THIS I WOULD LIKE TO KNOW—TWO YEARS LATER

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President Stoldt, Fellow Past Presidents, Members of the Illinois State Academy of Science, and Guests:

President Stoldt is to be congratulated on having read the addresses of four of the past presidents of the Illinois State Academy of Science. When she contacted the past presidents, Miss Stoldt mentioned that she wondered what they were doing since they were out of office. For my part, and I suspect the others can say the same, the time has been spent in doing some of the things that had been neglected. The regular duties of a regular job has kept each of us busy. During this last year I have wondered how I ever had time to attend the council meetings, answer the letters, make the plans, attend committee meetings, etc. that are required of a president. But this is not the subject that has been assigned.

"This I Would Like To Know—" was the subject of the Presidential address before this session in 1962. Several questions were asked and at that time it was thought that some could be answered in a short time. None has been answered. Some of these questions involve the fundamentals of plant science.

One question was that of, "Do auxin and auxin like compounds initiate or control the growth of cells, tissues, and organs?" Three years ago it was thought we might be close to the answer. Today we seem to be farther from the answer than we were then. In the meantime there is at least one plant scientist who insists that all is known about "auxinology" that can be known and we might as well turn our attention to some other subjects.

Some persons who were working on the initiation problems have become so involved with the problems of auxin transport that they have lost sight of the problem of "initate or control" function of auxin. Neither problem—initiation or transport—has become clearer in the last two years. Oh, yes, research has been published.

Another problem was mentioned two years ago. This was the matter of the control of cell division—either mitosis or meiosis. The question was asked, "Is there an inhibiting substances that will control the rythmn of cell division?" Is there some way to remove the inhibitor and thus speed up cell division? Of course other things must be in sufficient amount to support cell division.

This research has not progressed as rapidly as had been planned. There is the matter of purification

of the organic compounds used. There had to be a separation of artifacts from a real or at least a normal response. Subtle changes in the ultra structure must be studied before an answer is proposed. This research is being carried on and there is some evidence that there may be an answer.

This is getting so it sounds like some daytime T. V. shows. They never come to a conclusion. Much research is of just this nature only there is no script.

Another question was asked—the question of differentiation. There is evidence in both the vegetative and reproductive structures that some type of determination is going on. The matter of determining which cells will produce a branch or a leaf on a vegetative shoot has been reviewed many times in many different kinds of plants. The development of reproductive organs, with both sexes involved, in one larger organ of the higher plants, has been used to discuss the differentiation of cells, tissues and organs.

In plants, we have the mechanism for unlimited growth that presents a problem in determining just where differentiation takes place. It would be very convenient to divide the activities of a multicellular plant into three stages such as: cell division, morphogenesis, and diffentiation. In many plants these three stages may be going on at the same time in the same organ or tissue. If we think one stage leads to another then we have difficulty in drawing the limits or guidelines to separate the stages.

Research is being done in the matter of differentiation and the causes of differentiation. Several well known biologists are saying that now that we have the matter of the duplication of the gene worked out it is time we got to work on differentiation.

Two things are wrong with this idea. For one thing there are still some blind spots or areas of the unknown, at least unproven, in the theories of gene duplication. It is not true that we should start on development or differentiation but rather that we should continue our work. A number of Botanists have been working with the development of cells, tissues and organs for many years.

Some of the theories, that have been advanced in the last three years of reasearch, can be summed up in the following theories or combinations of these theories. The first might be the activator theory. A second might be the inhibitor theory, and last, we might consider the covered theory.

The activator theory indicates that there is some triggering device —chemical or physical—that we set in motion a sequence of events that will finally determine the development of a cell into the initial which will become evident only in the permanent tissue.

There is some experimental evidence that the "puff" structure of a chromosome is the response of hormone action and a percussor of metamorphosis in animals. It is obvious that this work was done on insects. The puff structure of the chromosome is studied in the giant

salivary chromosomes of insect larvae and the metamorphosis was studied in the larva-pupa development. Peter Karlson (1) has shown that there is an enzyme that controls the development of puffs on the giant chromosomes. The development from larva to pupa is seen just so many hours after the development of the puffs, and the puffs themselves will apear just so many hours after the injection of a hormone. Karlson suggests that the puffs are evidence that the DNA of the chromosome will be forming RNA. This RNA becomes the messenger RNA which activates the ribosomes in the cytoplasm which in turn will form enzymes with the proteins of the cell. This in turn becomes the activator of cellular activities including differentiation.

There are some interesting ideas expressed here. We might ask, as does Karlson, is hormone activity necessary for gene action or to initiate gene action? The genes are all present in all of the cells of a tissue, organ or individual for the development of all the different cells and tissues required. Then why do not all cells of a flower develop into the pollen mother cells or the megaspore mother cells? This hormone activation of the gene might be part of an answer. The hormone would be nessary to stimulate the DNA to produce the right RNA to produce the ribosomes to produce the right enzymes to produce microspore mother cells or megaspore mother cells. Are two hormones necessary, or are there hormones and associated vitamins, enzymes, coenzymes, or what have you, involved?

