er drops were allowed to fall vertically. The collisions took place in an electric field of 600 volt/cm. By moving the position of the hole in the bottom plate with respect to the point of collision, it was possible to capture *independently,* in the Faraday cage below, the large majority of either the larger or the smaller secondary drops.

We discovered that both of these fractions were highly and equally electrified but that the charges were of opposite sign. Specifically, the larger secondary drops acquired a charge of the same sign as that on the top plate, while the smaller drops had the same charge sign as the bottom plate. The larger drops always fall faster in the free atmosphere, so that once the direction of the inducing electric field E is specified, the arrangement of positive and negative charges is determined. Our measurements show that the induced free charges, Q, in electrostatic units on the oppositely charged fractions are

$$Q = 4 E_o a_o^2 \qquad (1)$$

where, in order to simplify the application of our measurements, we have approximated by assuming that a_0 is the radius of the larger drops *before* collision. The coefficient of Eq. 1 is dependent on the relative kinetic energy at collision; the value given applies to the conditions of our experiments, which we have selected as typical of heavy rain.

Since the larger raindrops always fall away from the slower-moving, smaller drops, the net result of disjections in an electric field is a separation of free charge and the establishment of a bipolar pair of free-charge sheets vertically spaced a few kilometers apart and serving as the upper and lower boundary of a volume distribution of a nearly neutral mixture of highly charged positive and negative drops. Moreover, the charge separation under the action of gravitational acceleration is always in such a direction as to neutralize any preexisting electric field. Usually this neutralization is not complete. It appears that the disjection electrification process is normally a stabilizing influence.

In considering the application of disjection electrifying processes to thunderstorms, one notices first that this electrification does not occur when atmospheric electric fields are absent, and therefore it plays little part in the initiation of thunderstorm electrification.

I have emphasized that the typical lightning discharge not only neutralizes the observed initial electric fields but frequently produces a reverse field that approximates the intensity of the initial field (*2*). During the main part of typical storms this electric field is due to a persistent free positive charge maintained at relatively low altitudes. This charge establishes a more or less continuous positive electric field above it so that at the altitudes where rain is formed and where disjection of colliding raindrops is frequent, the electric field is outward and positive. The resultant generated bipolar free charge sheets tend to neutralize this field, and free positive charge accumulates at the upper boundary of the rain-forming regions and free negative charge a few kilometers below.

Such a charge distribution results in quite rapid discharge of the positive free charge at the top and thus contributes to the maintenance of the fair weather field, whereas the negative charge at the lower altitude may build up to produce lightning. The net result is that the generated bipolar sheets are unstable and shortly degenerate into predominantly negative charge sheets somewhere near or below the freezing level. Wind shear also sometimes plays a part in converting bipolar sheets into effectively unipolar distributions of free charge. A qualitative evaluation of collisions and disjection of raindrops in the earth's persistent electric field during periods of electrical activity shows that disjections are extremely important. Their outstanding result is that charges of opposite sign are induced predominantly on the larger and smaller secondary drops; gravitational forces thereafter naturally account for the observed gross separations of free electricity necessary to produce lightning.

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Differentiation of Tobacco Plants from Single, Isolated Cells in Microcultures

Abstract. *Single cells isolated from pith callus of fresh stem of hybrid tobacco* (Nicotiana glutinosa ♂ × N. tabacum ♀) *gave rise to small colonies of cells in microcultures which upon transfer to agar medium produced clones of callus tissue. These single-cell clones differentiated roots, and shoots with green leaves, on a completely defined nutrient medium. The rooted shoots developed into normal plants after transfer to soil in pots in the greenhouse. Buds and flowers were produced by these plants.*

During the last 10 years evidence for the growth of fully differentiated and organized plants from free cells, either suspended in liquid medium or dispersed on semi-solid medium, has come from several different laboratories. The earlier reports emphasized the importance of substances like coconut milk in the medium (*1*). The same results have, in the last couple of years, been achieved on defined synthetic media (*2*). This work has thrown considerable light on problems of totipotency and morphogenesis, yet single cells of flowering plants removed from the vicinity of other cells and grown in complete isolation have failed to give rise to entire plants. We now report, for the first time, the differentiation of completely organized plants, capable of producing flowers, from single cells grown in isolation from all other cells and without the "nurse tissue" or the "conditioned medium."

