8 to 10 years. Under these conditions there is nothing certain except change. These meetings are educational, as I have mentioned. They are designed to provide information to keep the plant propagator the key man in the ornamental plant business. As someone once said, the beauty of a rose starts with the vitality of its roots. As an organization, pledged to the dissemination of information and the provision for guidance and assistance in the field of plant propagation, we here assembled dedicate this meeting.

I have several appointments to make before we begin the sessions. These are as follows: to the nominating committee, the three immediate past presidents, Harvey Templeton, chairman; Bill Snyder and Martin Van Hof. To the auditing committee, Ralph Shugert, chairman and Leslie Hancock. The resolutions committee, J. S. Wells, chairman and Wayne Loveless. If you gentlemen will prepare statements for presentation at the annual business meeting Saturday afternoon, I will appreciate it. Now, if you will turn to your program you will see that Vice President and program chairman John Roller, has arranged, at your suggestion, a round table panel discussion. Before we break up, I might also announce that anyone interested in joining the Society and has properly completed the necessary forms, see Peter Vermeulen, who is chairman of the Membership committee.

[*Editor's Note*: The members separated into three round-table discussions; one, Storage and care of cuttings, grafts, and established nursery stock, J. P. Mahlstede, moderator, Jack Hill, recorder; two, Sanitation and propagation — methods and materials, James Wells, moderator, F. O. Lanphear, recorder; and three, Cost control in propagation — lowering costs, George Rose, moderator, Wayne Lovelace, recorder. Summaries of the round-table discussions were presented Thursday evening.]

PRESIDENT MAHLSTEDE: The first paper this morning will be presented by Mr. Dale E. Herman, a graduate student at Purdue University.

THE EFFECT OF ETIOLATION UPON THE ROOTING OF CUTTINGS

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I. Introduction

Etiolation is known to play a definite role in enhancing the rooting potential of many plants. A number of references attest to this fact in the literature, several of which will be cited below.

Sachs (1864) noted that adventitious roots formed in great abundance in darkness on portions of stems of a variety of plant species, but that this phenomenon would not occur in light(23).

Mevius (1931) found that light inhibited the rooting of cuttings of *Tradescantia* species (14). Gardner (1937) successfully rooted cuttings of McIntosh apple made with the basal cut in an etiolated area (3). Reid (1922) and Blackie, Graham, and Stewart (1926) used similar methods as Gardner to affectively root a difficult clone of camphor (21, 1). Smith (1924) found that etiolation enhanced rooting in the genus *Clematis* (25). Etiolation was proven very beneficial in rooting avocado cuttings, according to Frolich (1961) and Johnston and Frolich (1957) (2, 10).

The process of etiolation is practiced most extensively in the various layering methods of vegetative propagation, especially in relation to the stooling and etiolation methods used at the East Malling Research Station. The advantage obtained in the rooting of various fruit and ornamental species by these methods is borne out in references by Regel (20), Knight et al (11, 12, 13), Garner and Hatcher (4), and Sinha and Vyvyan (24). We probably unconsciously take advantage of etiolation when we stick cuttings into a medium to root.

Although it is well established that etiolation does exert a pronounced effect in the rooting process of many plants, little progress has been made in elucidating the actual physiological and anatomical basis of such an effect. Therefore, this investigation was started using four different approaches.

1. A rooting study to verify enhanced rooting as a direct result of etiolation.
2. An anatomical study to determine any differences in the cells or tissues of etiolated stems as opposed to nonetiolated stems.
3. An investigation of the extractable auxins from etiolated tissues as compared with nonetiolated tissues.
4. A study to determine the level of extractable rooting "cofactors" in etiolated as compared with nonetiolated tissues.

Etiolation is the development of plants or plant parts in the absence of light. In this study, in contrast to most previous discussions on etiolation, we were interested only in the light falling on the actual tissue from which root initiation was to occur. Thus, this was only a localized etiolation or a tissue blanching.

II. Root Initiation Study

A. Methods

Red Kidney Bean, *Phaseolus vulgaris* L., and Chinese Hibiscus, *Hibiscus rosasinensis* L., were selected as test plants for the differential rooting study as well as other investigations. Three varieties of *Hibiscus* were used: (1) Cornell Red, a relatively easy rooting variety, (2) Ruth Wilcox, intermediate in rooting ability, and (3) Wilson's White, a difficult to root variety. The letters CR, RW, and WW will serve to designate the respective

varieties, and the letters RKB are used to designate red kidney bean.

The RKB's were planted in 4-inch plastic pots in a loamy clay soil mixed with one-third peat moss, 1 seed per pot. The etiolation treatment was begun an average of 18 days after the seeds were planted. By this time the first 2 unifoliate leaves were developed. The apical bud was pinched out of all plants to maintain a relatively equal photosynthetic surface on all plants.

Etiolation of the tissue was accomplished by wrapping the stems with black construction paper. The paper covered the tissue from the soil level to the second node, or the node from which the unifoliate leaves arise. Thus, the hypocotyl, cotyledonary or first node, and epicotyl tissue up to the second node were covered. One-half of the plants were left unwrapped as a control.

After an average of 3 weeks etiolation the black construction paper was removed and cuttings were taken slightly above the soil level. They were rooted in flats of perlite under intermittent mist without auxin treatment and the roots were counted at 4 and 8 day intervals. The average results of 7 replications are shown in Table I.

Table I — Effect of Etiolation Upon Root Initiation in Red Kidney Bean (*Phaseolus vulgaris*).

Rooting Period	Average Number of Roots per Cutting	
	Non Etiolated	Etiolated
4 days	3.0	16.3
8 days	19.4	36.0

As a final RKB rooting test, an auxin synergistic study was carried out. Forty etiolated and 40 nonetiolated cuttings were taken of which 20 from each group were treated with IBA using the concentrated dip method. Root counts were made at 4 and 6 day intervals and the results are shown in Table II.

Table II — The effect of Etiolation with and without Indolebutyric Acid (IBA) upon root initiation in Red Kidney Bean (*Phaseolus vulgaris*)

	Average Number of Roots per Cutting	
	Non Etiolated	Etiolated
Rooting Period 4 days		
Without IBA	4.2	31.0
With IBA*	1.4	125.4
Rooting Period 6 days		
Without IBA	27.3	39.4
With IBA	114.2	250.+

*IBA conc = 0 3gm in 100cc 50% ethanol, quick dip

Three experiments to study the effect of etiolation on the rooting of *Hibiscus* were conducted. Young, rather vigorous growing shoots were selected for etiolation, which was accomplished in the following manner. Except for the apical, primordial bud and adjacent unexpanded leaves, the 2 to 3 leaves were removed for an approximate distance of 4 inches below the bud. This area of the young shoot was then wrapped with black polyethylene plastic. After being wrapped for an average of 36 days, the new vegetative growth was several inches above the wrap. Cuttings were taken one-half inch above the base of the wrap and were treated with IBA, again using the concentrated dip method. The cuttings were placed in a plastic enclosed intermittent mist chamber and root counts made after an average of 26 days. Corresponding nonetiolated controls were taken the same day and in all respects treated the same. The average results from the 3 experiments with all 3 varieties are shown in Table III.

Table III — Effect of Etiolation upon Root Initiation in *Hibiscus rosasinensis* Varieties

Rooting Period-26 Days	Non Etiolated	Etiolated
Percent Rooting		
Wilson's White	6.7	13.3
Ruth Wilcox	88 3	93.3
Cornell Red	77.0	97.0
Ave. No. of Roots per Cutting		
Wilson's White	0.2	0.6
Ruth Wilcox	10.0	18.2
Cornell Red	6.1	10.5

B. Results and Discussion

The RKB results show conclusively that etiolation promotes root initiation. Table I shows that the rooting of the etiolated cuttings was over 5 times as great as that of the nonetiolated cuttings after 4 days rooting. After 8 days the rooting was still almost twice as great. Since few roots would be initiated after the 8th day, the 2:1 ratio would hold constant. Thus, etiolation renders a very prompt or initial response in the rooting process, which is evidenced in part by the much longer roots on the etiolated cuttings after 4 days. Etiolation not only strongly predisposes the plants to root formation, but root initials are actually formed prior to supplying of the conditions normally favorable for the rooting process. This was very evident as the wrap was removed, for the root primordia were already clearly visible on many of the cuttings.

Although the RKB generally roots to some extent even though no auxin or etiolation treatments are given, a final study

was carried out to determine the response of etiolated and nonetiolated cuttings to treatment with auxin. The results, shown in Table II, demonstrate that etiolation dramatically enhances the effectiveness of an auxin. The rooting of the etiolated cuttings was over 89 times greater than that of the nonetiolated cuttings after 4 days rooting. This seems to definitely indicate that some factor other than higher levels of endogenous auxin is involved. A possible interpretation is that the etiolated tissues contain a higher level of auxin synergists than the nonetiolated tissues.

The *Hibiscus*, according to Table III, was somewhat less responsive as a test plant. This, however, may be due to the fact that herbaceous plants often root quicker and more prolifically, numerically speaking, than hardwood or softwood cuttings of woody plants.

The WW variety is one that may parallel Hartmann and Kester's third grouping of plants in relation to the presence of root-promoting materials (7). Thus, an exogenous application of auxin gives little or no response due to the lack of other as yet unidentified factors—hormonal or nutritional or both. Even after etiolation of the cuttings, there is only a very slight response. However, the slight rooting edge held by the etiolated cuttings is also borne out in the percentage of cuttings rooted after 26 days. After 40 days rooting, 30% more of the etiolated cuttings were rooted as compared with the nonetiolated cuttings.

The most significant results from etiolation were shown by the intermediate rooting RW variety. The rooting obtained in the etiolated cuttings was nearly double that of the nonetiolated cuttings. One cannot fully account for the abnormally high rooting received in this variety. However, the high levels of auxin synergists which showed up in the RW tissues as indicated in the *Avena* first internode bioassays certainly adds positive evidence which may account for at least part of the response received. Reference will be made to this point in the discussion of the auxin synergistic study.

The CR variety tends to parallel Hartmann and Kester's second grouping of plants, in which the internal root-promoting substances of a hormonal or nutritional nature (suggested by Van Overbeek *et al.* for a red *Hibiscus rosasinensis* variety to be sugar and nitrogenous substances supplied by the leaves) (27) are present, but auxin is limiting. Thus, this variety roots well with an auxin application. One can note in Table III the consistently higher rooting obtained in the CR variety when etiolated. Also, significantly higher percentages of the etiolated as opposed to the nonetiolated cuttings of RW and CR were rooted after 26 days. An etiolation period of 36 days did not induce the production of root primordia visible to the naked eye, as in the RKB, but it certainly enhanced their rooting ability.

III. Anatomical Study

A. Methods

Microtechnique methods used in this study were based on those of Riker and Riker and Johansen (22, 9). One-half to one centimeter etiolated and nonetiolated stem sections were killed and fixed in formalin-aceto-alcohol (FFA) for 24 hours under partial vacuum. An ethyl alcohol-zylene dehydration schedule was used. After the tissues were embedded in paraffin blocks, they were softened in water and sectioned into 12 micron thick ribbons. Safranin and fast green were used to differentially stain the tissues. Individual stem cross-sections were then photographed.

