### Conclusion

Faced with these possibilities, what should we, as scientists, do? We are in some sense a privileged minority group, and all of us should be ready to exercise the grave responsibility which we all share, "to increase public understanding and appreciation of the importance and promise of the methods of science in human progress." These words are quoted from a statement of the objectives of this Association. A second objective of our organization is "to improve the effectiveness of science in the promotion of human welfare." These two should be the articles of our scientific creed in the years ahead. Furthermore, as scientists we should not lose our perspective but should recall the history of science and remember that it has survived pestilence, wars, and disaster and has surmounted barriers of race, religion, and language. Beyond this, it is even more important to recall, in a gray period of international tension, that all members of the human race, throughout its evolution and long history, have had a common opponent. This is inscrutable nature with her seemingly inexorable laws, her hosts of organisms and parasites, her hurricanes and catastrophic events of all kinds. For our human race the central problem is still that of understanding nature and attempting to control it. Here the thinking and tools of modern science have a great contribution to make. May we use them well.

Much of what I have said of warnings, of impacts and reactions, and of grave concern may have the ring of pessimism for the future as science moves swiftly ahead in one of the great adventures of the human mind. That this is not my intent can be made clear by a closing quotation from Carlyle's great satire *Sartor Resartus*. In this he attributes to his fictitious author, "Professor Teufelsdröckh of Weissnichtwo," these words, in the promethean spirit of which I share: "Man's unhappiness, as I construe, comes of his Greatness: it is because there is an Infinite in him, which with all his cunning he cannot quite bury under the Finite."

# Growth and Development of Cultured Plant Cells

## Biochemical and morphogenetic studies with cells yield new evidence on their metabolism and totipotency.

F. C. Steward, with M. O. Mapes, A. E. Kent, and R. D. Holsten

The rapid advances in molecular biology (perhaps more appropriately called the chemistry of biologically important compounds) have rightly focused attention upon the DNA of the nuclei of cells and upon its various derivatives expressed as RNA's of the cytoplasm (see *1*). The roles of the nucleic acids in maintaining the constancy of self-duplicating structures and in transmitting likenesses from cell to cell, or from generation to generation, are fundamental concepts of modern biology. Impressive as all this is, it should not obscure the fact that, despite all that has been learned about the apparatus in genes and in cellular organelles which prescribe cellular capabilities, there is a great deal yet to be learned concerning the means by which these potentialities are either evoked or suppressed.

The fertilized egg of a flowering plant was originally thought of as a unique cell, maintaining continuity from generation to generation, while the elaborate apparatus of the embryo sac and of the ovule was regarded as necessary to foster division of the zygote and early development of the plant. But, if each cell of the plant, derived in lineal descent from the zygote by equational division, has the genetic complement which is necessary to produce the whole organism, then, it appears that the zygote is not unique and the environment of the embryo sac may be replaced.

However, the facts of morphogenesis and differentiation indicate that diploid cells in a plant do vastly different things with what is presumably the same genetic information. The capacity to divide may be inherent in cambial cells of trees for over 4000 years, even though this capacity is expressed only intermittently. Some derivative cells of the cambium rapidly lose their protoplasmic contents and die as they differentiate; others, closely adjacent, remain alive, although quiescent, and fulfill their role as living cells in the plant body.

There must, then, be some mechanisms of control, some obvious restraints, which allow cells in the plant body to do only a small fraction of that of which they are capable. To explore these problems of regulation one must recognize that cells have organization vastly above the molecular level; one needs to know how cells, as integrated structures, are constrained to function as they do.

One should look to more complicated systems than those which it is now fashionable to study. Studies of the ribosome or the virus particle, even of the gene-enzyme-protein sequence, cannot provide a complete explanation. The individual cell becomes the lower limit of organization in terms of which these problems should be discussed, for it is the cell which divides, which grows and differentiates. And, freely suspended, single cells may give rise to whole organisms. Simple molecules may transmit the stimuli which evoke

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responses of the cell, and no doubt these stimuli work through the conventional apparatus described by "molecular biology." Nevertheless, the behavior of molecules cannot explain completely these essentially developmental processes; for the level of organization at which the definitive events or responses occur is far above the molecular.

What, then, directs the metabolic processes of cells away from the maintenance of a steady state and toward cell division, with its concomitant increases in cellular syntheses? This question can be attacked by investigating the conditions under which cells either grow or remain quiescent and the nature of the stimuli that evoke growth in otherwise resting or nondividing cells.

With this preamble, then, some recent developments in studies of plant cell growth and metabolism will be described. These concern the induction of division in cells of higher plants and their ability, once stimulated, to produce the complete organism. These developments have particular significance, since the essential stimuli are derived from the chemical environment of the young embryo, and the cells of the carrot (*Daucus carota* L.) that are freely suspended in liquid and respond most readily are derived from immature embryos. Since these contributions to cell biology are made with flowering plants, they have added interest because they help to close the gap between the ideas of molecular biology and the behavior of cells of higher organisms.

## Growth of Explants and Free Cells

It has been shown (*2*) that a small piece of preformed tissue, free from cambial or lateral root initials, when explanted aseptically to a suitable liquid medium (*3*), will grow rapidly but in an unorganized manner, and that this growth is largely the consequence of cell divisions which are induced by a coconut-milk supplement. However, when the carrot tissue has begun to proliferate, cells which are mechanically freed from the surface of the explant so that they are suspended in the liquid medium will also divide (*4*).

