

break' stage prior to grafting; then dried before actually grafting.

We have also used this method successfully with bench grafting *Prunus*, *Crataegus*, top fruit, *Malus*, *Magnolia* and *Hamamelis*.

THE ROLE OF AUXIN IN ROOT INITIATION IN CUTTINGS¹

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(Dedicated to Prof. Kurt Mendel, Rehovot, Israel,
on his 70th anniversary)

Abstract. Root formation in bean cuttings was investigated in terms of its pattern in various tissues, the presence of leaves, and accumulation and transport of sugars with regard to auxin treatments. It was found that root-forming ability of various types of cuttings was different, and that the presence of leaves was of prime importance in the expression of the auxin effect. IAA enhanced sugar accumulation at the base of the cutting concurrently with root formation, and increased the transport of ¹⁴C-labelled assimilates from the leaves in a basipetal direction. A general scheme for root formation is discussed and it is suggested that one of the roles of IAA in promoting rooting of bean cuttings is to increase sugar availability at the site of root formation.

INTRODUCTION

Since 1934, when the identity of the "root forming hormone" and endogenous auxin was first established by Went and Thimann, IAA and other synthetic auxins have been extensively used in promoting rooting of cuttings. The universality of auxin action is evident from Audus's analysis of 1240 sets of experiments with various stem cuttings (1): in most cases applied auxin improved rooting, 8.5% of the total did not respond to auxin, and only 5.5% were inhibited. On the other hand, it is generally found that plants which normally root with ease will usually respond readily to auxins, whereas poor rooters are much less responsive. This, together with the fact that there is a definite relationship between the presence of leaves and buds on a cutting and its capacity to root (10, 15), suggests that the

¹Thanks are due to Professor P. F. Wareing for his interest and hospitality at the Department of Botany, U.C.W., Aberystwyth, Wales, where this work was carried out.

auxin effect is connected with substances produced in leaves and buds

Apart from hormones and various co-factors that had been shown to affect root formation (3, 4, 6), the nutritional condition of the cutting is known to the horticulturist to be of prime importance. It was found that the number of roots on tomato cuttings was affected by the ratio of carbohydrates to nitrogen (9), and that starch was associated with the rooting ability (17). Several sugars were shown to have a positive effect on root initiation (16), and it was suggested that in certain cases the main function of the leaves in the process of rooting is to supply the cuttings with sugars and nitrogenous substances (15). It is of interest, in this respect, to note that the advantage of mist propagation of some plants is a result of the fact that the physiological activity of the leaves and the build-up of dry matter continue at a normal rate (8).

Although being extensively used, the role of auxin in promoting root formation is not well understood. It might directly affect the differentiation of the primordia, or increase the quantities of carbohydrates (and other nutrients) that are essential for root formation. This latter possibility was investigated in the present study.

MATERIALS AND METHODS

Plant material and rooting experiments. Seedling of dwarf bean, *Phaseolus vulgaris* L. cv. Canadian Wonder, were raised in John Innes No. 1 compost in a glasshouse. Eight to ten day old seedlings were used in all experiments. At this stage the seedlings had a fully-elongated hypocotyl, an epicotyl with the pair of primary leaves and an apical bud. Cuttings were prepared by removing the cotyledons and excising the hypocotyl 5 cm below the cotyledonary node. Except where mentioned otherwise, the blades of the two primary leaves were trimmed to a uniform area using a circular cutter of 3.7 cm in diameter (Fig. 1). Cuttings were introduced into glass vials containing distilled water or indole-3-acetic acid (IAA) at a specified concentration, the hypocotyl being completely immersed in the solution. The cuttings were transferred to a growth cabinet maintained in the dark at $24 \pm 1^\circ$ C. After a period of 24 hr treatment with the hormone, the cuttings were rinsed and transferred to vials containing tap water and 5 ppm H_3BO_3 and kept in a growth cabinet maintained at $22 \pm 1^\circ$ C and a 16 hr photoperiod.

Data on number of roots produced on each hypocotyl were recorded either 7 days after excision, when roots were completely visible, or 4 to 5 days after excision following staining of the primordia with aceto-carmin. In some cases other types of cuttings, prepared from older seedlings, were used, and these included: epicotyl cuttings (epicotyl excised 1 cm above cotyledons and primary leaves treated as above), internode cuttings (1st in-