B. Results and Discussion

The results of the anatomical study are illustrated in part by means of the photographs of nonetiolated and etiolated RKB

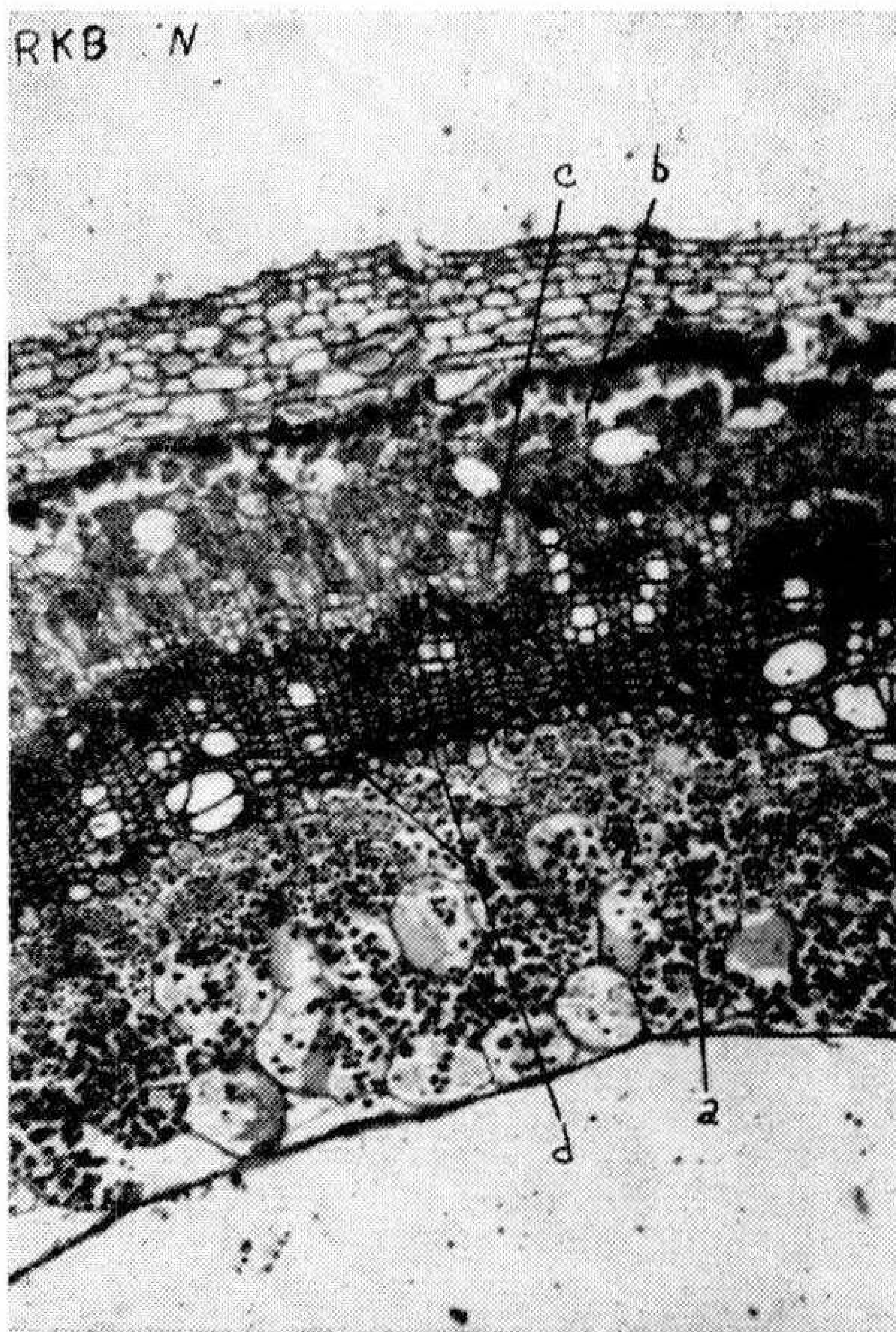

Figure 1. Cross section of a nonetiolated stem (hypocotyl) of *Phaseolus vulgaris*. Magnification — X 17, N — nonetiolated, RKB — Red Kidney Bean.

a. abundance of starch grains but a decrease in pith area and cell size.
b. pericycle fibers greater in number and slightly more lignified.
c. parenchyma cells less abundant and tissues more differentiated.
d. presence of increased amounts of cell wall deposits.

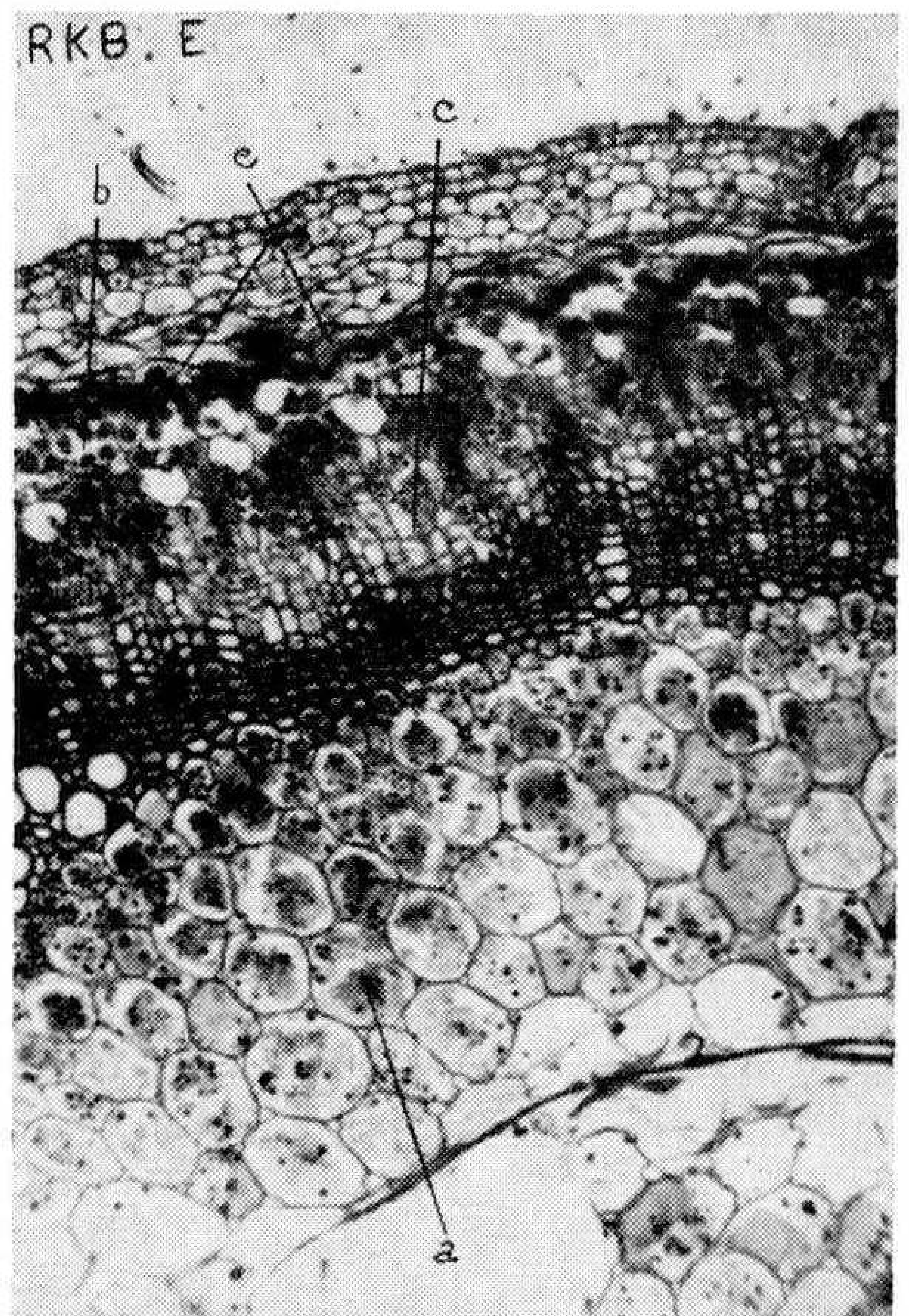

Figure 2. Cross section of an etiolated stem (hypocotyl) of *Phaseolus vulgaris*. Magnification — X 17, E — etiolated, RKB — Red Kidney Bean

a. reduction in quantity of starch grains but an increase in pith area and cell size.
b. pericycle fibers fewer in number and greater quantity of tissue and slightly less lignified.
c. abundance of parenchyma cells in a less differentiated condition.
d. reduction in amount of cell wall deposits.
e. indication of starch sheath.

and CR *Hibiscus* stem cross sections in Figures 1-2 and 3-4 respectively.

In reviewing the literature in relation to the anatomical basis of the effect of etiolation upon rooting, numerous papers by Priestley *et al.* cite evidence that a functional endodermis forms in etiolated shoots of various plants (17, 18, 19). Although this phenomenon was discussed in regard to totally etiolated plant tissues, as opposed to only a localized tissue etiolation in this study, it nevertheless warrants some attention. According to Priestley *et al.*, the secondary endodermis is characterized by the presence of the Casparian strip which becomes very suberized and impregnated with unsaturated fatty substances which harden as they oxidize. This suberin lamella renders the secondary endodermis relatively impermeable both to water and solutes. When the sap is retained within a functional endodermis only the parenchymatous tissues within that endodermis are capable of active growth. Since the endodermis completely retains the nutrient sap in the etiolated stem, it leads to an active formation of endogenous roots.

