We have therefore studied the cumulative effects of nerve impulses upon the membrane conductance of an isolated nerve fibre immersed in oil.

A typical experiment is given in the accompanying graph, Curve 1, and shows how the membrane conductance is affected by a short burst of activity. At time 0, a train of impulses of frequency 118 per sec. was initiated by means of a thyratron stimulator. At time 1·0 min. the stimulus was switched off and the membrane conductance again measured. By the end of the active period the membrane conductance had increased fourfold, but it returned rapidly to a steady level not very different from that which existed previously. These effects could have been produced by leakage of potassium from the active axon followed by a re-absorption during the period of recovery. But they might equally well have been due to some structural alteration in the membrane which did not depend upon a chemical change in the external medium. The second possibility was excluded by the fact that the time-course of recovery could be profoundly modified by dipping the nerve fibre into a large volume of sea-water for a few seconds. The effect of this test is shown by Curve 2, which was obtained in exactly the same manner as Curve 1, except that the axon was dipped into sea-water during the period *AB*. The resulting curve shows that immersion in sea-water caused the membrane conductance to return almost immediately to a value which was close to the final recovery level of Curve 1. In our view, this experiment and others of a similar kind prove beyond reasonable doubt that activity is associated with the leakage of a substance the effect of which on the nerve membrane is very like that of potassium.

The absolute amount of potassium lost by an active axon could be calculated on the assumption that the observed changes in conductance were wholly due to an increase in the potassium content of the external fluid. Eleven determinations of this kind were made and gave an average value of $1 \cdot 7 \times 10^{-12}$ for the number of moles of potassium which leak through 1 sq. cm. of membrane in one impulse. The charge of $1 \cdot 6 \times 10^{-7}$ coulomb carried by this number of potassium ions may be compared with the charge of the resting membrane. Estimated values of $1 \cdot 3 \mu F$ cm.$^{-2}$ for the membrane capacity and 60 mV. for the resting potential give the resting charge density as $7 \cdot 8 \times 10^{-8}$ coulomb cm.$^{-2}$. The amount of potassium lost in each impulse therefore appears to be more than sufficient to discharge the membrane capacity in the manner required by the membrane theory.

During the period of recovery, potassium appeared to be re-absorbed at a rate of approximately 3×10^{-10} mol. cm.$^{-2}$ sec.$^{-1}$ when its external concentration had increased threefold. This re-absorption may be thought of as an active process of a secretory type, but it can also be satisfactorily explained in terms of the type of Donnan equilibrium proposed by Boyle and Conway[8].

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Transformations of the Retinal Ganglionic Cells in Tissue Cultures

It is well known that axons of the retinal ganglionic cells are characterized by a strictly radial direction. They converge in the optic papilla similarly to the spokes of the wheel and make their further course by the way of the optic nerve and its chiasma.

The study of their regenerative process in tissue cultures in various vertebrates revealed a regularity which is of much interest and is obviously peculiar to the retinal ganglionic cells. The growth of regenerating axons in all organisms studied always develops in the same direction inside the explant as well as at their passage to the substratum of fibrin. It corresponds to the side along which the old axon passed. The course of the retinal axons is strictly polar. Under conditions of explantation, the growing fibres seem to push towards the exit of the optic nerve, that is, to the retinal papilla. This direction, as a rule, is maintained in all explants; in all and certainly in all vertebrate retinal ganglionic cells studied by us without exception and independently of the area from which the tissue was taken. Thus, according to the direction of the growing nerve fibres, it is quite possible to determine the side of the explant which in the organism was directed towards the exit of the optic nerve.

All the nerve fibres growing in the zone of growth of warm-blooded organisms (young rabbit, chick) are about the same size, diverge radially, branch, divide, form collaterals, cross and overlap one another. The fibres terminate in distal cones of growth. They exhibit many varicosities. Living nerve fibres are very delicate and highly refractive of light. They show *in vivo* a characteristic winding neurofibriallary striation.

The axons of the ganglionic cells in Amphibia (adult axolotl) growing in the zone of growth are distinguished by their thickness. They emerge radially from one of the sides of the explant, some of them showing a repeated dichotomic division. The length of such axons grown *in vitro* may reach 1 cm. The neurofibrillæ of the axons of the ganglionic cells of the retina in the axolotl subjected to vital study are recognized as straight, sharply outlined parallel bundles. The fibres of these cold-blooded organisms *in vitro* revealed a process of longitudinal splitting which often was incomplete, giving an illusive appearance of anastomoses or synditial connexions. In the old cultures there were still observed peculiar swellings, 'joints', along the course of the nerve fibre which, obviously, were of retrogressive character.