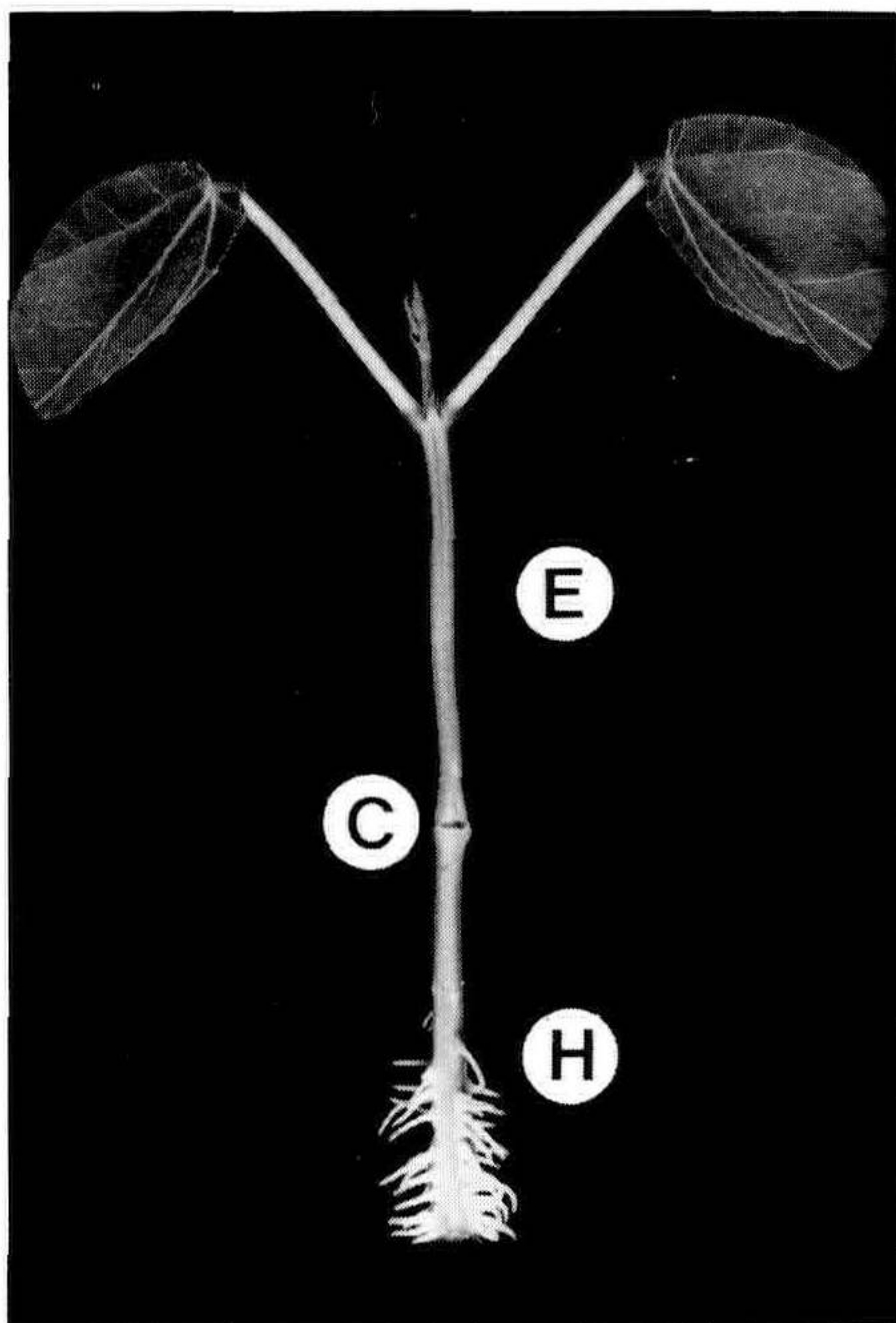


Fig. 1. Standard bean cutting used in the experiments, after 7 days in culture.

H = hypocotyl; E = epicotyl; C = cotyledonary node.

ternode excised 1 cm above the primary leaves node, the two side leaflets of the 1st trifoliate leaf were trimmed as mentioned and central leaflet discarded) and petiole cuttings (petiole of the primary leaf cut 1 cm above the node and leaf area reduced).

Uptake of IAA. Uptake and transport of IAA was followed in standard cuttings during the 24 hr period after excision, using the isotopically-labelled hormone (3-indolyl acetic acid 2-C¹⁴ ammonium salt). The label was counted in successive sections of the cuttings, after being extracted in hot 80% ethanol. Experiments were performed in an illuminated growth cabinet (as mentioned above).

Sugar accumulation. This was followed during a 4 day period after excision, in standard and in starved (seedling raised for the last 2 days before excision in the dark) cuttings. Hypocotyl sections were extracted in hot 80% ethanol and soluble sugars determined with the anthrone reagent. Experiments were performed in an illuminated growth cabinet.

Transport of ¹⁴C-labelled assimilates. One of the primary leaves was exposed to a pulse of ¹⁴CO₂ by sealing assimilation vials containing ¹⁴C-Na₂CO₃ onto the lower surface of the blade. The

radioactive CO₂ was released by addition of HCl, and the leaf was allowed to assimilate for 1 hr after which the vial was removed. The cuttings were left for an additional period of 23 hrs for transport, and successive sections were then extracted as described above. ¹⁴C-labelled assimilates were counted in the extract. All experiments were performed in the light.

RESULTS

Patterns of root formation. Since this study is concerned with the influence of IAA treatment on carbohydrate reserves, our first aim was to establish the relationship between leaves (as the source of assimilates), root formation and auxin.

The relative effect of the presence of leaves and buds is seen in Fig. 2. Leaves were the major factor in root formation, while the presence of the apical bud seemed to play a smaller role as judged from the control cuttings. Furthermore, the presence of leaves was essential for the expression of the auxin effect, since with leaves IAA increased root formation above the control (of intact cuttings). This is also illustrated by the data of Table 1 where the effect of leaves and IAA is shown for various types of cuttings. Partial or complete defoliation caused inhibition of root formation in hypocotyl and epicotyl cuttings.