A free cell in a homogeneous liquid medium, subjected to a symmetrical environment, might conceivably form a sphere if cells divided into two equal masses in accordance with the principle of Sachs and in accordance with Errera's principle that new walls are of minimum surface and tend to intersect the old at right angles. Free cells in liquid culture display a great range of individuality and exhibit a baffling array of growth forms and of division (*4*), even in a suspension of cells all derived from the same source. This fact emphasizes the diversity of behavior that is compatible with one genetic constitution when cells are free, in contrast to the comparative uniformity of cells which develop within the plant body. Thus, free cells in liquid culture exhibit intrinsic properties which, in similar cells within the plant body, are suppressed or limited by extrinsic factors.

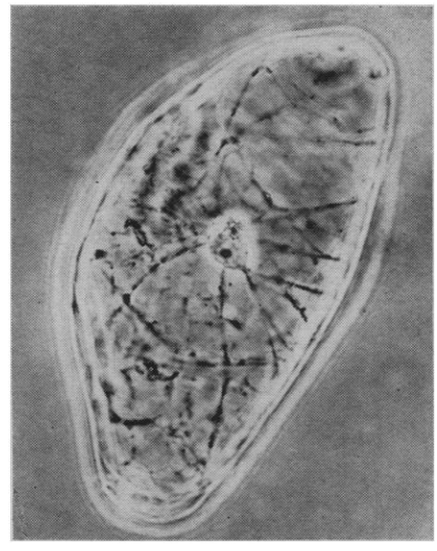

Fig. 1. Freely suspended carrot cell with inclusions. (Length approximately 300 $\mu$.)

From the studies of morphogenesis of cultured plant cells and tissues in this laboratory, the concept has emerged that two essential requirements must be met if cells are to exhibit their full totipotency. These are (i) that a cell be freed from organic connections with other cells, and (ii) that free cells be nourished by a medium which is fully competent to support their rapid growth and development.

## Development from Free Cells

Once freely suspended single cells have been obtained from tissue explants from the secondary phloem of carrot roots, they proceed to divide and to grow in the liquid medium. A typical freely suspended carrot cell with inclusions is shown in Fig. 1. Features of these cells are the very active protoplasmic streaming and the changes which are to be seen in the form of the cytoplasmic strands and in the position of the organelles. Cells and cell clusters which are withdrawn from the suspension show a variety of forms, some of which are remarkably similar to the early stages of carrot embryology (*5*). In the same suspension other cells form an unorganized mass of tissue. Some of these cell aggregates form organized areas or nodules from which roots are produced, and when a rooted nodule is transferred to agar it ultimately forms a shoot (*6*). The plantlet thus formed is in all respects a normal carrot plant which will mature and produce a storage organ, flowers, and seeds. The totipotency of cultured carrot cells is demonstrated by the fact that the life cycle of this biennial plant may now be maintained indefinitely, with cells from cultured explants of the secondary phloem of roots as the sole link between one vegetative growth cycle and the next (Fig. 2). The entire sequence has been carried through three complete cycles without loss of vigor (*7*).

Under the conditions in which living diploid cells exist in the plant body, their totipotency is obviously restricted. This restraint may even affect their ability to respond to the growth-inducing principles of coconut milk. It is possible that cells of immature embryos express their totipotency more readily than cells of the storage root do, because the embryonic cells have not, as yet, become so completely differentiated

To test this idea, embryos were dissected from the flowers of the wild carrot (Queen Anne's lace). When placed in the basal medium plus coconut milk, the embryos proliferated, and a dense suspension of cells from this proliferation was spread over an agar medium in a petri dish (Fig. 3*a*). On examination it was found that the resulting growth consisted of some large, vacuolated, undifferentiated cells which occurred either singly or in clusters, and of large numbers of embryo-like forms (Fig. 3, *a–d*) which recapitulated, in a surprisingly faithful fashion, the globular stage (Fig. 3*e*), the heart-shaped stage (Fig. 3*f*), and the torpedo and cotyledonary stages (Fig. 3, *g–i*) of normal plant embryogeny, and which eventually formed viable plants (Fig. 3, *j–l*) (*8*). In contrast to the undifferentiated cells, those cells

which gave rise to the embryoids were small and were densely packed with starch grains. The embryo cells on agar, in contrast to the single cells from the phloem explants, are more or less spherical and give rise to globular colonies. This is a quite different phenomenon from that by which dissociated cells of certain animals or their tissues may re-unite to form an organ or organism (*9*).

The frequency with which these cells embark upon organized development (Fig. 3, *a–d*) is most impressive. By counting small areas on a petri dish, and by calculating the total area covered by the suspension, it was determined that an aliquot of the cell suspension culture derived from a single carrot embryo yielded some 100,000 embryoids. The embryoid phenomenon has also been observed, although not in such profusion as in the wild carrot, in a strain of cultivated carrot which had been repeatedly grown to flowering from free cells derived from the phloem explants of the root. Thus, cells of a carrot embryo which are no longer in organic connection with it will grow like the zygote if they are appropriately nourished. However, certain strains of carrot seem to respond more readily than others do (*10*), and it may well be that the cells of the wild carrot are even more able to express their totipotency than are cells of the cultivated strains. Certainly the most precise morphological detail of the whole plant is recapitulated in the growth from cells (Fig. 3*l*).

Since large numbers of carrot cells can be caused to grow and to form plantlets, we now have a basis for the study of somatic variations and for the extension to the cells of flowering plants of techniques which have proved useful in bacterial genetics. Since somatic reduction occurs in the cultured cells (*11*), there is now reason to hope that haploid plants and their completely homozygous diploids may be obtained.

