year we tried it and I was just flabbergasted with the results. So that's what we did and we've been doing it every since.

VINCE BAILEY: I noticed in the pictures a few unrooted cuttings. Do you restick them?

LEONARD SAVELLA: Yes. If you restick unrooted cuttings indoors with heat those that are good will root very fast, probably in 2 weeks. And if you look in the back of the room, the one with the longest roots is the one that was restuck.

RALPH SHUGERT: Do you have cost figures on the three year cutting and grafts of the spruce?

LEONARD SAVELLA: No, like everybody else, I just do it. I don't know how much it costs me.

E. STROOMBEEK: I have two questions. Have you taken cuttings from different stock plants and where did the stock plants originate?

LEONARD SAVELLA: The stock plants were bought from Case Hoogendoorn. They are true Kosters. We have tried other varieties and we find Moerheim more difficult and the Hoopsi true to its name, it's tough.

BRUCE BRIGGS: Did you try some with pulling off and leaving the heal rather than cutting them off?

LEONARD SAVELLA: Yes, we tried pulling them, but we had better results with cutting them. It seemed that the right time to take them is just when the pulling stage is past.

BRUCE BRIGGS: We know that spruces root better when they have lots of free air. Can you root the same way inside the greenhouse having it completely open?

LEONARD SAVELLA: There again, I never tried it out, but getting the 85% outdoors, I thought I'd leave well enough alone.

PETER VERMEULEN: Did you find any difference on the position on the stock plant or between the lower cuttings and cuttings higher on the plant?

LEONARD SAVELLA: That's a good question, Pete. We took cuttings as far as we could reach.

HANS HESS: Was this a continual mist that you had on these cuttings or was it intermittent mist?

LEONARD SAVELLA: Intermittent. Here again, I never took the trouble of timing these things. I just put the mist on and when I thought it was enough I left them alone. Maybe if I figured this thing out, they wouldn't root.

MODERATOR PINNEY: Next, we shall learn about cell culture and plant propagation from Dr. John Mahlstede.

CELL CULTURE AND PLANT PROPAGATION

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Plant propagation, defined simply as the multiplication of plants by either sexual or asexual means involves the use of

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many and diverse techniques which you as plant propagators are quite familiar. The asexual propagation system that you use to supply that plant part with the missing organ or organs has as its basis the satisfaction of certain basic physiological processes founded on plant biochemistry and related to plant anatomy. There are many challenging and fascinating problems in plant reproduction. They are as diverse and intricate as the numerous enzymes, amino acids, proteins and auxins that control plant growth and development. Answers to these questions come slowly, and new break-throughs often represent the integration of many accumulated facts that are put together into a workable and sensible hypothesis. These are then assimilated by the resourceful horticulturist and applied directly to the solution of some propagation problem.

In 1963, Wetherell (2), of the University of Connecticut, described a system for growing whole plants from single cells. While the idea was not new and had been previously demonstrated by Steward (1), of Cornell University, the fact that vegetative cells of the type contained in the tissue systems of the cuttings you propagate, could be manipulated through a series of embryogenetic steps which approached that of the fertilized zygote of sexual propagation, was a breakthrough. By using a defined growing medium, callus tissue could be broken apart into single cells, and each single cell be induced to divide, differentiate and to eventually form an intact plant. The use of 2,4-D and adenine stimulated callusing. Kinetin substituted for adenine induced specialization and establishment of the familiar tissues systems and the formation of organs. Removal of the auxin 2,4-D stimulated embryo formation and the addition of coconut milk provided the impetus of after ripening and permitted the embryo to develop into a normal plant.

The technique appears simple, and it is for carrot. It is not simple for many of the types of plants which nurserymen grow and propagate.

We might pause a moment to reflect on the application of this technique to the problems that we have in propagation. The technique of tissue culture provides a tool by which we can study the effects of auxins and related balance systems on cell differentiation, growth and development. By providing precise conditions of environment, the effects of pH, aeration and many other conditions of the mirco-environment can be critically studied as they are related to the propagation techniques that we now apply grossly. As information is gathered, the propagator will become more deeply involved with refinements in the propagative skills and management practices he now employs. Propagation will be truly a science.

This trend has been evident since the founding of this Society. The development of mist propagation and the refinements and ingenuity applied to the regulative devices sequencing the application of water to best meet the physiological requirements of the cutting as well as the use of a form of tissue cul-

ture to index and develop pathogen-free plant material are other indications of the rapid progress which is taking place in the science of plant propagation. They further illustrate the importance of science to the plant propagator.

At Iowa State University we have been experimenting with the techniques of plant tissue culture for a period of four years. Cultures of carrot tissue were first used according to the procedures developed by Steward and Mapes. Chrysanthemum, geranium and Kolkwitzia are the plants currently under study in our laboratory. Our objectives, together with studying differentiation in these cell cultures are those of breeding and the development of new clones and cultivars.

Using *Kolkwitzia amabilis*, the common beauty bush, as an example, let me describe briefly our approach to the application of this technique. As you know, *Kolkwitzia amabilis*, and the variety *K. a. rosea* are the only forms in this genus. Any variation from these two would, in fact, represent a new clone.

In the spring of 1965, developing ovaries were collected from established plants at regular intervals. Developing embryos were then excised and cultured on a Marashige-Skoog medium with and without the addition of 2.5 mg/liter IAA, .2 mg/liter kinetin and 100mg/liter of yeast extract. When a solid medium was required, nine grams of agar was added to the basal medium. In one series of experiments the MS medium was further modified by the substitution of arginine and lysine for glycine, each alone as well as 1:4 and 4:1 proportions, all in equimolar concentrations to glycine.

