sion may I say that Hollies are a large group of plants, evergreen and deciduous, having red, black and even yellow fruits, some of them with smooth leaves, some with sharp spines. Their white flowers are generally inconspicuous. They are also dioecious, so be sure to select lots of females for fruit and a few boys to do their job and keep the girls happy.

Although they grow best in a rich well drained soil, they will thrive under a variety of conditions. If you select those hardy to your area, there is hardly a group of plants so well suited to such wide landscape use

MODERATOR SHUGERT: Thank you very much, Hans. The second speaker is Dr. Don F. Wetherell from the Department of Botany, University of Connecticut, Storrs, Connecticut.

GROWING WHOLE PLANTS FROM INDIVIDUAL CELLS

A Possble Propagation Technique for the Future.

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The age old practice of plant propagation has taught us that many of the organs of a mature plant can be separated from the rest of the plant and in a relatively short time will reconstruct the missing parts to reform an intact plant. This capacity for regeneration of parts must mean that at least some of the cells which compose these organs, carry all the information and metabolic tools necessary for the formation of an entire new plant, i.e., they must carry as much inherited information as the zygote which forms as the result of fertilization and which is the starting point for the embryonic plant contained in seeds. We call the possesson of all essential genetic information and metabolic machinery — totipotence. Biologists have long wondered whether such cells could be isolated from the protection of their tissues in the intact organ and still retain not only their ability to grow but also their totipotence. As long ago as the turn of the century intensive efforts were being made by Gottlieb Haberlandt, the famous German botanist and anatomist, to isolate single plant cells and make them grow under artificial conditions[1]. This ultimate refinement of plant propagation has been on the minds of botanists ever since but only very recently have we accumulated enough experience to permit us to test these ideas.

It was not until after the discovery of auxins in the 1930's and the subsequent recognition of their key role in growth and development, that we could carry out the first step, the culture of tissue masses on artificial culture media. Although auxin-dependent tissue cultures have now been common place for 20 years, the successful culture of single freely suspended cells (so called "cell culture") was not achieved until the mid 1950's. The

possibility of reconstructing intact plants from these cell cultures has only been demonstrated in the last few years. In this paper I will briefly outline the development of the knowledge of the hormones and their interplay in plant growth and then describe some of the recent researches in which the regeneration of shoots, roots or whole plants has been achieved starting from tissue-cultured cells.

When a wound occurs in plant tissue, masses of soft, so-called, parenchymatous tissue are quickly formed in the wounded area by the initiation of cell division and growth in cells which otherwise had ceased to grow and had settled down to a specialized existence as a part of a specialized tissue. If the wound is extensive then a large mass of wound tissue, or callus as it is often called, will form. This callus is at first characterized by thin walled rapidly growing cells, resembling in many ways the cells of the growing points of shoots or roots or of the cambium, but usually lacking the tissue organization exhibited in the normal growth centers. If the wound is not aggravated, there will occur in this callus, a return of certain cells to a degree of specialization which is familiar in maturing normal tissues. First one may observe a rather disorderly pattern of isolated elements of conductive tissue. Later orderly growth centers resembling root and shoot primordia may be formed, and under favorable conditions the regeneration of new shoots or roots may take place from the callus. Thus, it can be shown that the cells of the callus are totipotent.

There has been considerable speculation and a good deal of research over the nature of the stimulus which sets in motion the once quiet machinery of growth and cell division in wounded tissue. Attention has also often been focused on the conditions which bring about reorganization of the restraining forces which suppress the uncontrolled growth of the wound tissue and which direct its future development into normal tissue and organ patterns.

Soon after the discovery of the auxin hormone and the recognition of synthetic auxins, it was shown that the combined effects of wounding and auxin application resulted in a massive proliferation of wound tissue which assumed the appearance and proportion of a large shapeless tumor and as such dominated the nutritional supplies of the plant. These auxin-induced tumors strikingly resembled the pathological tumors of the crown gall disease and certain virus-caused diseases as well as the tumors which are known to form spontaneously on the stems of certain tobacco hybrids. The main difference being that the extent of growth of the artifically induced tumors was strictly dependent upon the supply of applied auxin while the other types required no auxin from outside sources. Much later it was shown that crown gall tumors were also dependent upon auxins but the cells of these tumors had somehow learned from the infecting bacterium to produce sufficient auxin to support the continued growth of the tumor. In short, it became clear that

auxin possessed the capacity to induce and maintain cell division and growth at least in wounded tissue and that a poorly specialized, relatively unorganized callus tissue was the result of such growth.

