

THE EFFECT OF BETA-INDOLE-ACETIC ACID UPON
ISOLATED PLANT EMBRYOS

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Embryos of three species of kidney bean (*Phaseolus multiflorus*, *P. vulgaris* and *P. nana*) were removed from their seed coats and cotyledons and grown on a sterile nutrient medium with or without the addition of beta-indole-acetic acid. The nutrient medium was made by diluting a stock salt solution* with nine times its volume of distilled water; to every hundred cc. of the dilute solution were added 2 grams of glucose, 1 gram of agar and 1 drop of 0.1% boric acid solution. The medium was poured into Petri dishes, vials or test-tubes and sterilized for at least an hour in an Arnold sterilizer. The embryos were removed with sterile forceps, and placed in or on the agar medium.

Embryos so treated begin to show growth in 2-3 days; on the fourth day both the hypocotyl and the radicle show marked elongation and the radicle has well-developed root hairs. While the rate of growth of the isolated embryos is slightly less than that of whole seeds germinated in the laboratory on moist paper, and the resulting plants are not as large as those from intact seeds, in all other respects the cultured embryos appear normal.

The effect of heteroauxin (Beta-Indole Acetic acid, obtained from the British Drug Houses Ltd.) upon these isolated embryos was tested. In a series of experiments, two sets of cultures were started at the same time; in one, the embryos were grown in the standard medium and in the other, embryos from seeds of similar origin and of approximately similar size were grown in the standard medium to which had been added either 7.5 mg. or 10 mg. of beta-indole-acetic acid per 100 cc. of medium. Out of 60 embryos grown in the medium with heteroauxin, 13 did not grow at all, 2 showed no difference from those grown in the auxin-free medium, and 45 showed marked abnormalities. These abnormalities consisted in (1) inhibition of growth of the radicle, (2) great enlargement of the diameter of the hypocotyl and (3) stimulation of the growth of secondary roots. Of 42 control plants, grown in the auxin-free medium, 7 did not grow, and 35 developed into normal, though small, plants, some of which were kept growing for more than a month.

The most conspicuous effect of beta-indole-acetic acid upon bean embryos is the swelling of the hypocotyl, which becomes apparent 3-5 days after the embryos are "planted," subsequently increasing to relatively enormous size, and in many cases rupturing the epidermis. Similar results have been obtained by Solacolu and Constantinesco¹ and by Gautheret² with plant embryos, and by Kraus, Brown and Hamner³ and Hamner and Kraus⁴ with young seedlings. In

* 1.416 gm. CaNO_3 ; 0.669 gm. KCl ; 0.136 gm. KH_2PO_4 ; 0.808 gm. KNO_3 ; 0.740 gm. MgSO_4 ; 0.034 gm. $\text{Fe}(\text{SO}_4)$, and 1000 ml. H_2O .

isolated *Phaseolus* embryos the swellings appear to be due both to the enlargement of cells beneath the epidermis and to their multiplication; there is also some indication that cell differentiation without any organization takes place. The mass consists of abnormally large cells of varied and peculiar shapes. Living cells scraped from the mass are oval, lemon-shaped or crescentic, with a single large vacuole; the nucleus is pressed against the cell wall and the cytoplasm is filled with aggregates of granules which are particularly numerous around the nucleus and which give a positive test for starch. A preliminary study of sections of these embryos fixed in Bouin and stained with crystal violet and erythrosin indicates that there are patches of vascular tissue within the cellular mass, and that in these patches the vascular elements have not their normal relationships.

The development of the primary roots is suppressed in the embryos grown in the medium with beta-indole-acetic acid, and that of the secondary roots is delayed, but ultimately stimulated. Secondary roots begin to project through the swellings in about two weeks in these embryos; in those grown in auxin-free medium they appear in a week or less. Sections of the swollen hypocotyls show the anlagen of the secondary roots growing out into the amorphous cellular mass; these appear on preliminary study to be normal in their development once that begins.

Embryos grown for four days on an auxin-free medium and then transferred to one containing 10 mg. per cent of beta-indole-acetic acid develop swellings in four days, while the growth of radicle and secondary roots, already started, continues apparently unchecked. Conversely, embryos grown for four days on a medium containing auxin, and then transferred to one without it, show no increase in the size of the swelling but no diminution of it, and after four days both the primary and the secondary roots begin to grow rapidly.

Attempts to culture cells taken from the swellings have so far been unsuccessful, but will be continued.

To test the effect of heteroauxin upon animal embryos, beta-indole-acetic acid was added in concentration of 10 mg. percent to the sea water in which the eggs of *Echinarachnius* had been fertilized and reared to the pluteus stage. No effect of the growth hormone in this concentration was evident. A series of experiments upon animal embryos with different concentrations of heteroauxin would be desirable, although the indications are that no effect is produced until the concentration is so great as to become lethal.

REFERENCES

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