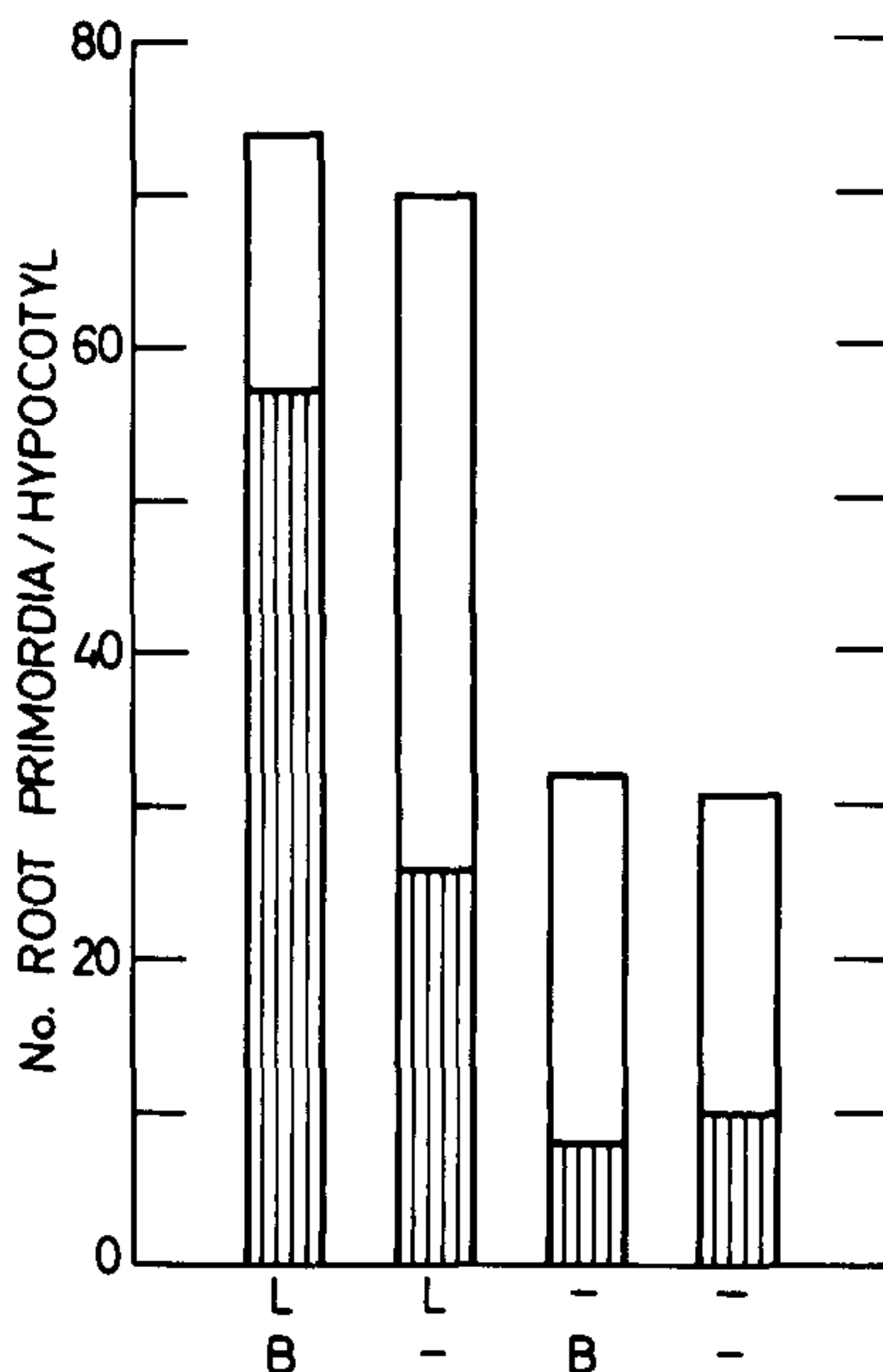


Fig. 2. The relative effect of the presence of leaves and apical bud on root formation in standard cuttings, in the control and with 5×10^{-5} M IAA. L=leaves (not reduced); B=apical bud; striated columns=control; white columns=addition with IAA

Table 1. The effect of leaves and IAA ($2 \times 10^{-5}M$) on the number of root primordia in various cuttings from 10 day-old seedlings (counted after 7 days in culture).

	No. of whole leaves	No. of root primordia per 5 cm cutting		
		Control	IAA	IAA, % of control
hypocotyl	2	66	88	133
	1	51	76	151
	0	19	33	177
epicotyl	2	28	93	336
	1	21	79	383
	0	13	21	170
petiole		6	73	1327

Due to intrinsic differences in the "rooting ability" between "leafy" hypocotyl, epicotyl and petiole cuttings, the combined effect of IAA and leaves was also different. Thus, percentagewise, the enhancement by auxin in hypocotyl cuttings was greater when leaves were absent, while in epicotyl cuttings the presence of leaves seemed to be more important. This difference is illustrated also in Fig. 3 with regard to the position of the root primordia. In hypocotyls, roots were formed in 4 distinct rows in association with the vascular tissue and auxin only increased the number of primordia. In epicotyls and petioles, where the arrangement of the vascular tissue differs, roots appeared to form at random (and auxin increased the number of primordia).

In another experiment older seedlings were used to include cuttings of the 1st internode. Fig. 4 shows the "root forming ability" of cuttings from different positions on the seedlings, proceeding from "root-like" tissue as the hypocotyl, to successive distant "shoot-like" tissues. Roots were formed abundantly in the hypocotyl even in the absence of auxin treatment, while auxin was found to be essential for the establishment of epicotyl and internode cuttings. Petioles treated with IAA had many primordia, although at this stage these had not fully developed into roots, while none were formed in the control.

Uptake and transport of IAA. Before going any further as to the mechanism of auxin effect, it was of interest to establish how the IAA was distributed in the cuttings under the experimental con-

