from the irradiated control stumps, but these outgrowths in all cases were simple cartilaginous spikes covered by skin. After a dosage of 3500 r or more, there was complete absence of regeneration on the control side, but all x-rayed limbs that were provided with unirradiated epidermis showed some hand regeneration. This included the experimental limbs in the groups that received 3500, 5000, 7500, or 10,000 r. There were at least 5 useful cases in each radiation group except in the group that received 10,000 r. All but one animal in the 10,000-r group died before regeneration had been completed. The type and amount of regeneration is shown for 4 representative cases in Fig. 1.

Appreciable growth potential remained in the 500-r series when all tissues were irradiated. When unirradiated epidermis has been added, the form of the regenerate is better and the amount of regeneration is greater. With dosages from 3500 to 10,000 r there was no outgrowth after both internal tissues and epidermis had been irradiated. However, there was some regeneration even from atrophied stumps whose internal tissues had received higher dosages, provided that they had been covered with unirradiated epidermis. Diminishing size of regenerate with increasing dosage may be accounted for in large part by the decreasing growth potential of the internal tissues. At 3500 r and above there was no outgrowth from controls and the stumps became smaller. From this dosage upward, any regeneration that occurred in the right limb must have resulted from the unirradiated epidermis.

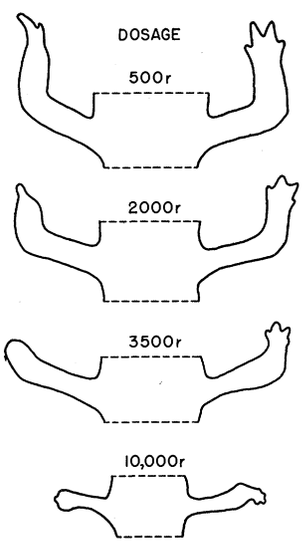

Fig. 1. Representative regenerates showing extent and type of regeneration after various x-ray dosages. The limbs were irradiated below the elbow. All limbs were amputated half way between the wrist and elbow. Left, regeneration after simple amputation through forearm; right, regeneration after amputation and removal of skin to a point above the elbow. The limbs on the right received unirradiated epidermis. The diagrams are drawings from photographs of the same magnification.

Some of the decrease in size of regenerates may result from increasingly deleterious effects of the x-rayed stumps upon the regenerates. This was quite apparent in the 10,000-r series, where failure of the epidermis to maintain a complete cover around and over the stump was observed. In spite of this there was regeneration of a recognizable hand on the one 10,000-r animal that lived.

The possibility that the unirradiated epidermis reactivated the internal tissues has been considered seriously (*16*), but all evidence is against it. As shown by Brunst (*12*), x-rayed limbs remain for years incapable of regeneration. There is additional counterevidence in the present work. The stumps of all x-rayed limbs lost some volume. The presence of an unirradiated epidermis does not reactivate to protect against this loss. In all cases the shrinkage of the stump was the same whether irradiated or unirradiated epidermis was present. This is evidence against reactivation.

Direct evidence that only the unirradiated tissue participates in regeneration was obtained by Umanski (*47*). After a dosage of 5000 r to hind limbs of black axolotls whose internal tissues are dark, followed by transplantation of forelimb skin from white axolotls and subsequent amputation, completely white forelimbs regenerated on the stumps of the hind limbs. Umanski suspected that the dermis was the source of the regeneration cells, but the hypothesis was not tested.

It is concluded that epidermis *can* serve as the only source of cells for a regenerating limb. It now appears likely that any limb tissue can serve as a source of regeneration cells. Whether one is the predominant source both in larvae and adults is not clear. Better regenerates were obtained from x-rayed stumps when normal whole skin was transplanted than when either muscle or bone was used (*18*).

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Action of Pectic Enzymes on Surface Cells of Living Brassica Roots

Robert's conclusion (*1*) that the root-hair wall consists of an inner layer of cellulose and an outer layer of calcium pectate continuous with corresponding layers in the hair-forming cell has been confirmed and denied (*2*). I obtained strong confirmatory evidence (*3*) from microchemical tests and by growing roots of *Brassica* seedlings in a variety of cultural solutions that were designed either to prevent or to stimulate calcification of the pectic layer. In this connection, the experiments with ammonium oxalate solutions were particularly convincing (*3*). A theory of root-hair development was formulated, that was based on the gradual hardening of the outer pectic layer to calcium pectate (*2–4*).

More recently, Ekdahl (*5*) considers the pectic and cellulosic substances to be uniformly distributed in the hair-wall and not separated into two distinct layers. In his opinion, calcification does not occur and hardening is due entirely to changes in the cellulosic substances.

Ekdahl's view still lacks direct proof and does not explain the fact that anything which prevents calcification also prevents root-hair formation. Furthermore, in denying the existence of an outer pectic layer or so-called middle lamella substance, his view fails to explain many well-known cellular phenomena such as the normal sloughing of root-cap cells, the formation of intercellular spaces, and the maceration of multicellular tissues by pathological organisms and chemical reagents.

The recent use of pectic enzymes in the maceration of plant material (*6*) suggested that they might be useful in the present problem. If an outer cementing layer of pectic material occurs in the epidermal cell walls, then it should dissolve on treatment of the root with pectic enzymes. To test the validity of this assumption, *Brassica* seedlings (*Brassica napus* var. oleifera), were grown in several different preparations of pectic enzymes

under the trade name Pectinol (*7*) in different concentrations and at different temperatures. Only one, namely, Pectinol 100 D, was found to be effective. *Brassica* roots were used because it was known that they produce abundant hairs in tap water (*2, 8*).