The growing retinal nerve fibres of the adult crucian showed *in vitro* a different modification. At the initial stage of growth they formed a herb-like bundle consisting of a mass of nerve fibres closely applied to one another. They passed to the zone of growth smoothly twisting and sometimes reaching also 1 cm. in length. Such a long bundle of nerve fibres reminded one of a small branched tree composed of fibres interlacing and crossing at a definite point (chiasma ?) and terminating in thin cones of growth. The neurofibrillæ in the axon clearly seen *in vitro* were twisted along their course corkscrew fashion, and showed an aspect of stretched spirals repeating the general outline of the nerve fibre.

The growth and regeneration of axons (and probably of the differentiation of the nerve cell in whole) are caused not so much by chemotaxis[1], the difference of electric potentials[2] or stereotropic conditions of the surroundings[3], as by the spatial polarity of the ganglionic cell itself. The polarity is due to its albuminous structure manifested in a definite orientation of the neurofibrillæ.

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Demonstration of Alkaloids in Solanaceous Meristems

A general concensus of opinion among investigators has agreed that Bouchardat's reagent is the most reliable among many tried for the cytochemical demonstration of alkaloids. It is a general alkaloid reagent, with no specificity, and consists of 1 per cent iodine dissolved in 1 per cent potassium iodide. It colours proteins very heavily. With practice, the red tint and fine grain of the alkaloid precipitate can be readily distinguished from the flocculent brown of the protein. Errera[1] devised the further expedient of treating parallel preparations with a 5 per cent solution of tartaric acid in alcohol. This dissolves out the alkaloids so that a clear distinction should be apparent in alkaloid-containing tissues with and without such pre-treatment. No difference will be apparent if the iodine precipitation is due to protein.

These devices enable a rapid and reliable determination of the distribution of alkaloids to be made with mature parenchymatous and similar tissues, where the alkaloid precipitates can be seen in the large vacuoles surrounded by the thin protein layers of the cytoplasm. In attempting to apply them to the root and stem meristems of solanaceous plants, serious difficulty was encountered. It was desired to determine as accurately as possible the moment of the first appearance of alkaloids in the germinating seedling, the resting embryo being itself alkaloid-free. The detection of minute amounts of alkaloid among the copious protein of the meristematic cells proved quite impracticable. Attempts at a micro-extraction with ammoniated chloroform, followed by a Vitali-Morin[2] treatment of the extract were not successful owing to the moisture contained in the tissue. It was found, however, that a good separation of alkaloid and protein could be achieved if the cellular membranes were first broken down. This was done by treating the tissue to be examined with ether. The small piece of excised tissue, root-tip, etc., is placed upon a microscope slide and surface dried with filter paper. One drop of ether is then dropped on it with a glass rod and allowed to evaporate. Evaporation of the ether must be complete, or iodine will be precipitated from the reagent as minute black crystals. When the ether is completely gone, the slide is transferred to the stage of a microscope and the tissue focused under a half-inch objective. One drop of Bouchardat's reagent is then applied from a glass rod and the tissue is kept under observation from the moment of application. If alkaloids are present, a red cloud diffuses out of the tissue and spreads for a short distance into the surrounding fluid. The brick-red colour and finely granular appearance are very characteristic and are due to the alkaloid which is now free to escape as water-soluble salts from the denatured proteins. The reaction is transient and the tissue should be kept under observation from the moment of application of the reagent. Tissues containing abundant alkaloid give dense reddish clouds of precipitate; after the extraction of the alkaloids with 5 per cent alcoholic tartaric acid no red cloud is produced.

Very small amounts of alkaloid associated with an excess of protein can be quickly and easily discovered in this way and minute quantities of tissue, such as a single meristem, suffice.

This technique has been applied to germinating seedlings of *Datura stramonium*, *D. stramonium* var. *inermis* and *D. tatula*, also to older roots of *Atropa belladonna*, with pleasingly definite results. *Datura* seedlings were germinated on washed sand and examined as soon as the radicle began to push through the testa, and at subsequent stages. Roots 1 and 2 mm. long were found to be entirely devoid of alkaloid: roots 3 mm. long gave a slight reaction and longer roots gave copious reactions. The cloud appeared from the surface of the treated root just behind the root-cap, that is, from the meristem proper. There was no reaction from the root-cap itself, nor, at first, from the elongation zone. In older roots this also began to give a reaction, but in roots carried on to a stage of starvation, the reaction disappeared even from the meristem. The results were identical for all three varieties of *Datura* used.

As soon as the cotyledons were expanded, the shoot meristem was also examined. A positive reaction for alkaloids was given by the smallest apex that it was possible to dissect out and also by the rudiments of the first two leaves. The cotyledons and the hypocotyl, intervening between the shoot and root meristems, gave no reaction at this stage. The amount of precipitate formed from the shoot apex was noticeably smaller than that from the root: but in *A. belladonna* seedlings, grown in sandy soil to a six-leaved stage, the relations were reversed. A copious red cloud was obtained from the dissected-out stem tip and little or none from that of the root.

These results confirm those of the older investigators of similar materials, and indicate that alkaloids appropriate to the species are very rapidly synthesized by cells in a phase of active metabolism and growth. The effectiveness of the ether treatment suggests that