Embryos were then cultured and segments of root, hypocotyl and epicotyl transferred to separate culture tubes and calluused. The developing callus mass was then broken into single cells (which is the only way in which mutations can be cloned) by the use of a chelating agent, Fe EDTA (ethylendiamine tetraacetic acid .01-.02 per cent). This chemical reduced the binding calcium of the middle lamella between cells. Separation was further activated by the use of magnetic stirrers and sterile Bellco flasks. These single cells were then transferred to a MS medium with IAA, kinetin and tyrosine. The individual study with lysine and arginine indicated that there was an optimum balance of these two amino acids as they affected the growth of the callus. Both ratios gave higher dry weights than either alone or glycine alone. From this it was obvious that each callus type may require its own organic nitrogen source, which can only be determined by trial and error.

By further subjecting these single cells to chemical mutagens, we hope, in the not-too-distant future to give to the nursery and arboretum inventory a whole new complex of *Kolwitzia* cutlivars . . . through a system of micro-propagation, a technique off the drawing boards of fantasy, but one that we hope will serve to further the science and practice of plant propagation . . . and a better living through plants.

LITERATURE CITED

1 Steward, F. C 1958. Growth and Organized Development of Cultured Cells. Amer. Jour. of Botany 45:693-703. 705-708, 709-713.
2. Wetherell, D. F. 1963. Growing Whole Plants from Individual Cells ,Proc. International Plant Popagators' Society 13:176-181.

HAROLD EPSTEIN: The meristem culture technique is already in use for orchids such as the desirable plants that everybody wants like the bright red cattleya and things like that and selling them as young plants for $7.50 and $8.50 apiece. Unfortunately, most of this is being done in France and the plants are exported to the rest of the world. France at this moment seems to have the production monopoly on meristem culture of orchids. So it's beyond the experimental stage.

DR. MAHLSTEDE: This is correct, but there is basically one difference between meristem culture and the type of technique that I discussed. We're getting down to the individual cell rather than a group of cells. And it's the process of differentiation that forms the apical meristem or root meristem that is already formed in the segment of the tissue that you have mentioned. It's essentially the same type of thing with the basic difference being the number of cells and the organization of the cells you start with.

DR. STOLTZ: Just a follow up on what you have said, John. We are also doing this type of work with orchids at the University of Kentucky. It does, of course, have the very basic difference that you pointed out.

JIM WELLS: John, have you been able to grow the single cells to complete plants yet?

DR. MAHLSTEDE: I was afraid you'd ask that, Jim. No we haven't. We have with carrot and with *Chrysanthemum* but not with the *Kolkwitzia*. In the past they have been using an agitated culture in which they take the Erlenmeyers and put them in a gently swishing solution. The single cells then are forced apart and land on the edge of the glass at the juncture of the back and forward movement, and from there they grow and develop. We've used flasks with a magnetic stirrer and this gently rotates — there is no violent action and then by the use of ETA the cells are gently split apart. And, of course, it's interesting how these cells develop when taken out of the framework of an entire plant part. And of course, we're doing exactly the same type in our propagation techniques. You and I, when we stick a cutting, try to provide an environment for it to grow. We are doing exactly the same type of thing on defined media using some of the results that Charlie has and some of the products he is using specifically in tissue culture and I'm sure that Len back here is doing the same thing. In other words it's a refined mirco-technique that I'm sure will have application in the future.

DICK STADTHERR: Have you irradiated any of these cells as yet?

DR. MAHLSTEDE: We've irradicated them, but we haven't set up this dosage yet. As you might imagine that it is pretty sensitive at this time. We've killed everything we've treated so far.

VOICE: Have you been able to find any chromosomes yet in these cells?

DR. MAHLSTEDE: Yes. but of course, with *Kolkwitzia* they are dfficult to find.

JIM ILGENFRITZ: John, do you have any data on the number of generations or divisions or the length of time before we begin to get differentiation from a single call?

DR. MAHLSTEDE: We haven't determined the exact number of divisions but certainly there are a great number of them.

MODERATOR TOM PINNEY: At this point we have asked five Canadian Nurserymen to discuss propagation of difficult, unusual and rare plants. At this time it gives me real pleasure to introduce the first of these gentlemen whom all of you I am sure know, Mr. Ray Halward of the Royal Botanical Gardens.

PROPAGATION OF DIFFICULT, UNUSUAL AND RARE PLANTS

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The plants included in this article are not necessarily difficult to root, but certainly could be classified as unusual, especially in our area. The *Davidia* or Dove Tree has been mentioned in previous papers. In 1958, Miss Mary Milton, former propagator of the Morris Arboretum, related its performance in the Philadlephia area. In 1960, Alfred Fordham, explained the best treatment for seed germination. My interest in *Davidia* was aroused in 1960, when I observed a 30′ specimen growing in Hamilton. It was imported in 1935 a 6′ plant from Daisy Hill Nurseries, Neury, Northern Ireland, and given some protection with evergreen boughs for four years. In 1947 it flowered for the first time, and has flowered every year since that time. I tried rooting softwood cuttings on two occasions and was unsuccessful. In the meantime, seed I had received from Denmark and the Arnold Arboretum in 1960, had germinated and were doing quite well, in an acid medium under lath house conditions. On July 14th this year I took the cuttings from the seedling plants which were over six feet high. The cuttings were six inches long and the large leaves were reduced in size. They were dipped in a mixture of one half Captan 50 W and one half Seradix No. 3 and they were put in boxes with a mixture of four sand and one peat, and put under intermittent mist with bottom heat supplied by a cable set at 70°.

In the first lot of 22 cuttings from the Danish source, only six were rooted by October 31st. In the second lot of all cuttings from the Arnold Arboretum's seedlings, five of which had