In young angiospermous stems the innermost layer of the cortex often contains abundant and large starch grains. Since it occupies the position where the endodermis would occur if it

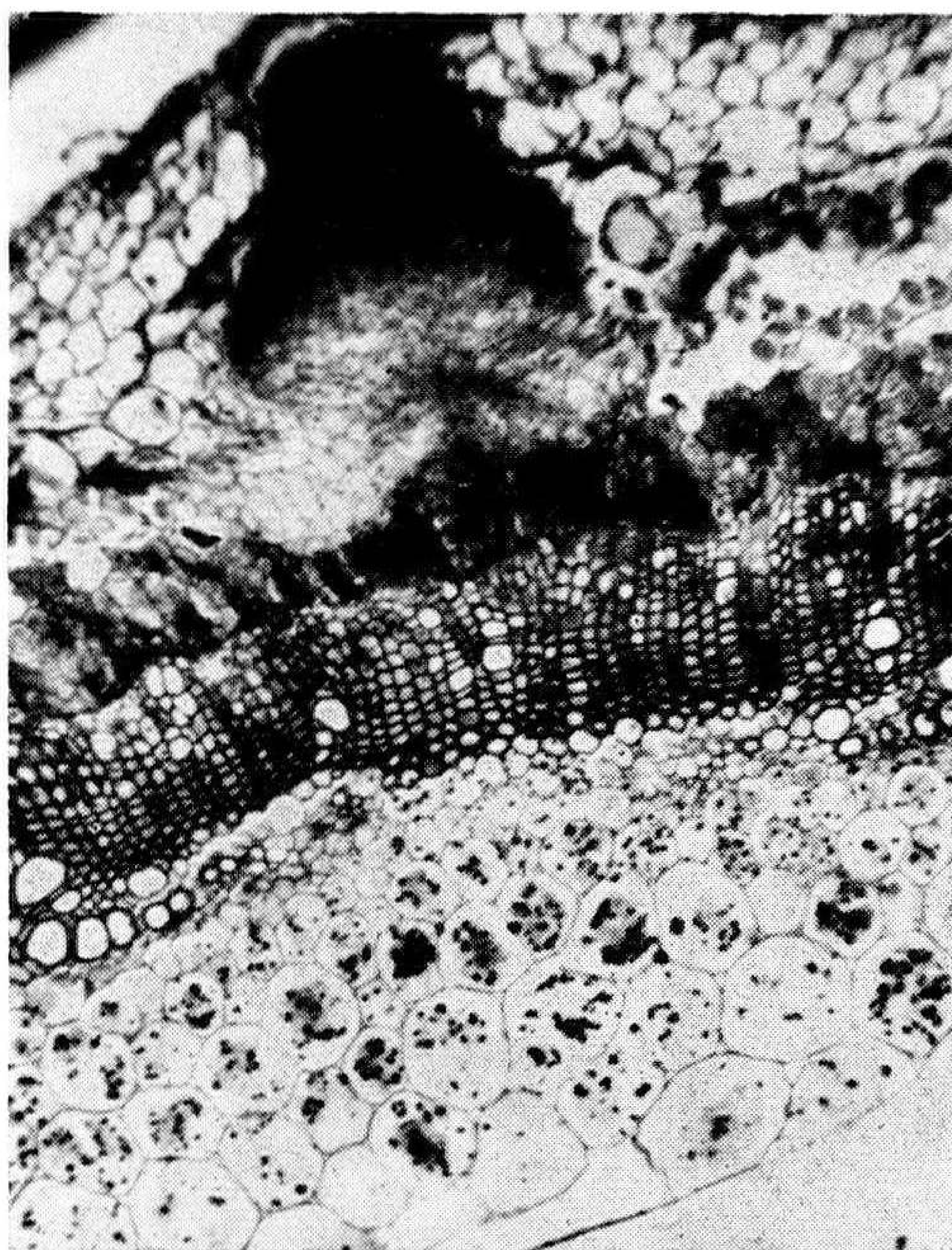

Figure 2a. Cross section of an etiolated stem of *Phaseolus vulgaris* showing an emerging adventitious root. Magnification — X 24, E — etiolated, RKB — Red Kidney Bean.

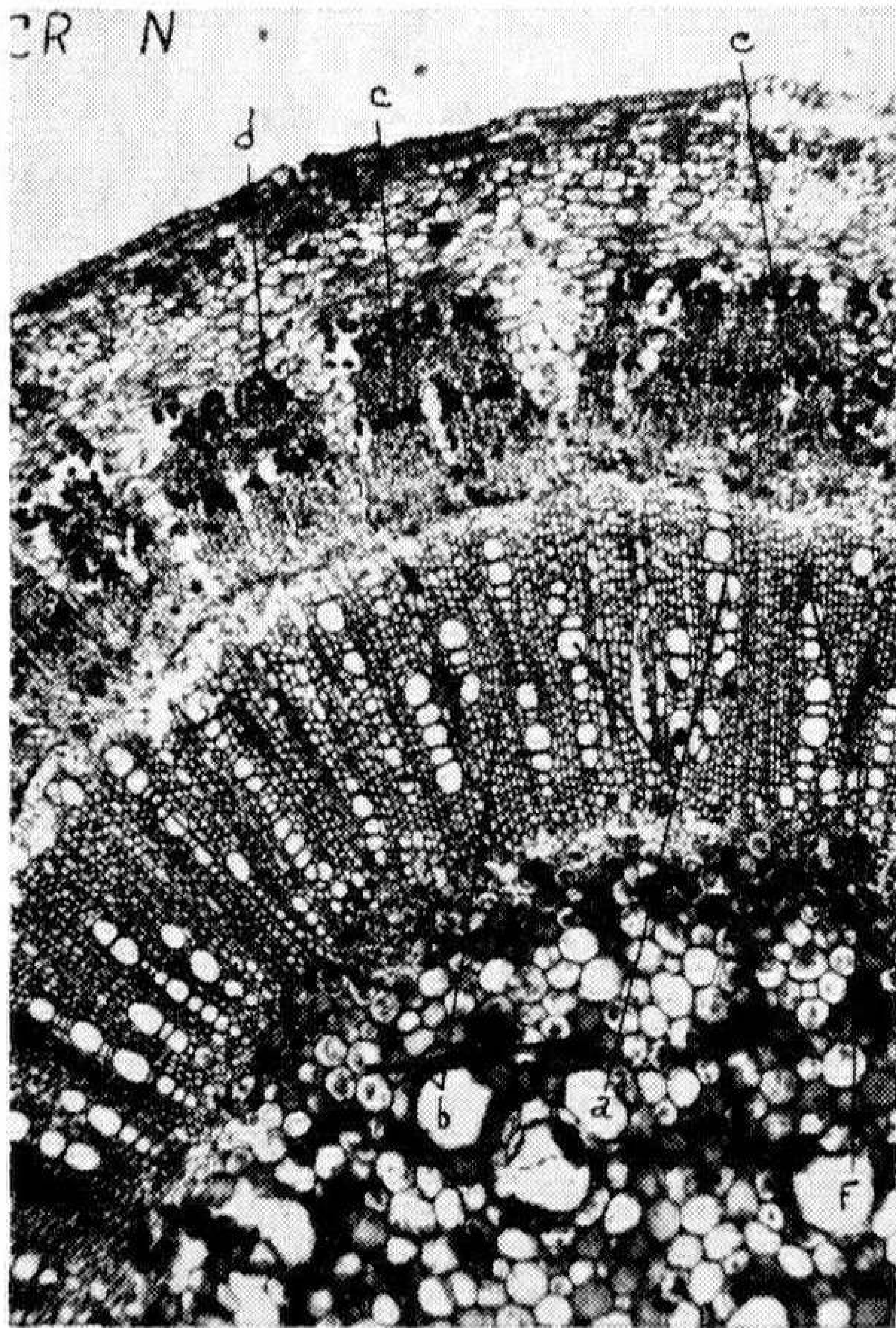

Figure 3. Cross section of a nonetiolated stem of CR variety of *Hibiscus rosasinensis*. Magnification — X 10, CR — Cornell Red, N — nonetiolated.

a. xylem more differentiated, vessels numerous.
b. vessels and other cells in xylem thicker walled.
c. phloem fibers more lignified.
d. pericycle fibers more lignified.
e. parenchyma cells less abundant and tissues more differentiated.
f. presence of increased amounts of cell wall deposits.

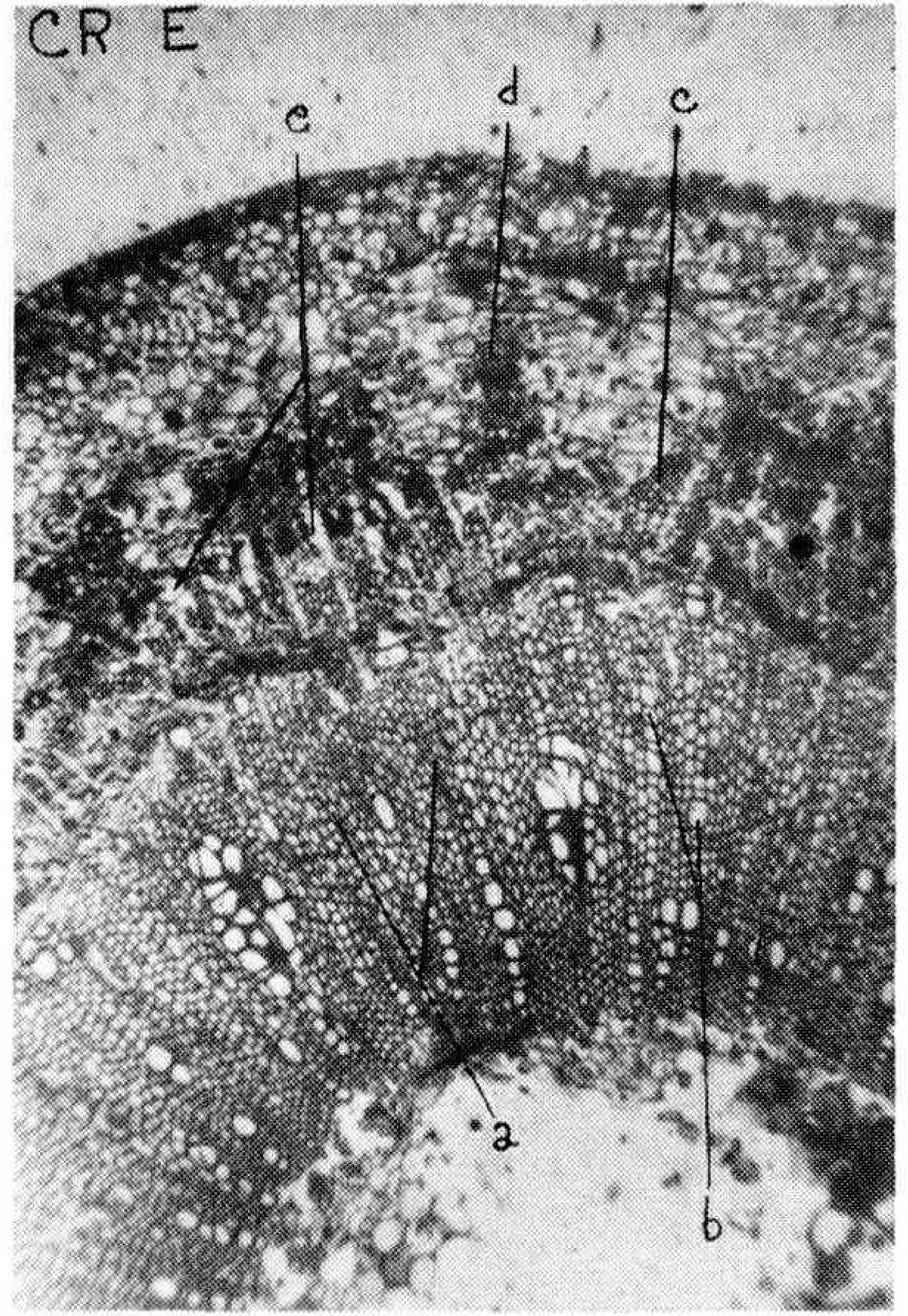

Figure 4. Cross section of an etiolated stem of CR variety of *Hibiscus rosasinensis*. Magnification — X 10, CR — Cornell Red, E — etiolated

a. xylem less differentiated, fewer vessels.
b. vessels and other cells in xylem thinner walled.
c. phloem fibers somewhat less lignified.
d. pericycle fibers somewhat less lignified.
e. parenchyma cells more abundant and greater quantity of tissue in less differentiated condition.

developed, the starch sheath, according to Guttenberg (6), is sometimes considered homologous with the endodermis.