An earlier communication (*3*) dealt with the growth and tissue formation from single, isolated tobacco cells in microculture. These single-cell clones of tissue failed to differentiate roots and shoots, possibly because the callus from which the single cells were isolated had been subcultured for about 8 years. Fresh callus was, therefore, isolated

from stem pith and was used as a source of single cells.

Tissue pieces, approximately 0.5 cm long and 0.25 cm in diameter, were removed aseptically from the stem pith of hybrid tobacco (*Nicotiana glutinosa* ♂ × *N. tabacum* ♀) and grown on agar D medium (*4*). After 4 to 6 weeks of growth, portions of callus produced (weighing 150 to 200 mg) were transferred to liquid C medium (same as D but without 2,4-D) on a reciprocating shaker (60 cy/min) in the dark. These dissociated into large numbers of single cells and groups of cells. Other media like Murashige and Skoog's (MS) (*5*) synthetic medium and D medium did not support growth and further dissociation of the fresh pith callus as well as the liquid C medium did. The period of rapid growth (as determined by increase in wet weight) and dissociation of the callus (number of single cells formed) coincided during the 9th to 14th days after transfer to the liquid medium. The capacity of the fresh pith callus for dissociation into single cells never equaled that of the old callus. Dissociated single cells from fresh pith callus showed greater variation in shape and size than cells from calluses several years old. Single cells were hand-picked with Pyrex glass micropipettes, under a dissecting microscope, from petri plates containing 0.2 to 0.5 ml of 9- to 14-day-old shake cultures and 15 to 20 ml of fresh liquid C medium. The drop containing fresh medium and the single cell was placed in a microculture which had been prepared in advance, and future observations were made according to the technique previously described (*3*).