Following germination, when they had attained a length of 1 to 3 millimeters, the roots of ten seedlings were placed through small holes in pieces of stiff, paraffin-coated paper and floated in tap water in small beakers, at 30°C. At the end of 6 hours, the floats were transferred to a filtered solution of Pectinol 100 D.

Great difficulty was experienced in keeping the roots alive. However, by dusting the seeds with Orthocide prior to germination and by means of the method described previously (*3*), it was found possible to grow roots in a 0.75-percent flowing solution of Pectinol 100 D in distilled water at 30°C. At the end of about 16 hours, only about half of the roots showed signs of growth. These roots measured 5 to 15 millimeters, about half the length of control roots in tap water. All treated roots had an unusual appearance characterized by the lack of hairs, discoloration, plasmolysis, separation of epidermal cells, and abnormal sloughing of root-cap tissue. Some epidermal cells were free at one or both ends (Fig. 1 A), others were twisted out of position, and still others had dropped from the root, leaving empty gaps in the epidermis. The walls of the separated cells were thin but distinct and gave a positive test for cellulose. The deformities were such that they could be explained on the basis of enzyme action on the outer, cementing pectic layer, leaving the cellulose layer intact.

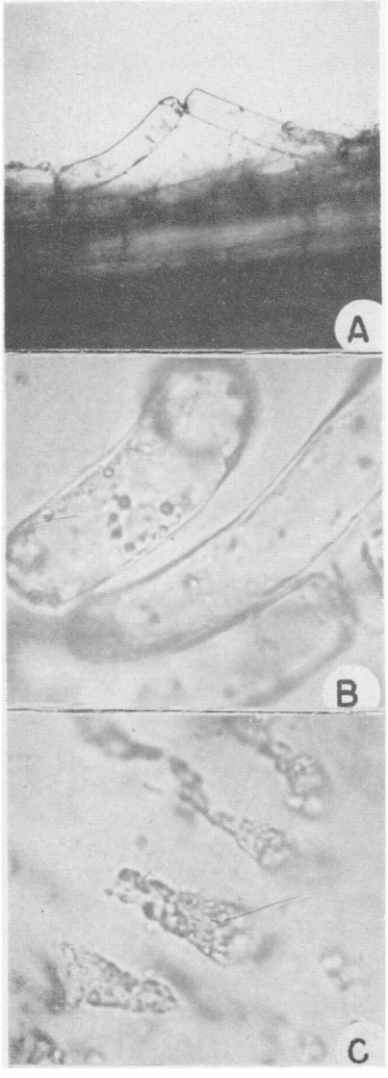

Fig. 1. *A*, epidermal cells of a root grown in a 0.75-percent flowing solution of Pectinol 100 D (×135). Separation of the cells indicates a change in the pectic layer. *B*, normal root-cap cells (×660). *C*, root-cap cells from the same treated root shown in *A*, showing complete dissolution of the cell walls (×660).

As in the earlier experiments with ammonium oxalate solutions (*3*), transfer of Pectinol-100-D-treated roots to a saturated solution of calcium sulfate resulted in resumption of normal growth, with the cessation of abnormalities and the immediate production of long hairs. That part of the root developed in the Pectinol 100 D solution remained unchanged. Some roots failed to respond, indicating irreparable injury to the apical meristem.

The condition of the root cap was of particular interest. In control roots, it formed a distinct, uniform covering over the root apex, the sloughed cells possessing healthy protoplasts and firm definite walls (Fig. 1B). In sharp contrast, the root caps of Pectinol-100-D-treated roots presented a discolored mass of partially or wholly macerated cells. In some cells the wall was thin, but distinct and definitely of cellulose, in others it was barely visible, and in still others it was dissolved completely (Fig. 1C).

The results of the experiments with pectic enzymes confirm earlier observations (*1–3*) of the occurrence of an outer layer of pectic material in the walls of the epidermal cells. If there were no definite cementing layer, it is inconceivable how individual cells could separate. The observations of recovered roots after removal to a calcium solution also corroborate the further view (*2–4, 8*) that hardening is the result of incorporation of calcium into the outer pectic layer of the elongating cell walls.

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Response of the Slime Mold to Electric Stimulus

There are four fundamental properties of protoplasm of peculiar interest to the student of the nervous system. These are (i) that a change in the physical or chemical environment of protoplasm brings about an alteration in the phase boundary of the protoplasm that can be identified by (ii) the change that is propagated through its substance, usually with direction, (iii) that protoplasm possesses the property of integrating, coordinating, or correlating all the events that occur at the phase boundary, and (iv) that protoplasm reacts in a characteristic manner to this chain of events.

Although something is known about these four properties, the exact mechanisms involved have not yet been unraveled. However, students of the nervous system have presented a great deal of evidence concerning the nature of the physical and chemical changes in the environment necessary to stimulate a neurone. In addition, a good deal of information is available concerning the propagation of the stimulus through protoplasm. In this last case, the advent of electrometric techniques has made it possible to define, with considerable accuracy, quantitatively and qualitatively, the electric aspects of the propagated impulse.

Since the great complexity of the nervous system in vertebrates and also in invertebrates makes analysis of the mechanisms involved exceedingly difficult, an attempt has been made to approach this problem in a very much simpler but still very complex living organism, the slime mold, *Physarum polycephalum* (*1*). Tasaki and Kamiya (*2*) have reported that the slime mold will respond to a tap stimulus and to an electric stimulus in much the same manner. Using a very high input impedance direct-current amplifier coupled through a counter electromotive force to the direct-current amplifier of a Dumont 304H oscilloscope, similar responses were obtained by us with some very striking differences.

Figure 1 is a photograph of the oscilloscope trace following a tap stimulus. This response is a graded response. A weak stimulus is sufficient to start it; it