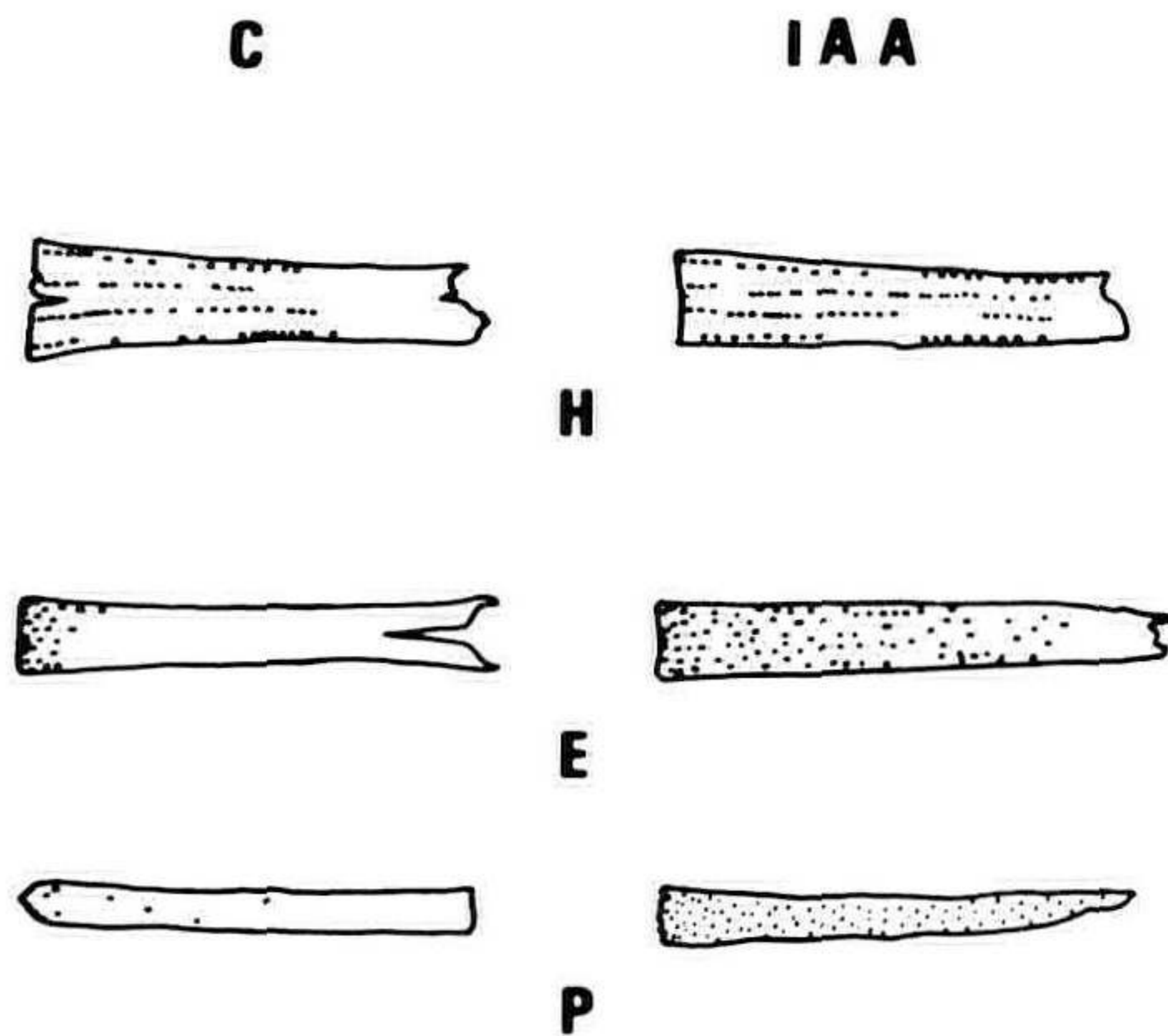


Fig. 3. The position of root primordia in 3 different types of cuttings (with leaves); in the control (C) and with $2 \times 10^{-5}M$ IAA. Redrawn from stained specimens.

H = hypocotyl; E = epicotyl; P = petiole.

ditions. This is of primary importance in any discussion of possible sites of IAA action.

Data of an experiment where cuttings were placed in solution of radioactive IAA are given in Table 2. The majority of the label was retained in the hypocotyl, to which it was applied, and only 10 to 13% was transported to other parts of the cuttings. However, this transported fraction reached the upper parts in the first 2 hr. Thus, at this early stage auxin was present in the transport system (epicotyl and petioles) as well as in the blades, where photosynthesis and carbohydrate interconversions are carried out.

Accumulation of sugars. After establishing that root formation was markedly inhibited in the absence of leaves, while being promoted with IAA, the possibility that these two phenomena are interrelated through the accumulation of carbohydrate at the base of the cutting was investigated.

In Fig. 5 results of an experiment in which sugar accumulation was followed during 4 days after excision of the cuttings, are shown. Sugars accumulated slowly in the base of control cuttings, the rise in accumulation was evident only after 24 hr. The IAA-treated cuttings evinced an initially smaller accumulation of sugars, but 12 hr after preparation of the cuttings the levels of sugars were found to be similar to those of control. Twenty four hours after the start of the experiment sugar content was considerably increased in the presence of auxin, the levels being almost twice those of control cuttings.

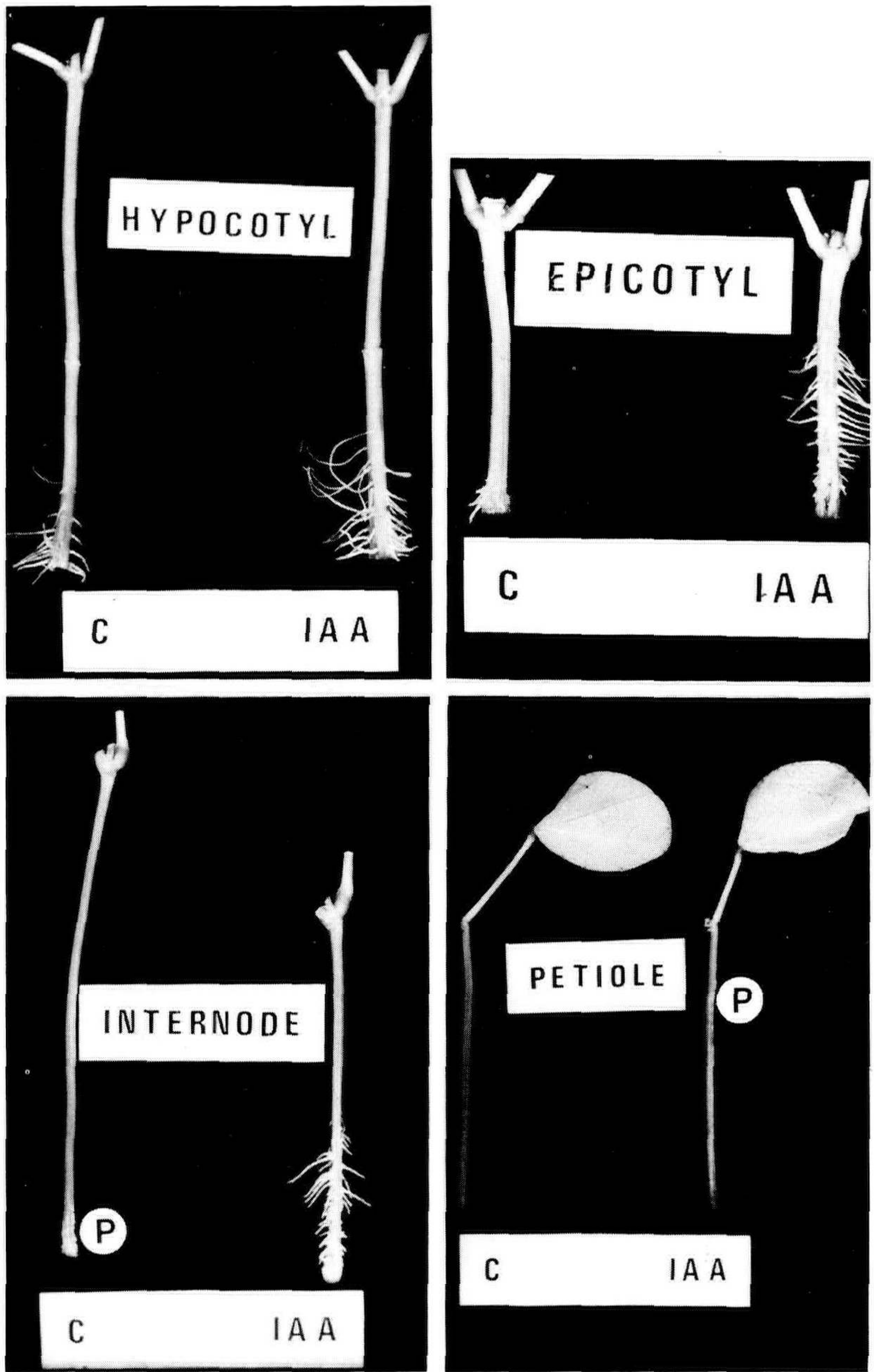


Fig. 4. Root formation in 4 different types of cuttings prepared from 21 day-old seedlings, in the control (C) and with $5 \times 10^{-5}M$ IAA. All cuttings possessed leaves at the time of rooting. P = root primordia.