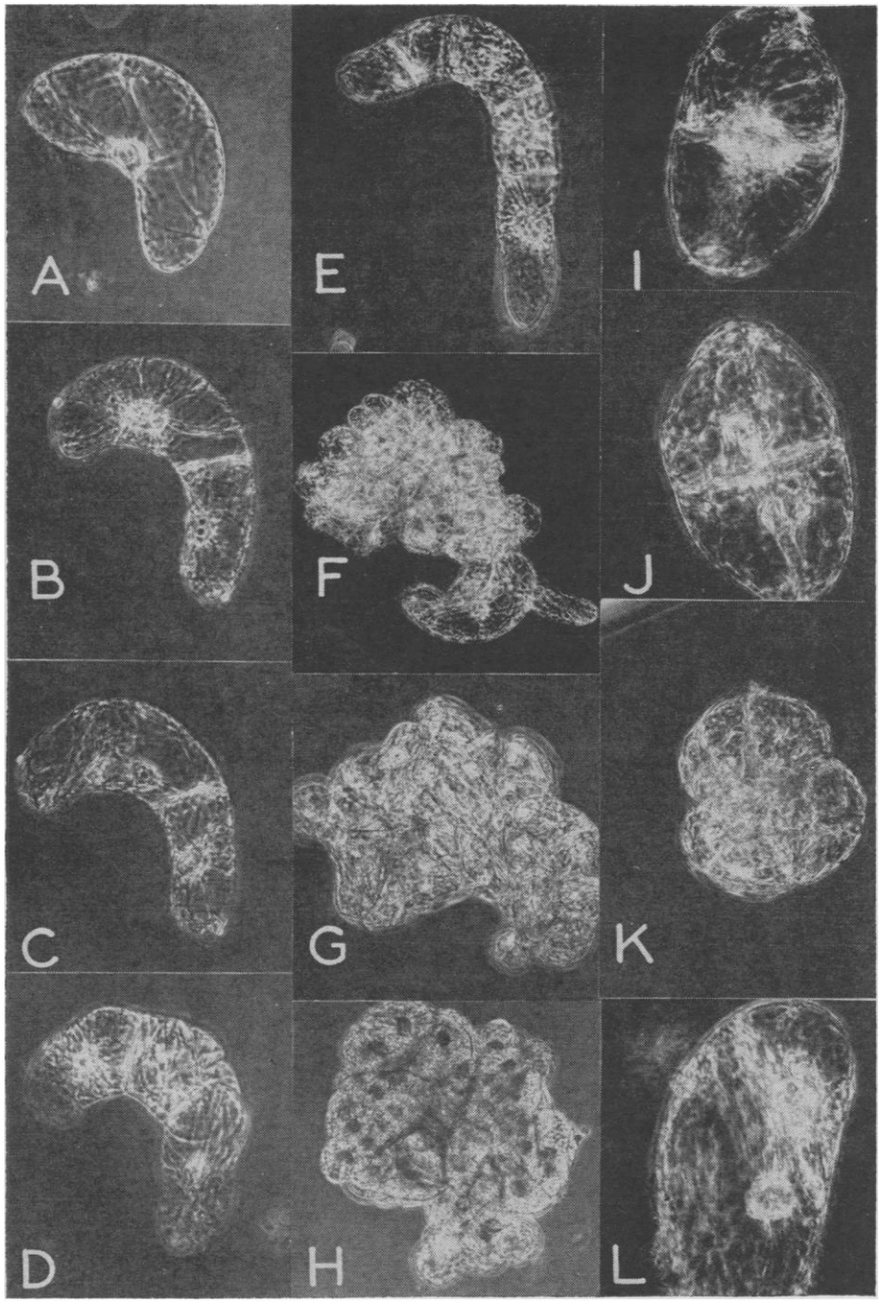

Fig. 1. Development of a mass of cells from a single cell of tobacco isolated in microculture from fresh-stem pith callus and variation in the pattern of cell division. *A*, Single cell 1 day after microculture (× 750). *B–H*, Stages in the formation of a mass of cells from the single cell in *A* (*B–D*, × 600; *E*, × 470; *F*, × 375; *G*, × 300; *H*, × 230). *I*, Another single cell in first division (× 750). *J*, Same, showing a quartet of cells (× 750). *K*, Mass of cells in the same series (× 300). *L*, Another cell after the first division; note the plane of cell wall formed (× 930).

A total of 150 single cells was isolated from the fresh pith callus; of these, 32 divided. Although the percentage of dividing cells could perhaps be increased by selecting smaller and more active cells, in none of the experiments did as many single cells from fresh pith callus divide as from calluses several years old. The single cells of tobacco showed the usual contents and some starch grains which were metabolized soon after the cell had been placed in the microculture (Fig. 1*A*). The first division, which gave rise to two cells (Fig. 1*B*), took place by the 4th day and was at right angles to the long axis of the cell (Fig. 1*I*).

The formation of an inclined wall at the end of the first division was also noticed (Fig. 1*L*). However, unlike the rigid sequence of cell divisions seen in single cells isolated from old calluses, here the second division was either parallel, or at right angles, to the first division giving rise to a filament (Fig. 1, *C* and *D*) or a quartet (Fig. 1*J*) of cells. Depending upon the next few divisions, a uniseriate filament (Fig. 1*E*) or a mass of cells was formed (Fig. 1*K*). The successive divisions in all planes resulted in an irregular and unorganized mass of 50 to 75 cells in 10 to 15 days (Fig. 1, *F–H*). These cell masses were established as clones of tissue when transferred to agar D medium in culture bottles (Fig. 2*A*). Fifteen such single-cell clones have been established from the 150 single cells initially isolated from the fresh pith callus.

At no stage did the pattern of division of the single cells of tobacco

grown in microcultures or suspension, or their derivatives, resemble or simulate any stages of normal embryogeny as is the case in carrot (*1*) and parsley (*6*).

Having obtained clones of single cells isolated from fresh pith callus, the next objective was to induce differentiation and organ formation in these clones. Although controlled shoot and root formation in some strains of tobacco pith callus has long been known (*7*), it was important to affirm whether the clones obtained from completely isolated single cells would also have the ability to produce roots and shoots if given the proper nutrition and other physiological conditions.