To usher in the era of tissue culture it remained only for a curious mind to conceive and test the idea that it might be possible to supply the nutritional requirements of these tumors artificially. This was first reported in 1939 by P. R. White[2], the now acknowledged "father of tissue culture," when he surface sterilized bits of tumor tissue from hybrid tobacco plants and placed them on sterile culture media containing minerals, sugar, and some vitamins. The transplant grew rapidly in these test tubes and continued to grow vigorously even after many generations of transplanting to subcultures. It soon became common practice to place seedlings or plant parts on auxin rich media to induce callus formation and then to transfer bits of this callus to test tubes containing a culture medium similar to White's. Many such tissue cultures have been kept growing by repeated sub-culturing, for nearly 20 years and thus have established a kind of immortality for the original donor plant.

However, the shapeless mass of callus in its test tube certainly is not recognizable as any part of a normal plant. One wonders, does it still possess the minimum essential genetic and metabolic control to re-establish itself in the original form of its donor plant? Reflecting on the regeneration of tissues and organs observed in the wound tissue still attached to a plant, one would hopefully expect similar behavior of cultured callus. Right from the beginning with White's cultured tobacco tumor tissue it became clear that at least some cultural tissues were totipotent and by one means or another (frequently as a result of simply aging) they would regenerate normal tissues, roots and shoots. However, until very recently, such regeneration has been too sporadic, too difficult to control or predict, to evoke much interest as either a means of propagation or as a laboratory tool to permit the study of conditions controlling regeneration.

Outstanding contributions leading to our present state of knowledge and interest in tissue cultures have been made by Folke Skoog of the University of Wisconsin and F. C. Steward of Cornell University. Working with tissue cultures and cultured stem segments of tobacco, during the late 1940's and early 1950's Skoog and his associate discovered an entirely new class of plant hormones, the kinins[3]. Adenine and kinetin, both members of this class, were shown to work in conjunction with auxins to greatly stimulate cell division, and more important to facilitate the regeneration of new shoot buds in much the same way that auxins alone evoke root primordia.

From Steward's laboratories in 1958 came a striking example of the regenerative powers of single cells produced and grown by tissue culture techniques[4]. Working with the commercial carrot, Steward and co-workers have shown that once quiescent

phloem storage tissue can be revitalized by certain constituents found in a liquid endosperm like coconut milk and that free cells sloughed from this proliferating phloem can readily be cultured and if given the proper conditions can reconstitute entire normal plants!

Our research at the University of Connecticut has added still another chapter to the continuing story of tissue cultures[5]. We have found in the wild carrot a most versatile and suitable test organism for the study of regeneration. Callus tissue cultures are obtained in the usual way by placing surface-sterilized seedlings or parts of mature organs on auxin-rich culture media in test tubes. The combined effects of wounding and high auxin level bring about extensive callus proliferation. We were pleased to find that this callus could be readily cultured on a simple medium all of the ingredients of which were known chemicals. The essential minerals, sucrose, an auxin and adenine or kinetin were all that was required. However, the uniqueness of this callus lies not in the ease of culture but in its amazing regenerative capacities. Individual cultured callus cells of this species were found to regenerate by recapitulating almost exactly the sequence of events which occurs in the growth and division of the zygote in the ovary of the fertilized flower, i.e., by first developing into a tiny embryo which in turn developed through the familiar stages of embryogenesis leading to a mature embryo. The cotyledonary node and the radicle are formed simultaneously and in their proper places on the globular embryo. The true shoot meristem is formed in the node and the radicle becomes a root meristem. This whole sequence of *regeneration takes place in the simple culture medium described* and more important, can be controlled by manipulating the concentrations of the two classes of hormones. A relatively high concentration of adenine and the auxin 2,4,D promotes a slow but uniformly unspecialized callus growth. If kinetin is used *in place of adenine, growth is much more rapid but the uniformi*ty of the callus is lost and some specialization takes place. Conductive tissue and pigmented cells appear scattered through the callus and a considerable portion of the callus enters the first stages of embryogenesis and progresses to the globular stage. Removing or lowering the concentration of the auxin initiates the rapid completion of embryo formation. The mature embryo so formed is apparently dormant for it develops no further until a small amount of coconut milk is added to the culture medium. (The use of coconut milk to stimulate the germination of excised seed embryos is a long established practice.)