Table 2. Uptake (by the hypocotyl) and transport (to other parts) of ^{14}C -labelled IAA in standard bean cuttings. The radiocarbon was counted in sections taken at various periods after the excision of the cuttings: A = 5 cm hypocotyl; B = 5 cm epicotyl; C = rest of epicotyl and apical bud; D = petioles of the 2 primary leaves; E = blades of primary leaves.

Hr after excision	DPM $\times 10^{-3}$ / 100 mg dry weight				
	A	B	C	D	E
2	232	28	11	6	6
4	430	44	15	10	8
8	825	84	31	21	14
24	1385	133	60	42	51

	DPM, % of total					
	A	B	C	D	E	B-E (% transported)
2	87.2	6.3	1.4	1.7	3.4	12.8
4	89.7	5.3	1.1	1.6	2.3	10.3
8	90.0	5.1	1.0	1.4	2.5	10.0
24	89.3	4.3	1.0	1.6	3.8	10.7

A comparison of sugar accumulation under the effect of IAA in green standard cuttings and in cuttings prepared from starved seedlings (Table 3) further substantiates the relationship between root formation and sugar accumulation. Sugars accumulated in the hypocotyl during the 24 hr period, and this accumulation was considerably greater in the presence of IAA in both types of cuttings. The level of sugars in IAA-treated starved cuttings was found to be higher than that of the control green cuttings. Percentagewise, the effect of IAA in starved cuttings was greater than in green ones, as a result of an initial lower sugar content in starved seedlings. In the same experiment, green cuttings had 56 roots (per hypocotyl) in the control and 76 roots in the IAA treatment, compared with starved cuttings that had 30 roots in the control and 66 roots with IAA. Thus, there was a close relationship between the enhancement of sugar accumulation and the formation of roots in the presence of auxin.

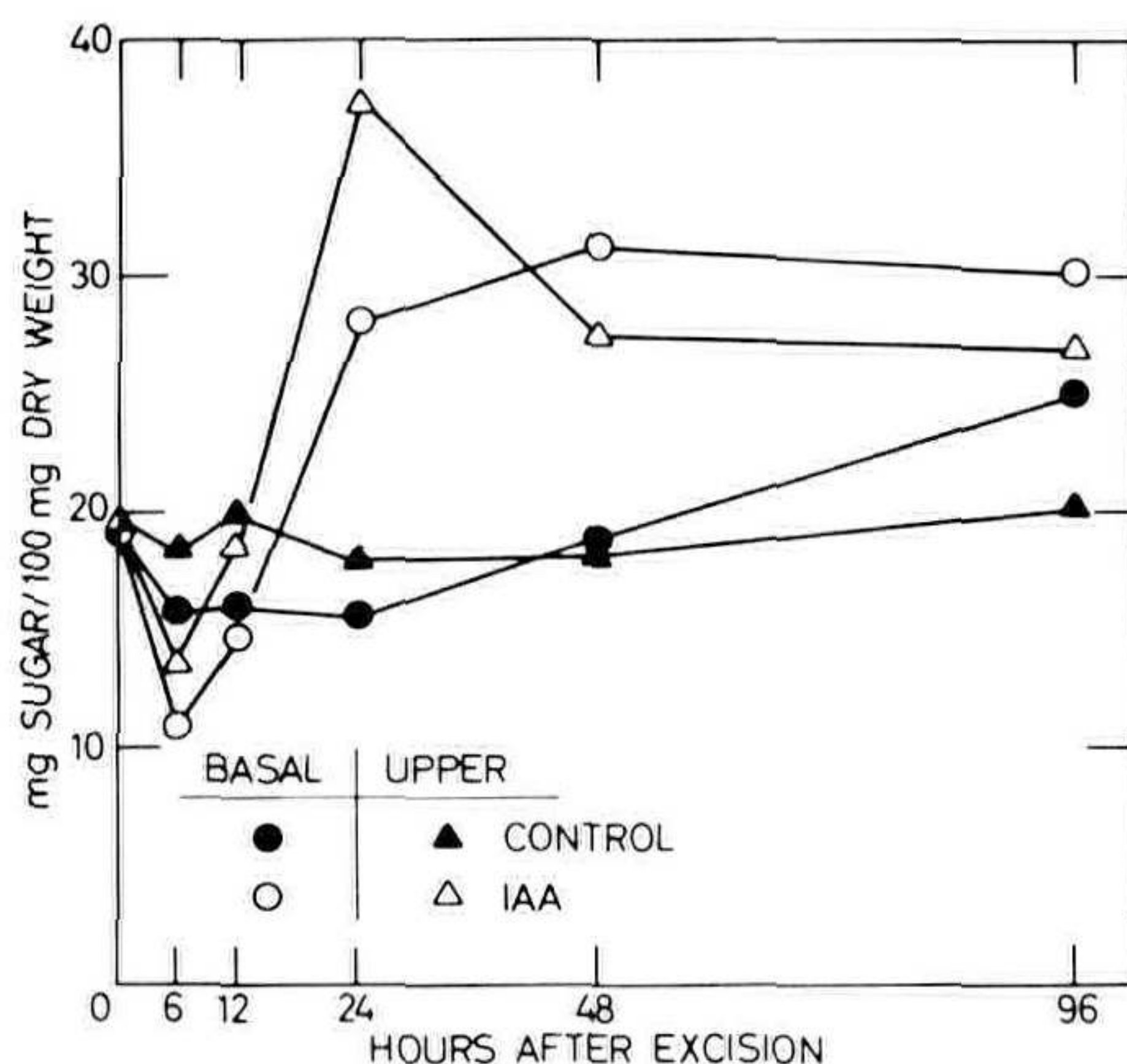


Fig. 5. Sugar accumulation in basal and upper segments of the hypocotyl during 4 days after excision of cuttings. Cuttings were treated during the first 24 hr either with water (C) or with 5×10^{-5} M IAA.