The anatomical study revealed a number of differences in the etiolated as opposed to the nonetiolated cells and tissues and these are summarized below.

1. A substantial reduction in starch in the etiolated RKB tissues as opposed to the nonetiolated tissues.
2. An endodermis with Casparian strip was not observed in either etiolated or nonetiolated RKB or *Hibiscus* tissues; however, there was an indication of a starch sheath in both etiolated and nonetiolated RKB tissues.
3. Presence of root primordia in the etiolated RKB stem cross sections, with none observed in the nonetiolated tissues.
4. A reduction in development of mechanical strengthening tissue, and a decrease in lignification in cells, e.g., in the

phloem and pericycle fibers, in etiolated stems of RKB as well as *Hibiscus*. The presence of greater amounts of cell wall deposits, e.g., cellulose and hemicellulose, in nonetiolated stems.

5. Greater pith area with larger cells in the etiolated RKB stem as opposed to the nonetiolated stem.
6. An increase in content of parenchyma tissue in etiolated cross sections of both RKB and *Hibiscus*.
7. The presence of thinner walled cells in etiolated tissues, e.g., vessels and fibers in xylem of *Hibiscus*.
8. An overall reduction in cell and tissue differentiation, e.g., a slight reduction in differentiation and total amount of vascular tissue, in the etiolated cross sections of both RKB and *Hibiscus* as compared with the nonetiolated cross sections.

In conclusion, several hypotheses are suggested:

1. With the difference noted in starch content, it may be possible that there is an increase in the level of soluble sugars in etiolated tissues.
2. The presence of increased amounts of parenchyma cells and a greater quantity of cells in a less differentiated condition may be very advantageous as a potential source of enhanced meristematic activity leading to root initiation. Secondly, it may result in a more efficient utilization of endogenous IAA, as well as exogenous applications. The slightly higher endogenous level of IAA which appears to be present in etiolated tissues may thus be of added significance.
3. A reduction in mechanical strengthening cells and tissues may render the egress of root primordia easier, as Kuster has advocated (13).
4. Doubt is expressed that these anatomical differences are substantial enough to account for the highly significant difference in rooting encountered in etiolated versus nonetiolated cuttings. Thus, the possible accumulation of some other unknown substance or auxin synergist in the localized etiolated area is by no means eliminated.

IV. Auxin Study

A. Methods

The extraction and bioassay techniques used in the comparative investigation of the extractable auxins from etiolated and nonetiolated tissues were based on the methods of Nitsch (15) and Nitsch and Nitsch (16).

1. Extraction

Etiolated and nonetiolated cuttings were taken, placed in a container with dry ice, and then lyophilized. Prior to freezing, the outer bark — the tissues from the vascular cambium outward — was removed from the *Hibiscus* stems and only this

outer bark cylinder, containing the phloem, was lyophilized. The entire RKB stem was lyophilized. A 0.5 gram sample of the dry, ground tissue was used for extraction with absolute methanol as the solvent. Three 40 minute extractions were made of each sample, and after each 40 minute period the extract was decanted and fresh solvent added to the tissue. For each extraction 25 cc. of methanol were used, giving a total of 75 cc. per sample. The extracts were then concentrated and evaporated just to the point of dryness and were taken up in 2 cc. of methanol, 1 cc. at a time.

2. Chromatography

The concentrated extracts were spotted on a 22½ x 18¼ inch sheet of number 3 Whatman chromatographic paper. The etiolated tissue was spotted on one-half and the nonetiolated on the adjacent half on a line across the width of the chromatogram 10 cm. from the top. The solvent system consisted of 8 parts isopropanol and 2 parts water (v/v). Using descending chromatography, the solvent was allowed to move down the paper for a distance of 30 cm. from the origin.

The chromatogram was prepared for bioassay by cutting 1-inch strips into 15 equal pieces, beginning at the origin or line of spotting and ending with the termination of the solvent front. A piece of the chromatogram which had been saturated with solvent and a piece taken from below the solvent front were used as controls. Each segment of chromatogram was placed in a corresponding test tube to which was added 2 cc. of citrate-K-phosphate buffer (pH 5.0) and 2% sucrose solution.

3. Bioassay

The straight growth tests described by Nitsch and Nitsch, (16) including the *Avena* coleoptile cylinder test and the *Avena* first internode (mesocotyl) test, were used for the bioassay of the auxins and inhibitors extracted from the stem tissues. Three days after planting variety Brighton oats, the coleoptile and first internode sections were approximately 2½ cm. in length and ready for use. Four mm. coleoptile sections were cut 3 mm. below the tip. In the first internode test the short coleoptiles produced on the dark grown seedlings were cut off at the node and discarded, and 4 mm. sections cut 2 mm. below the coleoptilar node.

In both tests 10 seedling sections were added to the tubes containing the chromatogram segments and incubation solution. After 20 to 24 hours incubation with rotation, the seedling sections were measured with a binocular microscope equipped with an ocular micrometer.

In addition to the usual straight growth tests, a test to determine the presence of auxin synergists was conducted. The procedure was the same as stated above with the addition that the incubation solution contained 1 x 10^{-8} M. IAA. This level of IAA was sufficient to stimulate growth but was considerably below the optimum.

B. Results and Discussion

The average results of 2 *Avena* coleoptile cylinder tests and 3 first internode (auxin synergistic) tests are expressed as histograms in Figures 5-8 and 13-16, respectively. Each column in the histograms represent the biological activity of one chromatogram section, starting with the base line of spotting on the left. The horizontal line across the histogram represents the growth of the controls. i.e., *Avena* sections in the buffer-sucrose solution with a piece of the chromatogram taken from above the solvent front and with one taken from the base which had been saturated with solvent. Columns above the control line indicate growth promotion, columns below the line indicate an inhibition of growth.

A comparative determination of the endogenous IAA auxin levels in etiolated as opposed to nonetiolated tissues was the primary objective at the onset of the investigation. Through color reaction or development of the "control" chromatograms, the R-f value for the synthetically prepared IAA extract was determined as approximately 0.79, or corresponding to chromatogram sections 12.0 to 13.7. This value corresponds very closely with the chromatographic position of the predominant growth promoting substance in the plant extracts, which occurs in the histograms from sections 11 to 13. The slight lag in the growth promotion or peak, which always occurred in section 12, is undoubtedly due to the plant extract, which tends to slow or hinder the movement of the auxin in the solvent system, compared to the movement of a synthetic preparation. Positive results were also received by spraying chromatograms of the native plant extracts with Salkowsky's reagent and also p-dimethylaminobenzaldehyde, but only after one gram samples of the tissue were used in extraction (5, 26). The color reactions specific for indole groups were verified by control chromatograms of synthetic IAA preparations. Thus, based on the above evidence, the predominant growth substance present in the plant extracts is probably IAA. However, its absolute identity was not finalized.

Histograms of the *Avena* coleoptile tests (Figures 5-8) show a striking correlation between the extracts of the growth

Figures 5 - 8 Histograms showing biological activity of chromatograms of etiolated and nonetiolated stem tissues of *Phaseolus vulgaris* and *Hibiscus rosasinensis*, var WW, RW, and CR 0.5 gram lyophilized tissue, extracted with methanol for 2 hours Extract chromatographed in isopropanol-water (8:2, v/v). Bioassay-*Avena* coleoptiles 4 mm long, taken 3 mm below tip Coleoptile control sections given concentrations 1 X 10-7 and 1 X 10-6 IAA grew to final lengths of 7.4 and 7.7 mm, respectively. Each histogram represents the average of 3 bioassays

E — etiolated
N — nonetiolated
RKB — red kidney bean
WW — Wilson's White
RW — Ruth Wilcox
CR — Cornell Red
IAA — indole-acetic acid