Out of the many single-cell clones, two were randomly selected for differentiation experiments. Since no differentiation was observed on C or D agar media (Fig. 2*A*), the synthetic MS medium in conjunction with indoleacetic acid (IAA) and kinetin, alone and in various combinations, was used. In the first series of experiments in the dark, shoots with small leaves developed in the MS medium containing 1.5 mg of IAA and 5 mg of kinetin per liter. No visible signs of differentiation were seen until the cultures were 8 to 10 weeks old, although the loose and friable callus did become firm and hard. Microscopic examination revealed small roots only in a medium containing 4 mg of IAA and 0.04 mg of kinetin per liter. These roots, however, could not be seen without teasing and dissecting out the callus.

Since it took nearly 3 months for the callus to differentiate into shoots and roots if stocks were used from agar D medium, in the next set of experiments stocks grown for 6 weeks on agar MS medium containing 1 mg/liter each of IAA and kinetin or 5 mg of IAA and 0.1 mg of kinetin per liter were tried. Vegetative buds arose from meristematic zones organized on the periphery of the callus tissue, and within 3 to 4 weeks shoots with leaves were seen in nearly all bottles (Fig. 2*B*). It appeared that IAA, at this stage, could perhaps be eliminated since kinetin (3 mg/liter) alone induced the desired differentiation. All bottles kept in the dark, or in a dark room with occasional short periods of low-intensity light, or in continuous, low-intensity light showed a large number of shoots with leaves. Light, therefore, was apparently not necessary for the induction of shoot formation, although the leaves were dark green and much better developed in light (Fig. 2*B*). After more than 8 weeks roots appeared at the base of vigorously growing shoots in all bottles with or without IAA, but only under continuous, low-intensity light (Fig. 2*C*). Roots rarely ever differentiated in a similar