Transport of ^{14}C -labelled assimilates. One of the mechanisms by which IAA can enhance sugar accumulation at the base of the cutting is by directly affecting its transport from the source where they are produced (assimilated in the leaves). This possibility was tested after feeding the leaves with radioactive carbon dioxide.

Data of one such experiment are presented in Table 4 from which it is evident that IAA markedly increased the transport of labelled assimilates to the base of the cutting (more than 4 times of control). This increased basipetal transport was due to the fact that assimilates were more readily mobilized from the upper parts (Table 5): 49.9% of the total counts assimilated were transported from the leaf in the control, as compared with 60.6% in the presence of IAA. The same holds true with regard to the percentage transported from upper parts of the cutting to the lower sections. This pattern of distribution of ^{14}C -labelled assimilates under the effect of auxin is even more clearly evident from Fig. 6.

DISCUSSION

Experimental data. The importance of leaves in promoting root initiation in bean hypocotyls and epicotyls (Fig. 2, Table 1) is in accordance with data of other studies on root formation (15). While in some studies buds also were found to be essential for rooting (3, 10), excision of the apical bud of bean cuttings seems to be less detrimental. As buds are regarded as the site of auxin production, it is assumed that in the present study endogenous auxin content was not a limiting factor for the formation of primordia. However, an exogenous supply of auxin enhanced root formation if leaves were

Table 3. Sugar content (80% ethanol-soluble) at the start of the experiment and after 24 hr in culture, in green and in starved cuttings.

	Green			Starved		
	0-time	24 hours		0-time	24 hours	
		control	IAA		control	IAA
mg glucose equiv. / 100 mg dry weight						
basal hypocotyl (0-25 mm)	16.0	19.5	30.1	9.7	12.2	20.9
upper " " (25-50 mm)	18.0	19.9	37.1	10.2	18.4	29.7
average	17.0	19.7	33.6	10.0	15.3	25.3
% of sugar content at 0-time						
basal hypocotyl	100	122	188	100	125	215
upper " "	100	111	206	100	180	391
average	100	116	198	100	153	253

present, thus showing that it was associated with substances derived from the leaves. There are reasons for believing that carbohydrates transported from the leaves are the main factor, rather than unknown "co-factors" of a hormonal nature.

The marked difference between tissues and organs of the same plant in their rooting ability (Fig. 3 and 4, Table 1) is a good illustration of the well known variability between species or even between varieties of the same species (3, 6). The fact that roots are formed abundantly in the hypocotyl might be related both to its intrinsic similarity to the root system and to the ease with which substances essential for rooting are supplied from the root system. Indeed, it is known that certain plants root better if cuttings are prepared from twigs closer to the root system (5).

Most of the radioactivity from labelled IAA remained at the base of the cutting (Table 2), and this is in accordance with other data of basal application of auxin (13). However, since a certain

Table 4. The effect of 5×10^{-5} M IAA on the transport of ^{14}C -labelled assimilates in standard cuttings.

	DPM x 10 ⁻³ / section		
	control	IAA	IAA, % of control
hypocotyl 0-50 mm	121	512	424
epicotyl 51-100 mm	169	438	260
whole cutting	909	1900	209

Table 5. Percentage distribution of ^{14}C -labelled assimilates in various regions of standard cuttings.

	% of total DPM in cutting	
	control	IAA
hypocotyl 0-25 mm	3.7	10.2
26-50 mm	3.8	11.6
Total for hypocotyl	7.5	21.8
epicotyl 51-75 mm	5.4	8.8
76-100 mm	4.6	4.6
Total for epicotyl	10.0	13.4
rest of cutting, except for treated leaf.	32.4	25.4
treated leaf	50.1	39.4

amount was transported to upper parts it is possible that the influence of auxin on root initiation is due to its combined effects at the site of root formation and on physiological processes in the hypocotyl and leaves.

Accumulation of sugars and soluble nitrogen compounds was found in cuttings (15) and in girdled stems (12) of *Hibiscus*, and this