HISTOGRAMS SHOWING BIOLOGICAL ACTIVITY
OF CHROMATOGRAMS OF STEM TISSUE

Bioassay - <u>Avena</u> Coleoptiles

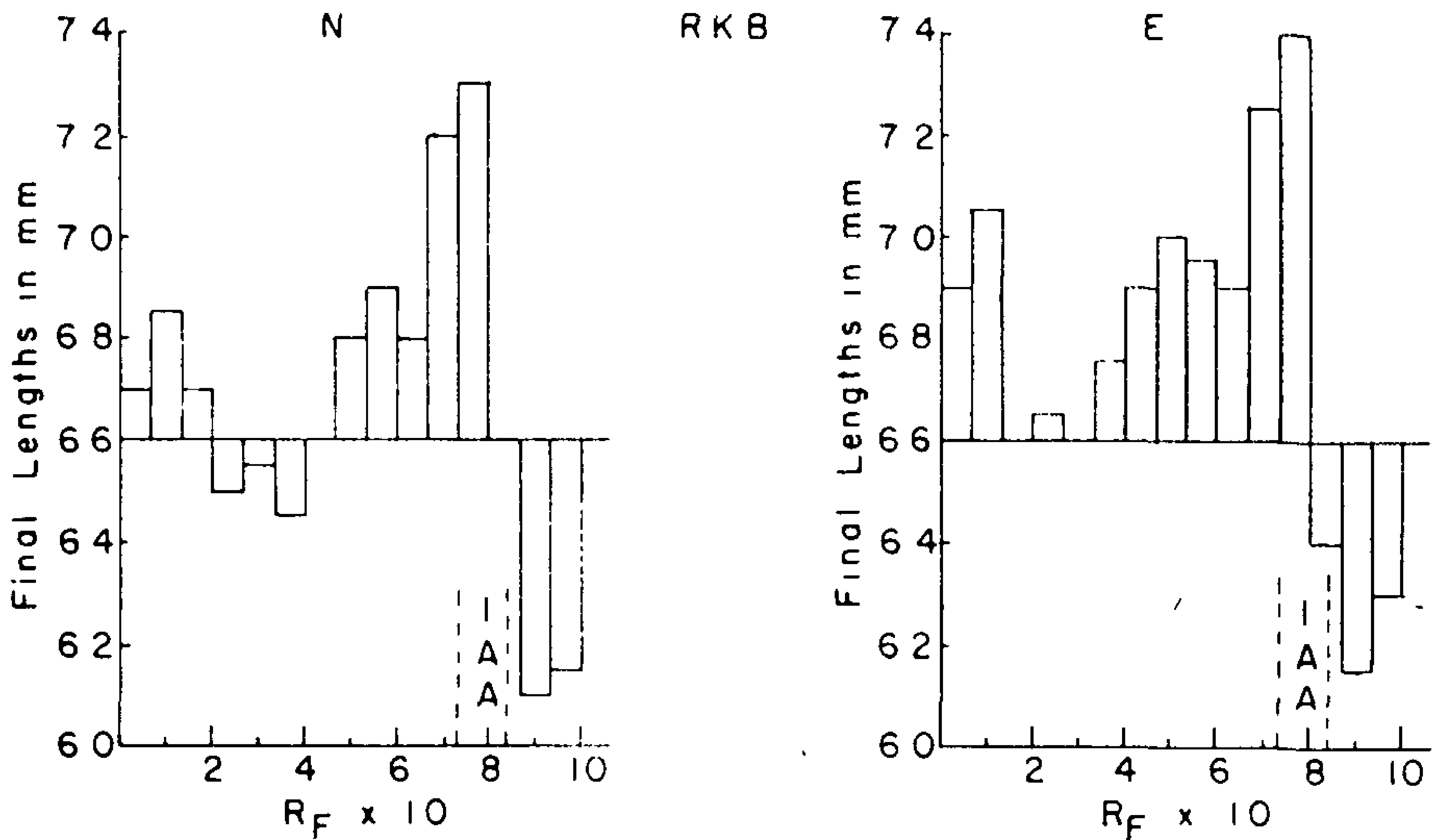

Figure 5

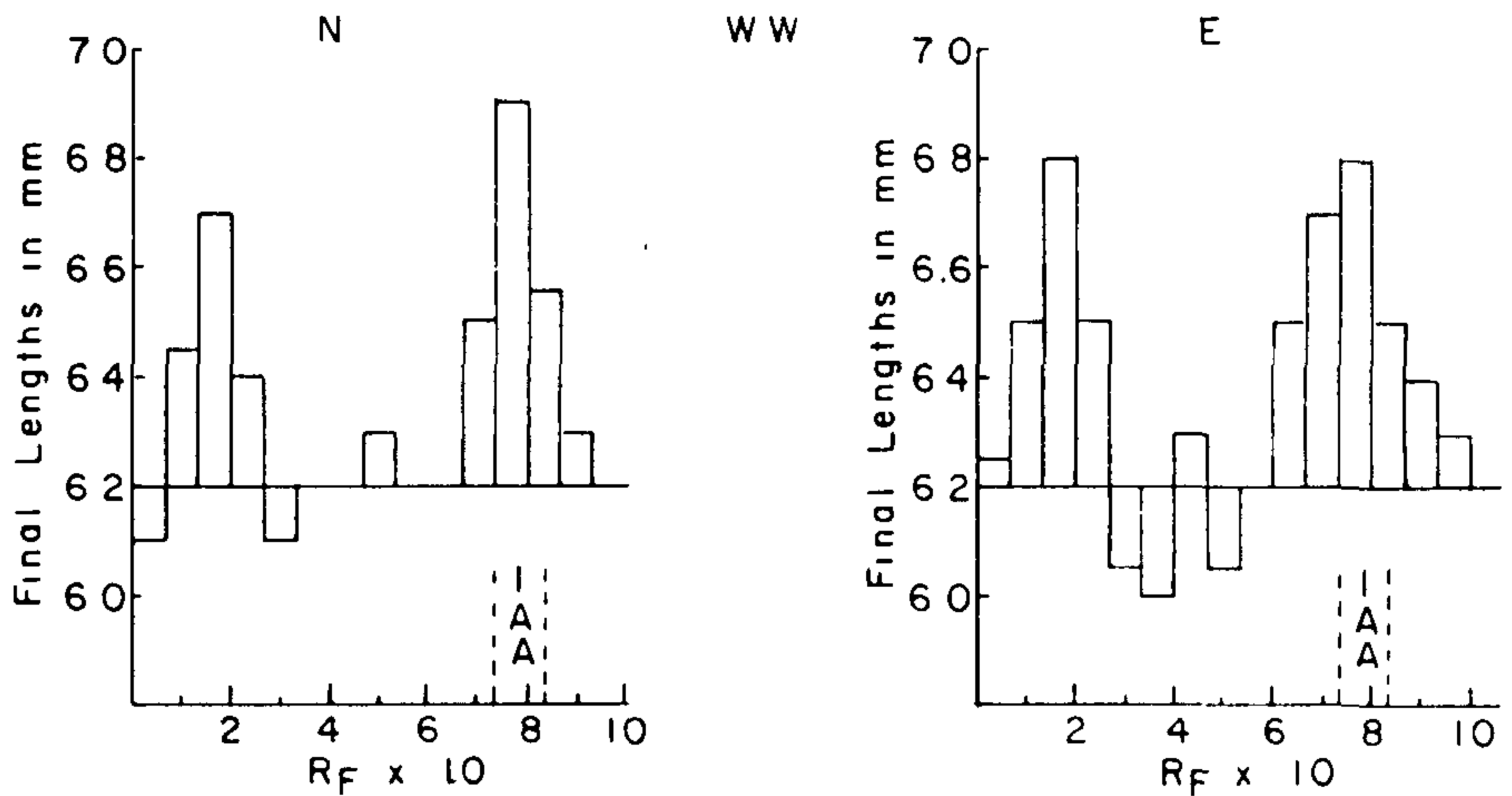

Figure 6

HISTOGRAMS SHOWING BIOLOGICAL ACTIVITY OF CHROMATOGRAMS OF STEM TISSUE

Bioassay - <u>Avena</u> Coleptiles

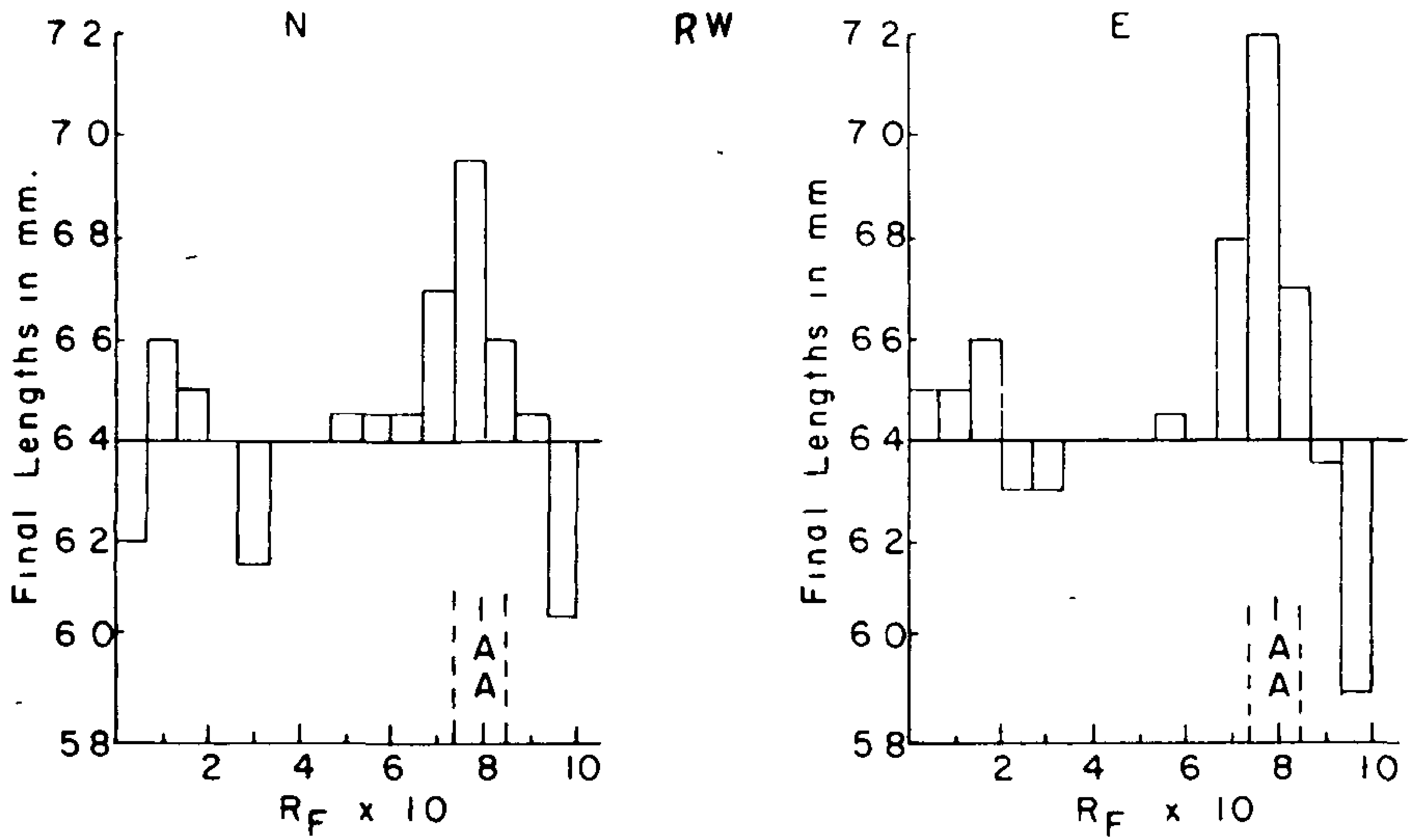

Figure 7

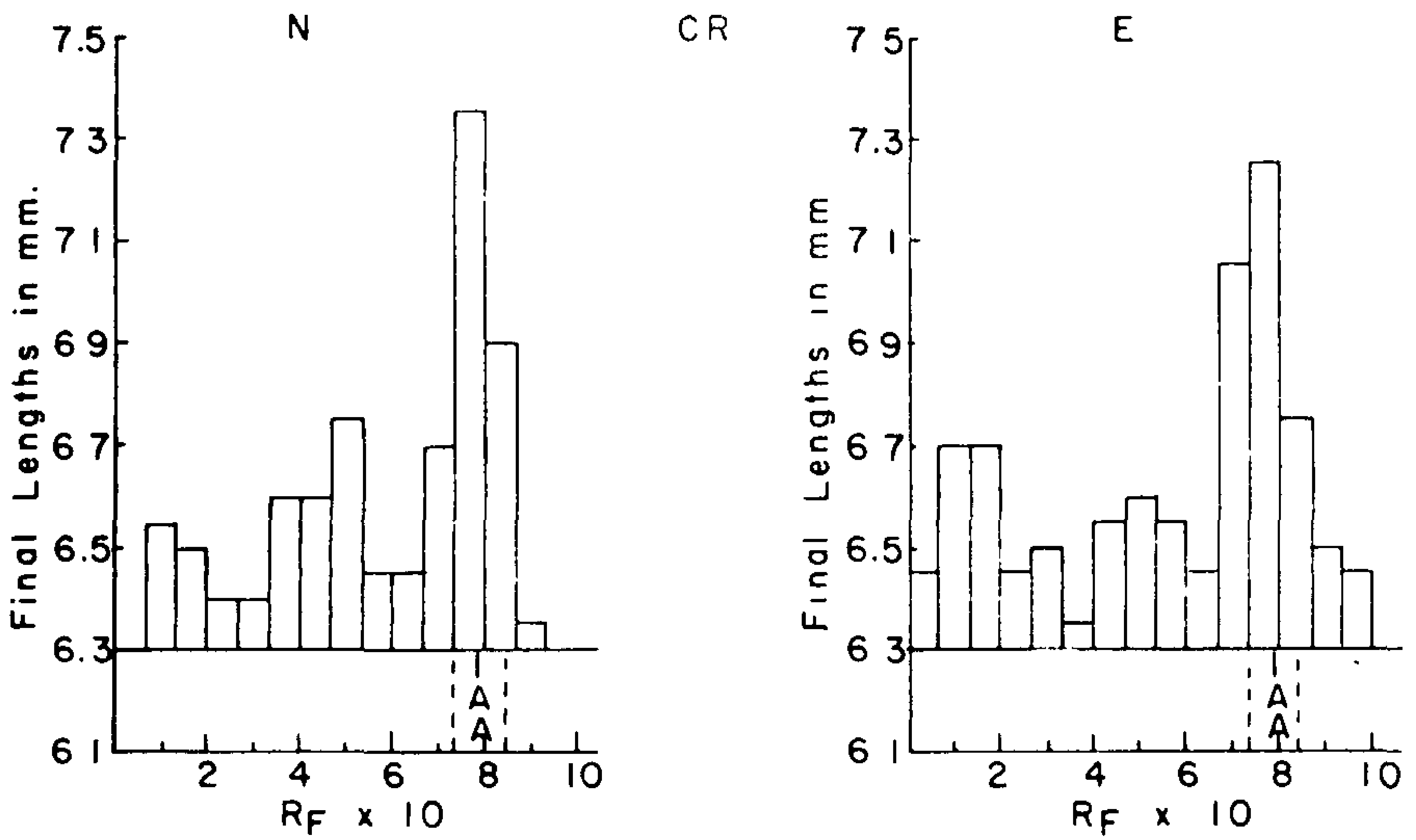

Figure 8

substances from the etiolated and nonetiolated stem tissues. However, growth promotion due to the presence of "IAA" is slightly greater in all cases from extracts of etiolated tissues. Thus, the endogenous concentration of "IAA" in etiolated tissues appears to be slightly higher than in the nonetiolated tissues.