## Cell Growth, Division, and Metabolism

The influence of the cell-division factors in coconut milk upon cell metabolism is, therefore, of great importance. Three contrasted states are of interest: (i) the resting cells as they exist in the intact carrot root; (ii) the actively metabolizing cells of the tissue explant, in which any growth that occurs is by cell enlargement, with a minimum of cell division; and (iii) the actively metabolizing cells which are stimulated by coconut milk to divide as rapidly as they can.

Here, then, is a system in which the stimuli in the medium, in addition to the nutrients of the basal medium, can be manipulated so that preformed cells will grow either by enlargement, with virtually no cell division—a form of growth which occurs in the presence of the basal medium alone—or by rapid cell division, with a minimum of enlargement, as in the presence of coconut milk. In the latter instance there is an increase in the number of cells per explant, and the average cell size is greatly reduced (*12*). When the tissue enters upon its "compound-interest" growth phase in the presence of coconut milk (see Fig. 4), it also acquires a greater measure of resistance or insensitivity to certain metabolic inhibitors (such as cyanide or carbon monoxide). At this time the growth of the tissue is particularly sensitive to compounds which tend to uncouple phosphorylation, such as the nitrocresols, and it is much more sensitive to these inhibitors than is similar tissue prior to the growth-inducing stimulus of the coconut milk (*13*). Various inhibitors which act to stop protein synthesis are now known, and if these are added to the medium at the outset, they prevent the expression of the coconut-milk effect (*5*). The action of these inhibitors suggests that enhanced protein synthesis is a prime factor in the growth-induction process.

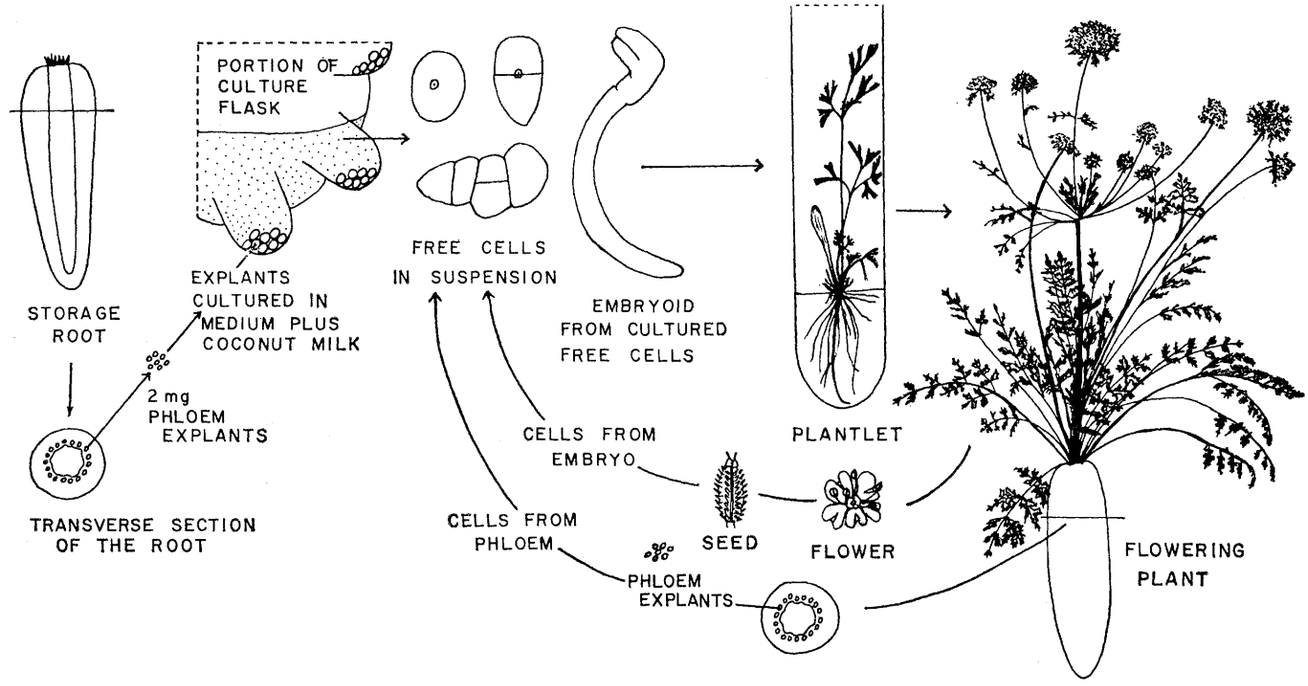


Fig. 2. Diagrammatic representation of the cycle of growth of the carrot plant; successive cycles of growth are linked through free, cultured cells derived from phloem, or from the embryo.