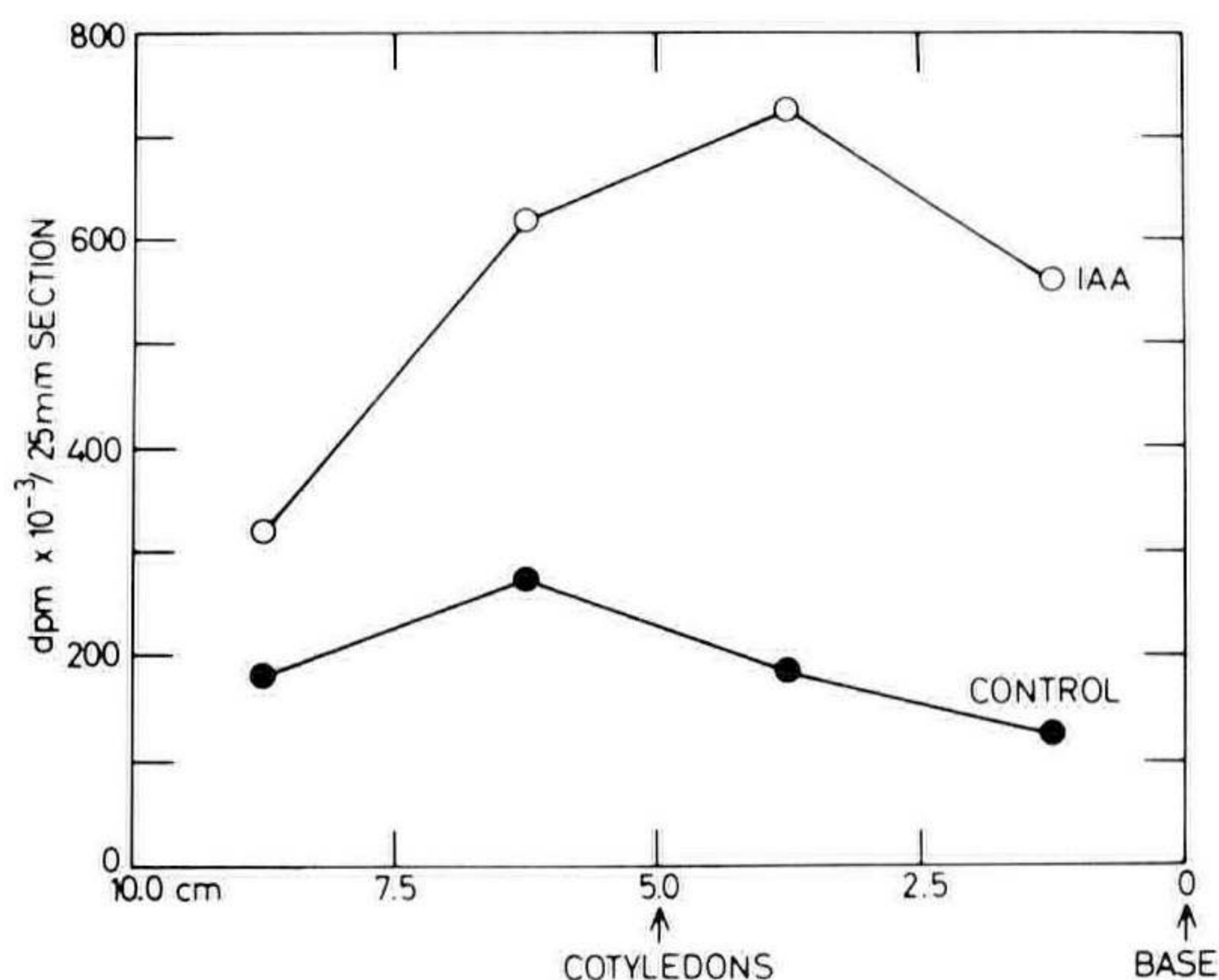


Fig. 6. Distribution of ^{14}C -labelled assimilates that were transported from the leaf during a 24 hr period, in the control and in the presence of $5 \times 10^{-5}\text{M}$ IAA. Radioactivity was measured in 2.5 cm sections from the upper part down to the base of the cutting.

was dependent on the presence of leaves and on environmental conditions. Treatment of bean cuttings with auxin resulted in a considerable accumulation of sugars at the base (Fig. 5, Table 3). Thus, IAA directly affected the availability of the sugars as well as root formation. The close relationship between the enhancement of sugar accumulation and root formation in starved cuttings (Table 3 and text) suggests that a certain minimal sugar content should be present in order to enable the development of a certain number of root primordia, and that this can be affected by IAA.

The auxin-induced sugar accumulation can be brought about by 4 different ways, or in combinations thereof:

1. By increasing the production of photosynthates in the leaves.
2. By increasing the rate of breakdown of carbohydrate reserves in leaves and in other tissues, *i.e.* affecting enzyme activity.
3. Increasing the rate of transport from the leaves to the zone of auxin application.
4. By increasing the demand for carbohydrates at the area of root formation, *i.e.* directly affecting primordia formation.

There is evidence in the literature to support any of these possibilities, although they were not investigated with regard to root formation in cuttings. Data of transport experiments (Tables 4 and 5, Fig. 6) show that the auxin-induced sugar accumulation is mediated, at least in part, by a direct effect on the transport. Hormone-directed transport of assimilates and minerals is now an