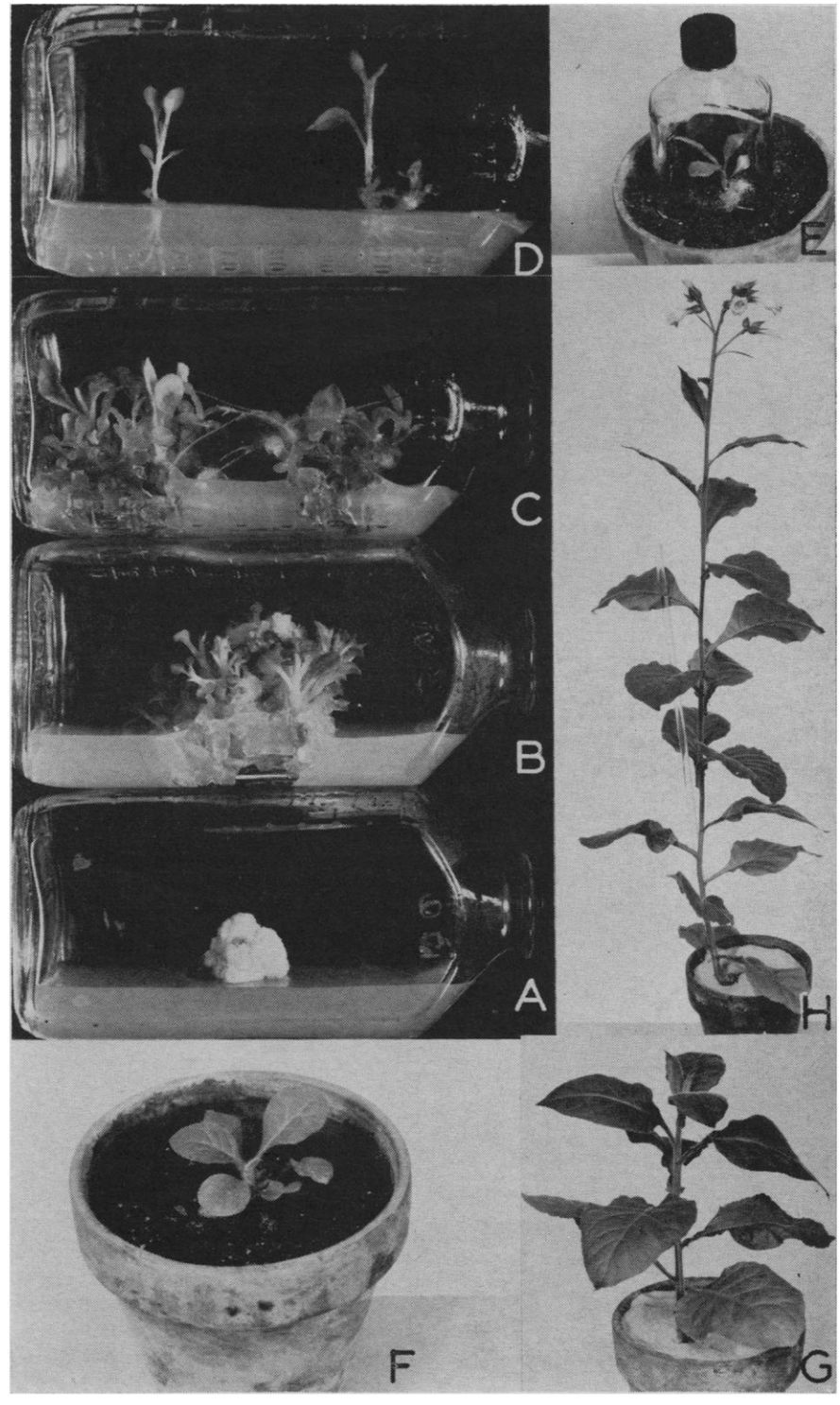

Fig. 2. Differentiation and plantlet formation in a single-cell clone of tobacco. *A*, Undifferentiated mass of callus. *B*, Differentiation of a large number of shoots with normal leaves. *C*, Root formation in plantlets. *D*, Plantlets with roots and shoots (*A* in D medium; *B* and *C* in MS medium; *D* in White's medium; all ⅔ natural size). *E*, Plantlet transferred to soil in greenhouse; inverted culture bottle retains high humidity around the young plant (⅓ natural size). *F–H*, Various stages in the further development and eventual flowering of the tobacco plants initially transferred from the culture bottles (*F*, ½ natural size; *G*, ⅓; *H*, ⅙).

set kept in the dark. Since it took more than 8 weeks for root development, many of the shoots were already turning brown. To overcome this browning and also to shorten the time required for root formation, healthy shoots from these bottles were transferred to bottles containing White's basal medium without any growth substances. Roots appeared at the end of each such shoot within 10 days (Fig. 2*D*). In this manner hundreds of tobacco plantlets with roots and shoots were obtained in vitro.

Finally these plantlets, both those grown directly from MS medium and those with roots produced on White's medium, were transferred to sterilized soil in pots in the greenhouse. The plantlets in the pots were covered by the top of the broken culture bottle for 10 to 15 days to keep the humidity high (Fig. 2*E*). More than a dozen such plants are now growing in the greenhouse (Fig. 2, *F* and *G*). Flower buds and regular flowers have also been produced by a number of these plants (Fig. 2*H*).

It has thus been demonstrated that from a completely isolated vegetative cell (single) of tobacco a whole plant capable of producing flowers can be obtained under controlled nutritional and physiological conditions. This demonstration confirms further the totipotency of single cells (at least in tobacco); such cells can grow, divide, and produce a colony of cells that can be induced to give rise to whole plants.

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Pituitary Gland: Enzymic Formation of Methanol from S-Adenosylmethionine

Abstract. *An enzyme has been found that can transform* S-*adenosylmethionine to methanol and* S-*adenosylhomocysteine. This enzyme is highly localized in the pituitary gland of several mammalian species.*