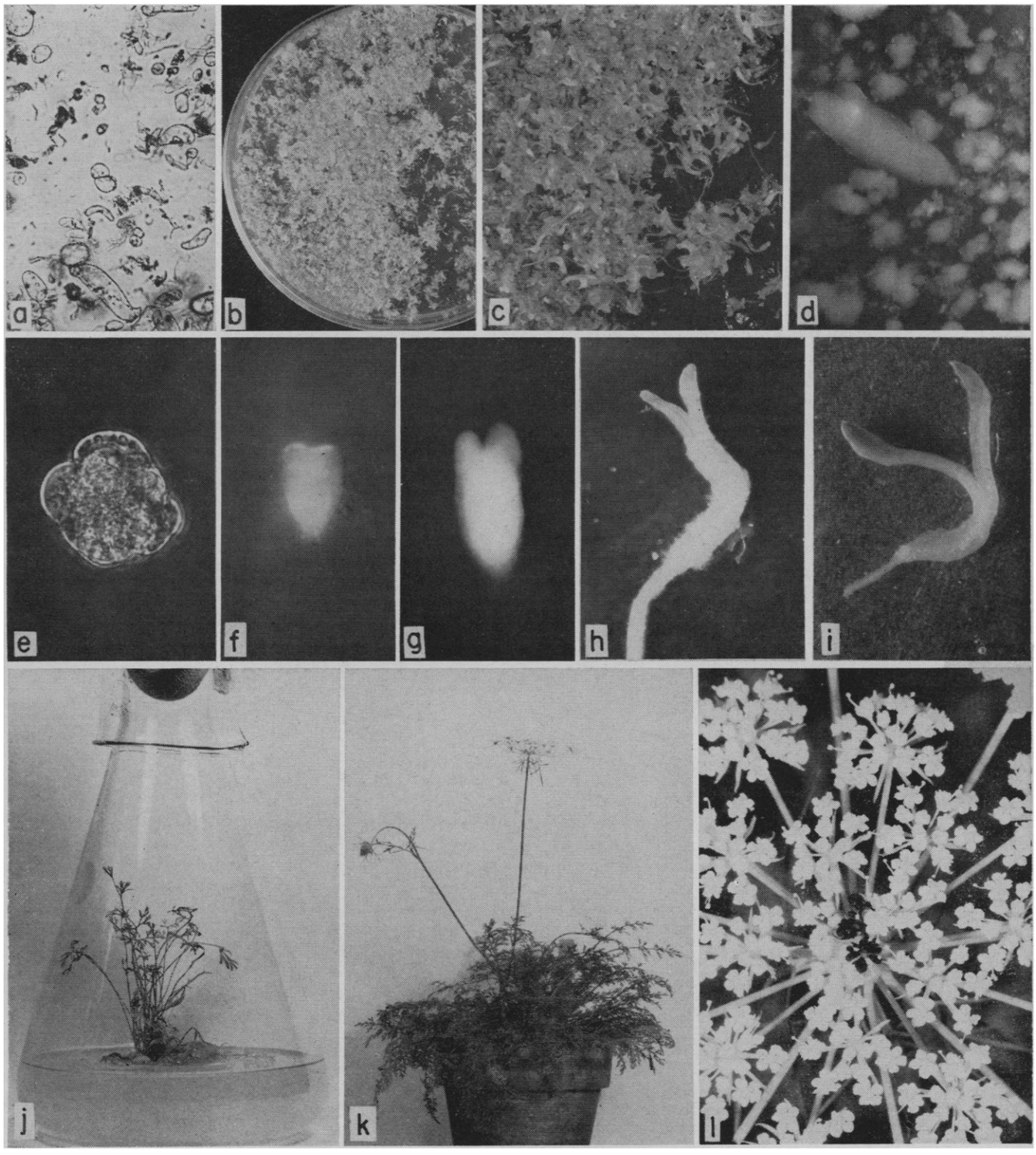


Fig. 3. Development of carrot plants from freely suspended cells of embryo origin. (*a*) Cultured carrot cells in liquid medium; the suspension is filtered through bolting silk. (*b*) Growth of a large number of embryoids on a petri dish on which a suspension of carrot cells was dispersed. An estimated 100,000 embryoids were on this plate, and all of them were derived from part of the cells from one embryo of the wild carrot. (*c*) Higher magnification of the plate shown in *b*. (*d*) Growth in a liquid medium of many units from a cell suspension similar to that shown in *a*. Many globular masses in various stages of development and one clear torpedo stage may be seen. (*e–i*) Selected stages of carrot embryogeny developed from free cells: (*e*) globular stage; (*f*) heart-shaped stage; (*g*) torpedo stage; (*h*, *i*) cotyledonary stages. (*j–l*) Stages in development of plants reared from embryoids grown, in turn, from cells: (*j*) plant on agar; (*k*) plant bearing inflorescences after 6 months' growth; (*l*) detail of inflorescence of Queen Anne's Lace (*Daucus carota* L.) on a plant reared from cells of embryo origin (see *a*). Note the few typical red flowers at the center of the inflorescence.

## Nucleic Acids, Protein Synthesis, and Growth Induction

The nucleic acid content and metabolism of the cells as they respond either to the basal medium or to the stimulus of the coconut milk becomes, therefore, of great interest in any attempt to explain fully the growth responses of the cultured tissue. Changes in the nucleic acid content of cultured cells have shed light on the mode of action of the factors in the coconut milk which stimulate cell division; they also bear on the factors that limit protein synthesis in the actively metabolizing cells before growth induction.

Studies of plant nucleic acids have generally been concerned with plants or plant parts that are undergoing differentiation during the normal growth cycle (*14*). In these studies the usual pattern is one of a decline in total nucleic acid content as differentiation proceeds. The developing tissues studied are progressing toward some determined structure that, once formed, will not show any further morphological or biochemical trends under normal conditions of growth. It is also important, however, to study the biochemical changes in cells which multiply with minimum differentiation.

With tissue-culture techniques (*15*) it has been possible to investigate the changes in nucleic acids during induction of growth of previously mature plant cells. With a slight modification of the method of Ogur and Rosen (*16*), nucleic acid determinations have been made on carrot phloem explants grown for a 2-week period in three types of liquid culture media (see legends to Figs. 4–7). A typical ultraviolet absorption spectrum for all samples extracted as nucleic acid by the procedure followed is shown in Fig. 8.