At an R-f of approximately 0.05 to 0.15 (chromatogram sections 2 and 3) an unknown auxin occurs. As shown by the degree of growth promotion, this auxin also tends to be present in slightly higher concentrations in the etiolated tissues. Inhibitory substances do not appear to be present in highly significant quantities in the stem tissue extracts, except in sections 14 and 15 of the RKB, where they occur in approximately equal quantities in both the etiolated and nonetiolated tissues. A series of *Avena* first internode (mesocotyl) bioassays was also run in which the results coincided very closely with those reported in the *Avena* coleoptile tests.

Because the root initiation studies indicated the definite presence of auxin synergists, and also since a substantial difference in the level of endogenous auxins in etiolated as compared with nonetiolated tissue was not found, the growth response due to auxin synergists was studied. When analyzing the histograms in Figures 13-16, one must remember that the entire level of growth or elongation has been raised due to the addition of an exogenous IAA supply. Thus, the peaks from endogenous auxin promotion appear to be somewhat masked. Likewise, one may note that the growth peaks due to auxin synergists, e.g., rooting cofactors, are now greater than the growth peaks from auxin itself. This phenomenon would be expected since the synergistic response due to addition of an exogenous supply of IAA would undoubtedly be greater in those test tubes previously lacking in auxin than in one already containing an endogenous supply. This observation may especially be noted in relation to chromatogram section 12, and, in fact, may serve as indirect evidence that this growth substance is "IAA."

Figures 13 - 16 Histograms showing biological activity of chromatograms of etiolated and nonetiolated stem tissues of *Phaseolus vulgaris,* and *Hibiscus rosasinensis,* var. WW, RW, and CR 0.5 gram lyophilized tissue, extracted with methanol for 2 hours Extract chromatographed in isopropanol-water (8:2, v/v) Bioassay-*Avena* first internodes 4 mm long, taken 2 mm. below the col*eopilar* node IAA, concentration 1 x 10^{-8}, was added to the sugar-buffer solution to study the growth promotion due to auxin synergism First internode control sections given concentrations 1 x 10^{-8} and 1 x 10^{-7} IAA grew to final lengths of 6.0 and 8.3 mm, respectively Each histogram represents the average of 3 bioassays

E — etiolated
N — nonetiolated
RKB — red kidney bean
WW — Wilson's White
RW — Ruth Wilcox
CR — Cornell Red
IAA — indole-acetic acid

HISTOGRAMS SHOWING BIOLOGICAL ACTIVITY OF CHROMATOGRAMS OF STEM TISSUE

Bioassay - <u>Avena</u> First Internodes (Auxin Synergistic Tests)

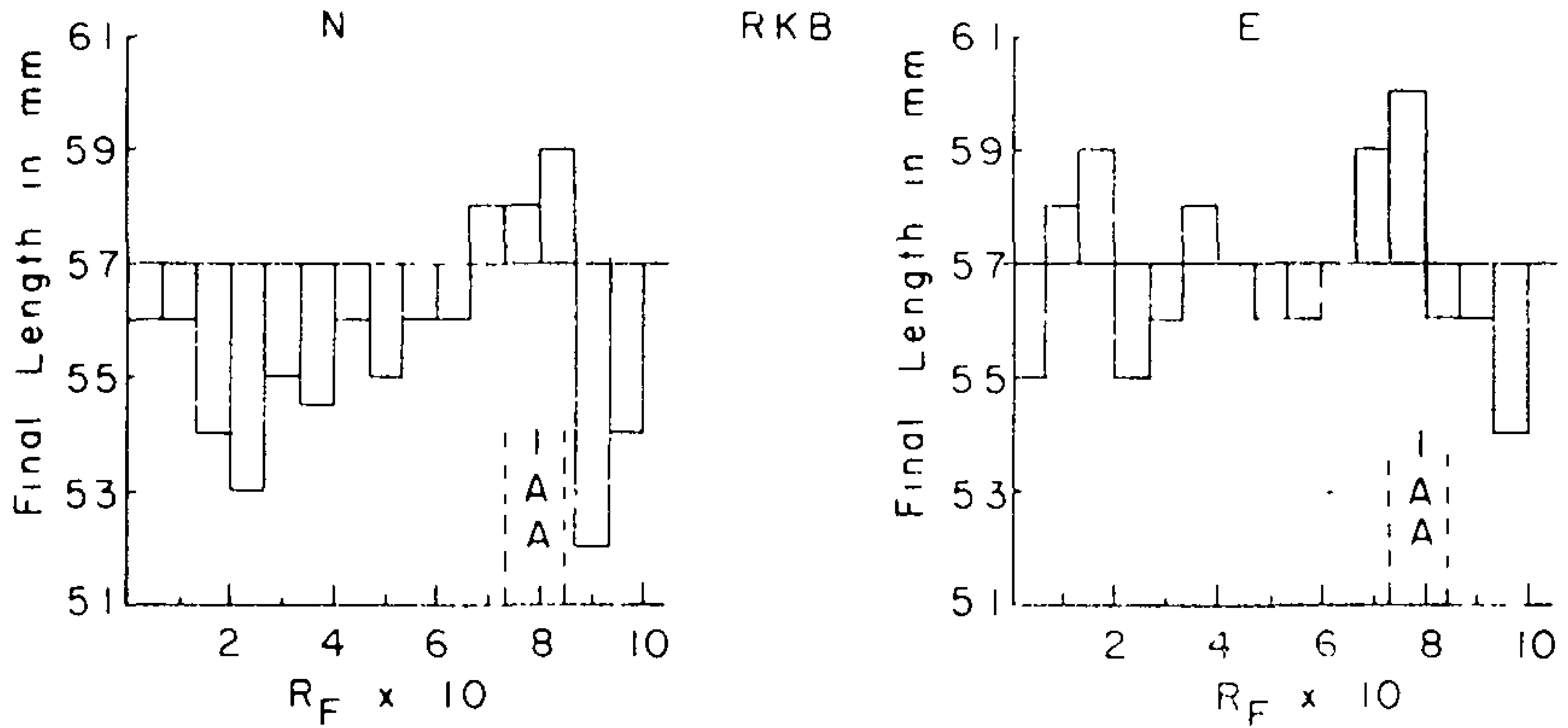

Figure 13

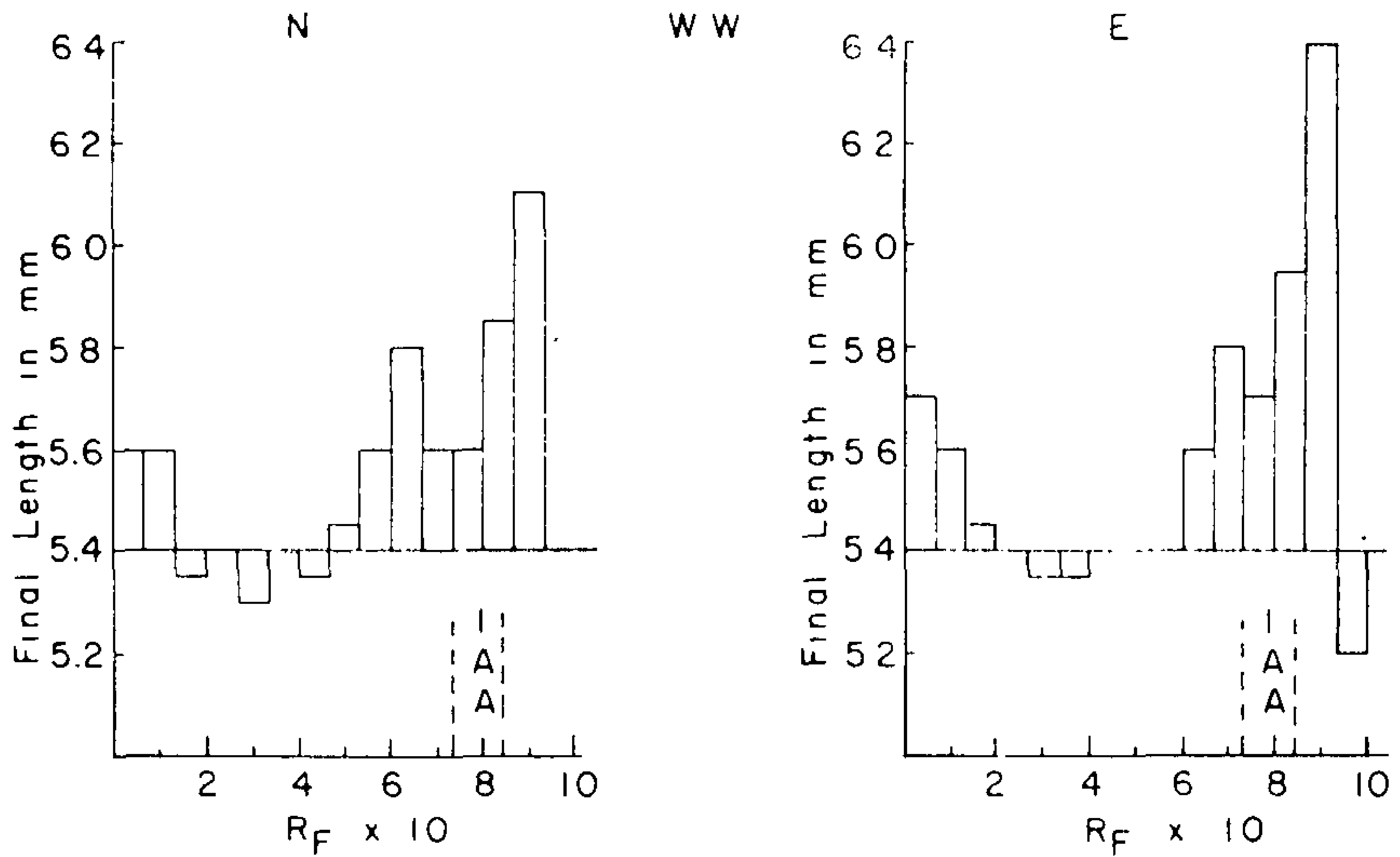

Figure 14

HISTOGRAMS SHOWING BIOLOGICAL ACTIVITY
OF CHROMATOGRAMS OF STEM TISSUE

Bioassay - Avena First Internodes
(Auxin Synergistic Tests)

