes apparent of applying the above method to the study of the influences which act upon a growing nerve. While at present it seems certain that the mere outgrowth of the fibers is largely independent of external stimuli, it is of course probable that in the body of the embryo there are many influences which guide the moving end and bring about contact with the proper end structure. The method here employed may be of value in analyzing these factors.