Once embryo-genesis has been initiated in a segment of callus it proceeds until nearly all of the cells of the callus or the free cells derived from it become involved in embryo formation. Thus, from a small piece of callus only one eighth of an inch in dimension it is possible to obtain hundreds of uniform normal embryos. These may be germinated on appropriate media, transferred to soil and grown as normal carrot seedlings. Thus,

through the careful use of auxins and kinins it has proved possible to both remove and reinstate the organizing forces which control normal growth and development. The carrot plant can be converted into a shapless mass of callus and stored in this condition, portions of the callus can be restored to the orderly form which we know as the wild carrot *Daucus Carota.*

The title of this paper and the purposes of this meeting obligates me to speculate upon the significance of these studies to the science of plant propagation. I do so fully aware that we are still a long way from understanding the process of regeneration even in the most favorable material, the wild carrot. We also do not yet know if the knowledge obtained from the study of these cultures can be applied sucsessfully to control regeneration in any other plant species. Therefore, at this time, we can only ask, if these techniques can be successfully applied to a broad spectrum of species, what advantages might be gained? If they cannot, and we are restricted to the study of a few favorable species, of what significance is this to plant propagators? First, let us answer the easier, less speculative question of the value of such studies to plant propagators. When we consider that in spite of centuries of wide-spread use of the propagation techniques and at least 50 years of scientific scrutiny of these techniques, we still know next to nothing about the physiology of regeneration of roots and shoots at the cellular level, we must welcome any favorable test organism which promises to shed light on these processes. It stands evident almost without saying, that the better our basic understanding of the details of root and shoot formation, the better will be our ability to control these processes to serve our needs. Unquestionably, wild carrot tissue cultures provide us with the best test organism yet discovered for studies of the complexities of regeneration.

Now to turn to the more speculative question of potential uses of tissue culture techniques in practical plant propagation. Four possibilities come to mind:

1. The use of tissue culture as a means of rapid propagation of new varieties. It should be possible, in a few months time, to produce thousands of uniform "true to type" plantlets from tissue cultured callus produced by auxin treatment of a single small piece of stem or root taken from a new variety.
2. Tissue culture techniques may enable us to propagate vegetatively, species which resist conventional techniques. Plants which are difficult to root in the cutting bench may respond more favorably to the more rigidly controlled environment possible with tissue cultures.
3. The production of new varieties through the use of mutation-inducing irradiation or mutagenic chemicals might become practical if tissue cultures were treated rather than intact plants. Attempts to induce new genetic forms by treating intact plants, seeds or even pollen grains with mutigens suffer greatly from the overgrowth of the mu-

tated cells in the highly competitive growing tissues or by the loss of new mutations because they are incompatible with the material tissue and therefore do not produce viable seed. In a system like the wild carrot tissue cultures, free-cell suspensions could be treated and all viable cells might be brought to the plantlet stage for further examination and testing of the induced changes.

4. Perhaps of more value to the breeder than to the propagator, would be the establishment of a tissue culture bank as an economical means of long term storage and maintenance of the germ plasm (the breeding stock) of the countless new varieties of plants. While no thorough genetic studies have yet been seen, we have good reason to believe that the plants which are regenerated from stored callus cultures are identical to the donor plant from which the original callus was obtained.

If any of these potential applications are worth achieving then plant propagators should keep one eye on developments in the field of tissue culture. The recent progress in this field, some of which have been described here, is certain to attract the attention of many new researchers. As a result, the rate of accumulation of new knowledge of the mechanism and control of regeneration should greatly increase. Thirty years ago, who among us would have believed that today commercial growers would be making routine use of chemical growth regulators or flash-lighting. It seems to me no more unlikely that the work we have described here will find its way into the practical procedures of the future.

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MODERATOR SHUGERT: Thank you very much, Dr. Wetherell, for a very stimulating talk. Our final speaker this afternoon is Dr. Don White from the Department of Horticulture, University of Minnesota.