Methyl-*S*-adenosylmethionine, labeled with C^{14}, has been found to be useful for assaying methyltransferase enzymes and detecting new methyltransferases. In measuring methyltransferase activity, C^{14}-methyl-*S*-adenosylmethionine is incubated with enzyme and substrate, the C^{14}-methylated product formed by the enzyme is extracted into a suitable solvent and the radioactivity is measured (*1*). The use of C^{14}-methyl-*S*-adenosylmethionine made it possible to demonstrate a new catecholamine, *N*-methyladrenaline, in mammalian adrenal gland (*2*) and the formation of a wide variety of unusual *O*-methylated catechols (*3*). In the search for new transferase enzymes, C^{14}-methyl-*S*-adenosylmethionine is incubated with tissue extracts, and the products formed from normally occurring substrates are identified. One such enzyme found was a nonspecific enzyme in rabbit lung that *N*-methylates serotonin, nornicotine, normorphine, and numerous other amines (*4*). We wish to describe a new enzyme that forms methanol from *S*-adenosylmethionine.

In a study of the tissue distribution of the adrenaline-forming enzyme (phenylethanalamine *N*-methyltransferase) we observed that the incubation of pituitary glands with C^{14}-methyl-*S*-adenosylmethionine resulted in the formation of a radioactive product that was extractable into organic solvents such as isoamyl alcohol. When the solvent was concentrated in a vacuum for identification by paper chromatography, a considerable portion of the radioactivity was lost, suggesting the presence of a volatile radioactive metabolite. To further characterize the enzyme and the metabolic product, C^{14}-methyl-*S*-adenosylmethionine was incubated with various subcellular fractions of the bovine pituitary gland (Table 1). After incubation the mixture was extracted with isoamyl alcohol and radioactivity was measured in portions of the extract before and after evaporation in a stream of warm air. After evaporation it was apparent that considerable amounts of the radioactivity had been lost. Heating the pituitary extract at 100°C destroyed all activity and thus demonstrated that the volatile product was formed enzymatically. The ability to form the volatile metabolite resided mainly in the soluble supernatant fraction. The nonvolatile metabolite was identified as methylhistamine by paper chromatography (*5*). This identification was not unexpected, for large amounts of endogenous histamine and histamine methyltransferase (*6*) are present in the posterior pituitary gland.

The enzyme that forms the volatile metabolic product was purified about 13-fold from bovine posterior pituitary by $(NH_4)_2SO_4$ precipitation, $Ca_3(PO_4)_2$ gel (negative adsorption), and alumina-C_γ gel chromatography. After incubation of a partially purified enzyme only the volatile metabolic product was detected.

One volatile metabolic product that might arise from *S*-adenosylmethionine is methanol. Its enzymic formation was examined as follows: After incubation of the partially purified enzyme with C^{14}-methyl-*S*-adenosylmethionine at *p*H

Table 1. Enzymatic formation of a volatile metabolite from C^{14}-methyl-*S*-adenosylmethionine in the bovine pituitary gland. Posterior pituitaries were homogenized with 10 volumes of cold distilled water, and the supernatant fraction was separated from the particulate by centrifugation at 80,000*g*. Various subcellular fractions obtained from 10 mg of pituitary gland were incubated with 37 nmole of C^{14}-methyl-*S*-adenosylmethionine (activity, 10^4 count/min) in 50 μl of 0.5*M* phosphate buffer (*p*H 7.9) in a final volume of 200 μl in a 15-ml, glass-stoppered centrifuge tube. After 60 minutes of incubation at 37°C, 0.5 ml of borate buffer (*p*H 10) and 6 ml of isoamyl alcohol were added and the mixture was shaken for 5 minutes. After centrifugation, 2-ml portions of isoamyl extract were transferred to vials. To one vial, 2 ml of ethanol and 10 ml of phosphor were added and the radioactivity was measured. The isoamyl alcohol in the other vial was evaporated to dryness is a stream of warm air, and radioactivity was measured after addition of 2 ml of ethanol, 2 ml of isoamyl alcohol, and 10 ml of phosphor. The radioactivity found in a 2-ml portion of isoamyl alcohol is reported.

Preparation	Radioactivity (count/min)	
	Before evaporation	After evaporation
Soluble supernatant fraction	1900	1280
Particulate fraction	49	25
Soluble supernatant fraction heated at 100°C (3 min)	58	50
$(NH_4)_2SO_4$ fraction from precipitate of posterior pituitary at 30 to 45% saturation (0.68 mg of protein)	895	60