The growth responses of the tissue in the basal medium and in basal medium with coconut-milk supplement are shown in Fig. 4. Tables 1 and 2 record the concomitant changes in nucleic acid content over the experimental period. To emphasize the time course of these relationships, the essential nucleic acid data are shown in the graphs in Figs. 5 and 6.

The nucleic acid content of the initial quiescent cells is low (Figs. 5 and 6, day 0), whereas the nucleic acid content in cells of the basal medium, which expand without division, becomes and remains very high (Fig. 5, from days 4 to 6 onward). The nucleic acid content of the cells which are about to divide in the presence of coconut milk reaches this high level but then falls steadily as divisions proceed (Fig. 6, from day 4 onward), and thereafter (by day 10) the content per cell reaches a constant level which is higher than the level in the initial quiescent cells. If the metabolism of the cell is not being canalized toward cell division, and if the rate of cellular metabolism varies from its low value in the initial storage organ to a much higher value even in cells that are not dividing (for example, the cells of the basal medium), then the total nucleic acid content of the cells of the explant may vary over a wide range, and it is maintained at a level (Fig. 5) much above that of the storage root.

Clearly, therefore, inability to synthesize nucleic acids is *not* the primary cause of any failure of the carrot cells to achieve their maximum rate of cell division and protein synthesis in the basal medium alone. On the contrary, lacking the stimulus to divide, the cell seems to store the nucleic acids which are being synthesized (Fig. 5). However, the response of the nucleic acid content to the stimulus for cell division provided by the coconut milk is quite apparent in Fig. 6. After the initial accumulation of nucleic acids, characteristic of what was originally called a "lag period" of growth (Fig. 4, days 1–4), which occurs prior to the stimulating effect of the coconut milk, the onset of cell division is accompanied by a rapid drop in the nucleic acid content per cell. In other words, it is only when the synthesis of nucleic acids occurs in cells which have access to the growth factors of the coconut milk that the nucleic acids are utilized for further growth and protein synthesis; or, conversely, cells which grow by enlargement alone (that is, without dividing) accumulate nucleic acids far in excess of their requirements for protein synthesis and growth. Thus it is clear that the coconut-milk growth factors, acting as stimuli to cell division, *do not act primarily to induce nucleic acid synthesis*; this can, and does, occur even when these factors are limiting. Where, then, may the coconut-milk growth factors be presumed to intervene?

In conventional views of protein synthesis (see *17* for review and further references), it is assumed that cellular amino acids are appropriately activated and form a complex with adenosine triphosphate (ATP). It is further assumed that this complex can then react with the requisite soluble RNA (sRNA), which becomes the carrier

Table 1. The nucleic acid content of explants and cells grown in modified White's basal medium.

| | Explant data | | | | | Cell data | | |
|---|---|---|---|---|---|---|---|---|
| Day | Average weight (mg) | Total nucleic acid ($\mu$g) | RNA ($\mu$g) | DNA ($\mu$g) | Total No. of cells per explant ($10^3$ cells) | Total nucleic acid ($10^{-5}$ $\mu$g) | RNA ($10^{-5}$ $\mu$g) | DNA ($10^{-5}$ $\mu$g) |
| 0 | 3.8 | 1.06 | 0.38 | 0.68 | 31.6 | 3.37 | 1.20 | 2.17 |
| 2 | 4.0 | 4.25 | 2.03 | 2.22 | 33.5 | 12.67 | 6.05 | 6.62 |
| 4 | 4.2 | 7.46 | 4.70 | 2.76 | 31.8 | 23.45 | 14.77 | 8.68 |
| 6 | 4.6 | 10.17 | 7.67 | 2.50 | 31.1 | 32.67 | 24.64 | 8.03 |
| 8 | 4.6 | 10.25 | 7.79 | 2.46 | 30.6 | 33.45 | 25.43 | 8.02 |
| 10 | 5.9 | 10.30 | 7.80 | 2.50 | 30.6 | 33.62 | 25.51 | 7.71 |
| 12 | 6.1 | 10.12 | 7.65 | 2.47 | 31.1 | 32.86 | 24.68 | 8.18 |
| 14 | 6.8 | 10.41 | 7.83 | 2.58 | 31.8 | 33.35 | 25.60 | 7.75 |

Table 2. The nucleic acid content of explants and cells grown in modified basal medium supplemented with casein hydrolyzate and coconut milk (10 percent).

| | Explant data | | | | | Cell data | | |
|---|---|---|---|---|---|---|---|---|
| Day | Average weight (mg) | Total nucleic acid ($\mu$g) | RNA ($\mu$g) | DNA ($\mu$g) | Total No. of cells per explant ($10^3$ cells) | Total nucleic acid ($10^{-5}$ $\mu$g) | RNA ($10^{-5}$ $\mu$g) | DNA ($10^{-5}$ $\mu$g) |
| 0 | 3.8 | 1.06 | 0.38 | 0.68 | 31.6 | 3.37 | 1.20 | 2.17 |
| 2 | 3.8 | 4.43 | 3.10 | 1.33 | 31.4 | 14.09 | 9.86 | 4.23 |
| 4 | 4.9 | 10.92 | 8.88 | 2.04 | 42.2 | 25.85 | 21.03 | 4.82 |
| 6 | 6.7 | 17.00 | 14.47 | 2.53 | 97.6 | 17.42 | 14.83 | 2.59 |
| 8 | 12.2 | 27.75 | 23.87 | 3.88 | 270.5 | 10.26 | 8.83 | 1.43 |
| 10 | 28.1 | 27.80 | 23.60 | 4.20 | 527.8 | 5.30 | 4.50 | 0.80 |
| 12 | 64.0 | 33.80 | 28.20 | 5.60 | 545.3 | 5.70 | 4.80 | 0.90 |
| 14 | 95.0 | 33.50 | 26.10 | 7.40 | 570.5 | 5.90 | 4.60 | 1.30 |