We have been using a piece of animal research to explain the initiation of the gametophyte generation of the flowering plants. This is about as remote as any thing can be but since we have gone this far suppose we take it to another step and ask if this could be applied to vegetative development as well. Could the origin of branch roots on a geranium cutting be initiated in the same manner? We know of a growth regulating substance involved here. It is the auxin-like substances.

Now we are back to the question asked in the address before this session in 1962. Does auxin initiate or regulate, or control or maintain the production of adventitious roots on apple stem cuttings? Could auxin be the hormone that will initate the production of a messenger RNA which will cause an enzyme to start the synthesis of a hormone causing the development of adventitious root? Is there an enzyme involved or can the messenger RNA cause the root initial to develop? If auxin is involved then it is more imperative than ever that we determine if there is an initiation or a regulatory function.

A second theory about morphogenesis and differentiation is the inhibitor theory. This theory would insist that since each cell has all the genes that would control all the differentiation of all the cells of the whole organism then there must be something that is inhibiting this action. The genes would be allowed to function only if the inhibitor were removed. This may appear to be a negative approach but it is

an approach that warrants some investigation. Inhibitors have been seen elsewhere in plant growth. Some chemicals have been proved to be stimulators under some conditions and inhibitors under other conditions. The simplest of these conditions might be the concentration of the chemical involved. In low concentration the chemical could become a stimulator and at a slightly higher concentration would be an inhibitor and at a still higher concentration it might prove to be lethal.

In much of the auxin work the idea of inhibitor is the idea that the inhibitor inhibits the normal growth action of the auxin. If a monophenolic compound will act in such a way that auxin can not do its proper job, and if this phenol were changed to a polyphenolic compound then auxin can function in the growth process. In this case, would the monophenolic compound be an inhibitor? Is the polyphenolic compound a growth stimulator? Do we need to define our terms? Is the enzyme that breaks down the auxin an inhibitor because less growth can take place if auxin is not present?

Then we are back to the same question again, "Is auxin the chemical that initiates cell division, cell elongation, etc., or is it a regulator of cell division and cell elongation, etc." Attempts at answering these questions were reported by Kefford (2) in a report of the 5th International Conference on Plant Regulation. On the subject of the Analysis of Development he reports, "The structures of a number of classes of compound which have been proposed as regulators have been described accurately or tentatively. The impression that plant development is the result of a developing pattern of interacting fields of those regulators has been built up. But this impression is not ready to be put to the test. The design of such tests probably depends as much upon advances in the analysis of development, down to the molecular mechanism for inter—and intracellular regulation of metabolism, as on defination of primary sites of the regulators in the cell." It has been demonstrated by workers at Oak Ridge that the dominant process in development might take place in cell enlargement rather than mitosis or in the total number of cells.

Mazia (3) of California has shown two levels of control. He differentiates between induction and maintenance of cell division. Induction may be regulated by a group of enzymes. Are any of these enzymes, or the absence of an enzyme, the inhibiting factor in the induction of mitosis? It has been pointed out by several workers that the control of the activities of a cell is the result of sequential enzyme activity. If one step in the sequence were omitted then the reaction would stop there. Then we might think that the development or differentiation of a cell might be stopped at various stages in the sequence and the cell would develop to its maximum under the sequence as far as it had gone. This could bring up the question, "What is the ultimate in development or differentiation?"

Much work has been done on the reversability of sex in Flowering Plants. Flowers that should have produced the female organs have

been changed into flowers producing male organs. Again it might be assumed that there is a sequential arrangement of enzymes that will control this development and in turn there must be a sequential arrangement of the genes of a cell to direct the production of the enzymes.

Postlthwait and Nelson (4) refer to switch points in the development of the ear of corn. By the use of certain genes the switch points could be changed. This switch could change the structure of the ear to resemble the structure of the tassel in corn. They cite work done by Heslop-Harrison which shows some such effect can be brought about by NAA. Here again we might ask if there is a hormone necessary for the expression of a gene.

Stern (2), of the University of Illinois, was prompted to warn that those who speculate on the molecular mechanisms of differentiation generally do not know of the inter—or intracellular metabolic activities going on in the cell. We need to get those who are working with plant growth regulators and those working with molecular mechanism to speak with a common language. Then we may advance our knowledge of differentiation.

The third theory was that the genes on the chromosome might be covered up by the histone layer on the outside of the chromosome. This histone layer would stop the action of the gene until such time as when the histone layer could be penetrated by the gene, then the gene, presumably be able to form the messenger RNA, could carry out the sequence of the cell functions. This theory, which was proposed some two years ago, has not received much support. It may be a modification of the first theory mentioned.

Three theories to explain differentiation of cells, tissues, and organs mean that do not know what is the state of affairs, or that there may be several ways in which determination and differentiation take place. These theories may not be all the possible theories. There may be others that will more nearly explain the observed actions of plants. It may be very foolish to expect any theory that can be applied to animal differentiation might also be applied to plant differentiation.

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