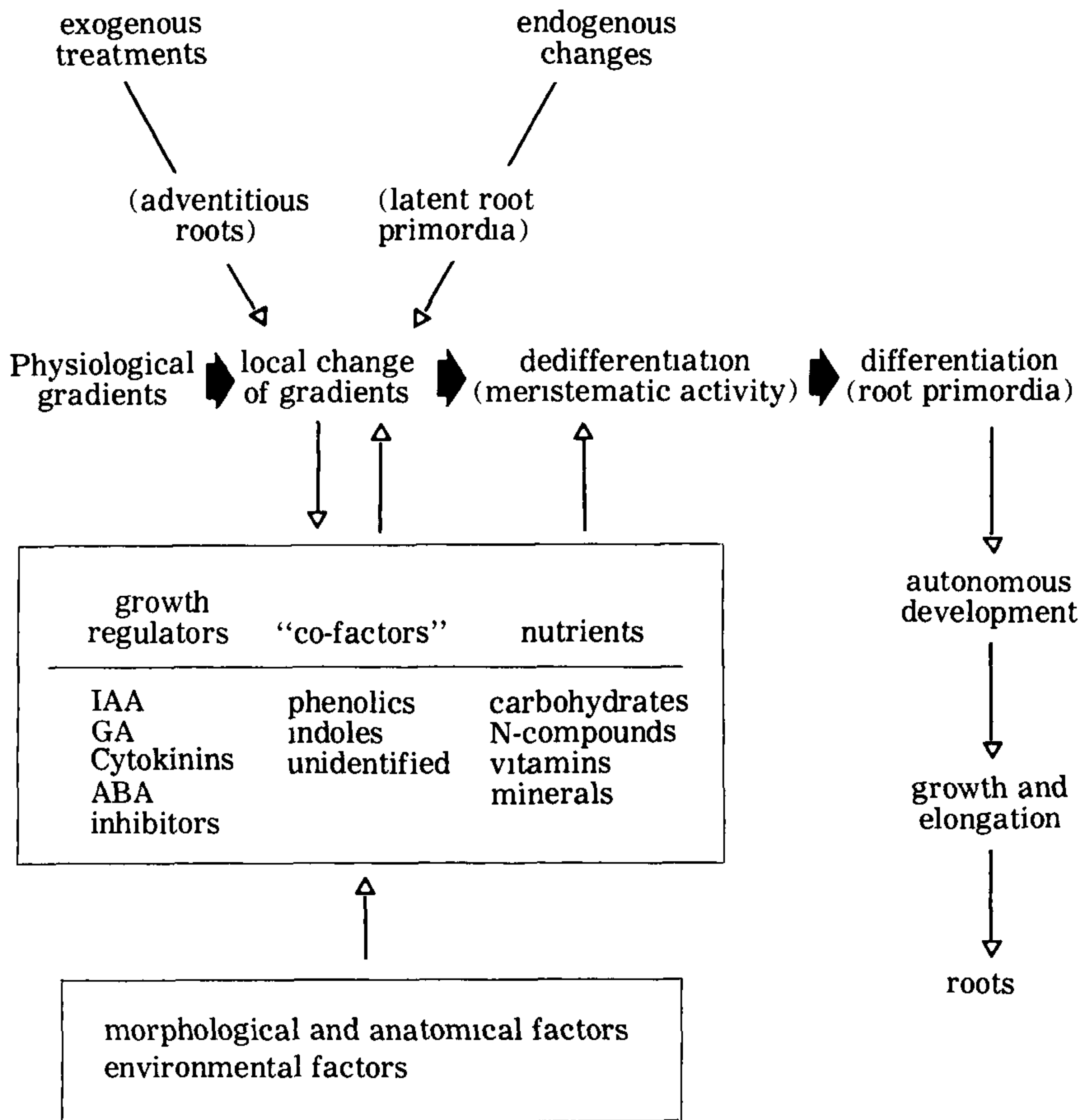


Fig. 7. General hypothetical scheme of root initiation in cuttings.

established fact (2, 11) although the underlying mechanism is still an open question. It has been suggested that hormone-induced transport involves a direct effect of the hormone on the transport system as such, and/or an affect on the developing "sink" (by increasing its metabolism). Data not presented here suggest that in bean cuttings both these mechanisms are involved, *i.e.* direct effect on the transport system and root formation.

CONCLUSIONS

1. Leaf area markedly affects root formation in the cuttings.
2. The expression of auxin-induced root formation is dependent on the area of leaves.

- 3 IAA increases sugar content at the site of root formation, with an equivalent increase in the number of root primordia.
4. IAA increases the transport of ^{14}C -labelled assimilates from the leaf to the base of the cutting.

It is suggested that one of the roles of IAA in promoting rooting of bean cuttings is by increasing sugar availability at the site of root formation.

GENERAL

Based on extensive experimental data as to the physiology of root formation (1, 4, 5, 7), a general, hypothetical, scheme of root initiation in cuttings can be presented (Fig. 7).

Intrinsic physiological gradients exist in any plant, organ and tissue as an integral part of their programmed development. The first step in the initiation of roots is a local change of gradients in the cutting. These can be brought about by either of two ways: (1) exogenous, horticultural, treatments (cutting, layering, girdling, etc.) that lead to the formation of adventitious roots, and (2) endogenous changes, resulting in the formation of latent ("preformed") root primordia. In the first case there is a change of one type of tissue to another and a continuous development, while the latter is complicated by a state of arrested growth of the primordia. Due to local change in the gradients, tissues in this area undergo a dedifferentiation process leading to meristematic activity. Root primordia are then formed, which at a certain stage attain an autonomous development, and finally roots emerge from the cutting. Levels and activities of growth regulators, co-factors and nutrients are apt to be influenced by the change in gradients, but at the same time can markedly affect these gradients and, thus, the differentiation process and the formation of roots. Superimposed on this, are morphological, anatomical and environmental factors.

Any hormonal treatment might affect root formation in two general ways: (1) directly, on the processes of dedifferentiation and differentiation, and (2) indirectly, by affecting certain steps of the overall metabolism. It is, thus, obvious that in a complex scheme like this all the factors are interrelated, and any one of them can be rate-limiting. Since the limiting factors are different in various plants and tissues (being part of its programmed development), the expression any given treatment in any given tissue will be dependent on the interplay between these factors.

LITERATURE CITED

1. Audus, L. J. 1959. *Plant Growth Substances*. Leonard Hill Ltd., London.

2. Davies, C. R. and P. F. Wareing. 1965. Auxin-directed transport of radiophosphorus in stems. *Planta* 65:139-156.
3. Fadl, M. S., and H. T. Hartmann. 1967. Relationship between seasonal changes in endogenous promoters and inhibitors in pear buds and cuttings bases and the rooting of pear hardwood cuttings. *Proc. Amer. Soc. Hort. Sci.*, 91:96-112.
4. Fernqvist, I. 1966. Studies on factors in adventitious root formation *Lantbrukshogskolan Annaler* 32:109-244.
5. Hartmann, H. T., and Dale E. Kester. 1968. *Plant Propagation: Principles and Practices*. 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.
6. Hess, C. E. 1963. Naturally-occurring substances which stimulate root initiation. *Col. Int. du Centre Nat. Recherche Sci.* No. 123:517-527 Paris.
7. Hess, C. E. 1969. Internal and external factors regulating root initiation. In W. J. Whittington ed., *Root Growth*. Proc. 15th Easter School, Nottingham. Butterworths, London.
8. Hess, C. E., and W. E. Snyder. 1955. A physiological comparison of the use of mist with other propagation procedures used in rooting cuttings *Rpt. 14th Int. Hort. Cong.*, pp. 1133-1139.
9. Kraus, E. J., and H. R. Kraybill. 1918. Vegetation and reproduction with special reference to the tomato. *Ore. Agr. Exp. Sta. Bul.* 149.
10. Lek, H. A. A. van der. 1925. Root development in woody cuttings. *Meded. Landbouwhoogesch. Wageningen* 38(1).
11. Seth, A., and P. F. Wareing. 1967. Hormone-directed transport of metabolites and its possible role in plant senescence. *J. Exp. Bot.* 18:65-77.
12. Stoltz, L. P., and C. E. Hess. 1966. The effect of girdling upon root initiation, auxin and rooting co-factors. *Proc. Amer. Soc. Hort. Sci.* 89:734-743.
13. Strydom, D. K., and H. T. Hartmann. 1960. Absorption, distribution and destruction of indoleacetic acid in plum stem cuttings. *Plant Physiol.* 35:435-442.
14. Thimann, K. V., and J. B. Koepfli. 1935. Identity of the growth-promoting and root-forming substances of plants. *Nature*, 135:101.
15. Van Overbeek, J., S. A. Gordon, and L. E. Gregory. 1946. An analysis of the function of the leaf in the process of root formation in cuttings. *Amer. J. Bot.* 33:100-107.
16. Went, F. W., and K. V. Thimann. 1937. *Phytohormones*. The Macmillan Company, New York.
17. Winkler, A. J. 1927. Some factors influencing the rooting of vine cuttings. *Hilgardia* 2:329-349.