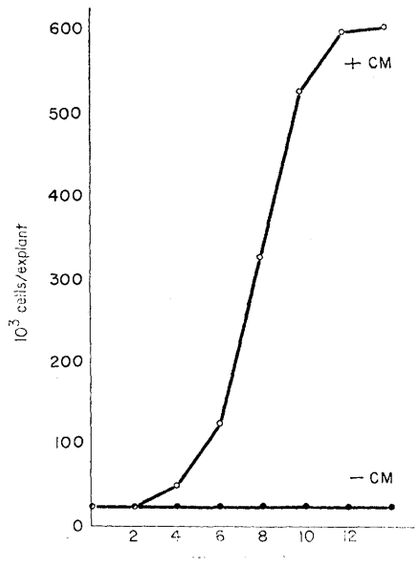


Fig. 4. The growth of explants from carrot phloem with and without coconut milk (*CM*) as a supplement to a modified White's basal medium, plotted against time. Growth is measured by the number of cells per explant. (Each of the initial explants weighed about 3.0 mg and contained approximately 30,000 cells.)

molecule that escorts the amino acid to the protein-synthesizing surface on the ribosomes of the cytoplasm. This protein-synthesizing surface, determined by a transient "messenger" RNA (*18*), receives the amino acid–sRNA complex and prescribes the correct linear order of amino acids on the template surface. Finally, by some as yet incompletely defined mechanism, the actual peptide bond synthesis occurs, and the newly formed protein molecule and the sRNA's vacate the template surface, leaving it available to repeat the process of protein synthesis.

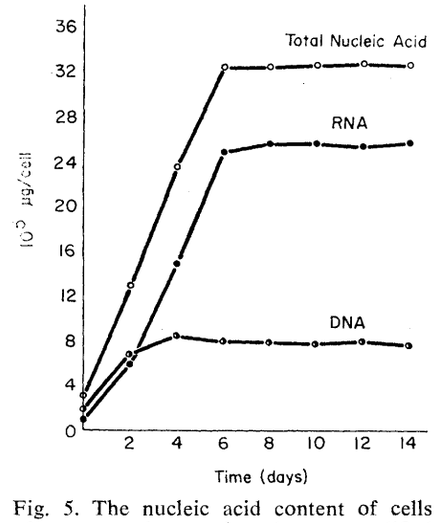


Fig. 5. The nucleic acid content of cells of carrot explants cultured on a modified White's basal medium, plotted against time.

Unquestionably, the tissue in the basal medium can make protein without the stimulus of coconut milk, for the soluble nitrogenous constituents of resting cells (potato as well as carrot), to the limited extent that they are available, are converted to protein even in the absence of the coconut-milk stimulus (*19*). One can, in fact, provide amino acids in the form of casein hydrolyzate and make sure that protein synthesis is not limited by an inadequate supply or by any inability of the tissues to synthesize them *de novo*. However, casein hydrolyzate will not of itself induce growth, nor will it stimulate the specific effect of coconut milk on the nucleic acids produced by the cells (Fig. 7).

There are, then, several steps at which the coconut-milk stimulus might, according to current views of protein synthesis, intervene in the events which occur in the general vicinity of the protein-synthesizing surface. First, there are the steps in the cytoplasm in which the amino acids are activated by appropriate enzymes and complexed with ATP, thereafter to combine with the appropriate sRNA, which escorts the amino acids to the ribosomal template. Other steps that might be facilitated are the actual formation of a messenger RNA, its complexing with the ribosome to provide the template for protein synthesis, or, finally, the actual peptide bond synthesis—that is, the so-called "zippering action" which unites the amino acids into a complete protein molecule after they are arranged in correct linear order on the template surface.

It appears that so large an effect on the total nucleic acid content of cells during the early stages of the response shown in Fig. 6 requires a concomitant effect on both ribosomal and sRNA fractions; but this does not preclude some effect of the coconut-milk stimulus on the structural organization of these respective fractions once they have been formed. Clearly, questions concerning the action of the growth stimuli on nucleic acids cannot yet be fully answered, especially since evidence in higher plants for the existence and duration of specific messenger RNA's is still limited (*20*).

The cultured cells of higher plants now provide a ready means of studying the mechanisms which determine how

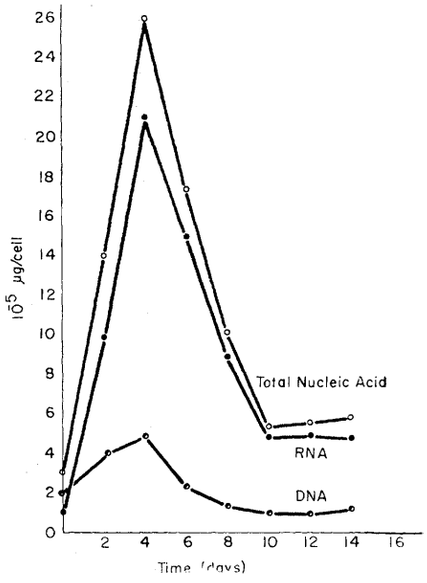


Fig. 6. Plot, against time, of the nucleic acid content of cells of carrot explants cultured on a modified White's basal medium supplemented with casein hydrolyzate (0.02 percent, wt/vol) and coconut milk (10 percent, by vol).

the nucleic acids, which can be synthesized far beyond the immediate requirement for them in nondividing cells, may become effective in protein synthesis and in growth.