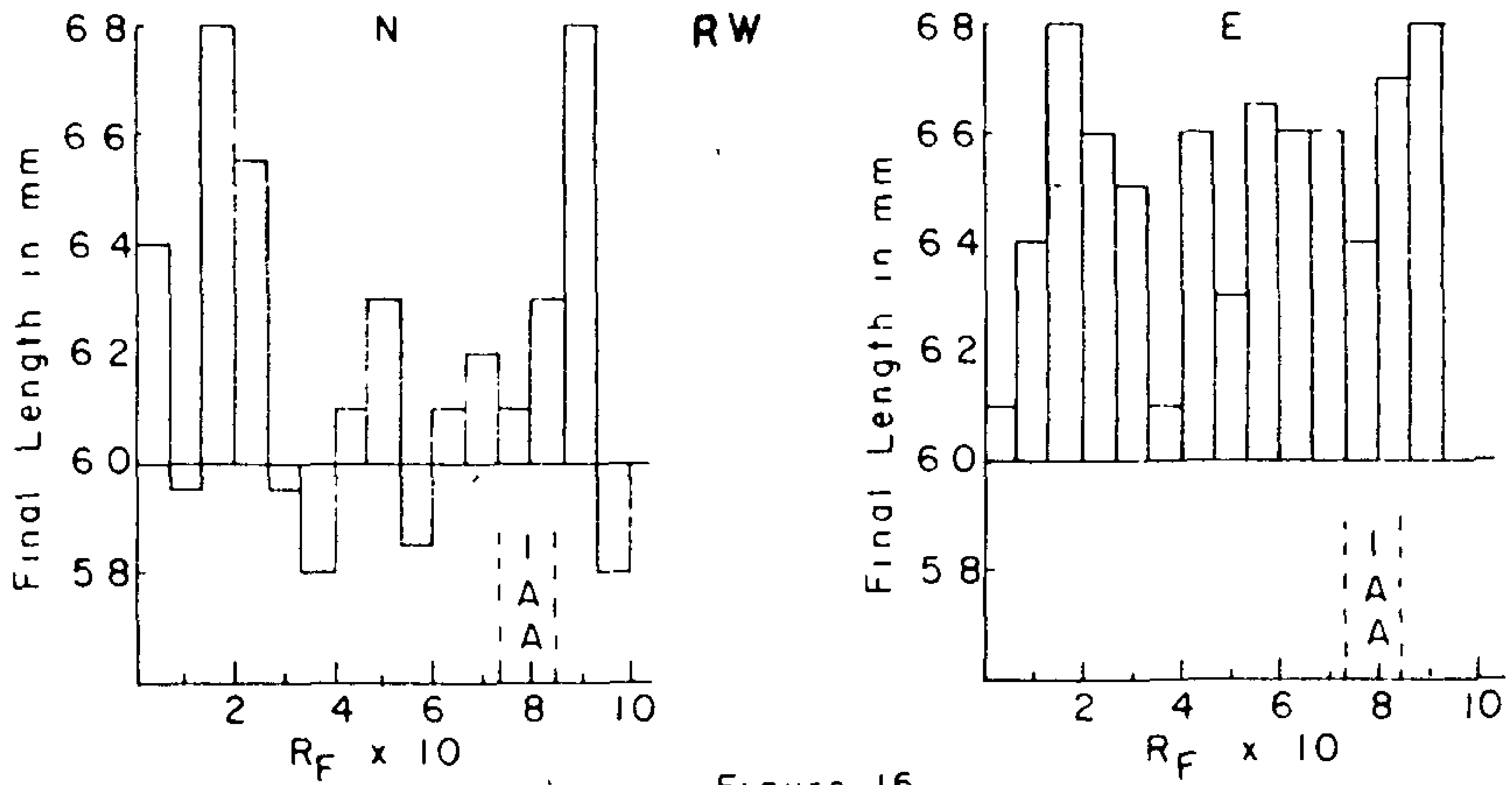

Figure 15

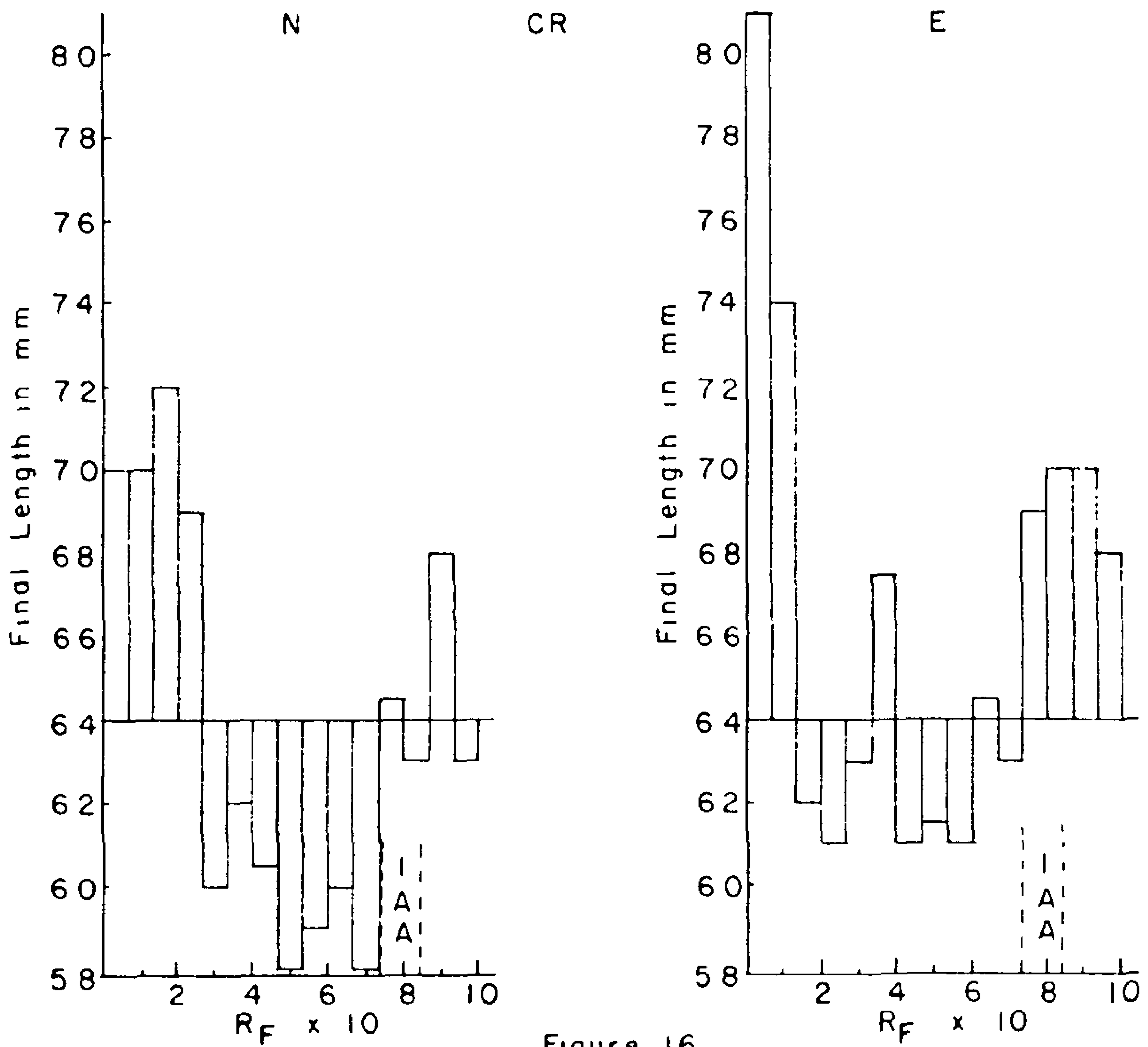

Figure 16

With the addition of an exogenous supply of IAA, the factors or variables involved have now been increased, and the data are indeed difficult to analyze. However, one can still note a significant correlation between the biological activity of the etiolated and nonetiolated tissue. There does appear to be slightly higher levels of auxin synergists present in the etiolated RKB and WW *Hibiscus* tissues as opposed to the nonetiolated tissues. In the RW variety, very high levels of auxin synergists are noted, which, as stated previously, may at least in part account for the very high rooting received in this variety. Significantly higher levels of auxin synergists are again evident in the etiolated tissues, which also holds true for the CR variety, where activity is very pronounced in the area of rooting cofactor 1.

V. Rooting "Cofactor" Study

A. Methods

Extraction and chromatography techniques used in this study of the extractable rooting "cofactors" from etiolated and nonetiolated tissues were similar to those used in the auxin study and will not be repeated.

In 1960 Hess developed a light grown mung bean rooting bioassay for the detection of the rooting "cofactors" (8). This bioassay is based on the initiation of adventitious roots in cuttings taken from mung bean (*Phaseolus aureus* Roxb.) seedlings. The seedlings are germinated and grown in vermiculite in the light chamber, and are ready for use in 9 days. Cuttings consist of the trifoliate bud, primary leaves, epicotyl, and 3 cm. of the hypocotyl. This light grown mung bean bioassay was selected for this study.

Ten cuttings were placed in each shell vial containing a chromatogram section and assayed with 4 cc. of a 5×10^{-6} M. solution of IAA. This solution was taken up by the cuttings in 24 hours and was then replaced with double distilled water. Roots were long enough to count in 5 days.

IAA, the natural plant auxin, is used because these root promoting substances, although unrelated to IAA, require its presence for maximum activity or expression. Thus, the root promoting substances apparently serve as cofactors of IAA.

Figures 17 - 18 Histograms showing biological activity of chromatograms of etiolated and nonetiolated stem tissues of *Phaseolus vulgaris* and *Hibiscus rosasinensis*, var WW. 0.5 gram lyophilized tissues, extracted with methanol for 2 hours Extract chromatographed in isopropanol-water (8:2, v/v). Bioassay-cuttings of light grown mung bean (*Phaseolus aureus*) seedlings. Cuttings consisted of the trifoliate bud, primary leaves, epicotyl, and 3 cm of the hypocotyl Average number of roots per cutting in the double distilled water control was 9.0 The hisotgrams represent the average results of 5 bioassay rooting tests.