It is important here to recognize that the problem of protein metabolism in plants is a far from simple one. It is indeed far less simple, even, than the commonly held and widely popularized

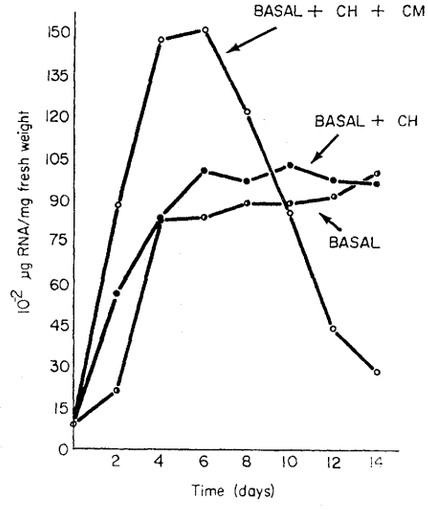


Fig. 7. Plot, against time, of the ribonucleic acid content per unit fresh weight of cultured carrot explants in a basal medium; in a basal medium supplemented with casein hydrolyzate (*CH*), and in a basal medium supplemented with casein hydrolyzate plus coconut milk (*CM*).

recent views might have one suppose. Ultimately, the control of protein synthesis does not rest alone with the genetic mechanism, nor is it merely the consequence of the preformation of DNA and RNA; it is obviously under the regulatory control of a variety of physiological stimuli which, when brought to bear, make cells grow. Even so, it is the "built-in capacity to grow" of the zygote, transmitted by equational divisions to the daughter cells, which enables the cells to respond to the growth stimuli. This response is remarkably complete; many mature cells can behave totipotently and reconstitute the whole organism.

### Totipotency: Its Expression and Control

If the observations here described have a more general meaning, it is that any diploid cell has all the propensities for the formation of the complete organism, and that these are controlled (evoked or suppressed) in part by chemical stimuli, chemicals which determine not only whether DNA and RNA are synthesized by cells but whether, having been so made, they can become effective in protein synthesis, cell division, and growth. The tightness of the controls that constrain living cells to become part of the permanent tissues of the plant, and the means by which this control is achieved through position in the plant body, is the crux of the problem of morphogenesis. The stimulation of otherwise mature cells to divide, though not to differentiate, and their autotrophic retention of this capacity, is the heart of the problem of tumorization. Moreover, a prime factor in determining whether a given cell produces an embryo or an unorganized mass of tissue is the degree to which it has become free from the constraints imposed by its neighboring cells, and this effect also must surely be mediated by chemical means. Thus, there are both intrinsic and extrinsic factors in cell growth and development.

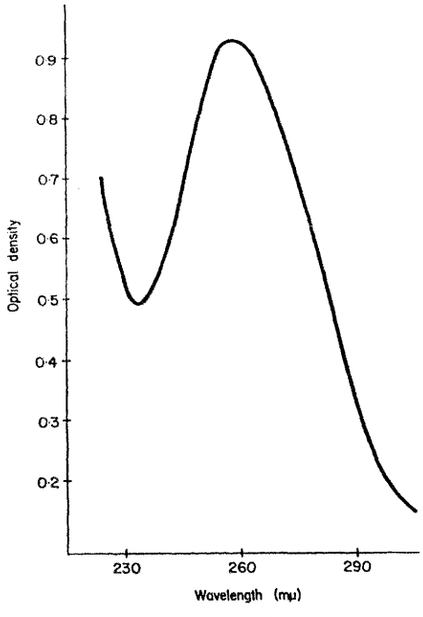


Fig. 8. Plot of the ultraviolet absorption spectrum of nucleic acid extracted from cultured carrot explants.

The best examples of totipotent cells in angiosperms are those derived from developing embryos, and the best known means of evoking cell division and totipotency is to furnish the near approximation, in coconut milk, of the chemical environment that exists in the ovule. The effect of the coconut milk, or of the normal environment of the ovule, is "epigenetic" in the sense that it is not among the qualities that are transmitted solely by genes in nuclei; it is part of the chemical apparatus which determines, in the milieu of cells, to what extent the cells are free to express their full genetically transmitted potentialities. In part, epigenetic stimuli express themselves in the great range of proteins formed at different stages in the morphogenetic development of plants, but an equally dramatic expression is the fact that the mechanism of protein synthesis, which is latent in the expanded cells exposed to a complete nutrient medium, must be "turned on" by the growth-inducing principles of coconut milk which impart to the cells the ability to divide. The growth-promoting or growth-regulating substances may well be found to act by activating particular parts of the chromosomes, thus emphasizing some special features of metabolism. The "puffing" chromosomes of certain insects during their morphogenesis (*21*) provide visible evidence in support of this suggestion, but unfortunately there is no comparable cytological evidence for the cells of higher plants.

Thus, the DNA of the nucleus may provide the coded information needed to construct the constituents of the cell and organism, but the initial thrust which growth requires must be furnished by the chemical environment of the cell. Nevertheless, it is the organization of a single totipotent cell that is so important. The course of growth, through time, may be modulated or controlled by environmental factors that in turn are mediated by chemical means through the control of cell division and cell enlargement. It becomes, then, as important to elucidate the morphogenetic controls and stimuli by which *differences* are determined as to elucidate the mechanisms of self-duplication by which *likenesses* are transmitted.

While it may seem simpler to deal with systems which lack some of the complications of higher plant cells, it is well to recognize that, if these complications are eliminated, the generality of any explanations that emerge is, to this extent, also limited. We have, therefore, in the investigation of the carrot-coconut milk system, a means of supplementing ideas that are now being drawn from work on simpler systems.

The processes of protein synthesis and growth must often go hand in hand. They are regulated not alone by nutrients and not alone by the DNA–RNA systems; they are additionally determined by the intervention of soluble molecules, such as those in coconut milk, which seem to be universally present in nutrient fluids of immature plant embryos, and which supply the link between the nucleic acids, protein synthesis, and growth. The cells synthesize protein as they grow; they grow as they synthesize protein.

#### References and Notes


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munication to the Botanical Society of America at Amherst, Mass., on 28 Aug. 1963 dealt more completely with the same subject [see F. C. Steward, M. O. Mapes, A. E. Kent, *Am. J. Botany* **46**, pt. 2, 618 (1963)]. On the same occasion D. F. Wetherell and W. Halperin described the similar formation of carrot plants from cells of the petiole and root of the wild carrot [D. F. Wetherell and W. Halperin, *Am. J. Botany* **46**, pt. 2, 219 (1963)]. A recent paper by H. Kato and M. Takeuchi [*Plant Cell Physiol. Tokyo* **4**, 243 (1963)] deals with the development of a callus on relatively large disks of carrot root. After these were extensively cultured (4 months), single cells were separated from the friable callus tissue, and these were observed to grow into plantlets in a manner which was very similar to normal embryogenesis. Thus, the growth of carrot plants from free cells is now being achieved in several different laboratories by different means.

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22. This article is based on work of this laboratory and, therefore, it does not give a comprehensive bibliography of the whole subject. A more general account of the earlier work is given in the proceedings of the 19th Growth Symposium [E. Rudnick, Ed., *Synthesis of Molecular and Cellular Structure* (Ronald Press, New York, 1961)]. Papers by de Ropp, Skoog, Torrey, Bergman, Muir, Hildebrandt, Tulecke, and others have contributed to special aspects of the problems here discussed; references to their work may be found in the papers cited. The work discussed has been supported by grants from the National Cancer Institute and from the National Institutes of General Medical Sciences of the U.S. Public Health Service. Other workers have contributed to this program, even though separate reference to their work is not made here. The participation of R. D. Holsten was supported in part by a Public Health Service fellowship from the National Institutes of General Medical Sciences.

# News and Comment

## Legislation: In First Session, 88th Congress Shows Itself to be More Critical Patron of Science

Whatever else can be said for the long-drawn-out and spottily productive first session of the 88th Congress, it was not an uneventful one for federal science. The legislators began treating federal science as if it were a genie which they themselves let out of the bottle some years ago and which, they noticed quite recently, had grown greatly and developed a somewhat overbearing manner. There are those in Congress who plainly want to cork it up again, but the majority reaction is to seek ways to make the genie more useful, efficient, and obedient.

Congress has not really been ignoring science and technology. The legislative history of the last two decades is littered with landmarks for science. Creation of the Atomic Energy Commission, the National Science Foundation, and the National Aeronautics and Space Administration and establishment of the machinery of advice and coordination for the Executive, with the new Office of Science and Technology as the point of focus, are cardinal examples. The escalating federal investment in health and medical research, notably through the National Institutes of Health, is another case in point.

But until last year, Congress as a whole seemed offhanded as well as openhanded in its treatment of science. Although science increasingly pervaded government agency operations and the total science budget climbed, there seemed to be little effective concern in Congress about the overall costs or consequences of federal science. In great part, this was due simply to the decentralized way in which Congress deals with the agencies through its Balkanized committee system.

As the annual R&D budget nudged $15 billion, a cloud of uneasiness settled over Congress, and this year a sort of anticyclone mood of economy, generated by the $100-billion federal budget, precipitated a change in the weather in Congress for science.

The attempt to put science in perspective followed three main lines of action: (i) Congress moved to inform itself and the public on the organization and conduct of federal science through exercise of its investigatory powers; (ii) a less strong but significant effort was launched to find ways to improve the advice on scientific matters which Congress gets independently of the executive branch; (iii) Congress played its trump card of fiscal power to apply pressure to science-agency budgets and policies.

While subjecting science to closer scrutiny, the 88th Congress has by no means shown itself to be an indiscriminate wielder of the economy axe. Passed during the first session were three notable education bills—one to aid in construction of academic facilities for colleges and universities, a similar bill to benefit medical schools and other institutions training persons in the health professions, and a bill designed to modernize vocational education and give it ampler federal support (*Science*, 20 December). In addition, Congress enacted two bills which will give the federal government significant new roles in two areas involving science—air pollution and work in behalf of the mentally retarded.

**Air Pollution.** The Clean Air Act of 1963 strengthens the existing federal program to control and prevent air pollution. A total of $90 million is authorized for the program over the 4 years 1964 through 1967. The money is to be used mainly to support research and training and for grants for establishing or improving local, state, and interstate agencies for air pollution control. Significantly, the act for the first time carries enforcement authority, which is keyed to a series of actions starting with consultations, and ascending through recommendations by the