E — etiolated
N — nonetiolated
RKB — red kidney bean
WW — Wilson's White
IAA — indole-acetic acid

HISTOGRAMS SHOWING BIOLOGICAL ACTIVITY OF CHROMATOGRAMS OF STEM TISSUES

Using 0.5 Gram in Extraction

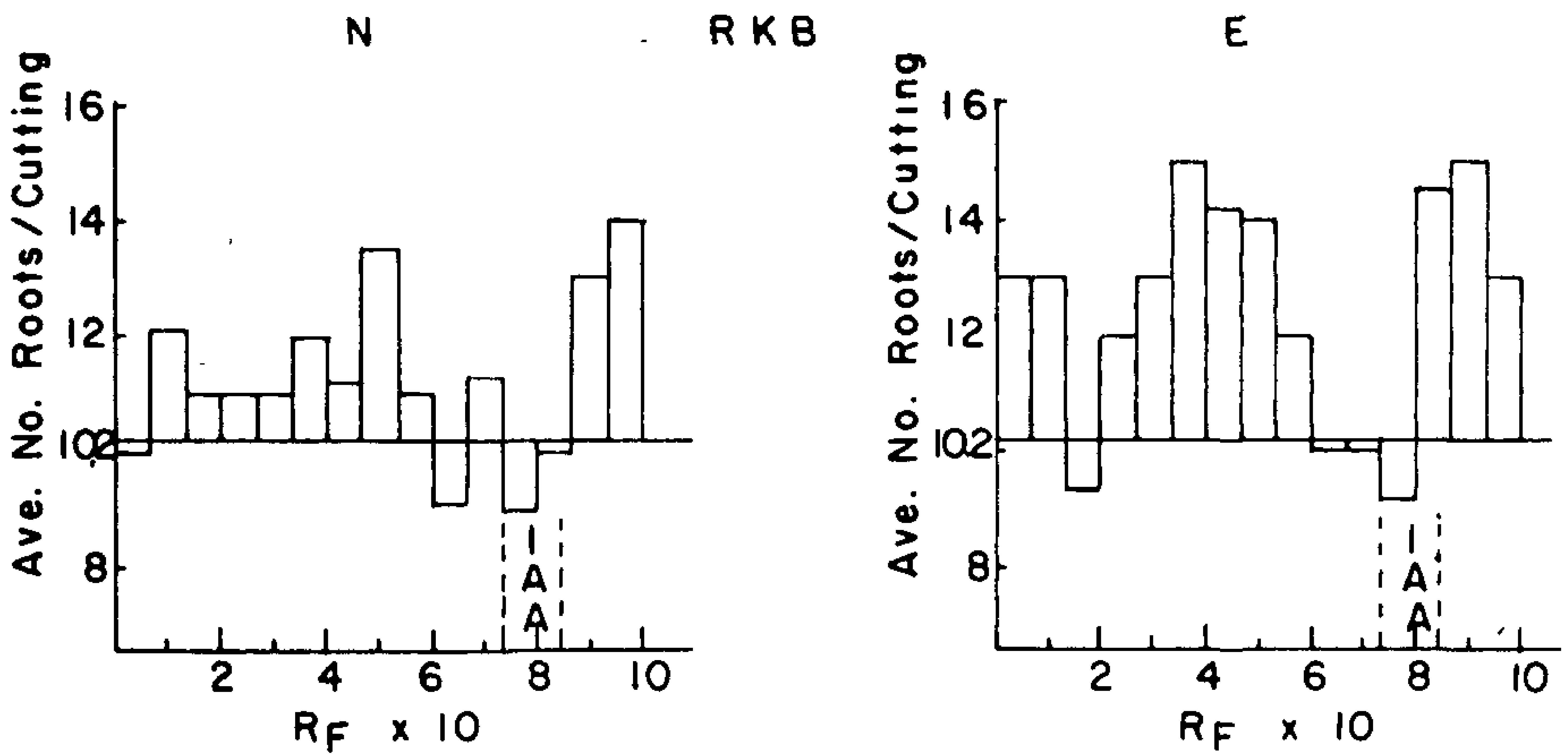

Figure 17

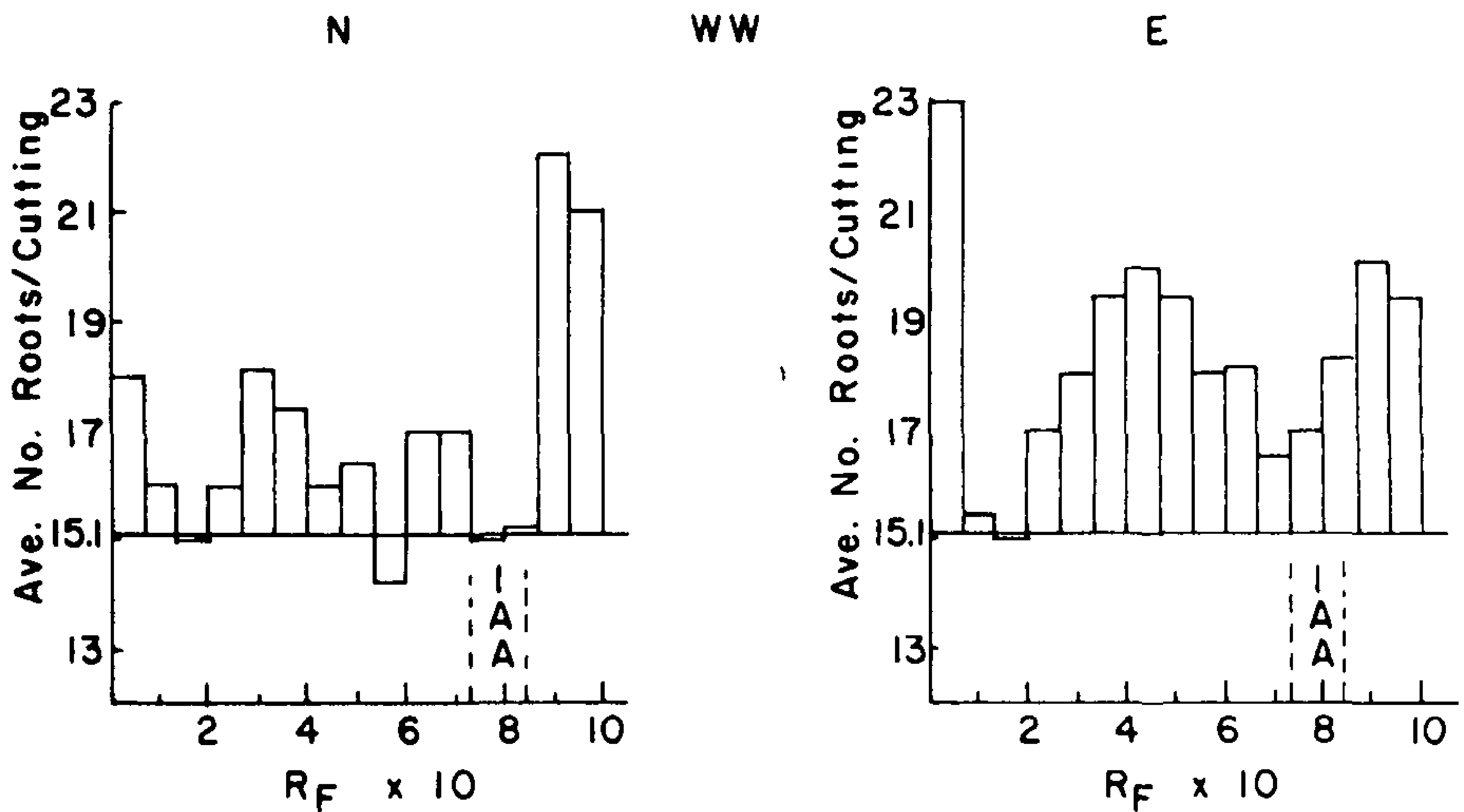

Figure 18

This supposition is supported by the fact that easy-to-root cuttings respond more to auxin application than do difficult-to-root cuttings. Mature and juvenile English ivy and easy-to-root CR and difficult-to-root WW *Hibiscus* are typical examples.

The results of the bioassay of the extractable rooting "cofactors" are expressed in the histograms in Figures 17-18. Higher levels of rooting cofactors with etiolation are apparent in the RKB and the WW *Hibiscus,* but the results with the CR and RW *Hibiscus* were not consistent. However, since these studies have been conducted, it has been found that cofactors 1, 2, and 3 are more soluble in 70% methanol than in absolute methanol. Thus, in using the absolute methanol we may have not been getting a complete extraction of the rooting cofactors, at least the first 3. This problem will be further investigated.

VI. Summary and Conclusions

Etiolation plays a definite role in enhancing root initiation in many plants. Using Red kidney bean and 3 varieties of Chinese *Hibiscus*, this physiological role was investigated by employing 4 different approaches, including differential rooting studies and anatomical, auxin, and rooting "cofactor" investigations. The etiolation treatment involved a localized blanching of the tissues from which root initiation was to occur. The basic conclusions derived from these studies were:

1. Root initiation studies significantly revealed a marked promotion in rooting incurred as a direct result of etiolation. Not only was there a higher numerical rooting per cutting, but the per cent of cuttings rooted was greater when the stems were etiolated prior to taking the cuttings.
2. The anatomical study revealed various anatomical modifications in cells and tissues of etiolated plant stems, e.g., decrease in starch content, mechanical strengthening tissues, cell wall thickness and cell wall deposits, and in total amount of vascular tissue; also an increase in parenchyma cells and a greater quantity of tissues in a less differentiated condition were present in the etiolated as opposed to the nonetiolated stems.
3. The investigation of the endogenous auxins showed a slightly higher auxin content in the etiolated tissues as opposed to the nonetiolated tissues.
4. An investigation of the extractable rooting "cofactors" indicated a somewhat higher rooting "cofactor" level in at least some etiolated as compared with nonetiolated plants. However, the relationship was not consistent and the postulation was made that some unknown substance(s) or auxin synergist(s) e.g., rooting "cofactors," accumulate in etiolated tissues, but which were not released in large enough quantities with the extraction solvents utilized in this study to be detected in the bioassays.

Also, the fact that there was a great response on the part of the etiolated cuttings to an exogenous auxin application indicates most strongly the presence of some other substance(s) which acts synergistically or as a cofactor in determining the overall rooting response.

It is evident from the preceding summary that the physiological role which etiolaton plays in the rooting process cannot be ascribed to any one factor. In the contrary, the marked increase in root initiation incurred from cuttings which are previously etiolated may be attributed to a complex of factors. These factors interact, some synergistically, in a final realization of the rooting response of a plant.

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PRESIDENT MAHLSTEDE: Thank you very much, Dale, for your very interesting paper. We will have a few minutes for questions.

MR. JAMES WELLS: How long were the *Hibiscus* and the beans etiolated?

MR. HERMAN: The *Hibiscus* were etiolated for five weeks and the beans, three weeks.

VOICE: Did you identify any of the compounds on the chromatograms?

MR. HERMAN: We have good evidence from R-f values and color reactions that the principal auxin was indoleacetic acid, but we have not crystallized it. We did not identify the other active areas as yet.

MR. PETER VERMEULEN: Will a short exposure to light eliminate the root promoting effects of etiolation?

DR. C. E. HESS: No, a short exposure will not eliminate the promotive effects of etiolation upon rooting. There are substantial changes in the structure and chemical make up of the stems during etiolation. It would take a period of several hours or more to remove the effects of etiolation. You may be thinking of Seymour Shapiro's paper about the growth of preformed root initials as are found in willow and poplar. If branches are placed in a moist, dark environment for approximately three days and then are exposed to a very short period of light, particularly red light, the outgrowth of the preformed roots is inhibited.

MR. AL LOWENFELS: How can etiolation be used practically?

MR. HERMAN: It is already used in layering.